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Determination and Analysis of Zearalenone in Oats from the Ulanqab Production Area

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Abstract

Using oat samples from 10 different cultivation areas in the Ulanqab production region as the study subjects, this study employed high-performance liquid chromatography–tandem mass spectrometry to establish a method for determining the content of zearalenone in oats and to analyze the zearalenone content in oat samples. Method validation demonstrated that the method exhibits good linearity, a wide linear range, a low detection limit, and high sensitivity. Detection results showed that among 30 oat samples from the Ulanqab production region, 29 had zearalenone contents below the detection limit (5 μg/kg); one sample exhibited a certain degree of mold growth due to continuous rainfall during the harvest period, resulting in a zearalenone content of 188 μg/kg, which exceeded the limit for zearalenone in grains and grain products specified in the national food safety standard for mycotoxin limits in food (60 μg/kg).

Keywords

Ulanqab; oats; zearalenone; high-performance liquid chromatography–mass spectrometry; determination and analysis.

1. INTRODUCTION

Oats are annual herbaceous plants belonging to the genus Avena in the family Poaceae, characterized by cold resistance, drought tolerance, high yield, and superior quality. They serve as a primary crop and a distinctive advantageous crop in ecologically fragile regions such as the arid and cold areas of northern and northwestern China [1-2]. Ulanqab is located at north latitude 41°, featuring a temperate continental climate, making it a prime region for oat cultivation, with its planting area accounting for 60% of Inner Mongolia's total [3]. As one of the main crops in Ulanqab City, oat-related products not only sell well in first-tier cities like Guangzhou, Shanghai, and Beijing but also reach markets in South Korea, Japan, Southeast Asia, Mongolia, and other countries and regions, positioning oats as a distinctive industry for rural revitalization and the acceleration of agricultural and rural modernization [4].

Zearalenone (ZEN), also known as F-2 toxin, is a toxic metabolite produced by Fusarium fungi, including Fusarium graminearum. Because the heterocyclic phenolic hydroxylgroup in its structure resembles that of estrogen, it is referred to as a fungal estroge; when it binds to estrogen receptors, it exhibits strong reproductive toxicity[5–6]. Its chemical formula is $C_{18}H_{22}O_5$; it is chemically stable and highly heat-resistant, requiring treatment at 110°C for 1h to achieve complete decomposition and loss of activity. Consequently, it is not readily broken down during normal processing and cooking and can accumulate in humans and livestock

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through the food chain, damaging the reproductive, immune, and hepatic systems of humans and animals, among other tissues [7–8].

China food safety standards set the limit for zearalenone in wheat, corn, and related products at 60μg/kg, while no specific standard has been established for the limit of zearalenone in oats [9]. China National Nutrition Plan (2030）explicitly proposes to strengthen quality and safety monitoring of specialty agricultural products. In 2023, the Ministry of Agriculture and Rural Affairs issued the Quality Standards Guidelines for the Whole Oat Industry Chain, which requires the inclusion of mycotoxins as core monitoring indicators from farm to table[10]. Researching and analyzing the content of zearalenone in oats from the Ulanqab production area is of great significance for improving local oat safety and quality testing standards, guiding local oat cultivation and production from the perspective of agricultural product quality and safety, promoting oat export trade, building the Original Flavor Ulanqab brand as a natural green organic product, and contributing to rural revitalization.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Materials: Oats currently in production in 10 cultivation area, totaling 30 portions.

Reagents: acetonitrile, methanol, formic acid, and acetic acid (chromatographic grade, Fisher); ammonia solution (chromatographic grade, Sinopharm Chemical Reagent Co., Ltd.); ZEN (50.00 μg/mL, Puritan Biosciences); 13C18-ZEN (12.46 μg/mL; Puritan Biosciences)

2.2. Instruments and Equipment

LCMC-8050 high-performance liquid chromatography–mass spectrometer (Shimadzu Corporation, Japan); Waters UPLC HSS T3 column (2.1mm×100mm, 1.8μm) (Waters Corporation,USA); TGL-21 high-speed refrigerated centrifuge (Sichuan Shuke Instrument Co., Ltd.); MultVortex multi-sample vortex mixer (Guangzhou Detai Instrument & Equipment Co., Ltd.); ME104 electronic balance (Shanghai Metro)

3. EMPIRICAL METHOD

3.1. Sample Pretreatment

Weigh accurately 5g of the sample (to 0.01 g) into a 50 mL centrifuge tube. Add 20 mL of an acetonitrile:water:formic acid solution (70:29:1, by volume), mix thoroughly for 1 min using a vortex mixer, and place the tube in a rotary shaker for 30 min of extraction. Transfer 1.0 mL of the extract to a 1.5 mL centrifuge tube and centrifuge at 10,000 rpm for 5 min. Accurately transfer 0.5 mL of the supernatant to another 1.5 mL centrifuge tube, add 1.0 mL of water, vortex-mix, and centrifuge at 10,000 rpm for 5 min at 4 °C; aspirate the supernatant and pass it through a 0.22 μm filter membrane. Aspirate 180 μL of the processed sample filtrate into a 300 μL inner tube, add 20 μL of the same isotopic mixed internal standard solution, vortex-mix, and await injection.

3.2. Chromatographic Conditions

Waters UPLC HSS T3 column (1.8 μm, 2.1 mm × 100 mm) chromatographic column, with 0.05% aqueous ammonia as mobile phase A and acetonitrile as mobile phase B, using gradient elution with gradient conditions shown in Table 1. The flow rate was 0.3 mL/min, column temperature was 40 °C, and multiple reaction monitoring (MRM) was employed.

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Table 1. Gradient Elution Program

Time(min)	$NH_3·H_2O$(%)	CH_3CN(%)
0	95	5
1.0	95	5
1.5	80	20
5.0	75	25
5.5	0	100
8.2	0	100
8.5	95	5
12	80	5

3.3. Sample Analysis

Sequentially into HPLC-MS/MS using an auto-sampler for detection. Plot a standard curve with concentration ratio as the x-axis and peak area ratio as the y-axis using Excel. Qualify based on retention time and relative abundance ratio of monitored ions, and calculate the content of zearalenone in the test sample using the prepared standard curve.

4. INTERPRETATION OF RESULT

4.1. Linearity of the method and detection limit

Using the series of concentrations of the zearalenone standard solution as the horizontal axis and peak area as the vertical axis, a standard curve was obtained, as shown in Table 2. The correlation coefficient exceeded 0.999, indicating good linearity; the detection limit was 5.0μg/kg, and the linear range was 0.01–5.0 ng/mL. The method features a wide linear range, a low detection limit, and high sensitivity, enabling it to meet the experimental requirement for the detection of low concentrations of zearalenone.

Table 2. Linear range, regression equation, correlation coefficient, and detection limit of the method

Analyte	Linearity range (ng/mL)	Regression equation	R^2	LOD (μg/kg)
ZEN	0.01-5.0	y=0.00651783x+0.00159258	0.9997	5.0

4.2. Recovery rate of the method

A spiked recovery experiment was conducted for zearalenone; the results are presented in Table 3. The results show that the spiked recovery rate for zearalenone ranged from 72.5% to 81.2%, falling within the 60–120% range and meeting the requirements for experimental detection.

Table 3. Spiked Recovery rate

Analyte	Sample weight (g)	Spiked concentration (μg/kg)	Recovery rate(%)
ZEN	5.02	51.2	72.5%
	5.02	51.2	72.0%
	5.05	50.6	81.2%

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4.3. Analysis of Test Results

This study measured the zearalenone content in 30 oat samples from the Ulanqab production area; the results are presented in Table 4. The results showed that among the 30 oat samples, 29 had zearalenone contents below the detection limit of 5.0 μg/kg, while one sample contained 188 μg/kg of zearalenone, exceeding the limit for zearalenone in grains and their products specified in the National Food Safety Standard GB 2761-2017, "Limits for mycotoxins in food" (60 μg/kg). A comparison of sampling information for the samples exceeding the limit revealed that Sample No.2 had become partially moldy during collection due to continuous rainfall.

Table 4. ZEN content in oats (μg/kg)

Sample Number	ZEN (μg/kg)	Sample Number	ZEN (μg/kg)
1	<5.0	16	<5.0
2	188	17	<5.0
3	<5.0	18	<5.0
4	<5.0	19	<5.0
5	<5.0	20	<5.0
6	<5.0	21	<5.0
7	<5.0	22	<5.0
8	<5.0	23	<5.0
9	<5.0	24	<5.0
10	<5.0	25	<5.0
11	<5.0	26	<5.0
12	<5.0	27	<5.0
13	<5.0	28	<5.0
14	<5.0	29	<5.0
15	<5.0	30	<5.0

5. CONCLUSION

Through the determination and analysis of zearalenone in oat samples from ten different cultivation areas within the Ulanqab production region, this study reached the following conclusions: The use of high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) for determining zearalenone content in oats provides a low detection limit, high sensitivity, excellent linear correlation, a wide linear range, and spiking recovery rates within the 60–120% range, meeting the detection requirements of national standards for zearalenone content. Based on the test results and analysis of outliers, it was found that the zearalenone content in oats from various cultivation areas in the Ulanqab region was consistently below the detection limit of 5.0 μg/kg; thus, the produced oats were not contaminated with zearalenone. In one sample of oats that underwent mold growth due to continuous rainy weather during the harvest season, the zearalenone content reached 188 μg/kg, which is three times the prescribed limit for grains and their products. This indicates that adequate ventilation and drying must be ensured during the oat harvest to prevent mold growth and subsequent infection by zearalenone and other mycotoxins. Since zearalenone can survive using residual grains from the previous season in the fields, thereby providing a source of fungi for the new season's crops and contaminating grains at the source of cultivation, continuous monitoring of cultivation areas with abnormal values is necessary thereafter, and local crop cultivation and production should be reasonably guided based on the test results.

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