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Application News

No. LCMSMS-102E

Liquid Chromatography Mass Spectrometry

Determination of Chlorpromazine and Diazepam in Pork by Ultra-High-Performance Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry

A method for the determination of chlorpromazine and diazepam in pork using Shimadzu Ultra-High-Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8040 was developed. The pork sample was first homogenized, extracted with ethyl acetate, and dried under nitrogen. The dried extract was reconstituted in methanol, and stored at -18°C for 5 min to remove grease. The sample was then injected for direct analysis. Using D6-chlorpromazine as the internal standard, the quantitative analysis was performed by the internal standard method under the electrospray ionization positive mode (ESI+) and multiple reaction ion monitoring (MRM). Based on 6 consecutive injections of 1 ng/mL and 5 ng/mL mixed standard solutions, the RSD% of retention time and peak area was 0.07-0.21% and 1.48-4.73% respectively, indicating good instrument precision. The calibration curve of chlorpromazine and diazepam exhibited good linearity over the concentration range of 0.1-50 µg/L, with correlation coefficients above 0.99. The method detection limit and limit of quantitation were 0.03 and 0.1 ng/mL respectively. The spike and recovery results of chlorpromazine and diazepam were good at the concentration of 1.0 and 5.0 µg/kg and the pork sample matrix did not interfere with the analysis.

Keywords: Chlorpromazine; Diazepam; Pork; Triple quadrupole mass spectrometer

■ Introduction

Phenothiazine tranquilizers, such as chlorpromazine and diazepam, are commonly used in veterinary medicine. These drugs produce anti-vomiting, anti-dizziness and indirect fattening effects and can also reduce animal mortality during transportation. With high fat solubility and easy accumulation in adipose tissue, these drugs can still be detected within several weeks to six months after drug discontinuation. Minute amount of phenothiazine residues in food of animal origin can cause leukopenia and agranulocytosis, thus leading to human liver and kidney lesions as well as conditions such as eye complications. Japan's regulation (Uniform Limit) states that neither diazepam nor chlorpromazine should be detected. At present, few analytical methods can be used for the detection of chlorpromazine and diazepam in animal-derived food; most studies focused on forensic testing of blood and urine, where the sample matrix is relatively complicated compared to food samples. Regarding the analysis of diazepam and chlorpromazine in food, China has a national standard "SN/T 2113-2008 Determination of Tranquillizer Drug Residues in Animal-origin Foodstuffs for Import and Export - LC-MS/MS Method.". Both the detection limits of chlorpromazine and diazepam in the national standard are 1.0 µg/kg. In this application note, a method for the rapid and accurate determination of chlorpromazine and diazepam in pork was established with reference to China's national standard. The use of Shimadzu Ultra-High-Performance Liquid Chromatograph Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8040 for the analysis of tranquillizer drugs in food can serve as a reference method for related inspection personnel.

■ Experimental

1.1. Instruments

Shimadzu Ultra-High-Performance Liquid Chromatography (UHPLC) Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8040 system was used. The specific configuration included LC-30AD×2 infusion pumps, DGU-20A5 Online Degasser, SIL-30AC Autosampler, CTO-30AC Column Oven, CBM-20A System Controller, Triple Quadrupole Mass Spectrometer LCMS-8040, and LabSolutions Ver. 5.50 Chromatography Workstation.

1.2. Analytical Conditions

Liquid chromatography (LC) conditions

- Analyzer: Nexera X2 System
- Chromatographic column: Shimadzu Shim-pack XR-ODS II, 2.0 mm (I.D.) x 75 mm (L) x 2.2 um
- Mobile phase: A-0.1% formic acid solution, 2 mM ammonium acetate solution; B-1% formic acid acetonitrile
- Flow rate: 0.4 mL/min
- Injection volume: 5µL
- Column temperature: 40°C
- Elution mode: gradient elution; with the initial concentration of phase B was 20%, Refer to Table 1 for the detailed gradient program.

Mass spectrometry (MS) conditions

- Analyzer: LCMS-8040
- Ion source: ESI, positive ion scan
- Ion source interface voltage: 4.5 kV
- Nebulizing gas: Nitrogen 3.0 L/min
- Drying gas: Nitrogen 15 L/min
- Collision gas: Argon
- DL temperature: 250°C
- Heating module temperature: 400°C
- Scan mode: Multiple Reaction Monitoring (MRM)
- Dwell time: 30 msec
- Pause time: 3 msec
- MRM parameters: Refer to Table 2

Table 1: Gradient Program

Time (min)	B.Conc.
3.00	90
4.00	90
4.10	20
6.00	Stop

Table 2: MRM Parameters

No.	Compound Name	CAS	Parent ion	Daughter ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	D_6-Chlorpromazine	1228182-46-4	325.10	92.20	-17	-21	-16
2	Diazepam	439-14-5	285.10	193.10* 154.10	-15 -15	-32 -28	-19 -29
3	Chlorpromazine	69-09-0	319.10	86.20* 58.20	-17 -17	-22 -37	-15 -21

Note: * represents Quantifier ion.

1.3. Standard Solutions and Sample Preparation

Preparation of standard solutions:

Appropriate amount of chlorpromazine, diazepam and D_6-chlorpromazine (IS) was weighed and diluted in methanol to obtain single standard stock solutions. A mixed standard stock solution at concentration of 100mg/L was prepared by adding appropriate amount of the single standard stock solutions, and subsequently diluted in methanol. Standard working solutions used for calibration were sequentially prepared by diluting 100mg/L stock solutions in mobile phase A.

Sample pretreatment method:

5.0 g of pork sample was accurately weighed in aa 50 mL centrifuge tube. 100 µL of 0.05 µg/mL D6-chlorpromazine solution, 10 mL of ethyl acetate, and 1 mL of 1 M NaOH solution were added to the pork sample. The mixture was homogenized for 2 min at 10,000 r/min and then centrifuged for 10 min at 5000 r/min. 1 mL of the supernatant was accurately drawn to a 10 mL glass test tube, and dried using nitrogen at 45°C. After reconstituting with 1mL of methanol, the capped tube was vortexed for 30 s and stored at -18°C for 5 min. The sample extract was then filtered using 0.22 µm millipore filter membrane to remove grease and injected for sample analysis.

■ Results and Discussion

2.1. MRM Chromatogram of the Standard Stock Solutions

See Figure 1 for the MRM chromatogram of the mixed standard stock solutions.

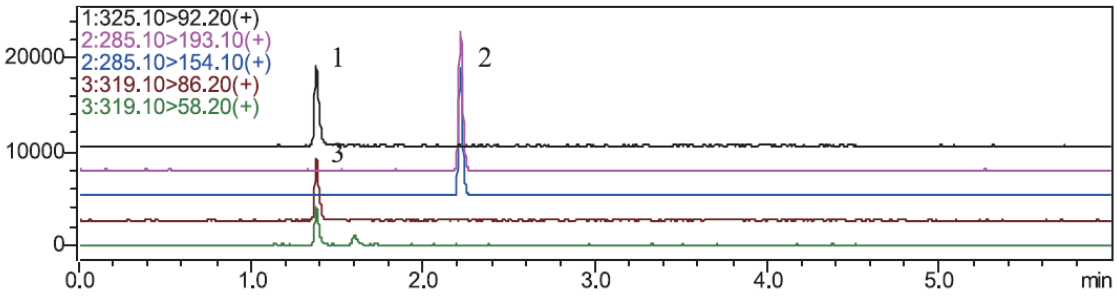

Figure 1: MRM Chromatogram of the Mixed Standard Sample (1. D_6-Chlorpromazine; 2. Diazepam; 3. Chlorpromazine)

2.2. Calibration and Linearity

Calibration, using the mixed standard stock solutions of diazepam and chlorpromazine at 0.1, 1, 5, 10 and 50 ng/mL with internal standard (D_6-chlorpromazine) concentration at 0.5 ng/mL, was performed using the internal standard method.

The calibration curves are plotted in Figures 2 and 3; diazepam and chlorpromazine exhibited good linearity. The linear equations, correlation coefficients as well as the method detection limit and limit of quantitation are shown in Table 3.

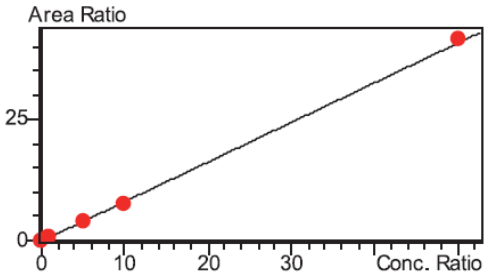

Figure 2: Standard Working Curve of Diazepam

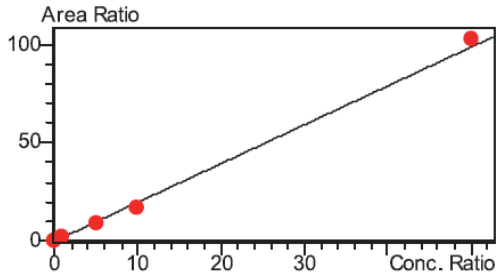

Figure 3: Standard Working Curve of Chlorpromazine

Table 3: Calibration Curve Parameters of Diazepam and Chlorpromazine

No.	Compound Name	Calibration curve	Correlation coefficient (r)	Detection limit (ng/mL)	Limit of quantitation (ng/mL)
1	Diazepam	Y = (0.815387)X + (-0.0272927)	0.9993	0.03	0.1
2	Chlorpromazine	Y = (1.97371)X + (-0.0414930)	0.9976	0.03	0.1

2.3. Precision Experiment

According to the 6 consecutive injections of mixed standard stock solutions at concentration of 1 ng/mL and 5 ng/mL, the RSD% of retention time and peak area were 0.07-0.21% and 1.48-4.73% respectively, showing good instrument precision.

2.4. Recovery and Matrix Effects

The blank pork sample was first pretreated according to the method in 1.3; the spiking concentration of diazepam and chlorpromazine was 1.0 µg/kg and 5.0 µg/kg respectively; two replicates for each concentration of each compound were investigated. The spiked recovery of diazepam and chlorpromazine are tabulated in Table 5. Figures 4 and 5 showed the chromatograms of blank pork sample and spiked pork sample (1.0 µg/kg) respectively. Based on the recovery results, the pork matrix pork did not interfere with the detection of diazepam and chlorpromazine.

Table 4: Repeatability Results of Retention Time and Peak Area (n=6)

Compound Name	RSD% (1 ng/mL)		RSD% (5 ng/mL)	
	R.T.	Area	R.T.	Area
Diazepam	0.10	4.73	0.07	1.48
Chlorpromazine	0.21	3.89	0.20	2.90

Table 5: Adding Standard Recovery Data

Recovery%	1.0 µg/kg		5.0 µg/kg	
Diazepam	98.1	107.0	90.7	100.7
Chlorpromazine	83.6	81.5	82.8	88.5

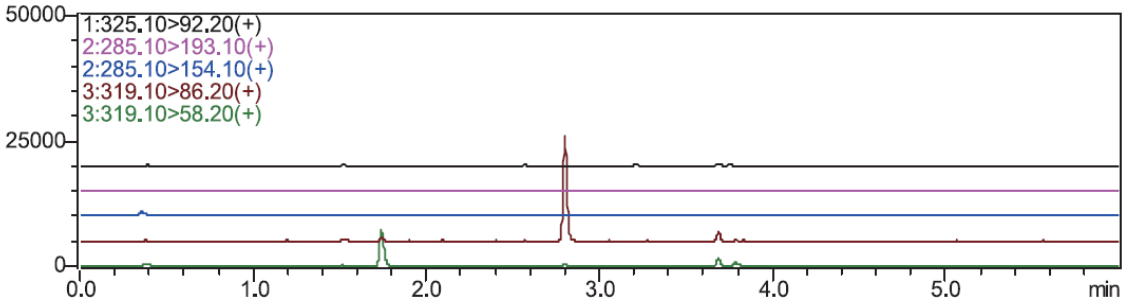

Figure 4: Chromatogram of the Matrix Blank Sample

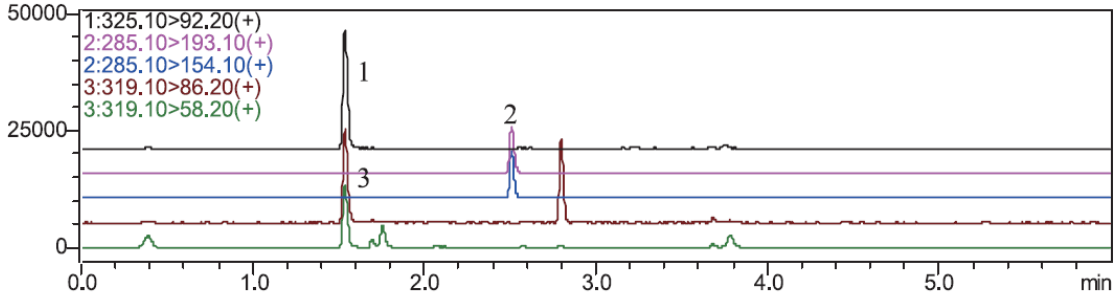

Figure 5: Chromatogram of the Adding Standard Matrix Sample (at 1.0 µg/kg, 1. D_6-Chlorpromazine;2. Diazepam; 3. Chlorpromazine)

■ Conclusion

A method for the determination of tranquilizers, diazepam and chlorpromazine, in pork was developed using Shimadzu Ultra-High-Performance Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8040. The isotope internal standard, D6-chlorpromazine, was employed for the quantitative analysis. These two tranquilizers exhibited good linearity within the concentration range of 0.1-50 µg/L; the spike recovery obtained at 1.0 and 5.0 µg/kg was good and showed no significant matrix interference. The RSD% of retention time and peak area were 0.07-0.21% and 1.48-4.73% respectively, indicating good instrument precision. The method detection limit meets the requirements stated in China's national standard.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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First Edition: July 2018