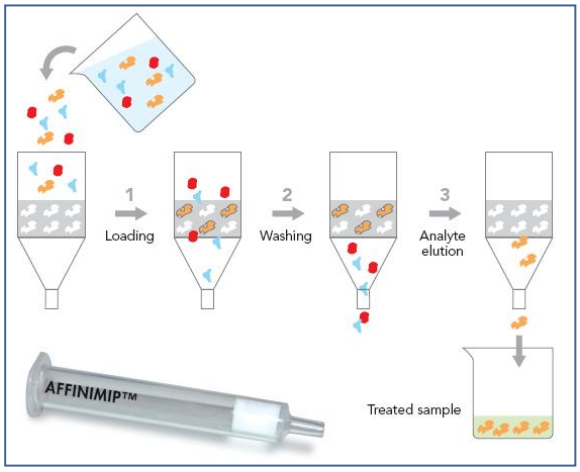
# SIMULTANEOUS Selective Solid Phase Extraction of Zearalenone and Fumonisins from Maize and Maize-based baby food products Using Molecularly Imprinted Polymers




## Introduction

Zearalenone (ZON) and Fumonisins are mycotoxins produced by *Fusarium* molds. Zearalenone (see figure 1) 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.

Fumonsins are known to cause animal diseases and is suspected human carcinogens. Fumonisin B1 (see figure 2) is the most abundant member of the family of Fumonisins while Fumonisin B2 (see figure 3) is more cytotoxic than fumonisin B1.

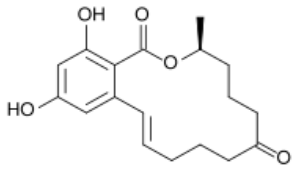



**Figure 1.** Chemical structure of Zearalenone, CAS N° 17924-92-4

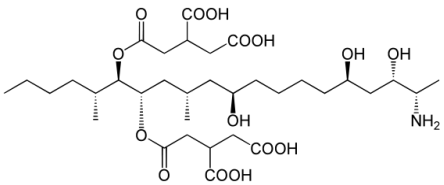



**Figure 2.** Chemical structure of Fumonisin B1, CAS N° 116355-83-0

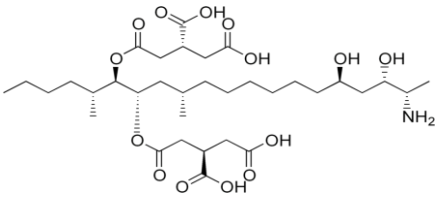



**Figure 3.** Chemical structure of Fumonisin B2, CAS N° 116355-84-1

Several countries have instituted Zearalenone and Fumonisins B1+B2 restrictions in foodstuffs. Member countries of the European Union have set maximum allowable levels of Zearalenone at 350µg/kg in unprocessed maize, 100µg/kg in maize for human consumption and 20µg/kg in maize based food for infants and young children and Fumonisins (B1+B2) at 4000µg/kg in unprocessed maize, 1000µg/kg in maize for human consumption, and 200µg/kg in maize based food for infants and young children (European Commission Regulation (EC) 1126/2007 [1]).

Several analytical methods have been developed for the determination of either Zearalenone or the Fumonisins. However, no method exists for the specific clean-up of Fumonisins **AND** Zearalenone prior to quantification. AFFINISEP has developed such a method.

To do so, we have developed a new class of intelligent polymers based on molecularly imprinted polymers specific to Zearalenone and Fumonisins. Molecularly Imprinted Polymer (MIP) is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. MIP has the advantage 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 and Fumonisins. This study



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describes the solid phase extraction of Zearalenone and Fumonisins from maize and maize-based baby food using a Molecularly Imprinted Polymer (MIP) SPE cartridge: AFFINIMIP® SPE FumoZON.

## Experimental conditions

### *Materials*

All reagents and chemicals were ACS grade quality or better. Zearalenone was obtained from Sigma Aldrich (Fluka). Fumonisin B1 was obtained from CFM Oscar Tropitzsch and Fumonisin B2 was obtained from LGC. Fumonisins B1 and B2 in Maize flour were obtained from Sigma Aldrich (Oekanal). Zearalenone in Maize was obtained from Sigma Aldrich (ERM-BC717). Cereal-based samples were purchased in supermarket.

### *Preparation of samples prior to SPE with AFFINIMIP® SPE FumoZON Cartridge*

25g of ground samples were extracted with 100 mL of Acetonitrile/Methanol/deionized Water (25/25/50, v/v/v) for 3 min using a blender. 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 FumoZON Cartridge. The details of each step are as follows:

- Condition the SPE cartridge with 2mL of Acetonitrile (ACN), then with 2mL of deionized Water
- Load 8mL of the loading solution
- Wash the cartridge with 8mL of deionized Water /Acetonitrile (60/40, v/v)
- Elute Zearalenone and Fumonisins with 2mL of Methanol containing 2% of Acetic Acid

The SPE procedure lasted approximately 30 minutes.
The elution fraction was then evaporated and dissolved in the mobile phase.

### *Analysis*

HPLC was performed on a ThermoFinnigan Surveyor Plus with a Thermo Hypersil Gold C18 column (50mm x 2.1mm). The separation was carried out at a flow rate of 0.2mL/min using a mobile phase of deionized Water-0.1% Formic acid/Acetonitrile (73/27, v/v) for Fumonisin B1 and Zearalenone and with a mobile phase of deionized Water-0.1% Formic acid/Acetonitrile (65/35, v/v) for Fumonisin B2. The detection system was a ThermoFinnigan MSQ Plus with an electrospray source (ESI).
The injection volume was 20µL. The quantification was done in selected ion monitoring at m/z 722 for Fumonisin B1 ($ESI^+$), m/z 705 for fumonisin B2 ($ESI^+$) and m/z 317 for Zearalenone ($ESI^-$).

### *Choice of the extraction solvent*

The composition of the solvent applied for extraction is a crucial parameter particularly when you have molecules with different properties. Several mixtures were tested on maize contaminated with Zearalenone or Fumonisins to determine the best extraction solvent.
The best result was obtained with a mixture Acetonitrile/Methanol/deionized Water (25/25/50, v/v/v).

### *Ion Suppression*

Ion suppression is caused by interferents that co-elute with the analyte of interest during analysis. This phenomenon can induce suppression or enhancement of the signal. The quantification is so erroneous. To evaluate the ion-suppression, blank maize-based baby food samples were cleaned up with AFFINIMIP® SPE FumoZON. The SPE extracts were spiked with a mixture of Fumonisin B1 and Zearalenone at 2 different concentrations. The standard calibration curves were compared to the matrix SPE extracts. The signal responses for the SPE extracts are very close to the signal responses obtained from the calibration sample (see Table 3). The use of AFFINIMIP® SPE FumoZON strongly reduces ion-suppression phenomena with a



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maximum of 15% observed for Fumonisins.

*Results*

**Table 1.** Recovery of Zearalenone, Fumonisins B1 and B2 in maize flour after AFFINIMIP® SPE FumoZON clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

| Sample | C° µg/kg | Mean µg/kg | Recoveries % | % $RSD_R$ |
|---|---|---|---|---|
| Zearalenone | 38 | 39.2 | 103.2 | 8.5 (n=8) |
| Fumonisin B1 | 2408 | 2002.2 | 83.1 | 10.3 (n=8) |
| Fumonisin B1 | 400 | 401.0 | 100.2 | - (n=2) |
| Fumonisin B2 | 630 | 684.6 | 108.7 | 11.5 (n=3) |

**Table 2.** Recovery of Zearalenone, Fumonisins B1 and B2 in maize-based baby food after AFFINIMIP® SPE FumoZON clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

| Sample | C° µg/kg | Mean µg/kg | Recoveries % | % $RSD_R$ |
|---|---|---|---|---|
| Zearalenone | 20 | 16.9 | 84.4 | 1.6 (n=4) |
| Fumonisin B1 | 200 | 168.6 | 84.3 | 1.4 (n=3) |
| Fumonisin B2 | 200 | 185.6 | 92.8 | 1.9 (n=3) |

**Table 3.** Ion suppression percentage obtained in Maize-based baby food (tested twice).

| Analyte | C° µg/kg | Ion suppression % |
|---|---|---|
| Zearalenone | 10 | 1%<br>5% |
| Zearalenone | 50 | 0%<br>5% |
| Fumonisin B1 | 100 | 8%<br>11% |
| Fumonisin B1 | 500 | 12%<br>14% |

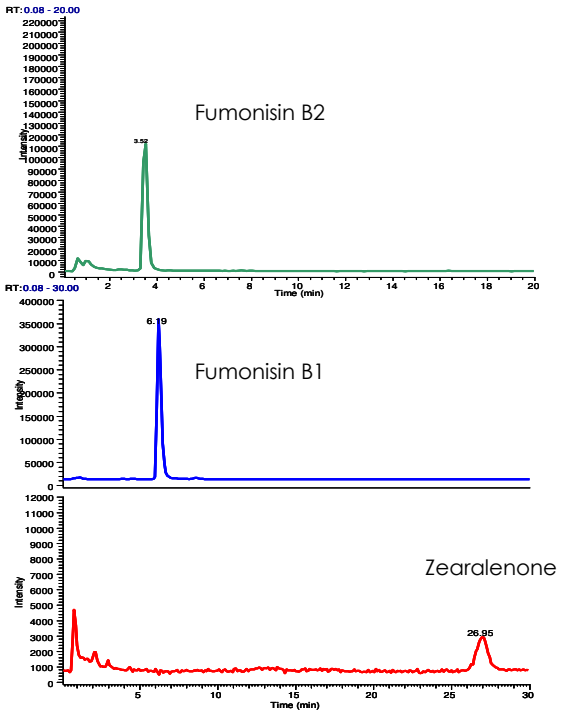


**Figure 4.** Chromatograms obtained after **AFFINIMIP® SPE FumoZON** Clean-up of a maize flour spiked at 38µg/kg with Zearalenone, 2408µk/kg with Fumonisin B1 and 630µg/kg with Fumonisin B2.

## Conclusion

The use of an AFFINIMIP® SPE FumoZON SPE cartridge is a simple, fast, sensitive and selective tool for the extraction of Fumonisins and Zearalenone from Maize products.

This method complies with the performance criteria for Fumonisins and Zearalenone established by the European Commission Regulation (EC) 401/2006 [2]. This regulation requires recovery values for Zearalenone higher than 60% for analysis done below 50µg/kg and for Fumonisins higher than 60% for analysis done below 500µg/kg and 70% for analysis done above 500µg/kg.

The use of AFFINIMIP® SPE Fumonisins and Zearalenone enables to obtain recoveries higher than 80% with low ion suppression. This method is well-suited for the analysis in maize products.



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## References

[1] Commission Regulation (EC) No. 1126/2007 of 28 September 2007, Official Journal of the European Union.

[2] Commission Regulation (EC) No. 401/2006 of 23 February 2006, Official Journal of the European Union.

## Related products

- **AFFINIMIP® SPE FumoZON**

Catalog number: FS109-02 for 25 columns
FS109-03 for 50 columns



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