

Qualitative Analysis of Essential Oils Using GC/MS with Hydrogen Carrier Gas and the Agilent HydroInert Source



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Abstract

Due to ongoing concerns with the price and availability of helium (He), many laboratories are looking for alternative carrier gases for their gas chromatography/mass spectrometry (GC/MS) methods. This application note describes the conversion of a typical GC/MS method for the qualitative analysis of flavor and fragrance compounds in essential oils from helium to hydrogen (H₂). The Agilent 8890 GC coupled with the Agilent 5977C GC/MSD system were used with hydrogen carrier gas and a new source that has been optimized for hydrogen operation—the Agilent HydroInert source. Unlike most conventional electron ionization (EI) sources, the HydroInert source provides excellent mass spectral fidelity for flavor compounds when using hydrogen. To further increase confidence in compound identification, deconvoluted mass spectra and linear retention indexes (RI) from Agilent MassHunter Unknowns Analysis software were searched against the NIST23 mass spectral library. Using the Agilent Method Translator tool, a column and chromatographic conditions for hydrogen were chosen that allowed reduction of the analysis time by a factor of 2.5 compared to the typical helium method. By proper selection of instrument configuration and operating conditions, the system with hydrogen carrier gas can generate results comparable to those with helium, but with significantly reduced run time.

Introduction

Essential oils are widely used as a source of flavors and fragrances in both food and nonfood consumer products. Quality control analysis of essential oils has long been challenging due to the hundreds of terpenes and terpenoid compounds that can be present in the oils. To address this, methods using high-resolution capillary GC combined with MS have typically been employed. Searching the acquired mass spectra against libraries of flavor and fragrance compounds can be performed for identification, but is usually insufficient because many compounds, especially isomers, give similar spectra. For this reason, RIs are often used as a complement to spectral matching for more dependable identifications. The measured RI of an unknown is used in conjunction with the results of the spectral library search to determine the best candidate for identification.

This application note describes the conversion of a typical GC/MS method for the qualitative analysis of flavor and fragrance compounds in essential oils from helium to hydrogen. The two methods are then applied to the analysis of two common essential oils—orange oil, of Brazilian origin, and neroli oil—for comparison. The hydrogen method was further evaluated using different EI source components to determine the optimal source for maintaining spectral fidelity.

The conversion of a method from helium to hydrogen carrier gas requires consideration of the chromatographic parameters such as column choice, column flow, amount injected, and temperature program rates.¹ In addition, it is important to consider the MS parameters of column flow rate and MS source configuration. All these considerations and parameters are included in the [Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion User Guide](#).¹

When determining the chromatographic parameters for the hydrogen method, it is desirable to have a method that has:

- Column dimensions that result in a high enough inlet pressure for accurate flow control
- Similar chromatographic resolution to the original helium method
- Maintenance of the same analyte elution order as the original helium method
- Use of a column flow near the optimum for the column diameter used
- Use of a column flow near the optimum for MS source sensitivity

The Agilent Method Translator tool²⁻⁴ is a calculator designed to greatly simplify this process. It was used in this application note.

For the MS parameters, the column flow rate should be kept within or near the optimum range of approximately 0.8 to 1.4 mL/min to maximize the MS response. Another important consideration is the choice of MS EI source hardware.

The concern is that some analytes undergo reactions with hydrogen in the source, changing their ion ratios and spectra, thus reducing their library match scores (LMS), and possibly resulting in misidentification. With the Agilent inert extractor source used in the Agilent 5977 series GC/MSD systems, this effect has been reduced in the past using an extractor lens with a larger diameter, such as 9 mm. However, this only partially addressed the problem, as many compounds such as nitro compounds and some terpenes and terpenoids still exhibited reactions. For this reason, Agilent developed the HydroInert source^{5,6}, which greatly reduces or eliminates these reactions. To evaluate the effectiveness of the HydroInert source, the qualitative analysis of the two oils was carried out with the helium method using the standard 3 mm inert extractor source and the hydrogen method using the standard HydroInert source, which is equipped by default with the 9 mm lens. In addition, the oils were analyzed with the hydrogen method and a conventional inert extractor source using both the 3 and 9 mm extractor lenses for comparison.

The next consideration is how to process the data files to obtain the mass spectrum of each oil component and search it against a library. In the past, this was done largely by obtaining the apex or average spectrum over the peak then subtracting a baseline spectrum taken next to the peak. The resulting spectrum was then searched against the spectral library. While this process is effective for handling a few peaks that were relatively well resolved, it becomes overwhelming with large numbers of peaks and/or overlapping peaks.

Fortunately, there is now a powerful solution for mass spectral identification called Agilent MassHunter Unknowns Analysis (MHUA), which is part of the Agilent MassHunter Quantitative Analysis software suite. MHUA uses spectral deconvolution to extract clean analyte spectra from the complex overlapping peaks. The deconvolution and library search processes are automated and take approximately 1 to 8 minutes per data file depending on the file size, library size, computer hardware, and so on. The result is cleaner spectra than with the previous approach, which therefore results in higher LMS and greater confidence in peak identifications.⁷

A second major feature of MHUA is the ability to calculate the RI for each peak. If the searched library has the appropriate reference RI values associated with spectral entries, the measured RI value for an unknown spectrum can be used to filter the spectral search results. This is especially important in the identification of essential oil components because of spectral similarities. RI values were used in this application note for this reason.

There are multiple mass spectral libraries available with RIs for flavor and fragrance compounds. For example, the Adams library⁸ has been used widely for many years for this analysis. Recently, NIST released a newer version of their mass spectral library (NIST23), which has numerous enhancements. Among them are the incorporation of the entire Adams library and the expansion of semistandard, nonpolar RI entries to cover all EI spectra. "Semistandard, nonpolar" refers to phases such as HP-5, DB-5, HP-5ms, and other 5% phenylmethyl silicone phases.⁹ The new RI values are either experimental values, if available, or artificial intelligence (AI)-generated values. Note that the AI-generated values have better accuracy than the previous "estimated" values. The new semistandard, nonpolar values are of specific interest here because this type of stationary phase is commonly used in essential oil analysis with GC/MS. Also, these are the NIST23 values currently usable with the RI function in MHUA. When the RI function of MHUA is used, the experimental semistandard, nonpolar RI values are used if available; if not, the AI-generated values are used. Therefore, NIST23 is the library used here.

Experimental

Column selection

For the reference helium method, the column and conditions chosen are like those frequently used in the past.^{8,10} A 30 m × 0.25 mm id, 0.25 μm Agilent J&W HP-5ms Ultra Inert (UI) column (part number 19091S-433UI) was used with an oven temperature program from 60 to 240 °C at 3 °C/min. Although some of the older methods¹⁰ used constant pressure control mode for column flow, constant flow mode is far better in terms of MS performance. A constant column flow rate of 1.0 mL/min of helium was used.

For the hydrogen method, a 20 m × 0.18 mm id, 0.18 μm Agilent J&W HP-5ms UI column (part number 19091S-577UI) was chosen. This column makes an excellent choice for several reasons:

- The column dimensions provide an inlet pressure with hydrogen that is high enough for accurate flow control.
- The column provides similar or better chromatographic resolution compared to the helium method.
- The phase ratio is the same, helping maintain the same analyte elution order as the original method.
- A column flow of hydrogen near the optimum for both chromatographic separation and MS source sensitivity can be used.

Method translation

The Method Translator tool is included as part of the Agilent MassHunter acquisition software or can be downloaded for standalone use from Agilent.com: <https://www.agilent.com/en/support/gas-chromatography/gccalculators>.

The free download includes the Method Translator, Vapor Volume Calculator, Pressure Flow Calculator, and Solvent Vent Calculator tools, which are all useful when developing GC methods.

After installation, the Method Translator is opened from an icon on the desktop. The opened Method Translator is shown in Figure 1. First, the chromatographic parameters of the original helium method are entered in the left column labeled Original Method Parameters. The carrier gas type (He), column dimensions, column outlet pressure, and oven temperature program ramp should be entered first. The column outlet flow is then entered. As shown in Figure 1, the other values such as phase ratio, inlet pressure, and so on, are calculated automatically.

Next, the carrier gas type (H₂), column dimensions, and column outlet pressure for the hydrogen method are entered in the right side of the calculator under the Calculated Method Parameters column.

After entry, the calculated hydrogen parameters are displayed. In the upper-left corner, the calculated speed gain is shown as 2.5877, meaning that the predicted retention times (RTs) with the hydrogen method would be a factor of approximately 2.6 smaller than the helium method. The calculated oven

ramp rate for hydrogen would be 7.763 °C/min. Note that it would be easier to have the oven ramp rate and the speed gain closer to 7.5 and 2.5, respectively. This can be done by selecting **Speed gain** and entering 2.5 into the field. The parameters are recalculated, resulting in the desired parameters. Figure 1 shows the results. Note that the calculated flow for the hydrogen method shown in Figure 1 is 0.84004 mL/min. Before using the method, retention time

locking (RTL) was used to make the RT of n-pentadecane in the hydrogen method precisely 2.5 times faster than that with the helium method. This made comparison of RTs easier. The resulting flow for the hydrogen method after RTL was 0.958 mL/min. For library searching, this step is not necessary as the RI calibration accounts for differences in flow.

Method Translator

Last file imported: C:\MS General Consulting\Old Sandra Flavors

Speed gain: 2.5000

Translate

Best Efficiency

Original Method Parameters		Calculated Method Parameters	
Gas: He		Gas: H2	
Length (m)	30 m	20 m	
Inner Diameter (µm)	250 µm	180 µm	
Film Thickness (µm)	0.25 µm	0.18 µm	
Phase Ratio	249.25	249.25	
Inlet Pressure (gauge)	8.2317 psi	7.596 psi	
Outlet Flow (mL/min)	1 mL/min	0.84004 mL/min	
Average Velocity (cm/s)	36.623 cm/sec	61.038 cm/sec	
Outlet Pressure (abs)	0 psi	0 psi	
Holdup Time	1.3653 min	0.54611 min	
Outlet Velocity (cm/s)	Infinity cm/sec	Infinity cm/sec	

#	Ramp Rate (°C/min)	Final Temp (°C)	Final Time (min)
Init		60	0
1	3	240	0

Total Run Time: 60.00 min

#	Ramp Rate (°C/min)	Final Temp (°C)	Final Time (min)
Init		60	0
1	7.5	240	0

Total Run Time: 24.00 min

Pressure Units: PSI

Original Column Capacity: 1.71

Translated Column Capacity: 0.61

The column capacity of the translated method is 36% of the original column capacity. You may need to adjust your injection volume.

Figure 1. Agilent Method Translator tool, used to determine method parameters for conversion of the helium method to hydrogen.

MS source hardware

The standard inert extractor source with the 3 mm extractor lens is an excellent choice for the helium method when analyzing flavor and fragrance compounds, and was used in this application note. For the hydrogen method, the HydroInert source with the 9 mm extractor lens was used, as it reduces in-source reactions with hydrogen and provides improved peak shape. The hydrogen method was also run with a conventional inert extractor source using both the 3 and 9 mm extractor lenses. These data were compared to that from the HydroInert and helium results to identify components of the oils that were most susceptible to in-source reactions by comparing their spectra and LMS values. Figure 2 shows the system configurations for the helium and hydrogen methods.

Chemicals and standards

In-house hydrogen with 99.9999% purity specification and low individual specifications on water and oxygen was used as the carrier gas for the hydrogen method. In-house helium with similar specifications was used as the carrier gas for the helium method.

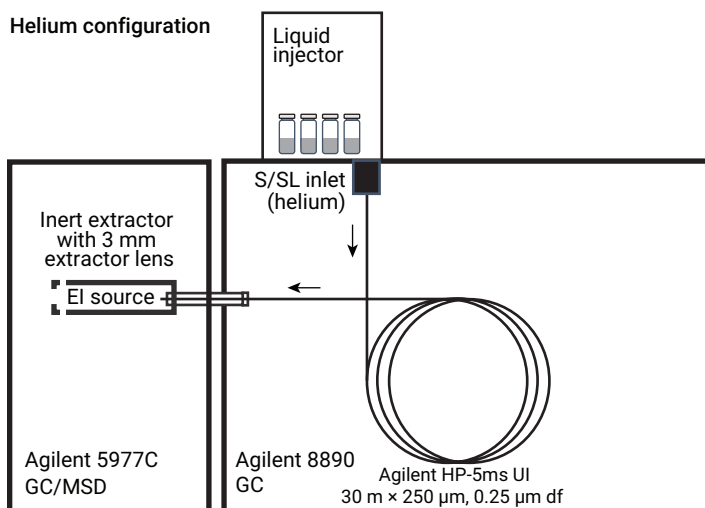
Cold-pressed orange oil (Brazil origin) and neroli oil (Morocco origin) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The oils were diluted to 20:1 (v:v) in ethanol.

A custom RI calibration standard consisting of all the n-alkanes from n-C₅ to n-C₄₀ plus n-C₄₄ was purchased from Ultra Scientific (now Agilent). All alkanes were at a concentration of 500 ng/μL in n-hexane except n-C₁₃, n-C₁₈, n-C₂₂, n-C₂₈, n-C₃₁, and n-C₃₉, which were at 1,000 ng/μL. The standard was then diluted to 50:1 (v:v) in isooctane.

Table 1. GC and MS conditions for helium and hydrogen methods.

Method Parameters		
	Helium Method	Hydrogen Method
Inlet	EPC split/splitless	
Mode	Split 25:1	
Column Flow	1.0 mL/min helium	0.958 mL/min hydrogen
Injection Volume	1.0 μL	
Inlet Temperature	250 °C	
Inlet Liner	Agilent universal low pressure drop UI liner with wool (p/n 5190-2295)	
Column	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 μm (p/n 19091S-433UI)	Agilent J&W HP-5ms UI, 20 m × 0.18 mm, 0.18 μm (p/n 19091S-577UI)
Column Temperature Program	60 °C (no hold) 3 °C/min to 240 °C (no hold)	60 °C (no hold) 7.5 °C/min to 240 °C (no hold)
Run Time	60 min	24 min
MSD Source	Agilent inert extractor (3 mm lens)	Agilent HydroInert source (9 mm lens)
Transfer Line Temperature	300 °C	
Ion Source Temperature	300 °C	
Quadrupole Temperature	150 °C	
EM, Gain Mode	0.1	
Mode	Scan 40 to 400 m/z	
TID, A/D Samples	TID ON, 8	TID ON, 4
Solvent Delay	2.2 min	0.88 min
Tune	etune.u	

Helium configuration



Hydrogen configuration

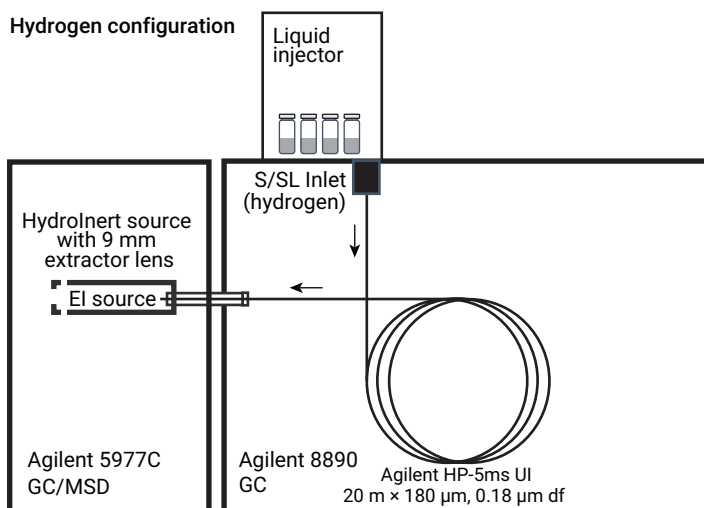


Figure 2. System configurations for the helium and hydrogen methods.

Results and discussion

Retention index calibration

The diluted RI calibration standard was run with both methods. The chromatograms are shown in Figure 3. Although the oil sample analysis is finished before n-C₂₆, the standard contains n-alkanes up to n-C₄₀. Therefore, the temperature ramps for both RI calibration methods were extended to 300 °C and held until n-C₄₀ eluted to prevent carryover peaks in subsequent chromatograms. The red arrow in each chromatogram indicates the normal end of run for the oil methods. For determining the RT of the RI calibration compounds, integrating the EIC for *m/z* 57 is preferred over the total ion chromatogram (TIC), as it has a better signal-to-noise ratio.

To use RI values in MHUA, a calibration file needs to be created for each method. The file can be created as a .csv file in Microsoft Excel, or as a text file in Microsoft Windows Notepad. Figure 4 shows the calibration .rtc files created in Notepad. The blue headers are not included in the files; they are included here to indicate the entry format. Each entry consists of the format name, CAS number, RI, and RT, and the associated text file is then saved with a .rtc extension in the filename. The text files are usually saved in either the library directory or the directory containing the data files.

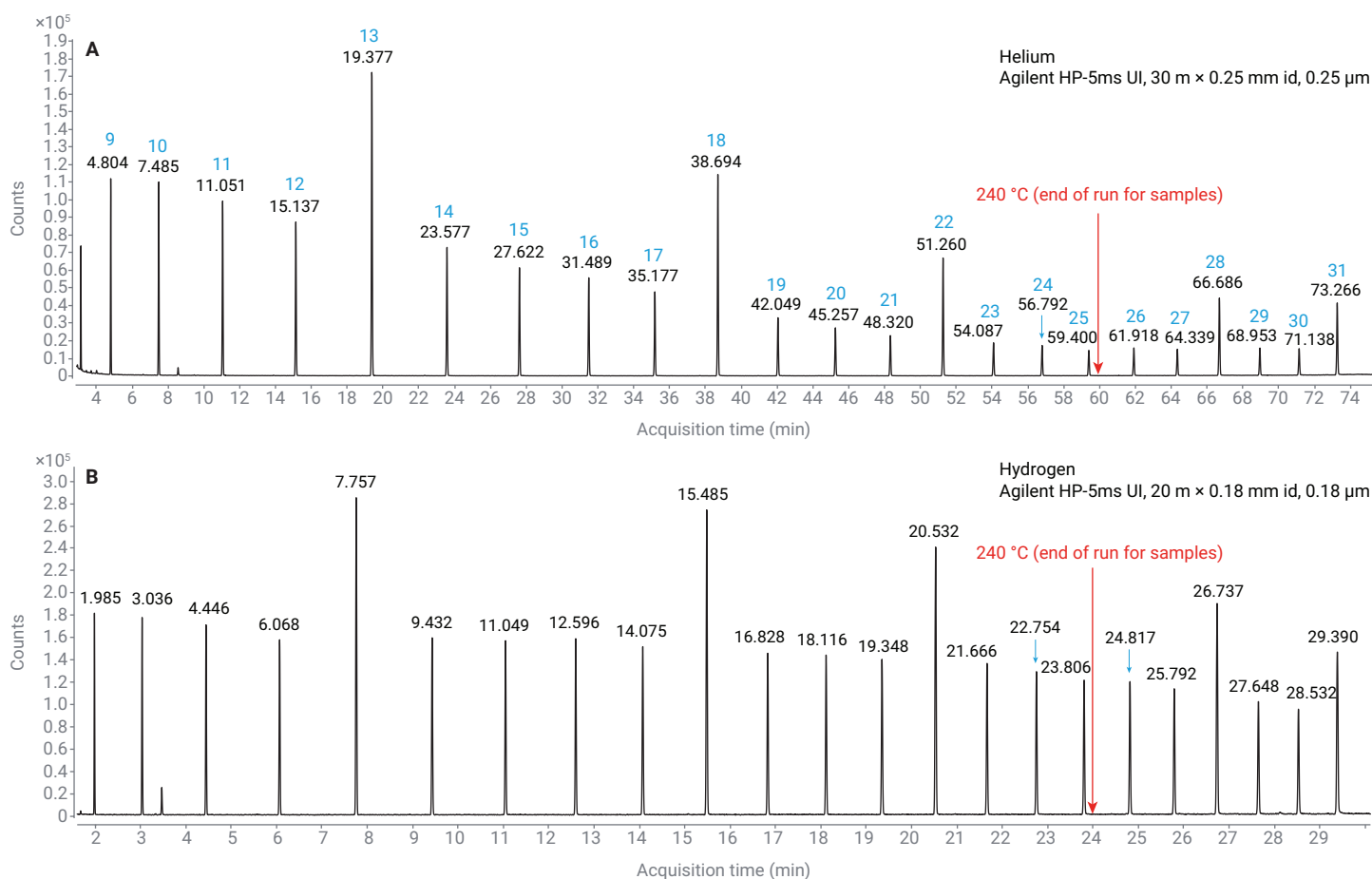


Figure 3. EICs at *m/z* 57 for the RI calibration standard. (A) Helium method; (B) hydrogen method.

Helium method

Name, CAS,	RI,	RT
n-C5,109-66-0	,500,	1.602
n-C6,110-54-3	,600,	1.801
n-C7,142-82-5	,700,	2.236
n-C8,111-65-9	,800,	3.139
n-C9,111-84-2	,900,	4.804
n-C10,124-18-5	,1000,	7.485
n-C11,1120-21-4	,1100,	11.050
n-C12,112-40-3	,1200,	15.137
n-C13,629-50-5	,1300,	19.377
n-C14,629-59-4	,1400,	23.577
n-C15,629-62-9	,1500,	27.622
n-C16,544-76-3	,1600,	31.489
n-C17,629-78-7	,1700,	35.117
n-C18,593-45-3	,1800,	38.694
n-C19,629-92-5	,1900,	42.049
n-C20,112-95-8	,2000,	45.257
n-C21,629-94-7	,2100,	48.320
n-C22,629-97-0	,2200,	51.260
n-C23,638-67-5	,2300,	54.087
n-C24,646-31-1	,2400,	56.792
n-C25,629-99-2	,2500,	59.400
n-C26,630-01-3	,2600,	61.918
n-C27,593-49-7	,2700,	64.339
n-C28,630-02-4	,2800,	66.686
n-C29,630-03-5	,2900,	68.953
n-C30,638-68-6	,3000,	71.138

Hydrogen method

Name, CAS,	RI,	RT
n-C5,109-66-0	,500,	0.694
n-C6,110-54-3	,600,	0.774
n-C7,142-82-5	,700,	0.946
n-C8,111-65-9	,800,	1.299
n-C9,111-84-2	,900,	1.985
n-C10,124-18-5	,1000,	3.036
n-C11,1120-21-4	,1100,	4.446
n-C12,112-40-3	,1200,	6.068
n-C13,629-50-5	,1300,	7.757
n-C14,629-59-4	,1400,	9.432
n-C15,629-62-9	,1500,	11.049
n-C16,544-76-3	,1600,	12.596
n-C17,629-78-7	,1700,	14.075
n-C18,593-45-3	,1800,	15.485
n-C19,629-92-5	,1900,	16.828
n-C20,112-95-8	,2000,	18.116
n-C21,629-94-7	,2100,	19.348
n-C22,629-97-0	,2200,	20.532
n-C23,638-67-5	,2300,	21.666
n-C24,646-31-1	,2400,	22.754
n-C25,629-99-2	,2500,	23.806
n-C26,630-01-3	,2600,	24.817
n-C27,593-49-7	,2700,	25.792
n-C28,630-02-4	,2800,	26.737
n-C29,630-03-5	,2900,	27.648
n-C30,638-68-6	,3000,	28.532

Figure 4. RI calibration text files (.rtc) used in Agilent MassHunter Unknowns Analysis.

Brazilian orange oil

Figure 5 compares the TICs obtained with the helium and hydrogen methods for the Brazilian orange oil sample. Figure 5A shows the complete time range of elution, and 5B is an expanded view to better compare the peak shapes and chromatographic resolution. As can be seen, by using the Method Translator technique, the relative elution order

of peaks is maintained, as is the resolution. Many of the larger peaks in the hydrogen chromatogram exhibited some fronting. This was predicted by the Method Translator tool, which indicated that the hydrogen setup has 36% of the original column capacity of the helium method. However, the chromatographic resolution is still about the same as the helium method.

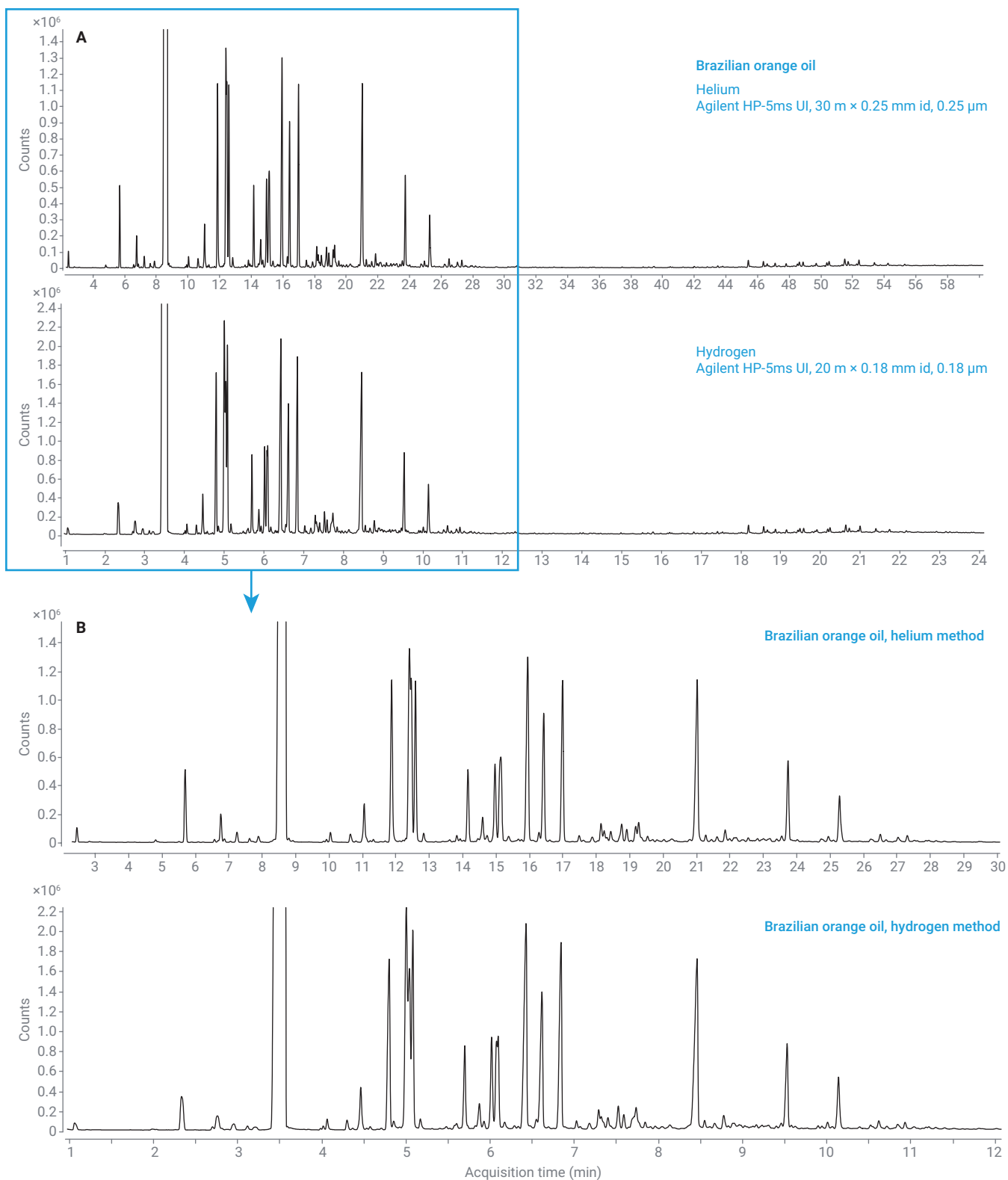


Figure 5. (A) Brazilian orange oil with helium and hydrogen methods showing full range of compound elution. (B) Expanded view of earlier elution time region.

Neroli oil

Figure 6 shows the TICs for the neroli oil run with the helium and hydrogen methods. As with the orange oil, the relative elution order of peaks is maintained, as is the resolution. The reduced column capacity of the hydrogen method is again evident in the increased fronting of the large peak at 4.6 minutes in the hydrogen chromatogram.

Peak identification with MassHunter Unknowns Analysis

The parameters used with MHUA are listed in Table 2. If the library has appropriate RI values for the spectrum entries, RIs can be used as a filter for library hits. The program uses RI if an RI calibration filename is entered in the RT calibration file box. A more detailed description for setting up and running an analysis is shown in the Appendix. Also, an excellent source of information about MHUA is available in a video on the Agilent YouTube channel.⁷

With the settings listed in Table 2 and a data file analyzed, the program will deconvolute the entire scan file and determine where each detectable peak (component) is. It will then take the deconvoluted (cleaned) spectrum of each component and search it against the library (NIST23). The library entry that best matches the spectrum of the component is checked to see if it exceeds the minimum match factor parameter of 70. If it does, it is next checked to see if the measured RI of the component falls within \pm the penalty free range, in this case ± 10 seconds. With the RT mismatch penalty set to Additive and the maximum RT penalty set to 100, if the difference between the measured RT and the library RI (converted to RT) is greater than 10 seconds, the entry is completely discarded. If the difference is less than 10 seconds and the LMS is greater than 70 and higher than the other possible hits, the hit is included in the results table.

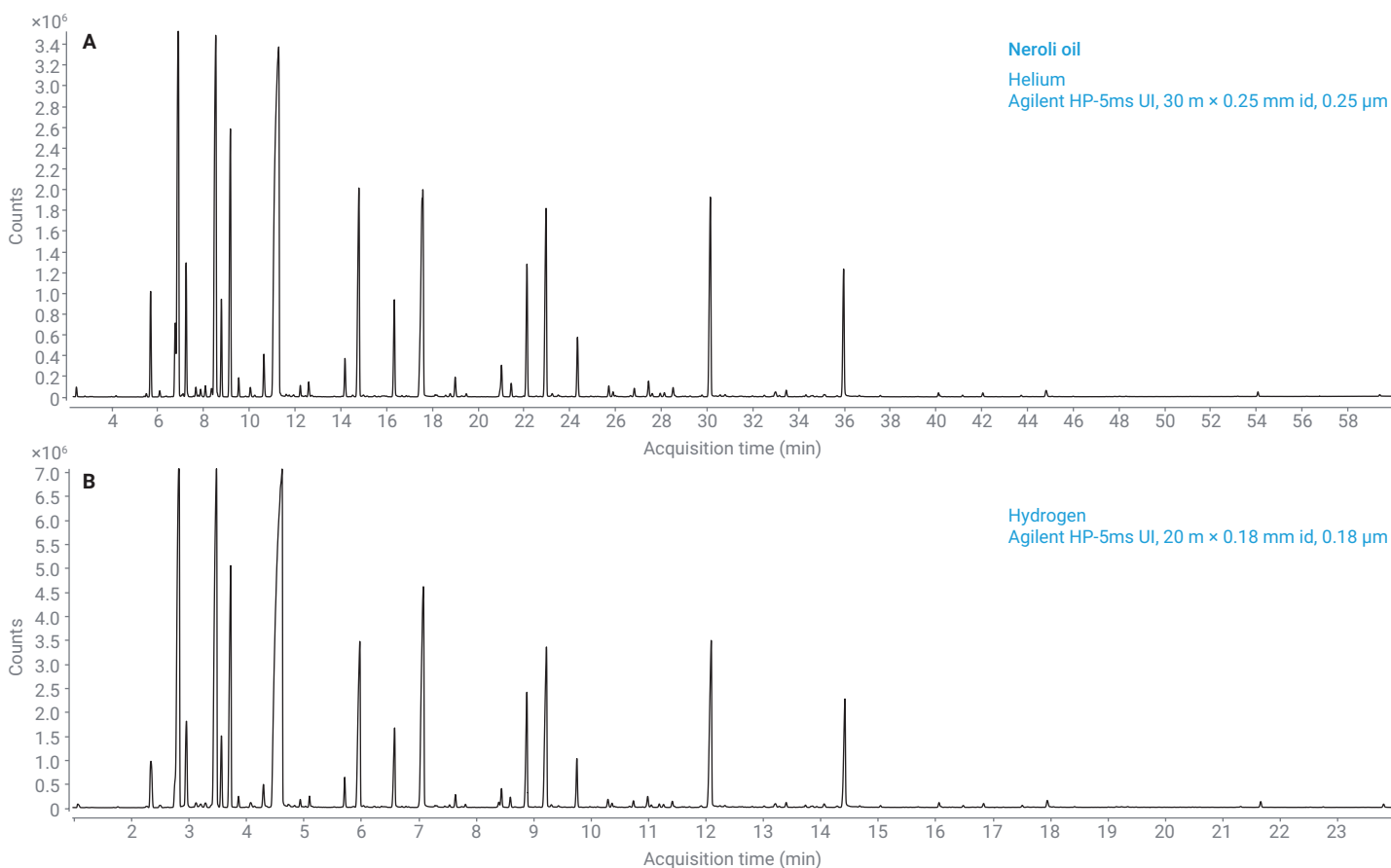


Figure 6. Neroli oil analyzed with (A) helium and (B) hydrogen methods.

Table 2. Agilent MassHunter Unknowns Analysis method parameters.

Parameter	Setting
RT Window Size Factor	10, 25, 50, 100, 200, 400, 600, 800
Library	NIST23.L
RT Penalty function	Trapezoidal
RT Range	10 s
Penalty Free Range	10 s
RT Mismatch Penalty	Additive
Maximum RT Penalty	100
Minimum Match Factor	70

Once the entire data file has been processed, the results can be reviewed. Figure 7 shows the results for the Brazilian orange oil with the helium method. Left-clicking on the compound name for one of the listed results displays the deconvoluted component spectrum head to tail with the library spectrum.

The selected component peak at 16.989 minutes is highlighted in red in the TIC chromatogram, which can be zoomed in for closer inspection. The five most abundant ions are extracted and overlaid in the box to the left of the spectrum display. This is to allow inspection of the peak shapes and apex retention times. If the apex RT or shape of one of the extracted ions is substantially different from the others, this suggests that there might be an interference, which should be considered when interpreting the identification.

In practice, reviewing the results consists of going down the list of hits and looking at the Match Factor (Library Match Score), Delta RI, and Base Peak Area. Using the peak at 16.989 as an example, the spectrum has a high-quality match for D-carvone of 98.2 listed, and the head-tail component and library spectra visually match well. The overlay of EICs of the principal ions all have the same shape and apex RT. The delta RI value of 2, which is the difference between the measured RI for the peak and that from the library, is small at 2. Finally, the base peak area and observed peak size in the TIC chromatogram indicate that the response is large enough to produce good-quality spectra. From these observations, the identification of D-carvone is confirmed with high confidence.

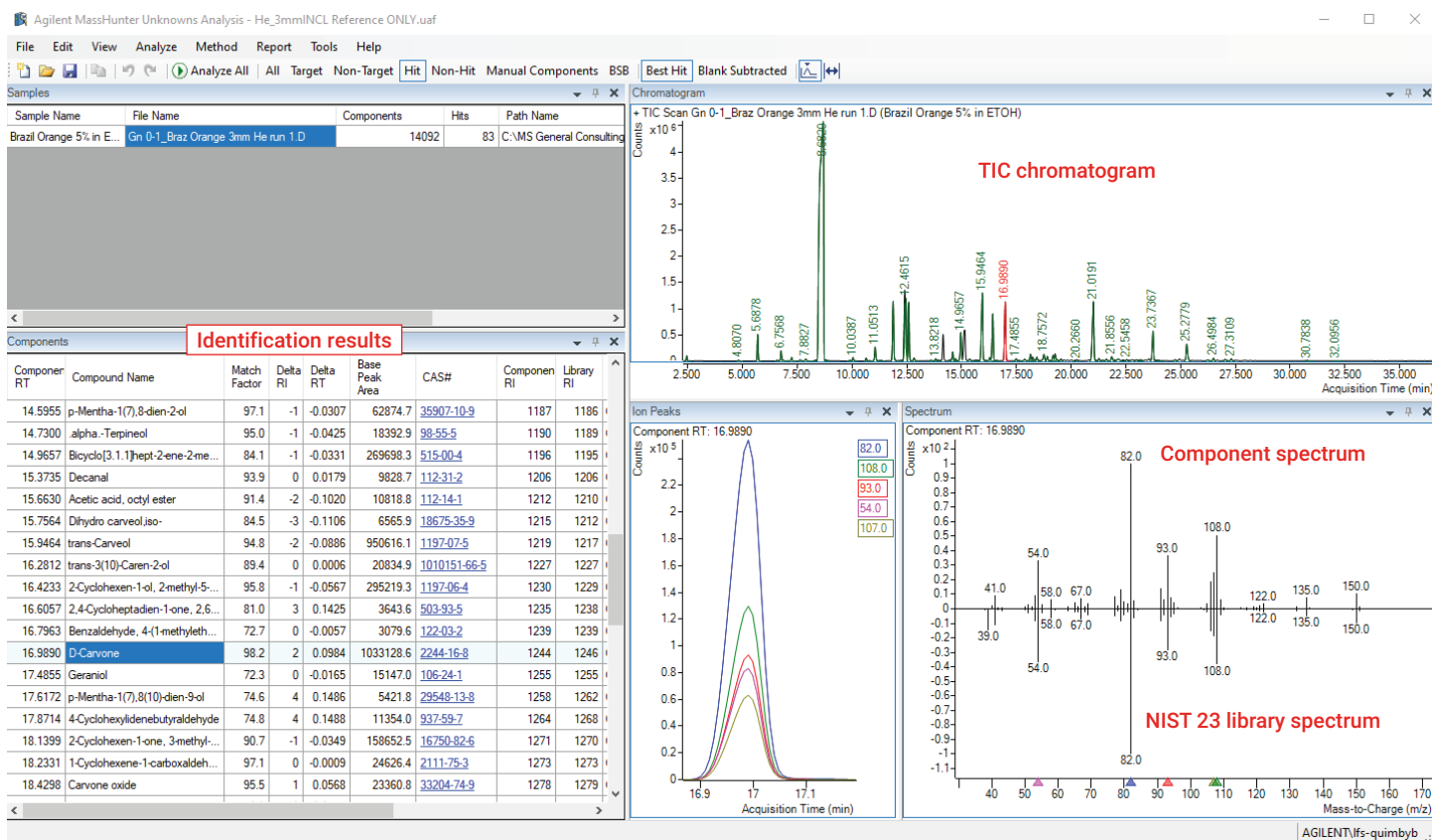


Figure 7. Agilent MassHunter Unknowns Analysis results for Brazilian orange oil run with the helium method.

In contrast, the peak at 17.6172, identified as p-mentha-1(7),8(10)-dien-9-ol, has a low LMS of 74.6, a larger delta RI of 4, one of the EICs has a noticeably different apex RT, and the base peak area is small—approximately 190 times smaller than the D-carvone peak. This would be a low-confidence identification. If the reviewer decides it should not be reported, the hit can be removed from the results by right-clicking the name and selecting **Delete Components/Hits**.

Components/Hits.

If an identification is questioned based on other information, the reviewer can right-click the name in the results table and select **Show Alternate Hits**. This will display a list of the other spectra in the library that also met the LMS and RI criteria, but with an LMS less than the listed Best Hit. This is useful as sometimes the LMS of the lesser hits is only a fraction of a point smaller. If desired, the reviewer can select one of the alternate hits and set it as the identification.

This review process is used to evaluate all the hits. Once completed, the reviewed analysis can be saved and a report can be generated if desired.

Evaluating in-source reactions with hydrogen

While the inert extractor source with the 3 mm lens is standard for use with helium, it is not the source of choice for use with hydrogen carrier. The metal surfaces inside the source tend to catalyze reactions between hydrogen and some analyte molecules in the source, resulting in peak tailing and spectral changes for some compounds. In the past, substituting the 9 mm extractor lens for the 3 mm lens was used as it reduced the tailing and spectral changes to some degree, but did not eliminate them. For this reason, the Hydrolnert source was developed.

In this section, the spectra of carvone oxide (CAS number 33204-74-9) obtained with hydrogen using the Hydrolnert source, the inert extractor source with the 3 and 9 mm lenses, and with helium are compared to illustrate the effects of source reactivity. Several other examples are presented in the Appendix.

Figure 8 shows the chromatograms and spectra of the carvone oxide peak with the helium and hydrogen methods under the optimized conditions. The library reference spectrum from NIST23 is shown upside down for comparison. With both methods, the deconvoluted spectra have high LMS values of > 95, demonstrating the excellent spectral fidelity provided by the Hydrolnert source with hydrogen carrier gas.

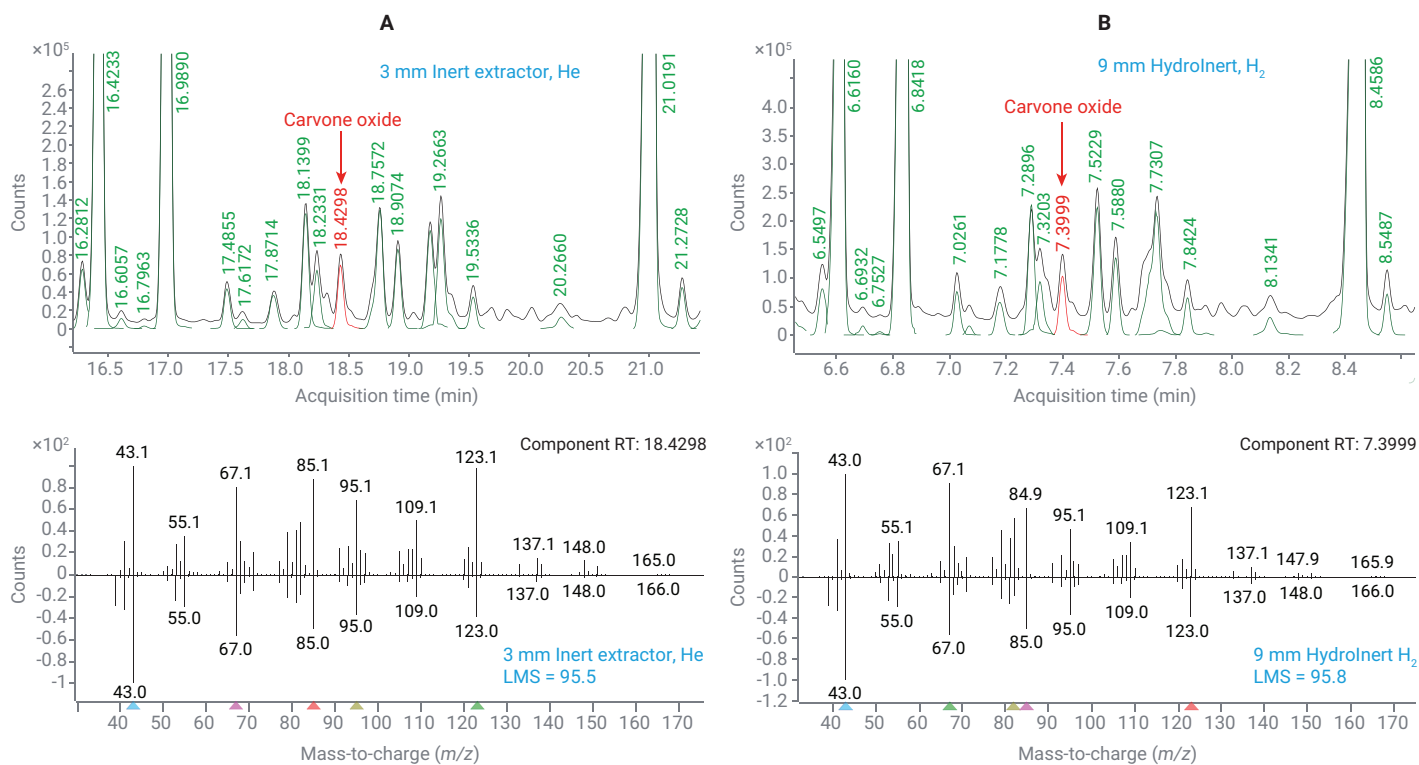


Figure 8. (A) Chromatogram and spectrum of the carvone oxide peak with the helium method. (B) Chromatogram and spectrum of the carvone oxide peak with the hydrogen method and Agilent Hydrolnert source.

For comparison, Figure 9 shows the spectra of the carvone oxide peak with hydrogen carrier using the HydroInert source and the inert extractor source with both the 9 and 3 mm extractor lenses.

With the 9 mm inert extractor lens shown in the spectrum in Figure 9B, the LMS value is still a respectable 91.2. However, there is clear evidence of some spectral changes. Most notably, the ions 82 and 108 have increased in abundance relative to the rest of the ions in the spectrum. While the degree of spectral fidelity is still useful, it demonstrates that in-source reactions, albeit limited, are occurring.

In contrast, the spectrum with the 3 mm inert extractor lens and hydrogen carrier is significantly changed. The spectrum is changed to the extent that the LMS for matching carvone oxide is below the 70 cutoff and thus is not listed, even in the alternate hits list. The search identified the peak as (2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)methanamine (CAS 61299-72-7), also known as pinane-3-(methylamine). Note that with an LMS value of 84.8 and a close RI match with a delta RI of only -1, this identification looks plausible but is incorrect.

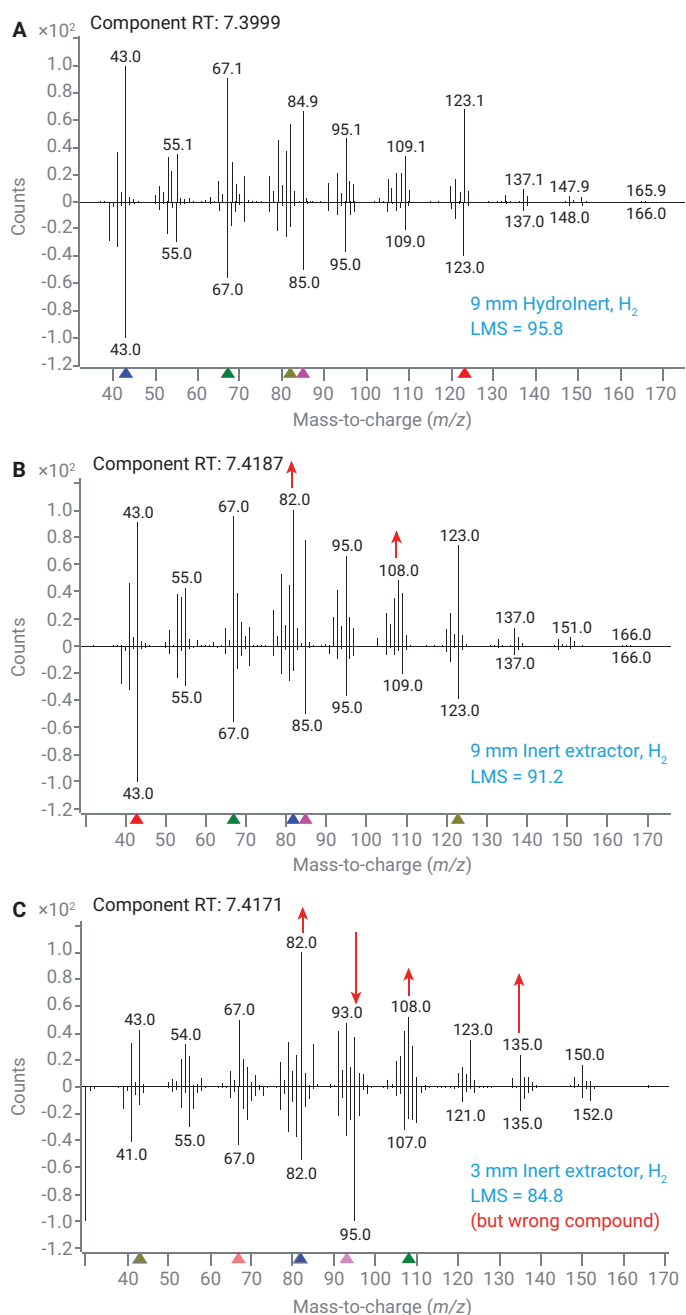


Figure 9. (A) Spectrum of the carvone oxide with hydrogen carrier and the Agilent HydroInert source. The reference spectrum is carvone oxide from NIST23. (B) Spectrum with an Agilent inert extractor source and a 9 mm lens. The reference spectrum is carvone oxide from NIST23. (C) Spectrum with an Agilent inert extractor source and a 3 mm lens. The reference spectrum is (2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)methanamine from NIST23.

To further investigate the nature of the in-source reaction with the 3 mm inert extractor source, the data file was reanalyzed with MHUA using the same parameters, except not using the RI match criteria. This would list the best hits solely on LMS. If the carvone oxide is reacting with hydrogen in the source to produce a reaction product, the spectral search may reveal what it is. Figure 10 shows the spectrum obtained with (A) the 3 mm lens at the carvone oxide RT compared with (B) that of the best match and (C) the carvone oxide library spectrum.

Note that the spectrum obtained with the 3 mm inert extractor source looks very much like a combination of that of carvone oxide and 3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone. Examining the structures in Figure 10, it appears that the epoxide structure of carvone oxide reacts with hydrogen to form the OH group.

This example clearly illustrates the perils of using a GC/MS source that allows reactions between hydrogen and analytes, and why the HydroInert source is the best choice when using hydrogen carrier gas. Several other examples are shown in the Appendix.

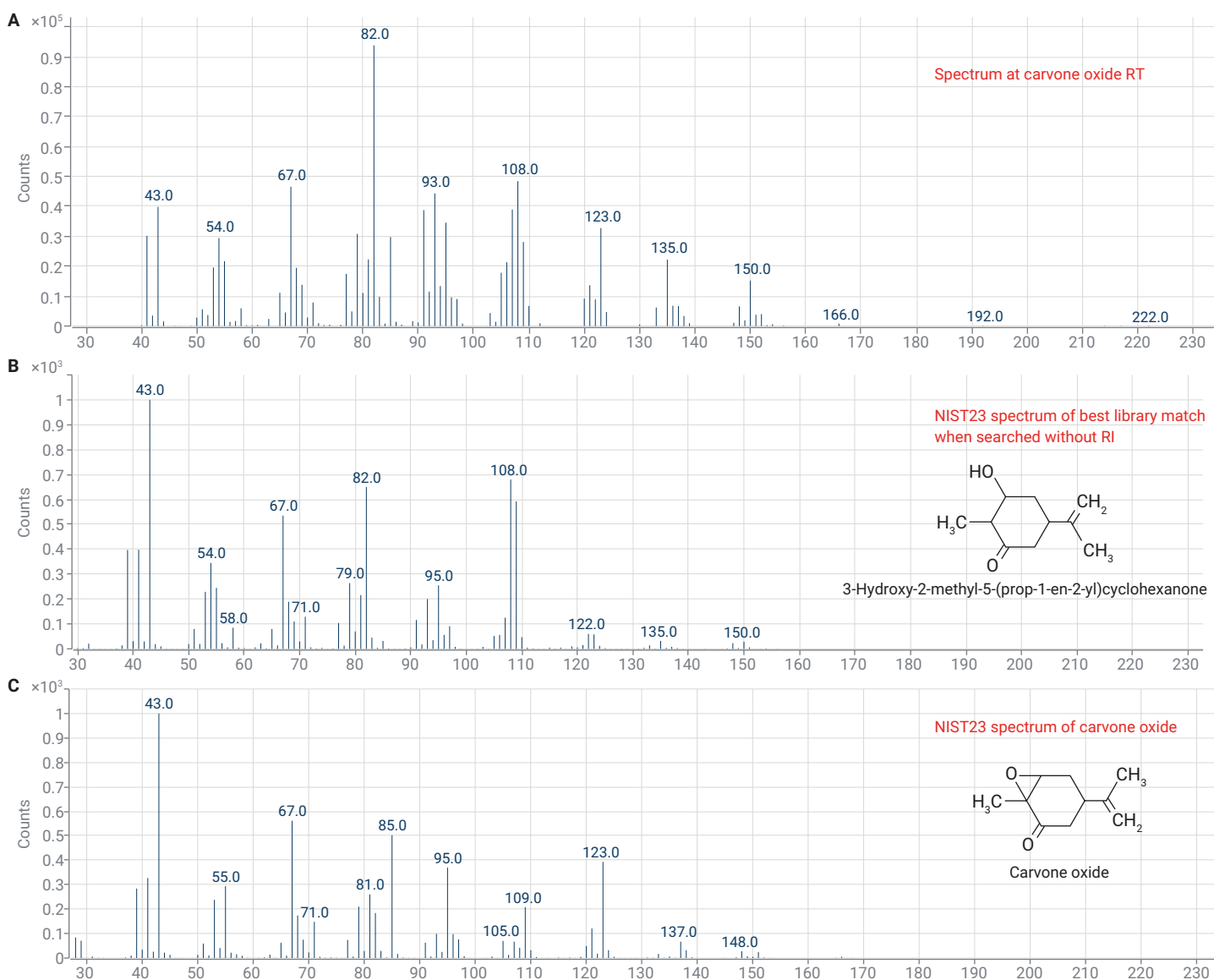


Figure 10. (A) Spectrum obtained with the 3 mm lens at the carvone oxide RT. (B) NIST23 library reference spectrum for the best match when searched without RI filtering, 3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone. (C) NIST23 library reference spectrum for carvone oxide.

Analysis results for Brazilian orange and neroli oils

Table 3 presents the results of the analysis of the Brazilian orange oil with both the helium method using the 3 mm inert extractor source and the hydrogen method using the 9 mm HydroInert source. Table 4 presents the results for neroli oil with the same methods.

The results were reviewed to address questions relevant to converting the method helium to hydrogen carrier gas:

- **RIs compared to NIST23:** The RIs measured with both methods closely matched those in the NIST23 library for most compounds. However, it should be recognized that the RI recognition window used in MassHunter Unknowns Analysis limits the maximum delta RI when listing hits. The second consideration is that if the NIST23 RI value is an AI-predicted value instead of a true experimental value, the errors, and thus the delta RIs, can be larger.
- **Comparing RIs between helium and hydrogen methods:** As seen in the columns listing the difference between the RI measured with helium and that with hydrogen (labeled RI He-RI H₂), the agreement of the RIs measured with both methods is good. The only exceptions are the earliest peaks and those such as linalool and D-limonene that are chromatographically overloaded, thus shifting their RT. This is one of the benefits of using the Method Translator tool to choose the chromatographic parameters for the hydrogen method, because it maintains the same relative elution order for analytes and RI calibrators between the two methods.

- **LMS versus NIST23:** The deconvolution process in general yields cleaner spectra, which results in improved LMS scores when compared to previous approaches. Looking at the column of LMS scores for the helium results, most of them are > 85. The smaller values can result from smaller responding compounds, overlapping peaks causing spectral interferences, or search results where the analyte is not in the library and an incorrect hit is listed.
- **Comparing LMS between helium and hydrogen methods:** The columns listing the difference between the LMS measured with helium and that with hydrogen (labeled LMS He-LMS H₂) shows that, in general, there is good agreement between the two methods. The exceptions where the hydrogen method values are significantly lower are either due to lower signal response or spectral interference from overlapping peaks. In general, the signal-to-noise ratios obtained with hydrogen are two to five times less than with helium, and this is reflected in lower LMS scores for the smallest peaks.

Table 3. Analysis results for the Brazilian orange oil with both the helium and hydrogen methods. (LR = low response; Int = interference.)

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H ₂	LMS He-LMS H ₂	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
Ethane, 1,1-diethoxy-	105-57-7	726	2.445	723	3	98	1.061	732	-6	96	-9	2	
Nonane	111-84-2	900	4.807	900	0	95	1.983	900	0	91	0	4	
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	932	5.688	933	-1	99	2.332	933	-1	97	0	2	
1-Heptanol	111-70-6	970	6.566	966	4	98	2.699	968	2	95	-2	2	
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	3387-41-5	974	6.757	973	1	98	2.759	974	0	98	-1	0	
beta-Myrcene	123-35-3	991	7.240	991	0	97	2.953	992	-1	95	-1	2	
Octanal	124-13-0	1,003	7.616	1,004	-1	98	3.116	1,006	-3	97	-2	1	
3-Carene	13466-78-9	1,011	7.883	1,011	0	98	3.211	1,012	-1	89	-1	9	
D-Limonene	5989-27-5	1,031	8.682	1,034	-3	99	3.562	1,037	-6	99	-3	0	
1-Methylbicyclo[2.2.1]heptan-exo-2-ol	766-25-6	1,039	8.796	1,037	2	76	3.593	1,039	0	74	-2	2	
trans-Sabinene hydrate	17699-16-0	1,070	9.837	1,066	4	74	3.985	1,067	3	77	-1	-3	
1-Octanol	111-87-5	1,070	9.919	1,068	2	98	4.016	1,070	0	97	-2	1	
cis-Linalool oxide	5989-33-3	1,074	10.039	1,072	2	94	4.063	1,073	1	94	-1	0	
trans-Linalool oxide (furanoid)	34995-77-2	1,086	10.630	1,088	-2	95	4.297	1,089	-3	94	-1	0	
Benzene, 1-methyl-4-(1-methylethenyl)-	1195-32-0	1,090	10.665	1,089	1	91	4.306	1,090	0	92	-1	-1	
Epoxy-myrcene, 6,7-	29414-55-9	1,090	10.804	1,093	-3	73	4.365	1,094	-4	79	-1	-6	
Linalool	78-70-6	1,099	11.051	1,100	-1	97	4.462	1,101	-2	95	-1	1	
Nonanal	124-19-6	1,104	11.232	1,104	0	96	4.533	1,105	-1	86	-1	10	LR
cis-Pinen-3-ol	1010292-85-2	1,108	11.324	1,107	1	81	4.574	1,108	0	73	-1	8	
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	7212-40-0	1,123	11.872	1,120	3	95	4.799	1,122	1	96	-2	-1	
5-Undecene, 4-methyl-	143185-91-5	1,132	12.217	1,129	3	74	4.925	1,130	2	71	-1	4	
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	1195-92-2	1,133	12.401	1,133	0	86	5.003	1,134	-1	90	-1	-4	
cis-p-Mentha-2,8-dien-1-ol	3886-78-0	1,133	12.462	1,135	-2	79	5.042	1,137	-4	89	-2	-10	
(+)-(E)-Limonene oxide	6909-30-4	1,139	12.586	1,138	1	97	5.082	1,139	0	98	-1	-1	
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	7299-41-4	1,144	12.826	1,143	1	95	5.171	1,145	-1	93	-2	1	
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, trans-	7299-40-3	1,161	13.610	1,163	-2	88	5.480	1,164	-3	83	-1	4	
Bicyclo[3.3.0]octan-2-one, 7-methylene-	1000151-92-1	1,166	13.822	1,168	-2	84	5.578	1,170	-4	64	-2	19	Int
1-Nonanol	143-08-8	1,173	13.921	1,170	3	98	5.603	1,171	2	96	-1	2	
Ethanone, 1-(4-methylphenyl)-	122-00-9	1,183	14.452	1,183	0	93	5.822	1,185	-2	95	-2	-2	
p-Mentha-1(7),8-dien-2-ol	35907-10-9	1,186	14.596	1,187	-1	97	5.873	1,188	-2	96	-1	1	
alpha-Terpineol	98-55-5	1,189	14.730	1,190	-1	95	5.928	1,191	-2	94	-1	1	
Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	515-00-4	1,195	14.966	1,196	-1	84	6.018	1,197	-2	86	-1	-2	
Decanal	112-31-2	1,206	15.374	1,206	0	94	6.173	1,206	0	93	0	1	
Acetic acid, octyl ester	112-14-1	1,210	15.663	1,212	-2	91	6.287	1,213	-3	79	-1	12	LR, Int
Dihydro carveol, iso-	18675-35-9	1,212	15.756	1,215	-3	85	6.334	1,216	-4	78	-1	7	
trans-Carveol	1197-07-5	1,217	15.946	1,219	-2	95	6.427	1,221	-4	96	-2	-1	
trans-3(10)-Caren-2-ol	1010151-66-5	1,227	16.281	1,227	0	89	6.550	1,229	-2	85	-2	4	
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	1197-06-4	1,229	16.423	1,230	-1	96	6.616	1,232	-3	97	-2	-1	
2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	503-93-5	1,238	16.606	1,235	3	81	6.693	1,237	1	73	-2	8	
Benzaldehyde, 4-(1-methylethyl)-	122-03-2	1,239	16.796	1,239	0	73	6.753	1,241	-2	58	-2	14	LR
D-Carvone	2244-16-8	1,246	16.989	1,244	2	98	6.842	1,246	0	98	-2	0	
Geraniol	106-24-1	1,255	17.486	1,255	0	72	7.026	1,257	-2	77	-2	-5	
p-Mentha-1(7),8(10)-dien-9-ol	29548-13-8	1,262	17.617	1,258	4	75	7.070	1,259	3	73	-1	2	

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H ₂	LMS He-LMS H ₂	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
4-Cyclohexylidenebutyraldehyde	937-59-7	1,268	17.871	1,264	4	75	7.178	1,266	2	76	-2	-2	
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	16750-82-6	1,270	18.140	1,271	-1	91	7.290	1,272	-2	83	-1	7	
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	2111-75-3	1,273	18.233	1,273	0	97	7.320	1,274	-1	84	-1	14	LR
Carvone oxide	33204-74-9	1,279	18.430	1,278	1	96	7.400	1,279	0	96	-1	0	
Pinocarvyl acetate, <i>cis</i> -	73366-18-4	1,285	18.757	1,285	0	80	7.523	1,286	-1	82	-1	-2	
Verbenyl acetate, <i>trans</i> -	1203-21-0	1,291	18.907	1,289	2	79	7.588	1,290	1	80	-1	0	
p-Mentha-1,8-dien-7-ol	536-59-4	1,297	19.266	1,297	0	95	7.731	1,298	-1	93	-1	2	
2-Propanol, 1-[(1-ethynylcyclohexyl)oxy]-	54644-17-6	1,303	19.534	1,304	-1	77	7.842	1,305	-2	79	-1	-1	
1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	1946-00-5	1,321	20.266	1,321	0	80	8.134	1,323	-2	84	-2	-4	
(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl acetate	81781-24-0	1,343	21.019	1,339	4	83	8.459	1,342	1	82	-3	1	
exo-2-Hydroxycineole acetate	57709-95-2	1,344	21.273	1,345	-1	86	8.549	1,347	-3	89	-2	-2	
1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-, formate	29621-55-4	1,356	21.612	1,353	3	87	8.664	1,354	2	90	-1	-4	
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, (1R- <i>cis</i>)-	7111-29-7	1,358	21.856	1,359	-1	80	8.775	1,361	-3	79	-2	1	
Copaene	3856-25-5	1,376	22.546	1,375	1	91	9.024	1,376	0	84	-1	6	
2-Methyl-4-(2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-ol	56763-65-6	1,406	23.737	1,404	2	83	9.528	1,406	0	85	-2	-2	
<i>cis</i> -β-Copaene	18252-44-3	1,432	24.728	1,428	4	93	9.897	1,429	3	89	-1	3	
Sesquicineole, 7-epi-1,2-dehydro-	149067-90-3	1,471	26.498	1,472	-1	78	10.618	1,473	-2	79	-1	-1	
3-Tetradecen-5-yne, (E)-	74744-44-8	1,488	27.032	1,485	3	72	10.842	1,487	1	73	-2	-1	
Valencene	4630-07-3	1,492	27.311	1,492	0	97	10.930	1,493	-1	96	-1	0	
Caryophyllene oxide	1139-30-6	1,581	30.784	1,582	-1	73	12.320	1,582	-1	81	0	-7	
1,5,9-Cyclododecanetriol	2938-55-8	2,007	45.413	2,005	2	72	18.190	2,006	1	76	-1	-4	
3-Eicosyne	61886-66-6	2,032	46.366	2,036	-4	75	18.573	2,037	-5	76	-1	-2	
Uvidin C, diacetate	1000501-90-0	2,107	48.516	2,107	0	75	19.432	2,107	0	73	0	2	
(9E,11E)-Octadecadienoic acid	544-71-8	2,237	52.406	2,241	-4	74	20.997	2,241	-4	73	0	2	
Incensole oxide, acetate	1000513-23-1	2,270	53.383	2,275	-5	72	21.395	2,276	-6	69	-1	3	

Table 4. Analysis results for neroli oil with both the helium and hydrogen methods. (LR = low response; Int = interference.)

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H ₂	LMS He-LMS H ₂	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
Ethane, 1,1-diethoxy-	105-57-7	726	2.445	723	3	98	1.060	732	-6	97	-9	1	
3-Hexen-1-ol	544-12-7	856	3.982	851	5	93	1.685	856	0	80	-5	13	LR
1-Hexanol	111-27-3	868	4.184	863	5	95	1.762	867	1	92	-4	3	
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	2867-05-2	929	5.501	926	3	98	2.258	926	3	92	0	6	
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	932	5.688	933	-1	98	2.334	933	-1	98	0	1	
Camphene	79-92-5	952	6.087	948	4	98	2.496	949	3	97	-1	1	
Benzaldehyde	100-52-7	962	6.380	959	3	98	2.628	961	1	96	-2	2	
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	18172-67-3	978	6.899	978	0	97	2.824	980	-2	98	-2	0	
m-Mentha-4,8-diene, (1S,3S)-(+)-	5208-51-5	983	7.047	984	-1	82	2.875	985	-2	77	-1	5	
beta-Myrcene	123-35-3	991	7.240	991	0	97	2.952	992	-1	97	-1	0	
Cyclohexane, 1-methylene-4-(1-methylethenyl)-	499-97-8	1,004	7.667	1,005	-1	98	3.121	1,006	-2	97	-1	1	
cis-Anhydrolinalool oxide	54750-69-5	1,007	7.775	1,008	-1	93	3.166	1,009	-2	79	-1	14	LR, Int
3-Carene	13466-78-9	1,011	7.874	1,011	0	97	3.203	1,012	-1	97	-1	1	
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	1,017	8.082	1,017	0	97	3.285	1,018	-1	97	-1	0	
D-Limonene	5989-27-5	1,031	8.536	1,029	2	99	3.476	1,031	0	99	-2	0	
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	3338-55-4	1,038	8.782	1,036	2	97	3.562	1,037	1	97	-1	0	
trans-beta-Ocimene	3779-61-1	1,049	9.174	1,047	2	97	3.724	1,049	0	97	-2	0	
gamma-Terpinene	99-85-4	1,060	9.540	1,058	2	99	3.860	1,058	2	99	0	0	
trans-Sabinene hydrate	17699-16-0	1,070	9.843	1,066	4	90	3.994	1,068	2	81	-2	9	
cis-Linalool oxide	5989-33-3	1,074	10.047	1,072	2	98	4.071	1,073	1	96	-1	2	
1,4-Undecadiene, (Z)-	55976-14-2	1,080	10.241	1,077	3	81	4.138	1,078	2	80	-1	1	
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9	1,088	10.638	1,088	0	98	4.294	1,089	-1	98	-1	0	
Linalool	78-70-6	1,099	11.285	1,106	-7	98	4.618	1,111	-12	98	-5	0	
Phenylethyl alcohol	60-12-8	1,116	11.611	1,114	2	98	4.725	1,117	-1	96	-3	1	
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	29803-82-5	1,122	11.938	1,122	0	93	4.837	1,124	-2	93	-2	0	
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	7216-56-0	1,131	12.231	1,129	2	99	4.935	1,130	1	99	-1	0	
2-Isopropylimidazole	36947-68-9	1,132	12.318	1,131	1	71	4.973	1,132	0	70	-1	1	
cis-p-Mentha-2,8-dien-1-ol	3886-78-0	1,133	12.460	1,134	-1	83	5.029	1,136	-3	82	-2	1	
Benzyl nitrile	140-29-4	1,144	12.594	1,138	6	98	5.095	1,140	4	94	-2	5	
Myroxide	28977-57-3	1,140	12.747	1,142	-2	92	5.144	1,143	-3	71	-1	20	LR, Int
Terpinen-4-ol	562-74-3	1,177	14.182	1,177	0	96	5.709	1,178	-1	96	-1	1	
Benzenemethanol, alpha,alpha,4-trimethyl-	1197-01-9	1,183	14.515	1,185	-2	91	5.864	1,187	-4	90	-2	1	
alpha-Terpineol	98-55-5	1,189	14.790	1,192	-3	99	5.972	1,194	-5	99	-2	0	
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	116-26-7	1,201	15.153	1,200	1	78	6.099	1,202	-1	79	-2	-2	
(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	206115-88-0	1,202	15.481	1,208	-6	88	6.229	1,210	-8	89	-2	-1	
Acetic acid, octyl ester	112-14-1	1,210	15.648	1,212	-2	92	6.285	1,213	-3	89	-1	3	
Benzofuran, 2-ethenyl-	7522-79-4	1,220	15.994	1,220	0	89	6.428	1,221	-1	86	-1	4	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	106-25-2	1,228	16.337	1,228	0	97	6.577	1,230	-2	98	-2	0	
Neral	106-26-3	1,240	16.860	1,241	-1	90	6.776	1,242	-2	88	-1	2	
Carvone	99-49-0	1,242	16.966	1,243	-1	87	6.821	1,245	-3	86	-2	2	
Linalyl acetate	115-95-7	1,257	17.587	1,258	-1	95	7.079	1,260	-3	92	-2	3	
Citral	5392-40-5	1,273	18.124	1,270	3	93	7.283	1,272	1	89	-2	4	

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H ₂	LMS He-LMS H ₂	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (Z)-	2142-94-1	1,282	18.575	1,281	1	89	7.452	1,282	0	89	-1	0	
Levo-bornyl acetate	5655-61-8	1,285	18.780	1,286	-1	99	7.534	1,287	-2	95	-1	4	
Indole	120-72-9	1,294	18.998	1,291	3	99	7.637	1,293	1	99	-2	0	
Benzene, (2-nitroethyl)-	6125-24-2	1,302	19.280	1,298	4	89	7.746	1,299	3	86	-1	3	
Geranyl formate	105-86-2	1,300	19.483	1,303	-3	91	7.811	1,303	-3	91	0	1	
δ-Elemene	20307-84-0	1,338	20.958	1,338	0	86	8.392	1,338	0	96	0	-10	
Methyl anthranilate	134-20-3	1,343	21.020	1,339	4	94	8.439	1,341	2	98	-2	-4	
alpha-Terpinyol acetate	80-26-2	1,350	21.445	1,349	1	97	8.593	1,350	0	96	-1	2	
6-Octen-1-ol, 3,7-dimethyl-, acetate	150-84-5	1,354	21.647	1,354	0	84	8.669	1,354	0	81	0	2	
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	141-12-8	1,364	22.133	1,366	-2	99	8.881	1,367	-3	99	-1	0	
Copaene	3856-25-5	1,376	22.543	1,375	1	82	9.026	1,376	0	80	-1	2	
Geranyl acetate	105-87-3	1,382	22.966	1,385	-3	98	9.222	1,387	-5	98	-2	0	
levo-β-Elemene	515-13-9	1,391	23.236	1,392	-1	96	9.307	1,393	-2	95	-1	1	
Benzoic acid, 2-amino-, ethyl ester	87-25-2	1,414	24.091	1,413	1	86	9.662	1,414	0	93	-1	-7	
Caryophyllene	87-44-5	1,419	24.340	1,419	0	99	9.751	1,420	-1	99	-1	0	
gamma-Elemene	29873-99-2	1,434	24.921	1,433	1	83	9.977	1,434	0	88	-1	-6	
Humulene	6753-98-6	1,454	25.707	1,453	1	92	10.293	1,453	1	91	0	1	
(E)-beta-Farnesene	18794-84-8	1,457	25.894	1,457	0	96	10.363	1,458	-1	96	-1	0	
Alloaromadendrene	25246-27-9	1,461	26.002	1,460	1	92	10.408	1,460	1	84	0	8	
gamma-Murolene	30021-74-0	1,477	26.657	1,476	1	93	10.669	1,476	1	91	0	2	
Germacrene D	23986-74-5	1,481	26.824	1,480	1	95	10.736	1,481	0	97	-1	-2	
Bicyclogermacrene	24703-35-3	1,496	27.445	1,496	0	98	10.985	1,496	0	97	0	1	
alpha-Murolene	10208-80-7	1,499	27.605	1,500	-1	96	11.048	1,500	-1	93	0	3	
alpha-Farnesene	502-61-4	1,508	27.955	1,509	-1	94	11.186	1,509	-1	96	0	-1	
γ-Cadinene	39029-41-9	1,513	28.137	1,513	0	97	11.261	1,514	-1	96	-1	1	
δ-Cadinene	483-76-1	1,524	28.519	1,523	1	97	11.413	1,524	0	97	-1	1	
α-Cadinene	24406-05-1	1,538	29.047	1,537	1	80	11.626	1,537	1	81	0	-2	
alpha-Calacorene	21391-99-1	1,542	29.254	1,542	0	94	11.710	1,543	-1	82	-1	13	LR
β-Germacrene	15423-57-1	1,557	29.779	1,556	1	96	11.915	1,556	1	87	0	10	Int
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	7212-44-4	1,564	30.146	1,565	-1	97	12.091	1,567	-3	97	-2	0	
Spathulenol	6750-60-3	1,576	30.574	1,576	0	93	12.244	1,577	-1	85	-1	9	
Caryophyllene oxide	1139-30-6	1,581	30.788	1,582	-1	92	12.329	1,583	-2	90	-1	2	
tau-Cadinol	5937-11-1	1,640	32.985	1,641	-1	96	13.198	1,641	-1	95	0	2	
δ-Cadinol	19435-97-3	1,645	33.163	1,646	-1	92	13.276	1,646	-1	88	0	4	
alpha-Cadinol	481-34-5	1,653	33.462	1,654	-1	96	13.396	1,654	-1	94	0	2	
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	483-78-3	1,674	34.205	1,675	-1	92	13.695	1,674	0	78	1	14	LR, Int
8-Heptadecene	2579-04-6	1,677	34.320	1,678	-1	95	13.731	1,677	0	94	1	2	
6,10-Dodecadien-1-ol, 3,7,11-trimethyl-	51411-24-6	1,692	34.795	1,691	1	81	13.928	1,690	2	87	1	-6	
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	3790-71-4	1,697	35.110	1,700	-3	92	14.058	1,699	-2	89	1	3	
trans-Farnesol	106-28-5	1,722	35.968	1,724	-2	97	14.418	1,724	-2	98	0	-1	
Farnesol, 2E, 6Z-	3879-60-5	1,742	36.652	1,743	-1	79	14.676	1,743	-1	78	0	1	
all-trans-Farnesyl acetate	4128-17-0	1,843	40.112	1,842	1	94	16.057	1,843	0	92	-1	3	
Cubitene	66723-19-1	1,878	41.166	1,874	4	82	16.479	1,874	4	78	0	3	
m-Camphorene	20016-73-3	1,960	43.728	1,952	8	95	17.508	1,953	7	93	-1	3	
p-Camphorene	20016-72-2	1,995	44.815	1,986	9	92	17.942	1,986	9	91	0	1	
Hexadecanoic acid, ethyl ester	628-97-7	1,993	45.083	1,995	-2	73	18.054	1,995	-2	75	0	-2	

Conclusion

Using the techniques described in this application note, a typical method for the qualitative analysis of essential oils was successfully converted to a method using hydrogen carrier gas. The resulting hydrogen method retains the same chromatographic resolution and relative elution order of the original method, but with a run time 2.5 times shorter. The column capacity with the new method is reduced, being a calculated 36% of the original method, so the amount injected may need adjustment in some cases.

The new hydrogen method was then applied to the analysis of two essential oils and found to generate results comparable to those from the helium method. The use of spectral deconvolution and retention index search filtering with Agilent MassHunter Unknowns Analysis gave improved search results that were faster than previous identification methods. The NIST23 library, with expanded content for essential oil components and RIs, allowed identification of a significant portion of the compounds present.

The Agilent HydroInert source is a key component in the successful conversion to hydrogen. Without it, in-source reactions were shown to degrade the spectra of some compounds to the point where they were misidentified.

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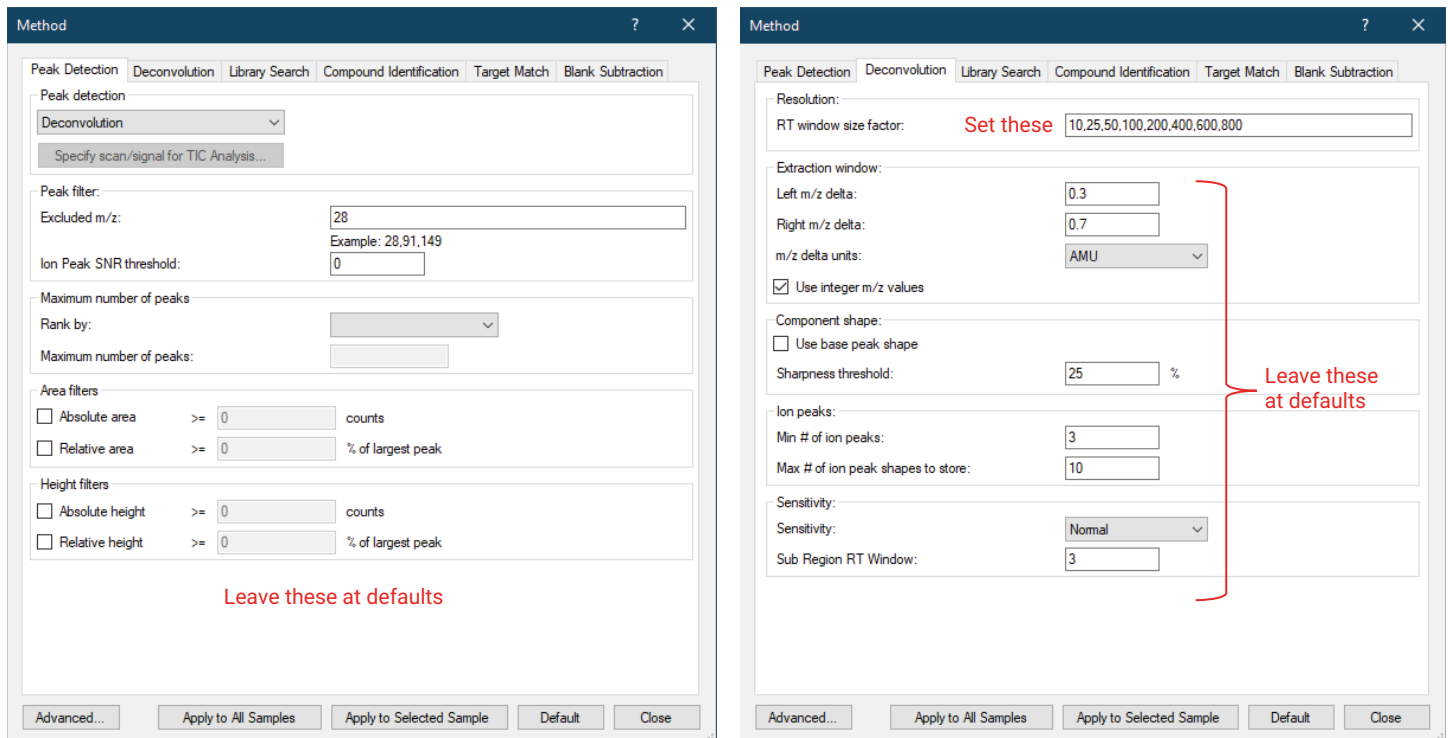
Appendix

Setting up MassHunter Unknowns Analysis

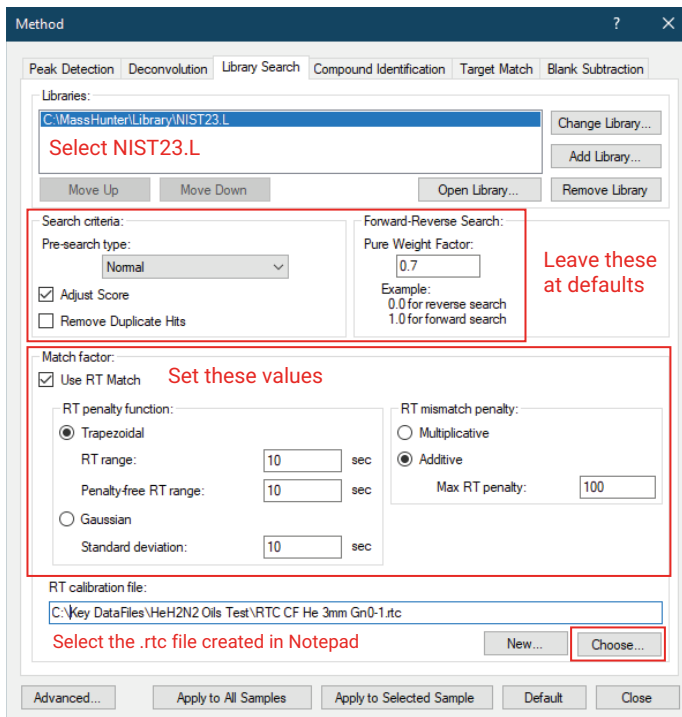
This section shows how to set up Agilent MassHunter Unknowns Analysis. The parameters shown here were used in this specific application. For other applications, different parameters can be used to optimize the process.

Locate the Agilent MassHunter Quantitative Analysis software folder under the Microsoft Windows Start menu and open the MassHunter Unknowns Analysis program.

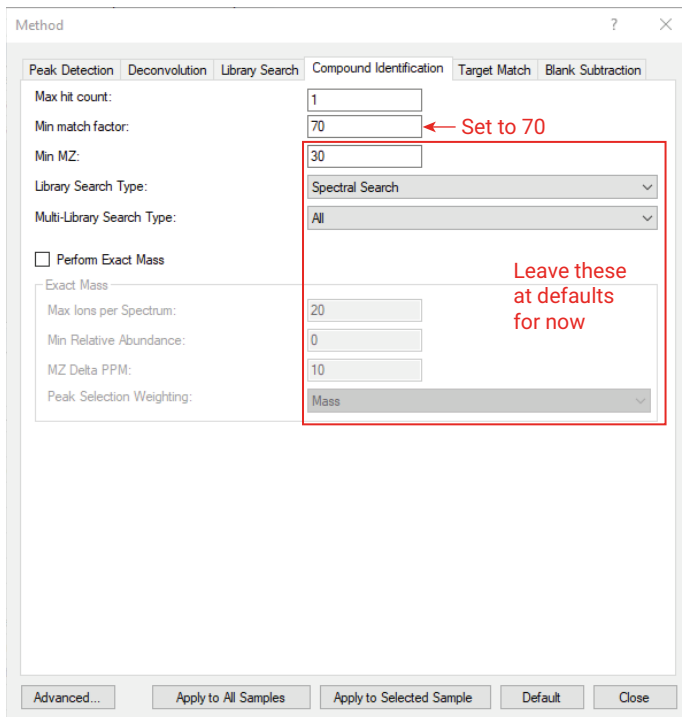
1. Click **File > New Analysis**. Navigate to the directory containing your data files.
2. Enter a file name for the analysis.
3. Click **File > Add Samples**. Select the data files that you want to process. You should see the TIC of your chromatogram appear.
4. Click **Method > Edit**. Set the parameters as shown in Appendix Figures 1 to 5.



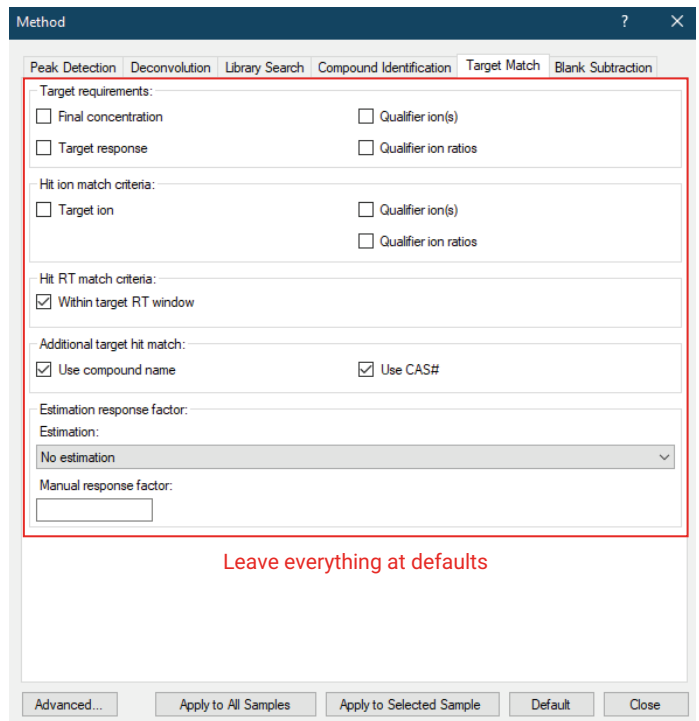
Appendix Figure 1. Setting the parameters for the Peak Detection and Deconvolution tabs in Agilent MassHunter Unknowns Analysis.



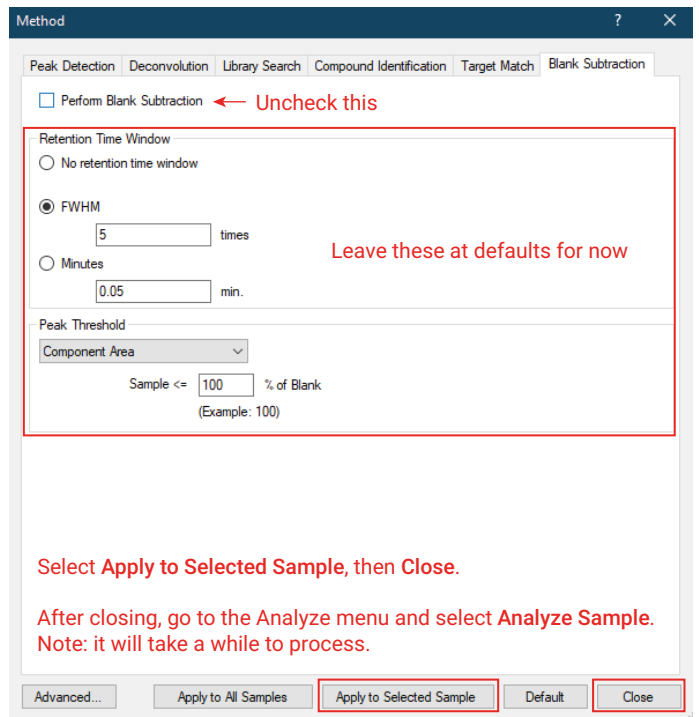
Appendix Figure 2. Setting the parameters for the Library Search tab in Agilent MassHunter Unknowns Analysis.



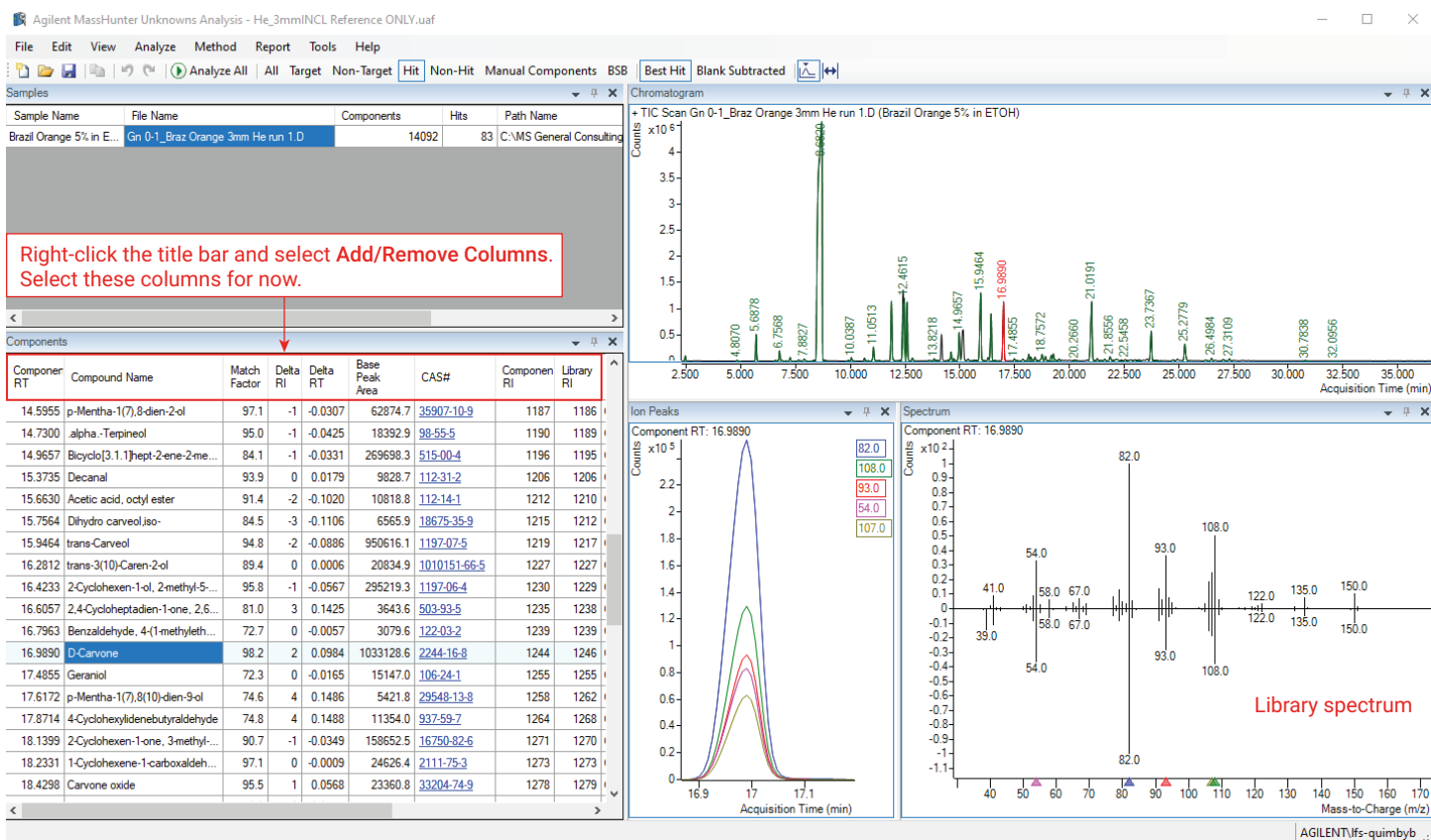
Appendix Figure 3. Setting the parameters for the Compound Identification tab in Agilent MassHunter Unknowns Analysis.



Appendix Figure 4. Setting the parameters for the Target Match tab in Agilent MassHunter Unknowns Analysis.



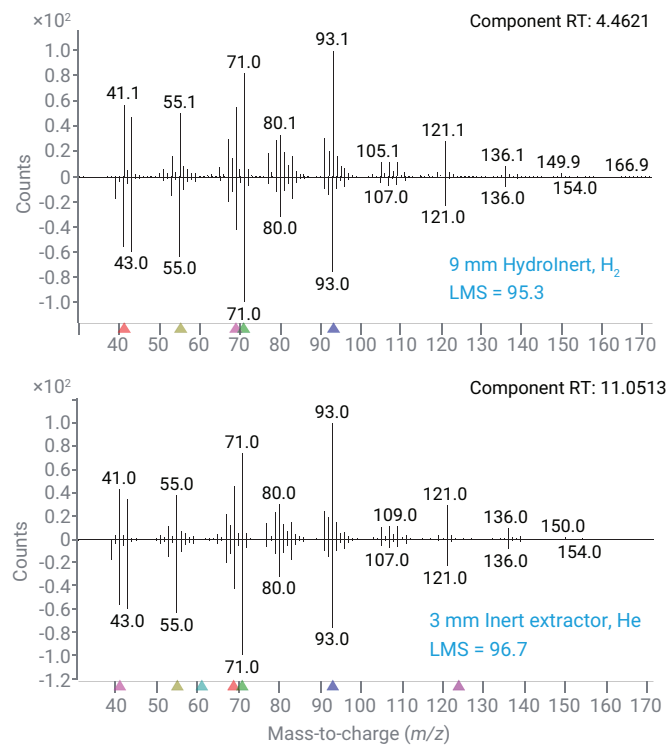
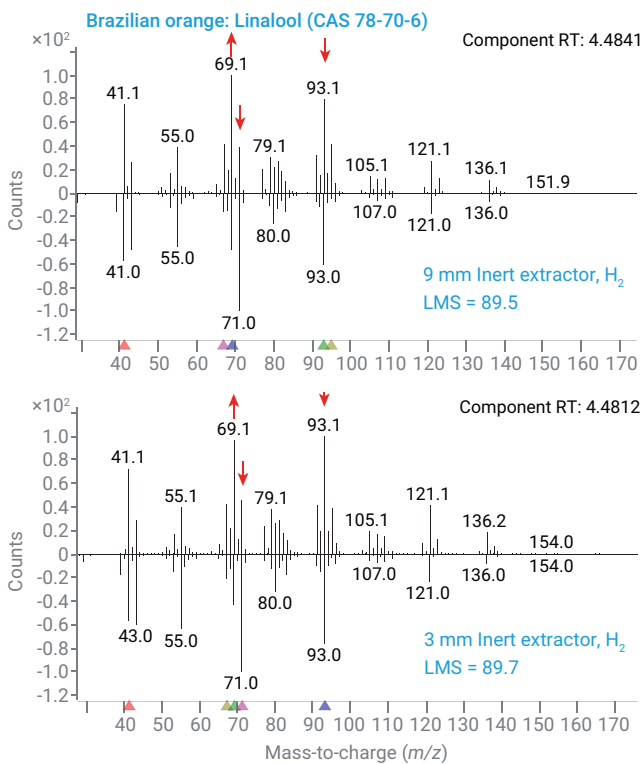
Appendix Figure 5. Setting the parameters for the Blank Subtraction tab in Agilent MassHunter Unknowns Analysis.



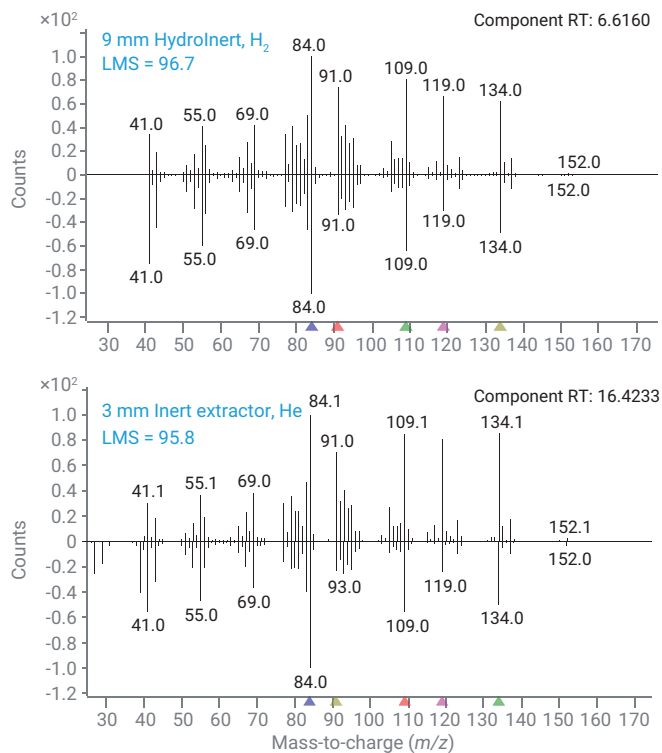
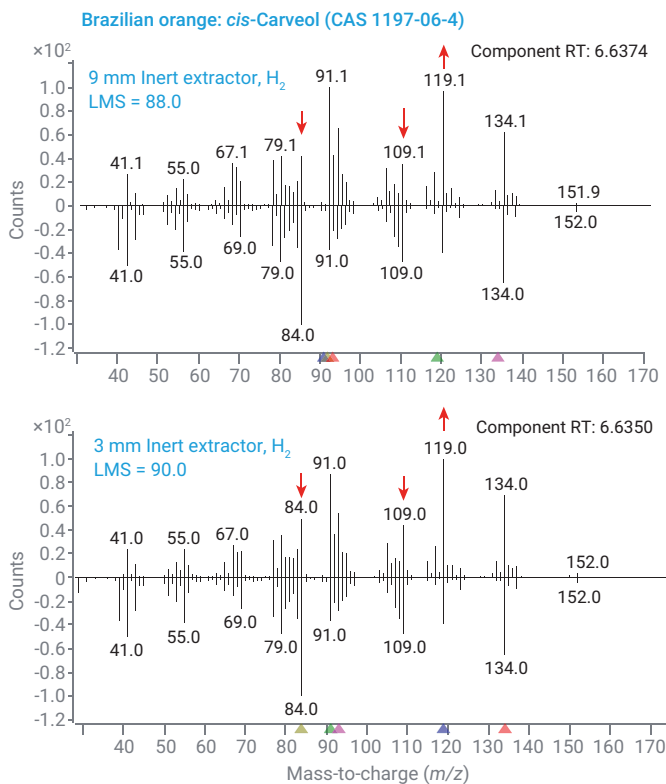
Appendix Figure 6. Example result of a deconvoluted/searched data file in Agilent MassHunter Unknowns Analysis.

More examples of in-source reactions eliminated with the HydroInert source

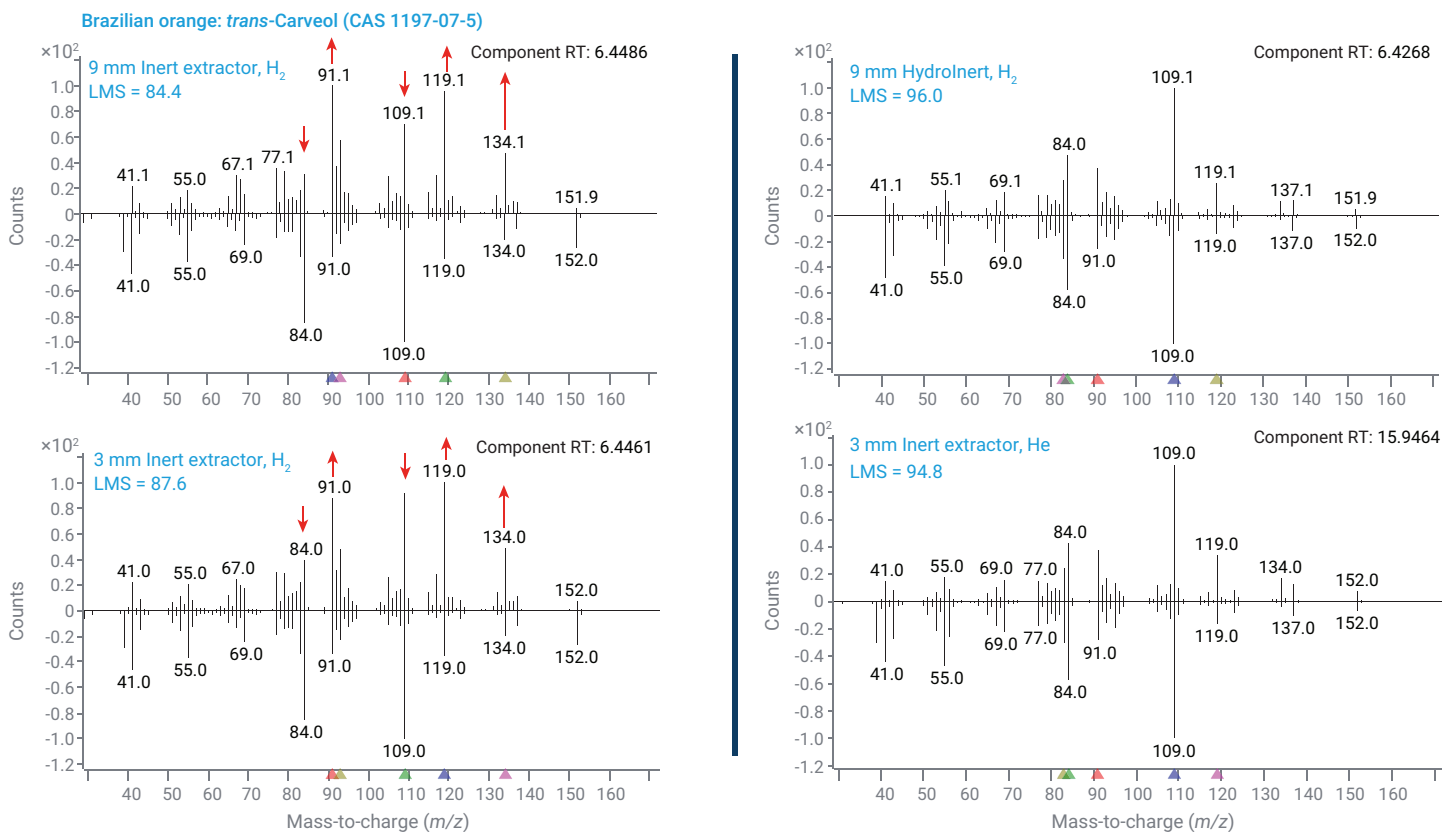
In this section, the spectra of several compounds from the Brazilian orange and neroli oils are examined. For each compound, the spectrum obtained using helium and the 3 mm inert extractor source is presented with the spectrum for each compound obtained using hydrogen with the 9 mm HydroInert source, and the inert extractor source with the 9 and 3 mm extractor lenses. As shown in Figures 7 to 11, changes in the hydrogen spectra without the HydroInert source are clear, resulting in reduced LMS values and reduced confidence in identification.



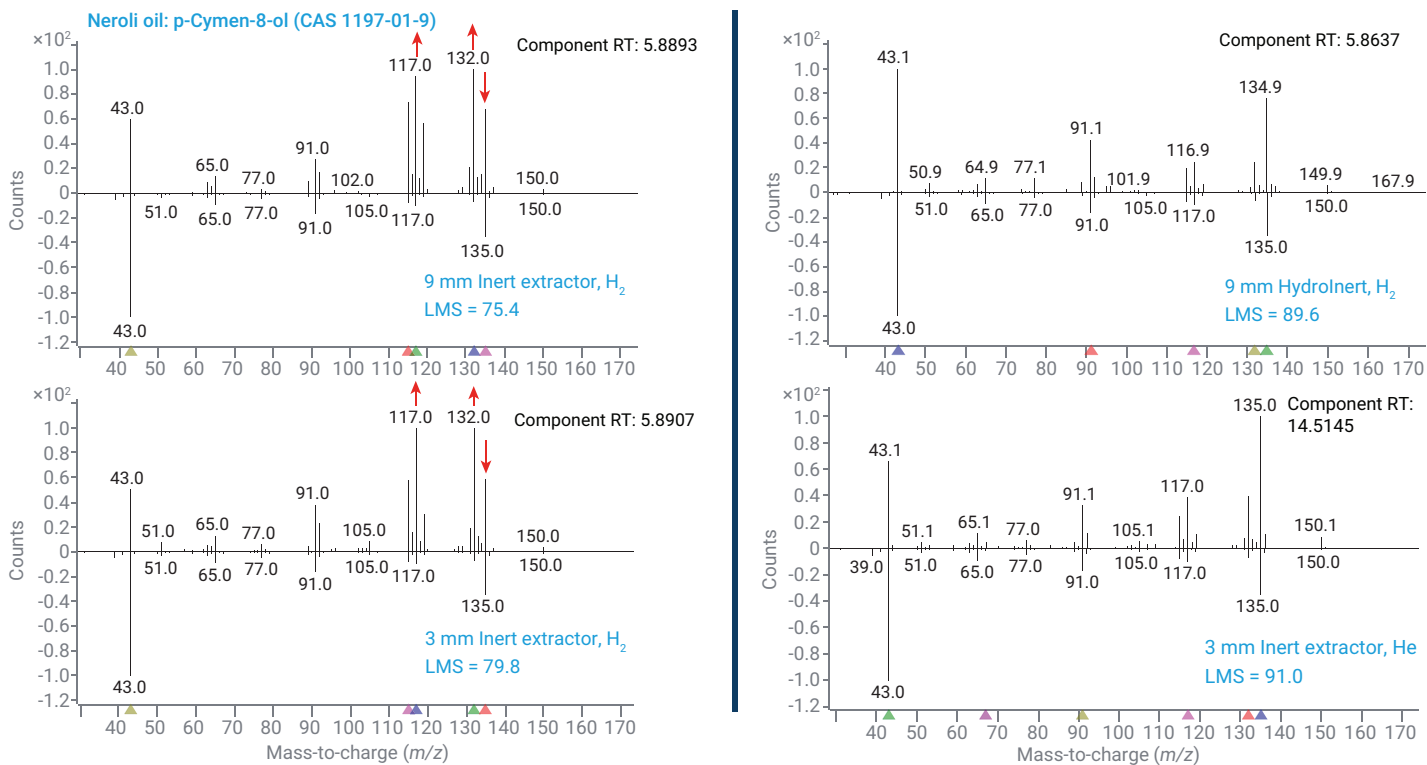
Appendix Figure 7. Linalool in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-Hydrolnert sources.



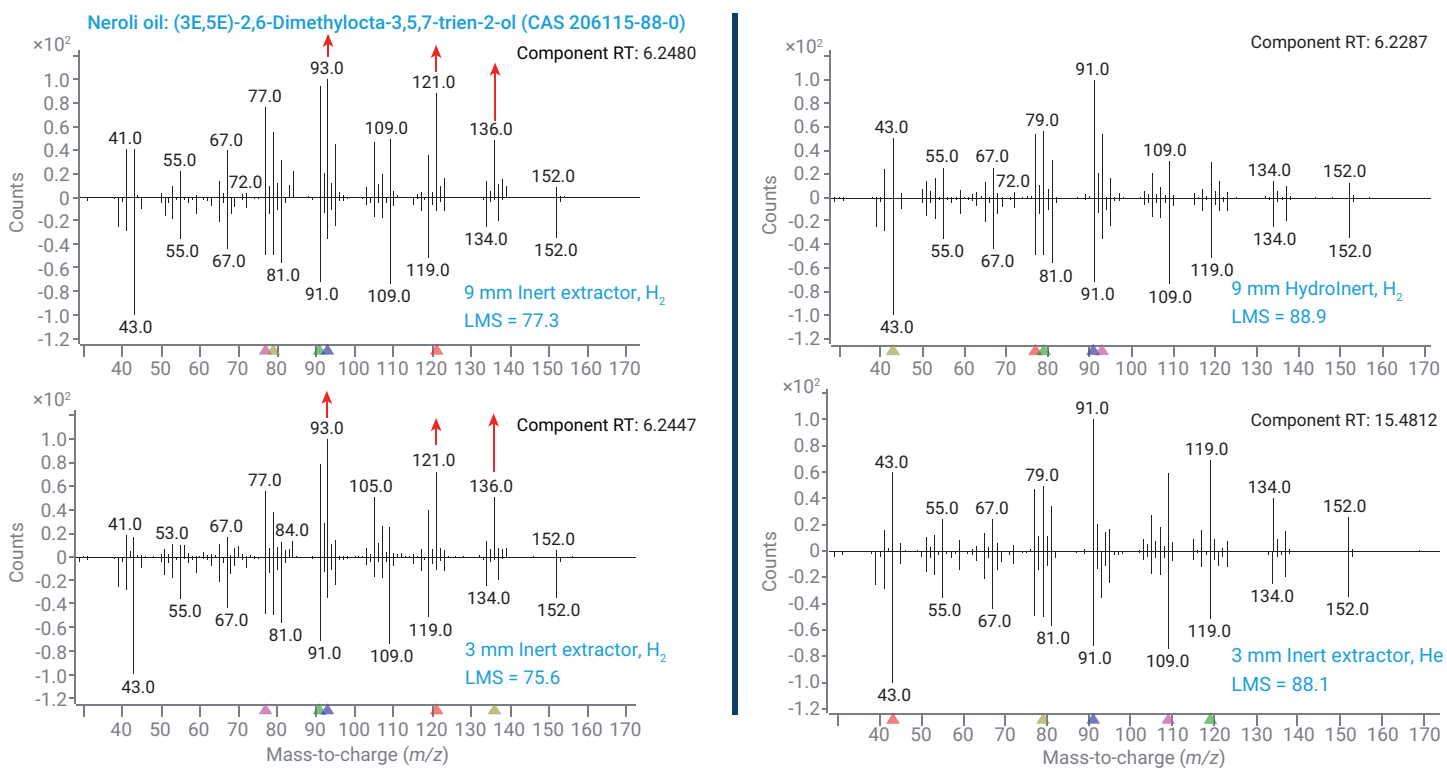
Appendix Figure 8. cis-Carveol in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-Hydrolnert sources.



Appendix Figure 9. *trans*-Carveol in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-Hydrolnert sources.



Appendix Figure 10. *p*-Cymen-8-ol in neroli oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-Hydrolnert sources.



Appendix Figure 11. (3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol in neroli oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.