Instruction of the Clean-up Process
Using SENSI*Column* IAC T2/HT2 3ml
Product Code: EFTE3165

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Clean-up of Commodity Extracts of Food and Feed Samples containing T2/HT2 via Immunoaffinity Chromatography
and
Subsequent Determination by HPLC with Precolumn Derivatization using 1-Anthroylnitrile and 4-Dimethylaminopyridin

Principle:
This instruction of T2/HT2 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 analyt and working range of column is followed.

Many pre-treatment methods for T2/HT2 in food and feed show low sensitivity because of interfering substances if problematic matrices are applied.

This method of content determination of T2/HT2 combines the high selectivity of an immunoaffinity column (IAC) with its potential to concentrate elute and additional step of purification of labelled T2/HT2 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 T2-toxin is given below.

Sample Preparation (Literature method given):
Samples which content of T2/HT2 are to be analyzed, e.g. cereals, are extracted by the method of Visconti et al.[1] using methanol-water (90/10 v/v) as extraction solvent. E.g. to 50g of sample are added a volume of 100ml of the extraction solvent and processed as cited.

Enrichment Step IAC:
2ml extract (1g sample equivalent if above mentioned example extraction is followed) is diluted with 18ml 10mM PBS, pH=7.2 resulting a total volume of 20ml and then applied in a reservoir on top of the **SENSI*Column* IAC T2/HT2 3ml** column.

If the example is followed the resulting organic solvent concentration of the diluted extract is 9% of methanol which is tolerated by the column. The flow rate is adjusted to lie 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 analyt 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 **SENSI*Column* IAC T2/HT2 3ml** column is reached.

Thus, to guarantee maximum efficiency in terms of maximum recovery rates using **SENSI*Column* IAC T2/HT2 3ml** column, please ensure that

1. organic solvent content in diluted extract which is applied on top of the column is not higher than 15%,

2. T2/HT2 load per column does not exceed a total of 1µg (see working range below).

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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 under pressure from bottom.

Elution:
Sample reservoir on top of the using **SENSI*Column* IAC T2/HT2 3ml** column is removed and an appropriate vial is placed below the affinity column. The bound toxins are eluted by using a total of 1.5ml of methanol as elution solvent. The elution process is performed in two steps to ensure complete release of analytes. First, a volume of 0.5ml elution solvent is applied. After that volume has passed through column half a minute is waited before the second portion of 1ml of elution solvent is eluted through the column. Remaining solvent solutions should be eluted by application of slight under- or overpressure. All methanol fractions are unified to give the column eluate.

The column eluate is concentrated to complete dryness (e.g. using VLM evaporator at 50°C under a permanent stream of nitrogen). The residue is treated with a mixture of 1-Anthroylnitrile and 4-Dimethylaminopyridin as reported[1] to give fluorescent labelled derivatives of T2 and HT2 which are then injected to the HPLC apparatus.

Column Characteristics:

A) Working Range and Recovery Rates of SENSI*Column* IAC T2/HT2 3ml Column:

Working Range of Column:	up to 1µg T2/HT2 per IAC
Zero Contamination of Column:	<1ng T2/HT2 (LOD of HPLC method)
Guaranteed Recovery Rates within the Working Range[(*)]:	
T2-Toxin:	>85%
HT2:	>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 SENSI*Column* IAC T2/HT2 3ml Column:

T2-Toxin:	85%
HT2:	100%
DON:	<1%

[(**)]   Recovery rates if a total of 1.5µg T2/HT2/DON (1:1:1 molar ratio) is analysed per column.

C) Capacity[(***)] of SENSI*Column* IAC T2/HT2 3ml Column:

Maximum Column Capacity:	3µg T2 Toxin

[(***)]   An excess of T2 Toxin, e.g. 5µg T2, 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 analyt equals maximum column capacity.

Analytical Method:
HPLC: Shimadzu; Column: Trentec Select RP C8 120 ODS3 5µm; 125x3,0mm with guard column; Mobile Phase A: acetonitrile / water (90/30, v/v); Mobile Phase B: acetonitrile / water (60/40, v/v); Gradient: 0.01min B 70%; 8min B 70%; 8.1min B 0%; 20min B 0%; 20.1min B 70%; Flow Rate: 0.5ml/min; Time of Analysis: 30min; Injector Volume: 100µl; Detection: λ_{EX} [nm]: 370nm; λ_{EM} [nm]: 470nm.

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Overall Method Characteristics (IAC and HPLC):

Measuring range is linear of 5ng to 100ng T2 pro injection (R2=0.999). Limit of detection is 1ng of T2 pro injection (3times of signal/noise ratio). HT2 characteristics are quite similar. If given dilution steps are obeyed T2/HT2 commodity contents totalling **0.05 to 1µg/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 T2 content:

(Calculation of HT2 content is analogous)

$$\frac{50g\ Sample}{100ml\ Extraction\ Solvent} \times \frac{2ml\ Extract}{1ml} \times 0.1ml\ injector\ volume = \textbf{0.1g Sample Equivalents}$$

$$\frac{\#\ \mu g\ injected\ T2}{Sample\ Equivalents\ [g]} = \mu g/g\ T2\ in\ e.g.\ cereal$$

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 deionised water. If necessary adjust pH to 7.4 (± 0.3) with 1N NaOH or 1N HCl

Chemicals:

- acetonitrile, HPLC grade
- methanol, HPLC grade

- deionised water

- dipotassium hydrogenphosphate, >98%
- potassium dihydrogenphosphate, >98%
- sodium chloride
- nitrogen gas 5.0 [Air Liquide M55763810] (to evaporate IAC-eluate)

Consumables:

- **SENSI*Column* IAC T2/HT2 3ml**

Elution Solvent:

methanol

OH Derivatization:

- 1-Anthroyl Nitrile [WAKO 017-12101]
- 4-Dimethylamino-pyridin [SIGMA-ALDRICH 29224-10G]
- Toluene [ROTH 7341.1]

Dilution Solvent:

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

Apparatus:

HPLC; Shimadzu; pump: LC-6A (2 pieces); autosampler: 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] "Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity clean-up and liquid chromatography with fluorescence detection", Angelo Visconti, Veronica Maria Teresa Lattanzio, Michelangelo Pascale, Miriam Haidukowski, *Journal of Chromatography A*, 1075 (2005) 151–158