

Application News

SGLC-LC/MS-033-EN

Triple Quadrupole Mass Spectrometer Shimadzu LCMS-8050
Shimadzu LC-30A

Analysis of Veterinary Drug Residues in Pork Using the SHIMSEN Styra HLB SPE Cartridge and Shim-pack GIST C18 UHPLC Column

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User Benefits

- ◆ Established an effective pretreatment method for food samples of animal origin.
- ◆ Realized simultaneous quantitative analysis of quinolones, sulfonamides, tetracyclines, nitrazoles, macrolides, glucocorticoids, Chloramphenicols and quinoethanol metabolites.
- ◆ SHIMSEN Styra HLB SPE cartridge allows users to extract a broad range of compounds using a single sorbent and generic methodology due to its hydrophilic-lipophilic balanced sorbent characteristics.

Introduction

In recent years, veterinary drugs have been widely used to prevent infection and to promote growth and development of animals. In order to ensure the safety of food of animal origin, regulatory authorities have set maximum residue limits, MRL's, for veterinary drugs in food for various tissues and animal types. In addition, regulators prohibit the usage of pharmacologically active compounds, which are at some point considered dangerous.

To improve selectivity and sensitivity of quantitative analysis, triple quadrupole mass spectrometers are widely used for the analysis of veterinary drugs in food^[1]. To improve the efficiency of sample preparation process, hydrophilic modified styrene polymer SPE sorbent was developed and used to extract a broad range of compounds from aqueous samples.

In this method, we realized a simultaneous quantitative analysis of 53 veterinary drugs in pork using Shimadzu LCMS-8050. The samples were purified by SHIMSEN Styra HLB SPE cartridge and separated by Shim-pack GIST C18 UHPLC column.

Analytical Conditions

The UHPLC and MS conditions are shown in Table 1.

Table 1 Analytical Conditions

UHPLC (LC-30A)	
Column	: Shim-pack GIST C18, 100 × 2.1 mm, 2 μm (P/N: 227-30001-04)
Flow rate	: 0.3 mL/min
Injection volume	: 5 μL
Column temperature	: 40 °C
Mobile phase A	: 0.1% Formic acid aqueous solution
Mobile phase B	: Methanol
Gradient program	: B conc. 10 % (0 min)-20 % (3 min)-35 % (8 min)-95 % (11 min)-10 % (12-15 min)
MS (LCMS-8050)	
Electron ionization mode	: ESI, positive/negative scan
Scan mode	: MRM
Heating gas	: 10 L/min
Nebulizing Gas	: 3 L/min
Drying gas	: 10 L/min
Interface temperature	: 300 °C
DL temperature	: 250 °C
Heat block temperature	: 400 °C

Compound List for Veterinary Drugs

Table 2, split into 3 parts, shows the compound list of 53 veterinary drugs used in this experiment.

Table 2 List of Veterinary Drugs (part 1)

No.	Veterinary drug	Precursor ion	Product ion	Q1 Pre Bias	CE	Q3 Pre Bias
1	Sulfacetyl	215.0	156.0	-10	-11	-27
		215.0	92.2	-11	-24	-14
2	Metronidazole	172.0	128.1	-11	-16	-20
		172.0	82.1	-11	-25	-30
3	Dimetridazole	142.0	96.1	-12	-17	-15
		142.0	95.2	-12	-23	-15
4	Sulfisomidine	279.0	124.1	-13	-22	-19
		279.0	186.0	-13	-17	-17
5	Sulfadiazine	251.0	156.1	-12	-16	-26
		251.0	108.1	-12	-23	-17
6	Sulfathiazole	255.9	156.0	-12	-15	-26
		255.9	108.1	-12	-23	-16
7	Tinidazole	247.9	121.1	-12	-16	-18
		247.9	128.1	-11	-21	-20
8	Sulfapyridine	249.9	156.0	-12	-16	-26
		249.9	108.1	-11	-25	-17



Fig. 1 SHIMSEN Styra SPE Cartridge and Shim-pack GIST C18 Column

Instrument and Materials

Instrument: Shimadzu LCMS-8050 equipped with LC-30A;
UHPLC Column: Shim-pack GIST C18, 100 × 2.1 mm, 2 μm (P/N: 227-30001-04);
SPE cartridge: SHIMSEN Styra HLB 60 mg/3 mL (P/N: 380-00855-03);
Filter: SHIMSEN Arc Disc HPTFE needle filter (P/N: 380-00341-05);
Vial: LabTotal Vial (P/N: 227-34001-01);
Pipet: SHIMSEN Pipet PMII-10 (P/N: 380-00751-02); SHIMSEN Pipet PMII-100 (P/N: 380-00751-04); SHIMSEN Pipet PMII-1000 (P/N: 380-00751-06).



Fig. 2 SHIMSEN Arc Disc Needle Filter, SHIMSEN Pipet Pipette and LabTotal Vial

Table 2 List of Veterinary Drugs (part 2)

No.	Veterinary drug	Prec- ursor lon	Prod- uct lon	Q1 Pre Bias	CE	Q3 Pre Bias
15	Tetracycline	444.9	410.1	-13	-20	-26
		444.9	410.2	-16	-20	-26
16	Ofloxacin	361.9	318.2	-10	-20	-30
		361.9	261.1	-10	-28	-25
17	Enoxacin	321.0	303.1	-11	-22	-30
		321.0	232.1	-11	-36	-22
18	Pefloxacin	334.0	316.1	-15	-22	-30
		334.0	290.2	-15	-19	-28
19	Sulfamethoxypyridazine	281.0	156.0	-13	-17	-26
		281.0	108.1	-13	-26	-16
20	Sulfamethazine	279.0	186.0	-13	-18	-17
		279.0	124.1	-13	-24	-19
21	Ornidazole	219.8	128.1	-10	-16	-20
		219.8	82.1	-11	-30	-30
22	Oxytetracycline	461.0	426.1	-10	-21	-18
		461.0	443.1	-13	-15	-19
23	Norfloxacin	319.9	302.1	-11	-22	-30
		319.9	276.1	-10	-18	-27
24	Ciprofloxacin	331.9	314.1	-11	-22	-30
		331.9	231.1	-11	-37	-22
25	Sulfameter	280.9	156.0	-10	-17	-14
		280.9	108.1	-10	-26	-16
26	Sulfachloropyridazine	284.9	156.1	-10	-15	-14
		284.9	108.1	-13	-25	-17
27	Enrofloxacin	360.0	316.2	-10	-20	-30
		360.0	342.1	-10	-23	-21
28	Darfloxacin	357.9	340.1	-10	-24	-21
		357.9	82.1	-10	-44	-30
29	Lomefloxacin	352.0	265.1	-10	-24	-25
		352.0	308.2	-10	-18	-30
30	Sulfamethoxazole	254.0	156.0	-12	-16	-25
		254.0	108.1	-12	-24	-17
31	Orbifloxacin	396.0	352.1	-11	-19	-22
		396.0	295.0	-11	-25	-29
32	Difloxacin	399.9	382.1	-11	-23	-24
		399.9	356.1	-11	-21	-15
33	Sulfamonomethoxine	281.0	156.0	-13	-18	-14
		281.0	108.1	-13	-27	-17
34	Sulfamethoxazole	268.0	156.0	-13	-14	-26
		268.0	113.1	-12	-16	-18
35	Sarafloxacin	386.0	368.1	-11	-23	-23
		386.0	342.1	-11	-20	-21
36	3-Methyl-quinoxaline-2-carboxylic Acid	189.1	143.2	-10	-17	-29
		189.1	145.3	-10	-15	-13
37	Sulfamethazine	310.9	156.1	-11	-21	-26
		310.9	108.1	-11	-29	-17
38	Sulfadoxine	310.9	156.0	-14	-18	-25
		310.9	108.1	-11	-27	-17

Table 2 List of Veterinary Drugs (part 3)

No.	Veterinary drug	Prec- ursor lon	Prod- uct lon	Q1 Pre Bias	CE	Q3 Pre Bias
39	Chlortetracycline	479.0	444.0	-13	-23	-28
		479.0	462.1	-13	-18	-30
40	Sparfloxacin	393.0	349.1	-11	-21	-22
		393.0	292.1	-11	-26	-28
41	Spiramycin	843.2	174.2	-22	-35	-29
		843.2	142.1	-22	-35	-12
42	Sulfaphenazole	315.0	158.1	-11	-29	-14
		315.0	160.1	-11	-22	-14
43	Tilmicosin	869.4	696.4	-20	-42	-32
		869.4	174.1	-20	-45	-30
44	Sulfaquinoxaline	300.8	156.0	-10	-17	-14
		300.8	108.0	-13	-26	-17
45	Oxolinic acid	262.0	244.0	-12	-20	-23
		262.0	216.0	-11	-29	-20
46	Sineptina	772.2	109.0	-22	-42	-17
		772.2	174.1	-22	-31	-15
47	Josamycin	828.3	109.0	-20	-45	-17
		828.3	174.2	-20	-33	-16
48	Flumequine	261.9	244.1	-12	-20	-23
		261.9	202.0	-12	-33	-18
49	Thiamphenicol	353.9	185.1	17	21	12
		353.9	290.1	17	13	14
50	Florfenicol	355.9	336.1	18	11	12
		355.9	185.1	17	20	12
51	Chloramphenicol	320.8	152.1	16	17	15
		320.8	257.1	16	11	12
52	Hydrocortisone	407.1	331.2	20	19	16
		407.1	297.2	20	32	20
53	Dexamethasone	437.1	361.2	22	19	17
		437.1	307.2	22	33	20

■ Sample preparation

Sample Extraction

Weigh 1.0 g pork sample into a 50 mL centrifuge tube, add 8 mL Mcllvaine-Na₂EDTA buffer*, vortex for 1 min to mix thoroughly, sonicate for 20 min, then centrifuge the samples at 8000 rpm for 2 min, collect the supernatant. Add 8 mL Mcllvaine-Na₂EDTA buffer* to the sample residue, then repeat the extraction process above for 1 time. Combine the supernatant, add 5 mL hexane, vortex for 1 min. Centrifuge the samples at 8000 rpm for 2 min, collect the below solution for latter SPE purification.

*Mcllvaine-Na₂EDTA buffer: Weigh 10.9 g Na₂HPO₄·12H₂O, 12.9 g citric acid, C₆H₈O₇·H₂O, and 37.2 g EDTA₂Na, dissolve them with water, then add water to 1000 mL, adjust pH to 5.0 with 1 mol/L NaOH aqueous solution.

Fig. 3 Sample Extraction Process

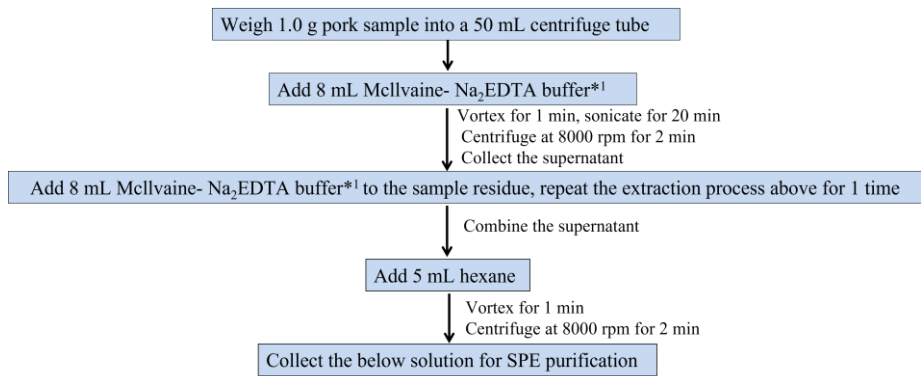
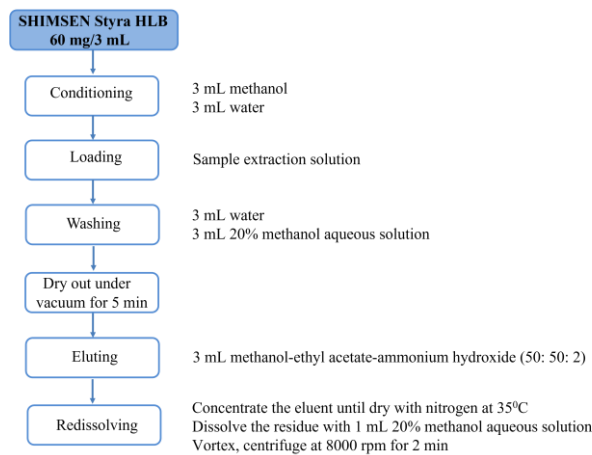


Fig. 4 Sample Purification Process



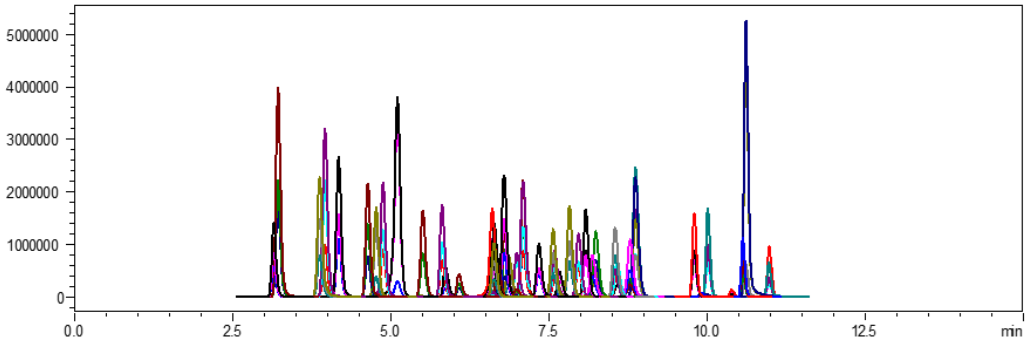
Sample Purification

Sample purification by SHIMSEN Styra HLB SPE cartridge.
Condition and equilibrate the SPE cartridges with 3 mL methanol followed by 3 mL water, discard the eluate.
Load the sample solution and discard the eluate.
Wash with 3 mL water followed by 3 mL 20% methanol aqueous solution, discard the eluate.
Dry out under vacuum (PN: RT-26077) for 5 min.
Elute with 3 mL methanol-ethyl acetate-ammonium hydroxide (V: V=50: 50: 2), collect the eluent.
Concentrate the eluent with nitrogen at 35 °C till nearly-dried, dissolve the residue with 1 mL 20% methanol aqueous solution, vortex to mix, then centrifuge the samples at 8000 rpm for 2 min, filter the sample solution through a 0.22 µm HPTFE needle filter for LC-MS/MS analysis.

MRM Chromatogram

Fig. 5 shows MRM chromatogram of 53 veterinary drugs standard mixture diluted to 10 µg/L.

Fig. 5 MRM Chromatogram of 53 Veterinary Drugs
Standard Mixture Solution (10 µg/L)



Spike and Recovery Test

A spike and recovery test was performed using pork extract to which 39 veterinary drugs mixture standard solution was spiked at 10.0 µg/kg per sample, and the recovery rate was evaluated. The results of recovery rate and reproducibility (%RSD) are shown in Table 3, which was split into 3 parts due to the limited space, and the break down of recovery rate is shown in Fig. 6.
Recovery rates were 60-90% for 49 of the 53 compounds. Good recovery rate and reproducibility were obtained without significant matrix inhibition.

Table 3 Recovery Rate and Reproducibility (%RSD, n=3) (part 1)

No.	Veterinary drug	Recovery (%)	RSD (%)
1	Sulfacetyl	78.22	1.00
2	Metronidazole	63.55	6.65
3	Dimetridazole	80.90	4.96
4	Sulfisomidine	79.68	1.30
5	Sulfadiazine	70.80	2.36
6	Sulfathiazole	75.93	7.60
7	Tinidazole	82.04	0.45
8	Sulfapyridine	83.00	0.98
9	Sulfamerazine	83.85	2.82

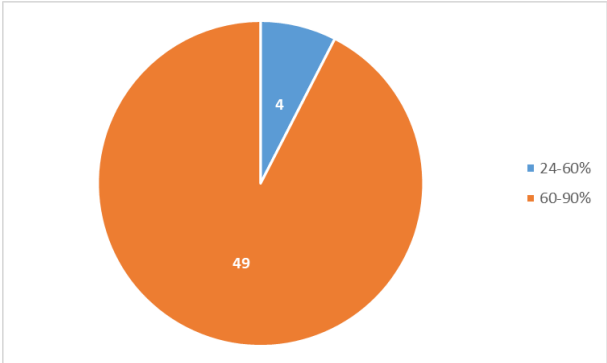
Table 3 Recovery Rate and Reproducibility (%RSD, n=3) (part 2)

No.	Veterinary drug	Recovery (%)	RSD (%)
10	Trimethoprim	78.76	1.74
11	Marbofloxacin	81.13	1.23
12	Fleroxacin	70.66	2.23
13	Sulfamethizole	63.63	1.71
14	Doxycycline	62.18	5.73
15	Tetracycline	78.65	0.50
16	Ofloxacin	70.52	0.83
17	Enoxacin	79.32	2.48
18	Pefloxacin	78.92	2.59
19	Sulfamethoxypyridazine	84.93	2.06
20	Sulfamethazine	76.80	6.56
21	Ornidazole	82.79	7.14
22	Oxytetracycline	73.18	2.13
23	Norfloxacin	71.29	2.30
24	Ciprofloxacin	76.33	3.00
25	Sulfameter	69.82	2.43
26	Sulfachloropyridazine	79.24	0.67
27	Enrofloxacin	75.54	3.06
28	Darfloxacin	81.14	0.77
29	Lomefloxacin	75.45	1.36
30	Sulfamethoxazole	81.90	0.51
31	Orbifloxacin	79.76	3.09

Table 3 Recovery Rate and Reproducibility (%RSD, n=3) (part 3)

No.	Veterinary drug	Recovery (%)	RSD (%)
32	Difloxacin	78.11	2.05
33	Sulfamonomethoxine	60.25	1.86
34	Sulfamethoxazole	74.00	1.97
35	Sarafloxacin	74.67	0.94
36	3-Methyl-quinoxaline-2-carboxylic Acid	79.49	0.88
37	Sulfamethazine	79.43	1.05
38	Sulfadoxine	24.20	14.09
39	Chlortetracycline	62.33	2.05
40	Sparfloxacin	62.54	7.11
41	Spiramycin	55.50	1.70
42	Sulfaphenazole	70.95	4.64
43	Tilmicosin	75.80	1.47
44	Sulfaquinolaxine	58.20	6.69
45	Oxolinic acid	65.60	6.20
46	Sineptina	62.77	3.42
47	Josamycin	66.10	3.15
48	Flumequine	46.96	3.47
49	Thiamphenicol	86.83	4.20
50	Florfenicol	87.75	1.78
51	Chloramphenicol	76.84	4.34
52	Hydrocortisone	90.89	4.36
53	Dexamethasone	72.58	2.63

Fig. 6 Breakdown of Recovery Rate



■ Conclusion

This method was established for the determination of 53 veterinary drugs in pork by using Shimadzu LCMS-8050. Samples were purified by SHIMSEN Styra HLB SPE cartridge and separated on Shim-pack GIST C18 (100 × 2.1 mm, 2 μm). The recoveries of 53 veterinary drugs at 10.0 μg/kg spiked level were between 24.20% and 90.89%, with the RSD varied from 0.45% to 14.09%, indicating that this method was acceptable for the quantitative analysis of quinolones, sulfonamides, tetracyclines, nitrazoles, macrolides, glucocorticoids, chloramphenicols and quinoethanol metabolites in pork samples.

<References>

- 1) Shima et al., poster presentation at the 114th Annual Meeting of the Japan Society for Food Hygiene and Safety Conference, High- speed Simultaneous Analysis of Veterinary Drugs in Meat by Combining STQ Method and LC/MS/MS (Pretreatment Edition)

<Related Applications>

1. Analysis of Veterinary Drugs in Chicken Tenders Using the Quadrupole Time-of-Flight Mass Spectrometer, 01-00461-EN

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