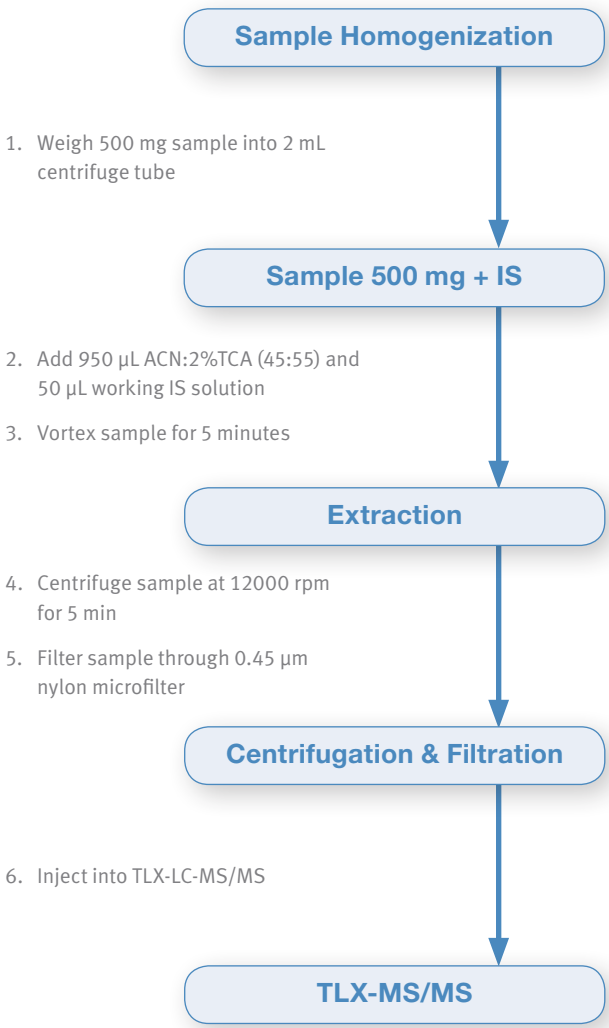
Automated Online Multi-Residue LC-MS/MS Method for the Determination of Antibiotics in Chicken Meat

Katerina Bousova, Klaus Mittendorf, Thermo Fisher Scientific Food Safety Response Center, Dreieich, Germany

Method 63646

Key Words
Antibiotics, Transcend TLX, TurboFlow Technology, TSQ Quantum Access MAX, Chicken meat, Food Safety

1. Schematic of Method



2. Introduction

Throughout the world, antibiotics are widely used for veterinary purposes to treat diseased animals, prevent diseases and promote growth. Due to inappropriate or excessive usage of antibiotics, residues of these compounds can be found in food and food products of animal origin. The use of antibiotics cannot be avoided; however, it is necessary to ensure the safety of food and food products for human consumption. For this reason, the European Commission has established maximum residue limits (MRLs) for antibiotics in animal tissue, milk and eggs in Council Regulation 2377/90/EC.[1] To detect and quantify antibiotics for regulatory purposes, laboratories need to utilize suitable analytical methods.

With the number of samples to be checked for the presence of antibiotic residues increasing, the need for multi-analyte methods that can efficiently handle high throughputs is growing as well. Generally, methods used for monitoring antibiotic residues can be classified in two groups: screening and confirmatory.

For fast *screening* of antibiotic residues, an immunoassay, microbiological assay or biosensor technique is typically used. Among the benefits are short analysis time, high sensitivity and selectivity for immunoassays, simplicity and automation. However, the disadvantages include the incidence of false-negative or false-positive results, the inability to distinguish between the different types of antibiotics and the possibility to provide only a semi-quantitative result for the total amount of drug residue.

The *confirmatory* quantitative methods are typically based on liquid chromatography coupled to mass spectrometry (LC/MS). This technique can also be used for screening and provides much higher sensitivity and greater specificity. The use of LC-MS/MS for screening was reported in a multi-residue semi-quantitative screening method for 39 drug residues covering eight drug classes in veal muscle.[2]

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This note describes a multi-residue, confirmatory method for the quantitative determination of antibiotics in chicken meat using the Thermo Scientific Transcend TLX system coupled to an LC-MS/MS. This method was developed on the basis of previous work concerning a confirmatory method for antibiotics in milk[3]. The Transcend TLX™ system powered by TurboFlow™ technology was used for online sample cleanup instead of lengthy offline solid-phase extraction (SPE). Combining the number of target compounds with the high sample throughput, this approach fulfills the demand for a fast and cost-effective multi-analyte method.

3. Scope and Application

This online TLX-MS/MS method can be applied to detect and quantify the presence of 36 compounds from seven different classes of antibiotics (aminoglycosides, sulfonamides, macrolides, quinolones, tetracyclines, lincosamides and trimethoprim) in chicken meat. This multi-residue method fulfills legislative requirements described in the EU Commission Decision 2002/657/EC[4].

4. Principle

The Transcend TLX system uses TurboFlow technology for online sample cleanup. Sample concentration, cleanup and analytical separation are carried out in a single run using a TurboFlow column connected to an analytical LC column. Macromolecules are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. Before introducing the sample extract onto the TurboFlow column, the sample is thoroughly homogenized and fortified with an internal standard, extracted with a solvent mixture of acetonitrile (ACN):2% trichloroacetic acid (TCA) (45:55) and centrifuged. Cleanup using the TLX system is optimized for maximum recovery of targeted compounds and minimal injection of co-extractives into the mass spectrometer. Identification of antibiotics is based on retention time, ion ratios using multiple reaction monitoring (MRM) of characteristic transition ions, and quantification using matrix-matched standards of one of the selected MRM ions.

5. Reagent List

5.1	Purified water, Thermo Scientific Barnstead Easypure II water system
5.2	Methanol, Optima, LC-MS grade
5.3	Water, LC-MS grade
5.4	Acetonitrile, Optima, LC-MS grade
5.5	Isopropanol, HPLC grade
5.6	Acetone, HPLC grade
5.7	Formic acid, extra pure, >98%
5.8	Heptafluorobutyric acid, 99%
5.9	Ammonia, extra pure, 35%
5.10	Trichloroacetic acid, extra pure, 99%

6. Calibration Standards

6.1 Standards

6.1.1	Chlortetracycline	Sigma-Aldrich®
6.1.2	Clarithromycin	Sigma-Aldrich
6.1.3	Clindamycin hydrochloride	Sigma-Aldrich
6.1.4	Cinoxacin	Sigma-Aldrich
6.1.5	Ciprofloxacin	Sigma-Aldrich
6.1.6	Danofloxacin	Sigma-Aldrich
6.1.7	Doxycycline hyclate	Sigma-Aldrich
6.1.8	Difloxacin	Sigma-Aldrich
6.1.9	Enoxacin	Sigma-Aldrich
6.1.10	Enrofloxacin	Sigma-Aldrich
6.1.11	Flumequine	Sigma-Aldrich
6.1.12	Josamycin	Sigma-Aldrich
6.1.13	Kanamycin	Sigma-Aldrich
6.1.14	Lincomycin hydrochloride monohydrate	Sigma-Aldrich
6.1.15	Lomefloxacin hydrochloride	Sigma-Aldrich
6.1.16	Marbofloxacin	Sigma-Aldrich
6.1.17	Nalidixic acid	Sigma-Aldrich
6.1.18	Neomycin	Sigma-Aldrich
6.1.19	Norfloxacin	Sigma-Aldrich
6.1.20	Ofloxacin	Sigma-Aldrich
6.1.21	Oleandomycin phosphate dehydrate	Dr. Ehrenstorfer
6.1.22	Oxolinic acid	Sigma-Aldrich
6.1.23	Oxytetracycline hydrochloride	Sigma-Aldrich
6.1.24	Sarafloxacin hydrochloride trihydrate	Sigma-Aldrich
6.1.25	Spiramycin	Sigma-Aldrich
6.1.26	Sulfadimethoxine	Sigma-Aldrich
6.1.27	Sulfadoxin	Sigma-Aldrich
6.1 28	Sulfaquinoxaline	Sigma-Aldrich
6.1.29	Sulfachlorpyridazine	Sigma-Aldrich
6.1.30	Sulfaclozine sodium	Dr. Ehrenstorfer
6.1.31	Sulfamethoxazole	Sigma-Aldrich
6.1.32	Tetracycline	Sigma-Aldrich
6.1.33	Tilmicosin	Sigma-Aldrich
6.1.34	Trimethoprim	Sigma-Aldrich
6.1.35	Tylosin tartrate	Sigma-Aldrich
6.1.36	Tylvalosin tartrate	FarmKemi

6.2 Internal Standard

6.2.1	Sulfaphenazole	Sigma-Aldrich

7. Standards Preparation

7.1 Stock standard solutions of veterinary drugs

Stock standard solutions (1000 µg/mL) are prepared individually by dissolving the analytes in methanol (lincosamides, macrolides, sulfonamides, tetracyclines and trimethoprim), in water (aminoglycosides) and in methanol with 2% 2M NH_4OH (quinolones). Solutions are stored at -20° C.

7.2 Working standard solution

The working 1000 µg/L calibration standard solution is prepared by dilution of individual stock standard solutions with acetonitrile. The solution should be prepared fresh each time before using.

7.3 Stock solution of internal standard

Stock solution of the internal standard (1000 µg/mL) is prepared by dilution of sulfaphenazole in methanol. Solution is stored at -20 °C.

7.4 Working standard solution of internal standard

The working solution of the internal standard (2000 µg/L) was prepared by dilution of stock standard solution (sulfaphenazole) with acetonitrile. Solution should be prepared fresh each time before using.

8. Apparatus

8.1	Transcend TLX-1 system
8.2	Thermo Scientific TSQ Quantum Access MAX triple quadrupole mass spectrometer
8.3	Fisher Science Education precision balance
8.4	Sartorius® analytical balance *(Sartorius GmbH, Germany)*
8.5	Barnstead™ Easypure™ II water system
8.6	Vortex shaker
8.7	Vortex universal cap
8.8	Waring® laboratory blender *(Waring Laboratory Science, USA)*
8.9	BRAND™ accu-jet® pipettor *(BRAND GmBh + Co. KG, Germany)*
8.10	Thermo Scientific Heraeus Fresco 17 microcentrifuge

9. Consumables

9.1	Thermo Scientific TurboFlow Cyclone P (50 × 0.5 mm) column
9.2	Thermo Scientific BetaSil Phenyl/Hexyl (50 × 2.1 mm, 3 µm) column
9.3	LC vials
9.4	LC caps
9.5	Thermo Scientific Finnpipette 100 – 1000 µL pipette
9.6	Finnpipette™ 20 – 200 µL pipette
9.7	Finnpipette 10 – 100 µL pipette
9.8	Finnpipette 500 – 5000 µL pipette
9.9	Finnpipette 1000 – 10 000 µL pipette
9.10	Pipette holder
9.11	Pipette tips 0.5–250 µL, 500/box
9.12	Pipette tips 1–5 mL, 75/box
9.13	Pipette tips 100–1000 µL, 200/box
9.14	Pipette tips 20000–10000 µL, 40/box
9.15	Pipette, Pasteur, soda lime glass 150 mm
9.16	Pipette suction device
9.17	Spatula, 18/10 steel
9.18	Spatula, nylon
9.19	Single-use syringes, 1 mL
9.20	Nylon syringe filter 0.45 µm, 17 mm
9.21	Vial rack (2 mL)
9.22	Centrifuge plastic tube (2 mL)
9.23	Rack for 50, 15, 2 and 0.5 mL tubes
9.24	Pipette tips 20000–10000 µL, 40/box

Glassware

9.25	Beaker, 50 mL
9.26	Beaker, 100 mL
9.27	Beaker, 25 mL
9.28	Volumetric flask, 25 mL
9.29	Volumetric flask, 10 mL
9.30	Volumetric flask, 5 mL
9.31	Volumetric flask, 100 mL
9.32	Glass pipette, 5 mL

10. Procedure

10.1 Sample Preparation

Approximately 150 g of the chicken sample is homogenized in a Waring laboratory blender for five minutes. Then, 500 mg is weighed into a 2 mL polypropylene tube. Working internal standard solution (50 µL) and solvent mixture ACN:2% TCA (45:55) (450 µL) are added to the sample. The sample is shaken for five minutes on the vortex and then centrifuged at 12000 rpm for five minutes. The supernatant is filtered through a nylon microfilter (0.45 µm pore size) directly into the LC vial and the sample is analyzed by TLX-MS/MS.

10.2 LC Conditions

LC analysis is performed on a Transcend TLX-1 system.

TurboFlow column:	TurboFlow Cyclone P (50 × 0.5 mm)
Analytical column:	BetaSil™ Phenyl/Hexyl (50 × 2.1 mm, 3 µm particle size)
Total run time:	19 minutes
Mobile phases:	A = 1 mM heptafluorobutyric acid and 0.5% formic acid in water B = 0.5% formic acid in acetonitrile/methanol (1/1) C = 2% methanol in water D = acetone/acetonitrile/isopropanol (20/40/40)

10.3 Injector Settings

Injector:	Thermo Scientific PAL injector with 100 µL volume injection syringe
Tray temperature:	10 °C
Cleaning solvents for the autosampler:	
Solvent 1:	Acetonitrile/water (20/80)
Solvent 2:	Acetone/acetonitrile/isopropanol - (20/40/40)
Pre-clean with solvent 1 [steps]:	3
Pre-clean with solvent 2 [steps]:	3
Pre-clean with sample [steps]:	1
Filling speed [µL/s]:	50
Filling strokes [steps]:	1
Injection port:	LC Vlv1 (TX channel)
Injection speed [µL/s]:	100
Pre-inject delay [ms]:	500
Post-inject delay [ms]:	500
Post-clean with solvent 1 [steps]:	5
Post-clean with solvent 2 [steps]:	5
Valve clean with solvent 1 [steps]:	5
Valve clean with solvent 2 [steps]:	5
Injection volume:	35 µL

Sample concentration, cleanup and analytical separation are carried out in a single run using an automated online sample preparation system, which includes the Transcend TLX system and Thermo Scientific Aria operating software. The sample is injected during the loading step by the loading pump and autosampler onto the TurboFlow column. During this step, macromolecules are removed while the target analytes are retained on the TurboFlow column based on their different chemical interactions. In the next step, the trapped analytes are transferred with the eluting pump, and an adequately strong solvent (eluent) in the loop onto the analytical LC column where the analytes are separated conventionally. While the separation on the analytical column is running, the loop is filled with the eluent and the TurboFlow column is washed and conditioned to be ready for the injection of the next sample. The TLX and LC conditions are set up in Aria™ software and presented in Table 1.

The analytical column is conditioned during the loading of the sample onto the TurboFlow column. The separation of the analytes on the analytical column is done by gradient (Table 1). To prevent the possibility of carryover and cross contamination, the injection syringe as well as the injection valve are washed with cleaning solvent 1 (acetonitrile/water - 20/80) and cleaning solvent 2 (acetone/acetonitrile/isopropanol - 20/40/40), five times before and five times after each injection.

Table 1. Gradient program table for TurboFlow system coupled with an analytical column

Step			TurboFlow column[a]					Cut-in loop		Analytical LC column[b]			
Description	Start [min]	Time [s]	Flow [mL/min]	A%	B%	C%	D%	Tee	Loop	Flow [mL/min]	Step	A%	B%
1.loading	0	60	1.5	-	-	100	-	-----	out	0.3	Step	100	-
2.transffering	1	60	0.2	100	-	-	-	T	in	0.6	Step	100	-
3.washing	2	60	1.5	-	-	50	50	-----	in	0.3	Step	100	-
4.washing	3	720	1.5	-	-	-	100	-----	in	0.3	Ramp	5	95
5.filling loop	15	120	1.5	50	50	-	-	-----	in	0.3	Step	5	95
6.equilibrating	17	120	1.5			100	-	-----	out	0.3	Step	100	-

[a]Mobile phases for the TurboFlow method:

A: 1mM heptafluorobutyric acid + 0.5% formic acid in water

B: 0.5% formic acid in acetonitrile/methanol – 1/1

C: 2% methanol in water

D: acetone/acetonitrile/isopropanol - 20/40/40

[b]Mobile phases for the analytical method:

A: 1mM heptafluorobutyric acid + 0.5% formic acid in water

B: 0.5% formic acid in acetonitrile/ methanol – 1/1

10.4 Mass Spectrometric Conditions

Mass spectrometric analysis is carried out using a TSQ Quantum Access MAX™ triple quadrupole system. Data acquisition for quantification and confirmation are performed in selected reaction monitoring (SRM) mode. All SRM traces (parent, qualifier and quantifier ion) are individually tuned for each target analyte by direct injection of the individual working standard solution (10 mg/mL). Data acquisition and processing is performed using Thermo Scientific Xcalibur 2.1 software.

Ionization mode:	Heated Electrospray (HESI)
Scan type:	SRM
Polarity:	Positive ion mode
Spray voltage [V]:	3500
Ion sweep gas pressure [arb]:	0
Vaporizer temperature [°C]:	400
Sheath gas pressure [arb]:	50
Aux gas pressure [arb]:	10
Capillary temperature [°C]:	370
Collision gas pressure [mTorr]:	0
Cycle time [s]:	0.6
Peak width:	Q1/Q3 the full width of a peak at half its maximum height (FWHM) of 0.70 Da

The parameters for SRM analysis for targeted compounds and internal standards are displayed in the Table 2.

11. Calculations

11.1 Identification

Identification of the antibiotics is indicated by the presence of transition ions (quantifier and qualifier) measured in SRM mode corresponding to the retention times (± 2.5%) of appropriate standards. In SRM mode, the measured peak area ratios for qualifier to quantifier ions should be in close agreement (according to EU Commission Decision 2002/657/EC) with those ratios of the standards, as shown in Table 3. The quantifier and qualifier ions were selected among the product ions produced by the fragmentation of the selected parent ion on the basis of the intensity and selectivity. A representative chromatogram is shown in Figure 1.

11.2 Quantification

For quantification, internal standardization is used to measure peak area ratios for matrix matched standards. Sulfaphenazole is used as the internal standard for all target antibiotics. Calibration curves are plotted as the relative peak areas (analyte versus the corresponding standard) as a function of the compound concentration. The antibiotic concentration in the samples is determined from the equation:

$$c_a = \frac{\frac{A_a}{A_{IS}} - b}{a}$$

c_a = antibiotic concentration in µg/kg

A_a = peak area of the antibiotic

A_{IS} = peak area of internal standard

b = y-intercept

a = slope of the calibration curve

12. Method Performance

The method was validated in-house according to the criteria for a quantitative method specified in EU Commission Decision 2002/657/EC[4]. The validation parameters were determined by spiking blank chicken meat at levels of 0.5, 1 and 1.5 times the MRL. For compounds without an MRL for chicken meat, samples were spiked at 10, 20 and 30 µg/kg for clindamycin, josamycin, clarithromycin, oleandomycin, tylvalosin, marbofloxacin, nalidixic acid, enoxacin, ofloxacin, lomefloxacin, norfloxacin, sarafloxacin and cinoxacin. The measured parameters were specificity, linear range, repeatability, accuracy, limit of detection (LOD), limit of quantification (LOQ), decision limit (CCα) and detection capability (CCβ).

12.1 Samples and Quality Control Materials

For preparation of matrix-matched calibration standards and spiked samples for validation, chicken meat was obtained from a local market and checked by repeated measurements to confirm that it was free of antibiotics. For determination of accuracy, a Food Analysis Performance Assessment Scheme (FAPAS®) test material T02174QC of fish muscle containing a certified amount of ciprofloxacin, which was obtained from the Food and Environmental Research Agency (York, United Kingdom) was used.

12.2 Selectivity

Using SRM, the specificity is confirmed based on the presence of the transition ions (quantifier and qualifier) at the correct retention times corresponding to those of the respective antibiotics. The measured peak area ratios of qualifier/quantifier are within the range defined in EU Commission Decision 2002/657/EC[4] when compared to the standards (Table 3).

12.3 Linearity & Calibration Curve

The linearity of calibration curves was assessed over the range from 0–400 µg/kg for all target compounds. In all cases, the correlation coefficients of linear functions had to be >0.99. The calibration curves were created from nine matrix-matched calibration standards, which were injected into each batch in duplicate.

12.4 Precision

Precision (repeatability) of the method was determined using independently spiked, blank samples at three different levels. In one day, the set of samples at three levels was measured with six repetitions. To determine between-day precision, two other sets at one level were measured with six repetitions over the next two days. The results for repeatability ranged from 4%–27% (Table 4).

12.5 Accuracy

Method accuracy was determined using independently spiked blank samples at three different levels. Accuracy was evaluated by comparing found values with standard additions in spikes. Recovery values ranged between 71%–120% (Table 5). Additionally, accuracy was established for ciprofloxacin by analyzing the certified reference material T02174QC, which was fish muscle. All measured concentrations of ciprofloxacin were within the acceptable range (Table 6).

12.6 LOD and LOQ

LOD and LOQ were estimated to be 3 and 10, respectively, by following the IUPAC approach of first analyzing the blank sample to establish noise levels and then estimating LODs and LOQs for signal/noise. The values for chicken meat are shown in Table 7 and, in all cases, they are under the level of MRL for all analytes that have an assigned MRL.

12.7 CCα and CCβ

Both CCα and CCβ were established by the calibration curve procedure according to ISO 11843[5]. The blank material fortified at and below the maximum residue limit (for analytes with MRL) or at and above the lowest possible level (for analytes without MRL) in equidistant steps was used. The calculated values are shown in Table 7.

13. Conclusion

This in-house validated method enables quantification of 36 residues from seven different classes of antibiotics in chicken meat. Although the 36 compounds come from different groups with widely varying polarities and solubilities, only one extraction procedure was used. The use of the Transcend TLX system and TurboFlow technology combined with TSQ triple quadrupole mass spectrometry detection for analytical separation saves a significant amount of time in sample preparation and increases sample throughput. Additionally, by using the Transcend TLX system very clean sample extracts enter the mass spectrometer so routine maintenance on the system, such as cleaning the ion source, is not required as often as with analytical methods in which the sample extracts are not cleaned but only diluted. The method was validated and fulfilled the requirements of the EU Commission Decision 2002/675/EC[4]; therefore, it can be recommended for enforcement of the legislation limits. Using this method, the control laboratory can measure up to 40 samples of chicken meat a day including sample preparation and measurement by instrument.

14. References

1. European Commission. 1994. Council Regulation (EEC) No. 2377/90 of 26 June 1990: laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, amending regulation no. 1430/94 of 22 June 1994. Off J Eur Comm. L156:23.
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3. Bousova, K.; Mittendorf, K. Multi-residue automated Turbulent Flow™ on-line LC-MS/MS method for the determination antibiotics in milk. Method number: 63551. Thermo Fisher Scientific, **2012**.
4. EU Commission Decision 2002/657/EC. Off. J. Eur. Commun. L221/8 (2002).
5. ISO 11843: Capability of detection (1997).

Table 2. LC-MS/MS parameters for selected reaction monitoring of analytes

Analyte	Molecular Weight	Precursor Ion	Quantifier Ion	CE for Quantifier Ion (V)	Qualifier Ion	CE for Qualifier Ion (V)	Tube Lens (V)
Kanamycin	484.5	485.28	163.10	25	324.15	15	90
Neomycin	614.6	615.34	161.03	29	163.11	31	101
Lincomycin	406.5	407.14	126.17	28	359.16	17	97
Clindamycin	425.0	425.14	126.17	28	377.17	18	86
Trimethoprim	290.3	291.10	230.10	23	261.09	24	93
Josamycin	828.0	828.43	173.99	30	109.10	34	18
Spiramycin	843.1	843.31	173.95	32	142.02	32	146
Tilmicosin	869.1	869.62	696.41	40	174.00	41	132
Tylosin	916.1	916.51	174.00	35	772.44	26	141
Clarithromycin	748.0	748.51	158.15	28	590.37	17	108
Oleandomycin	687.8	688.44	544.35	14	158.05	25	106
Tylvalosin	1042.3	1042.64	109.06	41	173.95	37	133
Sulfadimethoxine	310.3	311.03	156.06	21	108.13	27	88
Sulfamethoxazole	253.2	254.03	156.05	15	92.18	27	96
Sulfadoxin	310.3	311.04	156.04	18	108.14	26	88
Sulfaquinoxaline	300.3	301.04	156.02	17	92.16	28	92
Sulfachlorpyridazine	284.7	284.97	156.03	15	92.18	26	90
Sulfaclozine	284.7	284.96	92.16	29	108.12	26	87
Sulphafenazole (IS)	314.4	315.06	158.10	28	160.10	22	94
Oxytetracycline	460.4	461.11	426.10	18	426.10	18	93
Tetracycline	444.4	445.16	410.14	18	427.15	11	99
Chlortetracycline	478.8	479.09	444.08	22	462.11	16	98
Doxycycline	444.4	445.14	428.15	18	321.05	31	82
Marbofloxacin	362.4	363.11	72.30	22	320.06	14	97
Ciprofloxacin	331.3	332.08	314.10	18	288.11	22	89
Danofloxacin	357.4	358.11	340.14	24	314.15	16	99
Enrofloxacin	359.4	360.10	316.13	19	342.14	22	96
Difloxacin	399.4	400.10	382.11	21	356.1	19	98
Oxolinic acid	261.2	262.01	244.04	18	216.02	29	84
Flumequine	261.3	262.02	244.05	19	202.03	33	84
Nalidixic acid	232.2	233.04	215.08	15	187.05	25	77
Enoxacin	320.3	321.09	303.09	19	257.10	17	93
Ofloxacin	361.4	362.12	318.14	18	261.08	27	91
Lomefloxacin	351.3	352.10	265.09	23	308.13	15	100
Norfloxacin	319.3	320.07	302.08	22	276.1	16	94
Sarafloxacin	385.4	386.08	368.10	23	342.11	18	94
Cinoxacin	262.2	263.02	245.02	16	217.04	22	90

CE: Collision Energy

Table 3. Ion ratios (Qual/Quant) in matrix and in standard mixture (the agreement between ion ratios should be within the permitted tolerance, which is defined in EU Commission Decision 2002/657/EC)

Analyte	Ion Ratio (Std Mix)	Ion Ratio (Chicken Meat)	Difference (%)
Kanamycin	0.53	0.50	6.0
Neomycin	0.95	0.94	1.1
Lincomycin	0.09	0.09	0.0
Clindamycin	0.05	0.04	25.0
Trimethoprim	0.76	0.70	8.6
Josamycin	0.90	0.91	1.1
Spiramycin	0.17	0.21	19.0
Tilmicosin	0.88	0.88	0.0
Tylosin	0.23	0.23	0.0
Clarithromycin	0.62	0.61	1.6
Oleandomycin	0.65	0.71	8.5
Tylvalosin	0.55	0.50	10.0
Sulfadimethoxine	0.60	0.56	7.1
Sulfamethoxazole	0.31	0.30	3.3
Sulfadoxin	0.46	0.58	20.7
Sulfaquinoxaline	0.24	0.27	11.1
Sulfachlorpyridazine	0.44	0.46	4.3
Sulfaclozine	0.20	0.29	31.0
Sulfaphenazole (IS)	0.76	0.75	1.3
Oxytetracycline	0.13	0.10	30.0
Tetracycline	0.80	0.84	4.8
Chlortetracycline	0.48	0.42	14.3
Doxycycline	0.03	0.05	40.0
Marbofloxacin	0.79	0.61	29.5
Ciprofloxacin	0.13	0.14	7.1
Danofloxacin	0.06	0.03	50.0
Enrofloxacin	0.58	0.62	6.5
Difloxacin	0.58	0.69	15.9
Oxolinic acid	0.06	0.08	25.0
Flumequine	0.44	0.42	4.8
Nalidixic acid	0.30	0.32	6.3
Enoxacin	0.02	0.03	33.3
Ofloxacin	0.70	0.70	0.0
Lomefloxacin	0.58	0.64	9.4
Norfloxacin	0.05	0.09	44.4
Sarafloxacin	0.18	0.25	28.0
Cinoxacin	0.28	0.30	6.7

Table 4. Method intermediate precision as RSD (%) – 1 level – 3 sets with 6 replicates in 3 days and method repeatability expressed as RSD (%) and measured at 3 levels every time with 6 replicates Table 4. Method intermediate precision as RSD (%) – 1 level – 3 sets with 6 replicates in 3 days and method repeatability expressed as RSD (%) and measured at 3 levels every time with 6 replicates

Analyte	RSD (%) – spiking level 2			Chicken meat – RSD (%)		
	Day 1	Day 2	Day 3	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 3 (µg/kg)
Kanamycin	19	18	26	19	25	21
Neomycin	23	28	18	23	18	19
Lincomycin	4	13	6	4	10	10
Clindamycin	6	9	12	6	3	10
Trimethoprim	9	9	10	9	7	9
Josamycin	9	6	11	9	6	21
Spiramycin	8	31	10	8	10	21
Tilmicosin	7	7	6	7	7	9
Tylosin	9	16	4	9	7	19
Clarithromycin	11	14	5	11	6	12
Oleandomycin	13	24	15	13	10	10
Tylvalosin	15	17	11	15	6	16
Sulfadimethoxine	3	3	8	3	5	10
Sulfamethoxazole	7	8	3	7	10	5
Sulfadoxin	14	11	12	14	9	6
Sulfaquinoxaline	17	15	34	17	21	5
Sulfachlorpyridazine	8	10	17	8	8	13
Sulfaclozine	14	8	7	14	14	10
Oxytetracycline	27	6	16	27	13	11
Tetracycline	10	9	11	10	12	10
Chlortetracycline	13	4	17	13	19	12
Doxycycline	7	6	13	7	8	9
Marbofloxacin	9	15	19	9	12	18
Ciprofloxacin	10	3	12	10	8	8
Danofloxacin	5	3	7	5	3	9
Enrofloxacin	11	5	10	11	7	6
Difloxacin	4	4	9	4	8	10
Oxolinic acid	4	5	7	4	5	7
Flumequine	6	3	10	6	7	9
Nalidixic acid	6	9	9	6	6	8
Enoxacin	17	8	8	17	14	22
Ofloxacin	9	11	12	9	20	15
Lomefloxacin	27	18	16	27	19	16
Norfloxacin	11	8	10	11	7	16
Sarafloxacin	24	10	16	24	22	6
Cinoxacin	16	13	14	16	19	12

Table 5. Recoveries (%) for spiked samples of chicken meat at 3 different spike levels (6 replicates)

Analyte	Spiking levels			Chicken meat - REC (%)		
	Level 1 (µg/kg)	Level 2 (µg/kg)	Level 3 (µg/kg)	Level 1	Level 2	Level 3
Kanamycin	50	100	150	119	109	120
Neomycin	250	500	750	84	71	83
Lincomycin	50	100	150	104	94	102
Clindamycin	10	20	30	111	115	104
Trimethoprim	25	50	75	99	91	83
Josamycin	10	20	30	102	91	95
Spiramycin	100	200	300	108	102	92
Tilmicosin	37.5	75	112.5	115	105	102
Tylosin	50	100	150	86	84	82
Clarithromycin	10	20	30	101	105	98
Oleandomycin	10	20	30	116	93	92
Tylvalosin	10	20	30	91	101	99
Sulfadimethoxine	50	100	150	101	97	91
Sulfamethoxazole	50	100	150	113	108	96
Sulfadoxin	50	100	150	101	104	98
Sulfaquinoxaline	50	100	150	100	94	99
Sulfachlorpyridazine	50	100	150	109	102	94
Sulfaclozine	50	100	150	118	110	106
Oxytetracycline	50	100	150	115	109	114
Tetracycline	50	100	150	102	94	94
Chlortetracycline	50	100	150	96	85	87
Doxycycline	50	100	150	117	98	95
Marbofloxacin	10	20	30	104	105	106
Ciprofloxacin	50	100	150	101	114	103
Danofloxacin	100	200	300	116	108	109
Enrofloxacin	50	100	150	112	108	103
Difloxacin	150	300	450	106	105	102
Oxolinic acid	50	100	150	109	100	95
Flumequine	200	400	600	108	94	88
Nalidixic acid	10	20	30	118	103	99
Enoxacin	10	20	30	99	103	88
Ofloxacin	10	20	30	101	92	89
Lomefloxacin	10	20	30	98	100	94
Norfloxacin	10	20	30	101	112	101
Sarafloxacin	10	20	30	105	99	90
Cinoxacin	10	20	30	102	94	91

Table 6. Results of FAPAS quality control test material – fish muscle T02174QC – ciprofloxacin – c = 113 ± 50 µg/kg

Sample	concentration [found] (µg/kg)
Sample 1	90
Sample 2	103
Sample 3	107

Table 7. Limit of detection and quantification (LOD and LOQ), maximum residual limit (MRL) and limit of decision (CCα) and limit of capability (CCβ) for antibiotics in chicken meat

Analyte	MRL (µg/kg)	ccα (µg/kg)	ccβ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Kanamycin	100	121	143	10.0	25.0
Neomycin	500	602	704	40.0	120.0
Lincomycin	100	110	119	3.0	10.0
Clindamycin	-	1	2	0.3	1.0
Trimethoprim	50	57	64	1.0	3.0
Josamycin	-	2	4	0.3	1.0
Spiramycin	200	223	247	0.3	1.0
Tilmicosin	75	80	85	0.3	1.0
Tylosin	100	107	114	1.0	3.0
Clarithromycin	-	3	5	0.3	1.0
Oleandomycin	-	2	4	0.3	1.0
Tylvalosin	-	3	6	0.3	1.0
Sulfadimethoxine	100[a]	110	120	0.3	1.0
Sulfamethoxazole	100[a]	119	137	1.5	5.0
Sulfadoxin	100[a]	108	116	0.3	1.0
Sulfaquinoxaline	100[a]	111	122	0.3	1.0
Sulfachlorpyridazine	100[a]	111	122	10.0	25.0
Sulfaclozine	100[a]	116	132	3.0	10.0
Oxytetracycline	100	112	125	3.0	10.0
Tetracycline	100	115	130	3.0	10.0
Chlortetracycline	100	112	124	5.0	15.0
Doxycycline	100	110	120	1.0	3.0
Marbofloxacin	-	4	8	1.5	5.0
Ciprofloxacin	100[b]	104	109	0.3	1.0
Danofloxacin	200	217	233	0.3	1.0
Enrofloxacin	100[b]	108	116	0.3	1.0
Difloxacin	300	334	369	0.3	1.0
Oxolinic acid	100	109	118	0.3	1.0
Flumequine	400	438	476	0.3	1.0
Nalidixic acid	-	1	2	0.3	1.0
Enoxacin	-	2	5	0.3	1.0
Ofloxacin	-	2	4	0.3	1.0
Lomefloxacin	-	3	5	0.3	1.0
Norfloxacin	-	4	8	0.3	1.0
Sarafloxacin	-	3	5	0.3	1.0
Cinoxacin	-	3	5	1.0	3.0

[a] Expressed in form of sum-MRLs of all sulfonamides.

[b] Expressed in form of sum-MRLs of Enrofloxacine and its metabolite (Ciprofloxacine).

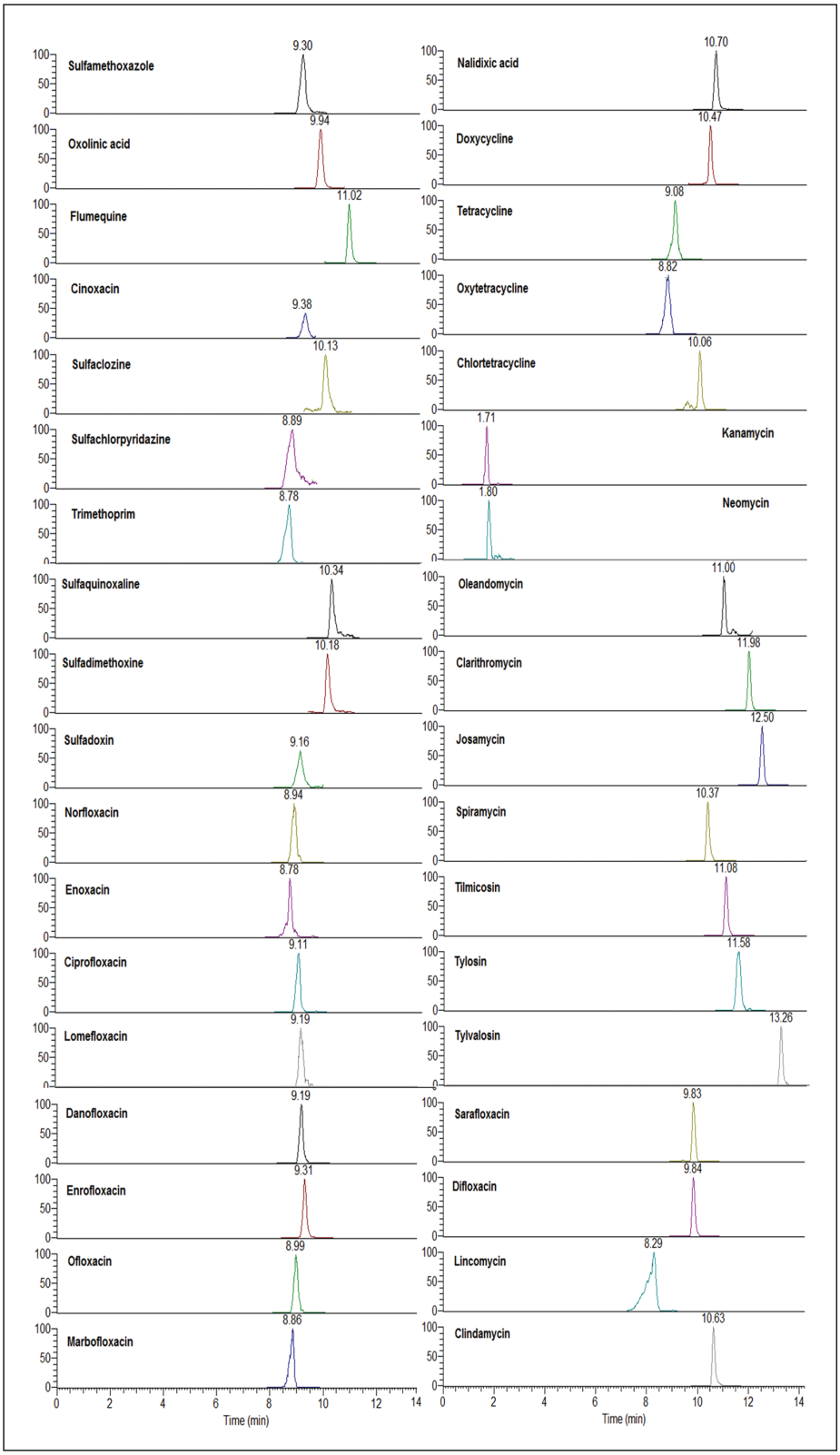

Figure 1. MRM chromatogram of chicken meat sample fortified with 36 antibiotics

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