

Supplementary Material

Antimicrobial residues in meat from chickens in Northeast Vietnam: analytical validation and pilot study for sampling optimisation

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Appendix 1.A: chromatographic gradient and LC/MS setup

Timetable of the elution gradient:

T (min)	Track A%	Track B%
0	90	10
4	70	30
5	70	30
7	30	70
10	30	70
10.1	5	95
11.1	5	95
11.2	90	10
15	90	10

A: Solution of 0.5 mmol/L Heptafluorobutyric acid (i.e. 0.0065%) + 4.75 mmol/L Pentafluoropropionic acid (i.e. 0.05%) in Optima water

B: Solution of 0.5mmol/L Heptafluorobutyric acid (i.e. 0.0065%)+ 4.75mmol/L Pentafluoropropionic acid (i.e. 0.05%) in Optima LC/MS Grade acetonitrile

Source conditions for Waters Xevo TQ-S:

- Type of scan: MRM (multiple reaction monitoring)
- Polarity: ES+
- Capillary voltage: 2.5 kV
- Source temp.: 150 °C
- Desolvation temp.: 500 °C
- Desolvation gas flow: 1000 L/hr
- Cone gas flow: 150 L/hr

Adjusted or Scheduled® MRM (Waters Xevo TQ-S):

- MRM detection window: 60 sec Target scan time: 2 sec
- Type of scan: MRM with MRM window adjusted for each transition at the expected retention time $\pm$ 30sec

Appendix 1.B: Retention times (RT), MRM transitions (Precursor ion > Product ions with Transition 1 and Transition 2) and mass spectrometer conditions used for each compound

	Transition	RT (min)	Precursor ion (m/z)2	Product ion (m/z)*	Cone voltage	Collision Energy	Dwell time (ms)
Sulfaguanidine	Tr1	2.03	215.0	156.0	30	14	25
	Tr2		215.0	108.0	30	20	25
Florfenicol amine	Tr1	2.84	248.1	230.0	20	10	25
	Tr2		248.1	130.1	20	22	25
Sulfadiazine	Tr1	2.96	251.0	108.1	52	20	25
	Tr2		251.0	156.0	52	12	25
Amoxicillin	Tr1	3.38	366.1	349.0	14	8	25
	Tr2		366.1	208.0	14	10	25
Sulfacetamide	Tr1	2.86	215.1	156.0	30	10	29
	Tr2		215.1	108.0	30	15	29
Sulfathiazole	Tr1	3.26	256.0	156.0	58	14	25
	Tr2		256.0	108.1	58	22	25
Sulfamerazine	Tr1	3.38	265.0	156.0	60	14	25
	Tr2		265.0	108.1	60	26	25
Desacetyl cephalirin	Tr1	2.44	382.0	112.0	40	22	25
	Tr2		382.0	152.0	40	24	25
Cephapirin	Tr1	3.68	424.1	291.9	16	14	25
	Tr2		424.1	151.9	16	22	25
Cefquinome	Tr1	3.91	529.4	134.0	34	12	25
	Tr2		529.4	125.0	34	52	25
Sulfadimerazine	Tr1	3.73	279.1	186.1	56	16	25
	Tr2		279.1	156.0	56	18	25
Lincomycin	Tr1	4.01	407.1	126.0	86	14	25
	Tr2		407.1	359.1	86	18	25
Cephalonium	Tr1	4.06	459.0	152.0	12	16	25
-	Tr2		459.0	337.0	12	8	25
Sulfamethoxypyridazine	Tr1	4.17	281.2	156.0	58	16	25
-	Tr2		281.2	108.1	58	22	25
Cephalexin	Tr1	4.60	348.0	158.0	2	6	25
-	Tr2		348.0	174.0	2	14	25
Ampicillin	Tr1	4.53	350.1	106.0	2	16	25
-	Tr2		350.1	160.1	2	10	25
Marbofloxacin	Tr1	4.55	363.1	345.0	20	26	25
-	Tr2		363.1	320.0	20	14	25
Norfloxacin	Tr1	4.61	320.1	302.0	66	20	25
-	Tr2		320.1	230.9	66	38	25
Thiamphenicol	Tr1	3.94	356.1	307.9	4	14	25
-	Tr2		358.0	310.0	4	14	25
Sulfamethisole	Tr1	4.20	271.0	156.0	56	14	25
-	Tr2		271.0	108.1	56	24	25
Trimethoprim	Tr1	4.73	291.1	123.1	30	24	25
-	Tr2		291.1	230.1	30	20	25
Ciprofloxacin	Tr1	4.75	332.1	314.0	2	20	25
-	Tr2		332.1	230.9	2	36	25
Oxytetracycline	Tr1	4.75	461.1	426.1	4	18	25
-	Tr2		461.1	443.0	4	12	25
Tildipirosin	Tr1	5.42	734.3	98.0	30	36	25
-	Tr2		734.3	174.0	30	36	25
Danofloxacin	Tr1	4.85	358.0	340.0	42	20	25
-	Tr2		358.0	255.0	42	34	25
Cefazolin	Tr1	4.42	455.2	322.9	2	10	25
-	Tr2		455.2	155.9	2	20	25
Enrofloxacin	Tr1	5.11	360.0	342.0	2	20	25
-	Tr2		360.0	286.0	2	32	25
Tetracycline	Tr1	5.19	445.0	410.0	2	18	25

	Tr2		445.0	154.0	2	26	25
Neospiramycin	Tr1	5.94	699.4	174.0	56	28	35
	Tr2		699.4	540.1	56	18	35
Sulfamonomethoxine	Tr1	4.69	281.1	156.0	36	16	25
	Tr2		281.1	108.0	36	26	25
Tulathromycin	Tr1	6.28	806.4	577.2	82	20	25
	Tr2		806.4	158.0	82	38	25
Sarafloxacin	Tr1	5.68	386.0	368.0	2	20	25
	Tr2		386.0	342.0	2	18	25
Difloxacin	Tr1	5.82	400.0	356.0	2	20	25
	Tr2		400.0	382.0	2	24	25
Cefoperazone	Tr1	5.27	646.4	143.0	22	34	25
	Tr2		646.4	530.0	22	10	25
Spiramycin	Tr1	6.65	843.4	174.0	60	36	28
	Tr2		843.4	142.0	60	34	28
Dapsone	Tr1	4.92	249.0	156.0	40	12	25
	Tr2		249.0	108.0	40	20	25
Cefuroxime	Tr1	4.92	447.1	385.9	16	12	25
	Tr2		447.1	341.9	16	12	25
Sulfachloropyridazine	Tr1	5.05	285.0	156.0	18	14	25
	Tr2		285.0	108.1	18	26	25
Sulfadoxine	Tr1	5.27	311.1	156.0	2	16	25
	Tr2		311.1	108.0	2	24	25
Chlortetracycline	Tr1	6.57	479.0	444.1	10	20	25
	Tr2		479.0	462.0	10	16	25
Doxycycline	Tr1	6.88	445.0	428.0	2	18	25
	Tr2		445.0	154.0	2	30	25
Ceftiofur	Tr1	6.81	524.0	240.9	6	16	25
	Tr2		524.0	125.1	6	58	25
Gamithromycin	Tr1	7.05	777.5	619.3	60	28	28
	Tr2		777.5	158.0	60	35	28
Tilmicosin	Tr1	7.13	869.7	174.1	58	40	25
	Tr2		869.7	696.4	58	40	25
Erythromycin	Tr1	7.25	734.5	158.1	30	30	35
	Tr2		734.5	576.4	30	20	35
Tylosin	Tr1	7.27	916.4	174.2	60	35	25
	Tr2		916.4	772.5	60	30	25
Sulfamethoxazole	Tr1	5.56	254.0	156.0	46	16	25
	Tr2		254.0	108.0	46	24	25
O-acetyltylosin	Tr1	7.40	958.7	174.2	60	35	19
	Tr2		958.7	814.5	60	30	19
Oxolinic acid	Tr1	6.09	262.1	244.0	56	20	25
	Tr2		262.1	215.9	56	24	25
Florfenicol	Tr1	5.59	358.0	241.0	12	16	25
	Tr2		360.0	243.0	12	16	25
Sulfaquinoxaline	Tr1	6.75	301.1	156.0	30	18	25
	Tr2		301.1	108.0	30	26	25
Tiamulin	Tr1	7.72	494.3	192.0	28	16	25
	Tr2		494.3	119.0	28	40	25
Sulfaclozine	Tr1	6.72	285.0	155.9	2	16	25
	Tr2		285.0	130.1	2	20	25
Sulfadimethoxine	Tr1	6.75	311.1	156.0	2	20	25
	Tr2		311.1	108.0	2	30	25
Valnemulin	Tr1	7.83	565.4	263.1	30	16	19
	Tr2		565.4	147.1	30	36	19
Josamycin	Tr1	7.88	828.5	174.2	12	30	25
	Tr2		828.5	109.1	12	48	25
Sulfaphenazole	Tr1	6.93	315.0	156.0	32	20	28
	Tr2		315.0	158.0	32	28	28
Penicillin G	Tr1	7.30	335.0	159.9	28	16	25

	Tr2		335.0	176.0	28	20	25
Tylvalosin	Tr1	8.11	1042.8	109.1	60	45	60
	Tr2		1042.8	174.2	60	38	60
Penicillin V	Tr1	7.58	351.1	160.0	28	12	35
	Tr2		351.1	114.0	28	32	35
Nalidixic acid	Tr1	7.35	233.1	186.9	50	26	25
	Tr2		233.1	215.0	50	20	25
Oxacillin	Tr1	7.74	402.1	160.0	4	14	25
	Tr2		402.1	243.0	4	14	25
Flumequine	Tr1	7.53	262.0	244.0	50	20	25
	Tr2		262.0	201.9	50	32	25
Nafcillin	Tr1	8.03	415.0	199.1	6	18	25
	Tr2		415.0	171.1	6	34	25
Cloxacillin	Tr1	7.94	436.0	160.1	2	10	25
	Tr2		436.0	277.0	2	10	25
Dicloxacillin	Tr1	8.21	470.3	160.0	22	10	25
	Tr2		470.3	310.8	22	15	25
Rifaximin	Tr1	8.34	786.5	754.5	22	20	25
	Tr2		786.5	151.1	22	38	25
Novobiocin	Tr1	9.05	613.2	189.1	52	34	25
	Tr2		613.2	218.1	52	12	25
Azithromycin	Tr1	6.81	749.3	591.4	24	24	25
	Tr2		749.3	158.1	24	34	25

Appendix 1.C: List of AMDs validated for residue testing based upon Dubreil et al. (2017) with their targeted validation concentration (CVal) and validation results for group A and group B drugs. Azithromycin was added to the panel

Group A:

		Transition 1					Transition 2						
Analyte	Cval (µg/kg) (21 samples spiked at 0.5MRL or 1 MRL)	T (Tr 1)	Fm (Tr 1)	CCβ transi 1	LD (µg/kg) Tansi 1	Sensitivity at Cval %	T (Tr 2)	Fm (Tr 2)	CCβ transi 2	LD (µg/kg) Tansi 2	Sensitivity at Cval %	CVr %	CVR %
PENICILLINS													
Amoxicillin	50	1.91E+02	2.17E+03	< Cval	4	100	8.99E+01	7.67E+02	< Cval	5	100.00	15.5	16.5
Ampicillin	25	1.29E+03	2.97E+04	< Cval	0.6	100	2.68E+03	1.90E+04	< Cval	5	100	12.8	17.4
Penicillin G	25	1.15E+02	5.06E+03	< Cval	0.3	100	1.04E+02	3.08E+03	< Cval	0.4	100	41.7	46.1
Penicillin V	25	5.45E+03	2.94E+03	> Cval	25	90	2.42E+03	2.73E+03	< Cval	19	90	43.6	51.4
Oxacillin	150	1.22E+02	1.57E+05	< Cval	0.1	100	1.30E+02	7.52E+05	< Cval	0.1	100	8.9	32.7
Cloxacillin	150	9.43E+01	1.69E+05	< Cval	0.04	100	1.59E+02	1.30E+05	< Cval	0.05	100	18.0	30.0
Nafcillin	150	4.19E+02	7.52E+05	< Cval	0.1	100	5.84E+02	4.27E+05	< Cval	0.2	100.0	8.0	9.2
Dicloxacillin	150	3.48E+01	1.21E+05	< Cval	0.02	100	1.91E+02	6.39E+04	< Cval	0.2	100.0	4.2	34.6
CEPHALOSPORINES													
Cefapirin	50	2.63E+02	-1.48E+03	> Cval	5	100	2.63E+02	-4.21E+02	> Cval	9	100	65.4	75.6
Desacetylcephapirin	50	1.82E+02	4.32E+03	< Cval	2	100	1.16E+04	3.34E+03	> Cval	155	0	10.9	12.5
Cefquinome	25	4.05E+01	2.36E+04	< Cval	0.2	100	1.11E+03	5.96E+03	< Cval	20	100	18.6	18.3
Cetalkonium	25	1.05E+02	2.00E+04	< Cval	0.5	100	7.76E+01	2.17E+04	< Cval	0.4	100	11.0	11.9
Cefazolin	25	7.58E+01	2.40E+04	< Cval	0.5	100	5.08E+03	2.00E+04	< Cval	39	100	4.9	5.1
Cefalexin	100	7.63E+00	6.78E+04	< Cval	0.01	100	9.75E+01	2.68E+04	< Cval	0.3	81	11.9	15.2
Ceftiofur	50	7.22E+01	5.42E+04	< Cval	0.1	100	6.85E+02	2.16E+04	< Cval	4	100	7.0	10.8
Cefoperazone	25	1.53E+02	2.19E+04	< Cval	0.8	100	6.32E+01	1.41E+04	< Cval	0.5	100	6.7	7.6
Cefuroxime	25	1.47E+02	2.75E+03	< Cval	3	100	5.45E+02	1.64E+03	< Cval	12	100	11.0	11.2
SULFONAMIDES													
Sulfaguanidine	100	3.09E+03	5.78E+03	< Cval	50	100	7.69E+02	1.48E+03	< Cval	33	100	20.3	20.4
Sulfadiazine	50	1.73E+03	6.38E+04	< Cval	3	100	4.47E+02	3.57E+05	< Cval	0.6	100	19.4	22.0
Sulfathiazole	50	8.74E+02	2.74E+04	< Cval	4	100	4.20E+02	1.08E+05	< Cval	0.5	100	12.4	14.5
Sulfadimerazine (Sulfadimidine)	50	1.67E+03	1.43E+05	< Cval	0.5	100	6.45E+02	5.32E+04	< Cval	0.8	100	17.4	18.9
Sulfamethoxyypyridazine	50	1.16E+03	1.32E+05	< Cval	1	100	4.81E+02	7.23E+04	< Cval	0.7	100	10.8	10.6
Sulfamonomethoxine	50	2.92E+02	1.19E+05	< Cval	0.3	100	4.86E+02	8.44E+04	< Cval	0.7	100	12.5	14.8
Sulfadoxine	50	3.01E+02	9.59E+05	< Cval	0.04	100	7.23E+02	4.50E+05	< Cval	0.2	100	11.5	12.0
Sulfaquinoxaline	50	6.43E+02	4.91E+05	< Cval	0.2	100	8.46E+02	2.11E+05	< Cval	0.4	100	17.2	17.2

Marbofloxacin	75	4.50E+02	5.98E+04	< Cval	1	100	4.26E+02	5.94E+04	< Cval	1	100	4.1	5.2
Norfloxacin	50	6.62E+03	1.45E+05	< Cval	3	100	1.69E+02	4.24E+04	< Cval	0.2	100	9.0	19.4
Ciprofloxacin	25	1.01E+03	8.26E+04	< Cval	0.2	100	1.35E+02	7.21E+04	< Cval	0.05	100	25.9	27.1
Danofloxacin	50	3.09E+03	2.57E+05	< Cval	2	100	3.09E+03	3.33E+04	< Cval	0.5	100	11.7	26.6
Enrofloxacin	25	6.15E+02	1.10E+05	< Cval	0.3	100	1.31E+02	2.08E+04	< Cval	0.3	100	11.5	16.0
Sarafloxacin	15	9.22E+02	5.94E+04	< Cval	1	100	2.84E+02	2.84E+04	< Cval	1	100	14.7	20.8
Difloxacin	150	1.37E+02	2.12E+05	< Cval	0.1	100	1.97E+02	3.92E+05	< Cval	0.1	100	10.0	13.1
Oxolinic acid	50	9.90E+02	1.07E+06	< Cval	0.2	100	8.99E+02	1.06E+05	< Cval	0.9	100	6.7	6.9
Nalidixic acid	25	1.06E+03	2.61E+05	< Cval	0.5	100	8.73E+02	1.87E+05	< Cval	0.5	100	10.6	15.9
Flumequine	100	5.41E+03	3.24E+05	< Cval	2	100	4.89E+03	2.17E+05	< Cval	1	100	12.3	24.3
PHENICOLS													
Florfenicol amine	50	5.06E+03	5.87E+04	< Cval	6	100	4.72E+03	2.53E+04	< Cval	14	100	13.7	13.9
Florfenicol	25	3.80E+02	5.62E+03	< Cval	4	100	1.16E+02	2.47E+03	< Cval	3	100	11.6	13.8
Thiamphenicol	25	7.75E+02	9.47E+03	< Cval	11	100	7.75E+02	5.73E+03	< Cval	6	100	7.8	8.8
PLEUROMOTULINES													
Tiamulin	50	4.91E+02	2.34E+06	< Cval	0.02	100	8.07E+02	4.63E+04	< Cval	0.1	100	12.1	39.8
Valnemulin	25	7.59E+01	4.63E+04	< Cval	0.1	100	3.12E+02	9.13E+03	< Cval	3	100	10.6	46.3
RIFAMICINE													
Rifaximin	25	6.90E+01	2.57E+04	< Cval	0.2	100	1.48E+02	6.49E+03	< Cval	2	100	33.0	32.3
DIAMINOPYRIMIDINE DERIVATIVES													
Trimethoprim	25	5.65E+02	5.08E+05	< Cval	0.02	100	9.88E+02	4.93E+05	< Cval	0.04	100	26.3	27.5
MISCELLANEOUS													
Novobiocine	25	4.50E+02	3.13E+04	< Cval	1	100	4.22E+01	1.72E+04	< Cval	0.3	100	14.1	27.4

Abbreviations: To estimate the detection capability CC_b , for each analyte, the **cut-off factor (F_m)** was determined using the mean and standard deviation of areas under the peak in the blank samples spiked at the pre-defined validation concentration C_{val} ($F_m = \text{mean} - 1.64 \text{ SD}$). Analysis of the background noise for each of the chromatograms corresponding to the transitions allowed for the calculation of **T**, the **threshold value** ($T = \text{mean} + 1.64 \text{ SD}$), related to the limit of detection of the method. Validation was successful if $F_m > T$ for the 2 transitions, demonstrating that CC_b is $< C_{val}$. Greyed boxes are transitions that did not pass the validation for this analyte. Precision was assessed by reporting repeatability (**CV_r** = intra-day variability) and reproducibility (**CV_R** = inter-day variability) as the coefficient of variation (CV%) calculated on all the Peak Areas of the spiked samples, i.e. 7 spiked samples per day yielding $n = 21$. The results show significant variability in the method for some of the analytes, but this variability is acceptable as this is a screening method which cannot be used for accurate quantification.