Instruction of the Clean-up Process Using *B-TeZ IAC Fumonisin 3ml*

Clean-up of Commodity Extracts of Food and Feed Samples containing Fumonisins (FB_1/FB_2) via Immunoaffinity Chromatography and Subsequent Determination by HPLC with Precolumn Derivatization using o-Phthaldialdehyde and 3-Mercaptopropionic Acid

Principle:
This instruction of fumonisins (FB_1 / FB_2) determination in food and feed focuses on the enrichment step of extract using immunoaffinity column (IAC) and quantification with HPLC.

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

Many pretreatment methods of fumonisins determination in food and feed, most of them which are based on solid phase extraction (SPE) with adsorbents or strong anion resins (SAX), show low sensitivity because of interfering substances if problematic matrices are applied.

This method of content determination of fumonisins combines the high selectivity of an immunoaffinity column (IAC) with its potential to concentrate elute and additional step of purification of derivatized fumonsins by HPLC column.

As said before, this instruction focuses on the handling with the IAC column. For the commodity extraction step a literature method is given. Please see below. The given apparatus (e.g. HPLC system) might serve as example among other possibilities. For your convenience, an example HPLC method for the analysis of fumonisins is given below.

Sample Preparation (Literature method given):
Samples which content of Fumonisins are to be analyzed, e.g. wheat, maize etc., are extracted by the method of Prioli et al.[1] using methanol-water (80/20 v/v) as extraction solvent. E.g. to 25g of sample are added a volume of 100ml of the extraction solvent and processed as cited.

Enrichment Step IAC:
1.6ml extract (0.4g sample equivalent if above mentioned example extraction is followed) is diluted with 10mM PBS, pH=7.2 resulting a total volume of 13ml and then applied in a reservoir on top of the ***B-TeZ IAC Fumonisin 3ml*** column. If the example is followed the resulting organic solvent concentration is 10% of methanol which is tolerated by the column. The flow rate lies between 1 to 3ml/min. According to application and contents expected the applied extract volumes could vary. In case of very low contents even extract volumes of 20ml may be diluted with buffer and applied without significant loss of analyte as long as resulting pH is fairly neutral and alcohol or acetonitrile content lies under 15%. If latter is not the case the extract must further be diluted with PBS until fore mentioned maximum allowed organic solvent content during enrichment step using the ***B-TeZ IAC Fumonisin 3ml*** column is reached.

Thus, to guarantee maximum efficiency in terms of maximum recovery rates using ***B-TeZ IAC Fumonisin 3ml*** column, please ensure that

1. solvent content in diluted extract is below 15%,
2. fumonisin load per column lies below 1µg (see working range below),
3. extract should be diluted with 10mM PBS rather than with 50mM PBS (low salt concentration of solution favors toxin binding to the antibody. The effect is not very strong, but might account for 10 to 30% less analyte in eluate if anorganic salt concentration in diluted extract exceeds that of a 10mM PBS 5fold and more).

Wash:
After whole sample has passed through the gel the latter is washed with 5ml of 10mM PBS. Remaining liquids in the gel are removed by applying either pressure from top of the column or underpressure from bottom.

Elution:
Sample reservoir on top of the using ***B-TeZ IAC Fumonisin 3ml*** column is removed and an appropriate vial is placed below the affinity column. The bound toxins are eluted by using a total of 3ml of methanol-acetic acid (98/2 v/v)(1) as elution solvent. The elution process is performed in two steps to ensure complete release of analytes. First, a volume of 1ml elution solvent is applied. After that volume has passed through column half a minute is waited before the second portion of 2ml of elutions solvent is eluted through the column. Remaining solvent solutions should be eluted by application of slight under- or overpressure. All methanolic-acetic acid fractions are unified to give the column eluate.

The column eluate is concentrated to complete dryness (e.g. using VLM evaporater at 50°C under a permanent stream of nitrogen(2)). The residue is redissolved in e.g. 1ml of acetonitrile-water (40/60 v/v)(3) as dilution solvent and an aliquot is derivatized in the auto injector prior to injection into the system.

Further explanations:

Acc. (1): If 100% methanol is used instead of recommended methanol-acetic acid (98/2 v/v) mixture as elution solvent a loss of up to 20% in recovery of fumonisins could be encountered compared to the recommended elution solvent because of incomplete release of these mycotoxins from the gel.

Acc. (2): No loss of analytes, despite of the presence of acetic acid, is observed in the evaporation process even though the eluate is evaporated to complete dryness.

Acc. (3): Please note, that the dilution solvent lacks acetic acid of HPLC solvent B, since the acid would suppress derivatization reaction.

Column Characteristics:

A) Working Range and Recovery Rates of *B-TeZ IAC Fumonsin 3ml* Column:

Working Range of Column:	up to 1000ng FB_1/FB_2 per IAC
Zero Contamination of Column:	<1ng FB_1/FB_2 (Det. Lim. of method)
Guaranteed Recovery Rates within the Working Range(*):	
Fumonisin B_1 (FB_1):	>85%
Fumonisin B_2 (FB_2):	>85%

(*) Recovery rates are confined to solvent content of diluted extract below 15% methanol or acetonitrile (see details under Enrichment Step).

B) Cross Reactivities(**) of *B-TeZ IAC Fumonsin 3ml* Column:

Fumonisin B_1 (FB_1):	100%
Fumonisin B_2 (FB_2):	130%

(**) Recovery rate of FB_2 divided by recovery rate of FB_1 if a total of 10µg FB_1/FB_2 (1:1) is analysed per column. Please notice that this quantity is near the capacity limit of column where binding sites of column are limited. For this reason cross reactivities are practically the same where fumonisin concentrations are within the given working range (antibody binding sites are in excess).

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C) Capacity(***) of *B-TeZ IAC Fumonisin 3ml* Column:

Maximum Column Capacity:	6µg Fumonisin B_1

(***) An excess of fumonisins, e.g. 10µg FB_1, in a small volume of 2ml PBS is incubated with the IAC for 5 minutes; then the IAC is washed with 2ml PBS and the nonbonded fraction is analyzed. The difference of added analyte and nonbonded analyte equals maximum column capacity.

Analytical Method:
HPLC: Shimadzu; Column: Trentec Reprosil-Pur RP C18 120 ODS3 5µm; 125x3,0mm with guard column; Mobile Phase A: acetonitrile / water / acetic acid (70/29/1 v/v/v); Mobile Phase B: acetonitrile / water / acetic acid (40/59/1 v/v/v); Gradient: 0.01min B 100%; 3min B 100%; 13min B 40%; 13.1min B 0%; 18min B 0%; 18.1min B 100%; 20min B 100%; Flow Rate: 0.5ml/min; Time of Analysis: 21min; Injector Volume: 100µl; Detection: λ_{EX} [nm]: 335nm; λ_{EM} [nm]: 440nm.

Precolumn Derivatization: Reaction Conditions: 2min at room temperature. Reagent: 0,67% (w/v) ortho-phthaldialdehyde (OPA), 1% 3-mercaptopropionic acid (3-MPA) (v/v), 80mM sodium borate in 17%methanol/water (v/v). Ratio of mixture: 100µl sample/standard and 200µl reagent.

Overall Method Characteristics (IAC and HPLC):
Measuring range is linear of 3ng to 200ng FB_1 pro injection (R2=0.999). Limit of detection is 1ng of FB_1 pro injection (3times of signal/noise ratio). FB_2 characteristics are quite similar. If given dilution steps are obeyed FB_1 /FB_2 commodity contents totaling **0.08 to 2.5µg/g** lie within the linear working range of the method (included working range of IAC column). If contents of used samples are higher than cited upper range smaller portion of extract should be applied on the IAC column and analysed to ensure maximum recovery rates.

Example Sample Calculation of FB_1 content:
(Calculation of FB_2 content is analogous)

A) Calculation of Sample Gramm Equivalents per HPLC injection:

$$\frac{25\text{g Sample}}{100\text{ml Extraction Solvent}} \times \frac{1.6\text{ml Extract}}{1\text{ml}} \times \frac{0.1\text{ml injector volume}}{} = 0.04\text{g Sample Equivalents}$$

B) Calculation of FB_1 contamination of examined commodity in ng/g:

$$\frac{\#\ \mu\text{g injected } FB_1}{\text{Sample Equivalents [g]}} = \mu\text{g/g } FB_1 \text{ in e.g. maize}$$

Buffer, Chemicals, Apparatus and Literature:

Phosphate Buffered Saline pH 7.4 (= 10mM PBS)

0.25g KH_2PO_4
1.45g K_2HPO_4
8.76g NaCl

Dissolve in 1L deionized water. If necessary adjust pH to 7.4 (± 0.3) with 1N NaOH or 1N HCl

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Chemicals:
•acetonitrile, HPLC grade

•methanol, HPLC grade
•acetic acid, 100% ultrapure
•deionized water
•dipotassium hydrogenphosphate, >98%
•potassium dihydrogenphosphate, >98%
•sodium chloride
•nitrogen gas 5.0 [Air Liquide M55763810] (to evaporate IAC-eluate)

Consumerables:
• ***B-TeZ IAC Fumonisin 3ml*** [Prod. No. BTFU314005]

Elution Solvent:
methanol-acetic acid (98/2 v/v):
Add 2ml acetic acid to 98ml methanol and mix.

Precolumn Derivatization:
•ortho-phthaldialdehyde, p.a.
•3-mercaptopropionic acid, 99%
•disodium tetraborate decahydrate, p.a.

Dilution Solvent:
acetonitrile-water (40/60 v/v):
Mix 40ml acetonitrile and 60ml water.

Apparatus:
HPLC; Shimadzu; pump: LC-6A (2 pieces); auto sampler: SIL 6B; fluorescence detector: RF-10AXL; data handling: CLASS LC10

Evaporator (with tripod) [VLM EVA EC1-S]

Vacuum SPE Manifold (BAKER spe-24G Column Processor – process up to 24 samples) [J.T. Baker 7208]

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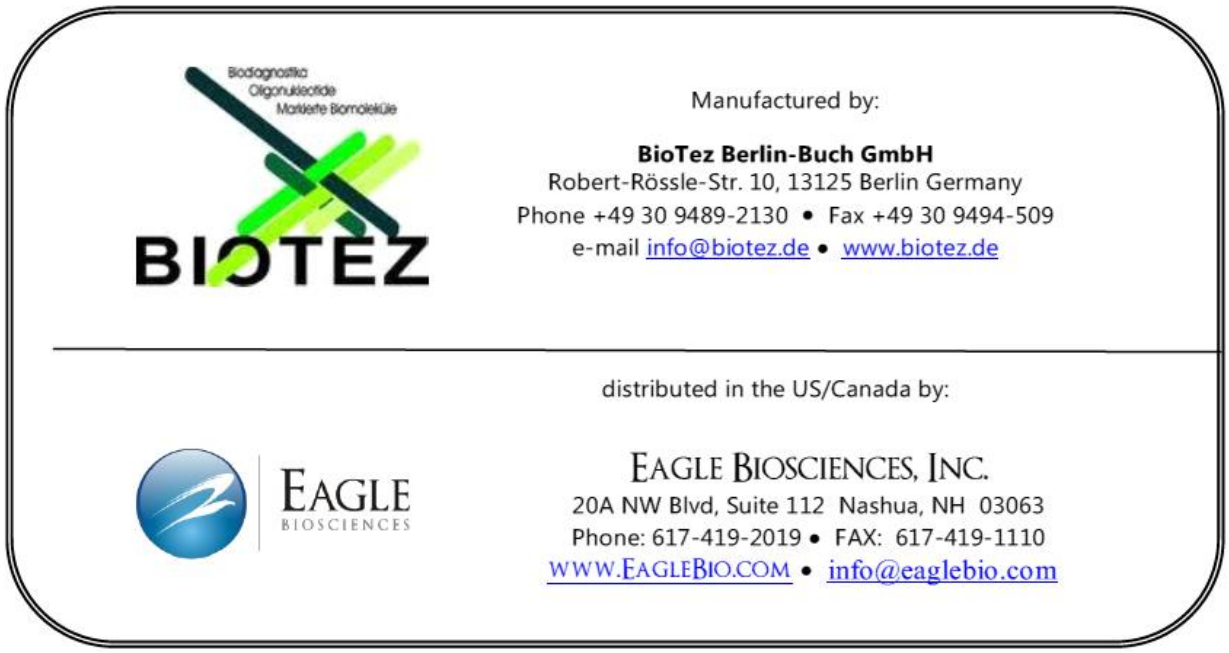
[1] "Improved fluorometric and chromatographic methods for the quantification of fumonisins B_1 , B_2 and B_3" Kelly Duncan, Scott Kruger, Nancy Zabe, Barb Kohn, Reginaldo Prioli, *Journal of Chromatography A*, **1998,** 815, 41–47



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