

Simultaneous Determination of Zearalenone and Aflatoxin B1 in Maize by High Performance Liquid Chromatography

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Abstract: This experiment established a method for the simultaneous detection of Zearalenone (ZEN) and Aflatoxin B1 (AFB1) in corn using High-Performance Liquid Chromatography with fluorescence detector. The sample was extracted with acetonitrile: 0.2% formic acid water (volume ratio: 80:20) and passed through a Prime HLB 3cc purification column. Liquid phase detection conditions: The chromatographic column is Agilent C18 (150mm×4.6mm, 5μm) The mobile phase is acetonitrile: water (volume ratio: 80:20). The retention time is qualitative and the external standard method is quantitative. The results showed that the correlation coefficients for ZEN 20~1000ng/mL and AFB1 1~50ng/mL were 0.9989 and 0.9976, respectively, and the detection limits were 5μg/kg and 0.8 μg/kg, with an average spiked recovery of 92.7% to 106.2%. This study combines the detection methods in the current two national standards into one, which can simultaneously achieve rapid and synchronous detection of ZEN and AFB1 in corn. The required extraction and detection time is reduced from 60 minutes to 30 minutes, which is 50% less than the original time. The detection results have high accuracy and good stability.

Keywords: Corn; Zearalenone (ZEN); Aflatoxin B1 (AFB1); High Performance Liquid Chromatography - fluorescence detector; Quick detection.

1. Introduction

Corn is an important food crop and raw material for food production, and can be processed into various food, feed, and industrial products. Due to the impact on the production and sales of high-quality corn in recent years, some food operating enterprises have accumulated a large amount of corn purchased. However, corn is extremely prone to mildew during transportation and storage, resulting in the production of mycotoxins such as zearalenone (ZEN) and aflatoxin B1 (AFB1). In particular, insufficient drying during harvest or excessive humidity and temperature during storage and transportation provide favorable conditions for the growth of these toxins [1]. Contamination of corn with harmful mycotoxins can lead to deterioration in its quality, leading to food safety issues. The human body can also be indirectly harmed by mycotoxins through the food chain transmission of food.

Mycotoxins are metabolites produced by fungal growth and can have harmful effects on humans and animals [2]. Globally, about 25% of grains are contaminated with mycotoxins every year, causing enormous economic losses to agriculture. Currently, more than 400 types of mycotoxins have been discovered, including common mycotoxins such as aflatoxin, zearalenone, deoxynivalenol (vomiting toxin), etc. [3-4]. Cereals and products such as corn, peanuts, rice, and wheat are prone to contamination by mycotoxins [5].

Establishing a method for the detection of mycotoxins in corn is of great significance for screening qualified corn raw materials, ensuring the health of humans, livestock, and poultry, and ensuring the safety of human, livestock, and poultry products. Currently, common mycotoxin detection methods include thin layer chromatography,

immunoassay, enzyme linked immunosorbent assay, high performance liquid chromatography, and high-performance liquid chromatography tandem mass spectrometry, with high performance liquid chromatography being the most common [6-8].

Based on the current national standard detection methods, this study combined the detection methods in the two national standards into one, optimized and improved the extraction and detection method and time. The same high-performance liquid chromatography fluorescence detection method was used to simultaneously detect the content of zearalenone and aflatoxin B1 in corn, providing a rapid and efficient detection method for the determination of zearalenone and aflatoxin B1 in corn.

2. Materials and Methods

2.1. Instruments and reagents

Agilent 1260 High Performance Liquid Chromatography with FLD Fluorescence Detector (Agilent, USA); Milli-Q Pure Water System (Millipore Company, USA); Electronic analytical balance (Aarhus Instruments Co., Ltd.); KQ-500 Ultrasonic Cleaning Machine: (Kunshan Ultrasonic Instrument Co., Ltd.); 5804R high-speed centrifuge (Eppendorf, Germany); UGC-24M nitrogen blowing instrument (Beijing Yousheng United Technology Co., Ltd.); LC-CQ-12F square solid phase extraction instrument (Shanghai Lichen Bangxi Instrument Technology Co., Ltd.); QL-901 Vortex Instrument (Haimen Qilin Bell Instrument Manufacturing Co., Ltd.); Zearalenone standard solution: aflatoxin B1 standard solution; Oasis PRiME HLB Xiaozhu (American Waters Company); Methanol (chromatographic grade), acetonitrile (chromatographic grade); The water used

in this experiment is pure water (with a resistivity of 18.25M Ω . cm).

2.2. Method

2.2.1. Sample pretreatment method

The corn purchased in the market is crushed and sieved, and (2.000 ± 0.050) g is accurately weighed into a 50mL centrifuge tube, and 10mL acetonitrile: deionized water (containing 0.2% formic acid) =80:20 is added for extraction. After mixing in the vortex for 30s, the supernatant is extracted after shaking for 20min, high-speed centrifugation at 5000r/min for 5min. Mount the Oasis PRiME HLB column on a pre-cleaned vacuum extraction device set to a minimum vacuum (approximately 2 psi). There is no need to perform the column activation step. Take about 0.4 mL of supernatant, pass it through the Oasis PRiME column, and discard the filtrate. Then take another 1 mL of the supernatant, pass it through the small column again, and collect the filtrate. After blowing in a mild nitrogen stream until almost dry, dissolve with 1.00 mL of mixed solution (methanol: water=50:50). After the vortex is mixed evenly, the mixed solution should pass through 0.22 μ m filter membrane, standby detection.

2.2.2. Instrument conditions

Chromatographic column: Agilent C18 chromatographic column, 150mm $\times$ 4.6 mm (inner diameter), particle size 5 μ m; Mobile phase: acetonitrile: water=80:20; Flow rate: 1.000mL/min; Detection wavelength: excitation wavelength 360 nm, emission wavelength 440 nm; Column temperature: 30 $^{\circ}$ C; Injection volume: 100 μ L. Qualitative retention time, quantitative external standard method.

2.2.3. Calculation formula

Calculate the content of various mycotoxins in corn protein powder samples according to the following formula:

Where: the content of mycotoxins (AFB₁, ZEN) in the sample in X(μ g/kg); C is the concentration of mycotoxin in the sample obtained from the standard curve (ng/mL); V is the constant volume (mL) of corn protein powder sample added with acetonitrile - 0.2% formic acid water; F is the concentration multiple; M is the weight of the sample taken (g).

2.2.4. Data processing

Data collection and processing are completed through the Chem Station workstation equipped with Agilent 1260 high-performance liquid chromatography. Each group of experiments was measured 3 times repeatedly. The data are the average of three parallel tests.

3. Results and Analysis

Table 1. Summary of Test Results

Indicator	ZEN result	AFB ₁ result
Standard curve of the method	$Y=0.0347X+0.9178$	$Y=1.2398X+3.1161$
Correlation coefficient	$R^2=0.9989$	$R^2=0.9976$
Linear range	20~1000ng/mL	1~100ng/mL
Method detection limit	5 μ g/kg	0.8 μ g/kg
Instrument precision	0.05%	0.07%
Addition recoveries	95.3%, 98.7%, 97.3%	96.0%, 92.7%, 106.2%

Note: In the standard curve, X is the mycotoxin concentration, mg/L; Y is the peak area, mV $\cdot$ min; RSD is the relative standard deviation.

3.1. Analysis of precision and accuracy results

Weigh and take a blank corn sample for recovery test, set 3 addition concentrations for each mycotoxin, and add ZEN 60 respectively μ g/kg, 120 μ g/kg, 300 μ g/kg and AFB₁ 20 μ g/kg, 40 μ g/kg, 100 μ g/kg, each added concentration was subjected to three parallel determinations, and processed according to the method in 2.2.1. The sample was loaded for testing, and the recovery rates of the two mycotoxins were determined. The addition and recovery test of mycotoxins using the established HPLC method showed that both mycotoxins achieved relatively stable recovery rates, ranging from 92.7% to 106.2%.

To further investigate the consistency of the entire process μ g/L of zearalenone and aflatoxin B₁ standard solution were continuously injected for 6 times, with each injection of 100 samples μ L50. Calculate precision and RSD based on peak area. The results showed that the RSDs for six repeated determinations were 0.05% and 0.07%, indicating that the fluorescence detector had a high precision.

3.2. Analysis of method peak time, linear range of standard curve, and detection limit

According to the selected chromatographic conditions, the prepared two-in-one mycotoxin standard working solution was tested on the computer. According to six repeated injections, the average peak time was obtained, which was 2.742 min for zearalenone and 3.575 min for aflatoxin B₁. Selected standard solution concentration series ZEN 20 μ g/L, 80 μ g/L, 200 μ g/L, 600 μ g/L, 1000 μ g/L and AFB₁ 1 μ g/L, 10 μ g/L, 20 μ g/L, 50 μ g/L, 100 μ g/L, with a wide linear range; The correlation coefficients of the standard curve are 0.9989 and 0.9976, respectively, which are greater than the requirement of a correlation coefficient of 0.9950; The slope of the standard curve shows that this method has a high sensitivity; The detection limit of 3 times the signal-to-noise ratio is 5 μ g/kg and 0.8 μ g/kg, the quantitative limit of 10 times the signal-to-noise ratio is 16 μ g/kg and 2.7 μ g/kg.

4. Conclusion

The high-performance liquid chromatograph fluorescence detector detection method established in this study can simultaneously detect zearalenone (ZEN) and aflatoxin B₁ (AFB₁) in corn. Compared to the detection methods in current national standards, this method achieves high spiking recovery, high accuracy and precision, good stability, simple sample pretreatment, and can shorten the detection time by half, greatly improving the detection efficiency of ZEN and AFB₁ in corn, which can meet the rapid and accurate detection of ZEN and AFB₁ in corn.

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