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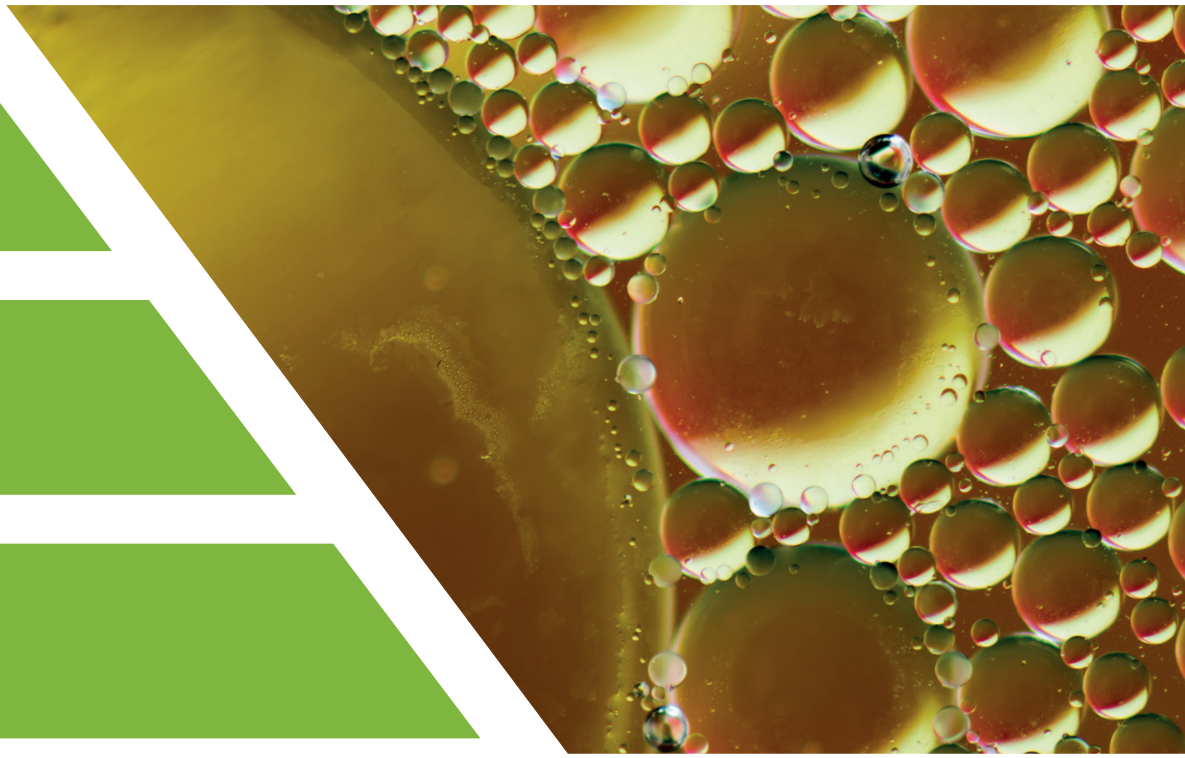

The Effect of Draw-out Lens Diameter on Sensitivity of **GC-MS analysis**

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Abstract

Increases in helium price combined with temporary shortages of the gas have prompted many GC users to look to alternative carrier gases for their GC and GC-MS analysis. Hydrogen is a cost-effective, viable alternative to helium with potential for improved chromatography and decreased analysis time, however there are concerns about use of hydrogen carrier gas for GC-MS because of some sensitivity effects when using hydrogen in place of helium.

This study investigated the effect of hydrogen and helium carrier gas across a range of column flow rates (1.0 – 2.0 cc/min), with different draw-out lenses (3mm vs 6mm) and selected ion monitoring (SIM) vs full scan detection for essential oil and volatile analysis.

Results showed that flow rate did affect resolution and signal-to-noise ratios, with results corresponding to the theoretical changes in carrier gas efficiency according to the van Deemter equation. The 6mm draw-out plate orifice used for hydrogen carrier gas did increase sensitivity compared to helium carrier gas with the standard 3mm draw-out plate, especially at higher flow rates. SIM detection, in combination with flow rate, also improved sensitivity, with hydrogen carrier gas eliciting a similar response to helium carrier gas when using SIM detection at higher flow rates.

This study demonstrates that simple adjustments to the GC-MS system can enable analysts to mitigate some of the negative effects that hydrogen can have on GC-MS detection and obtain like-for-like results with helium.

Introduction

Gas Chromatography (GC) is a well-established analytical technique used in industries including food and fragrance, petrochemical analysis and environmental analysis to name but a few and allows isolation and analysis of individual analytes within a complex mixture. GC coupled to mass spectrometry (GC-MS) results in a highly powerful and versatile hyphenated technique, which is typically used to identify compounds ranging from 40-500 m/z. Over the past few decades, helium has been the first choice carrier gas, owing to its inertness, good performance at reasonable carrier velocities and relatively cheap price.

Since 2001, helium has become increasingly expensive with a reported global increase in price of 500% between 2001 and 2016 (Fig 1 & 2).

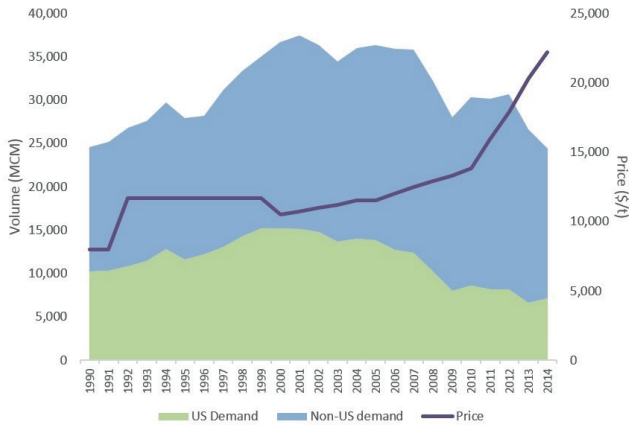

Figure 1:

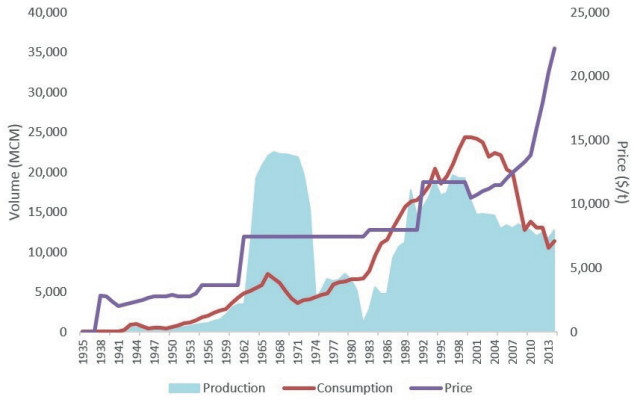

Figure 2:

In 2012-2013 the global helium shortage increased the number of GC users looking to switch to alternative carrier gases and improved availability of information on what is required to optimally switch to an alternative carrier gas. With many more people looking to switch methods to hydrogen, debate increased regarding the potential pitfalls of changing carrier gas, with a number of analysts unable to easily make the transition from helium to hydrogen for certain methods.

GC manufacturers also improved information regarding use of alternative carrier gases as well as developing new methods to reduce helium consumption such as redesigning the GC inlet (1) and developing automatic systems that switch to nitrogen when the GC is idle (2).

The vast increase in the price of helium corresponds

with a decline in US helium consumption since 2009 (Fig 1). Worldwide, however, consumption continues to rise, meaning that alternatives to helium, where feasible, are going to be sought as a way of reducing costs.

Hydrogen safety

Hydrogen is a reactive and explosive gas and has a lower explosion limit (LEL) of 4.1% in atmosphere. A small lab measuring 5m x 4m x 2.5m would require 2050L hydrogen to reach the LEL. Therefore, a large leak from a hydrogen cylinder containing around 9000 L at atmospheric pressure could easily reach the LEL in such a lab. A hydrogen generator producing 500cc/min would take almost 3 days to produce enough hydrogen to reach the LEL and the lab would need to be hermetically sealed. A hydrogen generator therefore provides enough gas to carry out analysis, but without the safety concerns associated with cylinders.

Improving Chromatography

Hydrogen is half as viscous as helium at the same temperature and pressure, while the diffusion of a sample within the two gases is similar, meaning that hydrogen travels through the GC column more quickly and offers faster analysis than helium. The Van Deemter curve (Figure 3) shows the relative efficiencies of hydrogen, helium and nitrogen at different flow rates and shows how hydrogen has superior column efficiency at higher flow rates. Using method translation software (3, 4), it is possible to model the effect of converting a method from helium to hydrogen in silico to see what time savings can be made and what changes to the method are required.

Some chromatographers are concerned about the time taken for the system to stabilize after switching carrier gas and the potential reduction in sensitivity sometimes reported when using hydrogen carrier gas. One hardware change that can be made to improve sensitivity when using hydrogen carrier gas is to increase the orifice size of the ion volume of the ion source.

Analysis of the essential oil mixture run using helium with a standard ion volume and hydrogen carrier gas using a larger diameter ion volume across a range of flow rates, stabilization time, peak resolution and signal to noise ratio were assessed.

Materials & Methods

Reagents and materials

Rosewood Essential oils extracts were provided by Prof. Lauro E. S. Barata, from UFOPA (Universidade Estadual do Oeste do Pará).

A volatile organic mixture was purchased from Sulpelco (EPA VOC Mix 2).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The ion source was baked-out using a slight modification of recommendations (p35-37) by Agilent technologies (5), with the source temperature set to 300°C and filament switched on for a period of 3 hours. To check stability of baseline, blank samples were run to ensure consistent background before any samples were analysed.

For Rosewood essential oil analysis, the temperature started at 60°C, increasing at a rate of 3°C/min to a maximum temperature of 210°C (total run time 50 minutes). A split ratio of 100:1 was used for essential oil injection.

All essential oil analysis was carried out using an Agilent Technologies GC-MS (Santa Clara, CA, USA), consisting of a 7890B GC with 5975 mass selective detector, equipped with an HP-5 capillary fused column (30m x 250 Qm I.D. x 0.25 Qm film thickness, 5% phenyl methyl siloxane).

For all analyses, the injector temperature was 270°C. Mass spectra were taken at 70eV ionisation energy in either full scan mode (40-500 amu) or selected ion monitoring mode (SIM) with a dwell time of 15 ms (Table 1). Helium carrier gas used was 99.9995% purity. Hydrogen carrier gas was 99.9999% pure and was supplied by a Peak Scientific (Inchinnan, UK) Precision Hydrogen Trace 500cc gas generator. Both carrier gases were used across a range of flow rates from 1.0 – 2.0 mL/min.

Analyses of samples run using helium carrier gas were acquired using the 5975 MSD with an inert 3mm Draw-out plate (G2589-20100). All samples run using hydrogen carrier gas were acquired using an inert 6mm Draw-out plate (G2589-20045). All other MSD conditions were identical.

Background stabilization was assessed by running a volatile organic mixture (EPA VOC Mix 2) for 7 days following change of carrier gas. Analysis of this mixture used the same GC-MS settings as essential oil analysis, except for the oven program which was as follows: 60°C, increasing at a rate of 10°C/min to a maximum temperature of 138°C (total run time 7.8 minutes).

Data Analysis

Data analysis was conducted using Agilent Masshunter qualitative software (B.07.00) and Enhanced Chemstation (E.02.011177) was used for data collection.

Results

The essential oil mixture was injected at three column flow rates; 1.0, 1.5 and 2.0 mLmin-1 to look at optimal vs. sub-optimal flow rates of both helium and hydrogen. The last compound in the mixture to elute, 1,3,5-trichloro benzene, was used to calculate the signal to noise ratio and resolution. All samples run using helium carrier gas used the 3mm draw-out lens, whereas all samples run using hydrogen carrier gas used the 6mm draw-out lens.

Effect of carrier gas on signal to noise

Signal to noise (S/N) and resolution (Rs) were calculated using 1,3,5-trichloro benzene, the last eluting peak of an essential oil mixture (Table 2). When running samples using helium carrier gas, an inverse relationship between carrier gas flow rate and signal to noise and resolution was found in full scan mode, with resolution dropping from 1988.3 at the optimal 1.0 mLmin-1 flow rate of helium to 864.9 at 2.0 mLmin-1 (Table 2). When running the sample using hydrogen carrier gas, the opposite relationship between carrier gas flow rate and S/N of the 1,3,5-trichloro benzene peak, with S/N increasing from 106.0 to 209.6 as column flow was increased (Table 2).

When running the same samples in SIM mode, there was little variation in S/N across flow rates when using helium carrier gas, with a change from 2690.4 to 2381.0 being seen when running samples at 1.0mLmin-1 and 2.0mLmin-1 respectively. However, when using hydrogen carrier gas, S/N improved greatly with carrier flow increase, with the S/N increasing from 798.8 at 1.0 mLmin-1 to 2359.3 at 2.0 mLmin-1, meaning that S/N results with hydrogen at higher flow rates were almost the same as results with helium.

These results appear to correspond to the relative efficiencies of helium and hydrogen at different linear velocities, with helium operating at 36.6 cms-1 at 1mL/min column flow, which is around its optimum, and corresponds to a 2.0 mLmin-1 column flow of hydrogen using the same column. When looking at the S/N results at the optimal flows for helium and hydrogen, in full scan mode, helium clearly gives better results with S/N 9.48 times higher than hydrogen. In SIM mode, however, S/N of 1,3,5-trichloro benzene using helium carrier gas is just 1.14 times higher than hydrogen, showing very similar performance.

Effect of carrier gas on resolution

Resolution of peaks was calculated using the following equation:

$$2[(t_R)_A - (t_R)_B]/(W_A + W_B)$$

t_R = Retention time; W = Peak width; A = Peak A; B = Peak B

In full scan mode, helium carrier gas followed a similar pattern to that seen regarding S/N, with resolution of the 1,3,5-trichloro benzene peak decreasing as carrier gas flow rate increased beyond the optimal velocity. When running samples using hydrogen, there was no clear relationship between peak resolution and flow rate, with the best resolution seen at the intermediate carrier flow of 1.5 mLmin-1. When comparing the optimal flow rates of each gas (1.0 - He and 2.0 - H2), peak resolution with helium carrier gas was almost double (1.9x) that of hydrogen (Table 2).

In SIM mode, peak resolution when using helium decreased relative to full scan resolution and was lower than peak resolution seen in hydrogen carrier gas (Table 2). Hydrogen resolution was vastly improved in SIM mode compared with Scan mode (1.9x) and at optimal flow rates, hydrogen gave improved resolution (1.76x) compared to helium.

Background stabilization

Results showed that the background was stable after 3 days, with repeated injections of the EPA VOC mixture being tested for 7 days (Fig 3).

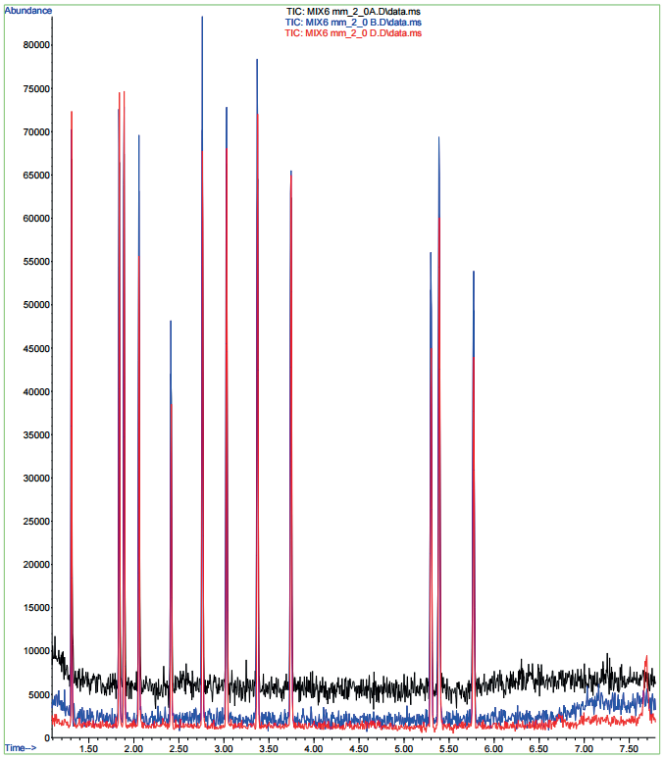

Figure 3:

Discussion

A number of applications now use hydrogen carrier gas as a viable alternative to helium in a range of analyses such as detailed hydrocarbon analysis, semi volatile analysis, blood alcohol content analysis and simulated distillation.

The van Deemter curve is key to understanding the effect of carrier gas velocity on efficiency. Helium and hydrogen have contrasting optimal gas velocities, with helium's optimum flow rate at around 35 cms-1 and hydrogen's optimum flow rate being around 50 cms-1.

The results of GC-MS performance when using helium and hydrogen carrier gas, comparing resolution and signal to noise, appear to correspond directly to the carrier gas flow rate relative to the optimal carrier gas velocity of each gas. At a column flow of 1 mLmin-1 helium, the average linear velocity is 36.6 cms-1, which is close to its optimum velocity. At this helium column flow, the best performance for both resolution and S/N were observed in full scan mode. SIM detection appeared to overcome some of the problems of reduced carrier gas efficiency of helium at higher velocities (44.8 cms-1 & 51.8 cms-1), with little difference found in either resolution or S/N across the range of flow rates tested.

Hydrogen has an optimum velocity of around 50 cms-1, which corresponds to the column flow of 2.0 mLmin-1. Similarly to helium, running samples at a sub-optimal flow rate affected resolution and S/N significantly in full scan mode, but in the case of hydrogen, also in SIM mode. Interestingly, in SIM mode, hydrogen carrier gas gave better peak resolution than helium. It appears that running in SIM mode largely eliminates background noise that can cause interference in full scan mode when using hydrogen. Even when following the recommendations for preparation of the system when switching to hydrogen, background signal will take at least three days to stabilise.

These results clearly demonstrate that hydrogen can be used for routine analysis of known compounds. When using full scan mode, analysts need to be aware that they are likely to see a 2-5 fold reduction in sensitivity. When using hydrogen carrier gas for GC-MS, it is essential to initially focus on reduction of factors which cause increased noise/background.

m/z	Dwell (ms)	m/z	Dwell (ms)	m/z	Dwell (ms)
53.0	15	59.0	15	68.0	15
80.0	15	93.0	15	105.0	15
107.0	15	108.0	15	111.0	15
119.0	15	120.0	15	121.0	15
133.0	15	136.0	15	139.0	15
147.0	15	154.0	15	155.0	15
161.0	15	175.0	15	187.0	15
189.0	15	204.0	15	205.0	15
212.0	15	220.0	15		

Table 1: SIM ions and dwell time for SIM detection.

Helium				
Flow rate / ml min^{-1}		Linear Velocity	Full Scan	SIM
RS	1.0	36.6	4.94	2.84
	1.5	44.8	4.32	2.91
	2.0	51.8	3.20	2.75
S/N	1.0	36.6	1988.3	2690.4
	1.5	44.8	1356.6	2818.6
	2.0	51.8	864.9	2381.0

Hydrogen				
Flow rate / ml min^{-1}		Linear Velocity	Full Scan	SIM
RS	1.0	54.4	2.96	3.20
	1.5	66.6	3.25	4.70
	2.0	76.9	2.60	5.00
S/N	1.0	54.4	106.0	798.8
	1.5	66.6	180.6	1502.8
	2.0	76.9	209.6	2359.3

Table 2: Results of resolution and signal to noise effects of helium and hydrogen carrier gas. Helium samples were detected with the 3mm draw-out plate, hydrogen samples were detected using the 6mm draw-out plate.

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