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Application News

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Food Safety Analysis / LCMS-8050

Analysis of Residual Chloramphenicol in Shrimp by LC/MS/MS with QuEChERS Sample Pre-treatment

❑ Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic that is widely used in the growth of animals and marine culture [1]. However, CAP can cause aplastic anaemia and is a possible carcinogen in humans [2]. Its use in livestock farming including fish and shrimps has been banned in European Union and many other countries. However, illegal use of CAP is still present due to its low cost and ready availability. As there is not a safe maximum residue level (MRL) determined in food for CAP, a highly selective and sensitive analytical method to detect CAP residues in complex matrices such as food samples is required. Here, we present a LC/MS/MS method for accurate and reliable quantitation of CAP in shrimp samples on LCMS-8050 with a heated ESI interface. The shrimp samples were pre-treated with a modified QuEChERs procedure. The method performance was evaluated with spiked samples and exhibits good accuracy, reproducibility, linearity and specificity over the concentration range from 0.005-20 ng/mL. The quantitation limit of the method with 5µL injection volume are determined to be 0.01 ng/mL, which is equivalent to 0.2 µg/kg of CAP in shrimp samples after incorporating a dilution factor of 20 from the sample pre-treatment procedure. This value satisfies the Minimum Required Performance Limit (MRPL) set at 0.3 µg/kg by the European Union.

❑ Experimental

Instrumental and analytical conditions

A high sensitivity LCMS-8050 (Shimadzu Corporation) was used in this work. The new heated ESI probe incorporated into LCMS-8050 improves desolvation efficiency, matrix removal and hence enhances the sensitivity of this instrument. The details of column, mobile phases and gradient program of LC separations and MS conditions are compiled into Table 1a and Table 1b.

Standards and samples

Chloramphenicol standard was purchased from Sigma Aldrich and QuEChERS sample pre-treatment kit was obtained from Restek. Fresh shrimp samples were purchased from the local supermarket. The procedure of sample preparation for shrimp samples using a modified QuEChERS procedure is illustrated in Figure 1. It includes spiking of standards into homogenized shrimp samples, extraction and clean-up with pre-weighed salts from QuEChERS kit. The supernatant collected after clean-up was diluted 10 times with ultrapure water, filtered and injected directly to the LC/MS/MS system.

Table 1a: LC/MS/MS analytical conditions (LC) for CAP analysis

LC Condition	
Column	Ascentis® Express C18, 100 x 2.1mm, 2.7µm
Mobile Phase	A: Water B: Acetonitrile
Elution Program	10% (0.0 to 1.0min) ➔ 95% (1.0 to 6.0min) ➔ 95% (6.0 to 10.0min) ➔ 10% (10.0min to 11.0min) ➔ 10% (11.0 to 13.0min)
Flow Rate	0.40 mL/min
Oven Temp.	40 ºC
Injection	5 µL

Table 1b: LC/MS/MS analytical conditions (MS) for CAP analysis

MS Interface Condition	
MS Mode	ESI, Negative
Block Temp.	400ºC
Interface Temp.	300ºC
DL Temp.	250ºC
Nebulizing Gas Flow	N_2, 2.0L/min
Drying Gas Flow	N_2, 10.0L/min
Heating Gas Flow	Zero air, 10.0L/min
CID Gas	Ar (270kPa)

[1] Weigh 10g homogenized sample in 50mL centrifuge tube

↓ Add 4mL water, 16mL acetonitrile and 20µl formic acid

[2] Add Q-sep Q100 Packet salt

↓ Shake vigorously, vortex for 20 mins and centrifuge at 6000rpm for 10 mins

[3] Transfer ACN supernatant into 20mL volumetric flask and wash with 4mL ACN

↓ Combine washing into volumetric flask and top up to mark

[4] Transfer ACN solution to 50mL centrifuge tube and add 20mL hexane, vortex for 5 mins

↓ Centrifuge at 6000rpm for 10 mins and discard hexane (top layer)

[5] Transfer 1mL ACN solution into 2mL QuEChERS dSPE tube, Cat. #26125

↓ Vortex 2 min and centrifuge 10min at 13000rpm

[6] Transfer 100µL extract to 1.5mL tube

↓

[7] Dilute with 900µL of ultrapure water

↓ Filter through 0.2µm Nylon syringe filter

[8] Analyze by Shimadzu LCMS-8050

Note :
- For pre-spiked recovery samples, spike 10µL of Chloramphenicol of known concentration in step 1.
- For post-spiked calibration curve, spike 10µL of Chloramphenicol of known concentration in step 6 and add 890µL of water instead in step 7.
- Total dilution factor: 20

Figure 1: Flow chart of shrimp sample pre-treatment

❑ Results and Discussion

Development of LC/MS/MS method

A MRM quantitation method for chloramphenicol was developed on LCMS-8050 using an ESI interface. The MRM optimization was performed in negative mode using an automated MRM optimization program with LabSolutions workstation. Two optimized MRM transitions for chloramphenicol were selected (Table 2), the first transition was used for quantitation and the second transition for confirmation.

Table 2: MRM transitions and voltage parameters of Chloramphenicol

Name	RT (min)	MRM Transition (m/z)		Voltage (V)		
		Precursor [M-H]-	Product	Q1 Pre Bias	CE	Q3 Pre Bias
CAP	3.326	321.0	152.2	21	16	30
			194.2	21	10	12

Calibration curve, linearity and accuracy

Two sets of standard samples were prepared by spiking in diluent and post-spiking in shrimp matrix obtained after sample clean-up. Each set included nine levels (triplicate) from 0.005 ng/mL to 20 ng/mL (Table 3). The processing of the calibration curves includes setting up a weighting method of 1/C. Linearity with R^2 greater than 0.999 were established as shown in Figure 2. The MRM chromatograms of standards spiked in diluent are shown in Figure 3. The accuracy of the MRM based quantitation method is between 95.3% and 112.7%.

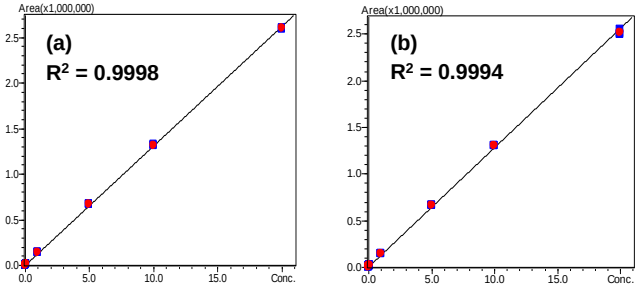

Figure 2: Calibration curves of chloramphenicol in diluent (a) and post-spiked shrimp matrix (b).

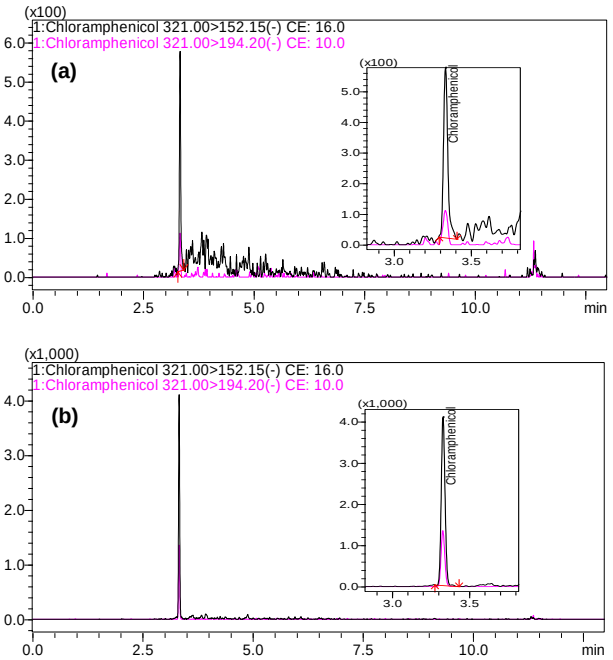

Figure 3: MRM chromatograms of chloramphenicol at 0.005 ng/mL (a) and 0.05 ng/mL (b) in diluent.

Matrix effect, recovery, repeatability and LOD/LOQ

Matrix effect of the MRM method was evaluated by comparison of peak areas obtained from calibration curve in diluent and in shrimp matrix. The calculation was based on the average area obtained from triplicate injections of every concentration from 0.01 ng/mL to 20 ng/mL.

It was noticed that the shrimp matrix blank contained an interference at the same retention time as chloramphenicol.

Table 3: Summary of performance evaluation for chloramphenicol

Concentration (ng/mL)	Area		Accuracy (%)		Matrix Effect (%)
	Neat	Post-spiked	Neat	Post-spiked	
0.010	1478	2506	112.7	95.3	169.5
0.025	3412	4445	104.1	98.7	130.2
0.050	7012	9010	107.0	117.5	128.6
0.100	14539	14428	110.9	103.0	99.2
1.000	136544	140032	104.1	108.5	102.6
5.000	670056	658068	102.2	102.7	98.2
10.00	1315274	1297491	100.3	101.3	98.6
20.00	2595906	2512621	99.0	98.2	96.8

Neuhaus et al. [3] reported also the observation of an interference MRM (m/z321>152) peak which appeared at the retention time as chloramphenicol. By using high resolution LC-TOF, we confirmed that the interference was not from chloramphenicol. Hence, matrix effect as well as recovery of chloramphenicol in the shrimp matrix were calculated after compensating the area from the interference [3]. The matrix effect of the method obtained is between 169.5% and 96.8% for the spiked concentration between 0.01 ng/mL and 20 ng/mL (Table 3).

Recovery of the sample pre-treatment method was determined by comparing the peak area obtained from pre-spike and post-spike shrimp samples. Shrimp samples were pre-spiked with 0.5, 2 and 20 ng/mL (final analyzed concentration was 0.025, 0.1 and 1 ng/mL respectively) of chloramphenicol and sample clean-up was done using the modified QuEChERS procedure. Each concentration level was repeated in triplicates over three days, a total of 9 samples being prepared and analyzed. Each sample was injected for three times and the average area of the three injections was used for recovery calculation. The recovery results are between 81% and 110% (Table 4).

Repeatability of the method was evaluated with standard samples in diluent and in post-spiked shrimp matrix. The concentrations used for repeatability studies were 0.005, 0.01 and 0.025 ng/mL and the RSD results for seven consecutive injections are tabulated in Table 5. The RSD values of chloramphenicol in diluent and shrimp matrix are below 6.3% for all three concentrations.

The LOD and LOQ of the method were determined directly from the calibration curve of chloramphenicol post-spiked in shrimp matrix. The LOQ of the method was found to be 0.01 ng/mL. The LOD was estimated to be or better than 0.005 ng/mL.

Table 4: Summary of recovery results (inter-day, n=3)

Concentration (ng/mL)	Recovery % (triplicates)			Average Recovery % (n=3)	Recovery %RSD (n=3)
	Day 1	Day 2	Day 3		
0.025	89.6	114.6	125.5	109.9	16.7
0.10	85.4	77.3	80.3	81.0	5.0
1.00	100.0	83.8	96.1	93.3	9.1

Table 5: Summary of repeatability results (n=7)

Concentration (ng/mL)	RSD%	
	Neat	Post-spiked
0.005	6.3	3.8
0.010	5.3	1.7
0.025	6.1	2.0

❑ Conclusions

A LC/MS/MS method with ESI had been developed and optimized for quantitative determination of chloramphenicol residue in shrimp samples using a modified QuEChERS procedure for sample pre-treatment. The QuEChERS procedure used for sample pre-treatment shows its advantages of being simpler, faster and very efficient. The detection and quantitation range for this analysis method was from 0.005 to 20 ng/mL with a 5µL injection volume. The LOQ of this method was determined to be 0.010 ng/mL, which is equivalent to 0.20 µg/kg of chloramphenicol in shrimp samples and satisfy the MRPL set at 0.3 µg/kg by the European Union. The performance of the method was evaluated thoroughly, including linearity, accuracy and repeatability. The results obtained from matrix effect and recovery indicated that the LC/MS/MS method with simple QuEChERS sample pre-treatment is highly sensitive and reliable in detection and quantitation of chloramphenicol in shrimp samples.

❑ References

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