Application Note

Food and Beverage Testing

Flavor and Fragrance GC/MS Analysis with Hydrogen Carrier Gas and the Agilent HydroInert Source

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Abstract

Flavors and fragrances are typically complex homogeneous mixtures used in a wide variety of consumer goods to produce smells and tastes. These components can be either natural or artificial and can be found in both food and non-food products. They consist of up to several hundred components with similar structures and chemical characteristics. High-efficiency techniques are commonly used for flavor and fragrance (F&F) analysis, such as gas chromatography/mass spectrometry (GC/MS).

Due to ongoing challenges with helium gas supply, laboratories around the world are trying to reduce their helium dependence by using different carrier gases while maintaining the quality of chromatographic results. Therefore, hydrogen is becoming the go-to carrier gas in GC/MS, due to its higher availability and the possibility to use hydrogen generators for its production. Hydrogen is also the best alternative to helium for GC applications, as it offers potential advantages in terms of chromatographic speed and resolution. However, hydrogen is not an inert gas like helium, and it may cause undesirable chemical reactions in the mass spectrometer electron ionization (EI) source. These reactions can lead to disturbed ion ratios in the mass spectrum, spectral infidelity, low library matching scores, peak tailing, and nonlinear calibration for some analytes. Therefore, a novel EI source for GC/MS and GC/MS/MS was developed and optimized for use with hydrogen as the carrier gas. The new source, the Agilent HydroInert source, was used in the system evaluated here for analyzing F&F mixtures.

Introduction

Identification of F&F mixtures is usually performed by GC/MS through a combination of chromatographic data and mass spectra. Therefore, good quality spectra that can properly match a library are critical for identifying the compounds of interest. Library spectra are most commonly acquired using helium as the carrier gas. Therefore, when using hydrogen as the carrier gas the match can be drastically affected for the compounds susceptible to the undesirable in-source chemical reactions.

The HydroInert source is a novel source based on the Agilent extractor source design. It is the source of choice when using hydrogen as the carrier gas, enabling performance similar to that observed with the extractor source. The advantages of the HydroInert source include minimized spectral distortion, improved sensitivity, and a superior high-boiler peak shape.

Experimental

Chemicals and reagents

A chemical mix containing different compounds, an orange essential oil, and a lemon essential oil were kindly provided by SACMAR S.R.L, Via Keplero 7, 20019 Settimo Milanese (MI), Italy.

Instrument and methods

It is important to recognize the differences with using hydrogen carrier. The Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion guide[5] provides detailed instructions for method conversion from helium to hydrogen carrier gas. The user guide outlines considerations and procedures for hydrogen safety necessary to make the transition to hydrogen carrier gas successful.

An Agilent 8890 GC system included:

- Split/splitless inlet
- Agilent Ultra Inert Low Pressure Drop Liner (part number 5190-2295)
- Agilent J&W DB-WAXetr, 30 m × 250 µm × 0.25 µm (part number 122-7332)

An Agilent 5977B GC/MSD included:

- HydroInert Source (part number G7078-67930 for 5977 GC/MSD)
- Extractor lens, 9 mm (standard with the HydroInert source)

Table 1. GC/MS method.

Agilent 8890 GC System			
Oven	°C/min	Hold (°C)	Hold (min)
		45	4
	8	220	12
	8	230	4
	Run time: 43.125 min		
Inlet (Split/Splitless)			
Liner	Ultra Inert Low Pressure Drop Liner (p/n 5190-2295)		
Temperature	210 °C		
Mode	Split		
Split Ratio	25:1		
Column			
Column	J&W DB-WAXetr, 30 m × 250 µm × 0.25 µm (p/n 122-7332)		
Mode	Constant flow		
Column Flow Settings	1.4 mL/min		
Agilent 5977B GC/MSD			
Source	HydroInert		
Acquisition Mode	Scan		
Tune	Etune		
Gain Factor	1		
Low Mass	40		
High Mass	250		
A/D Samples	4		
Threshold	150		
Source Temperature	280 °C		
Quad Temperature	150 °C		

Results and discussion

Peak shape

Hydrogen is probably the best alternative carrier gas for gas chromatography, allowing for the highest resolution when operating at the optimal linear velocity. However, when used with an MS detector, it can lead to pronounced peak tailing and spectral changes, making identification based on spectral matching problematic and affecting quantitation. Figure 1 shows the chromatograms of a mix of F&F compounds with different chemical characteristics acquired with the standard extractor source equipped with the HydroInert source (A) and a 3 mm extractor lens (B). Peak shape for all compounds, especially the later-eluting ones, is dramatically improved when using the HydroInert source. For example, the TIC peak corresponding to maltol is shown in Figure 2A. Tailing is substantially reduced when using th HydroInert source (top in green). Figure 2B shows excellent library matching of the convoluted spectrum of maltol on the top and the library spectrum as the mirror plot on the bottom. It is of note that the original method with helium as the carrier gas lasted 82 minutes. Translating the method to hydrogen enabled a factor-of-two speed gain, resulting in the analysis time of 41 minutes, while maintaining chromatographic resolution.

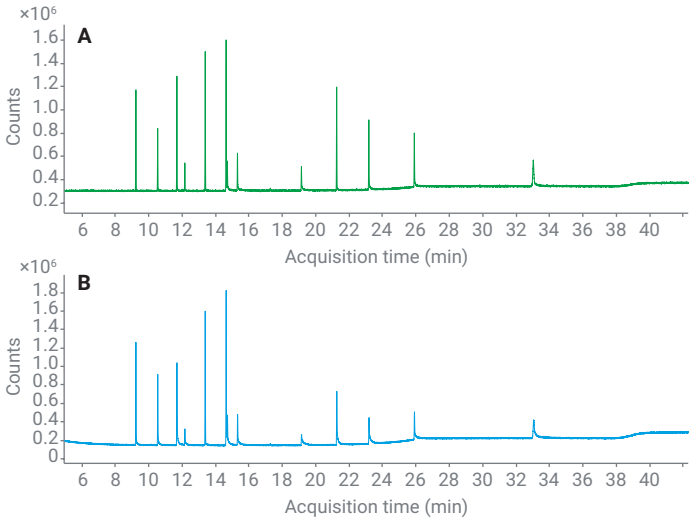

Figure 1. Noticeable improvement in the peak shape for F&F mixture with the Agilent HydroInert source (A) compared to the standard extractor source (3 mm) (B) with hydrogen carrier gas.

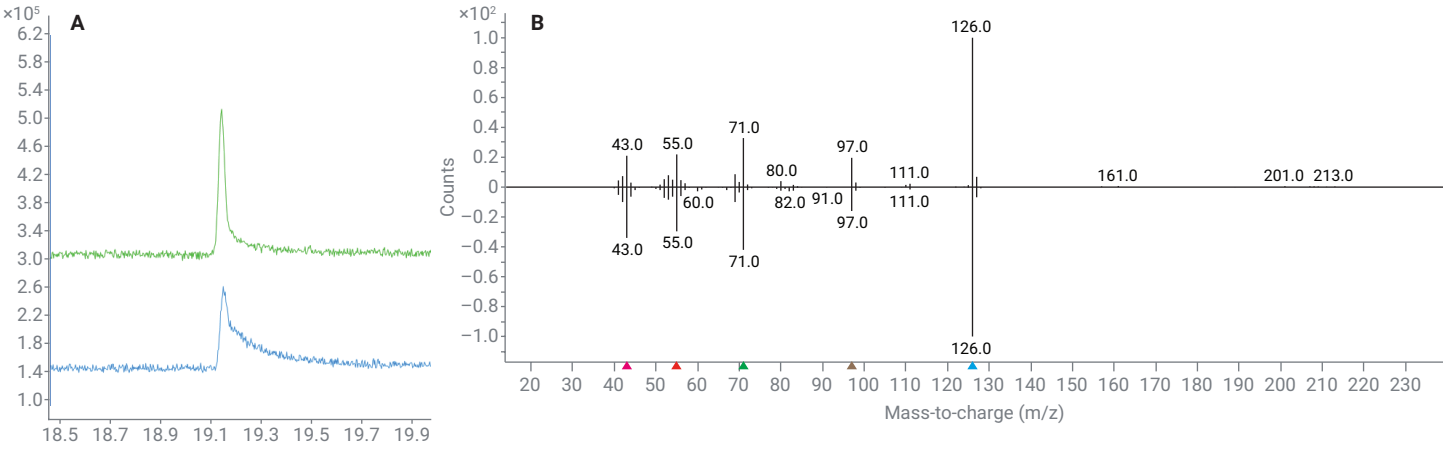

Figure 2. Maltol peak shape with the Agilent HydroInert source (9 mm, top) compared to a standard source (3 mm, bottom) acquired with hydrogen carrier gas (A). Deconvoluted mass spectrum of maltol acquired with the Agilent HydroInert source and the mirror plot of the refence library spectrum (B) demonstrating excellent library matching.

Library matching

The use of hydrogen as the carrier gas in MS can highly affect the spectra of some compounds, negatively impacting the library match score. Table 2 summarizes the library match scores observed for the F&F compounds with hydrogen carrier gas from the mixture, which are shown in the chromatogram in Figure 1. Agilent MassHunter Unknowns Analysis software was used for the identification of the 13 compounds present in the mix through spectral deconvolution. The Wiley Mass Spectra of Flavors and Fragrances of Natural and Synthetic Compounds library was used for compound identification based on spectral matching.

Table 2. Match score comparison between a standard source (equipped with a standard 3 mm lens) and the Agilent HydroInert source using hydrogen carrier gas. Highlighted in green are the values where the HydroInert source gives a better result.

Retention Time	Compound Name	Match Factor Standard Source	Match Factor HydroInert Source
9.23	Hex-(3Z)-enyl acetate	93.73	96.20
10.54	Hex-(3Z)-enol	96.88	96.68
11.69	Menthone	89.04	95.46
12.17	Isomenthone	83.65	96.06
13.38	Menthyl acetate	88.59	96.77
14.64	Menthol	90.89	97.70
14.71	Butyric acid	96.84	95.34
15.32	Butyric acid (2-methyl-)	96.08	94.26
19.15	Maltol	87.52	89.90
21.26	Decalactone (gamma-)	86.81	96.65
23.19	Sulfurol	90.25	97.15
25.91	Vanillin	93.98	95.91
33.04	Raspberry ketone	90.60	94.21

Overall, the HydroInert Source gives a substantially better match score. For the rest of the compounds that do not undergo undesirable interaction with hydrogen, the library match scores are comparable.

Real-world sample analysis: orange and lemon essential oils

Orange and lemon essential oils were analyzed under the same conditions as the standard mixture with hydrogen as the carrier gas (chromatograms are shown in Figure 3). Unknowns Analysis was used for compound identification (Table 3). The Unknowns Analysis data processing method was set for a minimum match score of 75 as the compound identification threshold. For this reason, compounds with lower library match scores did not appear in the table with the identified components.

Unknowns Analysis identified 42 compound hits for the orange essential oil and 54 for the lemon oil. Most of the identified compounds had a match score above 80 with hydrogen as the carrier gas (Table 3).

Figure 4 presents excellent peak shape for geranial dimethyl acetal even at lower concentrations, as well as a good spectral match with the library match score of 88.2.

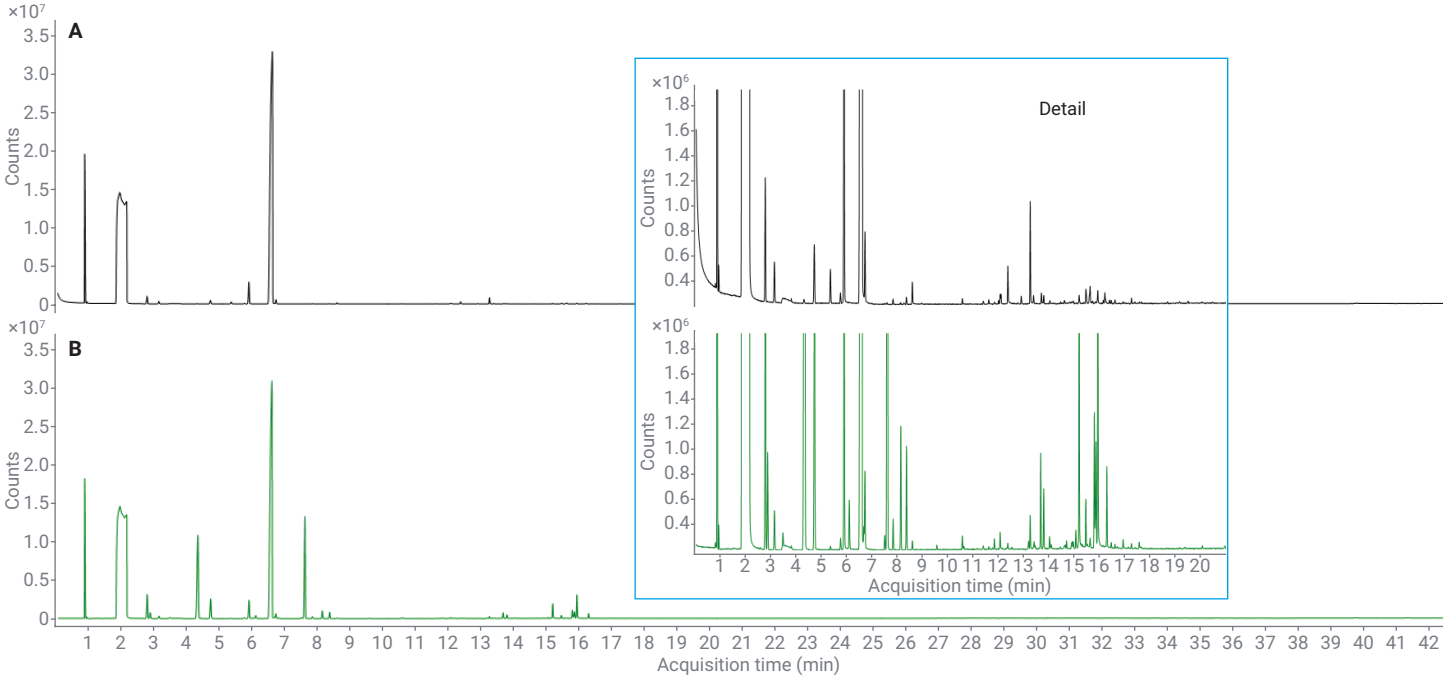

Figure 3. Total ion chromatogram of the real-world essential oil samples. Orange (A) and lemon essential oils (B) were analyzed with hydrogen carrier gas using the Agilent HydroInert source.

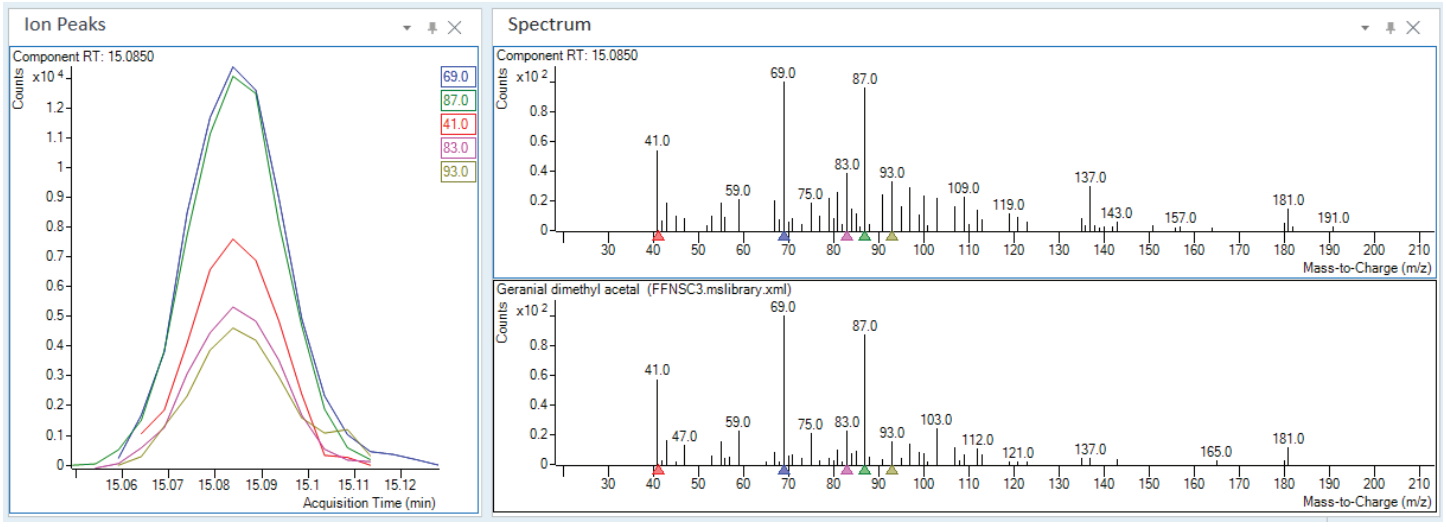

Figure 4. Peak shape, deconvoluted mass spectrum (top right), and the library spectrum (bottom right) for geranial dimethyl acetal detected in lemon essential oil.

Table 3. Compounds identified in the real-world essential oil samples using Agilent MassHunter Unknowns Analysis based on spectral deconvolution.

Orange		
RT	**Compound Name**	**Match Factor**
2.82	Pinene (alpha-)	98.32
3.18	Toluene	96.24
4.35	Sabinene	84.38
4.35	Pinene (beta-)	78.25
4.76	Phellandrene (beta-)	97.67
5.39	Carene (delta-3-)	97.39
5.79	Phellandrene (alpha-)	91.58
5.93	Myrcene	97.81
6.65	Limonene	97.73
6.76	Terpinene (gamma-)	94.82
7.87	Ocimene ((E)-, beta-)	83.73
8.40	Terpinene (alpha-)	88.37
8.63	Octanal (*n*-)	97.39
10.60	Nonanal (*n*-)	93.09
11.43	Pinene oxide (alpha-)	77.89
12.04	Acetate (octyl-)	90.95
12.10	Citronellal	90.73
12.13	Cubebene (alpha-)	81.72
12.40	Decanal (*n*-)	97.00
12.93	Muurola-4(14),5-diene (*cis*-)	85.46
13.28	Linalyl anthranilate	95.74
13.42	Octanol (*n*-)	93.27
13.73	Copaene (beta-)	90.26
13.82	Caryophyllene (E)-	92.36
14.06	Phytol acetate	77.26
15.22	Neral	85.76
15.48	Terpineol (alpha-)	90.36
15.62	Dodecanal (*n*-)	87.12
15.95	Geranial	88.76
16.23	Cadinene (delta-)	91.30
16.42	Hexanol (2-ethyl-)	78.15
16.49	Citronellyl formate	84.67
22.20	Sinensal (beta-)	84.11
23.33	Sinensal (alpha-)	75.25

Lemon		
RT	**Compound Name**	**Match Factor**
2.82	Pinene (alpha-)	98.34
2.92	Thujene (alpha-)	98.36
3.18	Toluene	97.33
3.52	2,2-Dimethyl-5-methylene norbornane	97.24
4.37	Pinene (beta-)	97.75
4.76	Sabinene	98.12
5.40	Carene (delta-3-)	84.34
5.80	Phellandrene (alpha-)	89.98
5.94	Myrcene	97.76
6.14	Terpinene (alpha-)	97.07
6.63	Limonene	97.86
6.71	Eucalyptol	86.44
6.76	Terpinene (gamma-)	93.51
7.54	Ocimene ((Z)-, beta-)	93.28
7.64	3-Methylapopinene	96.25
7.87	Ocimene ((E)-, beta-)	97.71
8.17	Cymene (*para*-)	98.16
8.40	Terpinolene	97.28
8.63	Octanal (*n*-)	94.84
9.60	Hept-5-en-2-one (6-methyl-)	91.48
10.60	Nonanal (*n*-)	95.38
11.43	Limonene oxide (*cis*-)	80.38
11.87	Sabinene hydrate (*trans*-)	91.53
12.10	Citronellal	94.30
12.40	Decanal (*n*-)	93.57
13.22	Menth-2-en-1-ol (*cis*-, *para*-)	80.72
13.28	Linalyl anthranilate	95.21
13.44	Bergamotene (alpha-, *trans*-)	86.10
13.70	Bergamotene (alpha-, *cis*-)	95.58
13.82	Caryophyllene ((E)-)	94.34
14.05	Terpinen-4-ol	86.16
14.12	Farnesene ((E)-, beta-)	80.23
14.72	Citral diethyl acetal	82.06
14.94	Citronellyl acetate	88.08
14.99	Farnesene ((Z)-, beta-)	78.05
15.09	Geranial dimethyl acetal	88.24
15.21	Neral	91.56
15.48	Terpineol (alpha-)	95.76
15.82	Bisabolene (beta-)	93.58
15.88	*cis*-Geranyl acetate	95.30
15.95	Geranial	93.83
16.31	Lavandulyl acetate	94.83
16.95	Nerol	87.28
17.59	Geraniol	79.31
22.10	Bisabolol (epi-alpha-)	78.09

Conclusion

In flavor and fragrances analysis, it is critical to have good spectral identification of the compounds of interest to ensure the quality of the products delivered. Due to lower helium availability, hydrogen is becoming the new carrier gas of choice in GC/MS. Although it has always been recognized as the best carrier gas for GC, hydrogen can bring some limitations with MS detection, including distorted library matching against spectral databases curated with helium.

The Agilent HydroInert source enables use of hydrogen carrier gas, a more sustainable alternative to helium, and improves the GC/MS performance with hydrogen carrier gas when compared to conventional EI sources.

This work demonstrates that the use of the HydroInert source overcame the problem of the spectral anomalies observed with hydrogen as the carrier gas. Results show an improved spectral match (more than 10%) for some compounds. A significant improvement in chromatographic peak shape was also observed for all compounds, and was especially pronounced for late-eluting ones.

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