Determination of Cyclopiazonic Acid in Chinese Yellow Wine by High-Performance Liquid Chromatography–Triple Quadrupole Mass Spectrometry

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Summary: We developed a detection method of cyclopiazonic acid (CPA), a mycotoxin in Chinese yellow rice wine, based on high-performance liquid chromatography and triple quadrupole mass spectrometry. Under optimized conditions, the limit of detection was 50 μg/L. CPA recovery rates in CPA-spiked Chinese yellow rice wine were 80.1% and 85.1% at 25 μg/L and 1,050 μg/L CPA, respectively (R > 0.9994). The relative standard deviation was 6.21–9.17%. Our developed method represents a rapid and accurate detection tool of CPA meeting minimum residue measurement requirements in Chinese yellow rice wine. The method will be widely available as a reference and be employed for Chinese official regulatory control purposes.

Keywords: Cyclopiazonic acid; HPLC/QQQ MS; Rice Wine

Introduction

Cyclopiazonic acid (CPA), an indole tetramic acid, is a toxic fungal secondary metabolite [1,2] produced by *Penicillium cyclopium*, *P. griseofulvum*, *P. camemberti*, *P. commune*, *Aspergillus flavus*, and *A. versicolor*. Studies have reported that this mycotoxin is present in grains, dried beans, grain-based products, and livestock feed contaminated with *Penicillin* and *Aspergillus* strains. While CPA and aflatoxin (total aflatoxins) are often detected in similar environmental conditions, CPA is more toxic [3-6]. According to animal studies, the oral administration of CPA to rats causes degenerative changes and necrosis to multiple organs (liver, spleen, exocrine and endocrine pancreas, kidney, salivary glands, myocardium, and skeletal muscle) and death in 1 – 5 d [7]. In rats, CPA has an LD_{50} of 2.3 mg/kg [8].

Chinese yellow rice wine is a traditional fermented wine, which is consumed as a beverage and used in food seasoning. Inappropriate practices during yellow rice wine fermentation processes increase the risk of fungal growth and toxin formation. Currently, common CPA detection methods include immunoassays [4], capillary electrophoresis [9], reverse ligand exchange chromatography [10], silica gel column chromatography [11], ion chromatography [12], and metal complex chromatography [13, 14]. However, the extraction steps of these methods are complicated, time-consuming, expensive, and require large volumes of toxic reagents such as chloroform or dichloromethane [15, 16].

In this study, we used high performance liquid chromatography (HPLC) as a CPA separation method and triple quadrupole mass spectrometry (QQQ MS) as a CPA detection method. Under optimized conditions, we measured the level of the mycotoxin in Chinese yellow rice wine.

Experimental

Materials and Instrumentation

All chemicals used in this study were of HPLC grade (CNW Technologies GmbH, Germany). CPA (purity >97%) was purchased from Sigma-Aldrich. Chinese yellow rice wine was obtained from a local market in Shaoxing city.

HPLC was performed with an Ultimate C18-WP column (5 μm, 4.6 mm × 250 mm, CNW) maintained at 35°C. The injection volume was 10 μl, and the mobile phases included A (aqueous 0.05 mol/L ammonium acetate: glacial acetic acid adjusted to pH 6.50 ± 0.05) and B (100% methanol). The A-to-B mobile phase ratio was 30/70 (v/v), with a flow rate of 0.3 ml/min.

Mass spectrometry was performed in an Agilent 1290 LC/MSD TraCp 6460 (CA, USA). QQQ MS conditions included an electrospray ionization (ESI) in negative mode, an atomizing air pressure of 275.8 kPa, a drying gas (N_2) flow rate of 11 L/min at 350°C, a nebulizer pressure of 50 PSI, a capillary voltage of 3,500 V, a sheath gas temperature of 350°C, and a nozzle voltage of 500 V. Selected reaction monitoring was used, with quantitative and qualitative ion pairs at m/z 335, 154 and 335, 140, respectively. Centrifugation was performed in a Hermle Model Z 200 A (Wehingen, Germany).

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Filtered, deionized water was obtained from a Milli-Q ultrapure water system (America Millipore). Other instruments included a MTN-2800D nitrogen concentrator (Tianjin Auto Science, China) and a HC-C18 solid phase extraction column (CNW, Germany).

Sample preparation

Chinese yellow rice wine (5 ml) was homogenized for 120 min with 5 ml of extraction solution (70% methanol and 30% of 1% $NaHCO_3$) and centrifuged for 15 min at 1,000×g. An aliquot (4 ml) of the resulting supernatant was mixed with 10 ml of hexane to remove lipids and centrifuged for 5 min at 10,000×g. This supernatant was diluted further with 20mL water and loaded on a solid phase extraction cartridge that had been prepared for use. Vacuum was employed to draw the sample through the cartridge at a flow rate of approximately 1mL per minute. The cartridge was dried with a stream of nitrogen, and the analytics were eluted with 5mL water/ methanol (1:1, v/v) at 1mL/min. An anion exchange column, a strong cation exchange column, and a reverse phase column were evaluated respectively. The solvent was evaporated under a stream of nitrogen gas at 50℃[17]. The internal standard was added in 1mL of mobile phase and the residue was dissolved with vortex mixing. The dissolved extract was passed through a microporous filter membrane (0.22 μm) (Millipore Corp., Bedford, Mass.) for HPLC/QQQ MS analysis. [17, 18]

Results and Discussion

Optimization of CPA purification

Immunoaffinity columns are ideal for the purification of specific CPAs. However, there are no suitable immunoaffinity columns for CPA purification. Therefore, we evaluated the performance (i.e., retention and recovery) of different columns, including an anion exchange column, a strong cation exchange column, and a reverse phase column. The results revealed that CPA recovery is 85% (77%), 73% (70%) and 69% (63%) respectively by anion exchange column, cation exchange column, and reverse phase column (Table-1). The anion exchange column was the most optimum column for CPA purification. The CPA phenolic groups formed strong interactions with the quaternary ammonium groups on the anion exchange column.

Optimization of HPLC/QQQ MS conditions

Two mobile phases were investigated in this study: aqueous acetonitrile and aqueous methanol. The highest analytical signal was obtained with aqueous ammonium acetate/methanol as the mobile phase. The addition of 0.005% aqueous ammonium acetate to the aqueous methanol phase increased signal strength. Matrix of Chinese yellow rice wine effects can be predicted. It is highly variable and difficult to control. The suppression or enhancement may be severity and nature for function of the concentration of the co-eluting matrix components. [10, 19].

Table-1: Optimization of CPA recovery.

Name	Recovery (%) Elution with MeOH	Recovery (%) Elution with Acetonitrile
Anion exchange column	85	77
Cation exchange column	73	70
Reverse phase column	69	63

Precursor and product ions of CPA were selected by full scan mass spectrometry using positive and negative electrospray ionization. Improved sensitivity was obtained in the negative ion mode. QQQ MS analyzes identified two characteristic peaks (m/z 154 and m/z 140). High abundance ions were selected as quantitative (m/z 335 >154) and qualitative (m/z 335>140) ions. Selective ion monitoring mass spectrometry provided a signal-to-noise area ratio of 3 and ensured accuracy in the results.

Validation of the HPLC/QQQ MS method

The calibration curve (Fig. 1) generated with different CPA standard concentrations (25, 50, 250, 350, and 1,050 μg/L) had a regression equation of Y = 12.717886X -184.530097 (R^2 = 0.9994). Limit of detection (LOD) and limit of quantification (LOQ) were measured based on a signal-to-noise ratio of 3 and 10, respectively. In this study, LOD was 50 μg/L and LOQ was 240 μg/L. To determine recovery rates, four blanks samples were each spiked with 50, 100, 200, and 500 μg/L CPA. The recovery rates were 80.1–85.1% (Table-2), with a relative standard deviation of 6.21% – 9.17%.

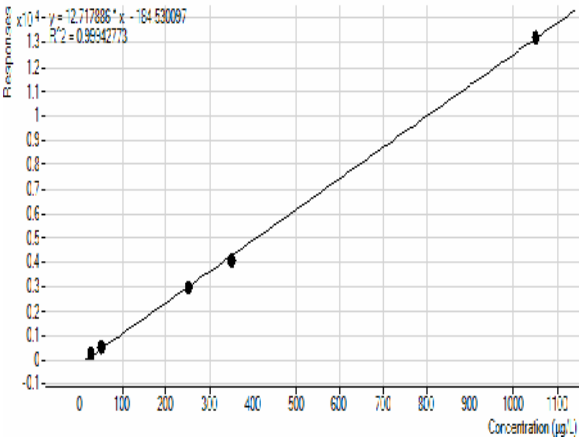

Fig. 1: Calibration curve for the cyclopiazonic acid.

Table-2: Recovery rates of cyclopiazonic acid (CPA) from CPA-spiked Chinese yellow rice wine.

Sample	Aging time (months)	Concentration of CPA added (μg/L)	Average recovery rate (%)	RSD (%)
1	12	50	80.1	6.21
	24	50		
	36	50		
2	12	100	83.2	7.77
	24	100		
	36	100		
3	12	200	85.1	8.19
	24	200		
	36	200		
4	12	200	81.9	9.17
	24	200		
	36	200		

RSD: relative standard deviation. Recovery experiments (n= 6)

Analysis of Chinese Yellow Rice Wine

Yellow rice wine purchased at a local Chinese market was analyzed using our developed method. The results revealed that the commercial yellow rice wine was CPA-negative. Yellow rice wine was spiked with 200 μg/L CPA and analyzed by HPLC/QQQ MS. The results showed that a visible quantitative ion peak, with a qualitative ion-to-quantitative ion relative abundance of <20% (Fig. 2). It is suitable for its intended test purpose. The goal of validation of the analytical procedure was demonstrated by analysis of spiked Chinese yellow rice wine. [19]

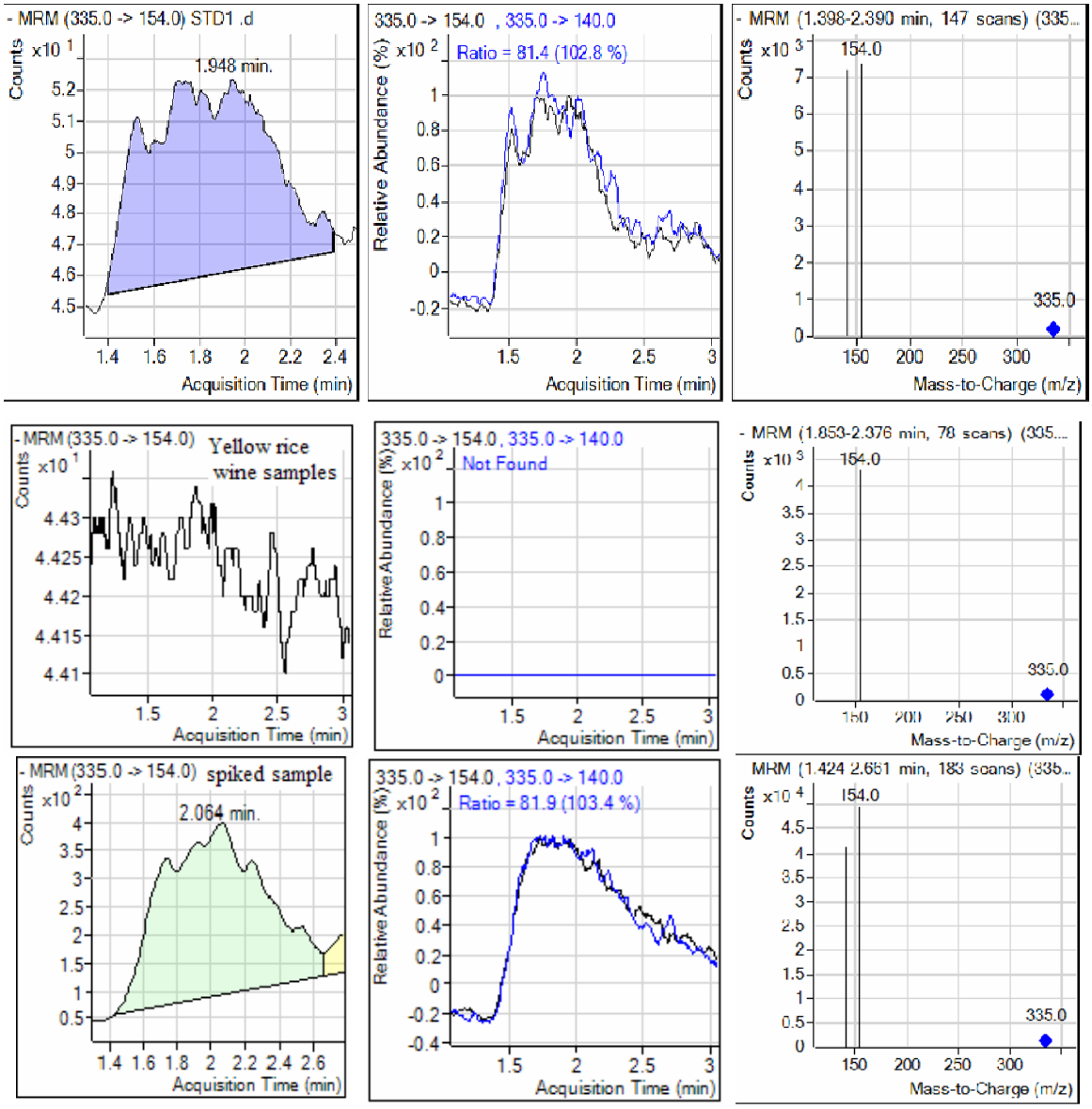

Fig. 2: Triple quadrupole mass spectrometry (QQQ MS) of CPA in commercial Chinese yellow rice wine.

Conclusions

Our validated method has sufficient sensitivity to detect the presence of CPA in Chinese yellow rice wine. Even though no maximum residue limits have been established, our method met the required performance criteria set by the Chinese Food and Drug Administration. The precision was good and the recovery rates were within the permissible range (80.1 – 85.1%). Therefore, our developed method can be used in the analyses of yellow rice wine and to support food safety agencies for regulatory control purposes.

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