Application Brief

Food Testing and Agriculture

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Analyzing 193 Veterinary Drug Residues in Livestock and Poultry Meat

Using Captiva EMR–Lipid HF with LC/MS/MS

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Abstract

This study establishes a method for the simultaneous determination of 193 veterinary drug residues in pork, beef, lamb, and chicken using Agilent Captiva EMR–Lipid HF passthrough cleanup combined with an Agilent 1290 Infinity II LC/6495C triple quadrupole MS (LC/TQ) system. Quantification was based on the use of matrix-matched calibration curves. The results show excellent calibration curve linearity for all targets with $R^2 > 0.99$ for the dynamic range of 0.5 to 100 ng/mL. The limit of quantification (LOQ) was 5 µg/kg and the limit of detection (LOD) was 2 µg/kg. At spiked concentrations of 5 to 200 µg/kg, the average spiked recovery rates for all veterinary drugs were between 50.0 and 120% in four kinds of meat, with < 20% RSDs, except for four veterinary drugs with < 50% recovery. The method was validated for multiresidue analysis of 193 veterinary drugs in livestock and poultry meat.

Introduction

The wide use of veterinary drugs in animal husbandry has generated increased public attention around veterinary drug residues in meat. Veterinary drug residues are regulated in food worldwide, including by the China National Food Safety Standard GB 31650 Maximum Residue Limits for Veterinary Drugs in Food.[1,2] This regulation specifies the maximum residue limits (MRLs) for veterinary drugs in animal-origin foods, and veterinary drugs tolerances that are allowed for use in animals as food sources. The establishment and implementation of the GB standards provide a legal basis for the detection of veterinary drug residues in food in China. Accordingly, the sensitive, reliable, and robust detection method for multiresidue analysis of veterinary drug residues in food is becoming critical for risk monitoring and assessment.

This application was developed based on the multiresidue method for veterinary drugs established by Wang *et al.*[3], targeted on multiclass and multiresidue veterinary drug analysis including quinolones, sulfonamides, β-lactams, macrolides, lincosamides, tetracyclines, amphenicols, benzimidazoles, avermectins, organophosphates, anticoccidials, antivirals, antipyretic analgesics, glucocorticoids, sedatives, and sex hormones, totaling 193 targets. The food matrices that were investigated in this study included popularly consumed livestock and poultry meat including pork, beef, lamb, and chicken. Meat samples were extracted through solvent extraction, and the crude extract was then cleaned up by a passthrough cleanup process using Captiva EMR–Lipid HF cartridges, which provided highly efficient and selective matrix cleanup for fatty meat matrices. The new Captiva EMR–Lipid HF cartridges also improved the sample elution flow. The improved flow allowed the fatty meat crude extract to flow through the cartridge, under gravity, within a reasonable time window. A 6495C LC/TQ system was used for sample analysis, providing the required detection limits.

Experimental

Reagents and food samples

The veterinary drug standard powders or stock solutions were purchased from Manhage (Shanghai) Biotechnology Co., Ltd. A mixed standard stock solution was made at 10 µg/mL in MeOH using individual stock solutions, and stored in a freezer at –20 °C.

McIlvaine-Na_2-EDTA buffer solution was made by dissolving 12.9 g of citric acid, 10.9 g of disodium hydrogen phosphate, and 39.2 g of Na_2-EDTA in 900 mL of water. The pH was adjusted to 5.0 ± 0.2 with 10 mol/L NaOH solution and diluted to 1,000 mL with water.

The pork, beef, lamb, and chicken samples were commercially available products. Fresh meat was cut into small pieces and frozen at –20 °C for 2 hours. The frozen sample chops were ground into fine powder and stored at –20 °C until ready for use.

Instrument and equipment

A 1290 Infinity II LC/6495C triple quadrupole MS (LC/TQ) system was used for the analysis. The chromatographic column used was an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (part number 959758-902).

Sample preparation consumables included Captiva EMR–Lipid HF 300 mg, 3 mL (part number 5610-2235), Agilent Captiva RC syringe filter 0.2 µm, 13 mm (part number 5190-5310), and Agilent ceramic homogenizers for 50 mL centrifuge tubes (part number 5982-9313).

Sample preparation

A meat homogenate sample was extracted using McIlvaine-Na_2-EDTA buffer and acidified acetonitrile (ACN). The sample was vortexed thoroughly and centrifuged. The crude extract was then loaded onto Captiva EMR–Lipid HF 3 mL cartridges for passthrough matrix cleanup. The sample eluent was diluted further with water, filtered, and injected onto the LC/TQ for analysis. The detailed sample preparation procedure is shown in Figure 1.

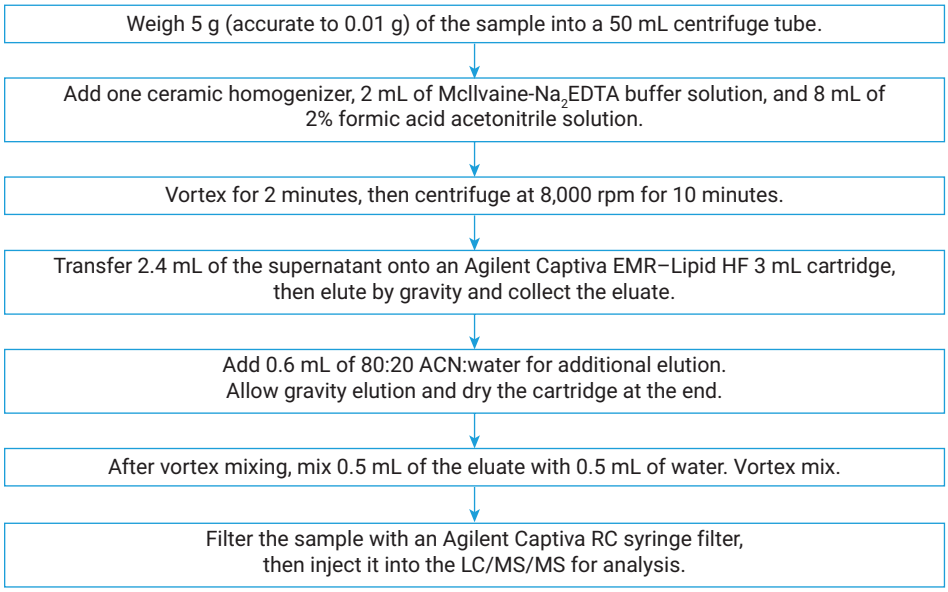

Figure 1. Sample preparation procedure for 193 veterinary drugs in pork, beef, lamb, and chicken.

Instrument method

The samples were run on a 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G7120A), an Agilent 1290 Infinity II multisampler (G7167B), and an Agilent 1290 Infinity II multicolumn thermostat column compartment (G7116B). The UHPLC system was coupled to an Agilent G6495C LC/TQ system equipped with an Agilent Jet Stream electrospray ion source. MassHunter workstation software was used for data acquisition and analysis.

Results and discussion

Calibration curve

Blank samples of pork, beef, lamb, and chicken were used to prepare matrix blank sample extracts for matrix-matched calibration curve standard spiking. A series of matrix-matched standards were then prepared by spiking standard spiking solutions at concentrations of 0.5, 1, 2, 10, 20, 50, and 100 ng/mL in a matrix blank extract. These matrix-matched standards were run on the LC/TQ instrument to generate calibration curves.

The results demonstrated excellent calibration curve linearity for 193 veterinary drugs in pork, beef, lamb, and chicken matrices within a dynamic range of 0.5 to 100 ng/mL, with a few exceptions for the dynamic range of 0.5 to 50 ng/mL. The correlation coefficients (R^2) were all greater than 0.99.

Table 1. HPLC conditions.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phase	A) water with 2 mM ammonium acetate and 0.1% formic acid B) MeOH
Gradient	Time (min) %B 0 5 0.5 5 2.0 15 5.0 30 10.0 40 18.0 70 22.0 100
Post Time	4.0 min

Table 2. MS conditions.

Parameter	Value
Gas Temperature	200 °C
Gas Flow	15 L/min
Nebulizer	30 psi
Sheath Gas Heater	375 °C
Sheath Gas Flow	12 L/min
Capillary	3,500 V (POS) 2,500 V (NEG)
Acquisition Mode	POS/NEG
Fragmentor	166 V
Data Acquisition	MRM as shown in Table 3

Table 3. Selected target analyte MRM conditions. For the complete MRM conditions, refer to Agilent Comprehensive Veterinary Drug dMRM Solution (G5368AA).

No.	Target	RT (min)	Parent Ion (*m/z*)	Product Ions (*m/z*)	CE (V)	Polarity
1	Enrofloxacin	8.49	360.0	342.1/316.2*	20/20	Positive
2	Ciprofloxacin	8.33	332.1	231.0/314.1*	42/20	Positive
3	Ofloxacin	7.65	362.0	318.1/261.1*	15/26	Positive
4	Norfloxacin	8.01	320.0	276.1/302.1*	15/20	Positive
5	Sulfamethazine	7.91	279.1	156.1/186.1*	16/15	Positive
6	Sulfamerazine	6.78	265.1	172.0/156.0*	21/10	Positive
7	Sulfathiazole	5.99	256.0	108.0/156.0*	21/10	Positive
8	Sulfadiazine	5.63	251.1	92.0/156.0*	10/38	Positive
9	Trimethoprim	7.08	291.1	123.0/230.1*	25/25	Positive
10	Cephalexin	7.94	348.0	140/158.0*	22/6	Positive
11	Cefaclor	7.42	368.0	106/174.0*	20/10	Positive
12	Cefapirin	5.84	424.0	152/292.0*	20/10	Positive
13	Cefodizime	12.78	585.0	262/304.0*	35/20	Positive
14	Tetracycline	8.27	445.2	427.1/410.0/154.0*	8/16/30	Positive
15	Oxytetracycline	8.6	461.2	443.1/426.0*	16/8	Positive
16	Chlortetracycline	11.89	479.1	462.0/444.0/154.0*	16/19/36	Positive
17	Doxycycline	13.84	445.1	154.0/321.0/428.0*	35/33/15	Positive
18	Avermectin B1	20.03	895.5	449.2/751.4*	50/45	Positive
19	Ivermectin	20.61	897.4	329.2/753.3*	60/46	Positive
20	Eprinomectin	19.88	936.4	490.3/352.2*	59/65	Positive
21	Doramectin	20.29	921.4	449.2/777.4*	55/45	Positive
22	Chlorpromazine	16.7	319.2	246.0/86.0*	20/15	Positive
23	Promethazine	15.32	285.1	71.1/86.1*	48/16	Positive
24	Diazepam	15.32	285.1	154/193.0*	25/32	Positive
25	Hydrocortisone	16.4	363.1	105.1/121.0*	55/30	Positive
26	Dexamethasone	17.14	393.1	147/355.0*	30/10	Positive
27	Testosterone	18.14	289.1	109/97.0*	35/10	Positive
28	Methyltestosterone	18.45	303.1	109.0/97.0*	30/35	Positive
29	Estradiol	17.46	255.0	133/159.0*	35/35	Positive
30	Estrone	17.48	271.0	91.0/77.0*	20/25	Positive
31	Progesterone	18.94	315.1	109.0/97.0*	55/65	Positive
32	Aldosterone	16.52	361.1	283/343.0*	30/35	Positive

* Quant ion

Method detection limit (MDL) and LOQ

Spiked matrix samples at different concentrations were prepared using the developed method. The LOD and LOQ of the method were determined based on the signal-to-noise ratio (S/N).

When the spiked concentration was 5 µg/kg, the S/N of the quantification ion chromatographic peak was greater than 10. Recovery and precision results satisfied the national standards criteria, except for chlorpromazine in beef and lamb, where the recovery was < 40%. Therefore, the LOQs for the 193 drugs were determined as 5 µg/kg. Similarly, the LOD was determined to be 2 µg/kg, given an S/N ≥ 3.

Recovery and precision

Three spiking levels samples in meat at 5, 100, and 200 µg/kg were prepared in six replicates for pork, beef, lamb, and chicken. The prepared matrix-spiked samples were quantified against the matrix-matched calibration curves in the corresponding food matrices. Figures 2A to 2H shows recovery and precision for all 193 targets at 5 µg/kg in four meat matrices (pork, beef, lamb, and chicken), which are classified based on the categories of targeted veterinary drugs.

The results indicate that for the 193 veterinary drugs in pork, beef, lamb, and chicken, acceptable recoveries (50 to 120%) and RSDs (< 20%) were achieved for all 193 targets, except four drugs with < 50% recovery. The exceptions included Cefdinir, Amprolium, Doxycycline, and Chlorpromazine. The MRM overlay chromatogram for the 193 veterinary drugs is shown in Figure 3.


A
Recovery for quinolone drugs
Pork
Beef
Lamb
Chicken
Average recovery % (n = 6)
120
100
80
60
40
20
0
Flumequine
Oxolinic acid
Nalidixic Acid
Enrofloxacin
Ciprofloxacin
Lomefloxacin
Ofloxacin
Norfloxacin
Sarafloxacin
Difloxacin
Danofloxacin
Cinoxacin
Fleroxacin
Gatifloxacin
Gemifloxacin
Marbofloxacin
Moxifloxacin
Orbifloxacin
Sparfloxacin
Tosufloxacin
Pefloxacin
B
Recovery for sulfonamide drugs
Pork
Beef
Lamb
Chicken
Average recovery % (n = 6)
140
120
100
80
60
40
20
0
Sulfamethazine
Sulfamerazine
Sulfapyridine
Sulfamethoxypyrid…
Sulfamethoxazole
Sulfadoxine
Sulfathiazole
Sulfamethizole
Sulfisoxazole
Sulfabenzamide
Sulfaphenazole
Sulfadiazine
Sulfaquinoxaline
Sulfachloropyridazi…
Sulfameter
Sulfisomidine
Sulfamonomethoxine
Sulfadimethoxine
Sulfanitran
Sulfapyrazole
Trimethoprim
Diaveridine
C
Recovery for β-lactam drugs
Pork
Beef
Lamb
Chicken
Average recovery % (n = 6)
140
120
100
80
60
40
20
0
Amoxicillin
Ampicillin
Penicilline G
Cloxacillin
Mecillinam
Pivampicillin
Penicillin V
Oxacillin
Dicloxacillin
Azlocillin
Methicillin
Phenethicillin
Piperacillin
Nafcillin
Sultamicillin
Cephalexin
Cefaclor
Cefapirin
Cefetamet…
Cefadroxil
Cefodizime
Cefathiamidine
Cefdinir
D
Recovery for anticoccidial and benzimidazole drugs
Pork
Beef
Lamb
Chicken
Average recovery % (n = 6)
140
120
100
80
60
40
20
0
Halofuginone
Clopidol
Amprolium
Robenidine
Decoquinate
Diclazuril
Ponazuril
Fenbendazole
Fenbendazole sulfone
Flubendazole
2-Amino-…
Mebendazole
5-…
Mebendazole-amine
Albendazole
Albendazole-Sulfone
Albendazole-Sulfoxide
Oxfendazole
Thiabendazole
Cambendazole
Oxibendazole

Figure 2. Recoveries of 193 veterinary drugs in pork, beef, lamb, and chicken. The sample spiking level was 5 μg/kg.

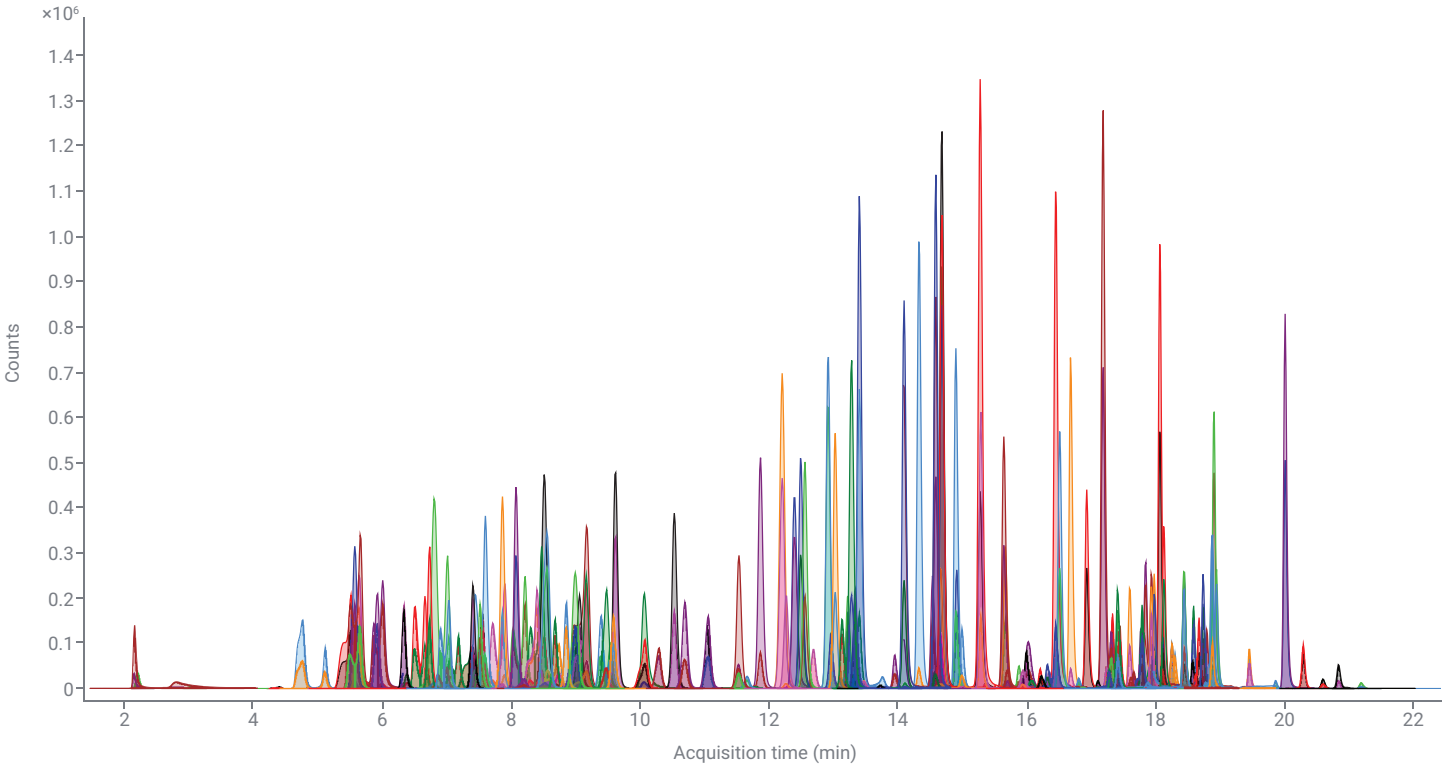

Figure 3. MRM chromatogram of 193 veterinary drugs (5 ng/mL).

Conclusion

This study used Agilent Captiva EMR–Lipid HF cartridges for matrix passthrough cleanup of meat samples after sample preliminary extraction. Captiva EMR–Lipid HF cartridges provided highly efficient and selective matrix removal without compromise in veterinary drug target recovery. The method demonstrated a simplified sample preparation method, saving time and effort. Combined with the high sensitivity provided by an Agilent 1290 Infinity II LC/6495C triple quadrupole mass spectrometry (LC/TQ) system, the method was validated for the analysis of 193 veterinary drugs in pork, beef, lamb, and chicken matrices using matrix-matched calibration curves.

References

1. GB 31650-2019 National Food Safety Standard: Maximum Residue Limits for Veterinary Drugs in Food.
2. GB 31650.1-2022 National Food Safety Standard: Maximum Residue Limits for 41 Veterinary Drugs in Food.
3. Wang, Y. L.; Ye, N.; Yin, H.; *et al*. Detection of 199 Drugs and Metabolites Residues in Livestock and Poultry Meat by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Chinese Journal of Veterinary Drugs* **2024**, *58(04)*, 50–61.

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