Determination of bongkrekic acid in rice noodles and Tremella mushrooms

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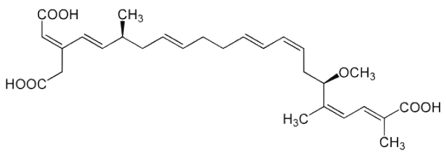
In this technical note, a LC-MS/MS method was developed for the quantitation of bongkrekic acid (BA) toxin in rice noodles and Tremella mushrooms. Using the SCIEX Triple Quad 4500 system, matrix spikes at 1 ng/g in rice noodle and Tremella samples showed mean recoveries ranging from 75% to 110% and mean precision ranging from 1.2%CV to 3.2%CV (Figure 1). Further, the calibration standards showed good linearity from 0.05 ng/mL to 10 ng/mL in vial. Overall, these results demonstrate a sensitive method to monitor for bongkrekic acid toxin in food using the SCIEX Triple Quad 4500 system.

Key benefits for the analysis of bongkrekic acid in food using the SCIEX Triple Quad 4500 system

- **Good method accuracy in food matrices:** Matrix spikes into rice noodles and Tremella mushrooms showed good mean recoveries (75-110%) and precision (%CV range: 1.2-3.2%)
- **Calibration curve performance:** The correlation coefficients for quantifier and qualifier transitions were greater than 0.998 over the range of 0.05 ng/mL to 10 ng/mL in solution



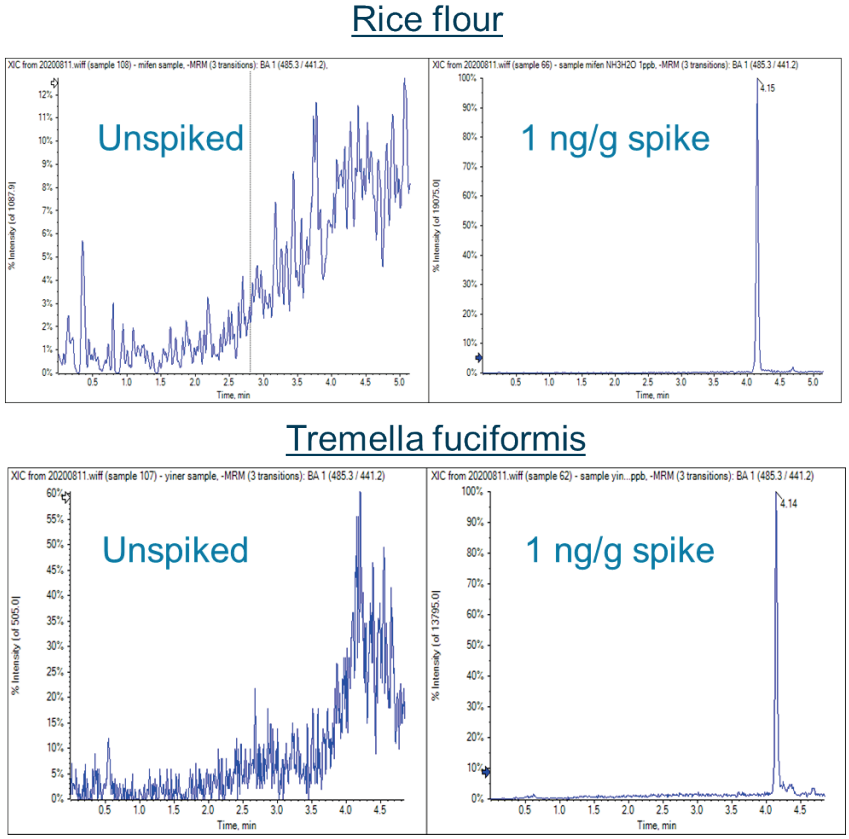


Figure 1. Extracted ion chromatograms (XICs) for matrix spikes into rice flour and Tremella fuciformis. Quantifier transition (m/z 485.3>441.2) shown for unspiked (blank) and 1 ng/g spike samples.

Introduction

Bongkrekic acid (BA) is a toxic metabolite formed by the bacteria, *Burholderia gladioli* pathovar *cocovenenans* (BGC).[1] Several BA-associated food poisoning events have been attributed to the consumption of contaminated rice noodles and mushrooms, particularly in China, southeast Asia and Africa.[2] BA poisoning has been shown to form in fermented cereal products, spoiled Tremella mushrooms and rice flour. BA is heat resistant and may persist during cooking. Symptoms of BA exposure include, nausea, vomiting, convulsions, shock as well as damage to the liver and brain nerve cells and kidney tissue. Reported mortality rates range from 30% to 100%.[2] The China national standard stipulates a BA level of less than 0.25 mg/kg in Tremella.[3,4]

The traditional BA analysis methods include liquid chromatography, thin layer chromatography (TLC) and UV spectroscopy. Compared with traditional analytical methods that have long detection times and poor accuracy performance, LC-MS/MS methods have good specificity, high sensitivity, fast analysis speeds, and are widely accepted by the food safety testing market.

Methods

Sample preparation: Crushed rice flour (5 g) or Tremella mushroom (2.5 g) was transferred to a 50 mL centrifuge tube to which 15 mL of 80:20 methanol/water (v/v) with 1% ammonia was added. The mixture was soaked for 1 hr and then sonicated for 30 min. An aliquot of the supernatant (3 mL of the rice flour extract or 6 mL of the Tremella mushroom extract) was transferred to a clean tube, concentrated to 1 mL using nitrogen gas and then filtered with 0.22 µm filter.

LC conditions. A SCIEX ExionLC system was used with the Phenomenex Synergi™ 4 µm Fusion-RP column (50 x 2 mm, P/N 00B-4424-B0) for chromatographic separation. Gradient conditions are described in Table 1. Mobile phase A was water with 0.01% formic acid and mobile phase B was acetonitrile. The flow was 0.45 mL/min, the injection volume was 6 µL and the column oven temperature was set to 40°C.

Table 1. LC gradient conditions for the analysis of bongkrekic acid using the SCIEX Triple Quad 4500 system.

Time (min)	Flow rate (mL/min)	Mobile phase B (%)
1	*0.45*	*10*
5	*0.45*	*95*
6.5	*0.45*	*95*
6.7	*0.45*	*10*
9	*0.45*	*10*

Instrumental conditions. Samples were analyzed using the SCIEX Triple Quad 4500 system operating in negative electrospray ionization mode. The source & gas parameters are presented in Table 2 and the compound-specific MRM parameters are presented in Table 3. The dwell time was 200 ms for both transitions.

Table 1: Source and gas parameters for the analysis of bongkrekic acid using the SCIEX Triple Quad 4500 system.

Parameter	Value
Polarity	*Negative*
Ion source gas 1	*50 psi*
Ion source gas 2	*55 psi*
Curtain gas	*20 psi*
Source temperature	*450°C*
Ion spray voltage	*-4500 V*
CAD gas	*Medium*

Table 2: Compound-specific MRM parameters used for analysis of bongkrekic acid using the SCIEX Triple Quad 4500 system.

Analyte	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)
Bongkrekic acid_1	*485.3*	*441.2*	*-30*	*-16*
Bongkrekic acid_2	*485.3*	*397.2*	*-30*	*-25*

Chromatographic performance

The XIC for the 1 ng/mL BA solvent standard (quantifier transition m/z 485.3>441.2) is shown in Figure 2. The XIC shows good analyte retention, separation from the void volume and symmetrical peak shape.

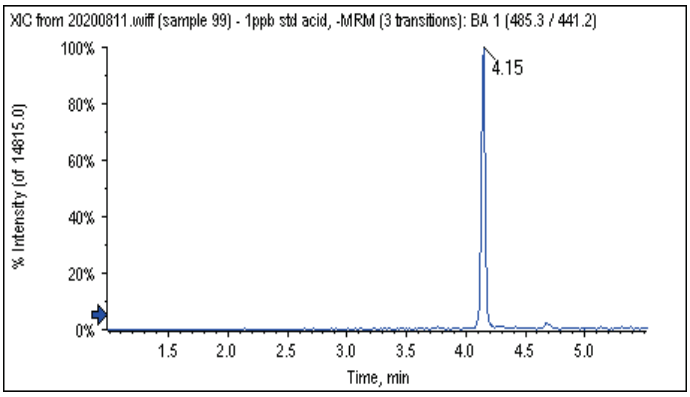


Figure 2. Extracted ion chromatogram (XIC) of a 1 ng/mL solvent-based calibration standard of bongkrekic acid using the SCIEX Triple Quad 4500 system. The quantifier transition (m/z 485.3>441.2) is shown.

Matrix spikes

Matrix spikes were performed in the rice flour and Tremella fuciformis samples at 0.5 ng/g, 1 ng/g and 10 ng/g (n=6). Spikes were performed prior to extraction and Table 4 shows the recovery and %CV for the two matrices. Overall, the mean recovery ranged from 75% to 110% with the precision ranging from 1.2%CV to 3.2%CV. The XICs for the 1 ng/g rice flour and Tremella matrix spikes are shown in Figure 1.

Table 4: Recovery and precision of 0.5 ng/g, 1 ng/g and 10 ng/g spikes of bongkrekic acid into rice noodles and Tremella (n=6)

Matrix spike (ng/g)	Rice noodles		Tremella	
	Recovery (%)	%CV	Recovery (%)	%CV
0.5	*102*	*3.0*	*96*	*1.5*
1	*110*	*2.9*	*90*	*3.2*
10	*97*	*1.2*	*75*	*1.2*

Calibration standard curve: Linear dynamic range

The solvent-based calibration standards showed good linear dynamic range for the both the quantifier (m/z 485.3>441.2) and qualifier (m/z 485.3>397.2) transitions of bongkrekic acid using the SCIEX Triple Quad 4500 system (Figure 3). The correlation coefficients for both transitions were greater than 0.998 from 0.05 ng/mL to 10 ng/mL.

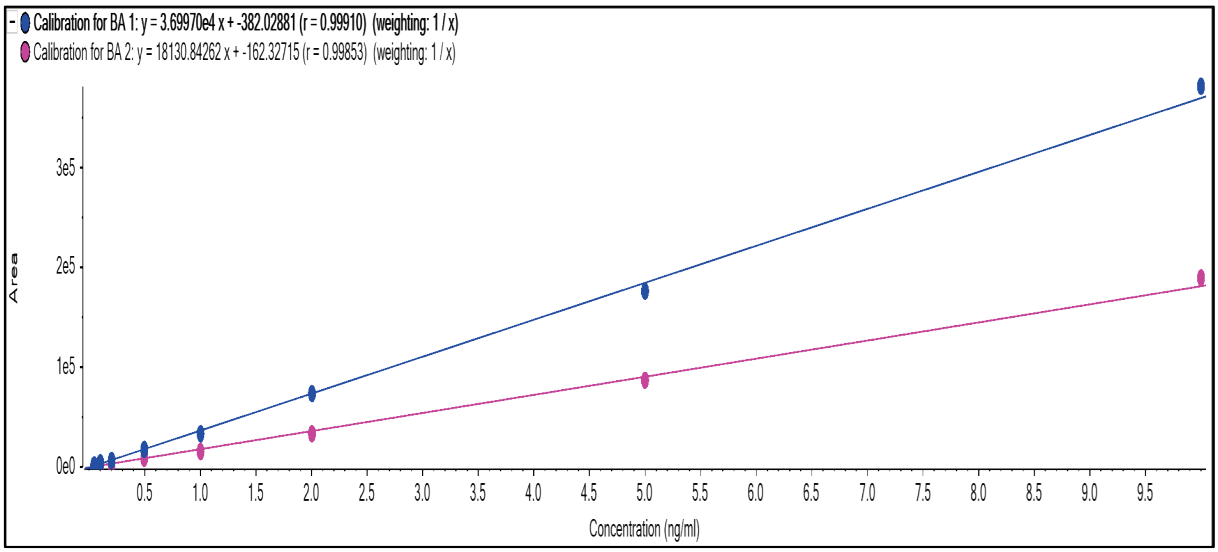


Figure 3. Calibration standard curve for the quantifier (m/z 485.3>441.2) and qualifier (m/z 485.3>397.2) transitions of bongkrekic acid using the SCIEX Triple Quad 4500 system.

Conclusions

This technical note demonstrated:

- A method for the analysis of bongkrekic acid toxin in rice noodles and Tremella mushrooms using the SCIEX Triple Quad 4500 system
- Good recovery and precision in rice noodle and Tremella mushroom samples with mean recoveries between 75-110% and %CVs between 1.2-3.2%
- Calibration curve linear dynamic range spanning 3 orders of magnitude from 0.05 ng/mL to 10 ng/mL

References

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