Determining Wine Authenticity: A Metabolomics Analysis Using UHPLC ESI/Q-TOF MS

Application Note

Authors

Shen Han, Jinhua Wang, Ying Liu, and Qi Zhou
Beijing Entry-Exit Bureau of Inspection and Quarantine
Beijing, China

Meiling Lu, Shan Zhou
Agilent Technologies China
Beijing, China

Abstract

This application note demonstrates the advantages of using an Agilent 1290 Infinity LC system combined with the Agilent 6530 Q-TOF LC/MS and multivariate statistical analysis for metabolic profiling of wines. This resulted in the differentiation of wines from different geographical areas including France and China, and it potentially allows for the identification of wine biomarkers.

Introduction

The chemical compositions of wines can vary by a wide range of factors. This is caused primarily by the variety of grapes, their growing conditions such as climate and soil, and the viticultural practices of the winemaker. In addition, the yeast strain, fermentation, and aging processes may also impact the taste and quality of wine. Chromatography coupled with accurate and high resolution mass spectrometry is currently a well accepted method for the metabolic profiling of wine. It is routinely used to analyze wine chemodiversity [1-3] and is beneficial for the discovery of wine biomarkers. This application note provides additional insight into the potential for improved quality control and authenticity assessments of wines. This study used ultra high performance liquid chromatography (UHPLC) with electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass spectrometry, as well as multivariate statistical analysis for wine profiling. The goal of this study was the discovery of characteristic features that could discriminate wines from different geographical regions of China and France.

Agilent Technologies

Experimental

Sample preparation

Wine samples previously filtered through a 0.22 µm membrane were subjected to UHPLC gradient elution followed by electrospray Q-TOF mass spectrometric detection in MS scan mode. Compounds were then extracted from the acquired data in MassHunter Qualitative Analysis software and extracted in an unbiased or non-targeted fashion, using the molecular feature extraction (MFE) algorithm. The extracted compounds were exported as a compound exchange file (.cef), then imported into Mass Profiler Professional (MPP) V.12.5, which is chemometric software for data alignment, filtering, statistical, and clustering analysis. The features in solvent blanks were excluded during MPP analysis. The data were initially filtered by removing the features that occurred in less than 25% of all samples. Additional filter steps were used to remove compounds that occurred in less than 60% of all files within at least one group, and having greater than 50% coefficient of variation (CV). The remaining compounds were analyzed by ANOVA for significant differences among groups of wines and visually displayed by Principal Component Analysis (PCA). The resultant characteristic markers were then subjected to targeted MS/MS analysis for further identification.

Approximately 200 wine samples were collected during entry/exit inspections from both France and China trading. There were at least six samples from each of the four major French Chateaux, with a wide range of vintages, from 1985 to current. There were three brands of Chinese wines, each containing 30 samples. All the Chinese wines were obtained directly from the manufacturers. Unknown wine samples were claimed as wines from one of the four French Chateaux with varying vintages, but required further confirmation.

Instrumentation

Instrument conditions

LC conditions

Instrumentation	Agilent 1290 Infinity binary pump with built-in degasser, 1290 Infinity Autosampler with temperature control, and 1290 Infinity Thermostatted Column Compartment
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm
Column temperature	30 °C
Mobile phase	Solvent A) 0.1% FA/5 mM CH_3COONH_4 in water Solvent B) 0.1% FA/5 mM CH_3COONH_4 in methanol/water (95:5)
Flow rate	0.4 mL/min
Injection volume	2 µL
Post time	3 minutes
Gradient elution profile	0–1 minutes, %B maintained at 1% 1–8 minutes, %B increasing from 1% to 15% 8–15 minutes, %B increasing from 15% to 45% 15–17 minutes, %B increasing from 45% to 90% 17–20 minutes, %B maintained at 90%

ESI-Q-TOF MS conditions

Instrumentation	Agilent 6530 Acurate-Mass Q-TOF LC/MS equipped with Agilent Dual Jet Stream Technology
Drying gas temperature	325 °C
Drying gas flow rate	11 L/min
Nebulizer gas pressure	45 psi
Sheath gas temperature	350 °C
Sheath gas flow rate	12 L/min
Capillary voltage	3,500 V (positive)/3,000 V (negative)
Nozzle voltage	500 V (positive)/1,000 V (negative)
Fragmentor voltage	130 V
Skimmer voltage	65 V
m/z scan range	100–1,100 for MS 50–1,000 for MS/MS
Acquisition rate	2 spec/sec for MS 3 spec/sec for MS/MS
Reference ions	121.0509 and 922.0098 (positive) 112.9856 and 1,033.9831 (negative)

Results and Discussion

Data repeatability

A number of compounds in wines can be eluted off an LC column with UHPLC reversed phase gradient elution followed by ESI-Q-TOF MS detection in full scan mode. They can be resolved by retention time, accurate *m/z*, or both. Overlapping two separate injections of the same sample (Figure 1) demonstrated that the repeatability in retention time was within 0.5% (RSD), whereas online calibration with reference ions maintained the mass accuracy within 5 ppm.

Data extraction

A molecular feature extraction algorithm was applied to the acquired data for the four groups of French wines. All ion species were related by corresponding coelution profiles. Each data file was imported into MPP software, aligned, and binned by retention time and *m/z*. Following data alignment, up to 32,116 and 43,236 entities were obtained for positive and negative ionization, respectively, which were subjected to further data filtering.

Data filtering

The data from above was filtered according to 60% occurrence frequency under at least one condition and sample variability with less than 50% coefficient of variation in each group. The remaining entities were further filtered by variance analysis (ANOVA, $p < 0.01$) and fold change ($FC > 2$). Fifty-five entities from positive ionization remained with significant differences among four groups. Extraction of these entities from the original total ion chromatograms demonstrated that these entities were mostly true compounds present in the samples, and their levels varied significantly among the groups. Example EICs are shown in Figure 2. These results suggest that these entities could be used as markers to differentiate wines from different sources.

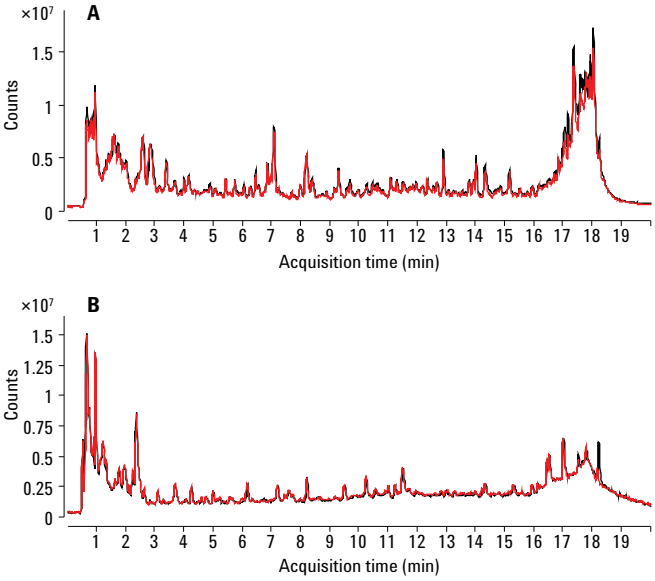

Figure 1. Typical total ion chromatograms obtained from both positive ESI and negative ESI modes. A. Positive ESI. B. Negative ESI.

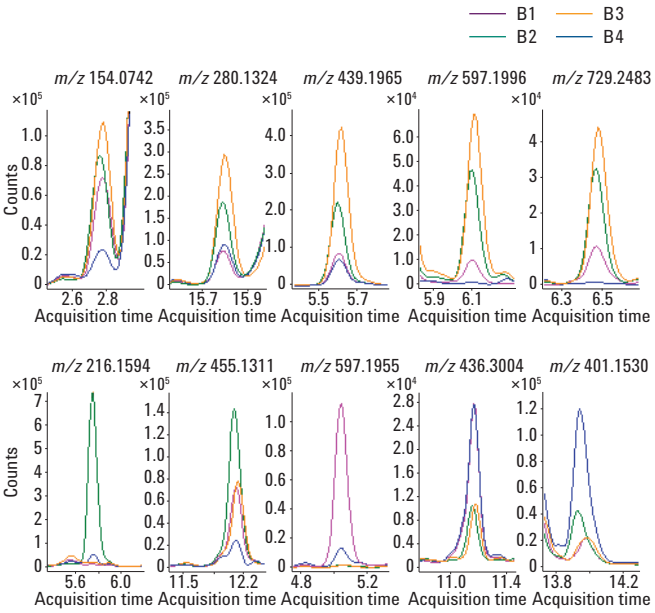

Figure 2. EIC of possible markers to discriminate wines from four French Chateaux. B2 and B3 can be differentiated from B1 and B4 by the five markers on the top; while B2 can be further distinguished from the rest three using m/z of 216.1594. B1 and B4 can be differed from each other at m/z of 597.1955 and 401.1530.

Principle component analysis (PCA)

PCA analysis of the 55 entities above demonstrated excellent separation of wines from four different French Chateaux (Figure 3). As the figure illustrates, B2 and B3 are close under components 1 and 2 (X, Y plane), but can be separated under components 2 and 3 (Y, Z plane). This indicates that B2 and B3 were relatively similar compared with other groups. It is also consistent with Figure 2 where most markers showed relatively low differences between B2 and B3 than other pairs.

Differentiation of wines from France and China

In order to determine whether the extracted entities can separate the collected wines of French Chateaux from the wines manufactured in China, two brands of wines from China were subjected to the same analysis. The principle component analysis (PCA) plot demonstrated that the Chinese wines were significantly different from French wines (Figure 4). One batch of wines (pink) from an unknown source were more similar to Chinese wine, although they were marketed as French wines from one of the Chateaux studied.

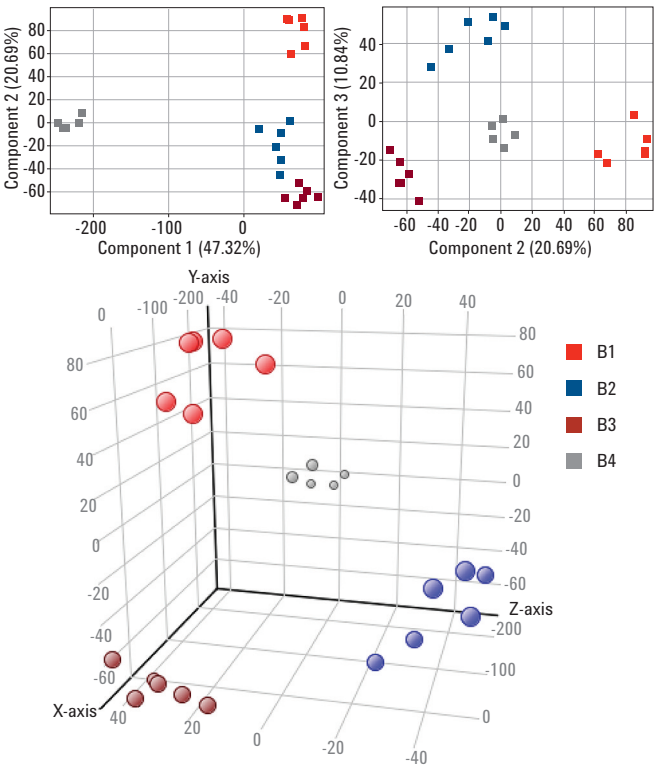

Figure 3. 2-D and 3-D principle component analysis (PCA) plots of four Chateaux (France) demonstrating a clear separation of the four groups based on 55 entities filtered by ANOVA (P < 0.01) and fold change (FC > 2).

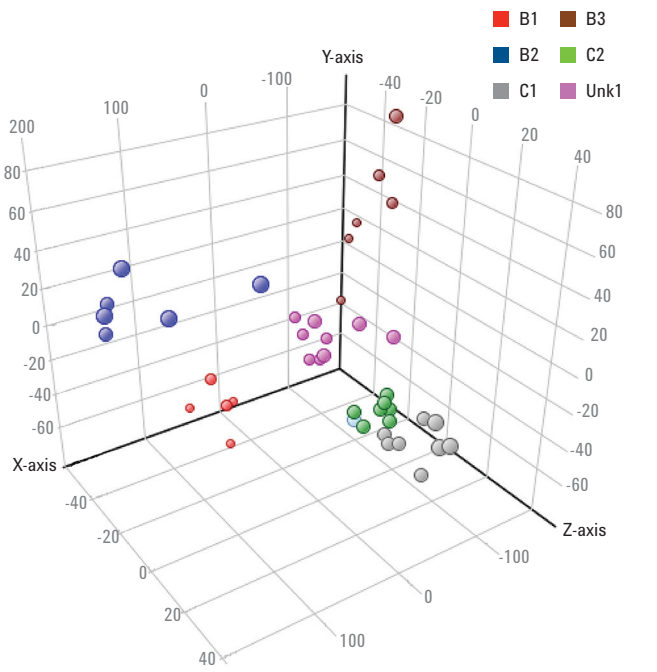

Figure 4. PCA analysis of French wines (B1-B3), Chinese wine (C1, C2), and one sample wine (Unk1), which source is unclear based on the extracted 55 entities after MPP analysis from four French wines.

Model creation and prediction

In order to improve the method's capability of identifying wines of unknown origin, a model based on partial least square differentiation analysis (PLSDA) was created using the extracted entities from French wines. As shown in Figure 5, the wines from four Chateaux were well separated from each other. The confusion matrix shown in Table 1 demonstrates that the prediction accuracy reached 100%. In this study, the average prediction confidence was about 78%, indicating that more samples are required to build a better model.

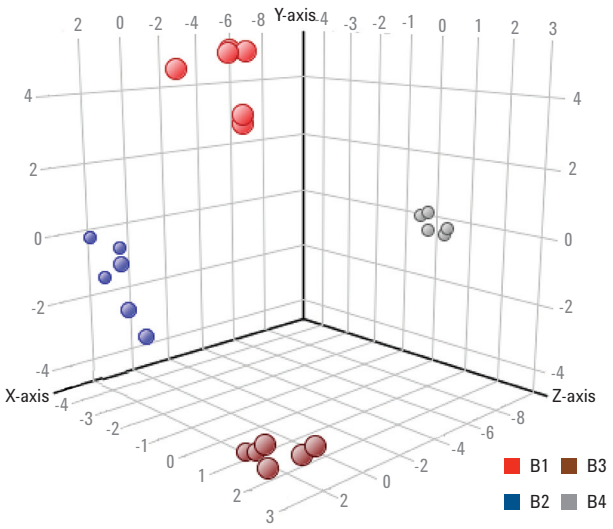

Figure 5. Predicted PLSDA model based on 55 extracted entities after MPP analysis.

Table 1. Prediction Accuracies of Wines Obtained from the PLSDA Model Indicated an Excellent Prediction Capability, With the Average Prediction Confidence Approximately 78%.

	B1	B2	B3	B4	Accuracy
(True) B1	6	0	0	0	100.00
(True) B2	0	6	0	0	100.00
(True) B3	0	0	6	0	100.00
(True) B4	0	0	0	5	100.00
Overall accuracy					100.00

Marker identification

The resultant significant entities were subjected to targeted MS/MS analysis. The acquired MS/MS spectra were imported into MassHunter Molecular Structure Correlator, for tentative identification of candidate compounds based on MS/MS fragments that matched compounds in the ChemSpider database. As shown in Figure 6, two potential structures were retrieved for one marker with a high compatibility score (> 90).

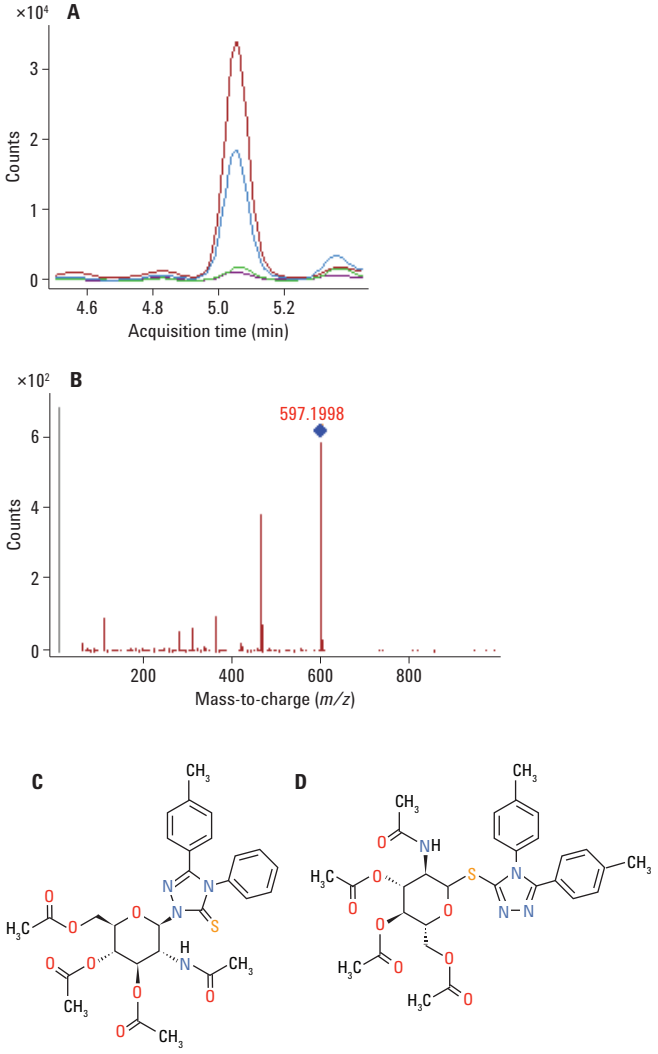

Figure 6. Tentative identification of one typical marker (m/z 597.1955, t_R 5.045 minutes). A) EIC of one marker (m/z 597.1955, t_R 5.045 minutes). B) Target MS/MS analysis of the marker; C,D) Molecular structure correlation suggesting two possible structures with compatibility score > 90.

Conclusions

This study demonstrates that accurate-mass Q-TOF LC/MS is a powerful technique for the metabolic profiling of wines from various sources. Data mining techniques such as molecular feature extraction in combination with chemometric software such as MPP help to rapidly and efficiently align and filter data. Significant markers were obtained among the wines from four major French Chateaux. The markers can be used to discriminate these four groups of wines, as well as differentiate two types of wines from Chinese sources. Assignment of an unknown wine was also consistent with the measurement conducted by other techniques. A PLSDA model was created, providing excellent accuracy and reasonable confidence. Involvement of more reference samples for the model building may further enhance the confidence during sample predicition using the built model. Tentative identification of markers with targeted MS/MS followed by molecular structure correlation generated some potential candidates, which are under further investigation.

References

1. L. Vaclavik, O. Lacina, J. Hajslova, J. Zeigenbaum *Anal. Chim. Acta*. 2011, **685**, 45.
2. A. Cuadros-Inostroza, P. Giavalisco, J. Hummel, *et al*. *Anal. Chem.*, 2010, **82**, 3573–3580.
3. F. Mandel, ASMS2011 Poster WP-222.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
March 20, 2014
5991-3335EN

Agilent Technologies