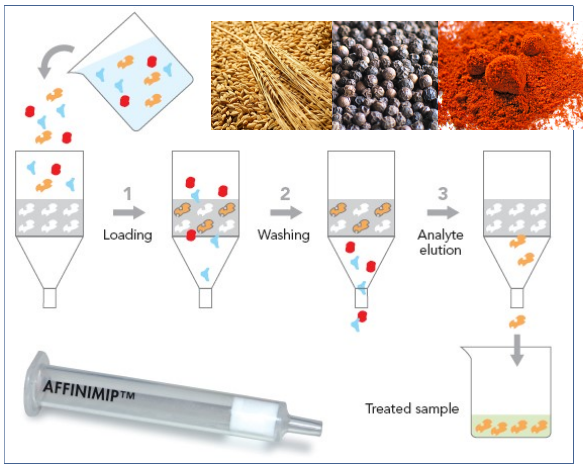
Selective Solid Phase Extraction of Ochratoxin A from Cereals and Spices Products Using Molecularly Imprinted Polymers



Introduction

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

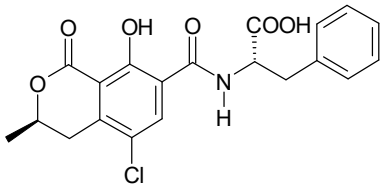


Figure 1. Chemical structure of Ochratoxin A, CAS N° 303-47-9.

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 showed in respect to the clean-up and pre-concentration of Ochratoxin A in different matrices (Wheat, Paprika, Pepper).

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 (10×2.1mm) 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 wheat matrices with 60/40 Acetonitrile/Water extraction solution

Preparation of samples prior to SPE

50g of finely ground wheat are mixed during 1 minute in a blender with 100mL of extraction solvent (60/40 Acetonitrile/deionized Water). The extract is filtered through a filter paper.

Then, 5mL of the extract is diluted with 5mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper, 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

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water

- Load 4mL of the loading solution (eq. 1g 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.

Purification procedure of Ochratoxin A from paprika matrices

Preparation of samples prior to SPE

10g of paprika are shaken during 30 minutes with 100mL of NaHCO3 1% in water. The extract is centrifuged for 30 minutes at 4000 rpm at room temperature then filtered through a filter paper.
25mL of the extract is diluted with 25mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper, 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 20mL of the loading solution (eq. 1g 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.

Purification procedure of Ochratoxin A from pepper matrices

Preparation of samples prior to SPE

10g of Pepper are shaken during 30 minutes with 100mL of NaHCO3 1% in water. The extract is filtered through a filter paper.

5mL of the extract is diluted with 5mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper, 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 5mL of the loading solution (eq. 0.25g of sample)
- Wash the cartridge with 5mL of 60/40 HCl solution pH=1, 0.1M /ACN (v/v)
- Wash the cartridge with 5mL of buffer ammonium formate pH=7.4, 30mM
- Dry 10 seconds or force the water down into the cartridge and out the bottom
- Wash the cartridge with 1mL of CHCl3
- Elute Ochratoxin A with 2mL of methanol (MeOH) containing 2% of acetic acid (v/v)

The elution fraction is then evaporated and dissolved in mobile phase. The SPE procedure lasts approximately 45 minutes.

Results

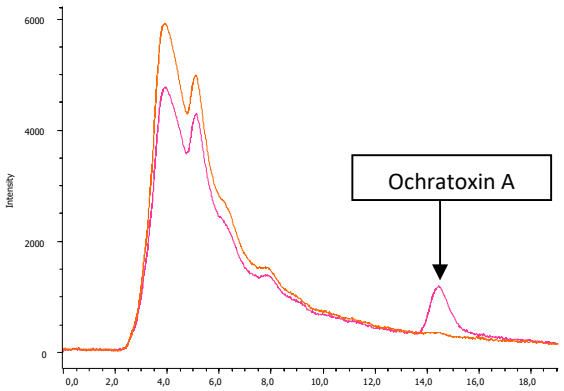

Figure 2. Chromatogram obtained after purification of wheat (spiked at 5µg / kg (pink) or not contaminated (orange)) with **AFFINIMIP® SPE Ochratoxin A**

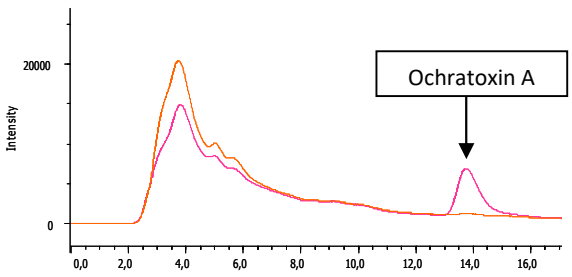

Figure 3. Chromatogram obtained after purification of paprika (spiked at 30µg / kg (pink) or not contaminated (orange)) with **AFFINIMIP® SPE Ochratoxin A**

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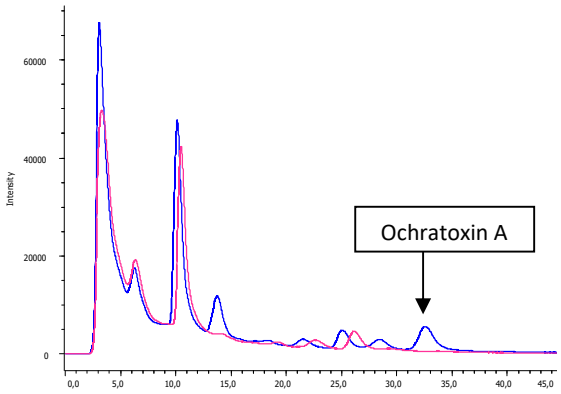

Figure 4. Chromatogram obtained after purification of pepper (spiked at 30µg / kg (blue) or no contaminated (pink)) with **AFFINIMIP® SPE Ochratoxin A**

Table 1. Recoveries of Ochratoxin A after **AFFINIMIP® SPE Ochratoxin A** Clean-up in different matrices.

Matrix	C° (µg/kg)	Recoveries %	% RSD
Wheat (n=6)	5	96.3	7.7
Paprika (n=4)	30	93.3	3.4
Grey Pepper (n=3)	30	73.3	5.7

Conclusion

The use of **AFFINIMIP® SPE Ochratoxin A** cartridge is a simple, fast, sensitive 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 values for Ochratoxin A higher than 70% for Ochratoxin A concentration values between 1-10µg/kg in foodstuffs with repeatability relative standard deviation lower than 20%. The use of **AFFINIMIP® SPE Ochratoxin A** enables to obtain recoveries above 80% with repeatability relative standard deviation below 10%.
This method is well-suited for the analysis of Ochratoxin A in cereal-based products or spices.

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
Catalog number: FS101-02 for 25 columns
Catalog number: FS101-03 for 50 columns

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