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Note d'application

Multiresidue LC-MS/MS Screening of Veterinary Drugs in Milk Using the Ostro Pass-through Sample Preparation Plate

Michael S. Young, Kim Van Tran

Waters Corporation

Abstract

This application note describes the use of the Ostro Plate for multi-residue screening for veterinary drugs in milk. Using this plate, milk samples are prepared using a few simple steps to effectively precipitate protein and remove potential interferences.

Benefits

- Rapid and simple sample preparation for efficient multiclass/multiresidue analysis
- Removal of potentially interfering phospholipids from prepared samples
- Fast, sensitive UPLC-MS/MS analysis

Introduction

The Ostro Pass-through Sample Preparation Plate allows for simple and rapid LC-MS determination of basic drugs and related compounds in biological fluids. The Ostro Plate provides a convenient platform for liquid extraction, protein precipitation, filtration, and subsequent removal of phospholipids from the resulting extract. This application note describes the use of the Ostro Plate for multi-residue screening for veterinary drugs in milk. Using this plate, milk samples are prepared using a few simple steps to effectively precipitate protein and remove potential interferences. As the extracted sample is passed through the plate the precipitated proteins are removed by filtration and phospholipid interferences are removed by retention to a proprietary sorbent. After this simple sample preparation, the reconstituted sample is analyzed using UPLC-MS/MS. To demonstrate the suitability of this method, representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, and beta-andrenergics.

Experimental

LC conditions

LC system:	ACQUITY UPLC
Column:	CORTECS UPLC CSH C_{18}+, 1.6 µm, 100 x 2.1 mm
Mobile phase A:	0.1% formic in water
Mobile phase B:	0.1% formic acid acetonitrile
Injection volume:	7 µL
Injection mode:	Partial loop injection
Column temp.:	30 °C
Weak needle wash:	10:90 acetonitrile:water (600µl)
Strong needle wash:	50:30:40 water:acetonitrile:IPA (200µl)
Seal wash:	10:90 acetonitrile:water

Gradient:

Time (min)	Flow (min)	%A	%B	Curve
Initial	0.4	85	15	Initial
2.5	0.4	60	406	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6

Time (min)	Flow (min)	%A	%B	Curve
7.0	0.4	85	15	6

MS conditions

Mass spectrometer:	Xevo TQ MS
Source temp.:	150°C
Desolvation temp.:	500°C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	30 L/hr
Collision gas flow:	0.15 ml/min
Data management:	MassLynxv4.1

Compound	MRM Transition	Cone (V)	Collision (eV)
Ciprofloxacin	*332.3>288.0*	28	18
	332.3>314.0	28	22
Chlortetracyline	*479.4>444.1*	25	25
	479.4>462.1	25	16
Enrofloxacin	*360.3>316.3*	30	25
	360.3>342.3	30	25
Erthromycin	*734.5>158.9*	25	30
	734.5>576.2	25	20
Lincomycin	*407.4>126.2*	30	25
	407.4> 359.4	30	20
Oxacillin	*402.1>159.9*	25	10
	402.1>243.1	25	15
Oxytetracycline	461.4>381.0	22	22
	461.4>426.3	22	20
Penicillin	335.5>160.2	20	15
	335.5>176.1	20	10
Ractopamine	*302.4>107.0*	22	25
	302.4>284.2	22	15
Salbutamol	240.4>48.1	20	25
	240.4>222.2	20	10
Sulfamerazine	265.3>92.1	25	25
	265.3>155.9	25	20
Sufamethazine	279.3>92.0	32	30
	279.3>186.1	32	15
Tetracyline	445.5>154.0	25	25
	445.5>410.0	25	20
Tylosin	*916.5>174.1*	55	35
	916.5>772.4	55	35

(MRM transition in italics used for quantitation)

Table 1. MRM transitions for this study with associated cone volatage and collision energy.

Sample preparation

Transfer 125 µL milk to a sample well (do not use a sample volume greater than 125 µL). Add 375 µL .2% formic acid in acetonitrile (ACN). Mix well with aspiration. Elute into collection plate well. Add 100 µL 200 mM ammonium formate in 50:50 methanol/ACN and mix well. Evaporate and reconstitute in 100 µL 25:75 ACN/25 mM aqueous ammonium formate. Figure 1 shows a typical setup for SPE using the Ostro Plate.

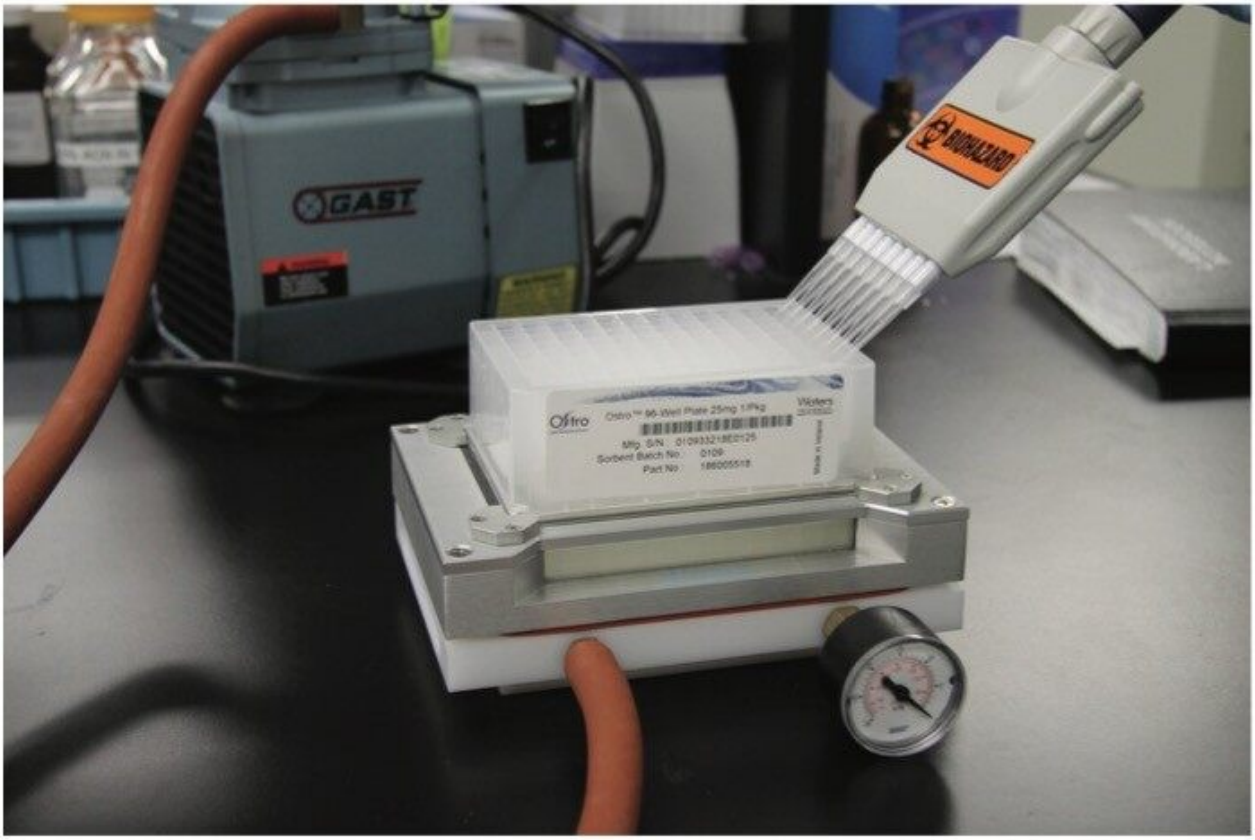

Figure 1. Typical setup for Ostro Pass-through Plate for analysis of milk.

Results and Discussion

Multiresidue Analysis

Compared to a previously published method[1] the Ostro Pass-through Plate procedure gives comparable results. However, the Ostro Plate procedure takes significantly less time and is much more suitable for high throughput analysis. A typical analyst can prepare a batch of ten or more samples in a few minutes compared with a few hours using prior methods. Figure 2 shows a typical LC-MS chromatogram obtained from analysis of a matrix matched standard of enrofloxacin at 10 µg/L. Although performance for most of the other compounds was similar, chlortetracycline, oxytetracycline and lincomycin were not quantifiable at the 10 µg/L level. An alternative method for tetracylines has recently been presented.[2] Table 2 shows recovery data observed for multiresidue milk analysis.

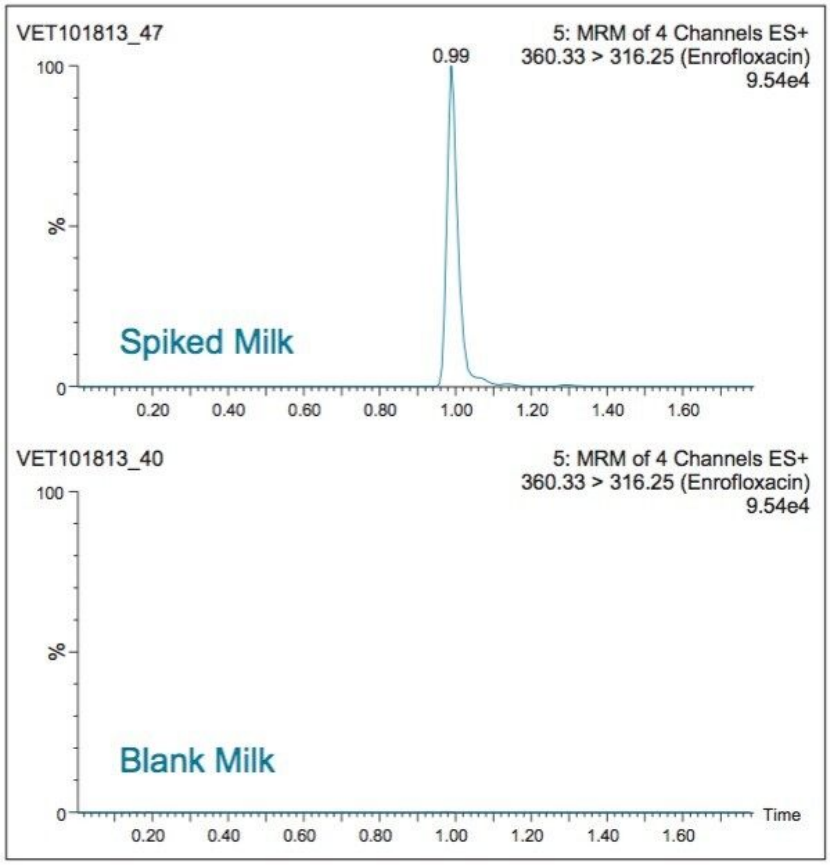

Figure 2. Enrofloxacin determined at 10 µg/L in milk using Ostro Pass-through Plate.

Compound	RT (min)	Spike Level (ppb)	% Recovery (% RSD)
Ciprofloxacin	0.87	10 100	81 (9) 82 (8)
Chlorotetracyline	1.54	10 100	bdl 37 (13)
Enrofloxacin	1.00	10 100	74 (9) 83 (7)
Erythromycin	*2.17*	10 100	71 (14) 50 (16)
Lincomycin	*0.60*	10 100	bdl 129 (18)
Oxacillin	*3.71*	10 100	73 (9) 71 (6)
Oxytetracycline	0.97	10 100	bdl 44 (5)
Penicillin	3.36	10 100	54 (14) 66 (8)
Ractopamine	0.92	10 100	80 (7) 87 (3)
Salbutamol	0.60	10 100	94 (13) 87 (15)
Sulfamerazine	1.45	10 100	49 (7) 54 (9)
Sulfamethazine	1.61	10 100	51 (14) 59 (11)
Tetracycline	1.06	10 100	42 (15) 52 (15)
Tylosin	2.39	10 100	70 (14) 57 (22)

Table 2. Recovery data (n = 6, bdl = below detection limit).

Phospholipid Removal

Milk contains a significant amount of phospholipids, approximately 1% of the total lipid content.[3] These phospholipids can interfere directly with late eluting compounds in LC separations. Also, the buildup of phospholipid residue has been shown to result in poor or deteriorating performance in reversed-phase LC separations.[4] Figure 3 shows UPLC-MS/MS analysis of milk samples using an MRM transition (*m/z* 184>184) chosen for identification of phospholipids. The lower trace shows the result obtained from a milk sample processed using the Ostro Pass-through Plate. The upper trace was obtained using protein precipitation with no SPE cleanup. The Ostro Plate was effective in removal of >98% of phospholipids.

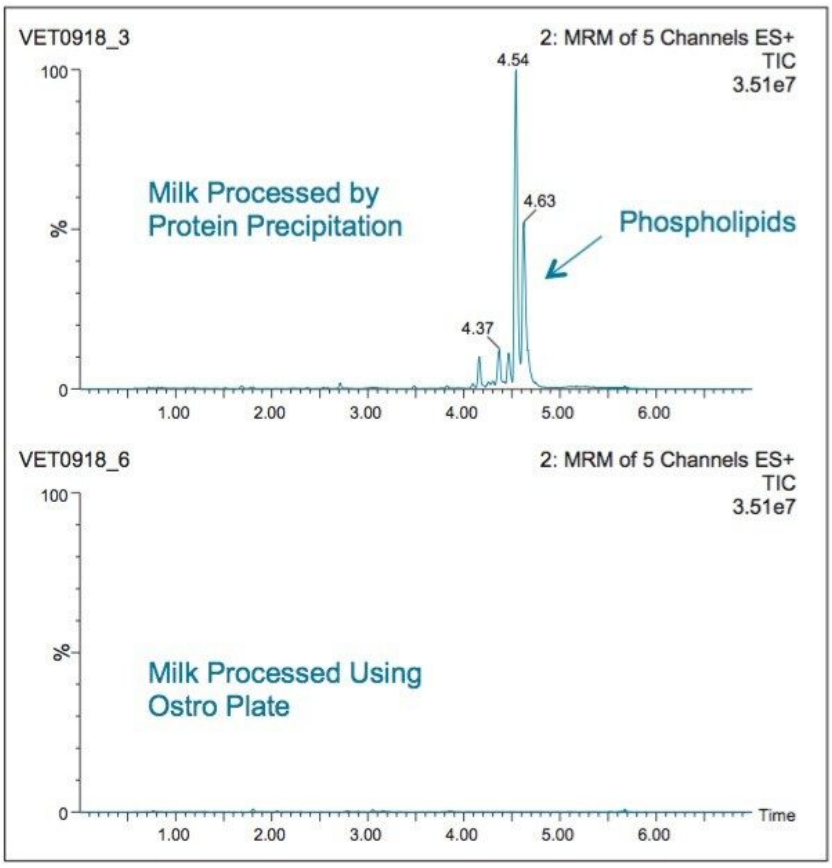

Figure 3. Removal of phospholipids from whole milk (3 % fat) using the Ostro Pass-through Plate.

Conclusion

- The Ostro Pass-through Sample Preparation Plate was demstrated for rapid multiclass extraction of veterinary drugs from milk.
- Acceptable performance for screening analysis was observed for all classes of compounds tested except for tetracyclines.
- The Ostro Pass-through Sample Preparation Plate was effective for nearly complete removal of phospholipids from milk extracts.

References

1. Optimized Extraction and Cleanup Protocols for LC-MS/MS Multi-Residue Determination of Veterinary Drugs in Edible Muscle Tissues, Waters Application Note 720004144en
2. HPLC/UV Determination of Tetracyclines in Milk Using Mixed-Mode SPE and e*x*tended *P*erformance [*XP*] 2.5 µm Columns, Waters Application Note 720004582en
3. Contarini, G and Povolo, M., Phospholipids in Milk Fat: Composition, Biological and Technological Significance, and Analytical Strategies, ***Int. J. Mol. Sci***, 14(2), 2808–2831(2013)
4. Eliminating Phospholipids in Drug Discovery Extractions Using a Fast, Generic Sample Clean-up Method, Waters Application Note 720004046en

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