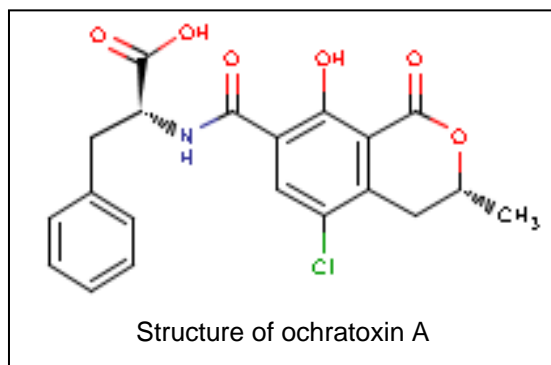


OCHRATOXIN



The mycotoxin ochratoxin A (OTA) is produced by different *Aspergillus*- and *Penicillium*-species in cereals, peanuts, grapes, raisins, wine, coffee, beer and spices typically during storage. Since OTA has or is suspected to have nephrotoxic, teratogenic, immunotoxic, and carcinogenic properties and investigations have shown that the above mentioned matrices sometimes contain large amounts of the toxin, appropriate maximum permissible values were set by the European Union. According to the matrix and the intended use, the limits are in the range of 0.5 to 10 ppb. In order to measure correspondingly sensitive and without interfering matrix effects, OTA is determined with fluorescence spectrometry after a clean up with immunoaffinity columns and post-column derivatization.

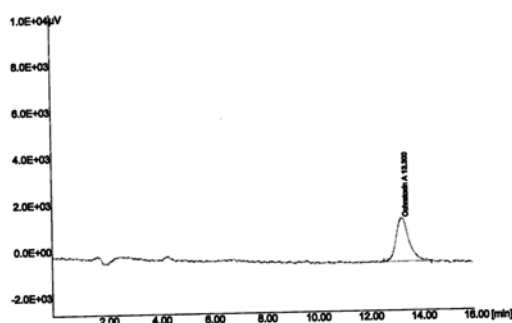


For this application LCTech supplies a suitable post-column derivatization system, the **PINNACLE PCX** from **PICKERING LABORATORIES**. The fully automated software-controlled system can be operated with any HPLC brand and is simply connected between HPLC and detector.

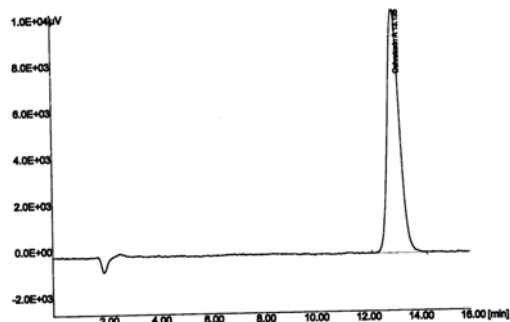
Method Description

After homogenization and extraction of the matrix, the crude extract is cleaned up with immunoaffinity columns (e. g. OtaCLEAN™ from LCTech). After separation on a reversed-phase column, the obtained column eluate is derivatized by the PINNACLE PCX system with sodium hydroxide or aqueous ammonia solution and measured via fluorescence detection.

Chromatograms



a) OTA standard without post-column derivatization



b) OTA standard with post-column derivatization;
each with 100 pg

Using post-column derivatization and depending on the fluorescence detector used, a significant increase in peak area is observed. OTA can be measured accurately down to 1 pg.

HPLC Conditions and Derivatization Parameters

HPLC	
Operating modus	Isocratic
Eluent	AcCN/MeOH/water/glacial acetic acid 5/55/40/1 (v/v/v/v)
Degassing	Helium- or vacuum-degassed
Flow rate	0.7 mL/min
HPLC column	RP C18 e. g. 150 x 4.6 mm with guard column

APPLICATION NOTE

Injection volume	100 µL
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Post-Column Derivatization	
Pinnacle PCX	Single reagent
Column oven	45 °C
Reactor volume	500 µL
Reactor temperature	40 °C
Reagent	1 N NaOH or 25 % aqueous ammonia solution
Reagent flow	0.3 mL/min
Detection	
Measurement	Fluorescence detection
Excitation wavelength	365 – 390 nm
Emission wavelength	440 nm
Flowcell	Analytic; pressure stable up to 7 bar

Literature

- 1) B. Zimmerli, R. Dick, *J. Chromatogr. B* **1995**, 666, 85 – 99.
- 2) A. Pittet, D. Torare, A. Huggett, R. Viani: Liquid Chromatographic Determination of Ochratoxin A in Pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure, *J. Agric Food Chem* **1996**, 44 (11), 3564-3569
- 3) A. Thellmann, W. Weber, *Deutsche Lebensmittelrundschau* **1997**, 93 (1), 1-3.
- 4) ISO 15141-2: **1998** Foodstuffs – Determination of ochratoxin A in cereals and cereal products – Part 2: High performance liquid chromatographic method with bicarbonate clean up.
- 5) S. Kastrup, U. Aulwurm, Analytik von Ochratoxin A in Lebensmitteln im ppt-Bereich, *GIT* **2002**, 9, 975 – 977.
- 6) COMMISSION REGULATION (EC) No **1881/2006** of **19 December 2006**
- 7) Information from the Bavarian State Office for Health and Food Safety available at http://www.lgl.bayern.de/de/left/fachinformationen/lebensmittel/mykotoxine_hoehstmengenregelung.htm

APPLICATION NOTE



Order Information

Order Number	Description
1153-1022	PINNACLE PCX – Single reagent; 500 µL reactor

For the cleanup of samples LCTech provides the 3 mL immunoaffinity columns OtaCLEAN™ or Afla-OtaCLEAN™ and additional useful accessories.

Order Number	Description
10515	OtaCLEAN™; 3 mL immunoaffinity cleanup columns for the ochratoxin A analysis; 25 columns/box
11022	Afla-OtaCLEAN™; 3 mL immunoaffinity cleanup columns for the combined aflatoxin and ochratoxin A analysis; 25 columns/box
10896	Reservoir, DURAN® glass for sample loading; reusable; labwasher-proof, with seal and screw-cap
11048	EluVac™ Vacuum manifold for immunoaffinity columns with large capacity (100 mL) reservoirs
11098	Sample rack/reservoirs (ideal in combination with sample reservoirs 10896)
11105	Sample rack for 4 mL Vials (Order No V0004)