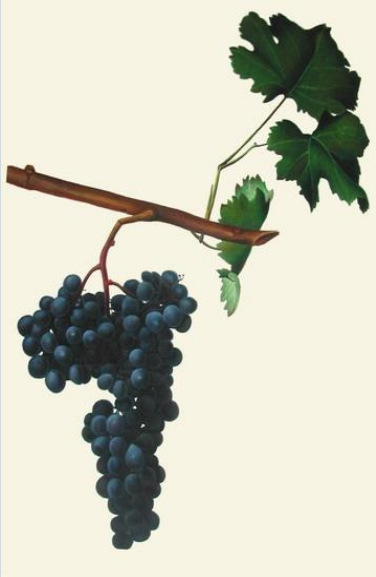
Ochratoxin A in wine: an exemplum of SACI performances

Keywords: Ochratoxin A, wine, no sample preparation, high sensitivity

Ochratoxin A (OTA) is a secondary metabolite of fungi *Penicillium verrucosum* and *Aspergillus ochraceus*. Toxic effects of OTA are renal damage with nephrotoxic effect, to interfere with mitochondrial respiratory function and pH homeostasis, and impaired organic anion transport. The toxin has also inhibition effect on tRNA-synthetase, enhanced lipid peroxidation *via* the generation of free radicals, can induce the formation of renal tumours. The fungi develop on grape, in particular in the harvest, in advantageous conditions of high humidity and temperature and OTA is transferred to the wine in winemaking. As a consequence dangerous contamination occurs in table grapes and wines. This has prompted adoption of regulatory limits in grape, grape juice and wine (CE Regulation n° 123/2005: maximum limits 0.002 ppm) which, in turn, implies the development of validated official analytical methods. In this study a new LC/MS-SACI method for OTA analysis in grape and wine performing reverse phase-LC/MS^3 analysis by direct injection of sample using internal standard ZAN, was developed.



Experimental conditions and settings:

Analyte: Ochratoxin A

Samples: Cabernet Sauvignon, Negroamaro and Valpolicella red samples commercially available.

Sample Preparation and Analytical Conditions: Wine samples were added of internal standard ZAN (zearalanone) in concentration 10 ng/mL and filtered by polypropylene 0.2 mm filter.

LC-MS/MS conditions: C18 2.1x100 mm; 3.5µm column was used. The chromatographic analysis was performed under gradient conditions.

The mobile phases were: (A) H2O/0.1 % formic acid/CH3COONa 0.6 mmol, and (B) CH3OH/0.1 % formic acid. Gradient program: 50 % A for 1 min, from 50 % to 20 % di A in 7 min, 20 % A for 4 min, from 20 % to 50 % A in 3 min, 50 % A for 3 min. Sample volume injected 20 µL; Flow 0.5 mL/min. Sample volume injected 20 µL; Flow 0.5 mL/min.

Instruments Employed&Settings:

- HCT ultra, Bruker Daltonics (Breman, Germany) .
- HPLC Dionex Ultimate 3000

LC-SACI mass spectra were obtained using multiple collisional dissociation (MS^3) approaches

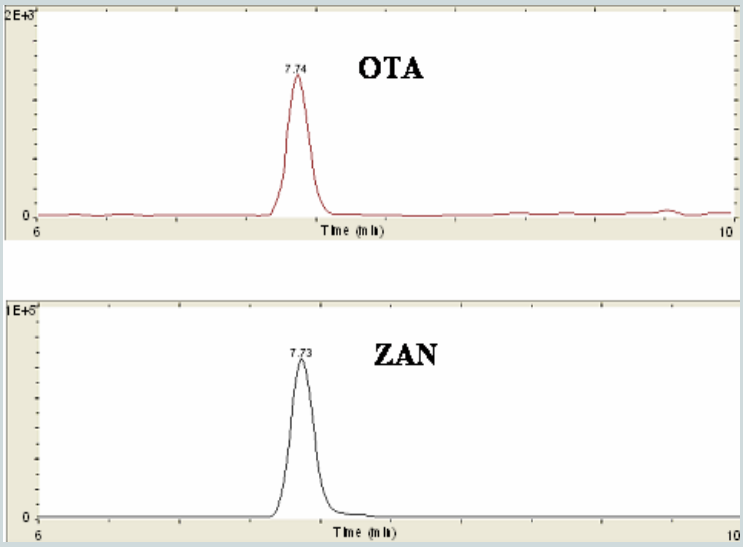
The isolation width of the precursor ion was 3 mass units. The collision energy applied to the precursor ion was 1V while (MS/MS) and the fragment ions obtained were fragmented by using 1.1V.

Experimental Results:

A new quantitative approach based on Liquid chromatography – Surface Activated Chemical Ionization (SACI) multistage fragmentation mass spectrometry (LC-SACI-MS^3) was performed to analyze Ochratoxin A (OTA) in wine. The sample was directly injected in the chromatographic column without any sample pre-concentration and purification step and the high flow gradient chromatographic conditions were used in order to avoid the matrix effect phenomenon. LC-MS^3 extracted ion chromatograms (Figure 1) relative to analysis of the OTA 0.1 ng/mL spiked wine sample for OTA (above) and ZAN 10ng/mL (below) are reported in Fig.1. SACI allows to detect OTA and ZAN by direct injection 20µL of wine samples; precursor ions and respective product ion of these compounds are reported in table 1.

	OTA	ZAN
MW*	403	320
[M+H]+ *	404	321
MS^2 Fragment ion *	358	303
MS^3 Fragment ion *	341, 239	163, 189, 207

Table 1: MW, precursor and product ions of OTA and ZAN. (* 35Cl-containing isotopic species)



Wine sample spiked 10ppb	Content found (ppb)
Cabernet Sauvignon	13.1
Valpolicella	9.2
Negroamaro	9.4

Table 2. Analyses of the three wine samples spiked with OTA 10 ng/mL.

Figure 1. EIC Chromatogram of MS^3 fragment ions

In Table 2 are reported analyses of three different wine samples spiked with OTA 10 ng/mL. Data not evidence significant effects of matrices.

Conclusions:

In the experimental conditions used SACI showed high efficiency in OTA ionization. The high sensitivity of method allowed to by-pass all steps of sample pre-concentration or purification and to reach a limit of quantification (LOD) at least 20-fold below the legal limit.

The limit of detection (LOD: **0.02 ng/mL**) and quantization (LOQ: **0.1 ng/mL**) of the approach lower with respect to legal limit (maximum LOD: 2 ng/mL) making possible its potential adoption for official screening purpose. Results achieved by analyzing 3 wine are shown and discussed.

The methods was found to be **highly robust**: analysis 10 times repeated of Cabernet Sauvignon spiked sample showed good repeatability of method with Cv% = 2.

Repeatability of method meets the acceptability criteria proposed from International Organization of Vine and Wine (O.I.V.) for analysis of OTA in red wine.

Acknowledgements and References:

Acknowledgements

1)ISB srl, via Fantoli 16/15, 20138 Milano.

2)CRA, Istituto Sperimentale per la Viticoltura, viale XXVIII aprile, 26 – 31015 Conegliano (TV), Italy

3)Bruker Daltonics S.r.l., Macerata, Italy.

References

This brochure is based on the publication: A New Sensitive and Selective Method for Analysis of Ochratoxin A (OTA) in Grape & Wine: Surface Activated Chemical Ionization (SACI) - Submitted -

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