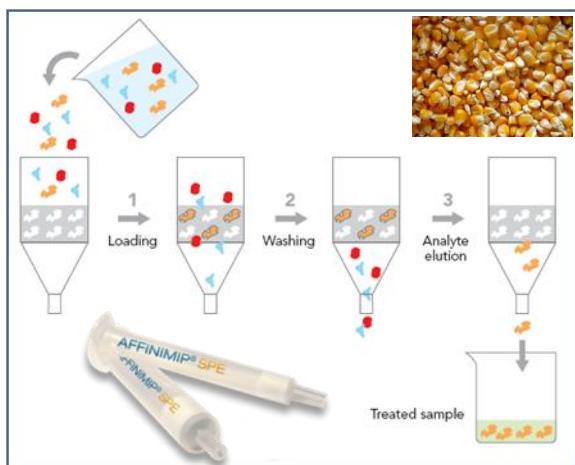


## Sample clean-up method for Deoxynivalenol (DON), 3-acetylDON and 15-acetylDON from Cereals using AFFINIMIP® SPE DEOXYNIVALENOL



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 the extraction of DON, 3-AcetylDON and 15-AcetylDON with a single hydro-organic extraction followed by a clean-up with **AFFINIMIP® SPE Deoxynivalenol** for a single quadrupole LC MS analysis. 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 and higher 60% for analysis done between 100 and 500µg/kg.

### Background

**Deoxynivalenol (DON)** also known as **Vomitoxin** and its analogues 3-Acetyl Deoxynivalenol (3-AcetylDON) and 15-Acetyl Deoxynivalenol (15-AcetylDON) are type B trichothecene mycotoxins 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.

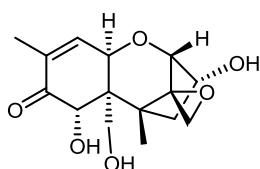


Figure 1. Chemical structure of DON, CAS N° 51481-10-8

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 Deoxynivalenol and its acetyl derivatives from complex matrices

**AFFINIMIP® SPE Deoxynivalenol** uses a new class of intelligent polymers based on molecularly imprinted polymers specific for Deoxynivalenol and its acetyl derivatives ensuring extremely clean extracts for an easy quantification.

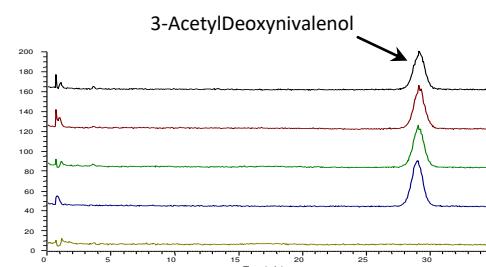
**AFFINIMIP® SPE** products remove matrix components and are chemically and thermally stable, compatible with all

### High recoveries on Cereals extracts

Recovery obtained for DON, 3-acetylDON and 15-acetylDON after **AFFINIMIP® SPE Deoxynivalenol** clean-up of Corn and relative standard deviation calculated from results generated under repeatability conditions (n=3).

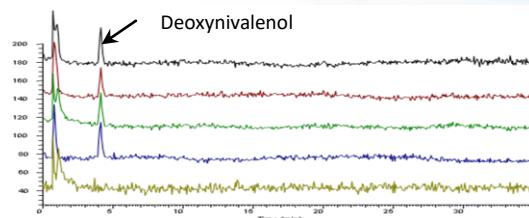
Compound	C° µg/kg	Mean µg/kg	R%	%RSDr
DON	800	653.7	81.7	0.3
3-AcetylDON	800	601.0	75.1	2.3
15-AcetylDON	800	641.8	80.2	3.4

### Easy analysis with a single quadrupole MS detector



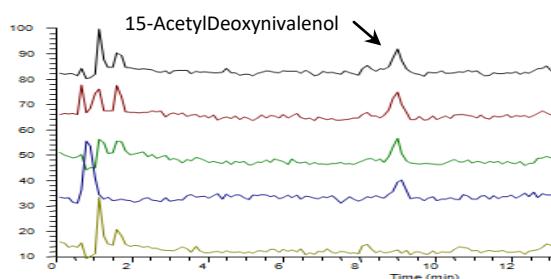
MS chromatograms obtained after hydro-organic extraction of 3-acetylDON from corn and clean-up with **AFFINIMIP® SPE Deoxynivalenol**:

- black, red and green: spiked with Deoxynivalenol at 800µg/kg
- dark yellow: not spiked
- blue: a standard solution of 3-AcetylDON at 200ng/mL is prepared by dilution of a 100µg/mL 3-AcetylDeoxynivalenol standard solution (reference : REA-3AcDON-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 Deoxynivalenol at 800 $\mu\text{g}/\text{kg}$
- dark yellow: not spiked
- blue: a standard solution of Deoxynivalenol at 200ng/mL is prepared by dilution of a 100 $\mu\text{g}/\text{mL}$  Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase



MS chromatograms obtained after hydro-organic extraction of 15-acetylDON from corn and clean-up with **AFFINIMIP® SPE Deoxynivalenol**:

- black, red and green: spiked with 15-AcetylDON at 800 $\mu\text{g}/\text{kg}$
- dark yellow: not spiked
- blue: a standard solution of DON at 200ng/mL is prepared by dilution of a 100 $\mu\text{g}/\text{mL}$  15-AcetylDeoxynivalenol standard solution (reference : REA-15AcDON-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 mixture was centrifuged at 2500 g for 15 minutes. After centrifugation, the supernatant was filtered through filter paper. This solution was then diluted 10 times using deionized water.

### *Solid phase extraction (SPE) protocol*

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 and its analogues 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) for Deoxynivalenol and 3-AcetylDeoxynivalenol and (90:10) for 15-AcetylDeoxynivalenol at a flow rate of 0.2mL/min.

The detection system was a Thermo Finnigan MSQ PLUS with an electrospray source. The quantification was done in selected ion monitoring at m/z: 265 (ESI<sup>+</sup>) for DON and 339(ESI<sup>+</sup>) for 3 and 15-acetylDON. The probe temperature was set at 350 °C; Cone: 75v. The injection volume was 20 $\mu\text{L}$ .

## Ordering information

### **AFFINIMIP® SPE Deoxynivalenol**

Catalog number	Description
<b>For food and baby food</b>	
FS117-02B	25 cartridges 6mL
FS117-03B	50 cartridges 6mL
<b>For feed</b>	
FS117-02B-200mg	25 cartridges 6mL
FS117-03B-200mg	50 cartridges 6mL