PICKERING LABORATORIES

CATALYST FOR SUCCESS

OCHRATOXIN A IN RED AND WHITE WINE WITH OTACLEAN™ AND ACCECLEAN™

INTRODUCTION

The mycotoxin ochratoxin A is found in many matrices and so is also found in grapes. As red and white wines are produced of grapes, the mycotoxin can be found in the final product. Therefore, a maximum limit for ochratoxin A in red, white and rosé wine and other wine and/or grape must based beverages by Commission Regulation (EC) No. 123/2005 was set at 2.0 µg/kg. Thus, these beverages must be analyzed for the mycotoxin content before they are sold on the market.

The standard procedure for analysis of ochratoxin A in matrices is to use immunoaffinity column cleanup. The cleanup may either be performed manually or much more convenient by an automated system. The following application note shows how wine samples can be processed with the OtaCLEAN™ immunoaffinity columns in combination with the fully automated sample preparation system AcceCLEAN™ producing high recovery rates and repeatability.

OtaCLEAN™ columns

AcceCLEAN™ system

CHEMICALS AND MATERIALS

For the analysis standard laboratory equipment such as beakers, funnels, filters, and stirrers are required. Furthermore, a centrifuge or a syringe with PVDF filters may be needed. The HPLC system should at least have an injection system (manually or automated), an isocratic pump, a column oven with guard and HPLC column, and a sensitive fluorescence detector. Using a post-column derivatization system further increases the sensitivity by a factor of 6 to 8, and the selectivity as interfering peaks are removed significantly.

PRODUCT DESCRIPTION	PART NUMBER
AcceCLEAN™	P/N 11020
Rack for 3 mL SPE-Cartridge	P/N 11631 (10 x)
Eluat Rack for 4 mL vials	P/N 11633 (10 x)
Vial with snap-on-lid, 30 mL	P/N V0030 (200 pcs/pkg)
Vials 4 mL	P/N V0004 (100 pcs/pkg)
OtaCLEAN™, 3 mL cartridge	P/N 10515 (25 pcs/pkg) P/N 12426 (100 pcs/bag P/N 12427 (500 pcs/bag)
PINNACLE PCX, single pump, 500 µL reactor	P/N 1153-1022

- Extraction solution, 5 % NaHCO3 with 1 % PEG 8000 (e. g. Sigma 4423)
- PBS buffer pH 7.2
- Water (HPLC grade)
- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- Glacial acetic acid p. a.
- Sodium hydroxide 1.0 N

PROCEDURE FOR RED AND WHITE WINE

Sample Preparation

Mix 10 mL of wine thoroughly with 10 mL of extraction solution for 3 min.

Pass the extract through a plaited filter.

10 mL of the filtered extract are diluted with 40 mL PBS-Puffer (pH 7.2). If there is any precipitation during mixing with the buffer, practically the sample volume has to be filtered by means of a syringe filter (PVDF) or centrifuged.

25 mL are applied on the immunoaffinity column by LCTech program 2 in the AcceCLEAN™ (25 mL, flow rate 2mL/min); for using the instrument please follow the manual.

Dilute or concentrate eluate to your requirements and measure by HPLC.

Chromatographic Conditions

Eluant: water/methanol/acetonitrile/glacial acetic acid 40/55/5/1 (v/v)

HPLC Column: 125 x 3 mm; RP C18; 3 µm; 120 Å

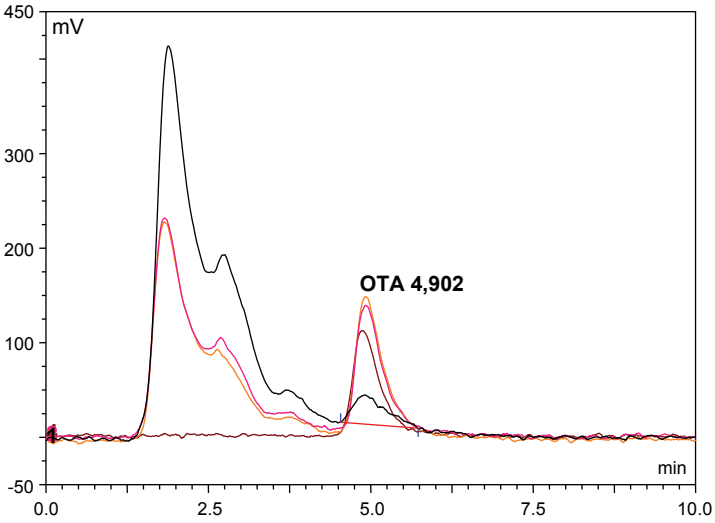
Flow Rate: 0.6 mL/min

Injection Volume: 10 – 100 µL

Column Temperature: 40 °C

Post-column Reagent: 1 N NaOH at 0.3 mL/min

FLD wavelength: 390 Ex; 440 Em (with derivatization)
335 Ex; 460 Em (no derivatization)



Overlayed chromatograms of a red wine (Pinot Noir); blank and spiked.

Black	Wine blank containing OTA below 1 ppb
Red	Wine sample spiked with 5 ppb after extraction
Orange	Wine sample spiked prior to extraction with 5 ppb
Brown	OTA standard (12.5 ng / 2 mL diluted to HPLC eluant to 5 ppb)

The recovery rates obtained at levels of 1 and 5 ppb were 99 ± 6 %.

PICKERING LABORATORIES
1280 Space Park Way / Mountain View, CA 94043
sales@pickeringlabs.com / support@pickeringlabs.com
800-654-3330 / 650-694-6700 / Fax: 650-968-0749