Development of ochratoxin a in cereal by chemiluminescence enzyme immunoassay

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Abstract: A rapid, simple and sensitive chemiluminescence enzyme immunoassay method (CLEIA) was established to detect ochratoxin A (OTA) in cereal. Optimal conditions including antibody dilution ratio and enzyme conjugate, ionic strength, pH value and organic solvent. Established indirect competition inhibition curve to determine the linear working range, detection limit and recovery rate. Results: The 50% inhibitory concentration and the detection limit of the CLEIA were 78.8 pg/mL and 14.86 pg/mL, respectively, with a linear range of 0.015-0.4 ng/mL. At 1~4 μg/kg fortified levels in wheat, mean recoveries ranged from 67.47% to 100.35%.

1. Introduction

Ochratoxin A (OTA) is a fungal toxin produced by several species of *Aspergillus* and *Penicillium*, with strong nephrotoxic, hepatotoxic, cytotoxic, immunotoxic and potentially carcinogenic. The International Agency for Research on Cancer (IARC) classified as class IIB carcinogens [1]. OTA widely exists in cereals, feed, juice, wine, beer and dried fruits. Pollution shows a wide range and low degree of characteristics [2]. In addition, it has accumulated toxicity [3], which harms the health of infants and young children through breast milk [4~5], but also have teratogenic effect [6]. So it is necessary to establish a sensitive and reliable detection method to ensure food safety.

In order to minimize the risks of OTA to consumers, the detection and monitoring of OTA is particularly important. At present, detection methods including thin layerchromatography (TLC) [7], liquid chromatography (LC) [8~9], enzyme-linked immunosorbent assay (ELISA) [10] and liquid chromatography mass spectrometry (LC-MS-MS) etc [11]. Although the instrument method is accurate, they are laborious, expensive, time-consuming and sample pretreatment cumbersome. In recent years, the limited standard of OTA is decreasing, so it is necessary to establish high sensitivity, specificity, simple and rapid detection method to quantify and confirm the OTA in food to ensure food safety. However, the detection sensitivity of chemiluminescence enzyme immunoassay is 10 ~ 10^2 magnitude orders than conventional ELISA [12].

2. Materials and methods

2.1. Instruments

Millipore Corporation, wellwash versa (Thermo scientific, USA), Luminoskan Ascent and its software (Thermo, USA), 96-well white polystyrene plates (Costar), incubator.

2.2. Apparatus
OTA antigen and anti-OTA monoclonal antibody were got from our own laboratory. Goat anti-mouse IgG-HRP was purchased from Sigma (St. Louis, USA), 0.01 M phosphate-buffered solution (PBS, pH 7.4). Luminol chemiluminescent substrate was purchased from Helisence (Shanghai, China).

2.3. CLEIA operation

- Chemiluminescent plate were coated with 120μL of OTA antigen per well for the night at 4°C. washed 5 times with PBST.
- Added 320μL 5% skim milk per well, which incubated for 3h at 37°C, after washing 4 times.
- Added 50μL standard solution or sample solution and 50μL antibody diluent per well at 37°C for 40min then washed 5 times.
- Added 100μL IgG-HRP per well for 40min at 37°C, after washing 5 times.
- Added 100μL Luminol chemiluminescent substrate per well and get the relative light unit (RLU).

2.4. Sample extraction and spiked

The corresponding levels of OTA standard are added to 5g crushed wheat sample and suspended in 25 mL of methanol-H₂O (70:30, v/v) for 30 min in sonication, after filtering, take 2 mL the filtrate then add 5 mL water solution, blending, filters again, collect the filtrate as a sample solution.

3. Results and discussion

3.1. Optimization of the antigen concentration and antibody dilution

The concentration of the coating antigen was optimized by the checkerboard titration, which the coated concentration between 0.25μg/mL to 4μg/mL. Figure.1 showed that 2μg/mL was the best concentration. Considered the RLU when selecting the antibody concentration, so choose the dilution of antibody from 1:1000 to 1:4000. The CLEIA showed the highest RLU max/IC₅₀ when the antibody dilution was 1:1000 in Fig.2.

![Figure 1. The antigen coated concentration effect of OTA](image)

![Figure 2. The effect of antibody dilution ratio](image)

3.2. Optimization of the enzyme conjugate

The RLU max / IC₅₀ ratio was used as a parameter to judge the impact of factors. Enzyme dilution proportion from 1:1500 to 1:6000, from the Fig.3 shows that with the enzyme concentration increase RLU max / IC₅₀ gradually reduce when the enzyme concentration at 1:1500, which shows the highest RLU max / IC₅₀.
Figure 3. The effect of enzyme dilution proportion

3.3. Optimization of the PBS concentration and pH value
Ionic concentration of the PBS from 5mM to 20mM, pH value from 6.5 to 8.5. The results demonstrate that optimum condition when the Ionic strength is 15 mM in Fig.4 and pH value is 8.0 in Fig.5.

3.4. Optimized the concentration of methanol
Use different percentage of methanol solution to dilute OTA standard solution and performed a CLEIA analysis, the results is 20% show in Figure.6.

3.5. Establishment of CLEIA standard curve
Based on the optimization results, the standard curve of OTA immunoassay was established in Figure.7. The linear regression equation was $Y = -55.228 +154.73$ ($R^2 = 0.9916$), the $IC_{50}$ was 78.8 pg / mL, the detection limit was 14.86 pg / mL.
3.6. Study on recovery of spiked samples
Table 1 shows that concentration of spiking and recovery ratio. In addition coefficient variation (CV) are less than 12%.

| Spiked level (ng/g) | Recovery ratio | CV (%) |
|---------------------|----------------|------|
| 1                   | 67.74          | 12   |
| 2                   | 89.53          | 8.6  |
| 4                   | 100.35         | 7.34 |

4. Conclusion
In this study, ochratoxin A was determined by indirect competitive chemiluminescence enzyme immunoassay. Provided rapid screening and quantitative analysis techniques for the detection of OTA in cereal and food. Due to the high sensitivity of this test, the luminous intensity is greatly affected by the experimental operation.

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