Improving Efficiency in the Forensics Laboratory: Introducing a New Controlled Substances Analyzer

Application Note

Authors

Sarah Keeling and Francis Diamond
NMS Labs
Willow Grove PA

Bruce Quimby
Agilent Technologies, Inc.
Wilmington DE

Abstract

Forensic chemists are faced with the challenge of analyzing a multitude of sample types to identify controlled substances and pharmaceuticals. Law enforcement depends on a laboratory's ability to identify not only the major components of the samples, but also relevant compounds present at lower levels. These samples can range from an unidentified white powder or botanical material to tablets, syringes, or charred pipe residues. Typically, these analyses are performed by full scan GC/MS with library search reports generated by database searching. Because the evidence obtained by law enforcement can encompass an ever widening variety of analytes including closely related compounds such as isomers and possible analogs, greater attention to detail is required in the analysis. The new novel psychoactive substances (NPS) and synthetic cannabinoids that have hit the streets contain many new isomers and analogs that are not readily differentiated by routine searching methods.

This system uses the following enhancements to increase the efficiency of analysis. Hydrogen is used as carrier gas to reduce operating costs. Retention time locking (RTL) is used to maintain precise retention time matching between multiple systems and the database. Backflush provides a mechanism to remove nonvolatile compounds by flow switching and redirecting them out the split vent. Finally, deconvolution reporting software (DRS) is used to identify drugs, even when present in complex mixtures or at trace levels. Results from the new system are compared to a typical helium system currently used for criminalistics analysis.

Agilent Technologies

Introduction

Crime labs routinely use GC/MS as the definitive technique to identify or confirm the presence of a controlled substance or drug. The current financial environment of crime labs requires forensic chemists to analyze for an ever expanding list of emerging drugs with fewer resources. Along with this financial strain, labs are faced with the increasing price of helium, with projected cost increases and scarcity rendering helium too costly to use. Another strain on the criminalistics system is the increased prevalence of complex sample types, such as synthetic cannabinoids and other botanical mixtures. A more efficient means of analysis is required to circumvent these issues.

Using hydrogen as a carrier gas is a viable alternative, as it is less expensive than helium and can be easily generated on site. To aid in shortening run time, the instrument is configured for fast oven ramp (using a 220 V power source) and an insert to reduce the volume of the oven, allowing for faster temperature programming. Equipping the system with backflush allows for the removal of nonvolatile material from the head of the column at the end of each run, preventing ghost peaks in subsequent chromatograms and reducing the need for column maintenance. The use of DRS coupled with the precise retention time control of RTL allows for more efficient data processing and provides the capability to distinguish between closely related compounds and to identify trace amounts of drug even in complex samples.

Traditional library searching involves simple search routines based on a large amount of a relatively pure substance being present in the chromatogram; however as samples become more complex in nature and contain multiple components, more labor intensive processes are required to perform the requisite identification. This involves more personnel time, greater training, and allows for subjective data manipulation that may be done in an inconsistent fashion. DRS is an automated method of analyzing data for the presence of specific compounds in a defined database. A report can be generated using predetermined set points to allow for consistency in data processing, reducing the possibility of human error and bias. This results in a more efficient analysis and a timelier reporting of results.

Experimental

Hardware configuration

The system is an Agilent 5975 GC/MS with an Agilent 7890A GC and an Agilent 7693 Automatic Liquid Sampler. It is configured to run on a 220 V power supply. It uses a thermal insulated oven insert. It is configured using purified hydrogen as the carrier gas supplied from a cylinder (UHP). It is additionally configured to use backflush with an AUX EPC module and a purged union (PUU). There is a restrictor to allow for proper application of pressure to control carrier gas flow into the mass spectrometer. The injection port is deactivated. The capillary column is a 10 m DB-5 with a 0.250 mm internal diameter and 0.25 µm film thickness. A diagram of the hardware is shown in Figure 1.

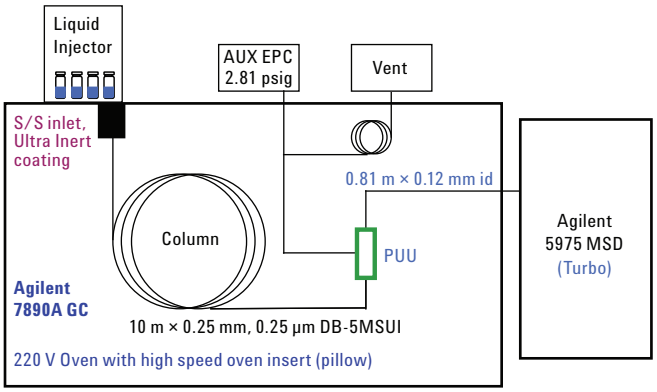

Figure 1. Diagram of hydrogen carrier gas GC/MS system configuration.

Software configuration

MSD Productivity Chemstation software was used for data acquisition and data analysis. Additionally, DRS was used for data processing. A database of compounds was generated consisting of drugs and controlled substances typically seen in case work. This database was compiled with data acquired using hydrogen as a carrier gas.

Data analysis settings

Various set points were evaluated, including flow, temperature, liner configuration, and injection mode. Using the results of the evaluation, a data acquisition method was optimized. Once in place, this method was used for retention time locking (RTL) and all subsequent data analyses.

RTL was employed to ensure that reproducible retention times were achieved every day of operation, even after column clipping or replacement. It also allowed precise matching of retention times with other instruments that were similarly configured and locked to the same compound.

A database of mass spectra was generated on the hydrogen system by extracting a series of reference standards. The spectra were verified by comparison to NIST11 and an in-house database. The standards were acquired on the RTL method, so precise retention times were recorded in the database. The mass spectra in the database were the extracted spectra generated using the AMDIS program. The use of AMDIS deconvolution results in clean spectra with interferences from column bleed and overlapping impurities removed. Each entry was added to the database with the CAS# when available, the chemical formula, the precise retention time (in minutes), and the compound name and synonyms. The retention time in seconds was entered as the retention index entry in the database, as required by DRS. The database contains 461 compounds.

Data acquisition parameters

Table 1 lists the experimental conditions comparing the hydrogen and helium systems used.

Table 1. Experimental Conditions Comparing the Hydrogen and Helium Systems Used

	Helium	**Hydrogen**
Inlet	EPC split/splitless	EPC split/splitless (deactivated)
Mode	Constant pressure	Constant flow
Injection type	Splitless	Pulsed splitless
Injection volume	1.0 µL	1.0 µL
Injection dispense speed	6,000 µL /min	6,000 µL /min
Inlet temperature	265 °C	265 °C
Pressure	19.973 psi	6.6078 psi
Total flow	55.452 mL/min	54.952 mL/min
Septum purge flow	3 mL/min	3 mL/min
Purge flow to split vent	50 mL/min at 0.3 minutes	50 mL/min at 1 minute
Injection pulse pressure		11 psi until 0.3 minutes
Sample overlap	Off	1.5 minutes before end of GC run
Gas type	Helium	Hydrogen
Oven		
Voltage (VAC)	240 V	220 V
Initial oven temperature	50 °C	50 °C
Initial oven hold	0 minutes	1 minute
Ramp rate	30 °C/min	60 °C/min
Final temperature	340 °C	325 °C
Final hold	6.83 minutes	4 minutes
Total run time	16.497 minutes	9.5833 minutes
Equilibration time	0.1 minutes	0.5 minutes

Table 1. (continued)

Column #1 (separation column)		
Type	DB-1	DB-5
Length	12 m	10 m
Diameter	0.200 mm	0.250 mm
Film thickness	0.33 µm	0.25 µm
Flow	2.4515 mL/min	1.9522 mL/min
Pressure	19.973 psi	6.6078 psi
Average velocity	90.224 cm/sec	54.454 cm/sec
Backflushing flow		–12.906 mL/min
Column #2 (MS restrictor)		
Type		Deactivated fused silica
Length		0.81 m (0.17 in transfer line)
Diameter		0.120 mm
Film thickness		0 µm
Pressure		2.8091 psi
Flow		2 mL/min
Average velocity		321.77 cm/sec
Flow 1 minute post run		9.428 mL/min
MSD		
Acquisition mode	Scan	Scan
Solvent delay	1.50 minutes	2.30 minutes
EMV mode	Relative	Gain Factor
Relative voltage	0	
Gain factor		1
Low mass	40.0 amu	40.0 amu
High mass	550.0 amu	570.0 amu
Threshold	250	50
Sample number	1	1
A/D samples	2	2
Vacuum pump	Performance turbo	Performance turbo
Quad temperature	150 °C	200 °C
Source temperature	230 °C	300 °C
Transfer line temperature	300 °C	300 °C

Study protocol

Three hundred eighty-four case samples originally analyzed on the helium system were reanalyzed on the hydrogen system to generate comparison data. These vials were evaporated at room temperature to dryness and reconstituted with ethyl acetate to the same volume. The reason for this was that the samples were originally dissolved in methylene chloride, which, if injected on the hydrogen system, may produce HCl gas and result in inlet and column problems. This represented over 1,200 individual drug findings, ranging from routine drug findings to newer synthetic compounds.

With the helium system, the data were processed originally using standard Chemstation qualitative analysis reports and, in some cases, manually enhanced searches including peak averaging, background subtraction, and extracted ion chromatograms using both in-house and commercially available databases. On the hydrogen system, DRS was used to process the data using the database generated in-house on this system. The DRS settings were determined experimentally, and those settings were used throughout the study, except for RT window adjustments for overloaded peaks.

Results and Discussion

General chromatographic challenges related to drug chemistry analysis

The data can be classified in the following fashion:

Scenario 1—Simple chromatograms with clearly defined chromatographic peaks
Compounds present at moderate amounts were generally identified in a straightforward fashion by both methods. This was true if the chromatographic peak was predominately a single compound.

Scenario 2—Column overloads
Because DRS searches for drugs in a specified retention window and penalizes the quality match if too far outside the window, when compounds were present at high levels, exceeding column capacity, additional steps were required to properly identify the compound if it had shifted out of the retention window. The AMDIS menu allows a larger or smaller window to be selected for individual compounds or for all compounds in the database. Additionally, searches can be performed in **Simple** mode, which does not penalize for mismatched retention times. Overloads are readily identified in a TIC and addressed accordingly. This is commonly seen when compounds are present in a sample at disproportionate amounts or because street samples are of unknown concentrations, aliquot volumes may be miscalculated.

Scenario 3—Trace amounts
When compounds were present at trace levels, normal search routines generally failed to identify the drugs. This may have been due to the data signal merging into the background signal or being masked by components present at larger amounts. DRS was easily able to make these identifications because it uses AMDIS deconvolution. The deconvolution algorithm internally generates extracted ion chromatograms for all masses in the scanned range and looks for masses with chromatographic peaks with the same shape and the same apex retention time. The cleaned spectra are then constructed by assigning the *m/z* value and abundance from those peaks with the same RT and shape.

Scenario 4—Interference
When multiple compounds elute as a chromatographic peak, routine library searching is not able to properly identify the individual components of the single peak. Normal Chemstation data processing requires manual intervention and subjective judgment to generate data confirming the presence of the individual components. DRS is capable of generating extracted spectra and properly indentifying the individual components without manual data manipulation. This will also allow for identification during periods of column bleed elution.

Scenario 5—Compounds with similar mass spectra and different retention times
Routine search algorithms can misidentify compounds with similar spectra because retention time is not factored into the quality match. DRS includes retention time when calculating the match factor, resulting in an identification made by both the fragmentation pattern and the RT. Isomers, analogs, and other closely related compounds are differentiated in this fashion.

Column backflushing

Samples containing heavy compounds that elute after the normal oven temperature program can cause problems with ghost peaks in subsequent runs. Mushroom extracts are an example. Figure 2A shows the chromatogram of a mushroom sample on the helium system, which does not have back-flushing. Without an extended post-run bakeout, the heavy materials show up as ghost peaks in the next run. Figure 2B shows a blank run, after the mushroom sample, containing several ghost peaks.

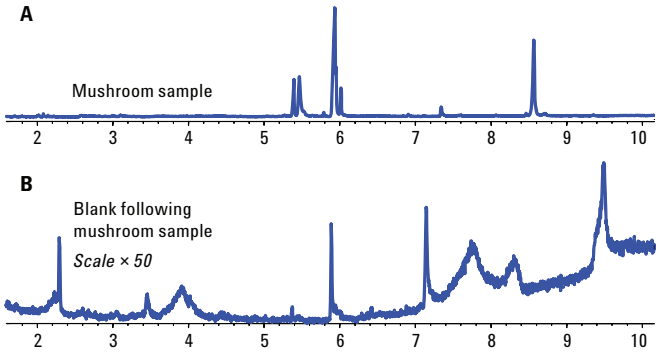

Figure 2. A) Mushroom sample with helium system. B) Blank following mushroom sample, showing ghost peaks.

Figure 3 contains the blank runs aquired immediately after running the mushroom extract. The chromatogram in Figure 3A shows a run with 0.5 minutes backflushing time. The broad ghost peak at approximately 8.3 minutes indicates that the backflushing time is not long enough to remove all the heavy materials. The chromatogram in Figure 3B shows that with 1.0 minute backflushing time, all ghost peaks are removed. Note that the small sharp peaks are impurities in the blank sample.

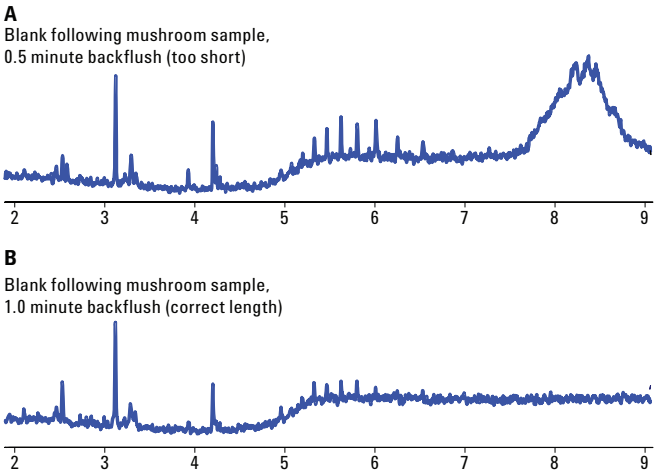

Figure 3. Backflushing results with hydrogen system. A) Blank following mushroom sample with 0.5 minutes backflush. B) Blank following mushroom sample with 1.0 minute backflush.

AMDIS deconvolution

Spectral deconvolution is an important part of the the new system. It gives much more consistent identifications across multiple users and can identify compounds even in severe overlap situations. Figure 4 shows the chromatogram of a standard used to test deconvolution.

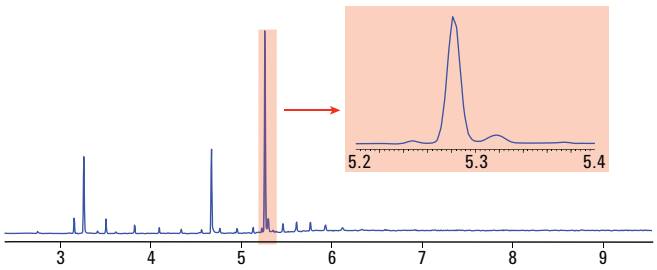

Figure 4. Chromatogram of a standard used to test deconvolution.

When either the apex spectrum or the average spectrum for the largest peak (5.28 minutes) was searched in the conventional way with PBM, the search report listed the first hit was Δ-9 THC with a 95 match. The second hit was hydrocodone, with a 35 match. A reasonable conclusion would be that the peak is THC. Figure 5 shows the average spectrum and the spectra of the two top hits from the library PBM search.

When DRS was applied to the same datafile, AMDIS deconvolution revealed that both Δ-9 THC and hydrocodone were present. Figure 6 shows extracted ion chromatograms for both compounds.

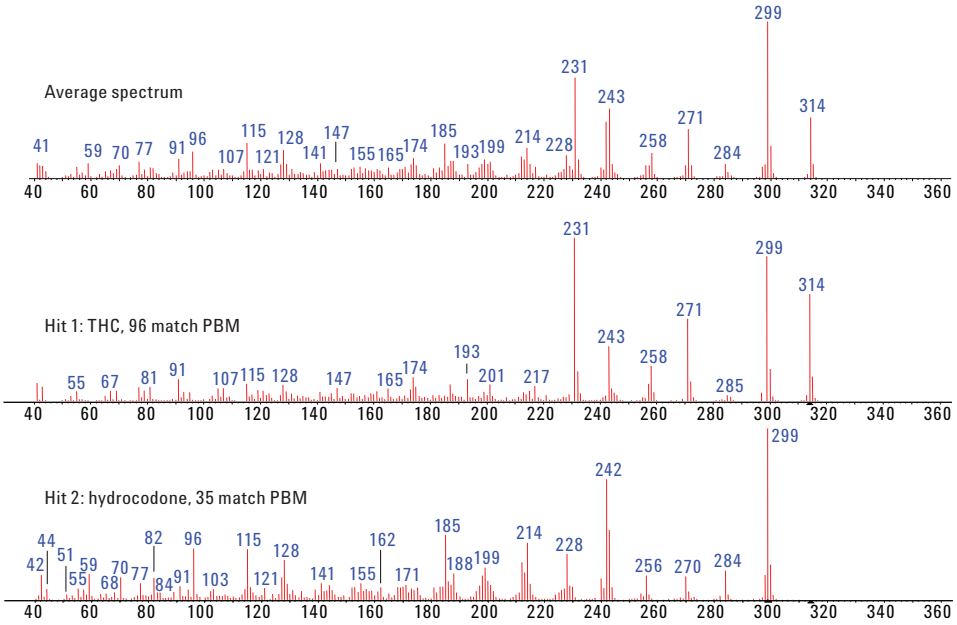

Figure 5. Average spectrum of peak at 5.28 minutes and the library spectra of the two top hits from the PBM search.

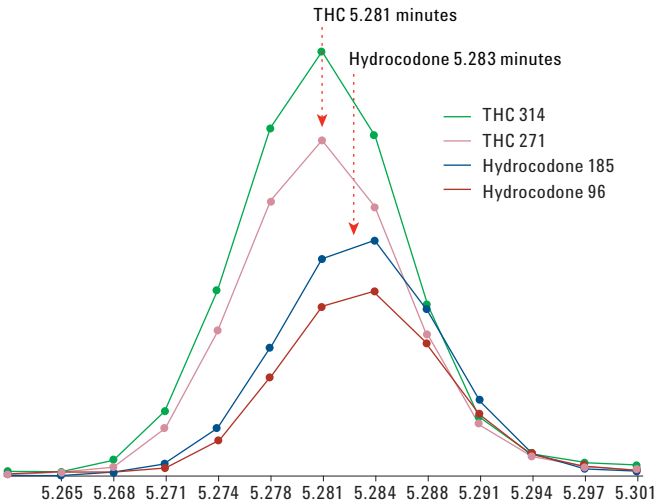

Figure 6. DRS reveals two severely overlapped compounds present at 5.28 minutes.

The retention times of the two compounds are only 0.002 minutes apart. Figure 7 shows the deconvoluted and library reference spectra for THC and hydrocodone. Note how well AMDIS deconvolution cleans the interfering ions from each spectra, resulting in a more accurate identification.

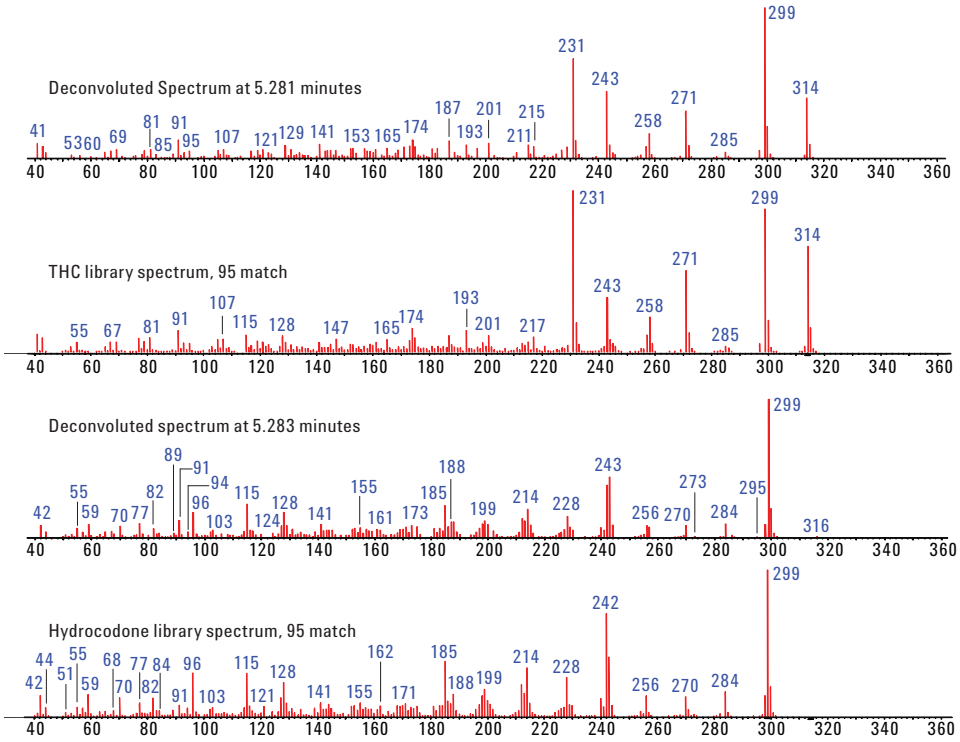

Figure 7. Deconvoluted and library reference spectra for THC and hydrocodone in the peak at 5.28 minutes.

Example analyses with hydrogen system

Street heroin samples typically contain a number of adulterants, byproducts of synthesis, and other naturally occurring alkaloids including 6-monoacetylmorphine (6-MAM), papaverine, and acetylcodeine. Table 2 shows an example of the DRS report from a typical street heroin sample.

The report lists the RT, CAS number, compound name, spectral match quality, and ΔRT for those compounds with a match quality > 60. The match factor is used by the analyst as an indication that a compound may be present. Using a match factor of 60 as a threshold, potential hits are indicated using the combined spectral similarity and RT match. The analyst uses this match factor as a guide to make a determination as to whether the indicated substance is positive. A factor < 60 indicates a poor spectral or RT match, and is not included in the search. It was observed that when a bona fide positive occured, the match factor generated exceeded a value of 80.

The ΔRT listing is the difference in seconds between the library reference RT value and the RT of the peak found in the sample.

Under the NIST section of the report, the first column is the reverse match value of the deconvoluted spectrum versus the NIST main library. The hit number is the position in the NIST search results list. High reverse match values and a low hit number helps confirm the identification. Note that benzocaine was the number 2 NIST hit. The number 1 NIST hit was N-acetyl benzocaine, which has a spectrum almost identical to benzocaine. The reverse match value for N-acetyl benzocaine was 96. This is a good example of the value of retention time locking. The spectrum AND RT of benzocaine match precisely those in the DRS database. N-acetyl benzocaine would have a different RT, and thus, is not the compound present. Note that the method used is set up only for qualitative identifications. If desired, the method can be calibrated for quantitative analysis as well, and the results would be combined into the DRS report.

Table 2. DRS Report from a Street Heroin Sample Run on the Hydrogen System

MSD deconvolution report

Sample name	ALA33	Adjacent peak subtraction = 1	
Data file	D:\MassHunter\GCMS\1\data\Heroin_Sample.D	Resolution = High	
Date/time	15:14 Wednesday, Oct 2 2013	Sensitivity = Medium	
		Shape requirements = Medium	

The NIST library was searched for the components that were found in the AMDIS target library.

			Amount (ng)		AMDIS		NIST	
RT	CAS no.	Compound name	ChemStation	AMDIS	Match	ΔRT (sec)	Reverse match	Hit no.
3.2850	51799327	N-Propylamphetamine			99	−0.4	90	1
3.8059	94097	Benzocaine			100	−0.5	95	2
4.2958	58082	Caffeine			100	−0.4	91	1
4.3613	137586	Lidocaine			99	−0.4	92	1
4.7037	3158858	10,11-Dihydrodibenz(b,f)(1,4)oxazepin-11-one			97	0.2	93	1
5.3308	6703271	Acetylcodeine			100	−0.2	95	1
5.3687	2784738	6-Monoacetylmorphine			100	0.2	95	1
5.4999	561273	Heroin			100	0.9	97	1
5.7215	58742	Papaverine			98	−0.2	94	1
6.2532	128621	Noscapine			92	−0.5	90	1

Figure 8 shows the TIC chromatogram from the street heroin sample.

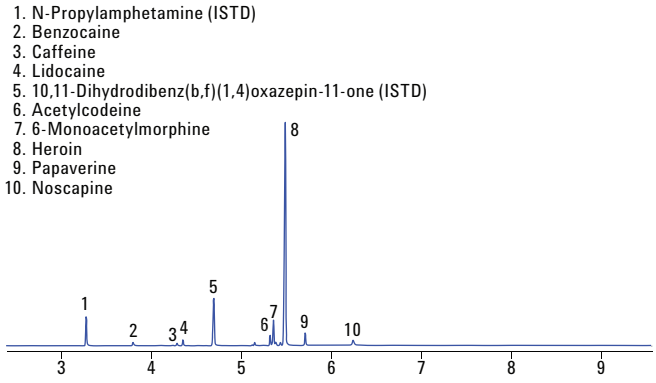

Figure 8. TIC of street heroin sample reported in Table 2.

The next example was a synthetic cannabinoid sample. Table 3 shows the DRS report for the sample.

Table 3. DRS Report for a Synthetic Cannabinoid Sample

MSD deconvolution report
Sample name 13137397-002-1B (1)
Data file C:\Datafiles\New_Case.D
Date/time 17:20 Friday, Mar 14 2014

Adjacent peak subtraction = 1
Resolution = High
Sensitivity = Medium
Shape requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

			Amount (ng)		AMDIS		NIST	
RT	**CAS no.**	**Compound name**	**ChemStation**	**AMDIS**	**Match**	**ΔRT (sec)**	**Reverse match**	**Hit no.**
3.2726	51799327	N-Propylamphetamine			99	−1.2	88	1
4.6894	3158858	10,11-Dihydrodibenz(b,f)(1,4)oxa zepin-11-one			98	−0.6	93	1
5.4175	999025137	XLR-11			100	0.6		
5.4175	74764526	Pyridine, 2,2'-(1,2-phenylene)bis-					75	1
5.4574	999027133	XLR-11 degradant			100	−0.2		
5.4574	0000	6-Cyclohexylamino-8-ethyl-3,3-dimethyl-3,4-dihydro-1H-thiopyrano[3,4-c]pyridine-5-carbonitrile					60	1
6.6462	1400742177	PB-22			100	0.8		
6.6462	864445396	2-(2-Methylphenyl)-1-(1-pentylindol-3-yl-)ethanone					85	1
6.9063	1400742417	5-Fluoro-PB-22			91	0.5		
6.9063	32741245	1,4-Naphthalenedione, 5,8-dihydroxy-2,3,7-trimethyl					81	1

DRS identified four synthetic cannabinoid compounds in the sample. XLR-11, XLR-11 degradant, PB-22, and 5-Fluoro-PB-22. These four compounds were not in the NIST 11 library. That is why the NIST section is empty for these four entries. The closest match from the NIST library is listed on the next line down. Figure 9 shows the chromatogram of the sample.

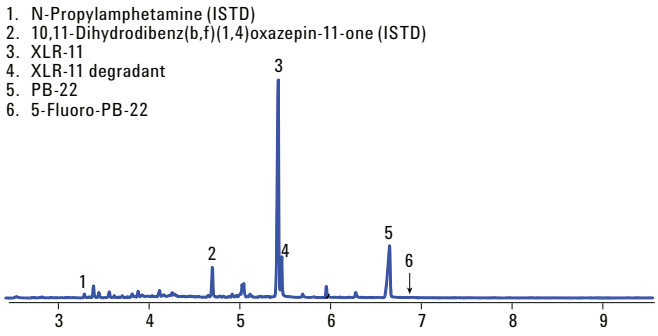

Figure 9. TIC of synthetic cannabinoid.

Comparison data by drug classification

One hundred heroin case samples were analyzed on both systems.

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Heroin	100	100	100
6-MAM	100	100	100
Acetylcodeine	100	100	100
Papaverine	86	86	24
Noscapine	19	19	16
Quinine	26	20	24

Of the 100 cases, heroin, 6-monoacetylmorphine, and acetylcodeine were identified on both instruments in all 100 samples. Eighty-six of these cases also contained papaverine. DRS was able to identify papaverine in all 86 cases, however, on the helium instrument, papaverine was identified in only 24 of the samples. Nine of these 24 required manual manipulation to get a satisfactory match. Additionally, there were 19 samples in which traces of papaverine were present in the helium data, but a sufficient match factor was not obtained with manual manipulation. Noscapine was present in 19 of the heroin cases and all samples were positive by DRS on the hydrogen instrument. Eleven of the cases were positive for noscapine using typical database search reports on the helium system, and an additional five samples were positive when manual searching was used. Finally, quinine is sometimes seen as an adulterant in heroin samples. Twenty-six of the cases analyzed were positive for quinine. Quinine was identified in 20 of the samples analyzed on the hydrogen system; however, five were missed by DRS due to poor chromatography. Twenty-four samples were positive for quinine on the helium instrument. The other two samples contained trace amounts of quinine that did not yield an acceptable match factor, even when manual data manipulation was performed.

Crime labs often see cases containing cocaine. Cocaine can be cut with a number of compounds. Levamisole is an anthelmintic veterinary drug frequently seen in cocaine samples.

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Cocaine	82	82	72
Levamisole	44	44	38

Eighty-two cases that contained cocaine were analyzed. The hydrogen instrument identified cocaine in all 82 cases. The data processing on the helium instrument gave a positive finding for cocaine in 72 of these cases. Of these 72, five cases required manual analysis. An additional five cases had traces of cocaine on the helium system, however, even with manual searching, an acceptable match could not be made. Forty-four of these cases also contained levamisole. The DRS was able to identify the levamisole in all of the samples. Levamisole was positively identified in 38 of the samples analyzed using the helium system. There were two cases in which traces of levamisole were present.

The compounds found in *papaver somniferum*, the opium poppy, are morphine, codeine, thebaine, papaverine, and noscapine. Opiods are derived from these compounds. For example, oxycodone can be synthesized from thebaine. Synthetic opiates, such as fentanyl and meperidine, have the same effects as natural opiates and opiods, but are not synthesized from the five components found in the poppy plant. These drugs are considered narcotics, are often prescribed for pain relief, but are abused for their ability to induce euphoria. Also included in this category are compounds such as methodone, buprenorphine, and naloxone. These drugs are given to treat opiate dependence.

Opiates/Opiods/Synthetic Opiates

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Oxycodone	102	102*	101
Codeine	7	7	7
Hydrocodone	9	9	5
Hydromorphone	5	5	0
Meperidine	4	4	4
Methadone	7	7	7
Morphine	26	11**	22
Fentanyl	1	1	1
Buprenorphine	8	8	8
Naloxone	5	5	5

* Oxycodone: seven of these positive finding required changes to the analysis settings

** Morphine: trace amounts

Oxycodone was present in 102 cases. The helium instrument identified the oxycodone in 101 of these cases. DRS and the hydrogen system initially reported 95 positive oxycodone findings. In the seven cases where oxycodone was not identified by DRS, it was due to poor chromatography in the sample. In these instances, oxycodone was present in low amounts, but exhibited excessive peak tailing, moving the peak out of the acceptable retention window. Relaxing the restrictions on retention time criteria allowed for successful identification. Morphine was present in a total of 26 cases. There were 11 positive morphine findings on the hydrogen system and 22 positive morphine findings on the helium system. Traces of morphine detected in these heroin cases on the helium instrument were not detected on the hydrogen instrument. The cause for this discrepancy was not determined, but occurred with trace amounts only. The four other cases were traces on the helium system that did not produce an adequate match. Both of the systems identified naloxone, buprenorphine, fentanyl, methadone, and meperidine in all of the positive cases. Seven codeine cases were analyzed. Both the hydrogen and helium systems identified codeine in all cases, however three of the helium cases required manual techniques for identification. Some reactivity was noted when using hydrogen. Some compounds can react in the presence of hydrogen and a metal catalyst at high temperatures. This behavior was observed in samples and standards containing codeine. A small amount of the codeine converted to hydrocodone. Of the seven codeine cases analyzed, four were positive for hydrocodone only on the hydrogen system. This was likely the reason for the discrepancy between the hydrogen and helium findings for hydrocodone. It is possible that this conversion also occurs when morphine samples are injected on the hydrogen instrument. Morphine was present in all five cases that were positive for hydromorphone on the hydrogen instrument. None of these cases were positive for hydromorphone on the helium instrument.

Synthetic cannabinoids are a relatively new trend in drug chemistry. These compounds generally have one of a few basic structures, such as naphthylindole, to which variations and substitutions are made, giving rise to a new drug. These compounds are generally seen sprayed onto a botanical material. Because the structures are very similar and samples can contain multiple isomers, retention time is key in the proper identification of these compounds.

Synthetic Cannabinoids

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
5-F-PB-22	2	2	2
AM-2201	1	1	1
JWH-018	4	4	4
JWH-022	1	1	0
JWH-250	3	3	2
PB-22	4	4	4
RCS-4	1	1	0
UR-144	1	1	0
URB-597*	1	1	1
URB-754	1	1	1
XLR-11	2	2	2

* Known artifact formation

Both systems were able to identify 5-F-PB-22, AM-2201, JWH-018, PB-22, URB-597 artifact, URB-754, and XLR-11 in all of the case samples in which they were present. One of the 5-F-PB-22 findings required data manipulation on the helium system to obtain an acceptable match. JWH-022, RCS-4, and UR-144 were identified using DRS, but missed by database searching on the helium system. In samples containing JWH-250, all were positively identified using DRS, however one sample was miss identified as JWH-302 on the helium system. JWH-250 and JWH-302 are isomers.

Another new trend appearing in crime labs is cases containing designer stimulants, including the novel psychoactive agents commonly referred to as bath salts. These are usually substituted phenethylamines and tryptamines, but may also be piperazines.

Designer Stimulants

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
5-MeO-DiPT	2	2	2
BTCP	2	2	2
BZP	3	3	3
MDA	1	1	0
MDEA	1	1	0
MDMA	2	2	1
MDPV	2	1	2
Methylone	1	1	1
TFMPP	3	3	3

DRS and database searching provided positive identifications in all cases containing 5-methoxy-diisopropyltryptamine (5-MeO-DiPT), benzothiophenylcyclohexylpiperdine (BTCP), benzylpiperazine (BZP), methylone, and trifluoromethylphenylpiperaize (TFMPP). Methylenedioxyamphetamine (MDA) and methylenedioxyethylamphetamine (MDEA) were identified on the hydrogen system, but not on the helium system. In both cases, there were trace amounts present on the helium system that did not give a suitable match. Methylenedioxymethamphetamine (MDMA) was identified in both cases using DRS, but only in one using database searching. Finally, methylenedioxypyrovalerone (MDPV) was present in two cases on the helium system, but was only identified in one sample on the hydrogen system.

Stimulant compounds are a popular group of drugs that can abused for weight loss or as performance enhancers in both sports and studying. Methamphetamine is a common street drug of abuse. Some stimulants are prescribed to treat ADHD.

Stimulants

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Amphetamine	12	12	12
Methamphetamine	13	13	13
Methylphenidate	4	4	4
Phentermine	10	10	10

In all of the samples analyzed, amphetamine, methamphetamine, methylphenidate, and phentermine were properly identified on both systems.

Tetrahydrocannabinol (THC) is the active ingredient in marijuana. Cannabinol and cannabidiol are both cannabinoids found in the marijuana plant. Unlike THC, neither are psychoactive. Cannabinol is a breakdown product of THC.

Cannabinoids

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
THC	12	12	11
Cannabidiol	6	6	5
Cannabinol	6	6	6

Cannabinol was present and identified on both systems in six cases. Cannabidiol was also present in six cases, however in one sample, the cannabidiol was present in trace amounts and an acceptable match was not obtained on the helium instrument. Twelve samples contained THC. The hydrogen system positively identified THC in these 12 cases. Eleven cases were positive on the helium instrument. One of these 11 required manual manipulation.

Lysergic acid diethylamide (LSD) and ketamine are sometimes seen in case samples. LSD is generally encountered as a perforated paper that has been coated with the drug. Ketamine was used as an anesthetic, but fell out of favor due to its dissociative effects. It is still used in veterinary practices.

Hallucinogens

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
LSD	2	2	2
Ketamine	1	1	1

The samples containing LSD and ketamine were identified on both systems.

Pharmaceutical preparations used to induce sleep and reduce anxiety are often encountered in forensic chemistry. These include, but are not limited to, benzodiazepines and barbiturates, though the prescription of barbiturates has significantly declined due to their addictiveness.

Sedatives/Hypnotics

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Alprazolam	43	43	42
Clonazepam	5	5	5
Diazepam	5	5	2
Methohexital	4	4	4
Pentobarbital	10	10	10
Zaleplon	4	4	2

Clonazepam, methohexital, and pentobarbital were identified correctly by both systems. In all 43 cases, the hydrogen system was able to identify alprazolam. The helium system missed alprazolam in one case. There were five samples that contained diazepam. The hydrogen system positively indentified diazepam in 100% of the samples. The helium system positively identified diazepam in two out of the five samples, and two more had diazepam present at trace levels. Finally, zaleplon was properly identified on the hydrogen system in 100% of the cases. It was properly identified on the helium system in 50% of the cases.

Antihistamines are often used to relieve allergy symptoms. They are available both over the counter and as prescriptions. Promethazine is most often seen in combination with codeine as a cough syrup.

Antihistamines

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Diphenhydramine	6	6	5
Doxylamine	4	4	1
Promethazine	4	4	4

Promethazine was identified four out of four times on both systems. Diphenhydramine was present in six cases analyzed. The hydrogen system identified diphenhydramine in all six samples. The helium instrument missed one diphenhydramine finding. Doxylamine was positively identified by DRS in the four samples in which it was present. On the helium system, however, only one doxylamine finding was reported. The remaining three cases were trace amounts that did not produce a suitable match.

Anabolic steroids are often abused to build muscle. Often, the abuse of anabolic steroids is accompanied by the use of drugs like clomiphene and tamoxifen, which regulate hormone levels for breast cancer or fertility treatment. Clenbuterol is used to treat asthma, but is often taken off-label for weight loss.

Steroids/Performance Enhancements

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Clenbuterol	1	1	0
Testosterone Propionate	1	1	1
Clomiphene	2	2	2
Tamoxifen	1	1	1

Testosterone propionate, clomiphene, and tamoxifen were identified on both instruments. One sample was positive for clenbuterol. The hydrogen system identified clenbuterol, but the helium system did not.

Analgesic drugs are given to relieve pain. Acetaminophen can be purchased over the counter, but is also seen as part of a pharmaceutical preparation in combination with oxycodone or hydrocodone.

Analgesics

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Nimesulide	1	1	1
Acetaminophen	39	39	29

Nimesulide was present in one sample. It was properly identified by both systems. Acetaminophen was present in 39 samples. It was properly identified in 100% of the samples by DRS. In one of these samples, the acetaminophen was overloaded and not originally identified by DRS. Adjusting the allowed retention time window corrected this, and acetaminophen was then called in this sample. On the helium instrument, acetaminophen was identified in 29 of 39 samples.

Antipsychotic compounds are not seen as frequently and most are not controlled, however, these pharmaceuticals are occasionally submitted as evidence.

Antipsychotics

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Quetiapine	3	3	3

Both systems properly identified quetiapine in three of three cases. When using database searching on helium, manual manipulation was required in two of the three cases.

The majority of street samples, excluding tablets and botanical materials, are cut with compounds to increase the weight and lower the purity. Adulterants are drugs themselves and are generally chosen because they have effects similar to the drug being cut, as opposed to diluents, which are often sugars or lactose added only to increase weight. Adding adulterants creates the impression that the sample is more pure than it actually is. For instance, caffeine may be added to a stimulant. Lidocaine or procaine may be added to a cocaine sample because each will create the same local anesthetic effect.

Common Adulterants

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Benzocaine	11	11	11
Caffeine	73	73	48
Diltiazem	61	60	52
Lidocaine	31	31	22
Phenacetin	3	3	2
Procaine	16	16	13
Xylazine	3	3	0
Hydroxyzine	4	4	0

Benzocaine was identified in all 11 samples on both systems. Caffeine was identified in all 73 samples on the hydrogen instrument. Data processing on the helium system failed to identify caffeine in 25 of these cases. Seven of these were trace amounts, where data manipulation did not yield a suitable match. Of 61 samples containing diltiazem, 60 were identified on the hydrogen system and 52 were identified on the helium system. One of these 52 diltiazem findings required manual manipulation. Lidocaine was properly identified in all 31 samples using DRS. Twenty-two were positive for lidocaine on the helium instrument. One of these 22 required manual manipulation. An additional four samples had traces of lidocaine that could not be positively identified on the helium system. Phenacetin was present in three cases. In all three of these cases, it was identified by the hydrogen system. In one of these cases, data processing on helium failed to identify it. Sixteen cases contained procaine. DRS identified procaine in all of these samples. The helium system properly identified procaine in 13 cases. Two of the three that were missed were due to trace amounts on the helium instrument. In three cases for xylazine and four cases for hydroxyzine, both were identified in the samples on the hydrogen instrument. Neither of these compounds were detected on the helium system. In two cases, there was a trace amount of hydroxyzine present, but a sufficient match could not be obtained.

Occasionally, compounds that do not necessarily fit into one of the above categories are encountered in a case.

Miscellaneous Findings

Compound	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Strychnine	10	10	10
Vitamin E	7	6	1
Nicotine	2	2	2

Nicotine and strychnine were properly identified on both instruments in 100% of the samples that contained them. One of the nicotine samples required manual data processing to make the identification on the helium instrument. Vitamin E was present in seven samples. It was identified in six of these seven on the hydrogen instrument. Trace amounts were present in the seventh sample, but it was not identified by DRS. One of the samples was positive for vitamin E on the helium system, and one more sample had a trace amount that could not be identified properly with manual data manipulation.

Negative Samples

	Total number of cases analyzed	Number of positive findings on hydrogen	Number of positive findings on helium
Negative	8	8	8

Eight samples that were negative were determined to be negative on both systems.

Time comparison

Data acquisition

The use of hydrogen allows for more efficient chromatographic separations in a shorter period of time. As a result of this, the combined run time and equilibration time allows for shorter cycle times. The use of sample overlap also improves this performance. Sample overlap prompts the autosampler to prepare samples for injection prior to system equilibration. Backflushing the column adds one minute to the total run time, but saves time compared to a long bakeout period by preventing ghost peaks from samples with late eluting matrix compounds.

Instrument	Run time
Hydrogen	13 minutes
Helium	21 minutes

Figure 10 demonstrates the shorter run time using hydrogen.

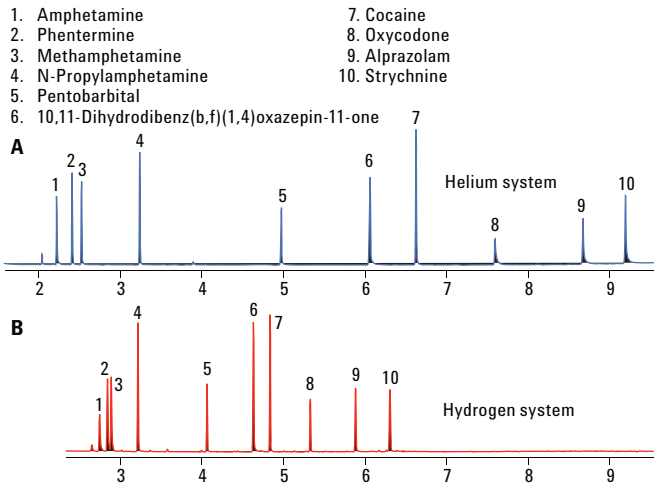

Figure 10. Chromatograms of test mix on helium system (A) and new hydrogen system (B).

Data processing
Two data processing scenarios were chosen. The first was a simple botanical sample containing THC and cannabinol. The second was a sample containing JWH-133, which coeluted with an internal standard. An analyst was timed processing the data using DRS and using standard ChemStation search routines. Additional manual data processing was required for the coeluting substances, including generation of extracted ion chromatograms and multiple spectral subtractions.

Data type	Processing time (Helium)	Processing time (Hydrogen)
Simple	49.2 seconds	18.3 seconds
Complex	2 minutes 44.4 seconds	20.1 seconds

Conclusions

Time savings and quality performance

There are two main savings provided by this instrument configuration. The first is run time. The cycle time for our helium general unknown screening method was approximately 21 minutes (this is injection to injection). For the hydrogen configured instrument, this time is reduced to approximately 13 minutes injection to injection. This is a 40% reduction in run time, which translates into an increase in efficiency and throughput in the production lab.

The second source of time savings is generated from the reduction in data processing time. For a simple sample, data processing using DRS takes approximately half the time as data processing in ChemStation. As the complexity in samples increases, more manual data processing and manipulation is required to generate acceptable data. The flaw in this process is that it is a labor intensive process that requires time and skilled personnel. Additionally, the data generated can be subjective based upon the analyst's bias. Alternatively, traces of material may be missed by inexperienced personnel. DRS has the capability of detecting various compounds in complex mixtures even at trace levels as demonstrated by the comparison data. Deconvolution can also properly analyze data containing chromatographic overloads through custom set points that can be applied to specific compounds. The process is performed objectively and consistently each time. All spectra are background subtracted and presented in an enhanced format that automatically removes background interferences. These data are generated in an automated fashion that does not require additional personnel time or expertise. Also, the DRS generated data factor in the established retention time of each identified component. This retention comparison is factored into the algorithm generated match factor for each finding. This retention data matching will serve to delineate the various isomeric forms that may be present in evidence. Typically used ChemStation options do not factor this additional dimension into the match factor. This eliminates the need for referencing retention times or reanalyzing reference material for verification.

DRS includes the ability to perform additional searching of the NIST libraries using the deconvolved spectra. This enhanced spectrum can be searched versus a NIST library free of interfering ions.

Additionally, coeluting substances are readily separated by DRS, whereas typical ChemStation processes require trial and error attempts to subtract the appropriate ions and manually generate cleaned spectra specific for each compound.

Cost savings

In addition to the savings made in instrument throughput and personnel time, further savings may be made. The hydrogen used as carrier gas is easily generated in-house by a hydrogen generator fueled by water and electricity. Even in cylinder form, hydrogen is readily available in highly purified forms. Helium supplies are dwindling and the price continues to rise. The cost of a cylinder of helium compared to the cost of a cylinder of hydrogen is approximately $200 for helium and $140 for hydrogen.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
June 15, 2014
5991-4536EN

Agilent Technologies