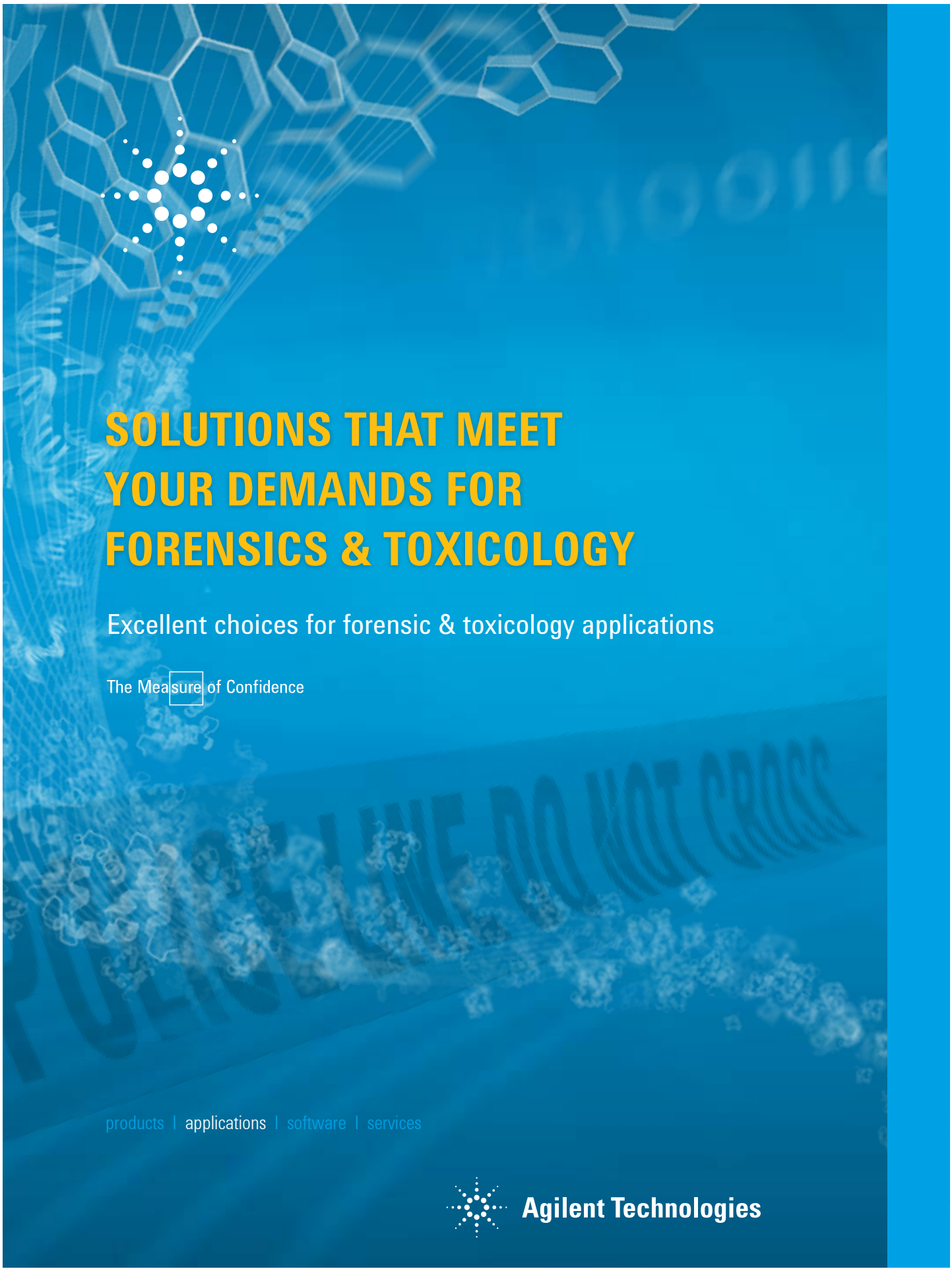

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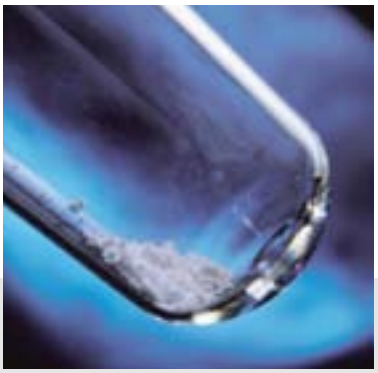

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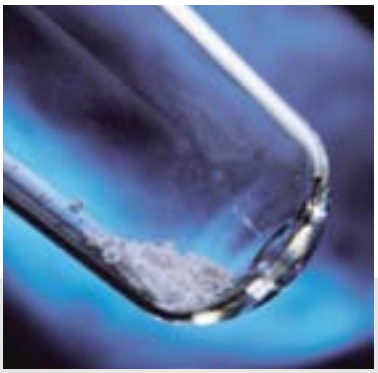

Applications by Technique

- ICP-MS
- LC/TOF & LC/QTOF
- LC/QQQ
- GC/QQQ
- LC/MS
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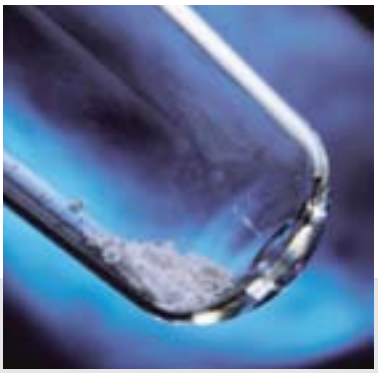
- Semiquantitative Analysis of Glass Fragments using Laser Ablation ICP-MS
- Analysis of Gunshot Residue by ICP-MS
- Introduction to Laser Ablation ICP-MS for the Analysis of Forensic Samples
- Methods for the Forensic Analysis of Adhesive Tape Samples by LA-ICP-MS
- Analysis of Forensic Glass Samples by Laser Ablation ICP-MS



Applications by Technique

ICP-MS

Semiquantitative Analysis of Glass Fragments using Laser Ablation ICP-MS

Application Brief

Forensic

Abstract

The 4500 ICP-MS equipped with a laser ablation system (LSX-100) was utilized to characterize and identify glass fragments from various sources. Rapid, semiquantitative analysis of the samples resulted in unique elemental "fingerprint" patterns that were used for sample identification. The method requires almost no sample preparation and sample consumption is limited. As a result, the remaining sample can be used to perform further tests if necessary.

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Introduction

When examining a glass fragment in a criminal case, the point in question is the identification or exclusion of the glass source. Historically, forensic comparison of glass samples has been limited to the comparison of physical properties of known and questioned samples, principally by the measurements of refractive index and density values. Due to advances in glass manufacturing technology, the range of the refractive indices of modern glass is narrowing, thereby potentially resulting in an increase of false positives[1]. In this study, an LSX-100 laser ablation system was connected to a standard 4500 ICP-MS enabling the direct multielement analysis of glass fragments. The elemental composition of a glass sample is a combination of major components, minor elements intentionally added to molten glass to enhance its physical properties, and trace levels of other elements which were present as contamination in raw materials.

Methods of elemental analysis are gaining popularity as forensic tools. The main disadvantage of many of these techniques for the analysis of glass is the required sample preparation: digestion/dissolution of the samples in HF. This sample preparation method is not only time consuming and requires extra safety precautions, but is also a destructive method, which in many cases may not be acceptable. LA-ICP-MS eliminates the need for extensive sample preparation, provides excellent detection limits, offers unmatched elemental coverage, and exhibits a wide dynamic range. An additional benefit of LA-ICP-MS is that the amount of sample used for a single determination is negligible and leaves the remaining sample available for further tests, if required.

Experimental

The 4500 ICP-MS was optimized using an NIST SRM 614 glass standard. The operating conditions for both the 4500 ICP-MS and the LSX-100 laser ablation system are listed in Table 1. Six glass samples of known origin were analyzed. The samples were washed with double distilled water, sonicated for 1 minute, and dried with isopropyl alcohol. The whole sample preparation process was less than 3 minutes. Three samples represented a class of flat glass: picture frame glass, window glass and a Pyrex glass from the laboratory. Three automotive glasses were also analyzed: 1991 Geo Metro, 1996 Dodge Avenger, and unidentified glass from the scene of an automobile accident.

Elemental ratios were used in developing the fingerprint patterns in order to eliminate variations in laser focus and in the extent of laser interaction with the sample surface. Another advantage to the use of elemental ratios is that quantitative calibration of the ICP-MS instrument is not required. However, if some estimate of the concentration were desired,

4500 ICP-MS	
RF Power	1.3kW
Interface	Ni cones
Plasma Gas	16 L/min
Auxiliary Gas	1.0 L/min
Carrier Gas	1.16 L/min
Sampling Depth	7 mm
Integration Time	0.6 sec/mass
LSX-100 Laser Ablation System	
Mode	Q-switched
Laser Power	1.5 mJ TEM00
Defocus	0.6 mm
Repetition Rate	20 MHz
Laser Scan Speed	0.03 mm/sec

Table 1.
Instrumental Parameters

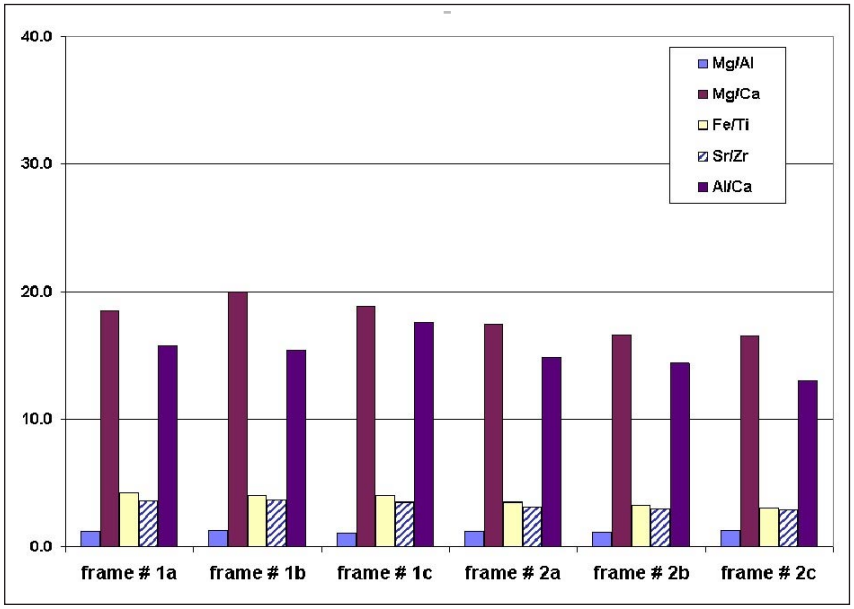

Figure 1.
Sample-to-Sampe and Intra-Sample Reproducibility

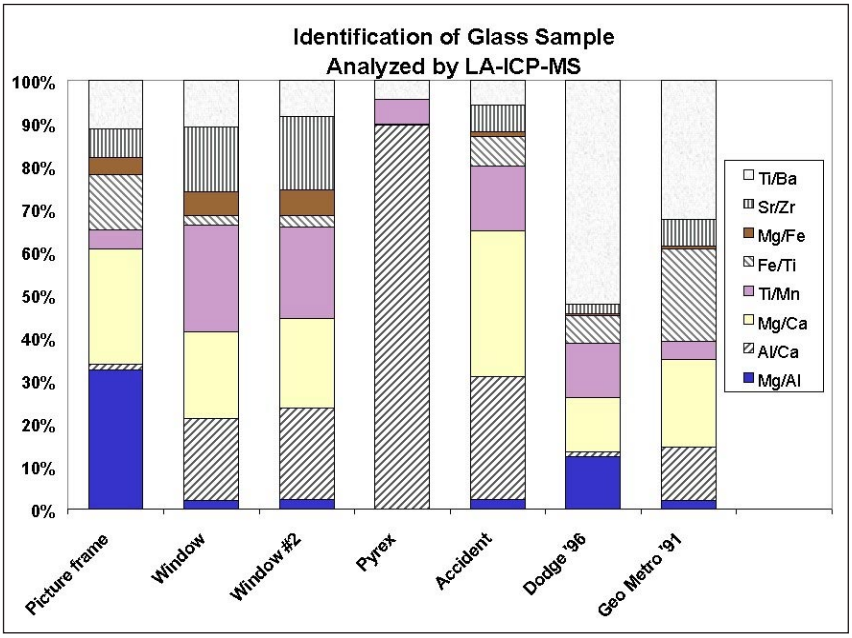

Figure 2.
The Graphical Representation of the Elemental Composition of Glass Samples

then the semiquantitative analysis feature of the 4500 ICP-MS software allows for the determination of over 70 elements during a single analysis in approximately 2 minutes.

Semiquantitative analysis requires quantitative calibration with a single standard and a minimum of only three elements which are not necessarily the analytes of interest. Approximate concentrations for all remaining elements in the periodic table can than be determined. Of course, true quantitative analytical results can also be obtained, if required.

Two different pieces of glass from each source were analyzed in triplicate. From all the elements, which were determined, 9 were identified as providing a distinct elemental fingerprint for the glasses examined: aluminum, barium, calcium, iron, magnesium, manganese, strontium, titanium and zirconium. The results, given in Figure 1, show excellent reproducibility between replicate analysis of a sample of glass from a picture frame (replicates a, b, and c) as well as between two different fragments of the same glass (frame #1 and frame #2). The results of the analysis of the six glasses are shown in Figure 2. Note that each sample has a distinct, identifiable elemental fingerprint pattern. A second fragment of the window glass (Window #2) was analyzed to once again confirm the unique nature of the fingerprint pattern and the reproducibility of the analysis.

Reference

[1] J.A. Buscaglia, Analytical Chimica Acta, 288 (1994) 17-24

Printed 4/2000
Publication number 5968-1953E

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Analysis of Gunshot Residue by ICP-MS

Agilent 4500 ICP-MS

Elzbieta (Ela) Bakowska
Peter B. Harrsch
Thomas J. Gluodenis, Jr.

Abstract

ICP-MS was successfully utilized for elemental analysis of gunshot residues (GSR). The concentrations of antimony, barium, and lead were determined from the GSR collection swab extract solutions. The capabilities of semiquantitative analysis were also demonstrated.

Introduction

The elemental analysis of GSR is being used as one of the tools in interpretation of the criminal event. Some answers to the question of "accepted uniqueness"[1] of GSR particles can be given by the determination of lead, antimony and barium with additional information provided by the determination of copper, zinc, and iron. Determination of antimony, barium, and lead from the hands of a suspect was originally performed by the Dermal Nitrate (paraffin cast) technique with diphenylamine used as the testing reagent. This technique detects nitrites in GSR. However, this technique also detects nitrites originating from other sources, such as urine, matches, fertilizer, and some pharmaceuticals. Thus, the diphenylamine test produces numerous false positives and has been abandoned as a means of detecting GSR[2]. The elemental analysis techniques which replaced the Dermal Nitrate method were neutron activation analysis (NAA) and graphite furnace atomic absorption spectrometry (GFAAS). Both of these techniques suffered from several limitations, especially as a tool for routine, rapid analysis of GSR samples. In the past several years, inductively coupled plasma mass spectrometry (ICP-MS) has gained wide acceptance for trace and ultratrace analysis of liquid and solid samples in variety of application fields, including judicial and regulatory arenas.

Experimental

GSR samples were collected from the hands of a person who had fired a 9 mm semiautomatic gun (Glock) with 9 mm ammunition (Federal Hydra Shok). The shooting was conducted outdoors, gun was handled two-handed, and the samples were collected approximately 40 minutes after the shooting. GSR samples and calibration solutions were placed on Q-tip cotton swabs (a pair of swabs for each sample and standard), placed in 15-mL polypropylene screw-top tubes and dried overnight. Sample preparation consisted of adding of 10.0 mL a 10% (v/v) nitric acid (Fisher Scientific, Optima grade) into each tube, recapping and vortexing for about 1 minute. The nitric acid solution was spiked with 50 µg/L each of indium (In) and bismuth (Bi) as internal standards[3]. The tubes with caps removed were placed in an oven set at 80°C for 2 hours. Solutions were mixed again, and centrifuged for 5 minutes for extract separation. The extract solution was transferred by pipetting into another polypropylene tube and analyzed.

The solutions were analyzed in unattended mode, employing the ASX-500 (CETAC) autosampler and the Agilent ChemStation software feature allowing for sequential analysis of the samples. The additional QA/QC software can be applied, to monitor the quality requirements of the analysis.

Table 1: Instrument Parameters

Agilent 4500 Series ICP-MS with ASX-500 Autosampler	
RF Power	1210 W
Nebulizer	cross-flow
Cones	Nickel
Sampling depth	8.4 mm
Plasma gas	16.0 L/min
Auxiliary gas	1.0 L/min
Carrier gas	1.16 L/min

The operating conditions for Agilent 4500 series ICP-MS instrument are listed in Table 1. Table 2 shows the acquisition parameters employed in quantitative analysis of the swabs.

Table 2: Acquisition Parameters Used for Quantitative Analysis

Monitored Masses	115, 118, 121, 123, 137, 138, 206, 207, 208, 209
Detector Mode	auto
Int.Time/point	0.1 s
Int. Time/mass	0.3 s
Number of Points/mass	3
Number of Repetitions:	3
Total Acquisition Time	16 s

Quantitation of element concentration was made using ^{115}In as internal standard for Sb and Ba. ^{209}Bi was used as the internal standard for lead (represented by the sum of its three major isotopes: ^{206}Pb + ^{207}Pb + ^{208}Pb). Lead has four naturally occurring isotopes at masses 204, 206, 207 and 208. Three major isotopes (206, 207, and 208), being products of different radio-decay processes, may vary in abundance, depending upon the source of lead. To minimize errors caused by the difference in isotopic distribution, the sum of the signals measured at three major isotopes is used and represented as the lead value. This approach was adapted from the USEPA methods utilizing ICP-MS for determination of lead in environmental samples[4]. Twelve-point calibration curves were created for all analytes. The calibration standard concentrations are listed in Table 3. The internal standard mix contained indium, and bismuth added at 0.05 µg level to all swabs.

The calibration curves for 121Sb, 138Ba and 208Pb are shown in Figure 1 through Figure 3, respectively.

Table 3: Calibration Standards for GSR Analysis (12-point Calibration)

Standard	Sb (µg)/swab	Ba (µg)/swab	Pb (µg)/swab
Blank (S-0)	0	0	0
S-1	0.01	0.05	0.05
S-2	0.02	0.10	0.10
S-3	0.03	0.15	0.15
S-4	0.04	0.20	0.20
S-5	0.05	0.25	0.25
S-6	0.10	0.50	0.50
S-7	0.15	0.75	0.75
S-8	0.20	1.00	1.00
S-9	0.50	2.50	2.50
S-10	1.00	5.00	5.00
S-11	2.00	10.0	10.0

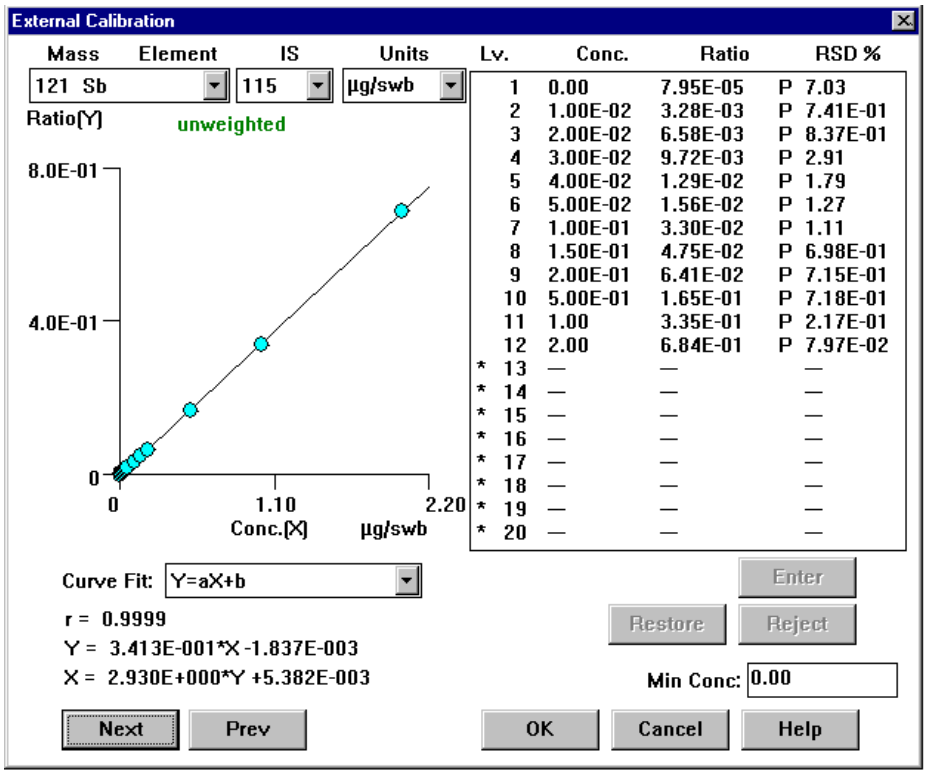

Figure 1. Calibration Curve: Antimony

The semiquantitative analysis of the samples was performed to demonstrate the unique capability of ICP-MS in providing fast and reliable information for over 70 elements, which can be present in the sample. This additional information can be used for further "fingerprinting" of GSR, similarly to the methods used in other forensic applications[5]. The acquisition parameters used in semiquantitative analysis are presented in Table 4.

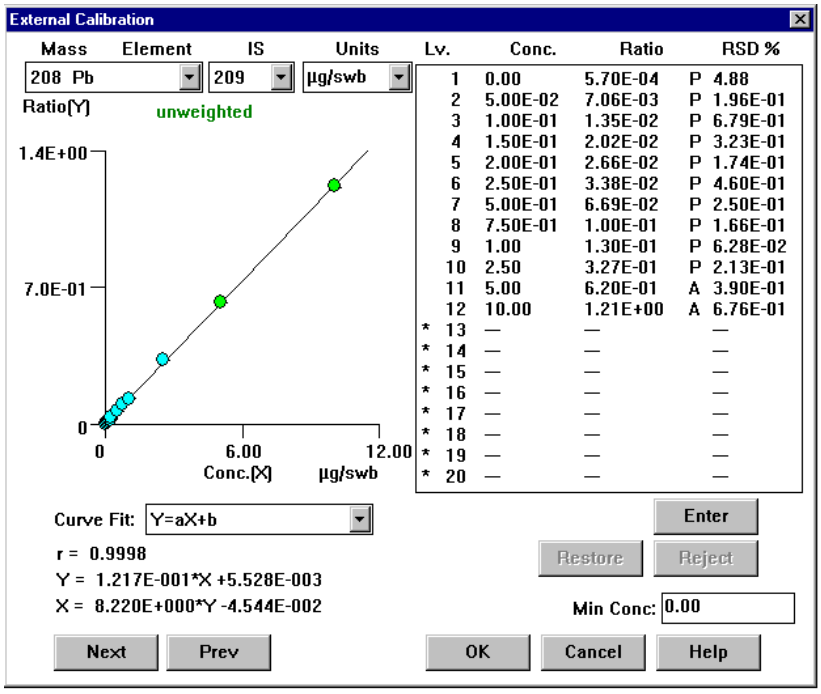

Figure 2. Calibration Curve: Barium

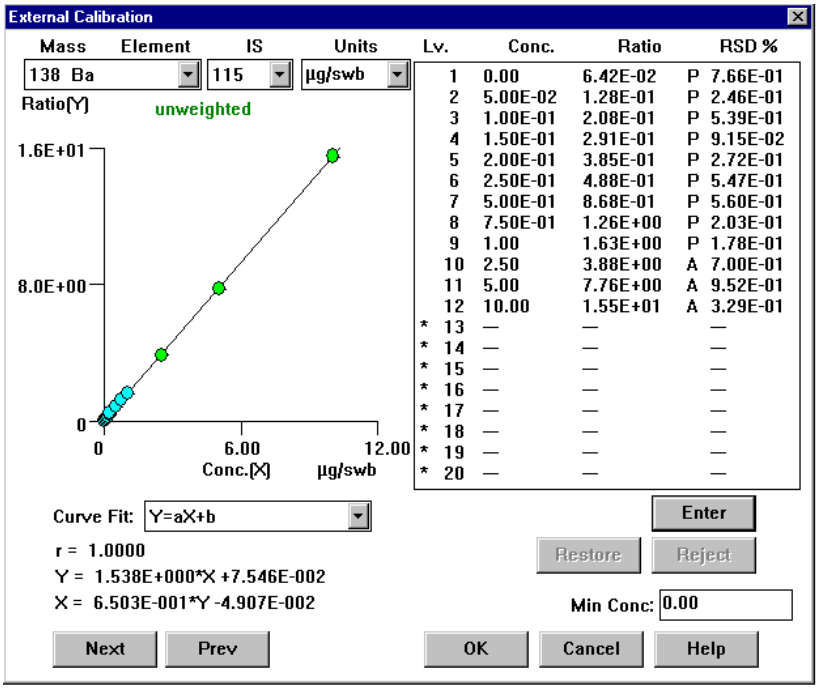

Figure 3. Calibration Curve: Lead

Table 4: Acquisition Parameters Used for Semiquantitative Analysis

Mass Ranges	6-11, 23-29, 39, 43-75, 77-78, 82-209, 232-238
Number of Masses	185
Detector Mode	auto
Int.Time/point	0.1 s
Int. Time/mass	0.6 s
No. of Points/mass	6
No. of Repetitions	1
Total Acq. Time	137 s

Results

Four samples were analyzed for the determination of Sb, Ba and Pb. They were swaps from the left hand palm, left hand's bottom, right hand palm, and right hand's bottom. They are labeled LP, LB, RP, and RB, respectively.

The results of the quantitative analysis of those four samples are presented in Table 5.

Table 5. Quantitative Analysis of GRS Samples

Sample	121Sb (µg)	138Ba (µg)	208Pb (µg)
LP	1.26	4.09	5.33
LB	0.27	0.91	1.50
RP	2.20	8.18	11.1
RB	0.12	0.40	0.91
Swab blank	0.007	<0.001	<0.001

The mass range, which includes the analytes of interest, is practically interference-free and monitoring either of the masses (121 or 123) for antimony will give the same results, especially since their relative abundances are almost equal. The isotope 121 was selected for reporting antimony results. For barium the two most abundant isotopes are 137 (11.2%) and 138 (71.7%). The latter offers more than six-fold higher signal, however it can

suffer from unpredictable elemental interferences from lanthanum and cerium. Most likely those interferences will be negligible in GRS samples, so the choice of isotope 138 is recommended. For routine analysis, only one isotope of antimony and one isotope of barium need to be quantified. As mentioned before, the lead value is calculated from a sum of signals collected at masses 206, 207 and 208 and represented as a value for isotope 208.

Figure 4 exemplifies the use of the semiquantitative analysis to verify that the signal measured at mass 138 was associated with barium. The software allows for measurement of the signals generated at the large mass range and subsequently fits a template corresponding to the natural abundance of the barium isotopes over the resulting signals. It is clear that there is a perfect fit between the experimental and theoretical values, thus the signal measured at mass 138 is a consequence of barium present in the solution.

Conclusions

ICP-MS was proven to be rapid and reliable analytical method for the determination of antimony, barium and lead in gun shot residue samples. The total analysis time (without sample preparation) was little over 2 minutes, with the actual data acquisition time equal to 16 seconds.

There are additional possible applications of ICP-MS for the evaluation of the origin of the GSR by the determination of other elements like copper, nickel and silver, and measurement of the isotopic ratios of lead.

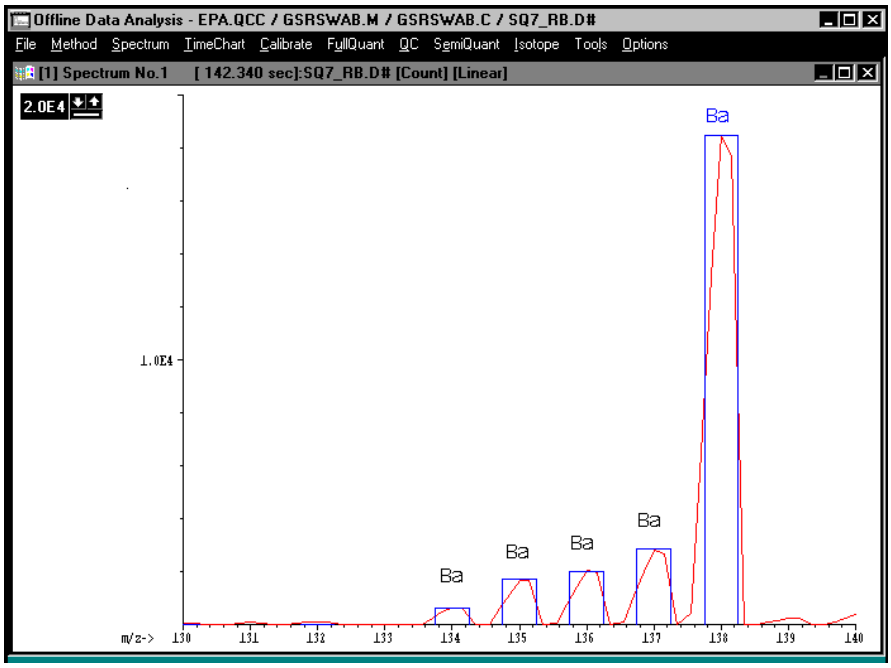

Figure 4. Isotopes of Barium: Semiquantitative Analysis

References

[1] A. Zeichner and N. Levin, J. Forensic Sci. 1997; 42(6), 1027-1028

[2] S.S. Krishnan, "Detection of Gunshot Residue: Present Status", in "Forensic Science Handbook", R. Saferstein, Ed., Prentice Hall, NJ, 1982, pp.139-183

[3] R. D. Koons, J. Forensic Sci. 1998; 43(4), 748 – 754

[4] US Environmental Protection Agency, EPA Method 200.8, Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma Mass Spectrometry, Version 5.4, 1994

[5] E. Bakowska, HP Application Brief, September 1998, Publ.No. (23) 5968-1953E

This application bulletin demonstrates feasibility of concept. Additional development and/or validation may be required for routine use.

Printed 3/2001
Publication number 5988-0532EN

Introduction to Laser Ablation ICP-MS for the Analysis of Forensic Samples

Application

Forensics

Author

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Abstract

Forensic scientists require reliable methodologies capable of determining the origin of inorganic materials found at the scene of a crime. Linking these materials to a suspect or suspects can result in the vital evidence needed to secure a successful prosecution. Due to the great variety, shape, and size of forensic material, there is a need for a flexible analytical tool capable of analyzing the trace element content of solid samples directly. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) enables identification and comparison of physical evidence, discriminating elemental and isotopic differences at the part per billion (ppb) level. In contrast to aqueous analysis, where significant amounts of material need to be destroyed in the analytical process, LA-ICP-MS is a micro-destructive technique. Often the total volume of sample ablated with this technique is <1 µg; sustaining the essential integrity of the original evidence, which may be extremely small, and enabling further measurements to corroborate the results.

Introduction

Today's quadrupole ICP mass spectrometers enable the analysis of elements across the periodic table at very high scanning speeds and with very low detection limits. Typically samples are introduced into an ICP-MS by aspirating a solution of the sample. Often liquid samples require little preparation, but without a solid sampling accessory, solid samples need to be dissolved. This process is time consuming and often requires the use of acid dissolution reagents and additional sample preparation apparatus. Adding hazardous chemicals, such as hydrofluoric acid to dissolve the sample, can give rise to matrix-based interferences forming in the plasma. Hazardous chemicals are also a potential source of contamination. In contrast, combining ICP-MS with the direct solid-sample introduction technique of laser ablation (LA) requires minimal sample preparation. LA-ICP-MS provides an excellent and relatively nondestructive technique for elemental analysis of forensic samples that are difficult to digest, or where small fragments or inclusions must be analyzed. LA-ICP-MS is particularly amenable to time-resolved analysis (TRA); enabling direct comparison of samples in three dimensions. Combining such flexible data handling capabilities with *in-situ* solid sampling enhances discriminating power; strengthening the analyst's ability to determine the similarities and differences within large data sets.

LA-ICP-MS

LA-ICP-MS is widely used to determine elements directly in solid samples with minimal sample preparation. It is a highly sensitive multi-element technique with a wide analytical dynamic range from the part per trillion (ppt) to the part per million (ppm) level in the solid. For this study, a Merchantek UP-213 (New Wave Research. Inc, USA) LA system was coupled to an Agilent 7500s ICP-MS. A schematic of the LA system is shown in Figure 1.

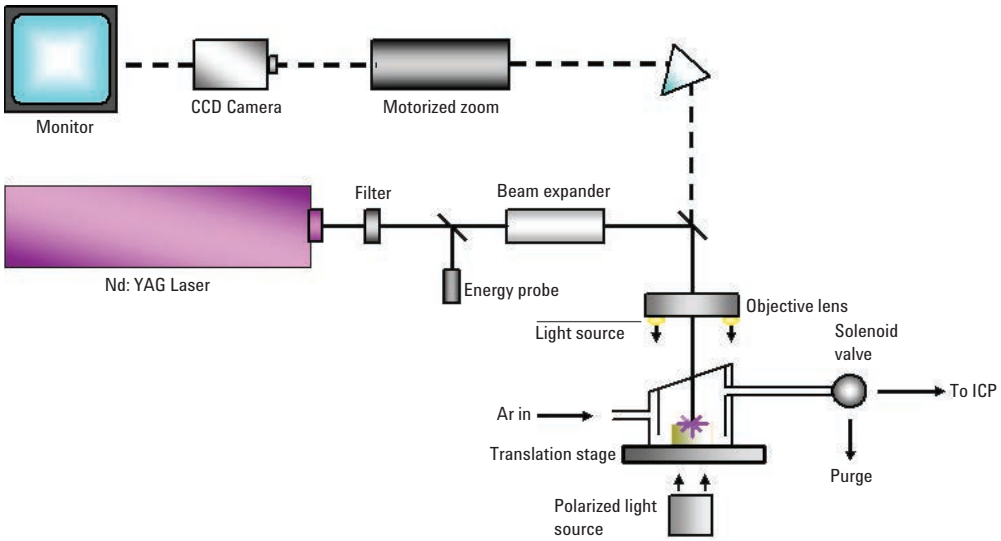

Figure 1. Schematic of Nd: YAG LA system (5th harmonic - 213 nm) for ICP-MS.

The sample surface is irradiated with deep-UV (213 nm) output from a frequency-quintupled Nd:YAG (neodymium doped yttrium aluminum garnet crystal) laser. The high-intensity pulsed ultraviolet (UV) beam is focused onto the sample surface in an ablation chamber or cell, which is purged with argon. The UV beam diameter can be accurately set by 12 software-controlled apertures to produce variable "spot" sizes from <5 µm to 300 µm depending on the application. The high-power, short-wavelength 213-nm laser couples directly with the sample matrix, with high absorption efficiency, reducing or eliminating plasma induced fractionation. The resultant laser-induced aerosol is then transported to the ICP in an argon carrier gas stream where it is decomposed, atomized and ionized, before extraction into the mass spectrometer vacuum system for analysis. Calibration is typically undertaken using a well-characterized synthetic solid material, such as NIST 612 Trace Elements in Glass or other suitable solid standard reference material (SRM).

Sample Analysis Using LA-ICP-MS

Generally, the ICP-MS is optimized by tuning the system during continuous ablation of a suitable SRM; examples of reference materials for glass and tape are given in Table 1. The Agilent ICP-MS can be optimized automatically using the AutoTune function of the Agilent ChemStation software. Often, tuning parameters for LA analysis are similar to those used for solution analysis. Tune parameters can be saved in a separate file for recall at a later date. If a SRM is available for the matrix being analyzed, it can be used to generate semi-quantitative response factors which are automatically stored in the ChemStation software. The sample can then be analyzed using a matrix element as the internal standard (IS). If an SRM is analyzed, the concentration of the IS is given and quantification is straightforward. However, for unknown samples, typical IS examples include the use of ^{13}C in polymer analysis and minor matrix isotopes in materials such as ceramics, stainless steel, and borosilicate glass, where the stoichiometry of the sample is known. While it is ideal to match the matrix of the standard to the sample, good semiquantitative data can be obtained for a wide range of matrices using a single set of response factors. This is because of the uniform response of the 7500 Series ICP-MS across the mass range, and the fine aerosol generated by the UV laser, which is more completely decomposed in the plasma, reducing matrix effects.

Table 1. Details of Forensic Standard Reference Materials

	Glass	Tape
Standard	NIST SRM 612: 50/µg/g nominal trace element concentration	BCR SRM 680: Trace elements in polyethylene
Matrix elements	Si (SiO_2), Na (Na_2O), Ca (CaO), Al (Al_2O_3)	
Source	National Institute of Standards and Technology, USA	Institute for Reference Materials and Measurements, Geel, Belgium

Software Controlled Operation

The LA software can be fully integrated into the Agilent ICP-MS ChemStation software for ease of setup and operation of the LA and ICP-MS. All laser parameters (for example, laser energy, frequency, purge valve position, sample viewing, stage positioning, and ablation pattern) can be controlled via the ICP-MS ChemStation PC. A high-magnification video system enables a full color, high-resolution image of the sample to be viewed directly on the ICP-MS monitor in real time, see Figure 2. The computer-controlled zoom feature and electronically-generated cross hairs aid sample positioning and can also be used to measure the size of any inclusions directly on the screen. Laser parameters can then be set accordingly, and during data acquisition, the laser power meter reading can be monitored on screen.

Forensic Applications

It is the task of the analyst to generate evidence based on trace elemental fingerprinting that can prove or disprove the source of the material. As a consequence, forensic materials presented for analysis by LA-ICP-MS could be anything from strands of hair to fibers of clothing.

For example, the glass used in the headlights and windows of automobiles is often unique to a manufacturer, and the elemental profile can be used to identify the marque, brand, or even year of manufacture of the vehicle. Trace element content offers far better discrimination than the traditional refractive index (RI) method. LA-ICP-MS provides a fast and simple means of characterizing glass fragments found on a suspect's clothing or at the location of an accident, without time-consuming sample preparation. Although the major and minor elemental composition of these glasses are very similar, and therefore are difficult or impossible to discriminate using traditional methods of characterization, these glasses may have trace elemental signatures (Figure 3) which enable accurate evaluation of differences by LA-ICP-MS.

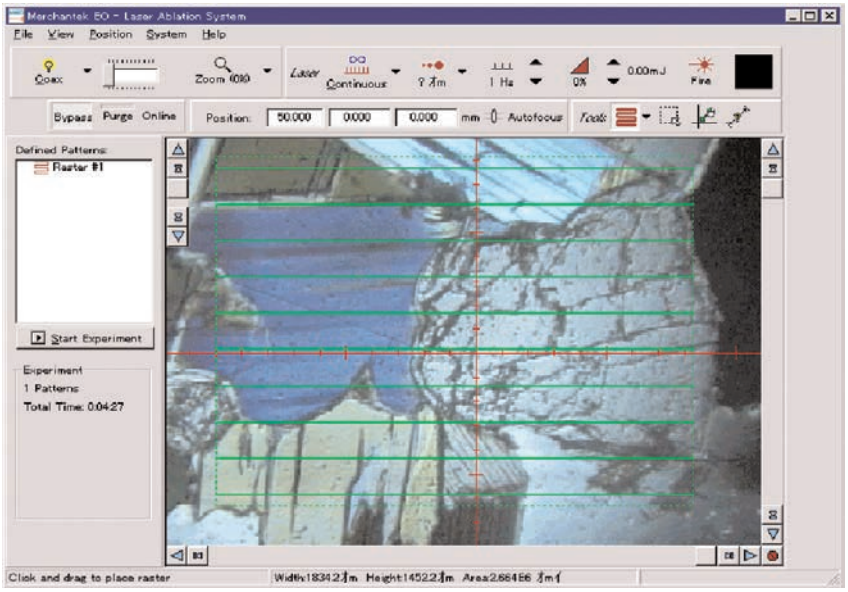

Figure 2. Screen capture showing a full color, high-resolution image of the sample.

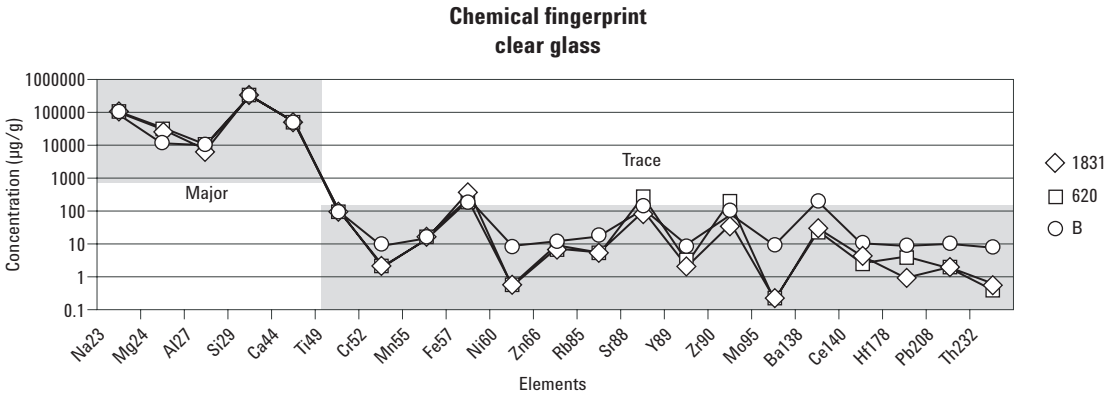

Figure 3. Elemental signature of clear glass.
The major elemental composition of these three glasses is similar (Na, Al, Si and Ca) while the trace elemental composition ≤100 µg/g (Cr, Ni, Rb, Sr, Y, Zr, Mo, Ba, Ce, Hf, Pb, Th) displays significant differences. Mg is an exception and at high concentration is often used as a discriminating element.

LA-ICP-MS can also be applied to other samples such as identifying inks on suspect documents, or element profiles of other scene of crime debris, including multi-layer paints, coatings on glass, bulk polymers, plastic bags, tape, and automobile parts. Figure 4 illustrates a sample of ballpoint pen ink after analysis using LA-ICP-MS. The ablated portion of the ink is clearly visible on the right side of the photograph.

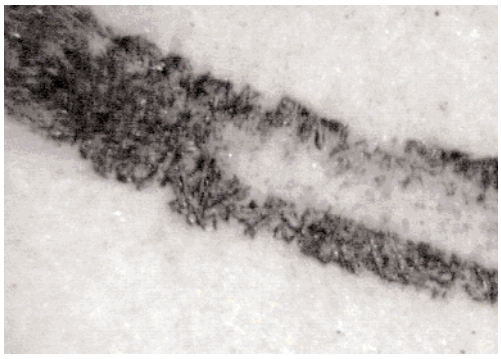

Figure 4. Magnified photograph of ballpoint pen ink sample after LA-ICP-MS sampling.

Data Manipulation

The data generated from LA-ICP-MS can be manipulated in real-time to enable the user to view the results of an analysis within seconds of data acquisition. Various optional software packages are available including:

- Glitter™ data reduction software
  Macquarie University - GEMOC [1]

GLITTER is an acronym for GEMOC Laser ICP-MS Total Trace Element Reduction. In addition to real-time on-line data reduction, GLITTER features a variety of plotting options, linked graphics and analysis tables, for simple presentation of the results. The ability to visualize results can aid users of forensic evidence in their understanding of the data.

- TriPlot Ternary plotting software
  Todd Thompson Software [2]

TriPlot produces a triangular plot of three variables that are plotted on the left, right and bottom sides of an equilateral triangle. Ternary plots are an effective way to discriminate subtle differences in sample populations, especially when multiple data point display is desirable as shown in the example in Figure 5.

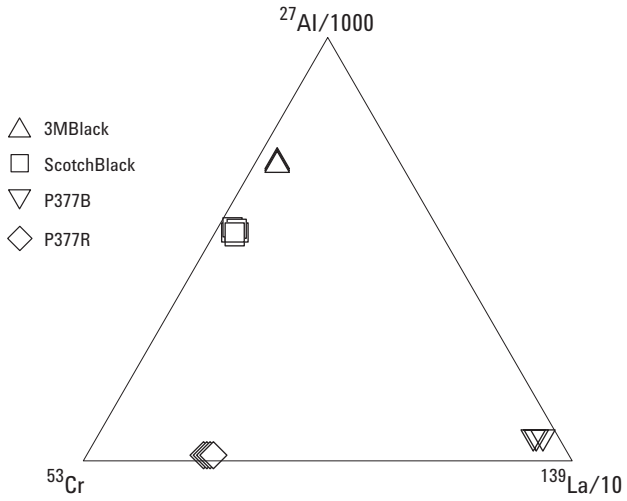

Figure 5. Ternary plot of adhesive tape data (integrated counts per second).

Conclusions

LA-ICP-MS is an effective tool for the analysis of a wide variety of forensic samples. This technique is particularly effective in overcoming the limitations associated with very small sample types or samples composed of chemically inert materials. The definitive "fingerprint" produced by LA-ICP-MS based on elemental and isotopic ratio data is used to qualify or disqualify the source of physical evidence. Often a clear visual representation of the data is produced using a suitable plotting program making it easier to discriminate samples.

Reference

1. Glitter™ data reduction software, Macquarie University - GEMOC
   http://www.es.mq.edu.au/GEMOC
2. TriPlot Ternary plotting sofware, Todd Thompson Software
   www.home.earthlink.net/~baedke/triplot

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Printed in the USA
September 9, 2004
5989-1565EN

Agilent Technologies

Methods for the Forensic Analysis of Adhesive Tape Samples by LA-ICP-MS

Application

Forensics

Author

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Abstract

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) offers great potential as a highly discriminatory technique for the analysis of forensic samples of adhesive tape. Four tape samples and a polyethylene standard were analyzed in this study. By ablating through multiple layers of alternating tape and adhesive glue, an elemental pattern that is unique to that specific tape can be obtained. Being able to present data clearly and unambigiously in a court of law is another consideration for the forensic scientist. Fortunately, LA-ICP-MS data can be presented using various plotting techniques, each designed to discriminate samples with similar visual, physical, and chemical characteristics. These attributes, combined with low levels of detection and high precision, explain the increasing acceptance of LA-ICP-MS for forensic investigation of tapes.

Introduction

Adhesive tape samples may be presented as crime scene evidence from various types of criminal activities: drugs, explosives, stolen articles, documents, etc. In such cases, the forensic scientists may be requested to compare the tape encountered at the crime scene with that found with a suspect or suspects. Traditional techniques for the analysis of tape include visual methods, Fourier transform infrared (FT-IR) for layers analysis, and x-ray fluorescence (XRF) for elemental analysis. Tapes from the same batch, from different manufacturers, of different color and/or morphology, can be discriminated effectively using these methods in many cases. However, for "in-type" discrimination (same brand, different batch and/or same color and matrix), a more rigorous chemical approach is necessary [1].

Standard techniques for the trace elemental analysis of these materials (polyethylene, polypropylene, acetate polymers) typically include time-consuming digestion procedures and hazardous waste by-products. Complete digestion and good trace element recoveries are not always guaranteed.

LA-ICP-MS is an alternative method offering many advantages over standard dissolution techniques. This application note will describe a procedure for the analysis, interpretation, and quantification of these sample types. Though this is a forensic application, there are clear benefits of this technique for environmental concerns.

Instrumentation

All the analyses for these experiments were undertaken using an Agilent 7500s ICP-MS. Solid sampling was achieved by introducing a stream of particles generated *in-situ* by direct coupling of a short ultraviolet (UV) laser with the sample surface into the ICP using a stable flow of argon gas. The laser system used was a New Wave Research (Fremont, CA) UP-213AI Nd:YAG operating at the 5th harmonic frequency (213 nm). Operating parameters for each experiment are given in Table 1. For more information on LA-ICP-MS, refer to application note 5989-1565EN [2].

Operating Parameters

Table 1. LA-ICP-MS Operating Conditions

Polyethylene standard			
Laser		**ICP-MS**	
Line ablation		RF Power:	1200 W
Spot size:	100 µm	Plasma gas:	14 L/min
Line length:	350 µm	Carrier gas:	0.8 L/min
Power:	1.2 mJ	Acquisition:	Time Resolved Analysis (TRA)
Stage speed:	20 µm/s	Integration:	50 ms
Pulse frequency:	10 Hz	Masses:	21
		Acquire time	180 s
Adhesive tape			
Laser		**ICP-MS**	
Spot ablation		Same as standard	
Spot size:	250 µm		
Power:	2.2 mJ		
Pulse frequency:	10 Hz		

Experimental

Calibration of the LA-ICP-MS was carried out using the following standard from Institute for Reference Materials and Measurements, Geel, Belgium.

BCR SRM 680: Trace elements in polyethylene

Adhesive tape samples were acquired from multiple sources. Two samples (P377R, red and P366B, blue) were supplied by VHG Labs, Inc., Manchester, NH. They were part of a group of industrial QC samples sent to the lab for digestion and subsequent aqueous analysis. Tan packing tape brand A and brand B, and 3M and Scotch black electrical tape were purchased at Walgreens, Fremont, CA. Both the tan adhesive tape samples and the black electrical tape samples were visually identical, but produced by two different manufacturers.

Both the polyethylene standard (BCR SRM 680) and the tape samples were attached to a petrographic slide (Figure 1) and placed in the standard UP sample cell for analysis. Tape samples were cut directly from the parent role. For sampling, at least 10 layers of tape were removed as a section from each roll. All tape samples were ablated continuously (250-µm spot) through multiple, alternating layers of the base polymer and sticky adhesive as illustrated in Figure 2. The data was imported into Glitter™ data reduction software for both qualitative and quantitative analysis.

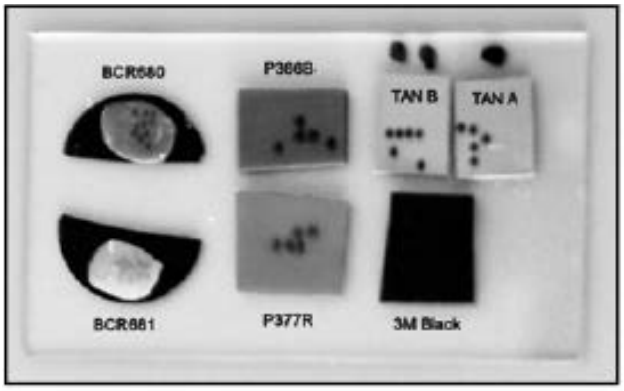

Figure 1. BCR standards and adhesive tape mounted on petrographic slide showing ablation craters.

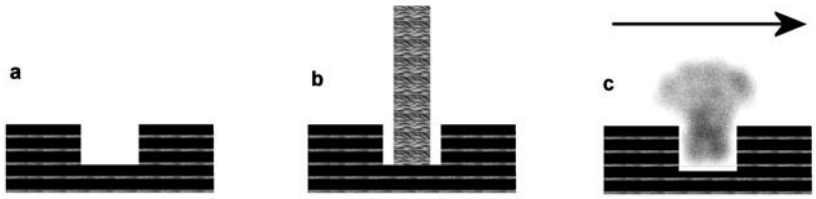

Figure 2. Graphical representation of the ablation process on layered tape samples. a) Layered tape sample after repetitive ablation b) generation of laser plume and c) subsequent removal of laser aerosol within an argon carrier gas stream (arrow).

Results

The six tape samples and a polyethylene standard (BCR SRM 680) were analyzed. Five repetitive analyses for each sample and standard were performed. The time-resolved layer analysis (Figure 3) was evaluated to determine the most appropriate way to integrate the data. By ablating through multiple layers of alternating tape and adhesive glue, a unique elemental pattern can be visualized. These elemental "wave-forms" appear to be interlaced or "out of phase" with one another. One set of elements (Al, Mn, Co, and Sb) appears to be associated with the tape backing material, as their signal rises immediately after the start of the ablation cycle. The second set of elements (Cr, Zr, La, Ce, and Pb) appears to be associated with the adhesive glue, as their signals trail the first set by 10 seconds. The lines continue out of phase for the remainder of the ablation cycle. These data were integrated over the entire period of ablation using the ^{13}C profile as a reference. The trace element concentrations for the P366B and P377R samples were calculated using the BCR SRM 680 (Table 2), and elemental relationships were characterized using a stacked bar graph (Figure 4).

In a previous study (Dobney et al) [1], tape samples were acid digested and the aqueous aerosol analyzed. It was determined that the polymeric base material (PP, PE, and PVC) was difficult to get into solution, was more prone to acid-based matrix interferences, and was chemically less interesting than the adhesive glue. Therefore, trace elements were quantified in the adhesive glue only. By ablating through multiple layers, it is possible to integrate the adhesive elements independently from the elements in the tape backing (data not shown) without any of the difficulties inherent with aqueous digestion.

Ternary plots compare the relationship between three components in a system. Each corner of the plot represents 100% of the labeled component. A data point in the center of the plot signifies that the sample is of equal composition for all three constituents. Ternary plots, more technical in nature compared to bar charts, can discriminate different sample types from one another as well as display sample reproducibility. Tight clustering of sample types describes good sampling precision and data reliability. Figure 5a describes the relationship between ^{27}Al, ^{121}Sb, and ^{137}Ba for the six tape samples characterized here. Although good separation is possible for four of the six tape samples, further discrimination (Figure 5b) is necessary to discriminate the remaining two Tan tape samples by changing the parameters of the ternary plot. In this way, clear separation may be accomplished between samples that are visually identical.

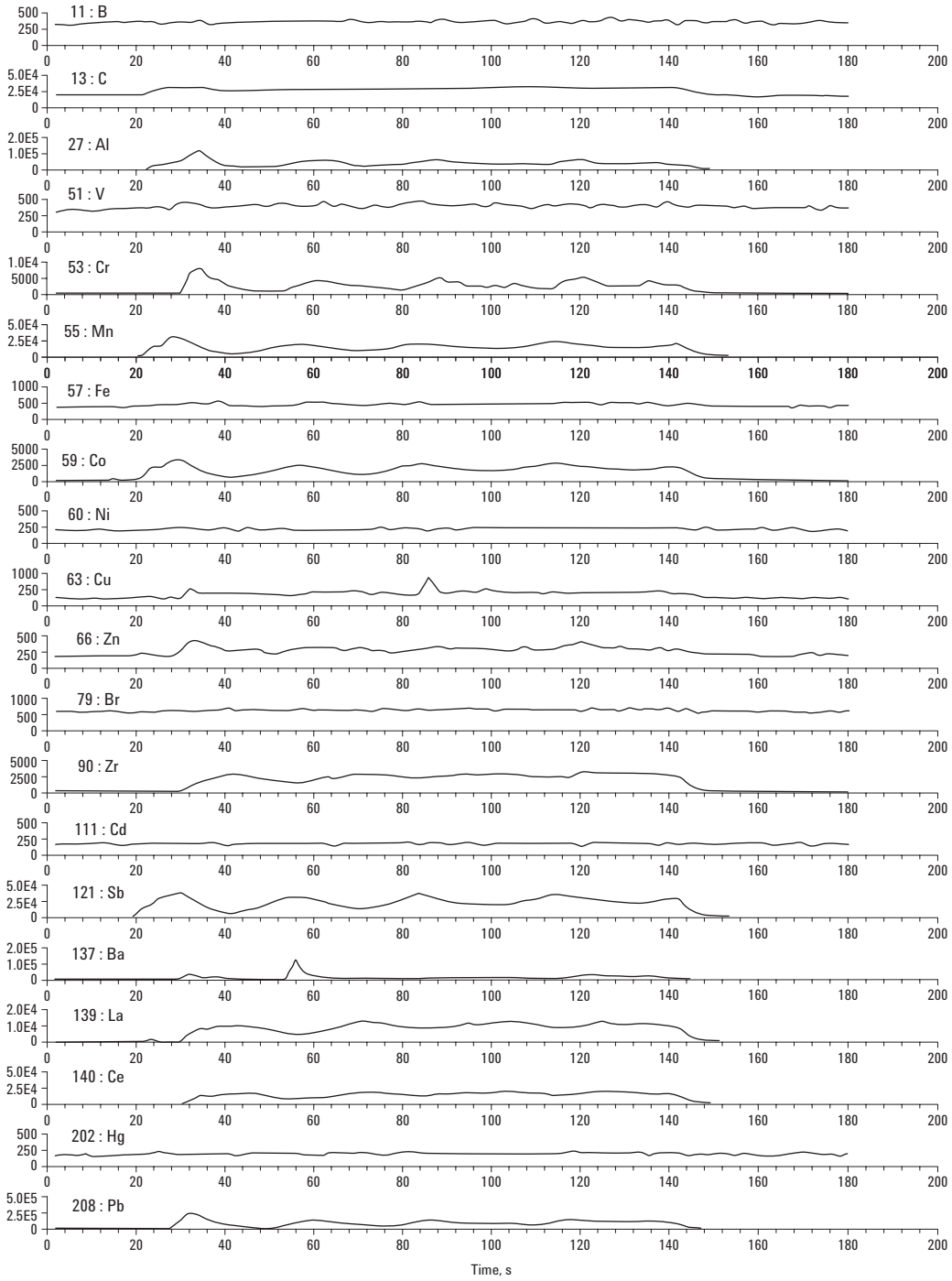

Figure 3. Time resolved layer analysis of colored (blue and red) electrical adhesive tape. Agilent ChemStation time resolved output format of a multi-element profile through successive tape layers. Notice how certain elemental signatures have delayed rise times. Elements within the tape matrix and the adhesive matrix are "out of phase" with respect to each other. Al, Mn, Co, and Sb rise with the onset of the ablation start point (t + 20s). Cr, Zr, La, Ce, and Pb first rise approximately 10 seconds later.

Table 2. Quantitative Analysis of Trace Elements in Polyethylene (BCR SRM 680), and the Two Tape Samples (P366B and P377R). Quantitative Data Was Reduced Using Glitter Data Reduction Software.

BCR

Element	Mean	SD	Agreement %
Al 27	51.4	0.4	100.7
Cr 53	114.5	0.1	99.9
Cu 63	118.4	1.9	99.5
Br 79	798.9	8.8	98.9
Cd 111	135.2	12.8	96.0
Sb 121	6.3	0.2	101.1
Ba 137	2639.0	132.8	97.1
Hg 202	24.4	0.7	96.5
Pb 208	107.3	3.2	99.7

P366B

Element	Mean	SD
Al 27	585.2	25.2
Cr 53	2.5	0.7
Cu 63	1016.7	32.5
Br 79	17.2	3.3
Cd 111	18.3	1.1
Sb 121	1162.2	22.1
Ba 137	9.8	2.7
Hg 202	1.8	0.3
Pb 208	2.2	0.7

P377R

Element	Mean	SD
Al 27	611.7	77.8
Cr 53	215.1	29.2
Cu 63	4.2	0.7
Br 79	30.9	15.4
Cd 111	22.9	4.3
Sb 121	1139.8	90.8
Ba 137	877.3	87.4
Hg 202	3.2	1.0
Pb 208	3067.8	397.2

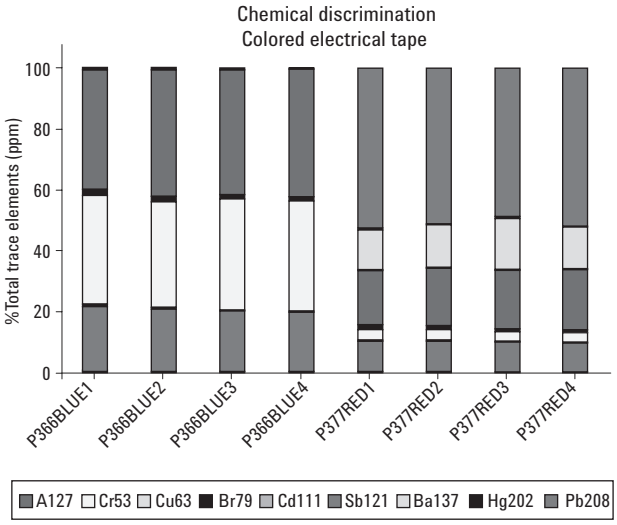

Figure 4. Stacked bar plot of two polypropylene tapes (blue and red) from the same manufacturer. The data in the chart is derived from Table 2.

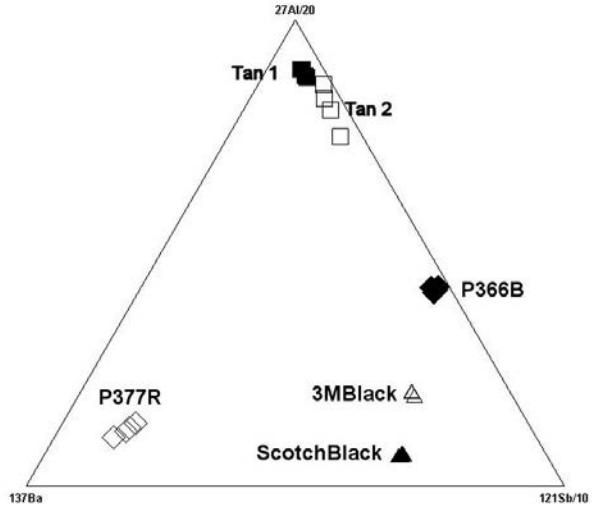

Figure 5a. Ternary Plot of Adhesive tape data (integrated counts per second). Ternary plots are an effective way to discriminate subtle differences in sample populations, especially when multiple data point display is desirable.

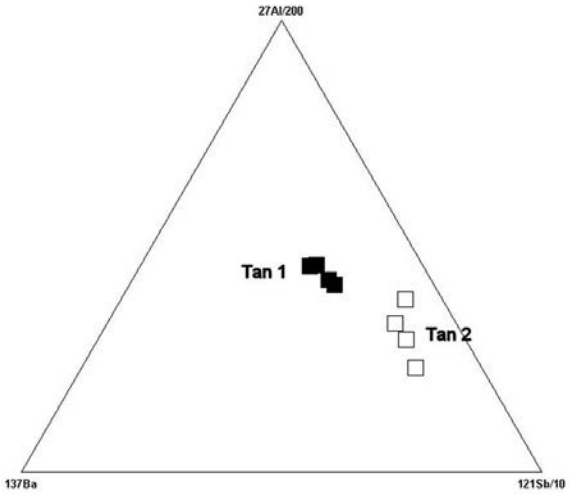

Figure 5b. By changing the parameters of ternary plots, it is possible to further discriminate subtle differences in chemistry between visually identical samples.

Conclusion

LA-ICP-MS is an *in-situ* analytical method capable of sampling through layered materials. Through the direct analysis of adhesive tape, three-dimensional chemical characterization is possible. Preliminary evidence suggests that there is an advantage to analyzing both the substrate and the adhesive in these samples.

Through the implementation of various plotting techniques, it is possible to discriminate samples with similar visual, physical, and chemical characteristics. By combining powerful, *in-situ* micro analysis, low levels of detection, high precision, and clear and easily understandable diagrams, LA-ICP-MS is becoming an increasingly important weapon in the arsenal of forensic science.

References

1. A. Dobney, W. Wiarda, P. de Joode and Gerard van der Peijl, Forensic Tape Investigations, Presentation at 2nd EAFS Meeting, 2000 Sept 24, Cracow.
2. Lawrence M. Neufeld, “Introduction to Laser Ablation ICP-MS for the Analysis of Forensic Samples”, Agilent Technologies publication 5989-1565EN www.agilent.com/chem.

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Printed in the USA
September 28, 2004
5989-1566EN

Analysis of Forensic Glass Samples by Laser Ablation ICP-MS

Application

Forensics

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Abstract

Physical evidence is often distributed widely when a crime is being committed. The smaller these suspect materials are, the more likely they will be transported from the crime scene undetected. When glass is shattered, the fragments created can be less than a few hundred microns (<0.2 mm). These fragments can become attached to clothing and embedded in shoes, "tagging" the criminal with a unique marker. However, as the major and minor elemental composition of modern glass is becoming more difficult to discriminate using traditional methods, new instrumentation is needed capable of resolving differences in the trace elemental profiles of similar glasses. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was evaluated and found to provide the accuracy, sensitivity and spatial resolution necessary for this application.

Introduction

Traditional methods of forensic glass analysis include the determination of a number of physical properties, including refractive index (RI), wet chemistry, scanning electron microscopy (SEM), x-ray fluorescence (XRF), and optical microscopy [1]. Although these techniques offer a high degree of differentiation with traditional glass, modern glass has a greater degree of chemical and physical similarity. The major and minor elemental composition and RI values of these new materials are becoming more difficult to discriminate. The histograms in Figures 1a and 1b show RI values for flat glass extracted from an FBI database for the periods of 1964 to 1979 and 1980 to 1997 respectively [2]. Comparison of the two charts clearly shows the reduced opportunity for intersample discrimination using this technique. Although the major and minor elemental composition of these glasses are very similar and therefore difficult or impossible to discriminate, using traditional methods of characterization, these glasses may have trace elemental signatures which are distinguishable by LA-ICP-MS.

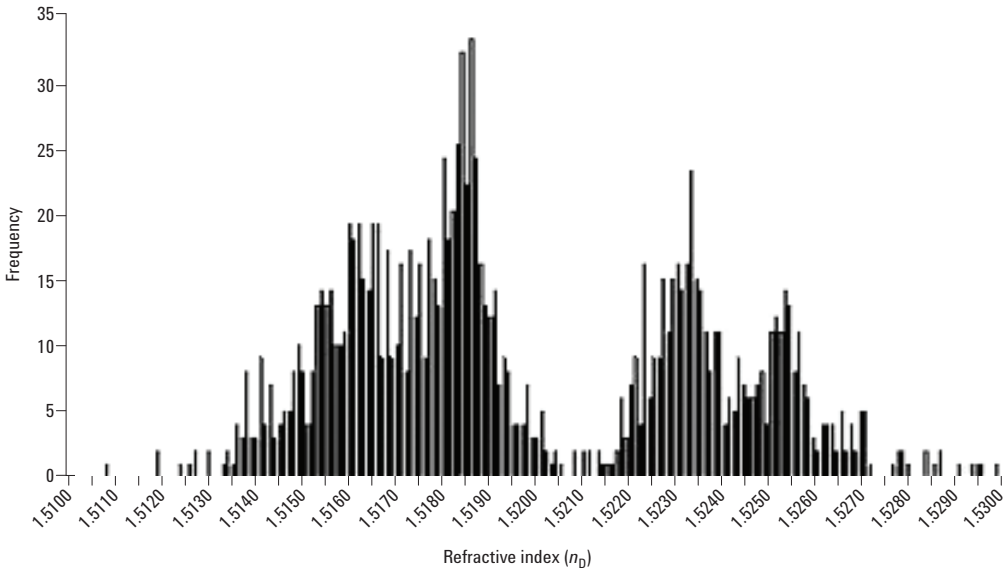

Figure 1a. Distribution of RI values from FBI database of flat glasses, 1964 to 1979.

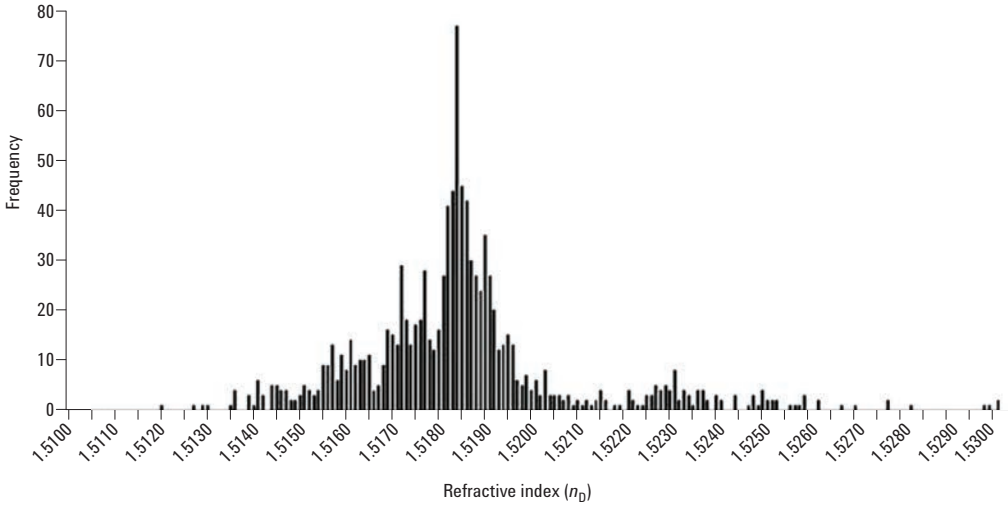

Figure 1b. Distribution of RI values from FBI database of flat glasses, 1980 to 1997.

Instrumentation

All the analyses for these experiments were undertaken using an Agilent 7500s ICP-MS. Solid sampling was achieved by introducing a stream of particles generated *in-situ* by direct coupling of a short ultraviolet (UV) laser with the sample surface into the ICP using a stable flow of argon gas. The laser system used was a New Wave Research (Fremont, CA) UP-213AI Nd:YAG operating at the 5th harmonic frequency (213 nm). Operating parameters for each experiment are given in Table 1. For more information on LA-ICP-MS, see Reference 3.

Operating Parameters

Glass Fragments

Table 1. LA-ICP-MS Operating Conditions

Laser		ICP-MS	
Line ablation		RF Power:	1200 W
Spot size:	100 µm	Plasma gas:	14 L/min
Line length:	350 µm	Carrier gas:	0.8 L/min
Power:	2 mJ	Acquisition:	Time Resolved Analysis (TRA)
Stage speed:	20 µm/s	Integration:	10 ms
Pulse frequency:	10 Hz	Masses:	36
		Acquire time	114 s

Experimental

Calibration of the LA-ICP-MS was carried out using the following standard, obtained from National Institute of Science and Technology (NIST), USA:

NIST SRM 612: 50 µg/g nominal trace element concentration.

Matrix elements: Si (SiO_2), Na (Na_2O), Ca (CaO), Al (Al_2O_3)

NIST soda lime glass standards (620, 621 and 1831) were used as surrogates for float glass (flat, clear glass) samples. It was therefore possible to check the accuracy and the precision of the calculated values by comparing them with the certified values given for the major elements (Table 2). Each sample was placed in a separate, sealed plastic bag and shattered. The small fragments (0.5 mm to 2 mm) were attached to a petrographic slide using double-sided graphite tape (Figure 2). This process was repeated for all the surrogates, as well as the three headlamp samples.

Table 2. NIST SRM 612 Major and Trace Multi-Element Results

Element	Na 23	Mg 24	Al 27	Ca 44	Ti 47	Cr 52	Mn 55	Fe 57	Ni 60	Zn 66
Mean, ppm	10.4%	79.07	1.1%	8.7%	49.5	39.8	38.5	57.7	38.8	38.6
SD	0.2%	2.51	0.0%	0.1%	2.52	0.30	0.49	2.99	2.02	1.50
%RSD	1.9	3.2	0.5	1.1	5.1	0.8	1.3	5.2	5.2	3.9
%Agreement	100.3	102.1	105.6	101.3	103.0	99.6	100.2	102.5	100.8	101.7
Element	**Rb 85**	**Sr 88**	**Y 89**	**Zr 90**	**Mo 95**	**Ba 38**	**Ce 140**	**Hf 178**	**Pb 208**	**Th 232**
Mean ppm	32.1	77.0	38.7	36.3	38.7	38.0	38.5	34.5	36.2	36.9
SD	0.42	0.94	0.95	0.97	0.81	0.60	0.53	1.01	5.06	1.15
%RSD	1.3	1.2	2.4	2.7	2.1	1.6	1.4	2.9	14.0	3.1
%Agreement	101.5	101.1	101.0	100.8	101.0	100.6	100.5	99.2	92.9	99.1

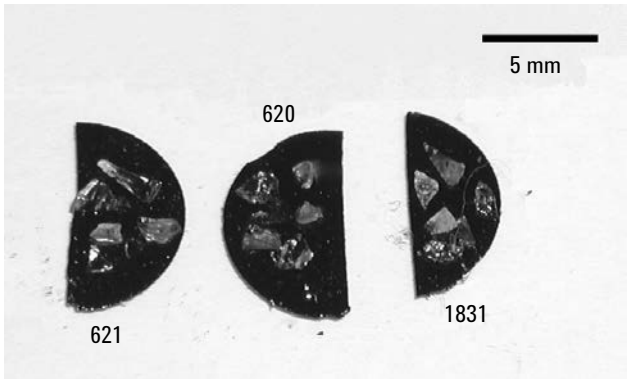

Figure 2. Sample mounting of glass fragments.

NIST 612 standard glass was used as a means of calibration and was analyzed repeatedly throughout the analysis procedure, bracketing each sample set. Each sample analysis was 115 seconds and consisted of a 20-second blank delay, a 60-second laser sampling period, followed by a 35-second washout period. Six repetitive data acquisitions over two separate lines were collected for each sample. The data was imported into Glitter™ data reduction software (Macquarie University - GEMOC). Analyte and blank regions were defined within the Signal Selection Screen (Figure 3) and quantitative values were determined. The mean and standard deviation (SD) for each sample was then calculated (Table 3a).

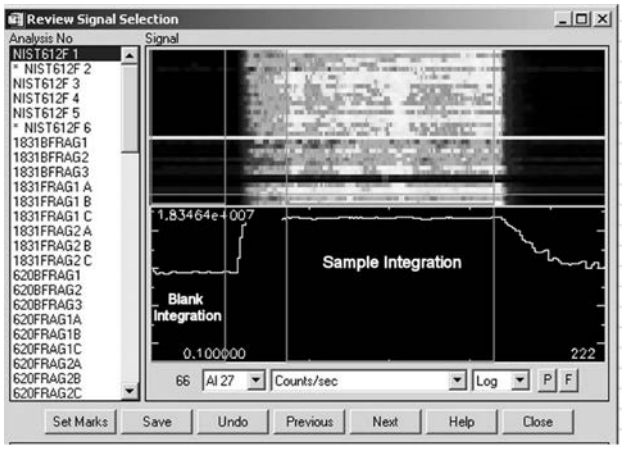

Figure 3. Signal selection screen, Glitter data reduction software. Traditionally used in geochronology, forensic data benefits from the ability of this software to enable easy isolation of changing data sets within a heterogeneous sample matrix. Each sample has its own associated blank, reducing memory effects.

Table 3a. Glass Data Obtained From the Analysis of Standard Glass Fragments
Unless Otherwise Noted all Data is in µg/g (ppm)

Element	NIST 620				NIST 621				NIST 1831			
	Mean (ppm)	SD (ppm)	RSD (%)	Agreement (%)	Mean (ppm)	SD (ppm)	RSD (%)	Agreement (%)	Mean (ppm)	SD (ppm)	RSD (%)	Agreement (%)
Na 23	10.8%	0.33%	3.1	100.7	9.3%	0.39%	4.2	98.0	9.8%	0.33%	3.4	100.9
Mg 24	3.1%	0.05%	1.5	140.2	0.2%	0.003%	1.5	136.1	2.9%	0.038%	1.3	137.3
Al 27	1.0%	0.02%	1.8	107.3	1.6%	0.01%	0.6	108.8	0.66%	0.004%	0.6	102.7
Ca 44	5.0%	0.09%	1.8	99.0	7.7%	0.11%	1.4	100.2	5.7%	0.064%	1.1	96.6
Ti 47	105	2.245	2.1	97.1	86	1.28	1.5	102.7	118	1.77	1.5	103.4
Cr 53	2.02	0.297	14.7	–	3.97	0.29	7.2	–	2.13	0.19	9.1	–
Mn 55	13.9	0.261	1.9	–	17.9	0.31	1.7	–	12.8	0.15	1.2	–
Fe 57	203	3.310	1.6	–	210	2.04	1.0	–	397	5.89	1.5	–
Ni 60	0.49	0.049	10.0	–	1.80	1.13	62.8	–	0.57	0.21	37.3	–
Zn 66	6.7	0.265	3.9	–	2.76	0.17	6.1	–	8.4	0.61	7.3	–
Rb 85	5.3	0.197	3.7	–	38.2	1.23	3.2	–	6.03	0.16	2.6	–
Sr 88	286	4.709	1.6	–	106	1.44	1.4	–	89.9	1.21	1.3	–
Y 89	2.99	0.043	1.5	–	2.63	0.05	1.9	–	2.05	0.04	1.8	–
Zr 90	198	4.291	2.2	–	62.7	0.96	1.5	120.9	39.5	0.93	2.3	–
Mo 95	0.19	0.022	11.6	–	2.34	0.13	5.7	–	0.18	0.01	6.9	–
Ba 138	22.5	0.156	0.7	–	84.7	7.41	0.9	–	30	0.54	1.8	–
Ce 140	2.50	0.036	1.4	–	2.09	0.03	1.4	–	4.35	0.08	1.8	–
Hf 178	4.30	0.106	2.5	–	1.51	0.02	1.1	–	0.97	0.03	3.5	–
Pb 208	1.97	0.138	7.0	–	14.5	0.96	6.6	–	1.94	0.10	5.1	–
Th 232	0.40	0.002	0.6	–	0.62	0.00	0.4	–	0.60	0.01	0.9	–

Table 3b. Glass Data Obtained from Headlight Fragments
Unless Otherwise Noted all Data is in µg/g (ppm)

Element	Fragment Sample A (Sylvania Headlamp H6024CB)			Fragment Sample B (Sylvania Headlamp H4656)			Fragment Sample C (Sylvania Headlamp 5006)		
	Mean (ppm)	SD (ppm)	RSD (%)	Mean (ppm)	SD (ppm)	RSD (%)	Mean (ppm)	SD (ppm)	RSD (%)
Na 23	3.5%	0.09%	2.64	34.9%	0.54%	1.54	3.59%	0.08%	2.36
Mg 24	41.78	0.37	0.88	70.6	0.96	1.36	62.8	0.55	0.88
Al 27	1.2%	0.04%	3.10	1.0%	0.01%	1.00	1.04%	0.01%	0.70
Ca 44	153	29.23	19.12	221	14.59	6.60	200	34.29	17.16
Ti 47	71	5.19	7.31	46.3	0.97	2.10	44.5	0.71	1.60
Cr 53	1.26	0.29	23.08	2.19	0.36	16.39	2.13	0.26	12.15
Mn 55	2.67	0.06	2.07	1.29	0.10	8.10	1.20	0.03	2.34
Fe 57	96	1.85	1.93	234	4.15	1.78	237	4.23	1.78
Ni 60	0.43	0.06	13.61	0.32	0.05	15.09	0.27	0.08	27.36
Zn 66	1.44	0.13	9.02	1.01	0.10	9.80	0.89	0.16	18.42
Rb 85	0.38	0.01	3.81	0.38	0.01	2.43	0.40	0.01	3.60
Sr 88	4.08	0.11	2.58	5.16	0.10	2.00	3.95	0.10	2.44
Y 89	9.42	1.27	13.50	0.92	0.03	2.84	0.86	0.03	3.38
Zr 90	5099	711.55	13.95	119	4.62	3.88	97	5.95	6.12
Mo 95	3.28	0.11	3.37	0.69	0.08	11.48	0.53	0.06	11.87
Ba 138	4.42	0.09	1.97	1.86	0.05	2.52	1.82	0.04	2.10
Ce 140	3.09	0.15	5.02	3.79	0.06	1.58	3.57	0.06	1.71
Hf 178	113	15.85	14.01	2.96	0.13	4.29	2.23	0.15	6.76
Pb 208	0.41	0.03	6.21	0.42	0.01	2.46	0.36	0.02	5.67
Th 232	1.74	0.22	12.71	0.29	0.01	2.08	0.25	0.01	2.93

Results

Discrimination of Clear Glass Fragments

Three sets of automobile headlamp fragments and three sets of NIST soda lime glass standard fragments were chosen as forensic sample surrogates for this study. All glass samples were colorless to the naked eye. Time resolved data was imported directly into Glitter data reduction software from the Agilent 7500s ICP-MS ChemStation software. Blank and sample integration areas were defined within the Signal Selection screen (Figure 3) and elemental concentrations were calculated using NIST 612 as the multi-element standard (Table 2). Though the glass fragments were typically <1 mm, elemental recoveries for the NIST certified values were very good and RSDs were <3% for many elements.

NIST soda-lime glass standards 620 (flat glass), 621 (container glass), and 1831 (sheet glass) were used to emulate samples. The good agreement between the certified values and the returned values support the efficacy of the method used. Though the Mg values are consistently high by approximately 40%, the data suggests that this is likely due to a problem with the calibration standard either because of an inhomogenous distribution of the element, or even possibly variation in the certified value. In this study, the value for Mg in NIST 612 was defined as 77.44 µg/g, 1σ 30.15 µg/g (Pearce, et al 1997) [4]. Another study (Gao, et al 2002) published the NIST 612 Mg value as 64 µg/g, 1σ 6 µg/g [5].

Forensic data must be presented in the most accurate and clearly understandable format. Jurors with little or no scientific background must be able to decipher subtle chemical differences between evidentiary materials. Consequently, we have presented our glass data in two discriminating formats: numerically and stacked bar graphs (Tables 3a and 3b and Figure 5). Stacked bar graphs are extremely effective in comparing different multi-component data sets. We have therefore included the quantitative mean values with 1σ SD (Tables 3a and 3b).

Like gel electrophoresis, banding patterns within an elemental data set are easy to visualize and differentiate. Stacked bar charts can clearly characterize the elemental nature of a unique sample type. Notice the clear and even banding pattern of NIST 612 (first bar Figure 5). In NIST 612, all elements with the exception of Sr (76 ppm) are nominally at equal concentration (50 ppm), which the banding pattern clearly portrays. The NIST glass serves not only as a quantitative standard, but also describes the effectiveness of the stacked bar chart in its ability to compare trace element constituents.

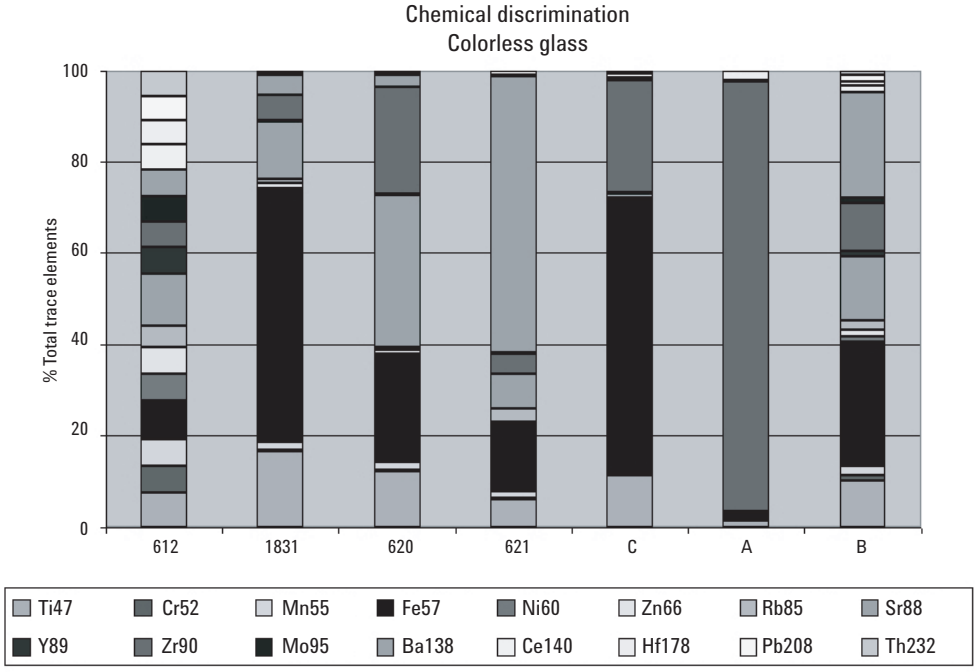

Figure 5:. The mean data from Table 3 is presented in a stacked bar chart format. This visual representation of the data aids data presentation in terms of clarity and relative simplicity.

Conclusion

LA-ICP-MS is an effective tool for the analysis of forensic glass samples. This technique is particularly useful in overcoming the limitations associated with very small sample types or samples composed of chemically inert materials.

Colorless glass fragments, indistinguishable to the naked eye and chemically identical at the ppm level, may be discriminated with good accuracy and precision, even at sub-millimeter dimensions. Due to the micro-destructive nature of this technique, forensic samples characterized by this method may also be available to alternative analysis if confirmation is required.

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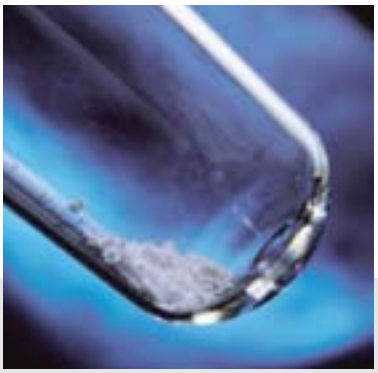
- An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensics/Toxicology Database
- The First Accurate Mass MS/MS Library for Forensics and Toxicology Using the Agilent 6500 Series Accurate Mass Q-TOF LC/MS
- Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS



Applications by Technique

LC/TOF & LC/QTOF

An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensics/Toxicology Database

Application Note

Forensics and Toxicology

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Abstract

A Forensic and Toxicological screening application kit has been developed for use with the Agilent TOF and Q-TOF Mass Spectrometers which contains an accurate mass database with a content of around 6700 analytes. The aim of the MassHunter Personal Forensics and Toxicology Database Kit is to provide a user with a sufficient starting point for the analysis of samples for which the ability to detect and identify from a large array of forensic and toxicological analytes is necessary. The combined system allows the user to create custom databases containing retention times of compounds of interest for smaller and more specific suites of analytes according to specific requirements. A test mix containing analytes of forensic interest, to demonstrate the functionality of the MassHunter Personal Forensics and Toxicology Database Kit, together with an example of a general screening method for common drugs of abuse is provided.

Agilent Technologies

Introduction

The application of high definition accurate mass spectrometers, such as time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF), to screening, discovery and confirmation in the areas of forensics and toxicology has become more desirable given the indiscriminant and non-targeted nature of their full spectral data capture. Indeed, given the highly accurate and sensitive mass measurement of modern TOF and Q-TOF instruments (sub 2-ppm mass accuracy, pg on-column sensitivity and high resolution) in combination with powerful software data mining tools, post acquisition screening techniques are easier to perform reliably with a higher number of analytes in one analytical method. The lists of potential toxins are large and typically depend on the area of analytical focus such as work-place drug testing, doping control, post-mortem toxicology, or explosives.

Accurate single-stage mass spectrometry (MS) mass measurements identify monoisotopic adducts to a high confirmatory degree, and databases can be built to accommodate various suites of forensic and toxicological analytes of interest. They are obtained from both TOF and Q-TOF LC/MS instruments. In contrast LC/MS/MS with a triple quadrupole MS in its most sensitive mode, multi-reaction monitoring (MRM), provides targeted screening and confirmation only.[1]

This application note describes the Agilent MassHunter Personal Forensics and Toxicology Database Kit for Forensic and Toxicological Screening and Identification which contains the accurate mass (AM) details for around 6700 analytes of forensic and toxicological interest. The content was gathered upon advice from many leading institutions and knowledge bases world-wide and contains information such as common names, monoisotopic mass, compound formulas, CAS & Chemspider IDs, chemical structure and in most cases the IUPAC nomenclature. In addition to accurate mass, the ability to add retention time for a chromatographic method to every analyte for extra search confirmation is a built-in functionality of the MassHunter Personal Compound and Library (PCDL) program interfaces. This allows accurate mass retention time (AMRT) data mining routines. Furthermore, an analyst can use the database content 'as is' for non-targeted screening or create smaller custom and more targeted databases from the read-only supplied database. Custom databases can be edited by changing entries, adding, and deleting entries and semi-automatically updating retention times for particular analytes and methods. [2] The analyst can create as many custom databases with LC-dependent retention times as needed.

This application note describes the typical use of the MassHunter Personal Forensics and Toxicology Database Kit through a few analytical screening work flow examples.

Experimental

The analysis results outlined in this application note were obtained using an Agilent 6230 Time-of-Flight LC/MS coupled to an Agilent 1200 SL Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter Workstation equipped with the [G6855AA] MassHunter Personal Forensics and Toxicology Database Kit.

Sample preparation

An ampoule from the LC/MS Toxicology Test Mix [p/n 5190-0470] which is included in the MassHunter Personal Forensics and Toxicology Database Kit [G6855AA] was opened and 10 µL of the 1 µg/mL (1 ppm) solution was diluted to a concentration of 100 ng/mL (100 ppb) using 990 µl of pure LC/MS grade methanol to create a clean solvent standard for method checkout purposes.

Table 1 outlines the composition of the LC/MS Toxicology Test Mix [p/n 5190-0470] which is intended to cover a wide and representative range of forensic analyte classes.

Table 1. LC/MS Toxicology Test Mix components (1 µg/ml)

Compound Name	Formula	Mass
3,4-Methylendioxyamphetamine (MDA)	$C_{10}H_{13}NO_2$	179.09463
3,4-Methylenedioxyethamphetamine (MDEA)	$C_{12}H_{17}NO_2$	207.12593
Alprazolam	$C_{17}H_{13}ClN_4$	308.08287
Clonazepam	$C_{15}H_{10}ClN_3O_3$	315.04107
Cocaine	$C_{17}H_{21}NO_4$	303.14706
Codeine	$C_{18}H_{21}NO_3$	299.15214
delta9-Tetrahydrocannabinol (THC)	$C_{21}H_{30}O_2$	314.22458
Diazepam	$C_{16}H_{13}ClN_2O$	284.07164
Heroin	$C_{21}H_{23}NO_5$	369.15762
Hydrocodone	$C_{18}H_{21}NO_3$	299.15214
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	320.01193
Meperidine (Pethidine)	$C_{15}H_{21}NO_2$	247.15723
Methadone	$C_{21}H_{27}NO$	309.20926
Methamphetamine	$C_{10}H_{15}N$	149.12045
Methylendioxymethamphetamine (MDMA)	$C_{11}H_{15}NO_2$	193.11028
Nitrazepam	$C_{15}H_{11}N_3O_3$	281.08004
Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.05091
Oxycodone	$C_{18}H_{21}NO_4$	315.14706
Phencyclidine (PCP)	$C_{17}H_{25}N$	243.1987
Phentermine	$C_{10}H_{15}N$	149.12045
Proadifen	$C_{23}H_{31}NO_2$	353.23548
Strychnine	$C_{21}H_{22}N_2O_2$	334.16813
Temazepam	$C_{16}H_{13}ClN_2O_2$	300.06656
Trazodone	$C_{19}H_{22}ClN_5O$	371.15129
Verapamil	$C_{27}H_{38}N_2O_4$	454.28316

Reagents and chemicals

Burdick & Jackson LC/MS grade acetonitrile together with de-ionized water (locally produced 18.1 MΩ) were used for mobile phases. Buffers were freshly prepared using a high purity source of formic acid and ammonium formate.

Instrument settings and MS acquisition method parameters

LC conditions

Column:	Zorbax Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm [p/n - 959764-902]
Column Temperature:	60 °C
Mobile Phase	A: 5 mM NH_4 formate/0.01% Formic acid in water B: 0.01% formic acid in acetonitrile
Flow Rate:	0.5 ml/min

Gradient program:

Time	A	B	Flow rate
Initial	90%	10%	0.5 ml/min
0.5 min	85%	15%	0.5 ml/min
3.0 min	50%	50%	0.5 ml/min
4.0min	5%	95%	0.5 ml/min
6.0min	5%	95%	0.5 ml/min

Injection volume:	1 µL (with 5 second needle wash in flushport)
Analysis time:	6.0 min
Post Time:	2.0 min
Overall Cycle time:	8.0 min

6230 TOF MS conditions

Source conditions:

Electrospray AP-ESI (using Agilent Jet Stream Technology):

Positive ionization polarity	
Sheath gas temperature and flow:	380°C, 12 L/min
Nozzle voltage:	500 V
Drying gas temperature and flow:	320°C, 8 L/min
Nebulizer gas pressure:	27 psi
Capillary voltage:	3750 V
Fragmentor voltage:	150 V

Electrospray AP-ESI:

Positive ionization polarity	
Drying gas temperature and flow:	350°C, 12 L/min
Nebulizer gas pressure:	30 psi
Capillary voltage:	2000 V
Fragmentor voltage:	150 V

MS acquisition method parameters:

Reference ion mass enabled:	121.050873, 922.009798
Acquisition mode:	MS1
Minimum mass value:	50 *m/z*
Maximum mass value:	1050 *m/z*
Scan rate:	3 Hz

All other instrument operating parameters were taken care of by Agilent's autotune functionality and subsequent mass calibration using standard settings.

Results and discussion

Fast and easy start up with Agilent LC/MS Toxicology Test Mix

The LC/MS Toxicology Test Mix [p/n 5190-0470] is included in the MassHunter Personal Forensics and Toxicology Database Kit [G6855AA] to rapidly implement the method and verify that acquisition and data analysis methodology is correctly set up. The LC/MS Toxicology Test Mix contains a representative range of components from 25 forensic analyte classes. (See Table 1). MS screening depends on accurate mass results from the TOF or Q-TOF. Therefore, the use of appropriate reference ions as outlined in the 'Experimental conditions' section obtains the most accurate results.

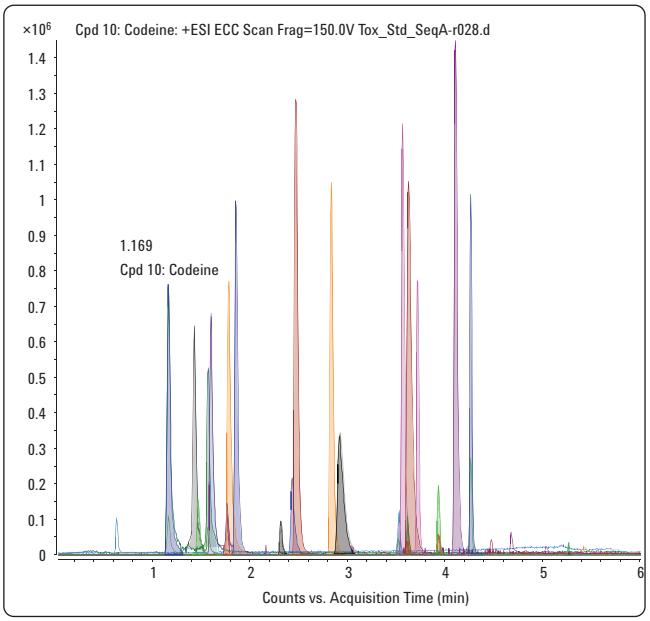


Figure 1. Extracted compound chromatogram of LC/MS Toxicology Test Mix.

In compliance with the methodology outlined in the experimental section, a 1-ul injection of the 100 ng/ml LC/MS Toxicology Test Mix equates to a 100 pg on-column injection amount. Figure 1 shows an overlay of the expected extracted compound chromatograms for the LC/MS Toxicology Test Mix. A standard method is included for TOF and Q-TOF as part of the MassHunter Personal Forensics and Toxicology Database Kit. These can be loaded so that all conditions are correct and the user can reproduce the analysis.

These methods are acquisition only methods and correspond to the instrument configuration as outlined in the experimental section of this application note. Appropriate settings must be manually input if a different instrument configuration is used. Similar results will demonstrate that the system is working properly.

Personal Compound Database and Library (PCDL) Software interface

Outline

An 'open database' dialog box appears after invoking the PCDL interface from the desktop icon. It is best to choose the pre-installed Forensic.cdb from the MassHunter\database directory. Figure 2 illustrates the single search view of the software interface. The screen shows a list of search results for 'amphetamine'. There are seven views available to the user, however, for the scope of this application note, only the first four (tabs to the left) that are directly applicable to AMRT functionality will be described. These views are switched on this flat user interface by clicking on the appropriate tab: Single Search, Batch Search, Batch Summary, or Edit Compounds.

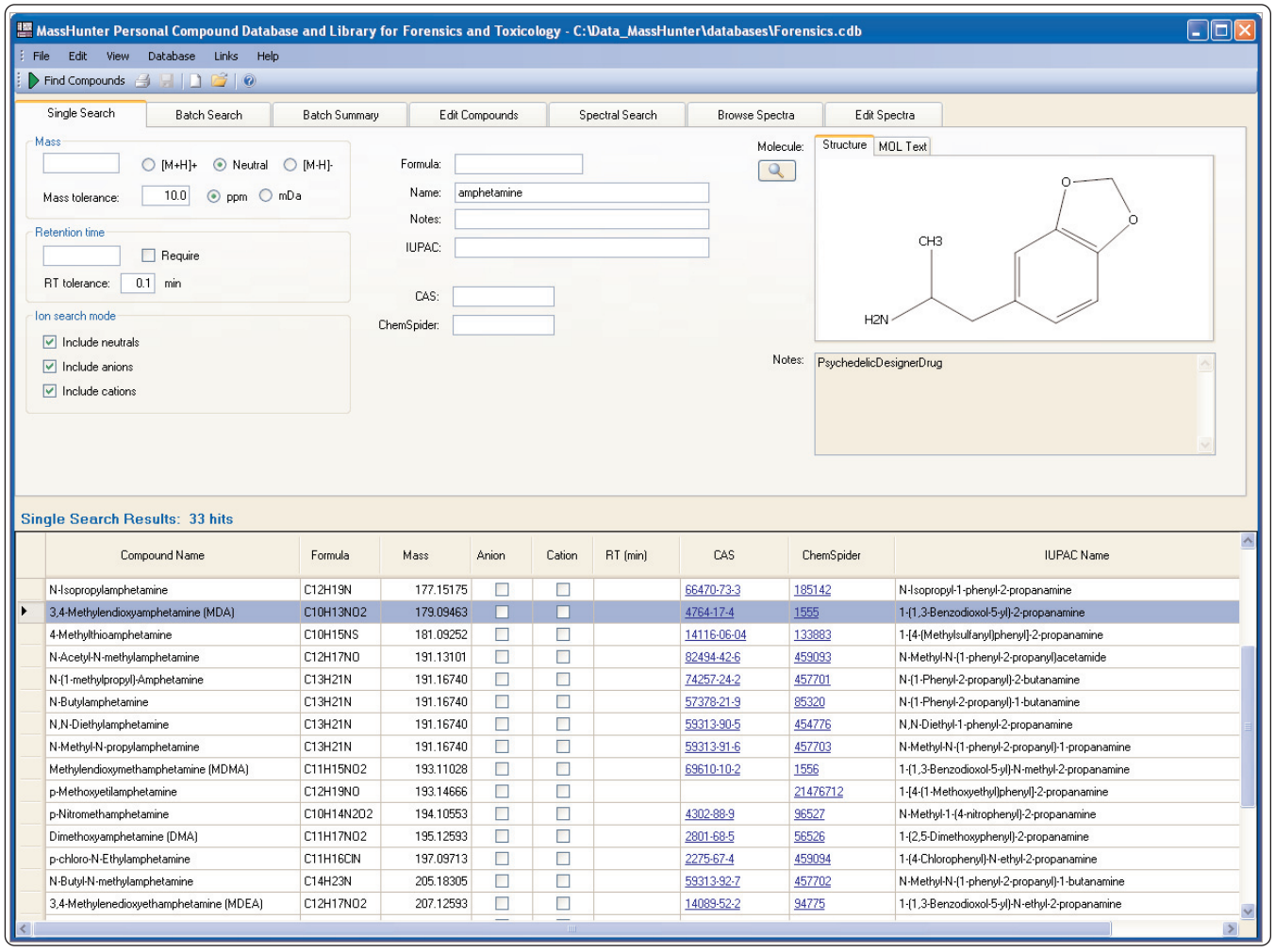


Figure 2 Single Manual Search view of the PCDL software interface.

Any field or combination of fields in the upper portion of the Single Search tab (Figure 2.) can be used to manually search the loaded database. Table 2 lists all available search fields from the PCDL single search view. The powerful search algorithm also handles partial names (eg. 'amph' will return all database entries containing this letter string.)

Note: To view the entire contents of the loaded database, a single search invoked with all empty search fields will allow the user to display the entire database content.

Table 2. All available search fields for PCDL single search.

Search Fields Available (Single Search View)	Value
Mass	Measured mass (*m/z*)
Retention time	(minutes)
Formula	Empirical Formula
Name	Common name of compound (or part thereof)
Notes	Compound class or description
IUPAC	IUPAC or commonly recognized compound name
CAS	Unique CAS number
ChemSpider	Unique ChemSpider ID

Workflow A. Manual (Single Mass Search)

Using PCDL Program

Single search would normally be used manually by obtaining a measured mass from a measured or observed spectrum in MassHunter Qualitative Analysis program and typing it in to the mass search field. Figure 3 illustrates this manual application of the MassHunter Qualitative Analysis program and PCDL single search capability for observed masses.

In this example, a compound peak was identified in MassHunter Qualitative Analysis program from positive polarity TOF data, the spectrum was extracted, and the observed mass of 244.205770 *m/z* was searched against the PCDL database (including cations) for [M+H]+ adducts using a mass tolerance of 10 ppm.

The search returns an accurate mass match with phencyclidine (PCP) and with a mass deviation (or delta mass) of 0.85 ppm between the measured and theoretical database values.

More detailed information of single search capability can be found in Agilent G6855AA MassHunter Personal Forensics and Toxicology Database and Kit Quick Start Guides [3,4] .

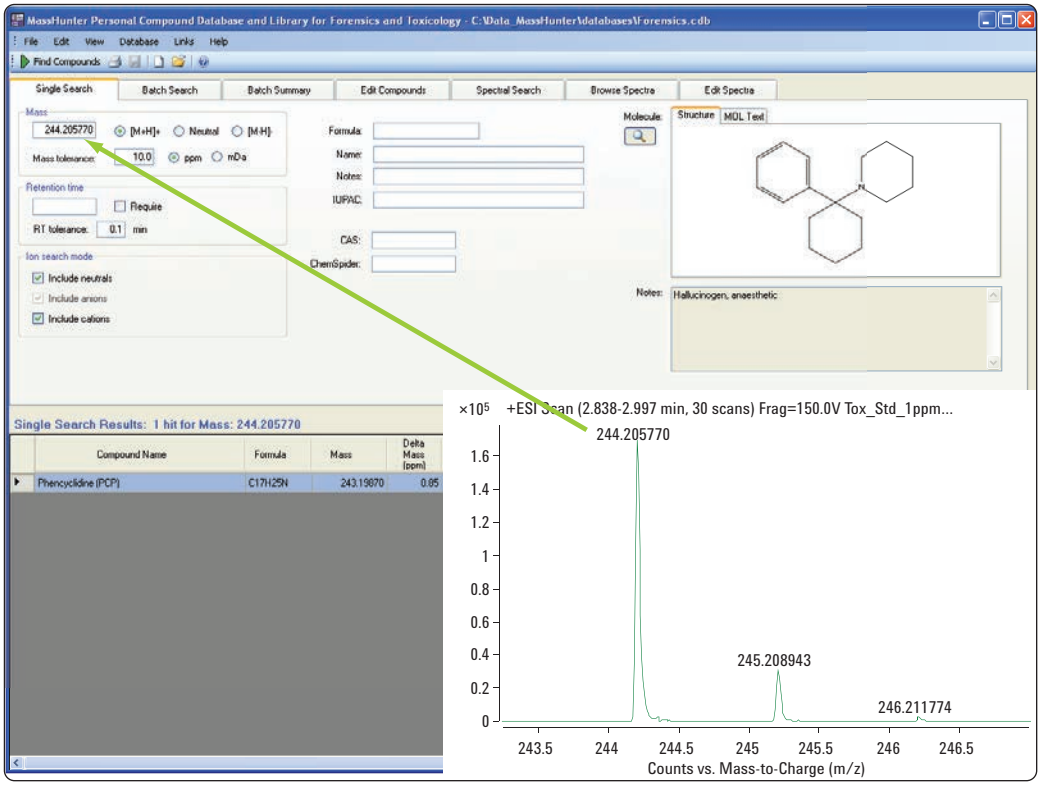


Figure 3. Manual search of observed mass.

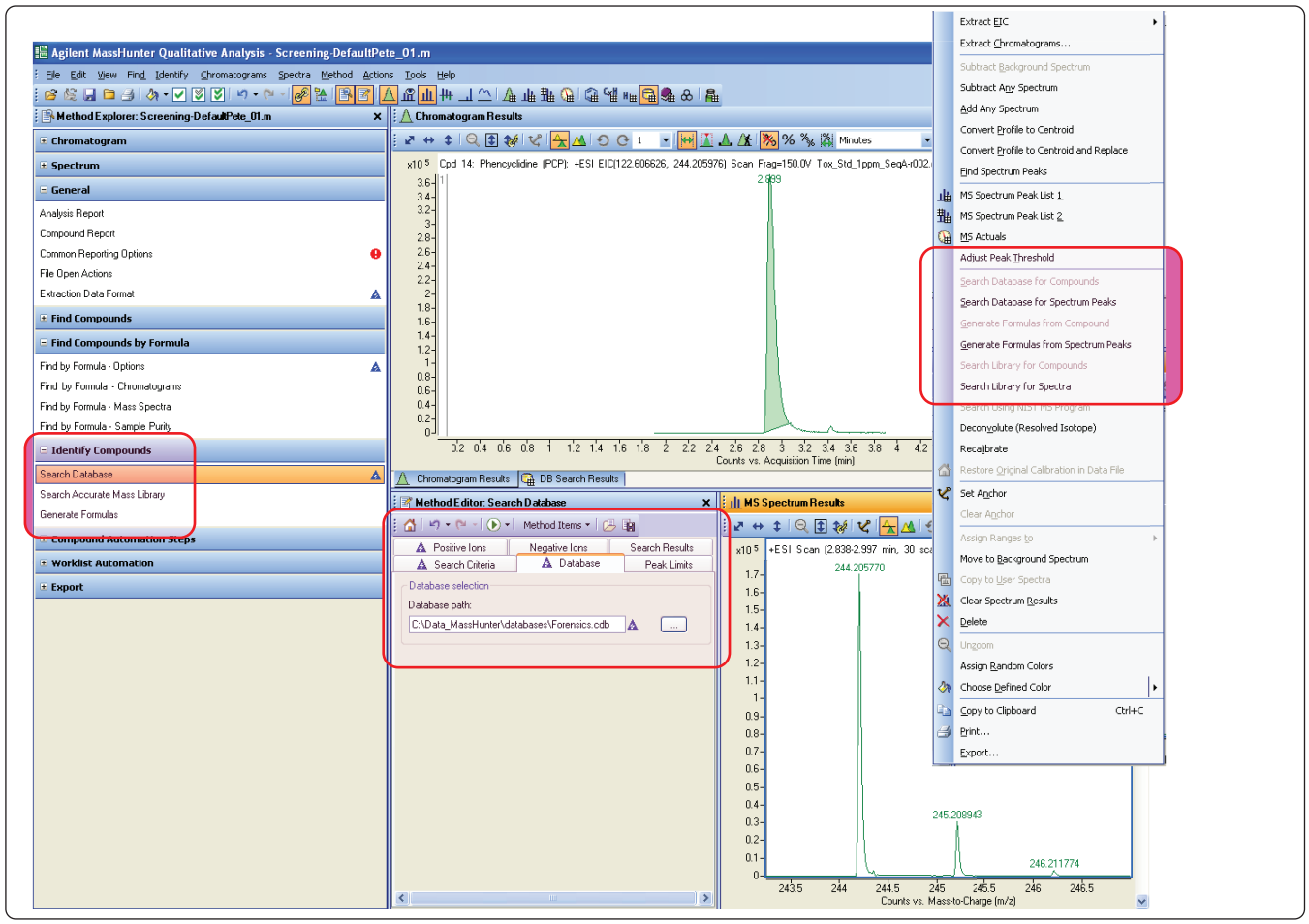


Figure 4a. Manual Search of observed mass using MassHunter Qualitative Analysis program.

Single manual search of database using MassHunter Qualitative Analysis program.

To obtain a seamless single spectral peak database search via MassHunter Qualitative Analysis program, the database must be specified in the qualitative analysis method editor. Compatible software versions are B.03.01 or higher. Figures 4a through 4d illustrate the settings used for this example.

Figure 4a shows the typical MassHunter Qualitative Analysis program view containing the chromatographic peak in question together with its manually extracted spectrum. On the left side of the screen shot, the 'Identify Compounds' method explorer options have been expanded and the 'Search Database' method editor was selected. In the method editor, the required AMRT database was specified as 'forensic.cdb'.

Figure 4b shows the mass tolerance window and the search criteria that can be selected, such as 'mass only' or 'mass with retention time'.

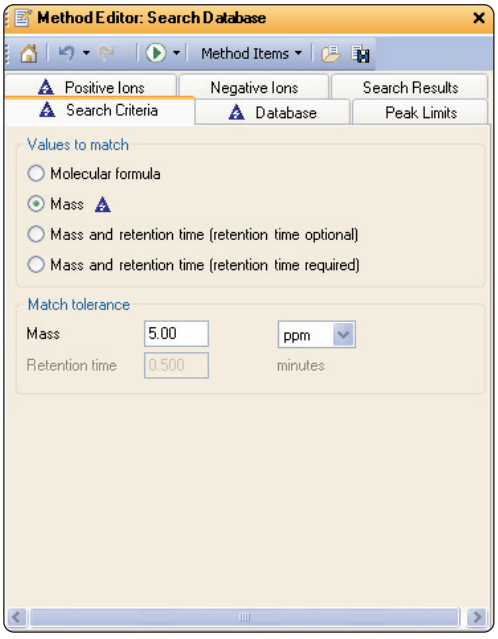


Figure 4b. Manual Search Criteria Settings.

Figure 4c illustrates more adduct and charge state options required for the database search.

Right-click in the spectrum window and a shortcut menu appears against the specified AMRT database (Figure 4a.) This menu has various options including 'Search database for spectrum peaks'. Selection of this option automatically invokes the database search. In Figure 4d the spectrum peak has been identified as PCP, with 0.87 ppm mass deviation and a spectral combined score of 99.36 out of 100 indicating extra confirmation of identity.

To calculate this score, three distinct score components were considered: Mass Match, Abundance Match, and Spacing Match with values of 99.61, 98.61, and 99.79, respectively. These are individually displayed in Figure 4d.

For trustworthy results, the software scores the database matches based on the similarity of each of the isotopic masses (Mass Match), isotope ratios (Abund Match), isotope spacing (Spacing Match), and optionally the retention time (RT Match).

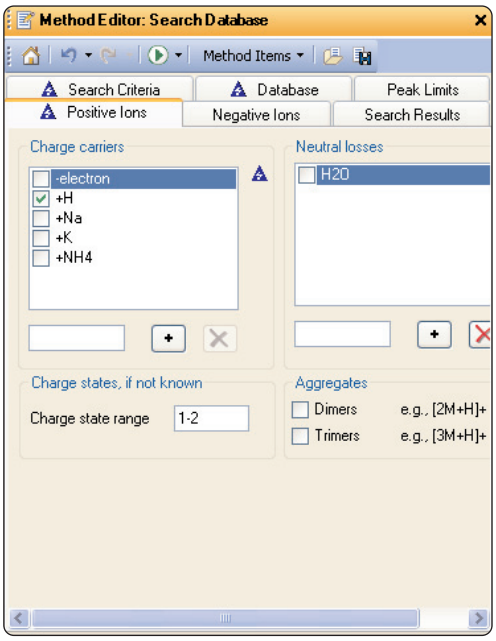


Figure 4c. Manual Search Adduct Selection.

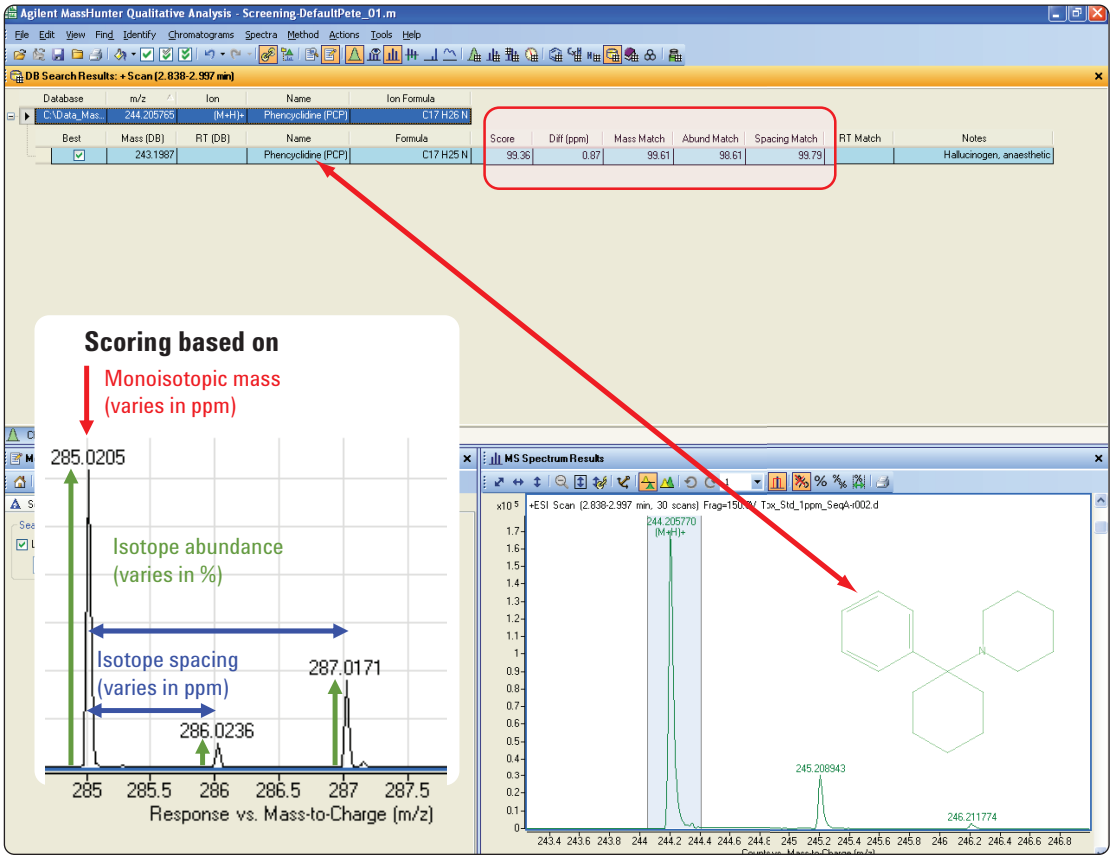


Figure 4d. Manual Database Search Results using MassHunter Qualitative Analysis program.

Isotope spacing is another important component of the scoring algorithm. The mass spacing from the M to the M+1 and M+2 isotopes can be measured with low-ppm accuracy. Any small mass shifts affect all isotopes equally, so this measurement is independent of overall mass axis shifts. This is outlined graphically in Figure 4d.

In this example, a single AMRT database result of phencyclidine (PCP) was returned, together with its structure which is optionally overlaid on the peak spectrum as shown in Figure 4d and can be displayed if selected in the reporting options.

More detailed information about MassHunter Qualitative analysis program database searching can be found in the MassHunter Qualitative Analysis Program Help Files or user guides [5].

Workflow B. Data mining using 'Molecular Feature Extractor' (MFE)

Batch PCDL searches (tabs 2 & 3) are designed for database searching and identification using an accurate mass list created from an automated data mining algorithm such as the Agilent Molecular feature extractor (MFE.) Such algorithms are extremely powerful, especially with complex data derived from difficult sample matrices, such as blood extracts. For the remainder of this application note, only batch searches invoked from inside the MassHunter Qualitative Analysis program interface will be outlined and described. For information on how to perform batch searches within the PCDL interface, please refer to the PCD application note [2].

Data mining algorithms such as MFE automatically search and 'mine' complex sets of single-stage MS data to determine and distinguish most likely and 'real' compound peaks from continuous background interferences. Combinations of adducts can be selected as part of the compound identification protocol to provide added assurance of compound validity.

Other data mining algorithms such as 'find by MS/MS' and 'find by Targeted MS/MS' are integral options included as part of the MassHunter Qualitative Analysis program software. The algorithms are dependent on the mode of operation and nature of the instrument being used. 'Find by Formula' compound search routines are described in the 'Workflow C' section of this application note.

For illustrative purposes, the LC/MS Toxicology Test Mix was analyzed under the conditions outlined in the experimental section. The data file was loaded into MassHunter Qualitative Analysis program. The 'Find by Molecular Feature' method editor was opened under the method explorer in the 'Find Compounds' section (see Figures 5a & 5b).

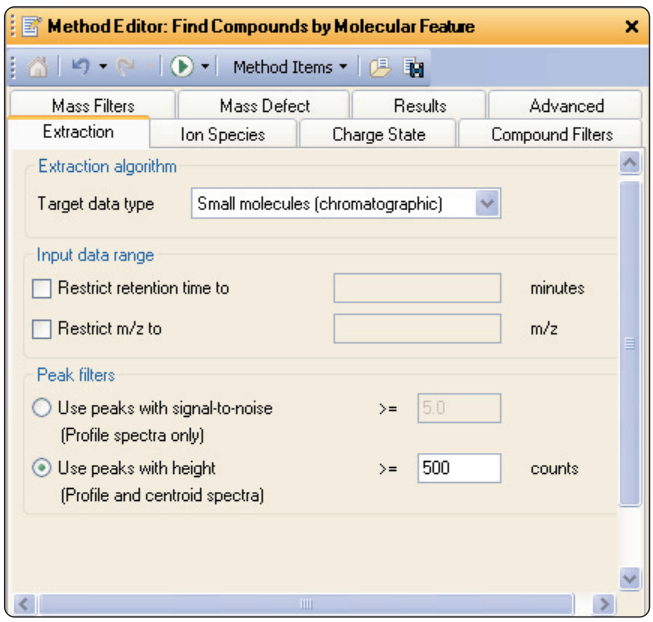


Figure 5a. MFE extraction parameters.

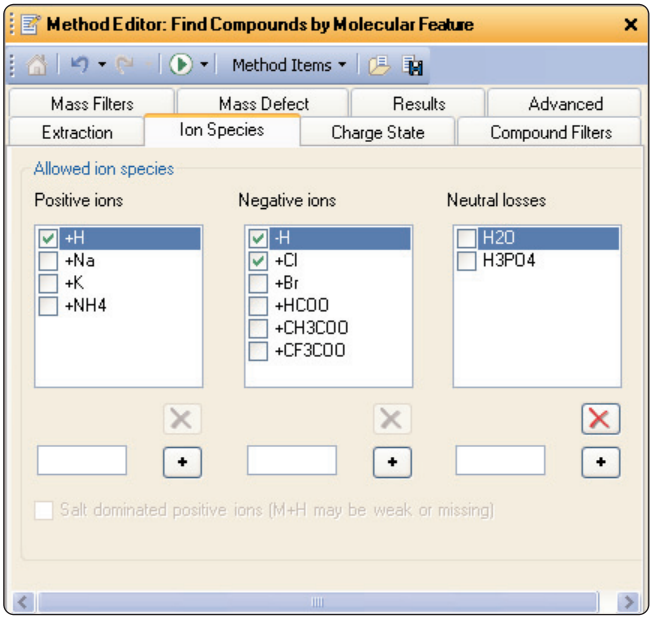


Figure 5b. MFE ion species setup.

A very aggressive setting of absolute peak height threshold (>500 counts) was used in this example (see Figure 5a), together with the small molecules algorithm (chromatographic) which yielded over 3000 possible compound hits. By raising this threshold amount, less abundant analytes may remain undetected. Conversely with a higher threshold the number of potential false positives are greatly reduced. Only [M+H]+ adducts were searched in this instance, however,

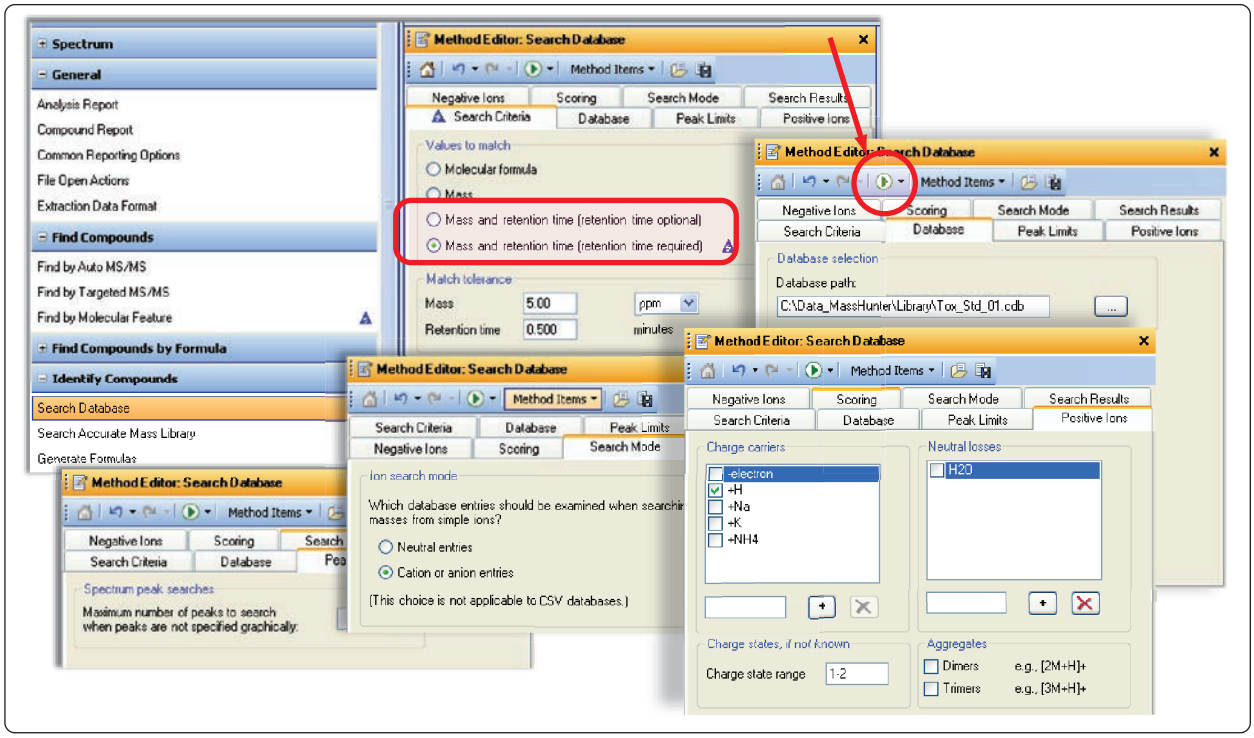


Figure 6. MFE compound database search settings.

further confidence could have been sought (see Figure 5b) by choosing additional adducts such as Na+ and NH4+.

No compound, mass filters or mass defect filters were specified for this search and a maximum charge state of 1 was specified in the MFE method setup. The next step after MFE search was to specify the forensic AMRT database (see Figure 6) in the identify compound/search database method editor, highlight all of the MFE-found compounds and search each compound against its content. A mass and retention time (RT) match was specified, since RT database values had already been pre-determined by analyzing individual standards and inserted into a customized compound database.

Figure 7 illustrates the results obtained from the MFE operation invoked by pressing the green 'process' button highlighted in the title bar of the MFE method editor (Figure 6).

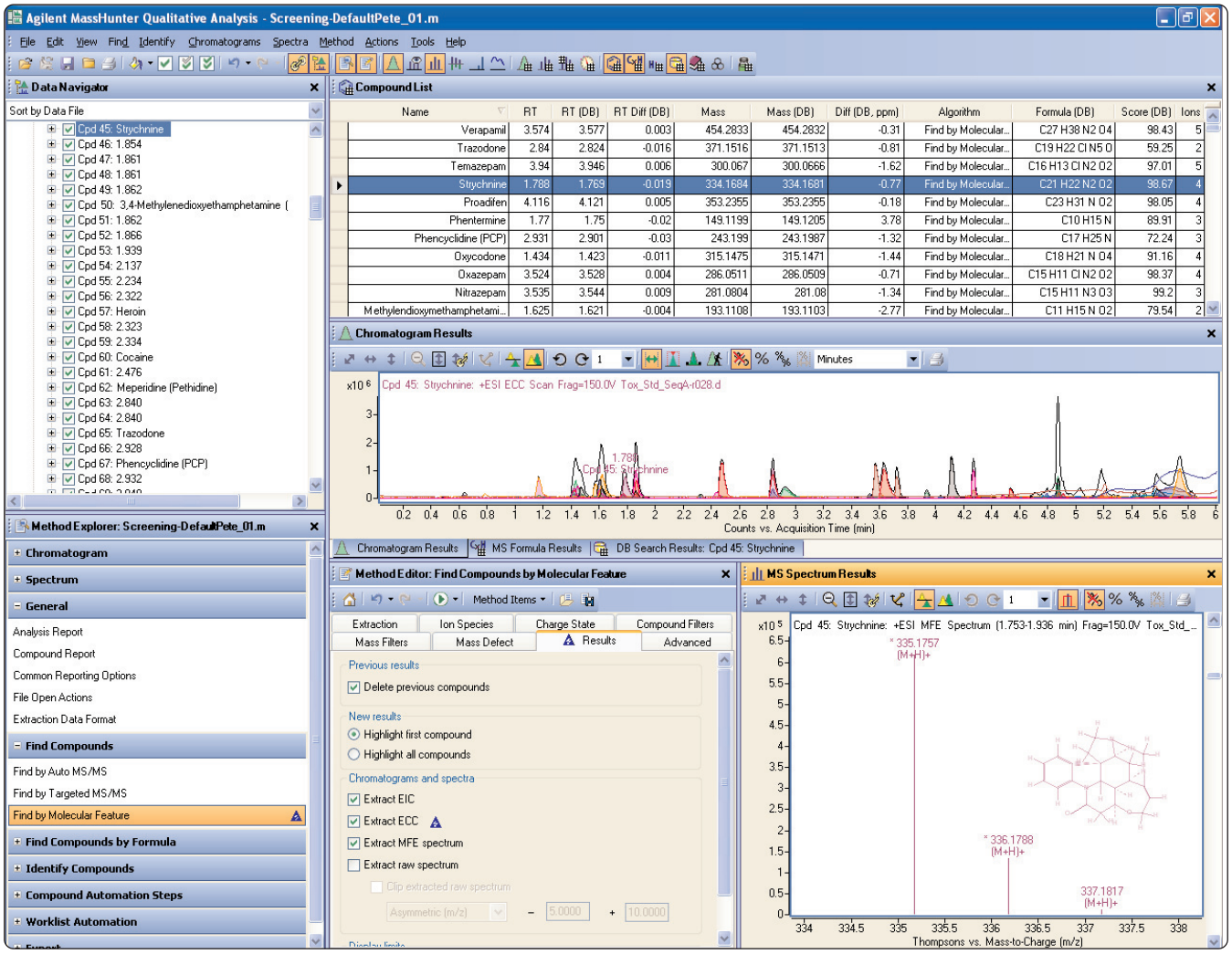


Figure 7. MFE compound database search results using MassHunter Qualitative Analysis program.

These results are detailed in Table 3 and show that all 25 compounds of the LC/MS Toxicology Test Mix were identified for this sample injection. This confirms that the data analysis settings for the find and identify steps are appropriate for the identification process. Many of the 3000+ compounds identified by MFE did not find any PCDL matches as expected and the data analysis option of excluding non-positives was used to report only the database hits.

Isobaric compounds such as codeine/hydrocodone and methamphetamine/phentermine were also correctly identified and distinguished automatically, by using the retention capability of the PCDL database and by inputting the predetermined retention time of each analyte for this chromatographic methodology as outlined in the Agilent G6855AA MassHunter Personal Forensics and Toxicology Database Quick Start Guide [3].

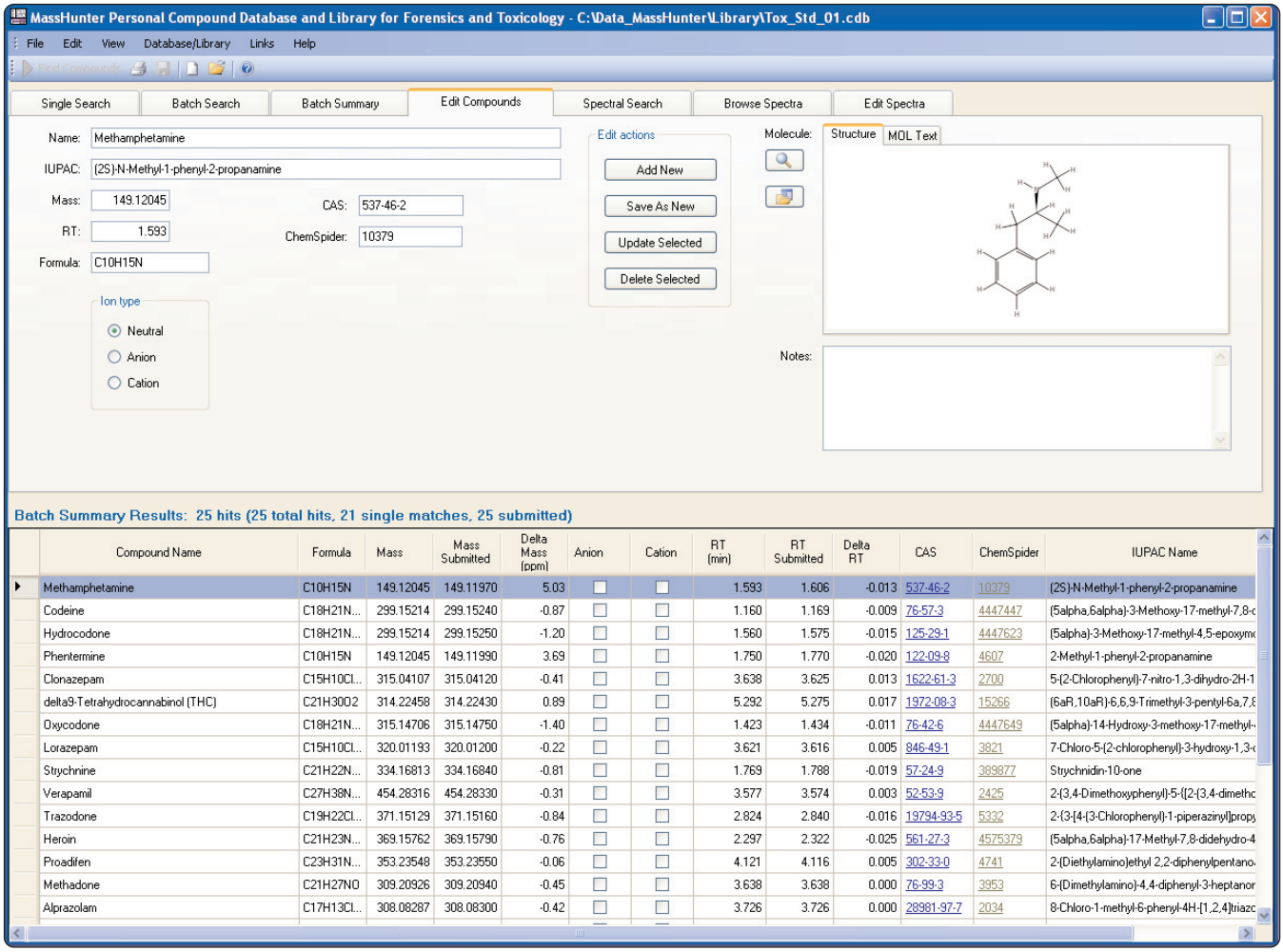
Table 3. MFE compound and database search results.

Name	RT	RT (DB)	RT Diff (DB)	Mass	Mass (DB)	Diff (DB, ppm)	Formula (DB)	Score (DB)
Verapamil	3.574	3.577	0.003	454.2833	454.2832	-0.31	$C_{27}H_{38}N_2O_4$	98.43
Trazodone	2.84	2.824	-0.016	371.1516	371.1513	-0.81	$C_{19}H_{22}ClN_5O$	59.25
Temazepam	3.94	3.946	0.006	300.067	300.0666	-1.62	$C_{16}H_{13}ClN_2O_2$	97.01
Strychnine	1.788	1.769	-0.019	334.1684	334.1681	-0.77	$C_{21}H_{22}N_2O_2$	98.67
Proadifen	4.116	4.121	0.005	353.2355	353.2355	-0.18	$C_{23}H_{31}NO_2$	98.05
Phentermine	1.77	1.75	-0.02	149.1199	149.1205	3.78	$C_{10}H_{15}N$	89.91
Phencyclidine (PCP)	2.931	2.901	-0.03	243.199	243.1987	-1.32	$C_{17}H_{25}N$	72.24
Oxycodone	1.434	1.423	-0.011	315.1475	315.1471	-1.44	$C_{18}H_{21}NO_4$	91.16
Oxazepam	3.524	3.528	0.004	286.0511	286.0509	-0.71	$C_{15}H_{11}ClN_2O_2$	98.37
Nitrazepam	3.535	3.544	0.009	281.0804	281.08	-1.34	$C_{15}H_{11}N_3O_3$	99.2
Methylendioxymethamphetamine (MDMA)	1.625	1.621	-0.004	193.1108	193.1103	-2.77	$C_{11}H_{15}NO_2$	79.54
Methamphetamine	1.606	1.593	-0.013	149.1197	149.1205	4.82	$C_{10}H_{15}N$	81.88
Methadone	3.638	3.638	0	309.2094	309.2093	-0.61	$C_{21}H_{27}NO$	99.67
Meperidine (Pethidine)	2.477	2.456	-0.021	247.1577	247.1572	-1.7	$C_{15}H_{21}NO_2$	97.91
Lorazepam	3.616	3.621	0.005	320.012	320.0119	-0.19	$C_{15}H_{10}Cl_2N_2O_2$	98.27
Hydrocodone	1.575	1.56	-0.015	299.1525	299.1521	-1.2	$C_{18}H_{21}NO_3$	85.2
Heroin	2.322	2.297	-0.025	369.1579	369.1576	-0.63	$C_{21}H_{23}NO_5$	98.97
Diazepam	4.272	4.275	0.003	284.072	284.0716	-1.36	$C_{16}H_{13}ClN_2O$	58.97
delta9-Tetrahydrocannabinol (THC)	5.275	5.292	0.017	314.2243	314.2246	0.94	$C_{21}H_30O_2$	94.83
Codeine	1.169	1.16	-0.009	299.1524	299.1521	-0.72	$C_{18}H_{21}NO_3$	72.49
Cocaine	2.44	2.418	-0.022	303.1475	303.1471	-1.29	$C_{17}H_{21}NO_4$	98.03
Clonazepam	3.625	3.638	0.013	315.0412	315.0411	-0.42	$C_{15}H_{10}ClN_3O_3$	98.72
Alprazolam	3.726	3.726	0	308.083	308.0829	-0.33	$C_{17}H_{13}ClN_4$	96.77
3,4-Methylenedioxyethamphetamine (MDEA)	1.862	1.846	-0.016	207.1263	207.1259	-1.8	$C_{12}H_{17}NO_2$	97.4
3,4-Methylendioxyamphetamine (MDA)	1.474	1.473	-0.001	179.095	179.0946	-2.23	$C_{10}H_{13}NO_2$	86.15

Customized databases with user-added retention times

One of the benefits of the Agilent Personal Forensics and Toxicology Database is that it can be saved to a user customized form. To create a read-write customizable database the user selects New Database from the PCDL File menu. The PCDL program then allows selection of an existing database and the naming of a new database. A description can also be given. When 'Create' is selected, the database with the new name contains all the entries of the selected database. In this way multiple custom or smaller, more targeted databases can be created depending on the analytes of interest. A technical note on the Pesticide PCD [2] shows how users can run standards with unique chromatographic conditions and easily update or insert retention times in their custom database.

Customizing and updating PCDL AMRT compound data is accomplished by using tab 4 (from left) of the PCDL program interface. This is shown in Figure 8, where the options of 'Add New', 'Save as New', 'Update Selected' and 'Delete Selected' are clearly present. When 'Allow Editing' is activated from the 'Database/Library' pull-down menu, any of the displayed information fields in the users' custom database can be changed, added to or deleted. Furthermore, the ability to insert '*.mol' molecular diagrams to any new database entry is possible from the 'Edit Compounds' tab.



Compound Name	Formula	Mass	Mass Submitted	Delta Mass (ppm)	Anion	Cation	RT (min)	RT Submitted	Delta RT	CAS	ChemSpider	IUPAC Name
Methamphetamine	C10H15N	149.12045	149.11970	5.03			1.593	1.606	-0.013	537-46-2	10379	(2S)-N-Methyl-1-phenyl-2-propanamine
Codeine	C18H21N...	299.15214	299.15240	-0.87			1.160	1.169	-0.009	76-57-3	4447447	(5alpha,6alpha)-3-Methoxy-17-methyl-7,8-d
Hydrocodone	C18H21N...	299.15214	299.15250	-1.20			1.560	1.575	-0.015	125-29-1	4447623	(5alpha)-3-Methoxy-17-methyl-4,5-epoxym
Phentermine	C10H15N	149.12045	149.11990	3.69			1.750	1.770	-0.020	122-09-8	4607	2-Methyl-1-phenyl-2-propanamine
Clonazepam	C15H10Cl...	315.04107	315.04120	-0.41			3.638	3.625	0.013	1622-61-3	2700	5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2H-1
delta9-Tetrahydrocannabinol (THC)	C21H30O2	314.22458	314.22430	0.89			5.292	5.275	0.017	1972-08-3	15266	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8
Oxycodone	C18H21N...	315.14706	315.14750	-1.40			1.423	1.434	-0.011	76-42-6	4447649	(5alpha)-14-Hydroxy-3-methoxy-17-methyl-
Lorazepam	C15H10Cl...	320.01193	320.01200	-0.22			3.621	3.616	0.005	846-49-1	3821	7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-d
Strychnine	C21H22N...	334.16813	334.16840	-0.81			1.769	1.788	-0.019	57-24-9	389877	Strychnidin-10-one
Verapamil	C27H38N...	454.28316	454.28330	-0.31			3.577	3.574	0.003	52-53-9	2425	2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimetho
Trazodone	C19H22Cl...	371.15129	371.15160	-0.84			2.824	2.840	-0.016	19794-93-5	5332	2-(3-[4-(3-Chlorophenyl)-1-piperazinyl]propy
Heroin	C21H23N...	369.15762	369.15790	-0.76			2.297	2.322	-0.025	561-27-3	4575379	(5alpha,6alpha)-17-Methyl-7,8-didehydro-4
Proadifen	C23H31N...	353.23548	353.23550	-0.06			4.121	4.116	0.005	302-33-0	4741	2-(Diethylamino)ethyl 2,2-diphenylpentano
Methadone	C21H27NO	309.20926	309.20940	-0.45			3.638	3.638	0.000	76-99-3	3953	6-(Dimethylamino)-4,4-diphenyl-3-heptanor
Alprazolam	C17H13Cl...	308.08287	308.08300	-0.42			3.726	3.726	0.000	28981-97-7	2034	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazo

Figure 8. Edit Compounds PCDL interface tab.

Workflow C. Data mining using 'Find by Formula' (FBF)

The 'Find by Formula' data-mining algorithm of the MassHunter Qualitative Analysis program uses a pre-defined empirical formula (or list of formulae) to search TOF and Q-TOF (MS) data files for evidence that peaks may be present. The PCDL-format databases can also be specified as the list of empirical formulae. Depending on the size and content of the database, FBF can take slightly longer than the MFE approach. However, FBF is highly accurate and sensitive especially at very low analyte concentration levels.

Figure 9 illustrates the results screen displayed after a 'Find by Formula' search has been undertaken using the LC/MS Toxicology Test Mix data file. All 25 compounds were matched with accurate mass, abundance and isotopic spacing in a combined score (shown) together with retention time. The DA method editor settings used for this FBF analysis are shown in Figure 10, where 'Tox_std_01.cdb' was a custom PCDL-format database.

When reporting the results, FBF assesses the chromatographic peak shape and isotopic match scores and returns the best match, even if there are several peaks displayed in the extracted compound chromatogram of similar mass.

Additional adducts [M+Na]+, [M+NH4]+ and [2M+H]+ were used during this FBF data screen. The extra information is displayed in the spectrum view and results table to provide added confirmatory evidence. Figure 9 shows the Temazepam spectrum which displays both [M+H]+ and [M+Na]+ adducts.

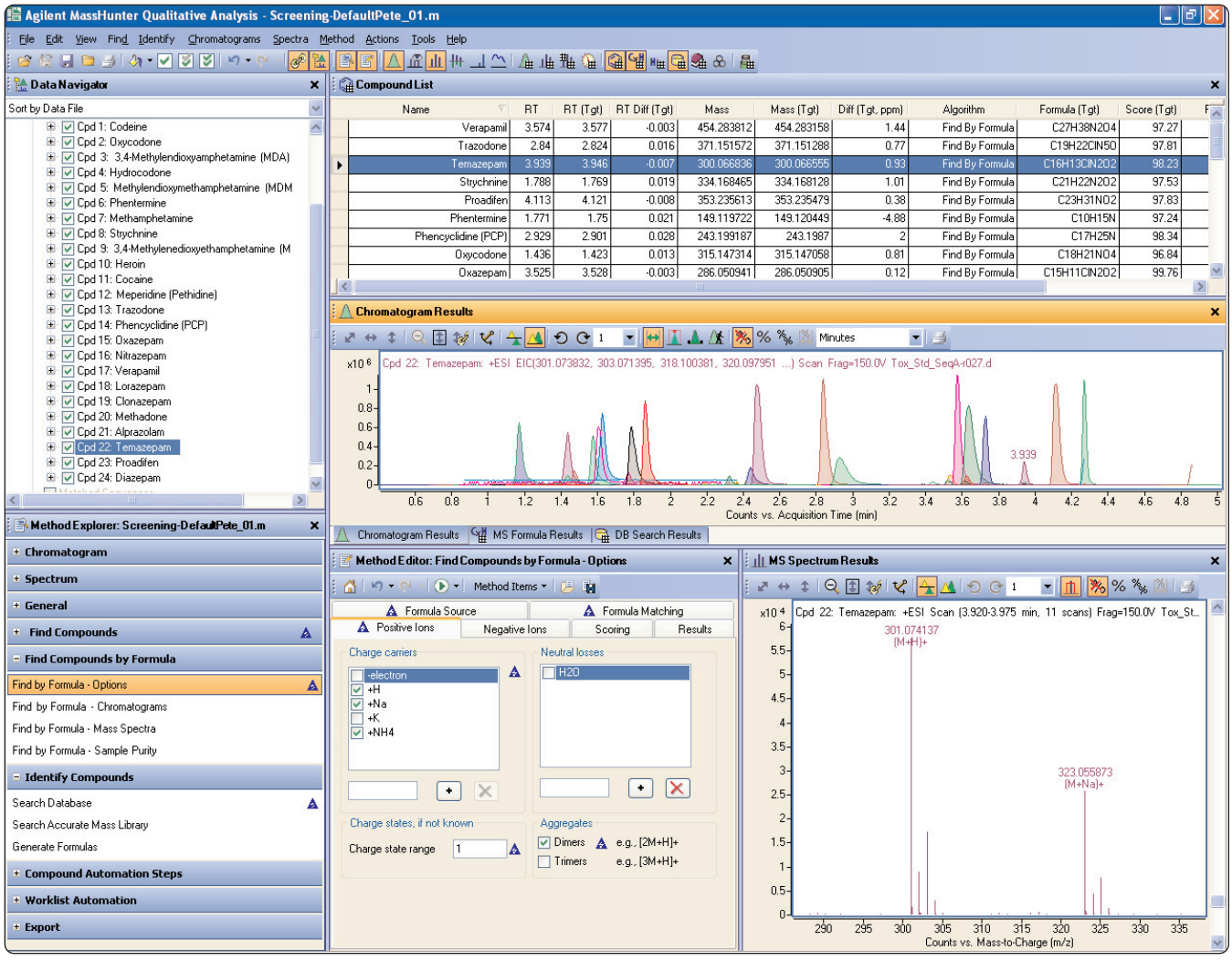


Figure 9. Find By Formula Database search results, MassHunter Qualitative Analysis program.

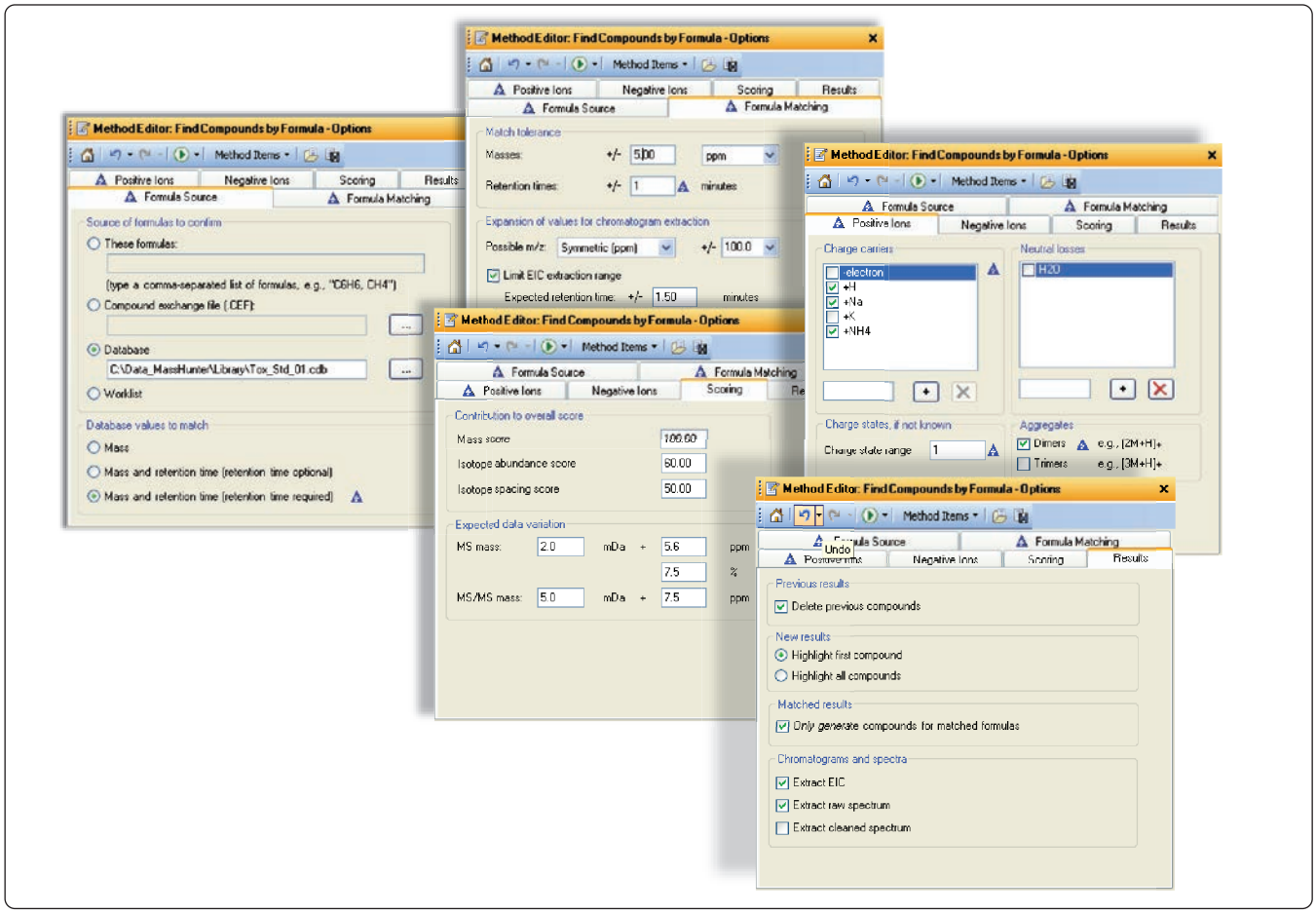


Figure 10. Find By Formula Database search - Method editor settings.

More in-depth information can be obtained from MassHunter Qualitative Analysis program Help files or Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide [5].

Reporting

Manual, MFE and FBF database searching all use the identical method of compound reporting options in the MassHunter Qualitative Analysis program software interface. Figure 11 details the reporting options which are based upon the standard compound report template 'CompoundReportWithIdentificationHits.xlsx'. Under the General section of the method explorer, the 'Common reporting options' link opens the corresponding method editor pane, shown on the left side of Figure 11. MassHunter Qualitative Analysis program treats search algorithm data and database searches as compound-centric data. Therefore, to report the results the appropriate compound report template must be chosen. In this example, the correct report template is displayed.

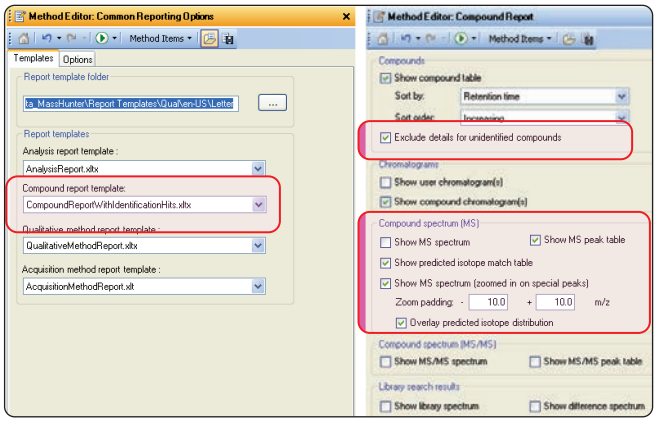


Figure 11. Common compound reporting options for Manual/MFE/FBF PCDL Searches.

More specific content can then be specified by choosing the information required for the Toxicology screen report using the 'Compound Report' options of the method editor (shown on the right in Figure 11).

Decisions about the report content are decided here. For example, if the check box for 'Exclude Details for Unidentified Compounds' is activated, then only positive PCDL identifications will be reported. The option to report compound extracted chromatograms, individual MS spectra, or summary results and individual compound tables is also determined from the compound report method editor.

Once all the correct settings have been achieved for the reporting of results, the green button (circled in Figure 12) activates the 'printing dialogue' window which gives various options for directing the output of the data file results. The user can choose to send results directly to a specified printer or save the results in excel format or public distribution format (pdf). Alternatively, the results report can be processed by choosing the 'Print Compound Report' option from the drop-down 'File' menu.

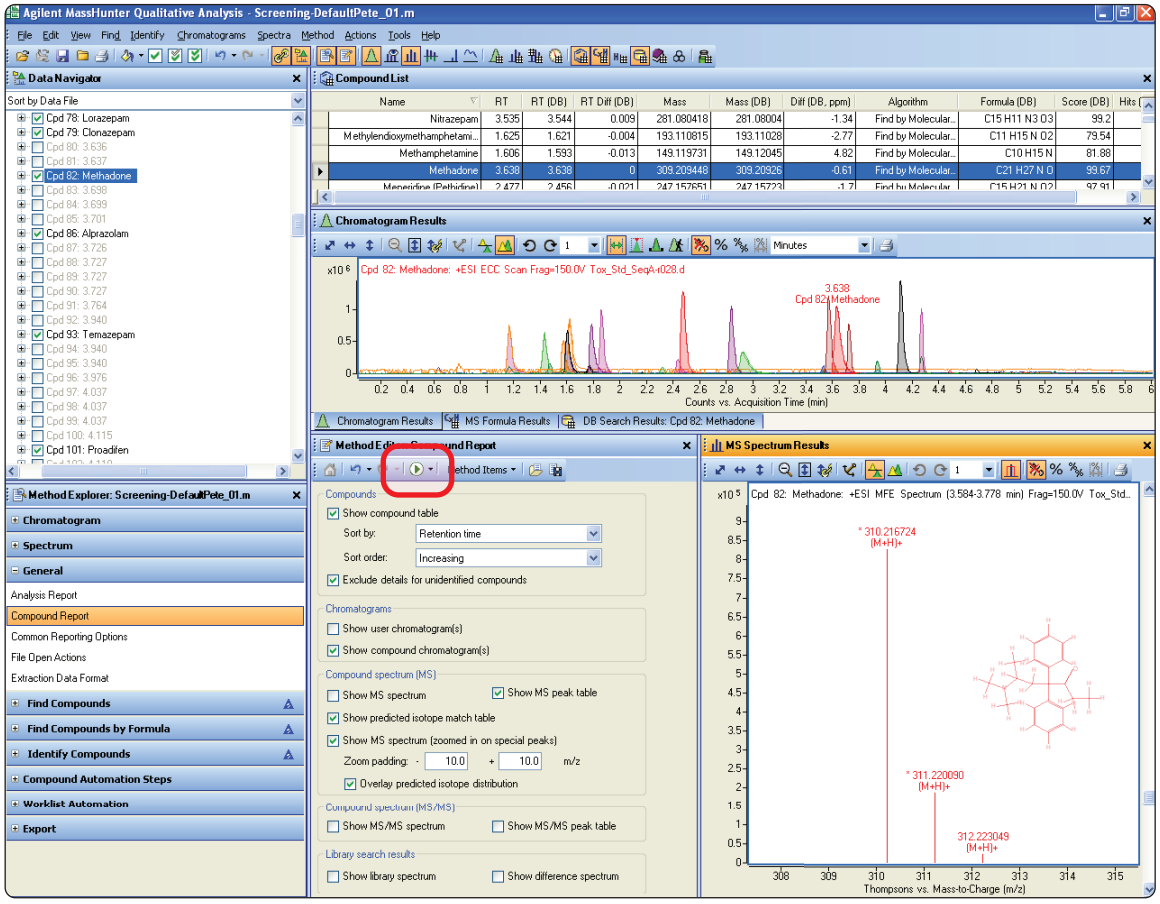


Figure 12. Compound Reporting for Manual/MFE/FBF PCDL Searches.

Figure 13 illustrates a typical report summary front page for the LC/MS Toxicology Test Mix.

Qualitative Compound Report

Data File	Tox_Std_SeqA-r028.d	**Sample Name**	Tox_Std_100pg
Sample Type	Sample	**Position**	Vial 2
Instrument Name	SEIKO-90500107	**User Name**	Pete Stone
Acq Method	PStone_Tox_Std_01.m	**Acquired Time**	7/17/2009 9:41:03 PM
IRM Calibration Status	Success	**DA Method**	Screening-DefaultPete_01.m
Comment			

Compound Table

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 10: Codeine	1.169	299.1524	Codeine	C18 H21 N O3	-0.72	2
Cpd 17: Oxycodone	1.434	315.1475	Oxycodone	C18 H21 N O4	-1.44	1
Cpd 23: 3,4-Methylendioxyamphetamine (MDA)	1.474	179.095	3,4-Methylendioxyamphetamine (MDA)	C10 H13 N O2	-2.23	1
Cpd 29: Hydrocodone	1.575	299.1525	Hydrocodone	C18 H21 N O3	-1.2	2
Cpd 34: Methamphetamine	1.606	149.1197	Methamphetamine	C10 H15 N	4.82	2
Cpd 39: Methylendioxymethampheta mine (MDMA)	1.625	193.1108	Methylendioxymethampheta mine (MDMA)	C11 H15 N O2	-2.77	1
Cpd 41: Phentermine	1.77	149.1199	Phentermine	C10 H15 N	3.78	2
Cpd 45: Strychnine	1.788	334.1684	Strychnine	C21 H22 N2 O2	-0.77	1
Cpd 50: 3,4-Methylenedioxyethamphetami ne (MDEA)	1.862	207.1263	3,4-Methylenedioxyethamphetami ne (MDEA)	C12 H17 N O2	-1.8	1
Cpd 57: Heroin	2.322	369.1579	Heroin	C21 H23 N O5	-0.63	1
Cpd 60: Cocaine	2.44	303.1475	Cocaine	C17 H21 N O4	-1.29	1
Cpd 62: Meperidine (Pethidine)	2.477	247.1577	Meperidine (Pethidine)	C15 H21 N O2	-1.7	1
Cpd 65: Trazodone	2.84	371.1516	Trazodone	C19 H22 Cl N5 O	-0.81	1
Cpd 67: Phencyclidine (PCP)	2.931	243.199	Phencyclidine (PCP)	C17 H25 N	-1.32	1
Cpd 73: Oxazepam	3.524	286.0511	Oxazepam	C15 H11 Cl N2 O2	-0.71	1
Cpd 74: Nitrazepam	3.535	281.0804	Nitrazepam	C15 H11 N3 O3	-1.34	1
Cpd 76: Verapamil	3.574	454.2833	Verapamil	C27 H38 N2 O4	-0.31	1
Cpd 78: Lorazepam	3.616	320.012	Lorazepam	C15 H10 Cl2 N2 O2	-0.19	1
Cpd 79: Clonazepam	3.625	315.0412	Clonazepam	C15 H10 Cl N3 O3	-0.42	1
Cpd 82: Methadone	3.638	309.2094	Methadone	C21 H27 N O	-0.61	1
Cpd 86: Alprazolam	3.726	308.083	Alprazolam	C17 H13 Cl N4	-0.33	1
Cpd 93: Temazepam	3.94	300.067	Temazepam	C16 H13 Cl N2 O2	-1.62	1
Cpd 101: Proadifen	4.116	353.2355	Proadifen	C23 H31 N O2	-0.18	1
Cpd 110: Diazepam	4.272	284.072	Diazepam	C16 H13 Cl N2 O	-1.36	1
Cpd 243: delta9-Tetrahydrocannabinol (THC)	5.275	314.2243	delta9-Tetrahydrocannabinol (THC)	C21 H30 O2	0.94	1

Compound Label	Name	RT	Algorithm	Mass
Cpd 10: Codeine	Codeine	1.169	Find by Molecular Feature	299.1524

Figure 13. Output Report from MFE/Database search.

Worklist Automation:

Once the analyst or operator has decided on the correct settings for all aspects of the data mining routines, the PCDL search options and reporting options (outlined in this application note) can be saved to one convenient data analysis method. This method can be used for repetitive and consistent data manipulation from week to week. This is achieved by choosing the 'Save As' option from the drop-down 'Method' menu in the MassHunter Qualitative Analysis program interface. This method will then open as the default DA method when the MassHunter Qualitative Analysis program is started until another DA method is saved or loaded.

An added advantage to saving reprocessing options is the 'Worklist Automation' functionality built into the MassHunter Qualitative Analysis program. Figure 14 outlines the setup of Worklist automation and specifically addresses a routine that would automatically interrogate a data file using MFE and PCDL database search followed by reporting of results to the specified printer or data file location.

In this example, a list of automatic data analysis steps are defined in order of operation, as they would be undertaken manually.

First, the sample data file is loaded, and all previous results (if any) are cleared. Next, the 'Find by MFE' routine according to the saved DA method setup is performed with the compound results searched against the PCDL database specified in the DA method. Finally, any results are automatically sent to a final report, the format of which has been determined and also saved to the DA method.

Two further steps must be performed to run such a worklist automation routine automatically during sample data acquisition.

First, the DA analysis method and the Worklist Automation routine must be saved into the acquisition method by using the 'Save As' option from the 'Method' menu and selecting the MassHunter acquisition method name. Once 'OK' is

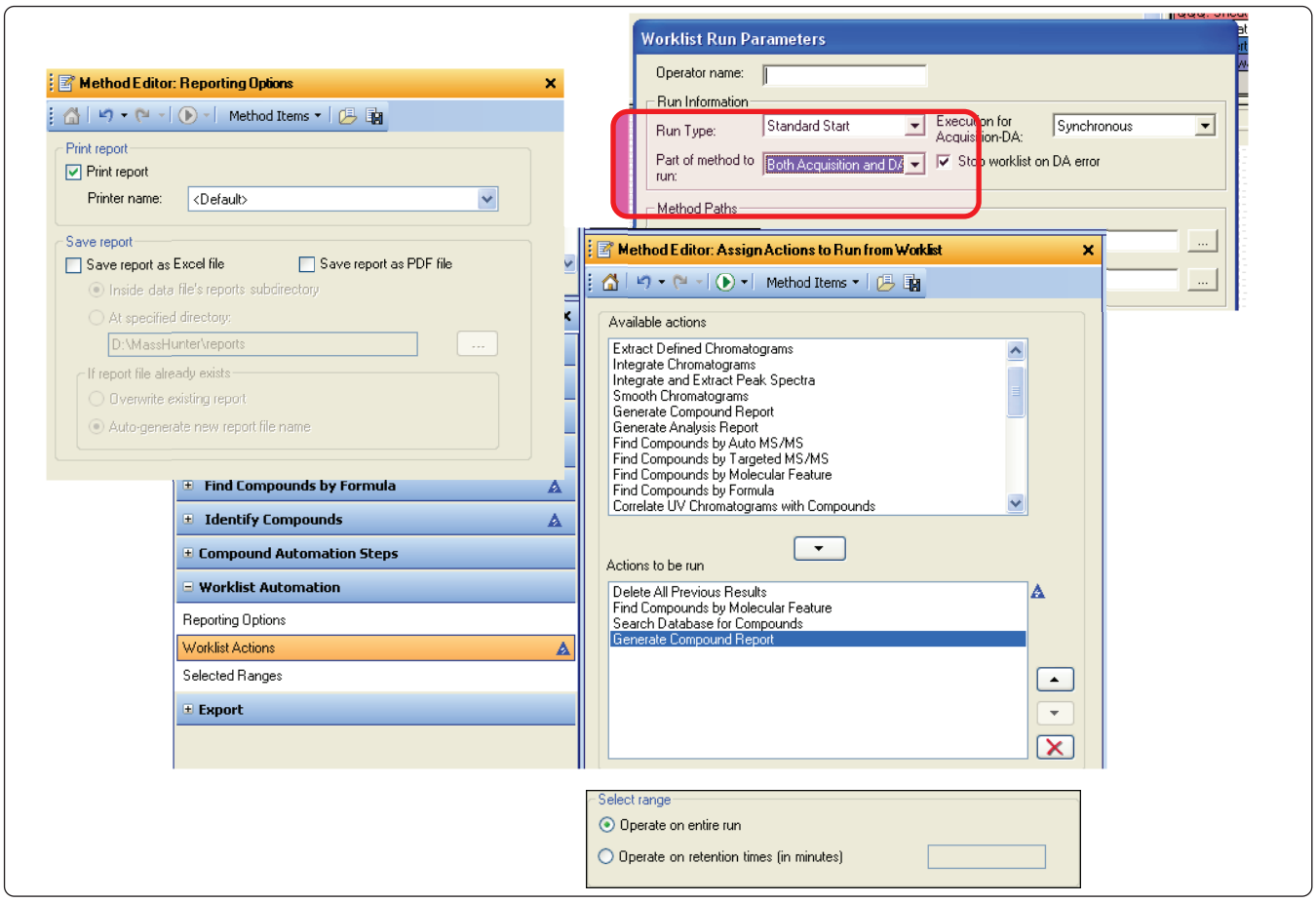


Figure 14. Worklist automation method setup.

selected, the data analysis method becomes an integral part of the Acquisition method.

Finally, to automatically perform Worklist Data Analysis during data acquisition, the 'Worklist Run Parameters' window must be opened from the 'Worklist' Menu of MassHunter Acquisition software. Figure 14 shows a screen capture of this window with the settings highlighted so that the DA routine will operate 'Parts of method to Run - Both Acquisition and DA'. The data analysis has the option to be run 'Synchronously' or 'Asyncronously'.

Conclusions

The Agilent MassHunter Personal Forensics and Toxicology Database Kit has been developed to provide comprehensive screening of samples for both targeted and non-targeted approaches. The database includes accurate mass data for around 6700 compounds of potential interest and gives the user flexibility in its use.

The MassHunter Personal Forensics and Toxicology Database Kit offers:

- Fast and easy startup of complex analyses
- A comprehensive database of around 6700 compounds including
  - Chemical structures, formulas and exact masses
  - Direct Chemical Internet links to PUBCHEM and ChemSpider
  - IUPAC names
  - The ability to create MS/MS spectral libraries
  - Complete customization with additions/deletions of retention time for chromatographic conditions developed by the user
- Results can be searched from within the PCDL software interface or directly from the MassHunter Qualitative Analysis program.
- Results can be data-mined with powerful searching tools, such as the Molecular Feature Extractor and Find by Formula
- Searches of the database can be partially or completely automated using MassHunter Qualitative Analysis program and the MassHunter Acquisition Worklist

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5. "Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide" Agilent Technologies Publication G3335-90060.

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Printed in the USA
August 3, 2009
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Database and Library Searching for Screening Toxins and Drugs-of-Abuse

The First Accurate Mass MS/MS Library for Forensics and Toxicology Using the Agilent 6500 Series Accurate Mass Q-TOF LC/MS

The Broecker, Herre, & Pragst Personal Compound Database and Library virtually eliminates false positives and provides confident identification without standards.

Screening and identifying the large number of compounds that are of concern to forensic scientists and toxicologists is a formidable undertaking. The Agilent 6500 Accurate Mass Q-TOF LC/MS with the Forensic and Toxicology Personal Compound Database and Library (PCDL) can screen and identify both the parent compound and resulting metabolites. There are over 7500 compounds in the database and over 2600 of them contain MS/MS spectra. Any of the Agilent Q-TOF LC/MS instruments can collect high resolution MS and MS/MS spectra with mass accuracies better than 3 ppm even for MS/MS fragments. Samples can be run and the database and library searched using Auto MS/MS and MassHunter Qualitative Analysis, which are powerful data mining tools that positively identify compounds with accurate mass of both precursor and fragment ion information.

Auto MS/MS precursor ions trigger MS/MS spectra to be collected under user defined conditions. All single MS ions detected are mined to determine if they represent compounds and if they do are searched against the database of compounds using exact molecular weight and the possible adducts. The MS/MS spectra are then searched for library matches and identified with both a forward and reverse score. Direct graphic and tabular inspection of the matches can be made. The power of the high quality data collected, data mining approaches, and the library allow a difficult task to be completed in hours versus days, with the confidence of a direct match from Agilent instrument to instrument.

Key Benefits

- Agilent 6500 Series Accurate Mass Q-TOF LC/MS provides the sensitivity needed with full spectra to determine toxins or drugs present in bodily fluids
- The Broecker, Herre & Pragst PCDL provides the greatest number of relevant compounds for screening and identification
- The database contains over 7500 compounds and metabolites with accurate mass MS/MS spectra for more than 2600 of them
- The library can identify a large number of compounds quickly
- False positives are virtually eliminated with confident identification of accurate MS/MS library search results
- Comprehensive workflows meet the needs of the specific analysis: Auto MS/MS for rapid screening Targeted MS/MS for focused analysis

Our measure is your success.

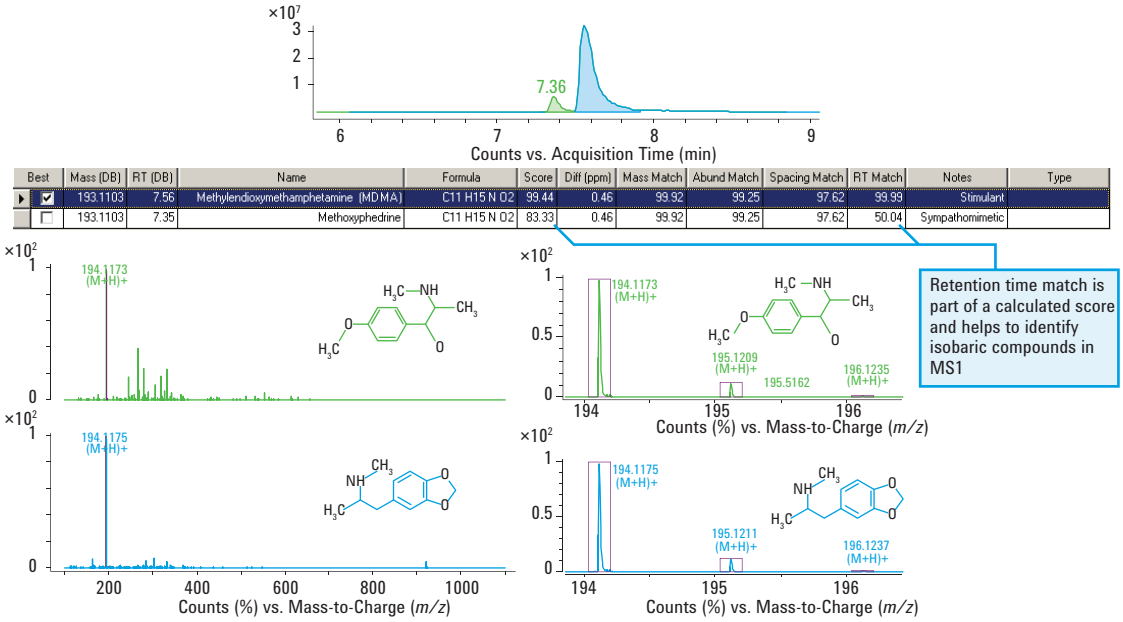

Figure 1: Single MS accurate mass data provides molecular formula but cannot determine isomers.

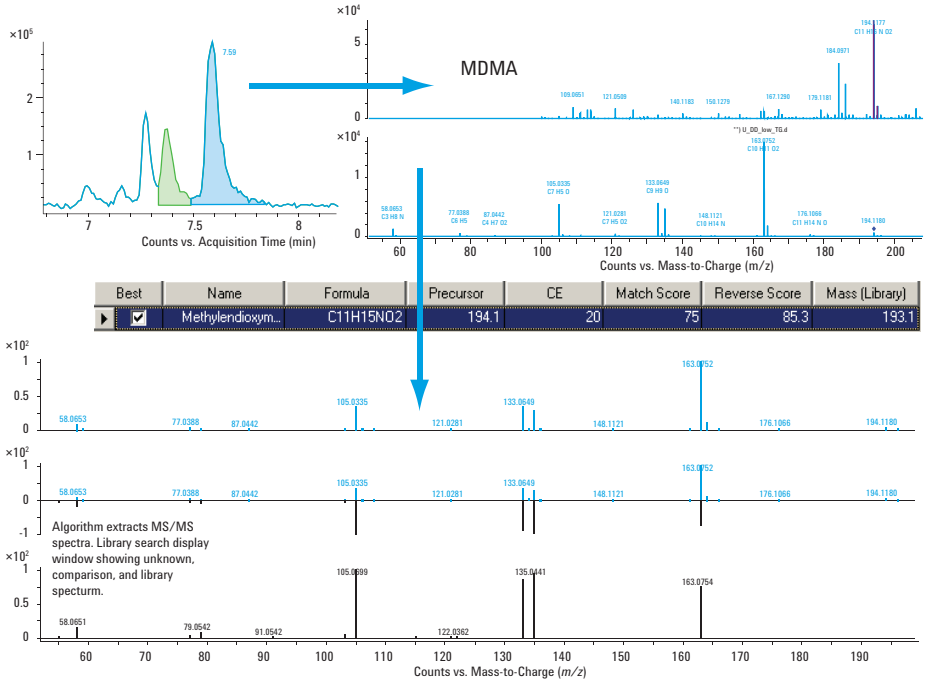

Figure 2: Detection of methoxyphedrine and MDMA isomers not distinguishable with a database search only without standards and retention time. With library, MDMA is readily identified.

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Printed in USA, December 2, 2010
5990-6941EN

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Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS

Application

Forensics

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Abstract

A key technique used in trace explosives analysis is HPLC with UV detection, following the guidelines set out in USEPA method 8330. Although sensitive for many target explosives, the method is limited by a lack of detector selectivity. This application note outlines the benefits and limitations of the use of liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) for the detection and quantitation of trace levels of these explosive residues.

Introduction

The identification of explosive residues in crime scene forensic investigation, environmental site remediation, and homeland security is an analysis of major significance to both public and regulatory authorities. The traditional and most commonly accepted method for the analysis of the nitroaromatic class of explosives is USEPA Method 8330. This method provides a sensitive UV-based analysis of 14 nitroaromatics and nitramines. However, due to the lack of selectivity provided by UV detection, confirmation of the species present requires the analysis to be performed on two analytical columns with different stationary phases.

The terrorist attacks on 9/11/2001, and subsequent attacks around the world have brought a new focus onto the identification and quantitation of explosive residues in crime scene investigation and homeland security. One of the front lines of homeland security, airport departure gates, uses sophisticated screening devices such as ion mobility spectrometers. These devices, though sensitive, face selectivity limitations in that they cannot determine the explosive species present. Additionally, terrorists are becoming increasingly erudite, as was seen in the attempt by Richard Reid in late December 2001. He used a peroxide-based explosive within his shoes, which was not detectable at trace levels using the analytical techniques commonly used for explosives analysis.

Inherent to the nature of explosive compounds is their instability, and propensity to breakdown. One of the best known and most common explosive compounds, trinitrotoluene (TNT) is reduced by bacteria to 2-amino-4,6-dinitrotoluene, (2-AMDNT) and 4-amino-2,6-dinitrotoluene (4-AMDNT); a metabolism that occurs also in plants and animals. Both of these compounds are markers for the former presence of TNT, and are also known to show severe toxicity and mutagenicity, making them important environmental markers.

An extensive search of the literature found several articles detailing the analysis of explosive materials using liquid chromatography (LC) [1-4]. However, of the 14 explosive materials of interest, very few, in particular TNT and RDX, were readily identified using mass spectrometry (MS) [5-7].

There still exists the requirement for a reliable and sensitive confirmatory technique of analysis for these explosive residues that can be performed on samples from a wide variety of sources. Liquid chromatography/mass spectrometry (LC/MS) provides an excellent tool for this analysis with the ability to couple the mass spectrometer to existing instruments performing USEPA method 8330. Furthermore, the choice of a mass selective detector (MSD) can provide confirmatory information previously required through the use of a second analytical column.

Accurate mass measurement, such as provided by the Agilent LC/MSD TOF time-of-flight mass spectrometer (LC/MSD TOF), greatly increases the confidence of identification because it inherently limits the possible number of candidate compounds. The better the precision and accuracy of the mass measurement, the fewer compounds are theoretically possible. This is particularly useful when needing to analyze samples from a variety of sources, each with their own potential interferences such as those encountered with explosives residues.

This application note demonstrates the utility of the LC/MSD TOF for the determination of low level explosives. The LC/MSD TOF provides accurate mass determination (better than 3 ppm) and linearity to three orders of magnitude, and thus is an excellent tool for the detection, confirmation, and quantitation of explosive compound residues.

The explosives studied are shown in Tables 1 and 2, including the chemical structure and theoretically calculated exact mass.

Table 1. Names, Abbreviations and Molecular Formulae of Explosives Studied

#	Name	Abbreviation	CAS no.	Molecular formula
1	Hexamethylenetriperoxidediamine	HMTD	NA	$C_6H_{12}N_2O_6$
2	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0	$C_4H_8N_8O_8$
3	Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4	$C_3H_6N_6O_6$
4	1,3,5-triamino-2,4,6-trinitrobenzene	TATB	3058-38-6	$C_6H_6N_6O_6$
5	Ethylene glycol dinitrate	EGDN	628-96-6	$C_2H_4N_2O_6$
6	1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4	$C_6H_3N_3O_6$
7	1,3-Dinitrobenzene	1,3-DNB	99-65-0	$C_6H_4N_2O_4$
8	Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8	$C_7H_5N_5O_8$
9	4-amino-2,6-dinitrotoluene	4A-DNT	1946-51-0	$C_7H_7N_3O_4$
10	Nitrobenzene	NB	98-95-3	$C_6H_5NO_2$
11	Nitroglycerin	NG	55-63-0	$C_3H_5N_3O_9$
12	2-amino-4,6-dinitrotoluene	2A-DNT	355-72-78-2	$C_7H_7N_3O_4$
13	2,4,6-Trinitrotoluene	TNT	118-96-7	$C_7H_5N_3O_6$
14	2,6-Dinitrotoluene	2,6-DNT	606-20-2	$C_7H_6N_2O_4$
15	2,4-Dinitrotoluene	2,4-DNT	121-14-2	$C_7H_6N_2O_4$
16	Hexanitrostilbene	HNS	19138-90-0	$C_{14}H_6N_6O_{12}$
17	2-Nitrotoluene	2-NT	88-72-2	$C_7H_7NO_2$
18	4-Nitrotoluene	4-NT	99-99-0	$C_7H_7NO_2$
19	Pentaerythritol tetranitrate	PETN	78-11-5	$C_5H_8N_4O_{12}$
20	3-Nitrotoluene	3-NT	99-08-1	$C_7H_7NO_2$
21	Triacetone triperoxide	TATP	NA	$C_9H_{18}O_6$
22	Carbamite	Carbamite	NA	$C_{17}H_{20}N2_O$

NA Not applicable

Table 2. Molecular Structures and Calculated Accurate Masses of Explosives Studied

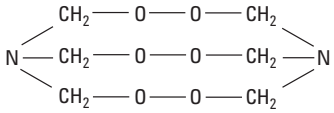
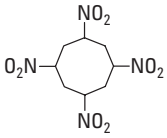
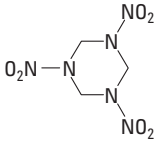
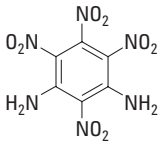
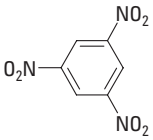
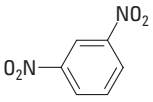
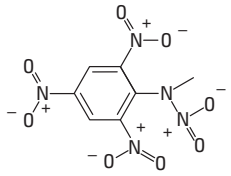
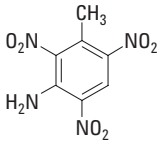
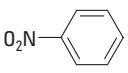
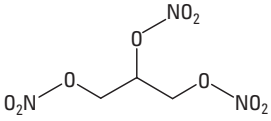
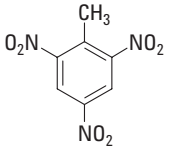
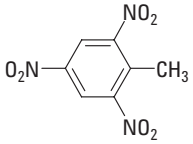
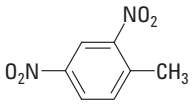
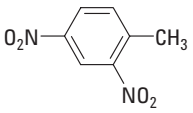
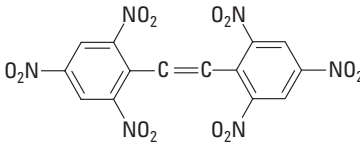
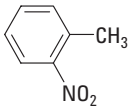
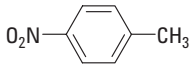
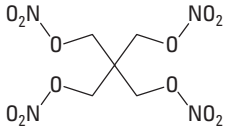
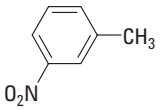
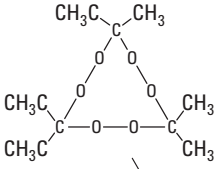
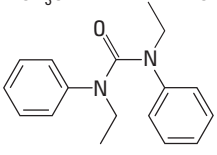
#	Name	Molecular formula	Structure	Molecular weight
1	HMTD	$C_6H_{12}N_2O_6$		208.0695
2	HMX	$C_4H_8N_8O_8$		296.0465
3	RDX	$C_3H_6N_6O_6$		222.0349
4	TATB	$C_6H_6N_6O_6$		258.0349
5	EGDN	$C_2H_4N_2O_6$		152.0069
6	1,3,5-TNB	$C_6H_3N_3O_6$		213.0022
7	1,3-DNB	$C_6H_4N_2O_4$		168.0171
8	Tetryl	$C_7H_5N_5O_8$		287.0138
9	4A-DNT	$C_7H_7N_3O_4$		197.0437
10	NB	$C_6H_5NO_2$		123.0320
11	NG	$C_3H_5N_3O_9$		227.0026

Table 2. Molecular Structures and Calculated Accurate Masses of Explosives Studied (continued)

#	Name	Molecular formula	Structure	Molecular weight
12	2A-DNT	$C_7H_7N_3O_4$		197.0437
13	TNT	$C_7H_5N_3O_6$		227.0178
14	2,6-DNT	$C_7H_6N_2O_4$		182.0328
15	2,4-DNT	$C_7H_6N_2O_4$		182.0328
16	HNS	$C_{14}H_6N_6O_{12}$		450.0044
17	2-NT	$C_7H_7NO_2$		137.0477
18	4-NT	$C_7H_7NO_2$		137.0477
19	PETN	$C_5H_8N_4O_{12}$		316.0139
20	3-NT	$C_7H_7NO_2$		137.0477
21	TATP	$C_9H_{18}O_6$		222.1103
22	Carbamite	$C_{17}H_{20}N_2O$		268.1576

Methodology

The work undertaken in this study was performed on an Agilent 1100 LC system consisting of: binary pump, autosampler, thermostatted column compartment, and the LC/MSD TOF.

LC Conditions

Solvents	Methanol and water		
Flow rate	0.9 mL/min		
Gradient	**Time (min)**	**% Methanol**	**% Water**
	0	60	40
	1	60	40
	15	92	8
	16	100	0
	18	100	0
	19	60	40
Post time	5 minutes		
Total run time	24 minutes		
Injection volume	10 µL, with needle wash		
Column temperature	40 °C		
Column	ZORBAX Extend-C18 4.6 mm × 250 mm, 5 µm p/n 770450-902		

MS Detection conditions

Ionization	APCI
Gas temperature	350 °C
Vaporizer temperature	325 °C
Drying gas flow	5 L/min
Nebulizer pressure	40 psig
PCI Corona current	4 µA
PCI Capillary voltage	4000 V
NCI Corona current	10 µA
NCI Capillary voltage	1500 V
Scan *m/z* range	70–1000
Fragmentor voltage	100 V
Storage mode	Profile
Skimmer	60 V
Oct RF	250 V

The experimental conditions listed above were optimized for sensitivity. Vaporizer temperature, drying gas temperature and flow rate, corona current, capillary voltage, and fragmentor voltage were all optimized.

A large increase in signal was observed by reducing the drying gas flow rate from 6 L/min to 5 L/min. This resulted in a 30% increase in signal area for more than 80% of the explosives under investigation.

Reference Mass Introduction with LC/MSD TOF

The Agilent LC/MSD TOF uses a reference mass in the generation of reliable accurate masses. The electrospray source for the LC/MSD TOF is a unique dual spray assembly that allows the simultaneous and constant introduction of a reference mass solution. When using APCI (atmospheric pressure chemical ionization), the reference masses must be introduced into the mobile phase post-column.

This was achieved via a low dead-volume tee connected prior to the APCI source with PEEK™ tubing. An isocratic pump was used to deliver the reference mix at a flow rate of 50 µL/min in positive ion mode and flow programmed from 70 µL/min to 150 µL/min in negative ion mode over the run time of 1 to 15 minutes. In order to ensure pulse-less reference mass introduction a rapid resolution column (ZORBAX SB-C18, 30 mm × 2.1 mm, 5 µm, part number 873700-902) was installed in the flow path, providing backpressure for the isocratic pump.

The reference mix was modified to suit the methodology, 25 µL of purine and 250 µL of HP-0921 was added to 250 mL of 90:10 methanol:water.

This enabled the use of the following reference masses:
Positive ion mode: 121.050873 and 922.009798
Negative ion mode: 119.036320, 966.000725, and 980.016375

In negative ion mode, with increasing organic mobile phase strength, the reference masses 966.000725 and 980.016375 decrease in intensity. By using the custom reference mass mix outlined above and the use of flow programming, sufficient abundance of the reference mass ions is maintained throughout the analytical run.

With the paucity of literature discussing the detection of explosives by LC/MS, the first step of development was evaluating component responses under both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) in both positive and negative ion modes. Table 3 lists the response characteristics for many of the compounds tested in this study and it clearly shows that no one ionization and detection technique is universally applicable.

Table 3. Detection Modes for Various Explosives

Compound	UV/Visible	ESI +	ESI -	APCI +	APCI -
HMTD	√	×	×	√	×
HMX	√	×	×	×	√
RDX	√	×	√	×	√
TATB	√	×	×	×	√
EGDN	×	×	×	×	P
1,3,5-TNB	√	×	√	×	√
1,3- DNB	√	×	×	×	√
Tetryl	√	×	×	×	√
4A-DNT	√	×	×	×	√
Nitrobenzene	√	×	×	×	P
Nitroglycerin	P	×	×	×	P
2A-DNT	√	×	×	×	√
TNT	√	×	√	×	√
2,6-DNT	√	×	×	×	√
2,4-DNT	√	×	√	×	√
HNS	√	×	×	×	√
2-NT	√	×	×	×	P
4-NT	√	×	×	×	P
PETN	P	×	√	×	√
3-NT	√	×	×	×	P
TATP	P	×	×	P	×
Carbamite	√	√	×	√	×

√ Good response

P Poor response

× No response

It was observed that negative APCI provided the best response for most explosives studied, and if run in positive APCI mode as well, additional components are detected. Negative APCI also has the advantage of being very selective, removing possible matrix interferences. The ability to couple UV detection prior to the mass spectrometer also provides a highly capable analysis for explosives. However, the major advantage of LC/MS over UV detection is the ability to detect the newer, more terrorist-friendly explosives such as TATP and HMTD in positive APCI mode. These peroxide explosives are reported to degrade when exposed to intense sources of UV light, such as what might be experienced in a UV detector.

A key parameter considered during the development of the method was the ability to transfer the HPLC method between different detectors. This precluded the use of nonvolatile buffers which would be detrimental to MS detection. Initial analyses investigated the use of buffers such as acetic acid, formic acid, ammonium acetate, and ammonium formate. While in many cases the chromatographic separation was improved, signal response was compromised. Using the high selectivity of the LC/MSD TOF, signal intensity was chosen as the key parameter to optimize. The addition of chloroform in APCI mode can also increase sensitivity with some explosive compounds; however, it was found in this study that the majority of compounds are best analyzed with no organic modifier present.

Various HPLC columns, mobile phase compositions, and gradients were also tested in this investigation. The conditions finally used were chosen for their selectivity, speed of analysis, and detection limits (DLs) attainable with MS.

Detection of Explosives Using the LC/MSD TOF

An overwhelming advantage of using the LC/MSD TOF for the trace level detection of any component is the confirmatory information that is provided through accurate mass measurement. An example of this mass accuracy is shown in Table 4, where observed masses and their deviations from the theoretical exact mass are shown.

The ability to closely match the expected mass and the observed mass provides the analyst with a very high level of confidence in the assignment given to a chromatographic peak.

Table 4. Theoretical Exact Mass, Observed Mass, Mass Error, and Limit of Quantitation (LOQ) Using the LC/MSD TOF

Compound	Monoisotopic mass	Adduct	Adduct mass	Observed mass	Mass error (ppm)	LOQ (µg/L)
HMTD	208.0695	$[M-H]^+$	207.0611	207.0612	0.18	30
HMX	296.0465	$[M+CHO_2]^-$	341.0447	341.0446	–0.11	10
RDX	222.0349	$[M+CHO_2]^-$	267.0330	267.0328	–1.07	0.5
TATB	258.0349	$[M-H]^-$	257.0276	257.0276	–.02	5
EGDN	152.0069	No response by TOFMS				
1,3,5-TNB	213.0022	$[M]^-$	213.0027	213.0026	–0.63	15
1,3-DNB	168.0171	$[M]^-$	168.0176	168.0175	–0.92	10
Tetryl	287.0138	$[M-NO_2]^-$	241.0214	241.0214	–0.24	5
4A-DNT	197.0437	$[M-H]^-$	196.0363	196.0362	–0.92	10
NB	123.0320	No response by TOFMS				
NG	227.0026	No response by TOFMS				
2A-DNT	197.0437	$[M-H]^-$	196.0363	196.0364	0.92	5
TNT	227.0178	$[M]^-$	227.0183	227.0178	–2.6	4
2,6-DNT	182.0328	$[M]^-$	182.0333	182.0331	–1.1	8
2,4-DNT	182.0328	$[M]^-$	182.0333	182.0331	–1.1	4
HNS	450.0044	$[M]^-$	450.0049	450.0042	–1.6	1
2-NT	137.0477	$[M-H]^-$	136.0404	136.0406	1.5	100
4-NT	137.0477	$[M-H]^-$	136.0404	136.0407	2.2	50
PETN	316.0139	$[M-NO_2 + CH_2O_2]^-$	316.0269	316.0267	–0.94	250
3-NT	137.0477	$[M]^-$	137.0482	137.0480	–1.7	5000
TATP	222.1103	Unassigned	ND	89.0597	ND	1000
Carbamite	268.1576	$[M+H]^+$	269.1659	269.1665	2.1	10

ND Not determined

A powerful result of accurate mass measurement was the ability to assign the ion formed by positive APCI of HMTD. The paper by Xu et al [8] assigned the ion observed for HMTD as being the $[M-1]^+$ species. It was not clear what the ion was and thus it was identified only as a loss of one mass unit. Using the accurate mass data obtained from the Agilent LC/MSD TOF, this ion can be assigned as the $[M-H]^+$ species, as the likely result of the multiple peroxide linkages in close association with a nitrogen atom. Note that the measured mass in Table 4 shows a loss of hydrogen. This is shown in Figure 1 below.

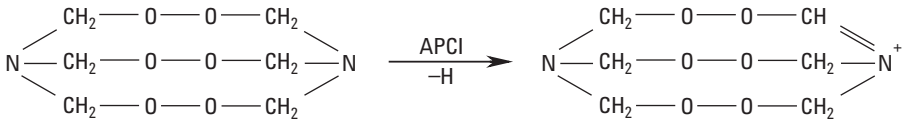


Figure 1. The theoretical positive ion formed from HMTD using APCI.

Further, a high degree of mass accuracy can increase the detection limit (DL), as noise is effectively reduced by narrowing the monitored mass range. This can be shown by observing the signal-to-noise (S/N) of RDX over a mass window of 0.1 amu (similar to what can be achieved on a single quadrupole system) and a mass window of 0.01 amu for a 1 µg/L (ppb) solution (Table 5).

Table 5. Calculated S/N for a 1 µg/L RDX at Different Mass Extraction Windows

Extracted ion range	Noise time range	Mean noise	P-P noise	Peak height	S/N (P-P)
267.0–267.1	3.509–3.692	21.4	57.0	285.5	5.0
267.03–267.04	3.509–3.692	5.2	21.0	245.5	11.7

A greater than two-fold increase in sensitivity is seen for these compounds. Figure 2 shows the reduction in noise that is observed with the extraction of a narrower mass range, a critical factor in confirmation when dealing with complex matrices.

An interesting observation that was made at higher concentrations was the dominance of a different adduct. This was a particular feature of HMX and RDX, whereby at high concentrations the adduct formed was $[M + CH_2O_2]^-$ instead of the otherwise observed $[M + CHO_2]^-$. This radical anion adduct could be explained by a charge exchange catalyzed by the very high concentration of ions/molecules in the APCI source. This split of signal would also explain the highly accurate mass measurement in spite of the high concentration that typically causes detector saturation and loss of accuracy.

Mass Accuracy with Concentration

The mass accuracy of the LC/MSD TOF was evaluated for four of the explosive compounds over a concentration range of 100,000 µg/L (100 ppm) to 1 µg/L (1 ppb) and is shown in Tables 6–9. The mass accuracy data was obtained from observing the mass spectral data at the apex of a plus/minus 1 amu extracted window of the accurate mass. The % RSD for each mass is reported and the mass error from the average mass. It should be noted that the error for the 100,000 µg/L HMX solution is for the previously mentioned $[CH_2O_2]^-$ adduct. Saturation of the detector at high concentrations is known to cause a loss of mass accuracy as shown in the results. For HMX and RDX the low concentration and low signal intensity resulted in a reduced mass accuracy as well. Higher signal intensity for the two other compounds, TNT and 2A-DNT, resulted in mass accuracy less than 2 ppm at the 1 µg/L concentration.

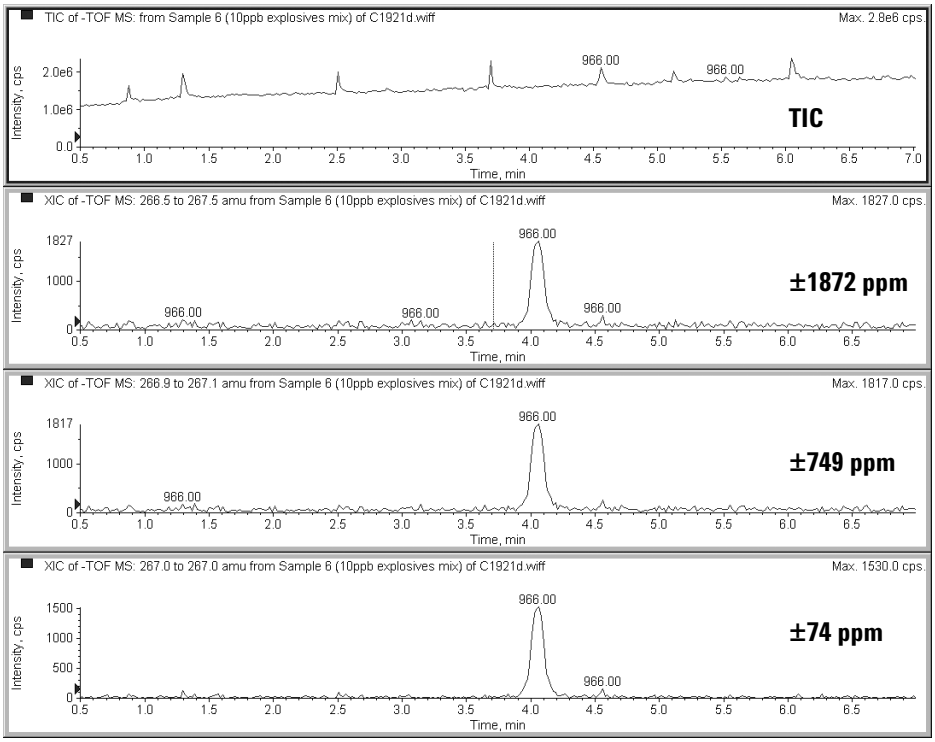


Figure 2. Effect of extracted ion range on noise of 10 µg/L RDX. The value given in each panel is the mass range extracted in parts per million (ppm) of expected exact mass of RDX.

Table 6. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for HMX

Replicate	1	10	100	1,000	10,000	100,000
1	341.0453	341.0444	341.0441	341.0449	341.0444	342.0668
2	341.0425	341.0461	341.0444	341.0445	341.0445	342.0645
3	341.0429	341.0446	341.0441	341.0446	341.0445	342.0628
4	341.0418	341.0445	341.0443	341.0444	341.0444	342.0651
5	341.0416	341.0457	341.0447	341.0443	341.0445	342.0600
Average	341.0428	341.0451	341.0443	341.0445	341.0445	342.0638
SD	0.0015	0.0008	0.0002	0.0002	0.0001	0.0026
Error (ppm)	–5.6	1.14	–1.2	–0.62	–0.62	34.61

Table 7. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for RDX

Replicate	1	10	100	1,000	10,000	100,000
1	267.036	267.0328	267.0345	267.0329	267.0324	267.0333
2	267.0357	267.033	267.0341	267.0331	267.0328	267.0336
3	267.0354	267.0314	267.0338	267.0331	267.0325	267.0335
4	267.0371	267.0326	267.033	267.0332	267.0327	267.0333
5	267.0297	267.0349	267.0334	267.0331	267.0322	267.0335
Average	267.0348	267.0329	267.0338	267.0331	267.0325	267.0334
SD	0.0029	0.0013	0.0006	0.0001	0.0002	0.0001
Error (ppm)	6.4	–0.69	2.7	–0.06	–2.2	1.2

Table 8. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for TNT

Replicate	1	10	100	1,000	10,000	100,000
1	227.0174	227.0180	227.0176	227.0177	227.0185	227.0457
2	227.0178	227.0162	227.0179	227.0176	227.0184	227.0416
3	227.0184	227.0173	227.0180	227.0177	227.0183	227.0346
4	227.0173	227.0170	227.0181	227.0177	227.0183	227.0360
5	227.0197	227.0193	227.0181	227.0176	227.0184	227.0318
Average	227.0181	227.0176	227.0179	227.0177	227.0184	227.0379
SD	0.0010	0.0012	0.0002	0.0001	0.0001	0.0056
Error (ppm)	–1.2	–3.5	–2.1	–3.01	0.072	86

Table 9. Mass Accuracy at Five Concentration Levels (1–100,000 µg/L) for 2A-DNT

Replicate	1	10	100	1,000	10,000	100,000
1	196.0375	196.0364	196.0361	196.0357	196.0399	196.0859
2	196.0371	196.0366	196.0361	196.0361	196.0397	196.0819
3	196.0360	196.0369	196.0364	196.0359	196.0397	196.0786
4	196.0358	196.0358	196.0368	196.0358	196.0390	196.0799
5	196.0368	196.0364	196.0364	196.0359	196.0394	196.0770
Average	196.0366	196.0364	196.0364	196.0359	196.0395	196.0807
SD	0.0007	0.0004	0.0003	0.0001	0.0004	0.0034
Error (ppm)	1.1	0.11	0.11	–2.4	16	230

Area Repeatability

Time-of-flight mass spectrometers have traditionally had a reputation as being unsuitable for quantitation and the provision of repeatable areas.

The area repeatability for the LC/MSD TOF was investigated at multiple levels for three of the explosive components. Generally, the LC/MSD TOF showed repeatability across five runs of better than 5% RSD. However, sometimes when approaching the LOQ, this would increase to a larger error. The area repeatability for RDX, TNT, and 2A-DNT for five injections at each concentration level analyzed are shown in Tables 10–12.

Table 10. RDX Concentration (µg/L)

Replicate	1	10	100	1,000	10,000	100,000
1	426	1890	12300	154000	2540000	14100000
2	642	1780	13000	143000	2450000	15200000
3	541	1820	13300	146000	2460000	15300000
4	659	2620	14000	141000	2330000	14900000
5	508	2760	13600	149000	2130000	14700000
Average	555.2	2174	13240	146600	2382000	14840000
SD	96	475	642	5128	159593	477493
%RSD	17.42	21.86	4.85	3.5	6.7	3.22

Table 11. TNT Concentration (µg/L)

Replicate	1	10	100	1,000	10,000	100,000
1	4760	16400	127000	1730000	20700000	74800000
2	4330	16600	134000	1700000	20600000	73300000
3	4490	16500	134000	1840000	20900000	71600000
4	4200	16200	134000	1790000	20400000	71300000
5	3990	16100	132000	1830000	19600000	71200000
Average	4354	16360	132200	1778000	20440000	72440000
SD	291	207	3033	61400	502991	1569394
%RSD	6.7	1.27	2.29	3.45	2.46	2.17

Table 12. 2A-DNT Concentration (µg/L)

Replicate	1	10	100	1,000	10,000	100,000
1	2300	7820	68400	779000	9720000	27600000
2	2440	9040	64500	807000	10400000	28800000
3	2340	8910	66200	862000	10400000	30800000
4	2250	8760	65900	849000	9690000	28400000
5	2350	7830	77800	940000	10100000	29600000
Average	2336	8472	68560	847400	10062000	29040000
SD	70	598	5350	61443	348310	1219836
%RSD	3.01	7.07	7.8	7.25	3.46	4.2

TOF Linearity

The linearity of the LC/MSD TOF was investigated for a range of the components in the mixture. Of the 10 components evaluated, most exhibited a linear regression coefficient of variation of greater than 0.998. Some of the compounds displayed excellent linearity across the four orders of magnitude. A linear dynamic range for this instrument is typically two-to-three orders of magnitude. As can be seen in the repeatability results for RDX, the area response is very linear between 10 and 10,000 µg/L. The 100,000 µg/L showed saturation and the 1 µg/L showed a less than 5x decrease in signal vs the nearly 10x for the other concentrations. Figures 3 to 5 show representative calibration curves for 3 of the 10 components evaluated.

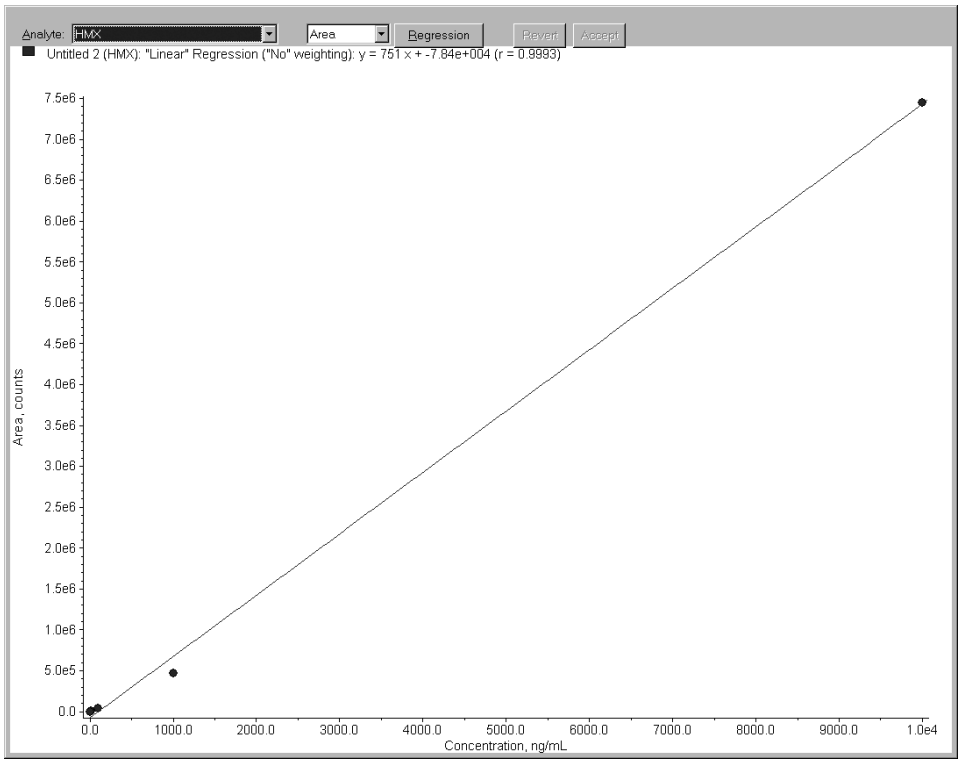


Figure 3. Calibration curve for HMX from 1 µg/L to 10,000 µg/L with MSD TOF.

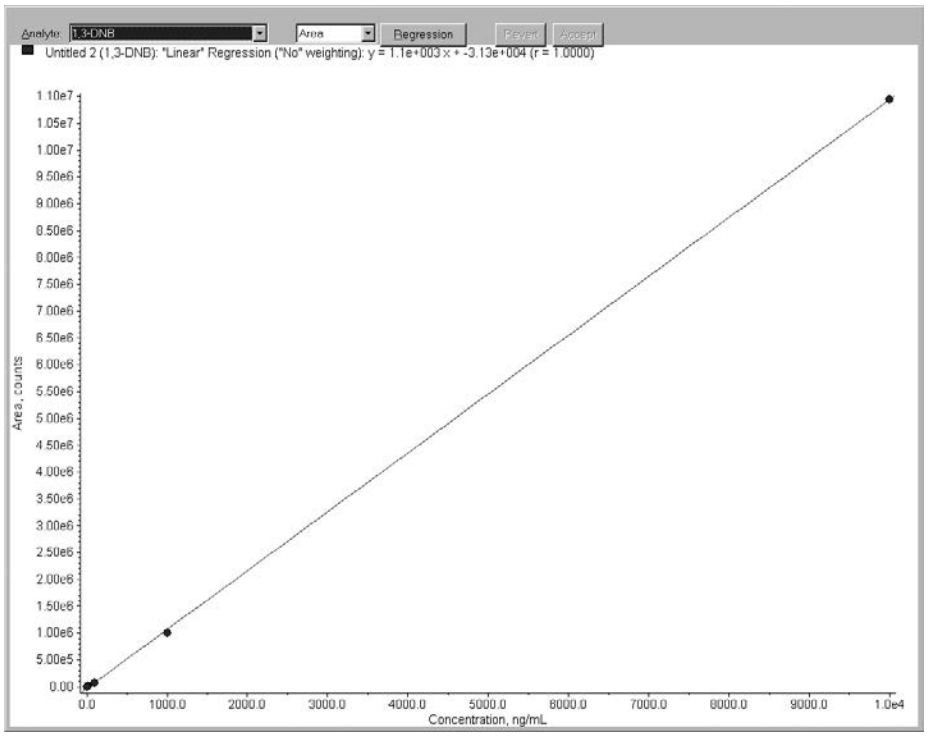


Figure 4. Calibration curve for 1,3-DNB from 1 µg/L to 10,000 µg/L with LC/MSD TOF.

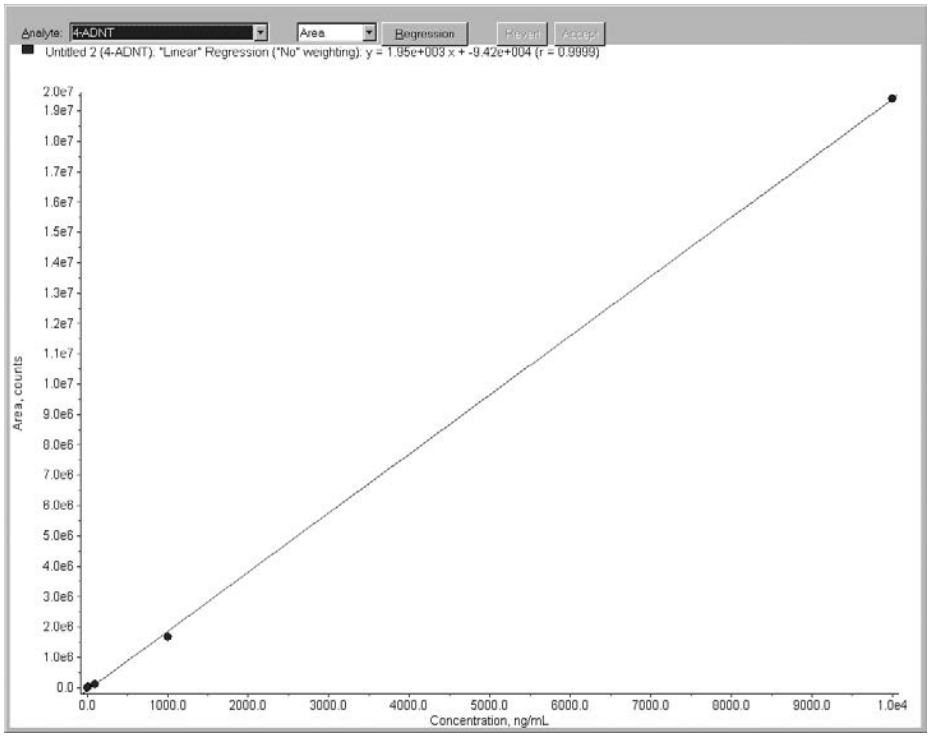


Figure 5. Calibration curve for 4A-DNT from 1 µg/L to 10,000 µg/L with LC/MSD TOF.

Chromatograms for four components are shown in Figure 6 at 10 µg/L with ±100-ppm extraction windows.

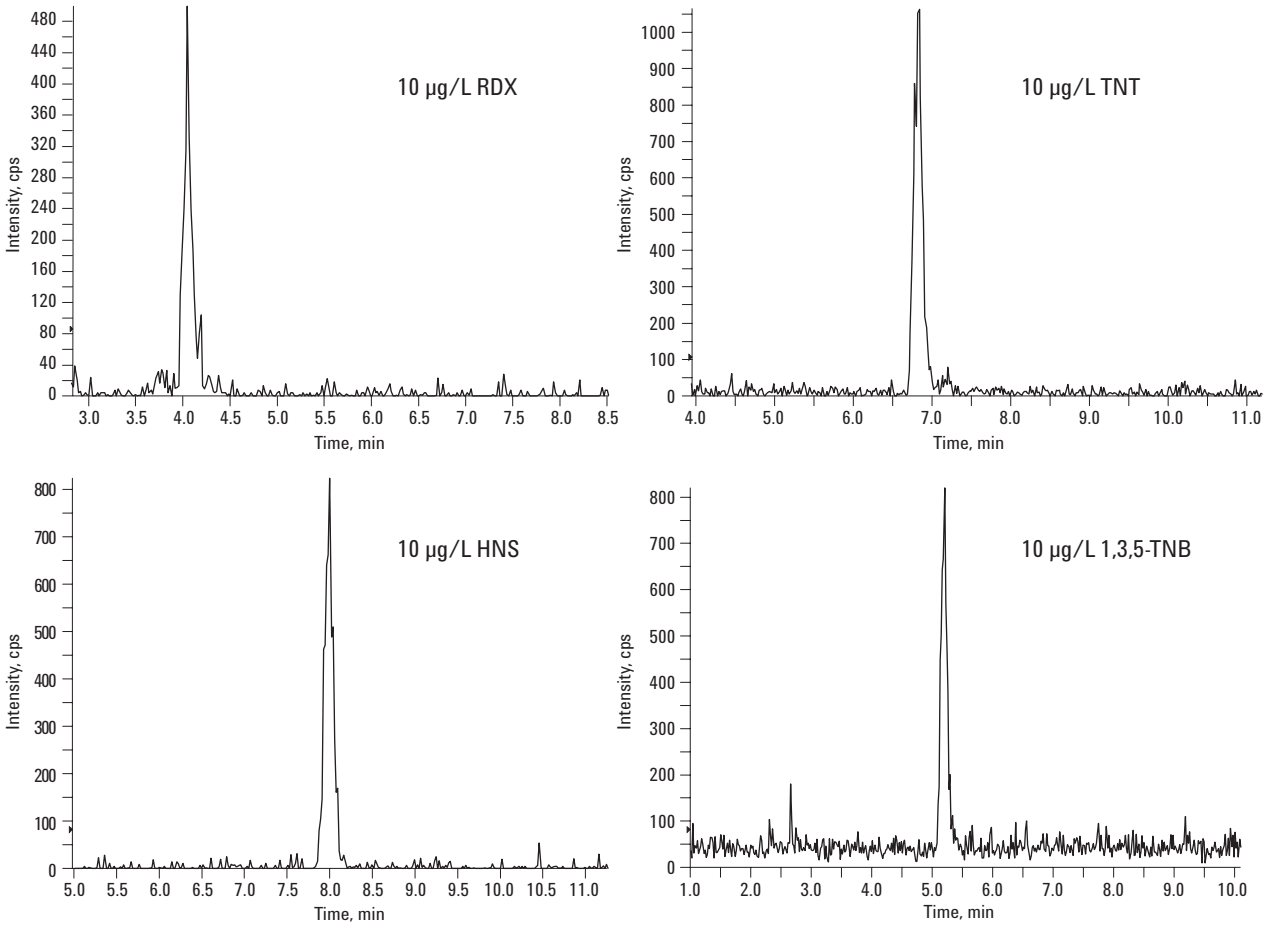


Figure 6. Representative chromatographic responses for four of the explosive compounds at the 10 µg/L concentration.

Spiked Recovery of Soil Samples

Figure 7 shows the results obtained from a soil spike of RDX.

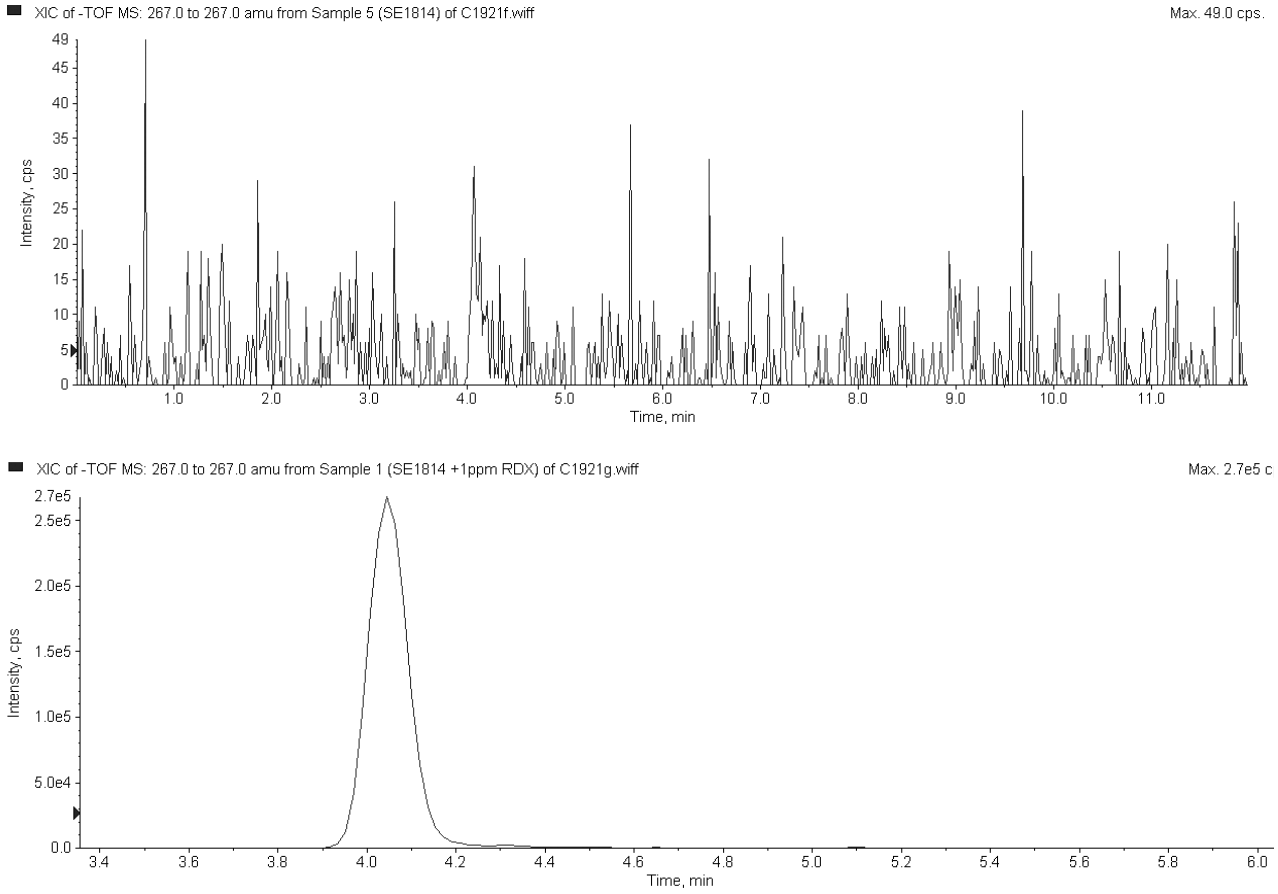


Figure 7. Soil sample SE1814 before and after spiking with 1 mg/L RDX. Extracted *m/z* 267.02-267.03

Table 13 gives the recoveries obtained when a dirty soil matrix is spiked with various explosives. The LC/MSD TOF provides a powerful tool in its ability to remove interference through the power of accurate mass measurements made at every scan.

Table 13. Spike and Recovery Levels for Three Soil Extracts

Soil sample ID	Spiked compound (amount)	Recovery
SE1814	RDX (1ppm)	115%
SE1814	RDX (0.1ppm)	120%
SE1816	2,4-Dinitrotoluene (1ppm)	75%
SE1947	1,3-dinitrobenzene (1ppm)	99%

Analysis of Crime Scene Samples

Two blind samples from archived crime evidence were analyzed with the Agilent LC/MSD TOF using the methodology developed in this study.

The first sample was treated as an unknown explosive. A small amount of material was dissolved in methanol and the resulting chromatogram in shown in Figure 8. The retention time of 6.8 minutes results in either two possibilities by retention time match, TNT or 2A-DNT (a TNT metabolite). By measuring the accurate mass of 227.0180 (Figure 9), it is a match for TNT with a radical union exact mass of 227.0183 (1.3 ppm mass error). Note that the $[M-H]^-$ ion is also observed and its measured mass of 226.0106 is only 0.18 ppm from the expected exact mass of this ion.

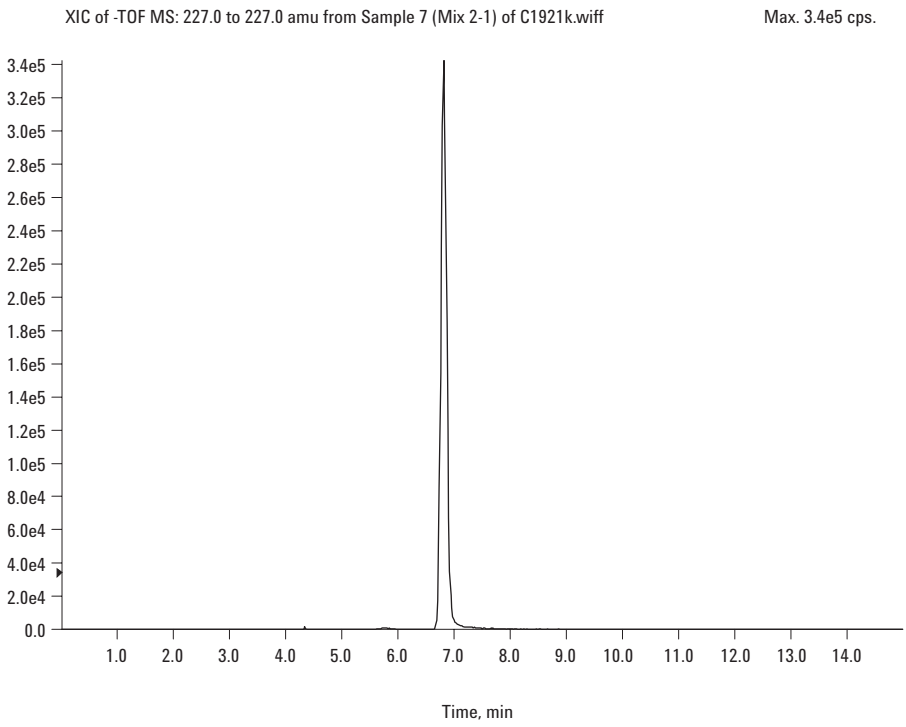


Figure 8. LC/MSD TOF Chromatogram of an unknown explosive material.

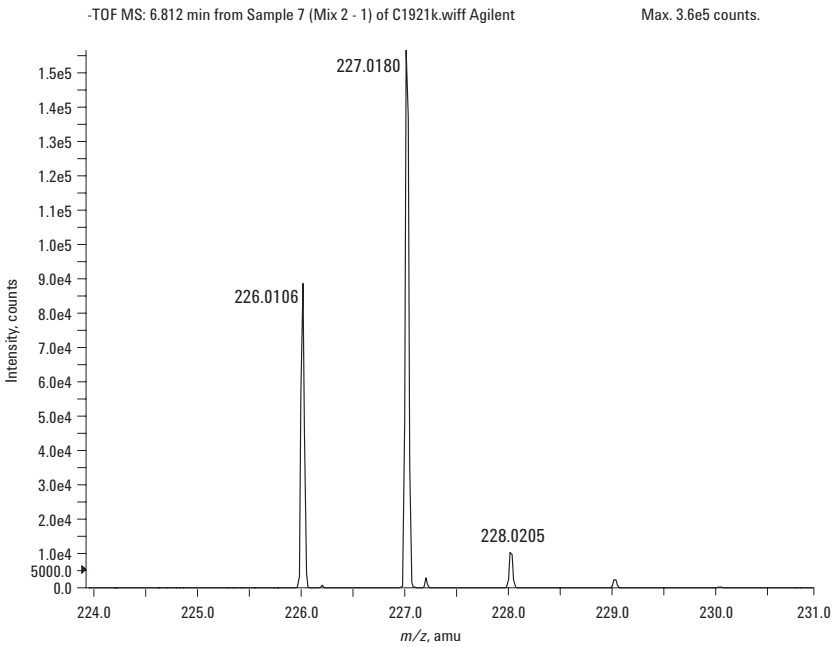


Figure 9. Mass Spectrum of an unknown explosive material.

The second sample was a soil extract to determine the possible presence of an explosive residue. By extracting all known accurate masses identified in this investigation within a 100 ppm mass window, one peak was identified at 4.0 minutes with a mass of 267.0331 *m/z*, which correlates to the presence of RDX with a mass error of 0.06 ppm for the formate adduct (Figure 10).

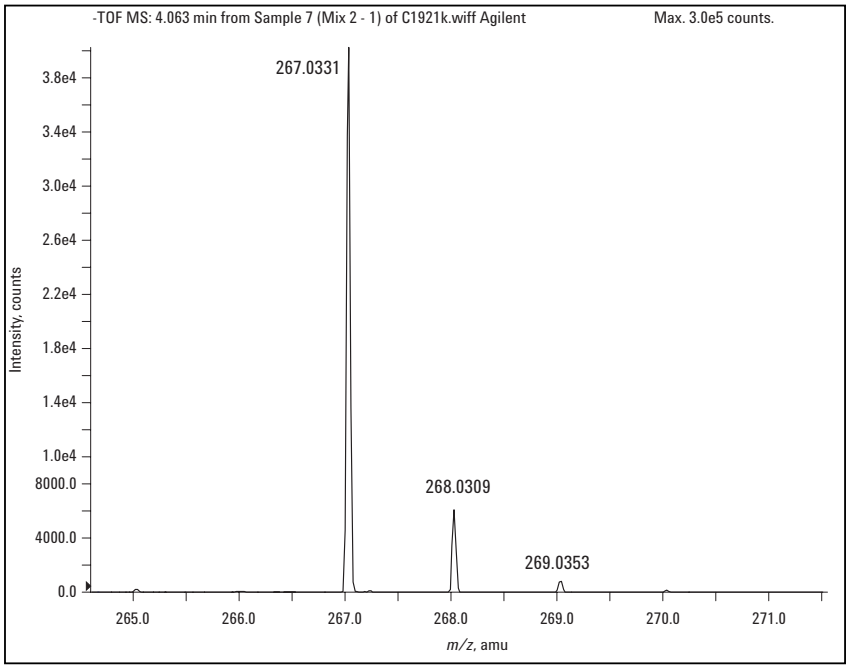


Figure 10. Confirmation of RDX in an explosive crime scene residue.

Summary

The detection of explosives has become a critical analysis in many countries from crime scene forensics to homeland security to environmental testing and remediation. The traditional method of analysis, USEPA method 8330 uses UV detection, which although for some components is sensitive, is nonselective and is prone to interference from the matrix.

The LC/MSD TOF, operated in APCI mode, has the advantage that all analyses take place in full scan mode, and hence any other components may be observed. This is coupled with a sensitivity that far exceeds UV detection as shown in Table 14. Additional confirmatory information and selectivity that is provided through the determination of the accurate mass provides a very powerful technique for the detection, identification and quantitation of explosive compounds.

This work has shown the Agilent LC/MSD TOF's ability to:

- Measure accurate masses within 3 ppm and often much better across a wide range of concentrations for many explosive compounds
- Obtain a high degree of selectivity, achieved with high resolution and accurate mass measurement at every scan
- Provide quantitative results
- Provide repeatability of response consistent with typical quantitative analysis
- Determines the identity of explosives in real samples with a high level of confidence

Table 14. LOQ for Explosives Using UV and LC/MSD TOF

Compound	UV	LC/MSD TOF
HMTD	10,000	30
HMX	1,000	10
RDX	100	0.5
TATB	1,000	5
EGDN	2,000	N.D.
1,3,5-TNB	3,000	3
1,3-DNB	500	2
Tetryl	500	5
4A-DNT	500	10
NB	800	N.D.
NG	500	N.D.
2A-DNT	500	5
TNT	200	4
2,6-DNT	400	8
2,4-DNT	400	4
HNS	500	1
2-NT	300	100
4-NT	200	50
PETN	1,000	250
3-NT	300	5000
TATP	10,000	1000
Carbamite	500	10

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- Analysis of TNT, RDX, and CL-20 by APCI LC/MS/MS
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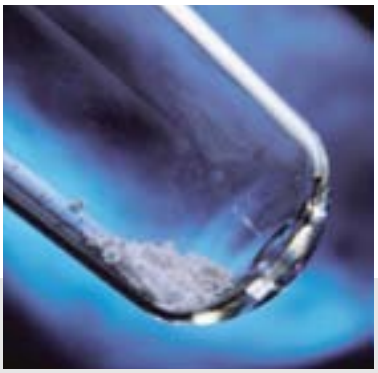
- Fast Analysis of Illicit Drug Residues on Currency using Agilent Poroshell 120



Applications by Technique

LC/QQQ

LC/MS Application Note

Number 18
April 2004

Analysis of TNT, RDX, and CL-20 by APCI LC/MS/MS

A. Colorado, Varian, Inc.

Introduction

The detection and characterization of explosives has gained the interest of various analytical laboratories and research groups around the world.

For the forensic community, trace analysis of explosive residues after arson and terrorism is of critical interest. Biologists and environmentalists monitor biotransformation of these high energy compounds when evaluating environmental contamination. Other groups, such as the munitions industry, continue to explore the synthesis of novel explosive materials. In all of these examples, investigators need an analytical methodology that is informative, sensitive, and selective as well as robust.

In this application note, LC negative ion APCI-MS/MS is used to characterize and detect trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20) (Figure 1).

Instrumentation

- Varian ProStar 430 AutoSampler
- Varian ProStar 210 Isocratic Solvent Delivery Module
- Varian 1200L LC/MS with APCI source

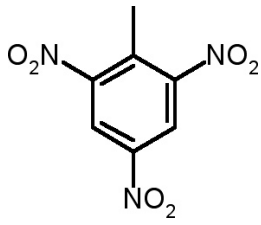
HPLC Conditions

Column	Pursuit C18, 5 µm, 150 x 4 mm (Varian Part No. 2000-150X40)
Mobile Phase	water:isopropanol:methanol at 60:30:10 and 0.1% chloroform (isocratic)
Flow	0.8 mL/min
Injection Volume	20 µL

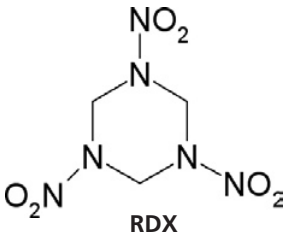
MS Parameters

APCI Torch Temp	450 °C
API Drying Gas	15 psi at 300 °C
API Nebulizing Gas	60 psi
Corona Current	5 µA
Capillary	40V
Housing	50 °C
Collision Gas	1.7 mTorr Argon

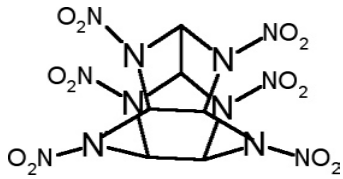
Compound Structures



TNT



RDX



CL-20

Figure 1. Structure of analyzed explosives.

MS/MS Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Retention Time (min)
TNT	227	210	8	4.6
RDX	257	46	6	2.6
CL-20	473	154	6	4.2

Results and Discussion

TNT, a nitroaromatic, readily undergoes charge exchange to create a radical anion in the source. Unfortunately, the chemical structures of RDX and CL-20 do not make them easily amenable to atmospheric pressure ionization without the aid of additives. For this analysis, chloroform was used as a source of chlorine for adduct ion formation.

TNT collisionally dissociates through two main fragmentation pathways (Figure 2). One pathway is the loss of 17 u (OH) producing a fragment at m/z 210. In the second pathway, TNT loses an NO functional group to yield a product ion at m/z 197.

CL-20 also yields two intense product ions (Figure 3). The major product ion is m/z 154 or a loss of 319 mass units ($C_5H_5O_8N_9$). Unlike TNT and CL-20, the RDX-chlorine adduct ion dissociates mainly to yield NO_2^- fragment ions (Figure 4).

All three explosives eluted in less than 5 minutes under isocratic conditions (Figure 5). TNT and RDX were well separated while CL-20 eluted close to TNT. MS/MS, however, adds an additional selective dimension by further separating the analytes according to their unique product ions. The table on page one shows the MS/MS transitions and retentions times for this analysis.

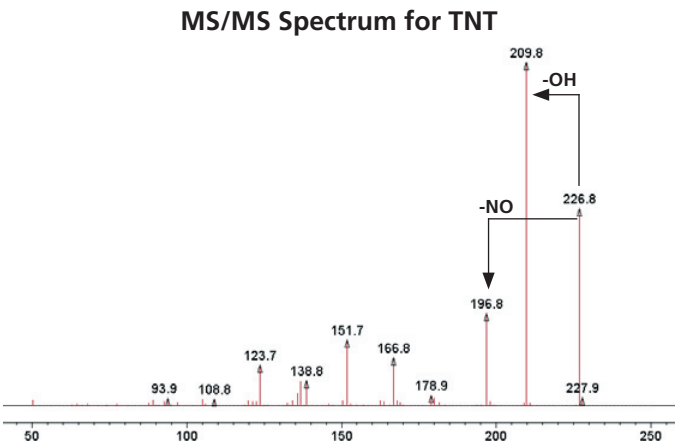


Figure 2. TNT dissociates two produce two intense product ions.

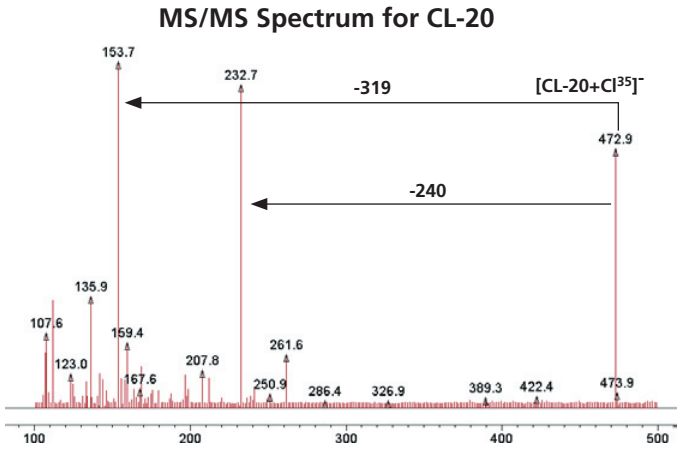


Figure 3. CL-20-chloride adduct also dissociates to produce two intense ions.

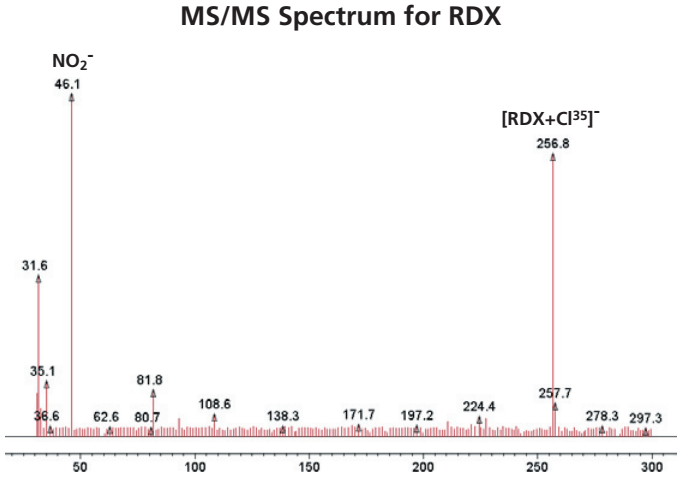


Figure 4. RDX-chloride adduct only yields one small product ion.

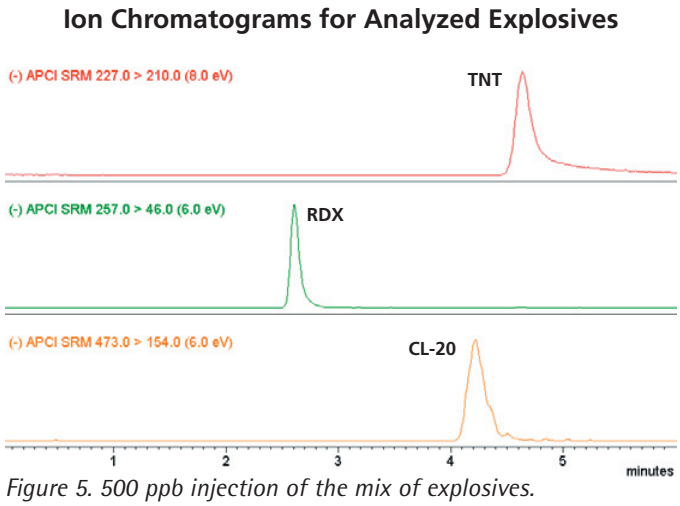


Figure 5. 500 ppb injection of the mix of explosives.

While 10 ppb was the lowest calibration point (Figure 6), single digit ppb levels can be easily attained with further optimization of ion source conditions (Figure 7).

The benefits of MS/MS are readily observable as the concentration of the explosives decrease. For example, a 1 ppb injection of CL-20 in SIM mode is not as discernable when compared to the MS/MS ion chromatogram at the same concentration (Figure 8).

Calibration Curves for Analyzed Explosives

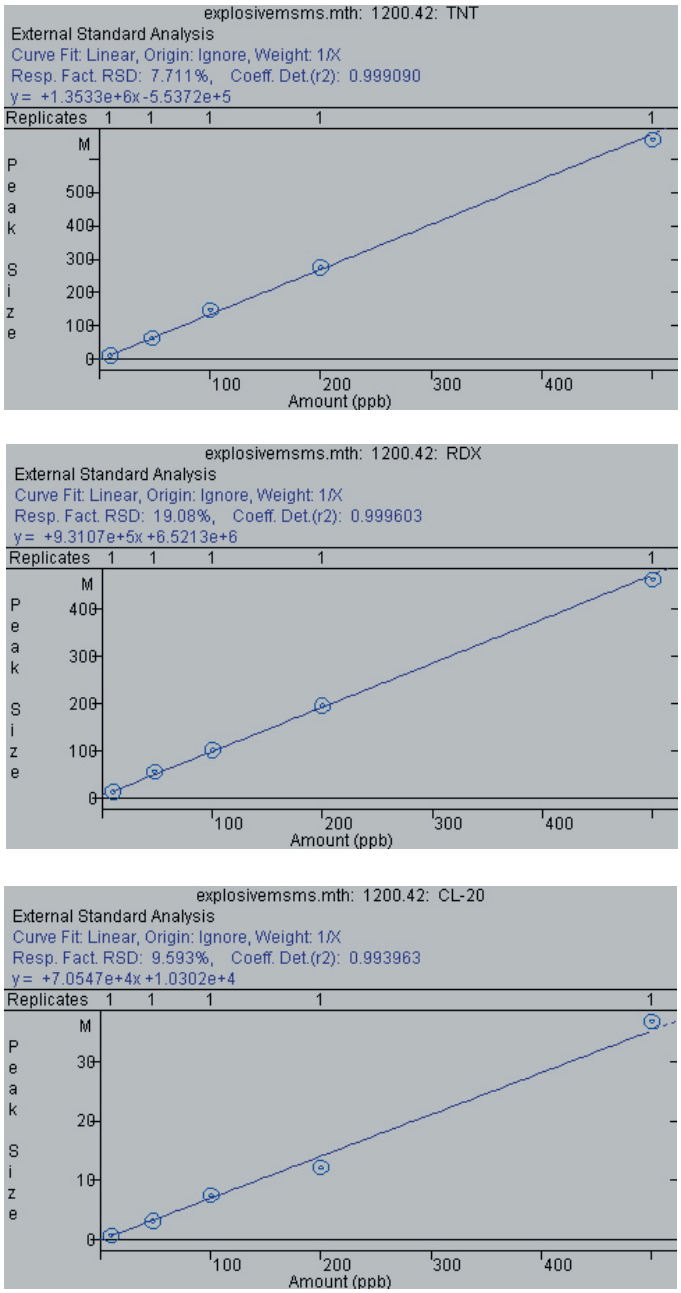


Figure 6. Good linearity was achieved for all compounds over a concentration of 10 ppb to 500 ppb.

LOD Study of Explosives Mix

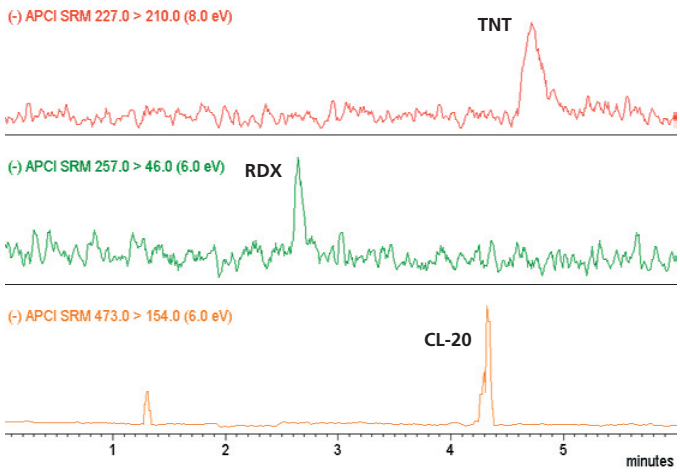


Figure 7. With a 1 ppb injection of explosives mix, TNT and RDX were at the LOD while the LOD for CL-20 could be significantly lower.

Comparison of SIM vs. MS/MS

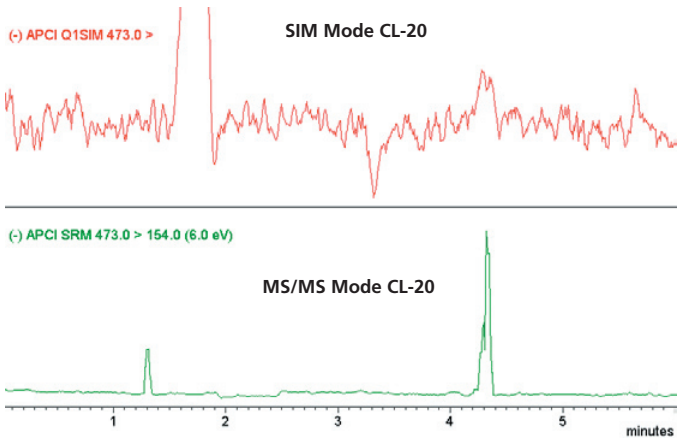


Figure 8. With a 1 ppb injection, baseline noise obscures the CL-20 peak in the SIM mode while an excellent signal-to-noise is achieved with MS/MS.

Conclusion

APCI-MS/MS is effective in the determination of explosives. The addition of an organochloro compound significantly enhances the detection limits of RDX and CL-20 through adduct ion formation. The added selectivity of MS/MS ensures reliable analysis of these compounds, especially at trace concentrations.

These data represent typical results.
For further information, contact your local Varian Sales Office.

An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using LC/QQQ MS/MS with a Dynamic MRM Transition Database

Application Note

Forensic and Toxicology

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Abstract

A Forensic and Toxicological screening application kit has been developed for use with the Agilent 6400 Series triple quadrupole (QQQ) LC/MS systems which contains a database of optimized MRM transitions for approximately 200 analytes of forensic and toxicological interest. The database content is mainly focused on controlled substances and drugs of abuse. The aim of this application kit is to provide a user with a solid starting point for building analysis methods where the ability to screen for a large array of forensic and toxicological analytes is necessary. Typical results obtained from such a method created by using the database are described using serial dilutions of a test mix containing analytes of forensic interest.

Agilent Technologies

Introduction

Lists of potential toxins and analytes of forensic interest can be extremely large and typically depend on the area of analytical screening focus (for example, workplace drug testing, doping control, postmortem toxicology, explosive residues, and so forth). Often, the concentration levels of such target analytes are challenging and low, which can be further impacted by a complex sample matrix or the quantity of sample obtained.

The most sensitive liquid chromatography/mass spectrometry (LC/MS) screening or quantitation techniques are those based around triple quadrupole (QQQ) LC/MS/MS instruments, where a second stage of MS (post fragmentation from a collision cell) acts as an effective method of eliminating background chemical noise that is not associated with the target precursor and fragment ions. This technique is commonly referred to as Multiple Reaction Monitoring (MRM.) Instruments using each quadrupole as targeted mass filters in this manner are an effective and widely accepted technique for forensic and toxicological studies of challenging sample matrices and concentration levels.

QQQ MS instruments, however, operate by focusing a finite amount of time on only one MRM transition before the next MRM transition is selected in turn. Once the complete list of target MRM transitions has been monitored, then the MRM list is repeated or cycled until the end of the chromatographic analysis or until a new retention time segment begins that contains different MRM transitions. The amount of finite time given to any specific MRM transition is referred to as dwell time and can be uniquely specified for every MRM transition.

The chromatographic consideration with regard to dwell time and overall MRM cycle time is one of peak width or resolution, normally referred to as full width at half maximum (FWHM). Statistically, higher numbers of data points measured across a chromatographic peak will provide more accurate and reproducible results. This means that the overall cycle time of the MRM target list must be sufficiently low to achieve this, relative to the particular chromatography used. Furthermore, each MRM transition dwell time must be high enough to output ion statistics of high quality and precision.

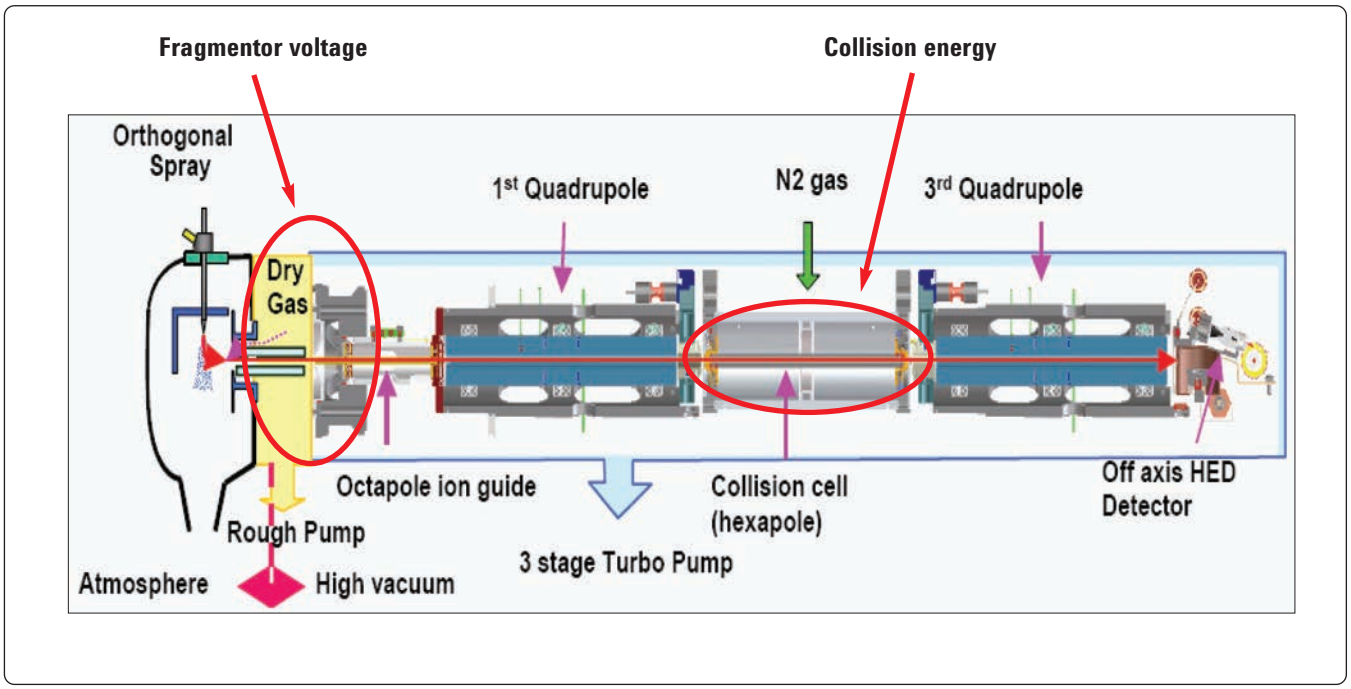

Figure 1. Two key optimized MRM transition settings.

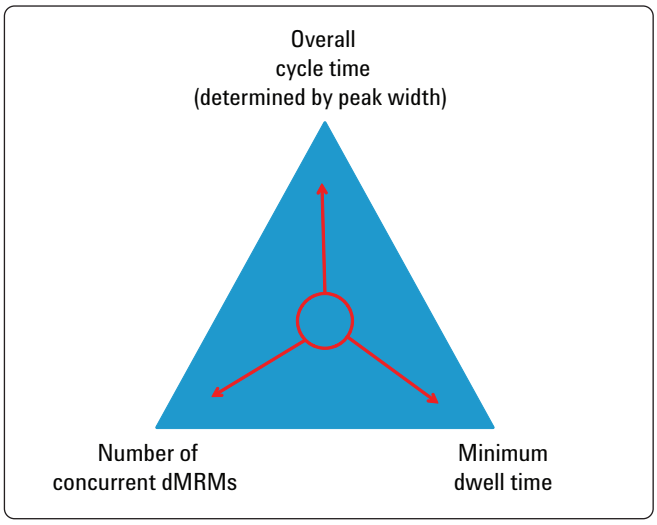

Figure 2. Compromise between cycle time, peak width, dwell time and number of MRM transitions.

Therefore, compromise between cycle time, dwell time and ultimately the total number of MRM transitions is often required especially with larger suites of analytes in a target screen assay (Figure 2). For this reason, Agilent Technologies introduced Dynamic MRM (dMRM) [1] functionality on the Agilent 6400 Series QQQ LC/MS system. Dynamic MRM is a technique where each ion transition has an associated retention time window (delta RT) where it is dynamically switched on and off without impacting a constant data cycle time. Since the complete list of ion transitions is unlikely to be cycled through at any given chromatographic retention time, then the result is normally higher dwell time for every transition and higher data quality when compared to normal MRM methods. Figure 3 graphically illustrates the Dynamic MRM principle.

Herein are described the results obtained from an analysis method using the Agilent MassHunter Forensic and Toxicological Dynamic MRM Database Kit (G1734AA) with optimized MRM transitions from the database inserted directly into the acquisition method. More detailed instruction on the creation of such methods are outlined in the G1734AA

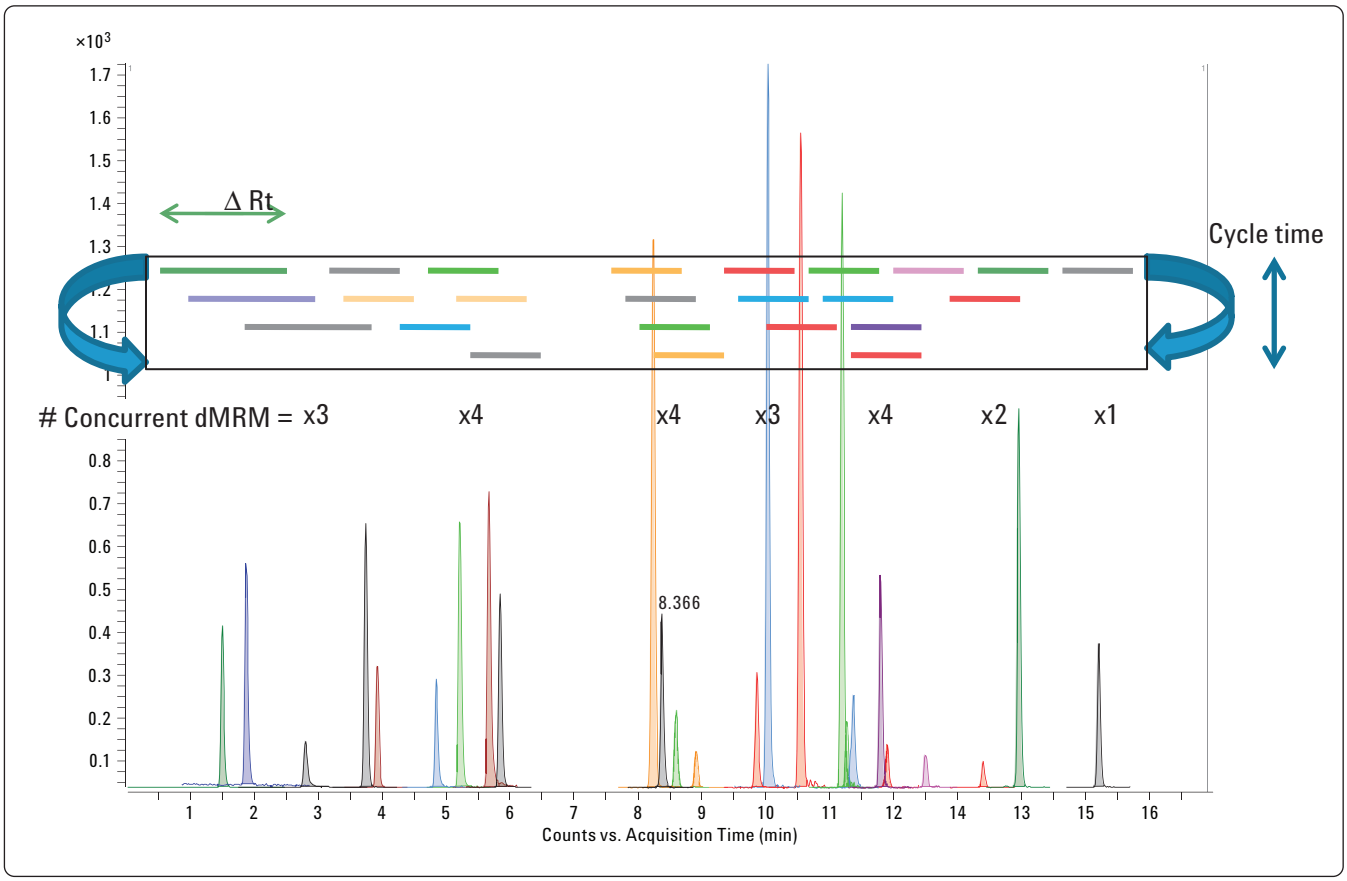

Figure 3. Illustration of Dynamic MRM principle.

MassHunter Forensic & Toxicology Dynamic MRM Database Kit Quick Start Guide [2]. Confirmatory evidence was obtained by using the two most abundant MRM transitions for use as quantifier and qualifier ions, the ratio of which are indicative of the analyte of interest. This application note aims to describe typical results using an LC/MS Forensic & Toxicology Test Mix.

Experimental

The analysis results outlined in this application note were obtained using an Agilent 6460 QQQ LC/MS coupled to an Agilent 1200SL Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter data system equipped with the MassHunter Optimizer program (Rev. B.02.01) and the [G1734AA] forensic & toxicology Dynamic MRM application kit.

Sample Preparation

An ampoule from the LC/MS Forensics & Toxicology Test Mix [p/n 5190-0470] which is included in the Forensic and Toxicology application kit [G1734AA] was opened and 100 µL of the 1 µg/mL (1ppm) solution was diluted to a concentration of 10 ng/mL (10 ppb) using 9.9 mL of pure LC/MS grade methanol to create a clean solvent standard for method checkout purposes.

Appropriate serial dilutions from the original LC/MS Forensic & Toxicology Test Mix were created for the purposes of quantitation. These are listed in Table 1.

Table 1. Dilution Series of LC/MS Forensic & Toxicology Test Mix

Data File	Type	Level	Vol. (uL)	Conc.	Units
LCMS_Forensic and Toxicology Test Mix 10fg.d	Cal	1	1	10	fg on-column
LCMS_Forensic and Toxicology Test Mix 25fg.d	Cal	2	1	25	fg on-column
LCMS_Forensic and Toxicology Test Mix 50fg.d	Cal	3	1	50	fg on-column
LCMS_Forensic and Toxicology Test Mix 100fg.d	Cal	4	1	100	fg on-column
LCMS_Forensic and Toxicology Test Mix 250fg.d	Cal	5	1	250	fg on-column
LCMS_Forensic and Toxicology Test Mix 500fg.d	Cal	6	1	500	fg on-column
LCMS_Forensic and Toxicology Test Mix 1pg.d	Cal	7	1	1000	fg on-column
LCMS_Forensic and Toxicology Test Mix 5pg.d	Cal	8	1	5000	fg on-column
LCMS_Forensic and Toxicology Test Mix 10pg.d	Cal	9	1	10000	fg on-column
LCMS_Forensic and Toxicology Test Mix 25pg.d	Cal	10	1	25000	fg on-column
LCMS_Forensic and Toxicology Test Mix 50pg.d	Cal	11	1	50000	fg on-column

Table 2 outlines the composition of the LC/MS Toxicology Test Mix [p/n 5190-0470] which is intended to cover a wide and representative range of forensic analyte classes.

Table 2. LC/MS Forensics & Toxicology Test Mix Components (1µg/mL)

Compound Name	Formula	Mass
3,4-Methylendioxyamphetamine (MDA)	$C_{10}H_{13}NO_2$	179.09463
3,4-Methylenedioxyethamphetamine (MDEA)	$C_{12}H_{17}NO_2$	207.12593
Alprazolam	$C_{17}H_{13}ClN_4$	308.08287
Clonazepam	$C_{15}H_{10}ClN_3O_3$	315.04107
Cocaine	$C_{17}H_{21}NO_4$	303.14706
Codeine	$C_{18}H_{21}NO_3$	299.15214
delta9-Tetrahydrocannabinol (THC)	$C_{21}H_{30}O_2$	314.22458
Diazepam	$C_{16}H_{13}ClN_{2O}$	284.07164
Heroin	$C_{21}H_{23}NO_5$	369.15762
Hydrocodone	$C_{18}H_{21}NO_3$	299.15214
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	320.01193
Meperidine (Pethidine)	$C_{15}H_{21}NO_2$	247.15723
Methadone	$C_{21}H_{27}NO$	309.20926
Methamphetamine	$C_{10}H_{15}N$	149.12045
Methylendioxymethamphetamine (MDMA)	$C_{11}H_{15}NO_2$	193.11028
Nitrazepam	$C_{15}H_{11}N_3O_3$	281.08004
Oxazepam	$C_{15}H_{11}ClN_2O_2$	286.05091
Oxycodone	$C_{18}H_{21}NO_4$	315.14706
Phencyclidine (PCP)	$C_{17}H_{25}N$	243.1987
Phentermine	$C_{10}H_{15}N$	149.12045
Proadifen	$C_{23}H_{31}NO_2$	353.23548
Strychnine	$C_{21}H_{22}N_2O_2$	334.16813
Temazepam	$C_{16}H_{13}ClN_2O_2$	300.06656
Trazodone	$C_{19}H_{22}ClN_5O$	371.15129
Verapamil	$C_{27}H_{38}N_2O_4$	454.28316

Reagents and Chemicals

Burdick & Jackson LC/MS grade acetonitrile together with deionized water (locally produced 18.1 MΩ) were used for mobile phases. Buffers were freshly prepared using a high purity source of formic acid and ammonium formate.

Instrumentation

LC Conditions

Column:	Agilent Zorbax Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm [p/n - 959764-902]
Column temperature:	60 °C
Mobile phase	A: 5 mM NH_4 formate/0.01% Formic acid in water B: 0.01% formic acid in acetonitrile
Flow rate:	0.5 mL/min

Gradient program:

Time (min)	A (%)	B (%)	Flow rate mL/min
Initial	90	10	0.5
0.5	85	15	0.5
3.0	50	50	0.5
4.0	5	95	0.5
6.0	5	95	0.5

Injection volume:	1 µL (with 5 second needle wash in flushport)
Analysis time:	6.0 min
Post time:	2.0 min
Overall cycle time:	8.0 min

6460 QQQ LC/MS Conditions

Source Conditions:
Electrospray AP-ESI (using Agilent Jet Stream Technology):

Positive ionization polarity

Sheath gas temperature and flow:	380 °C, 12 L/min
Nozzle voltage:	500 V
Drying gas temperature and flow:	320 °C, 8 L/min
Nebulizer gas pressure:	27 psi
Capillary voltage:	3750 V
Fragmentor voltage:	150 V

6410 QQQ LC/MS Conditions
(Results not included in this application note.)

Source Conditions:
Electrospray AP-ESI:

Positive ionization polarity

Drying gas temperature and flow:	350 °C, 12 L/min
Nebulizer gas pressure:	30 psi
Capillary voltage:	2000 V
Fragmentor voltage:	150 V

All other instrument operating parameters were taken care of by Agilent's autotune functionality and subsequent mass calibration using standard settings.

Dynamic MRM Acquisition Method Parameters

Table 3. Dynamic MRM Method Conditions

Compound name	ISTD?	Prec ion	MS1 res	Prod ion	MS2 res	Frag (V)	CE (V)	Rett ime	Ret window	Polarity
Codeine	–	300.2	Unit	165.1	Unit	158	45	1.11	0.4	Positive
Codeine	–	300.2	Unit	58.1	Unit	158	29	1.11	0.4	Positive
Oxycodone	–	316.2	Unit	298.1	Unit	143	17	1.285	0.4	Positive
Oxycodone	–	316.2	Unit	256.1	Unit	143	25	1.285	0.4	Positive
δ-Amphetamine	–	136.1	Unit	119.1	Unit	66	5	1.296	0.4	Positive
δ-Amphetamine	–	136.1	Unit	91	Unit	66	17	1.296	0.4	Positive
MDA	–	180.1	Unit	163	Unit	61	5	1.332	0.4	Positive
MDA	–	180.1	Unit	105	Unit	61	21	1.332	0.4	Positive
Hydrocodone	–	300.2	Unit	199	Unit	159	29	1.4	0.4	Positive
Hydrocodone	–	300.2	Unit	128	Unit	159	65	1.4	0.4	Positive
Methamphetamine	–	150.1	Unit	119	Unit	92	5	1.45	0.4	Positive
Methamphetamine	–	150.1	Unit	91	Unit	92	17	1.45	0.4	Positive
MDMA	–	194.1	Unit	163	Unit	97	9	1.468	0.4	Positive
MDMA	–	194.1	Unit	105	Unit	97	25	1.468	0.4	Positive
Strychnine	–	335.2	Unit	184	Unit	195	41	1.629	0.4	Positive
Strychnine	–	335.2	Unit	156	Unit	195	53	1.629	0.4	Positive
MDEA	–	208.1	Unit	163	Unit	107	9	1.735	0.4	Positive
MDEA		208.1	Unit	105	Unit	107	25	1.735	0.4	Positive
Heroine		370.2	Unit	268.1	Unit	149	37	2.256	0.4	Positive
Heroin		370.2	Unit	165	Unit	149	61	2.256	0.4	Positive
Cocaine		304.2	Unit	182.1	Unit	138	17	2.376	0.4	Positive
Cocaine		304.2	Unit	77	Unit	138	61	2.376	0.4	Positive
Meperidine		248.2	Unit	220.1	Unit	128	21	2.419	0.4	Positive
Meperidine		248.2	Unit	174.1	Unit	128	17	2.419	0.4	Positive
Trazodone		372.2	Unit	176	Unit	159	25	2.797	0.4	Positive
Trazodone		372.2	Unit	148	Unit	159	37	2.797	0.4	Positive
PCP		244.2	Unit	91	Unit	86	41	2.876	0.4	Positive
PCP	–	244.2	Unit	86.1	Unit	86	9	2.876	0.4	Positive
Oxazepam	–	287	Unit	269	Unit	150	12	3.53	0.4	Positive
Oxazepam	–	287	Unit	241	Unit	150	20	3.53	0.4	Positive
Nitrazepam	–	282.1	Unit	236.1	Unit	148	25	3.542	0.4	Positive
Nitrazepam	–	282.1	Unit	180	Unit	148	41	3.542	0.4	Positive
Verapamil	–	455.3	Unit	165	Unit	158	37	3.554	0.4	Positive
Verapamil	–	455.3	Unit	150	Unit	158	45	3.554	0.4	Positive
Methadone	–	310.2	Unit	265.1	Unit	112	9	3.61	0.4	Positive
Methadone	–	310.2	Unit	105	Unit	112	29	3.61	0.4	Positive
Lorazepam	–	321	Unit	275	Unit	102	21	3.626	0.4	Positive
Lorazepam	–	321	Unit	194	Unit	102	49	3.626	0.4	Positive
Alprazolam	–	309.1	Unit	281	Unit	179	25	3.727	0.4	Positive
Alprazolam	–	309.1	Unit	205	Unit	179	49	3.727	0.4	Positive
Temazepam	–	301.1	Unit	255.1	Unit	117	29	3.941	0.4	Positive

Table 3. Dynamic MRM Method Conditions (continued)

Compound name	ISTD?	Prec ion	MS1 res	Prod ion	MS2 res	Frag (V)	CE (V)	Rett ime	Ret window	Polarity
Temazepam	–	301.1	Unit	177	Unit	117	45	3.941	0.4	Positive
Proadifen	–	354.2	Unit	167	Unit	153	29	4.088	0.4	Positive
Proadifen	–	354.2	Unit	91.1	Unit	153	45	4.088	0.4	Positive
Diazepam	–	285.1	Unit	193	Unit	169	45	4.268	0.4	Positive
Diazepam	–	285.1	Unit	154	Unit	169	25	4.268	0.4	Positive
THC	–	315.2	Unit	193.2	Unit	150	20	5.277	0.4	Positive
THC	–	315.2	Unit	123.3	Unit	150	30	5.277	0.4	Positive

Results and discussion

Fast and easy startup with Agilent Test Mix

In order to rapidly implement and verify that acquisition and data analysis methodology is correctly set up, the LC/MS Forensics & Toxicology Test Mix [p/n 5190-0470] is included in the Forensic and Toxicology Dynamic MRM application kit [G1734AA] which contains a representative range of forensic analyte classes of 25 components (Table 2).

To create a method from first principles, the required transitions are selected from the database browser window (Figure 4). Once each selection has been made, the transitions are transferred to the acquisition method by clicking the 'Import' button to the bottom right of the browser window. An example of an acquisition method is illustrated in Figure 5.

Detailed information on this operation is contained in the MassHunter Forensic and Toxicology Dynamic MRM Database Kit Quick Start Guide [2].

Using the methodology outlined in the experimental section, a 1-uL injection of the 10 ng/mL LC/MS Forensics & Toxicology Test Mix equates to a 10 pg on-column injection amount. Figure 6 illustrates a typical overlay of extracted compound chromatograms for the test mix. A prepared method for QQQ is included in the application kit. When this method is loaded all conditions are correct and the user is able to reproduce the analysis.*

*These methods are acquisition-only and correspond to the instrument configuration as outlined in the experimental section of this application note. Appropriate settings must be manually input if a different instrument configuration is used. Similar results will demonstrate that the system is working properly.

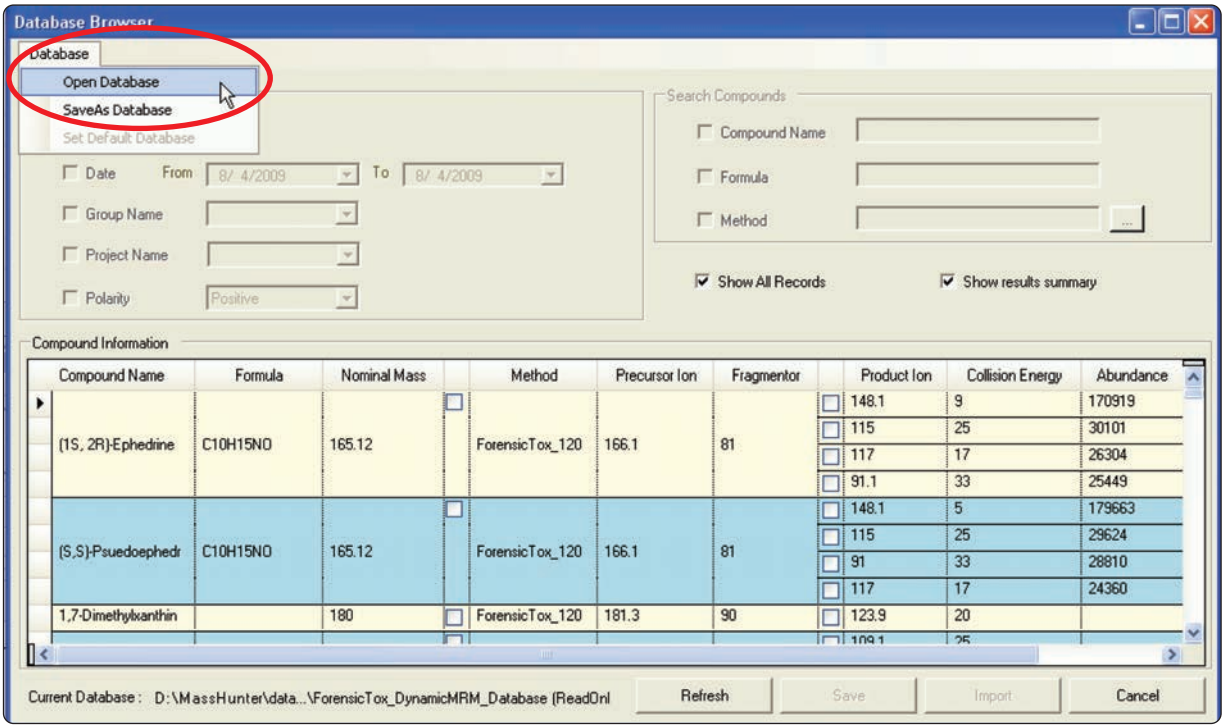

Figure 4. Compound MRM database browser containing 200 forensic analytes.

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	Polarity
Alprazolam	☐	309.1	Unit	281	Unit	179	25	3.715	1	Positive
Cocaine	☐	304.2	Unit	182.1	Unit	138	17	2.358	1	Positive
d-Amphetamine	☐	136.1	Unit	91	Unit	66	17	1.278	1	Positive
Diazepam	☐	285.1	Unit	154	Unit	169	25	4.269	1	Positive
Heroin	☐	370.2	Unit	165	Unit	149	61	2.236	1	Positive
Hydrocodone	☐	300.2	Unit	199	Unit	159	29	1.38	1	Positive
Lorazepam	☐	321	Unit	275	Unit	102	21	3.61	1	Positive
MDA	☐	180.1	Unit	163	Unit	61	5	1.311	1	Positive
MDEA	☐	208.1	Unit	163	Unit	107	9	1.72	1	Positive

Dynamic MRM Parameters

Cycle Time 500 ms

Figure 5. Scan segments table with Dynamic MRM transitions imported database browser.

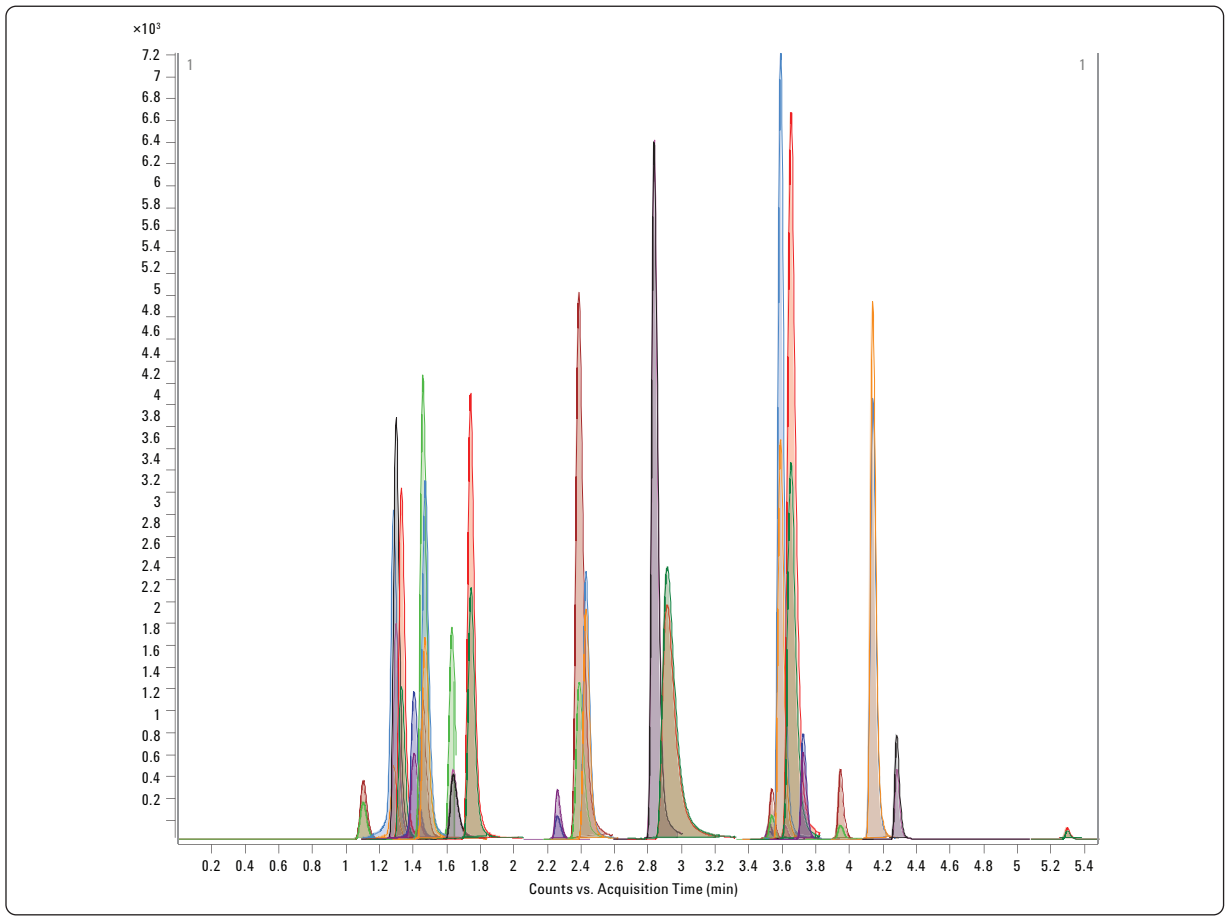

Figure 6. Example LC/MS Forensics and Toxicology test mix 10 pg on-column extracted ion chromatogram (overlay).

Quantitative analysis and standard curves

By using a Dynamic MRM acquisition method, the series of LC/MS Forensic and Toxicology Test Mix dilutions (Table 1) were analyzed according to the procedure outlined in the experimental section. All 50 Dynamic MRM transitions were used and Table 4 summarizes the results for the limits of detection and linearity of each component in the 25-component test mix.

Table 4. Limits of Detection and Calibration Linearity Results

Compound Name	Limit of Detection (fg on-column)	Linearity Correlation
3,4-Methylendioxyamphetamine (MDA)	50	0.99817
3,4-Methylenedioxyethamphetamine (MDEA)	10	0.99743
Alprazolam	50	0.99755
Clonazepam	100	0.99501
Cocaine	10	0.99755
Codeine	50	0.99841
δ9-Tetrahydrocannabinol (THC)	50	0.99869
Diazepam	10	0.99896
Heroin	25	0.99863
Hydrocodone	25	0.99493
Lorazepam	100	0.99601
Meperidine (Pethidine)	10	0.99687
Methadone	10	0.99666
Methamphetamine	10	0.98750
Methylendioxymethamphetamine (MDMA)	25	0.99217
Nitrazepam	25	0.99712
Oxazepam	250	0.99544
Oxycodone	50	0.99804
Phencyclidine (PCP)	25	0.99659
Phentermine	50	0.99898
Proadifen	<5	0.99772
Strychnine	50	0.99496
Temazepam	25	0.99751
Trazodone	<5	0.99777
Verapamil	<5	0.99787

Figures 7 through 10 illustrate the calibration curves through the range of 10-50000 fg on-column for six of the analytes from the LC/MS Forensic and Toxicology Test Mix.

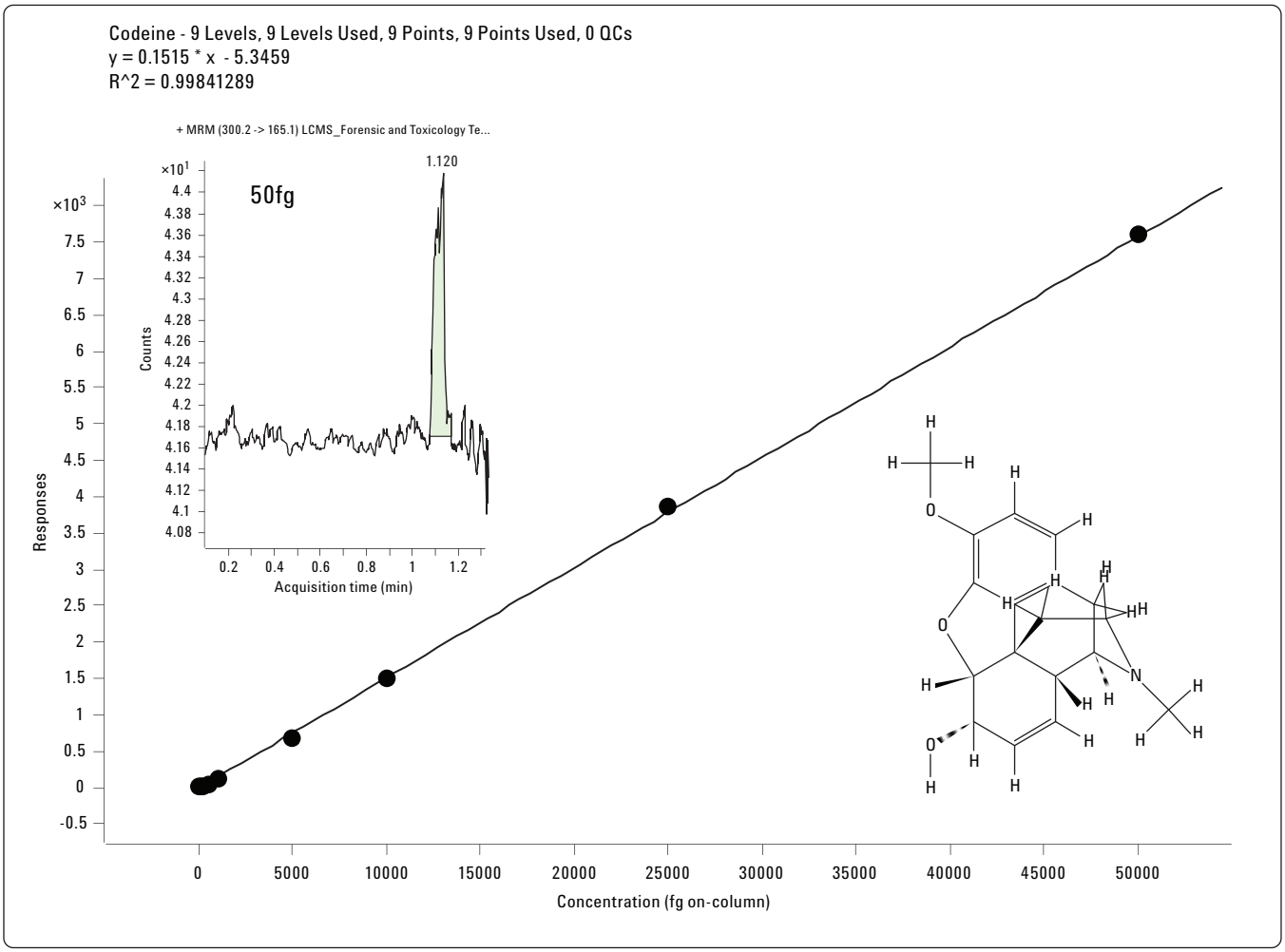

Figure 7. Calibration curve and LOD chromatogram, codeine.

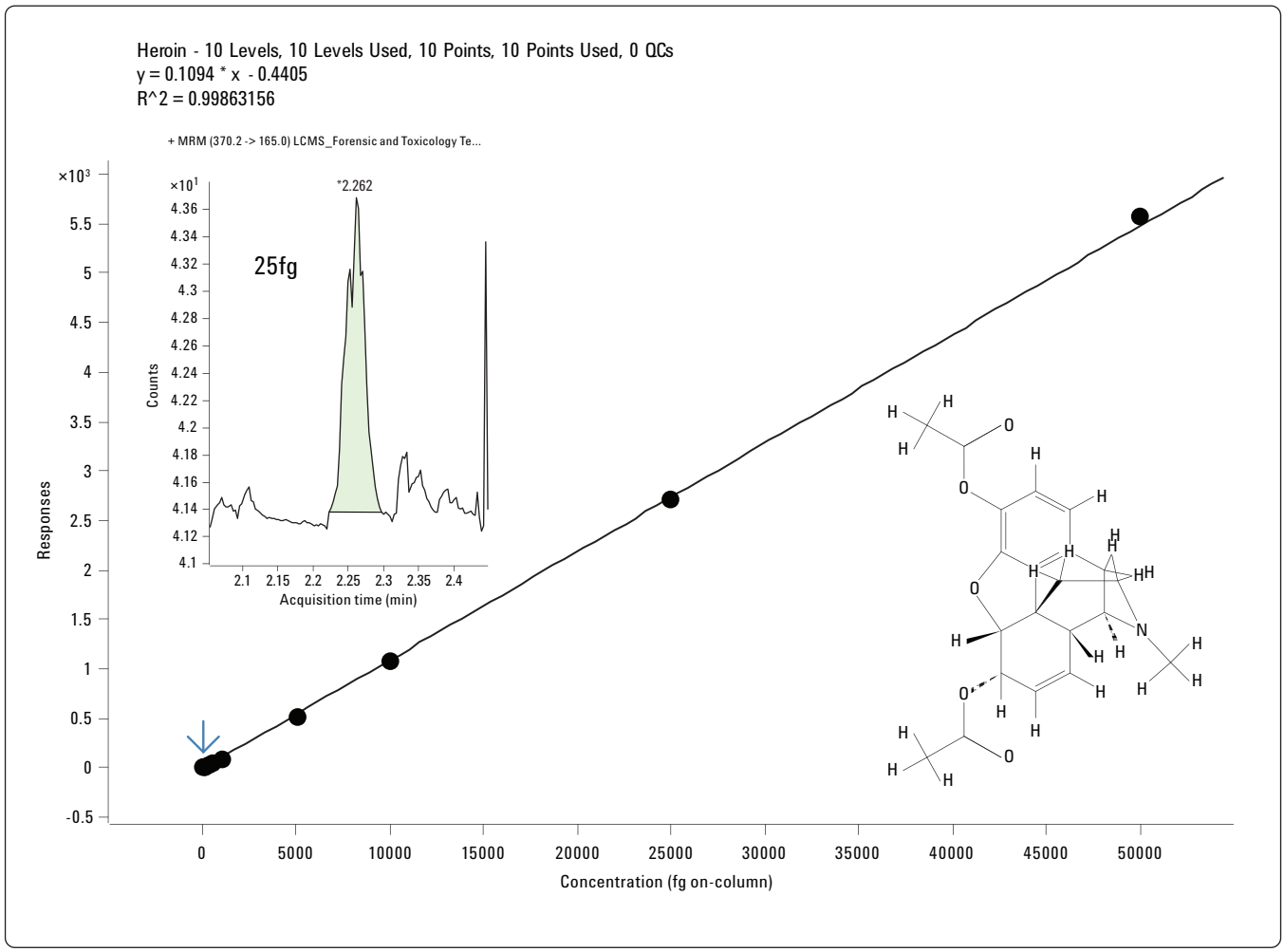

Figure 8. Calibration curve and LOD chromatogram, heroin.

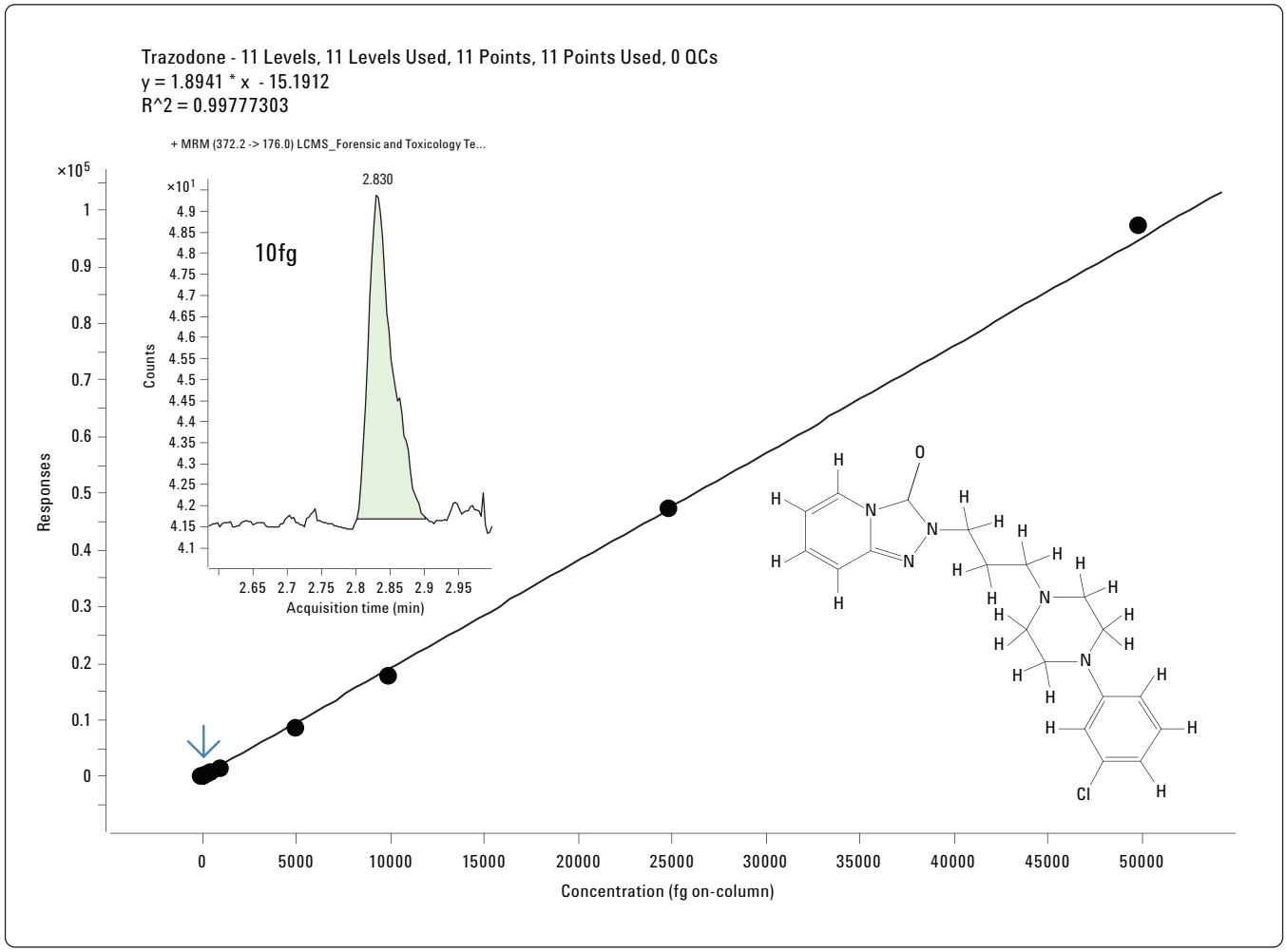

Figure 9. Calibration curve and LOD chromatogram, trazodone.

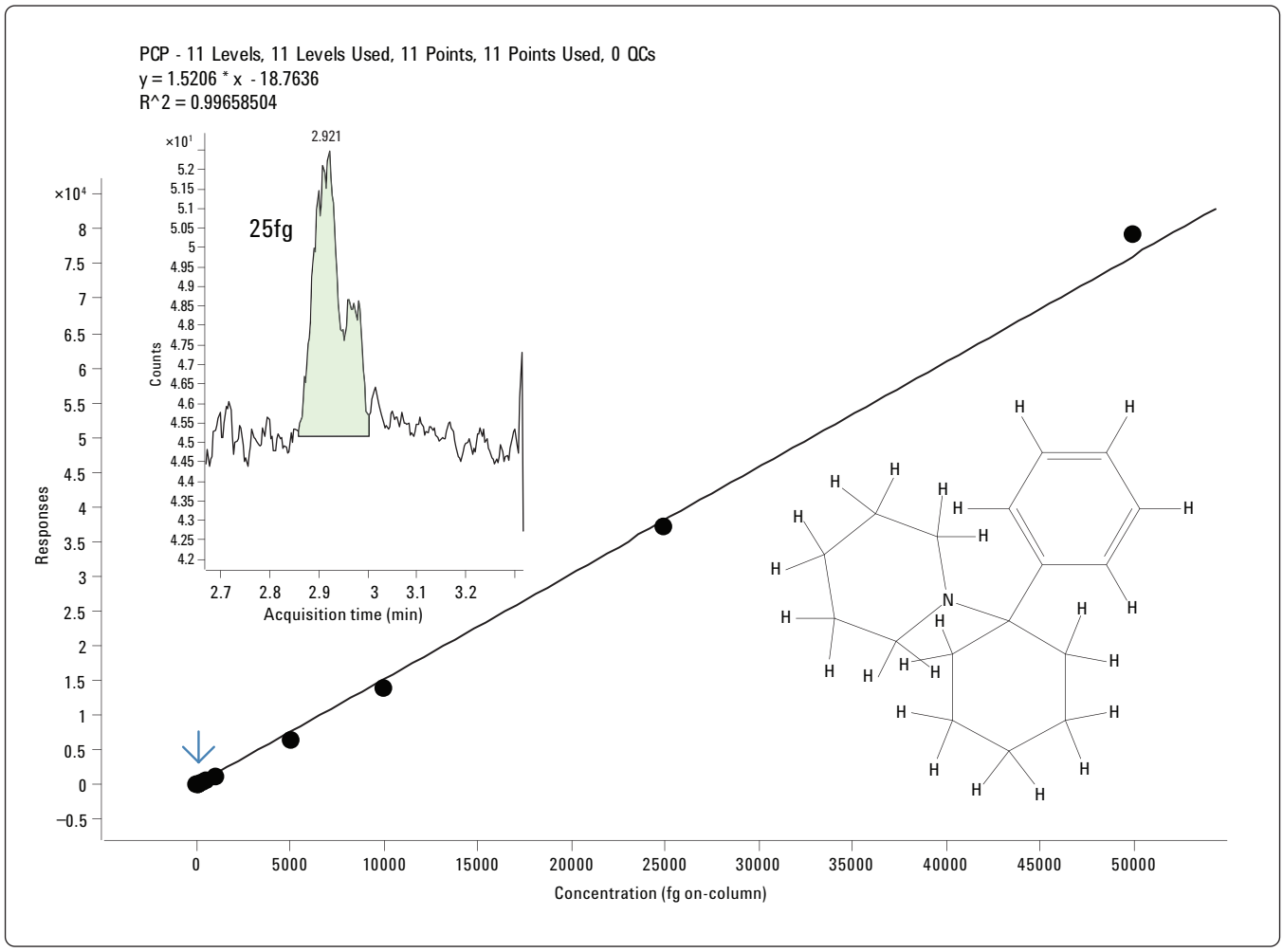

Figure 10. Calibration curve and LOD chromatogram, phencyclidine (PCP).

Conclusions

The Agilent MassHunter Forensic & Toxicology Dynamic MRM Database Kit provides a user with faster method development capability for 200 forensic analytes with up to 4 MRM transitions for each. These methods can be used equally for screening or for more focused and dedicated analyte quantitation dependant on specific needs.

This application note briefly outlines the type of results that could be obtained by using database optimized MRM parameters with the appropriate chromatography conditions and MS ion source settings.

The kit offers:

- Fast and easy startup of complex analyses.
- An optimized MRM transition database of approximately 200 forensic compounds.
- Completely customizable with additional optimized transitions to the database.
- Example chromatography with ready to use methods inclusive of test sample and chromatography column.
- Automatic re-optimization of transition parameters using the MassHunter Optimizer program for particular instrument conditions and method revalidation.

References

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Printed in the USA
November 18, 2009
5990-4254EN

Agilent Technologies

Fast Analysis of Illicit Drug Residues on Currency using Agilent Poroshell 120

Application Note

Forensics and Toxicology

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Abstract

Illicit drugs, like cocaine, are frequently found on US currency. While a more interesting perception might be that all bills were used to inhale the drug, the truth is much more mundane. Drug trafficking is thought to be the initial source of drug residues on a small percentage of bills, and because these compounds are fine powders, they are easily transferable from one surface to another. As money is processed through counting machines and automated teller machines (ATM), small amounts of drugs are readily transferred. An Agilent application note (Agilent Publication Number 5990-4254EN) details an application kit for the screening of 25 compounds considered in forensic and toxicology analyses using an Agilent 1200 Series LC system with an Agilent 6410 Triple Quadrupole LC/MS. In this work, an Agilent Poroshell 120 EC-C18 column is used to analyze 25 compounds found in the Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470). This ammonium formate/acetonitrile gradient analysis is scaled using faster flow rates to shorten analysis time and exploit the low back pressure of this superficially porous column. Calibration curves for each of the 25 compounds are generated, and as a demonstration of the method a $1 bill was extracted into methanol, analyzed and quantified.

Agilent Technologies

Introduction

The interest in superficially porous particles has led to discussions of method transfer from larger 5-µm totally porous particles, as well as from sub-2-µm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2-µm totally porous particles. This is due to short mass transfer distance and substantially narrower particle size distribution.

The benefit of transferring from larger particle columns is very significant time savings, because the superficially porous particles are optimally run at faster flow rates (usually double) and are able to achieve similar resolution with a much shorter column length [1-2]. Because analysts will likely change column length and flow rate when transferring from larger totally porous particles to superficially porous columns, calculations must be performed to proportionally scale a gradient method and preserve the chromatographic selectivity (Equation 1).

Equation 1

$$t_2 = \frac{t_1 \bullet d_2^2 \bullet L_2 \bullet F_1}{d_1^2 \bullet L_1 \bullet F_2}$$

Where:

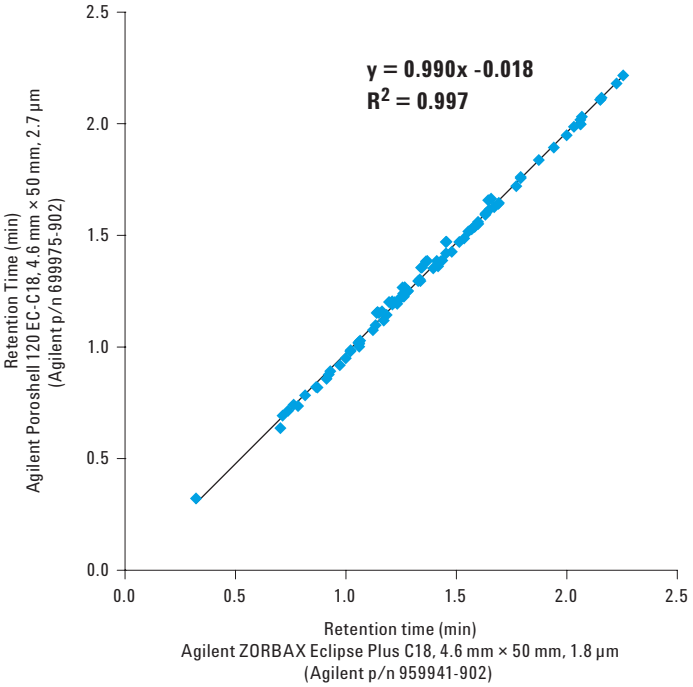
- *t_1 and t_2 are the original and new gradient times (min)*
- *d_1 and d_2 are the original and new column internal diameters (mm)*
- *L_1 and L_2 are the original and new column lengths (mm)*
- *F_1 and F_2 are the original and new flow rates (mL/min)*

In some cases, it may be useful to take advantage of the lower back pressure associated with superficially porous columns as compared to totally porous sub-2-µm columns. Depending upon operating conditions, the back pressure can be up to 50% less. This can give analysts the freedom to increase flow rates for higher throughput, or to increase column length to enhance resolution without exceeding the system pressure limits. Adjustments to flow rate and/or column length will require gradient scaling (Equation 1).

Method transfer can be especially easy, when columns like the superficially porous Agilent Poroshell 120 EC-C18 and totally porous Agilent ZORBAX Eclipse Plus C18 are manufactured to have similar bonding chemistries and use similar retention mechanisms. Figure 1 shows the similar retention of 90 compounds on Poroshell 120 EC-C18 and Eclipse Plus C18 columns using a generic gradient analysis with a variety of compounds from different chemical classifications. The high correlation coefficient (R^2) indicates a high degree of similarity between the interactions involved in the separation on the two C18 columns, while the slope ≈1 implies similar interaction strengths [3-4]. However, while many compounds give similar selectivity, it cannot be guaranteed that every application will transfer without adjustment.

This application note shows how a Poroshell 120 column can be used in a complex analysis, previously performed on a 1.8 µm column. This separation was demonstrated on Eclipse Plus in a previous Agilent application note (Publication Number 5990-4254EN) [5]. A 25-component LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) is used to illustrate the interchangeability between the two columns. Calibration curves for each of the 25 compounds on Poroshell 120 are constructed. A $1 bill is extracted in methanol to show significant presence of cocaine, as well as noticeable quantities of oxycodone, methamphetamine, PCP and THC. Trace amounts of several more illicit and prescription drugs can be detected also. Drug trafficking is assumed to be the cause for their initial presence on US currency, while ATM's and counting machines are likely the cause of their widespread presence [6]. Additionally, this gradient analysis is transferred to a Poroshell 120 SB-C18 column, which shows some selectivity differences; however it can be run at higher temperatures to allow for even faster flow rates and analysis times. Agilent Poroshell 120 columns are availabe with two different C18 phases in order to change selectivity and still have a C18 column choice. Flow rates were increased to reach 400 and 600 bar to show performance achievable on both conventional HPLC's and newer UHPLC's.

Agilent Poroshell 120 EC-C18 has Very Similar Selectivity to Agilent ZORBAX Eclipse Plus C18



Mobile phase:	A: 10 mM ammonium formate, pH 3 B: Acetonitrile
Gradient:	5% B at t_0 ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate:	2 mL/min
Sample:	1 µL of 1 mg/mL standard in H_2O

Furazolidone	Biphenyl	Acetanilide	DL phenylalanine	Oxybutynin chloride 1
Chloramphenicol	Acenaphthene	Fenoprofen	Doxepin hydrochloride	Diphenhydramine
Pyrimethamine	Methoxy naphthalene	Catechol	Ephedrine hydrochloride	Diflunisal
Sulfaquinoxaline	Anisole	Phenol	Loperamide	Nisoldipine
Sulfamonomethoxine	Dimethoxy benzene	Resorcial	Procaine hydrochloride	Diclofenac sodium salt
Nimopidin	Corticosterone	Hydroquinone	Fenoprofen calcium salt hydrate	Hydrocortisone
Sulfadimethoxine	Alpha hydroxyprogesterone	4 nitro phenol	Erythromycin	4 hydroxybenzoic acid
Sulfamethoxazole	Porgesterone	O cresol	Econazole nitrate	Procainamide hydrochloride
Sulfachloropyridazine	Alpha hydroxyprogesterone	P cresol	Gemfibrozil	Lidocaine
Sulfamethoxypyridazine	Prednisolone	3,4 dimethyl phenol	Beta estradiol	Terfenadine
Sulfamethizole	Mestranol	2,3 dimethyl phenol	Metoprolol	Terfenaine
Sulfamethazine	Deoxycorticosterone	2 nitro phenol	Prednisone	Chlortetracycline hydrochloride
Sulfamerazine	Progesterone	2,4 dimethyl phenol	Protriptyline	Chlorpheniramine maleate salt
Sulfathiazole	Chlorphenamine	2,5 dimethyl phenol	2-hydroxyhippuric acid	Chloramphenicol
Sulfadiazine	Berberine	1 napthol	Hydroxyisophthalic acid	Buspirone hydrochloride
Benzaldehyde	Impramithue	Imipramine hydrochloride	Flufenamic acid	Benzocaine
Iodobenzene	Norethindrone	D methionine	Pramoxine hydrochloride	Antipyrine
Phenanthrene	Phenacetin	3,4 dihydroxy-l-phenyl alanine	Naproxen	Acetylsalicyclic acid

Figure 1. Scatter plot of retention time of 90 compounds on Agilent Poroshell 120 EC-C18 versus Agilent Eclipse Plus C18.

Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent 6410 Triple Quadrupole LC/MS system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 5 mM ammonium formate with 0.01% formic acid, and B: acetonitrile with 0.01% formic acid. Gradient was 10% B at t_0, ramp to 15% B, ramp to 50% B, then ramp to 95% B and hold 95% B. Gradient times vary depending on column dimensions and flow rate (Table 1).
- G1367C Automatic Liquid Sampler (ALS) SL. Injection volume was 1.0 µL.
- G1316B Thermostated Column Compartment (TCC) SL with temperature set to 60 °C or 90 °C (on Poroshell 120 SB-C18 only).
- G6410A Triple Quadrupole LC/MS: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000 V, in dMRM mode, transitions found in Table 2.
- MassHunter versions B.02.01, B.02.00 and B.03.01 were used for data acquisition, qualitative and quantitative analyses respectively.

Three Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 695775-902)
- Agilent Poroshell 120 SB-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 685775-902)
- Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)

The compounds of interest are shown in Table 2, with their respective retention times on Poroshell 120 EC-C18 at 0.5 mL/min, and their qualitative and quantitative MRM transitions. Sample is a 1 µg/mL standard in methanol purchased from Agilent Technologies (LC/MS Toxicology Test Mixture, Agilent p/n 5190-0470). Serial dilutions in methanol were prepared for the calibration standards. The $1 bill sample was extracted in 7 mL of methanol and ultrasonicated for 30 min. Additionally, acetonitrile, formic acid and ammonium formate were purchased from Sigma Aldrich (Bellefont, PA). Methanol was purchased from Honeywell, Burdick and Jackson (Muskegon, MI). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

Table 1. HPLC Method Parameters for Various Columns and Conditions

Gradient and method parameters	2.1 × 100 mm 1.8-µm Agilent ZORBAX Eclipse Plus C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18
Flow rate (mL/min)	0.5	0.5	0.7	1.0	0.5	0.9	1.4
10% B (min)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15% B (min)	0.50	0.50	0.36	0.25	0.50	0.28	0.18
50% B (min)	3.00	3.00	2.14	1.50	3.00	1.67	1.07
95% B (min)	4.00	4.00	2.86	2.00	4.00	2.22	1.43
95% B (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
Stop time (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
Post run time (min)	2.00	2.00	1.43	1.00	2.00	1.11	0.71
Overall cycle time (min)	8.00	8.00	5.71	4.00	8.00	4.44	2.86
TCC temperature (°C)	60	60	60	60	90	90	90
Injection volume (µL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
System pressure (bar)	375	280	385	550	195	370	595

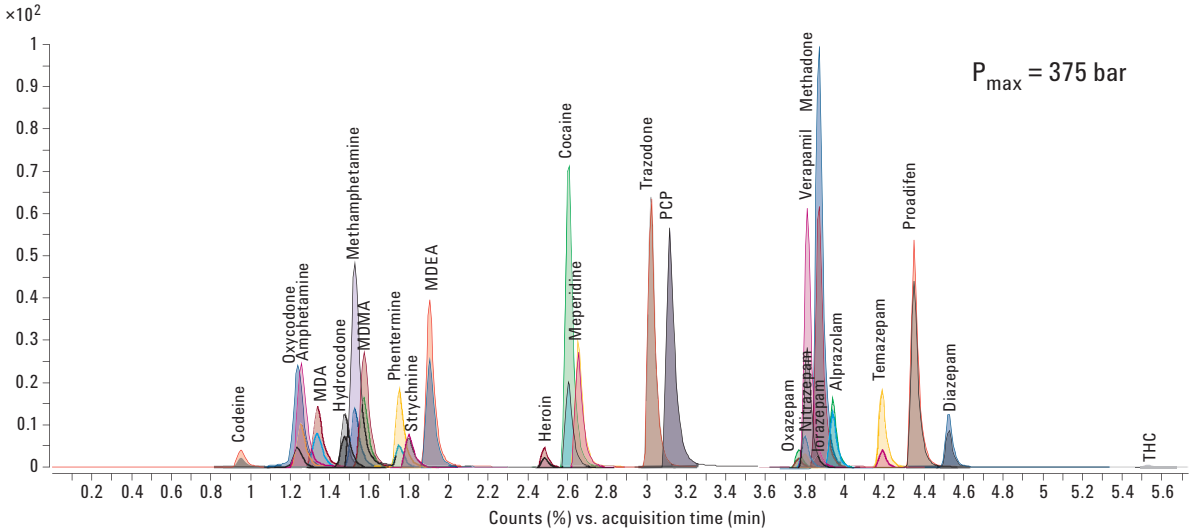
Table 2. MRM Transitions for 25 Compounds in Toxicology Test Mixture

Compound name	Precursor ion	Fragmentor voltage	Product ion 1	Collision energy 1	Product ion 2	Collision energy 2	Retention time (min)	Delta retention time
Codeine	300.2	158	165.1	45	58.1	29	0.89	0.4
Oxycodone	316.2	143	298.1	17	256.1	25	1.14	0.4
Amphetamine	136.1	66	119.1	5	91	17	1.19	0.4
MDA	180.1	61	163	5	105	21	1.25	0.4
Hydrocodone	300.2	159	199	29	128	65	1.34	0.4
Methamphetamine	150.1	92	119	5	91	17	1.43	0.4
MDMA	194.1	97	163	9	105	25	1.46	0.4
Strychnine	335.2	195	184	41	156	53	1.66	0.4
Phentermine	150	66	133	5	91	25	1.66	0.4
MDEA	208.1	107	163	9	105	25	1.8	0.4
Heroin	370.2	149	268.1	37	165	61	2.4	0.4
Cocaine	304.2	138	182.1	17	77	61	2.52	0.4
Meperidine	248.2	128	220.1	21	174.1	17	2.59	0.4
Trazodone	372.2	159	176	25	148	37	2.95	0.4
PCP	244.2	86	91	41	86.1	9	3.05	0.4
Oxazepam	287	150	269	12	241	20	3.66	0.4
Nitrazepam	282.1	148	236.1	25	180	41	3.66	0.4
Verapamil	455.3	158	165	37	150	45	3.75	0.4
Lorazepam	321	102	275	21	194	49	3.75	0.4
Methadone	310.2	112	265.1	9	105	29	3.83	0.4
Alprazolam	309.1	179	281	25	205	49	3.84	0.4
Temazepam	301.1	117	255.1	29	177	45	4.05	0.4
Proadifen	354.2	153	167	29	91.1	45	4.33	0.4
Diazepam	285.1	169	193	45	154	25	4.41	0.4
THC	315.2	150	193.2	20	123.3	30	5.4	0.4

Results and Discussion

Figure 2 shows the original method developed by P. Stone on an Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column. This analysis is accomplished in 6 min with a 2-min post run time at 375 bar. Figure 3 shows the same method with an Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm column. Analysis and post run time are identical to the Eclipse Plus method, while the system back pressure is reduced to 280 bar. While there are slight variations between elution patterns in Figures 2 and 3, overall selectivity is very similar, as would be predicted by Figure 1.

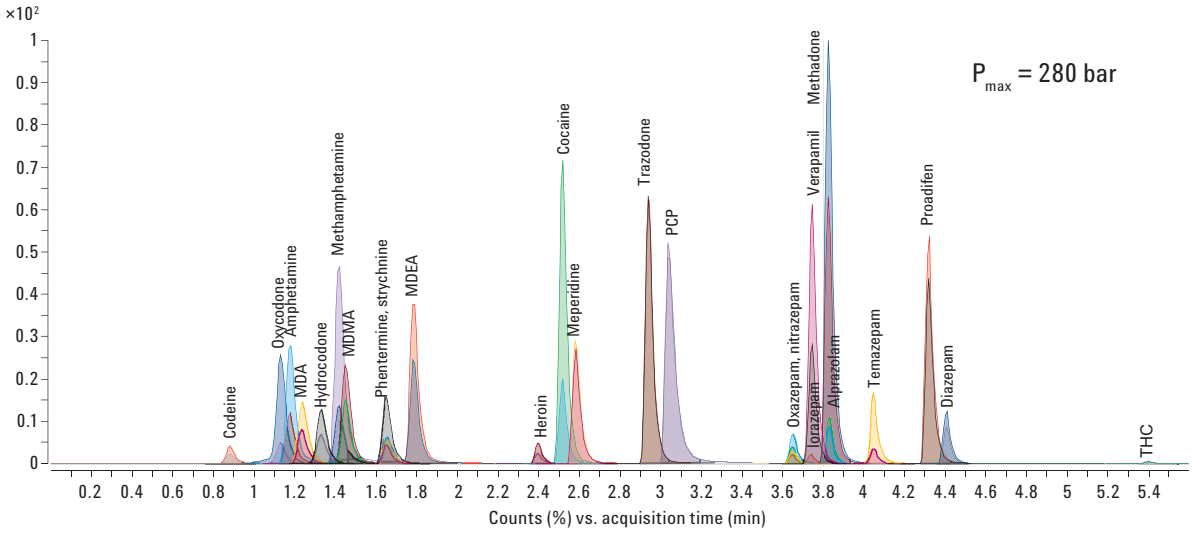
Original Toxicology Method on Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm (Agilent p/n 959764-902)



A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at t_0, ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

Figure 2. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent ZORBAX Eclipse Plus C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.

Original Toxicology Method on Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm (Agilent p/n 695775-902)



A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at t_0, ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

Figure 3. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent Poroshell 120 EC-C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.

Table 3 shows calibration data for all 25 compounds found in the Agilent LC/MS Toxicology Test Mixture on Poroshell 120. All compounds exhibit strong linear correlations, with R^2 > 0.9979. Calibration data was used to quantify a methanol-extracted US $1 bill sample; chromatographic and quantitative results are shown in Figure 4. A significant amount of cocaine was found on the dollar bill. Oxycodone, methamphetamine, PCP and THC were also detected. Smaller quantities of amphetamine, hydrocodone, MDMA, heroin, methadone and diazepam were also found. Quantities of these substances on US currency are consistent with previous findings [6-8].

Table 3. Calibration Data for 25 Toxicology Compounds on Poroshell 120

Compound name	Linear calibration curve	Correlation coefficient, R^2
Codeine	y = 25.4023 × + 3.1628	0.99990276
Oxycodone	y = 138.9535 × – 0.6269	0.99938632
Amphetamine	y = 196.3425 × + 50.1606	0.99987385
MDA	y = 121.2945 × + 180.2165	0.99945701
Hydrocodone	y = 72.1351 × – 8.1010	0.99964622
Methamphetamine	y = 286.7936 × + 429.4970	0.99789141
MDMA	y = 121.4217 × – 55.0435	0.99874569
Phentermine	y = 110.8083 × – 65.1028	0.99914972
Strychnine	y = 39.3465 × – 9.5339	0.99964358
MDEA	y = 200.4804 × – 14.2886	0.99980092
Heroin	y = 18.2969 × + 0.4442	0.99987634
Cocaine	y = 295.8654 × – 5.6261	0.99963342
Meperidine	y = 145.0367 × + 17.2273	0.99986118
Trazodone	y = 286.1986 × – 12.4408	0.99969366
PCP	y = 287.4395 × – 24.8090	0.99989199
Oxazepam	y = 14.7883 × – 0.4919	0.99900677
Nitrazepam	y = 49.1750 × + 69.2747	0.99876656
Verapamil	y = 273.3001 × + 17.3890	0.99986678
Lorazepam	y = 11.2911 × + 6.0687	0.99896851
Methadone	y = 439.7238 × – 6.7890	0.9997511
Alprazolam	y = 80.2721 × + 18.5435	0.99969734
Temazepam	y = 70.9899 × + 15.5246	0.99976598
Proadifen	y = 243.9474 × – 13.0696	0.99990655
Diazepam	y = 68.9622 × + 26.0608	0.99948978
THC	y = 3.1838 × – 2.7072	0.99801611

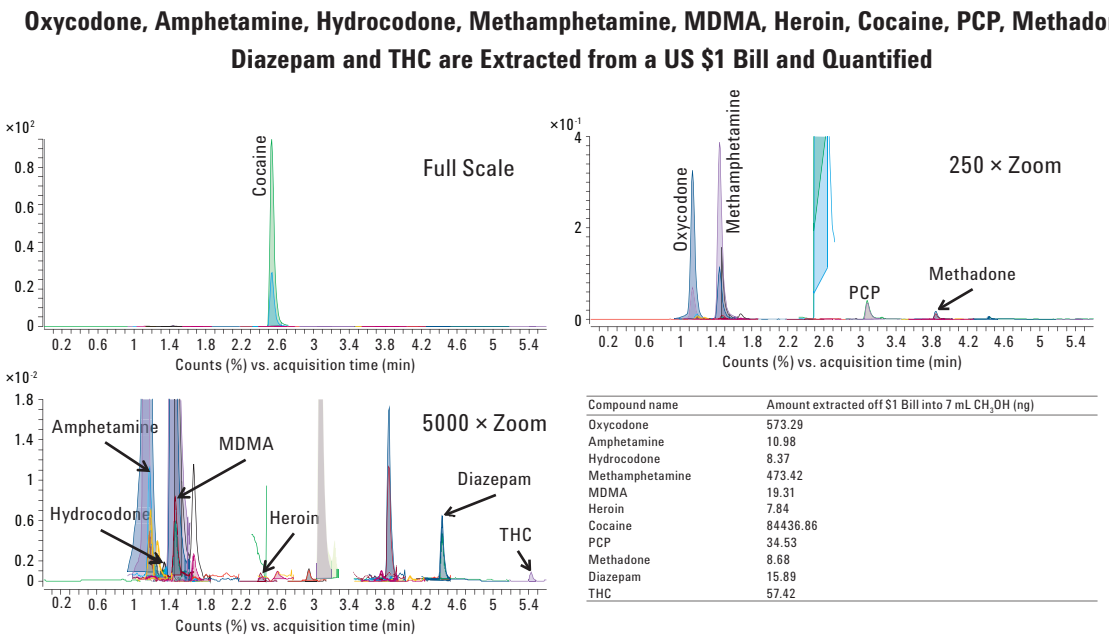


Figure 4. Chromatographic and quantitative results from a random US $1 bill sample extracted with 7 mL of methanol and ultrasonicated for 30 minutes.

Due to the low system back pressure generated with the Poroshell 120 column, the flow rate can be increased from 0.5 mL/min to 0.7 mL/min without exceeding 400 bar for use on a standard HPLC, or it can be increased to 1 mL/min without exceeding 600 bar for use on a UHPLC, as shown in Figure 5. The increased flow rate may be desirable when high throughput is important and when a UHPLC is available for use. Overall cycle time can be decreased by 2.3 minutes while keeping pressure below 400 bar, or by 4 minutes while keeping pressure below 600 bar (a 50% reduction in cycle time). Increasing the flow rate to this degree does cause some loss in resolution, but with MS detection this is not critical.

Significant Time Savings are Possible by Increasing Flow Rate with Agilent Poroshell 120 EC-C18 to LC System Pressure Limits, whether 400 or 600 bar

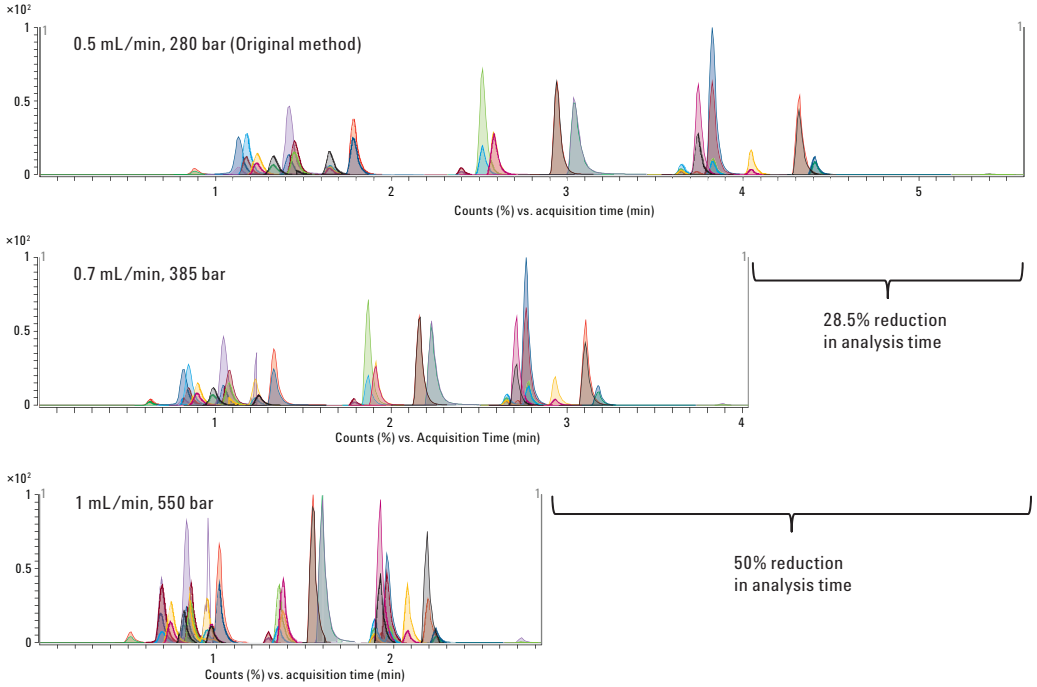


Figure 5. Overlay of Agilent Poroshell 120 EC-C18 toxicology analysis showing time savings by increasing flow rate to reach a 400 or 600 bar system limit.

Flow rate can be further increased by elevating temperature, thereby reducing mobile phase viscosity. The original method however was run at 60 °C, which is the maximum operating temperature for both Eclipse Plus C18 and Poroshell 120 EC-C18. In order to perform this analysis at a higher temperature, the column must be replaced with a Poroshell 120 SB-C18, which has a maximum operating temperature of 90 °C. Figure 6 shows the fast chromatography possible with Poroshell 120 SB-C18. With a 600 bar system pressure limit, it is possible to reduce run time by 64.3%, however this comes at the cost of reduced resolution. For an analysis as complex as this toxicology method, this loss of resolution and significant coelution will cost the analysts a reduction in data points across all peaks, therefore reducing the quality of the results. A simple solution may be to increae column length. A slight increase in column length from 100 mm to 150 mm will increase the resolution of all compounds. While the longer column cannot be run at quite as fast flow rates the analyst can still glean significant time savings by running it at its respective highest flow rate without exceeding system limitations.

Very Significant Time Savings are Possible by Increasing Temperature and Flow Rate with Agilent Poroshell 120 SB-C18 to LC System Pressure Limits, whether 400 or 600 bar

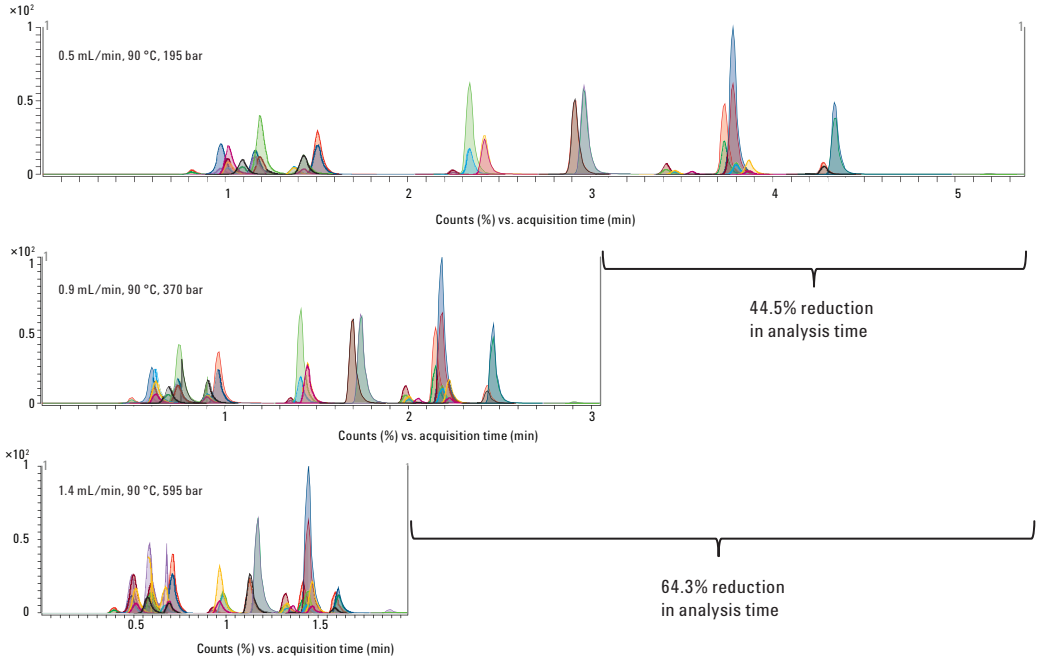


Figure 6. Overlay of Agilent Poroshell 120 SB-C18 toxicology analysis showing time savings by increasing temperature and flow rate to reach a 400 or 600 bar system limit.

Conclusion

A complex analysis of 25 toxicology compounds, that was originally performed on an Agilent ZORBAX Eclipse Plus C18 column, was easily carried out on a superficially porous Agilent Poroshell 120 EC-C18 column with high quality results and substantial time savings. Other complex analyses can likely be transferred from 1.8-µm Eclipse Plus C18 to Poroshell 120 EC-C18 of the same dimensions without method modification, due to very similar selectivity and efficiency. The lower back pressure of Poroshell 120's 2.7-µm particles can be exploited for productivity gains; faster flow rates may be used to shorten analysis time without exceeding system pressure limits for 400 bar HPLC's or higher pressure UHPLC's. This method was used to detect and quantify several drugs of abuse found on a $1 bill, including: cocaine, oxycodone, methamphetamine, PCP and THC.

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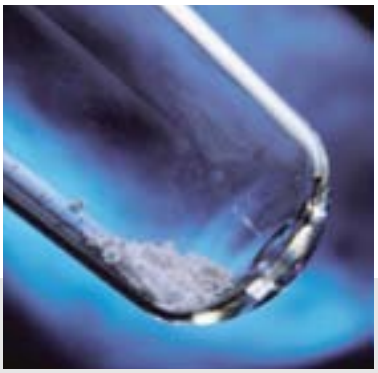
- Confirmation and Quantification of Synthetic Cannabinoids in Herbal Incense Blends by Triple Quadrupole GC/MS
- Detection of Gasoline in Fire Debris by GC/MS/MS



Applications by Technique

GC/QQQ

Confirmation and Quantification of Synthetic Cannabinoids in Herbal Incense Blends by Triple Quadrupole GC/MS

Application Note

Forensics

Authors

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Abstract

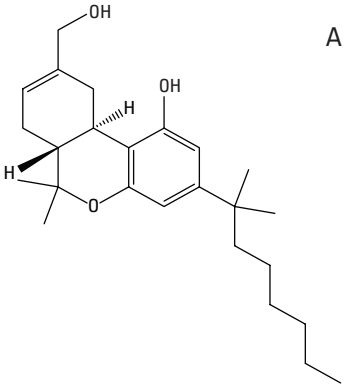
With the rapid and dangerous growth in popularity of herbal incense blends containing synthetic cannabinoids, today's forensic laboratories are challenged to confirm and quantify the controlled forms at trace levels in complex matrices with confidence. Here, a representative sample of 17 of the more than 30 known synthetic cannabinoids is analyzed to demonstrate the applicability of a Triple Quadrupole GC/MS method. The method's selectivity reduces matrix effects and improves signal-to-noise, significantly increasing confidence in analytical results. The method also eliminates the need for post data-acquisition processing such as mass spectral deconvolution.

Agilent Technologies

Introduction

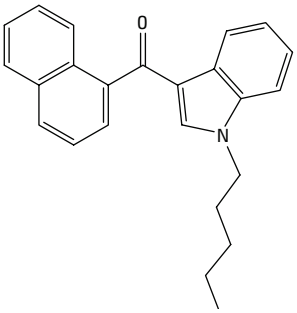
Synthetic cannabinoids are cannabinomimetic compounds originally synthesized for medical research. The rapid growth in use of these compounds by teens and young adults, and widespread availability in convenience stores, head shops, and the Internet is of serious concern in many countries including the U.S.

Synthetic cannabinoids fall into the three structural types shown in Figure 1. The first type (1A) possesses a structural scaffold similar to that of tetradydrocannabinol. The second type (1B) is synthetic napthoylindole analogues. The third type (1C) is phenylcyclohexyl moieties. A common motif inherent to most synthetic cannabinoids is a short aliphatic chain known to interact with the cannabinoid CB1 and CB2 receptors.



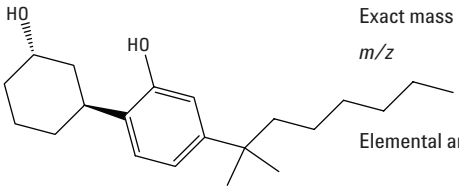
A **HU-210**

Chemical formula	$C_{25}H_{38}O_3$
Exact mass	386.2821
m/z	386.28 (100%) 387.29 (27.6%) 388.29 (4.3%)
Elemental analysis	C 77.68 H 9.91 O 12.42



B **JWH-018**

Chemical formula	$C_{24}H_{23}NO$
Exact mass	341.1780
m/z	341.18 (100%) 342.18 (26.3%) 343.18 (3.5%)



C **CP-47, 497 (C7) analogue**

Chemical formula	$C_{21}H_{34}O_2$
Exact mass	318.2559
m/z	318.26 (100%) 319.26 (23.2%) 320.26 (2.9%)
Elemental analysis	C 79.19 H 10.76 O 10.05

Figure 1. Synthetic cannabinoids fall into the three distinct structural patterns.

Synthetic cannabinoids are usually formulated in botanical matrices (Figure 2) and marketed for sale as herbal incense. Because they are surreptitiously labeled as *not for human consumption*, there is no oversight by the U.S. Food and Drug Administration (FDA). As such, there is no control over their manufacture, raw material quality, potency, and overall safety. The lack of homogeneity and variation in potency of these mixtures can lead to inadvertent overdosing with severe short-term complications including convulsions, anxiety attacks, elevated heart rate, increased blood pressure, vomiting, hallucinations, paranoia, and disorientation. Long-term health effects are unknown.

Figure 2. Synthetic cannabinoids are often formulated in botanical matrices.

Though many countries, including the U.S., have banned specific forms of these compounds, the large and growing number of synthetic cannabinoids has impeded their control. As soon as legislation is passed banning use of a specific form, a new one is synthesized and introduced. Due to the severe health risks and public threat associated with their use, the U.S Drug Enforcement Administration (DEA) exercised its emergency authority to control five specific synthetic cannabinoids for at least one year while it and the U.S. Department of Health and Human Services (DHHS) determine whether permanent control is warranted [1,2]. The DEA now controls:

- JWH-018
- JWH-073
- JWH-200
- CP-47,497 (C7)
- CP-47,497 (C8)

HU-210 is controlled under a previous DEA ruling. Over 20 uncontrolled forms remain and the number is growing.

Confirmation and quantification of synthetic cannabinoid analogs and homologs by single quadrupole gas chromatography/mass spectrometry (GC/MS) presents numerous analytical challenges. At the outset, the botanical matrix is surprisingly difficult to homogenize. Subsequent extraction requires a general approach because synthetic cannabinoids contain a variety of functional groups. However, a general approach extracts a large amount of matrix substances which in turn produce a complex chromatogram with a substantial number of peaks.

The blends often contain a mixture of synthetic cannabinoids which, due to their structural similarities and isomeric forms, co-elute producing overlapped mass spectra. Adding to the challenge, synthetic cannabinoids can be extremely potent and thus present at trace levels relative to the matrix. Though previously demonstrated as an effective and easy to replicate approach [3, 4], single quadrupole GC/MS analyses of these matrices yields very complex data that requires significant effort to interpret without the help of special post acquisition processing software, for example mass spectral deconvolution software.

In this application, a representative sample of an herbal blend is analyzed for the presence of synthetic cannabinoids to demonstrate the applicability of an alternative GC/MS/MS approach that offers enhanced selectivity and sensitivity, and that eliminates the need for mass spectral deconvolution.

Experimental

Reference standards and samples

Listed in Table 3, seventeen of the more than 30 known synthetic cannabinoids were chosen for the development of the GC/MS/MS method. These compounds were chosen to capture the structural diversity of synthetic cannabinoids found in popular herbal blends.

The herbal blends analyzed were EX 565, K2 Blondie, K4 Purple Haze, K3 XXX, Lunar Diamond, Zombie, and K2 Diamond.

Sample Preparation

Homogenization

The botanical material used as the carrier for synthetic cannabinoids, for example Damiana (*Tumera diffusa*), is soft and light. These properties make it difficult to crush into a homogenous form for representative sampling. For this analysis, approximately 500 mg of sample was ground between two 5 inch by 5 inch sheets of 100-grit sandpaper until a finely divided powder was obtained.

Extraction

The multiple functional groups associated with synthetic cannabinoids necessitate a generalized extraction approach. For this analysis, an acid/base combined extraction followed by centrifugation was employed. It is also possible to perform the extraction using methanol incubation. Either approach will extract substantial amounts of matrix components.

Using the acid/base approach, an aliquot of homogenized sample (50 – 100 mg) was acidified by adding 1 mL of de-ionized water, followed by three drops of 10% hydrochloric acid. Next, 1 mL of solvent (95% methylene chloride/5% isopropanol v/v) was added and the sample mixed. The sample was then centrifuged and the bottom solvent layer retained and set aside. Two drops of concentrated ammonium hydroxide and 1 mL of the solvent (95% methylene chloride/5% isopropanol v/v) were added to the remaining aqueous mixture (top layer). The sample was mixed and centrifuged again. The bottom solvent layer was removed, combined with the first bottom solvent layer collected, and then mixed briefly. The sample was then ready for GC/MS/MS analysis.

Derivatization

Some synthetic cannabinoids, for example HU-210, contain multiple, active, polar functional groups such as phenols and alcohols, which can make them much less amenable to GC/MS analysis. To enhance the chromatographic performance and sensitivity of the method for these compounds, derivatization with BSTFA (N,o-Bis (Trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) can be used to cap the functional groups and to produce more intense ions for identification and quantification. Derivatization is not required for the analysis presented in this application note.

GC/MS/MS Analysis

The GC/MS/MS analyses were performed on an Agilent 7000 Series Triple Quadrupole GC/MS system which couples the Agilent 7890A Gas Chromatograph with the Agilent 7000B Mass Spectrometer.

The Agilent 7890A Gas Chromatograph was equipped with a HP-5MS UI column. Table 1 lists the Gas Chromatograph run conditions.

The Agilent 7000B Mass Spectrometer was operated in electron impact ionization (EI) MS/MS mode using multiple reaction monitoring (MRM) for all analytes and reference standards. Table 2 lists the Mass Spectrometer operating conditions.

Table 1. Gas Chromatograph Run Conditions

Agilent 7890A Gas Chromatograph run conditions	
Column 1	HP-5MS UI (Agilent Santa Clara, CA)
Injection mode	Pulsed split-less
Inlet temperature	300 °C
Injection volume	1 mL
Carrier gas	Helium, constant flow mode, 1.2 mL/min
Oven program	80 °C (hold 0.17 min), then 30 °C/min to 300 °C (hold 0.5 min), then 5 °C/min to 340 °C (hold 5 min)
Transfer line temperature	325 °C

Table 2. Mass Spectrometer Operating Conditions

Agilent 7000B Mass Spectrometer operating conditions	
Tune	Autotune
Gain factor	50
Acquisition parameters	Electron impact ionization, multiple reaction monitoring
Collision gas	Nitrogen, 1.5 mL/min Helium quench gas 2.25 mL/min
Solvent delay	7.0 min
MS temperatures	Source 300 °C, Quadrupoles 150 °C

MRM transitions were developed empirically beginning with the collection of full-scan spectra from the reference standards, followed by product ion scanning to identify optimal precursor/product ion pairs for the analysis. Next, the collision cell energy was optimized to achieve the maximum ion intensity for each unique transition. Table 3 provides the analyte list with the associated precursor and product ions, and the optimized collision energies.

Table 3. Analyte List with Associated Precursor and Product Ions, Optimized Collision Energies, and Retention Times

Compound name	Precursor ion	Product ion	Collision energy	Retention time (min)
AM-694	435	232	27	10.918
AM-694	435	220	13	
CP-47-497-C8	377	191	29	7.967
CP-47-497-C8	377	167	33	
HU-211	530	446	13	9.306
HU-211	446	299	21	
JWH-015	327	310	10	10.684
JWH-015	310	268	23	
JWH-018	341	167	23	11.375
JWH-018	324	254	23	
JWH-073	327	167	23	10.875
JWH-073	310	254	23	
JWH-081	371	197	23	13.238
JWH-081	354	269	31	
JWH-122	338	268	23	12.226
JWH-122	298	181	12	
JWH-133	312	269	12	7.348
JWH-133	269	93	23	
JWH-200	384	100	23	14.373
JWH-200	100	56	17	
JWH-203	339	214	3	9.954
JWH-203	214	144	17	
JWH-250	335	214	3	10.007
JWH-250	214	144	17	
JWH-251	214	144	17	9.553
JWH-251	144	116	12	
JWH-398	375	201	23	12.539
JWH-398	318	189	23	
RCS-4	321	264	19	10.259
RCS-4	264	135	17	
RCS-8	254	158	13	12.463
RCS-8	254	144	19	
WIN55 212-3/2	100	70	13	14.373
WIN55 212-3/2	100	56	15	

Results and Discussion

In a GC/MS/MS MRM experiment, the target analyte is selectively isolated from the matrix. As shown in Figure 3, the first quadrupole mass filter isolates a single precursor ion which is allowed to pass into the collision cell. In the collision cell, the precursor ion is fragmented by a collision gas and an applied electrical voltage – a process called collision induced dissociation (CID). CID fragments the precursor ion into specific and predictable product ions. The second quadrupole mass filter is set to pass only the specific product ions designated by the user. The most intense ion, the quantifier ion, is used for quantification. The qualifier ion, when found in the correct abundance ratio with the quantifier, is used for confirmation.

Even if an interfering ion is inadvertently allowed to pass through the first quadrupole into the collision cell, the likelihood that the interfering ion would yield the same product ions as the analyte precursor ion is extremely low. In this manner, chemical noise is entirely separated from signal, increasing the signal-to-noise ratio and thus sensitivity.

Compared to performing selected ion monitoring (SIM) using a single quadrupole mass spectrometer, the MRM technique made possible by GC/MS/MS systems offers significantly improved selectivity and sensitivity for the detection of trace-level synthetic cannabinoids in complex matrices such as herbal incense blends.

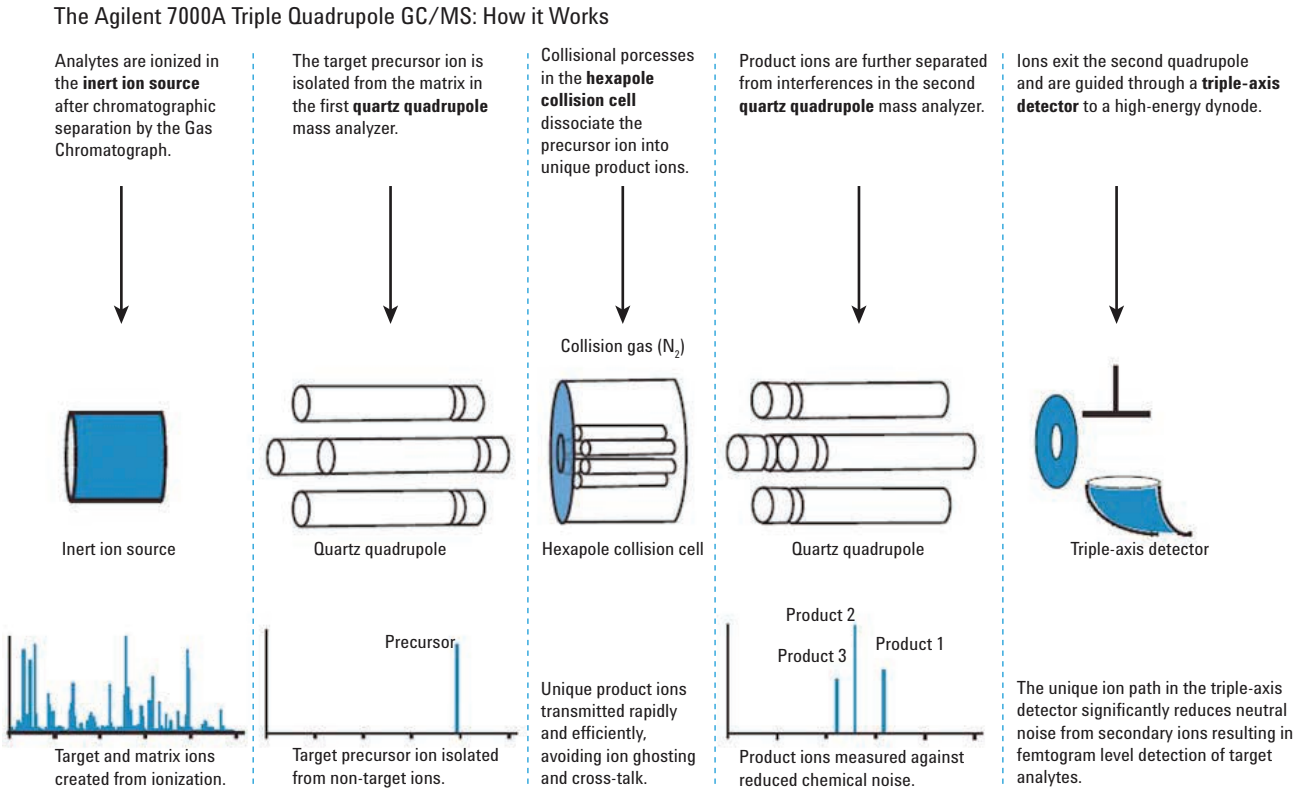


Figure 3. Multiple reaction monitoring (MRM) technique.

The MRM total ion chromatogram (TIC) for 100 ng/mL of the standard mixture is shown in Figure 4. All 17 of the synthetic cannabinoids chosen for analysis were found. Due to the high selectivity of the GC/MS/MS technique, chemical noise is negligible resulting in a very clean TIC.

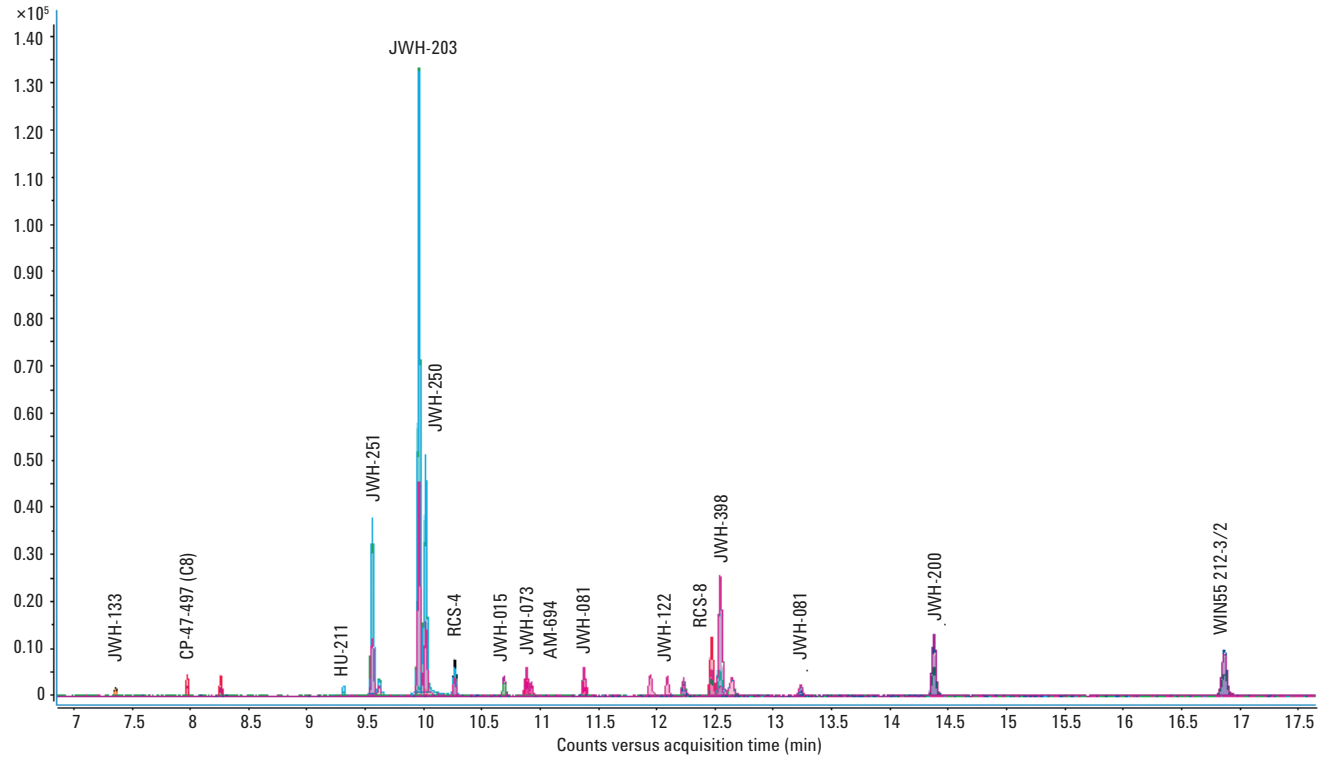


Figure 4. MRM total ion chromatogram for 100 ng/mL of the standard mixture. All 17 synthetic cannabinoid standards were easily found.

Calibration curves were then constructed over the range of 100 – 400 ppb by spiking blank extracted matrix with known reference standards. Replicate injections (n = 3) were made at 100 ppb, 200 ppb, and 400 ppb. The calibration curves for all analytes yielded an average correlation coefficient of linearity (r^2) of 0.99 with standard deviations of 0.012. The average RSD was 13%, 7%, and 6% at 100 ppb, 200 ppb, and 400 ppb, respectively. Levels of quantification as determined by a signal to noise ratio ≥ 10, were determined to range from 1 – 100 ppb in the heavy botanical matrix.

Figure 5 shows the calibration curves for two synthetic cannabinoids with very high activity, JWH-018 and JWH-073 at 100 ng/mL – 400 ng/mL. Typical chromatographic results, for example for JWH-018 at 100 ng/mL, are shown in Figure 6.

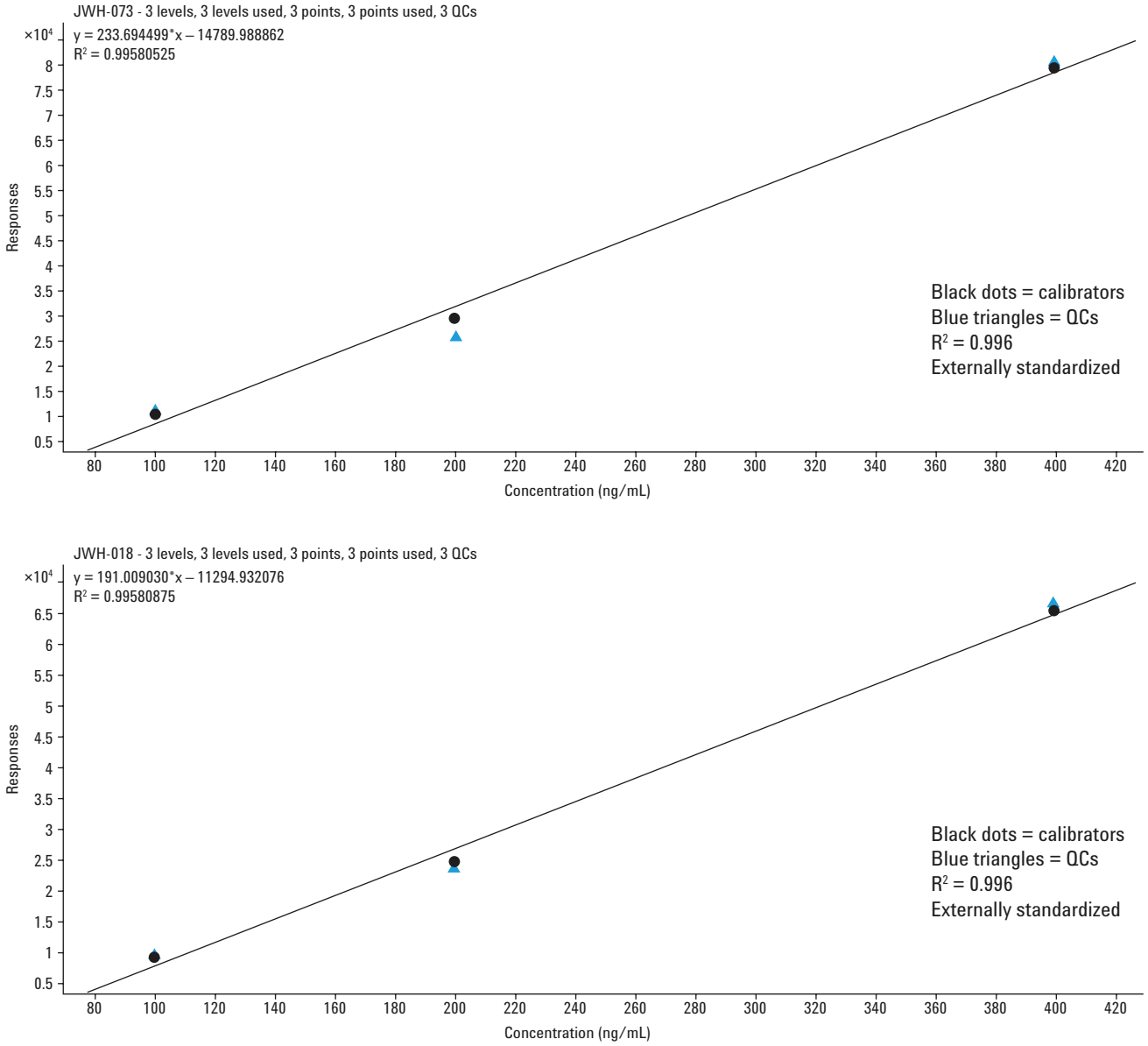


Figure 5. Calibration curves for JWH-018 and JWH-073 show the excellent linearity of the method.

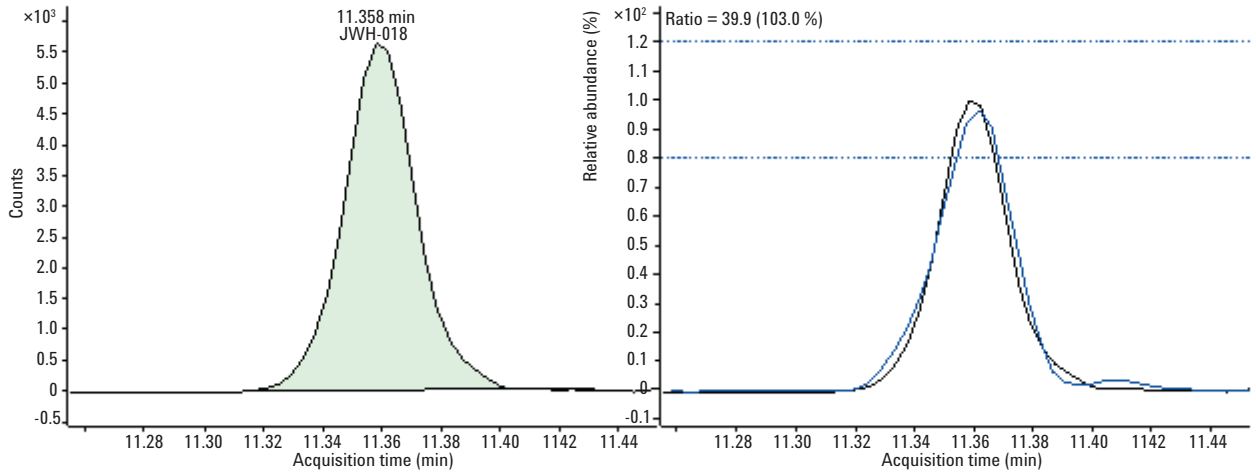


Figure 6. Results for JWH-018 at 100 ng/mL. The shaded peak shows the quantifier ion transition (324 to 254 m/z). The trace shows the qualifier ion transition (341 to 167 m/z) is within the criteria (horizontal lines) established for the method.

Demonstrating the wide variability of herbal blend formulations, JWH-073 and JWH-018 were detected in all of the blends at concentrations ranging from 50 to 150 ppb. Notably, K2 Blondie contained JWH-073 and JWH-018 at concentrations extrapolated to be as much as 1,000-fold higher based on area counts alone. All of the blends contained two or more synthetic cannabinoids as confirmed by correct ratio of the qualifying ion to that of the quantifying ion, and the expected retention time.

Conclusion

For the analysis of synthetic cannabinoids in herbal blends, the utility of triple quadrupole MS cannot be overstated. Its ability to negate matrix effects and improve signal-to-noise markedly increase confidence in analytical results. Compared to single quadrupole MS, triple quadrupole MS reduces false negatives and positives, and lowers detection limits, without need for additional post data acquisition processing such as mass spectral deconvolution and review, thereby providing a substantial time savings.

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Acknowledgement

The authors would like to thank the National Medical Services (NMS) Laboratory (Willow Grove, PA) for graciously providing the herbal blend extracts used in this study. The authors would also like to acknowledge Fran Diamond of NMS for the development of the sample preparation and extraction methodologies.

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Printed in the USA
September 14, 2011
5990-8987EN

Agilent Technologies

Detection of Gasoline in Fire Debris by GC/MS/MS

GC/MS

Varian Application Note

Number 62

Mark Froneman and Betty-Jayne Visser
Council for Scientific and Industrial Research, Pretoria, South Africa

Key Words: *MS/MS, Gasoline, Arson, Forensic*

Introduction

Samples of debris from fires are routinely analyzed for traces of hydrocarbon accelerants[1]. The method of analysis has been by gas chromatography with FID, once sample work-up is complete. More recently, mass spectrometry has been the method of detection used[2]. The switch to MS, instead of FID, was undertaken in order to eliminate the problems caused by the interference of pyrolysis products in the chromatograms obtained. The method of determination of hydrocarbon distillate type was by comparison of the sample chromatogram to that of standards. Individual mass chromatograms of key ions are typically plotted to make this comparison. However, a good comparison is not always possible as in many cases the sample is well burned, leading to residues of distillate and large amounts of pyrolysis products.

Discussion

In order to overcome the pyrolysis product interference and improve detection levels, MS/MS was utilized as the method of detection. As gasoline is one of the more common distillates used by arsonists, the identification of gasoline in fire debris samples was undertaken. Initially the ion of m/z 91, either as a parent, or daughter ion, was isolated and the MS/MS chromatograms for a variety of hydrocarbon distillates were obtained. The same analysis was then performed on fire debris samples. Initial results were successful and thus it was decided to improve the technique by isolating the masses as set out in Table 1. These masses are the molecular weights of the more abundant aromatic compounds found in the gasoline.

As gasoline is a distillate, the ratios of the isomers for each molecular weight are characteristic, and were thus compared. It was decided to compare the aromatic compounds, as these are the more characteristic compounds contained in gasoline.

The initial experimentation with m/z 91 showed that the use of resonance excitation did not provide enough fragmentation ions; therefore, non-resonance excitation was used throughout the mass ranges isolated. This method proved to be sufficient for all masses analyzed. The Toolkit Automated Method Development (AMD) feature was used to determine the CID voltage for each mass. Standards for the common aromatic compounds in gasoline were injected for this purpose.

Figure 1 shows a typical gasoline sample chromatogram using electron ionization GC/MS and a carpet sampled burned with gasoline as the accelerant. The difference in correlation of the two

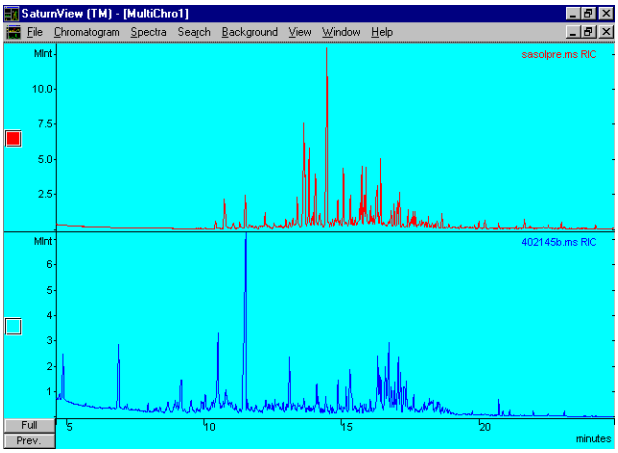

Figure 1: (Top) GC/MS analysis of gasoline sample. (Bottom) GC/MS analysis of carpet sample burned with gasoline.

samples is such that no positive conclusion that gasoline was used as an accelerant can be made. The pyrolysis products from the burned carpet contribute heavily to the chromatogram, as the gasoline residue is minute.

Gasoline contains many compounds that are aromatic and are alkylbenzenes. These compounds contain a predominant ion at m/z 91 and they can be screened by the presence of this ion from the rest of the aliphatic hydrocarbons. By choosing this ion as the parent ion for MS/MS analysis, a chromatographic pattern can be obtained for the gasoline and burned carpet samples. Figure 2 shows these chromatograms.

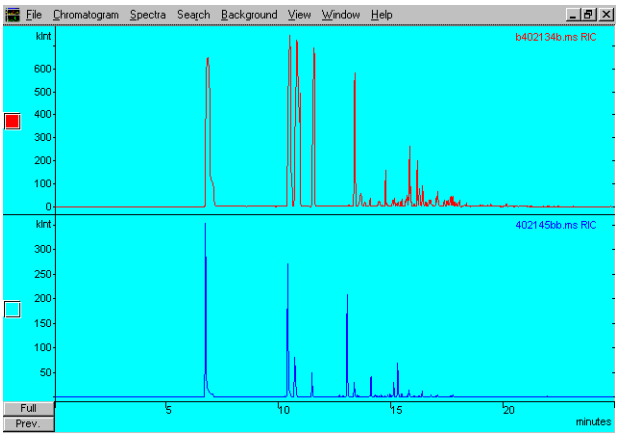

Figure 2: (Top) GC/MS/MS analysis of a gasoline sample using m/z 91 as the parent ion. (Bottom) GC/MS/MS analysis of a burned carpet sample using m/z 91 as the parent ion.

Note that the correlation is much better than that obtained using GC/MS, as in Figure 1.By taking this one step further, an even better correlation can be obtained. MS/MS is a time programmable feature throughout the chromatogram. This allows us to choose certain key compounds such as alkylbenzenes and alkyl substituted PAH's and analyze for these compounds and exclude all other matrix ions. In Figure 3 we can see that the correlation for this GC/MS/MS analysis is excellent and that the carpet was burned with gasoline.

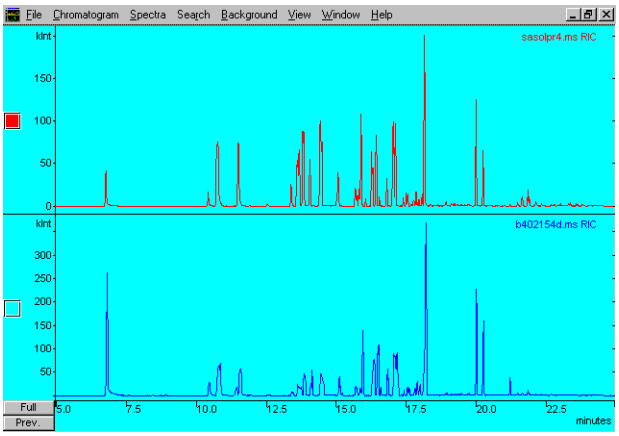

Figure 3: (top) Time programmed GC/MS/MS analysis of gasoline for aromatic compounds. (Bottom) Time programmed GC/MS/MS analysis of carpet sample burned with gasoline.

Experimental

Samples of fire debris were placed in oven bags with a charcoal absorbent strip. The bags were sealed and then heated to 60°C and maintained for 8 hours. After removal from the oven, the absorbent strip is removed, desorbed with carbon disulfide and the solution analyzed.

Gas Chromatograph

Column: DB-1 30M x 0.32mm ID x 0.25µm
Oven Program: 30°C for 6 minutes, program to 70°C at 7°C/min., program to 230°C at 10°C/min. and hold for 13 minutes.
Injector: 180°C

Mass Spectrometer

Scan range: 40-300
Scan rate: 0.71 Sec/Scan
Background mass: 35 u
Ion trap temperature: 120°C
Manifold temperature: 45°C
Transfer line temperature: 280°C
Window: 2 AMU for all compounds
RF level: 48 AMU for all compounds

Table 1: MS/MS Conditions

Mass	Non-Resonant Voltage	Compound
91	50	C1-Benzene
106	35	C2-Benzene
120	40	C3-Benzene
134	35	C4-Benzene
148	40	C5-Benzene
162	30	C6-Benzene
128	30	Naphthalene
142	30	C1-Naphthalene
156	30	C2-Naphthalene
170	30	C3-Naphthalene
178	30	Anthracene
192	30	C1-Anthracene
206	85	C2-Anthracene

Conclusion

The use of GC/MS/MS allows a direct comparison of standard compounds to burned material, in order to determine the type of accelerant used in a fire. An excellent correlation can be obtained, since the matrix compounds of the sample can be eliminated due to the high selectivity of GC/MS/MS. GC/MS/MS is an invaluable analytical technique in suspected arson cases.

Acknowledgement

We wish to thank Carl Feigel of Varian Chromatography Systems for his assistance during this work and for reviewing the manuscript.

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CRIMINALISTICS

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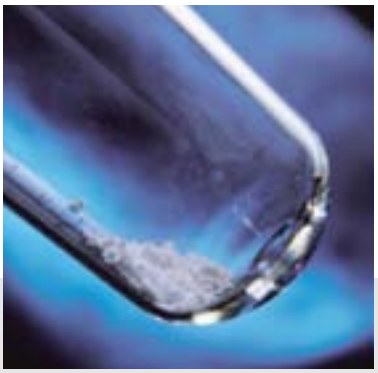
- HPLC Analysis of Explosives Using EPA Method 8330
- Fast analysis of ink dyes using the Agilent 1290 Infinity LC System coupled to Agilent 6140 single quadrupole LC/MS System for forensic analysis of ink pens and markers



Applications by Technique

LC/MS

HPLC Analysis of Explosives Using EPA Method 8330

LC

Varian Application Note

Number 24

Michael J. Lang
Varian Chromatography Systems

Key Words: Explosives, EPA, HPLC

Introduction

Since the end of the Cold War, various governments have begun to dismantle military installations and munitions plants in accordance with non-proliferation agreements and disarmament treaties. As a result of these efforts, major environmental problems are being discovered at many locations. Surrounding lands are found to be laden with explosive residues such as 2,4,6-trinitrotoluene (TNT) and associated nitroamine impurities. The highly toxic nature of many of these substances, coupled with their persistence in the environment, requires thorough characterization of contaminated areas.

Gas chromatography is often used to determine these substances. However, for the separation of thermally unstable and non-volatile compounds, high performance liquid chromatography (HPLC) with ultraviolet detection is ideally suited and offers adequate detection limits for nitroaromatics. HPLC methodology was first documented in the early 1980s by Bratin et. al.

The EPA Method 8330, first introduced in November 1990, is the most common way that explosives are analyzed. Analytes are able to be detected down to 2.5 ppb in water, soil. or sediment. The data generated in this report followed the EPA 8330 method. The EPA method documents co-elution of some of the compounds of interest. In order to identify and quantitate all explosive compounds, it was necessary to rerun the samples under different chromatographic conditions. This co-elution was observed by other investigators as well.

Complete separation of all compounds of interest in a single chromatographic run would improve sample throughput and decrease the cost of analysis.

Experimental

The HPLC Measurements were carried out on a Varian Star HPLC system which included: 9012 Gradient Solvent Delivery System, 9050 UV-VIS Detector, 9300 Refrigerated AutoSampler (fitted with a 20 uL loop), and a Star Chromatography Workstation.

The instrument conditions followed a modified version of EPA Method 8330 and are listed in Table 1. Standards were obtained from Accustandard and were diluted with water. A typical chromatogram showing the complete separation of these explosive compounds is shown in Figure 1. A typical calibration curve is shown in Figure 2.

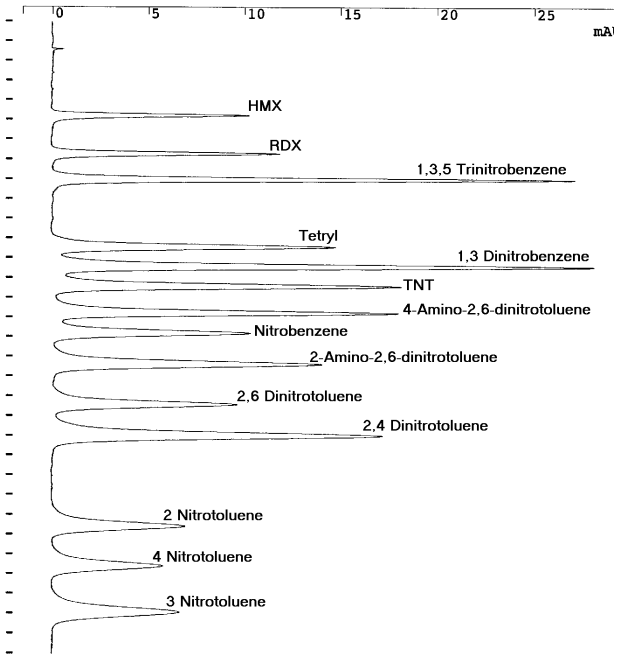


Figure 1. EPA Explosives at 10 ppb

Table 1

Columns	**30 mm CN in series with a 250 mm Bondesil C - 18**
Mobile Phase	**50% Methanol / 50% Water**
Flow Rate	**1.3 ml/min**
Detection	**254nm**
Sample	**4^{o} C in water**

Conclusions

The use of the unique combination of 30 mm Res Elut CN column in series with a 250 mm Bondesil C-18 column, produces complete separation of all explosives in a single run. This improves sample throughput, decreases analysis time, and eliminates the need for repeating injections on a second column. Reducing the sample injection volume from the typical 100 uL to 20 uL gave better peak shape and improved retention time reproducibility for early eluting compounds. The flow rate was also reduced from 2.0 mL/min to 1.3 mL/min without increasing the analysis time so solvent consumption was reduced. As illustrated in Figure 1, a complete separation of the 14 components in EPA Method 8330 is achieved. Table 2 shows the typical retention times under the chromatographic conditions outlined in this note. A second confirmation run, because of co-elution, is not required due to complete separation of the explosives in this modified method.

Excellent linearity of response is observed over the concentration range of 2.5 ppb to 1 ppm. Typical results of multi-level calibrations are shown in Figure 2 where the correlation coefficient is 0.998260.

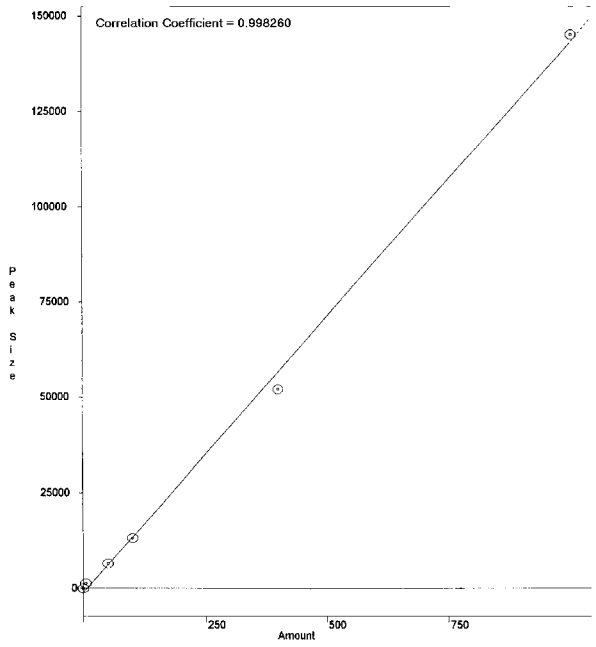


Figure 2. Calibration of TNT (1 ppm-2.5 ppb)

Table 2

Component	Time
HMX	4.585
RDX	6.450
1,3,5 Trinitrobenzene	7.757
Tetryl	10.937
1,3 Dinitrobenzene	11.931
TNT	12.905
4-Amino-2,6-dinitrotoluene	14.179
Nitrobenzene	14.974
2-Amino-2,6-dinitrotoluene	16.457
2,6 Dinitrotoluene	18.516
2,4 Dinitrotoluene	20.017
2 Nitrotoluene	24.394
4 Nitrotoluene	26.284
3 Nitrotoluene	28.466

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Fast analysis of ink dyes using the Agilent 1290 Infinity LC System coupled to Agilent 6140 single quadrupole LC/MS System for forensic analysis of ink pens and markers

Application Note

Forensics

Author

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Abstract

In forensics, the analysis of ink writings from documentation is required for authentication or crime analysis. Ten organic ink dye components typically found in ink pens were separated using the Agilent 1290 Infinity LC System and quantified using the Agilent 6140 single quadrupole LC/MS System. The dyes were separated in less than 3.5 min using a sub-2-µm, 30 mm column. Analysis of ink markings on paper from five black and five blue pens were matched to standard using retention time, mass-to-charge ratio and UV/Vis spectral matching. The results show that the pens can be distinguished from each other based on the percentage of ink dye content.

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Introduction

Determining the degradation and the source of ink play an important role during the forensic analysis of writings[1,2]. In this Application Note, ink source determination is demonstrated by comparing ink markings on paper from 10 pens against 10 external dye standards. The 10 external dye standards (Table 1) were separated on a sub-2-µm column and quantified using an Agilent 6140 single quadrupole LC/MS System. The recovery analysis of these dyes was performed from paper samples using an optimized extraction method. Ink markings on paper made by five black and five blue ink markers, ball point, and gel pens were matched with the standards using retention time (RT), mass-to-charge ratio (*m/z*) and UV/Vis spectra. Certain types of dyes were found to exist in different proportions in different pens. Therefore, the source of the ink can be linked to a specific type of pen in a relatively short amount of time using this method.

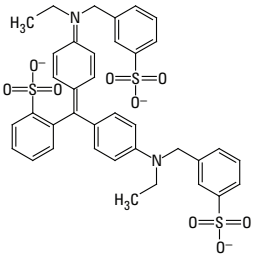
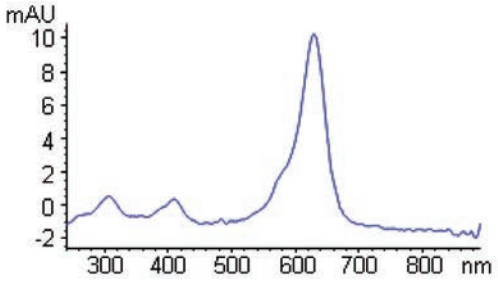
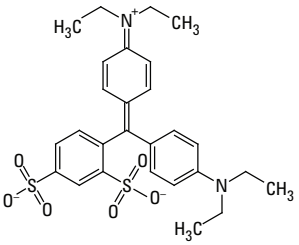
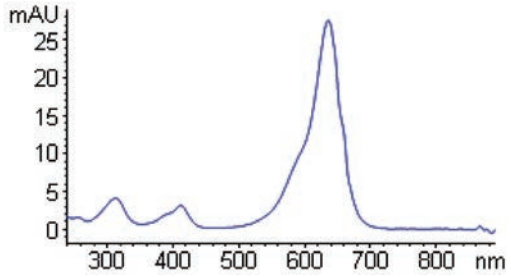
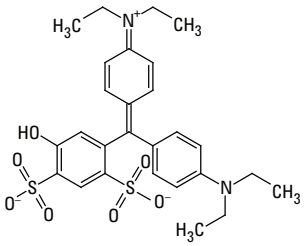
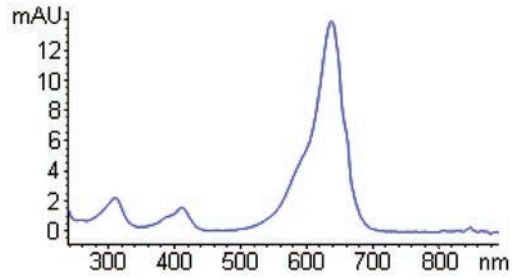
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Acid blue 9 (AB9)		 λ max: 630
Patent Blue VF (PBVF)		 λ max: 636
Patent Blue V (PBV)		 λ max: 636

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm. (continued)

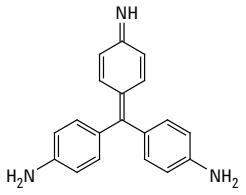
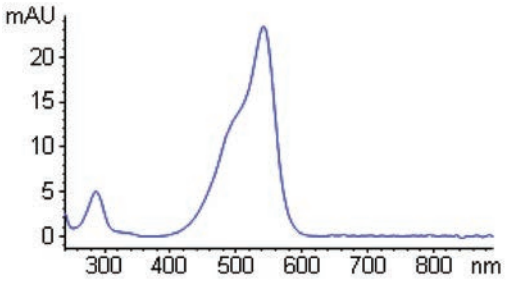
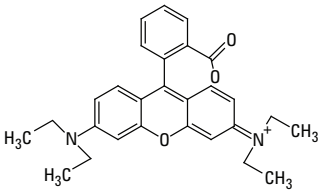
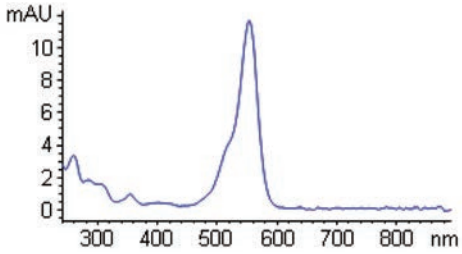
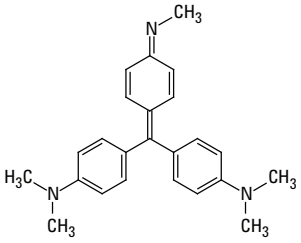
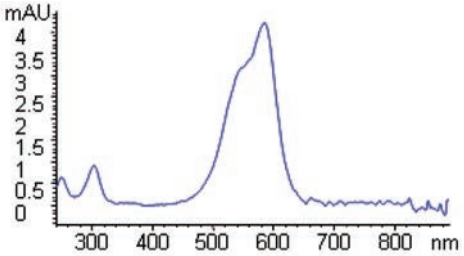
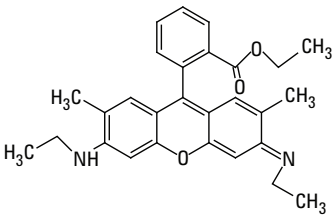
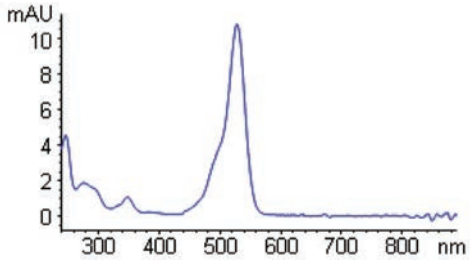
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Pararosaniline hydrochloride (PAH)		 λ max: 542
Rhodamine B (RB)		 λ max: 554
Methyl violet B base (MVB)		 λ max: 580
Rhodamine 6G (R6G)		 λ max: 528

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm. (continued)

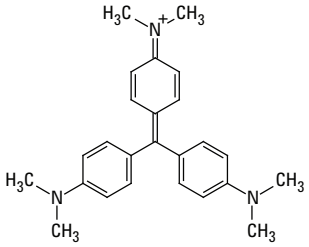
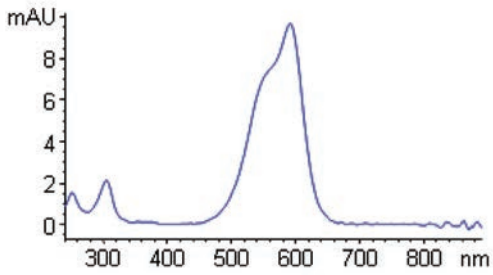
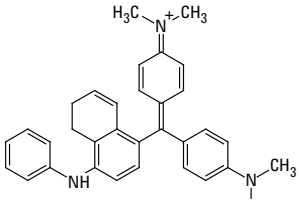
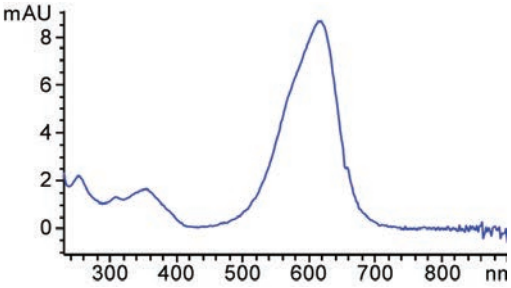
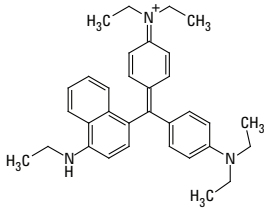
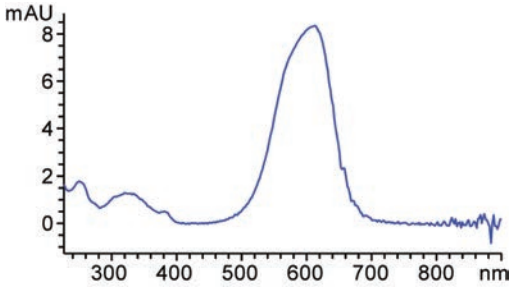
Ink Dye (abbreviation)	Structure	UV/Vis Spectra
Crystal Violet (CV)		 λ max: 592
Victoria blue b (VBB)		 λ max: 616
Victoria pure blue BO (VPBBO)		 λ max: 612

Table 1
Structures of 10 ink dyes used in the experiment along with the UV spectra from 230 nm to 900 nm.

Experimental

The 10 ink dye standards were purchased from Sigma Aldrich. Five black and five blue markers or ball point, gel pens were purchased from local stores for analysis. The mobile phase modifiers used were of LC-MS grade. Acetonitrile used was super gradient from Labscan.

Ten ink dye standard stock and linearity solutions: Standard stock solution was prepared in 100% methanol. Mixed linearity solution was prepared to the concentrations of 0.1 ppm, 0.5 ppm, 1 ppm, 2 ppm and 10 ppm in 50% mobile phase A and 50% mobile phase B. Six replicate experiments were performed using 0.5 ppm standard solution to obtain retention time and reproducibility values.

Recovery studies and extraction procedure: Dye mixture in the amount of 25 µL of 10 ppm (0.125 ppm) was added on 75 gram per square meter (gsm) paper and air dried. One milliliter of acetonitrile was added to the paper and vortexed for 30 sec followed by sonication for 10 sec. One milliliter of buffer A was then added followed by vortex for 30 sec and sonication for 1 min. The recovered amounts from the linearity results were compared against the expected amount of 0.125 ppm to determine the recovery percentage. The pens were used to fill a circle of 7-mm diameter on a paper. Samples were taken directly for analysis. Single ion monitoring (SIM) mode was used in the mass spectral acquisition.

Experimental Parameters	Details	
Column	Agilent ZORBAX SB-Aq 30 mm × 2.1 mm, 1.8 µm, p/n 824700-914; operated at 25 °C	
Mobile phase	Buffer A: Ammonium formate buffer pH 4.0 (190 µL of formic acid and 0.64 g of ammonium formate in 1L of water) Buffer B: 100% acetonitrile	
Step gradient run	Run time (min): 4.2 min 0 min – 20% B 0.01 min – 32% B 1.0 min – 34% B 1.1 min - 47% B 2.5 min – 50% B 2.6 min – 65% B 3.5 min – 75% B 3.6 min – 100% B 4.2 min – 100% B 4.3 min – 20% B 5.0 min – 20% B	
Flow	0.7 mL/min from 0 to 1 min 1.0 mL/min from 1 to 5 min	
Injection volume	1 µL, needle wash at flush port for 4 sec with 100% methanol	
Diode array detector (DAD) detection	Spectral acquisition at 2 nm step from 230 nm to 900 nm using Agilent 1200 Series DAD SL connected in series to an Agilent 1290 Infinity LC system	
Agilent 6140 single quadrupole LC/MS System	Drying gas	12.0 L/min
	Nebulizer pressure	40 psig
	Dry gas temperature	300 °C
	Capillary Voltage (+)	4000 V
	ESI Source: Positive mode	
	SIM mode, peak width 0.05 min	

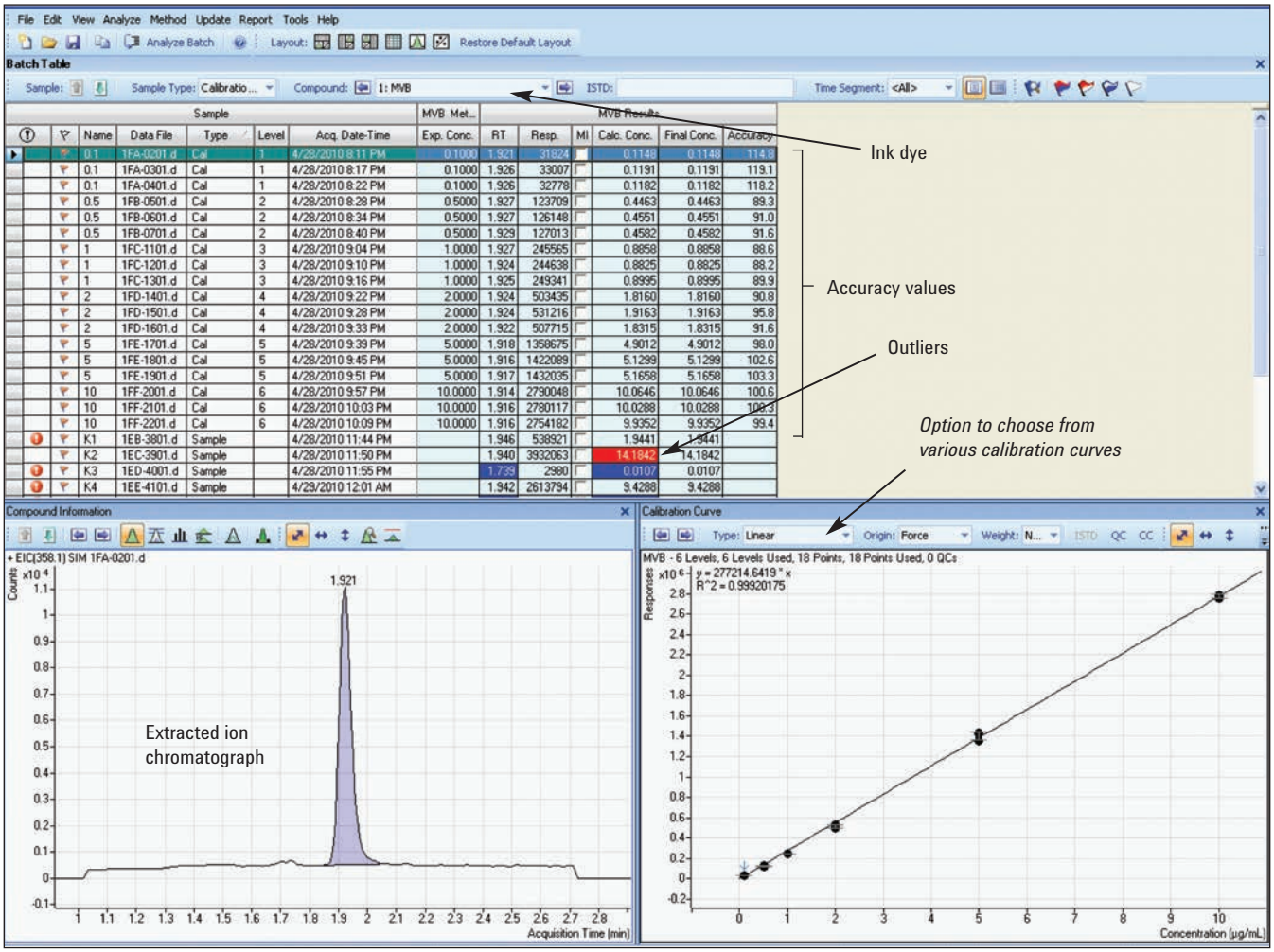


Figure 1
Snapshot of quantitative software for data analysis. Sample information, EIC and linearity curves are displayed in the same screen.

The data acquisition was performed using ChemStation B.04.02 software and the data files were converted online as a post acquisition step to MassHunter files using the MassHunter LC/SQ Integration Software (B.02.00). Data analysis was subsequently performed using MassHunter Quantitative Analysis software (B.03.01).

ChemStation data files were efficiently converted to MassHunter data files and all recovery and linearity data were processed using MassHunter Quantitative Analysis software (Figure 1).

Results and Discussion

A mixture of 10 ink dyes was analyzed using an Agilent 1290 Infinity LC system and an Agilent 6140 single quadrupole LC/MS System. All peaks were resolved well using a step gradient from 20% B to 100% B with a 30 mm Agilent ZORBAX SB-Aq, 1.8 µm column. The mobile phase (Buffer A) with pH 4.0 was found to be ideal to elute all ten ink dyes with good peak shape and resolution. The short gradient time ran from 20% B to 32% B and helped to separate PBV from PAH. The step gradient continued with partial isocratic steps of 32% B to 34% B and later of 47% B to 50% B. This helped to reduce the elution time of ink dyes from RB to CV, thereby reducing the overall run time. The specificity of the method was increased by operating the LC/MS in time programmed SIM mode. Here, three time segments were added in data acquisition: 0 – 1 min, 1–2.7 min, 2.7–5 min. This was done to contain specific molecular ions in each time segment (determined empirically) and to increase the dwell time (Table 2). Figure 2 shows the MS total ion chromatogram (SIM mode) for the 0.5 ppm standard mix of 10 ink dyes.

The Agilent 1290 Infinity DAD operates in the range of 190–640 nm while Agilent 1200 DAD SL has a specification range from 190–950 nm. Since some ink dyes have spectra that go beyond 640 nm, an Agilent 1200 Series DAD SL was connected to the Agilent 1290 Infinity LC System in series along with the Agilent 6140 single quadrupole LC/MS System. The advantages of MS-based detection are increased sensitivity and selectivity. These parameters along with UV-based detection and RT matching, provide accurate confirmation of dyes in pens.

The precision of the method (Table 2) using six replicates of 0.5 ppm solution show standard deviation (SD) for retention time to be less than 0.003 min and the RSD for area response to be less than 3.0. The linearity at six concentration levels shows the correlation coefficient (R^2) to be greater than 0.99. Recovery of the standard dyes from dried paper samples using the recovery procedure effectively extracted out all of the 10 ink dyes. The results from recovery experiments show a recovery range of 89% to 110% for all ten ink dyes from paper.

Ink dye analysis from pens

RT, *m/z* and UV/Vis spectral matches from standards were used to confirm the identity of the dyes from paper markings. Representative analysis results from two pens are shown in Figure 2. MVB, CV and VBB were identified in black pen 4 while VPBBO was present in blue pen 3. In the pen markings tested here, typically 5 out of 10 tested ink dye standards were found. These five dyes also were within the calibration range. The results in Table 3 show the specific ratios in which the 5 ink dyes occur in different pen markings. Analysis of the ratios of dyes present in the paper markings can possibly be traced to the origin of the pen. Nevertheless, there are some exemptions; for example, black pen 2 and 4 markings on paper show similar formulations of dyes while black pen 5 mark-

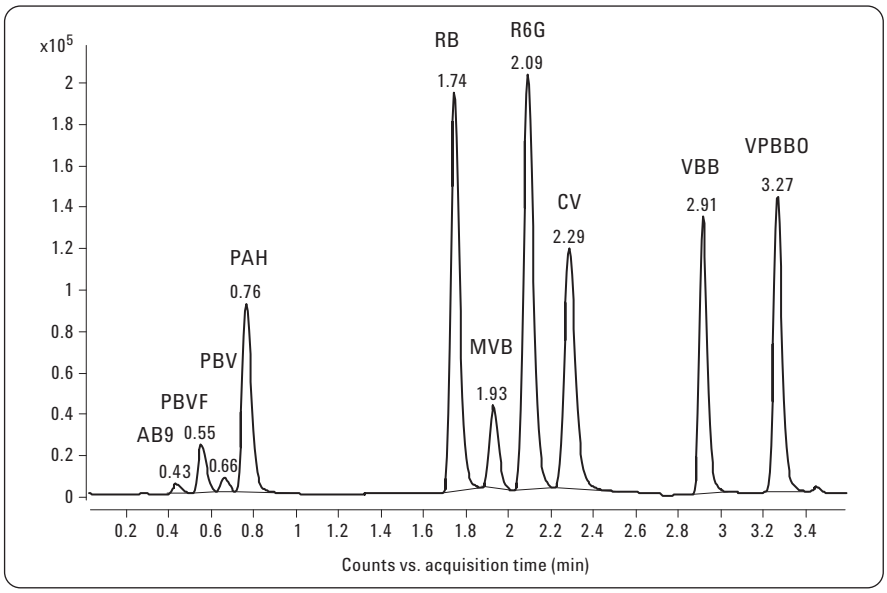


Figure 2
Total ion chromatogram (TIC) of the mixture of 10 ink dyes operated in time programmed SIM mode.

Abbreviated dye name	Molecular ion $(M+H)^+$	Fragmentor voltage (V)	Retention time (min)	SD of RT, n=6	RSD of Peak Area, n=6	Correlation Coefficient R^2	Average recovery % N=3
Time Segment: 0 – 1 min							
AB9	749.0	147	0.432	0.002	1.82	0.998	89
PBVF	545.0	123	0.552	0.002	1.63	0.991	106
PBV	561.0	96	0.660	0.002	1.24	0.992	110
PAH	288.1	120	0.762	0.002	2.08	0.998	106
Time Segment: 1 – 2.7 min							
RB	443.1	99	1.746	0.001	2.04	0.998	106
MVB	358.1	135	1.928	0.001	2.62	0.999	108
R6G	443.2	101	2.090	0.001	2.19	0.999	106
CV	372.2	156	2.284	0.001	1.78	0.998	104
Time segment: 2.7 – 5 min							
VBB	470.2	156	2.914	0.001	2.27	0.998	107
VPBBO	478.2	123	3.265	0.001	2.13	0.999	105

Table 2
Molecular ion, fragmentor voltage and retention time of 10 ink dyes acquired using SIM mode using time segments. The RT SD and area RSD were calculated from six replicate injections of 0.5 ppm standard solutions. The correlation coefficient represents the linearity samples at six concentration levels (0.1 – 10 ppm, three replicated each). Recovery of standards from paper ranged from 89% to 110%.

ings did not contain any of the ink dyes tested here. This suggests that additional dye standards are needed to make a comprehensive database for forensic analysis of documentation.

Conclusions

Ten ink dyes were separated in less than 3.5 min with excellent retention time reproducibility (SD < 0.003) while the area precision was less than RSD 3%. The recoveries of inks from paper ranged from 89 to 110%. Analyses of ink markings on paper from ten randomly selected pens mostly show five dyes in various combinations. This ratio of ink dyes helps to identify the origin of the pen. RT, *m/z* and UV/Vis spectral matching with external standards were used to confirm the identity of the compounds extracted from paper. The MassHunter LC/SQ Integration Software efficiently converted ChemStation files to MassHunter data files. The data processing was effectively performed on MassHunter Quantitative Analysis software. The combination of the Agilent 1290 Infinity LC System and Agilent 6140 single quadrupole LC/MS System is an efficient tool in forensic applications that include authentication, and crime analysis of documentations.

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Y. Liu, J. Yu, M. Xie, Y. Chen, G. Jiang, Y. Gao, "Studies on the degradation of blue gel pen dyes by ion-pairing high performance liquid chromatography and electrospray tandem mass spectrometry," *J. Chromatography A*, 1125: 95-103, 2006.

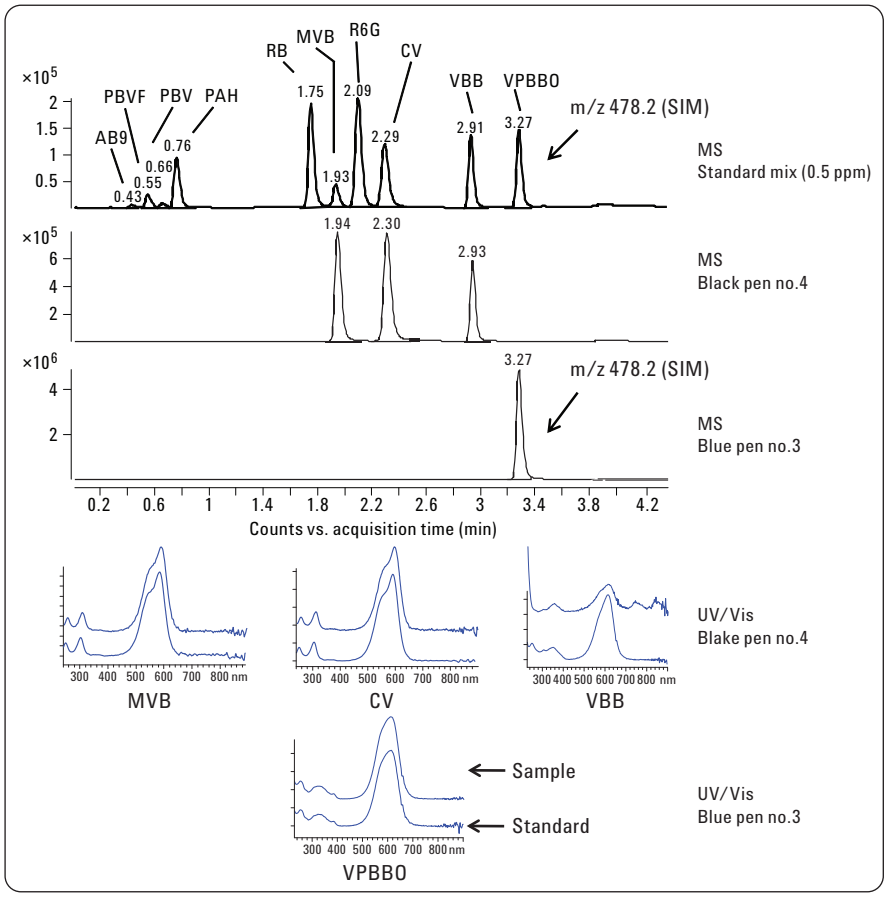

Figure 3
TIC showing the detection of MVB, CV and VBB dyes in black pen #4 and Blue pen #3 confirmed using *m/z* (SIM mode), RT and UV spectral matching. A small aberration at 656 nm seen in VBB and VPBBO spectra is the deuterium lamp peak.

Paper markings from pens	AB9	MVB	CV	VBB	VPBBO
Black pen 1	0	41	59	0	0
Black pen 2	0	63	23	14	0
Black pen 3	100	0	0	0	0
Black pen 4	0	62	22	16	0
Black pen 5	0	0	0	0	0
Blue pen 1	0	11	0	89	0
Blue pen 2	0	39	18	43	0
Blue pen 3	0	0	0	0	100
Blue pen 4	0	50	0	50	0
Blue pen 5	0	19	0	81	0

Table3
The ratio of five ink dyes that exists in paper markings from ten commercial black and blue pens.

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August 1, 2010
Publication Number 5990-6205EN

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- ATR FTIR imaging in forensic science
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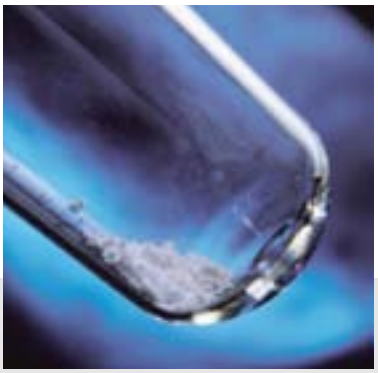

Applications by Technique

FTIR

ATR FTIR imaging in forensic science

Application Note

Author

Sergei G. Kazarian*, Camilla Ricci*, Simon Boyd**, Mustafa Kansiz**

* Imperial College UK

**Agilent Technologies, Inc.

Introduction

Conventional Fourier transform infrared (FTIR) spectroscopy and microscopy have been widely used in forensic laboratories for a number of years. This instrumentation has many potential applications for the analysis of forensic samples, including the identification of illicit drugs, fingerprints, gunshot residues, explosives, pharmaceuticals, and so on. Recent advances have allowed this technology to be extended further by using attenuated total reflection (ATR) FTIR spectroscopy/microspectroscopy. This has permitted the non-destructive measurement of different portions of a sample or even spectral mapping of its entire surface with little or no sample preparation. Most samples can simply be presented to the ATR surface in their present state.

A recent approach to acquiring infrared spectra of forensic samples involves the use of imaging array detectors. Imaging detectors allow for an examination of a sample's chemical distribution, making it possible to examine the heterogeneity of a sample. By using an n x n focal plane array (FPA†) detector (where n = 16, 32, 64 or 128), a grid of spectra is obtained in approximately the same amount of time that is required to acquire one spectrum with a single-element detector. For example, a 64 x 64 FPA† will simultaneously collect 4096 spectra from an image area of up to 2.5 x 3.5 mm^2 using a (ZnSe) imaging accessory. By simultaneously acquiring thousands of spectra within minutes, FPA† detectors provide information about the identification and concentration of specific compounds and their distribution in the measured field of view. An FPA† detector is superior to single-element detector technology (such as single point mapping), as it dramatically reduces the collection time required to obtain multiple spectra, and provides improved spatial resolution and signal-to-noise performance of obtained images[1].

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The same argument can be used for linear array mapping. Infrared ATR chemical imaging (both micro and macro) have increased the use of mid-IR spectroscopy in forensics, as they simplify sample preparation, are rapid and accurate, and provide reliable 2D chemical images that can be thought of as *chemical photography*[2].

We have recently demonstrated how ATR FTIR imaging can be used to identify or compare physical evidence in forensic analysis[3-5]. In this study, the use of ATR FTIR imaging in forensic science is demonstrated through the measurement of fingermark residues, both directly, and using a lifting medium. The detection of an exogenous substance (the drug paracetamol) on a suspect's fingertips is also demonstrated.

Instrumentation

All spectral images were recorded using a Cary FTIR spectrometer coupled to a large sample (LS) accessory and a Pike Vee-Max imaging ATR accessory. Direct fingermark imaging was performed using a Specac ATR accessory. Both of these accessories incorporated a ZnSe internal reflective element (IRE). Infrared images were collected with a 64 x 64 pixel liquid nitrogen cooled mercury cadmium telluride (MCT) FPA† detector in rapid scan mode.

NOTE: It is also possible to perform the above measurements with the Specac Imaging Golden Gate ATR Accessory, exclusive to Agilent. This accessory provides a preserved aspect ratio of 1:1, while providing increased spatial resolution of 10 µm for more sample detail with a field of view of up to 640 x 640 µm.

Table 1. Instrument parameters used in the collection of all images in this study

	Instrument Parameters	Settings
Detector	MCT 64 x 64 FPA	
Source	Mid-IR	Medium
ATR	Pike Vee-Max Specac ATR	
IRE	ZnSe	
Collection	Sample scans Background scans Resolution (cm^{-1}) Aperture Symmetry	16 16 8 open asym
Computation	Apodization type Zero filling factor	BH4 auto

Materials and reagents

Tape-lifted fingerprints were lifted from metal surfaces using a commercial gel (BVDA Gelatine Lifter), provided by the Home Office Scientific Development Branch (HOSDB). Paracetamol was obtained from capsules of commercially available Panadol.

Sample preparation

Fingermarks were laid directly on to the IRE of the ATR. In a second experiment, images containing trace amounts of paracetamol were obtained in the same way after transferring a small number of particles of the substance to the fingertip. Tape-lifted samples were obtained from fingermarks made on a metal door handle. The fingermarks were collected by placing a gel lift over the defined area of the door handle, smoothing the gel in place and then peeling it off. The tape was then firmly applied to the ATR surface to ensure homogenous contact.

Results and discussion

Macro ATR FTIR imaging and fingerprint analysis

The oldest method of personal identification for forensic purposes is fingerprint analysis. The ability to identify suspects from fingerprints left at a crime scene is possible due to the unique nature of the arrangement of ridges on each person's finger pads. ATR FTIR spectroscopic imaging with a ZnSe accessory offers a new and complementary means of studying the chemistry of fingerprints. A major feature of ATR FTIR imaging is its ability to provide spatially resolved chemical information.

As shown in Figure 1, direct imaging of a fingerpad can be quickly and simply obtained by monitoring the distribution of proteins. This chemical image was generated by integration of the area between 1700 and 1600 cm^{-1}, a region representative of the amide I band of proteins. The chemical image was collected with 16 co-added scans representing a collection time of just 13 seconds at a spatial resolution of ~50 µm.

The Resolutions Pro software can display chemical images of any wavenumber range with just one mouse click, thereby simplifying chemical analysis and data interpretation. Alternatively, a complete IR spectrum of any pixel can be displayed by clicking anywhere on the chemical image.

Current non-invasive methods of latent fingermark collection typically involve lifting fingermark residues from a surface using a lifting medium. The use of tape-lifting techniques is of paramount importance. They allow latent fingermarks to be collected from surfaces (such as door or mug handles, curved glass surfaces or computer screens) that are difficult to access when using powdering or other detection methods. The method also maintains the integrity of samples, allowing for further analysis or archiving purposes. Latent fingerprint analysis involves monitoring the distribution of sebaceous material captured within the fingerprint. Fingertip pads may accumulate sebaceous gland secretions due to frequent contact with regions rich in this gland, such as the face.

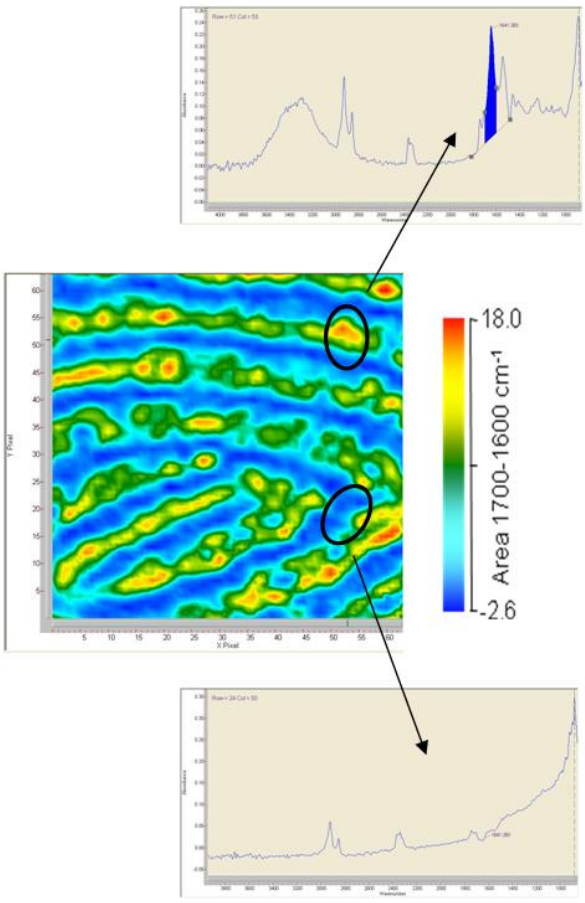

Figure 1. ATR FTIR chemical image and corresponding spectra of the protein distribution within a fingerpad surface. The size of the imaged area is approximately 3.2 × 4.5 mm^2. The image was collected with 16 co-added scans representing a collection time of 13 s at a spatial resolution of ~50 µm.

In this study we explored the use of a commercial gel (BVDA Gelatine Lifter) to collect fingermarks from the surface of a metal door handle[5,6]. Figure 2 illustrates the chemical image of one of these fingermarks based on the distribution of lipids. This image is based on the integrated area between 2855 and 2840 cm^{-1}, which correlates to the νC-H stretch of the sebaceous material captured within the fingerprint. This spectral region is typically used for chemical imaging of fingerprints because interference from the overlapping

absorbance of the substrate can reduce the quality of images at other wavelength ranges. Depth profiling using a variable angle ATR can aid to enhance the images even further by reducing interference from the lifting medium[6].

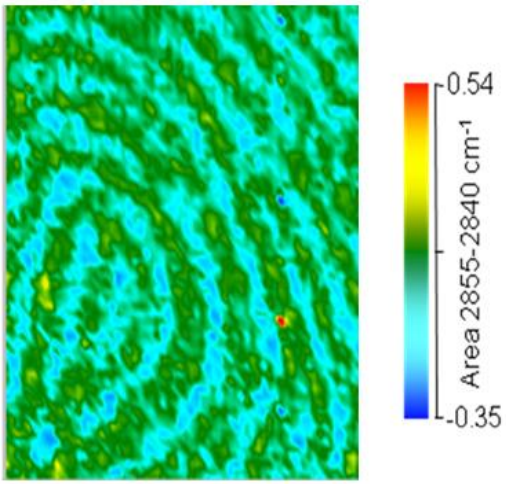

Figure 2. ATR FTIR image of a latent fingermark lifted from a door handle using BVDA Gelatine Lifter[7]. The scale bar on the right shows the integrated value of C-H stretch of sebaceous material between 2855—2840 cm^{-1}. The imaged area was collected with a Pike Vee-Max accessory at an angle of incidence of 44.6° and is approximately 4.3 × 5.9 mm^2.

Tape-lifted samples provide a means of obtaining fingerprints from inaccessible regions of a specimen and permit archiving of the samples.

Macro ATR FTIR imaging and homeland security

The applicability of ATR FTIR imaging to fingerprint analysis can be extended beyond its ability to provide fingerprint identification. The technique can also be used for homeland security applications such as linking a specific individual to a specific act through the detection of exogenous substances found on that person's hands[3,5]. One example involves the detection of trace drug materials that remain on a suspect's hands after drug handling. To model this scenario, the drug paracetamol (also known as acetaminophen) was intentionally handled, and the infrared spectra of contaminated fingerprints were acquired. The chemical image displayed in Figure 3 has been generated based on the absorbance at 1228 cm^{-1} (which is characteristic of a strong vPh-N absorbance band of paracetamol). The imaged area is ~4.3 × 5.9 mm^2 with a spatial resolution of ~50 µm. The highlighted paracetamol particle is of the order of 100 µm in size. Finer pixel configurations (for example, 128 x 128) or other internal reflective elements (for example, germanium) can be used to resolve particles down to 20 µm in size. The chemical image in Figure 3 demonstrates that ATR FTIR spectroscopy can locate and positively identify microscopic particles from a mixture of common materials found on an individual's finger.

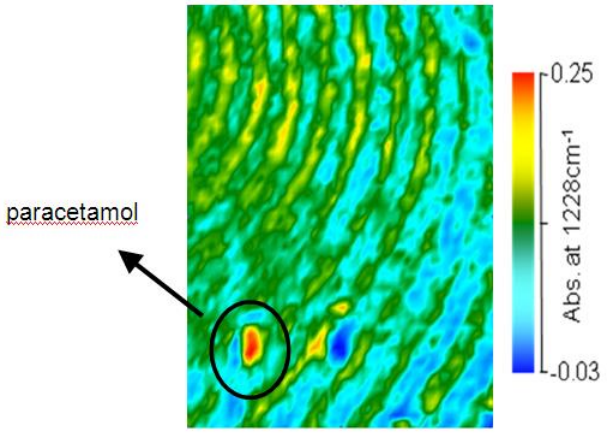

Figure 3. ATR FTIR image of the distribution of the vPh-N band (at 1228 cm^{-1}) on a fingerprint that is contaminated with paracetamol. The imaged area was collected with a Pike Vee-Max accessory at an angle of incidence of 44.6° and is approximately 4.3 × 5.9 mm^2.

Even trace amounts of exogenous substances (down to 20 µm particle size) can be easily located within a fingerprint using macro ATR FTIR imaging. Substances can be identified by comparison of extracted spectra with a library of known standards.

Conclusion

The use of macro ATR FTIR imaging spectroscopy in forensic science has been demonstrated through the measurement of fingermark residues, both directly, and through the use of a lifting medium. Macro ATR FTIR imaging spectroscopy permits fast and easy

analysis of fingerprints, even from regions of a specimen that are difficult to sample.

The technique is particularly useful as it is non-destructive and allows for archiving of tape-lifted samples. It also enables the detection of trace quantities (>20 µm particle size) of exogenous substances (such as drugs, pharmaceuticals, or explosives) on a suspect's fingertips or from tape-lifted samples recovered at a crime scene[3]. Agilent Cary FTIR spectrometers provide excellent infrared energy throughput when coupled to a macro ATR imaging accessory, translating to excellent signal-to-noise performance[7]. When coupled with an FPA[†] detector, this also allows for fast image acquisition at high spatial resolution.

The powerful Resolutions Pro software permits a wide range of analyses to be performed and allows for easy identification and spatial mapping of materials of interest.

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Published March, 2011
Publication Number si-01340

Agilent Technologies

Material analysis by infrared mapping: A case study using a multi-layer paint sample

Application Note

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Introduction

Agilent's 610 FTIR fourier transform infrared (FTIR) microscopes are routinely used for the analysis of heterogeneous materials. They provide an ability to characterize the spatial distribution of components as well as the ability to identify the specific chemical nature of a sample. Agilent's infrared microscopes can be used on both the microscopic and macroscopic scale using multiple measurement modes including:

- transmission
- reflection
- attenuated total reflectance (ATR)
- grazing angle reflection analysis
- 'large sample' mode using Agilent's large sampling side-port accessory

They are ideal for advanced materials characterization as they are simple to use, provide the best sensitivity and versatility, and can be customized to suit a desired area of analysis. By adding a motorized sample stage to an Agilent Cary 610 FTIR single-element detector microscope system, the capabilities can be extended to include automated infrared mapping analysis.

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Infrared mapping allows for multiple infrared spectra to be sequentially acquired from different spatially-resolved points on the same sample and provides both spectral and spatial information, thereby facilitating the study of within-sample chemical heterogeneity. Common infrared mapping applications in material sciences include simple material characterization, the analysis of the homogeneity of coating materials, the investigation of multi-layer sample interfaces such as polymer laminates and paint cross-sections, the automated screening of samples for defects or contamination, the characterization of the total reflectance of optical surfaces and other process control applications.

This paper highlights the simplicity and power of Agilent's Agilent Cary 610 infrared mapping microscope for the rapid and automated analysis of a multi-component paint sample.

Instrumentation

The infrared mapping experiment was conducted using a Cary 610 FTIR spectrometer, equipped with a 610 FTIR infrared microscope (containing a 250 micron single-element, narrow-band Mercury Cadmium Telluride detector and a motorized sample stage) operating under Resolutions Pro 5.0 software. A constant flow of dry air was used to purge the system, limiting the contributions from carbon dioxide and atmospheric water vapor.

The infrared map was collected in reflection-mode using a pre-loaded grid mapping template that was customized to collect a 19 × 19 grid (totaling 361 spectra) using a 20 µm step size from an area measuring 380 × 380 microns. The infrared spectra were sequentially recorded over the range of 4000–700 cm^{-1} at a spectral resolution of 8 cm^{-1} by co-adding 16 scans per point (~40 mins for the entire infrared map).

Sample preparation

The paint chip cross sections were prepared from vehicle paint fragments provided by a police forensic laboratory. Samples were mounted in a clear casting polyester resin, and then polished using a 12,000-mesh Micromesh polishing cloth. The embedded paint fragments were microtomed to a thickness of ~10 µm, and the samples transferred to a standard glass microscope slide that was covered with aluminum foil to allow for reflection/absorption analysis.

Results and discussion

Infrared mapping using Agilent's Cary 610 FTIR Microscope allows for the automated sequential acquisition of hundreds of high-quality infrared spectra from analytical samples. Using Resolutions Pro software, mapping experiments are extremely flexible. Users can either select individual spectral collection locations themselves or use one of several grid mapping templates that can be customized to a sample, saved and re-applied later. In this experiment, a paint fragment found at an automobile crime scene was embedded in a polymer resin, then microtomed to obtain an appropriate sample thickness. This sample was deposited onto the surface of a reflective infrared support slide which was then placed on the motorized stage of the microscope. A visual image of the paint sample was acquired, followed by the sequential collection of the 361 spectra (19 × 19 grid map; 380 × 380 µm area) using automated infrared mapping. The visual image of the sample and the spectral acquisition locations are shown in Figure 1. Each spectrum in the infrared map results from a spatial resolution of 20 µm.

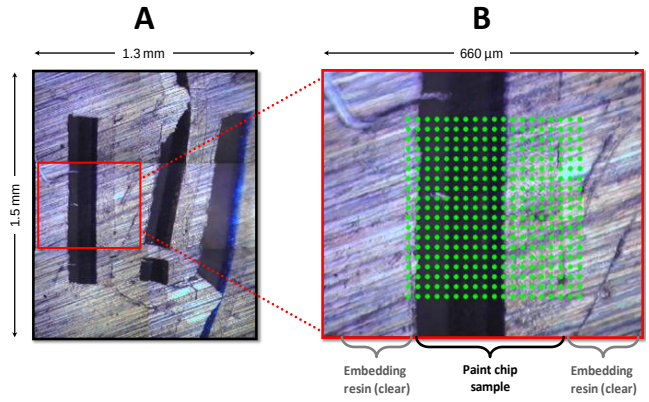


Figure 1. (A) Visual image of 3 sections of a paint chip sample (vertical bars), which were embedded into a polyester resin (clear). The reflective aluminum IR-slide upon which the samples are deposited can be seen through the resin. (B) Higher resolution view of a paint chip sample overlaid with the locations of spectral acquisition (represented by the grid of green circles). The overall area of analysis for the spectral map was 380 × 380 µm, yielding a total of 361 spectra.

The investigation and interpretation of the infrared data was simplified by several intuitive software features. For example, the grid of green circles that is overlaid on the surface of the visual image of the sample can be used to extract spatially resolved data. Simply clicking on a desired sample location (or multiple locations) will fill in the green circle(s) and will display the corresponding IR spectra in the software's 'spectrum' display panel. Spectral peaks of interest can then be compared or used for quantitative analysis, and the selected spectra can be overlaid or stacked to facilitate visual interpretation. Upon cursory visual examination of the forensic evidence in Figure 1, the vertical black strip appeared to be uniform in composition with only minimal variations. However, infrared investigation revealed that the sample is heterogeneous and composed of multiple spatially-resolved vertical layers. Exploratory investigation of the spectra in the map revealed the presence of four chemically distinct layers. In addition, the high spatial resolution of the infrared map allowed for the identification of localized areas with different chemical compositions within the stratified layers. Figure 2 illustrates selected absorbance spectra from the paint chip sample.

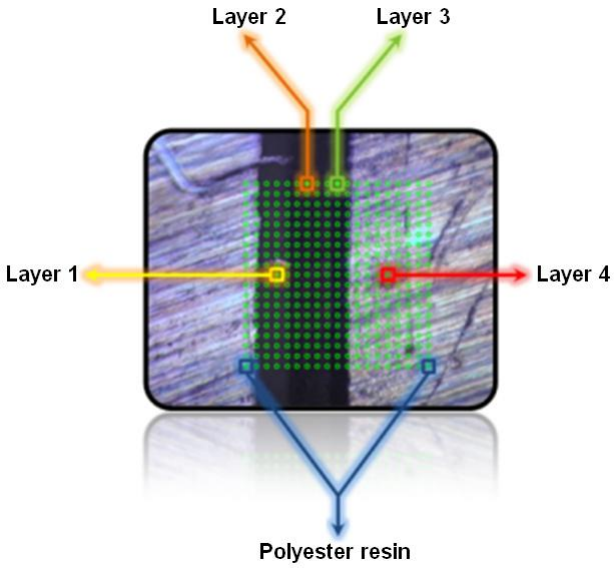
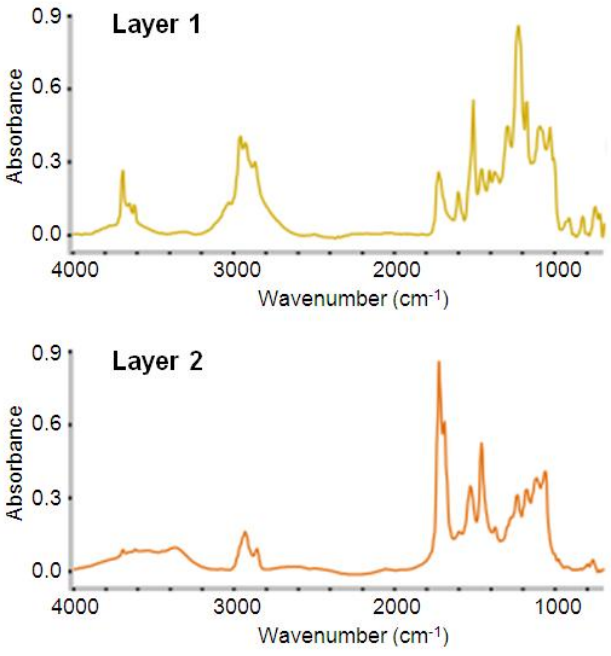
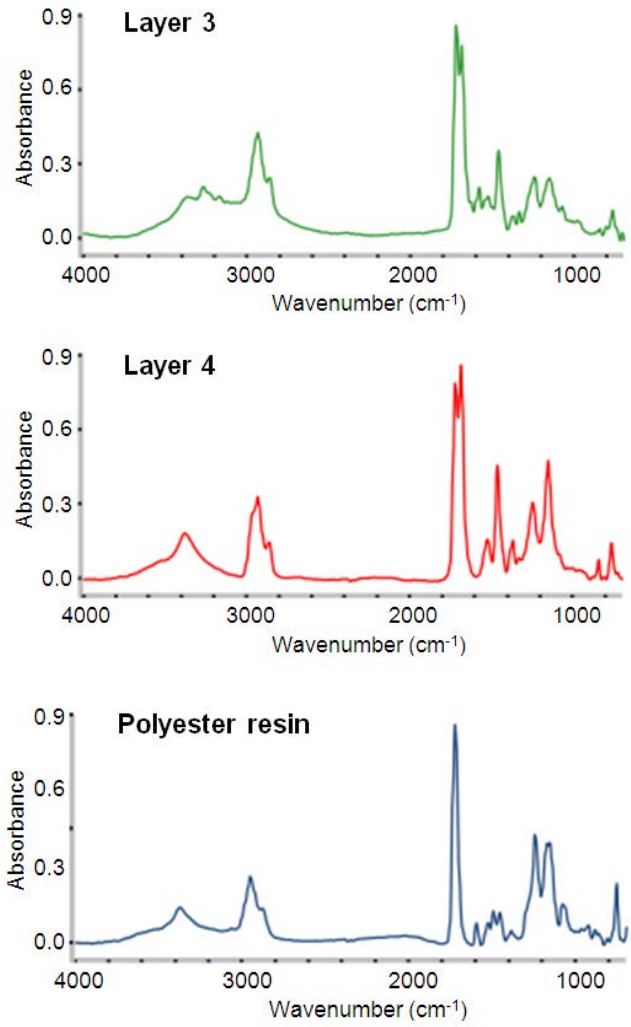


Figure 2. Representative FTIR spectra from the four layers of the paint chip sample as well as a spectrum of the embedding resin. Three of the spatially-resolved layers are in the black vertical bar, while one layer is transparent, as is the polyester resin. See layer spectra in the five images below.





The spectra in Figure 2 are visually distinct and contain sufficient information to allow for the characterization of each individual layer. Based on these spectra, forensic scientists are able to search spectral databases of paint and coating samples to identify the vehicle's make, model, year, and color. In this instance, the ability to detect trace materials in the evidence proved to be very useful in extending the knowledge of the sample's composition far beyond that which could have been obtained by in-bench FTIR experiments or by other analytical techniques.

Without a clear delineation of the layers, it is difficult to study the variations in sample chemistry across the infrared map by using the spectrum display alone. Resolutions Pro software makes it easy to view chemical differences across an entire infrared map of a sample. One means of probing a sample is to generate a feature image based on one or multiple spectral peaks (one or multiple functional groups of interest). A feature image assigns a color to the absorbance value of a selected peak (or spectral region) and plots the intensity across the infrared map to easily view spatially-resolved chemical differences on the visual image of a sample. The color red indicates a high absorbance value, while the color blue indicates a lower absorbance value. Figure 3 shows a feature image generated from a spectral peak that is unique to one layer of the paint chip. It is equally possible to view the feature image without displaying the locations of spectra acquisition, or to view it as a '3D' chemical image as shown in Figure 3.

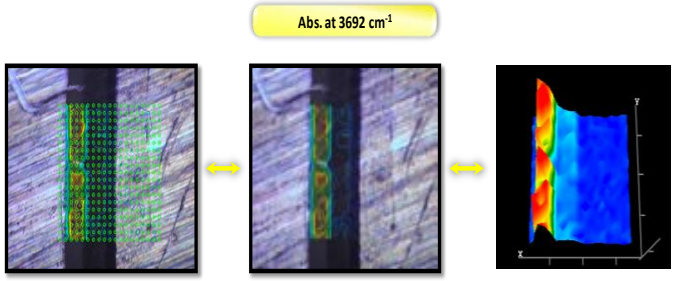


Figure 3. A feature image generated from a spectral peak that is unique to one layer of the paint chip (left), the same feature image shown without the spectral acquisition grid for clarity (center), and the 3-dimensional view of the feature image (right). These images were generated by plotting the intensity of the peak at 3692 cm^{-1} in the spectrum from each pixel across the entire infrared map.

Advantageously, feature images can be generated in real-time using any spectral range or absorbance peak to provide users with a better understanding of a sample's composition. Figure 4 illustrates the feature images generated from the four chemically distinct paint chip layers.

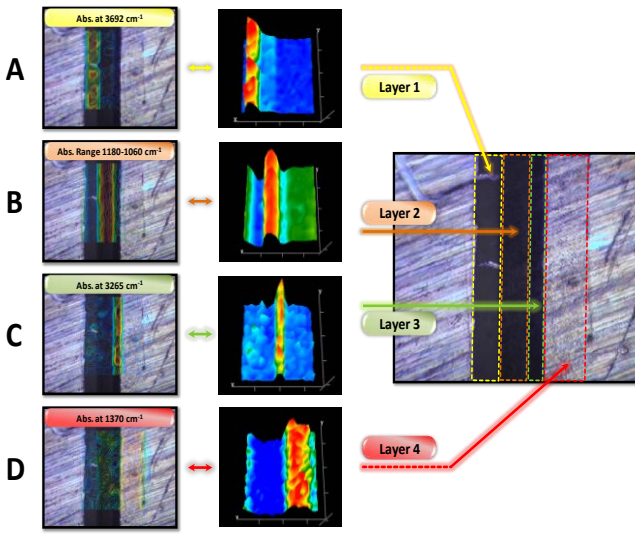


Figure 4. Feature images based on spectral peaks that are unique to each layer in the four-layer paint chip sample. The feature image in 'A' is based on the absorbance of the peak centered at 3692 cm^{-1}, which is primarily found in layer 1 of the paint chip; while the feature image in 'B' was generated from the absorbance peaks between 1180–1060 cm^{-1}, which are largely found in the second layer; 'C' shows the spatial distribution of the absorbance peak centered at 3265 cm^{-1}; while 'D' shows the feature image of the clear coating layer of the paint sample based on the absorbance at 1370 cm^{-1}. Legend for feature images: red = high intensity, green = medium intensity, blue = low intensity.

The chemical image display of the infrared mapping software was particularly useful to highlight the clear external coating of the paint sample, designated by layer 4 in Figure 4D. Depending on the visible contrast of a sample, it is occasionally easier to view the distribution of a selected spectral peak (or range) in different feature image views. From the feature images it is a simple task to estimate the approximate width of each stratified vertical layer; layer 1 is ~80 µm, layer 2 is ~80 µm, layer 3 is ~40 µm, while layer 4 is ~120 µm. It is equally possible to probe the heterogeneity within each layer for an improved characterization of the sample. For example, layer 1 in Figure 4A is not uniform in chemical composition and has a number of visible defects that can also be observed in the visible and feature images. With Resolutions Pro software, it is simple to investigate the chemical differences between adjacent spectra by displaying spectra simultaneously. However, for a more in-depth understanding of the samples' heterogeneity on the micro-scale, a higher spatial resolution infrared image would be required.

An alternate approach to acquiring IR spectra with a significantly higher spatial resolution involves the use of an infrared imaging system equipped with a focal plane array (FPA*) detector. An FPA-FTIR system would provide a superior means of investigating the subtle chemical differences found in each layer of the paint sample. Unlike infrared mapping using a single-element detector, an FPA* detector collects hundreds to thousands of spectra simultaneously within seconds, thereby providing dramatic savings in spectral acquisition time compared to infrared mapping techniques that perform sequential data collection. In practical terms, this infrared map required ~40 minutes acquisition time to collect 361 spectra for the area of 380 × 380 µm using a 20 µm spatial resolution; comparatively, Agilent's 128 × 128 FPA-FTIR system could acquire over 16,000 spectra with an identical signal-to-noise ratio from an area of 700 × 700 µm within a few seconds using an even higher spatial resolution of 5.5 µm per spectrum.

In addition, Agilent's FPA-FTIR imaging spectrometers have a number of easily user-changeable spatial resolution modes including: 1.1 µm (ATR Analysis), 5.5 µm, 11 µm, 22 µm and even larger sizes with pixel binning or macro imaging (for example, >40 µm). FPA-FTIR analysis would involve the same minimal sample preparation and could be used to reveal even the smallest features of the forensic evidence sample.

While this experiment focused on the characterization of a sample obtained from a crime scene, the application of FTIR microscopy and mapping in paint analysis extends far beyond forensic applications. They are commonly used for the characterization of historical art works, and for the development of conservation and preservation strategies for paintings and photographs. FTIR microscopy and mapping are equally important in the QC analysis of raw materials used in the manufacture of paints and inks, and are routinely applied to the analysis of resins, pigments, solvents and additives.

Conclusion

Agilent's Cary 610 FTIR Microscope provides the ability to collect high quality chemical information from multi-layer samples with a high spatial resolution. It provides an excellent means of probing a sample's chemistry as it can be used to visualize the relative distribution of specific components across a sample area of several centimeters. In this experiment, a 380 × 380 µm infrared map was automatically collected using a pre-defined acquisition grid to investigate the chemical heterogeneity of a paint chip sample. Four chemically distinct layers were resolved in the forensic evidence, including a miniscule layer measuring ~40 µm.

Feature images also were used to highlight each layer within the infrared map and to probe localized areas with varying chemical compositions within the stratified layers. The rapid nature and the simplicity of automated infrared mapping make it a key technique for the advanced characterization of material and polymer samples.

References

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Published March, 2011
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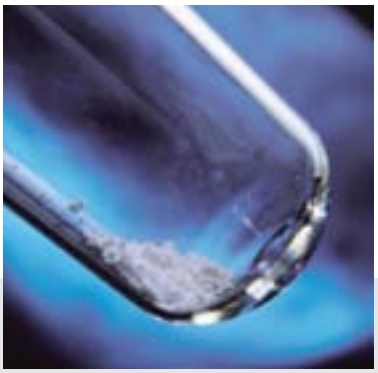
- Quick Explosives Identification using GC/MSD with TSP
- Determination of Synthetic Cannabinoids in Incense Products and Herbal Blends



Applications by Technique

GC/MS

Agilent Explosives ID solution

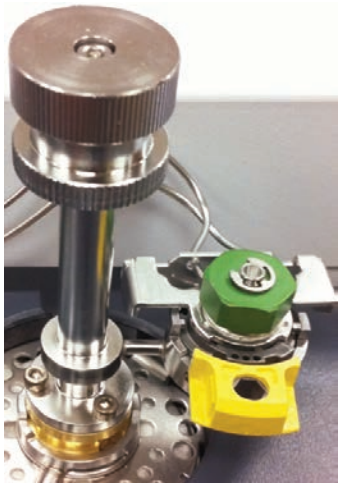
Quick Explosives Identification using GC/MSD with TSP

Forensics

The transportable Agilent 5975T GC/MSD, together with a rugged Thermal Separation Probe (TSP) provide fast, accurate identification. The TSP offers a rapid, rugged, and inexpensive approach with no sample preparation required for fast analysis of explosives. This fast analysis of explosives solution could be used in the lab and on-site mobile lab.

Description of Industry Application

There is increasing pressure to reduce time to identify explosives without sacrificing analytical quality. High explosives encountered in the forensic laboratory may be either pure or nearly pure compounds: nitroaromatics, nitrate esters, nitramines, or mixtures of these with or without other ingredients.



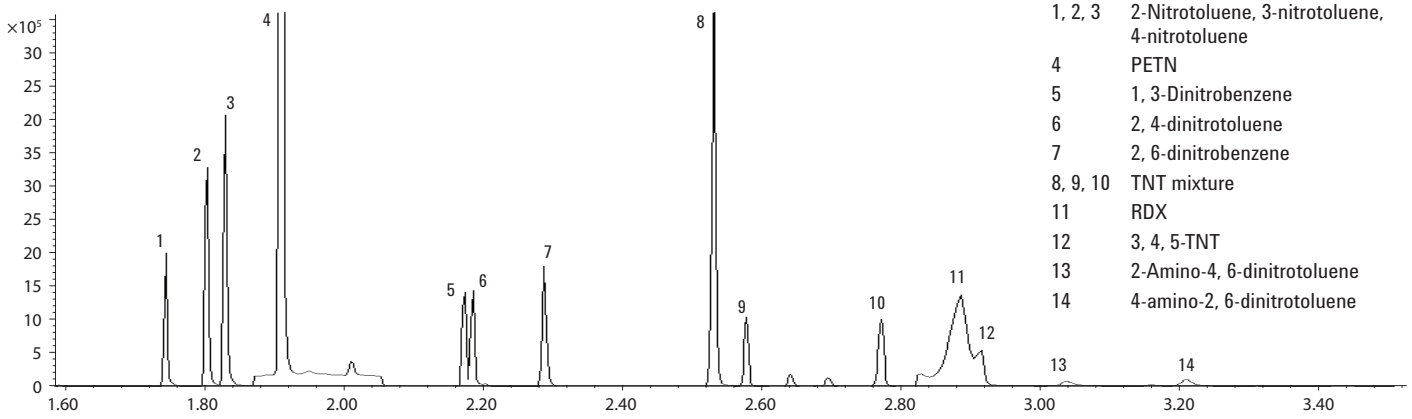
The Agilent 5975T Low Thermal Mass (LTM) with the Thermal Separation Probe (TSP) is the perfect instrument for this task either in the lab or in the field. The TSP requires little or no sample preparation, just measure the sample and start the run. The 5975T LTM GC/MSD utilizing short narrow-bore capillary columns with a quick ramp heating oven rate and fast cooling cycle provides, further improves run times to create an ultra-fast sample cycle.

Either when police find suspected explosives powders or after an explosion, the analysis can be made quickly. In either situation, they just take a small sample of powder or soil sample with a high concentration of explosives for quick measurement by 5975T and TSP, no sample preparation required, the results could be gotten within several minutes.

Key Benefits

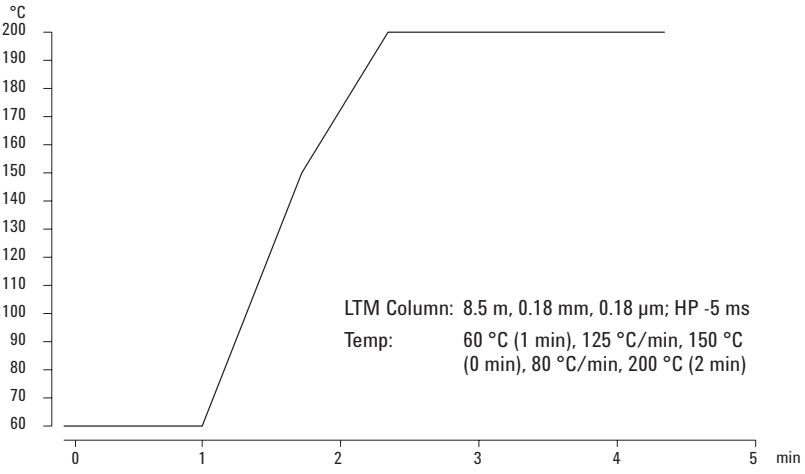
- On-site measurement with the Agilent 5975T GC/MS
- Agilent Thermal Separation Probe (TSP) minimizes sample preparation time
- Agilent 5975T Low Thermal Mass (LTM) GC/MS provides fast temperature ramp rates for short cycle times

Running time < 3 min!



Soil matrix, SQCI-011

1, 2, 3	2-Nitrotoluene, 3-nitrotoluene, 4-nitrotoluene
4	PETN
5	1, 3-Dinitrobenzene
6	2, 4-dinitrotoluene
7	2, 6-dinitrobenzene
8, 9, 10	TNT mixture
11	RDX
12	3, 4, 5-TNT
13	2-Amino-4, 6-dinitrotoluene
14	4-amino-2, 6-dinitrotoluene



LTM Column: 8.5 m, 0.18 mm, 0.18 µm; HP -5 ms

Temp: 60 °C (1 min), 125 °C/min, 150 °C (0 min), 80 °C/min, 200 °C (2 min)

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FORENSICS & TOXICOLOGY

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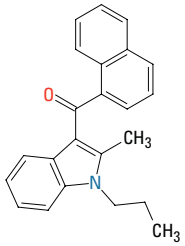

JW-015

The rapid proliferation of synthetic cannabinoid analogs and homologs in combination with the growth in popularity of synthetic cannabinoid use among teens and young adults is of serious concern. The structural similarity and isomeric forms of these cannabinoids in conjunction with the botanical substrate and the lack of reference materials for use in positive identifications present obstacles to analysis. Forensic laboratories are challenged to find trace-level cannabinoids in complex chromatographic data and identify the subtle differences between cannabinoid species that yield very similar retention times and mass spectra.

To help laboratories overcome these obstacles, Agilent Technologies, in collaboration with the Criminalistics Division of NMS Labs, has developed and validated an analytical method including a sample preparation and extraction protocol, as well as a supporting compendium and searchable mass spectral library of over 35 synthetic cannabinoids and their derivatives. The resulting method and library provides an effective and easy-to-replicate approach to the identification of synthetic cannabinoids in herbal incense blends by GC/MS. The compendium, library and all supporting electronic method files needed to perform the analysis are available from Agilent free-of-charge, at www.agilent.com/chem/cannabinoidcd.

Compounds

- JWH-015, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-133, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398
- HU-210, HU-211, HU-308, HU-331
- CB-25, CB-52
- CP47,497 (C7 analog), CP47,497 (C8 analog)
- CP55,940
- AM-694, AM-2201
- RCS-4, RCS-8
- WIN55,212-2, WIN55-212-3

Determination of Synthetic Cannabinoids in Incense Products and Herbal Blends

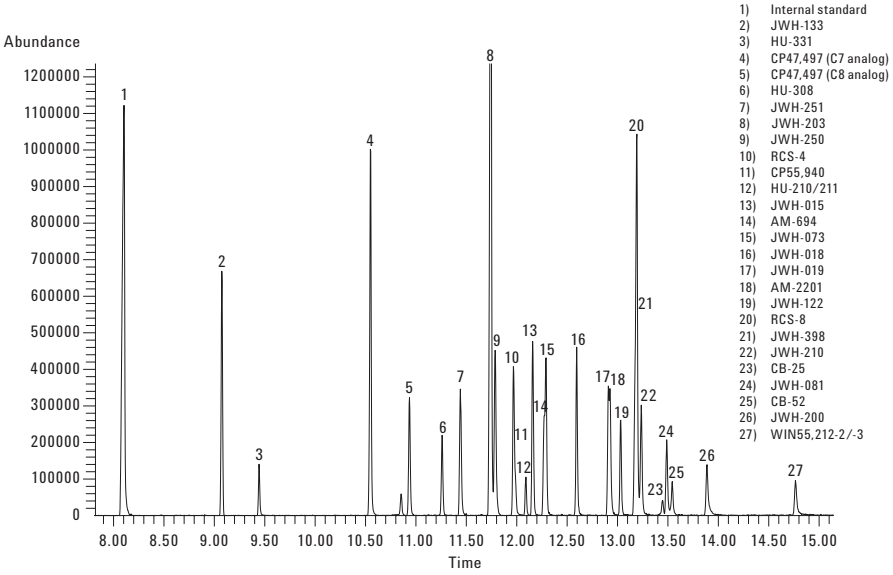

Figure 1. GC/MS Total Ion Chromatogram of the Synthetic Cannabinoids Incorporated in the Method.

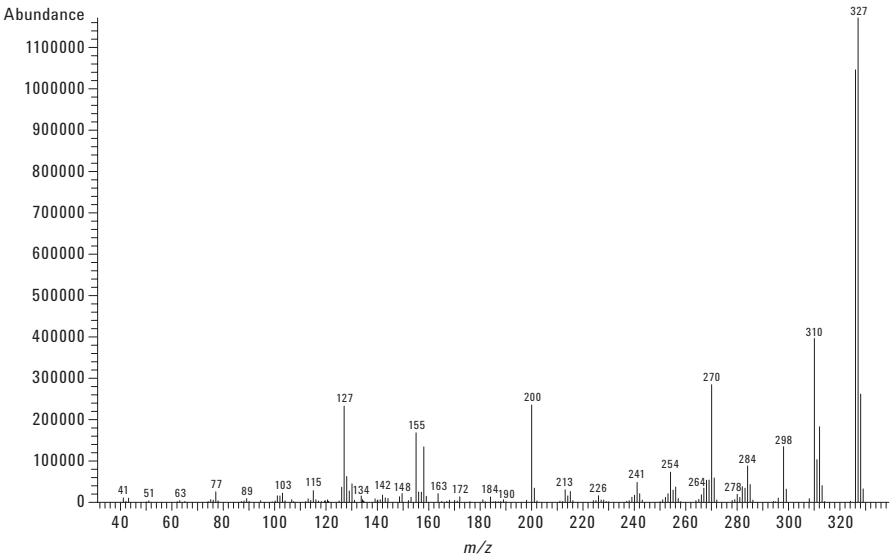

Figure 2. Mass Spectrum of JWH-015.

Key Benefits

Developed in collaboration with the Criminalistics Division of NMS Labs, an ASCLD accredited laboratory, a cd-rom is available, which contains:

- Validated analytical method, including sample preparation
- GC/MS library of synthetic cannabinoids
- Deconvolution Reporting Software (DRS) library is available to facilitate data interpretation
- Electronic method and library files for rapid start-up
- Compendium of synthetic cannabinoids with mass spectra

Learn more:
The compendium and mass spectral library can be requested at
www.agilent.com/chem/cannabinoidcd

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Published in USA, August 24, 2011
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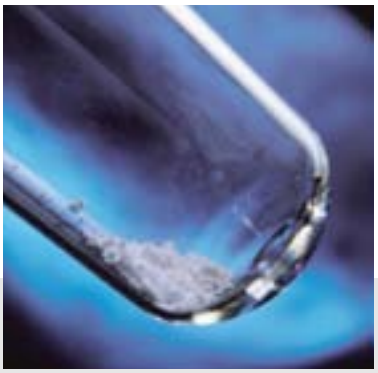

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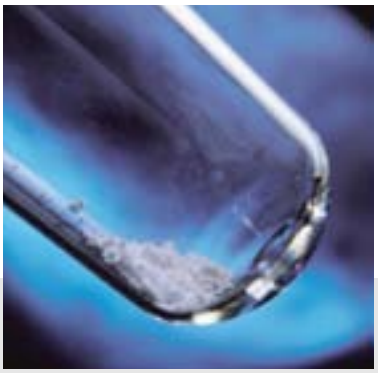
- Fast Analysis of Illicit Drug Residues on Currency using Agilent Poroshell 120



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- Analysis of TNT, RDX, and CL-20 by APCI LC/MS/MS
- Detection of Gasoline in Fire Debris by GC/MS/MS
- HPLC Analysis of Explosives Using EPA Method 8330
- An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensics/ Toxicology Database
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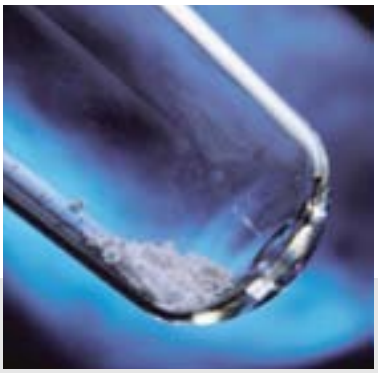
- The First Accurate Mass MS/MS Library for Forensics and Toxicology Using the Agilent 6500 Series Accurate Mass Q-TOF LC/MS
- Quick Explosives Identification using GC/MSD with TSP



Applications by Matrix

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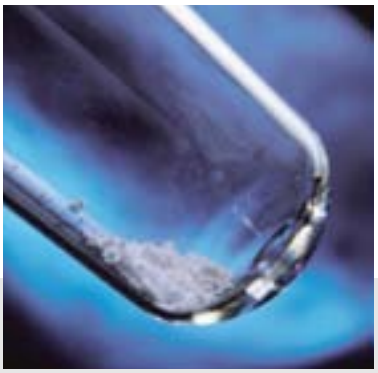

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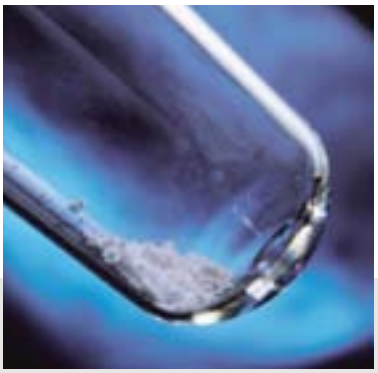

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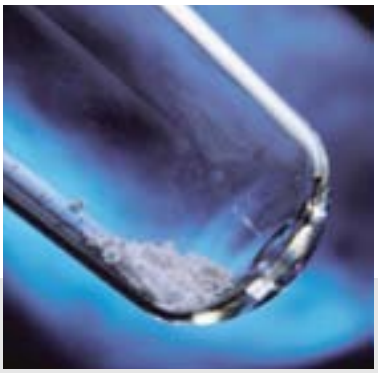

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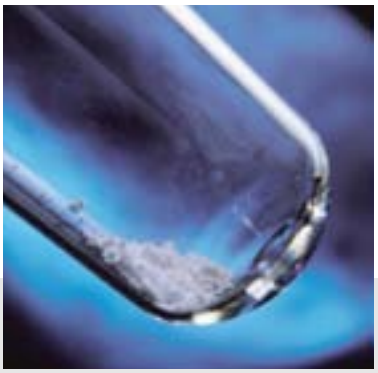

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Trace Analysis

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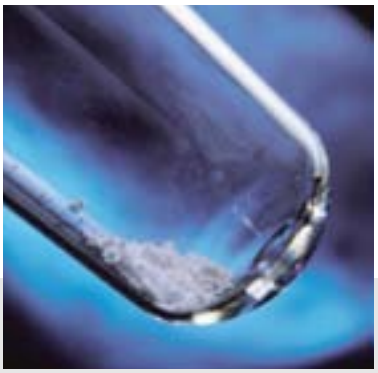

Productivity Tools

Improving Productivity and Extending Column Life with Backflush

Application Brief

Chin-Kai Meng

All Industries

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, μECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

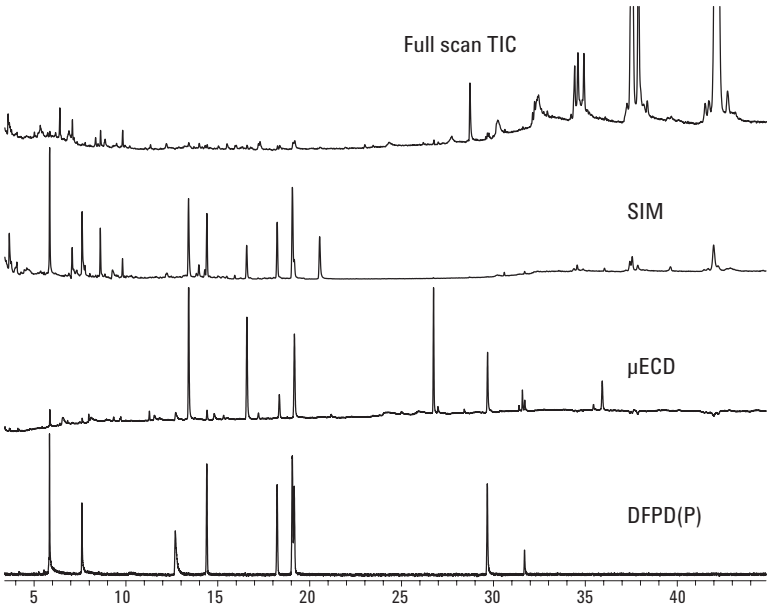

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

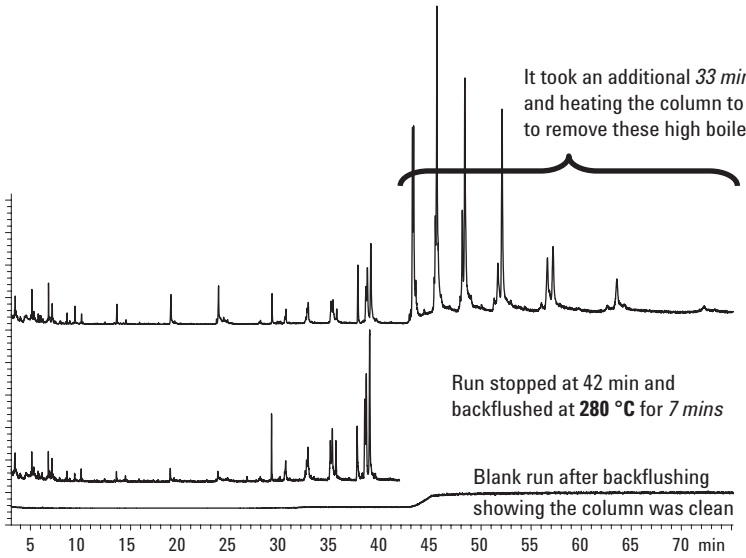

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Agilent Application Note, 5989-3299EN, July 2005.
2. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Application Note, 5989-5111EN, June 2006.

Acknowledgement

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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Printed in the USA
December 26, 2006
5989-6018EN

Improved Data Quality Through Automated Sample Preparation

Application Note

Authors

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Abstract

Sample preparation tasks can be extremely time-consuming and are often prone to errors, leading to poor reproducibility and accuracy. Many of these tasks, such as calibration curve generation, sample dilution, internal standard addition, or sample derivatization are performed daily, requiring significant resources as well. The Agilent 7696 Sample Prep WorkBench can perform many common sample prep tasks with better accuracy and precision than most manual methods, while using significantly fewer reagents and requiring less time from the operator. To demonstrate this, three sample preparation tasks were adapted for use on the Agilent 7696 Sample Prep WorkBench and yielded the same, if not better, results than the manual methods for accuracy and precision.

Agilent Technologies

Introduction

The Agilent 7696 Sample Prep WorkBench can perform many sample preparation tasks for either gas chromatographic (GC) or liquid chromatographic (LC) analyses. The Agilent 7696 Sample Prep WorkBench consists of two liquid dispensing modules, a single vial heater capable of reaching 80 °C, a single vial mixer, and barcode reader (Figure 1). This enables dilutions/aliquoting, liquid addition, heating for derivatization or digestion, liquid/liquid extractions, and sample mixing. Individual racks can also be heated and/or cooled. This sample preparation instrument can perform tasks with the same accuracy and precision as the Agilent 7693A Automatic Liquid Sampler only in an offline setting instead of on top of a GC [1]. Many sample preparation tasks such as sample dilution, calibration curve standard generation, and sample derivatization within both fields can be time consuming and resource intensive. Automating these procedures with the Agilent 7696 Sample Prep WorkBench therefore is beneficial in many ways.

Figure 1. The Agilent 7696 Sample Prep WorkBench.

A side-by-side comparison of manual and automated methods was performed for three common sample prep applications to demonstrate the improved data quality achieved through automated sample preparation. Sample dilution, calibration curve standard generation, and derivatizations were performed with success on the Agilent 7696 Sample Prep WorkBench.

Experimental

Three common sample preparation tasks were performed with the Agilent 7696 Sample Prep WorkBench. First, sample dilutions and internal standard additions were performed for analysis by both GC and LC. For the GC samples, 50 µL each of isooctane and a standard solution containing four analytes were added to an empty 2-mL autosampler vial. Additionally 0.5 µL of an internal standard solution (ISTD) containing three analytes was added to the vial. The solution was mixed using the onboard mixer before transferring the vials to a GC for analysis. The samples for LC followed a similar procedure. To an empty 2-mL autosampler vial, 187.5 µL of acetonitrile, 62.5 µL of a pesticide standard, and 125 µL of an ISTD were added. The sample was mixed before being transferred to an LC for analysis. For both of these sample dilutions, n=10.

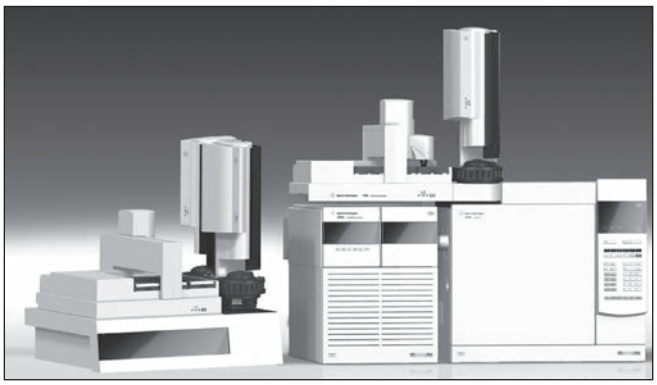

Figure 2. The Agilent 7696 Sample Prep WorkBench with a gas chromatograph and mass spectrometer.

Second, generic calibration curves for the GC were made in triplicate via linear dilution both manually in 10-mL volumetric flasks and with the Agilent 7696 Sample Prep WorkBench. To make the standards manually, small amounts of hexane was added to six clean, dry 10-mL volumetric flasks. Varying amounts of a stock solution containing five analytes at 5 mg/mL, ranging from 0.1 to 1 mL, were added using serological pipets. The flasks were diluted to the mark with hexane to yield concentrations of 50, 100, 200, 300, 400, and 500 ppm. For the automated method, 100 µL of hexane was added to six empty 2-mL autosampler vials. Again, varying amounts of the stock solution, ranging from 1 to 10 µL, was added to the vials yielding approximately the same concentrations.

Figure 3. The Agilent 7696 Sample Prep WorkBench with a liquid chromatograph.

Third, derivatization of fatty acids via silylation reaction was performed. For the manual prep, 100 µL of a silylating reagent was added to approximately 0.5 mL of a free fatty acid solution using an automatic pipettor. The solutions were heated to 70 °C using a heated block. The same derivatization was performed with the Agilent 7696 Sample Prep WorkBench using the single vial heater.

Results and Discussion

GC and LC Sample Dilution

For the 10 samples diluted for GC and LC analysis, the dispensed solvent, standard solution, and ISTD, was measured gravimetrically to determine the reproducibility of the dispensing action. Dispensing 50 µL with a 250 µL syringe results in a 0.5% relative standard deviation (RSD) for the 10 samples measured by weight. The samples were diluted within 1% accuracy, determined from the peak areas. The ISTD exhibited a slightly higher RSD. Dispensing 0.5 µL with a 25 µL syringe resulted in an RSD of 2% for the 10 samples. If a smaller syringe had been used to dispense the ISTD, a lower RSD, closer to that obtained when dispensing the solvent and standard, would have resulted. The added ISTD did not affect the accuracy of the diluted sample (Figure 4).

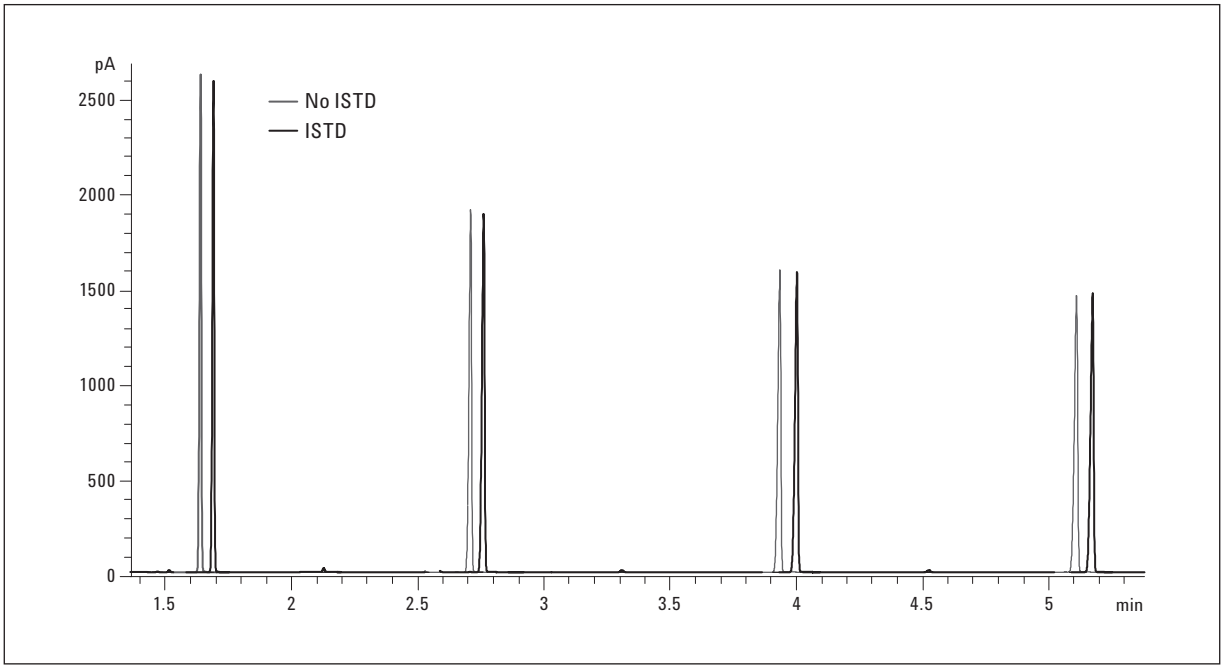

Figure 4. GC chromatograms (slightly offset) are shown for a standard solution dispensed and diluted with and without an ISTD added. No difference in peak areas are observed.

For the 10 samples diluted for LC analysis, similar results were obtained. Dispensing all three volumes with a 250 µL syringe resulted in a RSD of <0.5%, determined gravimetrically. By examining the peak areas after analysis, the dilutions were found to be accurate within 2% (Figure 5).

Calibration Curve Standard Preparation

Three sets of standards were made both manually and with the Agilent 7696 Sample Prep WorkBench. Comparing the three standard sets on the same plot highlighted the increased reproducibility of the Agilent 7696 Sample Prep WorkBench (Figure 6). While each individual curve yielded R^2 values of 0.999, when plotted together the R^2 value was reduced to 0.934 for the manually prepared standards. In contrast, the three curves prepared by the Agilent 7696 Sample prep WorkBench also yielded R^2 values of 0.999 for the individual curves, but when plotted together, the R^2 value was only reduced to 0.997.

Additionally, the relative response factor (RRF) was calculated for each set of standards. Calculating the RSD of the RRFs provides a measure of linearity and reproducibility. The individual calibration curves yielded good RSDs (<5%), demonstrating linear relationships. However, when comparing the three calibration curves together the superiority of the 7696 Sample Prep WorkBench made standards is evident. The average RSD of the RRFs for the three curves made manually was 16%; the three calibration curves made with the 7696 Sample Prep WorkBench gave an average RRF RSD of 4%.

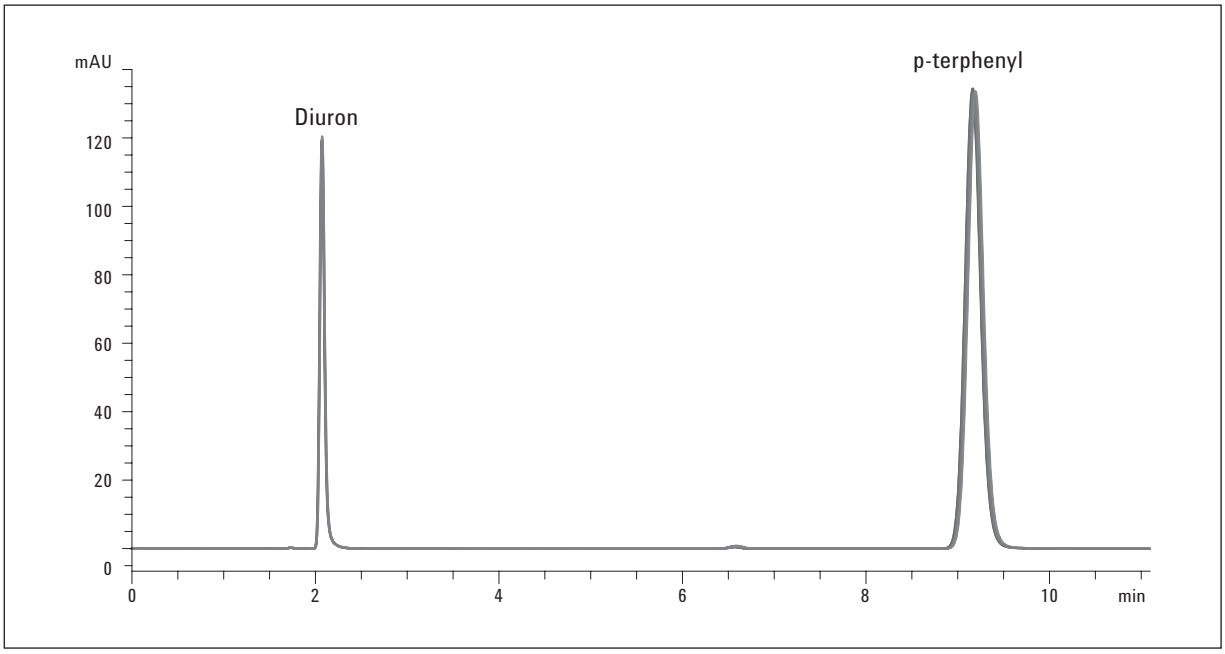

Figure 5. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

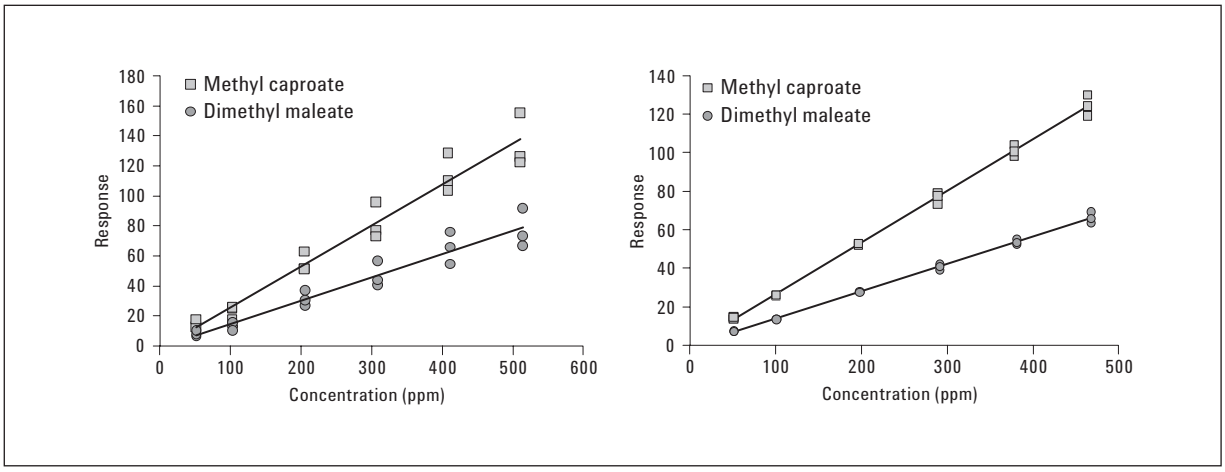

Figure 6. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with the Agilent 7696 Sample Prep WorkBench, are visibly more reproducible than the curves made manually on the left.

Fatty Acid Derivatization

For sample derivatization, identical results were obtained whether the sample was derivatized manually or with the Agilent 7696 Sample Prep WorkBench. For a set of four fatty acids, no discrimination was observed in either method when derivatizing with a silylating reagent (Table 1). However, as seen with other sample preparation tasks, the Agilent 7696 Sample Prep WorkBench is more reproducible in its liquid delivery. The RSD from the peak areas for the three samples prepared manually 0.9%. The RSD for the three samples prepared with the Agilent 7696 Sample Prep WorkBench was 0.7%.

Table 1. After normalizing the fatty acid peak areas to myristic acid, no discrimination was observed from automating the derivatization

Analyte	Ratio-manual	Ratio-automated
Capric acid	0.92	0.92
Capric acid	1.2	1.2
Myristic acid	1.0	1.0
Palmitic acid	1.1	1.1

Conclusions

The three sample preparation tasks presented in this application note highlight the increased reproducibility achieved by automation with the Agilent 7696 Sample Prep WorkBench. Sample dilutions are accurate and reproducible, calibration curve standards are more linear with fewer errors, and sample derivatizations can be performed without analyte discrimination. However, additional benefits can be reaped through sample prep automation with the Agilent 7696 Sample Prep WorkBench.

By automating calibration curve standard preparation, solvent and reagent usage is significantly reduced. Instead of using >60 mL of solvent to make up standards in 10-mL flasks, only 600 µL of solvent was used, excluding the wash vials. This can result in substantial cost savings for laboratories. Additionally, calibrations curve standards required approximately half the time to complete with the Agilent 7696 Sample Prep WorkBench, compared to making up the standards manually. While the other automated sample prep tasks require the same amount of time to complete as the manual methods, the Agilent 7696 Sample Prep WorkBench frees the operator to perform other tasks, such as experiment design or data analysis.

Overall there are many benefits to sample prep automation with the Agilent 7696 Sample Prep WorkBench. While freeing personnel to perform other tasks and reduced solvent usage are important, the largest benefit comes from the reproducibility and accuracy achieved with this system. The automated methods showed better reproducibility and accuracy with fewer errors, thereby improving the quality of the data.

Reference

1. Susanne Moyer, Dale Synder, Rebecca Veeneman, and Bill Wilson, "Typical Injection Performance for the Agilent 7693A Autoinjector," Agilent Technologies Publication 5990-4606EN.

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Printed in the USA
December 2, 2010
5990-6874EN

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Retention Time Locking: Concepts and Applications

Application

Gas Chromatography

December 1997

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Abstract

The concepts and applications of retention time locking (RTL) are described. RTL simplifies the process of transferring methods from chromatographic instrument to chromatographic instrument, column to column, and detector to detector. The analysis of impurities in styrene according to ASTM D 5135 is used to demonstrate the efficacy of the approach. Using RTL, the retention times matched within an average of 0.16% (0.02–0.03 minute) in constant pressure modes.

Key Words

Retention time locking, method validation, styrene analysis, ASTM D 5135, capillary gas chromatography, laboratory productivity

Introduction

Retention time is the fundamental qualitative measurement of chromatography. Most peak identification is performed by comparing the retention time of the unknown peak with that of a standard. It is much easier to identify peaks and validate methods if there is no variation in the retention time of each analyte.

However, shifts in retention time occur frequently. Routine maintenance procedures such as column trimming alter retention times. In a multi-instrument laboratory running duplicate methods, the retention times for each instrument will differ from each other, even when run under nominally identical conditions. These differences in retention times mean that each instrument must have a separate calibration and integration event table, making it time-consuming to transfer methods from one instrument to another. Differences in retention time also complicate comparison of data between instruments and over time.

Retention time locking (RTL) is the ability to very closely match chromatographic retention times in any Agilent 6890 gas chromatograph (GC) system to those in another 6890 GC system with the same nominal column.

There are several subtle effects that combine to cause retention time differences between similarly configured GC systems. Columns of the same part number can vary slightly in length, diameter, and film thickness.

GC pneumatics can have small variations in the actual inlet pressure applied at a given setpoint. The actual temperature of the GC oven also has minute but real deviations from the indicated value. The sum of these and other effects result in the observed retention time differences between similarly configured GC systems.

The pneumatics and oven temperature control of the 6890 GC have advanced the state of the art in GC hardware accuracy and precision. Agilent's advances in fused silica capillary column technology have resulted in highly reproducible column-to-column retention characteristics. With these advances, retention time precision for a given peak in a single GC setup is usually better than 0.01 minute. However, even with these advances in columns and instrument hardware, the sum of the effects mentioned above can cause retention time differences between identically configured GC systems of as much as 0.4 minute.

It would be impractical to control all of the instrument and column variables to a degree where retention time differences between similarly configured GC systems are removed. There is, however, a means of greatly reducing these differences. By making an adjustment in the inlet pressure, the retention times on a given GC setup can be closely matched to those of a similarly configured GC system. RTL is based on this principle. The process of RTL is to determine what adjustment in inlet pressure is necessary to achieve the desired match in retention times. Agilent RTL software (G2080AA), which integrates into the Agilent GC ChemStation (version A.05.02 or later), provides the tool required to determine the correct inlet pressure quickly and simply.

There are several advantages gained by using RTL in the laboratory. Peak identification becomes easier and more reliable. It is easier to compare data both between instruments and over time. Comparison of data when using different detectors for analyte identification is simplified. Transferring methods from instrument to instrument or laboratory to laboratory is easier because calibration time windows normally will not require readjustment. Validation of system performance is easier. With "locked" GC methods, the development and use of retention time data bases for unknown identification is much more straightforward.

To maintain a locked method, RTL should be performed whenever:

- The column is changed or trimmed
- The method is installed on a new instrument
- A detector of different outlet pressure is used
- System performance is validated
- Troubleshooting chromatographic problems

To lock a given method for the first-time or for the reasons below, one must first develop a retention time versus pressure (RT vs. P) calibration.

Even when using columns with the same part number (same id, stationary phase type, phase ratio, and same nominal length), separate/different locking calibration curves are needed when using:

- Systems with different column outlet pressures (FID/atmospheric, MSD/vacuum, AED/elevated)
- Columns differing from the "nominal" length by more than 15% (e.g., due to trimming)
- Systems where the predicted locking pressure falls outside the range of the current calibration

A specific solute (usually one found in the normal method calibration standard) must be chosen and then used for both developing the locking calibration and locking all future systems. The solute, or target peak, should be easily identifiable, symmetrical, and should elute in the most critical part of the chromatogram. Solutes that are very polar or subject to degradation should be avoided.

Once the target solute has been chosen and all other chromatographic parameters of the method have been determined, five calibration runs are performed. The runs are made at conditions identical to the nominal method except that four of the runs are made at different pressures. The pressures used are typically:

- Target pressure – 20%
- Target pressure – 10%
- Target pressure (nominal method pressure)
- Target pressure + 10%
- Target pressure + 20%

The retention time of the target compound is determined for each run. The resulting five pairs of inlet pressures and corresponding retention times are entered into the ChemStation software to generate an RTL calibration file.

Figure 1 shows the dialog box used to enter the calibration data. After the data is entered, a plot is displayed, as shown in figure 2. The maximum departure of the fitted curve from the data is given for both time and pressure. If the fit is acceptable, the retention time versus pressure calibration is stored and becomes part of the GC

method. This calibration need only be generated once. Subsequent users of the method can use this calibration when running the method on a similar instrument setup, regardless of location.

To relock a system or lock a new one:

1. Set up the method conditions and run a standard containing the target compound.
2. Enter the actual retention time of the target compound into the "(Re)Lock current method" dialog box (see figure 3).
3. Update the 6890 method with the new calculated pressure, and save the method.
4. Validate the retention time lock by injecting the standard at the new pressure, and compare the retention time obtained to the desired retention time.
5. Repeat steps 2 to 4, if necessary.

A Note on Constant Flow versus Constant Pressure Modes of EPC Operation

Many GC chromatographers prefer to use the "constant flow mode" of EPC operation. In this mode, inlet pressure increases automatically to maintain constant outlet flow rate as the oven temperature increases during the run. Constant flow mode reduces run time and ensures that flow-sensitive detectors see a constant column effluent flow.

The "constant pressure" mode of EPC operation is also popular. In this mode, the pressure remains constant during the run (outlet flow will decrease as temperature increases). For those wishing to reduce run time in constant pressure mode, a higher pressure can be chosen. For

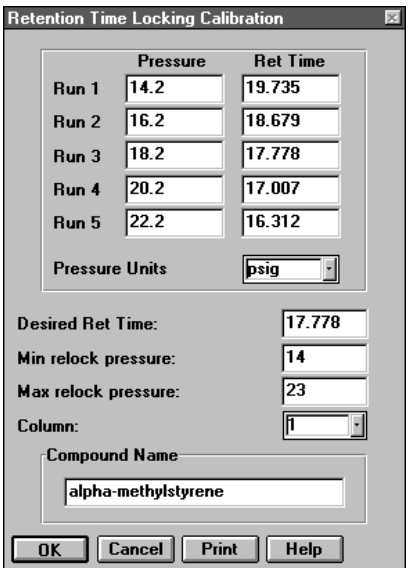

Figure 1. Dialog box used for entering retention time locking calibration data

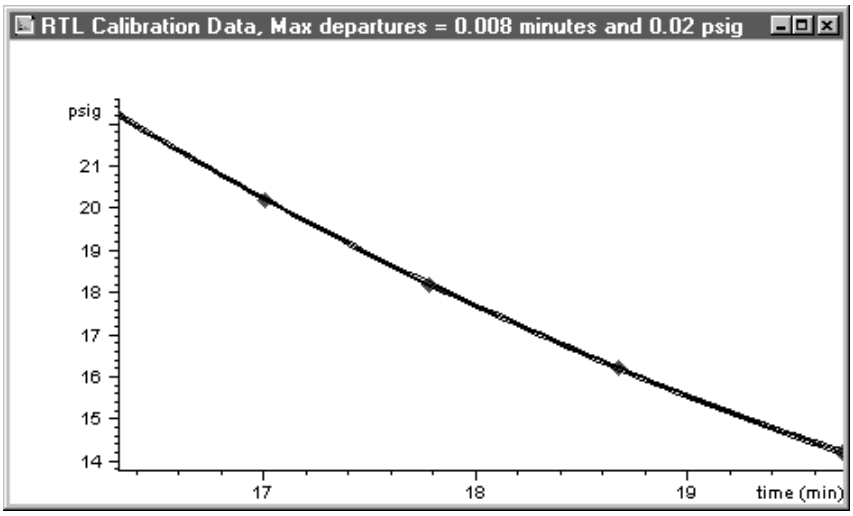

Figure 2. Plot of calibration data as displayed by RTL software

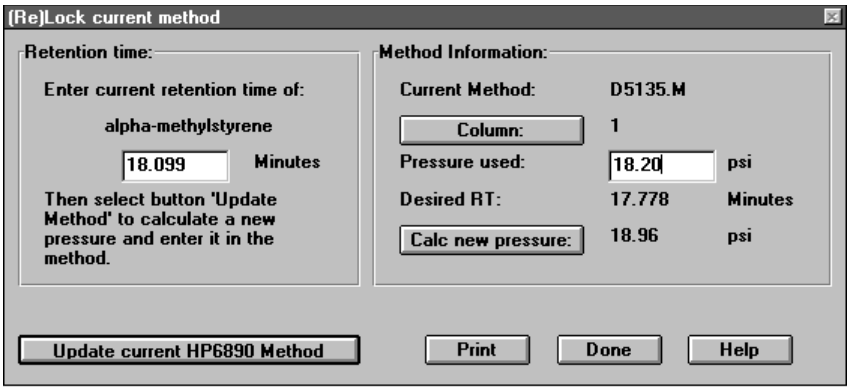

Figure 3. Dialog box used to calculate locking pressure and update the 6890 method

flow-sensitive detectors, one can set "constant column flow + makeup" via the 6890 keyboard or ChemStation. In this mode, the makeup flow is increased as the column flow decreases to keep the sum of the two constant.

The underlying theory of RTL predicts that constant pressure mode of EPC provides the closest matching of retention times. If one desires to compare data from systems with very different configurations, such as GC/FID to GC/MSD, it is best to use constant pressure mode. As can be seen from the styrene analysis data herein, retention time matching between systems of the same configuration (GC/FID, in this case) is still quite good in the constant flow mode.

This application note shows the use of RTL to lock retention times between multiple chromatographic instruments, columns, and detector types and demonstrates RTL in both constant flow and constant pressure modes.

Experimental

Two 6890 Series GC systems were used. Each system was equipped with:

- Electronic pneumatics control (EPC)
- Split/splitless inlet (250 °C, He carrier gas, split 80:1)
- Automatic liquid sampler
- GC ChemStation (version A.05.02)
- Flame ionization detector (FID)
- 60 m × 0.32 mm, 0.5 mm HP-INNOWax column (part no. 19091N-216)
- Temperature program: 80 °C (9 min), 5 °C/min to 150 °C

The inlet pressures/flows used are indicated with each chromatogram.

A third 6890 Series GC was also used. This system was equipped with an Agilent 5973 mass selective detector (MSD) and was used for peak identification. The GC-MSD chromatographic parameters used were the same as the GC systems noted above except for the inlet pressures as indicated.

Results and Discussion

GC-FID to GC-FID Locking

Figure 4 shows the original chromatogram (GC system 1) obtained from running a styrene sample under the conditions specified in ASTM D 5135.[1] Many of the typical impurities found in styrene are found here. The phenylacetylene peak represents about 60 ppm. The peaks are identified in table 1.

The sample was then run at four other pressures to collect the five data pairs for RTL calibration. Because this method was run in constant flow mode, the pressures entered into the RTL software were the initial pressures. The α-methylstyrene peak (peak 10) was chosen as the target compound. The calibration data are shown in figure 1.

The method conditions and RTL calibration were then moved to GC system 2, a different GC and column. The sample was run at the original method inlet pressure of 18.2 psi. The chromatogram obtained using this scouting run is overlaid on the original chromatogram in figure 5. The retention times shifted about 0.3 minute on the second GC. This is a typical result obtained when trying to replicate an analysis on a second instrument or with a second column.

The retention time of α-methylstyrene was entered into the RTL software

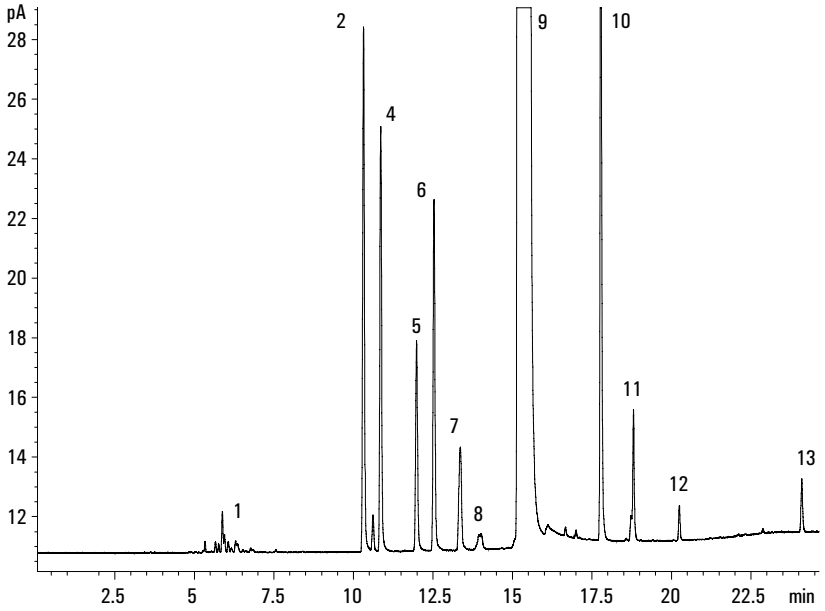

Figure 4. Styrene sample run on GC system 1 at 18.2 psi initial pressure, constant flow mode

dialog box on GC system 2, as shown in figure 3. The RTL software indicated the initial pressure should be modified from 18.2 psi to 18.96 psi. The new initial pressure was entered into the method and saved.

Figure 6 compares the chromatograms obtained from the original run and after locking retention times using the α-methylstyrene. Table 2 compares the retention times before and after using this approach. The retention times are now closely matched.

GC-FID to GC-MSD Locking

A second experiment was conducted to lock the original method from GC system 1 to the GC-MSD. This is useful for identification of unknown impurities that show up in the FID chromatogram. For example, there is a shoulder evident on the front side of the phenylacetylene peak in figure 4. It would simplify locating the impurity in the GC-MSD data if the retention times closely matched that of the GC-FID.

Because constant pressure mode is preferred when comparing data from FID and MSD systems, constant pressure mode was chosen, and the styrene sample was re-run on GC system 1 at 18.2 psi for reference.

The next step was to determine the chromatographic conditions to be used on the GC-MSD. The Agilent method translation software tool was used to calculate the conditions necessary to have the peaks elute in the identical order on the two systems.[2,3] Because the retention times need to match, the dead time and temperature program used for running the GC-MSD must be the same as the GC

Table 1. Peak Identities for Figure 4

Peak #	Name	Peak #	Name
1	Nonaromatics	8	p/m-Ethyltoluene
2	Ethylbenzene	9	Styrene
3	p-Xylene	10	α-Methylstyrene
4	m-Xylene	11	Phenylacetylene
5	i-Propylbenzene	12	β-Methylstyrene
6	o-Xylene	13	Benzaldehyde
7	n-Propylbenzene		

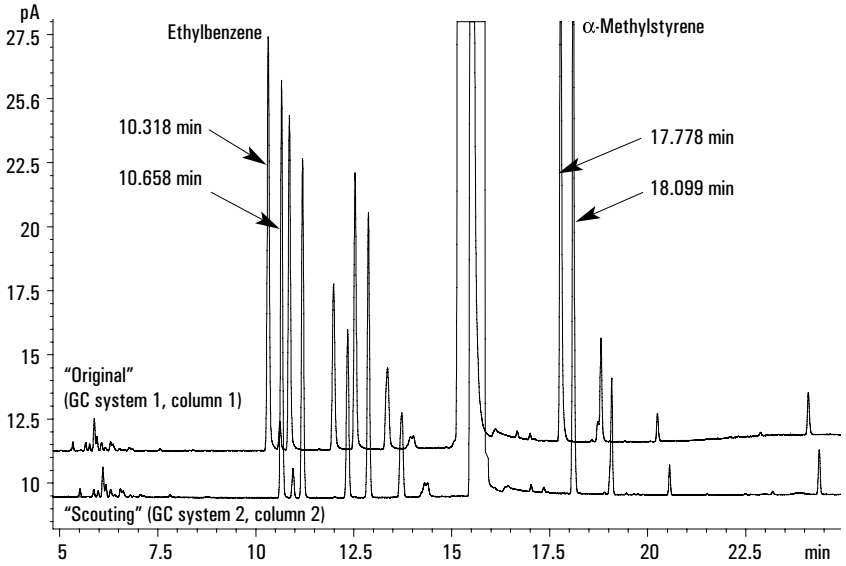

Figure 5. Comparison of original chromatogram on GC system 1 with GC system 2 before retention time locking

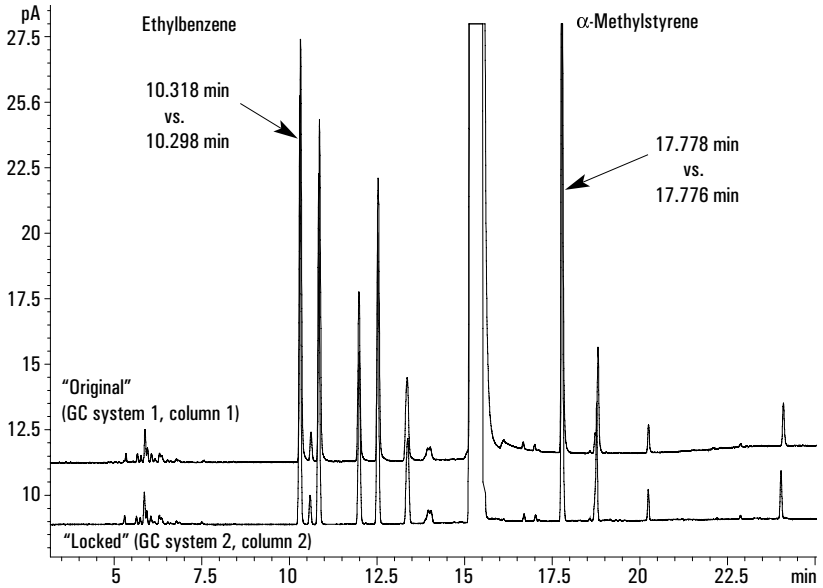

Figure 6. Comparison of original chromatogram on GC system 1 with GC System 2 after retention time locking

method. The pressure used, however, will be different due to the difference in column outlet pressure. The GC-MSD inlet pressure is calculated using the "none" mode of the method translation software (figure 7). In this mode, the holdup time between the two columns was forced to be identical to the GC-FID. This gives a speed gain of 1. The pressure calculated for use on the GC-MSD was 8.44 psi. Note that this calculated pressure is only the nominal pressure required to get similar retention times, not the exact locking pressure.

A different RTL calibration is required for GC-MSD because the outlet pressure is vacuum, and that of the FID is atmospheric pressure. Five runs were made on the GC-MSD system bracketing the 8.44 psi nominal method pressure. Because the GC-MSD used in this study was not equipped with RTL software, a dummy method was created in GC system 1 and the GC-MSD RTL calibration data was entered into it. A scouting run of the Styrene sample was made on the GC-MSD, and the α-methylstyrene retention time was used for locking. The locking inlet pressure calculated with the dummy method was 7.9 psi and was entered into the GC-MSD.

Figure 8 shows the resulting matched chromatograms from the GC-FID and GC-MSD. As seen in table 3, the retention times are now closely matched within 0.02 minute.

Figure 9 shows the MSD first choice of library search result of the impurity that created the shoulder on the front side of the Phenylacetylene peak. RTL ensured that this shoulder remained separated on the MSD system and eluted at the same time for easy comparison to the FID results.

Table 2. GC-FID Retention Times Before and After Locking for Styrene Impurities (Constant Flow Conditions). Chromatograms Shown in Figures 4, 5, and 6.

Component	Original Run GC 1/Column 1 18.2 psi	GC2–GC1 Before RTL	Scouting Run GC 2/Column 2 18.2 psi	GC2–GC1 After RTL	Locking Run GC 2/Column 2 19.0 psi
Ethylbenzene	10.318	0.340	10.658	–0.020	10.298
p-Xylene	10.616	0.333	10.949	–0.026	10.590
m-Xylene	10.858	0.337	11.195	–0.022	10.836
i-Propylbenzene	11.985	0.359	12.344	+0.005	11.990
o-Xylene	12.533	0.345	12.878	–0.012	12.521
n-Propylbenzene	13..360	0.364	13.724	–0.016	13.376
α-Methylstyrene*	17.778	0.321	18.099	–0.002	17.776
Phenylacetylene	18.806	0.275	19.081	–0.040	18.766
β-Methylstyrene	20.248	0.310	20.558	–0.006	20.242
Benzaldehyde	24.097	0.279	24.376	–0.069	24.028
Average Δ		0.326		0.028	

* Used in locking calculation

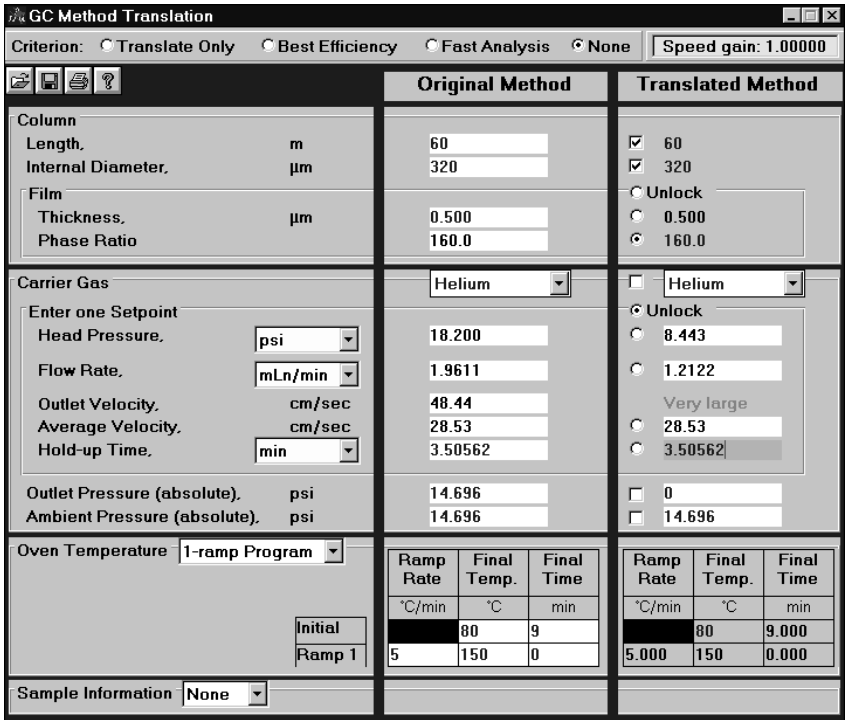

Figure 7. Method translation software provides scaled conditions for GC systems with different configurations

Conclusions

Retention time locking facilitates replicating results from instrument to instrument, from column to column, and from detector to detector by locking retention times. The retention times of a styrene sample analyzed according to ASTM D 5135 matched to within 0.06 minute after locking.

References

1. ASTM D 5135-95, "Analyses of Styrene by Capillary Gas Chromatography," Annual Book of Standards, Volume 06.04, ASTM, 100 Bar Harbor Drive, West Conshohocken, PA 19428 USA.
2. M. Klee and V. Giarrocco, "Predictable Translation of Capillary GC Methods for Fast GC" Agilent Technologies, Inc., Application Note 228-373, Publication 5965-7673E, March 1997.
3. GC Pressure/Flow Calculator for Windows, Version 2.0 and Method Translation Tool Version 2.0. Available at http://www.chem.agilent.com/servsup/usersoft/main.html.

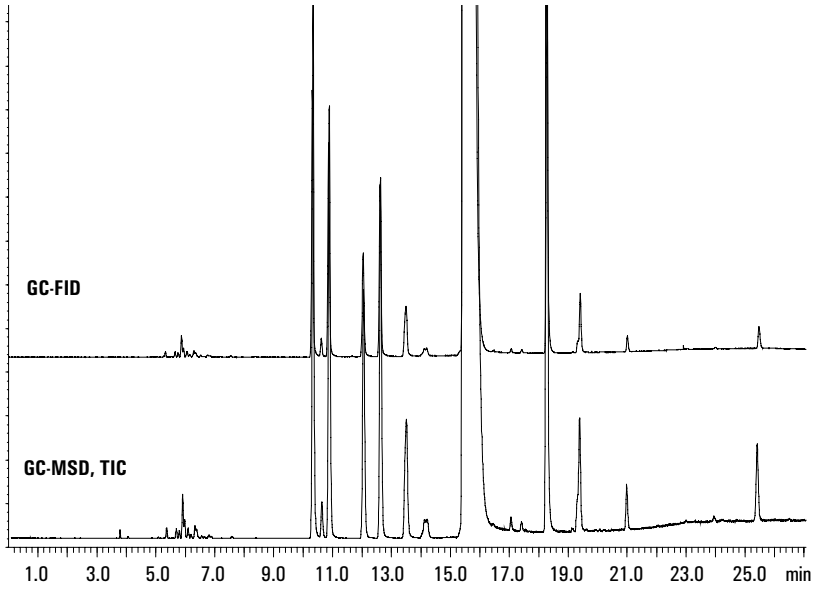

Figure 8. Comparison of chromatogram on GC system 1 with GC-MSD system after retention time locking, Constant Pressure Mode

Table 3. GC-FID vs. GC-MSD, Method Translated then Locked—Retention Times (Constant Pressure Conditions)

Component	GC-FID Original 18.2 psi	GC-MSD 7.9 psi	RT Difference min
Ethylbenzene	10.315	10.338	0.023
p-Xylene	10.620	10.642	0.022
m-Xylene	10.869	10.890	0.021
i-Propylbenzene	12.038	12.053	0.015
o-Xylene	12.613	12.630	0.017
n-Propylbenzene	13.492	13.508	0.016
a-Methylstyrene*	18.276	18.267	–0.009
Phenylacetylene	19.406	19.389	–0.017
b-Methylstyrene	21.008	20.987	–0.011
Benzaldehyde	25.475	25.415	–0.060
		Average	0.021

* Used in locking calculation

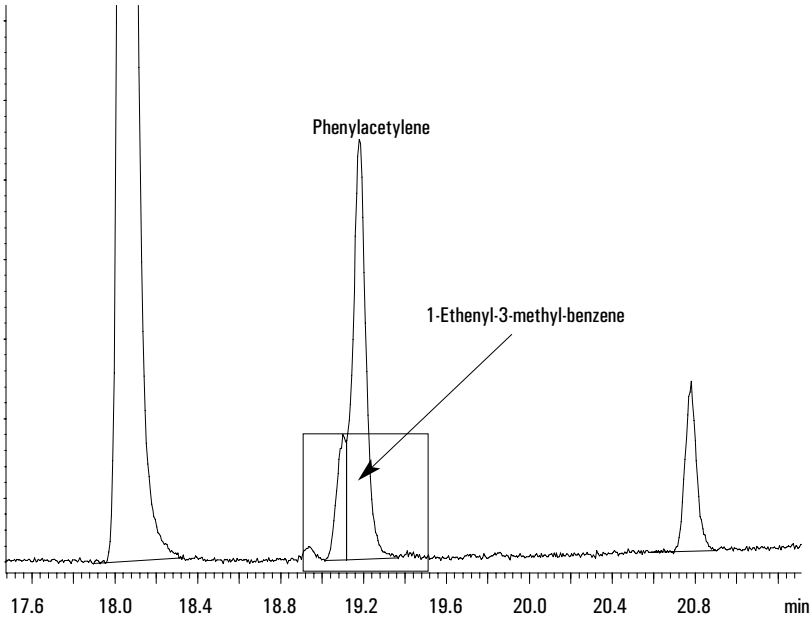

Figure 9. GC-MSD identification of impurity in shoulder of phenylacetylene peak

Printed in the USA 3/2000
5966-2469E

Agilent Technologies
Innovating the HP Way

Improving the Effectiveness of Method Translation for Fast and High Resolution Separations

Application

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Abstract

The increased availability of sub-2-micron (STM) columns and increased demand for methods friendly to mass spectrometers has led to strong trend toward conversion of existing HPLC methods to smaller diameter and smaller particle size columns. While the conversion is a simple mathematical exercise requiring the scaling flow rates, gradient times and injection volumes, many users observe less than perfect results. Here we look closely at the problem and propose calculations that improve the speed and/or resolution in a more predictable and beneficial way.

Introduction

Methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by replacing these columns with smaller dimension columns packed with smaller particle sizes. The potential benefits include reduced analysis time and solvent consumption, improved sensitivity and greater compatibility with mass spectrometer ionization sources.

Simplistically, a column of 250-mm length and containing 5-µm particles can be replaced by a 150-mm length column packed with 3-µm particles. If the ratio of length to particle size is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2, here about 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

LC/MS (Liquid Chromatography/Mass Spectrometry) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal LC/UV (UltraViolet UV/VIS optical detection) methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected on the smaller column, a proportional increase in peak response. We normally scale the injection mass to the size of the column,

though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column.

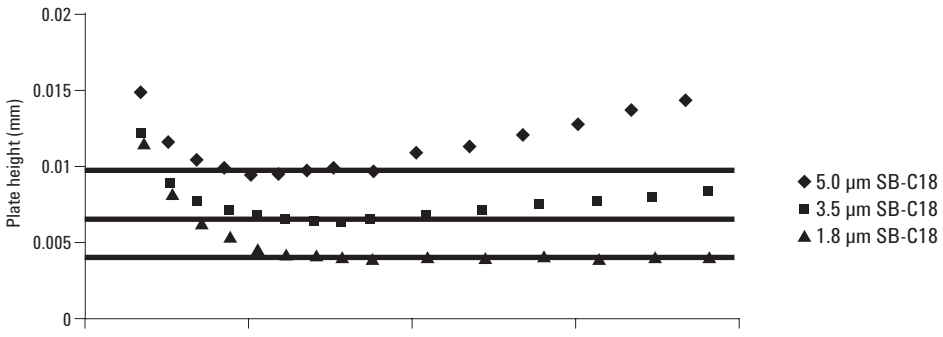
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. If one wishes to improve the outcome of the method conversion, though, there are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, or more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, this is often represented in van Deemter plots as shown in Figure 1, a modified version of the original plot [2].

In Figure 1 we observe that the linear velocity at which 5-µm materials are most efficient, under the conditions used by the authors, is about 1 mm/sec. For 3.5-µm materials the optimum linear velocity is about 1.7 mm/sec and has a less distinct optimum value, suggesting that 3.5-µm materials would give a more consistent column efficiency over a wider flow range. For the 1.8-µm materials, the minimum plate height, or maximum efficiency, is a broad range beginning at about 2 mm/sec and continuing past the range of the presented data. The practical application of this information is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity which results in a further reduction in analysis time. This increase in elution speed will decrease absolute peak width and may require the user to increase data acquisition rates and reduce signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings which will cause a measurable amount of bandspreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest



Lin. vel.	mm/sec	1	2	3	4	5
4.6 mm	mL/min	0.7	1.4	2.1	2.8	3.5
3 mm	mL/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	mL/min	0.14	0.29	0.44	0.58	0.73
1 mm	mL/min	0.033	0.066	0.1	0.133	0.166

Figure 1. van Deemter plot with various flow rates and particle sizes.

possible peak volumes. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of % change per minute, it is more useful to express it as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End}\% - \text{Start}\%)}{\#\text{Column volumes}} \right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C autosampler SL, with thermostatic temperature control
G1316B Thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 150 mm, 3.5 µm

Mobile phase conditions

Organic solvent:	Acetonitrile
Aqueous solvent:	25 mm phosphoric acid in Milli-Q water

Gradient Conditions

Gradient slope:	7.8% or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

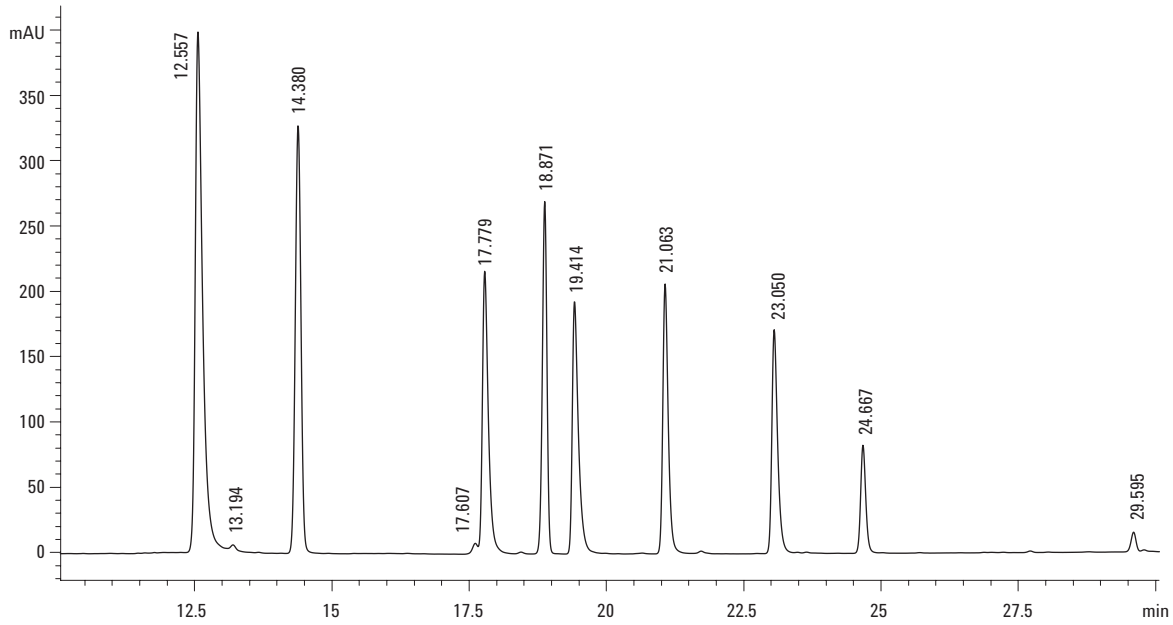
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

Results

The separation was initially performed on a standard 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column thermostatted to 25 °C (Figure 2) using conditions referenced in US EPA Method 555. The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm, 3.5-µm column (Figure 3). Solvent consumption is reduced from 60 mL to 15.5 mL per analysis.

The separation was then re-optimized for faster separation with the identical slope, 7.8%, by increasing the flow rate from 0.43 to 1.42 mL/min, and proportionately reducing the gradient time (Figure 4). Finally, increased resolution is demonstrated by keeping the original times used in Figure 3 with the increased flow rate (Figure 5). This yields a gradient with identical time but a reduced slope of 2.3%. The increased resolution of peaks 4 and 5 is readily apparent.

The conditions in Figure 4, 7.8% slope at increased linear velocity on 3.0 × 150 mm, 3.5-µm material, yield a separation with comparable resolution to the original 4.6 × 250 mm method, but with only a 12-minute total analysis time. This is excellent for



Conditions

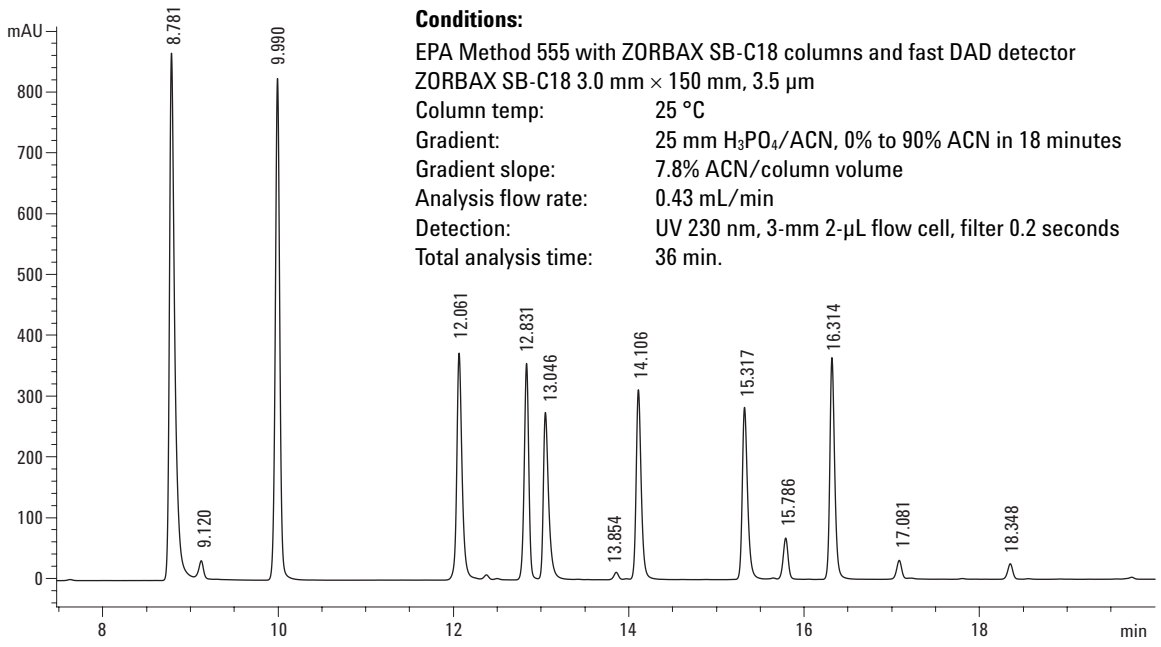
EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector
ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm

Column temp:	25 °C
Gradient:	10% to 90% ACN vs. 25 mM H_3PO_4
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min

Group A Compounds

Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 2. Gradient separation of herbicides on 4.6 × 250 mm 5-µm ZORBAX SB-C18.



Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector
ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 µm

Column temp:	25 °C
Gradient:	25 mm H_3PO_4/ACN, 0% to 90% ACN in 18 minutes
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min.

Figure 3. Gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

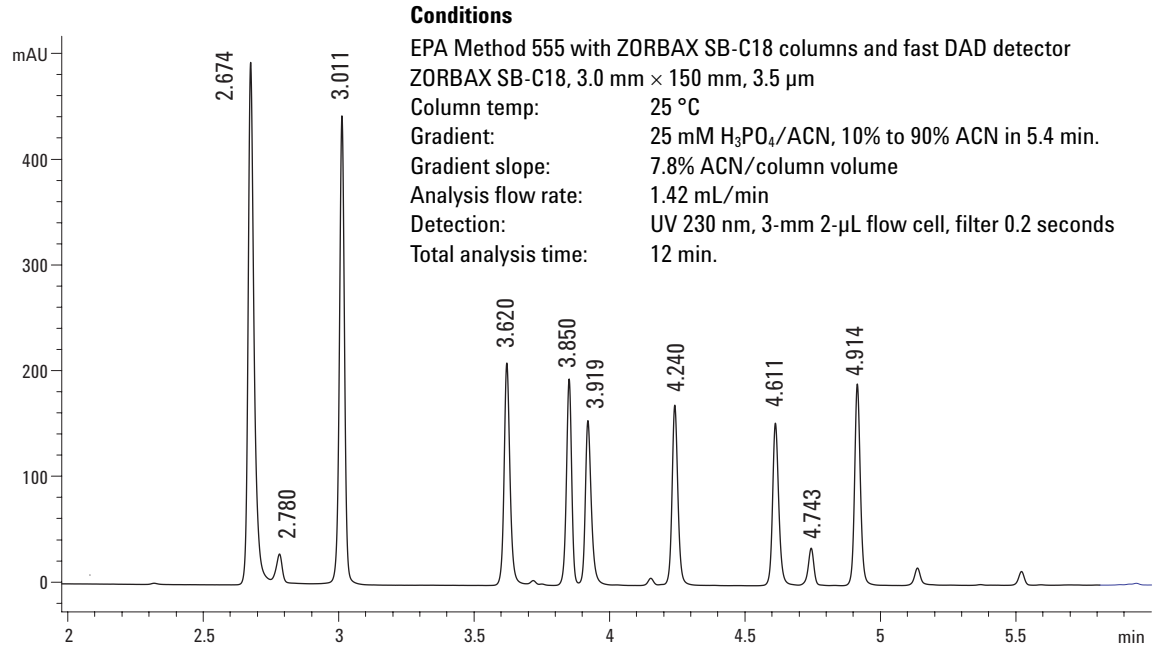


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

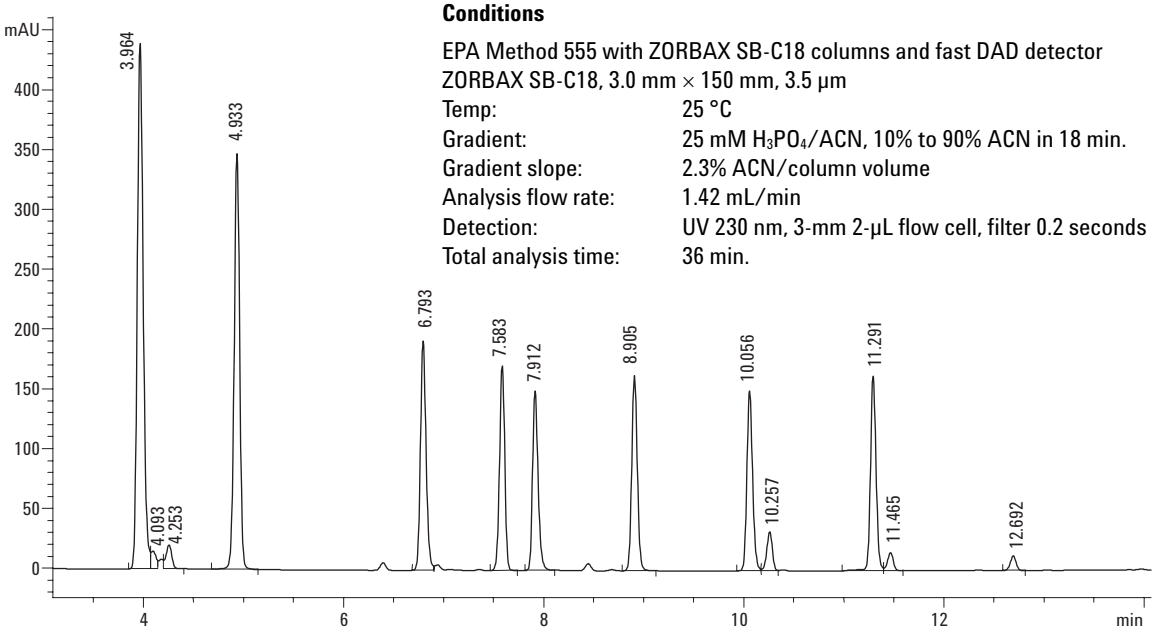


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

high throughput screening and quantitation of a large number of samples. Figure 5, with the gradient slope reduced to 2.3%, results in a high-resolution separation with a calculated R value of 3.3 vs. the standard 3.0 × 150 mm separation value of 1.9, for the critical pair seen in Figure 5 at 7.5 to 8 minutes.

In Table 1 the column has been replaced with a low dead volume connecting union in a system fitted with 0.12-mm id capillary tubing at all points of sample contact. A 1-µL injection of dilute actone is made to determine the bandspreading contribution of the system, with various flow cells. Multiple flow cells were tested, and the average result reported, where possible. The elution volume summarizes the total volume of all tubing in the system. While the absolute volume from the 2-µL to the 13-µL flow cells is 11 µL, we observe an increase of 15 to 16 µL because of the larger diameter inlet tubing integral to the larger volume flow cells.

Table 1. Volumetric Measurements of Various Flow Cells

Flow cell	Elution volume (µL)	Half height width (µL)	5 Sigma width (µL)
New SL 2 µL 3 mm	11	5	12
Micro 6 mm 1.7 µL (n = 2)	14	6	18
Semi-micro 6 mm 5 µL (n = 2)	13	6.5	18.5
Standard 10 mm 13 µL	26	11	26
New SL 10 mm 13 µL	27	11	25

Conclusion

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small and high resolution columns, will ensure good column efficiency which is critical to a successful translation of the method.

References

1. J. J. van Deemter, F. J. Zuiderweg, A. Klinkenberg, Chemical Engineering Science 1956, 5, 271–289
2. The Influence of Sub-Two Micron Particles on HPLC Performance, Agilent Technologies, application note 5989-9251EN, May 2003

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Printed in the USA
August 9, 2006
5989-5177EN

Improving GC-MS Method Robustness and Cycle Times Using Capillary Flow Technology and Backflushing

Application Note

Environmental

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Abstract

This application note demonstrates the customer benefits from using Capillary Flow Technology to provide backflushing of high-boiling materials in GC and GC/MS analyses. Benefits include reduction in chromatographic cycle times, a reduction in system column maintenance, and extended GC column life. If a GC/MS system is utilized, the author has experienced an increase in the number of samples analyzed before ion source maintenance is required.

Agilent Technologies

Introduction

A critical component of the GC/MS analysis of any sample that contains large amounts of matrix material is the sample preparation. Environmental samples such as soils and sediments require not only extraction, but may also require multiple cleanup steps in order to present as clean an extract as possible for injection in to the GC/MS system.

Any remaining matrix in the sample extract can have deleterious effects on the GC sample inlet, column, and the ion source of the mass spectrometer. Traditionally, these high-boiling matrix materials are removed from the capillary column by a long bake-out period after the analytes of interest have eluted. This long bake-out process causes thermal stress to the column and also drives the matrix material towards the ion source, where it will eventually affect system performance. Moreover, should any material remain in the column after the bake-out process, it can cause loss of chromatographic peak shape and retention time shifting of target analytes. This shifting of retention time is particularly troublesome if the mass spectrometer is being used in the selected ion monitoring (SIM) mode (as with a single quadrupole GC/MS) or in the multiple reaction monitoring (MRM) mode (as with a triple quadrupole GC/MS).

This paper demonstrates how high-boiling matrix materials can be removed from the column quickly and effectively – between sample injections – by using capillary flow technology and capillary column backflushing.

Figure 1 shows a schematic diagram of the GC/MS system used. The 15-m analytical column was connected to the EPC split/splitless inlet and a capillary flow technology two-way splitter (p/n G3180B or G1540 option number 889).

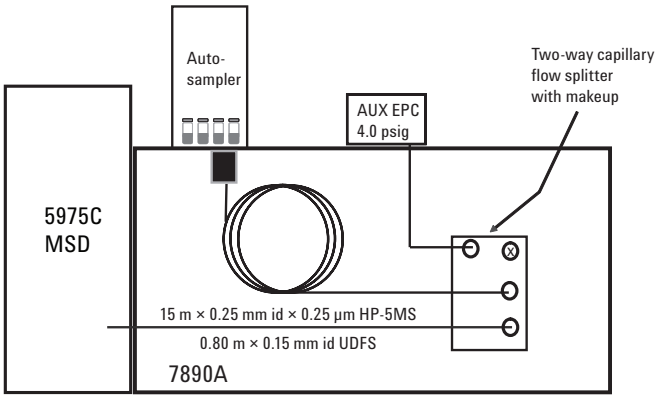

Figure 1. Schematic diagram of GC-MS system.

A short length of uncoated, deactivated fused silica (UDFS) capillary column is used as a restrictor between the splitter and the MS. Note carefully how the connections are made at the splitter. The X represents a port on the splitter plate that is closed off with a SilTite metal ferrule and stainless steel wire plug.

Backflushing in this example was accomplished during a post-run period by a combination of increasing oven temperature, reducing the inlet pressure of the analytical column, and increasing the pressure applied to the splitter plate.

Experimental

The full analytical conditions, both with and without post-run backflush set-points, are shown in Table 1.

Table 1. GC/MS Analysis Conditions

Gas chromatograph	Agilent 7890A
Columns	(1) 15.0 m × 0.25 µm id × 0.25 µm HP-5MS Ultra Inert (19091S-431SI) Inlet Front split/splitless, outlet 2-way Capillary Flow Device
	(2) 0.80 m × 0.15 mm id uncoated deactivated fused silica inlet two-way capillary flow device at 4.0 psig outlet vacuum
Carrier gas	Helium
Carrier gas mode	Constant pressure
Flow rate	17.18 psi
Injection port	EPC split/splitless
Autosampler	Agilent 7683A
Injection mode	Splitless, purge delay 0.5 min Purge flow 50.0 mL/min at 0.5 min
Injection volume	2.0 µL
Injection port liner	4 mm single-taper splitless liner (5181-3316)
Oven program °C (min)	70 (1) – 50 °C /min – 150 (0) 6 – 200 (0) – 16 – 280 (0) °C
Mass spectrometer	Agilent 5975C MSD
MS interface	280 °C
MS source	230 °C
MS quad 1	150 °C
Backflush conditions (1)	Post-run, 10 min, AUX 60 psig, oven 320 °C
Backflush conditions (2)	Post-run, 6 min, AUX 80 psig, oven 320 °C
Detection mode	EI full scan; mass range 40:550 amu
EI tune	Gain factor = 1

Results and Discussions

Experiment 1: No Backflushing Employed

In the first experiment, an extracted sediment sample was analyzed in full-scan mode to show the extent of the matrix problem. No backflushing was employed.

Before any sediment was injected, a system blank (no injection) followed by a 2-µL solvent blank was made. In the absence of the actual hexane solvent used to prepare the sediment extract, hexane that was not particularly clean was used. The TICs are shown overlaid in Figure 2, system blank in black, and solvent blank in gray. These chromatograms show that the system is free from high-boiling matrix material.

Following the blanks, a single injection of the sediment extract was made without backflushing; the TIC is shown in Figure 3. Note the very high abundance of the matrix and that when the analysis finishes, there is still a significant amount of matrix material to elute from the column.

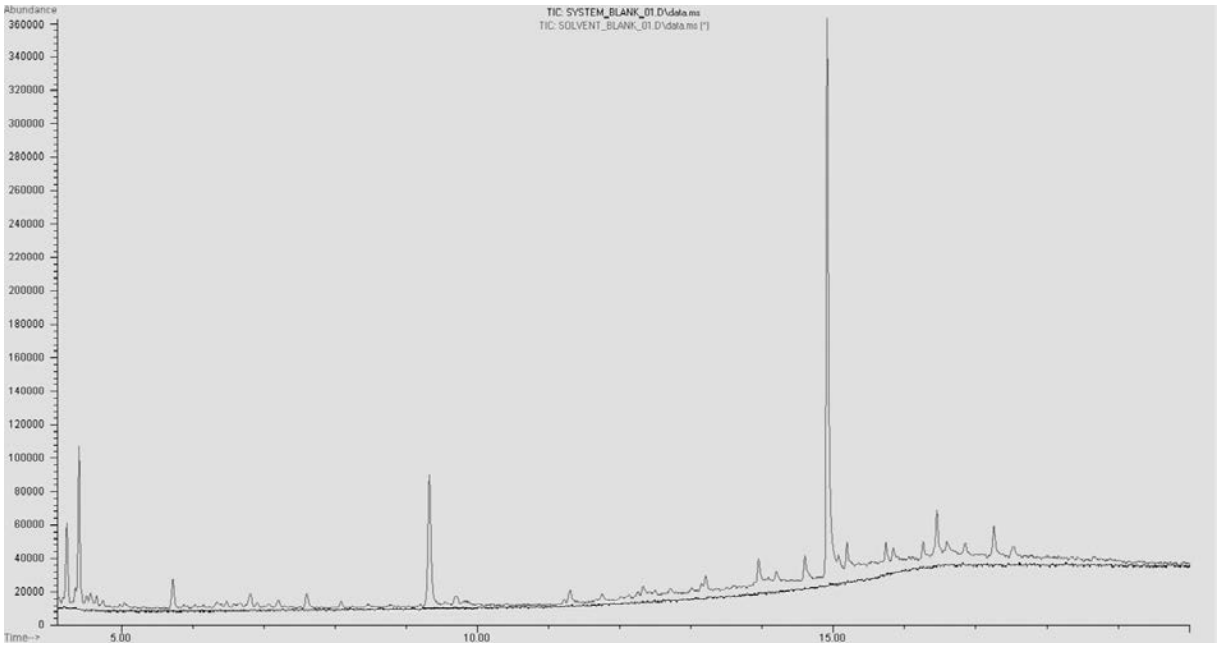

Figure 2. System blank and solvent blank TICs.

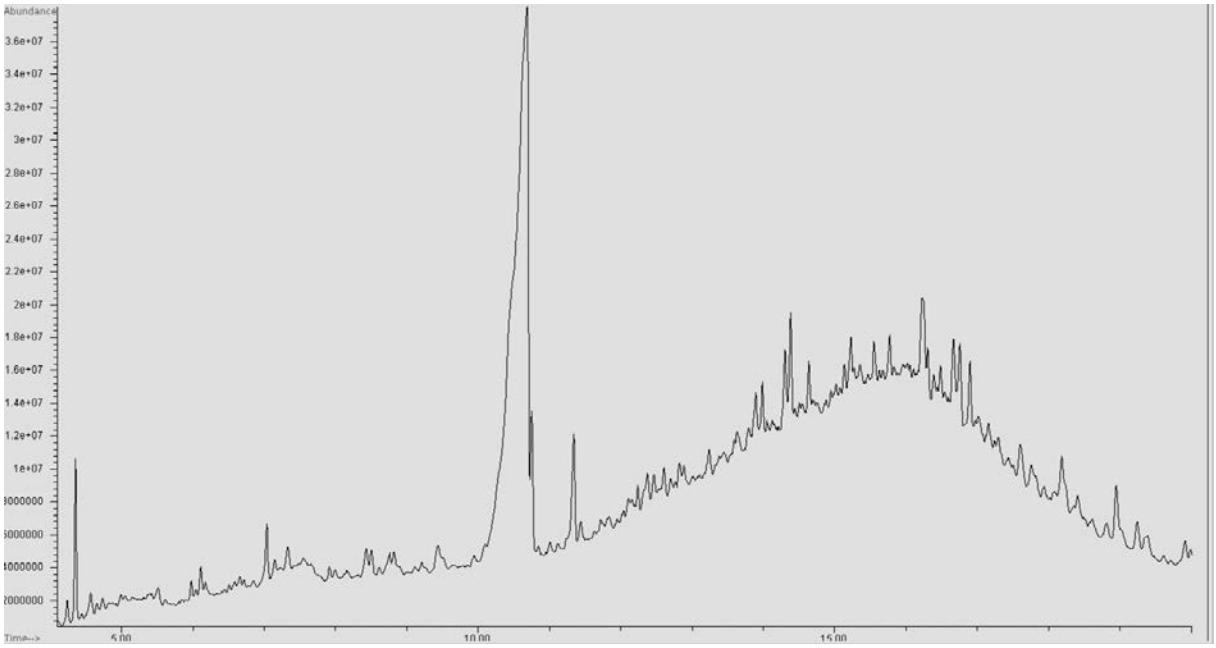

Figure 3. Sediment extract TIC.

The sediment extract injection was followed by a series of hexane blank injections. The first seven hexane blank TICs are shown overlaid in Figure 4 with the solvent blank before the sediment was injected into the GC/MS system.

Figure 5 shows that after the eighth solvent blank injection, the system has almost recovered to the level of background before the sediment sample was injected.

The original solvent blank TIC is shown in black, the eighth solvent blank TIC after the sediment injection is shown in gray.

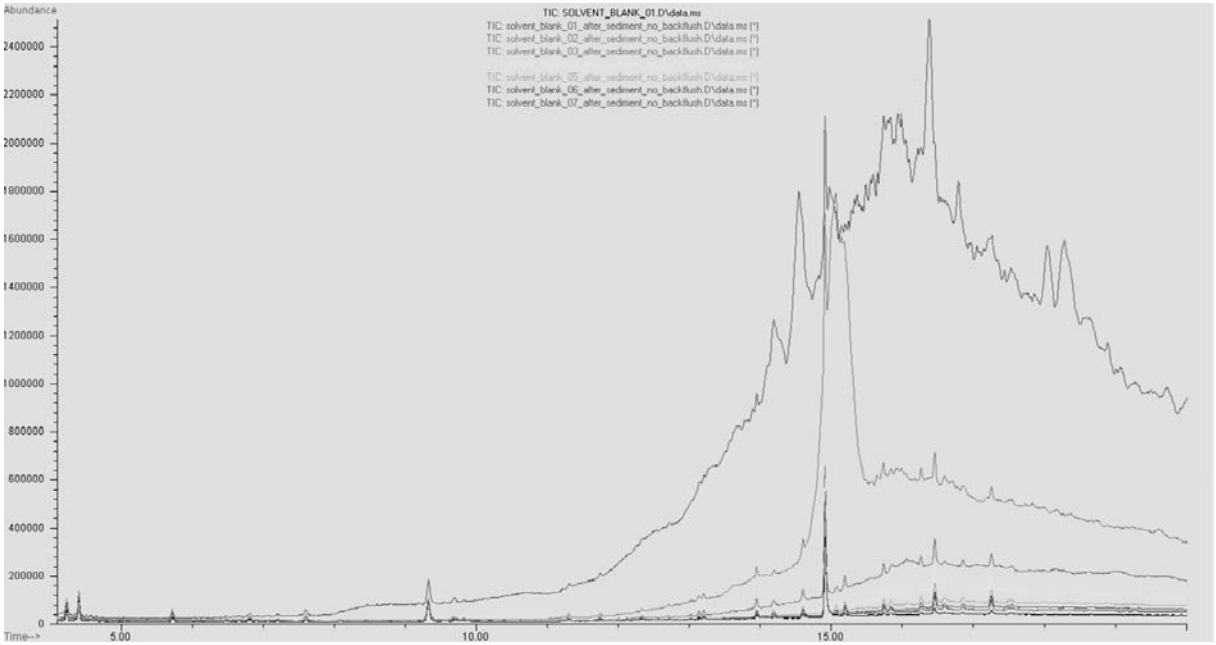

Figure 4. Successive solvent blank injections.

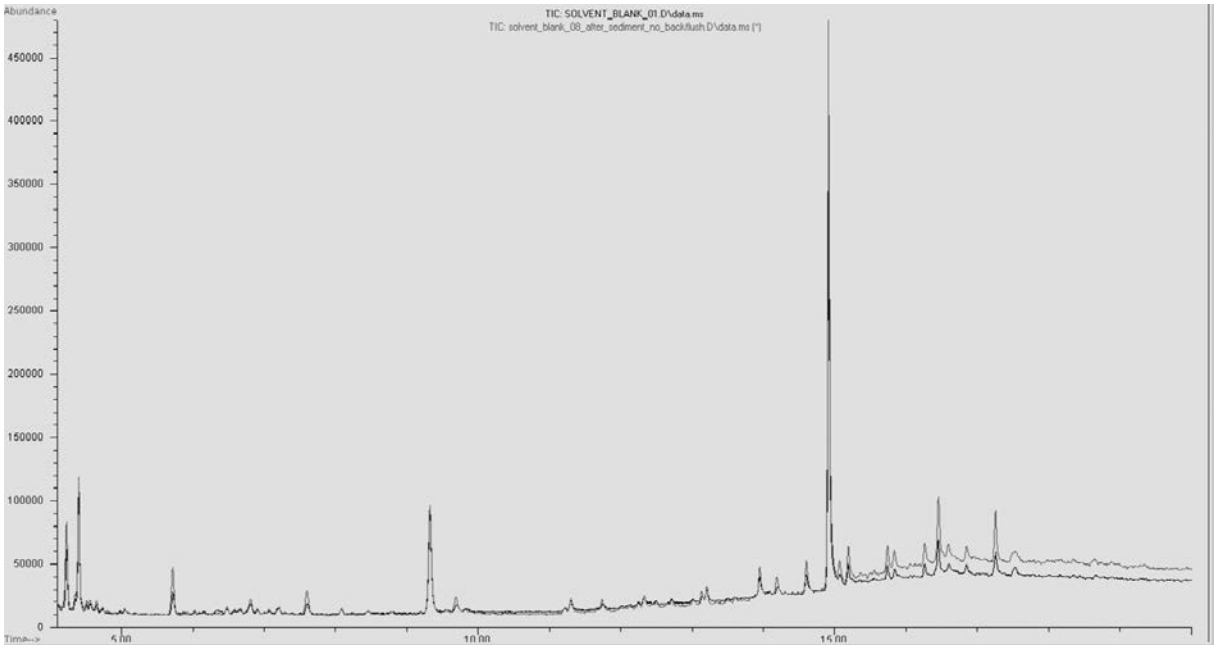

Figure 5. Eighth solvent blank and original solvent blank TICs

Experiment 2: Backflushing Employed

Backflushing was enabled during a post-run period by increasing column oven temperature, reducing the inlet pressure of the analytical column, and increasing the gas pressure applied to the splitter plate.

The 7890A instrument control software includes simple and easy-to-use screens to help set up post-run backflushing conditions. Figure 6 shows the configuration of columns and connections with the GC oven.

Figure 7 shows the actual backflushing conditions, namely the post-run oven temperature (320 °C), post-run inlet pressure for the analytical column (1 psig), post-run pressure applied to the splitter device (60 psig), and post-run time (10 minutes). The figure also shows the number of column-volumes of carrier gas that will backflush the analytical column.

Note that using the backflushing conditions shown in Figure 7 (320 °C, column pressure 1 psig, and splitter pressure 60 psig for 10 minutes), that 59.4 column volumes of carrier gas was used to backflush the column during the post-run period. This backflush time may have been more than necessary. Alternate conditions were also investigated and are presented later.

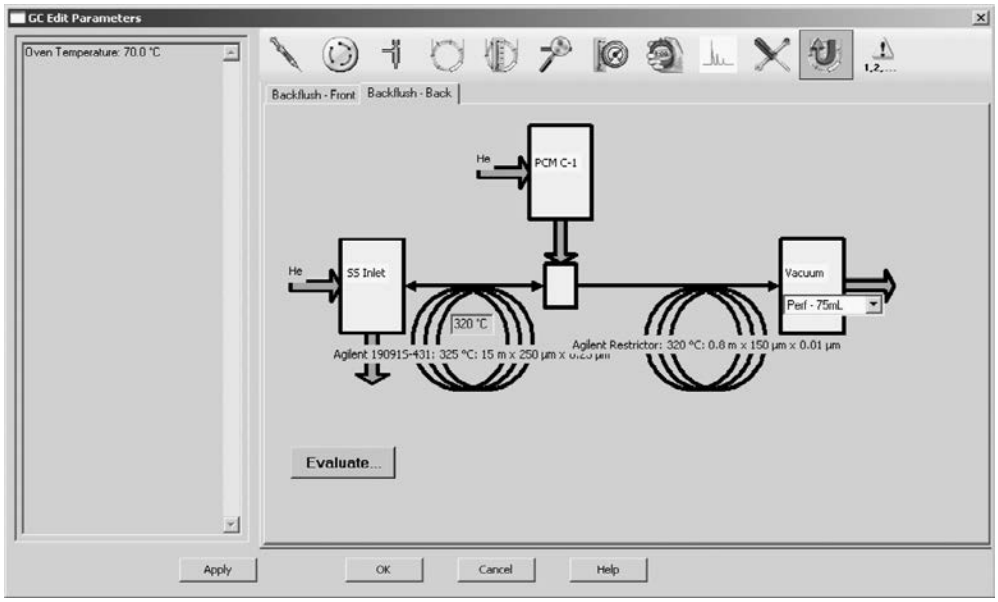

Figure 6. Post-run backflushing screen number 1.

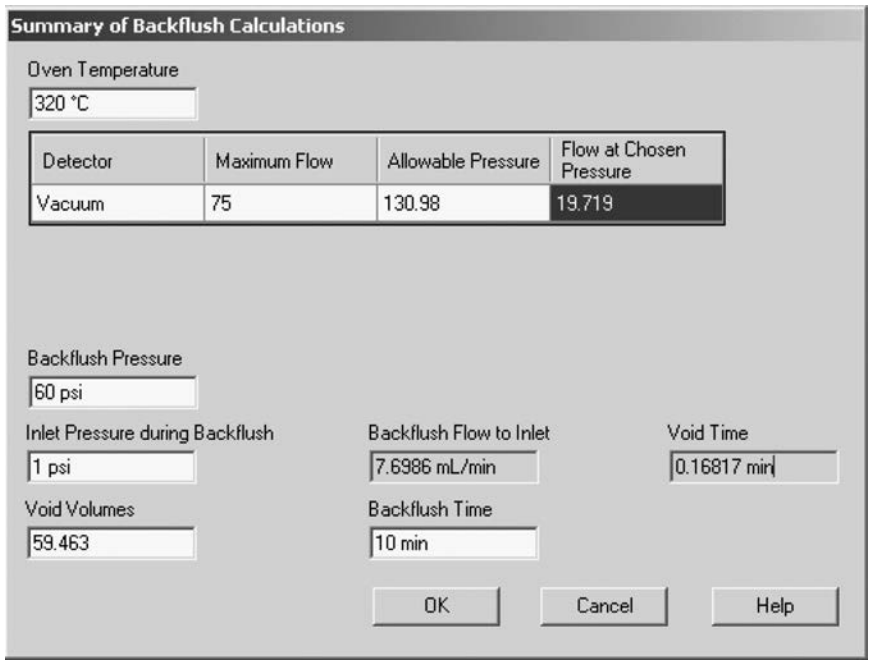

Figure 7. Post-run backflushing screen number 2.

Before applying the backflush conditions to the method the user is presented with a convenient summary of the back-flush conditions. See Figure 8.

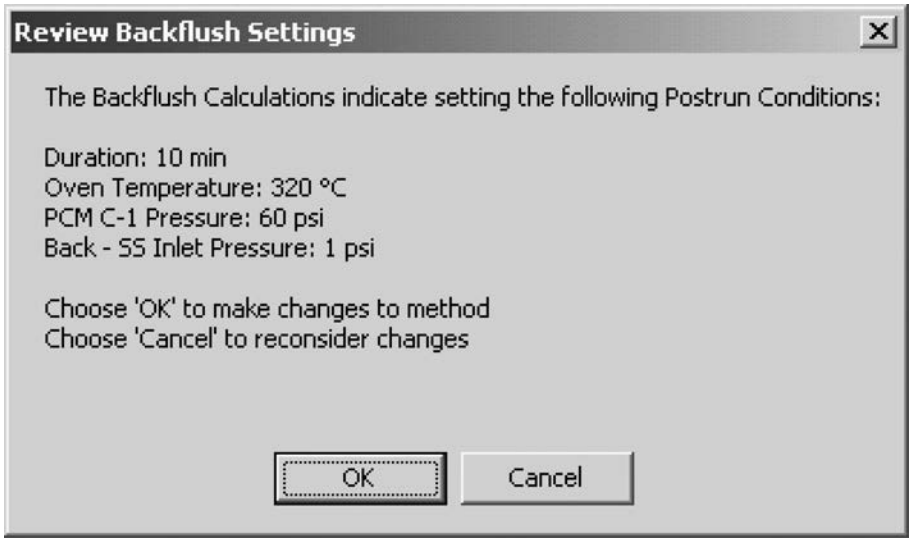

Figure 8. Post-run backflushing screen number 3.

Another injection of the sediment including backflush was made followed by a blank injection of solvent. Figure 9 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material had been effectively removed by backflushing.

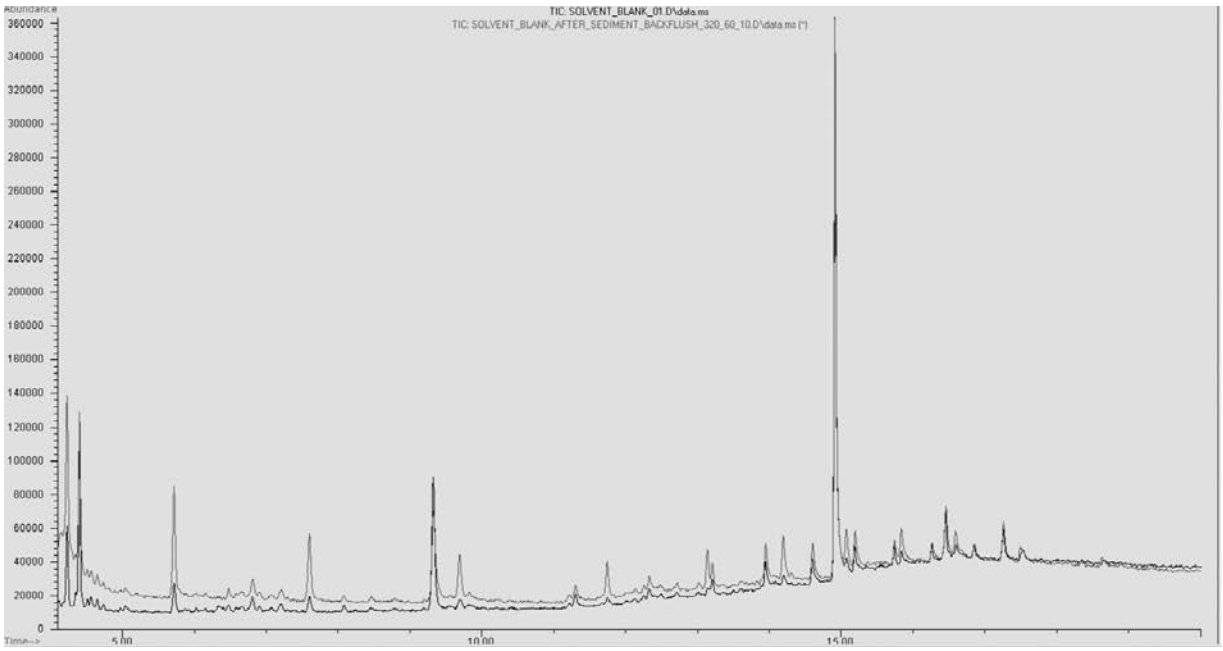

Figure 9. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (1).

Experiment 3: Backflushing Employed

In order to reduce cycle time for the method, the backflush conditions were modified by increasing the backflush pressure to 80 psig and holding for 6 minutes.

Note that using the backflushing conditions shown in Figure 10 (320 °C, column pressure 1 psig, and splitter pressure 80 psig for 6 minutes), that 46.6 column volumes of carrier gas was used to backflush the column during the post-run period.

Another injection of the sediment was made, followed by a blank injection of solvent. Figure 11 shows the overlaid TIC of the original solvent blank (black) overlaid on the solvent blank after the sediment injection (gray).

No evidence of any matrix material is indicated, demonstrating that all the high-boiling matrix material has been removed by backflushing with the more aggressive conditions as well. These conditions reduced the cycle time for this method 4 minutes compared to the backflushing conditions used in Experiment 1.

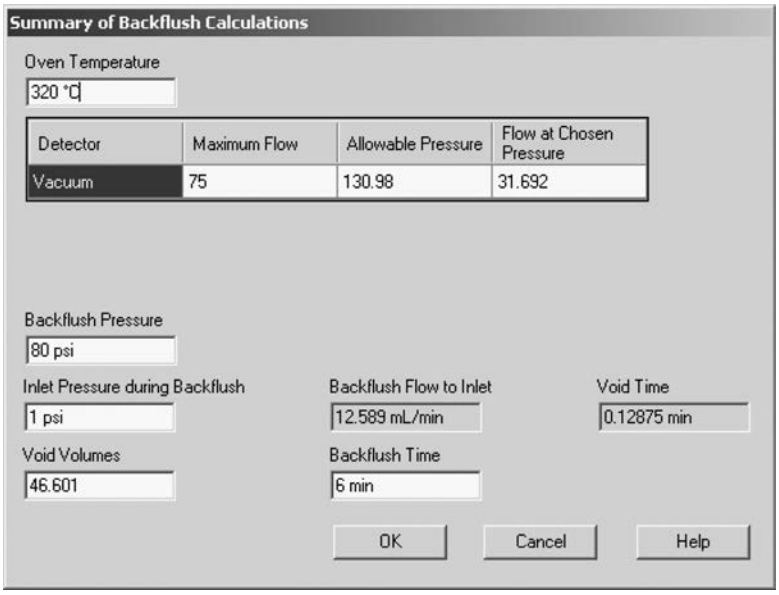

Figure 10. Post-run backflushing screen conditions number 2.

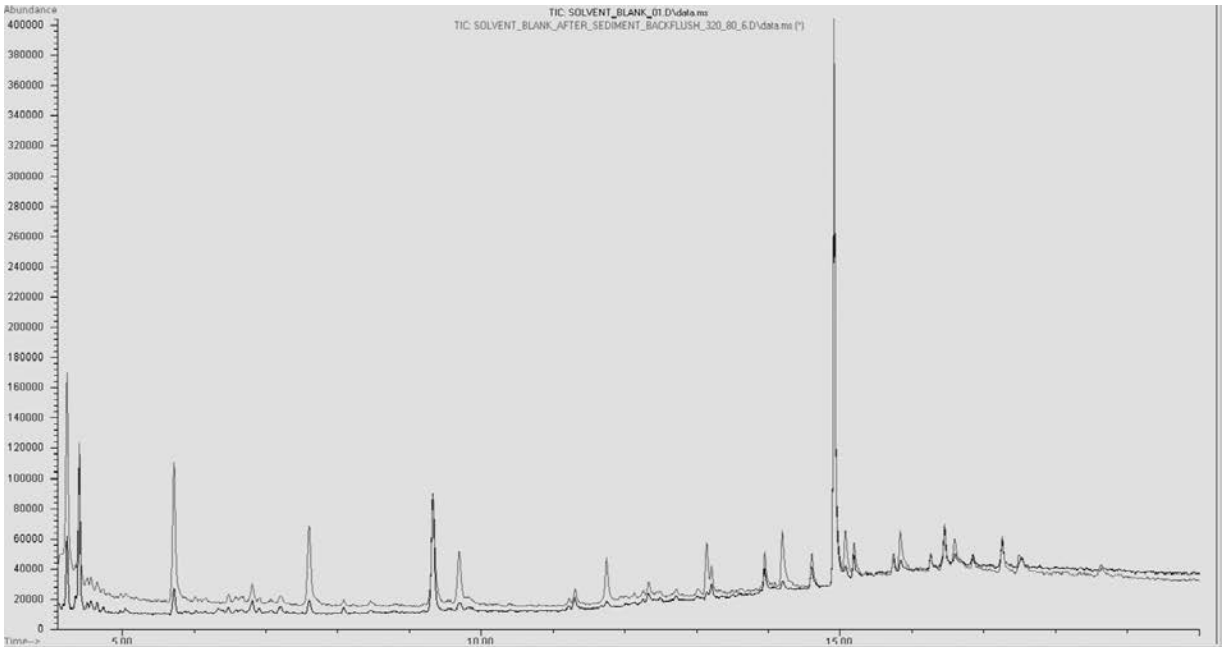

Figure 11. Original solvent blank TIC and solvent blank after sediment injection with post-run backflush (2).

Conclusions

Post-run backflushing was shown to effectively eliminate high-boiling sample matrix in a short amount of time. The major benefits of GC capillary column post-run backflushing include:

- Agilent's capillary flow technology and GC software enable easy and robust setup of GC backflushing.
- Compared to long bake-out periods with flow in the forward direction, a short period of backflushing can remove high-boiling matrix materials more effectively without contaminating the MS ion source.
- Chromatographic cycle time is reduced, columns stay clean, and the integrity of target analyte peak shapes and retention times are maintained.
- For this particular sediment extract the GC column was free of sample matrix after a backflush period of 6 minutes.
- Less system maintenance (ion source cleaning) is required.

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Published in the USA
January 30, 2009
5990-3367EN

Agilent Technologies

The 5973N inert MSD: Using Higher Ion Source Temperatures

Application

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Abstract

The new 5973N inert MSD and ChemStation software (G1701DA) offers the capability of operating the ion source at higher temperatures. This feature, combined with the improved inertness of the source, can provide the user with improvements in analysis, if exploited coherently. This application note provides advice and examples of how to explore the utility of ion source temperature.

Introduction

The default ion source temperature of 230 °C is commonly applied in electron impact (EI) ionization on the 5973 MSD platforms. The new Inert Source when used with the new revision of the ChemStation software (rev. DA) allows ion source temperature to be set to a maximum of 300 °C. As with all advances, there are advantages and disadvantages in operating at higher source temperatures. This note will address several general aspects in EI operation.

Tuning

Figures 1 and 2 show the results for autotuning the Inert Source at the standard 230 °C ion source temperature and the 300 °C temperature limit of the new source (quadrupole temperature 200 °C). The higher temperature for the source produces a perfluorotributylamine (PFTBA) spectrum that shows lower abundances of the higher mass fragments, which is not entirely unexpected. The *m/z* 219 fragment has dropped to an abundance comparable to the *m/z* 69 ion and the ion at *m/z* 502 has dropped about 50%. This is to be expected as the internal energy of the calibrating gas has increased. Note, however, that the isotopic ratios are maintained.

The user should also expect to see a higher background in the higher temperature tunes. A portion of the background will be due to ions associated with column bleed. Bleed, which usually condenses in the source, now is volatized and will appear as an increase in background and baseline.

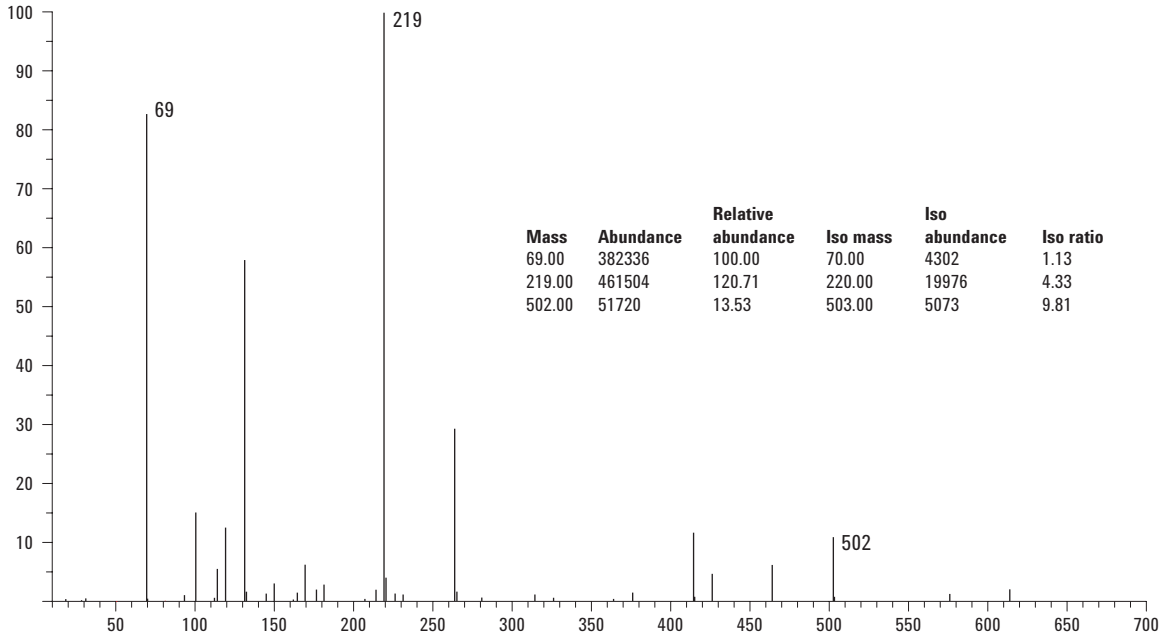

Figure 1. Autotune results for an ion source temperature of 230 °C.

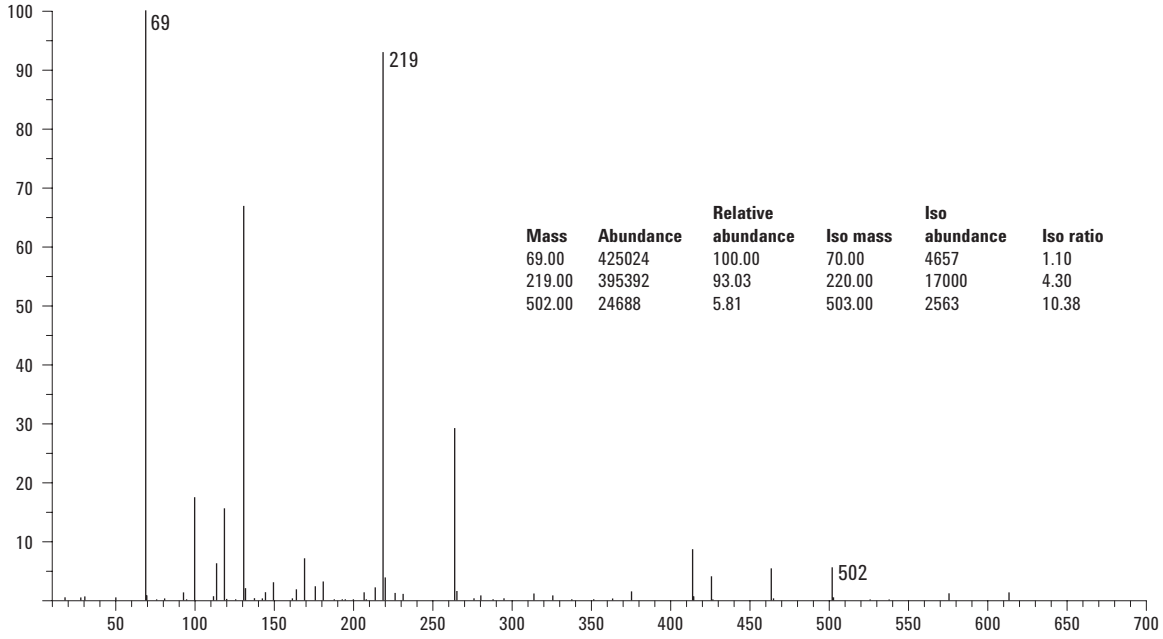

Figure 2. Autotune results for an ion source temperature of 300 °C.

Implications for Analytical Applications

Although the tuning compound showed a spectral change that favored more fragmentation, and all compounds could be expected to be influenced similarly, there are some advantages that can occur for less fragile compounds, especially those that have higher boiling points and are late eluting in GC. Analysis of the class of compounds known as "persistent organic pollutants" (POPs) is likely to benefit from higher source temperatures.

To illustrate the aspects that need to be examined, consider the six polychlorinated biphenyls (PCBs) acquired in full-scan and presented in Figure 3. The overlaid reconstructed total-ion-current chromatograms (RTICCs) suggest that the higher source temperature increases the total response for the later eluting PCBs but produces little enhancement for the early eluters. This could be due to more fragmentation and may not necessarily be useful if the increase in the RTIC is due to lower mass fragments since these lower mass ions are usually compromised by interferences. A calculation of the signal/noise (S/N) for the RTICCs shows that while there is an increase in signal at the source higher temperature, there is also an increase in the background noise and the result is a lower S/N ratio for the higher source temperature.

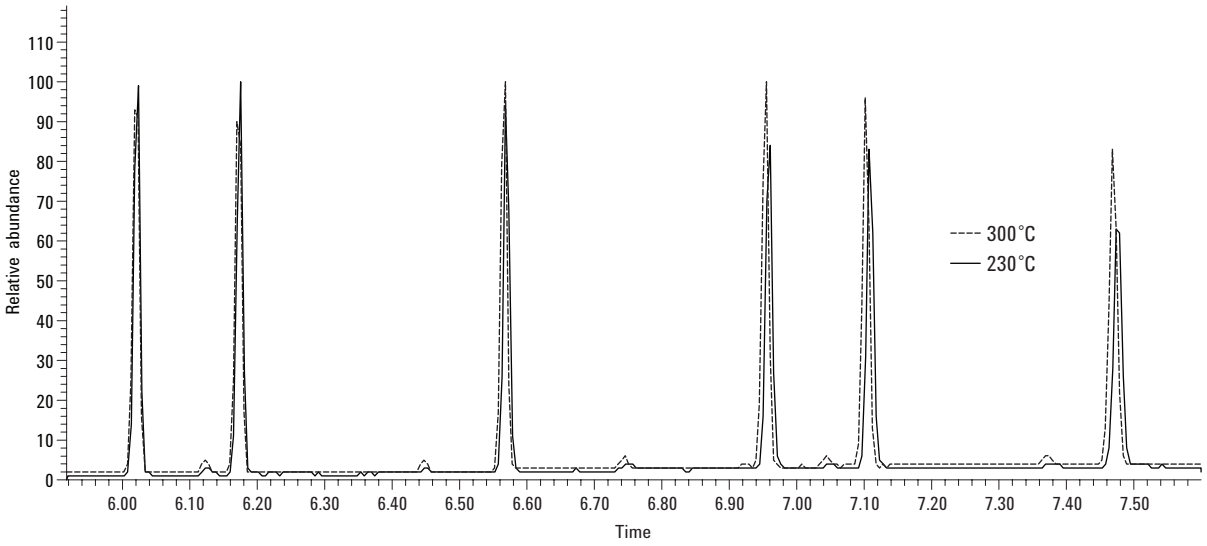

Figure 3. Overlaid RTICC of six PCBs acquired in full-scan (50–505 amu) at source temperatures of 230 °C and 300 °C. From left to right, or earlier to later, in the chromatogram, the PCBs consist of a Cl_3-Biphenyl, Cl_4-B, Cl_5-B, Cl_6-B, another Cl_6-B and a Cl_7-B.

Figure 4 shows the same analytes acquired in selected-ion-monitoring mode (SIM) using three ions for each component (M, M+2 or M–2, and M–70). The same trend appears with an enhancement apparent in signal for the later eluting PCBs but little increase for the earlier PCBs. Now, however, the RTIC for the SIM acquisition does show a higher S/N ratio for these later PCBs. As opposed to the full-scan acquisition, the SIM mode acquisition at higher source temperature does increase signal for the ions of interest and, because there was no increase in background, a useful S/N increase was obtained. As always, the guiding principle that an increase in signal is only useful if it exceeds the concomitant increase in background holds. This is clearly illustrated by the third PCB, the pentachlorobiphenyl (Cl_5–B). Figure 5 shows the behavior of the signal and background for the two source temperatures for one of the pentachlorobiphenyl confirming ions. The higher source temperature raises the signal and the background for this ion of interest over the lower temperature but fortunately signal increases faster than background. In this case, the background is due to column bleed components and is unavoidable but fortunately not very intense. This may or may not be the case in sample analysis.

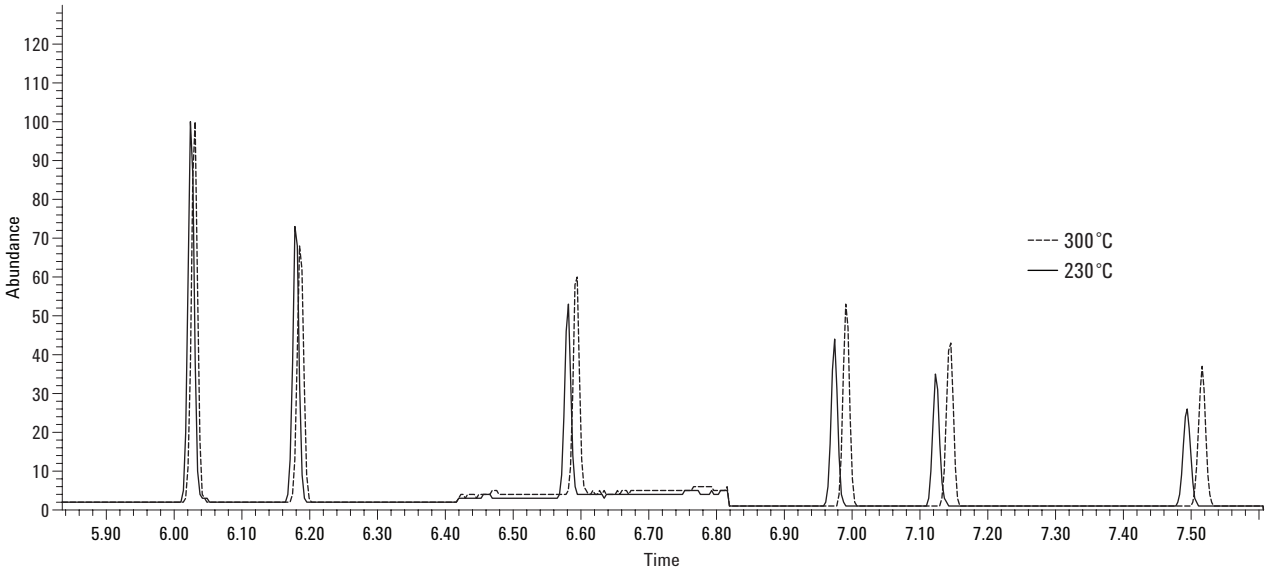

Figure 4. Overlaid RTICC of six PCBs acquired in SIM at source temperatures of 230 °C and 300 °C. From left to right, or earlier to later, in the chromatogram the PCBs consist of a Cl_3-Biphenyl, Cl_4-B, Cl_5-B, Cl_6-B, another Cl_6-B and a Cl_7-B.

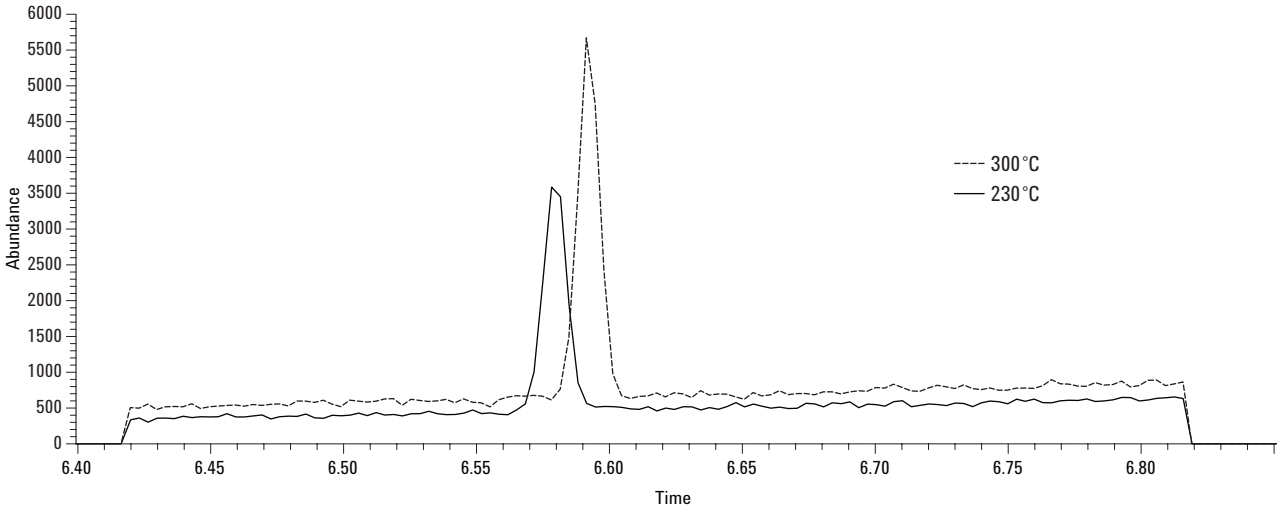

Figure 5. Overlaid extracted ion-current chromatograms of one ion (M-70) for the pentachlorobiphenyl acquired in SIM at source temperatures of 230 °C and 300 °C.

The detection limits for many late eluting, "high-boiling" compounds that will improve by implementing higher source temperatures (for example, PAHs, terphenyls, etc.). As an illustration of the enhancement for very "high-boiling" compounds, consider the 6-ring benzenoid hydrocarbon (PAH), coronene (CAS 191-07-1). This compound is difficult to determine due to low response and poor chromatography, although it is present in many sediment samples. Figure 6 shows overlaid RICCs for acquisitions of coronene at 230 °C and 300 °C. Although the peak area is the same, the enhanced Gaussian peak shape achieved at 300 °C improves detection.

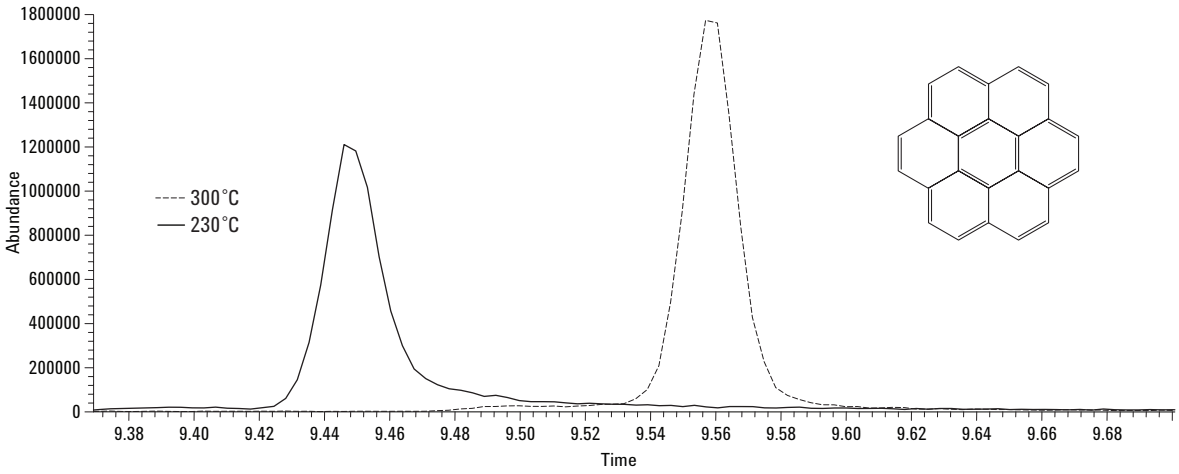

Figure 6. Overlaid extracted ion-current chromatograms of one ion (*m/z* 300) for coronene acquired in full scan at source temperatures of 230 °C, and 300 °C.

Source "Bakeout"

There may be considerable temptation to use the higher source temperature for source “cleaning” by “baking”. In other words, when the user notices a higher background in the source or a reduction in response, the ill-conceived approach of baking the source clean may come to mind. The result will be that “garbage” coating the source will be volatized further into the analyzer; the other lenses will get dirtier, as will the multiplier, etc. “Baking” is not a substitute for mechanical cleaning of the source. However, baking a source after a cleaning is a good approach and a macro that provides this option is given in Table 1. After a source has been cleaned, and the MS system pumped down and checked to be leak free, this macro can be implemented either manually or in a sequence. (Note that the temperature limits in the tune file need to be altered to 300 and 200 for source and quadrupole, respectively). Manually the bakeout is called from the command line in TOP by –

```
macro "bake.mac"  <enter>
bake 2  <enter>
```

The “2” calls for a 2 hour bakeout, and which can be set to anytime the user requires.

Copy the lines in Table 1 into Notepad and save the file as BAKE.MAC in the MSDCHEM\MSEXE directory. The “!” indicates a comment (line) which is not executed. Note that the temperature limits, which reside in the tune file, must be edited to allow the higher settings.

Table 1. ChemStation Macro for Baking the Source and Quadrupole After Source Maintenance

```
name Bake
! this macro sets the source and quad temps to their maximum and holds for a set period
parameter hours def 6                  ! default setting is 6 hours -this is customizable
msinsctl "mstemp QUAD, , , 200"        ! sets the quad temperature to bake at 200C
synchronize
msinsctl "mstemp SOURCE, , , 300"      ! sets the source temperature to bake at 300C
synchronize
SLEEP hours*60*60                      ! bakes for set period
msinsctl "mstemp QUAD, , , 150"        ! sets the quad temperature to operating temp at 150C
synchronize
msinsctl "mstemp SOURCE, , , 230"      ! sets the source temperature to operating temp at 230C
synchronize
return
```

Usually a source cleaning is executed at the end of the working day, and the system pumped down overnight for operation the next day. In this case, a "pumpdown sequence" is useful. After the system is confirmed to be leak-tight, this sequence is loaded and executed which bakes the source and quad overnight, then executes an Autotune, and then makes a few injections of a checkout standard to confirm system performance. In this way, the analyst returns the next day to review data about the system prior to beginning new analyses. An example of this is given in Figure 7.

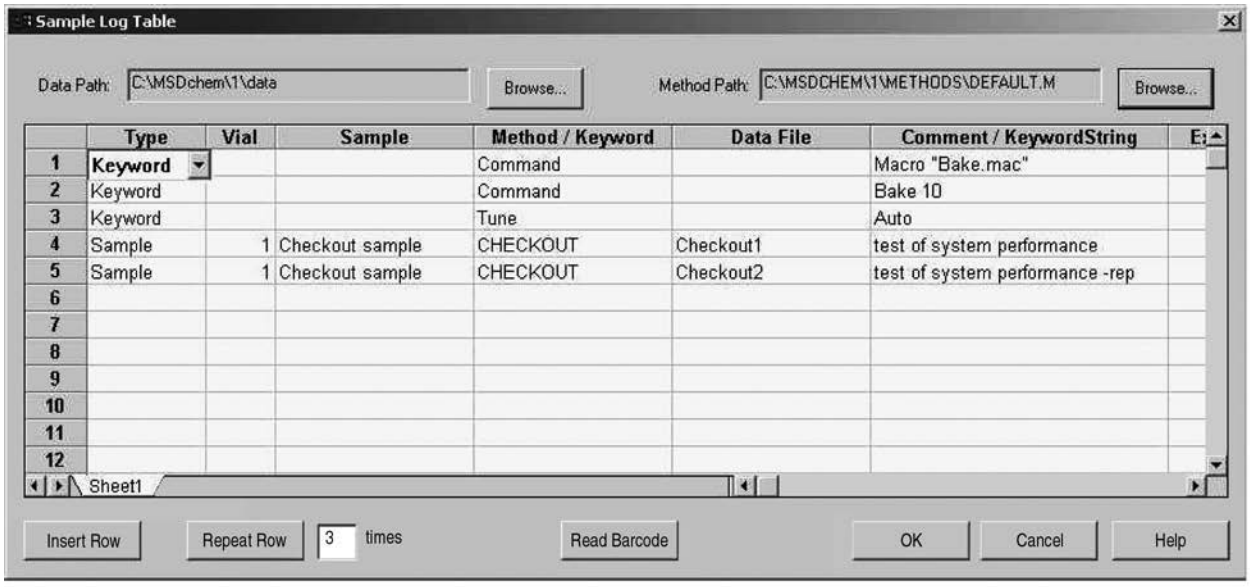

Figure 7. Pumpdown sequence table using source bakeout.

Line 1 Loads the Bake macro. Line 2 sets the bake time to 10 hours. After the bake, (Line 3) an autotune is executed. Lines 4 and 5 run the system performance method, CHECKOUT.M, on the system checkout standard. Note: after the system has been cleaned and leak-checked, the CHECKOUT.M method should be loaded, THEN this sequence should be run!

Conclusions

The increased source temperature limit available on the 5973N inert MSD can provide improved detection limits for common, late-eluting, recalcitrant compounds such as the POPs when properly applied. A requirement, that must be explored, is that the higher source temperatures do not increase compound fragmentation or reduce the intensity of the (useful) higher mass ions. These improvements are most likely to be realized in SIM acquisitions where the increased background that must result from higher source temperatures is not as likely to affect the signal.

This application note also describes a programmed bake-out of the source and quadrupole that can be automatically implemented after source cleaning. This bake-out provides a rapid lowering of the air-water background and can be used within the sequence table as part of the instrument performance checkout.

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Printed in the USA
February 10, 2004
5989-0678EN

Agilent Technologies

Fast and Ultra-fast Analysis with the Agilent 1200 Series Rapid Resolution LC System Compared to a Conventional Agilent 1100 Series LC System Using Sub 2-µm Particle Columns

Application Note

A. G. Huesgen

Abstract

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges and lower delay volume - predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns. This Application Note shows that shorter run times, shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained using the Agilent 1200 Series Rapid Resolution LC (RRLC) system.

Agilent Technologies

Introduction

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Increasingly more applications are carried out using LC/MS systems, therefore there is also a demand to use narrow-bore columns for full compatibility with most MS engines. Narrow-bore columns with an internal diameter of 2.1 mm and lower have high demands in respect to low delay volumes and dispersion volumes before and after the column. In the following experiment an example is given, showing how fast methods can be developed on an LC system taking advantage of higher pressure and temperature limits of state-of-the-art equipment. In addition, speed and performance comparisons are made between a conventional Agilent 1100 Series LC system and an Agilent 1200 Series Rapid Resolution LC system, using 4.6-mm ID columns and 2.-mm ID columns packed with 1.8-µm particles.

Experimental

An Agilent 1200 Series RRLC system was used with the following modules:

- Agilent 1200 Series binary pump SL with vacuum degasser for applications using 1.8-µm particle columns up to 150-mm length and with internal diameters from 2.1 to 4.6 mm
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with wide temperature range from 10 degrees below ambient up to 100 °C
- Agilent 1200 Series diode-array detector SL for 80-Hz operation, including new data protection tool
- ZORBAX SB C-18 columns with different internal diameters and 50-mm length, packed with 1.8-µm particles
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number G1316-68744)

An Agilent 1100 Series LC system was used with the following modules:

- Agilent 1100 Series binary pump with vacuum degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode-array detector B
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number 5065-9947)

Results and discussion

In the past the Agilent 1100 Series LC system was frequently used for fast and ultra-fast analysis[1]. The instrument is very well suited specifically for the analysis of compounds using short 4.6-mm ID column packed with 1.8-µm particles, and run times below one minute. Cycle times below two minutes were achieved. The Agilent 1200 Series RRLC system is a newly developed LC system with a wider pressure and temperature range, lower system delay volumes and improved noise for the DAD system. Due to these advancements, speed and performance have improved compared to an Agilent 1100 Series LC system, especially for columns with an internal diameter of 2.1 mm.

Experiments using a 4.6-mm ID column

Both instruments were set up in a standard configuration with mixers and 0.17-mm ID flow capillaries installed. Typically the same parameters can be used to optimize an LC method for speed and resolution. These parameters are flow rate, column temperature, gradient profile and other instrument-specific parameters such as switching the autosampler delay volume out of the flow path after the sample has reached the top of the column (ADVR=automatic delay volume reduction). Gradient changes can therefore reach the column much faster. A typical example of how a fast method can be developed is given in figure 1. The objective is to achieve fast cycle times and a minimum resolution of 2 for all peaks.

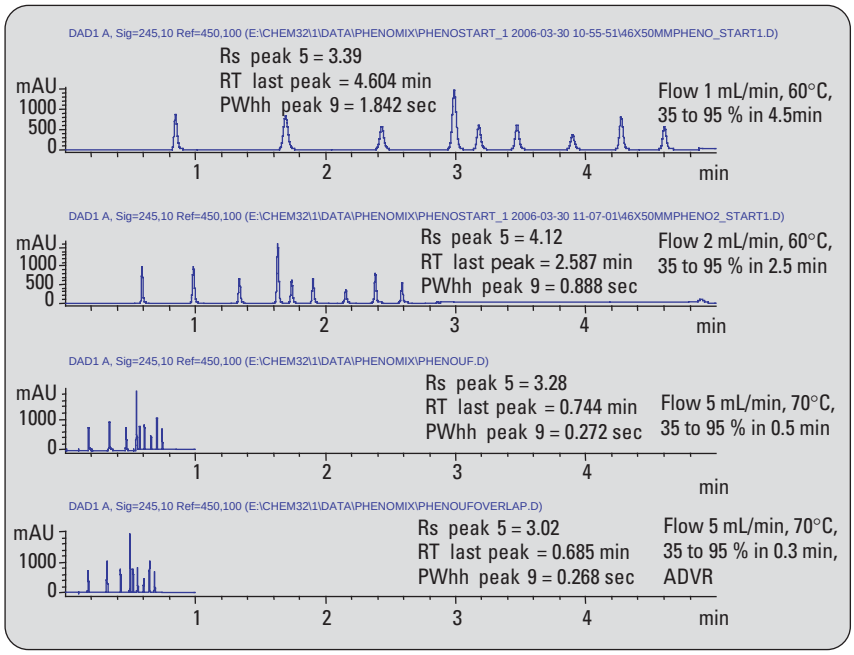

Figure 1
Method development of an ultra fast LC method.

Chromatographic conditions:

Test sample:	Set of 9 compounds; 100 ng/µL each; dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6.Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	50 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Pump:	Solvent A: H_2O + Solvent B: ACN Gradient: 35 to 95 % B using different profiles
Autosampler:	Injection volume: 1 µL Wash 5 sec for needle exterior flush out factor 20
Thermostatted column compartment:	Temperature: different temperatures
Diode array detector	B and diode-array detector SL: Signal: 245/10 nm Ref 450/100 nm

Optimization of all of the above-mentioned parameters on both systems resulted in the chromatograms shown in figure 2. The pressure limit of 400 bar on the Agilent 1100 Series LC system restricts the maximum possible flow. 5 mL/min flow was not possible, even though the column temperature was set to 80 °C, which is the upper limit for the 1100 Series column compartment. The Agilent 1200 Series RRLC system can be operated with up to 600 bar and up to 100 °C. Applying a flow rate of 5 mL/min can be done without reaching the 600 bar pressure limit at elevated temperatures. In addition, due to design changes, the noise level of the Agilent 1200 Series DAD SL has significantly improved compared to the Agilent 1100 Series DAD B.
The performance for both systems is shown in table 1.

Resolution and noise have improved with the Agilent 1200 Series RRLC system, whereas run and cycle times are comparable. The noise level of the 1200 Series RRLC system can be further reduced using the post column cooling device[2]. The device adapts the temperature of the column effluent to the temperature of the optical unit. This further reduces the noise level, especially if high flow rates and high temperatures are used. Another possibility to reduce cycle time is to enable the overlapped injection features, which is possible with both systems.

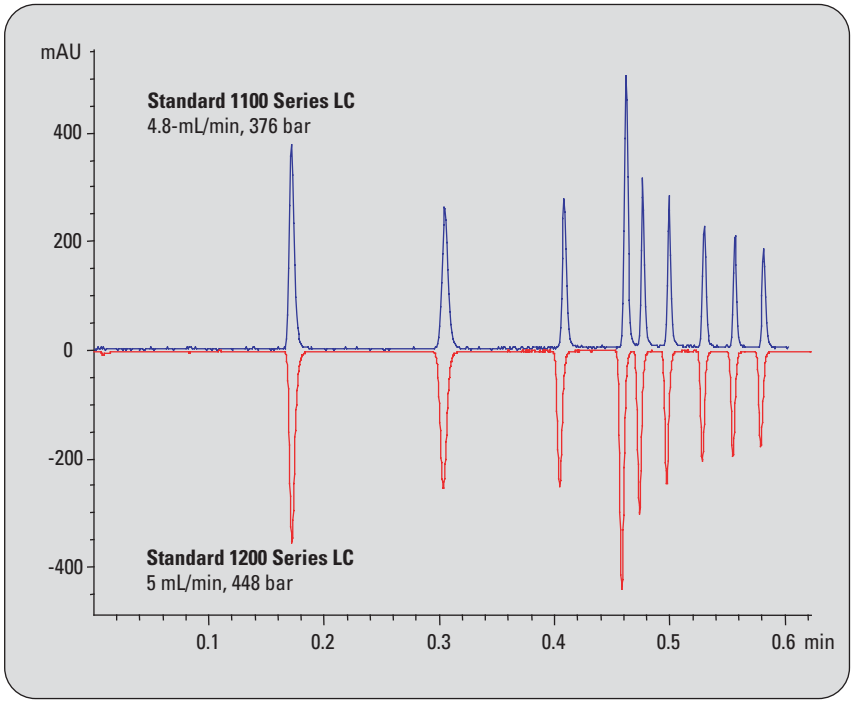

Figure 2
Standard Agilent 1200 Series RRLC system vs. Agilent 1100 Series LC system: analysis of phenone mix on 4.6-mm ID column packed with 1.8-µm particles.

Chromatographic conditions:

Test sample:	Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	50 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Pump:	Solvent A: H_2O, Solvent B: ACN Gradient: 35 to 95 % B in 0.3 min
Autosampler:	Injection volume: 1 µL Wash 5 sec for needle exterior, flush-out factor 20
Thermostatted column Compartment:	Temperature: 80 °C
Detector DAD B and DAD SL:	Signal: 245/10 nm Ref 450/100 nm

_Parameter	Standard 1100 Series 80 °C 4.8 mL/min	Standard 1200 Series 80 °C 5 mL/min
Flow rate	4.8 mL/min	5 mL/min
Run time	0.60 min	0.60 min
Cycle time	1 min 37 sec	1 min 37 sec
Rs Peak 5	2.22	2.30
PW1/2 peak 9	0.00378 min	0.00375 min
PW1/2 peak 1	0.00458 min	0.00486 min
Noise PtoP	6.2021mAU	0.7930 mAU
Backpressure	376 bar	448 bar
Injection volume	1 µL	1 µL
DAD data rate	20 Hz, path 10 mm	80 Hz, path 10 mm

Table 1
Performance comparison for 4.6-mm ID column.

Furthermore, column switching valves can be installed in the ovens, which provides even higher sample throughput using 2 columns for analysis. A sample is analyzed on the first column, while the second column is regenerated using a second pump. If the analysis on the first column is completed, the next injection can be immediately performed on the previously equilibrated second column.

Experiments using 2.1-mm ID column

Columns with an internal diameter of 2.1 mm and lower have high demands regarding low delay volumes and dispersion volumes before and after the column. Using columns with an internal diameter of 2.1 mm, the Agilent 1100 Series binary LC system must be optimized without using a mixer or only a mixer with a significantly smaller volume and capillaries with smaller IDs for all flow connections. Nevertheless, cycle times below 2 minutes could barely be achieved using columns packed with 1.8 µm particles and 50 mm length. This was mainly due to the pressure limitation of 400 bar for the Agilent 1100 Series LC system. In addition, the delay volume of the 1100 Series LC system is a drawback for fast run and equilibration times. With the introduction of the Agilent 1200 Series RRLC system this gap was closed. Now using narrow bore columns packed with 1.8-µm particles, run times below 0.5 min are possible, with higher flow rates and elevated temperatures. Both systems are compared using the same column and optimized instrument configurations. To allow for optimized conditions for both systems, the following set-ups were used:

Configuration of the Agilent 1100 Series LC system:

- The mixer was replaced by a short capillary with an internal diameter of 0.12 mm (Agilent part number G1312-67301)
- Seat and seat capillary were replaced by 0.12-mm ID parts (well-plate seat, Agilent part number G1367-87104, and seat capillary, Agilent part number G1313-87103)
- The capillary from the injector to the column compartment was replaced with a 0.12-mm ID capillary (Agilent part number 01090-87610)
- The 0.17-mm ID capillary from the column compartment to the column was exchanged with a capillary with an internal diameter of 0.12 mm (Agilent part number G1316-87303)
- The column was connected to the detector using the detector inlet capillary.
- A 1.7-µL cell with a path length of 6 mm was used as the detector cell.

Configuration of the Agilent 1200 Series RRLC system:

- The low delay volume configuration for the pump was set up with a 120-µL delay volume (mixer and damper were moved out of the flow path).
- Two flow capillaries were replaced with 0.12-mm ID capillaries, all included in the Agilent 1200 Series low dispersion kit (Agilent part number G1316-68744).
- The seat capillary was also replaced with a 0.12-mm ID capillary (included in kit Agilent part number G1316-68744)
- The DAD SL 2 µL flow cell with a 3-mm path length was used. The inlet capillary was directly connected to the column outlet.

The same 2.1 x 50 mm column was used for both systems. The flow rate was set so that the backpressure was close to the limit of each system. Automated delay volume reduction (ADVR) was selected in the injector setup screen for both systems. The injection volume was set to 1 µL for the Agilent 1100 Series LC system, and to 2 µL for the Agilent 1200 Series RRLC system to compensate for the lower path length of the 1200 Series 2-µL flow cell.

In figure 3 an overlay of the chromatograms obtained from both systems is shown. In table 2 the performance for both system is recorded.

The chromatograms in figure 3 clearly demonstrate the advantages of the Agilent 1200 Series RRLC system, using 2.1-mm ID columns, packed with 1.8-µm particles. Faster run times and cycle times are possible, due to the fact that higher flow rates can be obtained with the Agilent 1200 Series RRLC system. Table 2 indicates that the cycle time for the Agilent 1200 Series RRLC system is only half that of the Agilent 1100 Series LC system. In addition, the resolution of the 5th peak and also peak width at half height is significantly improved at higher flow rates.

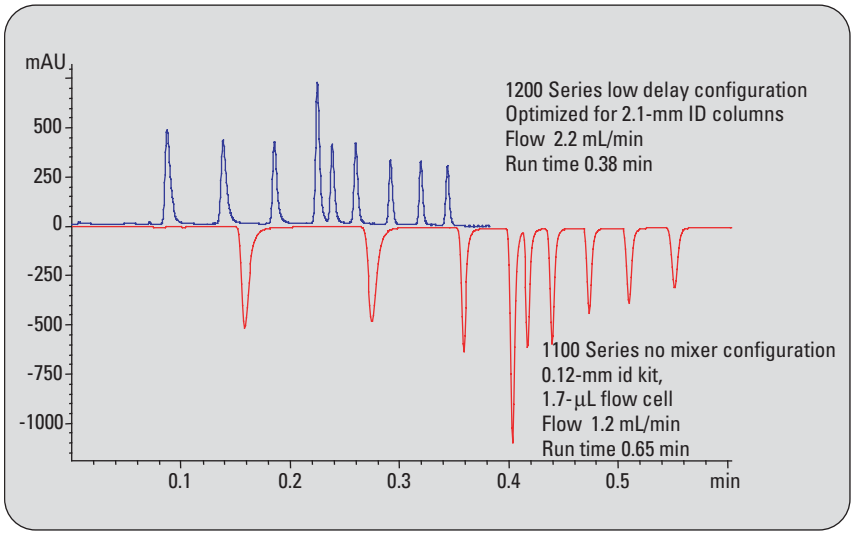

Figure 3
Analysis performed with a 2.1-mm ID column with the optimized Agilent 1200 Series RRLC system and the optimized Agilent 1100 Series LC system using automated delay volume reduction for both systems.

Chromatographic conditions:

Test sample:	Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	50 x 2.1 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Pump:	Solvent A: H_2O , Solvent B: ACN Gradient: 35 to 95 % B in 0.3 min
Autosampler:	Injection volume: 1 and 2 µL Wash 5 sec for needle exterior, flush out factor 20
Thermostatted column compartment:	Temperature: 80 and 95 °C
Detector DAD B and DAD SL:	Signal: 245/10 nm Ref 450/100 nm

Parameter	1100 Series, optimized, no mixer, ADVR, 80 °C	1200 Series, optimized, low delay volume configuration, ADVR, 95 °C
Flow rate	1.2 mL/min	2.2 mL/min
Run time	0.65 min	0.38 min
Cycle time	2 min 33 sec	1 min 16 sec
Rs Peak 5	1.86	2.15
PW1/2 peak 9	0.00556 min	0.00328 min
PW1/2 peak 1	0.00729 min	0.0049 min
Noise PtoP	0.1 mAU	0.2 mAU
Backpressure	370 bar	570 bar
Injection volume	1 µL	2 µL
DAD data rate	20 Hz, path 6 mm	80 Hz, path 3 mm

Table 2
Performance comparison using a 2.-mm ID column.

Conclusions

Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges. Predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns, an LC system like the Agilent 1200 Series RRLC system provides significantly lower delay volumes. Shorter run times and shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained.

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1.
Anabel Fandino, "Ultra-fast liquid chromatography using the Agilent 1100 Series HPLC system and 1.8-um ZORBAX SB C18 Rapid Resolution HT columns", *Agilent Application Note, publication number 5989-1603EN*, **2004.**

2.
A.G.Hüsgen, "Agilent 1200 Series column compartment SL with temperature control up to 100 °C and post-column cooling for lowest baseline noise", *Agilent Application Note, publication number 5989-5034EN*, **2006.**

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www.agilent.com/chem/1200rr

Published October 1, 2006
Publication Number 5989-5672EN

Agilent Technologies

Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns

Application Note

Michael Frank

Abstract

The need to increase the daily throughputs of LC systems is a constant desire. Now, with the Agilent 1200 Series Rapid Resolution LC system highest throughputs are possible, and in combination with the Agilent ZORBAX RRHT columns and the increased pressure and temperature range of the LC system, excellent chromatographic resolution can be achieved even at run times below one minute.

This Application Note describes the correct set-up of the instrument which is the key for optimal results with narrow bore columns, such as a 2.1 mm x 50 mm column packed with sub two micron particles. Peak capacities in the range of fifty in analysis times as short as 24 seconds and peak widths as narrow as 200 milliseconds are shown. The well-balanced use of all possible module options to achieve shortest cycle times with throughputs far beyond 1500 samples per day is described.

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Introduction

Particularly analytical service laboratories in the pharmaceutical industry, responsible for analyzing chemical libraries[1] or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates[2] are faced with the challenge to increase their throughput, but also to maintain a high chromatographic resolution. In 2003 Agilent Technologies introduced sub two micron particles in their RRHT column series. Because of the small particle size, the chromatographic resolution obtainable with these columns is superior to standard particle sizes such as 3.5 µm or even 5 µm. Due to a unique silica manufacturing process, Agilent ZORBAX RRHT columns show a significantly reduced backpressure, if compared to similar column dimensions of other manufacturers. Excellent chromatographic results are achieved in a very short analysis time with the Agilent 1200 Series Rapid Resolution LC system, which facilitates an increased pressure range and flow rates from 0.05 up to 5 mL/min using column diameters ranging from 2.1-mm id up to 4.6-mm id. This Application Note will focus on 2.1-mm id columns only.
Not only are the run times of the analyses important for high throughput, but also the overhead time. The Agilent 1200 Series Rapid Resolution LC system can be optimized to achieve highest throughputs with exceptionally good overall system performance.

Experimental

An important issue when dealing with narrow bore columns, especially in gradient mode where smallest peak widths can be achieved, is to have small extra column volumes. This also includes any volumes in front of the sampling device, because any volume after the solvent mixing point will increase the time for the gradient composition to reach the column. This results in an increased run time. The Agilent 1200 Series Rapid Resolution LC system can be reconfigured within a few minutes to provide appropriate system volumes for different column ids. Here, the pumps are set-up in the low delay volume configuration with an internal volume of approximately 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744). Consequently, only capillaries of 0.12 mm id are used beyond the injection valve. In the Agilent 1200 Series thermostatted column compartment SL the newly introduced low dispersion heat exchangers with 1.6 µL internal volume were used. In some experiments, the Agilent 1200 Series Rapid Resolution LC is set up for alternating column regeneration to achieve highest throughput using the ACR-capillary kit (G1316-68721) and 2.1-mm id columns[3]. The high pressure rated 2-position/10-port valve in the thermostatted column compartment was only placed into the flow path if alternating column regeneration was used indeed.

The instrument set-up is as follows (figure 1):

- Agilent 1200 Series binary pump SL with the new Agilent 1200 Series micro vacuum degasser
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration
- Agilent 1200 Series diode-array detector SL with a 2-µL/3-mm cell
- ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm

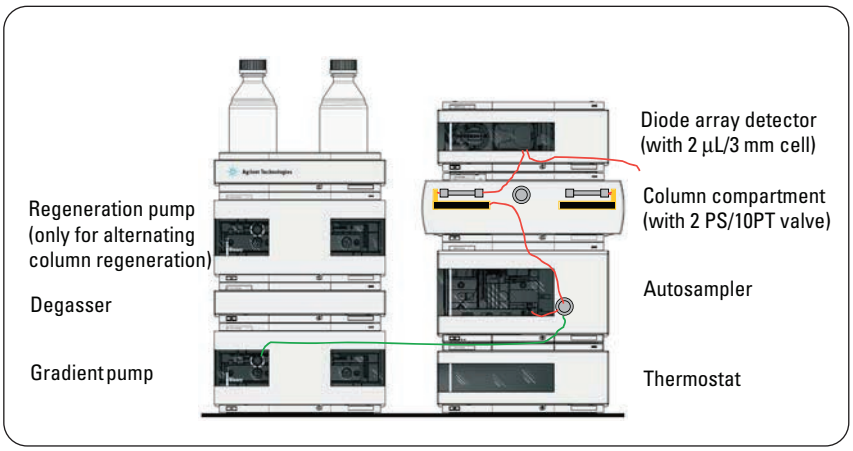

Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and lowest pump ripple. The pump hardware is significantly different from the standard binary pump. In the Agilent 1200 Series binary pump SL the pressure transducer is separate from the damper which has been modified to have a lower delay volume (pressure dependent ranging from 80-280 µL). In this study the pumps were used in the low delay volume configuration without the mixer and damper in the flow path. In contrast to the standard binary pump the pump heads of the binary pump SL have an additional damping coil (500 µL volume each) to allow damping in the low delay volume configuration. This does not add to the gradient delay volume because it is before the mixing point. Anyhow, pressure ripples are also strongly suppressed by the Electronic Damping Control (EDC). The pressure range of the pump and all other modules is increased to 600 bar.

Only one sample, the so-called "phenone-mix", was used in the course of this study to keep variations low. The sample consists of nine compounds: acetanilid, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. Unless otherwise stated, the concentration was 0.1 µg/µL for each compound except butyrophenone which was 0.2 µg/µL. The solvent was water-acetonitril 2:1.

Results and discussion

The most frequently sold particle size in chromatographic columns today is 5 µm. Of course, fast and ultra fast LC is also possible with columns packed with particles of these larger diameters – the reduced back pressure is even beneficial to allow higher flow rates. However, resolution will be sacrificed because conditions are usually far on the right side of the van-Deemter-optimum. Here, the big advantage of the RRHT columns with particles of less than 2 µm diameter is proven. The van Deemter optimum is shifted further to the right and the curve is much flatter at the onset because the "resistance of mass transfer" term is diminished (figure 2). In figure 3 the analysis on a 2.1-mm id column with 1.8-µm particles is compared to the linear scaled analysis on the same stationary phase but on 5 µm particles packed in a 4.6-mm id-column. The gain in resolution is obvious – from Rs = 2.1 up to Rs = 3.5 for the critical pair which matches the theoretically expected value of a 1.66 fold increase in resolution. Also note that there is a saving in solvent consumption of 8.6 mL in the "standard" HPLC analysis and only 1.8 mL in the ultra fast HPLC analysis.

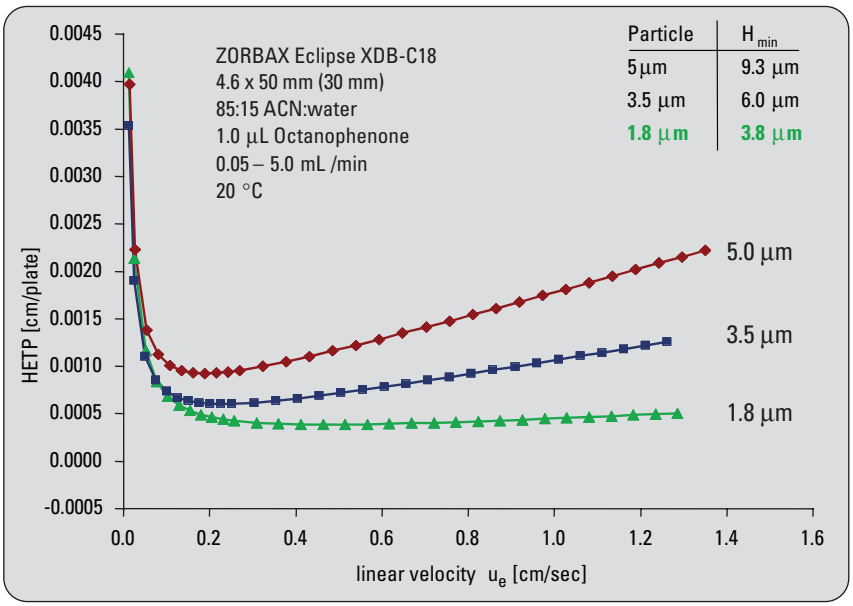

Figure 2
Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.

For gradient separation the dependencies of the capacity factor can be expressed as:

$$k^* = 0.87 \cdot tg \cdot \frac{F}{Vm \cdot \Delta\%B \cdot S}$$

(tg = gradient time, F = flow rate, Vm = column void volume, △%B = gradient steepness, S = solvent and solute dependent factor)

If the product of the gradient time and flow rate, the so-called gradient volume, is kept constant together with all other parameters, the gradient time might be decreased while the flow rate is increased. Thus, the capacity factors of two compounds will stay constant and if no large alteration of the plate height occurs, the resolution will not change significantly, either. The final point is the big advantage of the sub two micron particles – the van-Deemter curve is nearly flat on the right side of the minimum (figure 2) and flow rates can be increased with only little increase in plate heights. However, the equation is an empirical one and deviations may occur especially under extreme conditions.

With a two-step approach, highest gradient speeds with virtually no loss or only little loss in resolution can be achieved. In the first step, start from a medium temperature and begin to increase the flow rate up to the pressure maximum. Subsequently the temperature should be increased to lower the viscosity of the solvent and then the flow rate is increased again. It may be worthwhile to check the resolution with two identical gradients but with different temperatures to see the influence of the temperature change on the resolution which may be very compound dependent. In figure 4 the result of this approach is shown. A nearly 7-fold increase in separation speed could be achieved with still baseline separation of the critical pair before meeting the pressure and temperature limit (the maximum temperature is a function of flow, temperature, number of controlled Peltier elements and of the heat capacity of the solvent used).

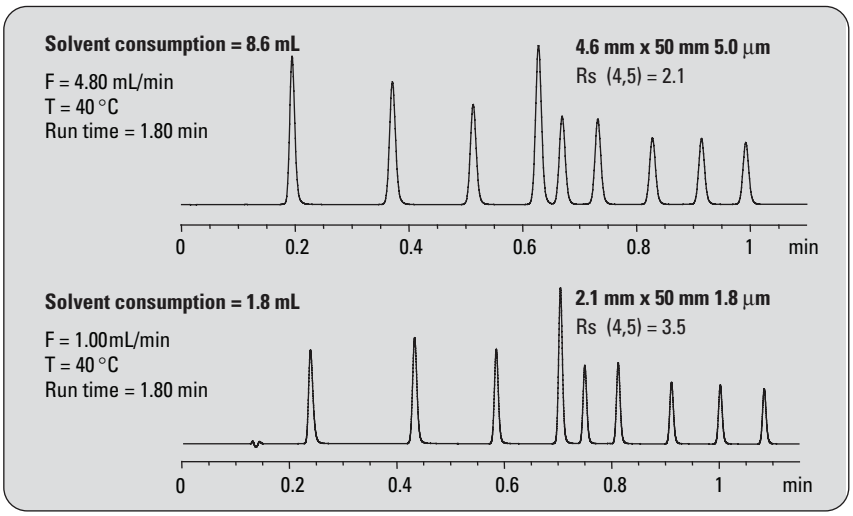

Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

Conditions:	4.6-mm id column used on standard Agilent 1200 system	
Solvent:	A = Water, B = ACN	
Temperature:	40 °C	
Column:	**2.1 mm x 50 mm, 1.8 µm**	**4.6 mm x 50 mm, 5.0 µm**
Flow:	1.0 mL/min	4.8 mL/min (scaled from 2.1 mm col.)
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 % B	1.11 min 35 % B
Stoptime:	1.15 min	1.15 min
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	245 nm (8), ref. 450 nm (80)
Peakwidth:	>0.0025 min (0.05 s res.time), 80 Hz	>0.01 min (>0.2 s), 20 Hz
Injection volume:	1 µL	5 µL (not scaled)

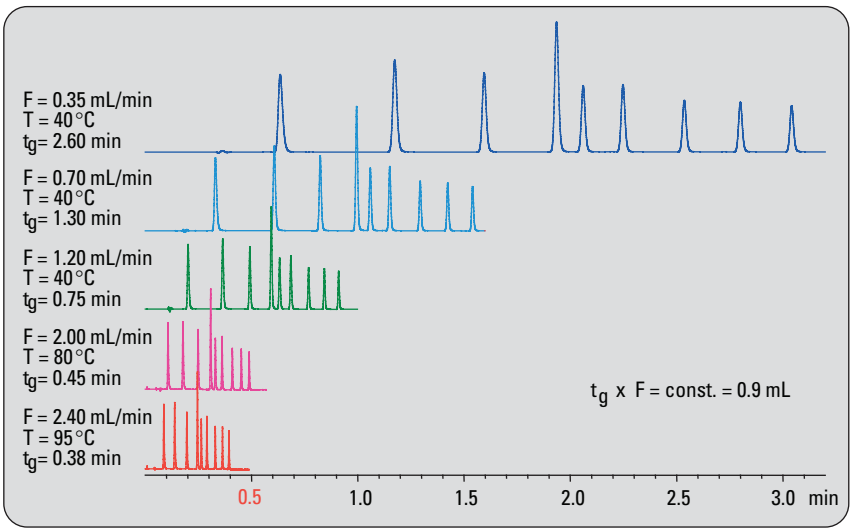

Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

Conditions:
Solvent: A = water, B = ACN
Temp.: 40 °C, 80 °C, 95 °C
Flow: 0.35, 0.70, 1.20, 2.00, 2.40 mL/min
Gradient: 0.00 min 35 %B
2.60 min 95 %B
3.20 min 95 %B
3.21 min 35 %B
Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL
Stop time: 3.20 min
Post time: 2.00 min
Wavelength: 245 nm (8), Ref. 450 nm (100)
Peak width: >0.0025 min (0.05 s response time), 80 Hz

The last chromatogram is enlarged in figure 5 and reveals the details of this separation. The first peak is eluted after only five seconds and peaks with a width at half height of less than 200 ms are achievable. Within twenty-four seconds nine compounds are separated with a peak capacity in the range of fifty.

Retention time precision at highest analysis speed

High analysis speed is meaningless without precision. One basic performance criteria for HPLC pumps is the precision of gradient formation measured by the precision of retention times of repeated gradients. However, the stability of the column temperature must also be taken into consideration, because temperature fluctuations will also influence the retention times of a given sample. In table 1 and figure 6 the results from the 10-fold repeated analysis of a standard sample are listed and since the deviation between individual runs is so small, the octanophenone peak is enlarged in a separate window. This sample contains compounds that are both not retained and refer to isocratically eluted compounds found at the starting conditions of the gradient, as well as highly unpolar and strongly retained compounds. The analyses

Conditions:

Solvent:	A = Water, B = ACN
Temp.:	40 °C, 80 °C
Flow:	0.35 mL/min, 1.20 mL/min, 2.0 mL/min
Gradient:	0.00 min 35%B
	2.60 min 95%B
	3.20 min 95%B
	3.21 min 35%B
	Time values for F = 0.35 mL/min.
	For all other flow rates times are
	scaled so that (time x flow) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Injection vol.:	1.0 µL

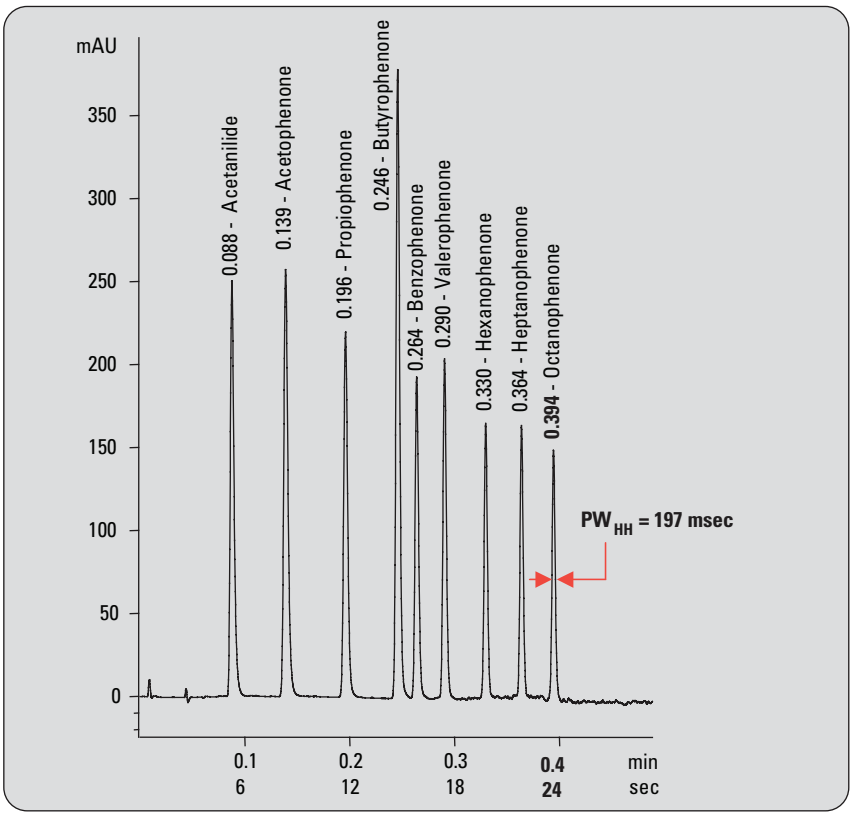

Figure 5
Separation of a nine compound mixture under ultra fast conditions.

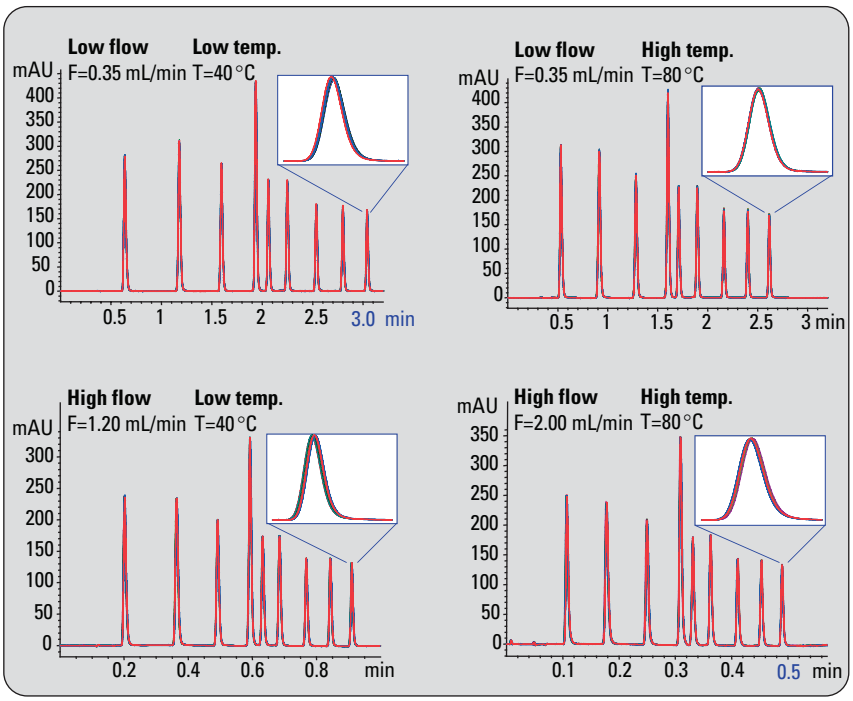

Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

were done at high and low flow rates as well as with high and low temperatures as in the examples shown earlier. In all cases the mean retention time precision is below 0.3 % RSD, which was the specification of the Agilent 1100 Series LC system. Of course, the results are also in line with the specifications for the new Agilent 1200 Series Rapid Resolution LC system which is < 0.07 % RSD or < 0.02 min SD, whichever is met first. At these high gradient speeds, the SD criteria are always met. The RSD criteria are also met for both fast-LC gradients of 2.6 min duration (0.35 mL/min flow rate). Even at ultra-fast gradient speeds, the retention time precisions are still below or only slightly higher than 0.1% RSD (table 1).

Improving the cycle-time

Not only is the gradient speed important when dealing with high-throughput analysis but furthermore the over all cycle time of the entire system, which is the time between two consecutive analyses. A good method to measure the cycle time is by using the time stamp the data file is assigned by the operating system of the computer. Clearly, optimizing the cycle time has some drawbacks. For example, extensive needle cleaning procedures are in contradiction with a high sampling speed. Table 2 gives an overview of important parameters influencing the cycle time. Using 1.8-µm particle size columns together with an optimized HPLC system very short run times can be achieved without sacrificing chromatographic resolution. Combining short run times together with low overhead times will result in a high daily throughput. In figure 7 the cycle time and daily throughput is shown for two

	0.35 mL/min, 40°C		0.35 mL/min, 80°C		1.20 mL/min, 40°C		2.00 mL/min, 80°C	
	SD	% RSD	SD	% RSD	SD	% RSD	SD	% RSD
Average	0.00107	0.067	0.00084	0.070	0.00048	0.098	0.00031	0.134

Table 1
Standard deviations (mAU) and %RSD (n=10) of the retention times under different chromatographic conditions in temperature and flow.

Module	Parameter	Effect on cycle time	Other effects
Pump	Low delay volume setting	Reduced retention times, run time can be shortened, reduced cycle time	Increased pressure ripple, slightly increased mixing noise if modifiers such as TFA are used.
Autosampler	Automatic Delay Volume Reduction (ADVR) – activated	Reduced delay volume, reduced retention times, run time can be shortened, reduced cycle time	Increased carry-over
	ADVR activated and Overlapped Injection (OI)	Enables parallel sampling, thus reduces the cycle time independently of the below listed settings (as long as the overall sampling speed does not exceed the gradient and post time)	Increased carry-over
	no OI – Needle Wash	Increased sampling time with increasing wash time	Reduced carry-over with longer needle wash time
	no OI – Equilibration time	Increased sampling time with increased equilibration time	Better injection precision with longer equilibration time
	no OI – Draw/Eject speed	Low speed causes increased sampling time	Low speed results in better injection precision
Column compartment	Alternating column regeneration	Saves column wash-out and equilibration time, reduces cycle time enormously	Additional hardware required, slightly increased extra column volume, slightly different retention times between columns possible
Detector	Pre-run and/or post-run balance	Increased cycle time	Baseline drifts possible if not applied
	Spectral data acquisition with high data rate, small band width and broad wavelength range large data files	Depending on computer power and additional processes running might increase cycle time because of writing speed	Reduced information content if no spectral data acquired or with lower resolution
Software	Data analysis with acquisition	Increased cycle time, depending on computer power and number of peaks	Data analysis has to be done offline is no set
	Save method with data	Slightly increased cycle time	Information is missing if method is not saved
	Execution of pre-run or post-run macros	Increased cycle time, depending on macro	Depending on macro
System	LC controlled over local network between computer and LC (and MS) only	Faster data and method transfer between computer and LC because of reduced net work traffic reduced cycle time	Additional hardware might be necessary (use independent acquisition computer)
	Number of detectors	More detectors produce a higher data amount and lower the data transfer speed, resulting in higher cycle times	More detectors higher information content

Table 2
Influence of various parameters on the overall cycle time.

different methods – both giving virtually the same resolution. The first method (0.45 min gradient) utilizes alternating column regeneration and high temperatures to allow high flow rates and speed optimized settings. A cycle time of 49 s could be achieved, resulting in a theoretical daily throughput of more than 1700 samples per day. The second method (0.90 min gradient) does not use high temperatures or alternating column regeneration and the time saving of some simple and often forgotten method options are shown. By optimizing these parameters the real cycle time gets as close to 8 s to the run time (stop time plus post time) and allows a daily throughput of more than 700 samples per day. By sub-optimal method set up this can easily drop to below 500 samples per day if options like automatic delay volume reduction, overlapped injection or offline data-analysis are not used.

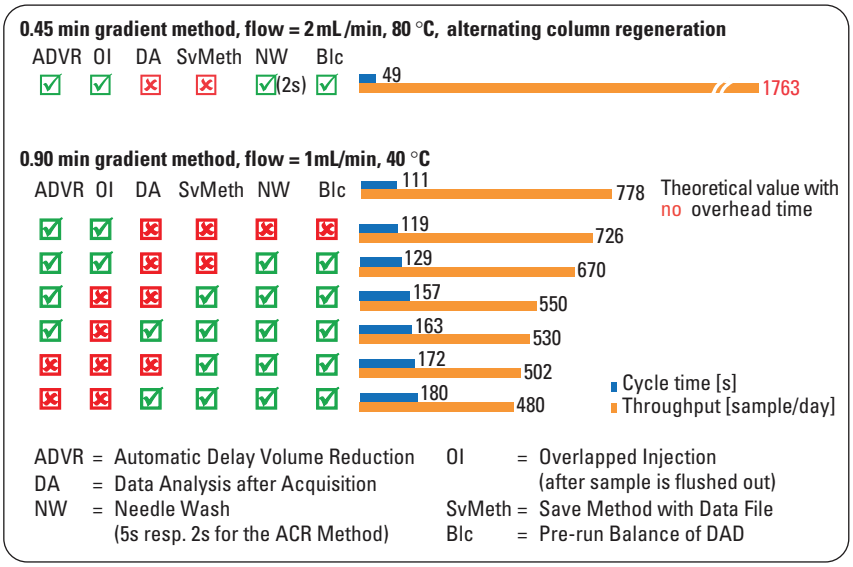

Figure 7
Cycle time and daily throughput optimization.

Chromatographic conditions:

Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	80 °C	
Flow:	2.0 mL/min	
ADVR:	Yes	
Gradient:	**Gradient-Pump**	**Regeneration-Pump**
	0.00 min 35 %B	0.00 min 35 %B
	0.45 min 95 %B	0.01 min 95 %B
	0.46 min 35 %B	0.11 min 95 %B
	0.57 min 35 %B	0.12 min 35 %B
Stoptime:	0.57 min	no limit
Posttime:	off	off
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	none	
Injection volume:	1.0 µL	
Injector:	Overlapped injection, 2 s needle wash, sample flush-out factor = 10, draw/eject speed = 100 µL/min	
Valve:	next position	

No Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	40 °C	
Flow:	1.0 mL/min	
ADVR:	Yes	No
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 %B	1.11 min 35 %B
Stoptime:	1.15 min	*1.40 min (add. 300 µL extra column volume, increased retention times)*
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	all, 190-500 nm, BW = 1 nm	
Injection volume:	1.0 µL	
Injector:	See figure 7, 2 s equilibration time	

Conclusion

The Agilent 1200 Series Rapid Resolution LC system is a powerful tool to achieve highest chromatographic resolutions and also highest throughputs. The extended pressure range allows the usage of columns packed with stationary phases with particles sizes below 2 µm, for example, Agilent RRHT columns with particle sizes of 1.8 µm. These columns not only allow an increase in linear flow rates with virtually no loss in resolution but also have an inherently higher resolution compared to 3.5 µm or even 5.0 µm particle sizes. The possibility to switch the pump into its low delay volume configuration allows the use of the entire bandwidth of today's widely used column ids – from 4.6 mm down to 2.1 mm and even 1.0 mm. As illustrated above, the system has uncompromised performance characteristics even at highest gradient speeds.

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www.agilent.com/chem/1200rr

Published June 15, 2010
Publication Number 5989-4502EN

Agilent Technologies

Combined EI and CI Using a Single Source

Technical Overview

Chris Sandy

Agilent Technologies

Introduction

The Agilent 5973x gas chromatograph/mass selective detectors (GC/MSDs) come with sources optimized for electron ionization (EI) and chemical ionization (CI). However, there are occasions where another ionization mode is desired without changing sources. This note demonstrates the capability of acquiring high-quality EI spectra with the CI source.

Data Acquisition

An Agilent 5973 inert MSD with a CI source was set up for the experiments. The following process was used to tune the MS:

1. Perform the CI autotune at the normal methane reagent gas flow rate (typically at a mass flow controller (MFC) setting of 20%).
2. Reduce the CI flow to 2%.
3. Set the emission current to 250 µa.
4. In Manual Tune, ramp the repeller from 0–5 volts for the mass 69 ion.
5. Set the repeller voltage to the maximum value.
6. Turn off the CI gas.
7. Save tune file.
8. Associate tune file with method.

Data was acquired in positive CI (PCI) and EI modes. Figure 1 shows the CI and EI total ion chromatograms using the CI source. The major and minor peaks are easily comparable in the two chromatograms.

Figure 2 shows the CI spectrum for Hexadecanolide (MW = 254) with the expected adduct ions for methane. Note the relatively large response for the 255 ion. As expected, there is little fragmentation due to the soft ionization.

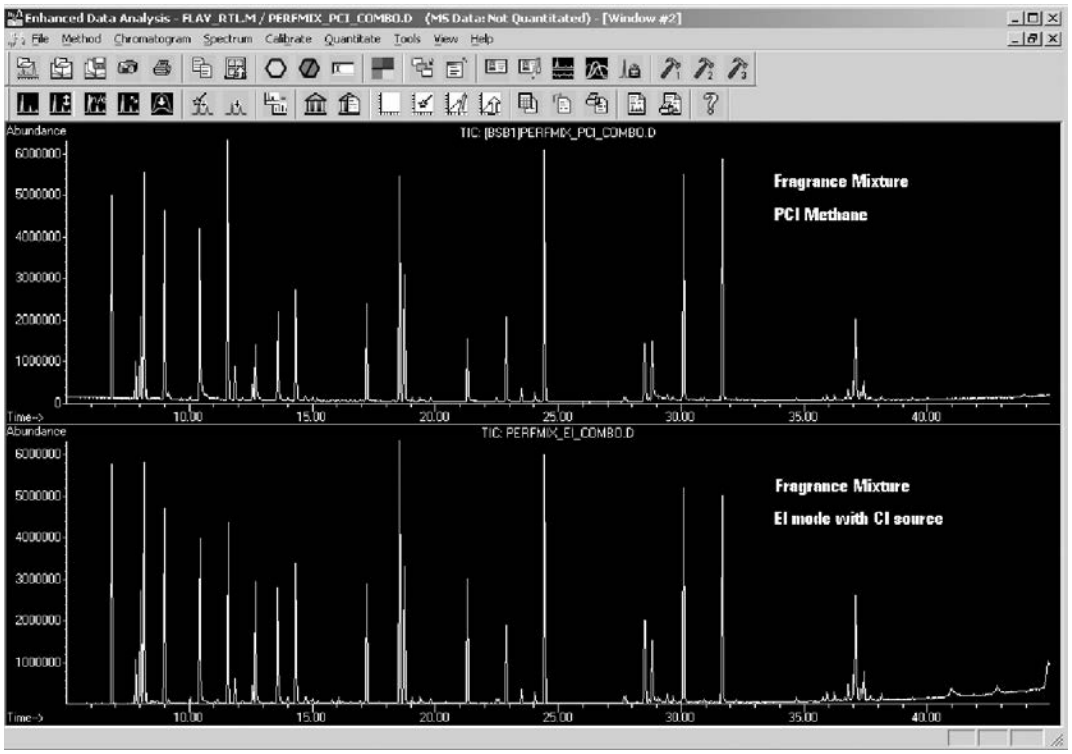

Figure 1. PCI and EI total ion chromatograms using the CI source.

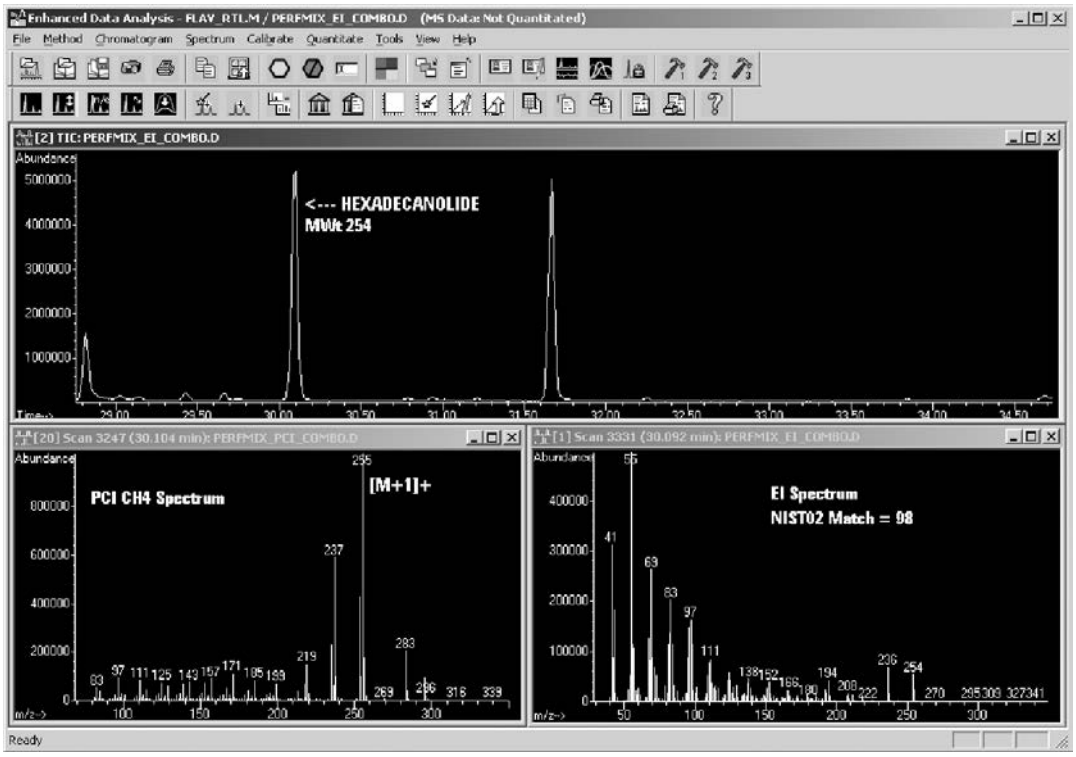

Figure 2. PCI and EI spectra for Hexadeconolide.

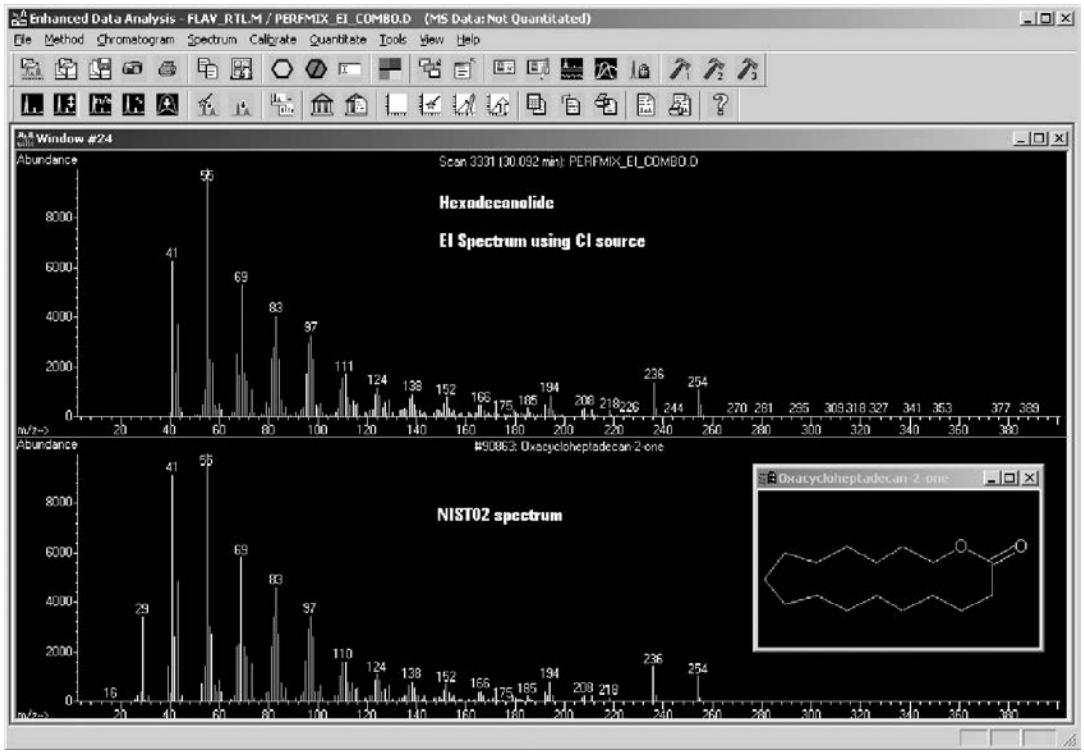

Figure 3. Acquired EI spectrum compared to the NIST02 library reference spectrum.

The EI data in Figure 3 shows much more fragmentation useful for compound identification. The response for 255 is relatively small. Using the NIST02 library, the EI reference spectra for Hexadecanolide (Oxacyclohelptadecan-2-one) was retrieved with a 98% quality match.

Summary

This data demonstrates the Agilent 5973 inert GC/MSD's ability to acquire high quality EI spectra using the CI source. The EI spectra can be searched against standard libraries for identification while the CI spectra provide molecular weight information. The ability to acquire both types of data without changing sources results in increased productivity.

For More Information

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Printed in the USA
January 30, 2004
5989-0595EN

Agilent Technologies

The Benefits of Achieving High Mass Accuracy at High Speed Using Agilent's TOF-MS Technology

Application Note

Edgar Naegele

Abstract

Measuring accurate molecular mass by mass spectrometry and calculating the corresponding empirical formula is an important step in the identification process of small molecules in a variety of application fields. Depending on the accuracy of mass measurement, significant empirical formulas can be calculated in low numbers. This Application Note will discuss the benefits of using the Agilent 6210 TOF mass spectrometer in combination with the Agilent 1200 Series Rapid Resolution LC system for compound identification in various applications.

Agilent Technologies

Introduction

Reliable empirical formula confirmation necessitates setting a mass accuracy limit, which takes the acceptable uncertainty of the accurate molecular mass measurement into consideration[1]. This results in more accurate mass measurement with decreasing relative mass error and requires fewer possibilities to consider for an empirical formula (table 1).

Mass accuracy [ppm]	Empirical formulae
100	138
50	67
25	32
10	15
5	7
2	2

Table 1
Mass accuracy vs. number of calculated empirical formulae for reserpine ($C_{33}H_{40}N_2O_9$ M=608.2734; within $C_{1\text{-}100}H_{2\text{-}200}N_{0\text{-}10}O_{0\text{-}10}$).

The current generation of comparably easy-to-use and inexpensive ESI orthogonal acceleration TOF (oaTOF) instruments are capable of handling this task. This was clearly demonstrated by a comparison study of different types of MS instruments, which are used for the determination of accurate mass of small molecules[2]. Innovations in TOF technology introduced during the past several years, like the orthogonal acceleration TOF technology with an analog-to-digital (ADC) converter, made this progress possible[3]. This Application Note will demonstrate the benefits of using the Agilent 6210 time-of-flight mass spectrometer in combination with the Agilent 1200 Series Rapid Resolution LC (RRLC) system and their impact on compound identification in various applications.

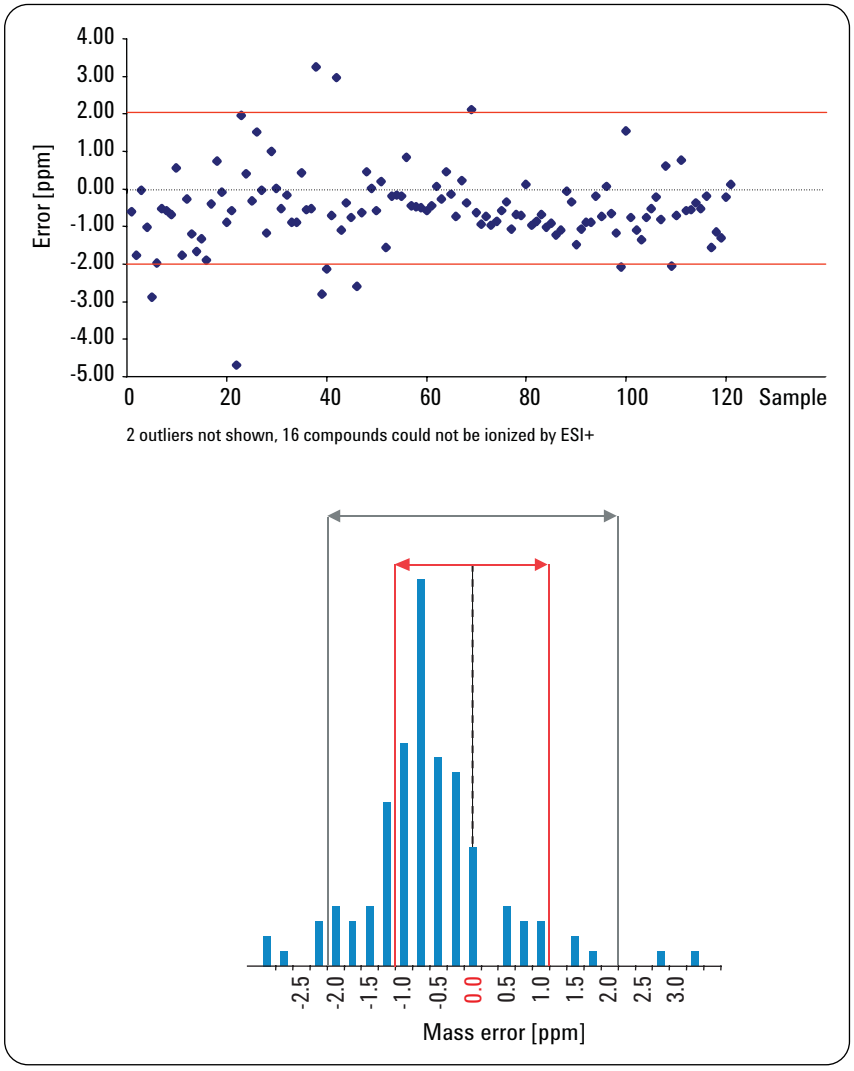

Figure 1
A) Mass accuracy errors as returned by an automatically generated report.
B) Histogram of the mass accuracy errors of the analysis of 140 real chemical library samples of a pharmaceutical company.

Results and discussion

When using a TOF mass spectrometer, attention is certainly focussed on the accurate mass. Figure 1A shows the achieved mass accuracy errors of the analysis of 140 members of a chemical library used in a screening campaign. More compelling is the histogram of these samples as shown in figure 1B. More than 71 % of the analyzed compounds have a mass accuracy error in the range of ± 1.0 ppm. This efficiency enables the chemist to narrow down the number of possible calculated empirical formulas for confirming the identity of a compound[4]. Analysis times below one

minute could be achieved, with high peak capacities above forty in just 39 seconds, both in the UV and in the MS chromatogram (figure 2) by using a method which includes alternating column regeneration, MS TOF data acquisition at 40 Hz, and DAD data acquisition at 80 Hz.

Application examples

- Analysis of complex samples with the MassHunter software, which allows extraction of molecular mass data and their detailed analysis[5] (figure 3).
- Detection and identification of minor impurities in pharmaceutical compounds generated during stability testing, production, formulation or storage of the final drug compound (Agilent publication numbers 5989-2348EN and 5989-5617EN).
- Statistical evaluation of achieved TOF mass accuracies with a real sample of less than 2 ppm (Agilent publication number 5989-3561EN).
- Simultaneous determination of metabolic stability and metabolite identification by high speed and high resolution (Agilent publication number 5989-5110EN).
- Automated screening of clinical body fluid samples for administered drugs (Agilent publica tion number 5989-5835EN).
- Identification of natural products from complex plant extracts (Agilent publication number 5989-4506EN).
- A complete overview of TOF applications is published in a compendium (Agilent publication number 5989-2549EN).

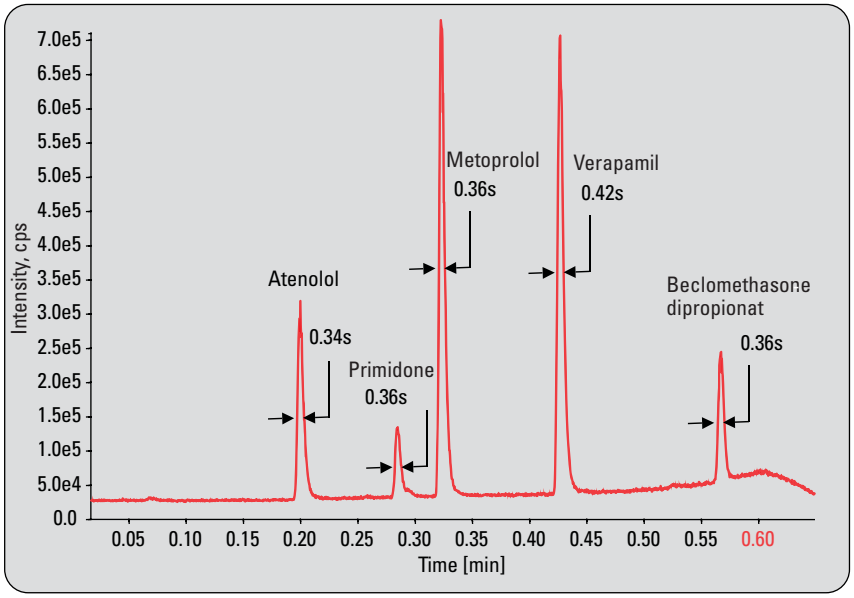

Figure 2
TIC chromatogram (40-Hz data rate of the 6210 TOF mass spectrometer, 80-Hz data rate of the DAD) with PWHH values for the TIC.

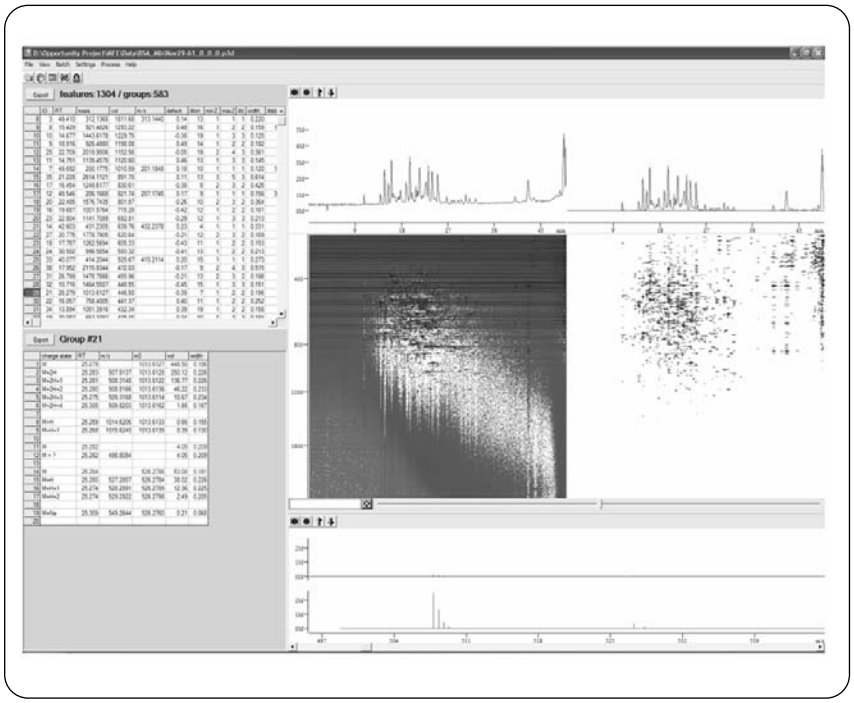

Figure 3
MassHunter software for analysis of complex samples.

Conclusion

- It is possible to rapidly acquire molecular mass data with highest mass accuracy in the single digit ppm error range with the Agilent 6210 TOF. This allows the unambiguous calculation of empirical formulas for compound confirmation.
- It is possible to measure mass differences with highest resolution with the Agilent 6210 TOF instrument. This allows the separation of compounds, which have a similar mass and distinguish between their empirical formulas.
- It is possible to acquire date with up to 40 Hz acquisition rate with the Agilent 6210 TOF. This permits the instrument to be used in ultra-fast LC separation applications.
- The principal benefits are accurate time-of-flight mass measurement, high resolution and high speed data acquisi tion, which can be used over a broad range of applications, such as library screening, screening of clinical samples, metabolite stability and metabolite identification, identification of minor impurities in drugs and natural product analysis.

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www.agilent.com/chem/tof

Published December 1, 2006
Publication Number 5989-5918EN

Agilent Technologies

Can "Deconvolution" Improve GC/MS Detectability?

Application Note

All Industries

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Abstract

This study uses 35 pesticides spiked in spinach extracts at the 50 ppb level to find the optimal AMDIS deconvolution settings. Additional advantages of using deconvolution versus MSD ChemStation, to find more compounds in an extract are also discussed.

The detectability of compounds in a complex matrix is significantly improved with deconvolution. This can also be viewed as better or increased sensitivity through improved selectivity versus the background.

Agilent's MSD ChemStation add-on - Deconvolution Reporting Software (DRS) runs AMDIS automatically to generate an easy-to-read quantitation report.

Agilent Technologies

Introduction

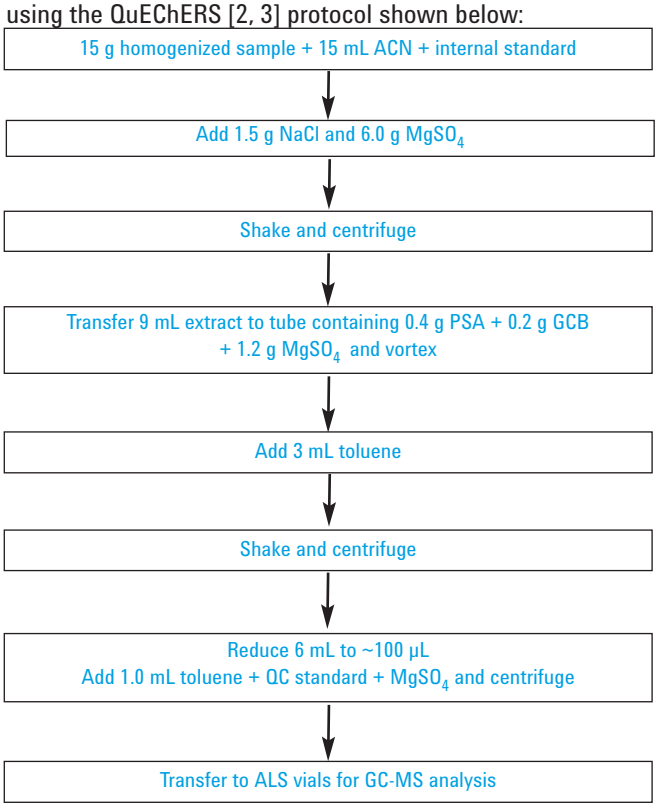
Instrument detectability is usually determined by the amount of sample injected, the responses from the detector and matrix interferences. The signal-to-noise ratio (S/N) can be used to gauge the sensitivity of an instrument in a clean sample. The presence of matrix alters this sensitivity due to a lack of selectivity between compounds of interest and background.

In a multiresidue analysis, the data reviewing process is also very important in confirming the hits found by the software and reviewing the integration and quantitation for accuracy.

Agilent Deconvolution Reporting Software (DRS) has been proven as a powerful data processing tool for finding trace compounds in complex matrices [1]. In this study, results from the Automated Mass spectral Deconvolution and Identification System (AMDIS), part of DRS is closely studied and compared to the results from ChemStation. The goal is to determine if deconvolution (DRS) can provide better results (detectability) than routine ChemStation data processing.

Experimental

Spinach extracts (see Acknowledgement) were prepared using the QuEChERS [2, 3] protocol shown below:



Thirty-five pesticides were spiked into spinach extract at 50 ppb (pg/µL).

Instrument parameters

GC: **7890A**

Autoinjector: 7693A
Retention gap: 2 m × 0.25 mm id Siltek capillary tubing
Column: HP-5MS UI (ultra inert), 15 m × 0.25 mm, 0.25 µm (from inlet to Purged Union) Agilent p/n 19091S-431 UI

Oven ramp:	Rate (°C/min)	Temp (°C)	Time (min)
Initial		100	1.6
Ramp 1	50	150	0
Ramp 2	6	200	0
Ramp 1	16	280	5

Run time: 20.933 min
Inlet: Multimode Inlet (MMI) at 17.73 psi (Retention Time Locked), constant pressure mode
RT locking: Chlorpyrifos-methyl locked to 8.297 min
Liner: Helix double taper, deactivated (Agilent p/n 5188-5398)
Injection mode: 2-µL cold splitless (fast injection)

Inlet temp. ramp:	Rate °C/min	Temp °C	Time min
Initial		50	0.01
Ramp 1	720	300	hold

Septum purge: 3 mL/min
Purged Union: 4 psi (PCM)
Split vent: 50 mL/min at 0.75 min
Gas saver: 20 mL/min after 4 min
Cryo on: Cryo use temperature 150 °C; time out at 15 min

Backflush

Postrun: 5 min
Oven: 280 °C
Purged Union: 70 psi
MMI: 2 psi
Restrictor: 0.7 m × 0.15 mm deactivated fused silica tubing (from Purged Union to MSD)

MSD: **5975C**

Solvent delay: 2.5 min
EMV mode: Gain Factor = 2
Mass Range: Full scan, 45-550
Threshold: 0
Sample number: 2 A/D Samples 4
Transfer Line: 280 °C
Source: 300 °C
Quad: 200 °C

Deconvolution

Deconvolution is a process for extracting ions from a complex total ion chromatogram (TIC), even with the target compound signal at trace levels. The software used for this technique is AMDIS developed by NIST (National Institute of Standards and Technology) [4].

As a review, let's look at the deconvolution process. AMDIS considers the peak shapes of all extracted ions and their apex retention times (RT). In this example, only some of the extracted ion chromatograms (EICs) are overlaid for clarity with the apex spectrum (Figure 1A).

Ion 160 EIC has the same RT as ions 50, 170 and 280, but has a different peak shape. Ion 185 has a different peak shape and an earlier RT. Ions 75 and 310 have similar peak shapes but they have different RTs.

Figure 1A

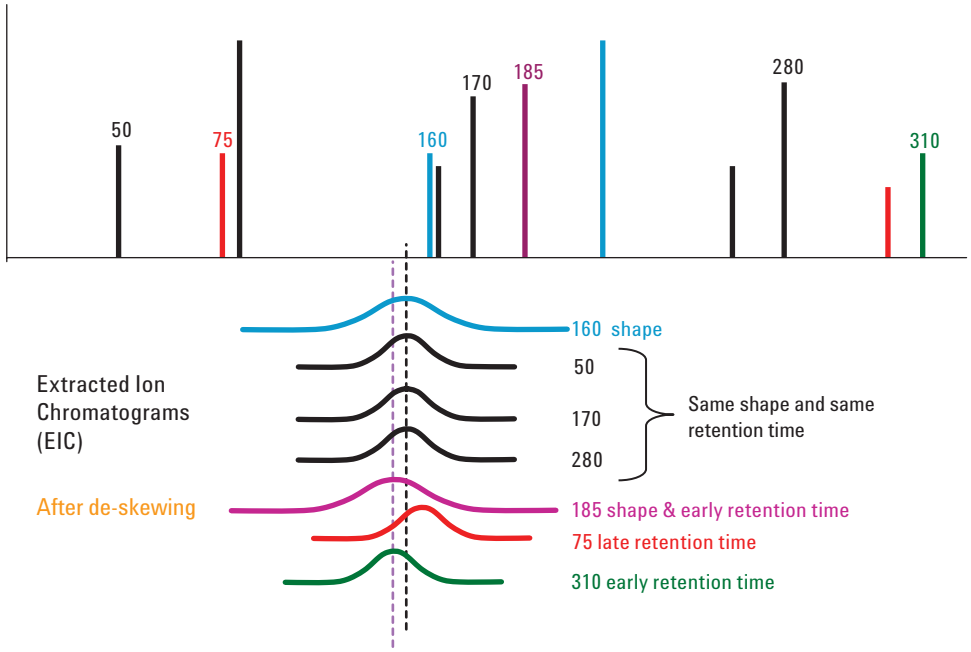


Figure 1B shows the EICs after the different peak shapes or RTs are eliminated from Figure 1A. Ions 50, 170, 280 and a few others remain.

Figure 1B

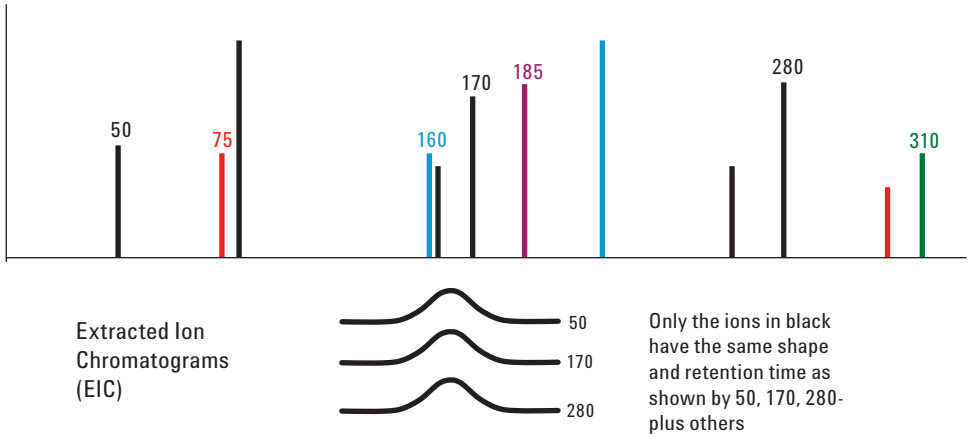


Figure 1A-1C. Simplified deconvolution process (continued).

Figure 1C shows all of the ions in black that have similar peak shapes and RTs, within the criteria set earlier by the analyst. These are grouped together and referred to as a component by AMDIS.

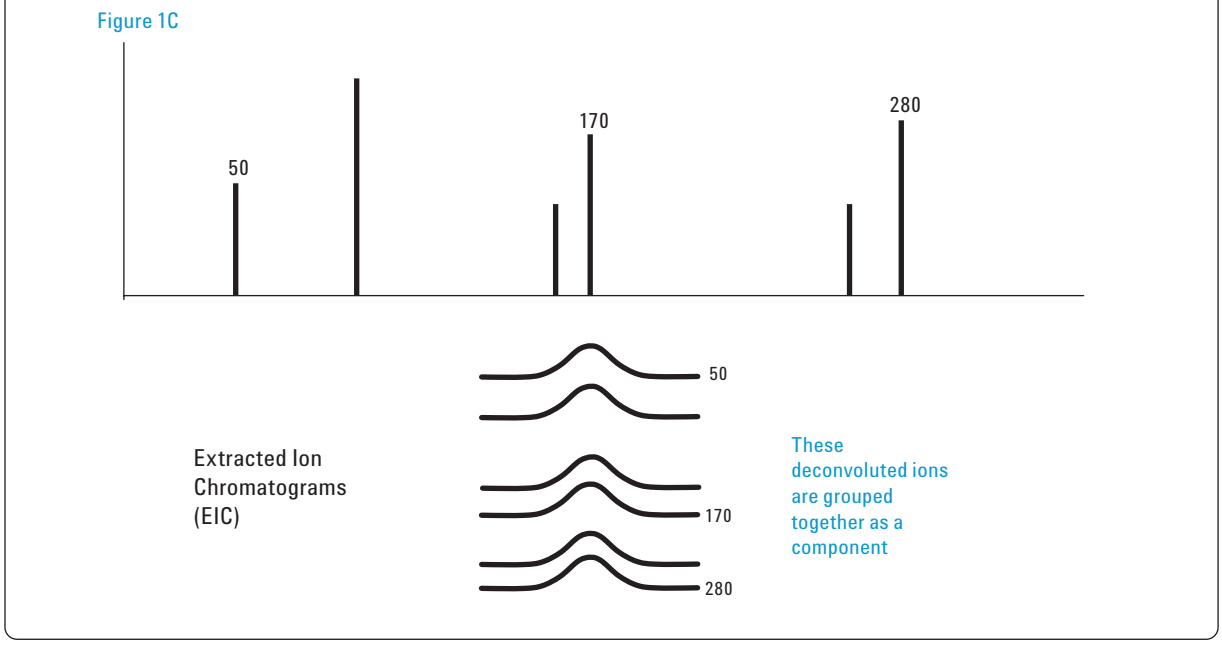


Figure 1A-1C. *Simplified deconvolution process (continued).*

Deconvolution finds the components from a complex TIC. Each component is searched against a retention time locking (RTL) library in AMDIS format. In addition to spectral matching, the locked RT can also be used as a criterion for hits. Depending on the match factor from the search, target compounds can be identified or flagged in a complex TIC. The power of deconvolution is appreciated while comparing the top two spectra in Figure 2. The raw scan or original nondeconvoluted scan is shown on top. The clean scan, that is the deconvoluted component, is shown in the middle. The bottom scan is the identified compound in the AMDIS library. Without deconvolution, the analyst would visually compare the background subtracted raw scan and library scans for confirmation. It would be very difficult, if not impossible, to say that Fenbuconazole, the target compound in this example, is present using that type of comparison.

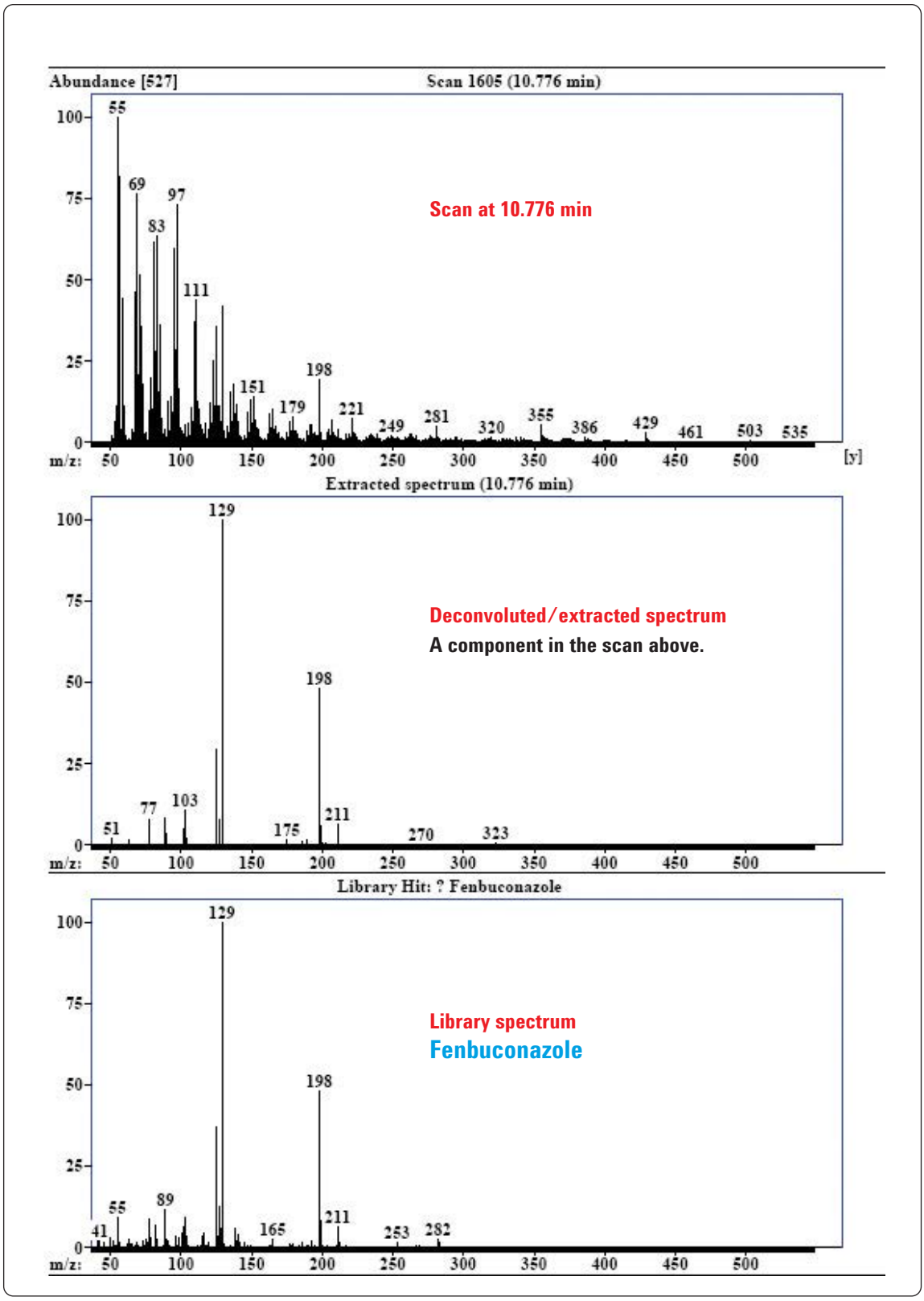


Figure 2. Comparison of raw, deconvoluted, and library spectra.

AMDIS Settings

Previous publications that discussed the power of using deconvolution to screen complex matrices, did not discuss specific AMDIS settings to define components [1, 5, 6]. In this study, several settings (that is, resolution, sensitivity, and shape requirements) are compared to find the maximum number of spiked compounds. The minimum match factor is set to 30 and the retention time window is limited to ± 30 seconds (RI window is set to 30) to qualify the hits from the retention time library search (Figure 3). The expected retention times of the compounds in the library database are obtained in acetone solvent without a retention gap. The samples in this study are in toluene solvent with a retention gap. Therefore, the retention time window is set wider than the normal 10 or 15 seconds, at ± 30 seconds.

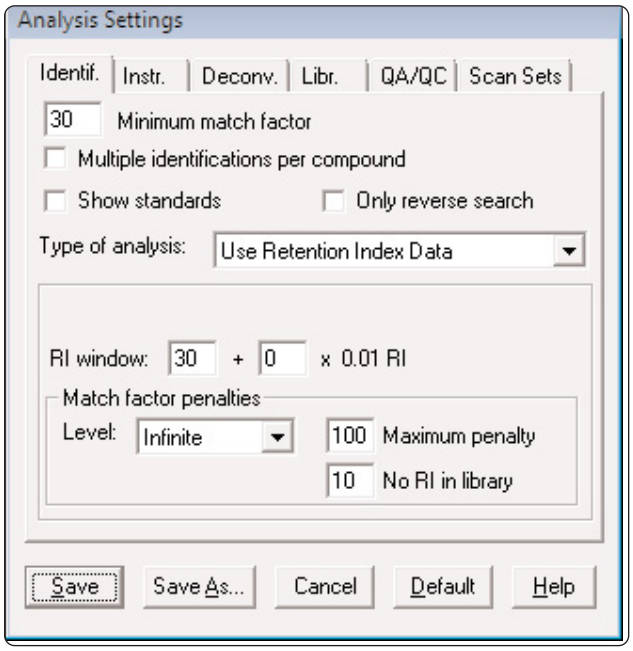


Figure 3. AMDIS identification settings.

Figures 4 and 5 describe some of the parameters in the AMDIS deconvolution tab. In this article, "1 M H M" means: adjacent peak subtraction = 1, resolution = medium, sensitivity = high, shape requirements = medium.

Settings can be optimized for chromatographic resolution, peak shape, retention time windows, acceptance criteria, and so forth. Settings can be saved to "ini" files. The chemist has control over the deconvolution and identification process by varying numerous AMDIS settings. Most of these parameter settings are not independent; so changing one parameter can affect another.

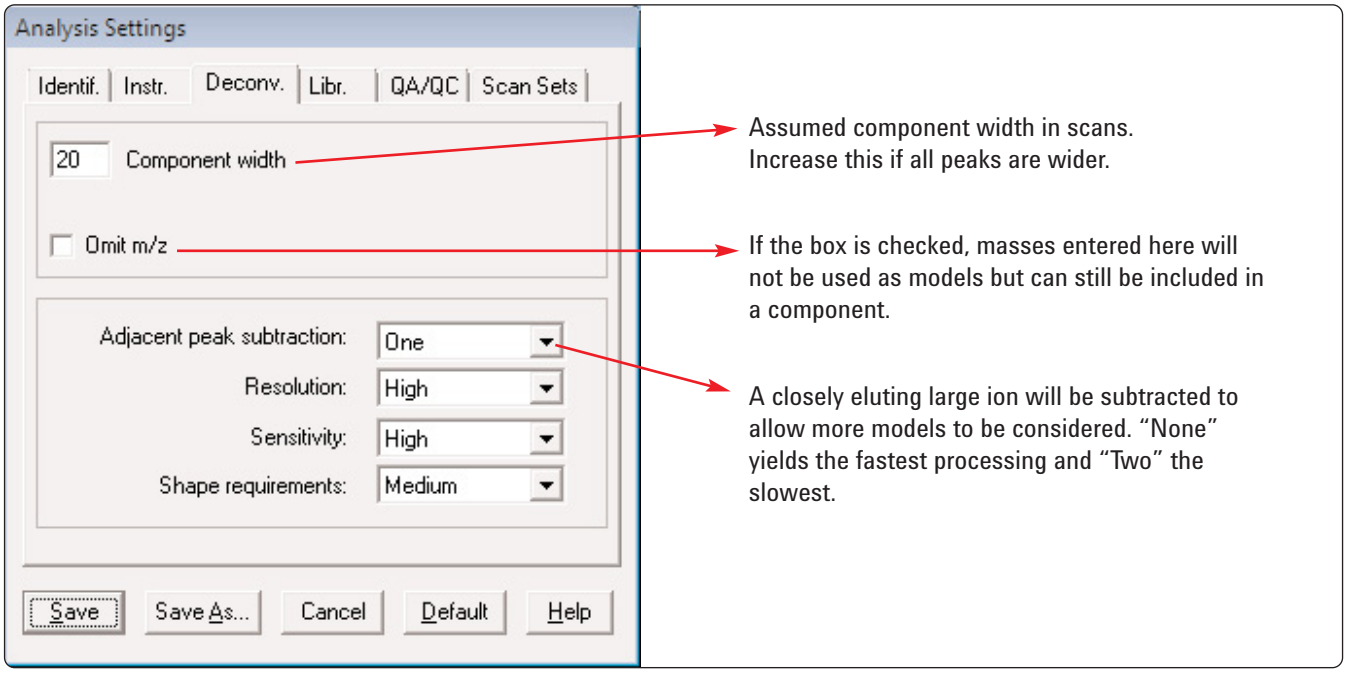


Figure 4. AMDIS deconvolution settings.

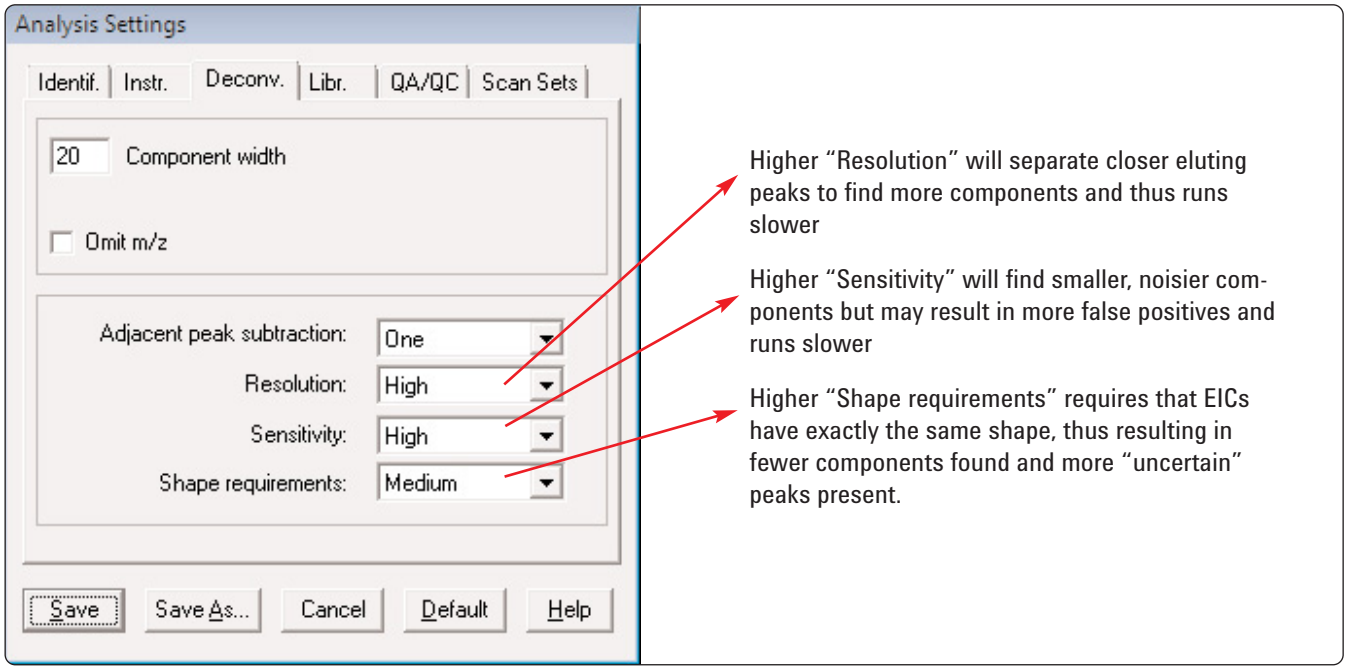


Figure 5. AMDIS deconvolution settings.

Results and Discussion

Deconvolution Settings

Figure 6 shows effects on match factors (y-axis) due to variation of adjacent peak subtraction and sensitivity across 35 pesticides (x-axis). This figure shows two things:

– The adjacent peak subtraction (1 or 2) makes little difference in match factor

– The sensitivity setting (very high and high) makes little difference in match factor

In the next few figures, the AMDIS setting is varied one at a time to observe the number of pesticides found. The reference point is the optimal setting (HHM) where the maximum number of hits were obtained.

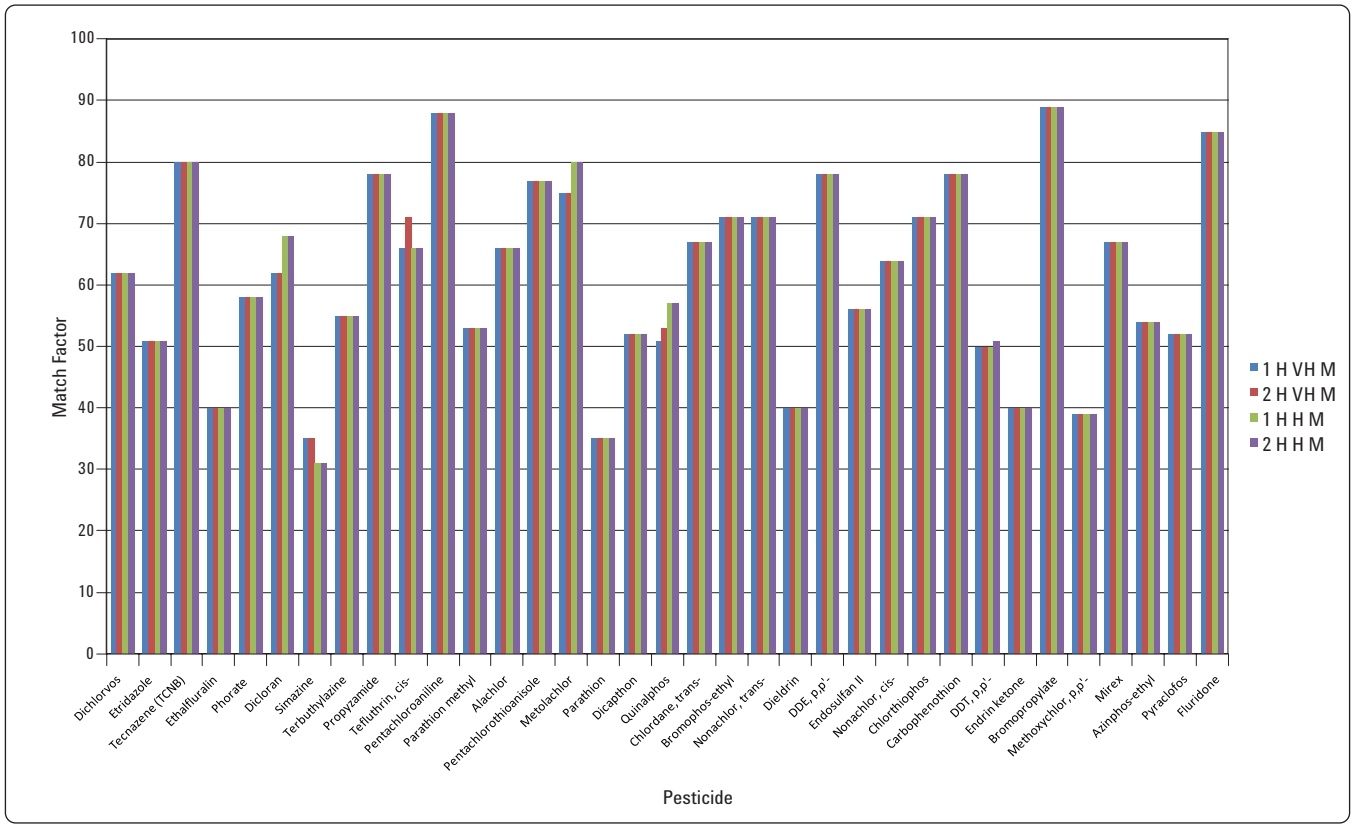


Figure 6. Comparison of match factors with four AMDIS settings.

Figure 7 shows that keeping the sensitivity and peak requirements the same, and lowering the resolution from H to M will find fewer targets. The number of targets found is in the yellow circle. A resolution setting of "low" yields even fewer targets.

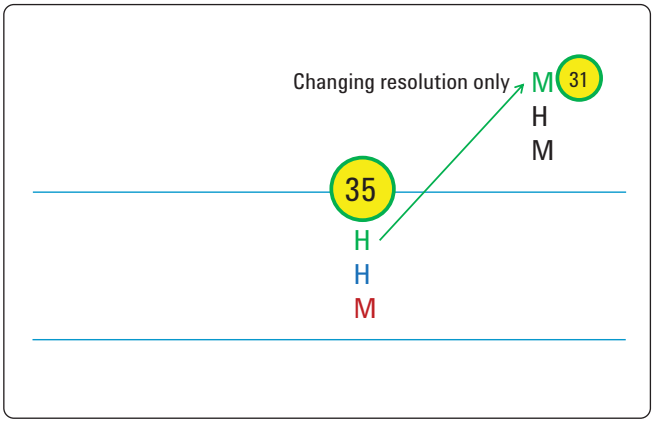


Figure 7. Number of compounds found by varying resolution.

Figure 8 shows that while keeping the resolution and peak requirement constant, lowering the sensitivity from H to M will find fewer targets. However, increasing the sensitivity from H to VH does not affect the number of targets found, similar to that in Figure 6.

Figure 9 shows that while keeping the resolution and sensitivity the same, lowering or increasing the peak shape requirement from M to L or H will find less targets.

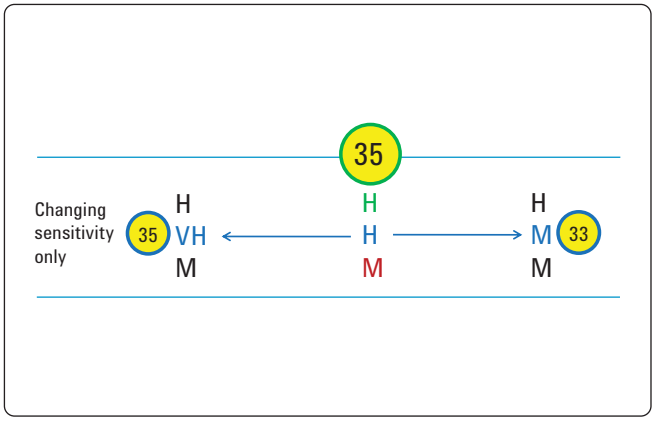


Figure 8. Number of compounds found by varying sensitivity.

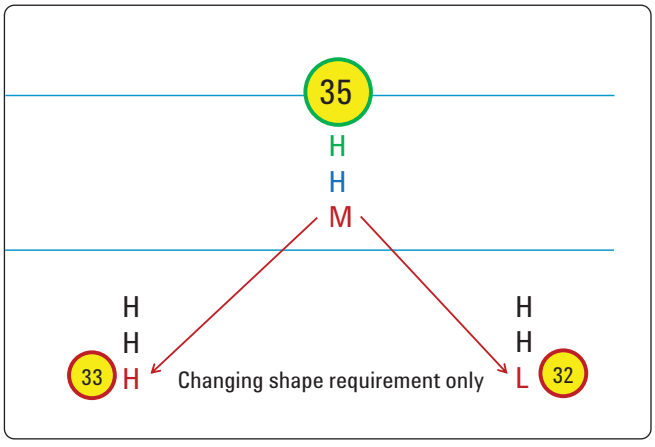


Figure 9. Number of compounds found by varying peak shape.

In addition to the number of targets found, we should look at the Average Match Factor (AMF) of all the targets found. The AMF is the number in the green triangle. Figure 10 shows that there is no significant variation in AMFs except in HHH mode (58.5) which is much lower than others (>61.6). This supports that HHM is still the optimal setting, considering processing speed and number of false positives.

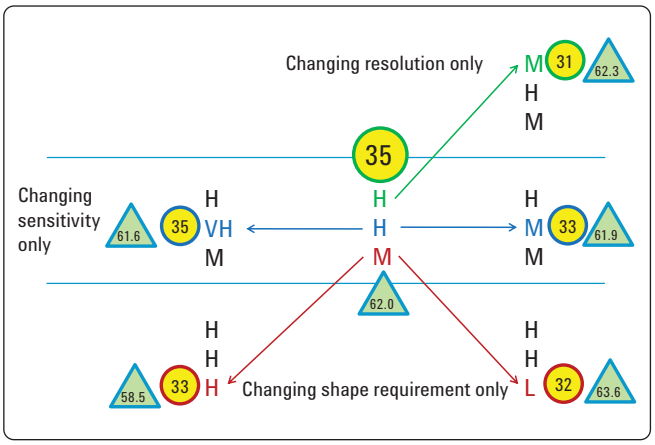


Figure 10. Comparison of average match factors with AMDIS settings.

ChemStation Quant settings

Figure 11 shows part of the "Edit Compound" screen in the MSD ChemStation. This shows the quant database for locating and confirming compounds using three ion ratios of each target analyte. The RT window is specified in the upper box and the ions and ion ratios are specified in the lower box.

As shown in Figure 11, the Extraction RT window is set to ± 0.5 min and the Qualifier Ion (Q1, Q2, and Q3), % Uncertainty is set to Absolute 50%. In ChemStation, the

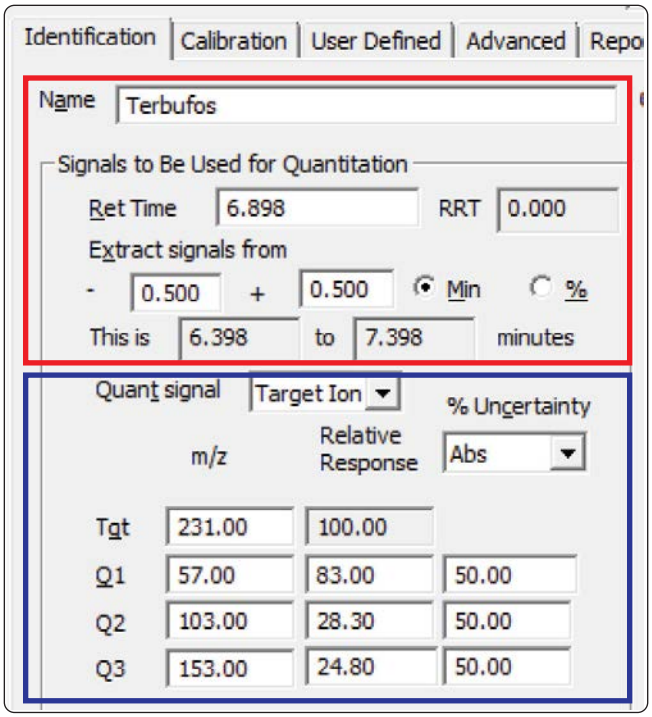


Figure 11. Target compound RT and ion setup.

target compound identification is based on four ions and three qualifier ion ratios. However, the target compound identification in AMDIS (Figure 2) was based on the full spectral library match which is more dependable.

Another key parameter in quantitation is the "Quantitation subtraction method" which is set to "Avg first and last" and not shown here.

Figure 12 is an overlay of four ions (Quant and Qualifiers) from ChemStation and the quant ion from AMDIS (in magenta).

Due to the chemical background, the four ions from ChemStation have offset and noisy baselines, which will affect the peak integration and proper quantitation results.

In comparison, the magenta trace is the deconvoluted quant ion from AMDIS. The chemical noise had been removed in the deconvolution process. It shows a flat baseline and accurate integration. There are other advantages of using deconvolution in GC/MS analysis as discussed below.

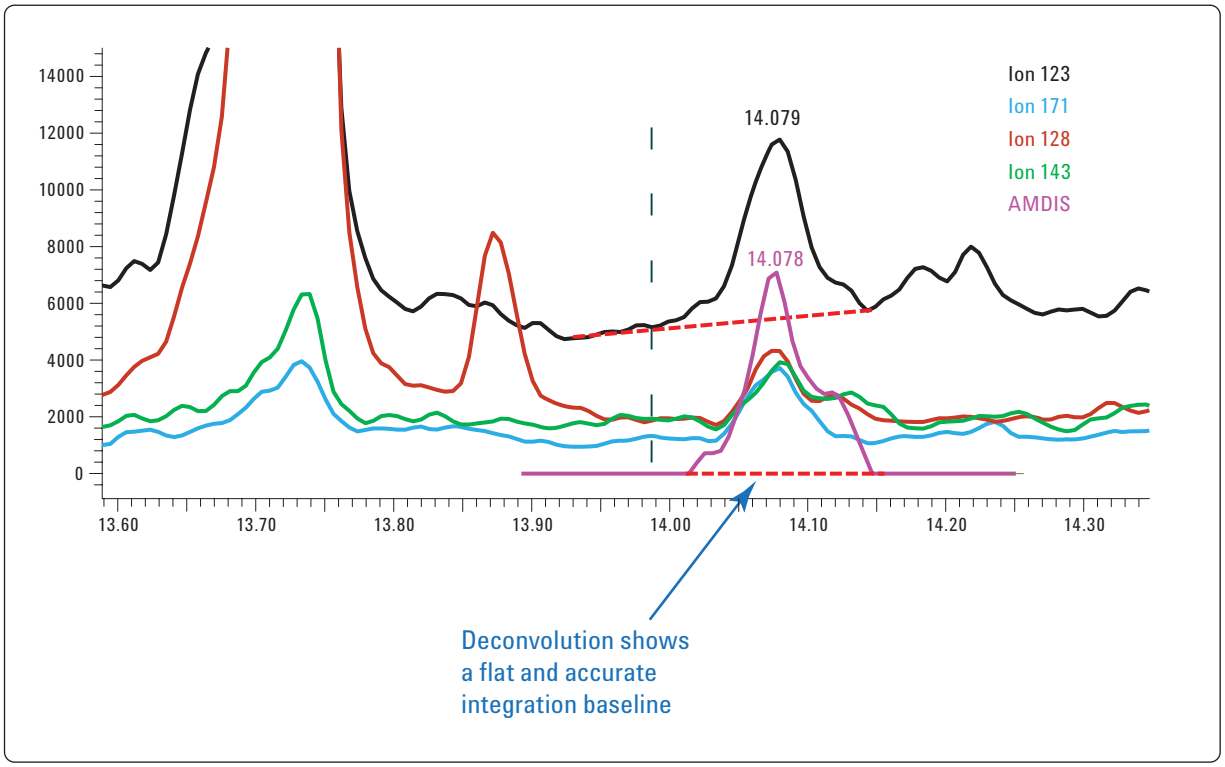


Figure 12. Target, qualifier and AMDIS deconvoluted EIC overlay.

Additional Advantages of Using Deconvolution

Finds more compounds than ChemStation does

In Figure 13, ChemStation did not integrate ion 109 (ChemStation target ion) at the expected RT, therefore, the compound was not found. AMDIS found Fonofos correctly, at 6.898 min. The qualifier ion ratios at this RT also match that required by ChemStation for identification.

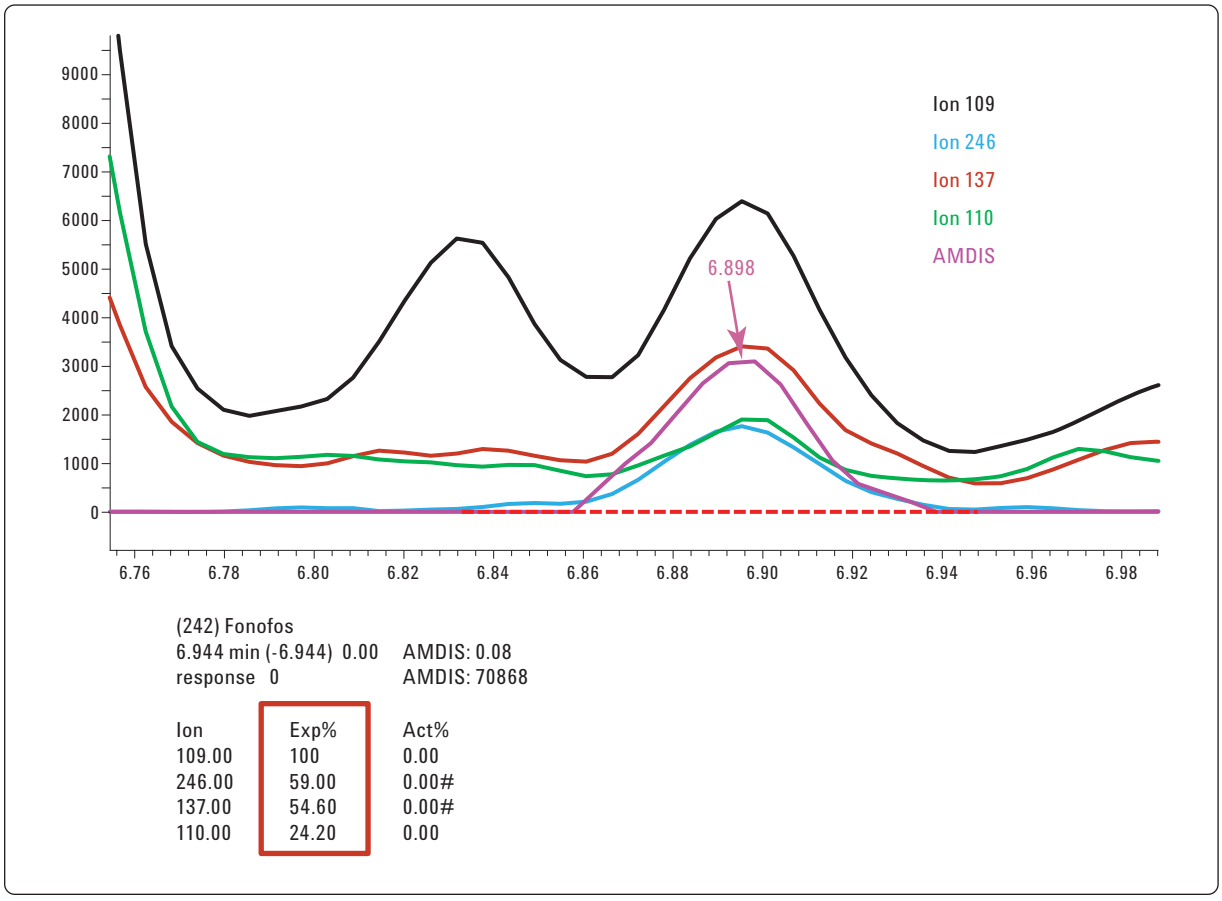


Figure 13. Target, qualifier and AMDIS deconvoluted EIC overlay.

Finds the correct peak

In Figure 14, from the size and location of the three qualifier ions, it is obvious that ChemStation picked the wrong peak (at RT = 4.067) to quantitate. However, AMDIS found a peak (at RT = 3.873) whose ion ratios are in agreement with the ChemStation qualifier ions. Again, this demonstrates that the AMDIS full-spectrum matching process is a more robust approach for identifing a compound in a complex matrix.

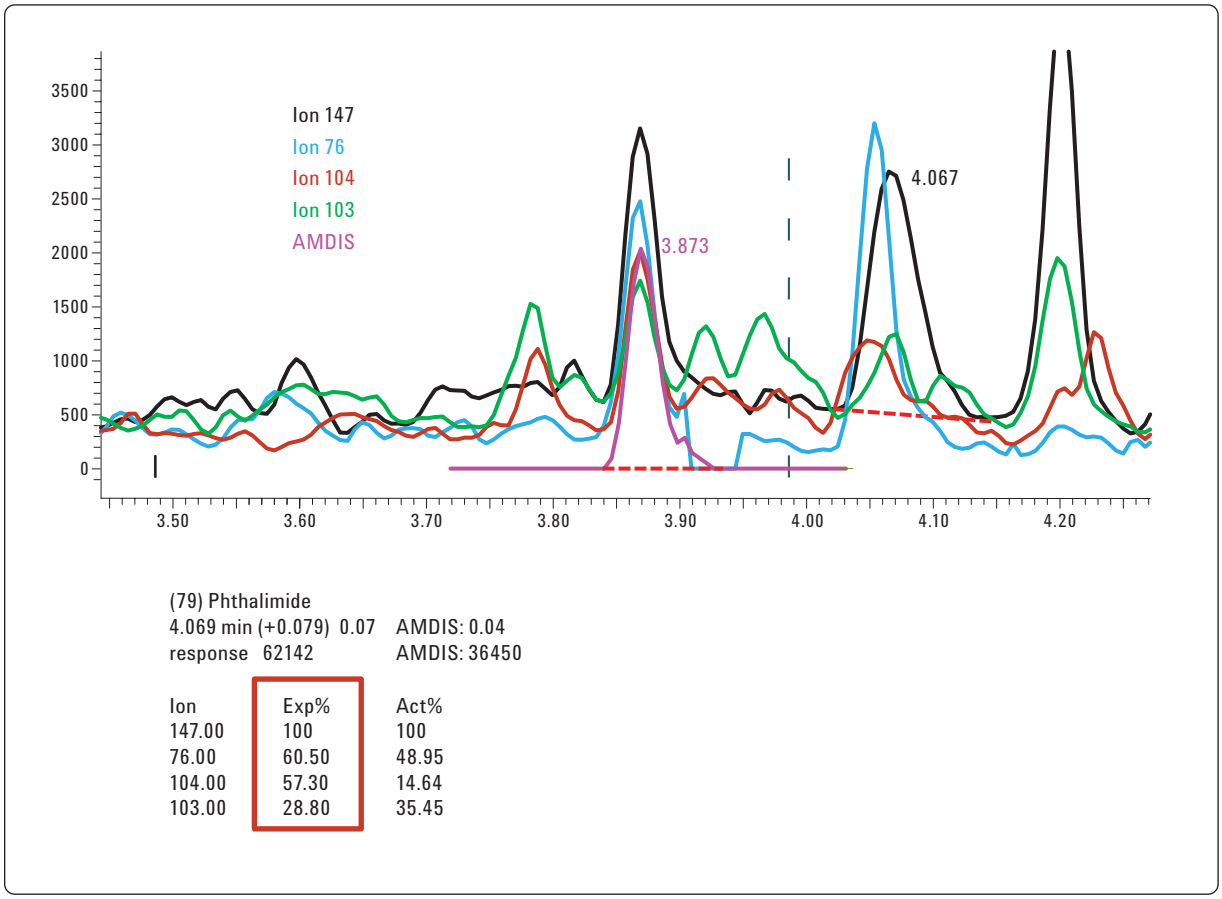


Figure 14. Target, qualifier and AMDIS deconvoluted EIC overlay.

Higher discrimination power than ChemStation

In Figure 15, the target ion (ion 235) is overwhelmed by the matrix background (shown as a large fronting peak). ChemStation was not able to differentiate the ion 235 contribution from the background or the compound; therefore it integrated the distorted peak. Due to the rising baseline, ChemStation integrated a large area of chemical background as the "target compound signal". On the other hand, AMDIS was able to deconvolute the compound signal away from the background ion and remove noise properly before the integration. This provides a more reliable quant result.

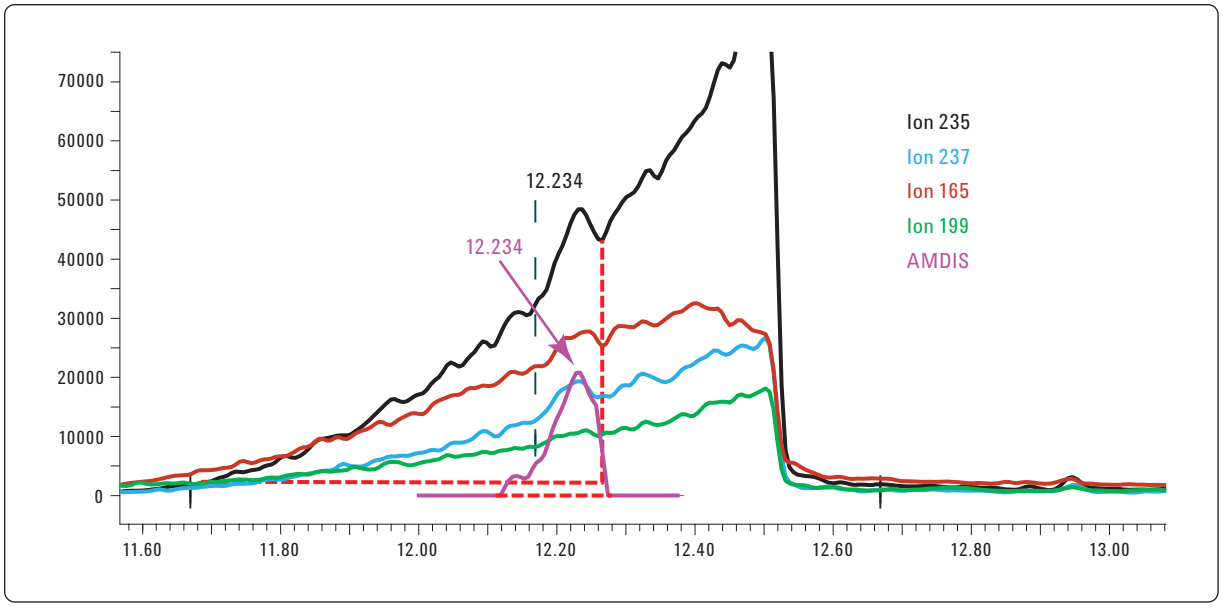


Figure 15. Target, qualifier and AMDIS deconvoluted EIC overlay.

Deconvoluted ion is noise-free, thus easier to integrate for more reliable quantitation results

In Figure 16, ChemStation and AMDIS found the same peak. Due to the noisy baseline, ChemStation drew the integration baseline (red dash line) incorrectly. Again, deconvolution removes chemical noise first, and can therefore, integrate the peak easily and reliably.

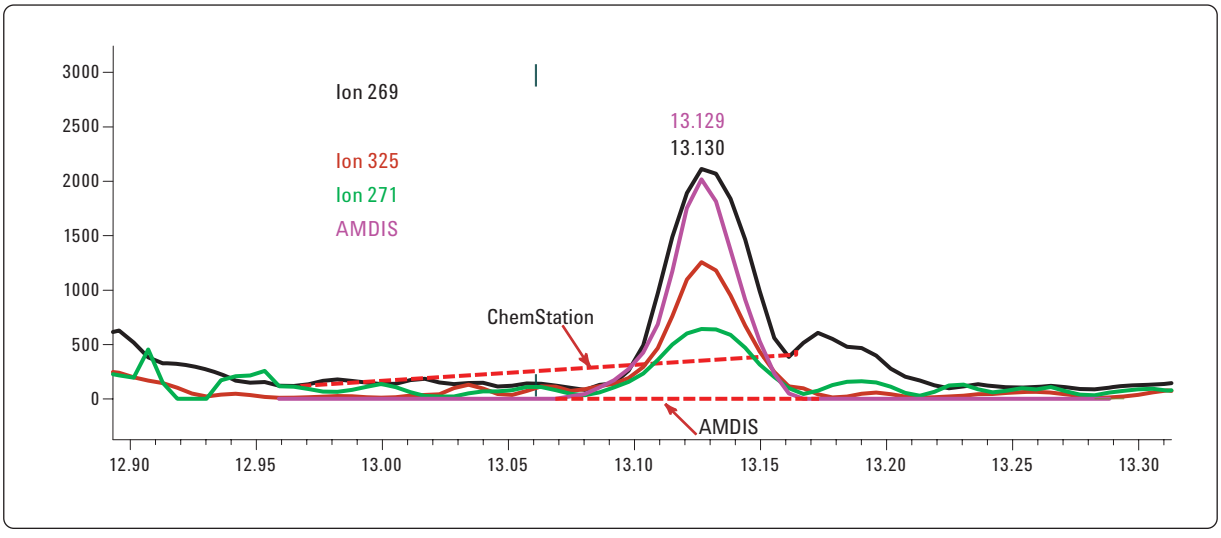


Figure 16. Target, qualifier and AMDIS deconvoluted EIC overlay.

Agilent's ChemStation add-on - Deconvolution Reporting Software (DRS) incorporates AMDIS deconvolution. Therefore, the above AMDIS advantages are automatically captured in DRS data processing which combines results from ChemStation, AMDIS, and NIST MS Search into one report.

Comparing number of compounds found between ChemStation and AMDIS

Figure 17 is a summary of the hits from ChemStation and AMDIS under four different settings, respectively. The blue bars represent the number of false positives and the red bars represent the number of actual target compounds found. On the left side of the graph, the settings of ChemStation are Ion Ratio Uncertainty. Although the absolute 30% and 50% increase the total number of compounds found, only about half of the 35 targets are found. The analyst is forced to review more hits and does not gain any additional information. The entire target list of 900+ compounds must be reviewed for false negatives. The right side of the graph shows that the four AMDIS settings gave similar results. In each case, all 35 targets were found with a reasonable number of false positives. There were no false negatives. The analyst must only review the positives, which is a significant time savings. This shows that AMDIS (DRS) is much more capable than ChemStation in finding target compounds in a complex matrix. AMDIS (DRS) provides better detectability and faster data processing.

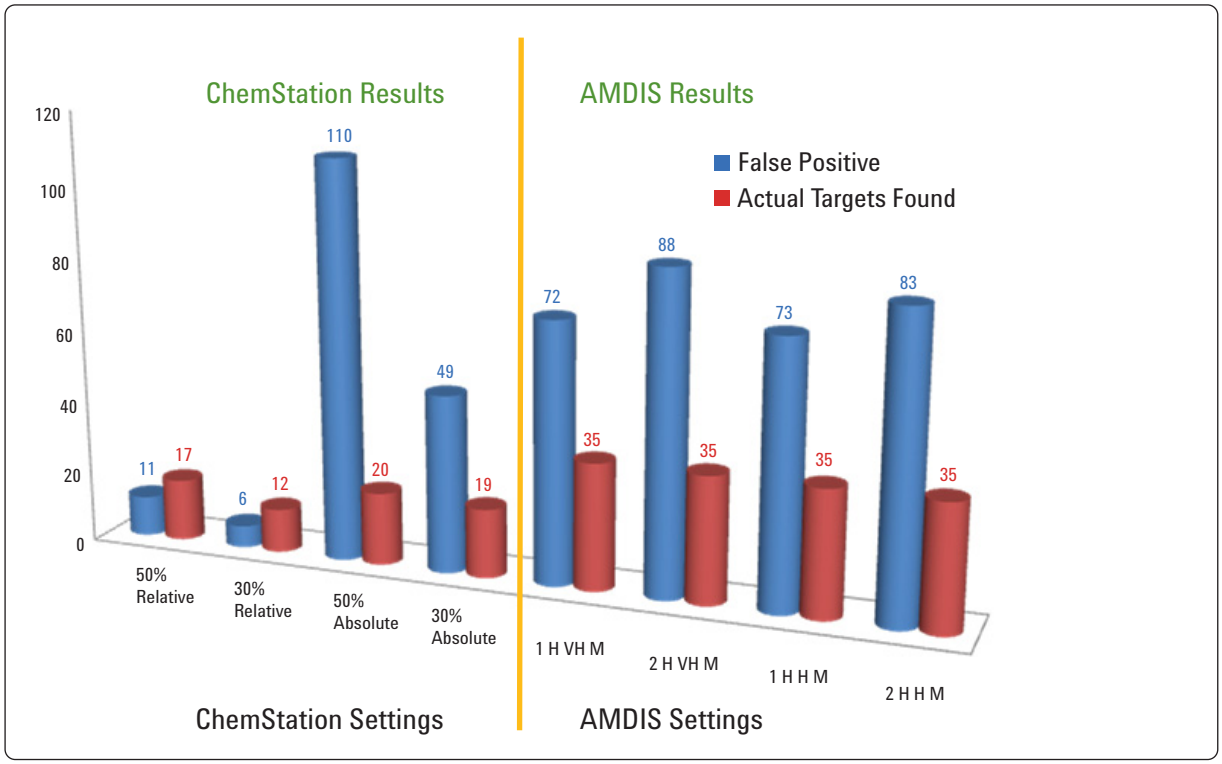


Figure 17. Overall comparison of AMDIS and MSD ChemStation compounds found.

Conclusions

- AMDIS finds more target compounds than ChemStation in a complex matrix. Deconvolution (DRS) provides a cleaned peak to integrate properly giving more reliable results.
- AMDIS did not miss any target compounds at the 50 ppb level using scan data. This minimizes the time an analyst must spend reviewing results.
- Confirmation of compounds is done in significantly less time with deconvoluted component spectra available.
- The detectability of compounds in a complex matrix is significantly improved with deconvolution. This can also be viewed as better or increased sensitivity through improved selectivity versus the background.
- Deconvolution Reporting Software (DRS) automates the deconvolution (AMDIS) process to produce an easy-to-read quantitation report.

Acknowledgement

The authors would like to thank Dr. Jon Wong (FDA-CFSAN, College Park, Maryland) for graciously provided samples for this study.

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