

Software

Flow-injection analysis (FIA) of wines using the Vanquish Neo UHPLC system coupled with the Orbitrap Exploris 480 mass spectrometer and Compound Discoverer software

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Keywords

Flow-injection, Vanquish Neo, Orbitrap
Exploris, Compound Discoverer

Goal

Development of a flow-injection analysis coupled with high-resolution mass spectrometry (FIA-HRMS) method for a qualitative analysis of wines from different varieties and regions.

Introduction

FIA-HRMS is a fast and powerful method that could be used for qualitative analysis of different matrices. In FIA-HRMS, a continuous flow of the sample is introduced into the mass spectrometer (MS), allowing for rapid and efficient analysis of complex mixtures. The high-resolution capabilities of the mass spectrometer enable accurate and precise detection of a wide range of compounds, providing a “fingerprint” of the sample. This technique is of utmost importance in various fields, including environmental monitoring,¹ pharmaceutical analysis,² metabolomics,³ and the food industry,^{4,5} as it enables researchers to detect and characterize numerous analytes with exceptional sensitivity—all with a single injection.

In this application note, an MS-based analytical method for qualitative analysis of wine from different regions and varieties is reported. FIA-HRMS was performed on a Thermo Scientific™ Vanquish™ Neo Ultra-High Performance Liquid Chromatography (UHPLC) system. Detection was performed on a Thermo Scientific™ Orbitrap Exploris™ 480 hybrid mass spectrometer with heated electrospray ionization (HESI) by polarity switching. Samples were directly injected with no prior chromatographic separation, enabling this method to be used for high-throughput applications.

Experimental

Sample preparation

Samples of wine (Table 1) were purchased at a local supermarket and diluted (1:25) with acetonitrile. A pooled sample (QC) was prepared by collecting aliquots of all the samples in the study and pooling them into a single sample and diluting it in a 1:25 ratio with acetonitrile, which was injected consistently throughout the batch. The diluted samples were transferred to a 96-well plate, sealed, and kept at 7°C in the autosampler prior to injection.

Flow-injection analysis

FIA was performed on a Vanquish Neo UHPLC system using direct injection mode at a micro-flow rate. No chromatographic column was employed in this method; however, the dimensions detailed in Table 2 were used to enable the method setup.

Table 1. List of wine samples according to their variety and country.

Type of wine	Country	Variety	Sample number
Red	South Africa	Malbec	1
Red	Argentina	Malbec	2
Red	Australia	Shiraz	3
Red	Spain	Rioja	4
White	South Africa	Chenin Blanc	5
White	Australia	Chardonnay	6
White	Australia	Sauvignon Blanc	7
White	Chile	Sauvignon Blanc	8
White	Italy	Pinot Grigio	9

Table 2. Separation column specifications.

Property	Value
Inner diameter	10 µm
Length	1 cm
Void volume	0.001 µL
Maximum pressure	1,450 bar
Maximum flow	100 µL/min
Maximum temperature	60°C
Maximum pressure change-up	1,000 bar/min
Maximum pressure change-down	1,000 bar/min

Load settings were set to “Keep Loop Inline.” Wash and Equilibration settings were set as described in Table 2. Fast Equilibration mode was toggled on.

Table 3. Wash and Equilibration settings.

Property	Value
Mode	Combined control
Flow	100 µL/min
Pressure	1,200 bar
Equilibration factor	2
Used % B	80%

The mobile phase composition consisted of H₂O (A) and Acetonitrile (B), at a constant flow rate of 50 µL/min, using an injection volume of 1 µL of diluted sample. The details of the analytical method are provided in Table 4.

Table 4. Gradient program.

Time (min)	Duration (min)	Flow (µL/min)	%B	Volume (µL)	No. of column volumes
0.0	Run				
0.0	0.0	50.0	80.0	0.0	0.0
0.3	0.3	50.0	80.0	15.0	15,000.0
0.3	Column wash				
0.3	0.0	50.0	80.0	0.0	0.0
0.4	0.1	50.0	80.0	5.0	5,000.0
0.4	Stop run				
0.4	Column equilibration				

After sample aspiration, an inner and outer needle and metering device wash was carried out using a strong wash solvent of 80:20 acetonitrile to water and a weak wash solvent of water, both containing 0.1% formic acid. Wash parameters are shown in Table 5.

Table 5. Outer needle wash.

Property	Value
Wash mode	After draw
Wash time (strong)	3 s
Wash speed (strong)	80 μ L/s
Wash time (weak)	5 s
Wash speed (weak)	80 μ L/s
Wash cycle time	Default

The parameter “air gaps” was not selected, while Vial Bottom Detection was toggled on. No temperature control was applied to the column compartment.

High-resolution mass spectrometry

Data were acquired on a benchtop hybrid Orbitrap Exploris 480 mass spectrometer with HESI operated in “Small Molecule” application mode. The infusion mode was set to “liquid chromatography.” The peak width was set to 6 s, while both advanced peak determination and mild trapping were not applied. The default charge state was set to 1. For internal mass calibration, the Thermo Scientific™ EASY-IC™ ion source was used in “Scan-to-Scan” mode. Thermo Scientific™ Xcalibur™ and Thermo Scientific™ AcquireX™ software method modification was toggled off. A summary of the MS source conditions is reported in Table 6. Data were acquired in Full Scan at a resolution of 120,000 (@ m/z 200) from m/z 100 to 1,000. The RF Lens was set to 70%, AGC “Standard” (1e6) and Maximum Injection Time set as “Auto.” Data were collected in profile mode with one micro-scan per scan. Polarity switching was applied.

Table 6. HESI source settings.

Property	Value
Ion source type	H-ESI
Spray voltage	Static
Positive ion (V)	3,000
Negative ion (V)	2,000
Gas mode	Static
Sheath gas (Arb)	25
Aux gas (Arb)	7
Sweep gas (Arb)	0
Ion transfer tube temp (°C)	320
Vaporizer temp (°C)	75

Data processing and analysis

Data were acquired using Xcalibur software version 4.5 and processed with the Thermo Scientific™ Compound Discoverer™ software version 3.3 SP2, using the workflow represented in Figure 1. Multivariate analysis was performed to assess significant differences among samples.

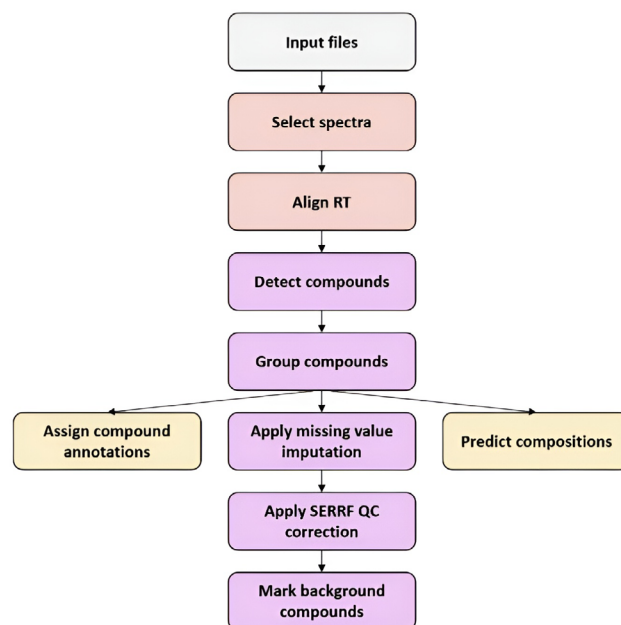


Figure 1. Workflow used on Compound Discoverer software.

Results and discussion

Samples were directly injected in technical replicates (n=4) without any chromatographic separation and detected by high-resolution mass spectrometry using polarity switching (positive and negative mode), allowing the acquisition of a fast and accurate “fingerprint” in only one minute per sample. Furthermore, data processing took 5 minutes 14 seconds in Compound Discoverer version 3.3 SP2 software for all collected samples and QCs. This method enables high-throughput analysis and a quick characterization of hundreds of samples per day and is accompanied by a fast data processing workflow.

A representative flowgram of wine samples is reported in Figure 2. The final table comprises a list of 274 compounds with high-quality peak shapes and great technical reproducibility (RSD% <30%). Both polarity modes were merged into a single table (Figure 3). Compounds and other artifacts present in the blank (solvent background) were filtered out from the compound table.

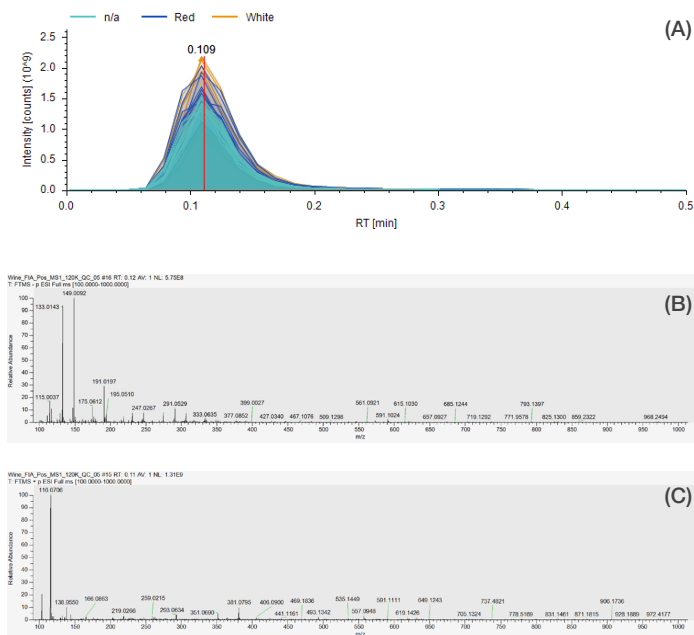


Figure 2. Typical extracted ion flowgram (*m/z* 133.0143) for red and white wine samples (A). Mass spectrum in negative ion mode (B) and positive ion mode (C).

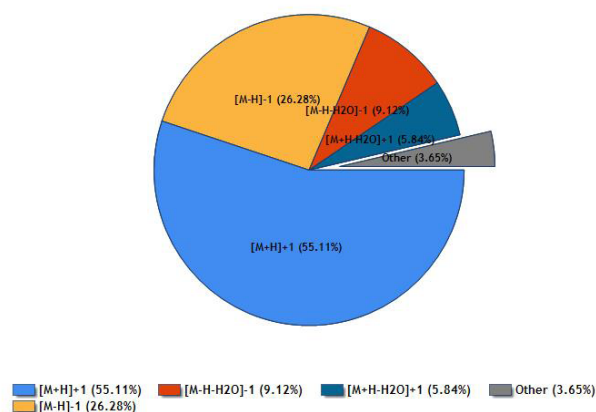


Figure 3. Percentage of compounds according to their reference ions.

A pooled quality control (QC) sample was injected multiple times across the batch to assess system performance and technical variability.

Based on multivariate analysis, it is possible to identify significantly different features that are associated with each wine type. In the principal component analysis (Figure 4), we can see a clear separation between wine types (4A), varieties (4B), and countries (4C).

These significant features can be exported as an “Inclusion list,” and MS² data can be acquired to annotate these compounds putatively.

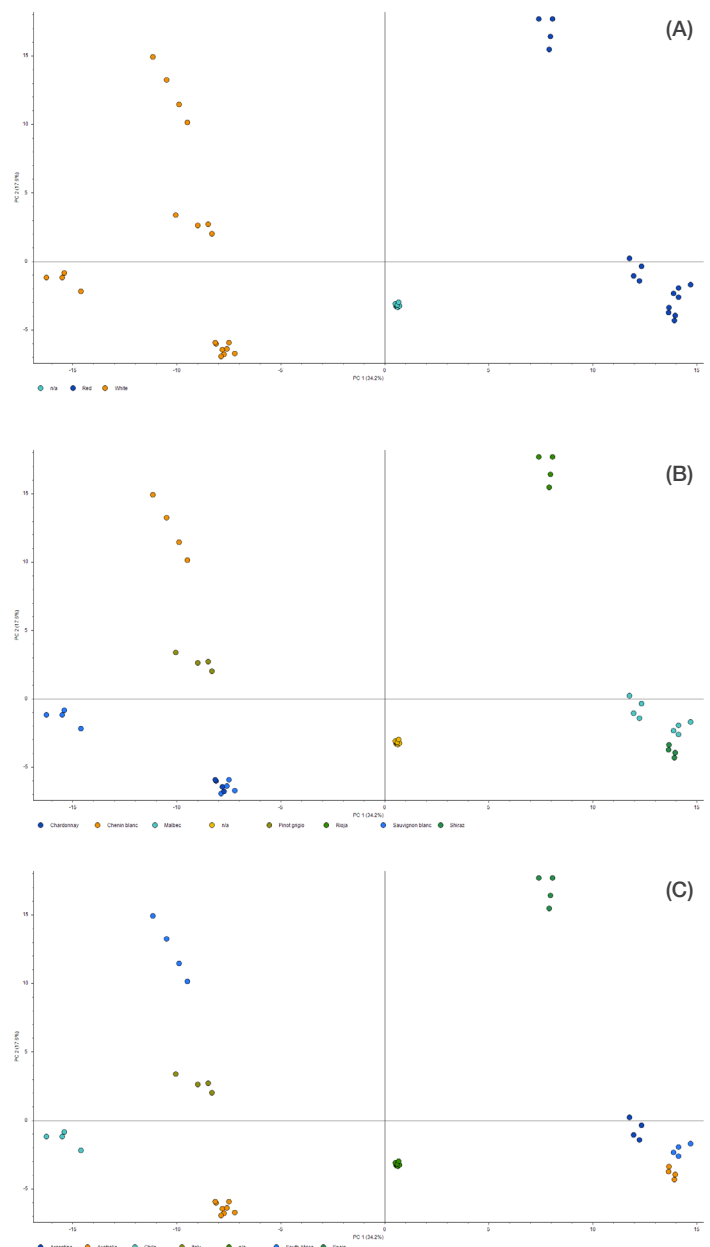


Figure 4. Principal component analysis performed on wine samples.

A heatmap comparing all the study samples is shown in Figure 5. It is possible to observe a clear separation between the wine types and their varieties.

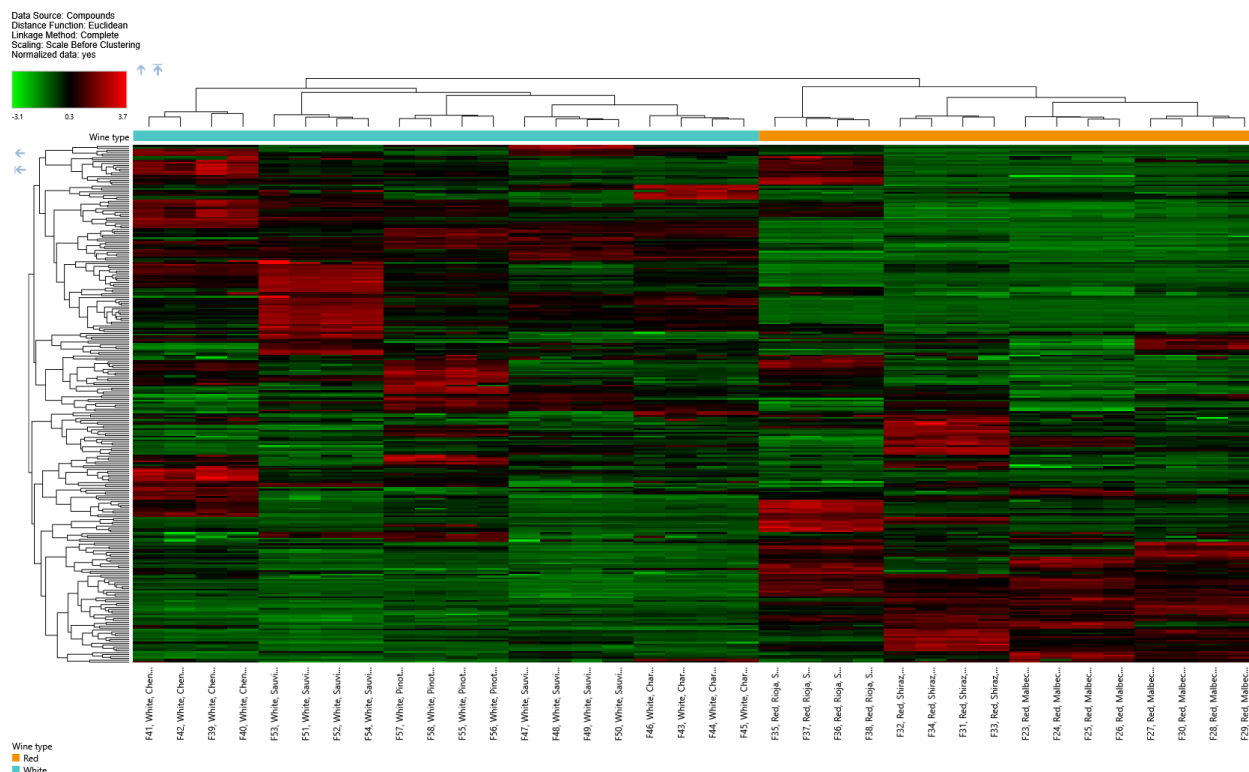


Figure 5. Heatmap comparison of the study samples.

Conclusions

The FIA-HRMS method implemented on a Vanquish Neo UHPLC system and Orbitrap Exploris 480 mass spectrometer, in combination with Compound Discoverer software, proved to be a fast, reliable, and accurate way to qualitatively assess differences between wine samples from different varieties and locations, providing an alternative to time-consuming classical chromatographic methods.

References

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