

Simple and Effective Clean-up for UPLC-MS/MS Determination of Veterinary Drug Residues in Egg

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APPLICATION BENEFITS

- Efficient, timesaving, multiclass/multiresidue methodology
- Simple, rapid, and effective sample clean-up suitable for a diverse range of analytes
- Fast, sensitive UPLC®-MS/MS analysis

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[ACQUITY UPLC® I-Class System](#)

[ACQUITY UPLC Columns](#)

[Xevo® TQ-S Mass Spectrometer](#)

[Oasis® PRiME HLB Cartridge for SPE Clean-up](#)

KEYWORDS

UPLC-MS/MS, Oasis PRiME HLB Cartridges, veterinary drugs, egg

OVERVIEW

In order to insure public health and safety, reliable analytical methods are necessary to determine veterinary drug residue levels in foods. The compounds of interest range from highly polar, water-soluble compounds to very non-polar, fat-soluble compounds. In order to maximize throughput and minimize costs it is desirable to determine the widest possible range of veterinary drug residues in food samples with a single analytical method. Eggs contain significant amounts of proteins, fats, and, lecithin (phospholipids). These components can be detrimental to good instrumental performance and should be reduced or eliminated prior to LC-MS analysis.

INTRODUCTION

Veterinary drugs are used in chicken farms to control diseases of laying hens. However, these compounds can be transferred to and accumulate in the eggs. The presence of veterinary drug residues in eggs is a potential health risk for the consumer because the residual drugs can provoke allergic reactions or induce pathogen resistance to antibiotics used in human medicine.¹ Sixteen representative veterinary drugs from twelve classes, most of which have MRLs established in US or China, were chosen for this study.^{1,2} Figure 1 presents the structures of a subset of these veterinary drugs.

Sample preparation is a challenging task for the multi-residue determination of veterinary drugs in eggs. The analyst must recover a wide variety of drug classes with different physico-chemical properties. Some of the target compounds may bind to proteins or other matrix components. Eggs are among the highest food sources of lecithin (phospholipids) and also have significant amounts of fats; these co-extracted substances can lead to interference and ion suppression in the LC-MS analysis, contamination of the analytical column, and other components of the UPLC system, and contamination of the mass spectrometer itself.

In this work, sample extraction, clean-up, and analysis methods were developed for UPLC-MS/MS determination of a wide variety of veterinary drugs in eggs. Samples were treated with an acidified acetonitrile/water solvent to precipitate proteins, release bound residues, and to extract the veterinary drugs of interest. Then, to remove fats and phospholipids, a simple pass-through clean-up was performed using a novel SPE device, the Oasis PRiME HLB Cartridge.

STANDARD COMPOUNDS

Sixteen veterinary drugs from different classes were chosen for this study. Table 1 lists their formulas, MWs, and MRLs established in USA or China.

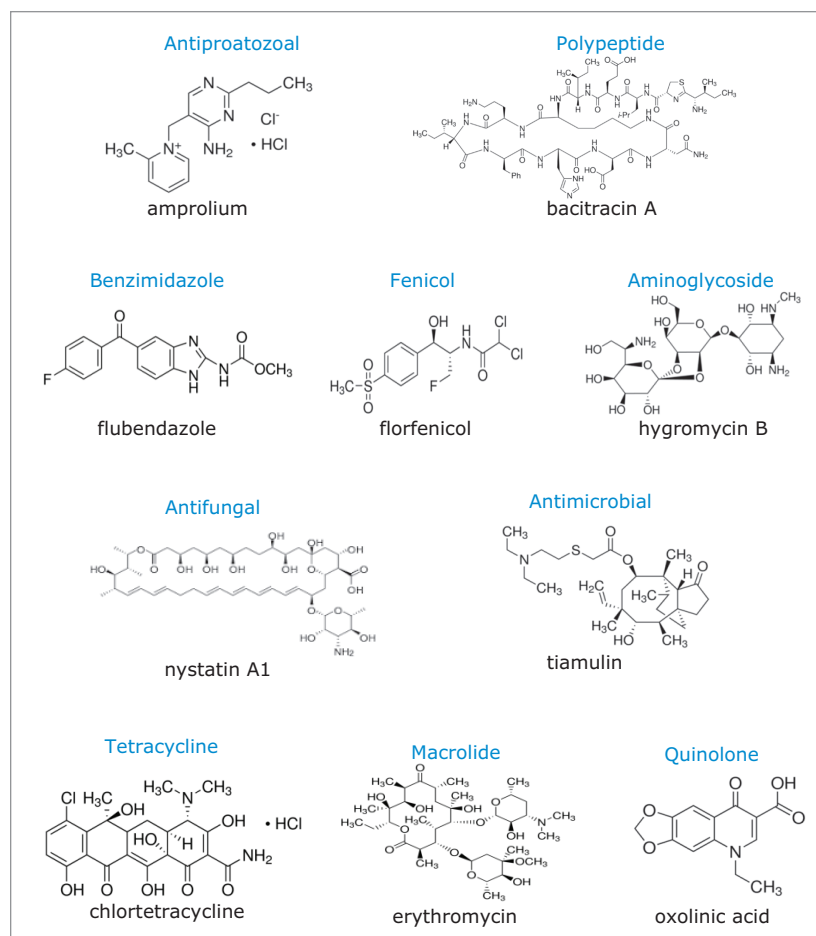


Figure 1. Structures of representative compounds from this study.

Compounds	Formula	Monoisotopic MW	Market	MRL (ng/g)
Amprolium	C ₁₄ H ₁₉ ClN ₄	278.130	USA	4000
Bacitracin A	C ₈₆ H ₁₀₃ N ₁₇ O ₁₆ S	1421.749	USA/China	500
Hygromycin in B	C ₂₀ H ₃₇ N ₃ O ₁₃	527.233	USA/China	No Residue Allowed
Nystatin A1	C ₄₇ H ₇₅ NO ₁₇	925.503	USA	No Residue Allowed
Colistin B	C ₅₂ H ₉₈ N ₁₆ O ₁₃	1154.750	China	300
Florfenicol	C ₁₂ H ₁₄ Cl ₂ FNO ₄ S	357.000	China	No Residue Allowed
Flubendazole	C ₁₆ H ₁₂ FN ₃ O ₃	313.086	China	400
Oxolinic Acid	C ₁₃ H ₁₁ NO ₅	261.063	China	50
Tiamulin	C ₂₈ H ₄₇ NO ₄ S	493.323	China	1000
Chlortetracycline	C ₂₂ H ₂₃ ClN ₂ O ₈	478.114	USA/China	400/200
Erythromycin	C ₃₇ H ₆₇ NO ₁₃	733.461	USA/China	25/150
Lincomycin	C ₁₈ H ₃₄ N ₂ O ₆ S	406.214	China	50
Oxytetracycline	C ₂₂ H ₂₄ N ₂ O ₉	460.148	China	200
Penicillin G	C ₁₆ H ₁₈ N ₂ O ₄ S	334.099	USA	No Residue Allowed
Tetracycline	C ₂₂ H ₂₄ N ₂ O ₈	444.153	China	200
Tylosin	C ₄₆ H ₇₇ NO ₁₇	915.519	USA/China	200

Table 1. Veterinary drugs in this study (Bacitracin, Colistin, and Nystatin all contain a mixture of more than two components; one major component was chosen for analysis).

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C ₁₈ , 2.1 x 100 mm, 1.7 µm (p/n 186003555)
Column temp.:	30 °C
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	The initial composition was 85% A and 15% B. Phase B was increased linearly to 40% in the first 2.5 min, and then linear ramp to 95% B in 1.4 min, maintained for 2.3 min, then returned to the initial composition and equilibrated for 2 min.

MS conditions

System:	Xevo TQ-S
Ionization mode:	ES+ (ES-for Florfenicol)
Capillary voltage:	3.00 kV (2.50 kV for negative mode)
Source temp.:	150 °C
Desolvation temp.:	600 °C
Cone gas flow:	150 L/hr
Desolvation gas flow:	1000 L/hr
Collision gas flow:	0.15 mL/min
Nebulizer gas flow:	7.00 Bar

Compounds	MRM Transition 1				MRM Transition 2		
	Precursor ion (m/z)	Product ion (m/z)	Cone (V)	Collision (eV)	Product ion (m/z)	Cone (V)	Collision (eV)
Amprolium	243.26	94.06	20	14	150.17	20	12
Bacitracin A	712.22	199.10	68	40	110.10	68	70
Hygromycin B	528.49	352.20	48	22	177.14	48	32
Nystatin A1	926.82	297.24	22	28	107.13	48	60
Colistin B	578.66	101.07	64	28	86.06	64	40
Florfenicol	356.03	335.96	52	10	184.94	52	22
Flubendazole	314.25	282.19	90	18	123.08	90	36
Oxolinic add	262.20	244.20	20	12	160.17	50	32
Tiamulin	494.45	119.10	40	42	192.17	40	20
Chlortetracycline	479.27	444.19	12	18	154.06	12	26
Erythromycin	734.72	158.08	48	26	576.52	48	18
Lincomycin	407.20	126.10	40	34	359.30	40	20
Oxytetracycline	461.36	426.22	20	18	201.07	64	36
Penicillin G	335.27	176.05	14	20	159.99	14	16
Tetracycline	445.30	410.20	40	21	154.00	40	26
Tylosin	916.88	174.13	80	36	101.10	45	45

Table 2. MRM transition parameters for 16 veterinary drugs.

Compounds	RT (min)	LOD (ng/g)	Linear range (ng/g)	R ²
2 Amprolium	0.61	0.5	80–40,000	0.998
9 Bacitracin A	2.52	1	10–5,000	0.992
1 Hygromycin B	0.48	4	4–1,000	0.990
15 Nystatin A1	3.30	10	40–1,000	0.992
4 Colistin B	1.73	30	90–600	0.990
10 Florfenicol	2.69	4	4–1,000	0.991
14 Flubendazole	3.29	0.5	8–240	0.993
11 Oxolinic acid	2.83	1	1–500	0.993
16 Tiamulin	3.88	0.5	20–1,000	0.990
8 Chlortetracycline	2.49	0.5	4–2,000	0.995
12 Erythromycin	2.95	0.5	0.5–250	0.995
3 Lincomycin	1.59	0.5	1–500	0.996
5 Oxytetracycline	1.89	0.5	4–2,000	0.995
6 Penicillin G	1.91	1	2–1,000	0.991
7 Tetracycline	2.04	0.5	4–2,000	0.994
13 Tylosin	3.06	0.5	20–800	0.991

Table 3. UPLC-MS retention times and calibration data.

Sample Preparation

Extraction: Two grams of homogenized whole chicken eggs were weighed into a 50 mL polypropylene centrifuge tube. Recovery samples were fortified with the appropriate amount of standards before 8 mL of 0.2% formic acid in 80:20 acetonitrile/water were added. The samples were vortexed for 30 s, placed on a mechanical shaker for 30 mins, and then centrifuged at 4500 rpm for 10 min. An aliquot of the supernatant was taken for the SPE clean-up.

Pass-through SPE clean-up: An Oasis PRiME HLB 3 cc Vac Cartridge, 60 mg, ([p/n 186008056](#)), was mounted on a precleaned vacuum manifold. Cartridge conditioning is not required and was not performed. A 0.5 mL aliquot of the supernatant was passed through the cartridge and collected using 1~2 psi vacuum. 0.2 mL of the collected extract was taken and diluted to 0.6 mL with aqueous 10 mM ammonium formate buffer (pH 4.5) prior to UPLC-MS/MS analysis. Figure 2 shows a typical chromatographic separation obtained for a matrix-matched standard.

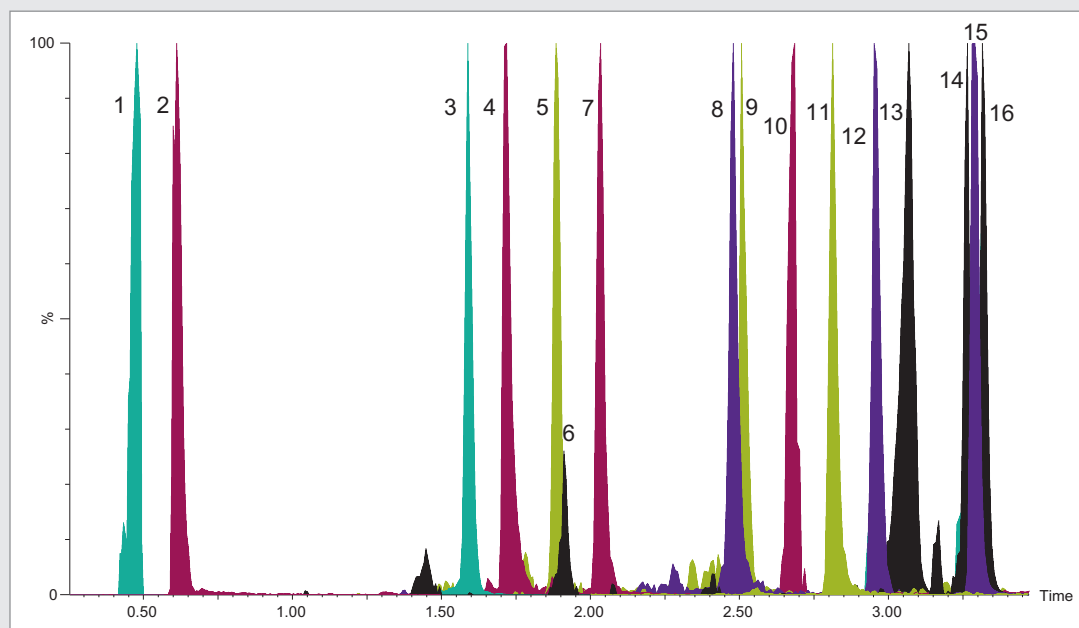


Figure 2. Overlay of MRM chromatograms of 16 veterinary drugs (matrix-matched standard at MRL level).

RESULTS AND DISCUSSION

OASIS PRiME HLB CARTRIDGE PASS-THROUGH CLEAN-UP

The Oasis PRiME HLB Cartridge was evaluated with respect to analyte recovery and phospholipids removal from egg matrix. The total method recoveries ranged from 50–97%. However, the Oasis PRiME HLB Cartridge clean-up contributes little to any method recovery losses. As shown in Figure 3, the measured recovery for the SPE clean-up step is better than 80% for all compounds, with recovery for most compounds greater than 90%.

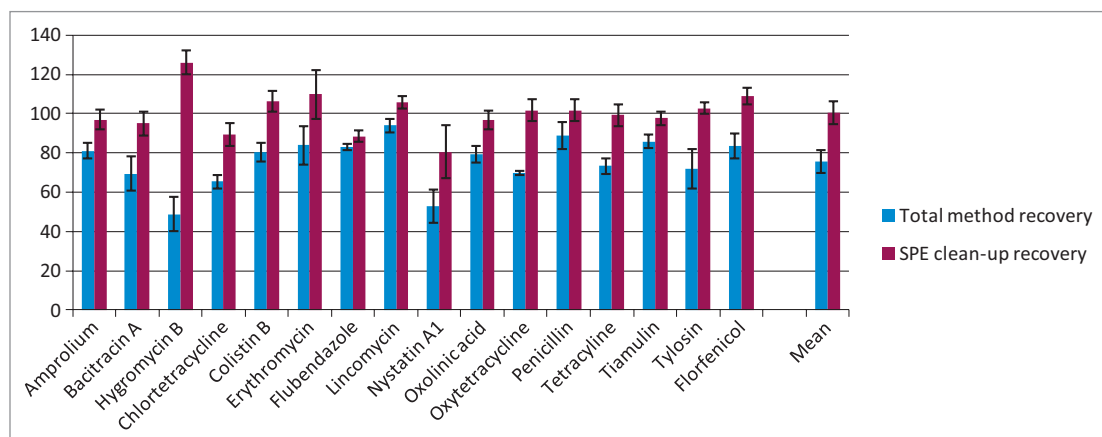


Figure 3. Recovery data for target veterinary drugs obtained using the Oasis PRiME HLB Cartridge clean-up procedure (at MRL level).

Whole eggs contain significant amounts of fat and are among the highest sources of dietary lecithin (phospholipids). The total lipid content of chicken egg is about 11% by weight (excluding the shell) and the phospholipids content is about 0.35%.⁴ Significant amounts of these potential interfering substances are extracted along with the target drugs in the initial sample preparation extraction step. Greater than 84% of total lipids were removed from the egg extract after pass-through clean-up with the Oasis PRiME HLB Cartridge. The clean-up step was even more effective for removal of phospholipids. Figure 4 shows that the Oasis PRiME HLB Cartridge clean-up removed greater than 95% of phospholipids from the egg extract.

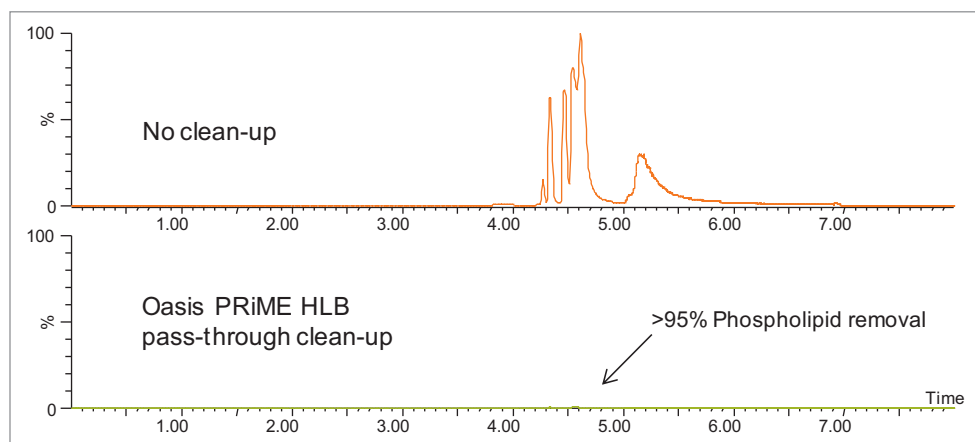


Figure 4. Effective removal of phospholipids from egg extracts with Oasis PRiME HLB clean-up.

METHOD RECOVERY AND PRECISION

Recovery studies were carried out at three concentration levels (0.4MRL, 1MRL, 2MRL), six replicates per level. Matrix-matched standard calibration curves were used. Figure 5 shows the results. Recovery was greater than 70% for most target compounds (>70%) except for Nystatin and Hygromycin. Reproducibility was acceptable (RSD<20%) for all compounds except for Hygromycin at 0.4 MRL (RSD=34%).

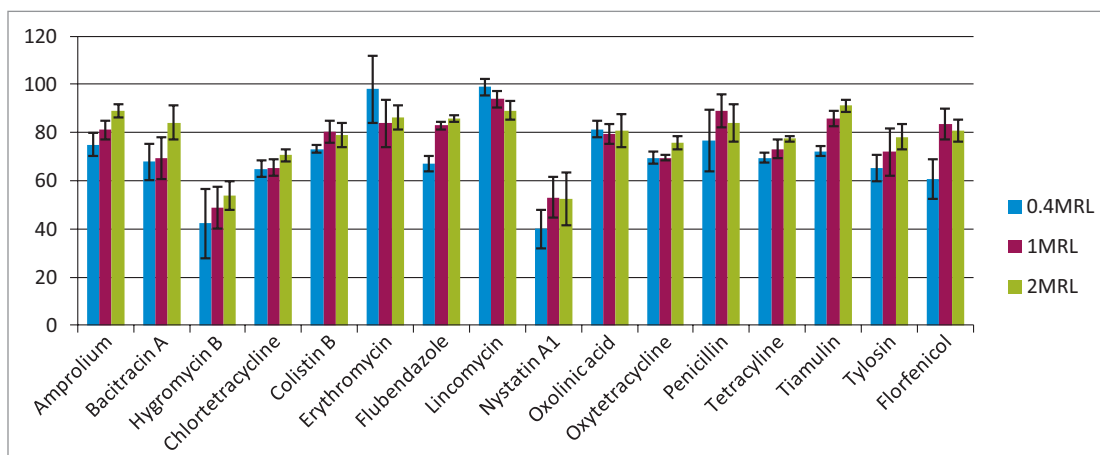


Figure 5. Summary of recovery data (blank eggs samples spiked at 0.4 MRL, 1MRL, 2MRL levels (Hygromycin B, Florfenicol, Penicillin G, and Nystatin A1 have no corresponding MRLs, so they were studied at 40, 100, 200 ppb levels).

CONCLUSIONS

- An analytical method has been developed for the simultaneous determination of several classes of veterinary drugs in eggs.
- A simple pass-through clean-up procedure using Oasis PRiME HLB Cartridge can remove more than 95% phospholipids from egg extracts.
- The Oasis PRiME HLB Cartridge clean-up procedure provided effective clean-up and good recoveries for the target veterinary drugs in egg.
- The ACQUITY UPLC I-Class System coupled with Xevo TQ-S MS offered good sensitivity for the veterinary drug residues in this study.

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