



March 2020

Ochratoxin A in Paprika and Chili Powder ~ Manual and Automated ~

Do you have a special matrix that we should test for mycotoxins? Please let us know and write an e-mail to: info@LCTech.de

Sample Preparation

MYCOTOXINS

Paprika and Chili Powder

Thousands of years ago, the indigenous peoples of South and Central America have used paprika and chili powder to season various dishes. Only after the discovery of America by Christopher Columbus in 1492, the paprika and chilies came to Europe. Nowadays they are indispensable as a spice in the kitchen. The chili is one of many different kinds of peppers. In 2017, almost 36 billion tonnes of paprika and chili powder were produced.

The alkaloid capsaicin is responsible for the pungent aroma of the chilies, which is found especially in the oily plant components of the fruits are present. The capsaicin remains dried in the powder or pod and has an even sharper effect by reducing the water content.

During the drying process or under incorrect storage conditions, mycotoxin-producing molds may form in the peppers and chilies. These can be toxic to humans in too large quantities. For this reason, regular checks are carried out when chilies and peppers are imported. The importers of peppers and chilies include Mexico, Indonesia and Spain.

Ochratoxin A in food and feed

Ochratoxin A is a naturally occurring mycotoxin produced by moulds of the genera *Aspergillus* and *Penicillium* as primary contamination. Ochratoxin A is often formed in food and animal feed as well as in paprika and chili powder.

In order to simplify the daily laboratory routine, LCTech has developed special solutions for the clean-up of ochratoxin A, which develops 3 mL OtaCLEAN immunoaffinity columns.

These achieve very good recovery rates even with difficult matrices, such as spices. In addition to the 3 mL format, they are also available as a practical 3 cm SMART version. Both are suitable for manual and automated processing, e.g. with the robotic system FREESTYLE SPE. Parameterize the application in the method of volume and flow rate - **Here we go.**

The OtaCLEAN SMART columns convince not only by their small size and a lower costs, e.g. reduced solvent consumption, shorter processing times and comparably good recovery rates.



OtaCLEAN and
OtaCLEAN SMART

Processing Protocol

Homogenise 10 g of paprika powder and add 1 g of sodium chloride. Extract the sample with 50 mL methanol/water (80/20 (v/v)) and 25 mL n-hexane in order to remove fat and oil. For high extraction efficiencies, continue the extraction for at least 10 minutes. Filtrate the raw extract and dilute 2 mL of n-hexane free phase with 12 mL PBS (contains 8 % Tween20).

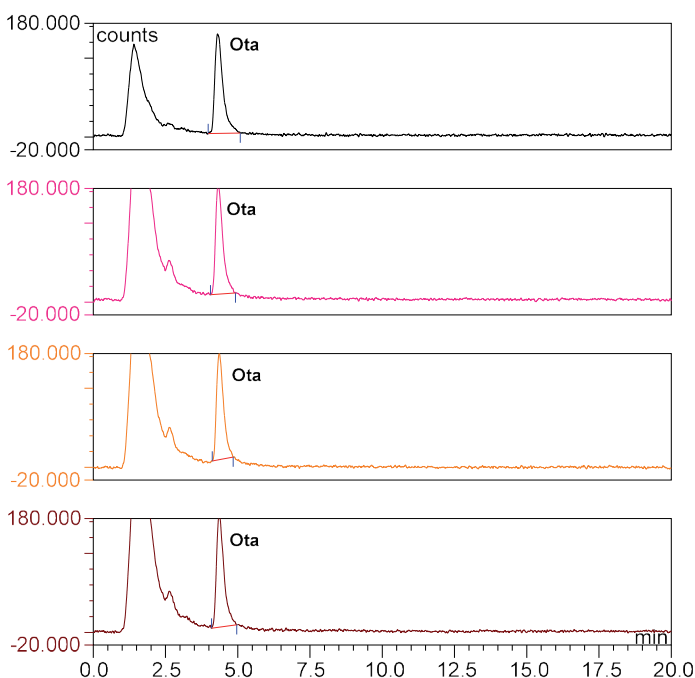
OtaCLEAN 3 mL column:

Load 14 mL of the sample (corresponds to 0.4 g matrix) onto an OtaCLEAN column. In order to remove unspecific matrix components and Tween residues efficiently, the column should be washed with 2 x 5 mL deionized water. Then elute, after drying, the OtaCLEAN column with 2 mL methanol. Make sure that the methanol 5 minutes into the column bed to achieve a complete denaturation of the antibodies and a release of the toxins.

OtaCLEAN SMART column:

At a flow rate of 1.5 mL/min, 2.8 mL of the sample (corresponds to 0.08 g matrix) is loaded onto the OtaCLEAN SMART column. Rinse the column with 2 x 1 mL deionized water at the same flow rate. The column is freed from liquid residues without an air flow. Elute the column with 0.4 mL methanol. Ensure that the methanol is allowed to act on the column bed for 5 minutes to completely break the antibody/toxin binding.

Chromatogram



Black = Paprika powder 10 ppb cleaned-up with OtaCLEAN
Red, Orange, Brown = Paprika powder 10 ppb cleaned-up with OtaCLEAN SMART

HPLC-Conditions

(Ochratoxin A)

Mycotoxin:	Ochratoxin A
HPLC:	isocratic
Column Oven:	40 °C
Separation Column:	EC125/3 Nucleosil 120-3 C-18
Flow Rate:	0.6 mL/min
Eluent:	HPLC-Water/Methanol/Acetonitrile (40/55/5 (v/v/v))+ 1% Acetic acid
Fluorescence Detection:	without Derivatisation
Excitation Wavelength:	335 nm
Emission Wavelength:	465 nm

Recovery Rates

Content of Ochratoxin A in Paprika and Chili Powder

Mycotoxin	100
Standard*	100
Recovery Rate** Pepper and chili powder, 10 ppb	96

*Standard is set = 100 %, **Corrected with non-spiked sample / The results comply with the performance specifications of EC 401/2006 (Section 4.3.1)

These LCTech Products were used:

OtaCLEAN
 Immunoaffinity Column for Ochratoxin A
 P/N 10515 / 11535

OtaCLEAN SMART
 Immunoaffinity Column for Ochratoxin A
 P/N 13346 / 13351

FREESTYLE SPE,
 Robotic System for Automated Sample Clean-up
 P/N 12663 / 12668