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Analysis of Veterinary Drugs in Chicken Tenders using the Quadrupole Time-of-Flight Mass Spectrometer

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1. Introduction

Currently, triple-quadrupole mass spectrometers are widely used for the analysis of veterinary drugs in food, because they provide highly selective and sensitive quantitative results. However, this method can only detect the envisaged target compounds, and there is a limit to the number of compounds that can be measured at one time. Therefore, this method has limited comprehensiveness for use in screening applications. Against this background, comprehensive analysis for veterinary drugs in full scan mode using a high-resolution mass spectrometer is attracting attention. In this poster, we report a case of using a quadrupole time-of-flight mass spectrometer to analyze veterinary drugs in chicken tenders.

2. Methods

2-1. Analysis Conditions

For the analysis of veterinary drugs, the method included in the LC/MS/MS Method Package Veterinary Drugs Ver. 2 was applied to the LCMS-9030 (Fig. 1). The HPLC and MS conditions are shown in Table 1.

Fig. 1 Nexera™ X3 and LCMS-9030

Table 1 Analysis Conditions

LC-MS method	
UHPLC (Nexera X3 system)	**MS (LCMS-9030)**
Column: Shim-pack™ Scepter C18-120 [Metal free column] (150 mmL×2.1 mm I.D., 2.7 µm, Shimadzu)	Ionization: ESI (Positive)
	TOF-MS: *m/z* 100-1000
Mobile phase A: 0.1 % Formic acid-Water	DL temp.: 250 °C
B: 0.1 % Formic acid-Acetonitrile	HB temp: 400 °C
Gradient program: B conc. 1 % (0 min)-15 % (1 min)-40 % (6 min)-100 % (10-15 min)-1 % (15.01-18 min)	Interface temp.: 250 °C
	Drying gas: 10 L/min
Flow rate: 0.2 mL/min	Nebulizing gas: 3.0 L/min
Column temp.: 40 °C	Heating gas: 10 L/min
Injection vol.: 2 µL (Co-injection 10 µL Water)	

2-2. Sample Preparation

Commercially available chicken tenders were used. Also, a mixture standard solution (Hayashi Pure Chemical Ind., Ltd. and FUJIFILM Wako Pure Chemical Corporation), which consist of sulfa drugs and quinolone drugs were used as the veterinary drugs for this analysis. The extraction and purification for chicken tenders were performed according to the STQ-LC method[1)] with repeated extraction developed by AiSTI SCIENCE Co., Ltd. The detailed preparation processes are shown in Fig. 2. In addition, by adding a fixed concentration of standard solution to the chicken tenders, the recovery rate for losses in the preparation process and matrix effects were also evaluated.

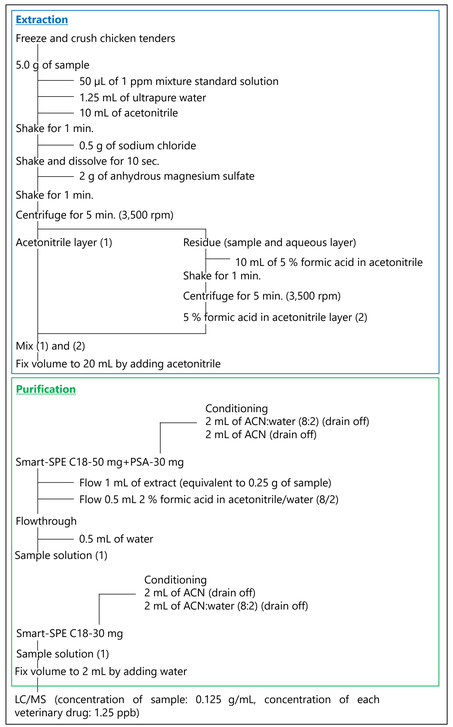

Fig. 2 Workflow for Sample Preparation

3. Results

3-1. Analysis by LCMS-9030

39 veterinary drug standard mixture diluted to 1.25 ppb, the chicken tenders extract pretreated with the veterinary drug mixture standard solution (veterinary drug concentration in the sample after pretreatment was 1.25 ppb), and the chicken tenders extract with no veterinary drug added as a blank were analyzed, respectively. Extracted ion chromatograms (XIC) of the 39 compounds in each are shown in Fig 3. The XIC drawing range was ±20 ppm or ±5 ppm. All 39 compounds were detected at a concentration of 1.25 ppb in the veterinary drug mixture standard solution and the veterinary drug-added chicken tenders extract. By narrowing the XIC drawing range from ±20 ppm to ±5 ppm for chicken tenders extract with no veterinary drug additives, chromatograms with less noise and fewer foreign peaks were obtained. The LCMS-9030 is a Q-TOF analyzer with high sensitivity that covers the lower limit of quantitation required for routine analysis, and its high mass accuracy enables chromatograms with few foreign peaks to be obtained.

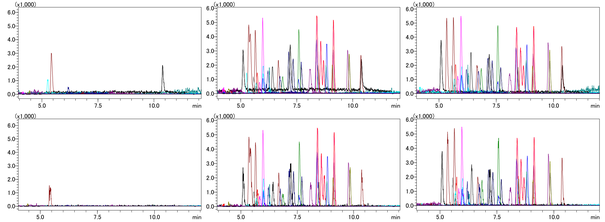

Fig. 3 Extracted ion chromatograms of 39 veterinary drug (XIC drawing range: top row: ±20 ppm, bottom row: ±5 ppm) (A) chicken tenders extract with no veterinary drug added, (B) chicken tenders extract with veterinary drug added, (C) mixed standard solution of veterinary drugs.

3-2. Linearity of Calibration Curve

Linearity of the calibration curve for each veterinary drug was evaluated by generating a 6-point calibration curve with the range 0.25-50 ppb in solvent and in chicken tenders extract. Both in solvent and in extract, linearity showed very good results (coefficient of determination R^2: 0.99 or more) for all compounds. Calibration curves for Sulfamethoxazole, a sulfa drug in solvent and in extract are shown in Fig. 4 as an example, and calibration ranges for all 39 compounds are shown in Table 2.

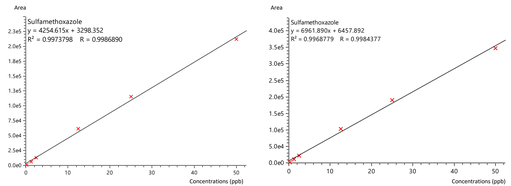

Fig. 4 Calibration Curve of Sulfamethoxazole (Left: in Solvent, Right: in chicken tenders Extract)

3-3. Spike and Recovery Test

A spike and recovery test was performed using chicken tenders extract to which 39 veterinary drugs mixture standard solution was spiked at 0.01 mg/kg per sample (concentration in pretreated sample solution was 1.25 ppb), and the recovery rate and mass error (n=6) were evaluated. The results of recovery rate, reproducibility (%RSD), and mass error are shown in Table 2, and the breakdown of recovery rate is shown in Fig. 5. Recovery rates were 70-120% for 32 of the 39 compounds. Good recovery rate and reproducibility were obtained without significant matrix inhibition, even in solutions containing high sample concentration.

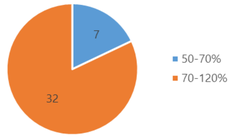

Fig. 5 Breakdown of Recovery Rate

Table 2 Linear Range, Recovery Rate, Reproducibility (%RSD) and Mass Error of 39 Veterinary Drugs

Compound	Calibration Range (ppb)		Recovery Rate (%)	%RSD	Mass Error (mDa)	Compound	Calibration Range (ppb)		Recovery Rate (%)	%RSD	Mass Error (mDa)
	in solvent	in chicken tenders extract					in solvent	in chicken tenders extract			
Ciprofloxacin	0.25-50	0.25-50	68.4	5.5	0.2	Sulfachlorpyridazine	0.25-50	0.25-50	125.8	8.0	0.6
Danofloxacin	0.25-50	0.25-50	90.4	5.6	1.0	Sulfadiazine	0.25-50	0.25-25	109.6	2.5	0.0
Diaveridine	0.25-50	0.25-50	92.8	3.0	0.7	Sulfadimethoxine	0.25-50	0.25-50	109.7	3.0	0.9
Difloxacin	0.25-50	0.25-50	70.1	3.7	1.4	Sulfadimidine	0.25-50	0.25-50	110.6	4.4	1.2
Enrofloxacin	0.25-50	0.25-50	116.1	12.1	1.3	Sulfadoxine	0.25-50	0.25-50	117.6	2.6	1.1
Flumequine	0.25-50	0.25-50	98.6	2.7	0.7	Sulfaethoxypyridazine	0.25-50	0.25-50	120.2	3.5	0.9
Marbofloxacin	0.25-50	0.25-50	61.9	5.0	1.0	Sulfamerazine	0.25-50	0.25-50	114.5	2.6	0.8
Miloxacin	0.25-50	0.25-50	80.3	4.3	0.6	Sulfamethoxazole	0.25-50	0.25-50	115.5	3.2	1.0
Nalidixic Acid	0.25-50	0.25-50	91.8	4.0	0.7	Sulfamethoxypyridazine	0.25-50	0.25-50	115.4	2.8	0.6
Norfloxacin	0.25-50	0.25-50	71.3	3.3	0.8	Sulfametoxydiazine	0.25-50	0.25-50	114.7	2.6	0.4
Ofloxacin	0.25-50	0.25-50	74.3	4.3	1.0	Sulfamonomethoxine	0.25-50	0.25-50	114.2	3.5	0.7
Orbifloxacin	0.25-50	0.25-50	68.5	4.1	1.2	Sulfapyridine	1.25-50	1.25-50	65.9	24.4	-0.3
Ormetoprim	0.25-50	0.25-25	97.1	2.8	0.8	Sulfaquinoxaline	0.25-50	0.25-50	112.3	1.9	0.6
Oxolinic Acid	0.25-50	0.25-50	105.2	4.0	0.7	Sulfathiazole	0.25-50	0.25-50	109.4	4.0	0.9
Piromidic acid	0.25-50	0.25-50	86.8	3.7	0.8	Sulfatroxazole	0.25-50	0.25-50	111.3	1.8	0.7
Pyrimethamine	0.25-50	0.25-50	94.3	3.3	0.7	Sulfisomidine	0.25-50	0.25-50	78.0	7.6	1.1
Sarafloxacin	0.25-50	0.25-50	73.4	7.7	0.7	Sulfisoxazole	0.25-50	0.25-50	114.7	2.4	0.7
Sulfabenzamide	0.25-50	0.25-50	108.6	3.2	0.5	Sulfisozole sodium	0.25-50	0.25-50	120.8	4.3	0.8
Sulfabromomethazine Na	0.25-50	0.25-50	111.9	3.4	1.2	Trimethoprim	0.25-50	0.25-50	84.2	2.2	0.8
Sulfacetamide	0.25-50	0.25-50	89.7	4.9	0.6						

4. Conclusion

- ✓ The STQ-LC method with repeated extraction made it possible to speed up and simplify the preparation process
- ✓ It enables comprehensive measurement of veterinary drugs by analysis using the LCMS-9030, which can obtain accurate mass.
- ✓ XIC with narrow *m/z* range can provide peaks with less noise and fewer contaminants.
- ✓ Analysis of pretreated chicken tenders samples using LCMS-9030 provided good results for spike recovery rate, reproducibility, and linearity.

<Reference>

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)