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APPLICATION NOTE 21904

Screening of veterinary drug residues in animal-derived foods with a Q Exactive Focus LC/MS system

Authors

Qian Peng, Bing Chen, Teng Guo
Thermo Fisher Scientific, Shanghai, China

Keywords

HyperSep Retain PEP SPE, Accucore VDX column, Q Exactive Focus, Orbitrap, veterinary drugs, drug residues, meat

Goal

To develop an SPE-LC/MS method for rapid screening and confirmation of 194 multi-class veterinary drug residues in animal-derived foods

Introduction

Veterinary drugs include quinolones, β-receptor agonists, sulfonamides, macrolides, hormones, chloramphenicol, cephalosporins, and penicillins. The simultaneous analysis of multi-class veterinary drugs in animal-derived foods reported in relevant standards and literature is still rare. The main reason for this is that there are hundreds of veterinary drugs and their metabolites, consisting of both water-soluble compounds with strong polarity and non-polar fat-soluble compounds with extremely different chemical properties. In addition, meat samples are a complex matrix, containing protein (15–25%), fat (5–25%), and phospholipids (1–3%). The development of an effective, rapid, and high-throughput sample preparation method for simultaneous screening and confirmation of multi-class veterinary drugs using LC-MS/MS is needed. A complete and reliable analytical method is imperative for residue screening of multi-class veterinary drugs in meat products to improve food safety supervision and to protect the public health and safety.

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Thermo Scientific™ HyperSep™ Retain PEP SPE is a porous polystyrene divinylbenzene (DVB) material with functionalized group modification, large capacity, and high purity. It can remove most of the protein, fat, and phospholipid interference in meat samples by a rapid purification method. The sample pretreatment process is simple, convenient, and fast. At the same time, it can effectively reduce the ion suppression in mass spectrometry, making the experimental data more stable and reliable, prolonging the life of the separation column, and reducing the maintenance cost of the instrument. Based on Core Enhanced Technology, Thermo Scientific™ Accucore™ VDX columns provide superior peak shape, fast separation, and high capacity. They offer slightly lower retention than conventional, fully porous C18 columns with a higher carbon load. The fast mass transfer in the core enhanced layer produces sharp and symmetric peaks–ideal conditions for sensitive mass detection.

This application note describes an LC-MS/MS method for rapid screening and confirmation of common veterinary drug residues in animal-derived foods. Acidified acetonitrile/water is used to extract and precipitate proteins. The HyperSep Retain PEP SPE cartridge is used for rapid and effective sample cleanup. The Accucore VDX column can separate various veterinary drugs effectively and the Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer can screen and confirm multi-class veterinary drug residues in the sample. The experimental results show that the detection rate of 194 veterinary drug residues in the matrix is greater than 84% when the concentration is 1.0 µg/kg and above 92% when the concentration is 5.0 µg/kg.

Experimental

Sample preparation and extraction

A 5 g sample was weighed into a 50 mL centrifuge tube, and 10 mL of water was added and mixed well. Then 5 mL of acetonitrile was added, and the mixture was vortexed for 5 min to be dispersed and mixed. Then the solution was centrifuged at 12,000 rpm for 5 min at 4 ºC. The supernatant was transferred to another 50 mL centrifuge tube, and the remaining portion was further extracted with an additional 5 mL of ACN. Both of the extracts were combined and mixed. Then, 4 g of Na_2SO_4 and 1 g of NaCl were added into the mixture, and the mixture was further vortexed. The solution was centrifuged at 6000 rpm for 4 min. Finally, 3 mL of supernatant was removed to be purified.

SPE step

A HyperSep Retain PEP SPE column, 60 mg 3 mL (P/N 60107-203), was used for solid phase extraction.

Equilibrate/condition:	3 mL methanol, 3 mL water
Sample load:	1 mL of the supernatant was passed through the column and discarded.
Collection:	2 mL of the supernatant was passed through the SPE column and collected.

The collected solution was blown to near dryness by nitrogen and brought to volume (1 mL) with pure water. Then it was centrifuged at 15,000 rpm for 5 min, and the supernatant was taken for mass spectrometry analysis.

Liquid chromatography method

A generic LC method was used for all samples.

Instrumentation:	Thermo Scientific™ UltiMate™ 3000 Rapid Separation LC (RSLC)
Column:	Thermo Scientific™ Accucore™ VDX 100 × 2.1 mm, 2.6 µm particle size (P/N VDX-102130)
Mobile phase A:	Positive: water + 0.1% formic acid; Negative: water + 0.1% ammonium hydroxide
Mobile phase B:	Positive: acetonitrile + 0.1% formic acid; Negative: acetonitrile 0.1% ammonia
Flow rate:	300 µL/min
Injection volume:	5 µL
Column temperature:	30 °C

Table 1. Gradient conditions

Time (min)	MP A	MP B	Flow (µL/min)
0.00	95	5	300
15.0	5	95	300
17.0	5	95	300
17.1	95	5	300
20.0	95	5	300

Mass spectrometry method

A generic full-scan method with data-dependent MS2 acquisition (FS-DDA) was used for all samples.

Parameter name	Setting
Spray voltage:	3500/3000 v (±)
Heater temperature:	400 °C
Capillary temperature:	350 °C
Sheath gas:	40 arb
Aux gas:	5 arb
Sweep gas:	1
Full Scan resolution:	70,000
Full Scan mass range:	100–1000 *m/z*
ddMS2 resolution:	17,500
MS isolation:	2.0 *m/z*

Software

Thermo Scientific™ TraceFinder™ software was used for data analysis.

Results and discussion

Figure 1 displays chromatograms of selected compounds from 5 ppb spiked pork.

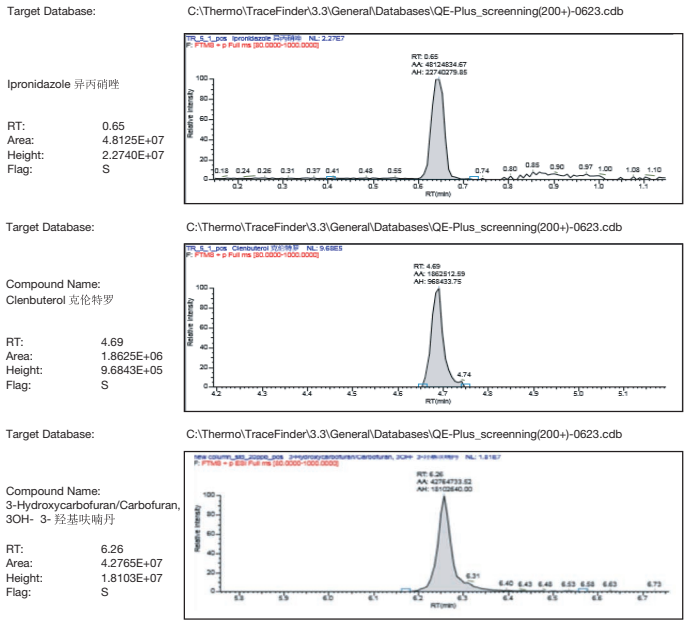

Figure 1. Example chromatograms

The detection rate of 194 veterinary drugs was measured (Figure 2). The results show that the detection rate of all compounds in the matrix was >84% when the concentration was 1.0 µg/kg. The detection rate of all compounds in the matrix was >92% when the concentration was 5.0 µg/kg.

The 194 veterinary drugs were added to the matrix of pork, pig liver, chicken, duck, etc. The recovery percentage of spiked concentrations of 1.0 µg/kg and 5.0 µg/kg was between 50 and 120%. The recovery data results for some of the compounds are listed in Table 2.

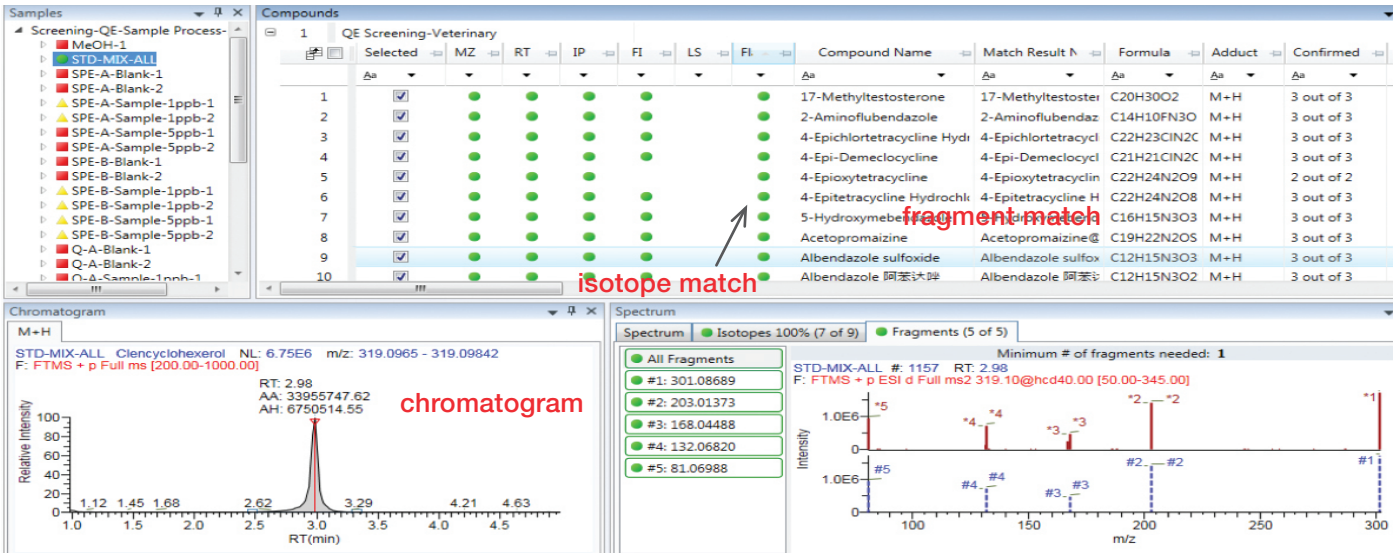

Figure 2. Screening chromatogram in TraceFinder software. A compound is confirmed with RT, isotope match, and fragment match.

Table 2. The average recovery data across pork, chicken, and duck. Recovery is calculated as follows: Recovery% = Peak area of spiked matrix sample undergoing extraction/Peak area of neat solution.

Compound	Type	Value of additional 1 ppb	Recovery % of additional 1 ppb	Value of additional 5 ppb	Recovery % of additional 5 ppb
17-Methyltestosterone	Hormone	0.68	68%	3	60%
Nandrolone	Hormone	1.1	110%	3	60%
Nandrolone-17-propionate	Hormone	0.5	50%	3.6	72%
Labetalol	Stimulant	0.52	52%	3.3	66%
Penbutolol	Stimulant	0.62	62%	3.3	66%
Phenylethanolamine A	Stimulant	0.54	54%	3	60%
Salmeterol	Stimulant	0.6	60%	3.9	78%
Terbutaline	Stimulant	0.5	50%	4.6	92%
Ciprofloxacin	Quinolone	0.55	55%	3.6	72%
Danooxacin	Quinolone	0.6	60%	3.3	66%
Difloxacin	Quinolone	0.56	56%	3.1	62%
Enoxacin	Quinolone	0.64	64%	3.8	76%
Enrofloxacin	Quinolone	0.51	51%	3	60%
Norfloxacin	Quinolone	0.6	60%	3.6	72%
Pefloxacin	Quinolone	0.59	59%	3.03	61%
Pipemidic acid	Quinolone	0.6	60%	4	80%
Azaperol	Sedative and anticonvulsant	0.53	53%	3.1	62%
Azaperone	Sedative and anticonvulsant	0.55	55%	3.3	66%
Albendazole-2-aminosulfone	Benzimidazole	0.72	72%	4.3	86%
Roxithromycin	Macrolide	0.54	54%	3.2	64%
Nafcillin	Penicillin	0.5	50%	3.9	78%
4-Epichlortetracycline hydrochloride	Tetracycline			3.3	66%
Methylprednisolone	Glucocorticoid	0.8	80%	3	60%
Thiamphenicol	Amphenicol	0.69	69%	4	80%
Difurazone	Nitrofuran	0.65	65%	3.7	74%
Dimetridazol	Nitrofuran	1	100%	5.5	110%
Diaveridine	Sulfonamide	0.61	61%	3.2	64%
Propylthiouracil	Thyroxine	0.63	63%	3.1	62%
Meloxicam	Anti-Inflammatory	0.63	63%	3.7	74%
Halofuginone	Anticoccidiol	0.5	50%	3.9	78%
Praziquantel	Antischistosomal	0.51	51%	3	60%
2-Aminoflubendazole	Other	0.74	74%	4.2	84%
Buquinolate	Other	0.7	70%	4.64	93%
Nequinate	Other	0.6	60%	3.9	78%
Premarin	Other	0.82	82%	3.2	64%

Conclusions

A method for rapid screening and confirmation of 194 multi-class veterinary drug residues in animal-derived food using SPE sample preparation and the Q Exactive LC/MS system has been developed. The detection rate of all compounds in the matrix was >84% when the concentration was 1.0 µg/kg and >92% when the concentration was 5.0 µg/kg. Compound recovery was between 50 and 150%.

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