A novel immunoassay for the detection of T-2/HT-2 toxins in oat and cereal mix

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Abstract

A-type trichothecene, T-2 and HT-2 toxins are fungal secondary metabolites produced by various species of *Fusarium*. T-2and HT-2 toxins cause acute and chronic toxicity, and inhibit protein synthesis and mitochondrial function. A competitive enzyme-linked immunosorbent assay (ELISA) for the detection of T-2/HT-2 toxins was developed. Rabbit polyclonal antibodies against T-2 and HT-2 were produced. The antibody produced showed better cross reactivity to HT-2 (67%) than the previous T-2 toxin assay developed in our lab (38%). Other closely related compounds such as T-2 Triol, T-2 Tetrol and verrucarol have little cross reactivity. The detection limit of the assay was 0.5 ppb with a working range of 0.5 – 16 ppb. A range of recoveries from two reference samples, oat (FAPAS) and cereal mix (ERM) were 87 – 89% and 99 – 103, respectively. Coefficient of variations (CV, %) were below 10% throughout the whole testing. This newly developed assay will be suitable to monitor Trichothecene toxins when HT-2 is at higher concentration in grains.

Introduction

T-2 and HT-2 toxins are type A trichothecene mycotoxins produced by *Fusarium* species such as *F. sporotrichioides*, *F. poae*, *F. equiseti* and *F. acuminatum*. T-2 toxin is a potent inhibitor of protein synthesis. T-2 toxin primarily attacks the immune system causing reduced leukocyte count, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection, and blastogenic transformation of lymphocytes. HT-2 toxin, which is a metabolite of T-2 toxin, contaminated crops were found and reported from several countries, Brazil, China, Finland, Germany, Norway, Sweden and UK. The levels of contaminations were in a range of 0.03 - 100 µg/kg (ppb) in barley, maize, oats, rice, rye and wheat. Various analytical instruments have been used to detect T-2 and HT-2 toxins such as gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography-fluorescence detector (HPLC-FLD), or liquid chromatography-mass spectrometry (LC-MS). However, these instrumental methods require additional procedures for analysis. GC method needs derivatization in order to increase volatility and sensitivity. LC method requires sample clean-up procedure. In addition, HPLC-FLD method requires further steps for the derivatization before analysis. In this study, a competitive ELISA was developed and used to determine T-2/HT-2 toxins in oat and cereal mix.

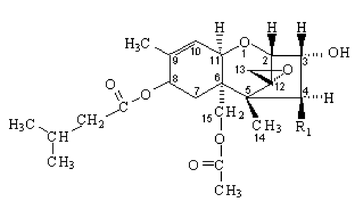


Figure 1. Structure of T-2 (R_1 = OAc) and HT-2 (R_1 = OH) toxins

Methodology

Polyclonal antibody production:

HT-2 toxin was coupled to a modified protein carrier, which served as an immunogen, and toxin was coupled to horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. These rabbits were shaved and injections were administered intradermally the back. The serum was collected monthly.

Assay performance:

Sheep anti-rabbit antibody was coated onto a 96-well microtiter plate.

The assay range is from 0.5 ppb to 16 ppb. The total assay run time is around 20 min.

Assay protocol:

50 µl of HRP conjugate was added to each well followed by 50 µl of calibrator or sample extract. Lastly 50 µl of antibody solution was added to the wells and mixed gently for 30 seconds and incubated for 10 minutes. The well contents were then decanted and washed 4 times with PBST wash solution. Substrate (100 µl) was added to the wells and incubated for 5 minutes. The reaction was stopped with 100 µl of Stop solution and the contents of the wells were read at 450nm-650nm.

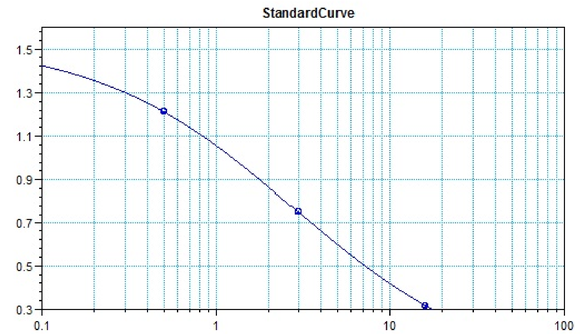
Sample preparation:

2 g sample + 0.5 g NaCl + 10 mL of 70% MeOH in H20 -> Mix vigorously for 3 min.

Centrifuge for 10 min at 5,000 rpm.

Take the supernatant, filter with glass fiber filter, and dilute if necessary then test.

Results



4-parameter curve fit: Y = (A – D)/(1 + (X/C)^B) + D

A	B	C	D	R^2
1.53	0.767	2.82	-0.00336	1

IC 50: 2.8 ppb, IC 85: 0.3 ppb

Figure 2. T-2 toxin standard curve.

Table 1 Determination of T-2 and HT-2 toxins in reference samples.

Sample	T-2 and HT-2 combined level (µg/kg)	Determination (µg/kg)	Recovery (%)
Oat (FAPAS, T2252)	319	283	89
Oat (FAPAS, T2261)	421	367	87
Cereal mix (I)	32	33	103
Cereal mix (II)	77	76	99

Table 2 Cross reactivity of T-2, HT-2 toxins and related compounds.

Toxin	Cross reactivity (%)
T-2	100
HT-2	67
T-2 Triol	1.6
T-2 Tetrol	<0.04
Verrucarol	<0.04

Discussion

A competitive ELISA method for the determination of T-2 and HT-2 toxins was developed. Cross reactivity of the assay to T-2 was 100%, and to HT-2 was 67%. This high cross reactivity to HT-2 enables the assay to determine HT-2 toxin as well as T-2 toxin from real samples. Since T-2 contaminated crops contain significant amounts of HT-2 toxin, this assay can be suitable to determine total amount of T-2 and HT-2 toxins combined. Four reference samples that were naturally contaminated with T-2 and HT-2 toxins were tested, and the recoveries of total amount of T-2 and HT-2 toxins combined ranged from 87 and 103%. These recoveries are better than those from our previous T-2 toxin ELISA kit. Since incubation times are relatively quick (10 min for antibody reaction and 5 min for substrate), using a multichannel pipette is strongly recommended for the assay to avoid high coefficient of variation (CV). A dilution step would be necessary for highly contaminated samples to get the OD values in the detection range (0.5 – 16 ppb). Although, matrix effect has not been tested yet, it would not be an issue due to high dilutions required to get within the detection range. An immunoassay developed for the detection of total T-2 and HT-2 toxins was successfully investigated. Further steps such as stability and stress tests will be conducted to make this kit to be commercialized.

References

E.E.Creppy. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters, 127, 19-28.

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