Profiling Flavors and Fragrances in Complex Matrices Using Linear Retention Indices Without Sample Preparation

Abstract

This Application Note describes a method for the analysis of flavor and fragrance compounds. The method deploys gas chromatography/mass spectrometry (GC/MS) using backflush and a thermal separation probe at constant flow with an Agilent Intuvo 9000 GC and an Agilent 5977B GC/MSD. Flavor and fragrance compounds in complex matrices such as soap, scented candles, toothpaste, cream liqueur, and fabric softener were analyzed without sample preparation. The samples were introduced into the GC/MSD system using the thermal separation probe. Identification was done using deconvoluted mass spectra and incorporating linear retention index results. In addition, a hyperlink was created to connect the compound name with a fragrance and flavor website to obtain organoleptic and cosmetic information.
Introduction

The Agilent Thermal Separation Probe (TSP) is ideal for fast GC/MS analysis of a variety of dirty liquid and solid samples in food testing, forensics, and environmental applications. Advantages of the thermal separation probe include:

- Little or no sample preparation required
- Greater flexibility with less risk than traditional direct sample probes
- Low risk of contamination or decrease in performance typically associated with traditional direct sample probes
- Control sample delivery by adjusting split flow ratios, eliminating the possibility of overload or contamination of the detector
- Temperature programming in the inlet and GC column for improved identification of multicomponent samples, which is not possible with traditional direct sample probes
- Two ways to use TSP with GC/MS systems: separate complex samples using a longer analytical column, or transfer the neat sample to the MS using a short deactivated capillary column

GC/MS has been used for many years for the analysis of fragrance and flavor compounds. GC/MS is probably the most powerful technique, and extended mass spectral libraries are available, but do not enable complete identification. In flavor and fragrance quality control, GC/MS with retention indices (RIs) is still frequently used as a complementary technique to GC/MS. Several libraries are available with RIs for many flavor and fragrance compounds. RIs are less dependent on operational parameters than absolute retention times, but they still depend significantly on the column type (stationary phase and supplier), temperature program, and, to a lesser extent, on the carrier gas velocity. Therefore, it is sometimes difficult to reproduce published RIs in different laboratories. Most companies in the flavor and fragrance industry use in-house methods based on historical choices of columns and conditions.

Agilent MassHunter Unknowns Analysis software uses a deconvolution process that helps identify compounds even when they are hidden under coeluting matrix compounds. The deconvolution process is automated, and takes approximately 1 to 2 minutes for a total ion chromatogram (TIC). The process not only allows analysts to get reliable and reproducible results fast, but also minimizes false positives and false negatives.

Because many retention data are already available as RIs, it was also evaluated whether these data could be transferred into absolute retention times that match with experimental data. In this study, two libraries with RIs were used: NIST 2017, and the Agilent flavor and fragrance RTL library.

The innovations in the Intuvo 9000 GC include:

- A direct heating system, which is faster, uses half the power, and takes half the bench space of a conventional air-bath oven
- Ferrule-free direct connections with a plug-and-play flowpath eliminate a major source of complexity and leaks.
- The unique disposable Intuvo Guard Chip eliminates the need for column trimming.

Experimental

Analyses were performed on an Intuvo 9000 GC equipped with a multimode inlet (MMI) and post column backflush. The MMI was set to 60 °C to introduce the TSP at a low temperature, thus avoiding loss of light components before the actual injection occurred. After introducing the TSP, the MMI temperature was ramped to 280 °C at 600 °C/min. Separation was done on an Agilent HP-5MS, 30 m × 0.25 mm id, 0.25 µm column (β = 250) (p/n 19091S-433-INT). Helium at approximately 65 kPa (9.43 psi) at constant flow was used as the carrier gas. Table 1 summarizes the analytical conditions.

Table 1. GC/MS analytical conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>HP-5MS, 30 m × 0.25 mm id, 0.25 µm (p/n 19091S-433-INT)</td>
</tr>
<tr>
<td>Injection</td>
<td>MMI, split ratio 100:1, 0.2 minutes 40 °C, then 900 °C/min to 300 °C</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Helium (13.4 psi), constant flow</td>
</tr>
<tr>
<td>RTL</td>
<td>Flow set to 1.46 mL/min to give a retention time of 32.000 minutes for n-hexadecane</td>
</tr>
<tr>
<td>Oven program</td>
<td>60 to 240 °C at 3 °C/min (60 minutes analysis time)</td>
</tr>
<tr>
<td>Guard Chip</td>
<td>Intuvo, multimode inlet</td>
</tr>
<tr>
<td>Temperature program</td>
<td>Track oven</td>
</tr>
<tr>
<td>Detection</td>
<td>MSD XTR 6 mm in scan mode (40 to 400 amu) solvent delay: 0 minutes transfer line: 300 °C</td>
</tr>
</tbody>
</table>
An alkanes mix from C₆ to C₄₄ was injected using the conditions described in Table 2 to generate the calibration retention time (CRT) file, and MassHunter was used to analyze the file and calculate library retention times.

Data were processed with MassHunter Unknowns Analysis software, using a deconvolution process and RIs from two libraries. The software calculates the library retention time using the CRT file and the RI from the different libraries, and calculates the difference between library retention time and real retention time. By applying the retention time filter and the minimum match factor, it was possible to eliminate misidentification.

**Transformation of RIs**

The linear retention index (LRI) requires that an n-alkane mixture is analyzed in the elution range of the analytes of interest. Each n-alkane is assigned an LRI value based on the number of carbon atoms multiplied by 100. For example, octane (n-C₈) is assigned an LRI of 800, nonane (n-C₉) is assigned an LRI of 900, decane (n-C₁₀) is assigned an LRI value of 1,000, and so on. The LRI of a given analyte was calculated according to where it elutes relative to the n-alkanes that eluted immediately before and after the analyte.

For temperature-programmed GC, the LRI is calculated by the following equation:

\[ I = 100 \times \left[ n + \frac{t_r(\text{unknown}) - t_r(n)}{t_r(N) - t_r(n)} \right] \]

where:

- \( I \) = Linear retention index
- \( n \) = Number of carbon atoms in the n-alkane eluting immediately before the analyte
- \( N \) = Number of carbon atoms in the n-alkane eluting immediately after the analyte
- \( t_r \) = Retention time.

From the retention index, the absolute retention time was calculated using the retention times of n-alkanes as reference compounds.

These absolute retention times are not the original retention times used for the retention index calculation, but calculated values. This means that retention times can be calculated from the RIs present in an existing database using the locked retention times for n-alkanes if the column dimensions and the temperature program are the same.

This study analyzed several samples with no sample preparation. Only a few milligrams were introduced into the microvial (vial volume 40 µL). The selected samples were chosen because of their organoleptic properties, and because it was not possible to inject directly into the GC port using a syringe due to the complex matrix.

**Results and discussion**

Figures 1 and 2 show chromatograms with identified compounds and TICs of toothpaste, fabric softener, scented candle, and cream liqueur samples. Figure 3 shows the library search results from the MassHunter Unknowns Analysis software. Also, we can use the information from the Web to find out if the identified compound has a relationship with the flavor and fragrance industry (see Figure 4). If the message *Sorry, your search: "Docosyl octyl ether" returned zero results.* is displayed, this compound has no relationship with flavor or fragrance ingredients.

### Table 2. MassHunter Unknowns Analysis method parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT window size factor</td>
<td>25, 50, 100, 200</td>
</tr>
<tr>
<td>Peak filter SNR threshold</td>
<td>5</td>
</tr>
<tr>
<td>Match factor (RT penalty)</td>
<td>Enable Trapezoidal RT range: 60 seconds Penalty free RT range: 30 seconds</td>
</tr>
<tr>
<td>Min match factor</td>
<td>75</td>
</tr>
<tr>
<td>Library search type</td>
<td>Spectral search</td>
</tr>
</tbody>
</table>
Figure 1. Toothpaste and fabric softener chromatograms showing identified compounds and TIC.
Figure 2. Scented candle and cream liqueur chromatograms showing identified compounds and TIC.
Figure 3. MassHunter Unknowns Analysis software enables the evaluation of an alternative using the match factor and retention time. This information can be sorted by the match factor or by the retention time difference between the component RT and the library RT.

Figure 4. Information about eugenol and stearyl alcohol obtained from the Web, and the hyperlink was used to create a MassHunter Unknown Analysis.
Figure 5. Analysis of the toothpaste sample. Some components were identified by the Agilent library, others were identified by NIST17. In both cases, the system calculates the retention time using the retention index from the libraries. Also, there are two hyperlinks: the hyperlink for the CAS number goes to the NIST webpage to get chemical information, and the hyperlink for the compound name goes to the Good Scents company webpage to get information about organoleptic and cosmetic properties, suppliers, safety data sheet, and so on.

Figure 6. In this table, the RT match penalty was disabled, resulting in lauryl alcohol being detected 10 times, all of them with a match factor over 84. But only one has the lower delta RT and the highest match factor. This feature made compound identification easier. When this feature was enabled, only one compound appeared in the search results.
Figure 7: MassHunter Unknowns Analysis software uses a deconvolution algorithm to separate two coeluting compounds. The undeconvoluted spectrum (A), compound deconvoluted spectrum (B), and library spectrum (C) are displayed in the same window to make data review easier.
Conclusions

A method was developed for the analysis of flavors and fragrances in complex matrices without sample preparation. A small quantity of sample was deposited into the microvial, and inserted in the inlet port. The method may be used for quality control analysis. The method is retention-time-locked using n-hexadecane as the locking standard. A retention index database, containing approximately 400 compounds, was modified from an existing method. This database can be used to identify constituents based on their absolute retention time under the locked conditions. The locked method also guarantees retention time stability as a function of time, between columns, and between instruments. Finally, it is shown that RIs for flavor compounds measured under specific operational conditions can be transferred into locked retention times using the locked retention times of n-alkanes. Thus, existing RI databases can be translated into locked retention time databases.

References

7. Sandy, C.; Butler, I. Incorporating Retention Index Results in Deconvoluted GC/MS Library Search Data, Agilent Technologies Data Sheet, publication number 5991-8221EN, 2017.

Appendix

Figure 8 shows the workflow for creating deconvoluted GC/MS libraries with LRI values, and processing sample data files in MassHunter Unknowns Analysis software. The full procedure can be found in the Agilent Technologies Data Sheet *Incorporating Retention Index Results in Deconvoluted GC/MS Library Search Data*, publication number 5991-8221EN.

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1. Acquire data files for the n-alkane standard mixture, reference standard mixtures, and test sample.

2. Use MH Unknows Analysis to deconvolute and library search the mass spectra of components in the n-alkane standard mixture against the NIST14 EI GC/MS library.

3. Add the deconvoluted n-alkane mass spectra to a user .xml library, and assign nominal LRI values to each entry.


5. Use MH Unknows Analysis to deconvolute and library search the standard mixture data files against the NIST14 EI GC/MS library using the .RTC file to calculate LRI values; then, create a user .xml library.

6. Process the sample data file in Unknowns Analysis using the user-created LRI library and NIST14.

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Figure 8. Workflow for creating deconvoluted GC/MS libraries with LRI values.
To provide qualitative information on the components of complex flavor and fragrance mixtures, user libraries can be searched in combination with commercially available GC/MS libraries (such as NIST17). Deploying retention time locking or linear RIs can improve confidence in analyte identification by reducing false positives. Mass spectral deconvolution provides better quality mass spectra of closely eluting/overlapping components when searching in GC/MS libraries.

To get more information about a selected compound, a hyperlink can be created in MassHunter Unknowns Analysis software.

1. Open the QuantAnalysis.exe.config file in the path: ProgramFiles\Agilent\MassHunter\Workstation\Quant\bin\ and edit the link to the desired URL. This file is read-only by default, so you need to disable this feature.
2. Open the file, and look for the line: <add key="Column.CASNumber. Action" value="URL:http://webbook.nist.gov/cgi/cbook.cgi?ID={0}">
3. Add the next line to create the new hyperlink into MassHunter for the compound name <add key="Column.CompoundName. Action" value="URL:http://www.thegoodscentscompany.com/search3.php?qName={0}">
4. Save the file.
5. Start MassHunter Unknowns Analysis software. Now, you have a new hyperlink in the compound name field that connects with the Web to get organoleptic and cosmetic information, data sheet, and so on (Figures 9 and 10).

Figure 9. Hyperlink connecting a compound name to a web page, in MassHunter Unknowns Analysis software.
Figure 10. Example of compound information from an associated webpage (http://www.thegoodscentscompany.com).