Fingerprint analysis of tea leaves by HS-SPME-GC×GC-QTOF.

Author application note: Daniela Peroni, JSB © 2016

Introduction

Comprehensive two-dimensional gas chromatography (GC×GC) provides very high resolution power and unmatched peak capacity. In addition, the 2D chromatograms are highly structured and allow linking the compound position in the 2D space to properties such as volatility and polarity. As a consequence, 2D patterns are very informative and specific and can be exploited for comparison and classification of complex samples. Here we show the use HS-SPME-GC×GC-QTOF for the fingerprinting of Earl Grey tea leaves from different commercial brands.

Experimental details

- Head-space SPME sampling of Earl Grey tea leaves from 7 different commercial brands.
- Agilent 7890A GC with a cryogen-free Zoex ZX2 thermal modulator and an Agilent 7200B QTOF detector.
- Data display and processing with the GC Image software.

Results and discussion

All samples generate very complex chromatographic profiles characterized by a large amount of compounds (Fig. 1). Many components are present at a very low level and would not be efficiently separated in one dimension by standard GC, leading to a significant loss of information. On the other hand, the 2D counterplots are very detailed. The 2D blob patterns show several hundred compounds as well as clear similarities/differences between the samples.

Fig. 1 – Examples of 2D chromatograms obtained for the different tea samples.

Table 1 – Degree of similarity calculated as template matching (%).

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>template 1</th>
<th>template 2</th>
<th>template 3</th>
<th>template 4</th>
<th>template 5</th>
<th>template 6</th>
<th>template 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.9</td>
<td>52.7</td>
<td>66.3</td>
<td>62.5</td>
<td>74.6</td>
<td>68.0</td>
<td>59.8</td>
<td></td>
</tr>
<tr>
<td>53.5</td>
<td>59.0</td>
<td>64.1</td>
<td>54.1</td>
<td>60.1</td>
<td>49.4</td>
<td>54.1</td>
<td></td>
</tr>
<tr>
<td>54.6</td>
<td>59.6</td>
<td>67.4</td>
<td>55.6</td>
<td>62.2</td>
<td>64.3</td>
<td>50.1</td>
<td></td>
</tr>
<tr>
<td>50.9</td>
<td>52.2</td>
<td>63.8</td>
<td>55.1</td>
<td>61.4</td>
<td>67.3</td>
<td>54.8</td>
<td></td>
</tr>
<tr>
<td>65.7</td>
<td>55.9</td>
<td>75.8</td>
<td>66.1</td>
<td>74.6</td>
<td>79.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Individual blob templates were created for each sample and applied to all the others for pair-wise comparison. Peak matching was based on retention times and MS spectrum. Some sample show high similarity while others are clearly very different (Table 1). Some compounds (e.g. 2-hexenal and benzyl alcohol) are found in all samples while others (e.g. benzyl benzoate or cinnamaldehyde) are specific for a certain sample.
Comprehensive fingerprinting

In a different approach we built a global template by adding cumulatively the blobs of all samples. The integration threshold was set in order to make the blob amount manageable (about 200 blobs in total). The measurements were performed with the QTOF in HR and EDR acquisition mode, respectively.

The matching percent of the comprehensive template for each sample provides an assessment of the samples complexity. Fig. 2 summarized the results obtained. As can be seen, the two acquisition modes are consistent. The matching ranges from 20% to 60%, indicating clearly the very significantly different nature of the tea leaves under exam. These very significant differences can be expressed in a more relevant way in terms of detection/absence (Table 2) or relative abundance (Fig. 3) of significant aroma-key compounds.

Table 2 – Examples of matched/unmatched (+/-) compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Sample 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longfolene</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citronellal</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linalool</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Estragole</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Geranyl isovalerate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Decanal</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Accurate mass

The accurate masses can be used to calculate the most likely formula for any MS fragment. The formula suggested can be compared to that expected for structure of the library match. This process can be applied to several fragments. If the errors obtained are very small for all fragments, identification confidence is greatly improved (Table 3).

Conclusions

- HS-SPME-GC×GC-QTOF allows detailed characterization of volatiles profiles of complex food matrices such as tea.
- Many minor potentially significant compounds are successfully separated from the highly complex matrix.
- The detailed and specific two-dimensional separation patterns are ideal for fingerprint analysis.
- The presence or absence of specific markers or aroma-key compounds can be used to differentiate or associate samples, e.g. in terms of geographical origin, treatment or sophistication.
- The high mass accuracy provided by the QTOF is very useful for identity confirmation and identification of unknowns.

Table 3 – Identity confirmation by multi-fragment mass accuracy evaluation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fragment (m/z)</th>
<th>Library match formula</th>
<th>Exact mass</th>
<th>Measured mass</th>
<th>Suggested formula</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citral</td>
<td>147</td>
<td>C10H16O+ (M+)</td>
<td>152,1196</td>
<td>152,1195</td>
<td>C10H16O+</td>
<td>0.35</td>
</tr>
<tr>
<td>3-Carene</td>
<td></td>
<td>C9H13+</td>
<td>123,1168</td>
<td>123,1171</td>
<td>C9H13+</td>
<td>2.46</td>
</tr>
<tr>
<td>Decanal</td>
<td></td>
<td>121,1012</td>
<td>121,1012</td>
<td>121,1010</td>
<td>C10H14+</td>
<td>1.89</td>
</tr>
<tr>
<td>Longfolene</td>
<td>159</td>
<td>C12H17+</td>
<td>161,1325</td>
<td>161,1324</td>
<td>C12H17+</td>
<td>0.42</td>
</tr>
</tbody>
</table>

HEAD OFFICE
JSB International
Tramstraat 15
5611 CM Eindhoven
T +31 (0) 40 251 47 53
F +31 (0) 40 251 47 58
INFO@GO-JSB.COM
WWW.GO-JSB.COM

SALES AND SERVICE

NETHERLANDS
Amstelweg 28
8239 DA Lelystad
T +31 (0) 32 087 00 18
F +31 (0) 32 087 00 19

BELGIUM
Grenstraat 7, Box 3
1920 Diegem
T +32 (0) 27219211
F +32 (0) 27207622

GERMANY, AUSTRIA, SWITZERLAND
Max-Planck-Strasse 4
D-47475 Kamp-Lintfort
T +49 (0) 28 42 9290 799
F +49 (0) 28 42 9732 638

UK & IRELAND
Cedar Court, Grove Park Business Est. White Waterway, Maidstone, Berks, SL6 3LW
T +44 (0) 16 208 522 48
F +44 (0) 70 394 006 78