

# Mycotoxins:



➤ Mycotoxins are dangerous in the food chain, that is why food and feed needs to be tested!

Mycotoxins (toxic mould fungus) are formed as poisonous metabolites.

Mycotoxins enter into the human food chain via contamination of plant-based foods. Grains and oil-containing seeds and nuts, such as corn, rice, peanuts, pistachios, sesame seeds, cotton seeds, dried fruits, milk, meat, spices, and cocoa beans etc. are particularly affected. Climate conditions have a big influence on contamination. Toxic mould fungus species are thus spread particularly in humid regions. Unfavourable harvesting and storage conditions can lead to increased mycotoxin exposure.

Mycotoxins can cause chronic and acute cases of poisoning. For example Aflatoxin B1 exhibits the highest toxicity of the discovered Aflatoxins and it is one of the strongest of all the mycotoxins. It has a strong genotoxic and carcinogenic effect, to which the liver is particularly susceptible. In addition to Aflatoxin B1, Aflatoxins G1, B2, and G2 are also amongst important fungi of the group that often occur together. M1 and M2 derivatives which are often found in milk are also important.

To protect consumers from illnesses caused by Mycotoxins, there are statutory limits in many countries.

With Clean-up Columns from BioTeZ you get excellent products for safe and reliable determination of mycotoxins.

BioTeZ Immunoaffinity Chromatography Clean-up columns are million times proven all over the world with Commodity Extracts of Food and Feed Samples containing Mycotoxins and subsequent HPLC or LC-MS/MS Analysis.

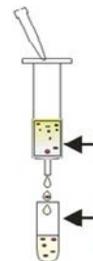
Choose premium quality, choose BioTeZ products!

## ➤ The principle of Immuno Affinity Clean-up Columns (IAC)

1. Extraction of the Analyte
2. Enrichment of the Analyte by using IAC
3. Elution of the Analyte
4. Detection

Details are in the Instruction of the IAC.

**Apply Sample    Wash Column    Elute Analyte**



**Ready for HPLC**

# Principle of IAC

## ➤ Extraction of the Analyte

Food and Feed are complex products and exact determination of single compound concentrations is seldom easy. Various extraction protocols with various gadgets and chemicals show different methods to set the targeted analyte free.

### *Sample for Extraction:*

- Weigh 25g thoroughly grinded sample (e.g. rice flour)
- Shake with 100ml **methanol-acetonitrile-water (25/25/50)** in an orbital shaker
- Centrifuge
- Take 4ml extract (contains mycotoxins 1g commodity) and **dilute with additional 16ml PBS**

**The interface to the next step (enrichment of the analyte by using IAC) is the organic solvent concentration.**



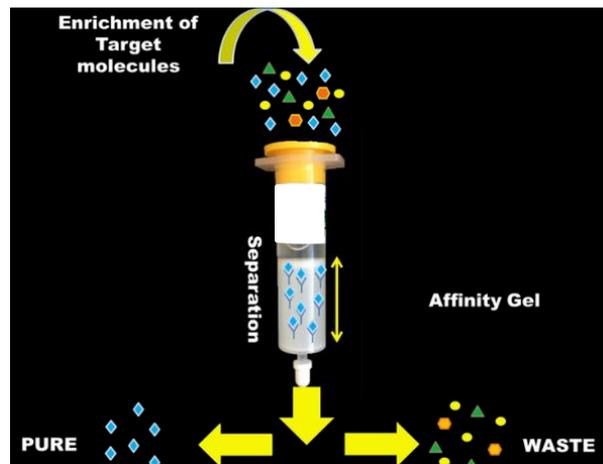
**Fazit:** The lab is free to use its extraction protocol, but the final organic solvent concentration of the extract being applied on top of the column should be limited to 10%. In some cases depending on the special column up to 20% are tolerated.

# Principle of IAC

## ➤ Enrichment of the Analyte

After extraction of the analyte from food or feed the matrix is still complex and contains still many disturbing compounds which disturb exact quantification. One excellent method to reduce disturbing compounds is to use IAC. Specific antibodies coupled to beads with high affinity to the analyte. The extract has to pass over the IAC. The analyte bind to the antibody and is immobilized. The column is washed with water, disturbing compounds are spilled away.

The analyte is during the wash immobilized on the IAC.



# Principle of IAC

## ➤ Elution of the Analyte

After wash the analyte is purified and immobilized on the IAC.

In an Elution step using an solvent (for example 3ml methanol) the antibody is denaturated and releases the analyte.

The Elution has to be done in a defined volume for recalculation of the concentration of the analyte in the sample.

Now the Eluate can used for detection. All detection methods are possible like HPLC, LC/MS-MS etc.



# Summary

## ➤ The principle of Immuno Affinity Clean-up Columns (IAC)

### 1. Extraction of the Analyte

Accepted laboratory extraction methods could be maintained. Full performance of the IAC column is given if pronounced criteria of organic solvent tolerance, elution process of analyte and working range of column is followed.

### 2. Enrichment of the Analyte by using IAC

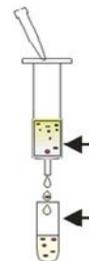
The IAC of Micotox contain highly specific antibodies coupled to beads to bind the analyte. Therefore the sample is applied to the IAC and the antibodies immobilize the analyte. Disturbing unwanted substances in the sample is washed away.

### 3. Elution

The bound analyte is released from the IAC by using a elution solvent like methanol. The eluate containing the analyte can now analyzed by HPLC or other methods.

Details are in the Instruction of the IAC.

### Apply Sample    Wash Column    Elute Analyte



**Ready for HPLC**

# Alternative Methods

## ➤ Other methods for sample Preparation

### **SPE columns an alternative to IAC ?**

For determination of analytes **Solid Phase Extraction (SPE) columns** were often used. This is a different technology. SPE columns are filled with “plastic” granulates which binds to disturbing components and reduces unwanted compounds in the sample. The binding process is unspecific.

In comparison to SPE method the IAC binds the analyte specifically. The SPE column binds unspecific to compounds.

### **Molecularly Imprinted Polymers** for Solid Phase Extraction

Polymer “plastic” granulates with imprinted specific binding motives follow the method of IAC, that means specific binding of the analyte, but this method isn’t working well in a daily routine work process. The affinity of Molecularly Imprinted Polymers to the target molecule is usually much lower than the affinity of antibodies.

### **Preparation without clean up columns**

This is possible, but the diagrams are disturbed and unclear. This leads to a lack of exactness.

**The best method of choice is using IAC!**

# Quality Statement



Immuno Affinity Clean-up Columns are manufactured with top quality standards to guarantee the highest quality and performance.

BioTeZ IAC are permanently tested following strong procedures. Comparisons with products of other market participants show and confirm the high premium quality of BioTeZ IAC for various commodities.

BioTeZ IAC show excellent capabilities:

- Excellent flow behaviour
- Extremely robust in handling and storage
- Easy enrichment guarantee
- High permeability
- Little abrasion
- No increased pressure required
- Long shelf life time
- Column size 3ml or 1ml or customized

#### WORKING RANGE OF COLUMN\*:

(applying 1g equivalents extract per column, see method above)

Aflatoxins

0.2 - 120\*\* $\mu$ g/kg

Ochratoxin A

0.2 - 20 $\mu$ g/kg

Zearalenon

2 - 500 $\mu$ g/kg

DON

10 - 3500 $\mu$ g/kg

Fumonisin

2 - 4000\*\* $\mu$ g/kg

I2/HT2

2 - 4000\*\* $\mu$ g/kg

\* within this range recovery rates above 85% are guaranteed

\*\* Sum parameter

## ➤ Product Portfolio

Standard pack units for IAC: Mini Pack 10 IAC, Box 30 IAC and Box 100 IAC

|            |  |                                |
|------------|--|--------------------------------|
| Mycotoxins | ELISA Aflatoxin B1   | 1 Microplate/96 determinations |
| Mycotoxins | ELISA Deoxynivalenol   | 1 Microplate/96 determinations |
| Mycotoxins | IAC Aflatoxin  | 30 columns                     |
| Mycotoxins | IAC Aflatoxin M1   | 30 columns                     |
| Mycotoxins | IAC Ochratoxin   | 30 columns                     |
| Mycotoxins | IAC Deoxynivalenol   | 30 columns                     |
| Mycotoxins | IAC Fumonisin  | 30 columns                     |
| Mycotoxins | IAC Zearalenon   | 30 columns                     |
| Mycotoxins | IAC T2/HT2   | 30 columns                     |
| Mycotoxins | IAC Combi Aflatoxin + Ochratoxin   | 30 columns                     |
| Mycotoxins | IAC Combi Aflatoxin + Ochratoxin + Zearalenon                                  | 30 columns                     |
| Mycotoxins | IAC Combi Deoxynivalenol + Zearalenon  | 30 columns                     |
| Mycotoxins | IAC Combi AOZDFT for LC-MS/MS (Afla, Ochra, Zeara, DON, Fumo, T2/HT2 in 1 IAC) | 30 columns                     |
| Mycotoxins | IAC Ergot Alcaluïdes   | 30 columns                     |
| Vitamins   | IAC Biotin   | 30 columns                     |
| Vitamins   | IAC Vitamin B12  | 30 columns                     |
| Vitamins   | IAC Folic Acid   | 30 columns                     |
| Hormons    | IAC Estradiol  | 30 columns                     |