Method for Selective Solid Phase Extraction of Ochratoxin using Molecularly Imprinted Polymers (MIP) and Automated Protocol

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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 products, coffee, beer, grape juice, wine, cacao products).

OTA exhibits toxicity in animals and mankind, including nephrotoxic, hepatotoxic, immunotoxic, teratogenic and carcinogenic effects, and represents therefore a serious health risk to lifestock and population. In order to limit these effects, European Regulation (EC) 1881/2006 sets maximum levels for OTA in foodstuffs (e.g. 5µg/Kg in raw cereal grains, 2µg/kg in wine).

The analysis of OTA in the commodities requires pre-treatment of the sample prior to high-performance liquid chromatography (HPLC)/ fluorescence[1] or MS-detection[2,3] to remove matrix components and enhance sensitivity.

This application note describes the efficiency of a **SPE cartridge based on molecularly imprinted polymer technologies (MIPs).** More precisely, the cartridge **AFFINIMIP™OTA (POLYINTELL)** is the **perfect tool** for the selective extraction of OTA from a complex matrix, especially for the clean-up and the pre-concentration of sample at trace levels. **AFFINIMIP™** OTA complies with the performance criteria for OTA analysis defined on the European Regulation (EC) 401/2006 with a recovery of OTA higher than 80% at 5µg/kg.

The purification described in this application note was performed on the **GILSON GX271 ASPEC** automate.

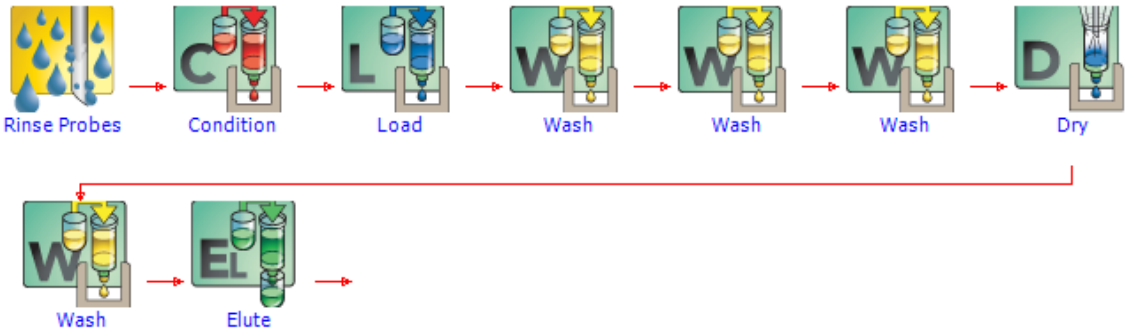
Sample preparation

50g wheat grains were ground during 2 min in a blender. Then the powder was blended with 100mL of acetonitrile/deionized water (60:40, v/v) during one minute to extract OTA. 5mL of the extract sample was diluted with 5mL of hydrochloric acid solution (HCl, pH=1). The solution is then filtered with a filter paper to obtain the loading solution

[1] *H. Valenta, J. Chromatogr. A 815 (1998) 75.*
[2] *P. Zöllner, A. Leitner, D. Lubda, K. Cabrera, W. Lindner, Chromatographia, 52 (2000) 818*
[3] *A. Leitner, P. Zöllner, A. Paolillo, J. Stroka, A. Papadopoulou-Bouraoui, S. Jaborek, E. Anklam, W. Lindner, Anal. Chim. Acta 453, (2002) 33.*

AFFINIMIP column clean up

Molecularly imprinted SPE columns (**AFFINIMIP**™ **OTA, POLYINTELL**) sealed with Gilson polypropylene caps were put in the **GILSON GX-271 ASPEC** automate**.** All clean-up steps are entirely controlled via **Gilson TRILUTION LH Software** according to the following summarized schematic:



The automated protocol is divided in the following steps:

- Initialization of SPE Racks (the mobile rack are placed above waste)
- Probe Rinsing with Water
- Conditioning of SPE column by deionized water (H_2O) with a flow of 5mL/min.
- Loading 4 ml of sample solution at a flow rate of 0.8mL/min
- Washing with 1mL HCl solution (pH=1) at a flow rate of 5mL/min
- Washing with 1mL solution HCl (pH=1)/Acetonitrile (60:40, v/v) at a flow rate of 5mL/min
- Washing with 10 ml of Water at a flow rate of 5mL/min
- Drying of the column bed with nitrogen stream during 5min
- Washing with 4mL of Acetonitrile-0.01% acetic acid.
- Eluting OTA with 2mL of methanol-2% acetic acid at a flow of 0.8mL/min

Then, the elution was evaporated and dissolved in the mobile phase solution before injection to the HPLC system. An alternative method to the evaporation of the elution sample could be the dilution to a fixed volume before the injection to HPLC.

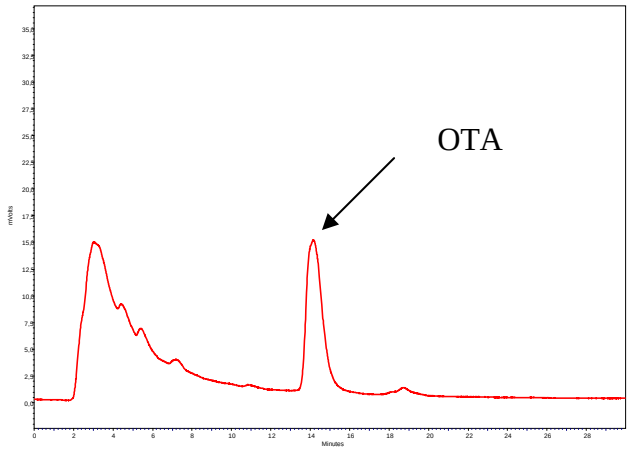
The clean-up steps took around 30min.

The throughput may be improved by using the **Gilson GX-274 ASPEC** automate which is able to clean-up 4 samples in parallel.

Analysis

The mobile phase consisted of a mixture of methanol/water/ 1% acetic acid (60:39:1, v/v) at a flow rate of 0.2 mL mL.min^{-1}. The injection volume was 20 µL. The analytical column was a polar endcapped C18 reverse phase Hypersil Gold (150×2.1 mm), protected by a Hypersil Gold (10×2.1 mm) guard column. The fluorescence detector was set to excitation/emission wavelengths of 333 and 460 nm, respectively

Results



Chromatogram obtained after purification of wheat (contaminated at 5µg/kg) with AFFINIMIP™ OTA.

Recoveries: 95%

Conclusion

This application note describes a simple, short and reliable pre-treatment method used for the quantitative analysis of OTA. This method was carried out with **AFFINIMIP™** OTA SPE cartridges in combination with a **GILSON GX-271 ASPEC automate** resulting in very good recoveries of OTA as well as unambiguous and reliable analysis (CV 1.7% vs CV 2.5% in manual mode).

This method is very well-suited for the control of Ochratoxin A in wheat samples.

Acknowledgements

We express our thanks to the **GILSON** team for putting at our disposal a GILSON automated system in order for us to finalize the validation of our new AFFINIMIP™ OTA cartridges. This collaboration also allowed us to validate our columns with GILSON a leading provider in the automated SPE platforms. Special thanks to **Mr. Fabrice Mangani.**

Finally; we would like to recognize the work of **Dr. Valérie Pichon; ESPCI Paris and her entire team** that worked in collaboration with Dr. **Sami Bayoudh (CSO/founder) and his entire team at POLYINTELL!**

If you wish to receive a FREE COLUMN STARTER KIT or for further information on the related product AFFINIMIP™ OTA *(ref# : PO-FS101-01C)* ***or on SPE automation please contact us:***

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