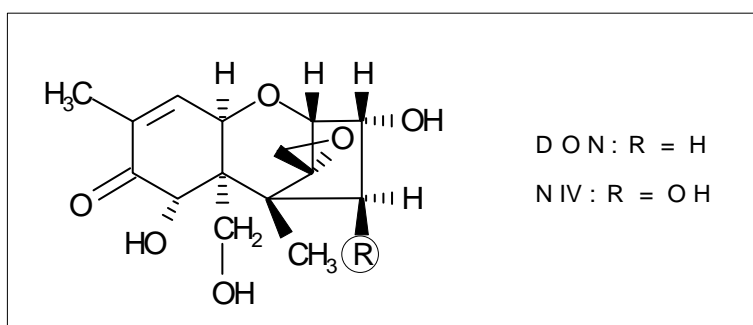


Trichothecene Analysis



Trichothecenes are mycotoxins produced by fungi of the species *Fusarium* and *Trichothecium*. In general the trichothecenes are divided in two classes, type A and B. At present only deoxynivalenol of the type B trichothecenes is regulated by law within the European Union; more regulated trichothecenes will follow. DON producing *Fusarium* strains are mainly found in more temperate zones and therefore accumulate in Europe and North America during suitable weather conditions during ear blossom, resulting in the so called 'Fusarium years' with elevated DON levels in cereals. Depending on the type of food, the maximum tolerable limits are in the range of 200 and 1750 ppb. In order to be able to measure DON at low ppb levels, it is recommended to use post-column derivatization, which in this case has a double specificity.



The structures of deoxynivalenol und nivalenol

For this application LCTech offers the post-column derivatization system **PINNACLE PCX** from **PICKERING Laboratories**.

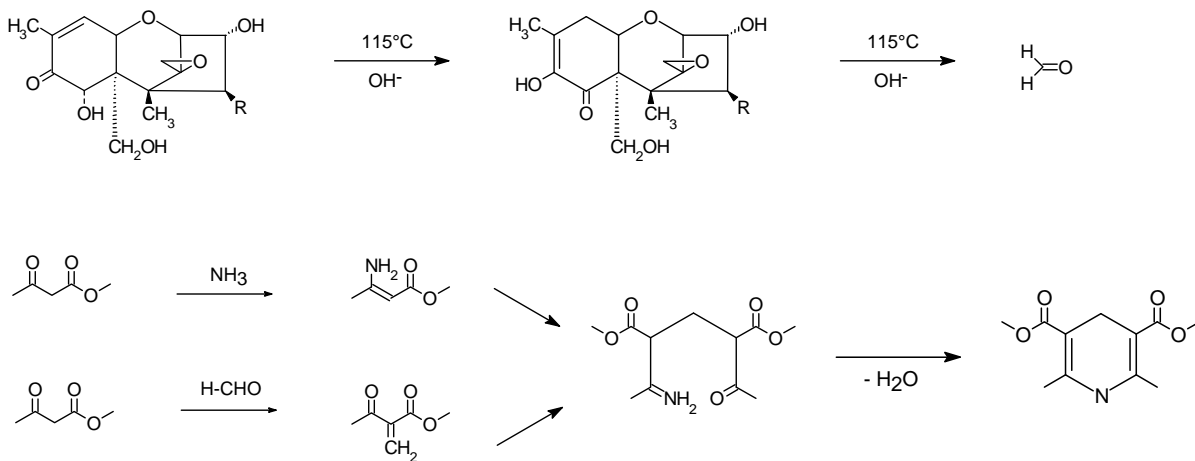


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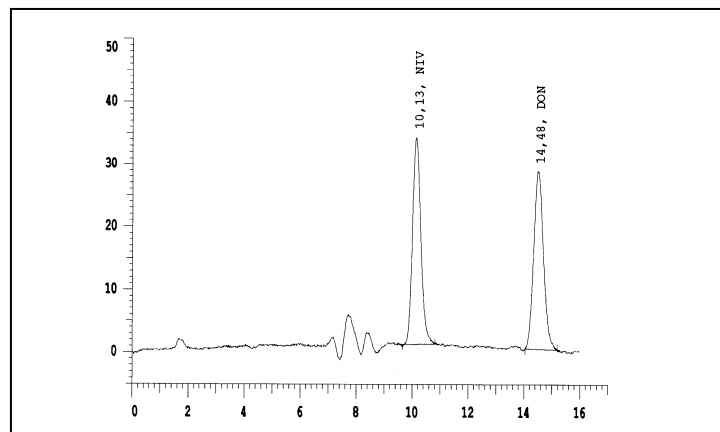
APPLICATION NOTE

Method Description

After homogenization and extraction of the sample, the crude extract is cleaned up with single-use columns. The deoxynivalenol and nivalenol in the column eluate are subsequently separated on a reversed phase column and derivatized via the PINNACLE PCX in a two-step reaction (see reaction scheme below). Technically the trichothecene is cleaved to formaldehyde in the first step. In the second step the formaldehyde reacts with ammonium acetate and methyl acetoacetate in a Hantzsch reaction to a fluorescing lutidine derivative. Afterwards the measurement is performed with a fluorescence spectrometry.



Chromatogram



Chromatogram of a DON/NIV standard (50 ng each)

APPLICATION NOTE



HPLC Conditions and Derivatization Parameters

HPLC	
Operating Mode	Gradient
Eluent	Acetic acid 0.01 M/acetonitrile (9/1, v/v)
Degassing	Helium- or vacuum-degassing
HPLC column	RP C18 with polar endcapping, 250 x 3 mm with guard
Flow rate	0.4 mL/min
Injection volume	Up to 200 µL
Post-Column Derivatization	
Pinnacle PCX	Dual-pump
Column oven	35 °C
Reactor volume	Special reactor
Reactor temperature	115 °C
Reagent 1	0.15 N NaOH
Reagent 2	2 M Ammonium acetate, 0.03 M methyl acetoacetate
Reagent flow	0.3 mL/min
Detection	
Detection mode	Fluorescence detection
Excitation wavelength	360 nm
Emissions wavelength	470 nm
Flowcell	Analytical; pressure stable up to 7 bar

APPLICATION NOTE

Literature

- 1) A. Sano, S. Matsutani, M. Suzuki, S. Takitani, *J. Chromatogr.*, **1987**, *410*, 427 – 436.
- 2) R. Schuhmacher, R. Krska, J. Weingaertner, M. Grasserbauer, *Fresenius J. Anal. Chem.* **1997**, *395*, 510 – 515.
- 3) R. Krska, *J. Chromatogr. A* **1998**, *815*, 49 – 57.
- 4) COMMISSION REGULATION (EC) No **1126/2007** of **28 September 2007**.
- 5) Information of the Bavarian State Office for Health and Food Safety available at http://www.lgl.bayern.de/de/left/fachinformationen/lebensmittel/mykotoxine_hoehstmengenregelung.htm

Order Information

Order number	Description
1153-1072	PINNACLE PCX – Dual-pump; special reactor