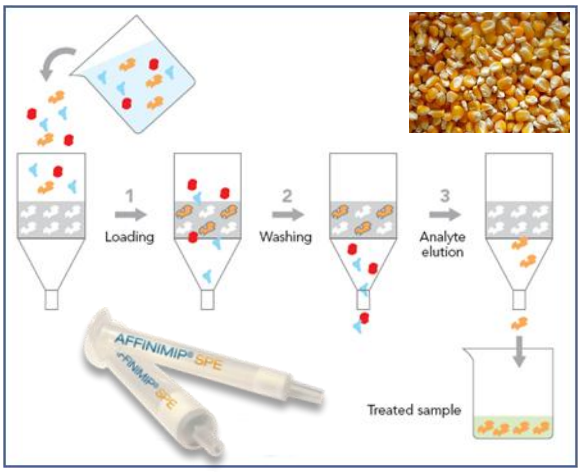
Sample clean-up method for Deoxynivalenol (DON), 3-acetylDON and 15-acetylDON from Cereals using AFFINIMIP® SPE DEOXYNIVALENOL



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.

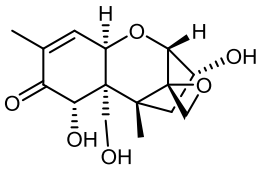


Figure 1. Chemical structure of DON, CAS N° 51481-10-8

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 solvents and cost-effective. For the tested matrices, the provided protocols require no further development.

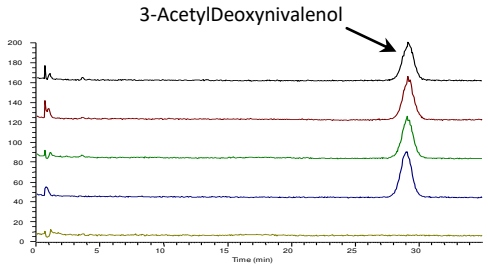
In this application note, the protocol of use shows that it is suitable for the extraction of DON, 3-AcetylDON and 15-AcetylDON with a single hydro-organic extraction followed by a clean-up with AFFINIMIP® SPE Deoxynivalenol for a single quadrupole LC MS analysis. High recovery yields were obtained demonstrating 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.

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

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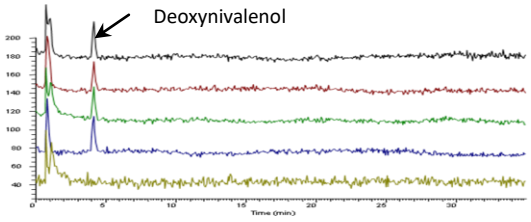
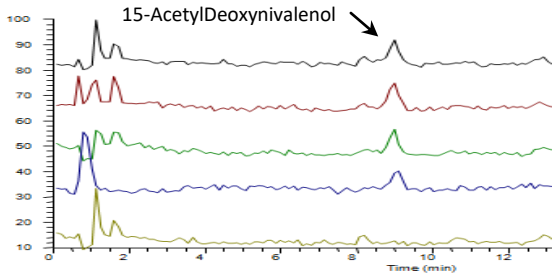


MS chromatograms obtained after hydro-organic extraction of DON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green: spiked with Deoxynivalenol at 800µg/kg
- dark yellow: not spiked
- blue: a standard solution of Deoxynivalenol 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 15-acetylDON from corn and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green: spiked with 15-AcetylDON at 800µg/kg
- dark yellow: not spiked
- blue: a standard solution of DON at 200ng/mL is prepared by dilution of a 100µg/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 $NaHCO_3$ 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^-) for DON and 339(ESI^+) for 3 and 15-acetylDON. The probe temperature was set at 350 ºC; Cone: 75v. The injection volume was 20µL.

Ordering information

AFFINIMIP® SPE Deoxynivalenol

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

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