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Application News

No. LCMSMS-137E

Liquid Chromatography Mass Spectrometry

Determination of Eight Tranquilizers in Pork by Ultra-High-Performance Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry

This application note presents a method for the determination of eight tranquilizers in pork using Shimadzu Ultra-High-Performance Liquid Chromatography (UHPLC) Nexera X2 coupled with Triple Quadrupole Mass Spectrometer LCMS-8040. With D6-chlorpromazine as the internal standard, this method separates and quantifies the tranquilizer residues (such as acepromazine, chlorpromazine, haloperidol, propionylpromazine, xylazine, azaperone and its metabolite azaperol, and β-receptor blocker carazolol) within 10 minutes. With the pork matrix added, these eight tranquilizers exhibited good linearity within the range of 0.1-50ng/mL; the correlation coefficient of standard curves was all above 0.997. The pork matrix was also used to prepare standard solutions at concentration of 0.5, 5 and 25 ng/mL for repeatability assessment. According to the results of 6 consecutive injections, the RSD of retention time and peak area was 0.02-2.23% and 0.81-8.34% respectively, indicating good instrument precision. The limit of quantitation of these eight tranquilizers in pork was 0.1 µg/kg, which was better than that stipulated in "GBT 20763-2006: Determination of Residues of Acepromazine, Chlorpromazine, Haloperidol, Propionylpromazine, Xylazine, Azaperone, Azaperol and Carazolol in Pork Kidney and Muscle Tissues - LC-Tandem MS Method. "

Keywords: Triple quadrupole mass spectrometry; Pork; Tranquilizers

■ Introduction

There are various groups of tranquilizers such as phenothiazines (e.g. chlorpromazine, acepromazine, propionylpromazine and xylazine) and butyrophenones (e.g. haloperidol and azaperone). Azaperol is a metabolite of azaperone. Carazolol, a β-receptor blocker, may cause bronchial spasm and nasal mucosal capillary vasoconstriction, thereby causing considerable damage to patients with asthma and allergic rhinitis. The mentioned tranquilizers cause weakness and sedative effects through inhibiting brain stem and cerebral cortex. At the same time, they cause some other side effects. European Union have prohibited the use of thiophene tranquilizers on edible animals. China has also listed chlorpromazine as a banned veterinary drug in edible animals.

A highly sensitive method for the determination of tranquilizer residues in pork sample matrix was established in this application note. By adopting a three-step extraction procedure consisting of alkalization, acidification and re-alkalization, this method can effectively remove any matrix interference from pork sample. These eight tranquilizers are alkaline compounds, whose partition would be either in the organic phase under alkaline conditions or aqueous phase under acidic conditions. Due to the low polarity of ether, the weak polar compounds, together with the target analytes (tranquilizer) in the pork matrix, would be extracted using ether in alkaline conditions. After the second extraction step (acidification), the removal of the ether layer would remove the weak polar interferents. The final step of alkalization involves the extraction of the target analytes to the ether layer. Finally, the ether extracts were dried under nitrogen and then reconstituted with the mobile phase for sample injection analysis. With D6-chlorpromazine as the internal standard, the limit of quantitation obtained using Shimadzu Nexera X2 UHPLC and Triple Quadrupole Mass Spectrometer LCMS-8040 can meet regulatory requirements. Therefore this rapid and sensitive method serves as a good reference for the inspection personnel of related industries.

■ Experimental

1.1. Instruments

Shimadzu Nexera X2 UHPLC and Triple Quadrupole Mass Spectrometer LCMS-8040 was used. The specific configuration included LC-30AD×2 infusion pumps, DGU-20A$_5$ 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

- Chromatographic column: Kinetex C18 (2.1 mm (I.D.) x 75 mm (L) x 2.6 µm
- Mobile phase: A: 2 mM ammonium formate solution, 0.1% formic acid
- B: Methanol
- Flow rate: 0.35 mL/min
- Column temperature: 40°C
- Injection volume: 5 µL
- Elution mode: gradient elution; the initial concentration of phase B was 20%, refer to Table 1 for the gradient program.

Table 1: Gradient Program

Time(min)	Module	Command	Value
1	Pumps	Pump B Conc.	20
2.5	Pumps	Pump B Conc.	32
4	Pumps	Pump B Conc.	55
6	Pumps	Pump B Conc.	55
6.1	Pumps	Pump B Conc.	95
7	Pumps	Pump B Conc.	95
7.1	Pumps	Pump B Conc.	20
10	Controller	Stop	

Mass spectrometry (MS) conditions

- Analyzer: LCMS-8040
- Ion source: ESI, positive ion scan
- Heating module temperature: 450°C
- DL temperature: 250°C
- Nebulizing gas flow rate: 3.0 L/min
- Heating gas flow rate: 15.0 L/min
- Ion source voltage: 4.5 kV
- Scan mode: Multiple Reaction Monitoring (MRM)
- MRM parameters: Refer to Table

Table 2: MRM Parameters

No.	Compound name	Precursor ion	Product ion	Q1 Pre Bias (V)	CE(V)	Q3 Pre Bias (V)
1	Azaperol	330.2	121.1* 321.2	-25 -25	-25 -15	-21 -21
2	Xylazine	221.1	90.1* 164.1	-30 -30	-22 -26	-16 -29
3	Azaperone	328.2	165.1* 121.2	-27 -27	-21 -23	-16 -21
4	Carazolol	299.1	166.2* 222.1	-16 -16	-21 -20	-21 -23
5	Haloperidol	376.1	123.1* 165.1	-29 -29	-42 -23	-21 -30
6	Acepromazine	327.1	58.1* 86.2	-17 -17	-36 -21	-22 -15
7	Propionylpromazine	341.1	58.1* 86.2	-18 -18	-39 -21	-22 -15
8	Chlorpromazine	319.1	58.1* 86.2	-17 -17	-38 -21	-22 -15
9	D6-chlorpromazine	325.1	92.2	-17	-21	-16

Note: * represents quantifier ion.

1.3. Preparation of Standard Working Solution

Standard drug substances: chlorpromazine, acepromazine, azaperone, azaperol, haloperidol, propionylpromazine, xylazine and carazolol (a total of 8 drugs) were used for this analysis. The internal standard used was D6-chlorpromazine.

Preparation of standard working solution: These 8 drugs were weighed and dissolved in absolute ethyl alcohol to obtain a 500 µg/mL mixed standard stock solution. The mixed standard stock solution was subsequently diluted by methanol-water (1:1) solution to get a 5 µg/mL mixed standard solution.

1.4. Sample Pretreatment

Sample was pretreated with reference to the sample preparation method listed in Part 7 of "GBT 20763-2006: Determination of Residues of Acepromazine, Chlorpromazine, Haloperidol, Propionylpromazine, Xylazine, Azaperone, Azaperol and Carazolol in Pork Kidney and Muscle Tissues - LC-Tandem MS Method". Ether was used as the extractant instead.

After drying under nitrogen, the pork extract was reconstituted in methanol-water (1:1) solution, which was then filtered using 0.22 µm organic filter membrane. The obtained solution was used to dilute the 5 µg/mL mixed standard solution to obtain a series of matrix spike standard solutions at concentrations of 50 ng/mL, 25 ng/mL, 5 ng/mL, 1 ng/mL, 0.5 ng/mL and 0.1 ng/mL. The concentration of D6-chlorpromazine (IS) used was 50 ng/mL.

■ Results and Discussion

2.1. MRM Chromatogram of Pork Matrix Spike Sample

The chromatogram of the blank pork sample and the chromatogram of the matrix spike standard solution (0.5 ng/mL) with internal standard are shown in Figure 1 and 2 respectively.

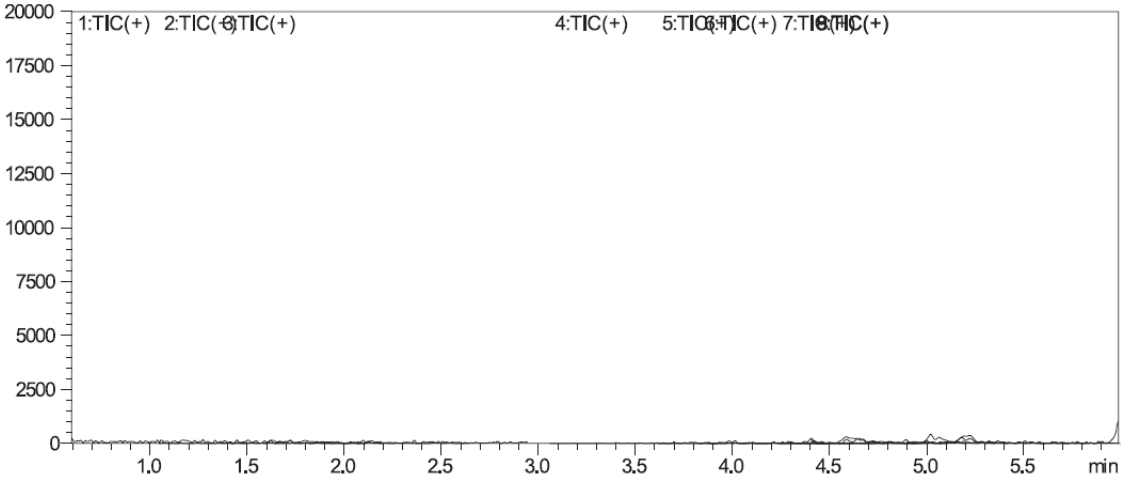

Figure 1: MRM Chromatogram of the Blank Pork Matrix Sample

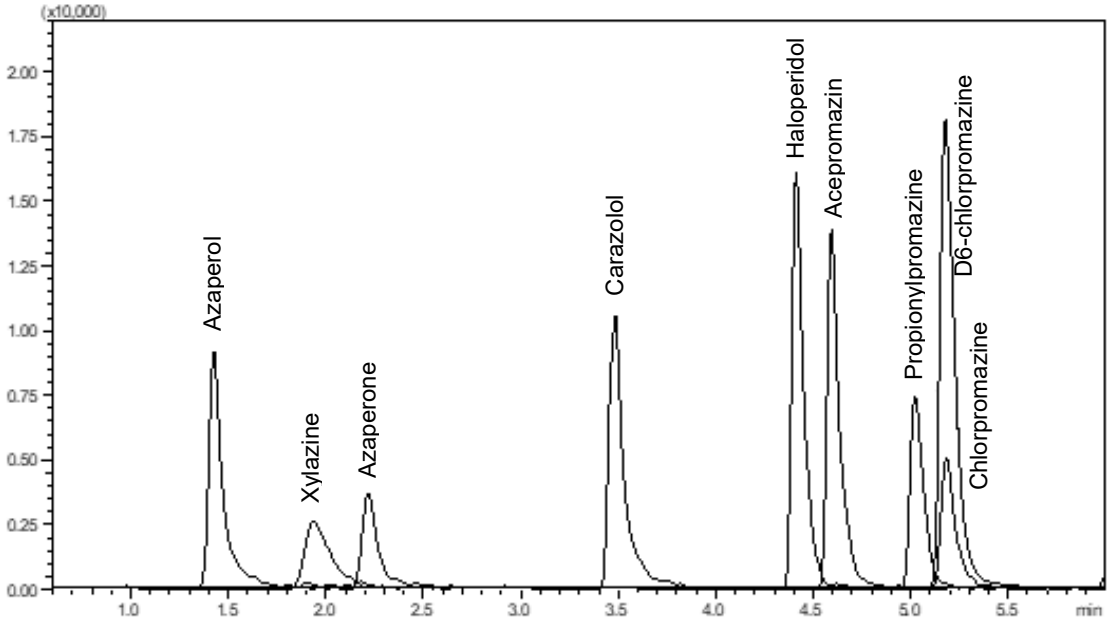

Figure 2: MRM Chromatogram of Eight Tranquilizers and Internal Standard in the Adding Standard Pork Matrix Sample (0.5 ng/mL)

2.2. Calibration and Linearity

Calibration was performed using a 6-point curve at concentrations of 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 25 ng/mL and 50 ng/mL. Internal standard method was used for quantitative analysis. The calibration curves of the 8 tranquilizers (i.e. acepromazine, chlorpromazine, haloperidol, propionylpromazine, xylazine, azaperone, azaperol and carazolol) are shown in Figures 3 – 10.

All calibration curves exhibited good linearity within the range of 0.1-50 ng/mL and the linear equations and correlation coefficients are shown in Table 3.

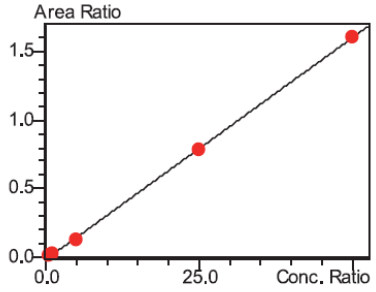

Figure 3: Calibration Curve of Azaperol

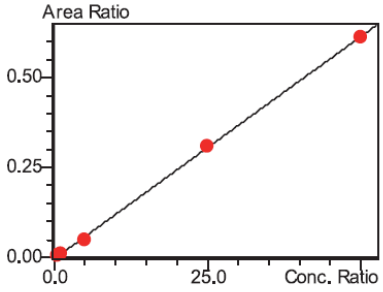

Figure 4: Calibration Curve of Xylazine

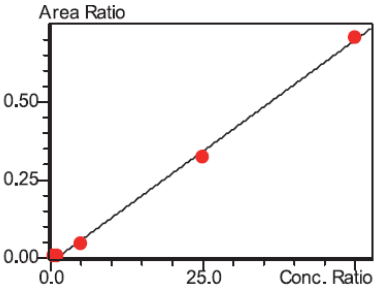

Figure 5: Calibration Curve of Azaperone

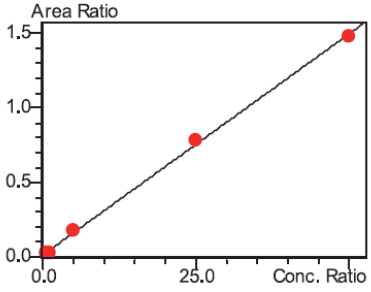

Figure 6: Calibration Curve of Carazolol

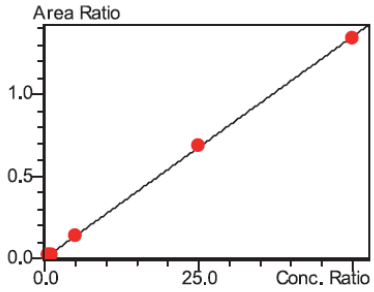

Figure 7: Calibration Curve of Haloperidol

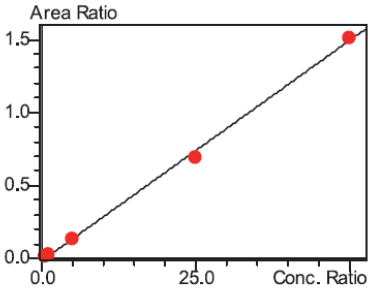

Figure 8: Calibration Curve of Acepromazine

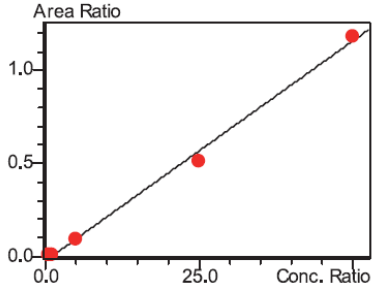

Figure 9: Calibration Curve of Propionylpromazine

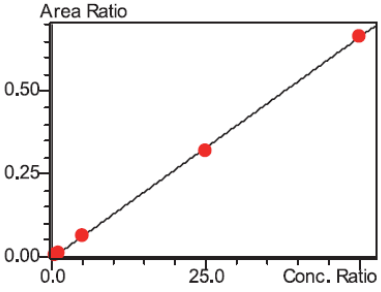

Figure 10: Calibration Curve of Chlorpromazine

Table 3: Calibration Curve Parameters of Eight Tranquilizers

No.	Compound name			
1	Azaperol	Y = (O.O3 2188O)X + (-0.0122110)	0.1~50	0.9998
2	Xylazine	Y = (O.O123129)X + (-0.00235905)	0.1~50	0.9999
3	Azaperone	Y = (O.O141913)X + (-0.0124341)	0.1~50	0.9990
4	Carazolol	Y = (O.O296O82)X + (0.0148377)	0.1~50	0.9997
5	Haloperidol	Y = (O.O26875 l)X + (0.00383163)	0.1~50	0.9999
6	Acepromazine	Y = (O.O3OO 782)X + (-0.0162544)	0.1~50	0.9991
7	Propionylpromazine	Y = (O.O242449)X + (-0.0261471)	0.1~50	0.9979
8	Chlorpromazine	Y = (O.O147256)X + (-0.0127699)	0.1~50	0.9998

2.3. Precision Experiment

Six consecutive injections of 0.5 ng/mL, 5 ng/mL and 25 ng/mL mixed standard solutions were conducted to evaluate the instrument precision. The repeatability results of retention time and peak area are tabulated (Table 4). The RSD of retention time and peak area at the three specified concentrations was 0.02-2.23% and 0.81-8.34% respectively, indicating good instrument precision.

2.4. Method Performance

The matrix spike solution of the 8 tranquilizers were prepared to obtain a sample injection concentration of 0.1 µg/kg via dilution with pork sample matrix. As shown in Figure 11, the lowest limit of quantitation (S/N=10, represented by LOQ) of these 8 tranquilizers reached 0.1 µg/kg.

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

No.	Compound name	RSD%	(0.5 ng/mL)	RSD%	(5 ng/mL)	RSD%	(25 ng/mL)
1	Azaperol	1.52	6.57	0.86	1.50	0.21	3.97
2	Xylazine	2.23	8.34	1.05	5.10	0.26	1.90
3	Azaperone	0.81	7.15	0.59	4.16	0.24	6.12
4	Carazolol	0.18	2.98	0.12	2.48	0.15	0.86
5	Haloperidol	0.06	4.34	0.02	2.96	0.09	1.67
6	Acepromazine	0.09	3.42	0.03	1.00	0.04	2.01
7	Propionylpromazine	0.05	5.24	0.03	0.81	0.64	3.49
8	Chlorpromazine	0.39	3.97	0.04	3.08	1.56	5.39

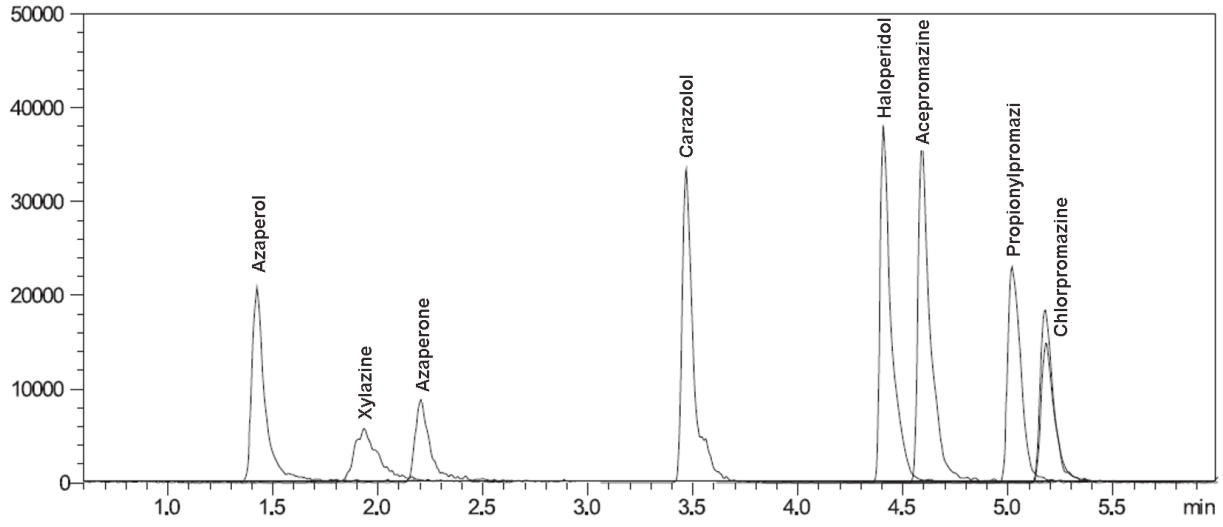

Figure 11: MRM Chromatogram of Eight Tranquilizers and Internal Standard in Matrix Spike Sample (0.1 µg/kg)

2.5. Spike and Recovery Experiment

The pork sample was pretreated according to the method listed in 1.4. Matrix spike solution was prepared where the spiking concentration was at a concentration of 1 µg/kg. 3 replicates (n = 3) were prepared to determine the spiked recovery and RSD. The results are tabulated (Table 5) and the spiked recovery ranged between 80.6 and 108%.

Table 5: Recovery Results of the Adding Standard Samples (n=3)

No.	Compound name	ng/mL	%	RSD%
1	Azaperol	0.978	97.8	7.1
2	Xylazine	0.908	90.8	7.3
3	Azaperone	0.884	88.4	2.3
4	Carazolol	1.04	104	5.2
5	Haloperidol	0.923	92.3	8.6
6	Acepromazine	0.806	80.6	3.8
7	Propionylpromazine	1.04	104	7.7
8	Chlorpromazine	1.08	108	3.1

Table 6: Information of Eight Tranquilizer Compounds

No.	Compound name	CAS
1	Azaperol	2804-05-9
2	Xylazine	7361-61-7
3	Azaperone	1649-18-9
4	Carazolol	57775-29-8
5	Haloperidol	52-86-8
6	Acepromazine	61-00-7
7	Propionylpromazine	7681-67-6
8	Chlorpromazine	50-53-3

■ Conclusion

Using Shimadzu Nexera X2 UHPLC coupled with Triple Quadrupole Mass Spectrometer LCMS-8040, a method for the determination of eight tranquilizers in pork was developed. This method separates and quantifies eight common tranquilizers (i.e. acepromazine, chlorpromazine, haloperidol, propionylpromazine, xylazine, azaperone, azaperol and carazolol) in 10min. All 8 analytes exhibited good linearity in the range of 0.1-50ng/mL with their correlation coefficient all above 0.997. The pork matrix was used to prepare standard working solutions at concentrations of 0.5, 5 and 25 ng/mL. Repeatability was evaluated by performing 6 consecutive injections at these 3 concentrations and the RSD of retention time and peak area was 0.02-2.23% and 0.81-8.34% respectively, indicating good instrument precision. The limit of quantitation of these eight tranquilizers in pork was 0.1µg/kg, which was better than that specified in GBT 20763-2006. With high sensitivity and good repeatability, this method is suitable for the trace detection of tranquilizers in pork samples.

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