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

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

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Introduction

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-ß-resorcyclic acid lactone] is a mycotoxin produced as a secondary metabolite of various *Fusarium* fungi (see Figure 1). ZON is also known as RAL, F-2 toxin or ZAR. Zearalenone (ZON) has been detected in a variety of cereal products such as wheat, maize, barley, oats, rice and sorghum as well as beer that has been produced with contaminated grains. ZON can be excreted in cow's milk after lactating cows are fed ZON in high doses (JECFA, 2000).

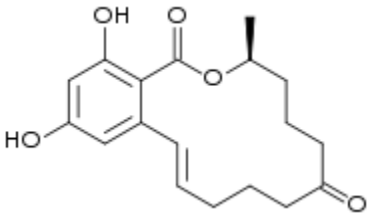

Figure 1. Chemical Structure of Zearalenone, CAS No. 17924-92-4

Exposure to ZON, in animals such as pigs and dairy cattle, has been known to cause estrogenic effects including infertility, reduced serum testosterone levels and sperm counts, reduced incidence of pregnancy and changes in progesterone levels. In addition, ZON can delay the breeding process and cost the producer significant economic and physical losses. As a result, maximum limits for ZON contamination have been established in a number of countries. Member countries of the European Union have set maximum allowable levels of ZON in different food products (European Commission Regulation (EC) 1881/2006).

Several analytical methods for the determination of Zearalenone have been reported in the literature, including thin-layer chromatography (Dawlatana et al., 1998), HPLC (Liao et al., 2009; Berthiller et al., 2005) and gas chromatography (Kinani et al., 2008). The analysis of ZON in agricultural products requires extensive extraction and post-extraction cleanup of the sample prior to analysis. These steps remove matrix interferents and enhance sensitivity. 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 ZON from wheat using a Molecularly Imprinted Polymer (MIP) SPE cartridge that is highly specific for Zearalenone (AFFINIMIP™ZON, POLYINTELL) and the Gilson GX-271 ASPEC™ (Figure 2). This method exceeds the recovery yields required by European Commission Regulation (EC) 401/2006.

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 samples were tested and certified as ZON free. Zearalenone standard was obtained from Sigma Aldrich (OEKANAL® Zearalenone standard in acetonitrile).

Preparation of Samples Prior to SPE with AFFINIMIP OTA Cartridge

Twenty-five grams of wheat grains were ground for two minutes in a blender to a powder. This powder was then mixed with 100 mL of acetonitrile/deionized water (75:25, v/v) for three minutes to extract the ZON. The extract was filtered through a folded filter paper and 5 mL of the filtrate was diluted with 15 mL of deionized water. This solution was used 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 ZON 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).

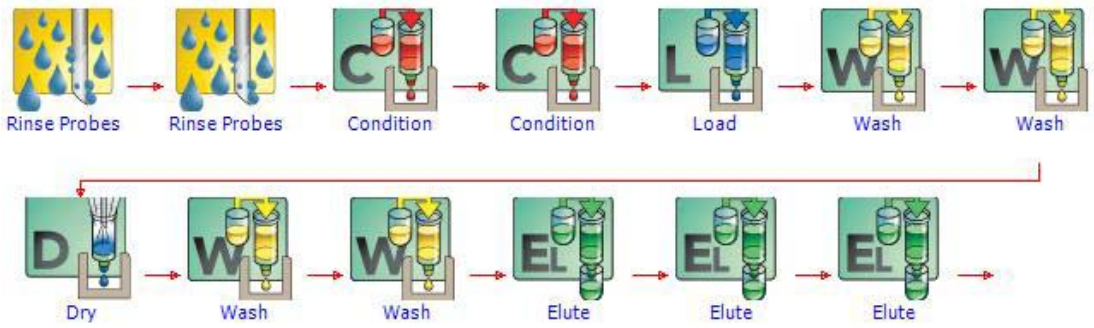

Figure 3. TRILUTION LH SPE Tasks for Extraction of Zearalenone (ZON) from Wheat Extract

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 5 mL of Acetonitrile (ACN) at a flow rate of 5 mL/min
- Condition cartridge with 5 mL of deionized water at a flow rate of 5 mL/min
- Load 10 mL of sample solution at a flow rate of 0.8 mL/min
- Wash with 4 mL water/acetonitrile (80:20, v/v) at a flow rate of 5 mL/min
- Wash with 2 mL of deionized water at a flow rate of 5 mL/min
- Dry column with nitrogen stream for 5 minutes
- Wash with 2 mL of acetonitrile at 5 mL/min
- Wash with 2 mL water/methanol (93:7, v/v) at a flow rate of 5 mL/min
- Elute ZON with 3 x 1 mL of methanol at a flow rate of 0.8 mL/min

Figure 4. Gilson Mobile Rack

The eluent was then evaporated using Nitrogen and dissolved in 500 µL 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.

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 (60:40, 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 275 and 450 nm, respectively. The injection volume was 20 µL.

Results

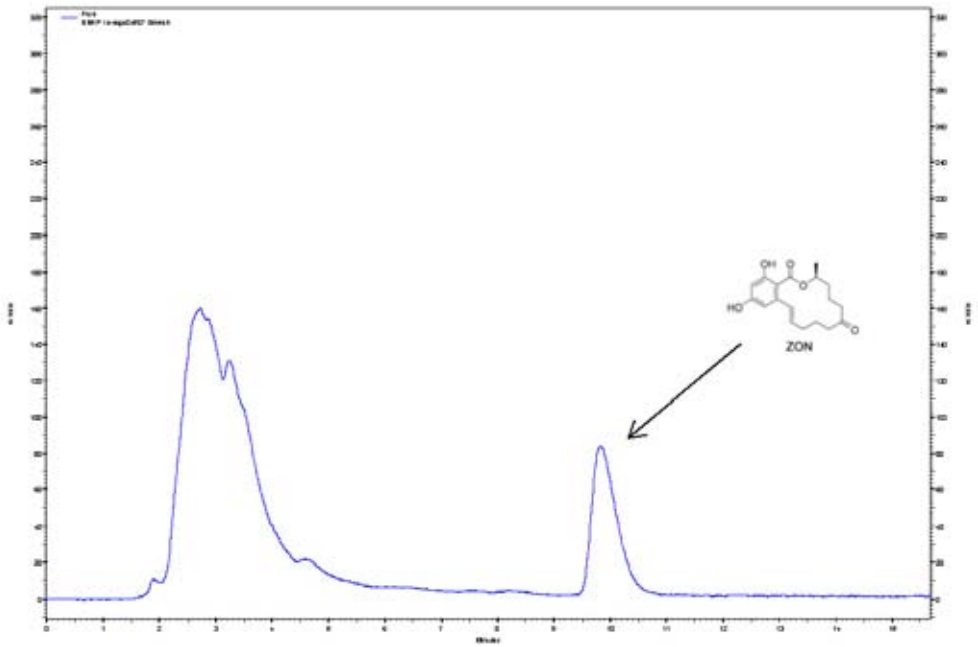

Figure 5. Chromatogram obtained after purification of wheat (contaminated at 75 µg/kg) with AFFINIMIP™ZON

Table 1. Recovery and Reproducibility of OTA (n=3) at a contamination level of 75 µg/kg in wheat after clean-up with AFFIMIP ZON Column.

	% Recovery	% CV
Gilson GX-271 ASPEC	95	6.7

Conclusion

The use of the MIP-based AFFINIMAP ZON SPE cartridge was a simple, fast, sensitive and selective tool for the extraction of ZON 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 could be further 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 ZON analysis established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for ZON in wheat of higher than 80% for analysis done above and below 50 µg/kg. Zearalenone recovery was 95%, with CVs of less than 7%. There was no ZON in any of the blanks tested and no carryover was observed between sample extracts. This method is well suited for the analysis of zearalenone in wheat samples.

References

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