



Polyphenol Analysis by CrossTOX® Clean-up of Wine, Mulled Wine and Spirits

Polyphenols - Wine-aging and Spirit Aromatisation



Introduction

Polyphenols are secondary plant metabolites that are ubiquitous as colorants, flavourings, attractants or defence mechanisms, attracting insects or protecting plant organs from being eaten. The function of polyphenols in nutrition is a more recent phenomenon. An example of polyphenols with potential health-promoting ability is resveratrol, which is found in various fruits, especially grapes and products made of them. Analysis of polyphenolic compounds in foods is a difficult approach because total phenolic analysis does not distinguish polyphenols from other phenolic components. For this purpose, a liquid chromatographic approach could be used, and in combination with mass spectrometry, the identification and quantification of polyphenols could be achieved. Good extraction and sample clean-up will help to overcome matrix interferences and enable simple, rapid and reliable analysis with low sample analysis costs and reduces maintenance costs for analytical devices.

3 Step Clean-up – Easy and Fast for *Polyphenol* Analysis from Various Matrices.

The nature of polyphenols not only as aroma compounds but also as an indicator of barrel-aged wine is a tool to determine the quality of wine in the context of food safety and regulation. This is the only way to unmask falsely marketed wine that has not been aged in barrels but is aromatised by the addition of polyphenols, suggesting higher quality products. For this purpose, we studied various wines and spirits to determine the content of certain polyphenols. Thanks to the individual polyphenol profile, alcoholic beverages can be characterised. This is like a “fingerprint” of the wine and gives us information about the time of barrel aging to gain aroma and flavor, which over the years increases the value enormously. Food fraud could be monitored thanks to this process, as the addition of flavors such as vanillin can be easily determined. Just load the sample onto the column. Wash the column to remove matrix interferences. Elute by adding methanol to the dried column. Ready for analysis!

- Easy, fast and selective 3-step clean-up
- Excellent matrix reduction
- Compatible with LC-MS/MS analysis
- Dedicated for high throughput analysis



CrossTOX® columns

Processing Protocol

Wine and spirits samples are diluted with HPLC water before being applied to the cartridge

- For *wine* samples, dilute 1 mL of wine with 4 mL of HPLC water
- For *spirits*, dilute 1 mL of the sample with 9 mL of HPLC water

The diluted sample is applied to the CrossTOX® column under constant flow (max. 2 mL / min). The column is washed with 2 mL of HPLC water and dried by an air purge to remove residual water from the column bed. The analytes are eluted with 1 mL of methanol incubated in the column bed for 5 minutes.

The eluate is collected and analysed by LC-MS/MS; to increase analytical sensitivity, the eluate can be concentrated at room temperature by a stream of nitrogen and then dissolved in LC solvent for analysis.



Chromatograms

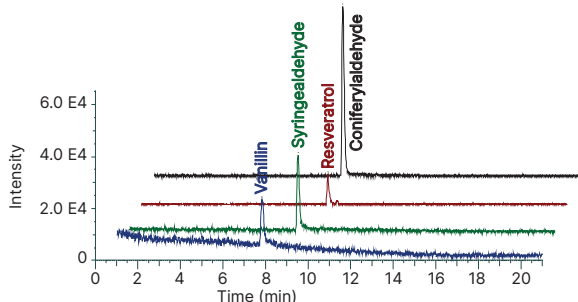


Fig. 1: The chromatographical analysis revealed good separation and identification of the selected polyphenols in all investigated matrices.

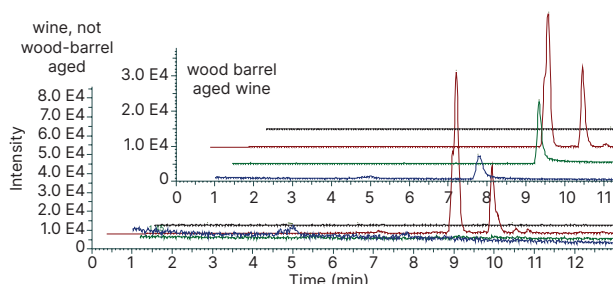


Fig. 2: Chromatographic comparison of barrel-aged and non-barrel-aged mulled wines.

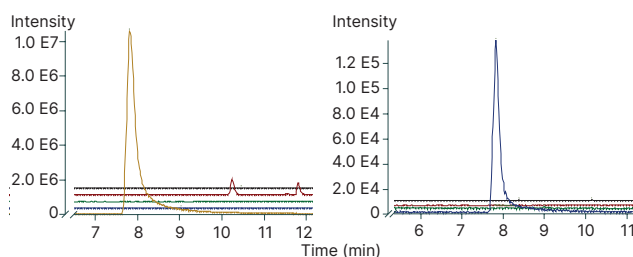


Fig. 3: Caramelle containing alcoholic beverage

Fig. 4: "Not aromatised" declared spirit named "pure". Case of food fraud!

Strong flavouring by vanillin can be found in various spirits, if the flavouring is not displayed, it could be food fraud.

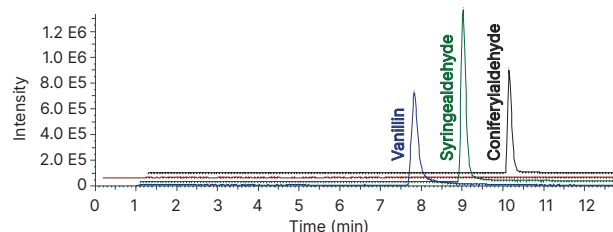


Fig. 5: Chromatogram of a wood barrel ageing rum.

Scotch and single malt whiskey spirits also show significant polyphenols, which in combination and ratio are dependent indicators of wood barrel ageing (data not shown). In addition, the individual ratio of polyphenols and the total amount reflect the individual aroma of the spirits and could be used as a guide to predict the value of spirits during the maturation process in combination with the original ingredients and the distilling process.

Analytical Parameters and Specifications

UHPLC-Vanquish MS/MS TSQ H-ESI mixed mode, UHPLC column Accucore Biphenyl 100 mm x 2.1 mm; 2.6 μ m with defender guard.

Column temperature was set to 38 °C, Eluent A: HPLC water/methanol (98 / 2 (v/v)) + 1 % acetic acid + 5 mM ammonium acetate. Eluent B: HPLC water/methanol (2 / 98 (v/v)) + 1 % acetic acid + 5 mM ammonium acetate. Flow rate 0.4 mL / min. MS settings: heated ESI, 3090 V positive / 3090 V negative ion voltage. Ion transfer tube temperature 325 °C, vaporiser temperature 350 °C, sheath gas 40 arb; aux gas 22 Arb; sweep gas 0 Arb. CID gas (agron) 1.5 Arb.

Time (min)	Eluent A (%)	Eluent B (%)	Curve
0-2	95	5	5
2-5	95	5	5
5-15	5	95	5
15-20	5	95	5
21-22	95	5	5
23-25	95	5	5

Table 1: UHPLC gradient conditions.

Conclusion

The analysis and profiling of wine products and spirits in terms of polyphenol content represents a new tool and an important analytical aid in the characterisation of spirit quality, furthermore the identification of false declaration and aromatisation can be analysed by an objective approach (fig. 3). The method can also be applied to wine products such as mulled wine and wine products specially produced for the Christmas season and allows the identification of flavouring additives or false declaration of these products (fig. 2).

These tools will help improve wine and spirit quality with regard to the official production process and ensure a higher level of food safety by detecting food fraud in beverages.

This LCTech product was used:

17900 CrossTOX®

100 pcs / package

Do you have a special request as to which matrix we should test for you?
Contact us by e-mail at: info@LCTech.de