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Application News

No.C99

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.
The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

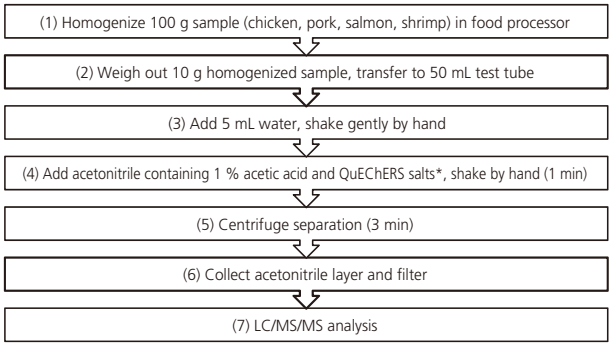
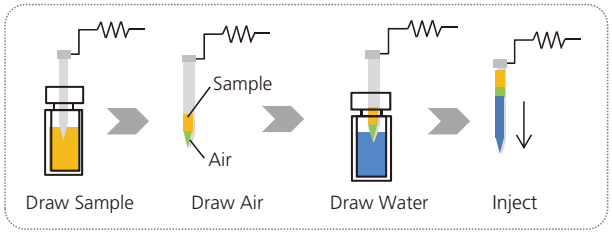


Fig. 1 Sample Preparation Procedure

Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).
To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.



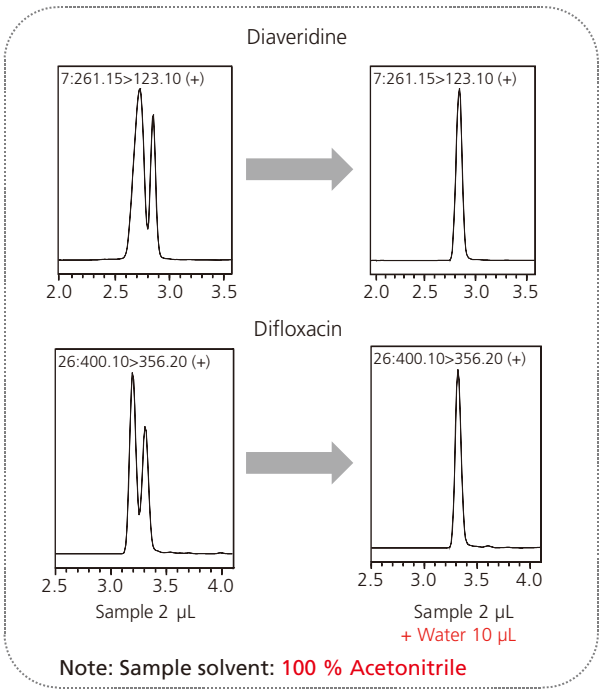


Fig. 2 Comparison of Peak Shape

MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).

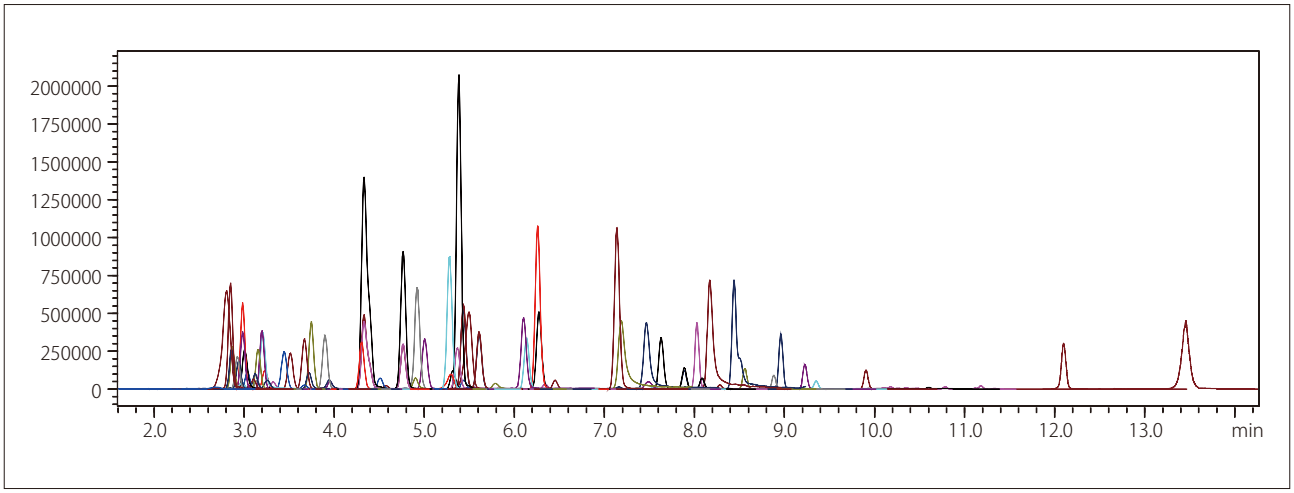


Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 µg/L pork extract solution with added standard solution)

Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Gentamicin	0.5	1	50
Sulfanilamide	1	1	50
Levamisole	0.05	0.05	50
Lincomycin	0.01	0.01	10
5-Propylsulfonyl-1-benzimidazole-2-amine	0.05	0.05	10
Diaveridine	0.01	0.01	10
Trimethoprim	0.02	0.02	20
Marbofloxacin	0.01	0.01	50
Sulfisomidine	0.02	0.02	20
Norfloxacin	0.5	0.5	50
Ormetoprim	0.02	0.02	10
Thiabendazole	0.01	0.01	10
Ciprofloxacin	0.05	0.5	10
Neospiramycin I	0.01	0.05	10
Danofloxacin	0.1	0.1	10
Enrofloxacin	0.05	0.1	50
Oxytetracycline	0.01	0.1	50
Xylazine	0.01	0.01	10
Orbifloxacin	0.05	0.05	50
Sulfacetamide	1	1	50
Clenbuterol	0.01	0.01	10
Tetracycline	0.05	0.01	50
Spiramycin I	0.01	0.01	50
Sarafloxacin	0.5	0.5	50
Difloxacin	0.05	0.1	50
Sulfadiazine	0.02	0.1	20
Sulfathiazole	0.02	0.1	20
Sulfapyridine	0.02	0.1	20
Carbadox	0.05	0.05	10
Pyrimethamine	0.02	0.02	20
Sulfamerazine	0.02	0.02	20
Chlortetracycline	0.1	0.1	50
Tilmicosin	0.1	0.1	50
Thiamphenicol	1	1	50
Sulfadimidine	0.02	0.02	20
Sulfametoxydiazine	0.01	0.02	10
Sulfamethoxypyridazine	0.02	0.02	20
Sulfisozole	0.01	0.01	50
Trichlorfon (DEP)	0.05	0.05	50
Sulfamonomethoxine	0.02	0.02	20
Furazolidone	1	1	50
Difurazone	0.05	0.05	50
Erythromycin A	0.01	0.01	50
Cefazolin	0.5	0.5	50

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Sulfachloropyridazine	0.02	0.02	20
Sulfadimethoxine	0.02	0.02	10
Tylosin	0.05	0.05	50
Sulfamethoxazole	0.02	0.1	10
Sulfaethoxypyridazine	0.02	0.02	10
Tiamulin	0.01	0.01	50
Florfenicol	0.5	10	50
2Acetylamino 5nitrothiazole	0.05	0.05	50
Sulfatroxazole	0.01	0.01	5
Leucomycin	0.01	0.01	50
Sulfisoxazole	0.01	0.05	50
Oxolinic acid	0.01	0.1	50
Chloramphenicol	0.5	1	50
Clorsulon	0.5	1	50
Sulfabenzamide	0.01	0.01	10
Ethopabate	0.01	0.01	10
Sulfadoxine	0.02	0.02	20
Sulfaquinoxaline	0.02	0.02	10
Prednisolone	0.1	0.05	20
Ofloxacin	0.5	0.5	50
Flubendazole	0.01	0.01	50
Methylprednisolone	0.5	0.5	50
Nalidixic acid	0.01	0.01	50
Dexamethasone	0.5	0.5	50
Flumequine	0.01	0.01	50
Benzylpenicillin	0.5	0.5	50
Sulfanitran	0.2	0.2	50
Sulfabromomethazine	0.01	0.01	50
betaTrenbolone	0.02	0.1	50
Emamectin B1a	0.01	0.01	50
alphaTrenbolone	0.02	0.1	50
Piromidic acid	0.01	0.05	50
Zeranol	1	0.1	50
Ketoprofen	0.01	0.05	50
Testosterone	0.01	0.05	10
Famphur	0.05	0.05	50
Fenobucarb (BPMC)	0.01	0.01	50
Clostebol	0.05	0.05	50
Dichlofenac	0.01	0.01	50
Melengestrol Acetate	0.05	0.05	50
Temephos (Abate)	0.01	0.5	50
Allethrin	0.1	1	50
Closantel	0.01	0.01	10
Monensin	0.01	0.01	10

(Unit: µg/L)

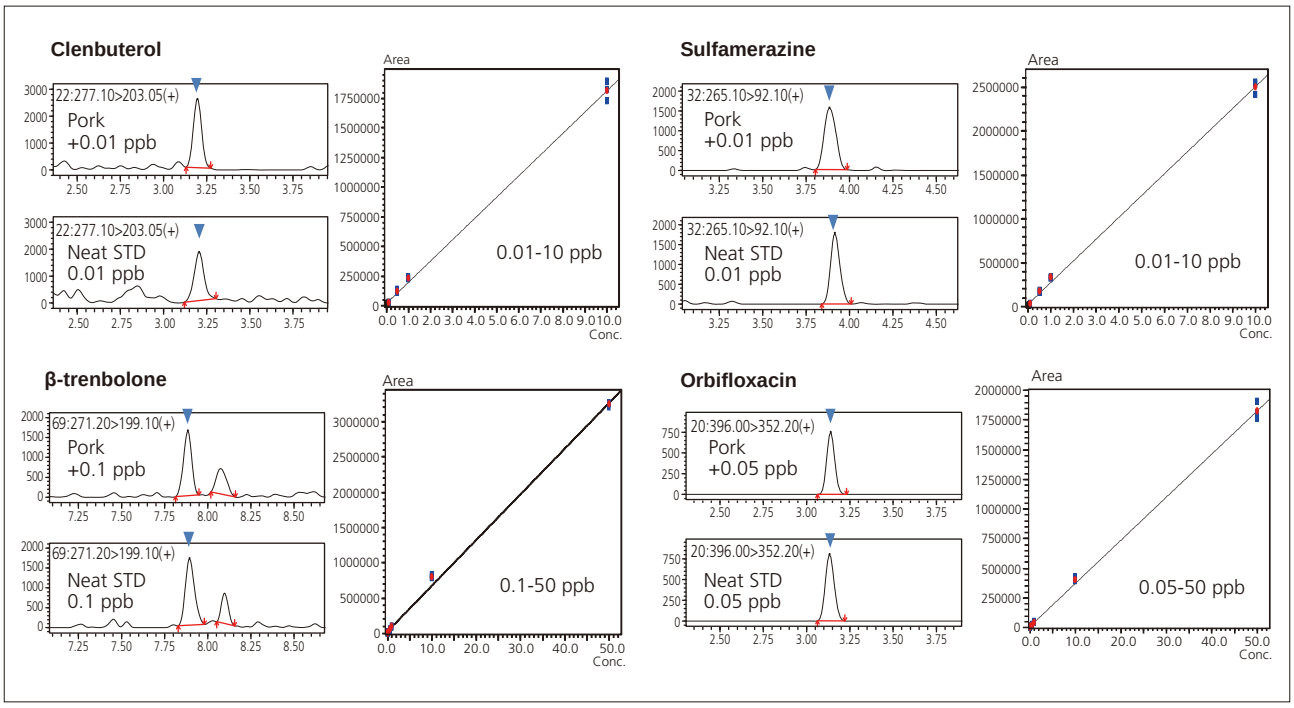


Fig. 4 MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds

Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 µg/L, after which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.

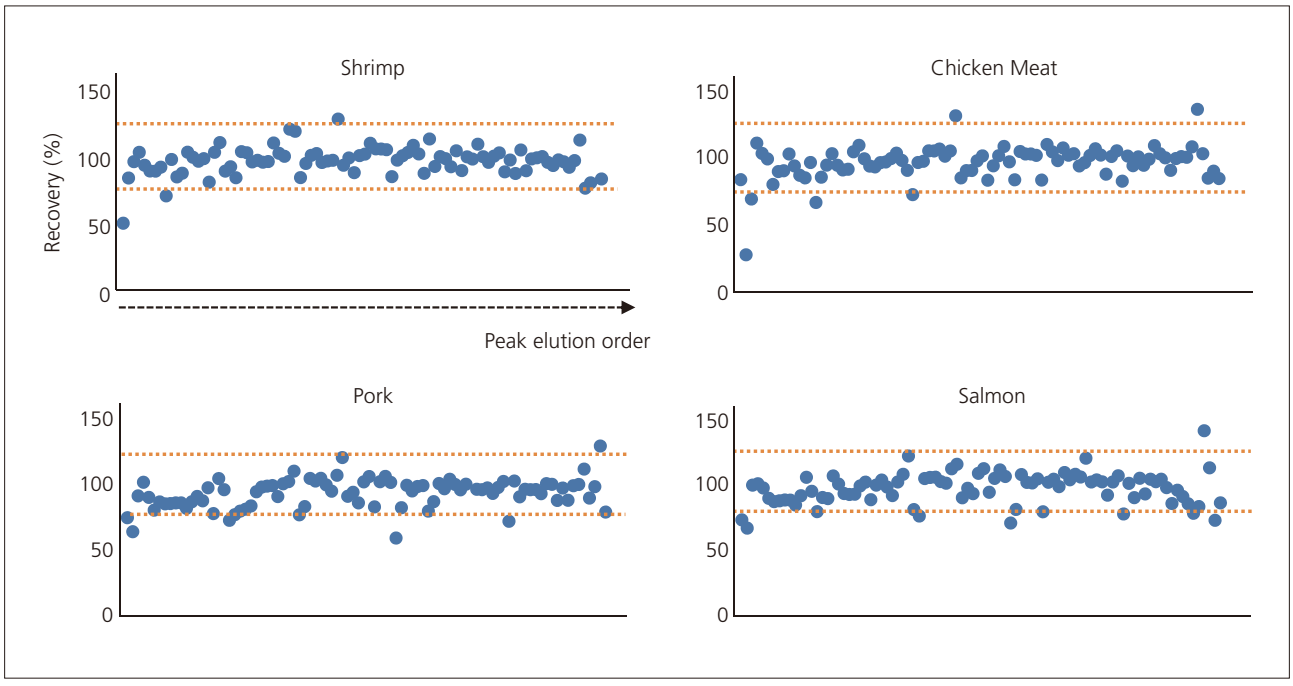


Fig. 5 Recoveries of Veterinary Drugs in Each of the Matrices

Acetonitrile Extraction Efficiency Using QuEChERS Method

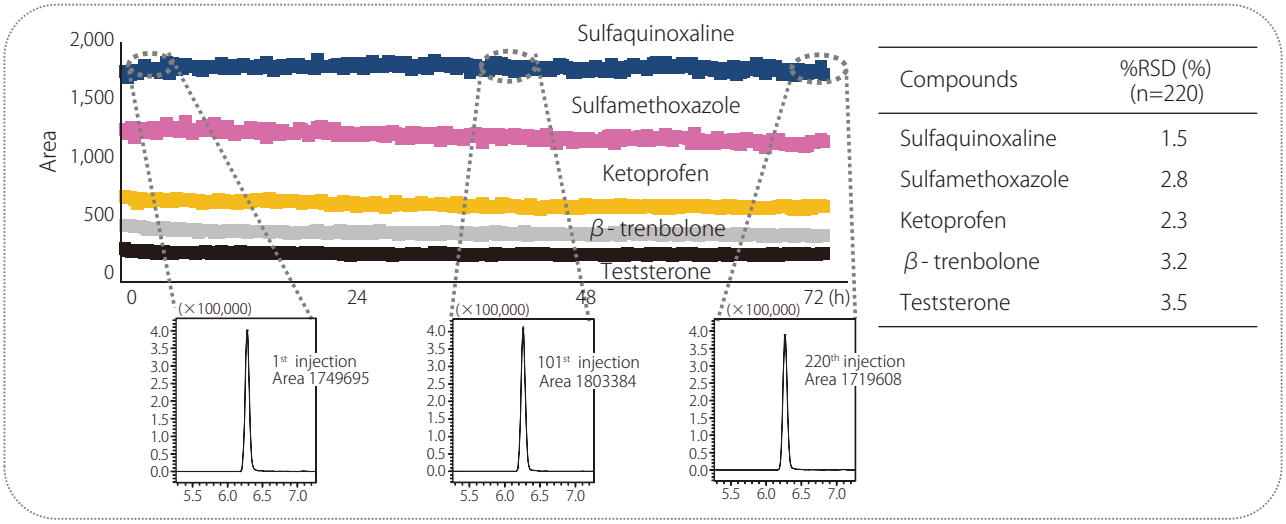
To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

Table 2 Recoveries (Pre-Spike)

Recovery	Without Matrix	With Matrix (Pork)	Compounds with Poor Recovery
< 50 %	17 (19 %)	13 (15 %)	Tetracyclines Quinolones
50 % - 70 %	1 (1 %)	8 (9 %)	
> 70 %	71 (80 %)	68 (76 %)	

Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.



Compounds	%RSD (%) (n=220)
Sulfaquinoxaline	1.5
Sulfamethoxazole	2.8
Ketoprofen	2.3
β - trenbolone	3.2
Testosterone	3.5

Fig. 6 Area Plot and %RSD of Typical Compounds with Continuous Analysis

Table 3 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (75 mm × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 0.1 % Formic Acid - Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL (2 µL sample solution + 10 µL water)
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +2.0 kV / -1.0 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

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