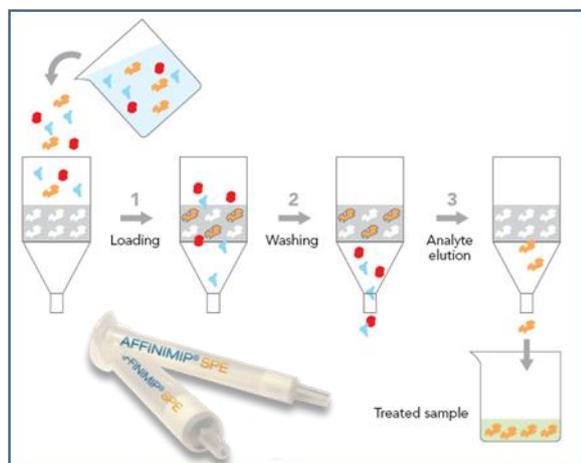


Selective Solid Phase Extraction of Deoxynivalenol Mycotoxin (DON) from Cereals using AFFINIMIP® SPE DEOXYNIVALENOL



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.

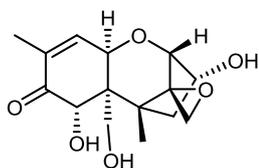


Figure 1. Chemical structure of DON, CAS N° 51481-10-8

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

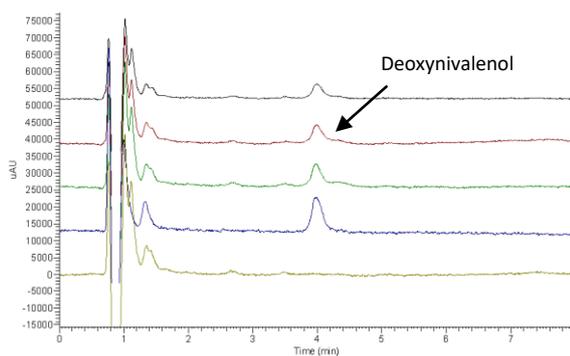
Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol clean-up and relative standard deviation calculated from results generated under **repeatability conditions**.

Matrix	Detection	C° µg/kg	Mean µg/kg	R%	%RSD _r
Corn (n=3)	UV	800	667.8	83.5	1.7
Corn (n=3)	MS	800	682.1	85.3	4.4

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

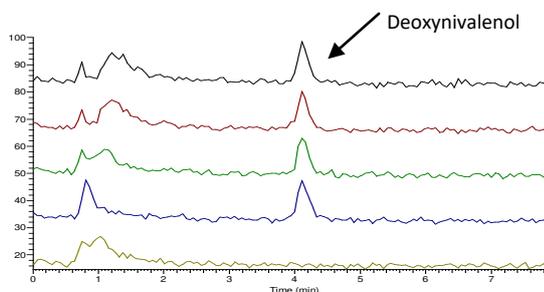
Matrix	Detection	C° µg/kg	Mean µg/kg	R%	%RSD _r
Corn (n=6)	UV	800	670.2	83.8	2.4
Corn (n=6)	MS	800	699.0	87.4	6.9

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**.

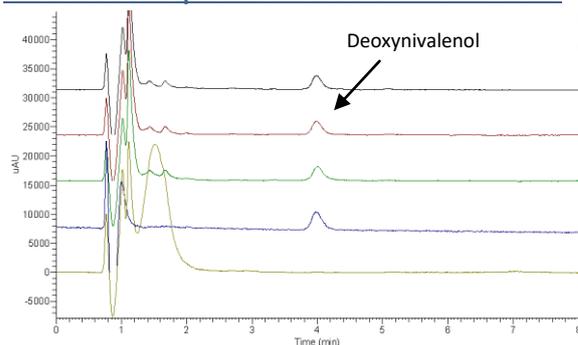
Matrix	Detection	C° µg/kg	Mean µg/kg	R%	%RSDr
Corn (n=3)	UV	800	623.4	78.0	1.4
Corn (n=3)	MS	800	642.7	80.3	3.4
Wheat (n=3)	MS	600	540.0	90.0	9.8

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol

Clean-up and relative standard deviation calculated from results generated under **reproducibility conditions**.

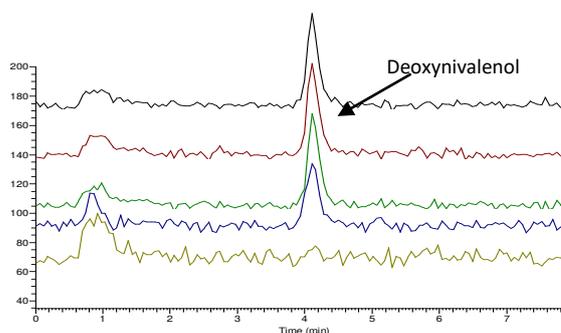
Matrix	Detection	C° µg/kg	Mean µg/kg	R%	%RSD _R
Corn (n=9)	UV	800	658.7	82.3	7.5
Corn (n=9)	MS	800	659.5	82.4	7.8

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 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 and a Thermo Finnigan MSQ PLUS with an electrospray source. The quantification was done in selected ion monitoring at m/z: 265 (ESI⁻). 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