

## SUPPLEMENTARY MATERIAL

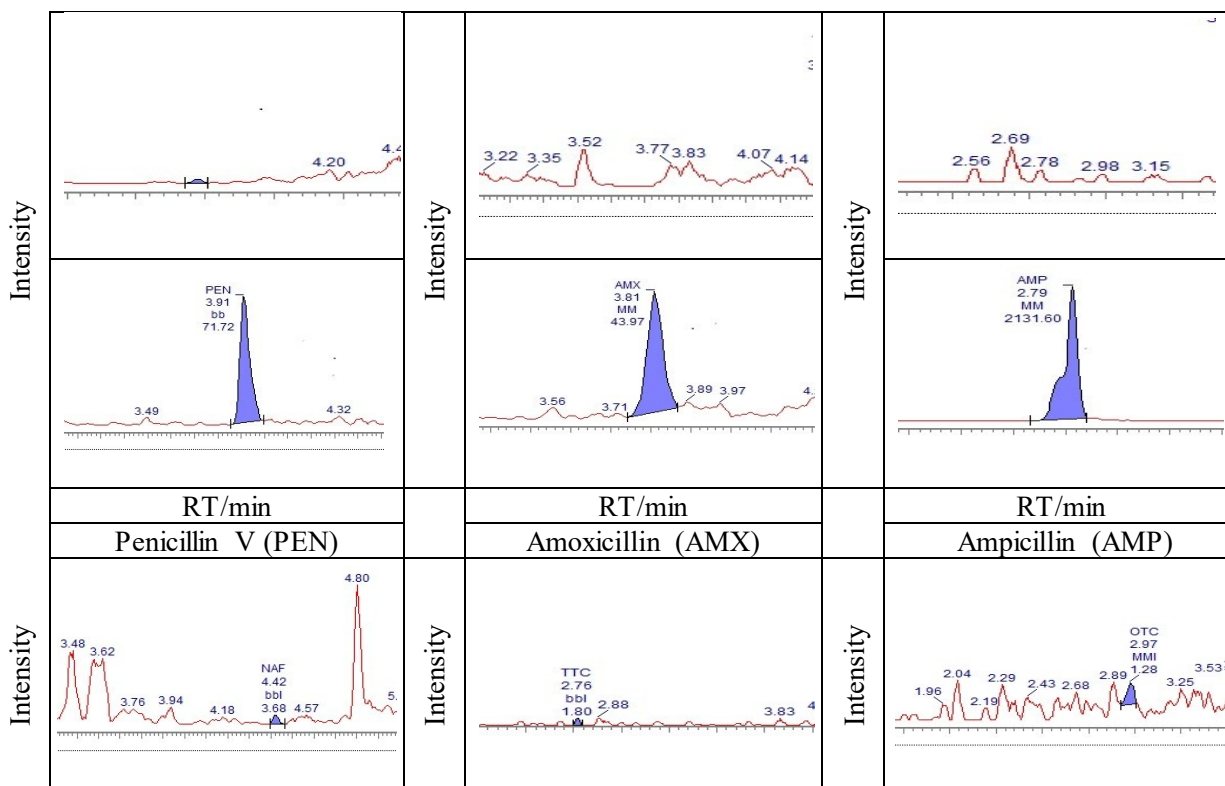
### MULTI-CLASS ANALYSIS OF ANTIMICROBIAL SUBSTANCES IN POULTRY FEED AT CROSS-CONTAMINATION LEVELS BY UHPLC-MS/MS – METHOD ESTABLISHMENT, VALIDATION, AND APPLICATION

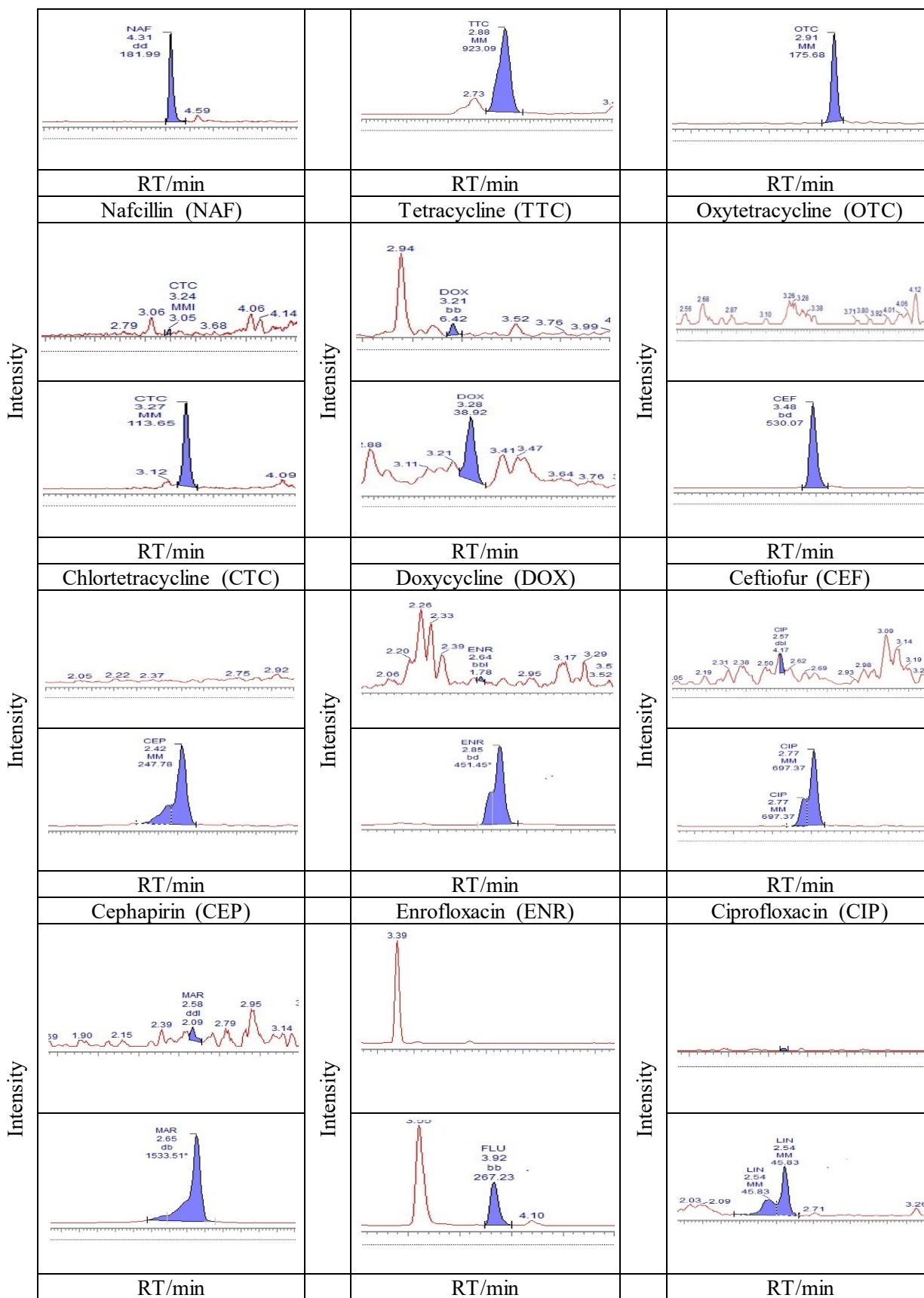
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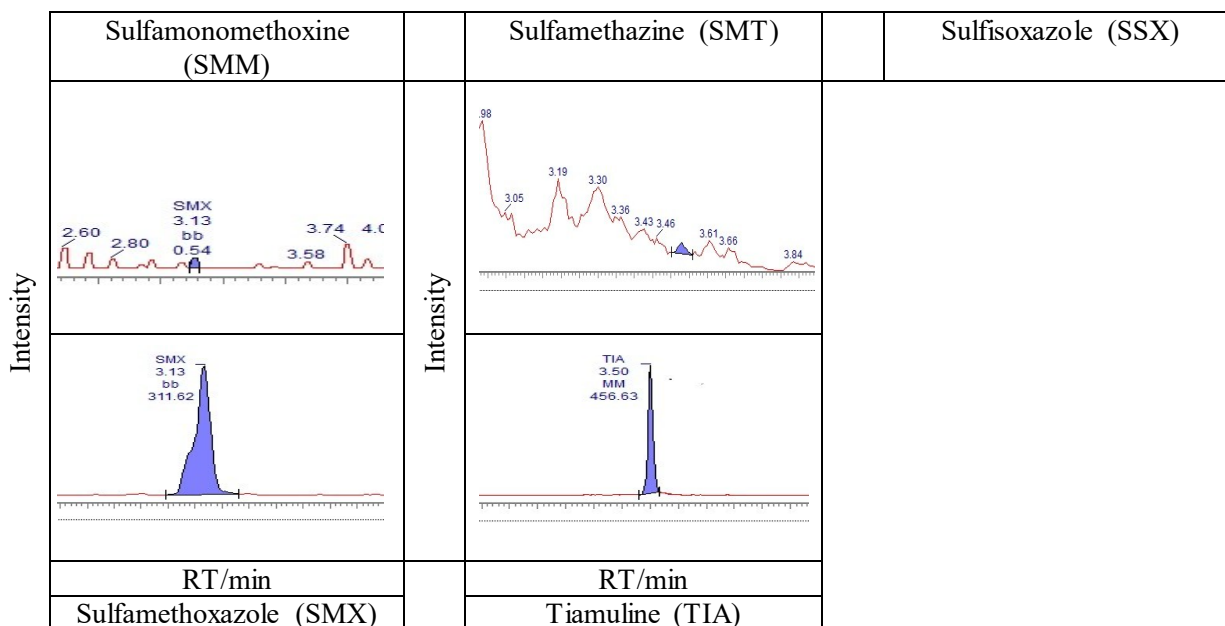
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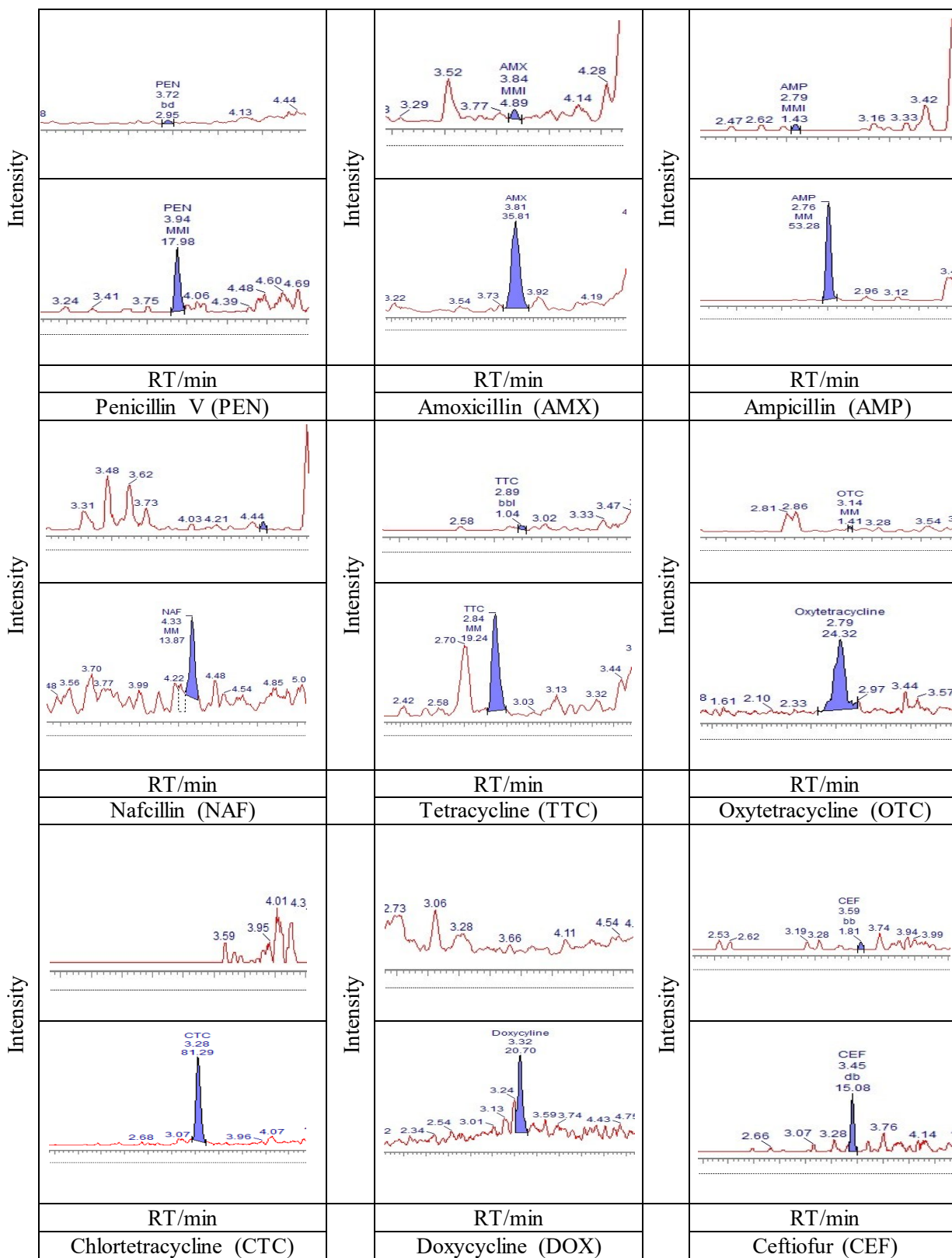


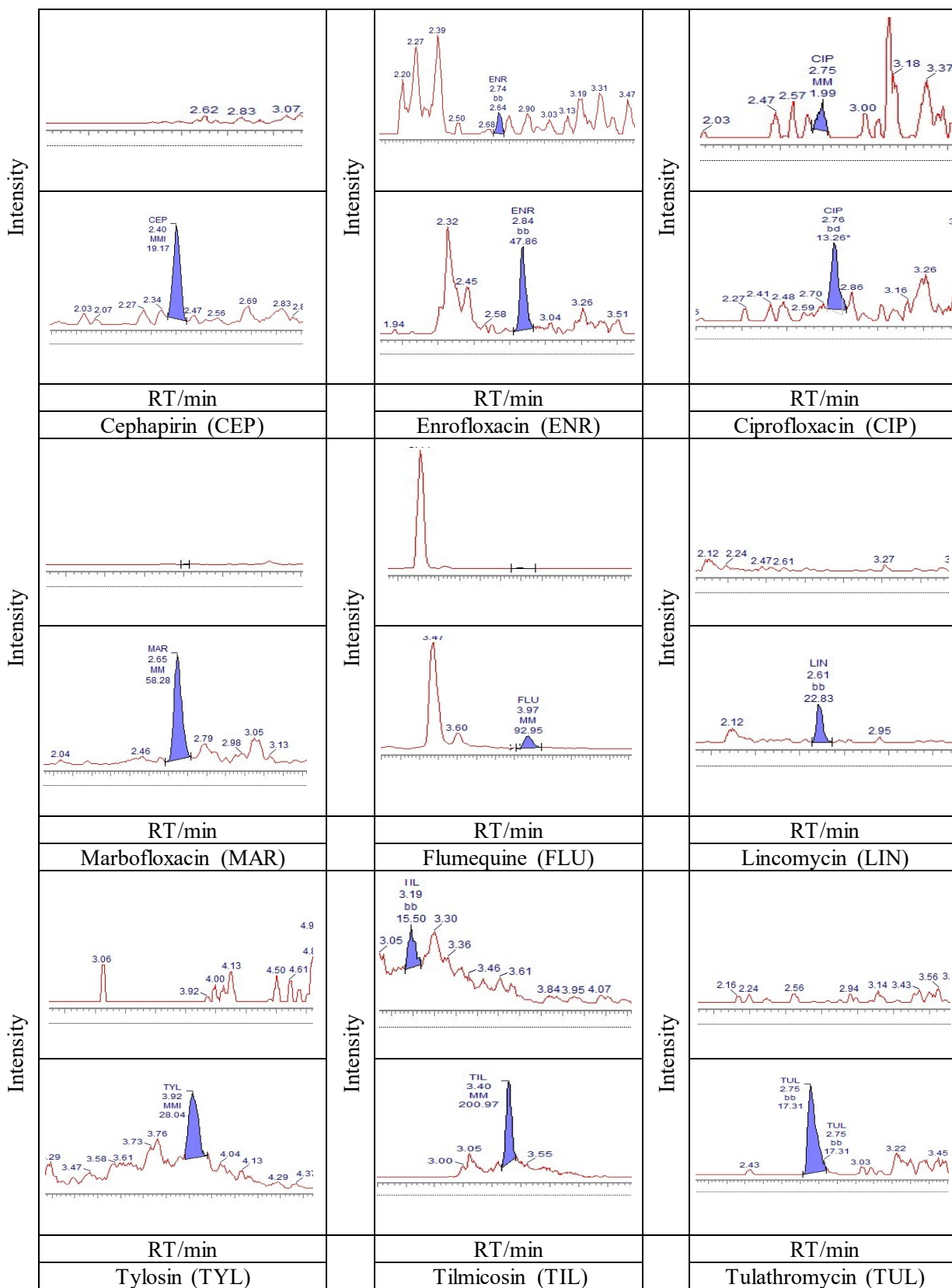


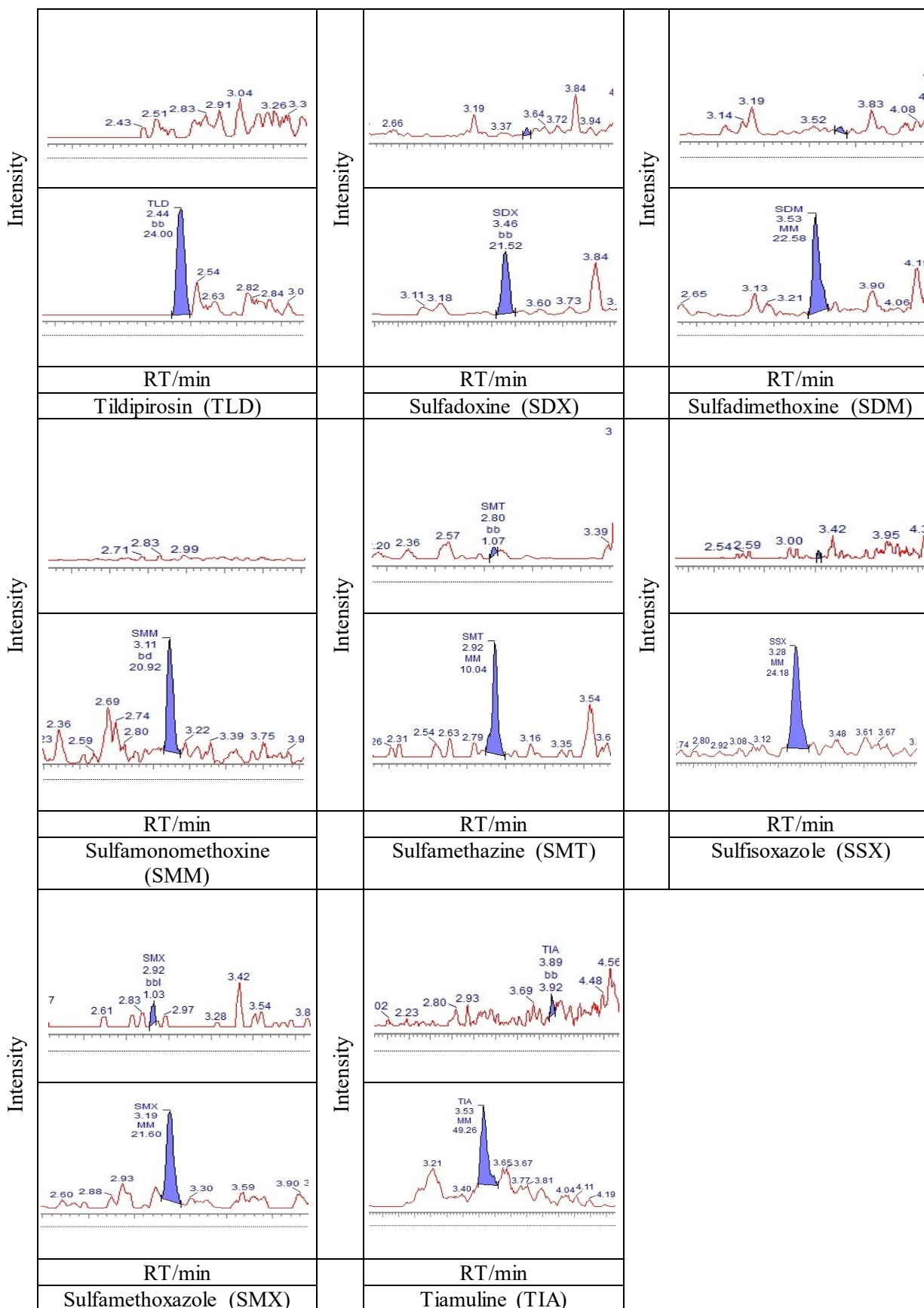
	<b>Marbofloxacin (MAR)</b>		<b>Flumequine (FLU)</b>		<b>Lincomycin (LIN)</b>
Intensity		Intensity		Intensity	
	RT/min		RT/min		RT/min
	Tylosin (TYL)		Tilmicosin (TIL)		Tulathromycin (TUL)
Intensity		Intensity		Intensity	
	RT/min		RT/min		RT/min
	Tildipirosin (TLD)		Sulfadoxine (SDX)		Sulfadimethoxine (SDM)
Intensity		Intensity		Intensity	
	RT/min		RT/min		RT/min



**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  $\mu\text{g kg}^{-1}$  (TIA), 25  $\mu\text{g kg}^{-1}$  (FLU, LIN, SDX, SDM, SMM, SMT, SSX, SMX), 50  $\mu\text{g kg}^{-1}$  (PEN, NAF, TTC, OTC, CTC, DOX, CEF, CEP, ENR, CIP, MAR, FLU, TYL, TIL, TLD) and 100  $\mu\text{g kg}^{-1}$  (AMP, AMX).







**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  $\mu\text{g kg}^{-1}$  (TIA), 25  $\mu\text{g kg}^{-1}$  (FLU, SDX, SDM, SMM, SMT, SSX, SMX), 50  $\mu\text{g kg}^{-1}$  (PEN, NAF, TTC, OTC, CTC, DOX, CEF, CEP, ENR, CIP, MAR, LIN TYL, TIL, TLD) and 100  $\mu\text{g kg}^{-1}$  (AMP, AMX).

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

Analyte	Spiking level $\mu\text{g kg}^{-1}$	Method 1			Method 2		
		$C_{\text{fresh}}^{\text{a}}$ $\mu\text{g kg}^{-1}$	$C_{\text{T1}}^{\text{b}}$ (%)	$C_{\text{T2}}^{\text{c}}$ (%)	$C_{\text{fresh}}^{\text{a}}$ $\mu\text{g kg}^{-1}$	$C_{\text{T1}}^{\text{b}}$ (%)	$C_{\text{T2}}^{\text{c}}$ (%)
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
Amoxicillin	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

<sup>a</sup>  $C_{\text{fresh}}$  - concentration of the freshly prepared extracts (N=5)<sup>b</sup>  $C_{T1}$  – remaining concentration of the analyte in aliquot stored 48 h at 4-6 °C, expressed in % RSD (N=5)<sup>c</sup>  $C_{T2}$  – 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 $\mu\text{g kg}^{-1}$	Method 1			Method 2		
		$C_{\text{fresh}}^{\text{a}}$ $\mu\text{g kg}^{-1}$	$C_{T1}^{\text{b}}$ (%)	$C_{T2}^{\text{c}}$ (%)	$C_{\text{fresh}}^{\text{a}}$ $\mu\text{g kg}^{-1}$	$C_{T1}^{\text{b}}$ (%)	$C_{T2}^{\text{***}}$ (%)
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

<sup>a</sup>  $C_{\text{fresh}}$  - concentration of the freshly prepared extracts (N=5)<sup>b</sup>  $C_{T1}$  – remaining concentration of the analyte in aliquot stored 48 h at 4-6 °C, expressed in % RSD (N=5)<sup>c</sup>  $C_{T2}$  – 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 ( $\mu\text{g kg}^{-1}$ )	Lincomycin ( $\mu\text{g kg}^{-1}$ )	Chlortetracycline ( $\mu\text{g kg}^{-1}$ )	Sulfamethazine ( $\mu\text{g kg}^{-1}$ )
Sample 21	Method 1	MF <sup>a</sup>		
	Method 2	MF <sup>a</sup>		
Sample 23	Method 1	MF <sup>a</sup>		54.2
	Method 2	MF <sup>a</sup>		69.3
Sample 40	Method 1			70.4
	Method 2			86.3
Sample 41	Method 1	MF <sup>a</sup>		121.7
	Method 2	MF <sup>a</sup>		131.1
Sample 42	Method 1		MF <sup>a</sup>	59.2
	Method 2		MF <sup>a</sup>	53.7
Sample 56	Method 1	>300 <sup>b</sup>		61.3
	Method 2	>300 <sup>b</sup>		58.6
Sample 63	Method 1	MF <sup>a</sup>		74.0
	Method 2	MF <sup>a</sup>		71.9
Sample 73	Method 1		158.9	119.5
	Method 2		156.7	130.8
Sample 83	Method 1			25.4
	Method 2			26.8
Sample 86	Method 1		MF <sup>a</sup>	36.9
	Method 2		MF <sup>a</sup>	30.0
Sample 90	Method 1			209.8
	Method 2			184.0
Sample 91	Method 1	297.5	MF <sup>a</sup>	77.6
	Method 2	285.2	MF <sup>a</sup>	79.6
Sample 92	Method 1	>300 <sup>b</sup>		41.7
	Method 2	>300 <sup>b</sup>		56.9
Sample 94	Method 1	MF <sup>a</sup>		277.1

	Method 2	MF <sup>a</sup>		301.8
Sample 95	Method 1	MF <sup>a</sup>		
	Method 2	MF <sup>a</sup>		
Sample 96	Method 1		MF <sup>a</sup>	>300 <sup>b</sup>
	Method 2		MF <sup>a</sup>	>300 <sup>b</sup>
	ML <sup>c</sup> (µg kg <sup>-1</sup> )	100	25	100
				25

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