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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-9030

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

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User Benefits

- It enables comprehensive measurement of veterinary drugs by full scan analysis using the LCMS-9030, which can obtain accurate mass.
- Extracted ion chromatograms (XIC) with narrow *m/z* range can provide peaks with less noise and fewer contaminants.
- It is possible to perform highly sensitive analysis that covers the lower limit of quantitation required for routine analysis.

■ Introduction

Veterinary drugs are used for various purposes such as prevention and treatment of diseases and growth promotion of livestock and farmed fish. While veterinary drugs enable a stable supply of livestock and seafood products, there is a problem of health risks due to drug residues. For this reason, each region and country has established maximum residue limits (MRLs) for veterinary drugs in food and strictly regulates them.

Currently, triple quadrupole mass spectrometers, which can perform quantitative analysis highly selectively and highly sensitively, are widely used for the analysis of veterinary drugs in food. 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.

This article introduces an example of comprehensive analysis of veterinary drugs in chicken tenders using a quadrupole time-of-flight mass spectrometer LCMS-9030 (Fig. 1).

Fig. 1 Exterior of Nexera™ X3 and LCMS -9030

■ Sample Preparation

Commercially available chicken tenders were used in this analysis. 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.

Extraction

Freeze and crush chicken tenders

5.0 g of sample

- 50 µL of 1 ppm mixture standard solution
- 1.25 mL of ultrapure water
- 10 mL of acetonitrile

Shake for 1 min.

- 0.5 g of sodium chloride

Shake and dissolve for 10 sec.

- 2 g of anhydrous magnesium sulfate

Shake for 1 min.

Centrifuge for 5 min. (3,500 rpm)

Acetonitrile layer (1)

Residue (sample and aqueous layer)

- 10 mL of 5 % formic acid in acetonitrile

Shake for 1 min.

Centrifuge for 5 min. (3,500 rpm)

5 % formic acid in acetonitrile layer (2)

Mix (1) and (2)

Fix volume to 20 mL by adding acetonitrile

Purification

Smart-SPE C18-50 mg+PSA-30 mg

Conditioning
- 2 mL of ACN:water (8:2) (drain off)
- 2 mL of ACN (drain off)

- Flow 1 mL of extract (equivalent to 0.25 g of sample)
- Flow 0.5 mL 2 % formic acid in acetonitrile/water (8/2)

Flowthrough

- 0.5 mL of water

Sample solution (1)

Smart-SPE C18-30 mg

Conditioning
- 2 mL of ACN (drain off)
- 2 mL of ACN:water (8:2) (drain off)

Sample solution (1)

Fix volume to 2 mL by adding water

LC/MS (concentration of sample: 0.125 g/mL, concentration of each veterinary drug: 1.25 ppb)

Fig. 2 Workflow for Sample Preparation

■ Analytical Conditions

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

■ Compound List for Veterinary Drugs

Table 2 shows the compound list of 39 veterinary drugs used in this experiment. Theoretical *m/z* values of compounds were calculated using LabSolutions Insight Explore™.

Table 1 Analytical Conditions

UHPLC (Nexera™ X3 system)	
Column:	Shim-pack™ Scepter C18-120 [Metal free column] (150 mmL × 2.1 mm I.D., 2.7 µm) P/N: 227-31073-03
Mobile Phase A:	0.1 % Formic acid-Water
Mobile Phase B:	0.1 % Formic acid-Acetonitrile
Gradient Program:	B conc. 1 % (0 min)-15 % (1 min)-40 % (6 min)-100 % (10-15 min)-1 % (15.01-18 min)
Flowrate:	0.2 mL/min
Injection Volume:	2 µL (Co-injection 10 µL Water)

MS (LCMS-9030)	
Ionization:	ESI (Positive)
TOF-MS:	*m/z* 100-1000
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Interface Temp.:	250 °C

Table 2 List of Veterinary Drugs

Compound	Molecular Formula	Selected Ion	*m/z*	Retention Time (min)
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	$[M+H]^+$	332.1405	5.700
Danofloxacin	$C_{19}H_{20}FN_3O_3$	$[M+H]^+$	358.1562	5.852
Diaveridine	$C_{13}H_{16}N_4O_2$	$[M+H]^+$	261.1346	5.302
Difloxacin	$C_{21}H_{19}F_2N_3O_3$	$[M+H]^+$	400.1467	6.766
Enrofloxacin	$C_{19}H_{22}FN_3O_3$	$[M+H]^+$	360.1718	6.088
Flumequine	$C_{14}H_{12}FNO_3$	$[M+H]^+$	262.0874	9.708
Marbofloxacin	$C_{17}H_{19}FN_4O_4$	$[M+H]^+$	363.1463	5.469
Miloxacin	$C_{12}H_9NO_6$	$[M+H]^+$	264.0503	8.598
Nalidixic Acid	$C_{12}H_{12}N_2O_3$	$[M+H]^+$	233.0921	9.633
Norfloxacin	$C_{16}H_{18}FN_3O_3$	$[M+H]^+$	320.1405	5.726
Ofloxacin	$C_{18}H_{20}FN_3O_4$	$[M+H]^+$	362.1511	5.620
Orbifloxacin	$C_{19}H_{20}F_3N_3O_3$	$[M+H]^+$	396.1530	6.318
Ormetoprim	$C_{14}H_{18}N_4O_2$	$[M+H]^+$	275.1503	5.886
Oxolinic Acid	$C_{13}H_{11}NO_5$	$[M+H]^+$	262.0710	8.638
Piromidic acid	$C_{14}H_{16}N_4O_3$	$[M+H]^+$	289.1295	10.235
Pyrimethamine	$C_{12}H_{13}ClN_4$	$[M+H]^+$	249.0902	7.568
Sarafloxacin	$C_{20}H_{17}F_2N_3O_3$	$[M+H]^+$	386.1311	6.649
Sulfabenzamide	$C_{13}H_{12}N_2O_3S$	$[M+H]^+$	277.0641	8.914
Sulfabromomethazine Na	$C_{12}H_{13}BrN_4O_2S$	$[M+H]^+$	357.0015	9.730
Sulfacetamide	$C_8H_{10}N_2O_3S$	$[M+H]^+$	215.0485	5.424
Sulfachlorpyridazine	$C_{10}H_9ClN_4O_2S$	$[M+H]^+$	285.0208	7.817
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	$[M+H]^+$	251.0597	5.751
Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	$[M+H]^+$	311.0809	8.980
Sulfadimidine	$C_{12}H_{14}N_4O_2S$	$[M+H]^+$	279.0910	6.929
Sulfadoxine	$C_{12}H_{14}N_4O_4S$	$[M+H]^+$	311.0809	8.120
Sulfaethoxypyridazine	$C_{12}H_{14}N_4O_3S$	$[M+H]^+$	295.0859	8.117
Sulfamerazine	$C_{11}H_{12}N_4O_2S$	$[M+H]^+$	265.0754	6.425
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	$[M+H]^+$	254.0594	8.187
Sulfamethoxypyridazine	$C_{11}H_{12}N_4O_3S$	$[M+H]^+$	281.0703	6.891
Sulfametoxydiazine	$C_{11}H_{12}N_4O_3S$	$[M+H]^+$	281.0703	7.328
Sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$	$[M+H]^+$	281.0703	7.432
Sulfapyridine	$C_{11}H_{11}N_3O_2S$	$[M+H]^+$	250.0645	6.018
Sulfaquinoxaline	$C_{14}H_{12}N_4O_2S$	$[M+H]^+$	301.0754	8.947
Sulfathiazole	$C_9H_9N_3O_2S_2$	$[M+H]^+$	256.0209	5.783
Sulfatroxazole	$C_{11}H_{13}N_3O_3S$	$[M+H]^+$	268.0750	8.311
Sulfisomidine	$C_{12}H_{14}N_4O_2S$	$[M+H]^+$	279.0910	4.998
Sulfisoxazole	$C_{11}H_{13}N_3O_3S$	$[M+H]^+$	268.0750	8.461
Sulfisozole sodium	$C_9H_8N_3NaO_3S$	$[M+H]^+$	240.0437	7.572
Trimethoprim	$C_{14}H_{18}N_4O_3$	$[M+H]^+$	291.1452	5.567

■ Full Scan Analysis by LCMS-9030

Full scan analysis was performed for the 39 veterinary drug standard mixture diluted to 1.25 ppb, veterinary drug added chicken tenders extract (concentration of each veterinary drug after sample preparation was 1.25 ppb), acetonitrile and veterinary drug-free chicken tenders extract as blank solution. Fig. 3 shows the extracted ion chromatogram (XIC) of each of the 39 compounds in each sample solution. The XICs were drawn with ± 20 ppm tolerance for theoretical *m/z* values.

All 39 compounds were detected at a concentration of 2.5 ppb from the veterinary drug added chicken tenders extract (Fig. 3-D). Furthermore, almost no contaminant signals were detected in blank solutions (Fig. 3-A, B), indicating that the LCMS-9030 has sufficient resolution for full scan analysis of veterinary drugs.

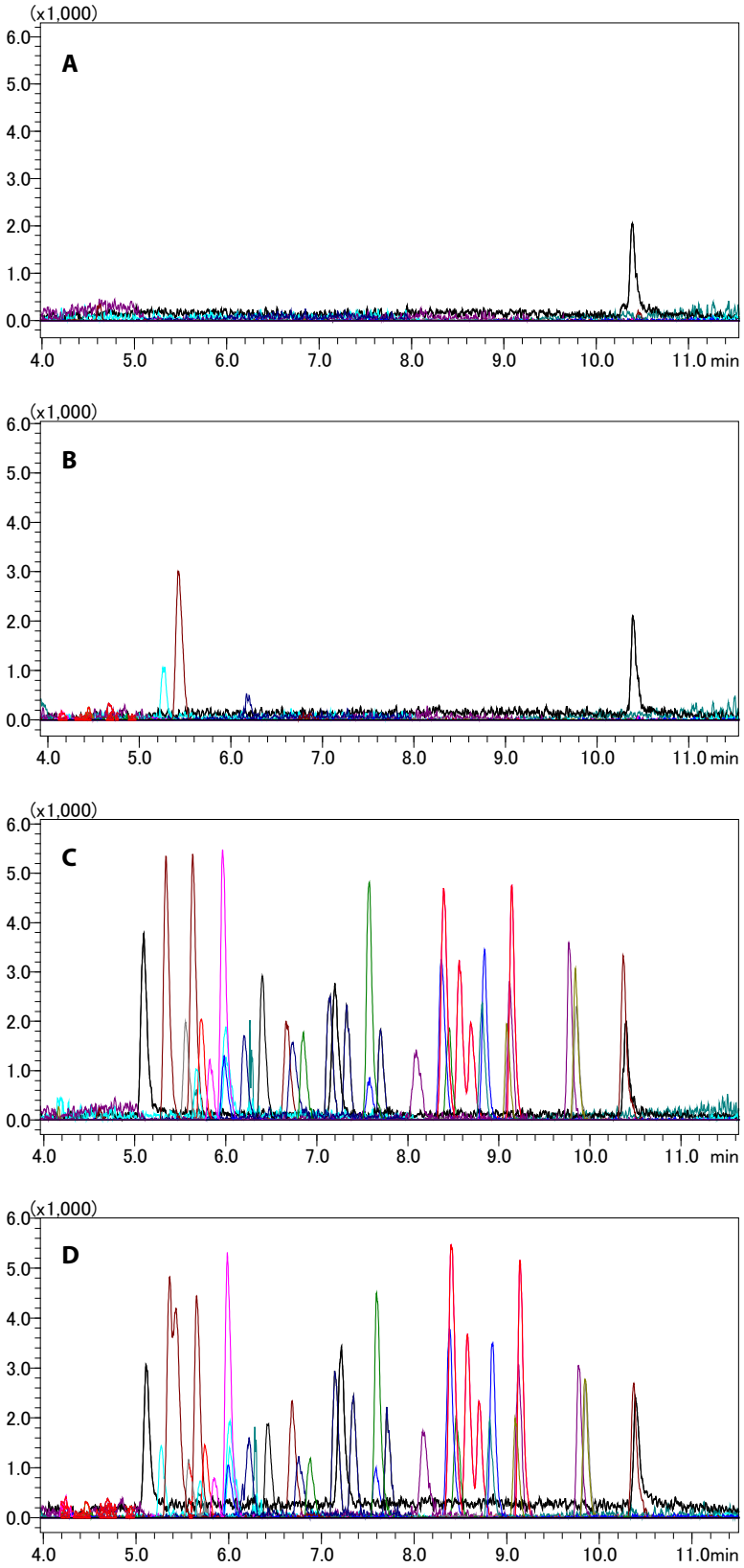

Fig. 3 Extracted Ion Chromatograms of 39 Compounds
(A) Acetonitrile, (B) Veterinary Drug-free Chicken Tenders Extract , (C) Mixture Standard Solution, (D) Veterinary Drug Added Chicken Tenders Extract

■ Linearity of Calibration Curve

Linearity of the calibration curve for each compound 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, and Enrofloxacin, a quinolone drug are shown respectively in Fig. 4 and 5 as an example, and calibration ranges for all 39 compounds are shown in Table 3.

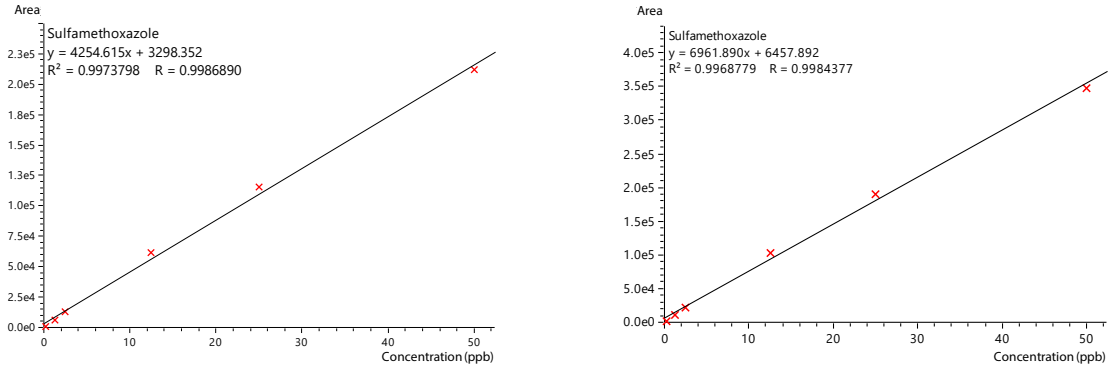

Fig. 4 Calibration Curve of Sulfamethoxazole (Left: in Solvent, Right: in Chicken Tenders Extract)

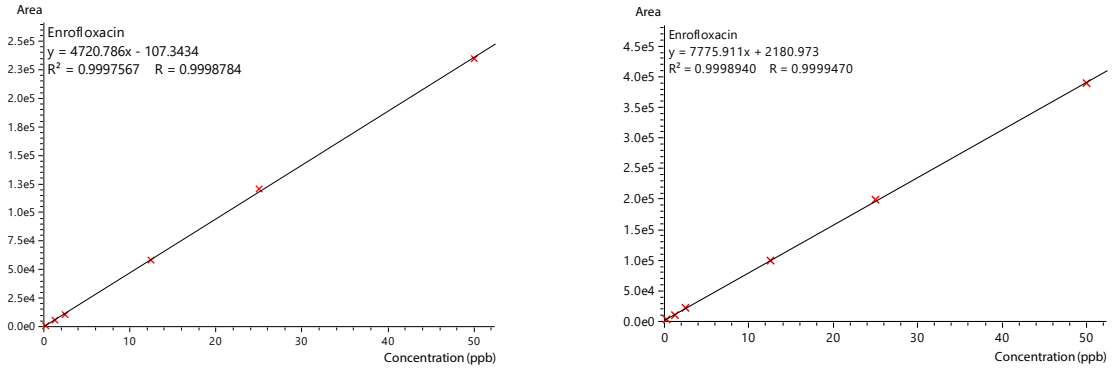

Fig. 5 Calibration Curve of Enrofloxacin (Left: in Solvent, Right: in Chicken Tenders Extract)

Table 3 Linear Range of 39 Compounds

Compound	Calibration Range (ppb)	
	in solvent	in chicken tenders extract
Ciprofloxacin	0.25-50	0.25-50
Danofloxacin	0.25-50	0.25-50
Diaveridine	0.25-50	0.25-50
Difloxacin	0.25-50	0.25-50
Enrofloxacin	0.25-50	0.25-50
Flumequine	0.25-50	0.25-50
Marbofloxacin	0.25-50	0.25-50
Miloxacin	0.25-50	0.25-50
Nalidixic Acid	0.25-50	0.25-50
Norfloxacin	0.25-50	0.25-50
Ofloxacin	0.25-50	0.25-50
Orbifloxacin	0.25-50	0.25-50
Ormetoprim	0.25-50	0.25-25
Oxolinic Acid	0.25-50	0.25-50
Piromidic acid	0.25-50	0.25-50
Pyrimethamine	0.25-50	0.25-50
Sarafloxacin	0.25-50	0.25-50
Sulfabenzamide	0.25-50	0.25-50
Sulfabromomethazine Na	0.25-50	0.25-50
Sulfacetamide	0.25-50	0.25-50

Compound	Calibration Range (ppb)	
	in solvent	in chicken tenders extract
Sulfachlorpyridazine	0.25-50	0.25-50
Sulfadiazine	0.25-50	0.25-25
Sulfadimethoxine	0.25-50	0.25-50
Sulfadimidine	0.25-50	0.25-50
Sulfadoxine	0.25-50	0.25-50
Sulfaethoxypyridazine	0.25-50	0.25-50
Sulfamerazine	0.25-50	0.25-50
Sulfamethoxazole	0.25-50	0.25-50
Sulfamethoxypyridazine	0.25-50	0.25-50
Sulfametoxydiazine	0.25-50	0.25-50
Sulfamonomethoxine	0.25-50	0.25-50
Sulfapyridine	1.25-50	1.25-50
Sulfaquinoxaline	0.25-50	0.25-50
Sulfathiazole	0.25-50	0.25-50
Sulfatroxazole	0.25-50	0.25-50
Sulfisomidine	0.25-50	0.25-50
Sulfisoxazole	0.25-50	0.25-50
Sulfisozole sodium	0.25-50	0.25-50
Trimethoprim	0.25-50	0.25-50

■ 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 4, and the breakdown of recovery rate is shown in Fig. 6.

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.

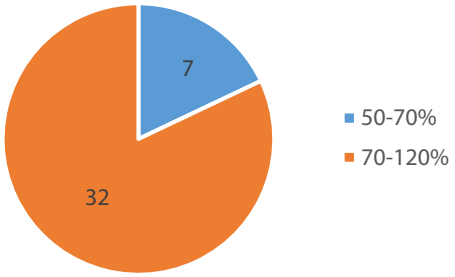

Fig. 6 Breakdown of Recovery Rate

Table 4 Recovery Rate, Reproducibility (%RSD) and Mass Error (n=6)

Compound	Recovery Rate (%)	%RSD	Mass Error (mDa)
Ciprofloxacin	68.4	5.5	0.2
Danofloxacin	90.4	5.6	1.0
Diaveridine	92.8	3.0	0.7
Difloxacin	70.1	3.7	1.4
Enrofloxacin	116.1	12.1	1.3
Flumequine	98.6	2.7	0.7
Marbofloxacin	61.9	5.0	1.0
Miloxacin	80.3	4.3	0.6
Nalidixic Acid	91.8	4.0	0.7
Norfloxacin	71.3	3.3	0.8
Ofloxacin	74.3	4.3	1.0
Orbifloxacin	68.5	4.1	1.2
Ormetoprim	97.1	2.8	0.8
Oxolinic Acid	105.2	4.0	0.7
Piromidic acid	86.8	3.7	0.8
Pyrimethamine	94.3	3.3	0.7
Sarafloxacin	73.4	7.7	0.7
Sulfabenzamide	108.6	3.2	0.5
Sulfabromomethazine Na	111.9	3.4	1.2
Sulfacetamide	89.7	4.9	0.6

Compound	Recovery Rate (%)	%RSD	Mass Error (mDa)
Sulfachlorpyridazine	125.8	8.0	0.6
Sulfadiazine	109.6	2.5	0.0
Sulfadimethoxine	109.7	3.0	0.9
Sulfadimidine	110.6	4.4	1.2
Sulfadoxine	117.6	2.6	1.1
Sulfaethoxypyridazine	120.2	3.5	0.9
Sulfamerazine	114.5	2.6	0.8
Sulfamethoxazole	115.5	3.2	1.0
Sulfamethoxypyridazine	115.4	2.8	0.6
Sulfametoxydiazine	114.7	2.6	0.4
Sulfamonomethoxine	114.2	3.5	0.7
Sulfapyridine	65.9	24.4	-0.3
Sulfaquinoxaline	112.3	1.9	0.6
Sulfathiazole	109.4	4.0	0.9
Sulfatroxazole	111.3	1.8	0.7
Sulfisomidine	78.0	7.6	1.1
Sulfisoxazole	114.7	2.4	0.7
Sulfisozole sodium	120.8	4.3	0.8
Trimethoprim	84.2	2.2	0.8

■ Conclusion

The STQ-LC method with repeated extraction made it possible to speed up and simplify the preparation process. Full scan analysis of pretreated chicken tenders samples using LCMS-9030 provided good results for spike recovery rate, reproducibility, and linearity. It was demonstrated that the analytical method introduced in this article enables “rapid, simple, and highly precise” analysis, and is useful for the analysis of veterinary drugs in food.

<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)

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