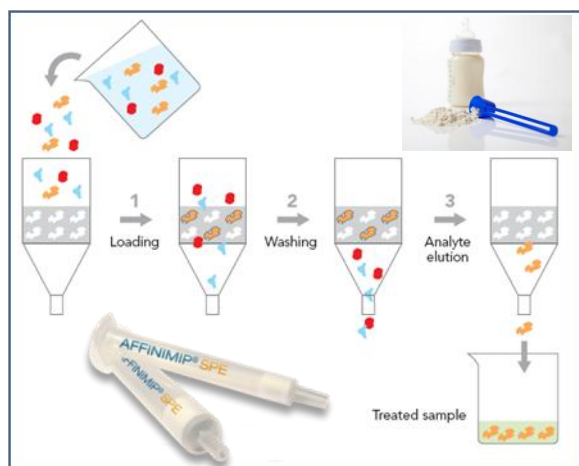


Sample clean-up method for Deoxynivalenol Mycotoxin (DON) from cereals based Babyfood using AFFINIMIP® SPE DEOXYNIVALENOL



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.

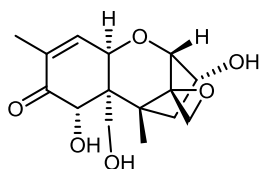


Figure 1. Chemical structure of Deoxynivalenol (DON), CAS N° 51481-10-8

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, 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 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.

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 60% for analysis done between 100µg/kg and 500µg/kg.

High Deoxynivalenol recoveries on cereals-based Babyfood extracts

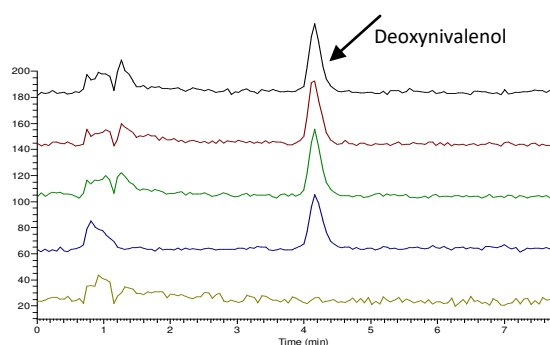
Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol clean-up and relative standard deviation calculated from results generated under **repeatability conditions**.

Matrix	C° µg/kg	Mean µg/kg	R%	%RSD _r
Babyfood (n=3)	150	136.5	91	0.4

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

Matrix	C° µg/kg	Mean µg/kg	R%	%RSD _R
Babyfood (n=6)	150	129.8	86.5	6.1

LC-MS Chromatograms demonstrate an efficient clean-up for DON extraction from Babyfood



MS chromatograms obtained after water extraction of Deoxynivalenol from cereals - based babyfoods and clean-up with AFFINIMIP® SPE Deoxynivalenol:

- black, red and green spiked with Deoxynivalenol at 150µg/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

Experimental conditions

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

150 ml of deionized water were added to 20g of cereals - based babyfood. This mixture was then placed in a beaker and left stirring 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.

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 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 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.

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

Standards solutions

Catalog number	Description
REA-DON-1mL	1mL of Deoxynivalenol standard solution at 100 µg/mL in acetonitrile
REA-3AcDON-1mL	1mL of 3-AcetylDeoxynivalenol standard solution at 100 µg/mL in acetonitrile
REA-15AcDON-1mL	1mL of 15-AcetylDeoxynivalenol standard solution at 100 µg/mL in acetonitrile

Ordering information

AFFINIMIP® SPE Deoxynivalenol