SHIMADZU

MP 178

Fast and Simlutaneous LC/MS/MS Analysis for Veterinary Drugs in Meat Combined with STQ method

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1. Introduction

Veterinary drugs are used for therapeutic and growth promotion purposes for animals or fishes. To provide assurance that food from animals is safe in regard to veterinary medicine residues, regulatory authorities have established Maximum Residue Limits (MRL) for certain drugs in target tissues and animal species. Veterinary drugs analysis commonly uses liquid chromatography coupled to mass spectrometer which is fast, highly sensitive and highly selective. This work describes the application of high-throughput LC-MS/MS system utilizing fast polarity switching. Faster, easier and high precision total workflow was investigated with QuEChERS method combined with solid-phase extraction cartridge to enhance purification efficiency.

Fig1. LC-MS/MS system (Nexera X2+LCMS-8060, Shimadzu Corporation.)

2. Methods and Pretreatment

Chicken, pork and beef were selected for recovery tests of veterinary drugs. Evaluation of analytical system and recovery test used 129 veterinary drugs spiked in meat (1.25 ppb in vial). Solid phase extraction Technique with QuEChERS method (STQ method) were processed using fully automated solid phase extraction system (ST-L400, AiSTI SCIENCE, Japan). LC and MS conditions are shown in Table1. ODS column and Biphenyl column were used to evaluate the peak shape of drugs.

Table1. LC and MS conditions

[LC] Nexera™ X2 System

	Method 1		Method 2	
Analytical Column	YMC-Triart C18 [Metal Free] (2.1 mmI.D.x 150 mmL., 3 μm)		Restek Raptor™ Biphenyl (2.1 mmI.D.x 100 mmL., 2.7 μm)	
Solvent A	0.1% formic acid – Water		0.5 mM ammonium formate+ 0.1% formic acid – Water	
Solvent B	0.1% formic acid – Acetonitrile		0.1% Formic – MeOH	
Gradient Program	Time (min)	%B	Time (min)	%B
	0.0	1	0.0	2
	1.0	15	12.50	100
	6.0	40	14.50	100
	10.0	100	14.60	2
	15.0	100	17.5	STOP
	15.1	1		
	18.0	STOP		
Flow Rate	0.2 mL/min		0.4 mL/min	
Column Temp	40 ℃		40 ℃	

[MS] LCMS-8060

Ionization	: ESI (Positive/Negative)	DL temp	: 250 ℃
Nebulizer Gas	: 2 L/min	HB temp	: 400 ℃
Interface temp	: 300 ℃	Heating Gas	: 10 L/min
Drying Gas	: 10 L/min		

Sample pretreatment

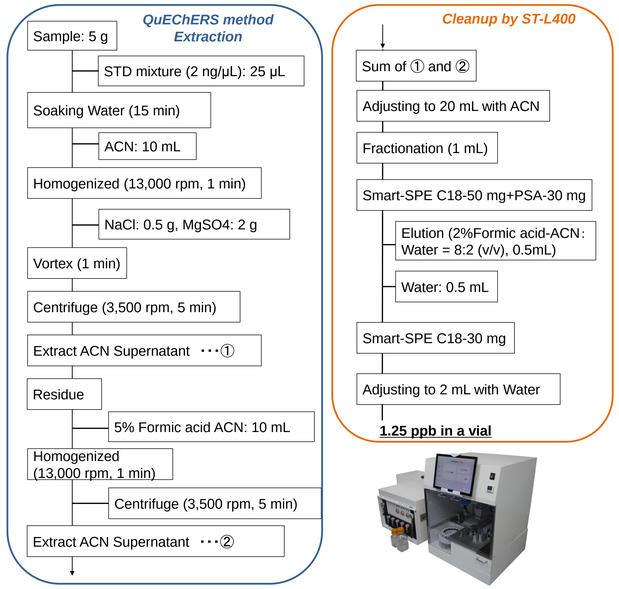

Fig2. Fully automated solid phase extraction system (ST-L400, AiSTI SCIENCE)

3. Results

Comparison of ODS column and Biphenyl column

In the Fig3 below, both ODS column and Biphenyl column measured all of 129 veterinary drugs from standard sample (10 ppb) within 18 minutes. MRM chromatogram of alpha- and beta- trenbolone, and sulfatroxazole and sulfisoxazole using ODS column and biphenyl column are shown in Fig4. Biphenyl column achieved good peak separation for alpha-trenbolone and beta-trenbolone. Conversely, ODS column sufficiently separated sulfatroxazole and sulfisoxazole. Adequate selection of column enables to establish accurate quantitative analytical system.

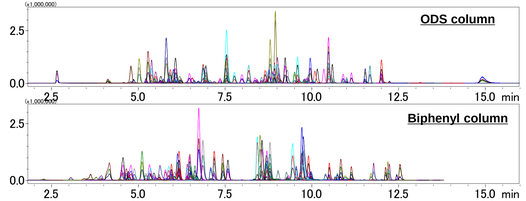

Fig3. MS Chromatogram of 129 veterinary drugs (10 ppb) using ODS and Biphenyl column

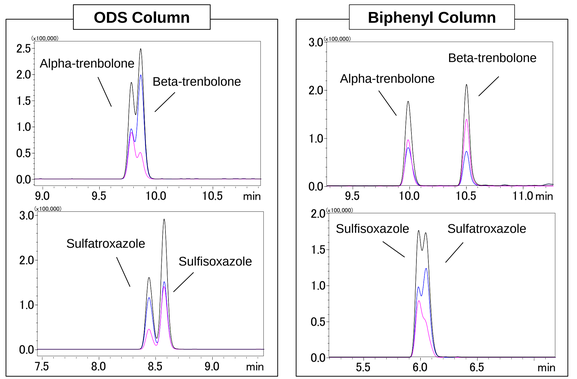

Fig4. MS Chromatogram of alpha- and beta- trenbolone, and sulfatroxazole and sulfisoxazole using ODS column and biphenyl column (STD, 10 ppb)

Recoveries of Veterinary Drugs in Chicken, Pork and Beef

Purified extract from chicken, pork and beef were assayed using LC-MS/MS using ODS column. The peak area of standard and post-spiked sample were compared for matrix effects. The peak area of pre-spiked sample and post-spiked sample were compared for recovery rates. The concentration of standard, pre-spiked and post-spiked sample were diluted at the concentration of 1.25 ppb. 94 drugs were obtained from each sample. The results indicated that 82% of the compounds in chicken, 85% of the compounds in pork and 84% of the compounds in beef were recovered from 70 to 120% (n=3). Table2 shows the typical result of matrix effects and recovery test from each samples. Stable and good recoveries were achieved with fully automated STQ method.

Table2. Typical results of matrix effects and recovery tests (n=3)

Compound	Chicken		Pork		Beef	
	Matrix Effect (%)	Recovery (%)	Matrix Effect (%)	Recovery (%)	Matrix Effect (%)	Recovery (%)
Altrenogest	87	79	78	92	80	82
Azaperone	101	83	97	86	99	87
Bromacil	94	90	92	92	115	87
Carazolol	99	78	90	98	101	88
Carprofen	100	76	104	71	84	102
Dicyclanil	94	84	91	83	74	89
Ethopabate	98	86	99	104	92	85
Famphur	106	79	89	93	94	91
Flubendazole	101	86	97	98	101	81
Flunixin	103	83	96	81	91	85
Josamycin	93	76	90	90	115	77
Mafoprazine	94	84	96	93	104	87
Mebendazole	100	86	101	88	95	91
Meloxicam	103	86	81	91	97	82
Menbutone	92	94	91	109	93	95
Miloxacin	119	74	94	89	94	88
Morantel	98	80	96	91	109	92
Nifurstyrenate	100	77	109	97	94	87
Oxibendazole	96	84	91	94	93	94
Praziquantel	101	82	92	89	95	89
Prifinium	96	86	95	95	99	91
PyrantelPamoate	98	88	100	91	98	98
Robenidine	106	82	91	92	83	71
Sulfabenzamide	110	84	89	92	93	89
Sulfabromomethazine Na	97	101	107	85	86	85
Sulfachlorpyridazine	103	78	86	92	96	79
Sulfadimethoxine	95	92	97	99	98	88
Sulfadimidine	99	87	92	92	100	80
Sulfadoxine	97	84	91	96	99	84
Sulfaethoxypyridazine	106	76	104	90	103	77
Sulfamerazine	94	81	94	92	72	94
Sulfamethoxazole	101	99	87	98	98	76
Sulfamonomethoxine	93	72	99	100	88	90
Sulfapyridine	95	86	95	91	72	92
Sulfaquinoxaline	96	91	99	98	92	80
Sulfathiazole	89	77	82	86	71	103
Sulfatroxazole	112	80	105	81	90	89
Sulfisoxazole	93	86	104	86	92	80
Thiamphenicol	105	85	80	113	78	107
Tiamulin	93	84	92	97	101	90
Tripelennamine	93	86	91	93	88	103
Valnemulin	108	86	100	100	98	94
Warfarin	101	81	99	86	86	95
Xylazine	102	85	91	96	87	83

5. Conclusion

- 129 veterinary drugs were detected using ODS column and Biphenyl column within 18 min
- 82% of the compounds in chicken, 85% of the compounds in pork and 84% of the compounds in beef were recovered between 70 to 120%.
- Fully automated solid phase extraction achieved minimized matrix effect with sufficient recovery rates