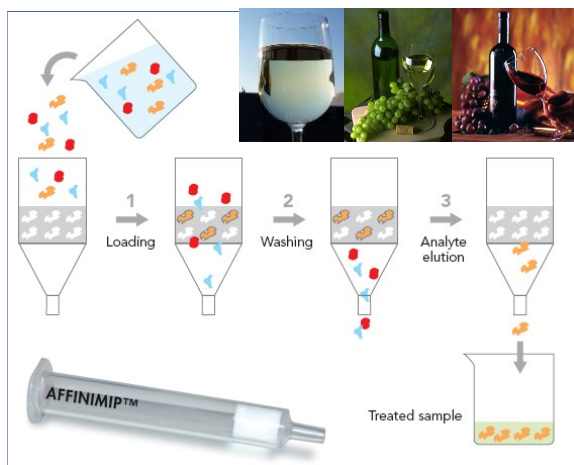


Selective Solid Phase Extraction of Ochratoxin A from Wine Products Using Molecularly Imprinted Polymers



Introduction

Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite of various *Aspergillus* and *Penicillium* fungi. It can be found on several commodities (e.g. cereals and cereal-based products, coffee, beer, grape juice, wine, cacao products, spices products).

Ochratoxin A exhibits toxicity in animals and mankind, including nephrotoxic, hepatotoxic, immunotoxic, teratogenic and carcinogenic effects and represents therefore a serious health risk to livestock and population. In order to limit these effects, European Regulation (EC) 1881/2006 sets maximum levels for Ochratoxin A in foodstuffs (e.g. 5µg/kg in raw cereal grains, 30µg/kg in spices).

The analysis of Ochratoxin A in the commodities requires pre-treatment of the sample prior to High Performance Liquid Chromatography (HPLC) combined with fluorescence detection or MS-detection to remove matrix components and enhance sensitivity.

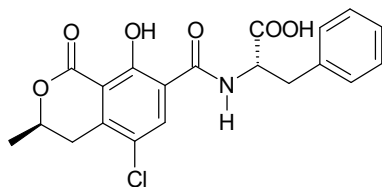


Figure 1. Chemical structure of Ochratoxin A, CAS N° 303-47-9.

In this application note, the efficiency of a method employing Molecularly Imprinted Polymer (MIP) as selective sorbents for solid-phase extraction (**AFFINIMIP® SPE Ochratoxin A**, AFFINISEP) is shown in respect to the

clean-up and pre-concentration of Ochratoxin A in different matrices (white wine, red wine).

Molecularly imprinted polymer (MIP) is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. MIP has the advantages to be not only highly selective and specific but also chemically and thermally stable, compatible with all solvents and cost-effective. This polymer is used as a powerful technique for clean-up and pre concentration applications of Ochratoxin A.

Experimental conditions

Materials

All reagents and chemicals were ACS grade quality or better. Ochratoxin A was obtained from Sigma Aldrich (Fluka). Samples were purchased in different supermarkets.

The SPE procedure used 3mL **AFFINIMIP® SPE Ochratoxin A** Cartridges.

Analysis

HPLC was performed on a Jasco System with a Thermo Hypersil Gold C18 column (150mm x 2.1mm) protected by a Hypersil Gold (10x2.1mm) guard column. Separation was carried out using a mobile phase of deionized water/acetic acid/MeOH (39/1/60, v/v) at a flow rate of 0.2mL/min. The detection system was a Jasco Model FP-2020 Fluorescence detector set to excitation/emission wavelengths of 333 and 460nm, respectively. The injection volume was 20µL.

Purification procedure of Ochratoxin A from wine matrices

Preparation of samples prior to SPE

10mL of wine is diluted with 10mL of HCl solution pH=1, 0.1M. This solution is used as the loading solution.

Solid phase extraction (SPE) protocol

The details of each step are as follows:

- Condition the SPE Cartridge with 4mL of Acetonitrile (ACN), then with 4mL of deionized water
- Load 2 to 10mL of the loading solution (eq. 1 to 5mL of sample)

- Wash the cartridge with 7mL of 60/40 HCl solution pH=1, 0.1M /ACN (v/v)
- Elute Ochratoxin A with 2mL of Methanol (MeOH) containing 2% of Acetic acid (v/v)

The elution fraction is then evaporated and dissolved in the mobile phase. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis. The SPE procedure lasts approximately 30 minutes.

Results

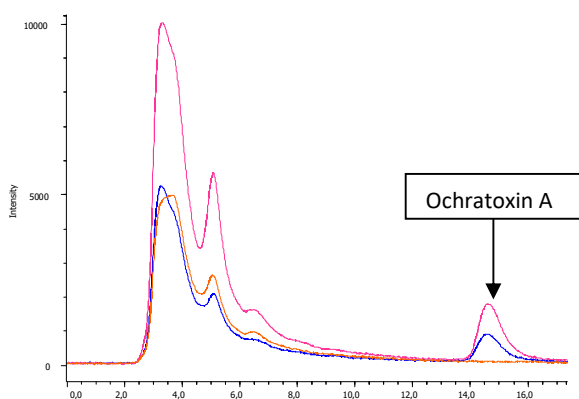


Figure 2. Chromatograms obtained after purification of white wine spiked at 2µg/kg (loading with 5mL (blue); loading with 10mL (pink) and after a loading of 5mL of not contaminated white wine (orange) with **AFFINIMIP®SPE Ochratoxin A**

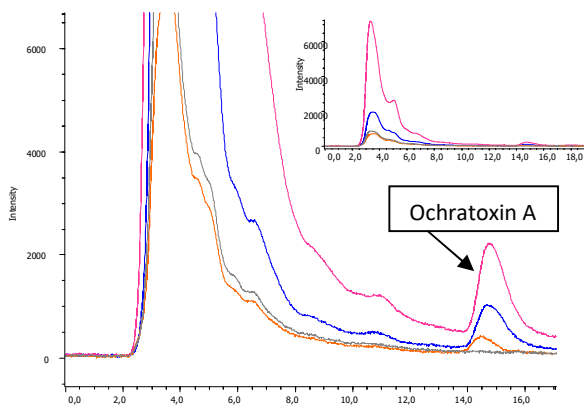


Figure 3. Chromatograms obtained after purification of red wine spiked at 2µg / kg (loading with 2mL (orange); loading with 5mL (blue); loading with 10mL (pink) and after a loading of 2mL of not contaminated red wine (grey) with **AFFINIMIP®SPE Ochratoxin A**

Table 1. Recoveries of Ochratoxin A after **AFFINIMIP®SPE Ochratoxin A** Clean-up in wine (white and red).

Matrix	C° (µg/kg)	Recoveries %	% RSD
White wine (n=10)	2	91.3	6.2
Red wine (n=4)	2	78.8	2.8

Conclusion

The use of **AFFINIMIP®SPE Ochratoxin A** cartridge is a simple, fast, efficient and selective tool for the extraction of Ochratoxin A. **AFFINIMIP®SPE Ochratoxin A** complies with the performance criteria for Ochratoxin A analysis defined on the European Regulation (EC) 401/2006. This regulation requires recovery yields for Ochratoxin A higher than 70% for OTA concentration values between 1-10µg/kg in foodstuffs with reproducibility relative standard deviation lower than 30%. The use of **AFFINIMIP®SPE Ochratoxin A** enables to obtain recoveries above 75% with reproducibility relative standard deviation below 10%.

This method is well-suited for the analysis of Ochratoxin A in wine.

References

Commission Regulation (EC) No. 1881/2006 of 19 December 2006, Official Journal of the European Union.

Commission Regulation (EC) No. 401/2006 of 23 February 2006, Official Journal of the European Union.

Related products

- **AFFINIMIP®SPE Ochratoxin A** (ref.: FS101-02 for 25 cartridges)
- **AFFINIMIP®SPE Patulin** (ref.: FS102-02 for 25 cartridges)
- **AFFINIMIP®SPE Zearalenone** (ref.: FS100-02)