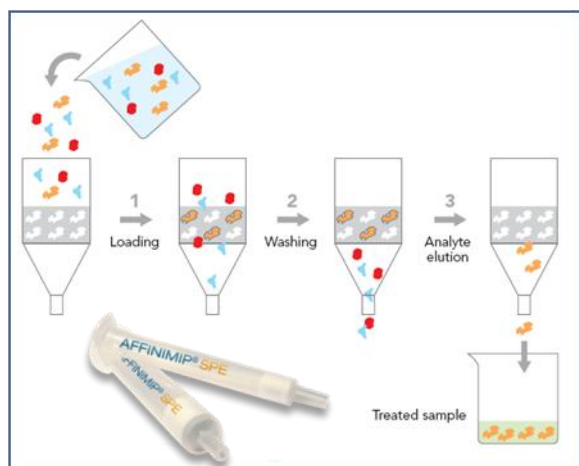


## Selective Solid Phase Extraction of Deoxynivalenol Mycotoxin (DON) from Cereals using AFFINIMIP® SPE DEOXYNIVALENOL



### Background

**Deoxynivalenol (DON)** also known as **Vomitoxin** is a type B trichothecene mycotoxin produced by various *Fusarium* fungi (see figure 1). These fungi grow mainly on cereals such as wheat, barley, oats, rye, and maize and it is a very common mycotoxin developed in grain.

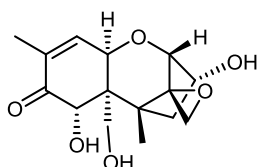


Figure 1. Chemical structure of DON, CAS N° 51481-10-8

In Europe, Regulation (EC) N°1126/2007 sets maximum levels for Deoxynivalenol mycotoxin in cereals respectively 1750µg/kg for unprocessed corn, wheat and oat, 1250µg/kg for others cereals, 750µg/kg for cereal flours and 200µg/kg for babyfood.

### AFFINIMIP® SPE Deoxynivalenol: highly selective clean up of DON from complex matrices

AFFINIMIP® SPE Deoxynivalenol uses a new class of intelligent polymers based on molecularly imprinted polymers specific for DON ensuring extremely clean extracts for an easy quantification by all chromatography techniques.

AFFINIMIP® SPE products remove matrix components and are chemically and thermally stable, compatible with all solvents and cost-effective. For the tested matrices, the provided protocols require no further development.

In this application note, the protocol of use shows that it is suitable for two different extractions methods of the mycotoxin, hydro-organic and water followed by a clean-up with AFFINIMIP® SPE Deoxynivalenol. High recovery yields were obtained demonstrating that these methods comply with the performance criteria established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for Deoxynivalenol higher than 70% for analysis done above 500µg/kg.

### Extraction with Hydro-organic solvents

#### High Deoxynivalenol recoveries on Cereals extracts done by hydro-organic solvents

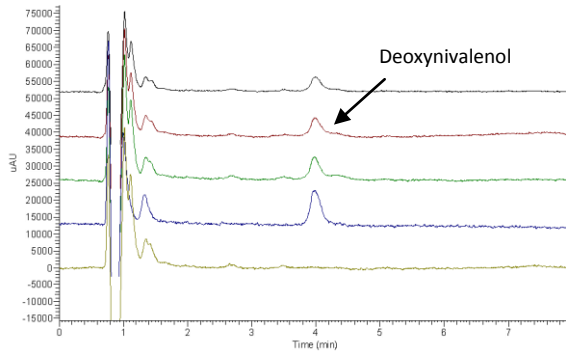
Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol clean-up and relative standard deviation calculated from results generated under **repeatability conditions**.

| Matrix        | Detection | C°<br>µg/kg | Mean<br>µg/kg | R%          | %RSD <sub>r</sub> |
|---------------|-----------|-------------|---------------|-------------|-------------------|
| Corn<br>(n=3) | UV        | 800         | 667.8         | <b>83.5</b> | 1.7               |
| Corn<br>(n=3) | MS        | 800         | 682.1         | <b>85.3</b> | 4.4               |

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol clean-up and relative standard deviation calculated from results generated under **reproducibility conditions**.

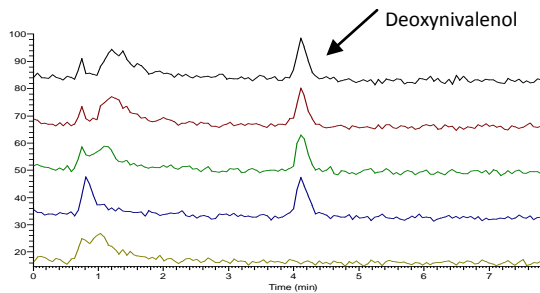
| Matrix        | Detectio<br>n | C°<br>µg/kg | Mean<br>µg/kg | R%          | %RSD <sub>R</sub> |
|---------------|---------------|-------------|---------------|-------------|-------------------|
| Corn<br>(n=6) | UV            | 800         | 670.2         | <b>83.8</b> | 2.4               |
| Corn<br>(n=6) | MS            | 800         | 699.0         | <b>87.4</b> | 6.9               |

**UV and LC-MS Chromatograms demonstrate an efficient clean-up for DON extraction from Cereals**



UV chromatograms obtained after HYDRO-ORGANIC extraction of DON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green spiked with DON at 800µg/kg ( )
- dark yellow not spiked
- blue, a standard solution of DON at 200ng/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase



MS chromatograms obtained after hydro-organic extraction of DON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green spiked with DON at 800µg/kg ( )
- dark yellow not spiked
- blue, a standard solution of DON at 200ng/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase

**Extraction with Water**

**High Deoxynivalenol recoveries in Cereals extracts with Water extraction**

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol Clean-up and relative standard deviation calculated from results generated under **repeatability conditions**.

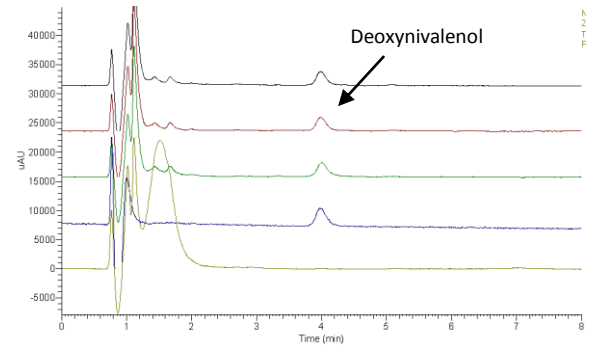
| Matrix     | Detection | C° µg/kg | Mean µg/kg | R%   | %RSDr |
|------------|-----------|----------|------------|------|-------|
| Corn (n=3) | UV        | 800      | 623.4      | 78.0 | 1.4   |
| Corn (n=3) | MS        | 800      | 642.7      | 80.3 | 3.4   |

| Wheat (n=3) | MS | 600 | 540.0 | 90.0 | 9.8 |
|-------------|----|-----|-------|------|-----|
|-------------|----|-----|-------|------|-----|

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol Clean-up and relative standard deviation calculated from results generated under **reproducibility conditions**.

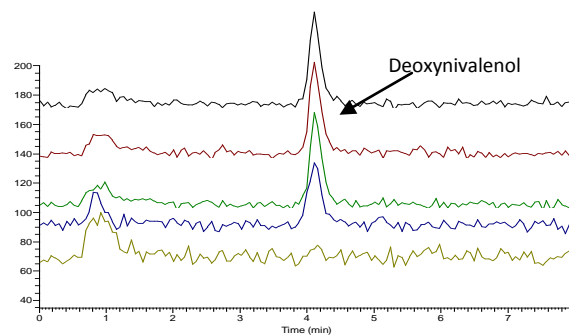
| Matrix     | Detection | C° µg/kg | Mean µg/kg | R%   | %RSD <sub>R</sub> |
|------------|-----------|----------|------------|------|-------------------|
| Corn (n=9) | UV        | 800      | 658.7      | 82.3 | 7.5               |
| Corn (n=9) | MS        | 800      | 659.5      | 82.4 | 7.8               |

**UV and LC-MS Chromatograms demonstrate an efficient clean-up for DON extraction from Cereals**



UV chromatograms obtained after WATER extraction of DON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green spiked with DON at 800µg/kg ( )
- dark yellow not spiked
- blue, a standard solution of DON at 200ng/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase



MS chromatograms obtained after hydro-organic extraction of DON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green spiked with DON at 800µg/kg ( )
- dark yellow not spiked
- blue, a standard solution of DON at 200ng/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase

## Experimental conditions

### Preparation of cereals with hydro-organic extraction prior to SPE with AFFINIMIP® SPE Deoxynivalenol Cartridge

20g of cereals were ground in a blender for 1 minute. Then, a solution of deionized water: acetonitrile (50:50) was added. This mixture was then ground for 2 additional minutes. After grinding, the mixture was placed in a beaker and left stirred under magnetic agitation for 30 minutes.

Then the whole mixture (both solid and liquid phases) was transferred in a centrifuge vial and centrifuged at 2500 rpm for 15 minutes. After centrifugation, the supernatant was filtered through filter paper. This solution was then diluted 10 times using deionized water.

### Preparation of cereal with water extraction prior to SPE with AFFINIMIP® SPE Deoxynivalenol Cartridge

20g of cereals were ground in a blender for 1 minute. Then, 80 ml of deionized water were added. This mixture was then ground for 2 additional minutes. After grinding the mixture was placed in a beaker and left stirred under magnetic agitation for 30 minutes.

Then the whole mixture (both solid and liquid phases) was transferred in a centrifuge vial and centrifuged at 2500 rpm for 15 minutes. After centrifugation the supernatant was filtered through filter paper. This solution was then diluted 5 times using deionized water.

### Solid phase extraction (SPE) protocol (common for both extraction methods)

The SPE procedure uses a 6mL AFFINIMIP® SPE Deoxynivalenol Cartridge (FS117-03B):

- Condition the SPE Cartridge with 2mL of Acetonitrile (ACN), then with 2mL of deionized water
- Load 6mL of the loading solution
- Wash the cartridge with 3mL of NaHCO<sub>3</sub> 1% in water
- Force the water down into the cartridge and out the bottom or apply vacuum 30 seconds
- Wash the cartridge with 1mL of diethyl ether
- Elute Deoxynivalenol with 4mL of Ethyl acetate

The SPE procedure lasts approximately 30 minutes. Then the elution fraction is evaporated and dissolved in water containing 0.1% formic acid.

## Analysis

HPLC was performed on a Thermo Finnigan Spectra System with a Thermo Hypersil Gold column (50mm x 2.1mm). The separation was carried out using a mobile phase of water containing 0.1% formic acid : acetonitrile (95:5) at a flow rate of 0.2mL/min.

The detection system was a Thermo Finnigan Spectra System Model UV6000LP set to 220nm and a Thermo Finnigan MSQ PLUS with an electrospray source. The quantification was done in selected ion monitoring at m/z: 265 (ESI<sup>-</sup>). The probe temperature was set at 350 °C; Cone: 75v. The injection volume was 20µL.

## Ordering information

### AFFINIMIP® SPE Deoxynivalenol

| Catalog number | Description       |
|----------------|-------------------|
| FS117-02B      | 25 cartridges 6mL |
| FS117-03B      | 50 cartridges 6mL |

## Reagents

| Catalog number | Description  |
|----------------|--|
| REA-DON-1mL    | 1mL of Deoxynivalenol standard solution at 100 µg/mL in acetonitrile |

## About AFFINISEP

Part of POLYINTELL, AFFINISEP develops, manufactures and markets innovative methods and products for sample preparation and selective extraction in various field such as food safety and quality, environmental , clinical chemistry, cosmetic, and pharmaceutical.

For more information or ordering: [contact@affinisep.com](mailto:contact@affinisep.com)  
Website: [www.polyintell.com](http://www.polyintell.com)

