Determination of illegal drugs for water-retaining in fresh meat by HPLC-MS/MS

1 Scope

The standard specifies the sample preparation and determination of illegal drugs of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol for water-retaining by LC-MS/MS in fresh meat.

2 Principle

The atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in the test sample were extracted from animal muscle tissues with phosphate buffer solution. The extracts were centrifuged and purified, and then determined by LC-MS/MS, quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagent used should be analytical grade, water is the first grade water prescribed by GB/T 6682.

3.1 Reagents

3.1.1 Methanol（CH_3OH）：HPLC grade.

3.1.2 Acetonitrile（CH_3CN）：HPLC grade.

3.1.3 Formic acid（HCOOH）：HPLC grade.

3.1.4 Acetic acid（CH_3COOH）：HPLC grade.

3.1.5 Normal hexane（C_6H_{14}）：HPLC grade.

3.1.6 Sodium hydroxide（NaOH）.

3.1.7 Mono potassium phosphate（KH_2PO_4）.

3.1.8 Ammonium hydroxide（$NH_3 \cdot H_2O$）.

3.2 Preparation of reagents

3.2.1 Sodium hydroxide solution (200g/L) : Weigh 20g sodium hydroxide (3.1.6) and dissolve it with appropriate amount of water. After cooling, dilute it with water to 100 mL and mix well.

3.2.2 Potassium dihydrogen phosphate buffer solution (0.1mol/L) : Weigh KH_2PO_4 (3.1.7) 13.6g, add water to dissolve it to nearly 1000 mL, use sodium hydroxide solution (3.2.1) to adjust the pH to 4.0, add water to 1000 mL and mix well.

3.2.3 Formic acid solution (2 mL/100 mL) : Transfer 2 mL formic acid (3.1.3) to a 100 mL volumetric flask, dilute with water and mix well.

3.2.4 Ammonium hydroxide - Methanol solution(2+98) : Transfer 2 mL of ammonium hydroxide(3.1.8), add methanol (3.1.1) to a 100 mL volumetric flask, dilute with water and mix well.

3.2.5 Acetic acid solution (0.1 mL/100 mL) : Transfer 1 mL acetic acid (3.1.4) to a 1000 mL volumetric flask, dilute with water and mix well.

3.2.6 Formic acid solution (0.1 mL/100 mL) : Transfer 1 mL formic acid (3.1.3) to a 1000 mL volumetric flask, dilute with water and mix well.

3.3 Standard

The purity of standard chemicals are more than 98% .

Tab.1 English name, CAS number, molecular formula, relative molecular weight and conversion coefficient of standard chemicals

No.	Compound	CAS **number**	Molecular formula	Relative molecular weight	Conversion factor
1	Atropine sulfate	55-48-1	$(C_{17}H_{23}NO_3)_2{\cdot}H_2SO_4$	676.82	0.855
2	Anisodamine	17659-49-3	$C_{17}H_{23}NO_4$	305.37	1.000
3	Scopolamine Hydrobromide	6533-68-2	$C_{17}H_{28}BrNO_7$	438.31	0.692
4	Procaine	59-46-1	$C_{13}H_{20}N_2O_2$	236.32	1.000
5	Lidocaine	137-58-6	$C_{14}H_{22}N_2O$	234.34	1.000
6	Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$	239.31	1.000

3.4 Preparation of standard solution

Standard stock solution（100 μg/mL）： Weigh about 10 mg atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol standard material (accurate to 0.01 mg) in 100 mL beaker, dissolve it with methanol, transfer it into the volumetric flask, and dilute with methanol to 100 mL.

3.5 Preparation of standard intermediate solution

Standard intermediate solution（1.00μg/mL）：Transfer standard stock solution1.00mL in 100 mL volumetric flask, dilute with methanol to a volume of 100 mL.

3.6 SPE Column

Oasis MCX cation exchange column, 60mg/3mL, or equivalent performance parameters.

4 Apparatus and equipment

4.1 High Performance Liquid Chromatography-Mass Spectrometer equipment: equipped with electrospray ionization source (ESI).

4.2 Analytical balance: sensibility reciprocal is 0.0001 g and 0.01 g respectively.

4.3 Centrifuge: ≥10000r/min.

4.4 Solid phase extraction device.

4.5 Vortex mixer.

4.6 Ultrasonic cleaner.

4.7 Pressure Blowing Concentrator.

4.8 pH meter.

5 Procedure

5.1 Sample preparation and storage

About 100 g of representative samples should be taken from all samples, then homogenized by the homogenizer, put in suitable clean container. After being sealed and labeled, the samples should be stored at below -18℃ in dark. Certain measures should be taken to prevent contamination of samples or decomposition of the residues during the sample preparation procedure.

5.2 Sample processing

5.2.1 Extract

Accurately 5 g test sample (accurate to 0.01 g) was weighed into a 50 mL centrifuge tube and 20 mL potassium dihydrogen phosphate buffer solution (0.1mol/L) (3.2.2)was added. After vortexing for 2 min and ultrasonicating for 15min, the mixture was centrifuged for 15 min at 12000r/min. The supernatant was poured into another centrifugal tube, and 20mL potassium dihydrogen phosphate buffer solution

(0.1mol/L) was added to the residue. The supernatants were combined into the 50 mL volumetric flask, and diluted with potassium dihydrogen phosphate buffer solution (0.1mol/L) to a volume of 100 mL.

5.2.2 Purification

Oasis MCX cationic exchange column was activated with 3 mL methanol (3.1.1), 3 mL water and 3 mL formic acid solution (3.2.3) and keep wet.5mL extracting solution(5.2.1) was transferred into the SPE column, and the column was washed with 3 mL formic acid(3.1.3) , 3 mL methanol (3.1.1), and 3mL normal hexane (3.1.5), and dried with pump for 2 minutes. The column was eluted with 5 mL ammonium hydroxide - Methanol solution (3.2.4). The elute solution was collected and dried by the N_2 at 40 ℃. 1mL formic acid solution (3.2.6) was added and the mixture was, vortexed for 0.5min filtered with 0.22μm filter membrane and injected into the LC/MS/MS system.

5.3 Preparation of matrix-based standard working curve

Weigh about 5g blank sample (accurate to 0.01 g) and placed in the 50 mL plunger centrifuge tube, and the standard intermediate solution (3.4.2) was added. The concentration was 0.500, 1.00, 2.00, 5.00, 10.0 and 50.0 ng/mL. After 5.2 steps, the sample was determined.

5.4 Apparatus operating condition

5.4.1 HPLC operating condition

a）Column: C_{18}，2.1×50mm 1.8μm or equivalent.

b）Column temperature：30℃；

c）Injection volume：5 μL；

d）Mobile phase: see Table 2.

Table 2 Mobile phase and gradient elution program

Time /min	Mobile phase A（Acetic acid solution 3.2.5）, %	Mobile phase B（acetonitrile）, %
0.0	10	90
1.0	10	90

3.0	17	83
3.5	90	10
4.0	10	90
5.0	10	90

e）Flow rate：0.4 mL/min；

5.4.2 MS/MS operating condition

lon source：electrospray ionization source（ESI）；Scan mode：Positive-ion mode；Monitor mode：multiple reaction monitoring (MRM)；Spray voltage：3000V；Main MS parameters of target compound are listed in Table 3.

Table 3 Multiple reaction monitoring (MRM) parameters

Compound	Parent ion	Product ion	Declustering potential / V	Collision energy/ V
Atropine	290	124*；93	90	22/30
Anisodamine	306	140*；122	80	25/30
Scopolamine	304	138*；156	80	18/20
Procaine	237	100*；120	80	16/30
Lidocaine	235	86*；58	80	20/40
Salbutamol	240	147.8*；222.0	80	25/15

Note：* The product ion is used for quantification

5.5 Qualitative determination

Under the same determination conditions the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of ±2.5% The variation range of the ion ratio between the qualitative ion for the unknown sample and the standard working solution at the similar concentration can not be out of range of Table 4. Then the corresponding analyte can be present in the sample.

Table 4 Maximum permitted tolerances for relative ion intensities

Relative intensity（%）	>50	>20~50	>10~20	≤10

Maximum permitted tolerances for relative ion intensities（%）	±20	±25	±30	±50

5.6 Quantitation determination

Under the optimized instrument working conditions, different matrix-based working standard solutions were injected. Using peak area as y-axis and the concentration as x-axis, the concentration of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in sample is quantified by standard calibration curve. The response of the atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in the sample solution should be in the linear range of the instrument detection.

5.7 Determination of sample solution

Using the sample solution in Part 5.2, the mass concentration of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in the sample were calculated by using the matrix-based external standard method.

6 Blank test

The operation of the blank test is the same as that described in the method of determination but with omission of sample addition.

7 Calculation and expression of the result

The calculation of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in the sample is according to Formula (1)

$$X = \frac{C \times V \times 1000}{m \times 1000} \times f \quad \text{.....(1)}$$

where：

X—the content of analyte in the test sample, μg/kg；

C—the concentration of analyte which is quantified by standard calibration curve, ng/mL；

v—the final volume of sample solution, mL；

m—the corresponding mass of test sample, g；

f—dilution ratio of sample solution.

The result was expressed as the arithmetic mean of two independent

determinations obtained under repeatability conditions and rounded to two decimal places.

8 Precision

The absolute difference of two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

9 Limit of quantitation

The limit of quantification is 0.5 μg/kg

10 Recovery

The recoveries of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol in fresh meat:

Spiked:0.5 μg/kg~5.0 μg/kg，Recovery:83.2%~100.3%.

Annex A

(Informative)

LC-MS/MS chromatogram of atropine, lidocaine, procaine, anisodamine, scopolamine and salbutamol

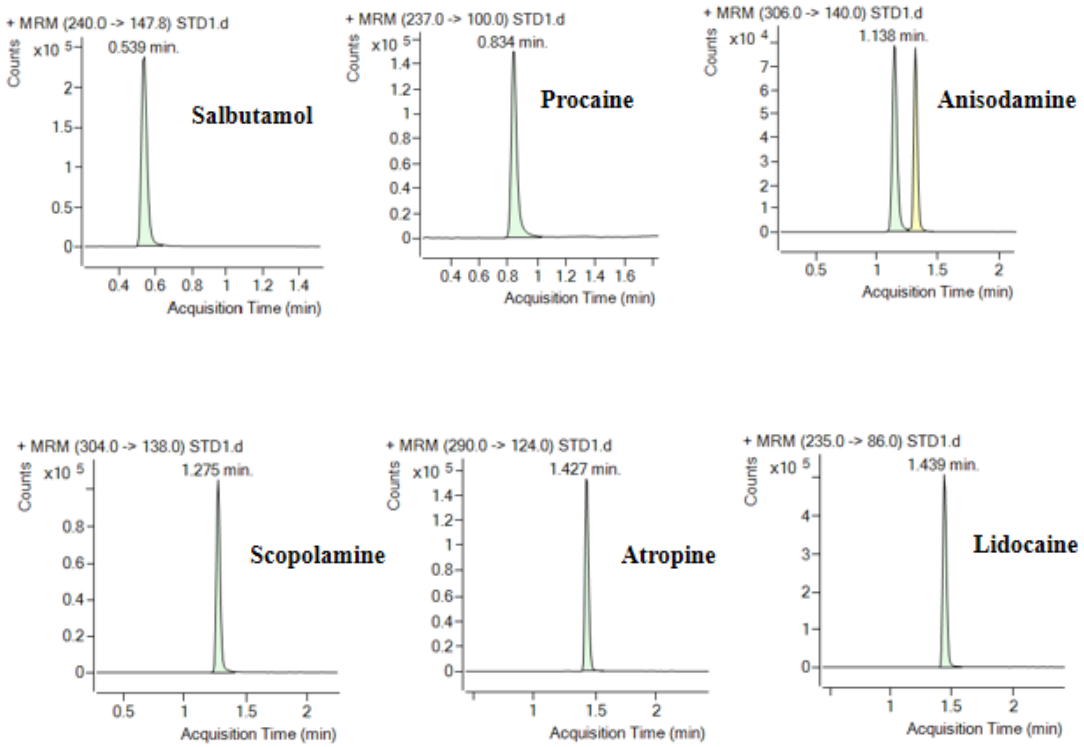

Fig.1 The MRM chromatograms of quantitative ion pairs for 6 compounds