An Automated Method for the Selective Solid Phase Extraction of Ochratoxin A from Wheat Using Molecularly Imprinted Polymers

Keywords: AFFINIMIP™ OTA, European Commission Regulation (EC) 401/2006, European Commission Regulation (EC) 1881/2006, Feeds, Food, GX-271 ASPEC, HPLC, MIP, Molecularly Imprinted Polymers, Ochratoxin A, OTA, Solid Phase Extraction, SPE, Wheat

This study was performed in collaboration with Dr. Valerie Pichon's team at ESPCI, Paris, FRANCE and POLYINTELL Intelligent Polymers, Val de Reuil, FRANCE

Introduction

Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite of various *Aspergillus* and *Penicillium* fungi (see Figure 1). OTA has been detected in cereal products (wheat, maize, barley and oats), coffee and coffee beans, beer, wine, grapes, peanuts and cocoa products. OTA has also been found in animal tissue after exposure to OTA-contaminated feed (Pohland et al, 1992; Clark and Snedeker, 2006).

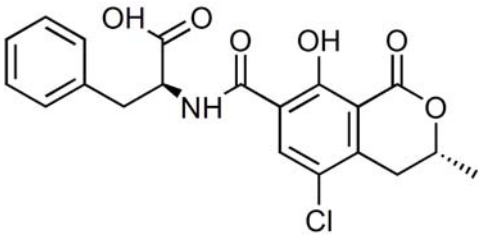

Figure 1. Chemical Structure of Ochratoxin A, CAS No. 303-47-9

Exposure to high levels of OTA or prolonged exposure to OTA have been linked to nephrotoxicity, hepatotoxicity and immunotoxicity in animals such as pigs and poultry. OTA is a teratogen and a suspected carcinogen. Humans are sensitive to OTA and ingestion has been linked to Balkan endemic nephropathy (Castegnaro et al., 1991). As a result, maximum limits for OTA contamination have been established in a number of countries. Member countries of the European Union have set maximum allowable levels of OTA at 5 µg/kg (5 ppb) in raw cereal grains, 3 µg/kg in cereal grains intended for human consumption and 2 µg/kg in wine (European Commission Regulation (EC) 1881/2006).

The analysis of OTA in agricultural products requires extensive extraction and post-extraction clean-up of the sample prior to analysis by HPLC with fluorescence detection or detection by mass spectrometry. These steps remove matrix interferents and enhance sensitivity (Valenta, 1998; Zöllner et al., 2000; Leitner et al, 2002). Molecularly Imprinted Polymers (MIPs) have been demonstrated to be very effective tools for the selective extraction of an analyte from a complex matrix such as a food product (Haginaka, 2009; Wei et al., 2007). This study describes the automated solid phase extraction (SPE) of OTA from wheat utilizing a Molecularly Imprinted Polymer (MIP) SPE cartridge that is highly specific for OTA (AFFINIMIP™OTA, POLYINTELL) and the Gilson GX-271 ASPEC™ System (Figure 2).

Figure 2. Gilson GX-271 ASPEC System with 406 Dual Syringe Pump (Part no. 2614008)

Experimental Conditions

Materials

All solvents were distilled in glass suitable for GC, HPLC, pesticide residues analysis and spectrophotometry. All reagents and chemicals were ACS grade quality or better. Wheat certified as OTA free was obtained from LGC (Teddington, UK). Wheat blank is also available from Sigma-Aldrich (catalog number BCR471). Ochratoxin A standard was obtained from Sigma Aldrich (OEKANAL® OTA solution in acetonitrile).

Preparation of Samples Prior to SPE with AFFINIMIP OTA Cartridge

Fifty grams of wheat grains were ground for 2 minutes in a blender to a powder. This powder was then mixed with 100 mL of acetonitrile/deionized water (60:40, v/v) for one minute to extract the OTA. Five milliliters of the extract was diluted with 5 mL of hydrochloric acid solution (HCl, pH=1). The solution was then filtered using filter paper and transferred to a test tube for MIP SPE extraction.

SPE Hardware

The Gilson GX-271 ASPEC System was configured as follows:

Description	Part numbers
GX-271 ASPEC w/ Dual 406 Syringe Pump	2614008
25 mL and 10 mL Syringes	25025346 and 25025345
406 Dual Adaption Kit for ASPEC plus 10 mL and 25 mL Plumbing Packages	2644708, 2644701 and 2644702
221x1.5x1.1mm BV Tapered Probe and Guide Assembly for 1.5 mm Probes	27067374 and 26046228
Rinse Stations	26034551 and 26034555
SPE Pressure Reg. Assembly, Plumbing pkg, GX-271 ASPEC 406 Dual Air/Gas, Plumbing pkg GX-271 ASPEC Air-Gas	25051376, 2644709 and 2644703
Locator Tray for five 20-Series Racks	26041033
DEC Accessory Kit for 3 mL SPE Cartridges	2604702
Rack Code 345 for 44 16 x150 mm Tubes	260440041
Code 61 Rack with glass bottles	2954715 and 2954663 (2)
Safety Shield Assembly, GX27X	2604706
TRILUTION® LH Software Package	21063020, 210630R20 and ORACLE10GXE

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 3 mL POLYINTELL AFFINIMAP OTA Cartridges. The cartridges were sealed using Gilson 3 mL Sealing Caps.

The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, Gilson TRILUTION LH Software(Figure 3).

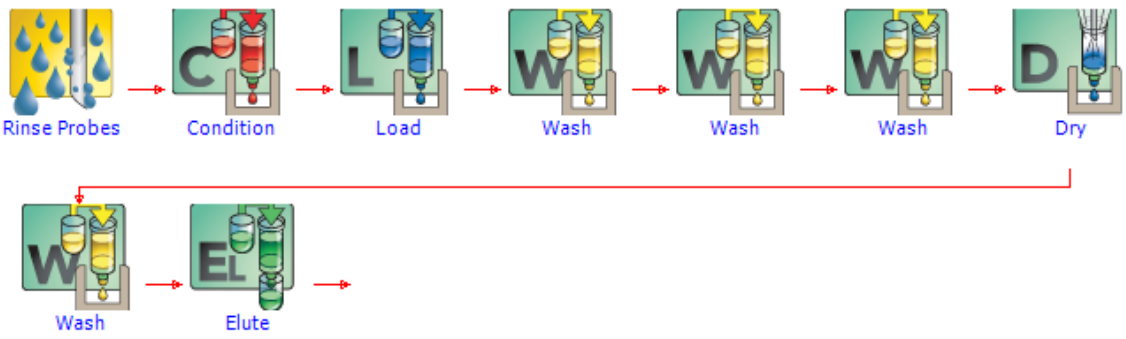

Figure 3. TRILUTION LH SPE Tasks for Extraction of OTA from Wheat Extract

The details of each step are as follows:

- Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 4)
- Rinse probe with deionized water
- Condition SPE Cartridge with 4 mL of deionized water at a flow rate of 5 mL/min
- Load 4 mL of sample extract at a flow rate of 0.8 mL/min
- Wash cartridge with 1 mL HCl solution (pH=1) at a flow rate of 5 mL/min
- Wash with 1 mL HCl (pH=1)/Acetonitrile (60:40, v/v) at a flow rate of 5 mL/min
- Wash with 10 mL of deionized water at a flow rate of 5 mL/min
- Dry column with nitrogen stream for 5 minutes
- Wash with 4 mL of acetonitrile-0.01% acetic acid at 5 mL/min
- Elute OTA with 2 mL of methanol-2% acetic acid at a flow rate of 0.8 mL/min

Figure 4. Gilson Mobile Rack

The eluent was then evaporated and dissolved in HPLC mobile phase before injection into the HPLC system. An alternative to the evaporation step could be the dilution of the sample to a fixed volume prior to injection. The SPE procedure had a throughput of approximately 30 minutes per sample. The throughput can be improved by utilizing the Gilson GX-274 ASPEC™ System which allows for the processing of four samples in parallel.

Analysis

HPLC Analysis was performed on a ThermoFinnigan Spectra System with a Thermo Hypersil GOLD™ polar endcapped C18 column (150 mm x 2.1 mm) with guard column (10 mm x 2.1 mm). Separation was accomplished using a mobile phase of methanol/water/1% acetic acid (60:39:1,v/v) at a flow rate of 0.2 mL/min. The detection system was a Jasco Model FP-2020 Fluorescence Detector set to excitation/emission wavelengths of 333 and 460 nm, respectively. The injection volume was 20 µL.

Results

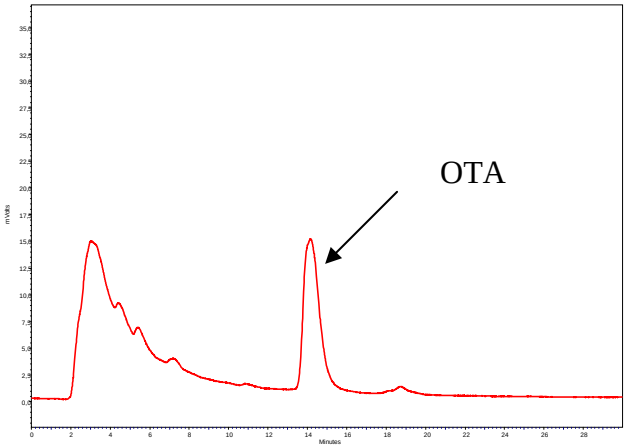

Figure 5. Chromatogram of OTA (5 µg/kg) in Wheat Extract After AFFINIMIP™ OTA Clean-up

Table 1. Recovery and Reproducibility of OTA (n=3) at a contamination level of 5 µg/kg in wheat after clean-up with AFFIMIP OTA Column. Manual versus automated (GX-271 ASPEC) clean-up were compared.

	% Recovery	% CV
Gilson GX-271 ASPEC	90.5	1.7
Manual	90.4	2.5

Conclusion

The use of the MIP-based AFFINIMAP OTA SPE cartridge was a simple, fast, sensitive and selective tool for the extraction of OTA from wheat samples. This cartridge readily lends itself to automation of the SPE protocol using the Gilson GX-271 ASPEC system. Automation of the SPE process improved reproducibility and increased sample throughput over the manual method. Sample throughput was approximately 30 minutes per sample. This could be improved using the Gilson GX-274 ASPEC which allows for the processing of four sample extracts in parallel. Automation also allows one to easily optimize extraction conditions for different matrices and decreases the possibility of errors that can occur when using manual SPE methods.

This method complies with the performance criteria for OTA analysis established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for OTA in wheat of higher than 80% at 5 µg/kg. OTA recovery was 90%, with CVs of less than 2%. There was no OTA in any of the blanks tested and no carryover was observed between sample extracts. This method is well suited for the analysis of ochratoxin A in wheat samples.

References

Castegnaro, M, Maru, V., Petkova-Bocharova, T., Nikolov, I and Bartsch, H. (1991). Concentrations of ochratoxin A in the urine of endemic nephropathy patients and controls in Bulgaria : lack of detection of 4-hydroxyochratoxin. **IARC Sci Publ. 115**:165-169.

Clark, H.A. and Snedeker, S.M. (2006). Ochratoxin A: its cancer risk and potential for exposure. **Journal of Toxicology and Environmental Health. Part B, Critical Reviews 9** (3) 265-296.

Haginaka, J. (2009). Molecularly imprinted polymers as affinity-based separation media for sample preparation. **Journal of Separation Science 32** (10):1548-1565.

Leitner, A., Zöllner, P., Paolillo, A., Stroka, J., Papadopoulo-Bouraoui, A., Jaborek, S., Anklam, E. and Linder, W. (2002). Comparison of methods for the determination of ochratoxin A in wine. **Anal. Chim. Acta 453**: 33-41.

Pohland, A.E., Nesheim, S. And Friedman, L. (1992). Ochratoxin A: a review. **Pure & Appl. Chem. 64** (7): 1029-1046.

Valenta, H. (1998). Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. **Journal of Chromatography A 815** (1): 75-92.

Wei, Y, Qiu, L and Yu, J.C.C. (2007). Molecularly imprinted solid phase extraction in a syringe needle packed with polypyrrole-encapsulated carbon nanotubes for determination of ochratoxin A in red wine. **Food Science and Technology International 13** (5): 375-380.

Zöllner, P., Leitner, A., Lubda, D., Cabrera, K. And Lindner, W. (2000). Application of a Chromolith Speed ROD RP-18e hplc column: determination of ochratoxin A in different wines by high performance liquid chromatography-tandem mass spectrometry. **Chromatographia 52** (11/12): 818-820.