Application Note

Forensics

Evaluation of Hydrogen Carrier Gas and the Agilent HydroInert Source for Forensic Street Drug Analysis

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Abstract

Common screening steps to determine the presence or absence of a controlled substance in a suspected drug sample require the ability to separate the major components into discreet individual compounds. Forensic chemists have routinely used capillary gas chromatography (GC) with mass selective detectors (MSD) for this purpose. Helium (He) is an inert gas that has historically been the preferred carrier gas for gas chromatography/mass spectrometry (GC/MS). Due to its chemical and physical characteristics, high-resolution chromatographic separations can be achieved with minimal analyte interactions. In recent years, there has been difficulty in the availability and increased cost of securing a routine, ultra-high purity (UHP) helium supply for GC and GC/MS users. Hydrogen (H_2) has become a secondary option for GC/MS; however, hydrogen does have disadvantages based on its reactivity with some analytes, reduced sensitivity, increased peak tailing, and reduced spectral fidelity when compared to helium-generated reference spectra. The purpose of this study is to demonstrate the ability of the Agilent HydroInert source to be successfully incorporated into the current forensic workflow on an existing GC/MS system. This application note outlines the process of translating a GC/MS forensic drug screening method from using helium carrier gas to using hydrogen carrier gas by applying the Agilent HydroInert source. The method also incorporates best practices and difficulties associated with this transition.

Introduction

Within the last decade, there has been an increase in the difficulty of procuring UHP helium in the quantities required for full laboratory operations, as well as a drastic increase in the overall cost of UHP helium tanks. GCs with atmospheric detectors often use alternative carrier gases such as nitrogen, argon, and hydrogen. However, when the GC is coupled to an MS under a high vacuum, parameters based on a mean free path of ion molecules, inertness, vacuum, low background, and high sensitivity come into view. Based on these parameter limitations of nitrogen and argon, hydrogen is the practical alternative. Nonetheless, hydrogen does have disadvantages that may cause a GC/MS analyst to re-evaluate the urgency to convert to a hydrogen carrier gas system. This study reduces some of the unknown factors and provides an effective foundation for the screening and identification of illicit street drugs using the HydroInert source and hydrogen carrier gas.

One of the first steps in migrating to a hydrogen method and choosing a column configuration is determining how much hydrogen flow your GC/MS system can effectively and safely handle. The recommended maximum column flow rate of hydrogen for a turbo pump is ~ 2.0 mL/min, and a diffusion pump is not supported for hydrogen carrier or an extractor source.[1] Hydrogen is not as viscous as helium, meaning lower head pressures are used to produce flow rates similar to those of helium. This usually results in a column configuration change to a column with a smaller diameter, thicker film, or longer length, or various combinations of all three. Ideally, if the column phase ratio can be carried over from the original helium method to the new hydrogen method, similar chromatographic patterns and analyte elution order should be achieved.[2] This prevents the need to determine and relearn the elution order for analytes of interest or the separation of common interferences. For optimal MS sensitivity, flow rates usually range between 0.8 and 1.2 mL/min.[1] To assist with these parameter changes, Agilent has online tools to help determine the resultant flow and column parameters when attempting to change column dimensions. The Agilent Pressure Flow Calculator and Method Translator tools are available to download free from the Agilent website and are also available in Agilent OpenLab software or Agilent MassHunter Acquisition software.

To optimize performance, Agilent also recommends the use of a 9 mm (or 6 mm) drawout or extractor lens, depending on the version of source, to be installed in the source body when using hydrogen as the carrier gas.[1] The combination of heat, presence of hydrogen, and metal source components can cause in-source reactions. Enlarging the orifice of the drawout or extractor lens reduces the surface area of the lens and helps to reduce the possibility of analyte reactions on the metal surface. The Agilent stainless steel, inert, and inert extractor sources come with a 3 mm lens, as standard for most helium applications. Table 1 lists the respective Agilent part numbers for the different lenses, depending on the source type of an existing instrument. Also, the HydroInert source employs a proprietary material, reducing catalytic activity and tailing in the source, thus minimizing reactivity. An example of a common in-source reaction using hydrogen carrier is the conversion of nitrobenzene through a hydrogenation reaction to aniline, depicted in Figure 1. Due to the structural similarities and elemental composition, similar ion fragments are found in both spectra but the ion ratios are different, causing a problem with the library match score (LMS). This reaction persists even with a 9 mm drawout or extractor lens.

Table 1. Agilent source-specific part numbers for drawout and extractor lenses.

Source Design	Part Number		
	3 mm	6 mm	9 mm
Stainless Steel Source—Drawout	05971-20134	G3163-20530	–
Inert Source—Drawout	G2589-20100	G2589-20045	G3440-20022
Inert Extractor Source—Extractor	G3870-20444	G3870-20448	G3870-20449
Hydroinert Source—Extractor	G7078-20906	G7078-20908	G7078-20909

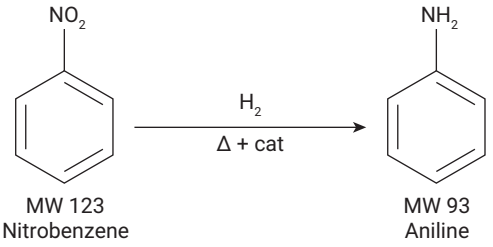


Figure 1. Hydrogenation in-source reaction of nitrobenzene to aniline.

However, as Figure 2 illustrates, due to improvements to the HydroInert source, the in-source hydrogenation reaction is minimized, generating an increase in spectral fidelity and resulting in a higher LMS for nitrobenzene with reduced tailing. Other examples of the spectral differences between the inert extractor and HydroInert sources are displayed in Figures 3 and 4 for two common drugs of abuse. The degree of variation in LMS is compound and source dependent. The HydroInert source can accommodate a 6 and 3 mm extractor lens, possibly resulting in an increase in signal-to-noise ratio (S/N) of target analytes, but also increasing the possibility of reactivity of other compounds.[1] More testing needs to be performed to determine the best configuration based on a target compound's response and reactivity compared to the recommended 9 mm extractor lens. Building off the nonreactive HydroInert source, multiple applications that are routinely helium based have been developed under hydrogen carrier gas using the HydroInert source.[3–6]

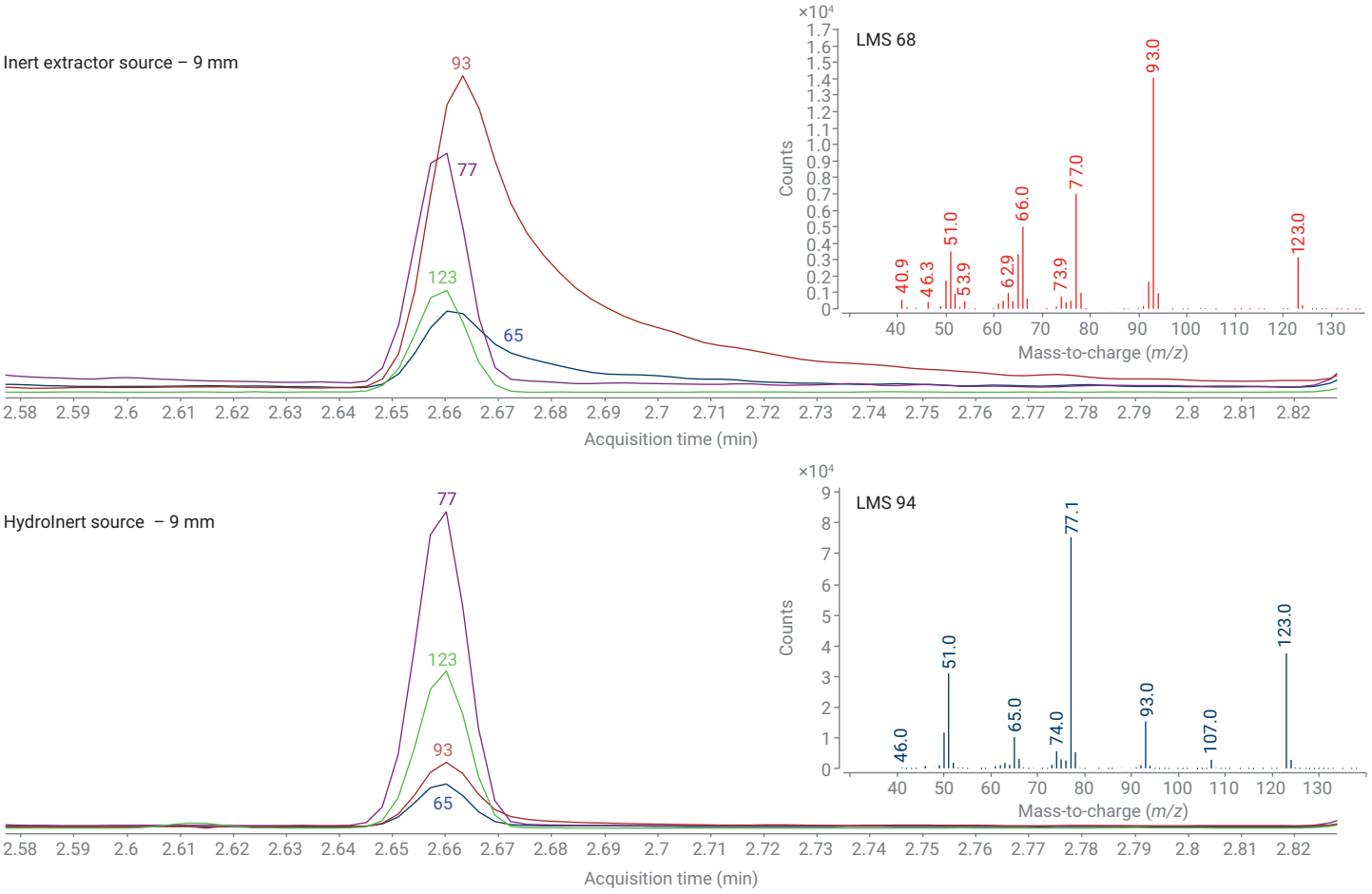


Figure 2. Nitrobenzene spectra generated on an Agilent inert extractor source and the HydroInert source using hydrogen carrier gas (LMS based on NIST20 library search results).

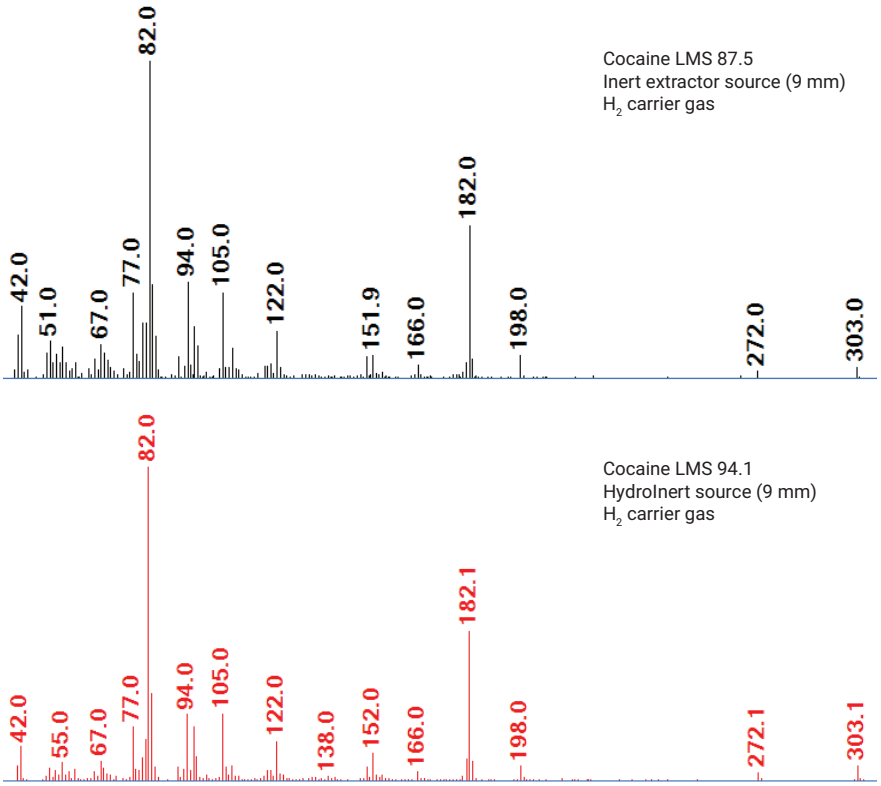


Figure 3. Spectral differences of cocaine on the Agilent inert extractor source and HydroInert source using hydrogen carrier gas. (1 ng on column, atune, deconvoluted extracted spectra, NIST20 library.)

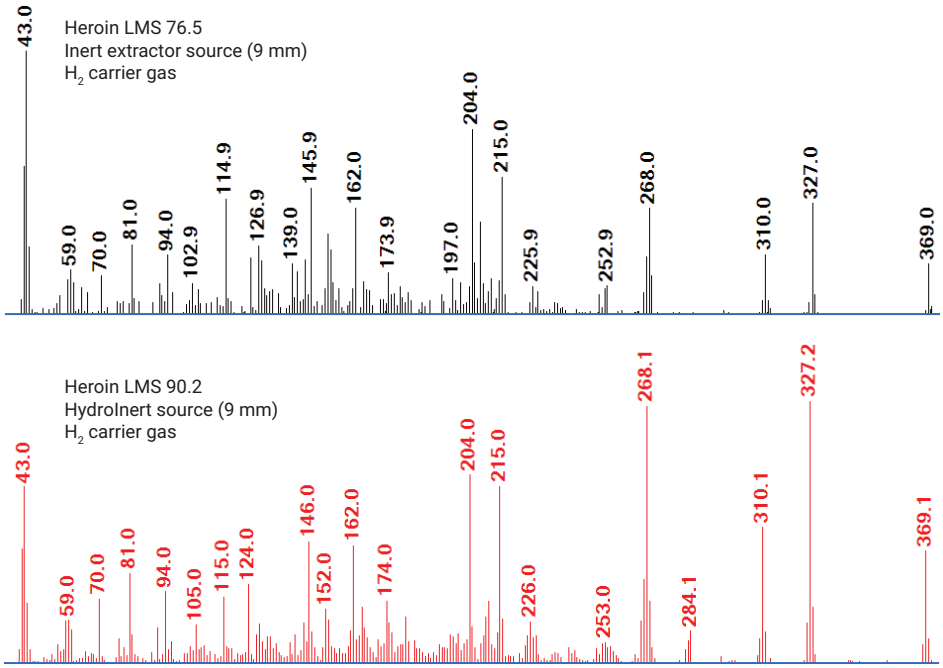


Figure 4. Spectral differences of heroin on the Agilent inert extractor source and HydroInert source using hydrogen carrier gas (1 ng on column, atune, deconvoluted extracted spectra, NIST20 library.)

This work outlines the process of converting a drug screening method from using helium carrier gas to using hydrogen carrier gas by applying the HydroInert source on an existing Agilent 5977B GC/MSD and an Agilent 8890 GC system. Approximately 120 forensic street drug case samples were analyzed using the hydrogen method with an additional ~ 120 drug standards. The GC/MS data acquisition was controlled by Agilent MassHunter acquisition software, with data analysis performed in MassHunter Unknowns Analysis software using deconvolution. The NIST20 and SWGDRUG 3.8 mass spectral libraries were used for identification of components that generated an LMS ≥ 70.

Gas chromatography/mass spectrometry parameters under hydrogen

The MS employed was a 5977B GC/MSD system, equipped with a high-performance turbo pump. The pumping capacity for hydrogen is approximately half that of helium, limiting the choice of columns and achievable linear velocities (hydrogen average linear velocity is 30 to 55 cm/s). To minimize the method development process, the Agilent Method Translator tool was used to translate an existing drug screening method from using helium carrier gas to a using hydrogen carrier gas. Figure 5 portrays the process and resulting parameter calculations needed for the method conversion. The flow rate conversion from helium to hydrogen increased the flow to 1.2 mL/min of hydrogen (red boxes). Since this is the upper limit of optimal MS flow and exceeded the average linear velocity of hydrogen, the flow rate was reduced to 1.0 mL/min. The oven ramp was translated to 33 and 44 °C/min, respectively. However, to optimize resolution of early-eluting phenethylamines and specific later-eluting opiates, benzodiazepines, and buprenorphine, the initial oven temperature was lowered to 55 °C and the second oven ramp was decreased to 40 °C/min. These changes resulted in a final run time of 10.23 minutes, shown in Table 2. Although minor changes deviated from the original method translation, the Method Translator tool saved multiple hours (and possibly days) of trial-and-error injections. Once the hydrogen method was finalized, it was retention time locked (RTL) on phencyclidine (PCP) at 5.94 minutes.

Data for both methods were generated on an 8890 GC split/splitless inlet (noninert) and acquired on an Agilent J&W DB-5ms Ultra Inert (UI) 20 m × 0.18 mm, 0.18 µm analytical column (part number 121-5522UI).

MS data acquired under helium carrier were generated on the inert extractor source with a 3 mm extraction lens, while all hydrogen mass spectral data were generated using the HydroInert source equipped with a 9 mm extractor lens.

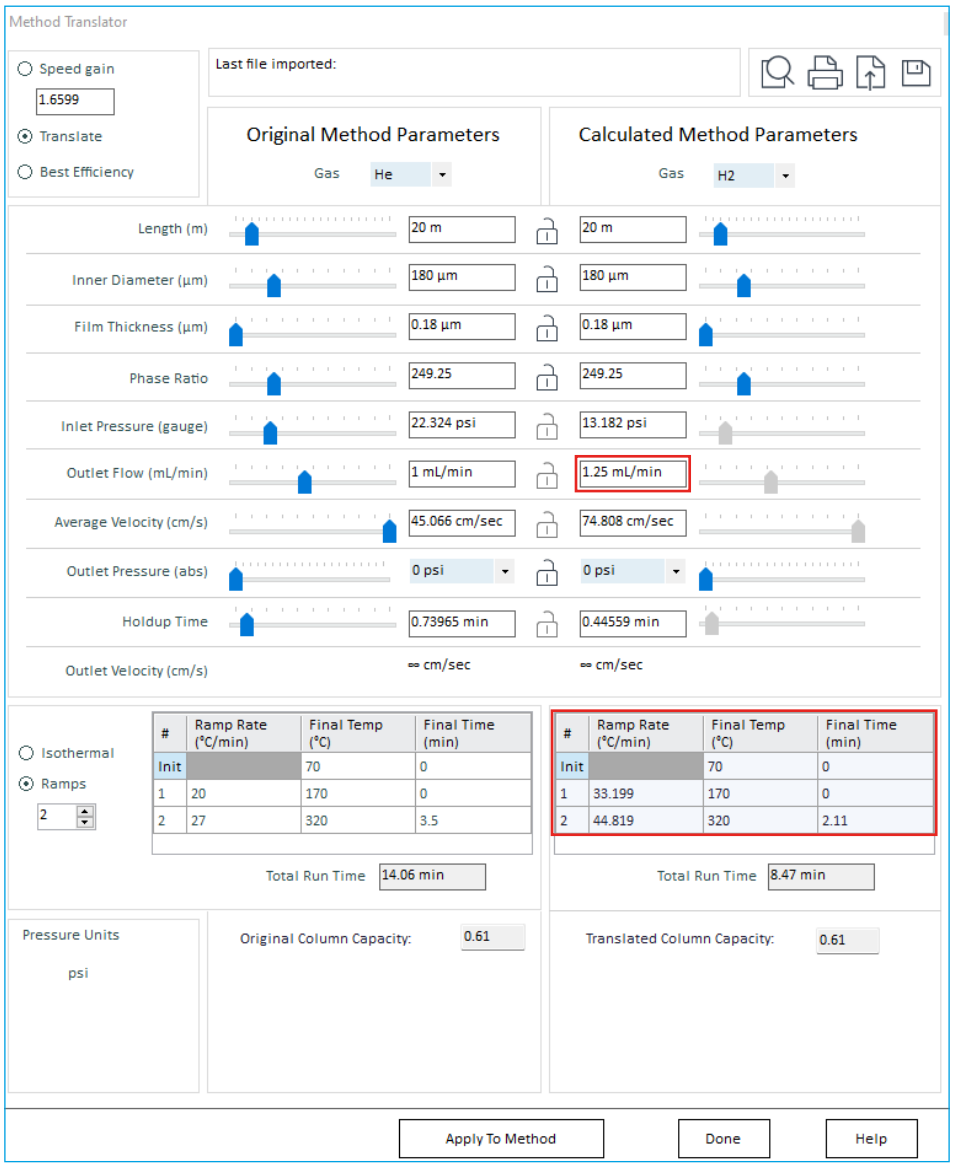


Figure 5. Agilent Method Translator tool converting the helium method to a hydrogen method.

Table 2. Method parameters for the hydrogen method using the Agilent HydroInert source.

Parameter	Value
Injection Source	Autosampler tower/tray
Injection Volume	0.5 to 1 µL
GC Split/Splitless Inlet	260 °C, split mode 10:1 and 20:1
Inlet Liner	Agilent splitless UI fritted inlet liner, single taper, bottom frit (p/n 5190-5112)
Septum Purge	3 mL/min
Temperature Program	55 °C (hold 0.2 min) 33 °C/min to 170 °C (hold 0.2 min) 40 °C/min to 320 °C (hold 2.3 min) 10.23 min run time
Analytical Column	Agilent J&W DB-5ms UI, 20 m × 0.18 mm, 0.18 µm (p/n 121-5522UI)
Column Flow	H_2 at 1 mL/min constant flow
Initial Inlet Pressure	9.3 psi
Linear Velocity	66 cm/s
MS Transfer Line Temperature	285 °C
Ion Source Temperature	285 °C; atune, etune, and stune at 230 °C
Quadrupole Temperature	150 °C
Scan Range	40 to 550 *m/z*
Gain	etune: 1; atune: 2; stune: 3
Threshold	0
A/D Samples	2[1]

Tune stability and stability of HydroInert source deactivation

All case samples and standards data were generated under atune, etune, and stune algorithms for comparisons of LMS using the NIST20 and SWDRG libraries. An initial comparison of source temperatures to analyte response and peak shape was performed before this study (data not presented), and 285 °C was determined to be the optimal overall temperature using atune and etune. However, stune, which is used by some forensic laboratories, was run at 230 °C for consistency and legacy reasons. During this study, all three tune algorithms were run at the beginning of the week before running samples for the week.

Based on the nitrobenzene hydrogenation reaction on a non-HydroInert source, and the reduced reaction exhibited on the HydroInert source (Figure 2), 0.5 µL nitrobenzene (0.5 mg/mL) was injected every 50 to 100 injections to determine if source deactivation would remain stable and consistent over the course of this study, encompassing over ~ 1,800 injections of case samples and standards.

Spectral fidelity and analyte response comparison between helium and hydrogen

As previously stated, the hydrogen method was translated from an existing helium method. All standards and case samples were run under the helium method on the inert extractor source, under all three tune algorithms. This was done to create a foundation for spectral comparisons and changes that may occur based on the algorithm when comparing LMS to spectra produced by the HydroInert source.

To determine the variability in analyte response based on helium and hydrogen environments, a series of case samples and standards were analyzed under both helium (inert extractor source) and hydrogen (HydroInert source) methods, with their respective electron multiplier gain set to 1.[1]

Effect of chlorinated solvent on the gas chromatography/mass spectrometry system

Dichloromethane and chloroform are common solvents used in sample preparation for forensic street drug analysis due to their solubility with many drugs. Powders, residues, and trace samples are also frequently diluted with methanol (MeOH) as a quick and easy sample preparation. To reflect real-world samples and mimic the forensic workflow and sample preparation processes, 36 methanolic dilutions and 80 dichloromethane (DCM) acid/base extracts were acquired. Samples were identified individually as DCM 1 to 80 and MeOH 1 to 36. Each sample was injected in triplicate, and data were generated under each tune algorithm on the HydroInert source, along with ~ 120 drug standards and mixtures, culminating in over ~ 1,800 injections on the HydroInert source and GC inlet.

To maintain accurate and reproducible data and to determine when to perform maintenance on the HydroInert source, the Agilent test standard for J&W DB-1 and DB-5 columns (part number 200-0310) was employed. The eight-analyte composition probes the column for resolution characteristics, efficiency, and inertness, as well as exercising the overall flow path.[7] The test standard was analyzed at the beginning of each sequence and inserted every 100 injections, along with the nitrobenzene standard. Indications of active sites or a contaminated flow path would result in broadened peaks, loss of analyte response, peak tailing, retention time shifts, or loss of an analyte completely.

Injection mode differences (split/splitless)

Personal communications with various forensic chemists from around the region indicated that they had experienced inlet reactions and

breakdown products when injecting opiates in splitless mode while operating with hydrogen carrier gas. To detect this phenomenon, morphine (Cayman Chemical, part number ISO60147) and codeine (Cayman Chemical, part number ISO60141) were injected in the helium method in both split and splitless modes, and the data were compared to identical injections performed in the hydrogen method. Both the helium and hydrogen methods were run in split mode with a 20:1 split (~ 50 µg on column), splitless mode (~ 1,000 µg on column), and pulsed splitless mode, while injecting 1 µL of codeine (1 mg/mL) and 1 µL of morphine (1 mg/mL) in the inlet. High concentrations were purposefully used, as most forensic laboratories do not know the concentration levels of their unknown samples and frequently inject large concentrations on a single injection.

Results and discussion

Gas chromatography/mass spectrometry parameters under helium

The Method Translator tool produced a hydrogen carrier method with a run time ~ 1.7 times faster than that of the original helium method. However, after optimizing the carrier flow rate and adjusting the oven ramp slightly, the final hydrogen method was ~ 1.4 times faster than the helium method and maintained similar elution patterns and peak resolutions while decreasing the run time by ~ 3 minutes, as depicted in Figure 6. The hydrogen method also produced baseline separation of the phenylamines (amphetamine, phentermine, and methamphetamine) and delivered reliable resolution of multiple synthetic opiates, benzodiazepines, and fentanyl analogs with an adequate run time to capture strychnine and buprenorphine before the end of the run.

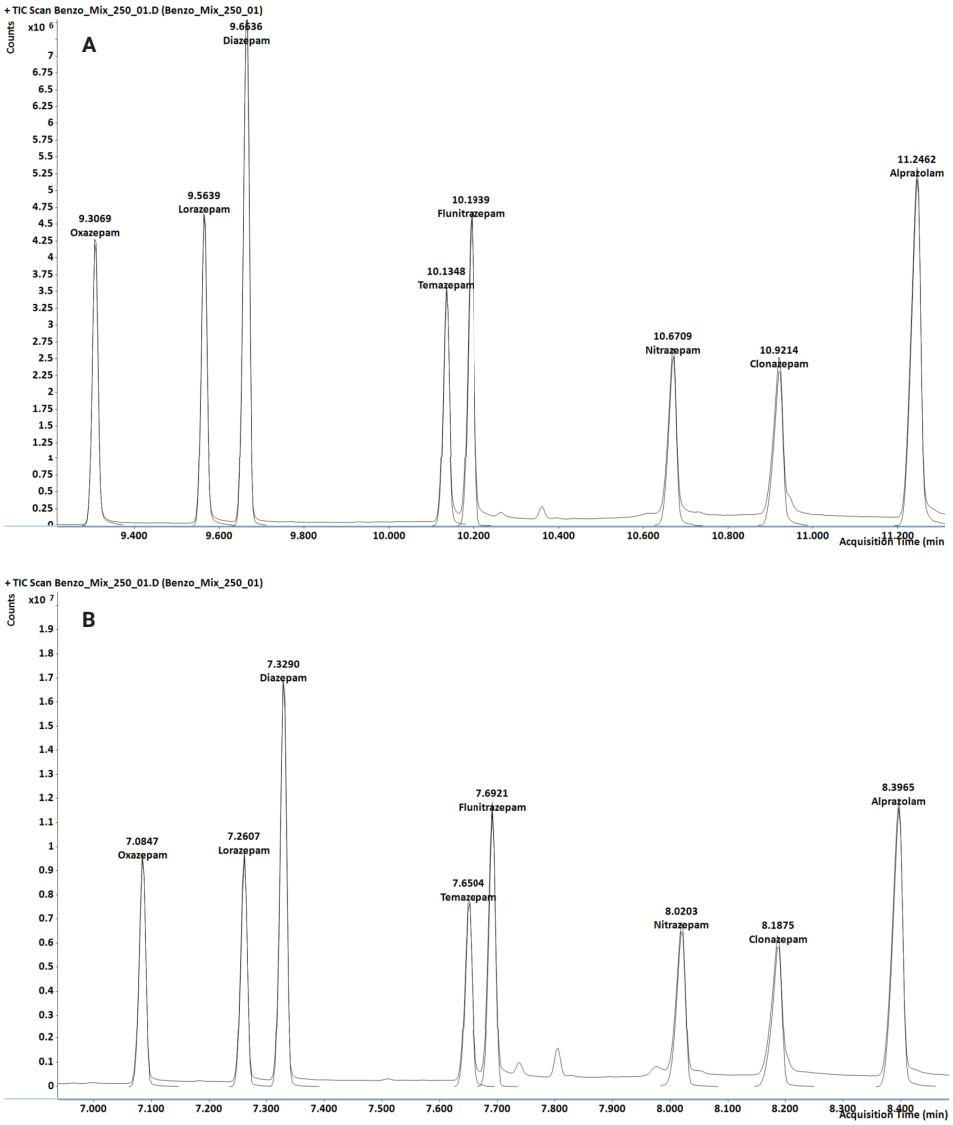


Figure 6. Benzodiazepine mix (Cerilliant, part number B-033). (A) Resolution under helium method with Agilent inert extractor source; (B) resolution under hydrogen method with Agilent HydroInert source.

Tune stability and stability of HydroInert source deactivation

As previously stated, all standards and case samples were run under atune, etune, and stune parameters. Note that to produce consistent and reproducible results, the system must be equilibrated under the hydrogen carrier before running samples or the validation process. Time will vary based on condition of the system (older existing system, newly purchased), even if the tunes are passing their respective tune criteria. This study began in July and was completed in November of the same year. The least number of variations in the tune parameters shown in Table 3 were from September to November. This does not invalidate the data generated in July and August, because LMS and spectral fidelity were reproducible throughout the study; however, tune parameters will change and stabilize over time based on the data in this work. This is to be expected since hydrogen is a reducing gas and interacts with all the surfaces it comes into contact with, including the solvent, matrix, and target analytes.[1]

Table 3. Tune parameter ranges over a seven-month period on the Agilent HydroInert source after ~ 1,800 injections.

Tune Parameter	atune	etune	stune
Repeller	34.9	4.7 to 0.5*	19.96
Extractor	NA	−1.9 to −0.4	NA
EM Voltage	998 to 867*	912 to 782*	1,037 to 976*
Isotopic Fidelity**	1.3 to 1.1, 4.4 to 4.3, 10.9 to 9.3**	1.1 to 1.2, 4.4 to 4.6, 9.6 to 10.4**	1.1 to 1.2, 4.2 to 5, 9.1 to 12.9**
Gain Factor	0.33 to 0.47	0.10 to 0.19	0.4 to 1.8

* Started high; came down over seven months.

** *m/z* ratios 69/70, 219/220, and 502/503 respectively.

The stune algorithm has been used as the foundational tuning parameters over multiple generations of instruments. However, the stune algorithm reduces overall source sensitivity to many analytes while maintaining reproducible spectral fidelity and ion ratios. In this study, case sample data generated under stune identified all the main targeted analytes. However, as depicted in the database spreadsheets in Figures 7 and 8, many of the low-responding analytes were not detected when compared to the same data being generated under the atune or etune algorithms. The lack of detection of low-level components could fail to help substantiate the presence of other identified compounds detected in the chromatogram (for example, 4-ANPP, fluorofentanyl isomers, and phenethyl 4-ANPP).

All data analysis in this work used MassHunter Unknowns Analysis software with deconvolution and had an LMS cutoff ≥ 70. All the analytes detected in Figure 7 had an LMS ≥ 70 for the helium and hydrogen methods acquired under the atune criteria. Standards were not run to confirm the presence of medetomidine, fluorofentanyl isomers, or phenethyl 4-ANPP, but these analytes were similarly detected in many of the ~ 120 case samples analyzed under atune and etune algorithms with corresponding retention times. Different gain factors were used for each type of algorithm (see Table 2).

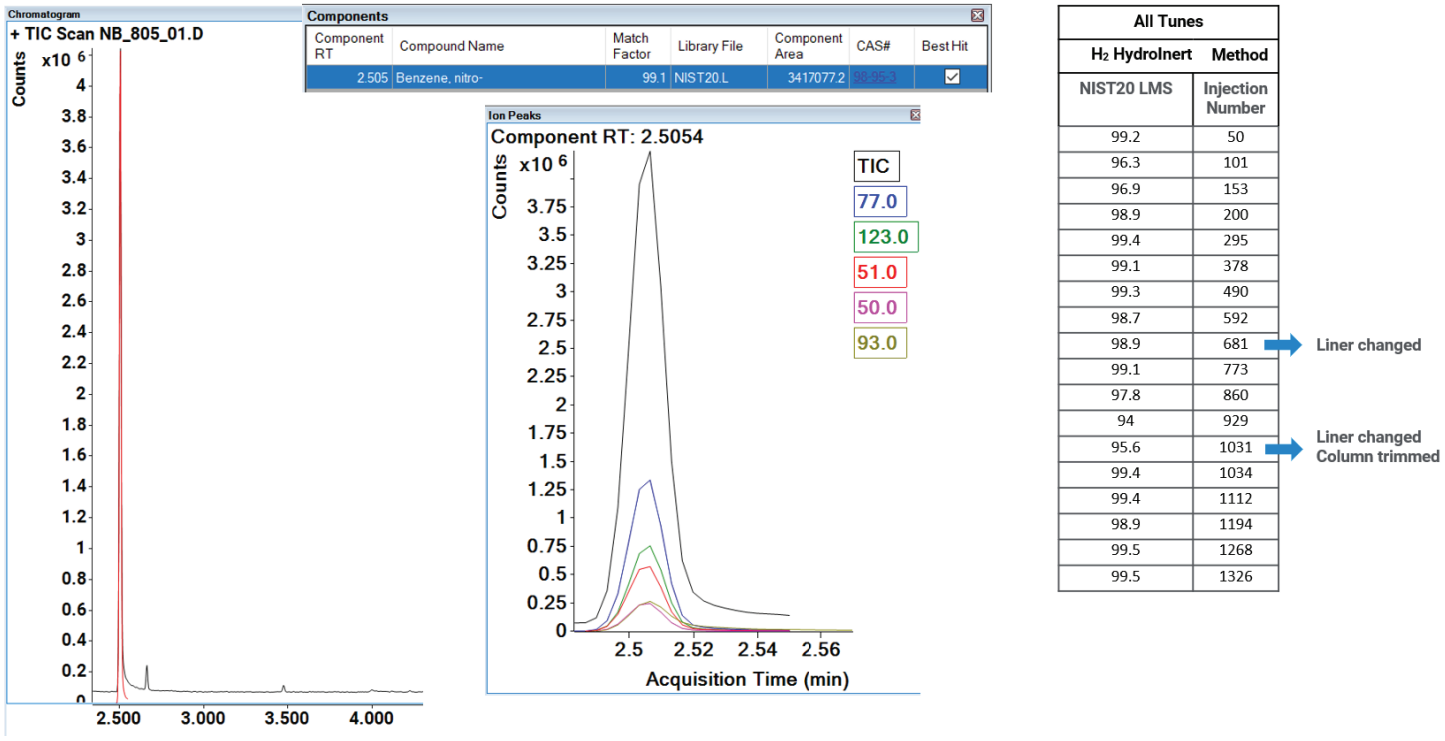
				Hydrogen Carrier Data Set										
Sample ID	Atune			Helium GC/MS Method		NIST20 LMS			SWGDRUG LMS					
		Compound RT	Internal Standard	NIST20 LMS	SWGRUG LMS	Injection 1	Injection 2	Injection 3	Injection 1	Injection 2	Injection 3	NIST20	SWGDRUG 3.8	
	Compounds		X=DH O=PA			Match (%)	Match (%)	Match (%)	Match (%)	Match (%)	Match (%)	Average	Average	Difference
DCM_14	Caffeine	7.363	X O	99.3	98.7	99.2	99.1	99.2	98.6	98.8	98.8	99.2	98.7	0.4
	Benadryl	7.514		94	93	92.6	91.9	89.9	91.8	90.4	88.2	91.5	90.1	1.3
	Medetomidine	7.793		85.7	88.4	81.8	66.4	64.4	85.6	73.9	71.3	70.9	76.9	-6.1
	Xylazine	7.991		99.1	98.4	99.2	98.9	98.9	98.9	98.6	98.5	99.0	98.7	0.3
	Cocaine	8.830		92.9	92.6	94.6	90.8	87.7	94.9	91.7	88.2	91.0	91.6	-0.6
	4-Anilino-N-Phenethylpiperidine	9.694		89.4	86.7	90.2	87.4	85.8	89.1	86.8	87.5	87.8	87.8	0.0
	Diacetylmorphine	10.213		81.5	80.3	86.2	77.8	76.5	86.2	79.5	77.7	80.2	81.1	-1.0
	Fluorofentanyl isomer	10.364		69.8	70.7	86.9	80	76.9	87.3	80.5	76.5	81.3	81.4	-0.2
	Fentanyl	10.476		94.4	93.2	93.8	95.3	93.5	92.8	95.3	92.6	94.2	93.6	0.6
	Quinine	10.758		79.5	81.8	81	70.9	74.3	78.3	74.4	74.7	75.4	75.8	-0.4
	Phenethyl 4-ANPP	11.943		NE	85.8	NE	NE	NE	85.8	74	75		78.3	

Figure 7. Extracted DCM case sample number 14 entry from data spreadsheet listing analytes detected and their respective LMS based on atune criteria. (NE = no entry.)

Sample ID	Stune			Hydrogen Carrier Data Set										
				Helium GC/MS Method		NIST20 LMS			SWGDRUG LMS					
		Compound RT	Internal Standard	NIST20 LMS	SWGRUG LMS	Injection 1	Injection 2	Injection 3	Injection 1	Injection 2	Injection 3	NIST20	SWGDRUG 3.8	
	Compounds		X=DH O=PA			Match (%)	Match (%)	Match (%)	Match (%)	Match (%)	Match (%)	Average	Average	Difference
DCM_14	Caffeine	7.367	X O	99.6	99.4	97.9	98	98.1	97.4	97.7	97.7	98.0	97.6	0.4
	Benadryl	7.516		96	96	80.9	77	79.2	77.9	80.3	77.2	79.0	78.5	0.6
	Medetomidine	7.793		71.2	74.6	ND	ND	ND	ND	ND	ND			
	Xylazine	7.994		98.4	98.8	95	93.9	96	95	93.9	95.9	95.0	94.9	0.0
	Cocaine	8.835		92.7	93.2	ND	ND	70.6	ND	ND	69.9	70.6	69.9	0.7
	4-Anilino-N-Phenethylpiperidine	9.698		85.5	85.7	70.2	ND	ND	70	ND	ND	70.2	70.0	0.2
	Diacetylmorphine			ND	ND	ND	ND	ND	ND	ND	ND			
	Fluorofentanyl isomer			ND	ND	ND	ND	ND	ND	ND	ND			
	Fentanyl	10.482		94.3	93.5	85.1	84.8	86.4	85	83.4	85.4	85.4	84.6	0.8
	Quinine	10.770		75.5	78.3	ND	ND	ND	ND	ND	ND			
	Phenethyl 4-ANPP			ND	ND	ND	ND	ND	ND	ND	ND			

Figure 8. Extracted DCM case sample number 14 entry from data spreadsheet listing analytes detected and their respective LMS based on stune criteria. (ND = not detected.)

When using the HydroInert source and running real-world street drug samples, the length of time that the deactivation lasts and whether it changes over time is unknown. To determine the short-term answer to these questions, nitrobenzene was used, as it is a known reactive compound under hydrogen carrier. Figure 9 illustrates the results of 18 nitrobenzene injections over the course of ~ 1,400 injections. Based on the LMS scores and a review of the spectral data, the HydroInert source deactivation appears to have remained stable throughout the study. The lowest LMS of 94 occurred immediately before the liner was changed and the entrance to the column was cut (~ 6 cm) due to the column test standard failing peak shape and response criteria following a liner change.



All Tunes	
H_2 HydroInert	Method
NIST20 LMS	Injection Number
99.2	50
96.3	101
96.9	153
98.9	200
99.4	295
99.1	378
99.3	490
98.7	592
98.9	681
99.1	773
97.8	860
94	929
95.6	1031
99.4	1034
99.4	1112
98.9	1194
99.5	1268
99.5	1326

Liner changed

Liner changed
Column trimmed

Figure 9. Nitrobenzene injections generated throughout ~ 1,300 injections with minimum LMS ≥ 94.

Spectral fidelity and analyte response comparison between helium and hydrogen

Tables 4 and 5 are populated with various analytes from DCM and methanolic extracts and were chosen randomly from the spreadsheets of the case samples database. To maintain a comparative approach during data acquisition, both tables represent analytes acquired under atune, a gain setting of 1, and integrated using MassHunter Unknowns Analysis software deconvolution and the Agile2 integrator. The largest LMS variation between NIST20 and SWGDRUG 3.8 libraries for the same compound using the Agilent search algorithm under helium and hydrogen methods was 2.4. The hydrogen method with the HydroInert source demonstrated equivalent and better LMS for most analytes than the standard helium method with inert extractor source. Compared to the loss of spectral fidelity discussed previously, when generated on an inert extractor hydrogen carrier method, the HydroInert source maintained or slightly improved spectral fidelity compared to spectral entries in the commercial NIST20 or publicly available SWGDRUG 3.8 libraries. Also, some analytes produced a similar signal response on the HydroInert source compared to the inert extractor helium method, while others responded two to three times more on the HydroInert source than the inert extractor source.

Table 4. Analyte LMS and responses under helium carrier gas method with 3 mm Agilent inert extractor source.

Analyte	NIST20 LMS	SWGDRUG 3.8 LMS	Component Area Counts
Meperidine	98.9	97.4	4,915,190
Methadone	95.2	92.9	5,384,547
Codeine	98.3	97.3	5,166,966
Hydrocodone	99.1	97.2	6,063,934
Oxycodone	98.5	96.9	5,248,754
4-ANPP	97.3	95.7	2,175,883
Cocaine	99.0	98.6	6,066,265
Cocaethylene	98.7	96.3	6,200,280
Benzoylecgonine	95.9	96.1	1,424,026
Ethylone	87.6	90.0	2,024,200
Buprenorphine (HCl)	98.7	97.6	11,668,192

Table 5. Analyte LMS and responses under hydrogen carrier gas method with 9 mm Agilent HydroInert source.

Analyte	NIST20 LMS	SWGDRUG 3.8 LMS	Component Area Counts
Meperidine	99.4	98.5	8,308,348
Methadone	96.2	93.8	8,772,967
Codeine	99.3	98.2	5,710,259
Hydrocodone	99.4	97.7	6,877,950
Oxycodone	98.5	97.6	4,282,091
4-ANPP	98	97	3,629,448
Cocaine	99.2	98.9	11,626,484
Cocaethylene	99.0	97.0	12,131,391
Benzoylecgonine	97.9	97.9	4,241,150
Ethylone	96.6	96.1	1,969,345
Buprenorphine (HCl)	99.0	97.4	12,633,590

Effect of chlorinated solvent on gas chromatography/mass spectrometry system

The possibility of forming HCl when using chlorinated solvents like DCM can be reduced by running inlet temperatures < 280 °C. In this study, the hydrogen method used a 260 °C set point for the inlet. Chromatography and spectral quality did not appear to be affected by running DCM as a solvent for many of the samples. However, near the end of the study, an interesting observation was made. At ~ 1,030 injections, the entrance of the column had to be trimmed to bring back chromatography, even after first replacing the liner. This is an unusual event based on the number of samples run on the column. Based on face-to-face discussions, many forensic drug chemistry analysts do not cut the entrance of the column for months, or until thousands of samples have passed through the inlet and column. This early maintenance task could be the result of combining a chlorinated solvent and hydrogen carrier gas into a hot metal injection port, producing HCl or other active compounds, but this could not be determined in this study. Liner replacement appears to be consistent with previous work regarding street drug samples being changed every ~ 600 injections depending on matrix and sample preparation, as illustrated in Figure 10.[7] Septa were changed every 300 to 350 injections using Agilent preholed inlet septa (part number 5183-4757).

Figure 10. Agilent UI splitless liner (part number 5190-5112) after ~ 700 injections of case samples consisting of DCM and methanol extracts. Agilent preholed inlet septum (part number 5183-4757) is shown with ~ 300 to 350 injections.

Injection mode differences (split/splitless)

Figure 11 shows codeine injected in split mode with a 20:1 split ratio into the inert extractor source (in the helium method), resulting in a single codeine peak. Figure 12 illustrates the splitless mode injection into the same system, resulting in additional background peaks and a morphinan alkaloid isomer found in opium poppy, but no additional codeine or opiate isomers. A pressure-pulsed splitless injection generated similar results. Figure 13 shows the 20:1 split injection on the HydroInert source (in the hydrogen method), producing an equivalent outcome to the helium method split mode result. However, the splitless injection of codeine under the HydroInert source produced a large hydrocodone peak (structural isomer of codeine), along with an increased morphinan alkaloid isomer peak, as seen in Figure 14. It is possible that codeine underwent an isomerization reaction to produce hydrocodone in the inlet; however, the purpose of this study was only to identify the differences between injection modes, rather than explore formation pathways. A pressure-pulsed splitless injection produced a likewise intense hydrocodone and morphinan isomer peak. Injecting morphine in splitless mode into the HydroInert source produced hydromorphone (a structural isomer of morphine), which could have been produced via a hydrogenation reaction. Inlet parameters were not optimized to determine specific inlet parameter settings to gauge the point at which reactions/formations occur in splitless mode. Moreover, not all split flow settings were investigated and/or optimized. The analyst needs to be aware of the possibilities of inlet reactions as well as source reactions when developing and validating a drug method using hydrogen carrier gas.

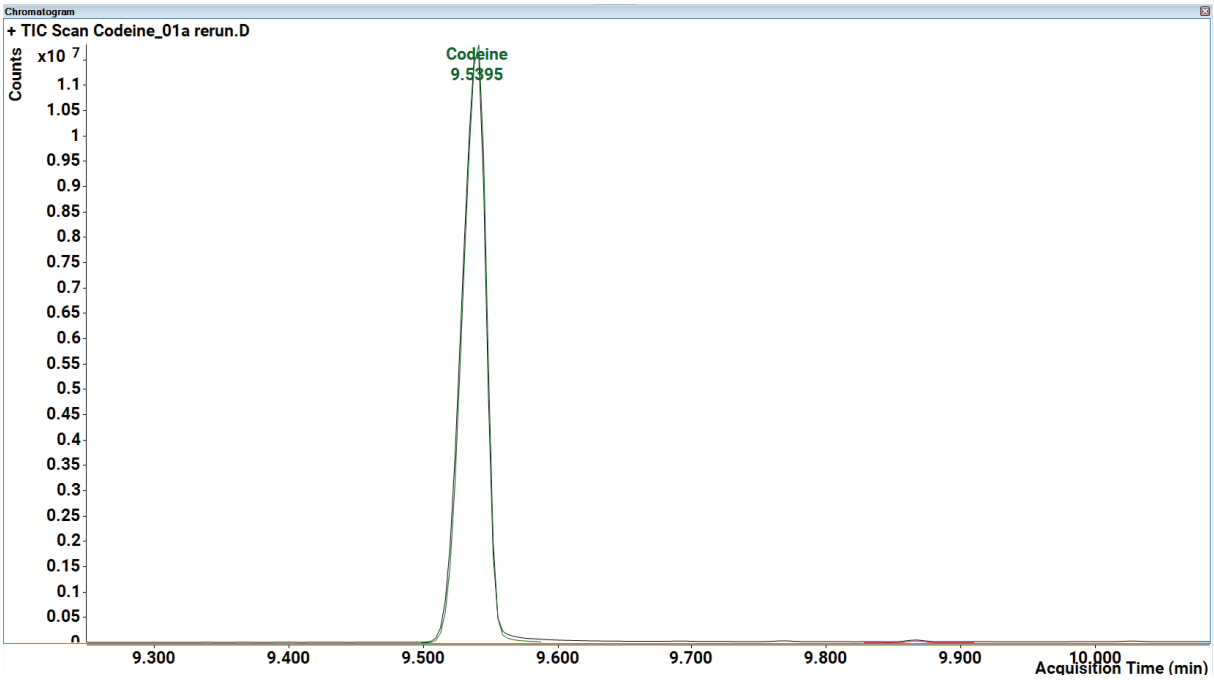


Figure 11. Split injection (20:1) of 1 mg/mL codeine standard (50 µg on column) in the helium method with the Agilent inert extractor source.

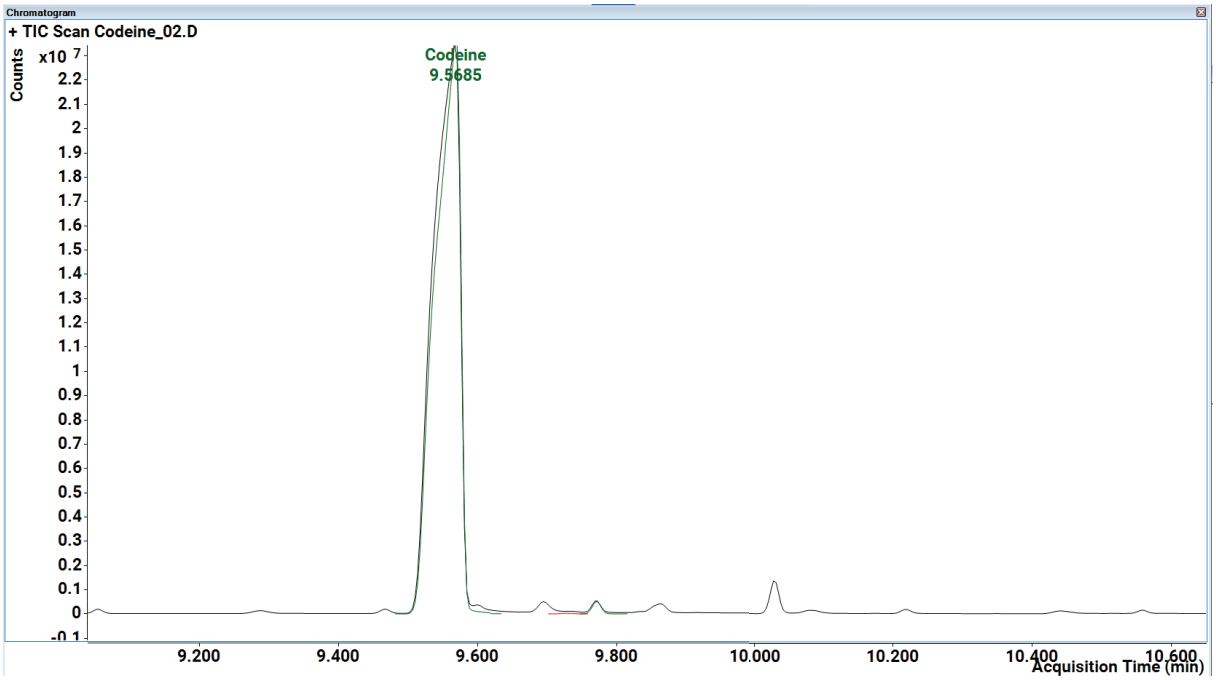


Figure 12. Splitless injection of 1 mg/mL codeine standard (~ 1,000 µg on column) in the helium method with the Agilent inert extractor source.

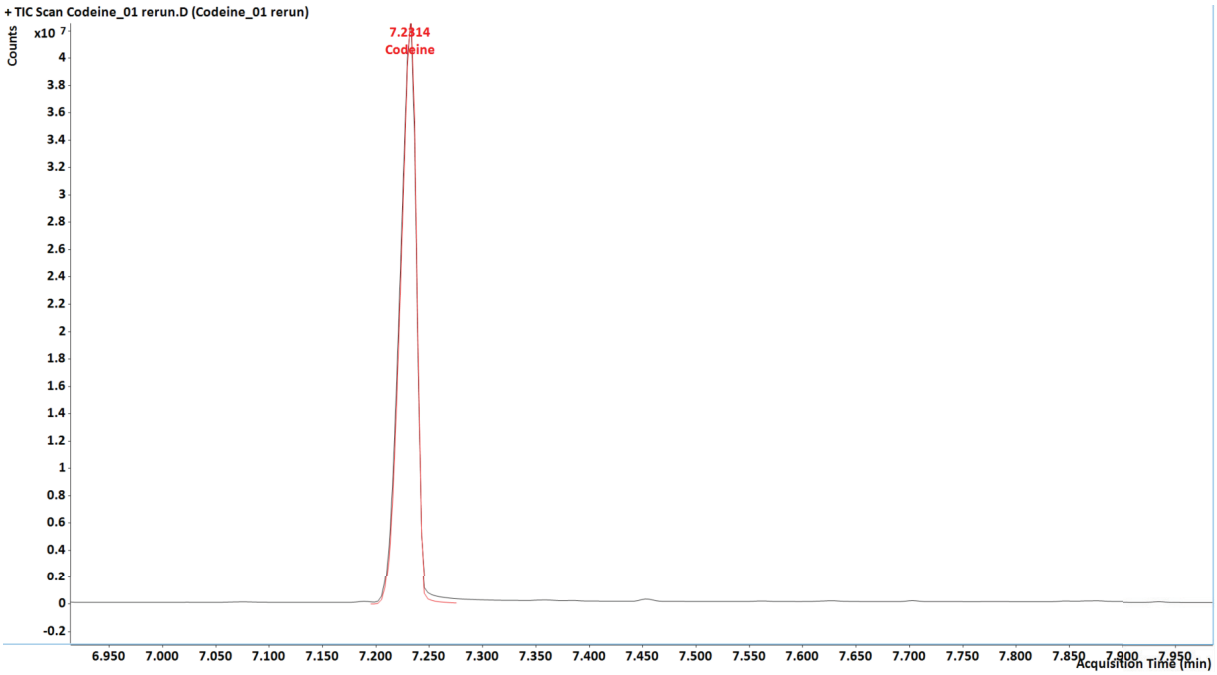


Figure 13. Split injection (20:1) of 1 mg/mL codeine standard (50 µg on column) in the hydrogen method with the Agilent HydroInert source.

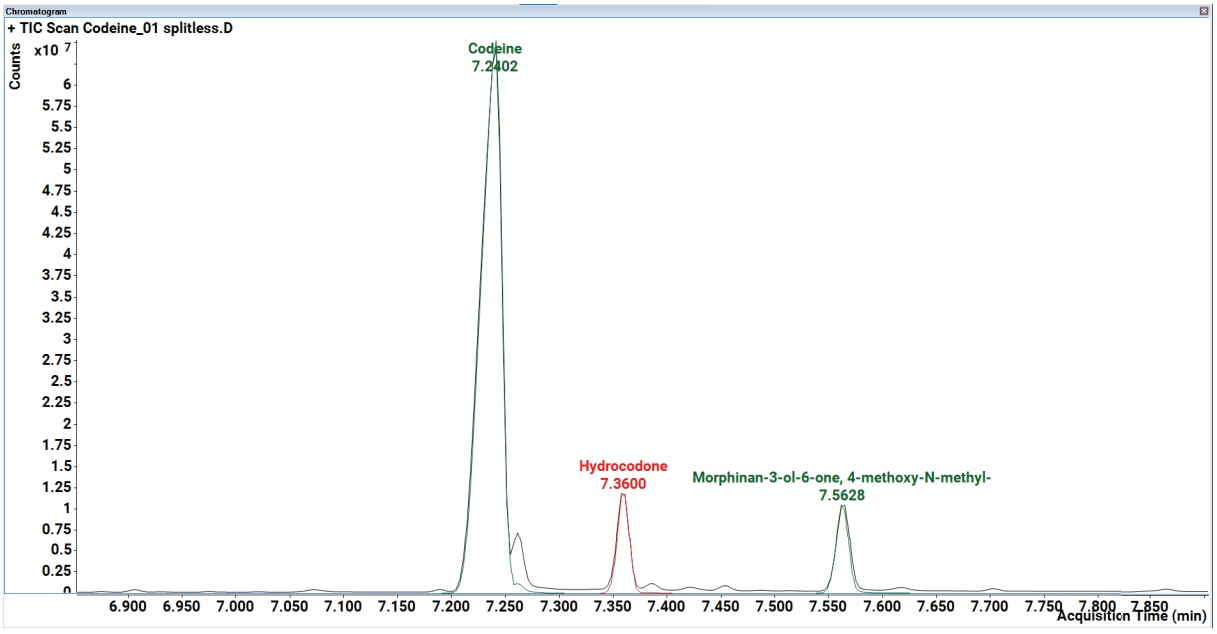


Figure 14. Splitless injection of 1 mg/mL codeine standard (~ 1,000 µg on column) in the hydrogen method with the Agilent HydroInert source.

Conclusion

Although helium is the preferred choice of carrier gas, this study demonstrates that hydrogen can be considered as carrier gas if using the Agilent HydroInert source. The HydroInert source was shown to increase spectral fidelity for many compounds and produce similar LMS when compared to a helium carrier gas method using the Agilent inert extractor system. The HydroInert source maintained its deactivated surface throughout the study and delivered excellent spectral quality for the very reactive compound nitrobenzene. Dichloromethane and methanol solvents did not seem to negatively affect chromatography or the stability of the overall chromatographic system; however, cutting ~ 6 cm off the column entrance after ~ 1,000 injections illustrates the emergence of reactions upon sample introduction sooner than anticipated in most helium GC/MS system environments. This study demonstrates the best overall practices including specific MS source considerations and acquisition parameters necessary to make the transition to hydrogen carrier gas more successful. When analyzing any true unknown, it is highly recommended that a secondary orthogonal technique with hydrogen-generated GC/MS data is used. Hydrogen carrier gas always comes with the risk of reacting with the solvent, sample matrix, and any analytes contained in the sample to produce unconventional spectra or interact with the chromatographic system. Time needs to be allotted to adjust to these challenges when developing a hydrogen GC/MS method through the validation process.

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