

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

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### APPLICATION 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

### WATERS SOLUTIONS

ACQUITY UPLC®

Xevo® TQ MS

CORTECS® UPLC 1.6 µm Columns

Ostro™ Pass-through  
Sample Preparation Plate

### KEY WORDS

Milk, UPLC-MS/MS, veterinary drugs, phospholipids

### 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 <sub>18</sub> +, 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 (600 µL)			
Strong needle wash:	50:30:40 water: acetonitrile: IPA (200 µL)			
Seal wash:	10:90 acetonitrile:water			
Gradient:				
Time (min)	Flow (mL/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
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:	MassLynx® v4.1

Table 1 summarizes the MRM transitions and instrument parameters used for this study.

Compound	MRM Transition	Cone (V)	Collision (eV)
Ciprofloxacin	<i>332.3&gt;288.0</i>	28	18
	332.3>314.0	28	22
Chlortetracycline	<i>479.4&gt;444.1</i>	25	25
	479.4>462.1	25	16
Enrofloxacin	<i>360.3&gt;316.3</i>	30	25
	360.3>342.3	30	25
Erythromycin	<i>734.5&gt;158.9</i>	25	30
	734.5>576.2	25	20
Lincomycin	<i>407.4&gt;126.2</i>	30	25
	407.4>359.4	30	20
Oxacillin	<i>402.1&gt;159.9</i>	25	10
	402.1>243.1	25	15
Oxytetracycline	<i>461.4&gt;381.0</i>	22	22
	461.4>426.3	22	20
Penicillin	<i>335.5&gt;160.2</i>	20	15
	335.5>176.1	20	10
Ractopamine	<i>302.4&gt;107.0</i>	22	25
	302.4>284.2	22	15
Salbutamol	<i>240.4&gt;48.1</i>	20	25
	240.4>222.2	20	10
Sulfamerazine	<i>265.3&gt;92.1</i>	25	25
	265.3>155.9	25	20
Sufamethazine	<i>279.3&gt;92.0</i>	32	30
	279.3>186.1	32	15
Tetracycline	<i>445.5&gt;154.0</i>	25	25
	445.5>410.0	25	20
Tylosin	<i>916.5&gt;174.1</i>	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 voltage and collision energy.

## Sample preparation

Transfer 125  $\mu\text{L}$  milk to a sample well (do not use a sample volume greater than 125  $\mu\text{L}$ ). Add 375  $\mu\text{L}$  0.2% formic acid in acetonitrile (ACN). Mix well with aspiration. Elute into collection plate well. Add 100  $\mu\text{L}$  200 mM ammonium formate in 50:50 methanol/ACN and mix well. Evaporate and reconstitute in 100  $\mu\text{L}$  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,<sup>1</sup> 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  $\mu\text{g}/\text{L}$ . Although performance for most of the other compounds was similar, chlortetracycline, oxytetracycline and lincomycin were not quantifiable at the 10  $\mu\text{g}/\text{L}$  level. An alternative method for tetracyclines has recently been presented.<sup>2</sup> Table 2 shows recovery data observed for multiresidue milk analysis.

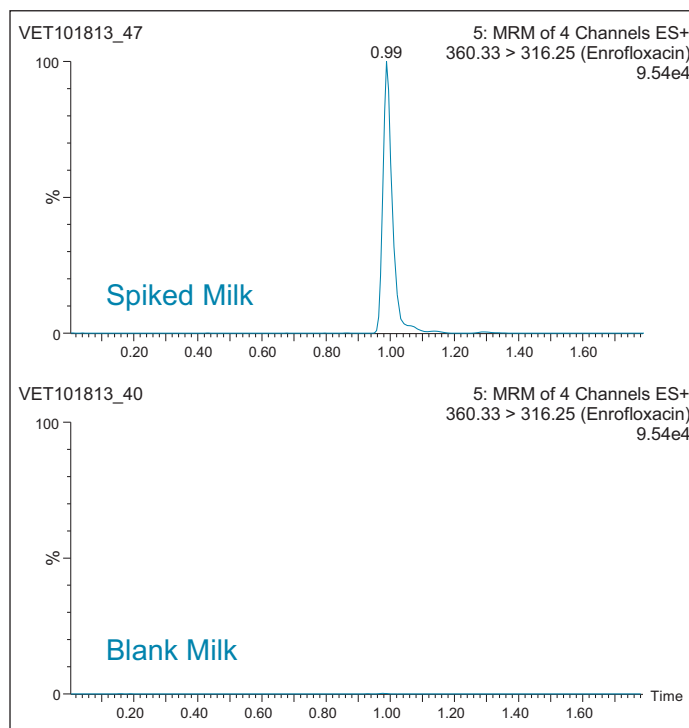


Figure 2. Enrofloxacin determined at 10  $\mu\text{g}/\text{L}$  in milk using Ostro Pass-through Plate.

Compound	RT (min)	Spike Level (ppb)	% Recovery (% RSD)
Ciprofloxacin	0.87	10	81 (9)
		100	82 (8)
Chlorotetracycline	1.54	10	bdl
		100	37 (13)
Enrofloxacin	1.00	10	74 (9)
		100	83 (7)
Erythromycin	2.17	10	71 (14)
		100	50 (16)
Lincomycin	0.60	10	bdl
		100	129 (18)
Oxacillin	3.71	10	73 (9)
		100	71 (6)
Oxytetracycline	0.97	10	bdl
		100	44 (5)
Penicillin	3.36	10	54 (14)
		100	66 (8)
Ractopamine	0.92	10	80 (7)
		100	87 (3)
Salbutamol	0.60	10	94 (13)
		100	87 (15)
Sulfamerazine	1.45	10	49 (7)
		100	54 (9)
Sulfamethazine	1.61	10	51 (14)
		100	59 (11)
Tetracycline	1.06	10	42 (15)
		100	52 (15)
Tylosin	2.39	10	70 (14)
		100	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.<sup>3</sup> 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.<sup>4</sup> 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.

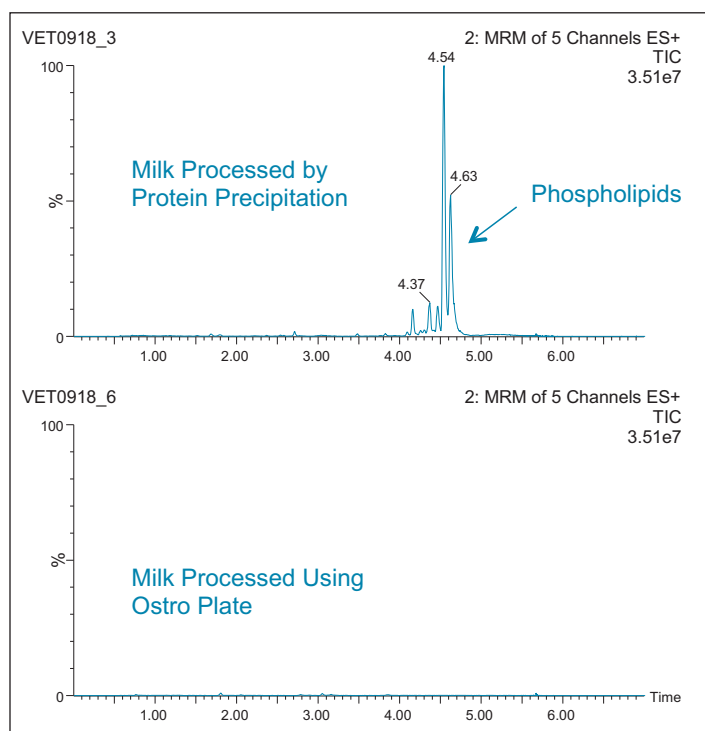


Figure 3. Removal of phospholipids from whole milk (3 % fat) using the Ostro Pass-through Plate.



## CONCLUSIONS

- The Ostro Pass-through Sample Preparation Plate was demonstrated 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

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