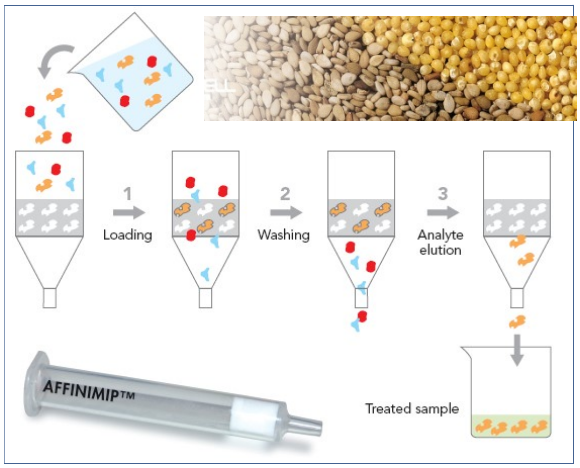
Selective Solid Phase Extraction of Zearalenone from cereals products, cereal-based foods and babyfood for infants and children using Molecularly Imprinted Polymers



Introduction

Zearalenone [6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone; ZON] is a mycotoxin produced as a secondary metabolite of various Fusarium fungi (see figure 1). Zearalenone is known to cause estrogenic effects at relatively low levels, including infertility, reduced serum testosterone levels and sperm counts, reduced incidence of pregnancy, and change in progesterone levels. In addition, Zearalenone can delay the breeding process and cost the producer significant economic and physical losses.

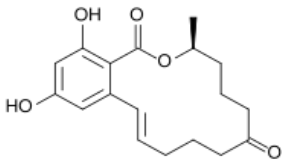


Figure 1. Chemical structure of Zearalenone, CAS N° 17924-92-4.

In Europe, Regulation (EC) 1881/2006 sets maximum levels for Zearalenone in foodstuffs. Several analytical methods for the determination of Zearalenone have been reported in the literature, including thin-layer chromatography, high-performance liquid chromatography (HPLC), gas-chromatography, and enzyme-linked immuno-sorbent assay.

In this application note, the efficiency of a method employing molecularly imprinted polymer (MIP) as selective sorbents for solid-phase extraction (**AFFINIMIP®SPE Zearalenone**, AFFINISEP is showed in respect to the clean-up and pre-concentration of Zearalenone in different matrices (Rice, Maize, Cereal-based babyfood).

Molecularly imprinting 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 Zearalenone.

Experimental conditions

Materials

All reagents and chemicals were ACS grade quality or better. Zearalenone was obtained from Sigma Aldrich (Fluka). Cereal-based samples were purchased in different supermarkets.

Preparation of samples prior to SPE

25g of ground cereal-based samples were extracted with 100 mL of acetonitrile/deionized water (75/25, v/v) for 3 min. The extract was filtered through a folded filter paper and 10 mL of the filtrate were diluted with 10 mL of deionized water. Then, this solution was filtered through a filter paper.

This solution was used as the loading solution.

Solid phase extraction (SPE) protocol

The SPE procedure used a 3mL **AFFINIMIP®SPE Zearalenone** cartridge. The details of each step are as follows:

- Condition the SPE Cartridge with 4mL of acetonitrile (ACN), then with 4mL of deionized water
- Load 12mL of the loading solution (eq. 1.5g of sample)
- Wash the cartridge with 4mL of 58/2/40 deionized water/acetic acid/ACN (v/v/v)
- Elute Zearalenone with 2mL of methanol (MeOH) containing 2% of acetic acid (v/v)

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

AFFINISEP
Bd Sonopa| Pôle d'innovation des Couronnes |76650 Petit couronne | FRANCE
E-mail : contact@affinisep.com | Website : www.affinisep.com

Analysis

HPLC was performed on a ThermoFinnigan Spectra System with a Thermo Hypersil Gold C18 column (150mm x 4.6mm). Separation was carried out using a mobile phase of deionized water/MeOH (40/60, v/v) at a flow rate of 1mL/min. The detection system was a Jasco Model FP-2020 Fluorescence detector set to excitation/emission wavelengths of 275 and 450nm, respectively. The injection volume was 100µL.

Results

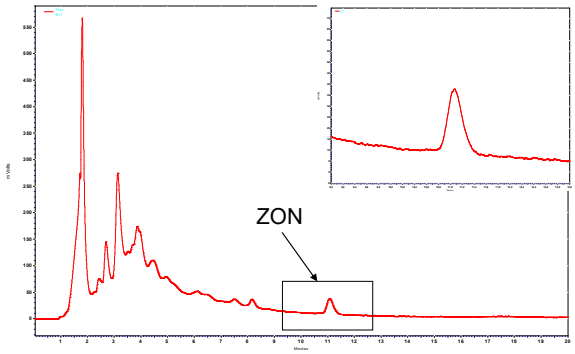


Figure 2. Chromatogram obtained after purification of Maize (contamined at 41 µg / kg) with **AFFINIMIP®SPE Zearalenone**.

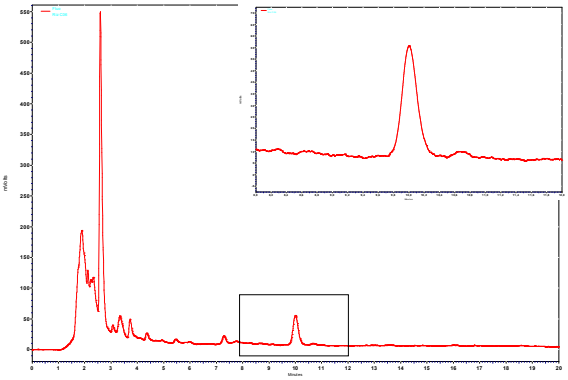


Figure 3. Chromatogram obtained after purification of Rice (contamined at 41 µg / kg) with **AFFINIMIP®SPE Zearalenone**.

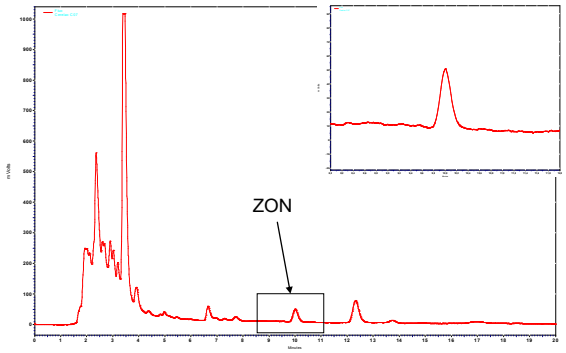


Figure 4. Chromatogram obtained after purification of Cereal-based babyfood (contamined at 41µg / kg) with **AFFINIMIP®SPE Zearalenone** (after dilution by 2 of the elution fraction with water).

Table 1. Recoveries of Zearalenone at a contamination level of 41µg / kg after **AFFINIMIP®SPE Zearalenone** Clean-up in different matrices.

Matrix	Recoveries %	% RSD
Maize (n=9)	86	8
Cereal-based babyfood (n=5)	80	3

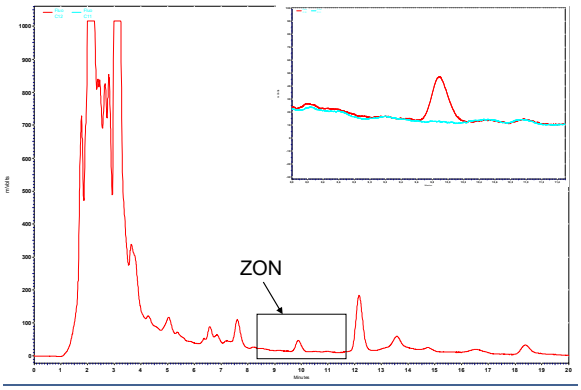


Figure5. Chromatograms obtained after purification of Cereal-based babyfood (contamined at 10µg/kg (red) or 0µg/kg (blue)) with **AFFINIMIP®SPE Zearalenone** (after evaporation of the elution fraction and dissolution in 1mL of the mobile phase).

Conclusion

The use of **AFFINIMIP®SPE Zearalenone** cartridge is a simple, fast, sensitive and selective tool for the extraction of Zearalenone.

In Europe, Regulation (EC) 1881/2006 sets maximum levels for Zearalenone in foodstuffs.

AFFINIMIP®SPE Zearalenone permits to work at 10µg/kg. **AFFINIMIP®SPE Zearalenone** is well-adapted for analysis of cereal-based foods and baby foods for infants and young children were the maximum levels allowed is 20µg/kg.

This method complies with the performance criteria for Zearalenone established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for Zearalenone higher than 60% for Zearalenone concentration values lower than 50µg/kg in foodstuffs. The use of **AFFINIMIP®SPE Zearalenone** enables to obtain recoveries above 75%.

This method is well-suited for the analysis of Zearalenone in cereal-based products.

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.

AFFINISEP
Bd Sonopa| Pôle d'innovation des Couronnes |76650 Petit couronne | FRANCE
E-mail : contact@affinisep.com | Website : www.affinisep.com