

# DETERMINATION OF DEOXYNIVALENOL IN PASTA SAMPLES BY HPLC/DAD

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## INTRODUCTION

Deoxynivalenol (DON) is one of the most frequently detected mycotoxin contaminants in wheat and wheat products worldwide. It is a secondary metabolite produced by fungi from the *Fusarium* genus (*Fusarium graminearum* and *Fusarium culmorum*). In many countries a legal limit of 750 µg/kg of DON in pasta is established. This level of contamination in feedstuffs can cause serious health problems and diseases (alimentary toxic aleukia, ATA). Deoxynivalenol (DON), also known as Vomitoxin (VOM), is a type B trichothecene, an epoxy-sesquiterpenoid.

## PROCEDURE

### Extraction

Sample: 25 g of grained sample

Extraction solvent:

200 ml deionized water/HPLC grade

Blending: high speed for 2 min.

Filtration: Whatman No 42 filter paper.

Clean-up: immunoaffinity column. DONPREP<sup>®</sup>, Biopharm

The purified methanol extract is evaporated to dryness and the residue is reconstituted with 0.5ml water

### HPLC chromatography

Apparatus: Agilent 1200 (Germany)

Column: Zorbax SB-Aq C18, 250 × 4.6 mm, 5 µm (Agilent)

Eluent: acetonitrile (A)/water solution (B) (10:90, v/v)

Flow: 1.2ml/min

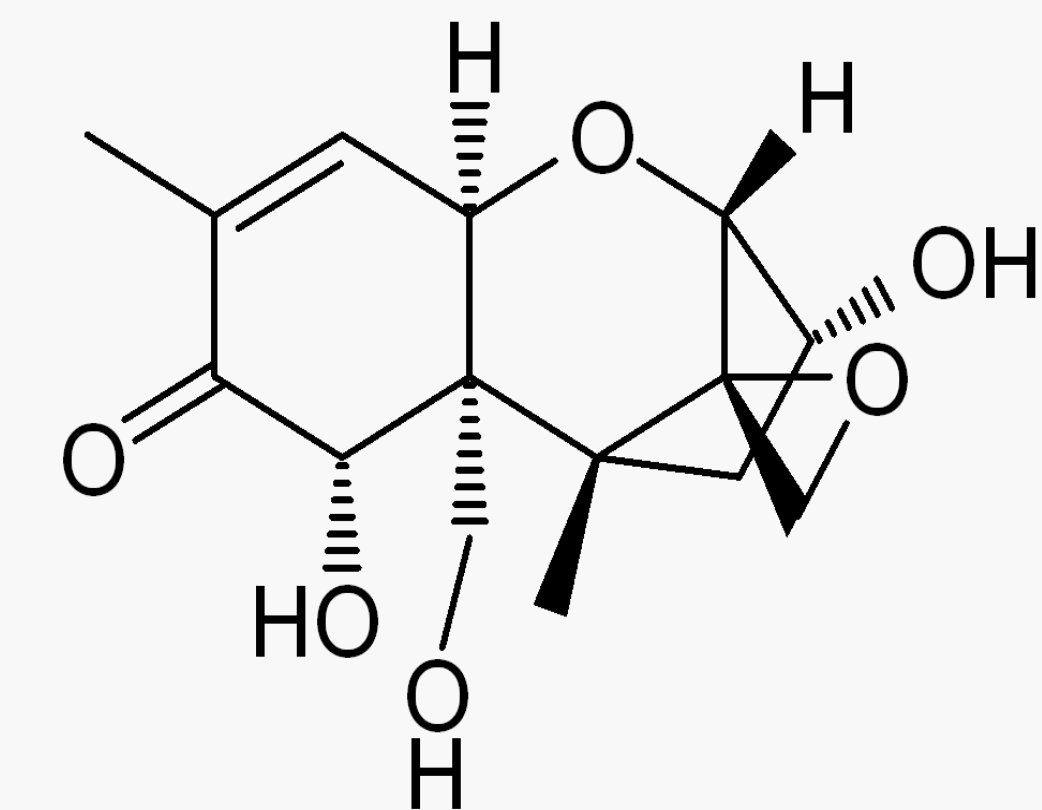
Gradient: 0min-90% B, 20min-20% B, 22min-20%B

Post time: 3min

Detection: DAD λ = 218 nm

## RESULTS

	Range ng/ml	Equation	LOD (µg/kg)	LOQ (µg/kg)	C (µg/kg)	Avr. Rec %	Precision RSD % (n=5)
DON	25-1000	y=0.06935x+1.22836	7	25	50	85.6 (RSD 6.81%)	4.5
		R <sup>2</sup>			200	87.6 (RSD 6.81%)	
		0.9993			1250	93.2 (RSD 6.81%)	
CRM	FAPAS T2226						
		Theoretical value	640 (421-859) µg/kg				
		Experimental value	710 µg/kg				
		Trueness	11.1%				



IUPAC name: (3α,7α)-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one

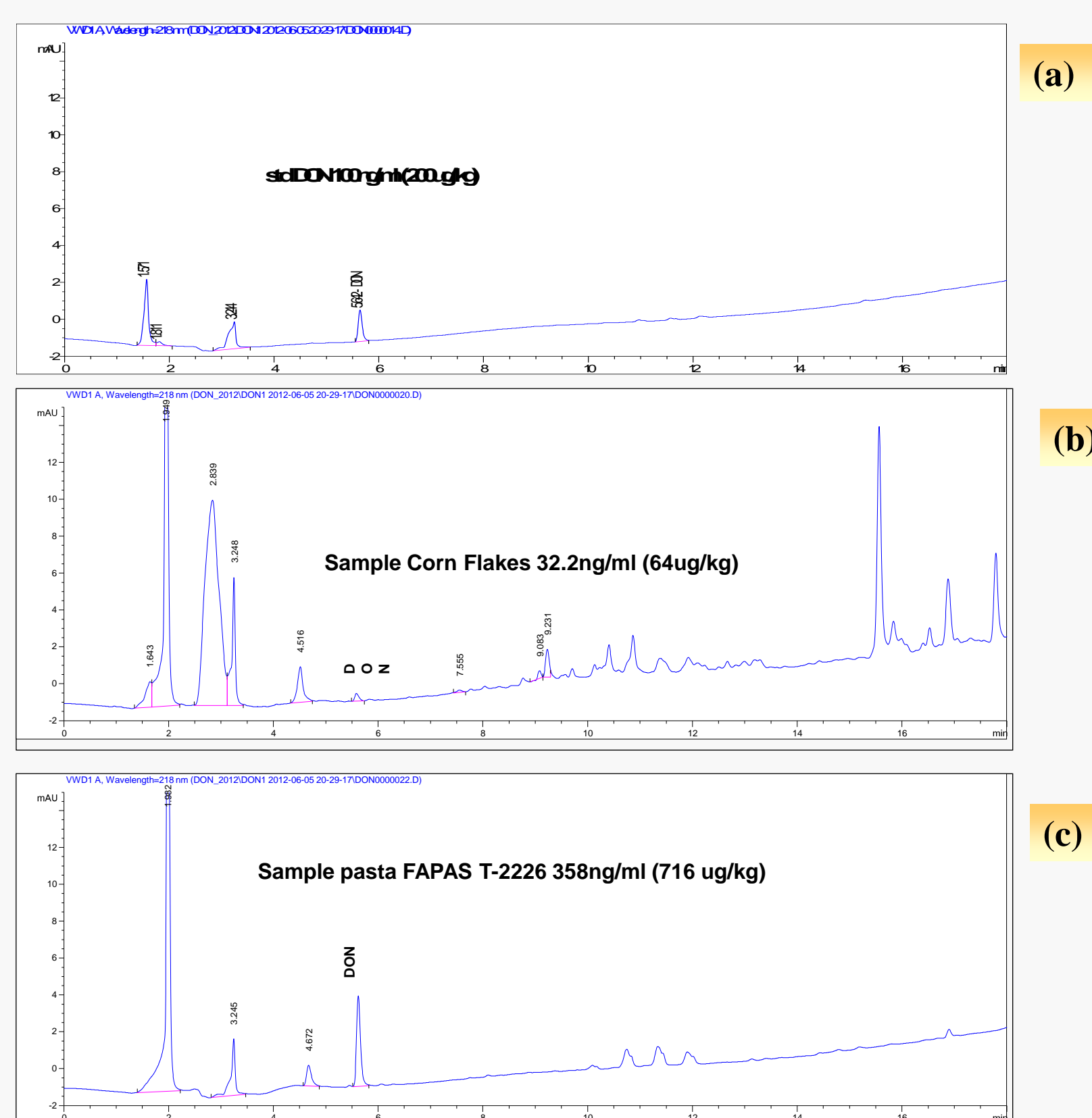


Fig. 1. Chromatograms of DON standard (a), real sample (b) and CRM (c)

The investigated pasta samples were contaminated by three species fungi from the genus *Fusarium*: *F. graminearum*, *F. culmorum* and *F. verticillioides* (Fig. 2)



Fig. 2. *Fusarium* sp.: *F. graminearum* (a), *F. culmorum* (b) and *F. verticillioides* (c)

## CONCLUSION

A procedure for determination of a DON in pasta and wheat-based food using immunoaffinity columns clean up and HPLC/DAD has been described. This work also includes an application to samples obtained from the retail market. Out of 12 samples 4 were found to be contaminated with DON but content in all the samples was below the MRL. The occurrence of DON in pasta samples demonstrates the importance of an effective control of these products by governmental authorities and industries.