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Simultaneous determination of 130 veterinary drugs in pork using ultra performance liquid chromatograph-tandem mass spectrometry

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Simultaneous determination of 130 veterinary drugs in pork using ultra performance liquid chromatograph-tandem mass spectrometry

Overview

In the present study, a rapid, sensitive and specific method was developed to quantitatively detect 130 veterinary drugs residues in pork, including sulfonamides, quinolones, tetracyclines and β-receptor agonists, etc. with ultra-performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS).

Introduction

It has been reported that veterinary drugs have been widely used in animal derived food production. However, it is difficult to remove residues of veterinary drugs remained in food, especially antibiotics, which are the most commonly residues in meat product. In the long run, the residual status of antibiotics will be harmful to human beings, even to leading acute diseases. Therefore, it is imperative to establish efficacy methods to detect the concentration of residues in animal derived food to evaluate possible biological risks.

Methods

Sample extraction and purification :

The residues of veterinary drugs were extracted with acetonitrile from pork via QuEChERS extraction tube, cleaned up by QuEChERS clean-up tube and concentrated to dryness under N2. Then the residues were dissolved in 1 mL of mobile phase and the separation was performed on a Shim-pack column by gradient elution. The analysis was detected with LCMS-8050 under positive and negative electrospray ionization modes at the same time and the quantitation was performed using multiple reaction monitoring (MRM).

Analysis conditions

LC condition	
Column	: Shim-pack GIST (2.0 mm I.D.×100 mm L., 2.1 μm) ;
Mobile phase	: A-water (contain 0.1% formaic acid and 2 mmol/L ammonium acetate); B-methanol ;
Binary gradient	: 10%B (0 min)-10%B (0.5 min)-30%B (3.0 min)-70%B (8.0 min)-100%B (10.0-13.0 min)- 10%B (13.1 min)- Stop (16 min);
Flow rate	: 0.3 mL/min;
Column temperature	: 40 °C;
Injection volume	: 10 μL.
MS condition	
Ion type	: ESI;
Scan mode	: MRM;
Interface temp.	: 300 °C
DL temp.	: 250 °C
Heating block temp.	: 400 °C
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
Detector voltage	: Tuning result
Dwell time	: 10 ms

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Figure 1. Shimadzu LCMS-8050

Result

Total 22 categories, 130 veterinary drugs were detected in pork matrix (Table 1).

Table 1. List of veterinary drugss in pork matrix

Compound	Number	Compound	Number
Sulfonamide	22	Benzimidazole	12
Quinolones	19	Glucocorticoid	9
Tetracycline	3	Fungicide	1
Chloramphenicol	1	Mental medicine	4
β-Receptor agonist	13	β-Zearalenol	1
Non-steroidal anti-inflammatory drugss	3	Olaquindox metabolite	1
Penicillin	3	Quinoline	1
Hypoglycemic	9	Macrolides	6
Nitroimidazole	5	Estrogen	1
Cephalosporins	2	Androgen	4
Insecticide	8	Progesterone	2

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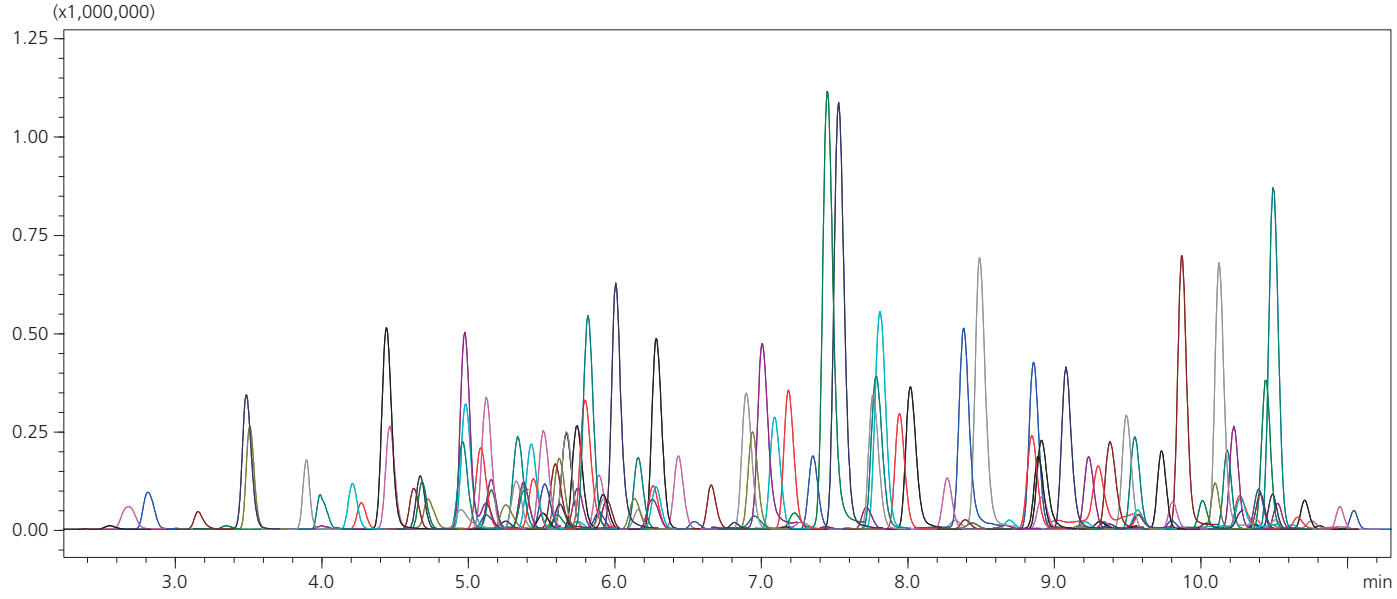

Figure 2. MRM chromatogram of 1 ng/mL standard solution

Blank matrix, which was obtained by the method mentioned above was used to prepare different standard solutions. The MRM chromatogram of standard solution (1 ng/mL) was shown in Figure 2. It took 16 minutes per one LC-MS/MS analysis with high sensitive detection. As can be seen in Table 2 and Figure 3, good linearities were obtained with the relative coefficients of the representative veterinary drugs greater than 0.997.

Table2. Linearity of representative veterinary drugs

Compound	Range	Accuracy (%)	Coefficient (r)
Sibutro	0.2~20	98.8~102.2	0.9999
Sulfadiazine	0.2~20	97.7~103.2	0.9996
Pipemidic acid	0.2~20	97.9~102.7	0.9998
Sulfathiazole	0.2~20	97.3~102.5	0.9998
Marbofloxacin	0.2~20	96.5~102.4	0.9997
Fleroxacin	0.2~20	97.8~102.2	0.9999
Ornidazole	0.2~20	98.8~101.2	0.9999
Clentro	0.2~20	98.7~102.8	0.9998
Cefoperazone	0.5~20	96.9~104.7	0.9992
Azithromycin	0.2~20	95.8~105.0	0.9992
Methotrexate	0.2~20	88.2~108.5	0.9970
Ceftiofur	0.2~20	94.3~108.1	0.9985
Chlorpromazine	0.2~20	93.5~107.6	0.9981
Betamethasone	0.2~20	91.9~106.2	0.9984

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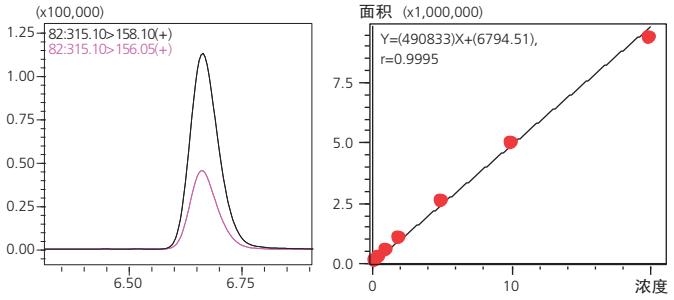
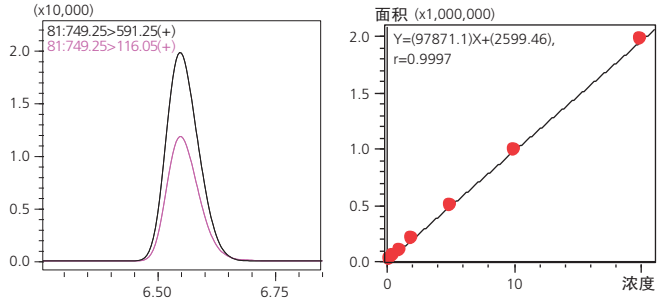
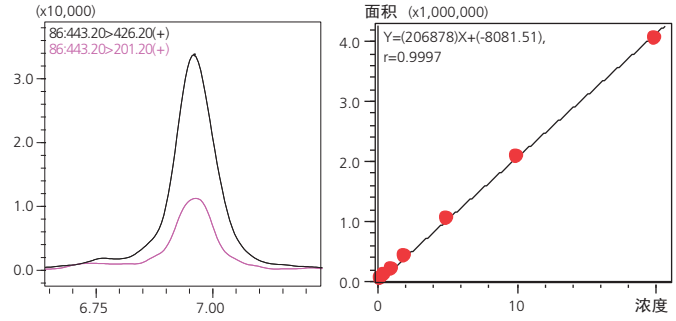
Sulfaphenazole



Azithromycin



Methotrexate



Betamethasone

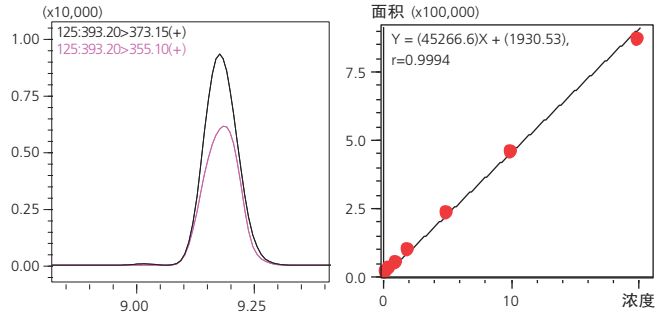


Figure 3. Representative MRM chromatograms and calibration curve (sulfaphenazole, azithromycin, Methotrexate and Betamethasone)

As shown in Figure 4 and 5, the recovery test were carried out using blank pork spiked with all 130 veterinary drugs at levels of 1 µg/kg, 5 µg/kg and 10 µg/kg for 3 replicates. The results indicated that at the levels of 5 µg/kg and 10 µg/kg, more than 55% of the compound recovery rate were between 81 and 120% with RSDs below 10% at the spiking level. Even with a spiked concentration of 1 µg/kg, only 1% of compounds have a recovery greater than 120%.

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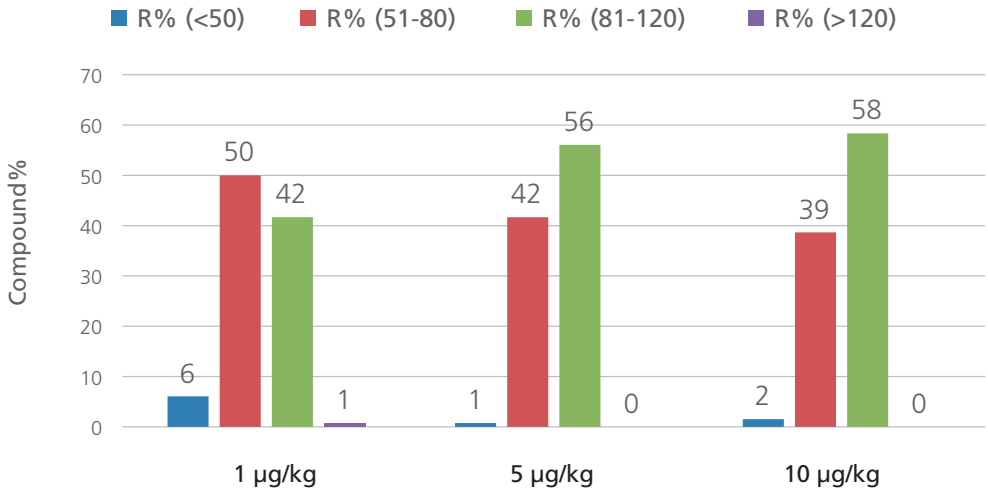

Figure 4. Statistics on the recovery rate of different spiked concentrations in pork

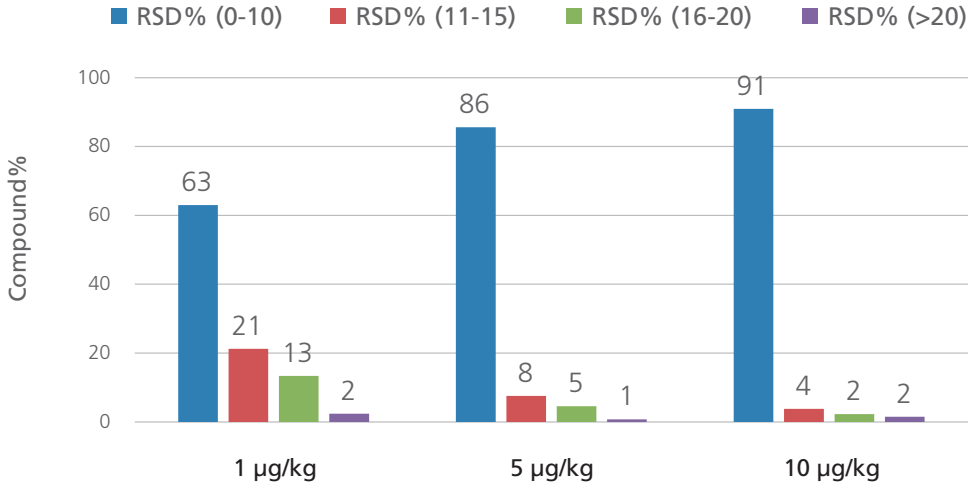

Figure 5. Reproducibility statistics (n=3)

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Conclusions

In this study, a method to detect 130 veterinary drugs in pork was established by LCMS-8050. The method is based on extraction of veterinary drugs from samples using acetonitrile as extraction solvent and the QuEChERS method for sample purification. The analysis was detected with LCMS-8050 under positive and negative electrospray ionization modes at the same time. The results showed that good linearities were obtained with the relative coefficients of the 130 standard solutions greater than 0.992. The inter-day precision (RSD) of retention time and area across three concentrations were 0.03~0.31% and 0.29~10.88%, respectively. The recovery rates in the range of 40.39~121.14% were achieved with added concentration as 1, 5 and 10 ng/mL. All this figures demonstrated that a validation of the high sensitivity, high selectivity, easy automation analytical method could be successfully used for the determination of multiple veterinary drugs residues in animal-derived food samples.

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