Application News

No. LCMSMS-268E

Liquid Chromatography Mass Spectrometry

Determination of Quinolone Antibiotic Residues in Chicken by Ultra-High-Performance Liquid Chromatography Coupled with Triple Quadrupole Mass Spectrometry

Abstract: A method was developed for the determination of 12 quinolone antibiotics in chicken using Shimadzu Ultra-High-Performance Liquid Chromatography (UHPLC) Nexera X2 coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The analysis of 12 antibiotics was completed within 9 min and the correlation coefficients of the calibration curves were all above 0.997. The mixed standard solutions with various concentrations of antibiotics were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds were 0.03-0.27% and 1.13-4.93%, respectively, and the precision of the instrument was good. The range of matrix spike recovery was 91.90 - 108.60% at different concentrations. The method can be applied to the simultaneous detection of 12 quinolone antibiotic residues in chicken.

Key Words: Triple Quadrupole Mass Spectrometry, Quinolone, chicken, antibiotics

■ Introduction

Quinolones (QNs) are synthetic drugs with broad-spectrum bactericidal effect. Due to their strong antibacterial activity and wide spectrum range, they are widely used in the prevention and treatment of various infectious diseases in human beings, poultry and livestock. However, drug overdose or improper use will lead to a high level of QNs residues in animals, especially for food-producing animals.
In addition to the immediate and direct toxic effects of QNs on human body, the long-term consumption of animal-derived food containing QNs can readily induce drug resistance, thus affecting the clinical efficacy of QNs on human body. Therefore, the issue of QN residues has raise more and more concerns. The U.S., Japan, E.U. and China have regulated the maximum residue limit of QNs in food and it varies according to the different classification, properties and characteristics of QNs and is in the range of 10-6000 µg/kg.

Chicken is a meat widely consumed in China, so the determination of QNs residues in chicken is of great significance. High performance liquid chromatography – tandem mass spectrometry is a rapid developing analytical technology in recent years. With its ability to perform highly sensitive and selective quantitative and qualitative analyses as well as provide high accuracy for antibiotic compounds in complex matrices, it is the preferred technique for ultra-trace residue analysis. A method was established in this application note for determination of 12 QNs antibiotics in chicken using a Shimadzu UHPLC Nexera X2 coupled with Triple Quadrupole Mass Spectrometer LCMS-8045.

■ Experimental

1.1 Instruments

The experiment employed Shimadzu UHPLC Nexera X2 and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are LC-30AD×2 infusion pump, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

1.2 Analytical Conditions

Liquid Chromatography (LC) Conditions

- Column: Shim-pack GISS 2.1 mm I.D.× 100 mm L, 1.9 µm
- Mobile phase:
- Mobile Phase A-0.2% formic acid in water;
- Mobile Phase B-acetonitrile/methanol (6:4)
- Flow rate: 0.4 mL/min
- Column temperature: 40 °C
- Injection volume: 10 µL
- Elution method: gradient elution. With the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for gradient program.

Table 1: Gradient program

Time (min)	Module	Command	Value (%)
4.50	Pumps	Pump B Conc.	40
4.60	Pumps	Pump B Conc.	95
5.50	Pumps	Pump B Conc.	95
5.60	Pumps	Pump B Conc.	10
9.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

- Analytical Instruments: LCMS-8045
- Ionization mode: ESI (+)
- Heating gas: Air 10.0 L/min
- Nebulizing gas: Nitrogen 3.0 L/min
- Drying gas: Nitrogen 10.0 L/min
- Collision gas: Argon
- Source temperature: 300 °C
- DL temperature: 250 °C
- Heater temperature: 400 °C
- Scan mode: Multiple reaction monitoring (MRM)
- Pause time: 3 msec
- MRM parameters: Refer to Table 2

Table 2: MRM optimized parameters

No.	Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Pipemidic Acid	51940-44-4	304.1	286.1*	-16	-21	-20
				215.1	-30	-38	-24
2	Enoxacin	84294-96-2	321.1	303.1*	-13	-22	-11
				232.1	-13	-35	-16
3	Ofloxacin	82419-36-1	362.1	318.2*	-12	-21	-22
				261.1	-12	-29	-18
4	Norfloxacin	70458-96-7	320.0	302.1*	-11	-22	-21
				231.1	-11	-39	-24
5	Pefloxacin	149676-40-4	334.1	316.1*	-28	-23	-22
				290.1	-13	-18	-14
6	Ciprofloxacin	93107-08-5	332.0	314.1*	-11	-16	-24
				231.0	-11	-37	-25
7	Lomefloxacin	98079-52-8	352.1	265.1*	-16	-25	28
				308.1	-16	-17	-21
8	Danofloxacin	119478-55-6	358.1	340.1*	-14	-24	-12
				255.1	-14	-40	-17
9	Enrofloxacin	93106-60-6	360.1	316.2*	-12	-20	-11
				342.1	-12	-20	-11
10	Cinoxacin	28657-80-9	263.0	245.0*	-28	-15	-26
				217.0	-28	-23	-22
11	Oxolinic Acid	14698-29-4	262.1	244.0*	-17	-18	-26
				216.1	-30	-28	-23
12	Flumequine	42835-25-6	262.1	244.1*	-16	-16	-17
				202.0	-16	-36	-22

Note: * indicates quantifier ion

1.3 Standard Solution Preparation

Quinolone standards were weighed and dissolved in methanol to prepare mixed standard stock solutions of 1.0mg/ml. The mixed standard solutions were stored at -18°C. Accurate volumes of mixed standard stock solution were added to blank chicken extract solutions to prepare mixed standard working solutions with concentrations of 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL.

1.4 Sample Preparation Method

Chicken samples were prepared with reference to the national standard GB/T 21312-2007 “Analysis of 14 Quinolone in Food of Animal Origin by High Performance Liquid Chromatography Tandem Mass Spectrometry”.
5.0g (accurate to 0.1g) of homogeneous chicken sample was weighed in a 50mL polypropylene centrifuge tube. 20mL of 0.1m/L EDTA-Mcllvaine buffer solution was added. The mixture was vortexed, ultrasonically extracted for 10 mins and centrifuged at 10,000r/min for 5 mins. The extraction was repeated 3 times in total and the supernatant was combined.

SPE clean-up was performed using HLB SPE cartridges (200mg, 6mL). The SPE was first activated with 6mL of methanol and 6mL of water before use. 6mL of extracted supernatant was added to the SPE column and rinse with 2mL of 5% methanol solution. The filtrate was discarded and the SPE column drained. Elution of SPE was carried out with 6mL of methanol. The eluate was collected and completely dried with nitrogen. The dried extract was reconstituted with 1ml of mobile phase and vortexed. The reconstituted extracts were filtered through a 0.22µm filter membrane and injected into LC-MS/MS for analysis.

■ Results and Discussion

2.1 MS scan and product ion scan of standard samples

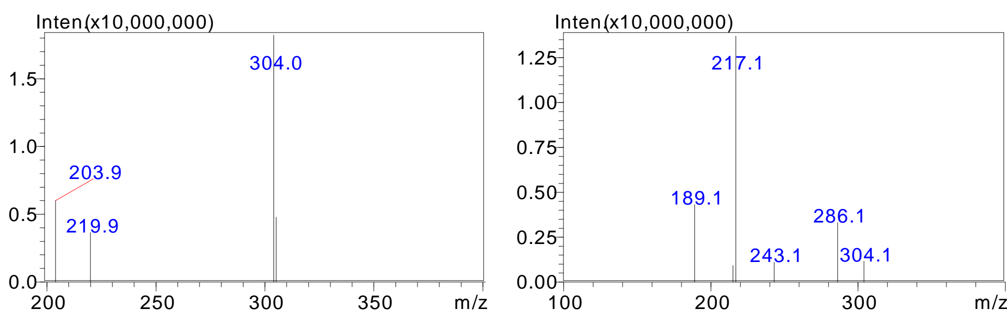

Figure 1: Q1 MS Scan (left) and product ion scan (CE value-20 V, right) of pipemidic acid

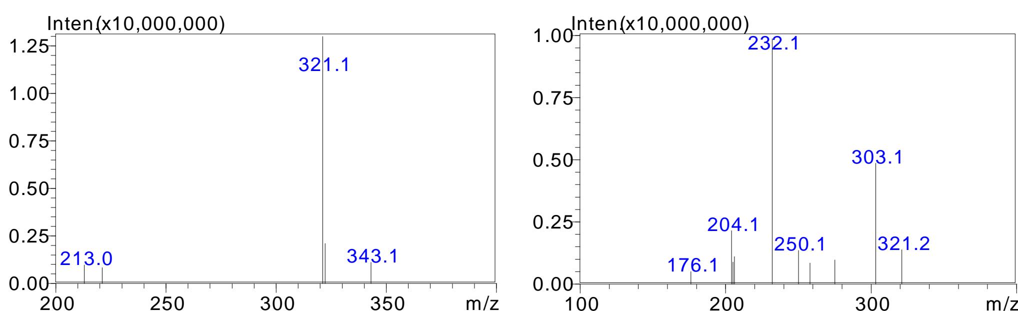

Figure 2: Q1 MS scan (left) and product ion scan (CE value-30 V, right) of enoxacin

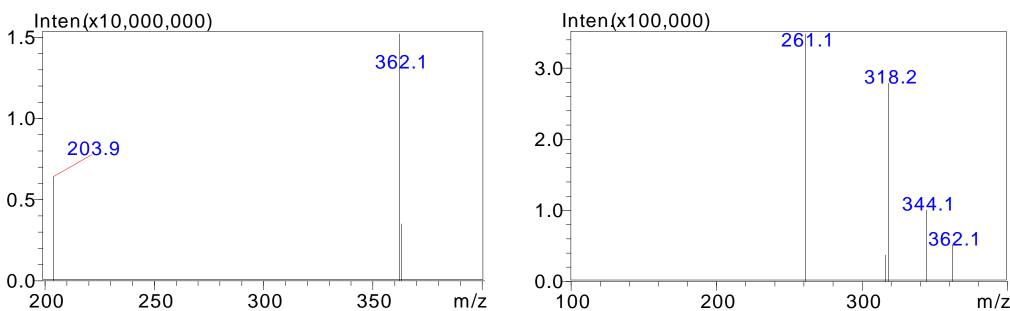

Figure 3: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ofloxacin

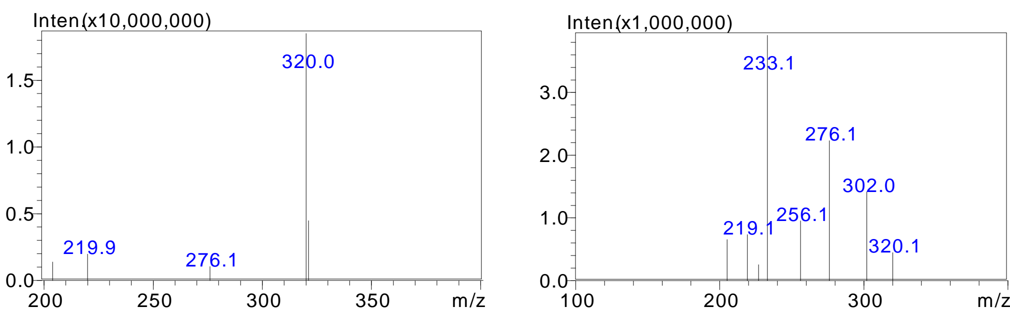

Figure 4: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of norfloxacin

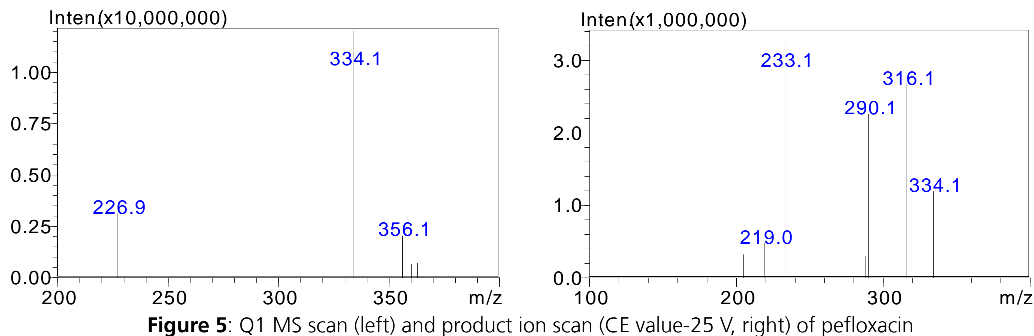

Figure 5: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of pefloxacin

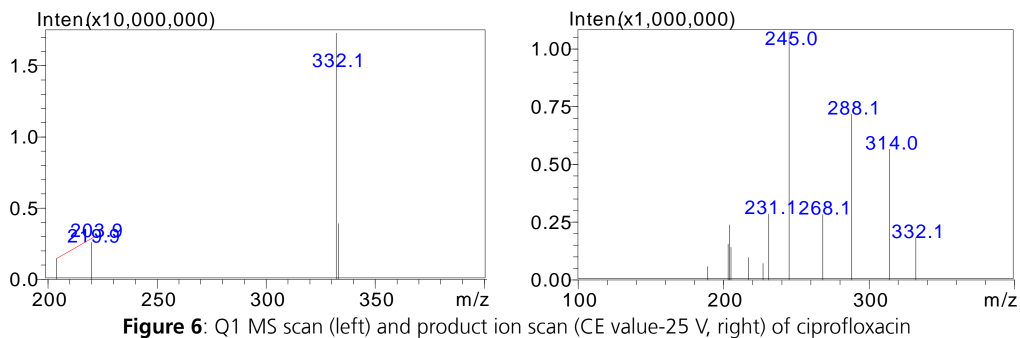

Figure 6: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ciprofloxacin

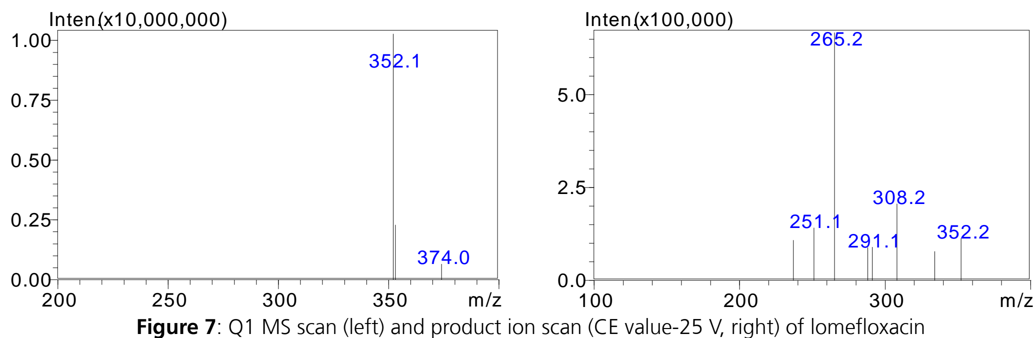

Figure 7: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of lomefloxacin

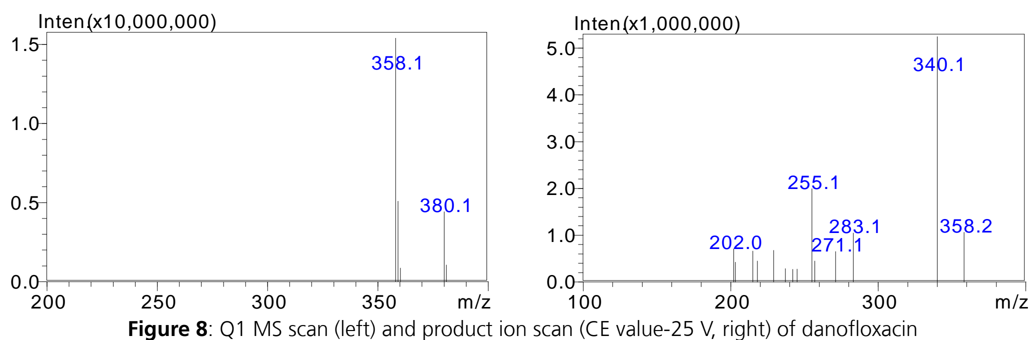

Figure 8: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of danofloxacin

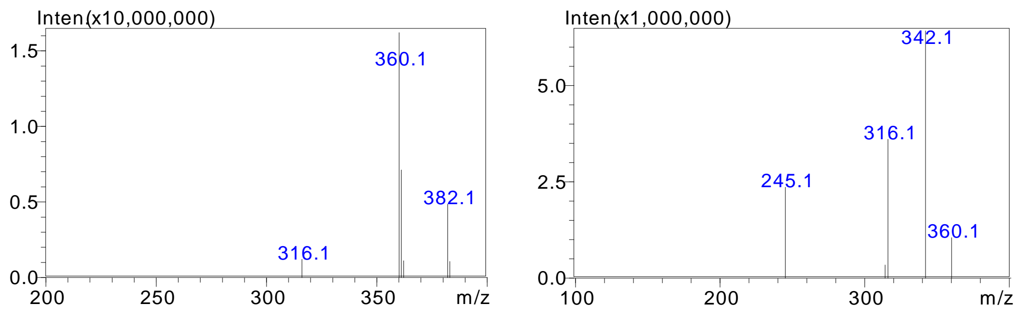

Figure 9: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of enrofloxacin

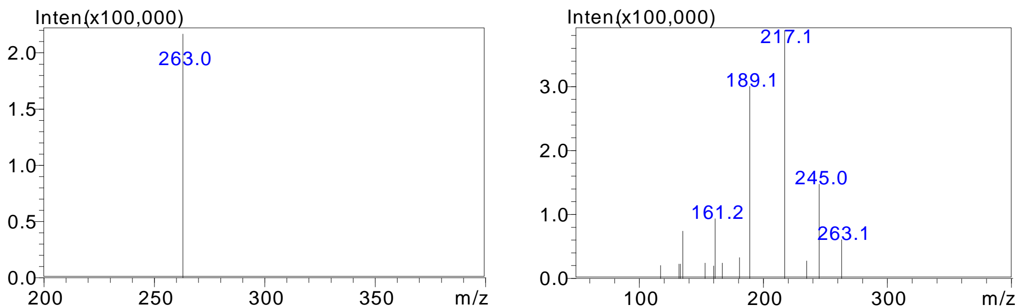

Figure 10: Q1 MS scan (left) and product ion scan (CE value-30 V, right) of cinoxacin

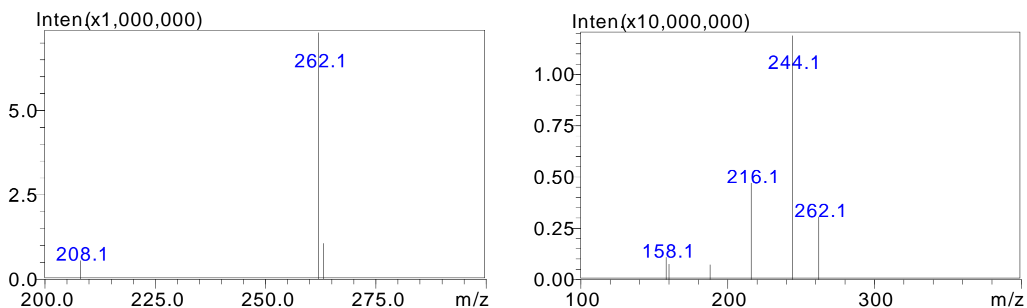

Figure 11: Q1 MS scan (left) and product ion scan (CE value-25 V, right) of oxolinic acid

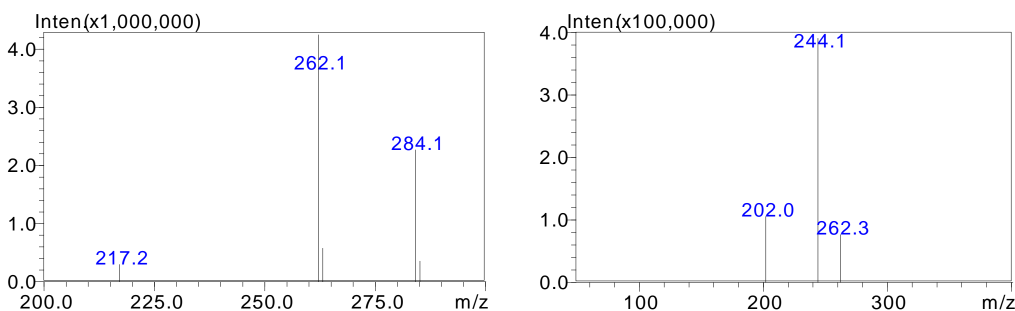

Figure 12: Q1 MS scan (left) and product ion scan (CE value-35 V, right) of flumequine

2.2 MRM Chromatograms of 12 Quinolone Standard Solutions (1 ng/mL)

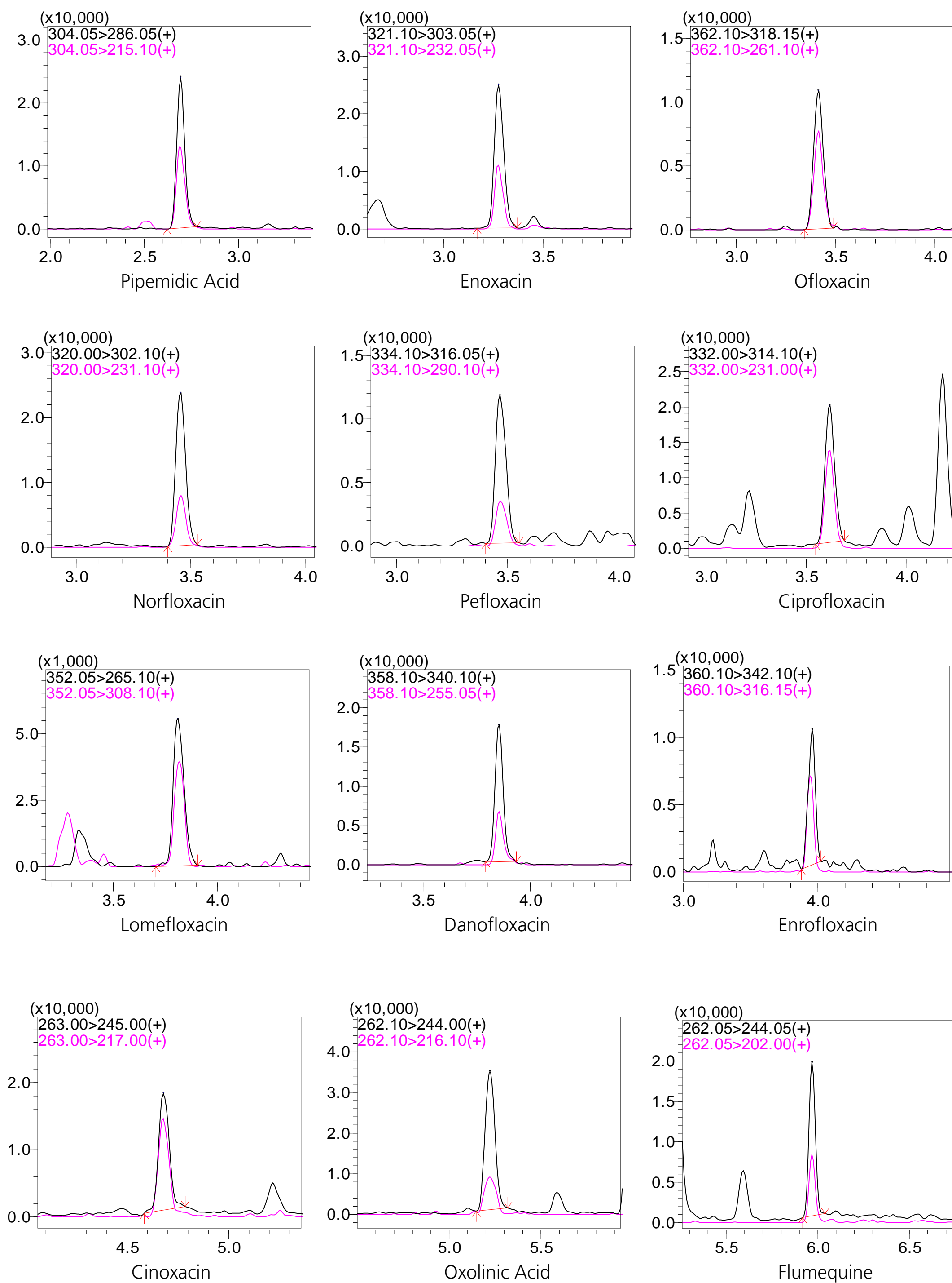

Figure 13: MRM chromatograms of 12 quinolones (1 ng/mL)

2.3 Calibration and linearity

About 60mL of combined liquid extract was collected after the extraction of 5.0g chicken sample with EDTA-McIlvaine buffer solution. The combined liquid extract went through similar SPE clean-up as indicated in Section 1.4 and was reconstituted to give 10mL of blank chicken extraction solution. The mixed standard calibration solutions of concentrations 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL were prepared by diluting appropriate amounts of the standard stock solution (1mg/mL) with the blank chicken extract solution.

A calibration curve was plotted showing concentration of working solution against peak area (see Figure 14 below). The linearity was good and the linear equation and correlation coefficient were shown in Table 3.

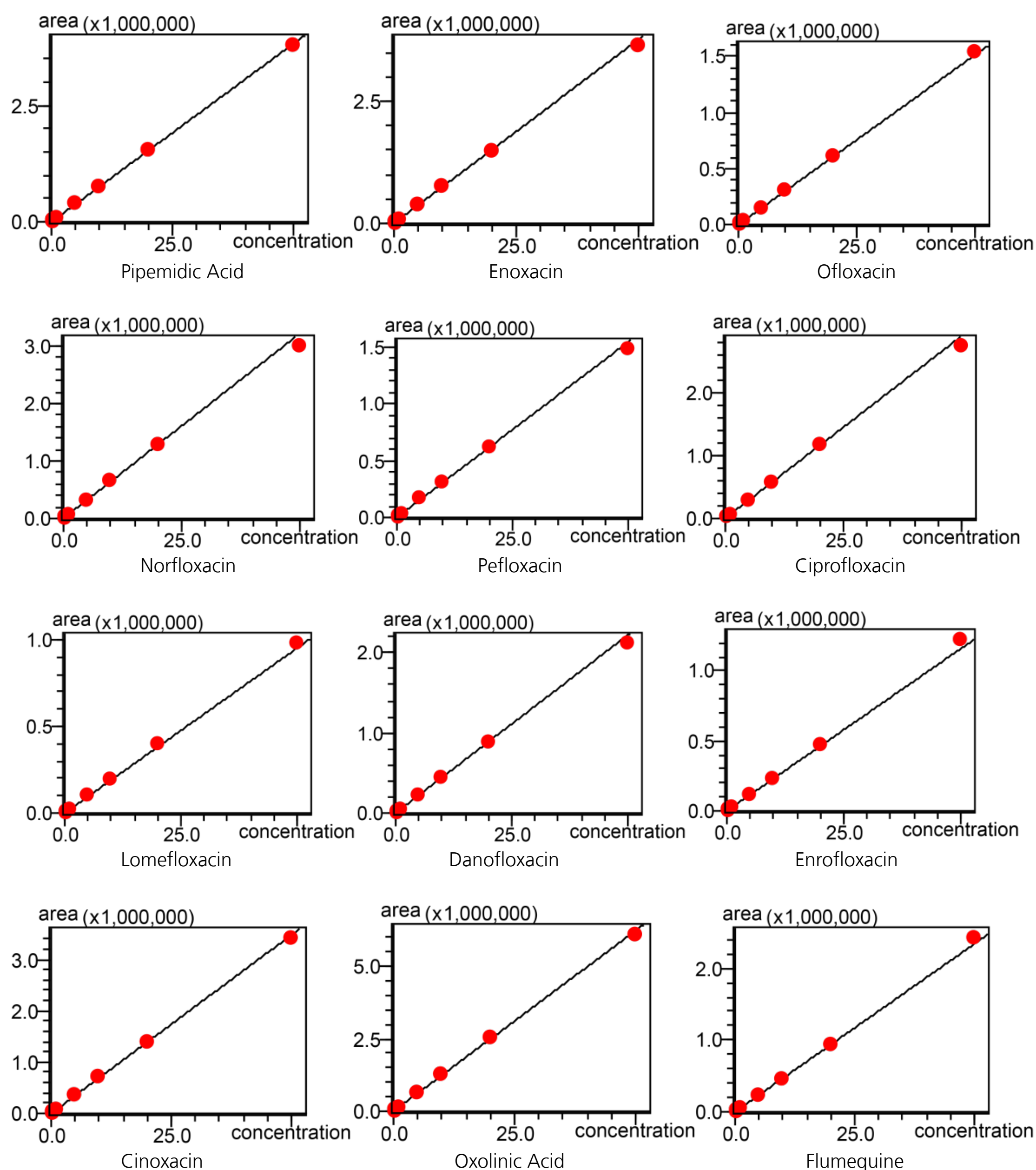

Figure 14: Standard curves of 12 quinolones

Table 3: Parameters of calibration curve

No.	Compound Name	Calibration curve	Linear range (ng/mL)	Correlation Coefficient (r)	Accuracy %
1	Pipemidic Acid	Y = (76616.3) X + (-2517.68)	0.2-50	0.9998	96.1-104.1
2	Enoxacin	Y = (75235.4) X + (832.686)	0.2-50	0.9996	87.9-109.4
3	Ofloxacin	Y = (30271.5) X + (-849.705)	0.2-50	0.9998	90.9-106.6
4	Norfloxacin	Y = (64434.1) X + (1680.13)	0.2-50	0.9988	85.7-112.4
5	Pefloxacin	Y = (30801.7) X + (579.667)	0.2-50	0.9990	89.5-109.8
6	Ciprofloxacin	Y = (58040.6) X + (4785.98)	0.2-50	0.9990	90.7-114.2
7	Lomefloxacin	Y = (19629.0) X + (-749.499)	0.2-50	0.9997	89.8-107.5
8	Danofloxacin	Y = (44434.3) X + (911.073)	0.2-50	0.9995	91.1-110.6
9	Enrofloxacin	Y = (23178.6) X + (76.5354)	0.2-50	0.9975	87.2-111.3
10	Cinoxacin	Y = (69528.6) X + (284.320)	0.2-50	0.9995	92.1-111.6
11	Oxolinic acid	Y = (124807) X + (4369.16)	0.2-50	0.9999	87.5-113.0
12	Flumequine	Y = (18436.0) X + (814.851)	0.2-50	0.9987	91.2-108.2

2.4 Precision Test

The retention times and peak areas of the mixed standard working solutions at different concentrations (6 replicates for each concentration) were determined to evaluate the precision of the instrument. The repeatability results of retention time and peak area are shown in Table 4.

The results indicate that the relative standard deviations of retention time and peak area of standard samples at different concentrations are 0.03-0.27 % and 1.13-4.93% respectively, showing good precision of the instrument.

Table 4: Repeatability and sensitivity results of retention time and peak area

Compound Name	RSD% (0.2 ng/mL)		RSD% (10 ng/mL)		RSD% (50 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
Pipemidic Acid	0.23	4.93	0.21	2.54	0.24	1.85
Enoxacin	0.16	3.73	0.18	1.21	0.19	1.79
Ofloxacin	0.27	4.93	0.17	1.59	0.18	1.66
Norfloxacin	0.17	4.91	0.16	1.92	0.19	1.13
Pefloxacin	0.16	4.77	0.17	2.06	0.19	2.01
Ciprofloxacin	0.07	3.88	0.15	1.34	0.18	2.29
Lomefloxacin	0.15	3.37	0.15	1.70	0.18	1.45
Danofloxacin	0.17	3.23	0.15	2.61	0.16	3.74
Enrofloxacin	0.23	4.88	0.13	3.16	0.15	1.81
Cinoxacin	0.10	3.32	0.11	1.40	0.10	1.34
Oxolinic acid	0.09	2.15	0.09	1.69	0.07	1.44
Flumequine	0.04	3.43	0.03	3.37	0.03	2.00

2.5 Sensitivity Test

To determine the sensitivity of the instrument, a low concentration (0.2ng/mL) mixed standard antibiotics solution was prepared and analyzed according to the analytical conditions in Section 1.2. The signal-to-noise (S/N) ratio, limit of detection (LOD) and limit of quantitation (LOQ) was determined with the use of RMS calculation method and LabSolutions Ver. 5.86. The S/N ratio, LOD and LOQ of the 12 compounds are shown in Table 5.

2.6 Matrix Spike Test

A matrix spike using the blank chicken sample was prepared according to Section 1.4 to give spiked sample at concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL. 3 replicates were tested and the average results are shown in Table 6.

Table 5: Signal-to-noise ratio (s/n), LOD and LOQ

Compound	Concentration Level (ng/mL)	S/N	Limit of Detection (ng/mL)	Limit of Quantification (ng/mL)
Pipemidic Acid	0.20	15.43	0.04	0.13
Enoxacin	0.20	31.11	0.02	0.07
Ofloxacin	0.20	21.89	0.03	0.10
Norfloxacin	0.20	39.04	0.02	0.06
Pefloxacin	0.20	19.21	0.04	0.12
Ciprofloxacin	0.20	56.02	0.01	0.04
Lomefloxacin	0.20	39.68	0.02	0.06
Danofloxacin	0.20	29.48	0.02	0.07
Enrofloxacin	0.20	13.34	0.05	0.16
Cinoxacin	0.20	29.57	0.07	0.21
Oxolinic acid	0.20	44.84	0.02	0.05
Flumequine	0.20	18.66	0.03	0.09

Table 6: Test results of matrix spike

No.	Compound Name	Spiked Sample Concentration (0.5 ng/mL)		Spiked Sample Concentration (10 ng/mL)		Spiked Sample Concentration (40 ng/mL)	
		Average (ng/mL)	Recovery (%)	Average (ng/mL)	Recovery (%)	Average (ng/mL)	Recovery (%)
1	Pipemidic Acid	0.54	108.60	10.22	102.20	39.02	97.55
2	Enoxacin	0.50	100.23	9.72	97.23	37.34	93.38
3	Ofloxacin	0.51	101.93	9.59	95.90	38.84	97.08
4	Norfloxacin	0.48	96.35	9.82	98.20	37.16	92.90
5	Pefloxacin	0.47	94.08	10.01	100.08	37.37	93.43
6	Ciprofloxacin	0.47	94.23	9.90	99.03	37.12	92.83
7	Lomefloxacin	0.46	93.70	9.33	93.40	38.51	96.30
8	Danofloxacin	0.47	93.05	9.72	97.20	36.77	91.90
9	Enrofloxacin	0.48	95.85	9.86	98.63	39.70	99.25
10	Cinoxacin	0.48	95.78	9.82	98.25	37.71	94.28
11	Oxolinic acid	0.50	100.65	10.24	102.45	39.36	98.43
12	Flumequine	0.50	100.50	10.05	100.60	39.40	98.50

The test results indicate that the spike recovery of the 12 samples of antibiotics are 91.90–108.60%.

■ Conclusion

A method was established for the determination of quinolone antibiotics in chicken using Shimadzu UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. This method analyzed 12 antibiotics within 9 min, and the correlation coefficients of the calibration curve are all above 0.997. The mixed standard antibiotics solutions at concentrations of 0.2 ng/mL, 10 ng/mL and 50 ng/mL were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds are 0.03-0.27 % and 1.13-4.93% respectively, showing good precision of the instrument. The chicken sample matrix at spiked concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL were tested in 3 replicates, and the spike recovery was 91.90-108.60%. The described method is fast and ensures high sensitivity and excellent reproducibility. It can be used for the determination of various antibiotic residues in chicken.

ULTRA FAST MASS SPECTROMETRY

LCMS-8040

LCMS-8045

LCMS-8050

LCMS-8060

LCMS-2020

Q-TOF LCMS-9030

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