MJCCA9 –

Received: June 5, 2025
Accepted: October 9, 2025

ISSN 1857-5552
e-ISSN 1857-5625
DOI: 10.20450/mjcce.2025.3166
Original scientific paper

SUPPLEMENTARY MATERIAL

MULTI-CLASS ANALYSIS OF ANTIMICROBIAL SUBSTANCES IN POULTRY FEED AT CROSS-CONTAMINATION LEVELS BY UHPLC-MS/MS – METHOD ESTABLISHMENT, VALIDATION, AND APPLICATION

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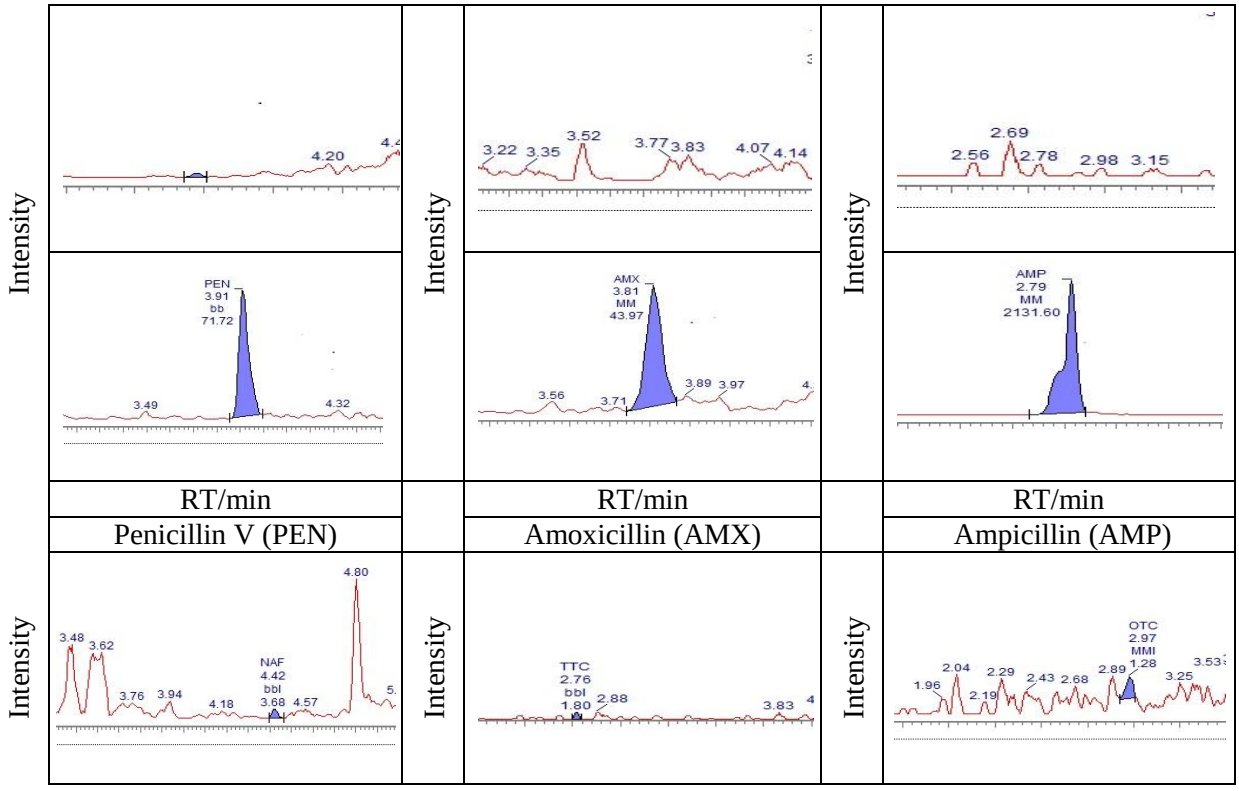
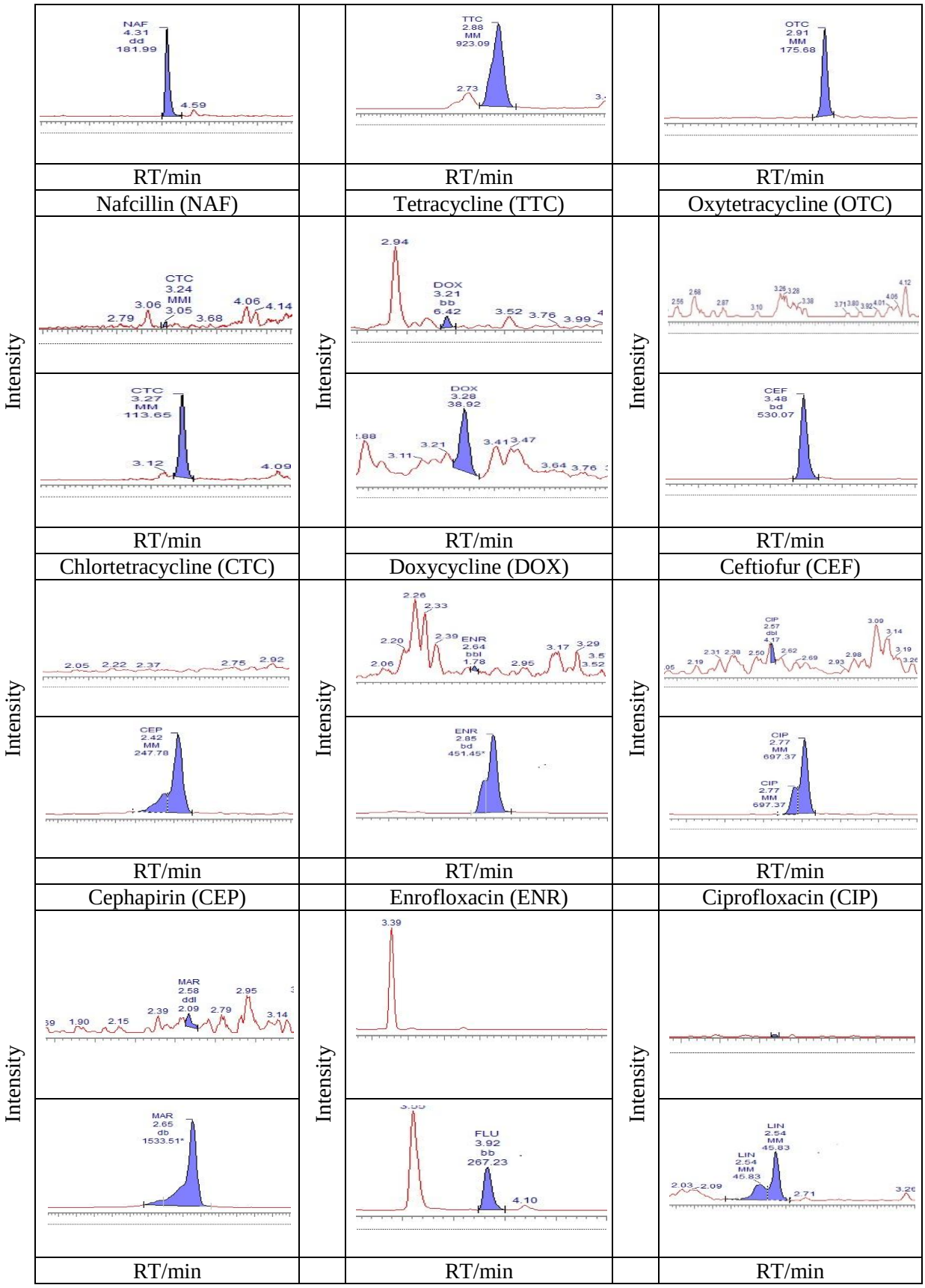
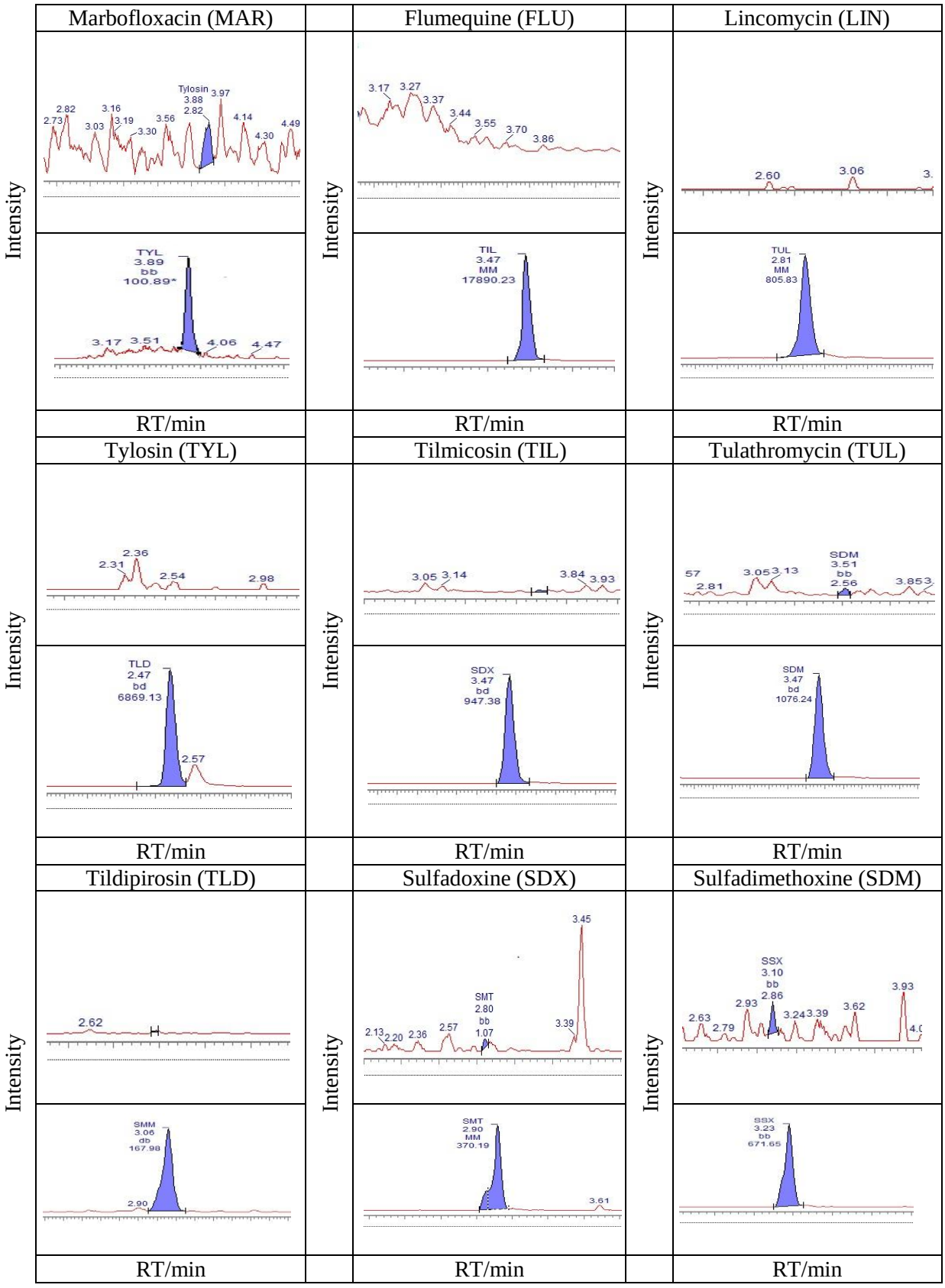



NAF
4.31
dd
181.99
4.59
TTC
2.88
MM
923.09
2.73
OTC
2.91
MM
175.68
RT/min
RT/min
RT/min
Nafcillin (NAF)
Tetracycline (TTC)
Oxytetracycline (OTC)
Intensity
CTC
3.24
MMI
2.79
3.06
3.05
3.68
4.06
4.14
DOX
3.21
bb
6.42
2.94
3.52
3.76
3.99
CTC
3.27
MM
113.65
3.12
4.09
DOX
3.28
38.92
3.11
3.21
3.41
3.47
3.64
3.76
CEF
3.48
bd
530.07
RT/min
RT/min
RT/min
Chlortetracycline (CTC)
Doxycycline (DOX)
Ceftiofur (CEF)
2.05
2.22
2.37
2.75
2.92
CEP
2.42
MM
247.78
2.26
2.33
2.20
2.39
ENR
2.64
bbi
1.78
2.06
2.95
3.17
3.29
ENR
2.85
bd
451.45*
CIP
2.57
dbi
4.17
3.09
3.14
CIP
2.77
MM
697.37
CIP
2.77
MM
697.37
RT/min
RT/min
RT/min
Cephapirin (CEP)
Enrofloxacin (ENR)
Ciprofloxacin (CIP)
MAR
2.58
ddi
2.09
1.90
2.15
2.39
2.79
2.95
3.14
3.39
MAR
2.65
db
1533.51*
FLU
3.92
bb
267.23
4.10
LIN
2.54
MM
45.83
LIN
2.54
MM
45.83
2.03
2.09
2.71
RT/min
RT/min
RT/min


Marbofloxacin (MAR)
Flumequine (FLU)
Lincomycin (LIN)
Intensity
Tylosin 3.97
3.88
2.82
2.82
2.73
3.03
3.16
3.19
3.30
3.56
4.14
4.30
4.49
TYL
3.89
bb
100.89*
3.17
3.51
4.06
4.47
3.17
3.27
3.37
3.44
3.55
3.70
3.86
TIL
3.47
MM
17890.23
2.60
3.06
TUL
2.81
MM
805.83
RT/min
Tylosin (TYL)
Tilmicosin (TIL)
Tulathromycin (TUL)
2.36
2.31
2.54
2.98
TLD
2.47
bd
6869.13
2.57
3.05
3.14
3.84
3.93
SDX
3.47
bd
947.38
57
2.81
3.05
3.13
SDM
3.51
bb
2.56
3.853
SDM
3.47
bd
1076.24
Tildipirosin (TLD)
Sulfadoxine (SDX)
Sulfadimethoxine (SDM)
2.62
SMM
3.06
db
167.98
2.90
2.13
2.20
2.36
2.57
SMT
2.80
bb
1.07
3.39
3.45
SMT
2.90
MM
370.19
3.61
2.63
2.79
2.93
SSX
3.10
bb
2.86
3.24
3.39
3.62
3.93
SSX
3.23
bb
671.65

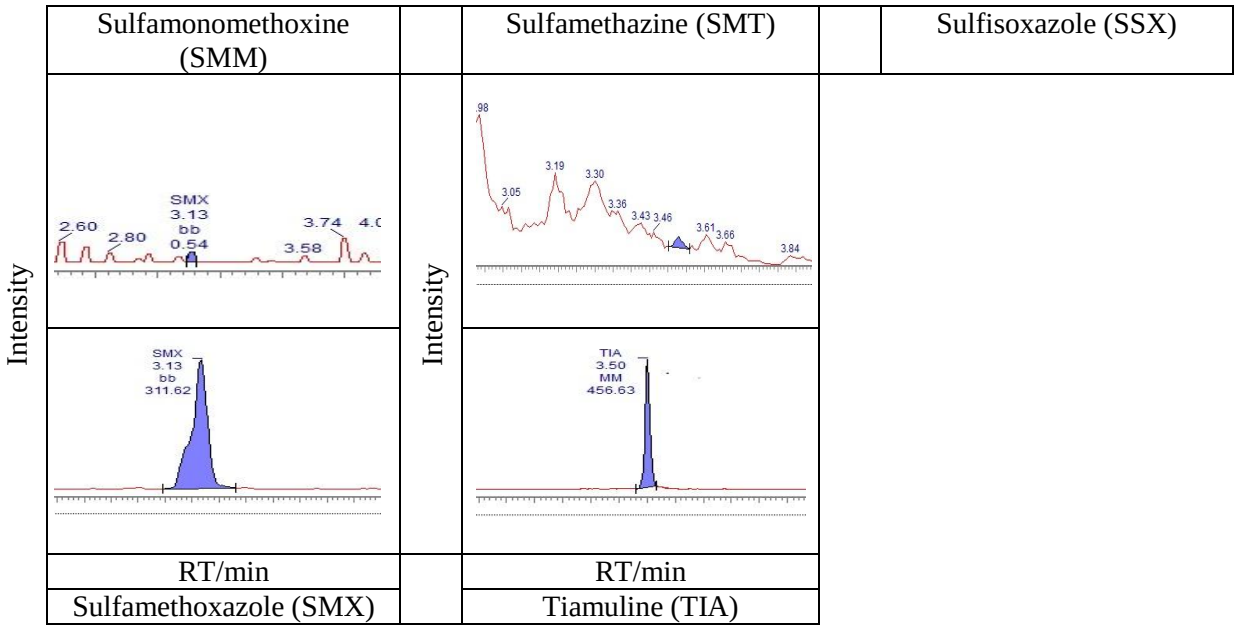
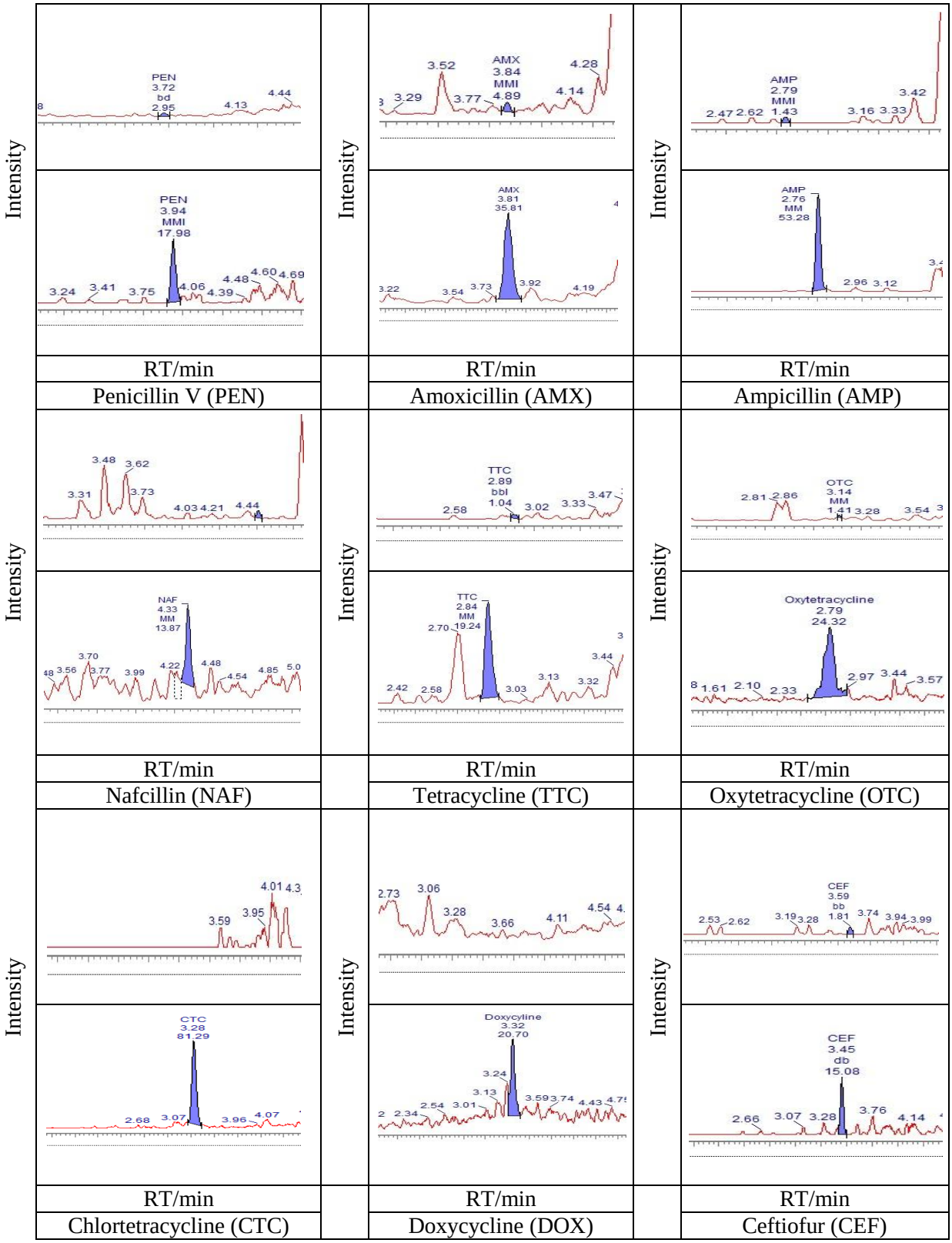
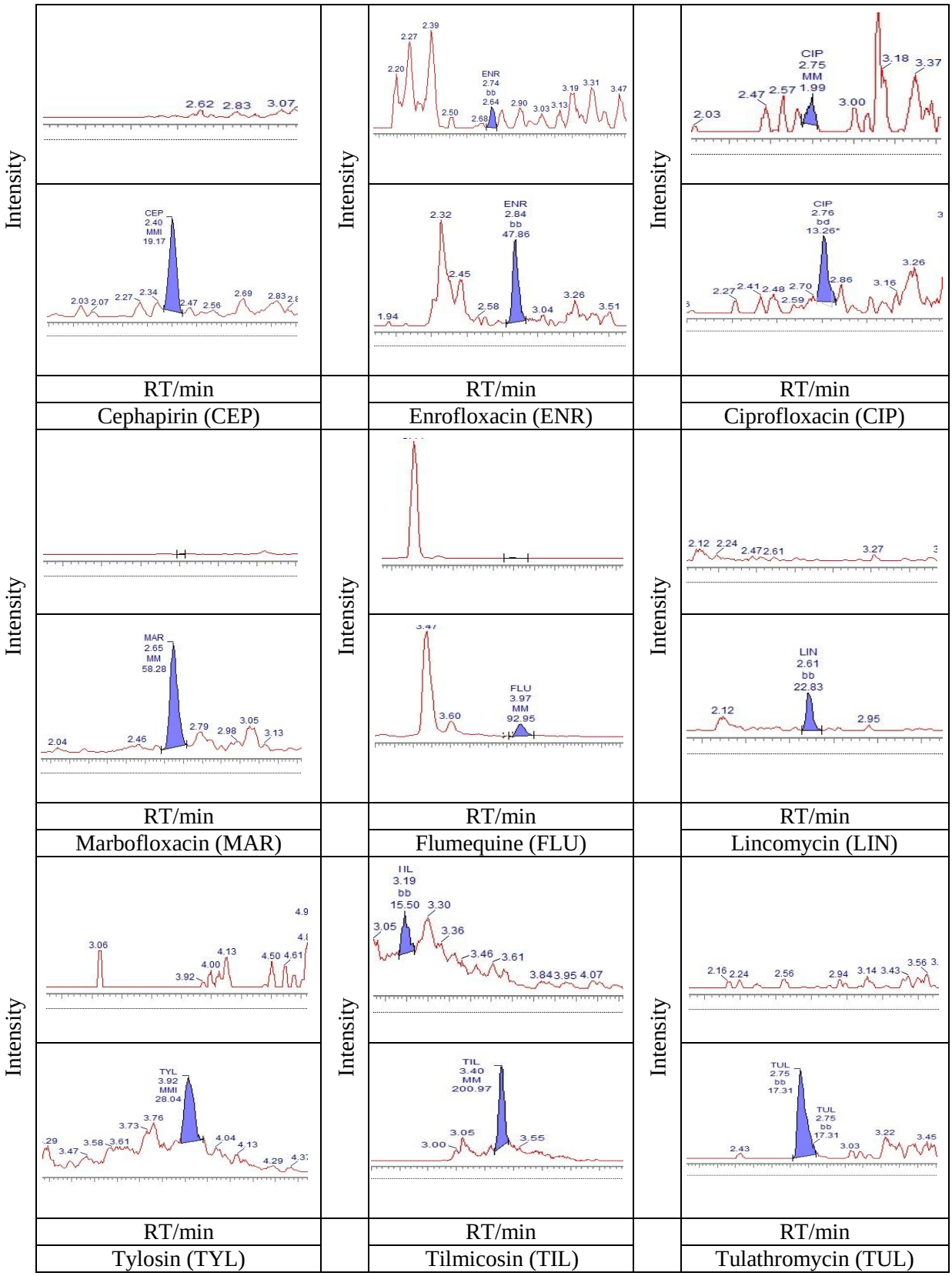


Fig. 1S. Chromatograms of the quantification ions applying Method 1 for the blank feed sample (upper figure) and spiked analytes at the limit of quantification (lower figure): 10 µg kg^{-1} (TIA), 25 µg kg^{-1} (FLU, LIN, SDX, SDM, SMM, SMT, SSX, SMX), 50 µg kg^{-1} (PEN, NAF, TTC, OTC, CTC, DOX, CEF, CEP, ENR, CIP, MAR, FLU, TYL, TIL, TLD) and 100 µg kg^{-1} (AMP, AMX).


Intensity
PEN
3.72
bd
2.95
4.13
4.44
PEN
3.94
MMI
17.98
3.24
3.41
3.75
4.06
4.39
4.48
4.60
4.69
RT/min
Penicillin V (PEN)
Intensity
3.52
AMX
3.84
MMI
4.89
3.29
3.77
4.14
4.28
AMX
3.81
35.81
3.22
3.54
3.73
3.92
4.19
RT/min
Amoxicillin (AMX)
Intensity
AMP
2.79
MMI
1.43
2.47
2.62
3.16
3.33
3.42
AMP
2.76
MM
53.28
2.96
3.12
RT/min
Ampicillin (AMP)
Intensity
3.48
3.62
3.31
3.73
4.03
4.21
4.44
NAF
4.33
MM
13.87
3.70
3.56
3.77
3.99
4.22
4.48
4.54
4.85
RT/min
Nafcillin (NAF)
Intensity
TTC
2.89
bbl
1.04
2.58
3.02
3.33
3.47
TTC
2.84
MM
19.24
2.70
2.42
2.58
3.03
3.13
3.32
3.44
RT/min
Tetracycline (TTC)
Intensity
OTC
3.14
MM
1.41
2.81
2.86
3.28
3.54
Oxytetracycline
2.79
24.32
1.61
2.10
2.33
2.97
3.44
3.57
RT/min
Oxytetracycline (OTC)
Intensity
4.01
3.59
3.95
CTC
3.28
81.29
2.68
3.07
3.96
4.07
RT/min
Chlortetracycline (CTC)
Intensity
2.73
3.06
3.28
3.66
4.11
4.54
Doxycyline
3.32
20.70
3.24
3.13
2.34
2.54
3.01
3.59
3.74
4.43
RT/min
Doxycycline (DOX)
Intensity
CEF
3.59
bb
1.81
2.53
2.62
3.19
3.28
3.74
3.94
3.99
CEF
3.45
db
15.08
2.66
3.07
3.28
3.76
4.14
RT/min
Ceftiofur (CEF)


Intensity
2.62 2.83 3.07
CEP
2.40
MM
19.17
2.03 2.07 2.27 2.34 2.47 2.56 2.69 2.83
RT/min
Cephapirin (CEP)
Intensity
2.20 2.27 2.39 2.50 2.68
ENR
2.74
bb
2.64
2.90 3.03 3.13 3.19 3.31 3.47
2.32
ENR
2.84
bb
47.86
2.45 2.58 3.04 3.26 3.51
1.94
RT/min
Enrofloxacin (ENR)
Intensity
2.03
CIP
2.75
MM
1.99
2.47 2.57 3.00 3.18 3.37
CIP
2.76
bd
13.26*
2.27 2.41 2.48 2.70 2.59 2.86 3.16 3.26
RT/min
Ciprofloxacin (CIP)
Intensity
MAR
2.65
MM
58.28
2.04 2.46 2.79 2.98 3.05 3.13
RT/min
Marbofloxacin (MAR)
Intensity
3.60
FLU
3.97
MM
92.95
RT/min
Flumequine (FLU)
Intensity
2.12 2.24 2.47 2.61 3.27
LIN
2.61
bb
22.83
2.12 2.95
RT/min
Lincomycin (LIN)
Intensity
3.06 3.92 4.00 4.13 4.50 4.61
TYL
3.92
MMI
28.04
3.47 3.58 3.61 3.73 3.76 4.04 4.13 4.29
RT/min
Tylosin (TYL)
Intensity
TIL
3.19
bb
15.50
3.05 3.30 3.36 3.46 3.61 3.84 3.95 4.07
TIL
3.40
MM
200.97
3.00 3.05 3.55
RT/min
Tilmicosin (TIL)
Intensity
2.16 2.24 2.56 2.94 3.14 3.43 3.56
TUL
2.75
bb
17.31
TUL
2.75
bb
17.31
2.43 3.03 3.22 3.45
RT/min
Tulathromycin (TUL)

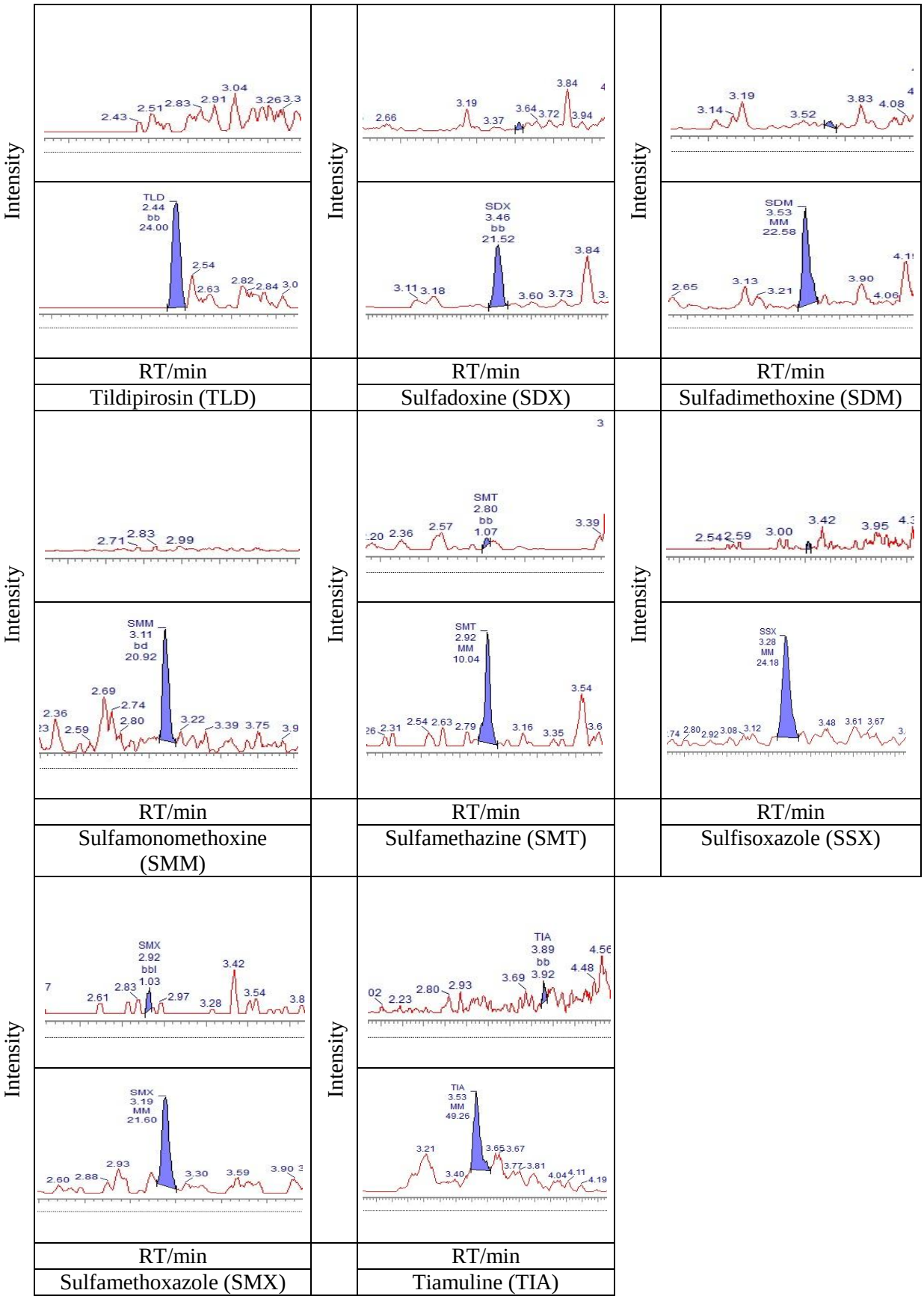

Intensity
TLD
2.44
bb
24.00
RT/min
Tildipirosin (TLD)
Intensity
SDX
3.46
bb
21.52
RT/min
Sulfadoxine (SDX)
Intensity
SDM
3.53
MM
22.58
RT/min
Sulfadimethoxine (SDM)
Intensity
SMM
3.11
bd
20.92
RT/min
Sulfamonomethoxine (SMM)
Intensity
SMT
2.92
MM
10.04
RT/min
Sulfamethazine (SMT)
Intensity
SSX
3.28
MM
24.18
RT/min
Sulfisoxazole (SSX)
Intensity
SMX
3.19
MM
21.60
RT/min
Sulfamethoxazole (SMX)
Intensity
TIA
3.53
MM
49.26
RT/min
Tiamuline (TIA)

Fig. 2S. Chromatograms of the quantification ions applying Method 2 for the blank feed sample (upper figure) and spiked analytes at the limit of quantification (lower figure): 10 µg kg^{-1} (TIA), 25 µg kg^{-1} (FLU, SDX, SDM, SMM, SMT, SSX, SMX), 50 µg kg^{-1} (PEN, NAF, TTC, OTC, CTC, DOX, CEF, CEP, ENR, CIP, MAR, LIN TYL, TIL, TLD) and 100 µg kg^{-1} (AMP, AMX).

Table 1S. Results from the short-term stability study

Analyte	Spiking level µg kg^{-1}	Method 1			Method 2		
		C_{fresh}[a] µg kg^{-1}	C_{T1}[b] (%)	C_{T2}[c] (%)	C_{fresh}[a] µg kg^{-1}	C_{T1}[b] (%)	C_{T2}[c] (%)
Penicillin V	50	51.1	94.70	94.07	45.1	94.05	100.68
	100	101.2	94.46	94.65	90.9	95.81	98.46
	150	149.3	96.51	97.83	137.9	96.94	97.32
Amoxycillin	100	104.8	88.03	91.40	88.2	89.67	104.22
	150	146.8	93.16	92.34	140.7	93.44	100.12
	200	190.1	95.10	95.75	187.2	94.07	100.18
Ampicillin	100	98.2	90.89	94.78	95.8	92.08	94.35
	150	154.2	95.39	92.45	143.0	92.64	98.45
	200	197.4	93.54	95.96	186.8	91.95	98.42
Nafcillin	50	53.3	86.12	92.57	50.1	93.12	92.12
	100	103.5	88.28	95.48	98.3	96.28	97.71
	150	152.7	94.98	94.39	144.2	94.35	99.67
Tetracycline	50	47.7	101.44	97.19	51.4	95.91	95.70
	100	101.2	100.93	101.77	96.3	96.16	97.34
	150	151.10	101.74	99.33	143.2	95.58	100.97
Oxytetracycline	50	49.8	97.17	98.20	45.4	97.26	99.18
	100	97.2	99.06	98.73	92.6	96.87	98.02
	150	147.6	98.71	99.27	142.6	97.12	101.99
Chlortetracycline	50	51.6	101.50	99.08	46.3	93.40	98.48
	100	96.2	102.22	96.88	94.1	97.04	102.65
	150	146.7	103.19	97.53	142.8	96.23	101.95
Doxycycline	50	50.9	98.85	96.39	45.7	93.22	101.33
	100	101.9	97.64	97.87	91.4	94.98	99.31
	150	149.9	100.79	98.80	143.1	96.00	99.16
Ceftiofur	50	51.4	94.08	94.37	47.7	91.14	95.67
	100	101.2	93.78	93.18	97.6	93.11	101.70
	150	151.1	95.14	94.53	142.5	93.30	100.34
Cefapirin	50	50.1	92.41	91.74	46.9	91.35	102.87
	100	101.6	94.25	93.06	93.2	95.36	103.13
	150	149.5	93.48	91.98	141.3	95.88	98.79
Enrofloxacin	50	48.7	103.07	100.26	45.7	95.80	99.77
	100	94.7	102.09	99.78	94.6	96.18	101.37
	150	148.2	101.99	101.10	143.6	97.64	100.90
Ciprofloxacin	50	50.8	94.40	100.45	45.2	97.17	103.87
	100	100.8	98.70	99.30	95.6	97.89	98.69
	150	145.6	98.68	101.49	144.5	99.35	102.67
Marbofloxacin	50	51.0	98.76	98.17	50.9	97.80	94.33
	100	102.6	94.85	99.13	96.4	96.19	102.64
	150	146.6	99.56	99.42	142.7	96.87	103.00
Flumequine	25	24.6	97.34	97.99	23.7	95.43	98.72
	50	49.9	101.02	100.22	46.9	96.81	97.78
	100	97.8	99.85	100.10	93.2	95.29	100.42
Lincomycin	25	25.4	97.86	96.86	/	/	/
	50	49.6	100.87	101.62	46.8	96.15	94.93
	100	98.5	101.00	101.40	92.2	97.17	94.13

	150	/	/	/	99.8	96.24	97.98

[a] C_{fresh} - concentration of the freshly prepared extracts (N=5)
[b] C_{T1} – remaining concentration of the analyte in aliquot stored 48 h at 4-6 °C, expressed in % RSD (N=5)
[c] C_{T2} – remaining concentration of the analyte in aliquot stored 48 h at less than -20 °C, expressed in % RSD (N=5)

Table 1S Results from the short-term stability study (continued)

Analyte	Spiking level µg kg^{-1}	**Method 1**			**Method 2**		
		C_{fresh}[a] µg kg^{-1}	C_{T1}[b] (%)	C_{T2}[c] (%)	C_{fresh}[a] µg kg^{-1}	C_{T1}[b] (%)	C_{T2}*** (%)
Tylosin	50	48.5	102.63	99.78	49.1	95.50	97.09
	100	93.9	101.72	97.32	92.7	97.59	97.01
	150	147.2	103.22	101.80	142.8	96.94	99.48
Tilmicosin	50	53.01	92.59	95.61	48.1	94.80	95.36
	100	102.2	99.31	102.80	90.9	95.18	103.64
	150	153.4	99.39	100.84	144.8	95.63	101.37
Tulathromycin	50	49.3	102.20	100.21	46.6	95.84	100.88
	100	97.2	102.98	101.70	93.3	97.42	100.56
	150	146.8	101.46	99.00	143.8	96.99	100.67
Tildipirosin	50	52.9	95.58	96.75	47.2	95.80	94.72
	100	102.8	96.67	96.26	94.4	95.28	101.09
	150	156.7	97.69	99.30	144.4	96.60	101.11
Sulfadoxine	25	23.8	101.90	100.05	24.0	95.70	99.04
	50	50.6	100.71	99.22	46.2	96.64	103.23
	100	102.8	97.36	99.58	98.4	99.61	98.32
Sulfadimetoxine	25	24.5	103.85	98.17	24.6	95.91	102.37
	50	50.6	101.00	100.43	95.7	94.09	98.50
	100	100.7	100.41	99.37	98.9	94.70	99.24
Sulfamonometoxine	25	26.8	95.71	96.35	23.5	96.83	99.13
	50	51.5	97.27	98.63	45.3	98.52	104.20
	100	100.4	99.83	100.20	95.8	97.30	100.42
Sulfamethazin	25	24.0	102.55	99.14	23.9	96.11	98.73
	50	49.3	102.51	98.92	48.5	97.72	99.12
	100	97.5	102.37	101.57	95.6	97.08	103.49
Sulfisoxazole	25	23.9	100.23	98.67	24.1	96.34	98.96
	50	50.1	99.20	99.76	45.4	97.15	101.34
	100	98.9	99.04	98.92	97.3	96.58	99.93
Sulfametoxazole	25	24.6	98.36	100.31	25.6	97.80	98.32
	50	49.1	102.44	100.95	48.0	96.62	102.62
	100	98.6	102.57	98.26	97.4	97.23	97.88
Tiamulin	10	9.8	94.34	99.25	9.9	94.48	102.32
	50	52.5	95.29	99.12	49.3	95.45	96.95
	100	102.2	98.68	101.20	97.7	96.52	99.60

[a] C_{fresh} - concentration of the freshly prepared extracts (N=5)
[b] C_{T1} – remaining concentration of the analyte in aliquot stored 48 h at 4-6 °C, expressed in % RSD (N=5)
[c] C_{T2} – remaining concentration of the analyte in aliquot stored 48 h at less than -20 °C, expressed in % RSD (N=5)

Table 2S. Results from analysis of feed samples with determined presence of antimicrobial substances.

Analyte		Tylosin ($\mu g\ kg^{-1}$)	Lincomycin ($\mu g\ kg^{-1}$)	Chlortetracycline ($\mu g\ kg^{-1}$)	Sulfamethazine ($\mu g\ kg^{-1}$)
Sample 21	Method 1	MF[a]			
	Method 2	MF[a]			
Sample 23	Method 1	MF[a]			54.2
	Method 2	MF[a]			69.3
Sample 40	Method 1				70.4
	Method 2				86.3
Sample 41	Method 1	MF[a]			121.7
	Method 2	MF[a]			131.1
Sample 42	Method 1		MF[a]		59.2
	Method 2		MF[a]		53.7
Sample 56	Method 1	>300[b]		61.3	24.1
	Method 2	>300[b]		58.6	25.6
Sample 63	Method 1	MF[a]			74.0
	Method 2	MF[a]			71.9
Sample 73	Method 1		158.9	119.5	59.6
	Method 2		156.7	130.8	63.3
Sample 83	Method 1				25.4
	Method 2				26.8
Sample 86	Method 1		MF[a]		36.9
	Method 2		MF[a]		30.0
Sample 90	Method 1			209.8	
	Method 2			184.0	
Sample 91	Method 1	297.5	MF[a]		77.6
	Method 2	285.2	MF[a]		79.6
Sample 92	Method 1	>300[b]			41.7
	Method 2	>300[b]			56.9
Sample 94	Method 1	MF[a]			277.1

	Method 2	MF[a]			301.8
Sample 95	Method 1	MF[a]			
	Method 2	MF[a]			
Sample 96	Method 1		MF[a]		>300[b]
	Method 2		MF[a]		>300[b]
ML [c] (µg kg^{-1})		100	25	100	25

[a] MF – likely medicated feed; [b] concentration above the upper method limit; [c] ML= maximum limits set by the regulation.[21]