



December 2019

## Ochratoxin A in Mulled Wine ~ 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](mailto:info@LCTech.de)

### Sample Preparation

### MYCOTOXINS

#### Mulled Wine

Christmas music, roasted almonds, bratwurst and: Mulled wine. No Christmas market can do without it. It makes the icy winter endurable and is as much a part of Advent in Germany like gingerbread, fairy lights and "Jingle Bells". Mulled wine is made exclusively from red wine or white wine and is mainly flavoured with cinnamon, cloves, orange peels, cardamom and star anise.

The wine contained in the mulled wine is produced by the fermentation process from white or red grapes. If this fermentation or previous storage of the grapes results in improper processing or storage, mycotoxins may develop. In addition, during the drying process of the spices processed in mulled wine, mycotoxins may also be formed. Too many of these can be toxic to humans.

#### Immunoaffinity Column OtaCLEAN for Ochratoxin A



Immunoaffinity Column OtaCLEAN

LCTech supports laboratories worldwide in the field of sample clean-up in food and feed analysis so that mulled wine of good quality can continue to be enjoyed at Christmas markets.

Especially for the clean-up of ochratoxin A we offer the immunoaffinity columns OtaCLEAN, a solution that convinces with very good recoveries even with difficult matrices.

In addition to the 3 mL format, they are also available as a practical 3 cm SMART version and are suitable for manual as well as automated processing, e.g. with the robotic system FREESTYLE SPE.

Simply prepare your mulled wine sample according to the extraction and clean-up protocol on the following page, fill the racks with the samples, configure the desired method in the software and press START - while the system is now processing sample by sample, you have time for other important laboratory tasks.

## Processing Protocol

Add 10 mL polyethylene glycol (1 %) and NaHCO<sub>3</sub> (5 %) to 10 mL of mulled wine and mix the sample.

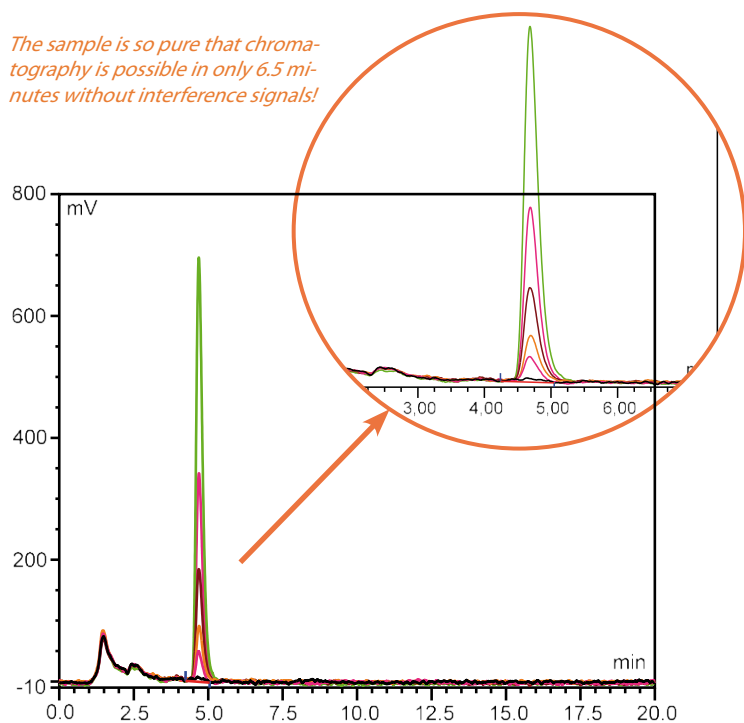
Filter the raw extract and dilute 12 mL of the mixture with 48 mL PBS.

Load the sample (50 mL correspond to 5 g matrix) onto the OtaCLEAN column. Then rinse the column twice with 5 mL deionized water. Dry the column with a short flush of air.

Elute the toxin with 2 mL methanol. Make sure that the column bed incubates with methanol for 5 minutes to completely denature the antibodies.

## Chromatogram

*The sample is so pure that chromatography is possible in only 6.5 minutes without interference signals!*



Black = Mulled Wine not spiked  
Red = Mulled Wine 0.5 ppb  
Orange = Mulled Wine 1 ppb

Brown = Mulled Wine 2 ppb  
Red = Mulled Wine 4 ppb  
Green = Mulled Wine 8 ppb

## Conclusion

Due to the high matrix clean-up an extremely high measuring sensitivity (<0.1 ppb for this matrix) is possible.

The chromatograms show that the use of LCTech immunoaffinity columns OtaCLEAN achieves good recovery rates even in the highly contaminated sample material.

This, combined with the high reproducibility, makes the columns very effective for use.

## HPLC-Conditions (Ochratoxin A)

| Mycotoxin:              | Ochratoxin A   |
|-------------------------|--|
| HPLC:                   | isocratic  |
| Column Oven:            | 40 °C  |
| Separation Column:      | RP EC 125/3 nucleosil 120-3 C18  |
| Flow Rate:              | 0.6 mL/min   |
| Eluent:                 | HPLC-Water/<br>Methanol/Acetonitrile<br>(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 Mulled Wine)

| Mycotoxin                               | Ochratoxin A |
|---|--------------|
| Standard*                               | 100          |
| Recovery Rate**<br>Mulled Wine, 0.5 ppb | 95           |
| Recovery Rate**<br>Mulled Wine, 1 ppb   | 91           |
| Recovery Rate**<br>Mulled Wine, 2 ppb   | 90           |
| Recovery Rate**<br>Mulled Wine, 4 ppb   | 93           |
| Recovery Rate**<br>Mulled Wine, 8 ppb   | 87           |

\*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

FREESTYLE SPE, Robotic System for Automated Sample  
Preparation  
P/N 12663 / 12668