[APPLICATION NOTE]

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A Simple Cleanup Protocol Using a Novel SPE Device for UPLC-MS/MS Analysis of Multi-Residue Veterinary Drugs in Milk

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APPLICATION BENEFITS

- Enable simultaneously determination of multi-class of veterinary drugs using an innovative solid phase extraction device
- Simple, fast, pass-through SPE cleanup prior to UPLC-MS/MS analysis
- The matrix interference from fatty/non-polar materials and phospholipids are removed together in one straightforward SPE cleanup for longer column life and less maintenance of the mass spectrometer

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® TQ-S Mass Spectrometer

ACQUITY UPLC BEH C_{18} Column

Oasis® PRiME HLB 3 cc 60 mg cartridges

TruView™ LCMS Certified Vials

MassLynx® v4.1 data system with Quanpedia™ database

KEY WORDS

Oasis PRiME HLB, multi-residue, veterinary drug, SPE, milk, UPLC-MS/MS

SUMMARY

In this experiment a new solid phase extraction (SPE) device, the Oasis PRiME HLB Cartridge, was used in the sample preparation of milk samples as a cleanup method for multi-residue veterinary drug analysis. The initial extraction and protein precipitation was done by adding acidified acetonitrile. The extract was cleaned up by pass-thru SPE using the Oasis PRiME HLB Cartridge prior to UPLC-MS/MS analysis. Sample extraction, chromatographic and mass parameters were all optimized. As a result, within the ranges of 0.1 to 10.0 µg/mL spiking concentrations, 9 classes of 72 veterinary drugs including sulfonamides, fluoroquinolones, β-agonists, macrolides, glucocorticoids, amphenicols, β-lactams, cephalosporins, penicillin, and tetracyclines, the percent recoveries are all within 50% to 130%, and the RSD <20% (n=5). This method is simple, rapid, and accurate, suitable for multi-residue veterinary drug analysis of milk.

INTRODUCTION

Many veterinary drugs are used to treat animals grown for human consumption. The presence of excessive amounts of drug residues in animal products such as milk may represent a health hazard. Therefore, effective and reliable analytical methods are required to identify and quantify drug residues in animal products. Among the frequently used veterinary drugs in the animal farms are sulfonamides, fluoroquinolones, β-agonists, macrolides, glucocorticoids, amphenicols, β-lactams, cephalosporins, and tetracyclines. Drug residues in an animal's bloodstream can be introduced into the milk of lactating animals and eventually transferred to humans by consumption of the milk. Among the consequences are possible allergic reactions and the induced side effect of drug resistance. Therefore the monitoring of residual veterinary drugs of milk plays a significant role in the assurance of food safety of dairy products. Currently the published methods from official agencies and literatures are individual methods based on individual classes of compounds. The goal of combining these individual methods into one multi-class method is difficult to accomplish due to the unavailability of a robust universal sample preparation procedure. Creating one single LC-MS/MS instrumental method for multiple classes of veterinary drugs poses certain degree of challenge as well.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Injection volume:	5 µL
Temperature:	45 °C
Mobile phase A:	10 mM ammonium acetate in water (pH 5.0)
Mobile phase B:	10 mM ammonium acetate in methanol
Flow rate:	0.45 mL/min
Gradient:	2 %B initial and hold to 0.25 minutes, linear gradient to 99 %B at 12.25 minutes, hold to 13.0 minutes, back to 2 %B at 13.01 minutes, hold and re-equilibrate until 17 minutes

MS conditions for UPLC

Instrument:	Xevo TQ-S
Mode:	Electrospray (ES+ and ES-)
Capillary:	3.5 kV
Source temp.:	150 °C
Cone gas:	150 L/hr
Desolvation temp.:	600 °C
Desolvation gas:	1000 L/hr
Collision gas (Argon):	0.15 mL/min

UPLC-MS/MS cone and collision parameters, as well as MRM transitions used for this study are presented in Table 1.

This method utilizes Waters'® new and novel Oasis PRiME HLB Solid Phase Extraction Device. This new SPE can retain the majority of phospholipids and fats in milk. By combining with protein precipitation technique, it can effectively remove most interference from the milk matrix.

Using the Xevo TQ-S System and including the veterinary drug analysis parameters in the Quanpedia Database establishes a highly efficient total solution for the multi-residue analysis of veterinary drugs in milk.

Sample preparation

Sample extraction:

In 1 mL of milk, add 4 mL of 0.2% formic acid (FA) in acetonitrile (ACN), mix well. Centrifuge for 5 min at 10,000 rpm. Aliquots of the supernatant are used for SPE cleanup.

Solid phase extraction (SPE) cleanup:

Prepare the 3 cc Oasis PRiME HLB Cartridge (p/n 186008056) by passing through 3 mL 0.2% FA in ACN. Note: this conditioning step is only required to facilitate subsequent gravity loading, it is not necessary if a sample is processed with minimal vacuum.

Pass the supernatant through the cartridge and collect. Evaporate to dryness under a gentle nitrogen stream. Reconstitute the solution in 1 mL 5% methanol in water. Filter the extract and transfer to a vial for UPLC-MS/MS analysis.

Name	Ion Mode	Precursor (*m/z*)	CV (V)	Product (*m/z*)	CE (V)	RT (min)
Cimaterol	ESI+	220.1	25	143.0	24	2.2
	ESI+	220.1	25	160.1	15	
Clenbuterol	ESI+	277.1	25	140.0	46	4.65
	ESI+	277.1	25	168.1	25	
Ractopamine	ESI+	302.2	25	164.1	15	4.21
	ESI+	302.2	25	284.2	12	
Salbutamol	ESI+	240.2	25	148.1	20	2.46
	ESI+	240.2	25	222.1	12	
Terbutaline	ESI+	226.1	25	107.0	26	2.33
	ESI+	226.1	25	125.0	26	
Tulobuterol	ESI+	228.2	30	118.0	25	5.11
	ESI+	228.2	30	154.1	15	
Zilpaterol	ESI+	262.2	25	185.1	22	2.41
	ESI+	262.2	25	202.1	18	
Clindamycin	ESI+	425.2	20	125.9	25	7.52
	ESI+	425.2	20	377.2	18	
Erythromycin	ESI+	734.5	30	158.1	30	7.74
	ESI+	734.5	30	576.5	20	
Kitasamycin	ESI+	786.4	20	108.9	35	
	ESI+	786.4	20	174.0	30	
Lincomycin	ESI+	407.4	40	126.2	25	3.86
	ESI+	407.4	40	359.4	20	
Spiramycin	ESI+	422.2	30	101.0	20	6.36
	ESI+	422.2	30	174.1	20	
Tilmicosin	ESI+	869.5	25	174.2	45	7.17
	ESI+	869.5	25	696.5	40	
Tylosin	ESI+	916.5	60	101.1	45	7.89
	ESI+	916.5	60	174.1	40	
Oxytetracycline	ESI+	460.7	34	426.2	18	4.28
	ESI+	460.7	34	444.2	18	
Tetracycline	ESI+	444.7	30	410.3	18	4.16
	ESI+	444.7	30	427.3	14	
Sulfabenzamide	ESI+	277.1	30	92.0	25	3.61
	ESI+	277.1	30	156.0	15	
Sulfachlorpyridazine	ESI+	285.0	20	92.0	28	3.77
	ESI+	285.0	20	155.9	15	
Sulfaclozine	ESI+	285.0	20	92.0	28	4.61
	ESI+	285.0	20	155.9	15	
Sulfadiazine	ESI+	251.0	30	92.0	27	2.34
	ESI+	251.0	30	156.0	15	
Sulfadimethoxine	ESI+	311.1	36	92.0	32	5.2
	ESI+	311.1	36	156.0	20	
Sulfaguanidine	ESI+	215.0	20	91.8	22	0.96
	ESI+	215.0	20	156.0	13	
Sulfamerazine	ESI+	265.1	35	92.0	25	2.99
	ESI+	265.1	35	156.0	15	
Sulfameter	ESI+	281.0	20	91.8	27	3.34
	ESI+	281.0	20	155.9	15	
Sulfamethazine	ESI+	279.1	35	124.1	25	3.61
	ESI+	279.1	35	186.0	15	
Sulfamethizole	ESI+	271.1	30	92.0	25	3.31
	ESI+	271.1	30	156.0	15	
Sulfamethoxazole	ESI+	254.1	30	92.0	25	3.92
	ESI+	254.1	30	156.0	15	
Sulfamethoxypyridazine	ESI+	281.1	35	92.0	25	4.03
	ESI+	281.1	35	156.0	15	
Sulfamonomethoxine	ESI+	281.0	35	92.0	35	3.7
	ESI+	281.0	35	156.0	22	
Sulfamoxol	ESI+	268.0	20	91.8	26	3.47
	ESI+	268.0	20	155.9	15	
Sulfanilacetamide	ESI+	215.0	20	91.8	22	1.71
	ESI+	215.0	20	156.0	13	
Sulfaphenazole	ESI+	315.0	20	108.0	25	4.85
	ESI+	315.0	20	156.0	18	
Sulfapyridine	ESI+	250.0	33	108.0	25	2.87
	ESI+	250.0	33	156.0	16	
Sulfaquinoxaline	ESI+	301.1	32	92.2	30	5.38
	ESI+	301.1	32	156.1	16	
Sulfapyridine	ESI+	250.0	33	108.0	25	2.87
	ESI+	250.0	33	156.0	16	
Sulfaquinoxaline	ESI+	301.1	32	92.2	30	5.38
	ESI+	301.1	32	156.1	16	
Sulfathiazole	ESI+	256.0	31	92.0	25	2.68
	ESI+	256.0	31	156.0	15	
Sulfisomidine	ESI+	279.1	20	123.9	20	2.54
	ESI+	279.1	20	186.0	15	
Sulfisoxazole	ESI+	268.0	30	92.0	28	3.91
	ESI+	268.0	30	156.0	13	
Trimethoprim	ESI+	291.3	40	123.0	30	3.79
	ESI+	291.3	40	230.2	30	
Cinoxacin	ESI+	263.2	35	189.1	30	4.33
	ESI+	263.2	35	245.1	15	
Ciprofloxacin	ESI+	332.1	42	288.1	18	4.13
	ESI+	332.1	42	314.1	22	
Danofloxacin	ESI+	358.2	38	96.0	25	4.31
	ESI+	358.2	38	314.1	20	
Difloxacin	ESI+	400.3	30	356.2	20	5.39
	ESI+	400.3	30	382.2	20	
Enoxacin	ESI+	321.1	40	232.0	30	3.86
	ESI+	321.1	40	303.1	35	
Enrofloxacin	ESI+	360.3	25	316.3	20	4.85
	ESI+	360.3	25	342.3	20	
Flumequine	ESI+	262.1	35	202.0	35	6.67
	ESI+	262.1	35	244.0	15	
Lomefloxacin	ESI+	352.1	39	265.1	22	4.32
	ESI+	352.1	39	308.1	16	
Marbofloxacin	ESI+	363.1	35	72.0	20	3.69
	ESI+	363.1	35	320.0	15	
Nalidixic acid	ESI+	233.1	30	187.0	25	6.33
	ESI+	233.1	30	215.0	15	
Norfloxacin	ESI+	320.1	40	233.0	25	4.00
	ESI+	320.1	40	276.1	20	
Ofloxacin	ESI+	362.3	25	261.3	30	3.99
	ESI+	362.3	25	318.3	20	
Orbifloxacin	ESI+	396.1	40	295.1	22	4.39
	ESI+					
Oxolinic acid	ESI+	262.0	32	216.0	30	5.44
	ESI+	262.0	32	244.0	19	
Pefloxacin	ESI+	334.1	42	290.1	19	4.45
	ESI+	334.1	42	316.1	19	
Sarafloxacin	ESI+	386.2	45	299.1	27	4.59
	ESI+	386.2	45	342.1	18	
Chloramphenicol	ESI-	321.2	25	152.2	15	4.94
	ESI-	321.2	25	257.2	10	
Florfenicol	ESI-	356.0	30	185.0	17	4.07
	ESI-	356.0	30	336.0	10	
Thiamphenicol	ESI-	354.1	20	184.9	20	3.20
	ESI-	354.1	20	290.0	12	
Amoxicillin	ESI+	366.2	27	114.0	20	1.37
	ESI+	366.2	27	349.1	8	
Penicillin V	ESI+	351.1	23	114.0	35	6.24
	ESI+	351.1	23	160.1	10	
Betamethasone	ESI-	361.2	40	307.2	18	7.78
	ESI-	361.2	40	325.2	20	
Cortisone	ESI+	361.3	40	163.1	25	6.93
	ESI+	361.3	40	342.2	20	
Dexamethasone	ESI+	393.3	20	355.2	10	7.83
	ESI+	393.3	20	373.2	10	
Hydrocortisone	ESI+	363.4	35	121.1	25	7.19
	ESI+	363.4	35	327.3	15	
Meprednisone	ESI+	373.2	20	355.1	11	7.78
	ESI+	373.2	20	357.1	12	
Methylprednisolone	ESI+	375.2	25	357.3	10	7.92
	ESI+					
Prednisolone	ESI+	361.2	25	147.0	20	7.21
	ESI+	361.2	25	343.2	10	
Triamcinolone	ESI+	435.4	25	397.3	15	5.3
	ESI+	435.4	25	415.3	5	
Triamcinolone acetonide	ESI+	395.4	30	357.0	30	7.95
	ESI+	395.4	30	375.0	10	
Cefalexin	ESI+	348.2	40	139.9	35	3.36
	ESI+	348.2	40	158.0	20	
Cefotaxime	ESI+	456.1	30	167.0	20	3.43
	ESI+	456.1	30	396.2	10	
Ceftiofur	ESI+	524.2	35	241.1	16	5.25
	ESI+					
Cephapirin	ESI+	424.2	35	152.0	20	3.68
	ESI+	424.2	35	292.2	16	
Ceftiofur	ESI+	524.2	35	241.1	16	5.25
	ESI+					
Cephapirin	ESI+	424.2	35	152.0	20	3.68
	ESI+	424.2	35	292.2	16	

Table. 1 Analyte MS parameters and retention time.

RESULTS AND DISCUSSION

The optimization of sample preparation

Milk matrix is complicated. It contains large amounts of proteins and phospholipids, which interferes with the detection of target analytes. It is essential to clean up the complex matrix of milk and to release the analytes from the effect of matrix.

For the initial extraction and protein precipitation, 3:1 and 4:1 ratios of acetonitrile and milk were evaluated at both 0.2% and 1.0% formic acid concentrations. Results indicated that 1.0% formic acid in acetonitrile and milk at ratio of 4:1 gives the best effect of protein precipitation. However, 1% formic in acetonitrile has negative impact on the recoveries especially for the basic analytes like sulfonamides (see Figure 1). This could be because the more acidic condition gives rise to a higher degree of ionization of the sulfonamides with resulting solubility decrease in the high organic solvent. Therefore, 0.2% formic acid in acetonitrile mixed with milk at a 4:1 ratio was chosen for the final extraction and protein precipitation.

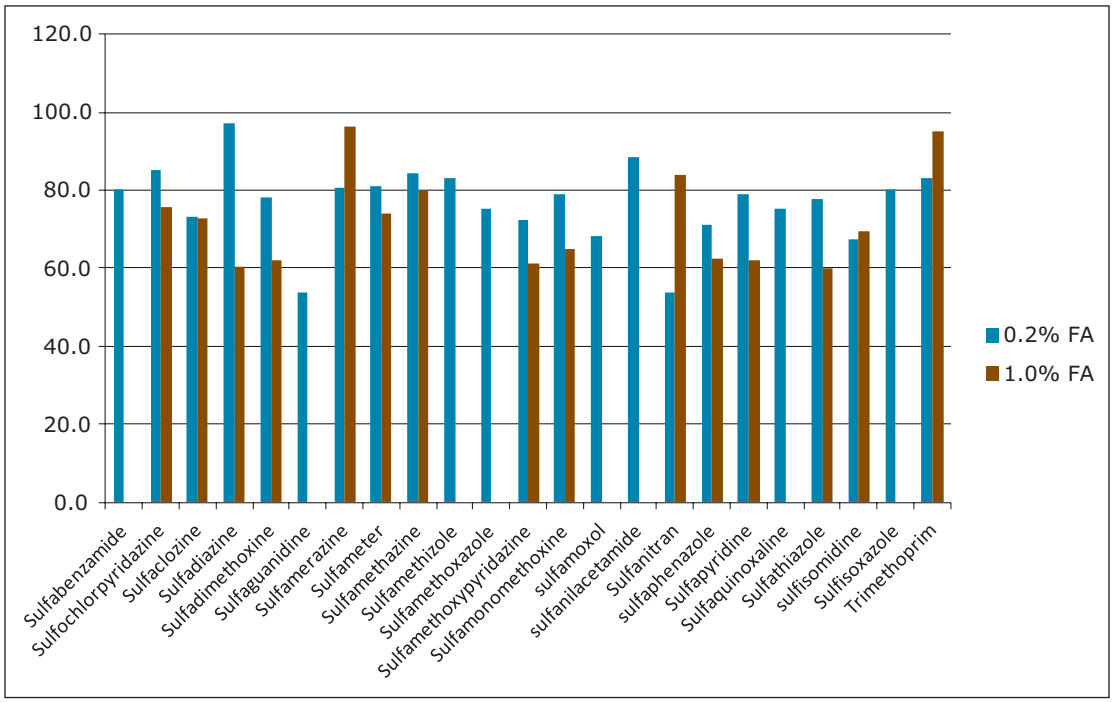

Figure 1. The comparison of recovery of sulfonamides for using 0.2% and 1.0% formic acid.

The large amount of phospholipids in milk not only becomes matrix interference for target analyte analysis, but also increases the cost and time of instrument maintenance. The use of Oasis PRiME HLB can remove the fat and phospholipids in sample matrix. As a result, the numbers of samples that could be analyzed are greatly increased before maintenance. In Figure 2, the effect of phospholipids removal by using SPE cleanup is compared to the milk sample only by protein precipitation. The sample after protein precipitation still has significant phospholipids present in the matrix that are mostly removed by the SPE cleanup.

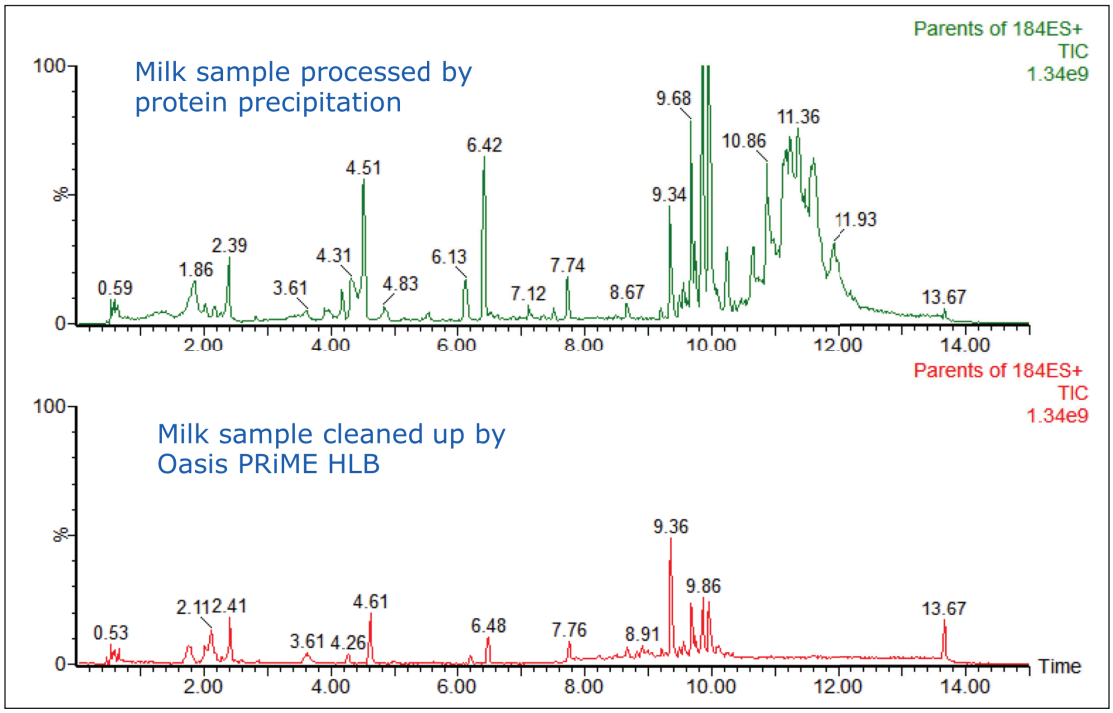

Figure 2. The chromatograms of phospholipids removal between milk samples processed by protein precipitation and cleanup by Oasis PRiME HLB.

The optimization of instrument condition

Developing a method for multi-residue analysis takes significant time and effort. Often there are significant differences in methods among the laboratories that developed them. This will lead to the deviations of testing results among the laboratories. This experiment builds the UPLC-MS/MS method using the Quanpedia Database, which contains LC-MS/MS methods of more than 1000 compounds including most of the veterinary drugs that are required in food safety testing. Waters Quanpedia database provides the ready-to-use instrument conditions that include: the liquid chromatographic parameters for each compound, mass parameters, and the quantitation method. This significantly cuts down the time of method development, also greatly reduces the level of potential error and the difficulty of method development. As a result, it decreases the amounts of work, time, resource spent for the laboratories.

Analyte recovery and precision

The recovery was determined by spiking known concentrations of the analyte drugs into blank milk. The spiking concentrations were 0.1 µg/L, 0.5 µg/L, 1.0 µg/L, and 10.0 µg/L. Five replicates were analyzed at each level. All the samples were processed according to the method described previously. The concentrations are calculated using matrix-match calibration curve. The average recoveries and precision for each spiking level are listed in Table 2.

Name	0.1 ug/L		0.5 ug/L		1.0 ug/L		10.0 ug/L		Matrix Effect at 10.0 ug/L
	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	Recovery (%)	RSD (%) n=5	
Cimaterol	95.0	2.5	94.0	8.5	77.2	18.0	98.1	3.2	0.17
Clenbuterol	81.0	15.6	84.4	3.5	92.6	5.3	113.0	5.8	0.11
Ractopamine	93.8	7.3	92.4	7.7	95.7	11.4	121.3	1.0	0.03
Salbutamol	93.8	5.5	93.2	6.9	90.0	3.3	111.3	2.6	0.11
Terbutaline	90.4	7.2	90.4	4.8	97.9	11.2	108.0	3.0	0.14
Tulobuterol	89.4	5.5	84.8	4.9	92.7	9.9	113.7	1.0	0.16
Zilpaterol	90.2	9.8	79.6	8.8	72.1	10.0	94.9	1.5	0.27
Clindamycin	–	–	111.2	12.6	73.0	18.0	86.5	5.4	0.10
Erythromycin	–	–	–	–	–	–	81.7	9.7	0.96
Kitasamycin	–	–	53.2	11.8	66.2	8.1	80.1	2.2	0.12
Lincomycin	85.6	9.0	71.2	7.1	70.8	3.2	70.8	3.2	0.25
Spiramycin	–	–	–	–	60.3	17.5	71.1	6.2	0.77
Tilmicosin	60.0	6.1	63.6	4.1	50.0	5.5	91.6	9.6	1.01
Tylosin	58.0	13.1	55.2	9.4	63.2	2.7	73.4	11.9	0.11
Oxytetracycline	–	–	75.2	17.1	72.0	8.9	69.0	3.1	0.08
Tetracycline	57.2	18.3	42.0	17.2	44.0	13.1	59.1	3.3	0.17
Sulfabenzamide	80.8	19.0	80.4	6.9	80.2	4.2	67.1	6.4	0.06
Sulfachlor–pyridazine	62.4	8.9	86.0	10.5	75.9	16.3	70.4	5.5	0.01
Sulfaclozine	61.6	30.6	76.4	22.9	72.9	6.4	71.1	13.0	0.04
Sulfadiazine	–	–	126.0	6.2	60.5	11.1	69.4	2.6	0.02
Sulfa–dimethoxine	96.8	6.3	78.8	8.5	62.2	9.5	80.0	6.7	0.00
Sulfaguanidine	90.4	10.2	69.6	13.4	53.8	13.0	70.9	11.5	0.46
Sulfamerazine	87.4	8.3	87.2	8.2	96.4	15.9	116.7	2.2	1.13
Sulfameter	–	–	80.4	8.7	74.1	1.7	75.5	5.4	0.51
Sulfamethazine	93.0	13.4	85.2	11.3	79.7	9.4	84.5	10.4	0.06
Sulfamethizole	82.4	36.9	74.8	7.0	83.2	11.5	72.8	5.6	0.04
Sulfa–methoxazole	74.8	12.4	77.2	9.1	75.2	2.9	63.6	5.4	0.05
Sulfamethoxy–pyridazine	73.4	11.1	79.2	17.4	61.4	17.4	73.4	8.7	0.04
Sulfamono–methoxine	93.4	10.7	80.8	2.8	64.8	9.0	69.9	5.6	0.05
Sulfamoxol	76.0	17.7	70.0	7.3	68.2	4.1	51.3	3.7	0.06
Sulfanil–acetamide	108.0	4.1	95.6	7.2	88.6	7.6	78.6	4.7	0.12
Sulfaphenazole	72.4	24.3	68.8	17.3	62.4	20.0	74.4	14.4	0.03
Sulfapyridine	87.4	17.0	78.0	1.8	62.1	1.4	70.3	3.5	0.10
Sulfa–quinoxaline	72.4	15.2	73.6	17.1	64.5	3.5	68.9	6.6	0.04
Sulfathiazole	93.0	7.4	82.8	10.9	60.2	0.7	69.0	0.8	0.06
Sulfisomidine	87.2	3.8	80.8	10.3	69.5	4.2	79.6	5.4	0.13
Sulfisoxazole	71.0	20.3	92.8	9.2	80.2	7.8	66.5	7.0	0.15
Trimethoprim	77.6	25.2	82.4	6.0	95.0	11.5	113.7	1.8	0.23
Cinoxacin	89.8	30.0	94.8	13.6	75.3	7.7	113.7	5.9	0.12
Ciprofloxacin	–	–	90.4	31.3	87.1	17.3	86.3	13.9	0.53
Danofloxacin	73.2	16.1	64.4	8.9	12.9	62.4	104.1	14.0	0.31
Difloxacin	49.6	11.5	61.6	15.6	66.6	13.5	88.7	11.4	0.47
Enoxacin	–	–	–	–	78.9	15.1	91.1	12.7	0.45
Enrofloxacin	82.0	6.7	74.4	7.0	77.3	3.2	106.5	11.6	0.54
Flumequine	69.4	8.4	79.6	5.1	75.0	8.9	92.3	3.1	0.11
Lomefloxacin	66.0	16.8	66.0	6.4	67.8	5.3	105.6	10.0	0.16
Marbofloxacin	–	–	67.2	8.0	85.8	7.9	99.9	9.8	0.65
Nalidixic acid	75.6	9.1	82.8	2.2	86.1	8.4	106.5	6.4	0.27
Norfloxacin	68.8	14.6	70.4	16.4	62.6	4.6	92.9	18.1	0.24
Ofloxacin	92.0	9.1	91.6	8.7	70.4	17.0	86.3	13.9	0.45
Orbifloxacin	88.8	11.2	78.8	4.9	74.8	16.3	100.7	2.9	0.24
Oxolinic acid	79.4	11.5	79.6	6.5	97.3	7.2	118.7	4.0	0.08
Pefloxacin	65.8	14.9	70.0	6.1	75.4	10.2	87.4	7.3	0.69
Sarafloxacin	79.6	8.0	71.6	5.4	83.4	8.3	91.7	10.8	0.19
Chloramphenicol	85.4	16.8	97.2	12.4	80.2	15.0	113.7	2.7	0.03
Florfenicol	95.2	26.3	96.0	12.1	69.8	16.9	101.5	10.6	0.05
Thiamphenicol	40.0	18.3	68.0	39.6	63.2	4.7	123.3	3.4	0.18
Amoxicillin	–	–	–	–			54.1	3.2	0.15
Penicillin v	–	–	103.2	4.9	100.0	9.7	72.0	17.2	0.50
Betamethasone	–	–	–	–	58.0	15.7	84.3	1.9	0.02
Cortisone	118.4	13.2	92.8	7.6	71.5	16.2	86.1	2.8	0.12
Dexamethasone	–	–	–	–	72.8	13.3	82.6	6.4	0.18
Hydrocortisone	–	–	100.4	11.1	71.9	13.0	83.4	3.9	0.17
Meprednisone	–	–	78.0	10.9	79.6	7.6	83.0	3.0	0.01
Methyl–prednisolone	–	–	85.6	8.6	84.8	12.4	82.3	12.0	0.14
Prednisolone	–	–	74.4	12.7	84.8	12.4	84.8	5.3	0.08
Triamcinolone	–	–	–	–	–	–	84.7	13.4	0.55
Triamcinolone acetonide	70.2	7.5	79.6	10.3	61.7	18.7	101.2	7.6	0.38
Cefalexin	–	–	–	–	–	–	63.1	18.8	0.49
Cefotaxime	97.5	24.5	77.2	47.7	79.2	13.8	75.6	9.4	0.11
Ceftiofur	76.0	33.6	70.4	7.1	69.4	10.7	77.0	8.4	0.11
Cephapirin	–	–	46.8	21.3	71.5	15.4	91.5	13.2	0.09

Table. 2 The spike recoveries and precision (%RSD) of antibiotics in milk.

CONCLUSIONS

- An analytical method was created for determination of multi-residue veterinary drugs in milk including 72 compounds in 9 drug classes.
- Reasonable recoveries were obtained in the range of 50% to 130% with precision (RSD) <20% (n=5) for all compounds.
- The Oasis PRiME HLB Cartridge was shown to effectively remove phospholipids and fats from milk. The sample preparation is simple, effective, and suitable for handling large numbers of samples in daily routine analysis.
- Waters Quanpedia Database contains all the liquid chromatographic methods, mass parameters, and quantitation method for veterinary drug analysis. It was very useful for developing this method.

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