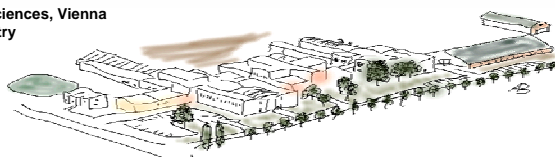


Monoclonal antibody-based indirect competitive ELISA for the detection of T-2 toxin in wheat, maize, and baby food



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Introduction

Mycotoxins are toxic secondary metabolites of fungi produced mainly by *Aspergillus*, *Penicillium* and *Fusarium* genera. The determination of mycotoxins in food and animal feed is of great importance since they represent a relevant source of danger to human and animal health causing food- and feed-borne intoxication. Trichothecenes are a group of mycotoxins produced by *Fusarium* spp. and are classified in Type A and in Type B trichothecenes. The most prevalent Type B trichothecenes are deoxynivalenol (DON), nivalenol (NIV), and 3- and 15-acetyldeoxynivalenol (3-AcDON and 15-AcDON), and Type-A trichothecenes are T-2 and HT-2 toxin, which are mainly found on wheat, maize, barley, and oat.

Mycotoxins are ubiquitous and therefore testing of products is required to keep our food and feed safe. For this purpose, sensitive and reliable tests are needed to detect contaminations. Therefore, an indirect competitive enzyme linked immunosorbent assay (ELISA) was developed using a T-2 specific monoclonal antibody produced in-house for the detection of T-2 toxin in wheat, maize, and baby food. The test stability and solvent resistance properties were assayed and a test validation study was performed determining the parameters sensitivity, specificity, precision, recovery and accuracy.

Experimental



Sample preparation:

Spiking blank samples:

- Adding 1 µg/L T-2 toxin to filtered extracts before further concentrated by evaporation to obtain an end concentration of 10 µg/L T-2 toxin
- Adding 10 µg/L T-2 toxin to grinded samples before drying in the desiccator over night and extraction

Extraction:

- Methanol/distilled water in a ratio of 75:25 (v/v). The extracts were then diluted with distilled water to an end methanol concentration of 25% before testing with the developed ELISA procedure.

Test procedure:

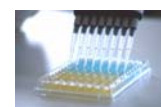
Coating: T-2-BSA [1mg/mL] was diluted to a concentration of 0.02 µg/L in coating buffer (pH 9.6) and 200 µL/well was applied to the microtiter plate. → overnight incubation at 4 °C

Blocking: 400 µL/well of a 0.5% skimmed milk powder solution → incubation at 37 °C for two hours

Standard/Sample: T-2 standard solution (100 ppm) was diluted 1:400 [250 µg/L] and was further diluted in 1:4 steps in assay buffer. 150 µL/well of the standard solution and 50 µL/well of the monoclonal T-2-B1 antibody (1:10 000) was added to each well. As blank, pure assay buffer was used. → incubation at room temperature on a shaker for one hour.

Antimouse-HRP: 200 µL of 1:30 000 with assay buffer diluted antimouse-HRP was applied. → incubation at room temperature on a shaker for one hour.

Substrate solution: 200 µL of a TMB stock solution was added, following by an incubation time of 15 minutes. Reaction was stopped by adding 50 µL 1M sulphuric acid. The absorbance was read at 450 nm.



Results and Discussion

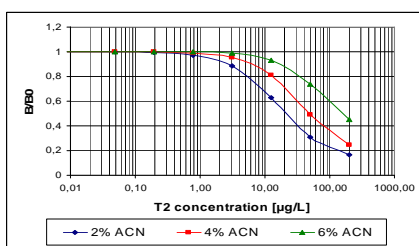


Figure 1: T-2 standard curves with different acetonitrile concentrations

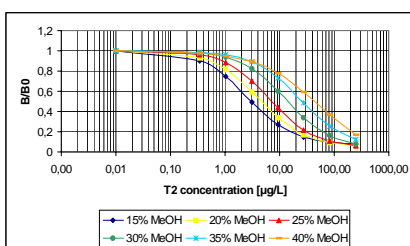


Figure 2: T-2 standard curves with different methanol concentrations

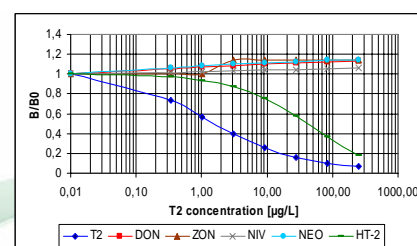


Figure 3: Cross reactivity study with different mycotoxins

For solvent resistance studies, different concentrations of acetonitrile and methanol were tested. Adequate sensitivity with an IC₅₀ value of 146.7 µg/kg T-2 toxin was established with 4% acetonitrile in assay buffer. An IC₅₀ value of 26.6 mg/kg T-2 toxin was achieved with 25% methanol in assay buffer.

The developed ELISA showed only a cross-reactivity of 5.3% to HT-2 toxin and met all requirements in terms of sensitivity. The assay may therefore be used for quantification since the cross-reactivity does not exceed 10%.

For validation studies, the determination of the T-2 toxin content in spiked samples of baby food, wheat and maize as well as one naturally contaminated wheat sample was performed. The results are shown in Table 1.

| Validation parameters | Baby food matrix | Maize matrix | Wheat matrix |
|---|------------------|--------------|--------------|
| LOD [µg/kg] | 0.4 | 4.2 | 2.5 |
| LOQ [µg/kg] | 1.2 | 12.5 | 7.5 |
| Recovery [%] | 92 - 142 | 53 - 160 | 95 - 209 |
| Intraassay variation s ₂ [µg/kg] | 0.6 - 56 | 2.5 - 73 | 0.9 - 4.4 |
| Coefficient variation [%] | 13.4 - 219.1 | 53.7 - 243.6 | 7.8 - 29.3 |
| Interassay variation (s) ₂ [µg/kg] | 0.5 - 74.2 | 0.1 - 13.4 | 1.5 - 52.2 |
| Coefficient variation [%] | 10.1 - 254.7 | 1.5 - 32.1 | 18.3 - 271.6 |

Table 1: Summary of validation parameters

Conclusion

It can be concluded that the developed ELISA showed high sensitivity in baby food matrix and adequate sensitivity in maize and wheat matrix. A reliable quantitative determination of T-2 toxin could only be achieved with T-2 toxin concentrations ranging from 10-40 µg/kg.

Higher contaminated samples had to be diluted. The benefit that no sample clean up is required makes the ELISA a rapid method compared to alternative methods such as GC-MS, with an overall incubation time of the method of 75 minutes.

References

Grossalber, K., Development of Immunodiagnostic methods - ELISA and LFD- for T2-toxin and hazelnut; Diploma Thesis, 2006



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