Volatile Profiling in Wine Using Gas Chromatography Mass Spectrometry with Thermal Desorption

Application Note

Food sensory

Abstract

Wine aroma is an important characteristic and may be related to certain specific parameters such as raw material, production process, and so forth, that impart specific aromas to the wines. Gas chromatography-mass spectrometry with thermal desorption (GC/MS-TD) parameters were optimized to profile volatile compounds from wines. Sample preparation involved extraction of an 8-mL wine sample into a headspace vial with 2 g sodium chloride and internal standard (IS). A preconditioned SPE cartridge was used to adsorb released volatile compounds while heating the vial on a magnetic stirrer for 40 minutes at 80 °C. A SPE cartridge loaded with volatiles was inserted into a TD tube, and the tube was kept in an autosampler for analysis. The GC/MS-TD parameters, desorption time, desorption temperature, low and high trap temperatures, and so forth, were optimized. The profile of 225 volatile compounds of Indian wines was qualitatively analyzed by GC/MS-TD. The targeted deconvolution was used to identify a large number of volatile compounds in wine samples in a shorter time. Wine contains esters (67), alcohols (61), aldehydes (19), terpenes (19), organic acids (18), ketones (14), ethers (7), phenols (5), lactones (4), Pyrazines (3), and others (8). Based on the wine aroma profile of 15 Indian wines, these wines could be differentiated into three groups. Thirteen of the wines could be placed into a single group, whereas Cinsaut and Gewurztraminer showed significantly different concentrations of volatile compounds, and formed individual groups of wines.
**Introduction**

The wine aroma profile is important as it contributes to the quality of the final product. Aroma compounds are closely connected with sensory attributes, which are crucial in determining consumer acceptability. In terms of volatile compounds, wine is one of the most complex beverages [1]. More than 800 volatile compounds such as alcohols, acids, esters, ethers, ketones, terpenes, aldehydes, and so forth, are found in wine and identified with a wide range of concentrations. How many, and what types of volatile compounds are present depends on many factors such as the vineyard’s geographical site, which is related to soil and climate characteristics [5], grape variety [6], yeast strain, and technical conditions during wine making [2].

Usually, volatile compounds in wine are present in different concentrations ranging from mg/L down to a few ng/L, and several sampling techniques are used for analysis (isolation) of these volatile compounds. Of the many methods of extracting volatiles from wine, liquid-liquid extraction (LLE) [3] is primarily used for the fractionation of free and bound volatile compounds [4]. Some other modern volatile extraction techniques used for volatiles analysis of grapes and wines include:

- Static headspace [5]
- Stir bar sorptive extraction (SBSE) [6]
- Solid phase microextraction (SPME) [7]
- Purge and trap/dynamic headspace, and so forth

SBSE is a volatile extraction technique developed in 1999 [8]. It is a highly sensitive technique for trace and ultra-trace analyses, and uses a magnetic stir bar coated with polydimethylsiloxane (PDMS). This application note describes the optimization of the SBSE technique using a SPE cartridge (SPE-td cartridge) in a thermal desorption system coupled with GC-MS for screening wine samples for large numbers of volatile compounds. This optimized method was applied to screen volatiles from different wine samples. This application note demonstrates the use and need of software-based identification in large-scale screening methods.

**Experimental**

**Instrumentation**

TD system series 2 UNITY assembled with autosampler series 2 ULTRA (Markes International, LLANTRISANT, RCT, UK. The TD tube containing the SPE cartridge (Markes p/n C-SPTD) (loaded with volatile compounds) was kept in the autosampler. The desorption of volatiles was performed at 180 °C for 20 minutes. All volatiles and semivolatiles were passed from the autosampler to the TD trap unit using a transfer line (maintained at 200 °C). All volatiles were trapped by a cold trap, which was kept at –20 °C using an electronic cooling system (Pelletier cooler). After that, the cold trap was heated to 275 °C at the rate of 60 °C/sec, and maintained at that temperature for 5 minutes. The desorbed vapours were transferred to the analytical column through a transfer line maintained at 200 °C, and analyzed using GC/MS.

**Table 1. Instrumental Conditions for the Analysis of Volatiles in Wine**

<table>
<thead>
<tr>
<th>GC conditions</th>
<th>Flow (mL/min)</th>
<th>Oven ramp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column HP-INNOWAX 60 m × 0.25 mm, 0.25 µm (p/n 19091N-136)</td>
<td>1.3</td>
<td>40 °C (1 minute hold) –5 °C/min to 250 °C (24 minutes hold)</td>
</tr>
<tr>
<td>Total run time (minutes)</td>
<td>67</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MS conditions</th>
<th>Ion source temperature (°C)</th>
<th>Electron voltage (eV)</th>
<th>Quadrupole temperature (°C)</th>
<th>EM gain</th>
<th>EM volts</th>
<th>Scan range (m/z)</th>
<th>Tuning</th>
<th>Interface temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>230</td>
<td>–70</td>
<td>150</td>
<td></td>
<td>2035</td>
<td>30 m/z – 300 m/z</td>
<td></td>
<td>240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thermal desorption conditions</th>
<th>Autosampler</th>
<th>Desorption time (minutes)</th>
<th>Desorption temperature (°C)</th>
<th>Low trap temperature (°C)</th>
<th>High trap temperature (°C)</th>
<th>Trap hold (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>180</td>
<td>–20°C</td>
<td>275°C</td>
<td>5</td>
</tr>
</tbody>
</table>
Sample extraction

The 8-mL wine sample was drawn into a 20-mL glass head-space vial. A 3-octanol internal standard (150 µg/L), and 2 g of NaCl were added. A SPE cartridge (for adsorbing volatile compounds) and magnetic stirrer (for shaking) were placed in the mixture. After crimping the vial, it was heated for 40 minutes at 80 °C, followed by cooling for 30 minutes at room temperature. The SPE cartridge with the volatile compounds was wiped with a lint-free tissue paper, and transferred to an empty glass TD tube prior to analysis.

Table 2. Optimization Parameters to Get Better Abundance and Repeatability

<table>
<thead>
<tr>
<th>S. no</th>
<th>Parameter in optimization</th>
<th>Function</th>
<th>Condition</th>
<th>Optimized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desorption time</td>
<td>Time required to desorb volatiles from Spe-TD</td>
<td>5, 10, 15, 20, 25 minutes</td>
<td>20 minutes</td>
</tr>
<tr>
<td>2</td>
<td>Cold trap temperature</td>
<td>Concentrating volatiles from Spe-Td</td>
<td>+10, 0, −10, −20, −30</td>
<td>−20 °C</td>
</tr>
<tr>
<td>3</td>
<td>High trap temperature</td>
<td>Temperature to transfer concentrated volatiles to column</td>
<td>250, 275, 300, 325, 350, 375 °C</td>
<td>250 °C</td>
</tr>
<tr>
<td>4</td>
<td>Volume of wine for extraction</td>
<td>Get better abundance</td>
<td>4, 6, 8, 10 mL</td>
<td>6 mL</td>
</tr>
<tr>
<td>5</td>
<td>Spe-TD soaking time with stirring</td>
<td>Spe-TD exposure time to wine to adsorb</td>
<td>10, 20, 30, 40, 50, 60, 70 minutes</td>
<td>40 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Sodium chloride addition</td>
<td>Increase absorption by ionization</td>
<td>0.5, 1, 1.5, 2, 2.5, 3.0 g</td>
<td>2 g</td>
</tr>
<tr>
<td>7</td>
<td>Heating cartridge while extraction</td>
<td>To increase absorption capability</td>
<td>40, 50, 60, 70, 80, 90 °C</td>
<td>80 °C</td>
</tr>
</tbody>
</table>

Figure 1. Effect of desorption time for sample tube on increase in the response of the volatile compounds from wine.
Figure 2. Effect of different cold trap temperatures on adsorption.

Figure 3. Effect of different cold trap temperatures on desorption.
Figure 4. Effect of percentage of NaCl addition and compound response.

Figure 5. Effect of shaking up time for SPE-td during extraction.
Data analysis

The optimized method for analysis of volatile compounds could generate a very complex chromatogram with a large number of compounds (225), eluting in a shorter run time of 67 minutes. Manual integration of these samples and their identification could limit up to 70 compounds because identification of complex coelutions was ambiguous. The major disadvantage of manual integration is that integration of one such sample requires more than 8 hours, and analysis of batches of samples will be a herculean task. Using targeted deconvolution, the integration of one sample could be processed within 10 minutes with identification of the compounds against the targeted library of 370 compounds. The same processing could also be done by AMDIS. This tool uses the NIST11 spectral database for identification, which contains thousands of mass spectra of compounds other than target compounds. During the processing of the samples, it is possible to have a library hit for a nontarget or a false identification of a compound, therefore, manual review of the peaks is essential. In an analysis using targeted deconvolution software (Agilent MassHunter quan. Tool B.06), only target compounds are identified, because the processing is done against the target library, which is more reliable.
Figure 8. Target deconvolution tool for wine volatile screening.

The Compounds at a Glance feature of Agilent MassHunter Software (Figure 9) quickly differentiates matching the compounds analyzed in the respective batch, based on qualifier, purity, and retention time.

Figure 9. Compound at a glance for filtering out qualifying peaks.
Practical application of the method to the real wine samples

The method was applied to different Indian wine samples, and gave satisfactory performance for volatiles analysis. Tentative identification and quantification of volatile compounds was done using targeted deconvolution software by putting a match factor minimum of 50% against a self-generated in-house library for 370 volatile compounds. Tentative concentration of the identified compounds was calculated against the concentration of 3-octanol.

Conclusion

The volatiles profiling of Indian wines was done using GC/MS-TD. In addition, for the first time, step-by-step optimization of extraction and thermal desorption GC/MS parameters was accomplished and reported for the analysis of large numbers of volatile compounds including compounds with low abundance, such as pyrazines. With the help of this optimized method and targeted deconvolution software, 225 compounds could be tentatively identified from 15 different wine varieties. The method seems to be more efficient than headspace and HS-SPME analysis, in terms of number of compounds identified and the comparatively lower cost of the technique. With the help of target deconvolution software, compounds with complex coelutions could also be satisfactorily identified. Thus, the method described in this paper is a better option than other currently used methods. Based on the analysis of wines for volatiles profiling and statistical analysis of the results, different wines could be easily distinguished from each other.
References


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