

Reduce your mycotoxins analysis time : comparison of HPLC/MS/MS and UPLC/MS/MS methods for the quantification of 8 trichothecenes in cereals and feeds

Mickaël HYBOIS*, Stéphane LE TEXIER, Gabriel MORICE, Renaud LE BOUQUIN, Claude CHARRETEUR

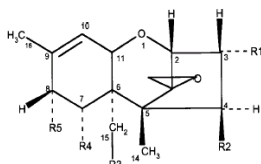
LAREAL, Laboratoire de Chimie Alimentaire, Post Box 234, 56006 Vannes Cedex, France

Phone: 00 33 2 97 48 49 80, Fax: 00 33 2 97 48 49 81, contact@lareal.com, www.lareal.com

* corresponding author : mhybois@lareal.evl.s.net

PROCEDURE

Trichothecenes are a large group of tetracyclic sesquiterpenoid mycotoxins.



General structure of trichothecenes

SAMPLE EXTRACTION

Extraction solvent : acetonitrile / water (84/16)
 Mechanical stirring : 2 hours at room temperature
 Clean Up : TRICU#225 or TRICU#227 columns (LIBIOS)

The purified extract is evaporated to dryness and the residue is reconstituted with the Internal Standards solution.

HPLC - UPLC / MS / MS

Apparatus : Alliance 2695 HPLC (WATERS), Acquity UPLC (WATERS), Quattro Micro (MICROMASS)

Columns : Atlantis dC18, 4.6x250 mm, 5µ (HPLC - WATERS)
 HSS T3, 2.1x150 mm, 1.8µ (UPLC - WATERS)

Eluent : gradient of two eluents : water with sodium acetate and acetonitrile/methanol mix with sodium acetate

Detection : positive ESI mode for type A - trichothecenes
 negative ESI mode for type B - trichothecenes

Matrix effects are minimized by using three internal standards (isotopically labelled compounds : Deoxynivalenol, T2 toxin and HT2 toxin)

SUMMARY

Trichothecenes are a large group of mycotoxins produced by various *Fusarium* fungi. These mycotoxins have extremely toxic effects on animal and human health.

LAREAL, feed and food analytical laboratory, has many years of experience in mycotoxins analysis (fumonisins, aflatoxins, ochratoxin A, zearalenone, patulin and trichothecenes, ...). The future mycotoxins legislation in Europe will induce an increase of mycotoxins analysis.

Currently, trichothecenes are analysed by HPLC/MS or GC/MS, but the arrival of Ultra Performance Liquid Chromatography (UPLC) has permitted the development of a rapid method : UPLC - Electrospray ionisation - Tandem mass spectrometry

In this work, we compare the separation, the sensibility, the analysis time and the quantification of 8 trichothecenes (3 type A : Diacetoxyscirpenol, T2 toxin, HT2 toxin and 5 type B: Deoxynivalenol, Nivalenol, Fusarenone X, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol) by HPLC and UPLC methods.

CONCLUSION

Analysis time : significant reduction with UPLC
 UPLC 4 times faster

Chromatographic separation : the same good separation
 HPLC & UPLC : resolution of 0.9 between peaks of 3 and 15 - acetyldeoxynivalenol

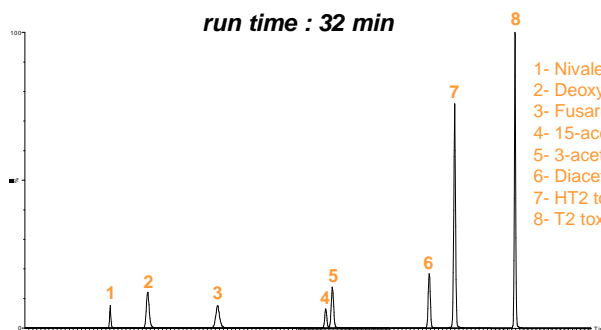
Quantification : no significant variation
 Intra-lab reproducibility (RSD, %) of Deoxynivalenol, in Quality Control sample (wheat):
 - HPLC: 8.8 % (mean= 185 µg/kg, n=10)
 - UPLC: 9.0 % (mean= 191 µg/kg, n=20)

Sensibility : better with UPLC

Productivity of the mass spectrometer: better with UPLC
 HPLC : 1.8 sample / hour
 UPLC : 7.5 samples / hour

HPLC

run time : 32 min



UPLC

run time : 8 min

