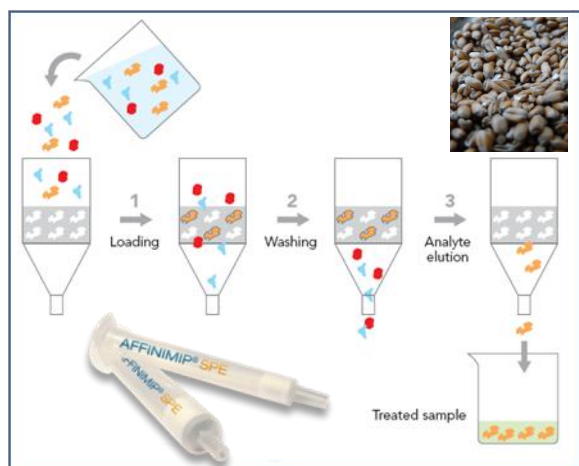


Sample clean-up method for Deoxynivalenol Mycotoxin analysis in Animal feed using AFFINIMIP® SPE DEOXYNIVALENOL



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.

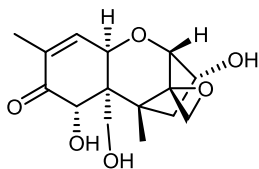


Figure 1. Chemical structure of DON, CAS N° 51481-10-8

In Europe, Regulation (EC) N°576/2006 sets maximum levels in feed materials for Deoxynivalenol mycotoxin respectively 8mg/kg for cereals and cereals products, 12mg/kg for maize by-products.

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 Deoxynivalenol and analogues 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

high recovery yields were obtained and 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 than 60% for analysis done between 100 and 500µg/kg.

High Deoxynivalenol recoveries for feed analyses (Wheat and Whiskas)

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol Clean-up and relative standard deviation calculated from results generated under **repeatability conditions (n=3)**.

Feed Matrices	C° mg/kg	Mean mg/kg	R%	%RSDr
Wheat	6	5.7	94	0.1
Whiskas	0.8	0.73	91	2.4

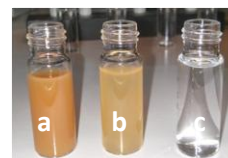
High capacity cartridges

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol Clean-up of Whiskas matrix spiked with different concentrations of Deoxynivalenol.

C° mg/kg	R%
4	84
6	83
8	87
10	90

The above table shows that also at different concentrations the **recovery efficiency remains constant**.

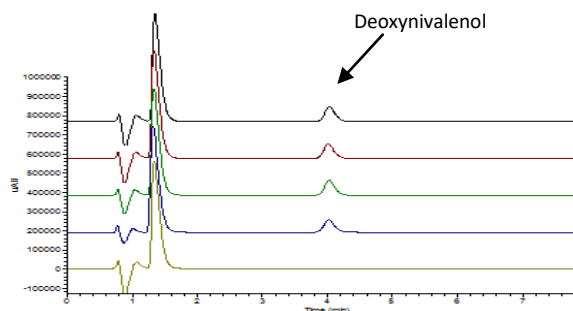
Efficient clean-up for Deoxynivalenol extraction from Feed



Analysis of Whiskas:

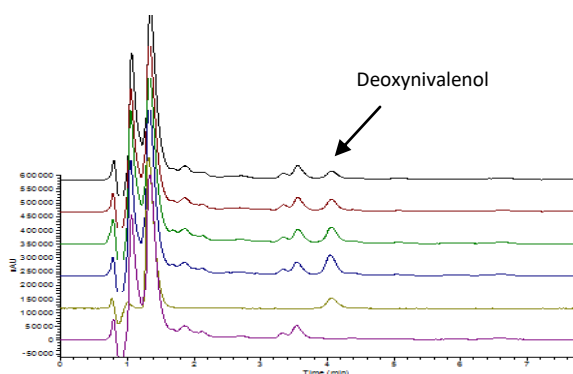
- a- Extraction solution with water
- b- Loading solution
- c- Elution solution

UV chromatograms of the analyses



UV chromatograms obtained after water extraction of Deoxynivalenol from animal feed (wheat) and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green: spiked with DON at 6mg/kg
- dark yellow: not spiked
- blue: a standard solution of Deoxynivalenol at 1µg/mL prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) with the mobile phase.



UV chromatograms obtained after water extraction of Deoxynivalenol from animal feed (Whiskas) and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red, green and blue: spiked with DON respectively at 4mg/kg, 6mg/kg, 8mg/kg and 10mg/kg
- purple not spiked
- dark yellow: a standard solution of DON at 1000ng/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 water extraction prior to SPE with AFFINIMIP® SPE Deoxynivalenol cartridge

20g of animal feed 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 was centrifuged at 2500 g 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

The SPE procedure uses a 6mL AFFINIMIP® SPE Deoxynivalenol Cartridge (FS117-03B-200mg): Condition the SPE Cartridge with 2mL of Acetonitrile (ACN), then with 2mL of deionized water

- Load 2mL of the loading solution
- Wash the cartridge with 3mL of NaHCO₃ 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. 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