

Ochratoxin A in nutmeg Cleaned up with *OtaCLEAN*



Mould toxins in spices: Nutmeg

During the drying process, nutmeg can easily be contaminated by moulds and thus also contain toxins that are harmful to health. In 2021, 11 complaints were claimed in context to mycotoxins and nutmegs to be traded in European trade.

These were always the toxins aflatoxin and ochratoxin A. In the first quarter of 2022 alone, 5 mycotoxin findings were reported in nutmegs that were also to be imported into the EU, mainly due to exceeded ochratoxin A levels.

OtaCLEAN - IAC column for the analysis of ochratoxin A

The columns are available in the practical 3 mL format or in the miniaturised SMART format and are suitable for manual or automatic processing

A monoclonal antibody specially developed for this field of application guarantees best results even with difficult matrices and enables efficient reduction of matrix components to perform sensitive analysis. This sample purification technique is suitable for both HPLC with fluorescence detection and analysis by mass spectrometry.

Processing protocol

Mix 10 g homogenised nutmeg with 2 g sodium chloride. For the extraction use 100 mL methanol/water (80/20 (v/v)). During the extraction process add 50 mL n-hexane to remove fats and oils efficiently. An extraction of at least 15 minutes is recommended. Filter the crude extract and then centrifuge it to achieve optimal separation of the methanolic lower phase from the n-hexane phase.

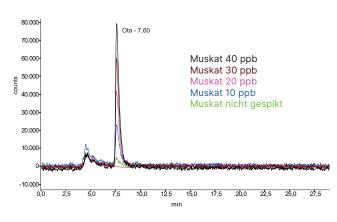
Mix 2 mL (equivalent to 0.2 g matrix) of the upper methanolic phase with 12 mL PBS buffer containing 8 % Tween20 and load onto the OtaCLEAN immunoaffinity column. Successively rinse the Sample vessel with 2 \times 5 mL deionised water and load the rinse solution onto the OtaCLEAN column as well. After washing dry the column with a stream of air. Add 2 mL methanol to the column and seal it after the methanol has flowed into the column bed. Make sure that it is allowed to incubate for at least 5 minutes to ensure complete denaturation and thus elution of the toxin. Collect the eluate in a 2 mL measuring cylinder.







Chromatograms



Comparison of the chromatograms of the non-spiked nutmeg sample (green) with the 10 ppb (blue), 20 ppb (pink); 30 ppb (brown) and 40 ppb (black) spiked nutmeg sample.

Due to the specific affinity of the OtaCLEAN column, the toxin can be selectively concentrated from the matrix without interfering with the chromatography. The selective, highly specific clean-up shows a chromatographic picture like an analytical standard. This allows a easy and fast analytical interpretation of the results.

| Conditions | | |
|---------------------------|---|--|
| HPLC | Isocrate | |
| Column oven | 40 °C | |
| Separation column | RP EC 125/3 nucleosil 120-3 C18 | |
| Flow rate, Running medium | 0.6 mL/min; HPLC-water/methanol/ acetonitril (40/55/5 (v/v/v)+1% acetic acid) | |
| Fluorescencedetection | Without derivatistion | |
| Excitation wavelength | 335 nm | |
| Emmission wavelength | 465 nm | |

| Recovery rates | | |
|----------------|--------------|--|
| | Ochratoxin A | |
| Standard | 100 | |
| 10 ppb | 86 | |
| 20 ppb | 89 | |
| 30 ppb | 87 | |
| 40 ppb | 88 | |

^{*} Standard was set = 100% set

Conclusion

The OtaCLEAN column offers a wide range of applications in food and feed with best clean-up and good recovery rates. In addition to considerable matrix tolerance, highly selective binding of analytes is possible. This enables the highest measurement sensitivity from feed to baby food, but also spices, medicinal herbs and other matrices such as coffee, liquorice and much more.

Chromatography times are significantly reduced and little matrix interference allows faster and more efficient analysis with best results.

This LCTech product was used:

515 OtaCLEAN 25 per package

Do you have a special request as to which matrix we should test for you?

Contact us by e-mail at: info@LCTech.de



^{**} Corrected with non-spiked sample / The results are in accordance with the performance specifications of EC 401 / 2006 (section 4.3.1).