Determination of T-2 toxin by chemiluminescence enzyme immunoassay

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Abstract. T-2 toxin is a mycotoxin that is harmful to humans and animals. Chemiluminescence enzyme-linked immunosorbent assay (CLEIA) was established for the detection of T-2 toxin. In this study, the antigen coated concentration, antibody dilution proportion, goat anti-mouse IgG-HRP dilution proportion, the concentration of organic solvents, pH value and ionic concentration of the buffer were optimized to obtain the best reaction condition. The experiment have drawn the following conclusions, the 50% inhibitory concentration (IC₅₀) of CLEIA was 0.94 ng/ml. The standard was added 1.25 ng~5 ng to wheat flour and added 1 ng~5 ng to corn flour. The recovery ratios were 83%~87% and 86%~107%, the relative standard deviations (RSD) were 3%~10% and 2%~6%, respectively.

1. Introduction
T-2 toxin belongs to trichothecenes, a four-ring sesquiterpenoid mycotoxin, which is metabolized by a variety of fungi, such as fusarium, tricinctum and sporotrichiella[1]. T-2 toxin is a white needle-like crystal that is insoluble in water, but dissolving in methanol, acetoneethyl acetate, ethanol, acetone and other organic solvents. The property of T-2 toxin is stable, the toxicity will not be reduced at room temperature for 6 to 7 years or 100~120 temperature for 1~2 h, but the toxicity will lose in alkaline conditions[2]. Figure 1 shows the chemical structure of T-2 toxin.

Figure 1. The chemical structure of T-2 toxin

In 1968, Bamburg firstly isolated and purified T-2 toxin and determined its chemical structure[3]. T-2 toxin is a typical and most toxic trichloroethylene[4]. The toxicity of T-2 toxin is mainly cytotoxicity and immune system toxicity, and the current research mainly focuses on cytotoxicity, which mainly affects the digestive system, nervous system and reproductive system, thus causing damage to skin, liver cells and lowering the production performance[5].

At present, the detection of T-2 toxins mainly focuses on agricultural products such as feed, grain and oats. The common detection methods are liquid chromatography (LC)[6,7], gas chromatography (GC)[8,9], Chromatography-mass spectrometry[10,11] and immunoassay[12]. Although these methods are accurate and sensitive, they are time-consuming and unsuitable for rapid screening. It is necessary to
establish sensitive, reliable and simple detection method for T-2 toxin. This paper established a chemiluminescent enzyme-linked immunosorbent assay for the detection of T-2 toxin in cereals.

2. Materials and methods

2.1. Reagents and instruments
Goat anti-mouse IgG-HRP and methanol (AR grade) were purchased from sigma. Mouse anti-T-2 monoclonal antibody was obtained from our own laboratory. The chemiluminescence substrate was purchased from Helisence. Samples were purchased from a local market. Wellwash versa (Thermo scientific, USA), Luminoskan ascent (Thermo, USA), 5415D High-speed Centrifuge (Eppendorf, Germany), 96-well white polystyrene plates (NUNC, Denmark).

2.2. Determination of T-2 toxin by CLEIA procedure
Coating: 96-wells white polystyrene plates coated by adding 120 uL per well of T-2 antigen, incubated at 37°C for 2h, then washed four times with PBST and blocked by adding 330μL 5% skim milk powder to each well, which incubated at 37°C for 2h. After the plates have been washed 4 times with PBST, 50 uL T-2 standard solution or sample solution and 50 uL of antibody dilution were added to each well, the plate was incubated for 40 minutes at 37°C. After washing 5 times with PBST, 100μL goat anti-mouse IgG-HRP dilution solution was added each well, incubating condition as the former. In the last step, 100 uL of chemiluminescent substrate was added to each well for measuring the luminescence value (RLU).

2.3. CLEIA optimization
The antigen coated concentration, the dilution of antibody, the concentration of goat anti mouse IgG-HRP, PH value, the concentration of organic solvents and ionic strength of the buffer were optimized, respectively. The RLU/IC50 ratio was used as a parameter to judge the impact of factors.

2.4. Establishment of standard curve
The T-2 toxin standard reserve solution was diluted into a series of concentration standard solutions. The luminescence value was determined by experimenting according to the competition step under optimized optimal conditions. The logarithm of 10 × (T-2 toxin standard solution concentration) was abscissa and conjugation rate (%) was ordinate, the competitive inhibition curve was established.

2.5. Sample preparation
The T-2 standard was added to the 1g of grain sample, 4 mL of methanol–H₂O (80: 20, v/v) was added to grain sample for extraction. Then the mixture was vortexed for 10 minutes, ultrasound for 30 minutes and centrifuged at 5000 rpm for 5 minutes 4°C. Then the supernatant was taken and diluted with water.

3. Results and discussion

3.1. Optimization of antigen coated concentration and antibody dilution proportion
The antigen coated concentration ranged from 0.25μg/mL to 4μg/mL was optimized by the checkerboard titration, the antibody dilution proportion ranged from 1:200 to 1:12800. Considering the RLU, the optimization of antigen coated concentration was 1μg/mL, the antibody dilution Proportion ranged from 1:400 to 1:3200 were optimized by the indirect competitive. According to value of RLU/IC50, when the antibody diluted to 1:400, RLU/IC50 was the highest. The results were shown in figure 2(a) and figure 2(b).
3.2. Optimized goat anti-mouse IgG-HRP dilution proportion and the concentration of organic solvents

The goat anti-mouse IgG-HRP dilution proportion ranged from 1:2000 to 1:8000. The concentration range of organic solution (methanol-water, V/V) was 5%-30%. We can know that the best dilution proportion of goat anti-mouse IgG-HRP was 1:2000, and the optimum condition of the concentration of organic solvent was 20%, these results were shown in figure 3(a) and figure 3(b).

3.3. Optimized the pH value and the ionic strength of the buffer

The pH value of the buffer was 5.5-9.0, the ionic strength of the buffer (PBS) was evaluated from 1mM to 20mM, respectively. The result showed that the optimum condition of pH value was 7.0, and the optimum ionic strength of the buffer was 15mM, respectively in the figure 4(a) and figure 4(b).
3.4. Establishment of the Standard Curve

According to the optimized parameters, figure 5 was shown the standard curve for T-2 toxin based on CLEIA. We can calculate the IC50 was 0.94ng/mL from the calibration curves, and the linear working range was 0.25-5ng/mL.

3.5. The standard addition recovery experiments.

The wheat meal samples were added with T-2 toxin at concentrations of 1.25 ng/ml, 2.5 ng/ml and 5 ng/ml; corn flour samples were added with T-2 toxin at concentrations of 1 ng/ml, 2.5 ng/ml and 5 ng/ml, respectively, and then they were analyzed by using the optimized CLEIA. The recovery ratios and RSD values for wheat meal and corn flour were listed in table 1. The recovery values of wheat meal samples ranged from 83%-87%, the recovery values of corn flour samples ranged from 86%-107%, and all RSD values were less than 10%. It shows that the method has high recovery efficiency and good reproducibility.

| Sample       | Adding standard (ng/mL) | Average detection (ng/mL) | Recovery ratio (%) | RSD value (%) |
|--------------|-------------------------|----------------------------|-------------------|--------------|
| Wheat meal   | 5                       | 4.37                       | 87                | 4            |
|              | 2.5                     | 2.10                       | 84                | 10           |
|              | 1.25                    | 1.04                       | 83                | 3            |
|              | 5                       | 5.33                       | 107               | 2            |
| Corn flour   | 2.5                     | 2.68                       | 107               | 6            |
|              | 1                       | 0.86                       | 86                | 2            |

Figure 4(a). The effect of the PH value

Figure 4(b). The effect of the ionic strength

Figure 5. CLEIA calibration curves for T-2 toxin
4. Conclusion
The CLEIA method has proven to be a very useful method for the detection of T-2 toxins in cereals. The preparation of sample was simple and practical, it was performed with wide linear range and lower limit of detection. The method would be more sensitive to the detection of T-2 toxin and be suitable for the detection of T-2 toxin in food.

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