Analysis of Grape Volatiles by Solid Phase Microextraction and Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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Key Words: GCxGC-TOFMS, SPME, Food and Flavors

1. Introduction

Volatile composition is one of the most important factors to determine wine character and quality. Several studies have recognized a relationship between the wine varietal character, and the grape and musts volatile compounds, namely terpenoids (Wilson et al., 1986) and aromatic alcohols (Rocha et al., 2000). Volatile compounds appear in the free and/or glycosidically linked forms. These precursors have been reported as glycosides having the aroma compounds as their aglycons. They may be released during winemaking by acid and/or enzymatic treatments.

The aim of this work was to study the varietal volatile composition of mature crushed grape berries from Fernão Pires (white) and Baga (red) grape varieties, from the Portuguese Bairrada appellation. Baga is the main variety from the Bairrada appellation, an ancient winemaking region in Portugal. This variety represents 92% of the red vineyard, and 80% of the overall Bairrada vineyard, covering 15,000 ha, with a mean wine production of 450,000 hL. Fernão Pires is a variety that is spread throughout the Portuguese appellations, and represents 70% of the white vineyard in the Bairrada appellation. Due to the considerable importance of volatile monoterpenoids and sesquiterpenoids to flavor and varietal character of Vitus vinifera varieties, particular attention was devoted to these compounds.

Grape volatiles represent a very complex matrix. For the purpose of mass spectral identification, good gas chromatographic separation (GC) is crucial. Comprehensive two-dimensional gas chromatography (GCxGC) employs two orthogonal mechanisms on apolar and polar columns to separate the constituents of the sample. Therefore, the separation potential is greatly enhanced compared to one-dimensional GC. Time-of-flight mass spectrometry (TOFMS) provides full mass spectra and identification based on comparison with NIST library spectra.

2. Experimental Conditions

Samples

Vitis vinifera var. healthy-state Fernão Pires (white) and Baga (red) grapes were collected from the experimental vineyard propriety of Estação Vitivinícola da Bairrada (EVB), the Vine and Wine Research Institute of the Bairrada appellation. The grape berries were transported immediately under refrigeration to the laboratory and were stored at -80°C until they were analyzed.

Sample Preparation

50 g of frozen grapes were ground, mixed with 8 g NaCl, and transferred to a 125 ml amber solid phase microextraction (SPME) vial. A Teflon stirrer was placed in the suspension. The sample in the vial was conditioned and stirred in a 40°C warm-water bath for 1 hour. Subsequently, 1 hour headspace SPME sorption was performed on a 65 µm Carbowax/divinylbenzene fiber (Supelco, USA).

Analysis Conditions

GC Parameters:
Agilent 6890N Gas chromatograph equipped with a LECO GCxGC Thermal Modulator and Secondary Oven

Injection:
Manual injection of SPME fiber,
Splitless 5 min, 250°C

Primary Column:
Equity-5 60 m x 0.25 mm x 1 µm (Supelco, USA)

Secondary Column:
Supelcowax 2.5 m x 0.1 mm , 0.1 µm (Supelco, USA)

Carrier Gas:
Helium, 1 ml/min, constant flow

Primary Oven Program:
40°C, 1 min, 5°C/min to 260°C, 15 min

Secondary Oven Program:
45°C, 1 min, 5°C/min to 265°C, 15 min

Modulator Temp Offset (above primary oven): 30°C
Modulation Time: 4 s
Hot Pulse Time: 0.8 s
Transfer Line Temp: 250°C
Total Run Time: 64 min

MS Parameters: LECO Pegasus® 4D GCxGC-TOFMS
Ionization: Electron Ionization at 70 eV
Source Temp: 220°C
Stored Mass Range: 33-350 u
Acquisition Rate: 125 spectra/s

Data Processing: LECO ChromaTOF® software

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3. Results and Discussion

A sample of white grapes was analyzed and the chromatogram was processed with automated Peak Finding and Deconvolution algorithms. A Classification feature of ChromaTOF software was used to apply the processing only for selected areas in the contour plot, where the analytes of interest eluted, and also to exclude many peaks not of interest (for example, peaks associated with column bleed). Using Classifications, both data processing time and the number of "extra" peaks in the peak table are reduced. Despite the fact that classification removed most of the "bleed" peaks, this approach did not help for those "bleed" peaks eluted close to the analytes of interest. These had to be removed from the peak table by sorting according to typical unique masses.

As a result of Peak Find processing at a minimum signal-to-noise (S/N) of 500, 1562 peaks were found in the sample. Additional exclusion of cca 500 "bleed" peaks from the table and filtering according to library match factor (similarity>850) resulted in 301 identified compounds. Many structural classes were present in the grapes volatile fraction: alkanes, alcohols, aldehydes, ketones, esters, acids, lactones, phenols, terpenes, sesquiterpenes and their derivatives.

Identifications of the most abundant peaks are shown in Figures 1-4 in different zoomed sections of the contour plot. The sample was obviously very complex and contained a high number of compounds differing in volatility and polarity. This sample dimensionality matches well to the GCxGC separation technique, which employs orthogonal separation mechanisms of volatility and polarity.

In Figure 5 the identical sections of the contour plot are shown for white (top) and red (bottom) grapes, so that the differences can be easily recognized. The differences observed were mostly quantitative; few qualitative differences were found. The corresponding peaks are marked by white circles in Figure 5.

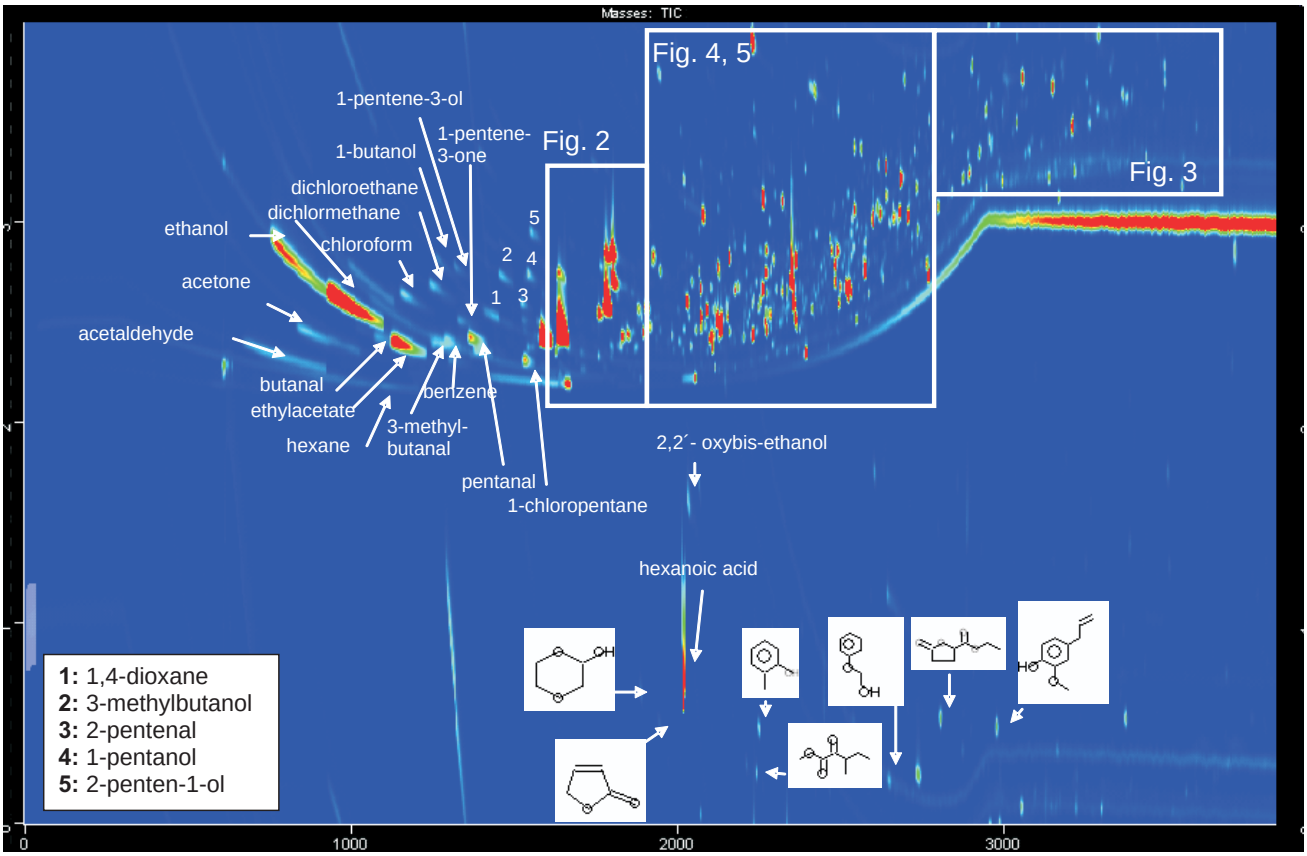

Figure 1. Contour plot from headspace SPME GCxGC-TOFMS of a white grape sample.

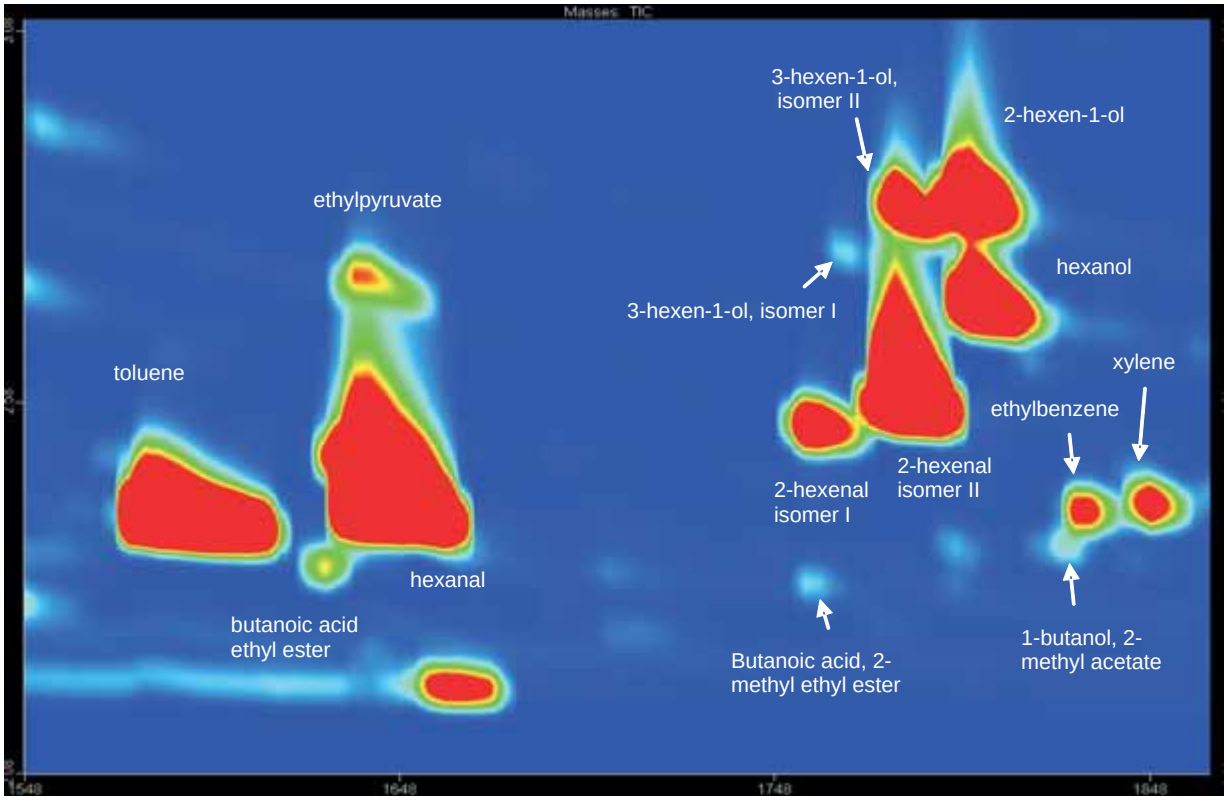

Figure 2. Zoomed section of contour plot from Figure 1—white grapes.

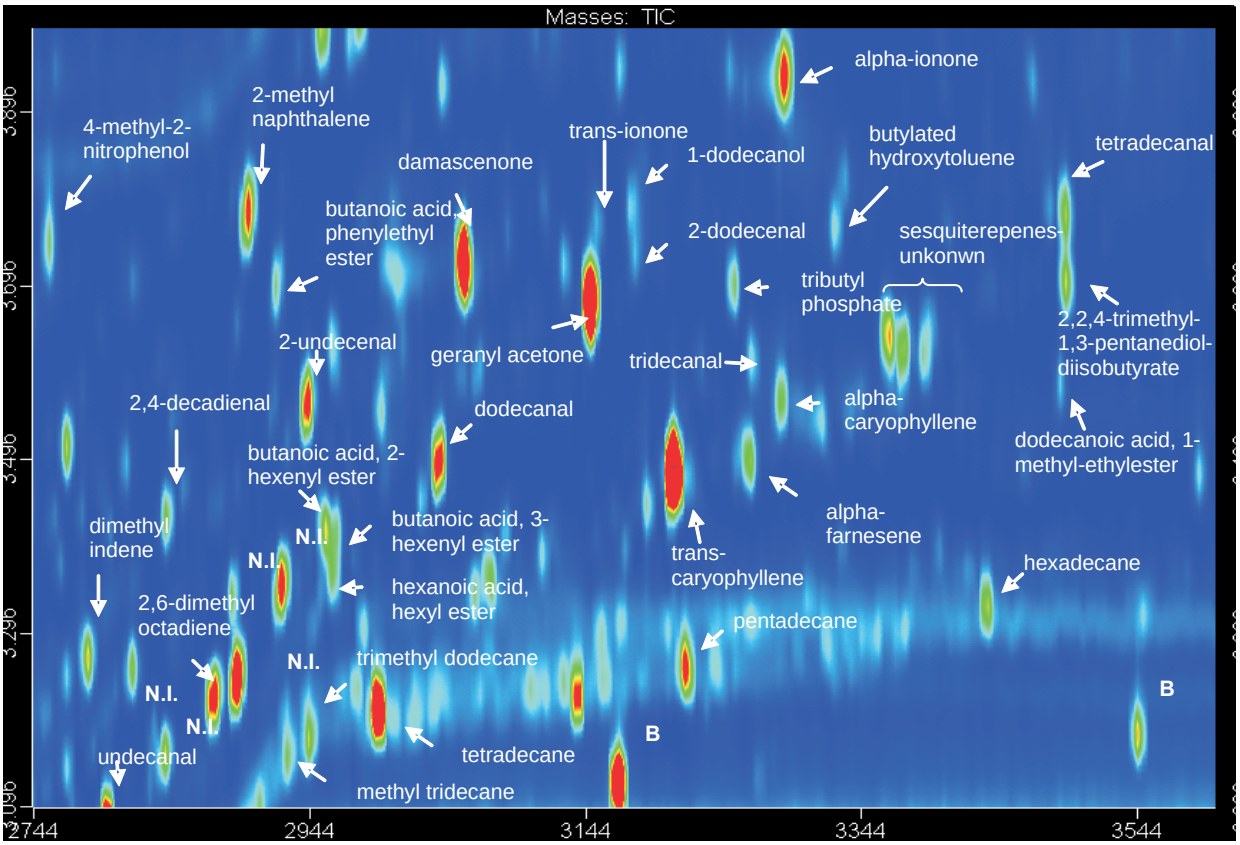

Figure 3. Zoomed section of contour plot from Figure 1—white grapes, N.I. = not identified, B = "bleed" peaks.

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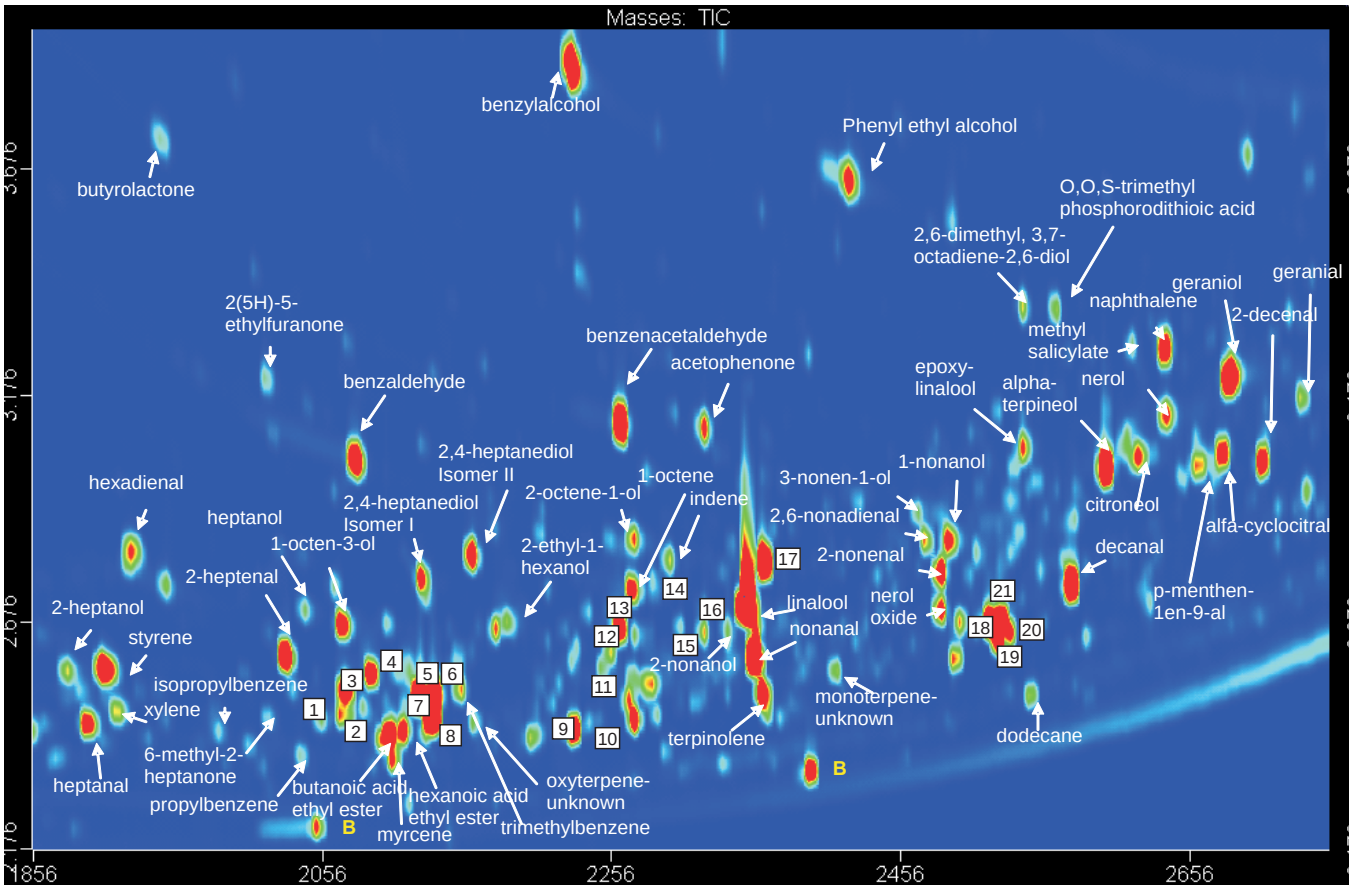


Figure 4. Zoomed section of contour plot from Figure 1—white grapes.

1: 2-propyl benzene, 2: 1-ethyl-2-methyl benzene, 3: 1-octen-3-one, 4: 5-heptene-2-one, 5: 3-hexene-1-ol acetate, 6: 2-hexene-1-ol acetate, 7: octanal, 8: acetic acid hexyl ester, 9: limonene, 10: ocimene, 11: 2,2,6-trimethyl cyclohexanon, 12: 2,6-dimethyl octadiene, 13: 2-octenal, 14: 3,5-octadiene-2-on, 15: isophoron, 16: linalool oxide, 17: 3,7-dimethyl-1,5,7-octatriene-3-ol, 18: butanoic acid, 3-hexenyl ester, 19: octanoic acid, ethyl ester, 20: butanoic acid, hexyl ester, 21: butanoic acid, 2-hexenyl ester, B- bleed peaks

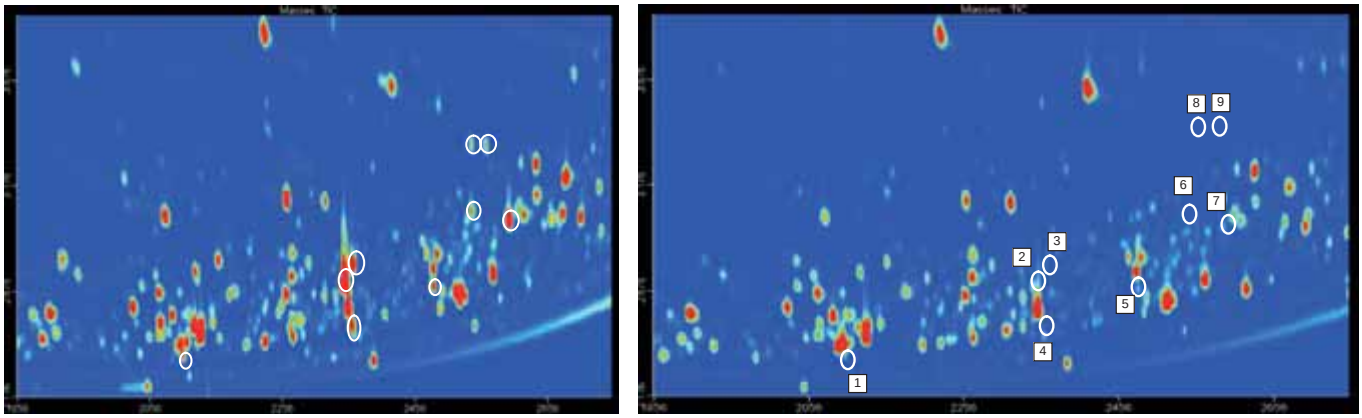
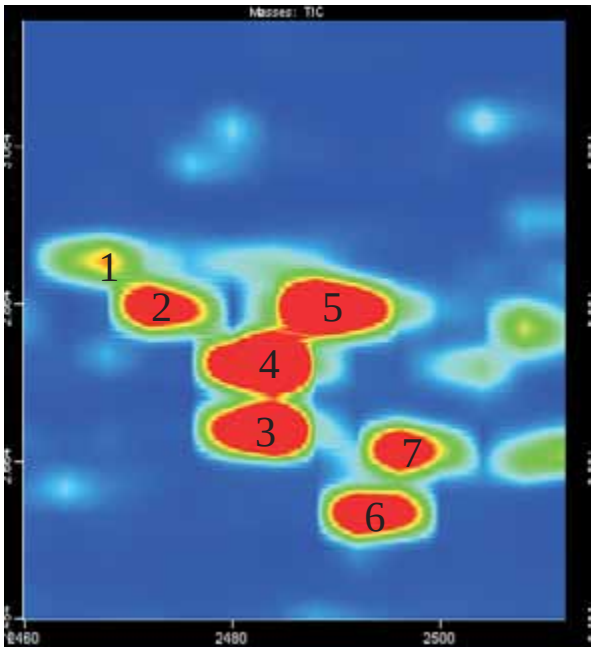


Figure 5. Zoomed section of contour plot from Figure 1—comparison of white (top) and red (bottom) grapes.

Some of the compounds present in white grapes are missing (or significantly lower in concentration) in the red grapes: 1: beta-myrcene; 2: linalool; 3: 3,7-dimethyl-1,5,7-octatrien-3-ol; 4: terpinolene; 5: nerol oxide; 6: epoxylinalol; 7: alpha-terpineol (p-menth-1-en-8-ol); 8: 2,6-dimethyl-3,7-octadiene-2,6-diol; 9: phosphorodithioic acid, O,O,S-trimethyl ester



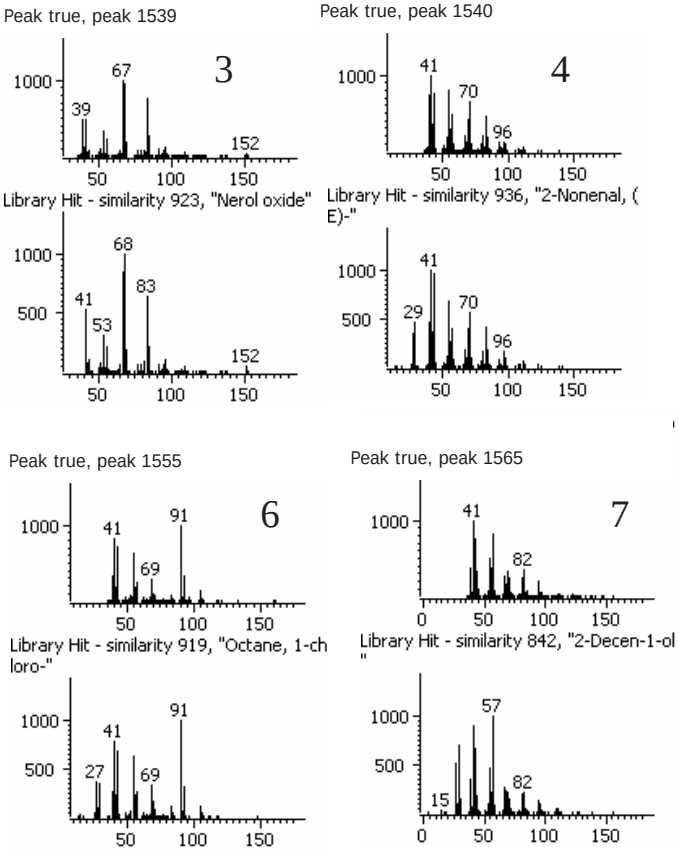


Figure 6. Example of a GCxGC separation for a complex part of the chromatogram.

Compounds shown in the contour plot identified as: 1: 3-nonen-1-ol; 2: 2,6-nonadienal; 3:nerol oxide; 4: 2-nonenal; 5: 1-nonanol; 6: 1-chlorooctane; 7: 2-decen-1-ol. TOFMS spectra (top) and NIST library spectra (bottom) are compared.

In Figure 6 an example of a separation of a complex part of the chromatogram is shown. It is clear that GCxGC adds extra separation for some critical pairs. In the particular group of compounds shown in Figure 6, nerol-oxide (3) and 2-nonenal (4) peaks lay on the same vertical lines, i.e. coelute on the Equity-5 column. However, these two compounds exhibit different polarity and therefore are separated on the wax column in the second dimension. Similarly, partial coelution of 1-chlorooctane and 2-decen-1-ol (6,7) is resolved by GCxGC technique. Resulting mass spectra are very clean and allow good identification.

4. Conclusions

Grapes samples (Vitis vinifera var.) were analyzed using headspace SPME GCxGC-TOFMS. The sample was processed using Automated Peak Find at a S/N level of 500, and modulated bleed and solvent peaks were removed. As a result, 301 compounds were identified with NIST library similarities higher than 850, comprising chemical groups of alkanes, alcohols, aldehydes, ketones, esters, acids, lactones, phenols, terpenes, sesquiterpenes, and their derivatives.

GCxGC was found to be an effective tool to separate the components in a very complex mixture of grapes volatiles. In many cases, critical coelutions on the Equity-5 column were resolved by adding the second dimension polarity separation on the Supelcowax column.

5. Acknowledgement

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