

Multiclass Residue Analysis of Veterinary Drugs in Pork and Hen Eggs Using an Agilent Ultivo Triple Quadrupole LC/MS System

Authors

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Introduction

Veterinary drugs, consisting mainly of antimicrobials and hormones, have been widely used to treat or prevent diseases and enhance growth and feed efficiency. There have been world-wide concerns that veterinary drug residues remain in the products of animal origin at the time of consumption that can cause allergic reactions and antibiotic resistance. To protect public health, the US FDA published final rules to regulate antimicrobial drug usage with the Veterinary Feed Directive in June 2015¹. The EU regulation is described in legislative proposal 2014/0257(COD)², and would repeal and replace Directive 2001/82/EC. The new regulation aims to address the goals determined by the impact assessment, and address the public health risk of antimicrobial resistance. In China, to set maximum veterinary drug limits in animal-derived foods³, the Minister of Agriculture published Announcement 235³. Depending on analytes and matrices, the accurate quantitation requirements range from parts-per-trillion to parts-per-billion levels. Due to the complexity of the matrices and characteristics of different classes of veterinary drugs, such as hydrophobicity and stability under different pH, it is challenging to detect multiple classes of analytes in one run. A typical standard method covers one class or a small cohort of similar classes of veterinary drugs. To effectively monitor veterinary drugs and protect public health, the ability to detect multiple classes of analytes in one run is highly desired.

The Agilent Ultivo triple quadrupole LC/MS system fits well in the scheme. The Ultivo is designed to address many challenges faced by routine production labs, especially in the environmental and food safety arenas. Several revolutionary innovations are incorporated into the system, making it much smaller than previous generations of triple quadrupole systems, without sacrificing performance. Superior ion transmission efficiency is achieved with the dual hexapole design. Polarity switching becomes faster with hardware and electronics improvements. Moreover, Ultivo reduces the need for user intervention for system maintenance, making the system operation and maintenance manageable for nonexpert MS users with the implementation of VacShield and removable detector.

This Application Note demonstrates an analytical screening method that could analyze 151 veterinary drugs across 27 classes in pork and hen eggs using an Ultivo Triple Quadrupole LC/MS in combination with an Agilent 1290 Infinity II UHPLC. We also demonstrate the wide applicability of the Agilent EMR—Lipid sample preparation kit. EMR—Lipid efficiently removed high-lipid contents from matrices of animal origin, and provided a fair recovery for the veterinary drugs.

Experimental

Standards and Reagents

Veterinary drug standards were purchased from Dr. Ehrenstorfer GmbH, WITEGA laboratorien Berlin-Adlershof GmbH, Toronto Research Chemicals (TRC) or AccuStandard, Inc. Table 1 lists the 151 compounds covered in this solution. Ultrapure water (>18.2 M Ω , Milli-Q water system), acetonitrile (LC-MS grade, Fluka) and formic acid (~98 %, for mass spectrometry, Fluka) were used for mobile phase preparation. Acetonitrile (HPLC grade, Sigma-Aldrich), dimethyl sulfoxide (DMSO, >99.9 %, Aldrich), ammonium acetate (\geq 98 %, Sigma-Aldrich) and formic acid (~98 %, HPLC grade, Fluka) were used for sample preparation.

110871-86-8

Sparfloxacin

Table 1. 151 Veterinary drugs analyz	zed
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Classification	Name	CAS	Classification	Name	CAS
	Cimaterol	54239-37-1		Beclomethasone	4419-39-0
	Clenbuterol	21898-19-1		Betamethasone	378-44-9
	Clorprenaline	3811-25-4		Dexamethasone	50-02-2
	Penbutolol	38363-40-5		Flumethasone	2135-17-3
β-Agonists	Propanolol	318-98-9		Hydrocortisone	50-23-7
	Ractopamine	90274-24-1	Glucocorticosteroids	Methylprednisolone	83-43-2
	Salbutamol	18559-94-9		Prednisolone	50-24-8
	Terbutaline	23031-32-5		Prednisone	53-03-2
	Tulobuterol	56776-01-3		Triamcinolone	124-94-7
	Mengestrol Acetate	595-33-5		Triamcinolone acetonide	76-25-5
Androgono	Methyltestosterone	58-18-4		Acetylisovaleryltylosin/tylvalosin	63409-12-1
Androgens	Nadrolone/19-nortestosterone	434-22-0		Erythromycin	59319-72-1
	Testosterone	58-22-0	Manualistan	Kitasamycin/eucomycin	1392-21-8
	2-Aminoflubendazole	82050-13-3	Macrolldes	Oleandomycin	7060-74-4
	5-Hydroxymebendazole	60254-95-7		Tilmicosin	108050-54-0
	Albendazole	54965-21-8		Tylosin	74610-55-2
	Albendazole sulfone	75184-71-3		Dimetridazole	551-92-8
	Albendazole sulfoxide	54029-12-8		Dimetridazole-OH (HMMNI)	936-05-0
	Albendazole-2-aminosulfone	80983-34-2	Nitroimidazoles	Metronidazole	443-48-1
	Cambendazole	26097-80-3		Metronidazole-OH	4812-40-2
	Fenbantel	58306-30-2		Ronidazole	7681-76-7
	Fenbendazole	43210-67-9	Peptides	Virginiamycin M1	211411-53-0
Benzimidazoles	Flubendazole	31430-15-6		Ciprofloxacin	85721-33-1
	Mebendazole	31431-39-7		Danofloxacin	112398-08-0
	Mebendazole-amine	52329-60-9		Difloxacin	98106-17-3
	Oxfendazole	53716-50-0		Enoxacin	74011-58-8
	Oxfendazole sulfone/	54029-20-8		Enrofloxacin	93106-60-6
	fenbendazole sulfone	04027200		Fleroxacin	79660-72-3
	Oxibendazole	20559-55-1		Flumequine	42835-25-6
	Thiabendazole	148-79-8		Lomefloxacin	98079-51-7
	Thiabendazole-5-hydroxy	948-71-0	Quinciones	Nalidixic acid	389-08-2
	Triclabendazole	68786-66-3		Norfloxacin	70458-96-7
Chloramphenicols	Chloramphenicol	56-75-7		Ofloxacin	82419-36-1
	Florfenicol	73231-34-2		Orbifloxacin	113617-63-3
	Thiamphenicol	15318-45-3		Oxolinic acid	14698-29-4
				Pefloxacin	70458-92-3
				Sarafloxacin	98105-99-8

Classification	Name	CAS
	Sulfabenzamide	127-71-9
	Sulfacetamide	144-80-9
	Sulfachloropyridazine	80-32-0
	Sulfaclozine	102-65-8
	Sulfadiazine	68-35-9
	Sulfadimethoxine	122-11-2
	Sulfadoxine	2447-57-6
	Sulfaguanidine	57-67-0
	Sulfamerazine	127-79-7
	Sulfameter/sulfamethoxydiazine	651-06-9
	Sulfamethazine/sulfadimidine	57-68-1
Sulfonamides	Sulfamethizole	144-82-1
	Sulfamethoxazole	723-46-6
	Sulfamethoxypyridazine	80-35-3
	Sulfamonomethoxine	1220-83-3
	Sulfamoxole	729-99-7
	Sulfaphenazole	526-08-9
	Sulfapyridine	144-83-2
	Sulfaquinoxaline	59-40-5
	Sulfathiazole	72-14-0
	Sulfisomidine	515-64-0
	Sulfisoxazole/sulfafurazole	127-69-5
	Trimethoprim	738-70-5
	Chlortetracycline	57-62-5
Tetracyclines	Doxycycline	564-25-0
	Tetracycline	60-54-8
	Crystal Violet/basic violet 3	548-62-9
Triphenylmethanes	Leucomalachite green	129-73-7
	Malachite green	569-64-2
Diterpene	Valnemulin	133868-46-9
Dhan athianin a	Chlorpromazine	50-53-3
Phenothiazines	Xylazine	7361-61-7
Quinoxalines	Olaquindox	23696-28-8
Trematocides	Nitroxynil	1689-89-0
	Clopidol	2971-90-6
	Diclazuril	101831-37-2
	Ethopabate	59-06-3
	Halofuginone	55837-20-2
A	Nicarbazine	330-95-0
Anticoccidiosis	Robenidine	25875-50-7
	Toltrazuril	69004-03-1
	Toltrazuril sulfone	69004-04-2
	Toltrazuril sulfoxide	69004-15-5
	Zoalene	148-01-6

Classification	Name	CAS
Antivirus	Amantadine	768-94-5
	Carbofuran	1563-66-2
	Chlordimeform	6164-98-3
	Coumaphos	56-72-4
	Dichlorvos	62-73-7
Pesticides	Fenthion sulfoxide	3761-41-9
	Malathion	121-75-5
	Pentachlorophenol (PCP)	131-52-2
	Propetamphos	31218-83-4
	Trichlorfon	52-68-6
Lincosamide	Lincomycin	7179-49-9
Democraci	Dapson	80-08-0
Dapsones	N-Acetyl dapsone	565-20-8
Organia agida	Heptadecafluorooctanesulfonic acid (PFOS)	1763-23-1
Organic acids	Perfluorooctanoic acid (PFOA)	335-67-1
	4,6-Dinitro-o-cresol (DNOC)	534-52-1
Nitros	Nitrovin	2315-20-0
	Sodium nitrophenolate/4-nitrophenol	100-02-7/63317-67-9
Tranquilizor	Azaperol	2804-05-9
	Azaperone	1649-18-9
	4-Acetylamino antipyrine	83-15-8
	4-Formylaminoantipyrine	1672-58-8
	Flufenamic acid	530-78-9
	Flunixin	42461-84-7
N	Indoprofen	31842-01-0
Non-steroid	Ketoprofen	22071-15-4
drugs (NSAIDs)	Mefenamic acid	61-68-7
	Meloxicam	71125-38-7
	Piroxicam	36322-90-4
	Sulindac	38194-50-2
	Tenoxicam	59804-37-4
	Tolmetin	26171-23-3
Parasiticide	Levamisole	14769-73-4
Olaquindox	3-Methyl quinoxaline-2-caboxylic acid	74003-63-7
Olaquinuox	Quinoxaline-2-carboxylic acid	879-65-2

Sample Preparation

- Weigh 5 ±0.05 g of homogenized sample into a 50-mL centrifuge tube (spike standards for calibration samples and quality control samples).
- 2. Add 1.0 mL of water and two ceramic homogenizers (p/n 5982-9313), then mix the samples on a mechanical shaker (for hen eggs, skip this step).
- 3. Add 10 mL of 5 % formic acid in acetonitrile, manually shake vigorously to disperse the samples, then mix the samples on a mechanical shaker for 5 minutes (2,000 rpm).
- 4. Centrifuge at 4,000 rpm for 5 minutes.
- EMR Activation: Add 5.0 mL of 5 mM ammonium acetate buffer (for hen eggs, add 3.0 mL) to a 15-mL EMR—Lipid dSPE tube (p/n 5982-1010), and vortex immediately at least 30–60 seconds (2,000 rpm). Ensure that all EMR sorbent is well hydrated (emulsion formed).
- 6. Transfer 5.0 mL (for eggs, transfer 7.0 mL) of supernatant to the activated EMR—Lipid dSPE tube.
- 7. Vortex immediately for 2 minutes (2,000 rpm).
- 8. Centrifuge at 4,000 rpm for 5 minutes.
- Add two ceramic homogenizers (p/n 5982-9313) into a new 50-mL centrifuge tube, decant the entire extract into it, and add the contents of one polish pouch (p/n 5982-0102), and shake aggressively.
- 10. Vortex immediately for 2 minutes (2,000 rpm).
- 11. Centrifuge at 4,000 rpm for 5 minutes.
- 12. Transfer 2.0 mL of the upper layer into a glass tube, add 50.0 μ L of DMSO, then concentrate to constant volume (40 °C, ~50 μ L) under nitrogen.
- 13. Reconstitute with 0.950 mL of 15 % ACN in water, and vortex for 1 minute (2,000 rpm).
- 14. Transfer the solution to a 2.0-mL centrifuge tube, centrifuge at 10,000 rpm for 5 minutes, then transfer the upper clear solution into an injection vial for LC/MS analysis.

Instrument parameters

Table 2. LC Parameters

	LC Parameters		
Column	Agilent ZORBAX Eclipse plus C18, 3.0 × 150 mm, 1.8 μm (p/n 959759-302)		
Injection volume	15 µL		
Column temperature	40 °C		
Flow rate	0.5 mL/min		
Mobile phase	A) 0.2 % formic acid in water B) 0.2 % formic acid in acetonitrile		
Gradient	Time (min) %A %B 0 98 2 0.5 98 2 1.8 85 15 3.5 80 20 6 75 25 7 70 30 11 65 35 16 0 100 26 0 100		
Run time	26 minutes		
Post run time	4 minutes		
Needle wash	Wash mode: flash port Needle wash solvent: 95 % acetonitrile/water Needle wash time: At least 15 seconds		
MS Parameters			
Ionization mode	Positive/negative		
Scan type	Dynamic MRM		
Gas temperature	200 °C		
Gas flow	11 L/min		
Nebulizer	35 psi		
Sheath gas temperature	275 °C		
Sheath gas flow	11 L/min		
Capillary	3,000 (positive); 3,500 (negative)		
Nozzle voltage	200 (positive); 1,500 (negative)		

Results and Discussion

Detection Sensitivity

A set of nine matrix-spiked calibration standards (0.1 ng/g, 0.2 ng/g, 0.5 ng/g, 1.0 ng/g, 2.0 ng/g, 5.0 ng/g, 10 ng/g, 20 ng/g, and 40 ng/g) were analyzed consecutively. Calibration curves were generated for each analyte with linear fitting, where 87 % of compounds in pork had $R^2 \ge 0.990$, while 89 % of compounds in egg had $R^2 \ge 0.990$.

Figure 1 shows calibration curves obtained in egg for eight compounds that belong to eight different chemical classes: albendazole, beclomethasone, chloramphenicol, tetracycline, oleandomycin, enoxacin, sulfachloropyridazine, and clenbuterol.



Figure 1. Calibration curves of albendazole (A), beclomethasone (B), chloramphenicol (C), tetracycline (D), oleandomycin (E), enoxacin (F), sulfachloropyridazine (G), and clenbuterol (H) in egg on an Agilent Ultivo triple quadrupole LC/MS.

Ultivo showed very high analytical sensitivity. The majority of the compounds could be detected at 2 ng/g spiked level in matrix. For example, 93 % of compounds could be detected in egg with a signal-to-noise ratio (S/N) greater than 10 for both qualifier and quantifier. Figure 2 illustrates a sample chromatogram of 2 ng/g spiked in egg.

Figure 3 shows the percentages of compounds that have a S/N > 10 for both qualifier and quantifier at different concentrations in matrix.



Figure 2. Example chromatogram of an egg sample fortified with 151 veterinary drugs at 2 ng/g.



Figure 3. Percentages of compounds with a S/N >10 for both qualifier and quantifier at different concentration in matrix.

Method Precision

The repeatability at 5 ng/g spike level was investigated with seven replicates. Figure 4 shows the percentage distribution of %RSD at 5 μ g/kg spiking level in pork and hen egg (n = 7). Good repeatability results were achieved; 92 % of compounds had %RSD within 20 %.

8 % 7 % 9 % 76 %

%RSD in egg at 5 μ g/kg concentration

%RSD in pork at 5 µg/kg concentration



Figure 4. Distribution of %RSD in pork and hen egg at $5 \mu g/kg$ spiking level (n = 7).

Conclusions

A quick and easy method was developed to analyze 151 veterinary drugs across 27 classes in pork and hen eggs. The analytical sensitivity, accuracy, and precision proved that the Agilent Ultivo Triple Quadrupole LC/MS is a viable solution for veterinary drugs analysis.

References

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