New rapid clean-up device for ochratoxin A

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Summary

High Performance Liquid Chromatography (HPLC) with fluorescence detection (FLD) and Thin Layer Chromatography (TLC) are widely used analytical methods for the determination of Ochratoxin A (OTA). As normally the crude extract cannot be used directly for these analyses, a clean-up step is necessary. Commonly used clean-up procedures comprise immunoaffinity columns (IAC), liquid liquid extraction (LLE) and solid phase extraction (SPE). The performance of a new single step clean-up column Mycosep® 229 Ochra from Romerlabs® was tested in matrices often contaminated with OTA like green coffee beans, raisins and various kinds of cereals. In all these matrices an average recovery of more than 90 % (range 2.6-92 µg/kg; n=24) of the added OTA could be obtained. Additionally, a certified reference material (CRM), OTA in wheat, was tested with the column. An average recovery of 96.2 % (n=9) was achieved for the CRM proving the applicability and high accuracy of the developed method.

Key words: ochratoxin A, sample clean-up, high performance liquid chromatography, food analysis, Mycosep®

Introduction

Ochratoxin A (OTA) was first discovered in 1965 and isolated from a culture of Aspergillus ochraceus ¹, and was subsequently found in Aspergillus carbonarius, Aspergillus niger and Penicillium verrucosum, Penicillium cyclopium etc.

All these different strains have different optimal conditions for producing OTA. For example, Penicillium verrucosum is mostly responsible for OTA contamination on crops in the colder climatic zones such as Scandinavia and Canada, while Aspergillus
ochraceus is more common in warmer regions such as Australia and the former Yugoslavia. OTA has been listed as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). In several animals species OTA causes renal toxicity, nephropathy and immunosuppression. In rodents, it also induces DNA damage in vivo and in vitro.

It has been suggested that OTA causes endemic nephropathy and urothelial tumours, but the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the data available are not sufficient to support these arguments and that other nephrotoxic agents may also be involved.

Two widely used analytical methods for the determination of OTA are HPLC-FLD and TLC. Often the crude extracts cannot be used directly for these analyses. Hence, a clean up step is necessary. The most commonly used clean-up methods compromise the use of immunoaffinity columns (IAC), liquid liquid extraction (LLE) and solid phase extraction (SPE).

A new developed clean-up device, containing a mixture of polymers and non-polar absorbents, from Romerlabs is available in two formats: the Mycosep and the Multisep format (syringe format). But in contrast to conventional SPE this column does not need to be conditioned and the clean-up is a done in one step. The extract is sucked (Multisep format) or pressed (Mycosep format) through the column and the collected eluate is used for further analysis. The performance of this single step clean-up device was investigated.

Materials and Methods

Twenty five g of ground sample were extracted for 90 min with 100 mL of acetonitrile/water (84/16, v/v) in an Erlenmeyer flask on an orbital shaker. After filtering and acidifying the extract to 1 % acetic acid about 6 mL were filled into the culture tube of the Mycosep Ochra column (Romerlabs, Union, USA). The column was pushed down into the culture tube until 4 mL of purified extract could be removed from top. The purified extract was brought to dryness under a gentle stream of air. The residue was dissolved in 0.5 mL mobile phase.

HPLC analyses were performed with an HP1100 series equipment (Hewlett Packard, Waldbronn, Germany) consisting of quaternary pump, column oven, and autosampler. The fluorescence detector was a HP1046A (Hewlett Packard, Waldbronn, Germany). Chromatographic separation was carried out at 20 °C with acetonitrile/water/acetic acid (40+60+1) on a Hypersil ODS 2.0’100mm 3μ column (Agilent, Palo Alto, USA).

The performance of Mycosep Ochra column was tested with maize, wheat, green
coffee beans, raisins and BCR472, a certified reference material (CRM) from IRMM (Geel, Belgium). BCR472 is a naturally contaminated wheat sample with a certified value of 8.2 μg/kg (uncertainty 1.0 μg/kg).

A stock solution (26.5 μg/mL) with crystalline OTA (Biopure, Tulln, Austria) in acetonitrile was prepared and used for spiking experiments and an external calibration. Blank wheat was spiked at two levels 2.6 μg/kg and 26.2 μg/kg. In addition standard addition was done with the CRM. Two levels where added one with + 2.6 μg/kg and one with + 13.1 μg/kg, resulting in 10.8 and 21.3 μg/kg. Maize, green coffee beans and raisins were spiked in 8 equidistant levels from 2.6 μg/kg up to 91 μg/kg.

**Results and Discussion**

The HPLC-FLD method achieved a limit of detection of 4.1 ng/mL corresponding to 2.1 μg/kg (Calculated according to the guideline of the German Standard DIN 32645 (May 1994) where the limit of detection derives from the Y-intercept and its confidence interval after linear regression). The performance of this method was mainly limited by the used fluorescence detector. A well separated OTA peak with a retention time of about 9.5 min was achieved (Fig. 1). The recovery rates for maize, green coffee beans and raisins were calculated by plotting the spiked concentration against the found one. The slope of the resulting correlation line corresponds to the recovery rate (Table 1).

![Chromatograms of a maize samples after clean-up with Mycosep® 229.](image)

The results for the wheat samples are summarized in Table 2. The lower level of wheat shows a recovery rate of only 78.8 % but it has to be taken into account that this level is below the limit of quantification.
Good repeatability of results for the spiked wheat sample and the CRM sample (Table 2) demonstrates the good precision of the method and its applicability for naturally contaminated samples.

In conclusion this preliminary validation study demonstrates the great potential of this new Mycosep® Ochra clean-up column from RomerLabs® as an alternative clean-up method to the most frequently used immuno affinity technique.

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References

2) Environmental Health Criteria 105, 1990, World Health Organization, Geneva, Switzerland