Enhanced Essential Oils Analysis Using a High Performance Benchtop GC/MS System

Application Note

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Abstract
A gas chromatograph (GC) coupled with a mass spectrometer (MS) forms a powerful tool for high quality quantitative and qualitative analysis. However, GC/MS analysis of complex mixtures such as essential oils often is a time-consuming task due to limitations in techniques and instrumentation. Faster analyses of these mixtures are possible by coupling the 5973A mass selective detector (MSD) with a 100 µm ID GC column. The faster scan rate of the 5973A MSD takes advantage of the 100 µm ID column's higher resolution. In a comparison of lemon oil analyses using 100 µm ID and 250 µm ID columns, the 100 µm columns yielded significantly faster results. A few modifications to standard operating parameters are required; method translation software, which can be downloaded from the web site, facilitates this process. Sample throughput using 100 µm columns may be increased up to four-fold using faster oven ramping, though this can result in a minor, progressive loss of resolution. Faster oven ramp rates may also lead to a change in elution order, but the MSD ensures correct peak identification.

Key Words
gas chromatography, mass spectrometry, fast GC, lab productivity, flavors analysis, essential oils analysis, fragrance analysis, 100 µm columns, method translation software

Introduction
For two decades, GC/MS systems have been renowned for their rugged reliability and high quality results. While recent developments have focused on improvements to sensitivity, many laboratories also express the need for enhanced GC performance and quicker turnaround times. Increased laboratory productivity through more efficient techniques and instrumentation results in lower operating costs and higher revenues.

Analyses of flavors, fragrances, and other essential oils can be particularly complex and time-consuming. These samples are typically comprised of numerous components, many of which are structural or geometric isomers that often co-elute when analyzed using standard GC columns. High resolution capillary columns and slow oven temperature ramps are commonly used in GC/MS analyses.
to separate and identify these complex samples, resulting in analysis times of 15 to 60 minutes or longer. However, some analyses do not require as stringent parameters as others. For instance, flavor and fragrance analyses generally do not push instrument detection limits. Split injections are typical, and the dynamic range needed is a moderate two to three orders of magnitude. Furthermore, the highest mass compound to be analyzed is usually less than 300 amu. The capabilities of a mass spectrometer render it a good detector for these analyses.

Columns for GC/MS analyses typically have inner diameters (ID) ranging from 200 to 320 µm. However, the qualities of 100 µm columns make them more suitable for essential oils analyses. The higher resolution achievable with these columns can considerably shorten the analysis time with little loss in separation power. The limited sample capacity of these columns is sufficient for essential oils analyses, which typically do not require a wide range in sample amounts. In the past, one barrier to using 100 µm columns was the detector’s relatively slow scan speed. These very narrow bore columns require scan speeds approaching 3000 amu per second to acquire enough scans across the eluting GC peak to yield a usable mass spectrum. This paper presents data showing the current generation Agilent Technologies benchtop mass selective detector (5973A) has the required scan speeds to take advantage of the enhanced resolution offered by the 100 µm columns and accelerate essential oils analysis.

**Experimental Design**

**GC/MSD System**

All experiments were performed using an 6890 GC equipped with a standard 110 V oven and a split/splitless injection port with standard pressure pneumatics. The 6890 series has several features to facilitate faster GC methods, including:

- a 100 and 150 psi EPC split/splitless inlet
- automated split ratios to 7500:1
- fast detector sampling rates (0.1–200 Hz)
- fast oven ramping rates

The experiment utilized the 5973A MSD turbomolecular pump version (the G1099A MSD) operating in electron impact mode, but this analysis may also be run using the diffusion pump version (the G1098A MSD). Several factors are responsible for the improved performance of this MSD over its predecessors, among them:

- improved electronics, including an optimized logarithmic amplifier operating at twice the normal speed
- a gold-plated quartz quadrupole that yields better mass spectral peak shape
- an enhanced detector that increases the signal-to-noise even at high scan speeds

Two columns were used in the experiment: a 250 µm ID × 30 m × 0.25 µm film thickness HP-5 MS column (part number 19091S-433), and a 100 µm × 10 m × 0.34 µm film thickness HP-5 column (part number 19091S-003), both paired with the MSD in capillary direct mode.

Method parameters used for the two columns are listed in Table 1. Although the same sample was used for both columns, method parameters for the 100 µm ID × 10 m × 0.34 µm HP-5 column were altered slightly to preserve chromatographic resolution and data integrity. Specifically:

- the split ratio was increased to avoid overloading the smaller bore column
- the scan cycle rate was increased to sample the narrower chromatographic peaks accurately and to avoid “tilting” the spectrum during acquisition
- the final oven temperature was increased for the 20°C/min and 50°C/min runs to allow the last component to elute during the oven ramp
- the GC/MSD interface temperature was increased due to the higher final oven temperature
- the solvent delay was altered depending on the oven ramp rate chosen (e.g., the solvent delay was 2 minutes for 5°C/min and 1 minute for 50°C/min)
The program offers several modes of method translation. The “translate only” option maintains the relative efficiency of the current method, but alters the column dimensions, carrier gas type, outlet pressure and/or phase ratio as required. In contrast, the “best efficiency” option calculates the optimal temperature and flow rate to achieve the greatest separation efficiency for most compounds based on user-defined conditions. The “fast analysis” option achieves twice the speed of the “best efficiency” mode by altering the head pressure and oven temperature. Finally, the “none” option allows the user complete flexibility with all test parameters. Particulars of the method translator are discussed in other publications.4

This experiment utilized the “translate only” option to convert the original method used with the 250 µm column to a similar method for the 100 µm column. This software proposed a linear velocity of 39 cm/sec for the 100 µm column and suggested that...
the oven ramp rate for the column could be raised to 55°C/minute. However, this exceeded the controlled oven ramp rate for this instrument, and the maximum controllable ramp rate of 50°C/minute was substituted.

Results and Discussion

Figure 1 compares the chromatograms of both columns with a similar linear velocity and oven temperature ramp. For both samples, the oven program rate is 5°C/minute and the analysis time for the last major peak is 17 minutes, while trace components continue to elute for 24 minutes. The phase in the 100 µm column is HP-5, but the phase in the 250 µm column is HP-5MS. Note the similarity of the chromatographic patterns, despite the slightly different stationary phases.

Faster Analyses

Figure 2 illustrates the effects of the enhanced parameters suggested by the methods translator software on the 100 µm column (top). More importantly, it points out the major advantage of using 100 µm columns and faster oven ramp rates. At 50°C/min, the last major peak in the 100 µm column elutes in under 3.2 minutes, and the last components elute in under 4 minutes. The analysis using the 100 µm column is virtually finished before the first sample peak elutes from the 250 µm column (shown in the lower chromatogram). This is over five times the analysis speed achieved with the 250 µm column. Even considering the cool down time of the oven, it is still possible to run three to four times as many
samples on the 100 µm column as on the 250 µm column in the same amount of time. Differences in this accelerated chromatogram will be discussed later in this paper.

This dramatic increase in analysis speed when using a mass selective detector is a direct result of the faster scan speeds achievable by the 5973A MSD. In spite of the narrower chromatographic peak widths obtained when using the 100 µm column, the 5973A MSD will scan fast enough to accurately reflect the peak shape. In Figure 3, the chromatographic peak width at half height is about 0.005 minutes (300 milliseconds), and the baseline-to-baseline width is about 1 second. However, with the MSD operating at close to 10 scans per second, nine to 10 scans across the peak are acquired as the compound elutes, preserving the chromatographic integrity.

**Spectral Quality**

The 5973A MSD can scan quickly enough to meet the demands of a 100 µm column, but what is the quality of spectra obtained under these conditions? This question can be answered by a comparison of spectral data obtained for a given compound eluting under the two different chromatographic methods. A compound, trans-caryophyllene, from a later-eluting group of compounds (comprised of five major peaks) was selected to compare spectral data. Refer to the lower chromatogram in Figure 2 (after 13 minutes) to observe the group with the following elution order: neryl acetate, geranyl acetate, trans-caryophyllene, α-bergamotene and β-bisabolene.

The mass spectrum for the trans-caryophyllene peak, eluting at a retention time of 14.58 minutes for the separation with the 250 µm HP-5MS column, is shown in the upper part of Figure 4. Trans-caryophyllene is a minor component representing approximately 0.3% of the sample on an area percent basis. Yet, the spectrum is very strong, all isotopes are present, and the spectral skewing is minimal. Moreover, the data matched perfectly with the trans-caryophyllene spectra contained in the Wiley spectral library.
Figure 5 shows a mass spectrum from the same region of the chromatogram using data from the 100 µm column where the same five peaks are present, but the elution order has changed (this phenomenon is discussed later in this paper). Once again, the mass spectrum of the smallest of the five peaks, trans-caryophyllene, retention time 2.98 minutes, is displayed in the upper window. Even though this spectrum was taken at eight times the scan speed of the spectrum shown in Figure 4, it is virtually identical; and once again, the spectrum is a perfect match with the Wiley library spectra.

Elution Order Changes

As previously noted, the elution order of certain peaks differed between the 100 µm and 250 µm columns. To explore this further, more analyses were run on the 100 µm column at various oven ramp rates. The results are summarized in Figure 6. The peak identities on the bottom chromatogram are Z-citral, E-citral, neryl acetate, geranyl acetate, trans-caryophyllene, α-bergamotene and β-bisabolene. The oven ramp rates are, from bottom to top, 5, 10, 25 and 50°C/minute.

The chromatograms are scaled so that the first and last peaks appear at the same point in the window. By examining the mass spectrum
of each peak, the variation of elution order with respect to oven ramp rate may be determined. It is clear that trans-caryophyllene elutes earlier than $\alpha$-bergamotene when using 5 or 10°C/minute oven ramp rates, elutes later when using a 50°C/minute ramp rate, and co-elutes when the oven ramp rate is 25°C/minute. A major advantage of the MSD is its ability to sort out elution order changes and avoid misidentification of the peak.

**Resolving Power**

To investigate the resolving power of the columns, a mixture of n-tetradecane, n-pentadecane and n-hexadecane in n-hexane was analyzed. This analysis was run in selected ion monitoring (SIM) mode using a single ion, mass 57.05, with a dwell time of 50 milliseconds. The shorter scan cycle time of the SIM improved the accuracy of the chromatographic peak width and retention time determinations. Standard calculations were performed to characterize the resolution by determining the Trennzahl (or separation number) values listed in Table 2.

The Trennzahl is a measure of the resolving power of the column suitable for use under temperature programming conditions: the higher the number, the better the resolving power. At the same oven ramp rate, the 100 µm column seems to have somewhat better resolving power, but as the oven ramp rate increases, the resolving power decreases to below that of the 250 µm column run at 5°C/minute.

The loss of resolution may also be detected in the chromatograms of the lemon oil sample. Figure 7 shows the peaks due to neryl acetate and geranyl acetate. The top chromatogram results from the 100 µm column at 50°C/minute ramp rate, and the lower chromatogram is from the 250 µm column at 5°C/minute. The displays have been scaled so that the two peaks are the same distance apart. It is clear that the faster analysis on the 100 µm column has reduced resolution compared to the slower analysis on the 250 µm column. Nonetheless, the resolution obtained on the 100 µm column at the faster oven ramp rate is more than sufficient to separate these two compounds.

**Table 2. Resolving Power of 100 µm and 250 µm Columns Using Homologous Normal Hydrocarbons and Varying Oven Ramp Rates**

<table>
<thead>
<tr>
<th>Column ID</th>
<th>Oven Ramp Rate</th>
<th>C14-C15</th>
<th>C15-C16</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 µm</td>
<td>5°C/min</td>
<td>34.1</td>
<td>32.1</td>
</tr>
<tr>
<td>100 µm</td>
<td>5°C/min</td>
<td>36.1</td>
<td>34.1</td>
</tr>
<tr>
<td>100 µm</td>
<td>10°C/min</td>
<td>30.0</td>
<td>29.0</td>
</tr>
<tr>
<td>100 µm</td>
<td>25°C/min</td>
<td>29.5</td>
<td>26.7</td>
</tr>
<tr>
<td>100 µm</td>
<td>50°C/min</td>
<td>21.5</td>
<td>20.2</td>
</tr>
</tbody>
</table>

**Figure 7. Chromatographic resolution at the fastest analysis conditions.**
Summary
The fast scan rate of the 5973A MSD takes advantage of the higher resolution of 100 µm ID columns to speed up analysis times for complex mixtures such as flavors, fragrances, and essential oils. Faster oven ramp rates used on the 100 µm ID columns increased sample throughput by a factor of three to four over 250 µm ID columns. Some modifications to standard operating parameters are required to achieve this increase, but the free method translator software facilitates this transition.

The greatly enhanced speed of analysis achievable with 100 µm ID columns does not come without a few tradeoffs. Faster oven ramp rates can result in a minor loss of resolution and may cause peak reversals. However, one advantage of the MSD is its ability to identify chromatographic peaks in the event of elution order changes. Taken together, the 5973A MSD paired with 100 µm ID columns provide the speed and resolution required for complex mixture analysis.

To Obtain the Method Translation Software
The method translation software can be downloaded from the Chemical Analysis Group section of the web site at: www.chem.agilent.com/cag/servsup/usersoft/main.html

References

