Supplementary Material

TRANSFER OF A MULTICLASS METHOD FOR OVER 60 ANTIBIOTICS IN FOOD FROM HIGH RESOLUTION TO LOW RESOLUTION MASS SPECTROMETRY

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Table S1- Validation plan in bovine muscle

Analyte spiking level (µg kg^{-1})	Concentration of analyte solution (µg mL^{-1})	Added volume of analyte solution (µL)	IS spiking level (µg kg^{-1})	Concentration of IS solution (µg mL^{-1})	Added volume of IS solution (µL)	Number of fortified samples/day	Final volume (mL)	Dilution
10	1	15	10	1	15	4	1.5	1
33	1	50	10	1	15	4	1.5	1
100	1	150	10	1	15	4	1.5	1
150	1	225	10	1	15	4	1.5	1
333[a]	10	50	100	10	15	4	15	10
1000[a]	10	150	100	10	15	4	15	10
1500[a]	10	225	100	10	15	4	15	10

[a] Starting from 333 µg kg^{-1}, for practical reasons (added volumes), only the analytes with MRL > 100 µg kg^{-1} have been added and the final extract diluted 1:10 prior to the injection in the LC system

Table S2- Validation plan in milk

Analyte spiking level (µg kg^{-1})	Concentration of analyte solution (µg mL^{-1})	Added volume of analyte solution (µL)	IS spiking level (µg kg^{-1})	Concentration of IS solution (µg mL^{-1})	Added volume of IS solution (µL)	Number of fortified samples/day	Final volume (mL)	Dilution
2[a]	0.1	30	10	1	15	4	1.5	1
10	1	15	10	1	15	4	1.5	1
33	1	50	10	1	15	4	1.5	1
100	1	150	10	1	15	4	1.5	1
150	1	225	10	1	15	4	1.5	1
333[b]	10	50	100	10	15	4	15	10

[a] This concentration was done only for amoxicillin, ampicillin and penicillin G; [b] For practical reasons (added volumes), only the analytes with MRL > 100 µg kg^{-1} have been added and the final extract diluted 1:10 prior to the injection in the LC system

Table S3- Linearity studies in matrix (matrix-matched curves)

Antibiotic class	Analyte	Linearity range: muscle ($\mu g\ kg^{-1}$)	Linearity range: milk ($\mu g\ kg^{-1}$)
Amphenicols	Florfenicolamine	10-150	2-150
	Thiamphenicol	10-150	2-150
	Florfenicol	33-150	10-150
Beta lactams	Amoxicillin	2-150	2-150
	Ampicillin	2-150	2-150
	Cefacetrile	33-150	100-150
	Cefalexin	2-150	2-150
	Cefalonium	2-150	10-150
	Cephapirin	2-150	2-150
	Cefazolin	2-150	10-150
	Cefoperazone	2-150	2-150
	Cefquinome	2-150	10-150
	Ceftiofur	2-150	2-150
	Cloxacillin	2-150	2-150
	Desacetylcephapirin	2-150	2-150
	Dicloxacillin	2-150	10-150
	Nafcillin	2-150	2-150
	Oxacillin	2-150	10-150
	Penicillin G	2-150	10-150
	Penicillin V	2-150	2-150
Quinolones	Ciprofloxacin	10-150	10-150
	Danofloxacin	10-150	10-150
	Difloxacin	10-150	10-150
	Enrofloxacin	10-150	10-150
	Flumequine	10-150	10-150
	Marbofloxacin	10-150	10-150
	Nalidixic acid	Not evaluated	10-150
	Norfloxacin	Not evaluated	10-150
	Oxolinic acid	10-150	10-150

	Sarafloxacin	10-150	10-150
Macrolides	3-O-Acetyltylosin	2-150	2-150
	Erythromycin	10-150	2-150
	Gamithromycin	2-150	10-150
	Neospiramycin	2-150	2-150
	Spiramycin	10-150	2-150
	Tylosin A	10-150	2-150
	Tildipirosin	2-150	10-100
	Tilmicosin	10-150	10-150
	Tylvalosin	10-150	2-150
	Tulathromycin marker	2-150	10-100
	Tulathromycin	10-150	10-150
Sulfonamides	Sulfadiazine	2-150	2-150
	Sulfadimethoxine	2-150	2-150
	Sulfaguanidine	33-150	2-150
	Sulfamerazine	10-150	2-150
	Sulfamethazine	2-150	2-150
	Sulfamethoxazole	2-150	10-150
	Sulfamonomethoxine	2-150	2-150
	Sulfanilamide	33-100	2-150
	Sulfapyridine	2-150	2-150
	Sulfaquinoxaline	10-150	2-150
	Sulfathiazole	2-150	2-150
Diamino pymidines	Trimethoprim	2-150	10-150
Tetracyclines	4-epi-chlortetracycline	10-150	2-150
	4-epi-oxytetracycline	2-150	2-150
	4-epi-tetracycline	2-150	2-150
	Doxycycline	2-150	2-150
	Chlortetracycline	10-150	2-150
	Oxytetracycline	2-150	2-150
	Tetracycline	10-150	10-150
Lincosamides	Lincomycin	2-150	10-150

Rifampicins	Rifaximin	10-150	2-150
Pleuromutilins	Tiamulin	2-150	2-150
	Valnemulin	10-150	2-150

Table S4- Calibration data (matrix-matched curves) of six representative analytes

Concentration (µg kg^{-1})	Muscle			Milk		
Danofloxacin	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**
2	-	-	-	-	-	-
10	2207097	12	20	1732977	11	12
33	6301830	34	4.1	5532588	36	7
100	19501848	106	6.3	16212978	105	5
150	27271102	149	-0.9	23084541	149	-1
R^2	0.998			0.9988		
Slope	183505			154760		
intercept	302955			234367		
Doxycycline	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**
2	113808	1.7	-15	64792	1.8	-8.9
10	613831	9.1	-8.7	305648	8.6	-14
33	2064390	31	-6.9	1086503	31	-7.4
100	6827497	102	1.6	3431807	97	-3.5
150	9970481	148	-1.1	5361000	151	0.5
R^2	0.9995			0.9993		
Slope	67208			35564		
intercept	-39377			-40255		
Oxacillin	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**
2	134310	1.9	-2.7	-	-	-
10	689885	10	0	70005	10	4
33	2384304	35	4.7	247580	37	12
100	6900456	100	0	691866	103	3
150	10364762	150	0.1	1006262	150	0
R^2	0.9999			0.9991		
Slope	68998			6712		
intercept	19900			9825		

Spiramycin	Mean peak area[a]	Back-calculated concentration[b] (µg kg^{-1})	Deviation (%)[c]	Mean peak area[a]	Back-calculated concentration[b] (µg kg^{-1})	Deviation (%)[c]
2	-	-	-	52546	2.4	20
10	533578	10	2.4	211964	9.7	-3.3
33	1620556	31	-5.8	788059	36	9.0
100	5355690	103	2.8	2343962	107	7.0
150	7728987	148	-1.1	3222096	147	-2.0
R^2	0.9991			0.9970		
Slope	52105			21915		
intercept	-5605			25611		
Sulfamethazine	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**
2	201038	2.0	1.5	74659	1.9	-6.0
10	1132098	11	14	421374	11	6.1
33	3525227	36	7.9	1493884	38	14
100	10728282	108	8.3	4114759	104	3.6
150	14534896	147	-2.1	5921403	149	-0.6
R^2	0.9961			0.9987		
Slope	99023			39714		
intercept	151609			51741		
Tiamulin	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**	**Mean peak area[a]**	**Back-calculated concentration[b] (µg kg^{-1})**	**Deviation (%)[c]**
2	1266776	1.7	-15	843106	2.1	7.0
10	8166183	11	9.5	3941489	10	0.1
33	24718486	33	0.5	13038241	33	0.3
100	78910016	106	5.8	39452787	100	0.2
150	109836949	147	-1.8	59075828	150	0
R^2	0.9979			1.0000		
Slope	745604			393844		
intercept	490855			27922		

[a] Each matrix-matched point was injected three times; [b]Measured (calculated) concentration using the calibration curve ($C_{measured}$); [c]Deviation (%) of the back-calculated concentration from the true one: $C_{measured}$-C_{true}) x 100/C_{true}. In the relevant region the deviation should not be more than ±20% [32]

Table S5 – MRL (µg kg^{-1}), decision limits (µg kg^{-1}) and detection capabilities (µg kg^{-1}) of the 64 tested antibiotics in the main food-producing species

	Analyte	Bovine			Porcine			poultry			Ovi-caprine		
		MRL	CCα	CCβ	MRL	CCα	CCβ	MRL	CCα	CCβ	MRL	CCα	CCβ
Amphenicols	Florfenicolamine	200	220	242	300	330	363	100	110	121	200	220	242
	Florfenicol	200	252	319	300	379	478	100	126	159	200	252	319
	Thiamphenicol	50	59	70	50	59	70	50	59	70	50	59	70
Beta lactams	Amoxicillin	50	55	61	50	55	61	50	55	61	50	55	61
	Ampicillin	50	57	64	50	57	64	50	57	64	50	57	64
	Cefacetrile	-	33	33	-	33	33	-	33	33	-	33	33
	Cefalexin	200	228	259	-	10	10	-	10	10	-	10	10
	Cefalonium	-	10	10	-	10	10	-	10	10	-	10	10
	Cephapirin	50	56	62	-	10	10	-	10	10	-	10	10
	Cefazolin	-	10	10	-	10	10	-	10	10	-	10	10
	Cefoperazone	-	10	10	-	10	10	-	10	10	-	10	10
	Cefquinome	50	56	63	50	56	63	-	10	10	-	10	10
	Ceftiofur	1000	1159	1343	1000	1159	1343	-	10	10	1000	1159	1343
	Cloxacillin	300	354	418	300	354	418	300	354	418	300	354	418
	Desacetylcephapirin	50	56	63	-	10	10	-	10	10	-	10	10
	Dicloxacillin	300	349	406	300	349	406	300	349	406	300	349	406
	Nafcillin	300	340	386	-	10	10	-	10	10	300	340	386
	Oxacillin	300	354	418	300	354	418	300	354	418	300	354	418
	Penicillin G	50	57	66	50	57	66	50	57	66	50	57	66
	Penicillin V	-	10	10	25	30	35	25	30	35	-	10	10
Quinolones	Oxolinic Acid	100	111	123	100	111	123	100	111	123	100	111	123
	Ciprofloxacin	100	114	129	100	114	129	100	114	129	100	114	129
	Danofloxacin	200	224	250	100	112	125	200	224	250	200	224	250
	Difloxacin	400	449	503	400	449	503	300	336	377	400	449	503
	Enrofloxacin	100	111	123	100	111	123	100	111	123	100	111	123
	Flumequine	200	224	251	200	224	251	400	448	502	200	224	251

Quinolones	Marbofloxacin	150	167	186	150	167	186	-	10	10	-	10	10
	Sarafloxacin	-	10	10	-	10	10	-	10	10	-	10	10
	Nalidixic Acid	-	10	10	-	10	10	-	10	10	-	10	10
	Norfloxacin	-	10	10	-	10	10	-	10	10	-	10	10
Macrolides	3-O-Acetyltylosin	-	10	10	50	63	80	-	10	10	-	10	10
	Erytromycin A	200	228	260	200	228	260	200	228	260	200	228	260
	Gamithromycin	-	10	10	100	112	125	-	10	10	50	56	62
	Neospiramycin	200	252	319	250	316	398	200	252	319	-	10	10
	Spiramycin	200	256	327	250	320	409	200	256	327	-	10	10
	Tildipirosin	400	466	542	1200	1397	1626	-	10	10	400	466	542
	Tilmicosin	50	58	68	50	58	68	75	87	102	50	58	68
	Tylosin A	100	125	155	100	125	155	100	125	155	100	125	155
	Tylvalosin	-	10	10	50	66	86	-	10	10	-	10	10
	Tulathromycin	300	379	478	800	1010	1275	-	10	10	450	568	717
	Tulathromycin Marker	300	342	391	800	913	1042	-	10	10	450	513	586
Sulfonamides	Sulfaquinoxaline	100	113	127	100	113	127	100	113	127	100	113	127
	Sulfadiazine	100	113	129	100	113	129	100	113	129	100	113	129
	Sulfadimethoxine	100	113	128	100	113	128	100	113	128	100	113	128
	Sulfaguanidine	100	116	135	100	116	135	100	116	135	100	116	135
	Sulfamerazine	100	110	120	100	110	120	100	110	120	100	110	120
	Sulfamethazine	100	112	125	100	112	125	100	112	125	100	112	125
	Sulfamethoxazole	100	116	134	100	116	134	100	116	134	100	116	134
	Sulfamonomethoxine	100	112	126	100	112	126	100	112	126	100	112	126
	Sulfanilamide	100	115	131	100	115	131	100	115	131	100	115	131
	Sulfapyridine	100	112	125	100	112	125	100	112	125	100	112	125
	Sulfathiazole	100	113	128	100	113	128	100	113	128	100	113	128
Diamino pymidines	Trimethoprim	50	56	64	50	56	64	50	56	64	50	56	64
Tetracyclines	Chlortetracycline	100	113	127	100	113	127	100	113	127	100	113	127
	Doxycycline	100	116	134	100	116	134	100	116	134	100	116	134
	Epi-chlortetracycline	100	121	147	100	121	147	100	121	147	100	121	147
	Epi-oxytetracycline	100	121	147	100	121	147	100	121	147	100	121	147

	Epi-tetracycline	100	115	132	100	115	132	100	115	132	100	115	132
	Oxytetracycline	100	113	128	100	113	128	100	113	128	100	113	128
	Tetracycline	100	115	133	100	115	133	100	115	133	100	115	133
Lincosamides	Lincomycin	100	112	125	100	112	125	100	112	125	100	112	125
Rifampicins	Rifaximin	-	10	10	-	10	10	-	10	10	-	10	10
Pleuromutilins	Tiamulin	-	10	10	100	121	147	100	121	147	-	10	10
	Valnemulin	-	10	10	50	Not applicable		-	10	10	-	10	10

Table S6 –MRL (µg kg^{-1}), decision limits (µg kg^{-1}) and detection capabilities (µg kg^{-1}) of the 64 antibiotics in aquaculture, rabbits, horses and milk

Class	Analyte	Aquaculture			Rabbit			*Equidae*			Milk		
		MRL	CCα	CCβ	MRL	CCα	CCβ	MRL	CCα	CCβ	MRL	CCα	CCβ
Amphenicols	Florfenicolamine	1000	1100	1210	100	110	121	100	110	121	-	10	10
	Florfenicol	1000	1262	1594	100	126	159	100	126	159	-	10	10
	Thiamphenicol	50	59	70	50	59	70	50	59	70	50	61	74
Beta-lactams	Amoxicillin	50	55	61	50	55	61	50	55	61	4	4.4	4.8
	Ampicillin	50	57	64	50	57	64	50	57	64	4	4.7	5.4
	Cefacetrile	-	33	33	-	33	33	-	33	33	125	Not applicable	
	Cefalexin	200	228	259	-	10	10	-	10	10	100	111	123
	Cefalonium	-	10	10	-	10	10	-	10	10	20	23	26
	Cephapirin	-	10	10	-	10	10	-	10	10	60	72	86
	Cefazolin	-	10	10	-	10	10	-	10	10	50	61	76
	Cefoperazone	-	10	10	-	10	10	-	10	10	50	65	84
	Cefquinome	-	10	10	-	10	10	50	56	63	20	26	34
	Ceftiofur	-	10	10	1000	1159	1343	1000	1159	1343	100	112	125
	Cloxacillin	300	354	418	300	354	418	300	354	418	30	36	43
	Desacetylcephapirin	-	10	10	-	10	10	-	10	10	60	66	72
	Dicloxacillin	300	349	406	300	349	406	300	349	406	30	36	44
	Nafcillin	-	10	10	-	10	10	-	10	10	30	33	36
	Oxacillin	300	354	418	300	354	418	300	354	418	30	34	39
	Penicillin G	50	57	66	50	57	66	50	57	66	4	Not applicable	
	Penicillin V	-	10	10	-	10	10	-	10	10	-	10	10
Quinolones	Oxolinic Acid	100	111	123	100	111	123	100	111	123	-	10	10
	Ciprofloxacin	100	114	129	100	114	129	100	114	129	100	112	126
	Danofloxacin	100	112	125	100	112	125	100	112	125	30	32	35
	Difloxacin	300	336	377	300	336	377	300	336	377	-	10	10
	Enrofloxacin	100	111	123	100	111	123	100	111	123	100	110	120
	Flumequine	600	672	752	200	224	251	200	224	251	50	54	58

	Marbofloxacin	-	10	10	-	10	10	-	10	10	75	81	88
	Sarafloxacin	30	34	38	-	10	10	-	10	10	-	10	10
	Nalidixic Acid	-	10	10	-	10	10	-	10	10	-	10	10
	Norfloxacin	-	10	10	-	10	10	-	10	10	-	10	10
Macrolides	3-O-Acetyltylosin	-	10	10	-	10	10	-	10	10	-	10	10
	Erytromycin A	200	228	260	200	228	260	200	228	260	40	48	57
	Gamithromycin	-	10	10	-	10	10	-	10	10	-	10	10
	Neospiramycin	-	10	10	-	10	10	-	10	10	200	239	286
	Spiramycin	-	10	10	-	10	10	-	10	10	200	225	253
	Tildipirosin	-	10	10	-	10	10	-	10	10	-	10	10
	Tilmicosin	50	58	68	50	58	68	50	58	68	50	54	57
	Tylosin A	100	125	155	100	125	155	100	125	155	50	54	58
	Tylvalosin	-	10	10	-	10	10	-	10	10	-	10	10
	Tulathromycin	-	10	10	-	10	10	-	10	10	-	10	10
	Tulathromycin Marker	-	10	10	-	10	10	-	10	10	-	10	10
Sulfonamides	Sulfaquinoxaline	100	113	127	100	113	127	100	113	127	100	108	116
	Sulfadiazine	100	113	129	100	113	129	100	113	129	100	114	130
	Sulfadimethoxine	100	113	128	100	113	128	100	113	128	100	106	113
	Sulfaguanidine	100	116	135	100	116	135	100	116	135	100	123	151
	Sulfamerazine	100	110	120	100	110	120	100	110	120	100	114	131
	Sulfamethazine	100	112	125	100	112	125	100	112	125	100	123	151
	Sulfamethoxazole	100	116	134	100	116	134	100	116	134	100	111	123
	Sulfamonomethoxine	100	112	126	100	112	126	100	112	126	100	116	135
	Sulfanilamide	100	115	131	100	115	131	100	115	131	100	120	143
	Sulfapyridine	100	112	125	100	112	125	100	112	125	100	116	134
	Sulfathiazole	100	113	128	100	113	128	100	113	128	100	114	129
Diamino pymidines	Trimethoprim	50	56	64	50	56	64	100	113	127	50	54	58
Tetracyclines	Chlortetracycline	100	113	127	100	113	127	100	113	127	100	112	125
	Doxycycline	100	116	134	100	116	134	100	116	134	-	10	10
	Epichlortetracycline	100	121	147	100	121	147	100	121	147	100	118	139
	Epioxytetracycline	100	121	147	100	121	147	100	121	147	100	125	155

	Epitetracycline	100	115	132	100	115	132	100	115	132	100	112	126
	Oxytetracycline	100	113	128	100	113	128	100	113	128	100	109	118
	Tetracycline	100	115	133	100	115	133	100	115	133	100	108	117
Lincosamides	Lincomycin	100	112	125	100	112	125	100	112	125	150	161	172
Rifampicins	Rifaximin	-	10	10	-	10	10	-	10	10	60	69	80
Pleuromutilins	Tiamulin	-	10	10	100	121	147	-	10	10	-	10	10
	Valnemulin	-	10	10	50	Not applicable		-	10	10	-	10	10

Table S7 – Estimated LODs and LOQs based on the observed accuracy at the first and at the second validation level

	Muscle		Milk	
Analyte[a]	**LOD ($\mu g\ kg^{-1}$)**	**LOQ ($\mu g\ kg^{-1}$)**	**LOD ($\mu g\ kg^{-1}$)**	**LOQ ($\mu g\ kg^{-1}$)**
All analytes except the antibiotics listed below	10	10	10	10
Tulathromycin	10	10	10	10
Valnemulin	10	-	10	10
Cefacetrile	33	33	33	-
Tulathromycin marker	10	10	10	-
Amoxicillin	10	10	2	2
Ampicillin	10	10	2	2
Penicillin G	10	10	2	10

[a]For valnemulin (muscle) and cefacetrile and tulathromycin marker (milk) the method can be used only for screening purposes (inadequate accuracy);

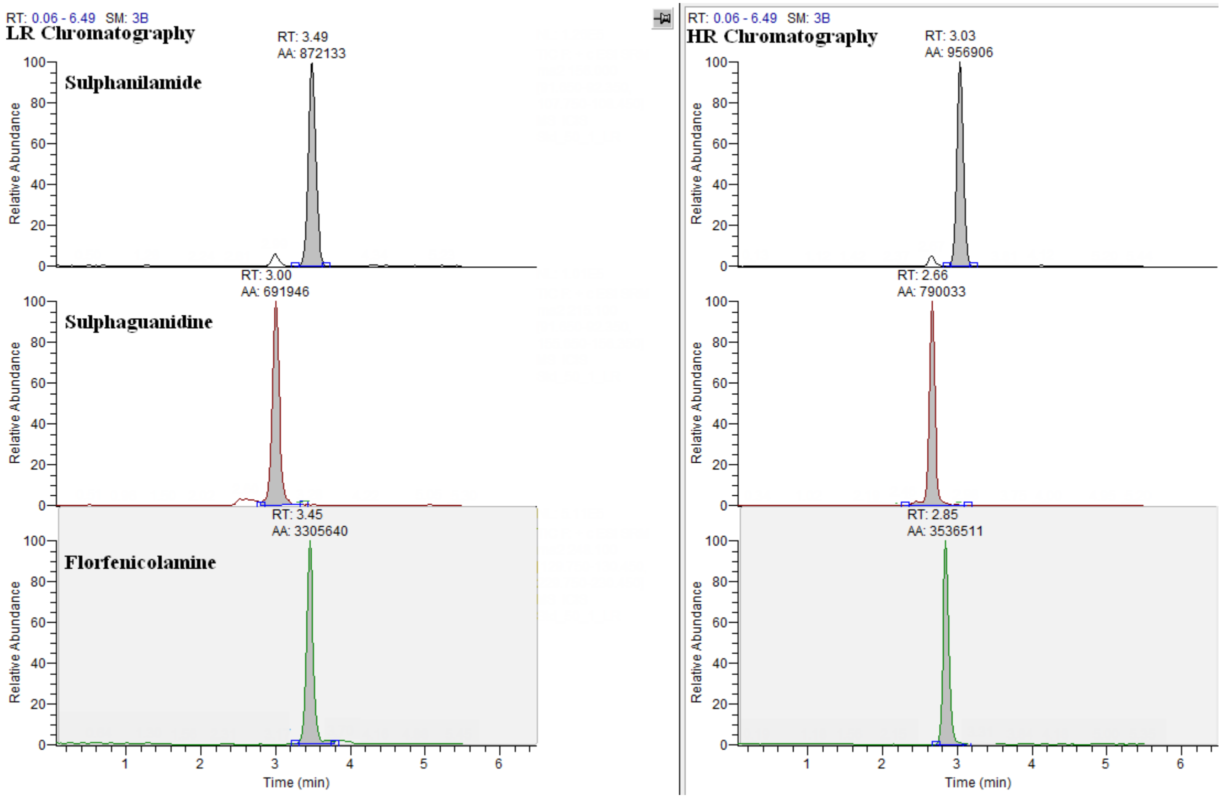

Figure S1- LC-QqQ chromatograms of the three more polar analytes (sulfanilamide, sulfaguanidine and florfenicolamine) with different % MeOH at the beginning of the gradient: 2% (left) and 5% (right)

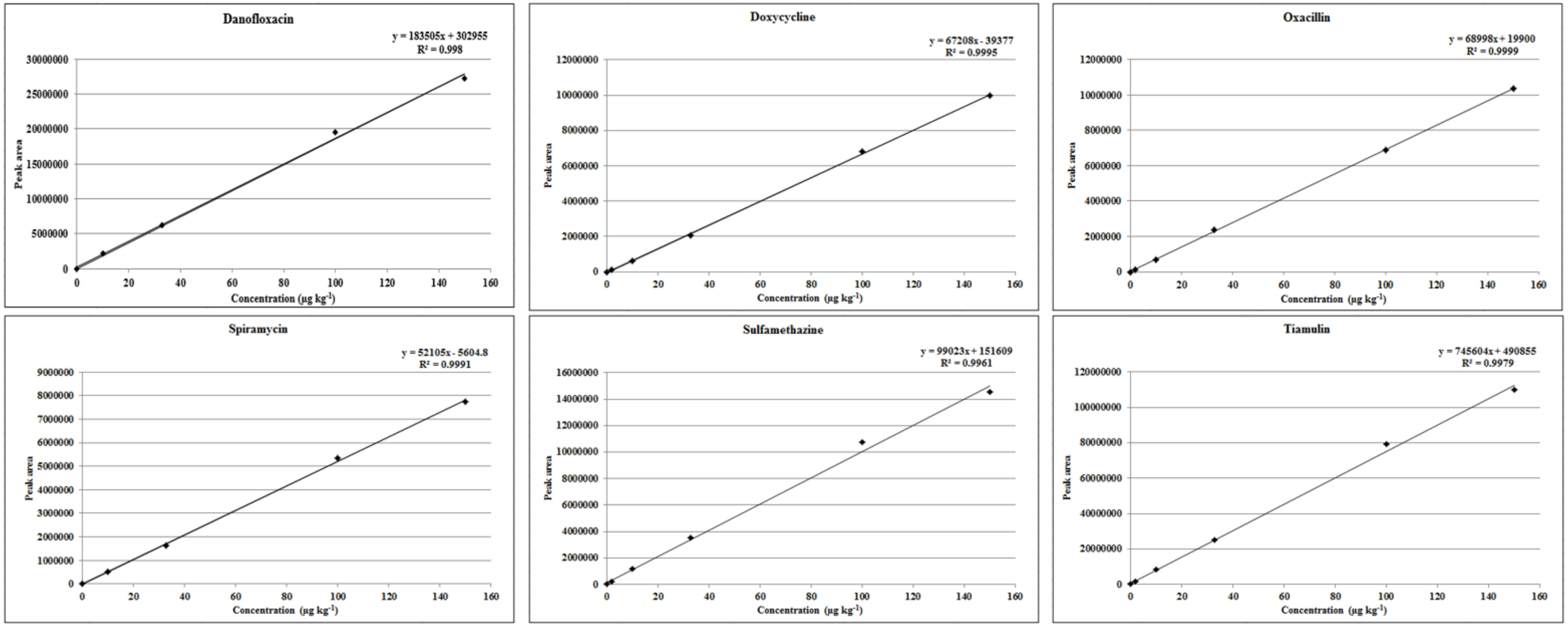

Figure S2 – Matrix-matched calibration curves of danofloxacin, doxycycline, oxacillin, spiramycin, sulfamethazine and tiamulin in meat

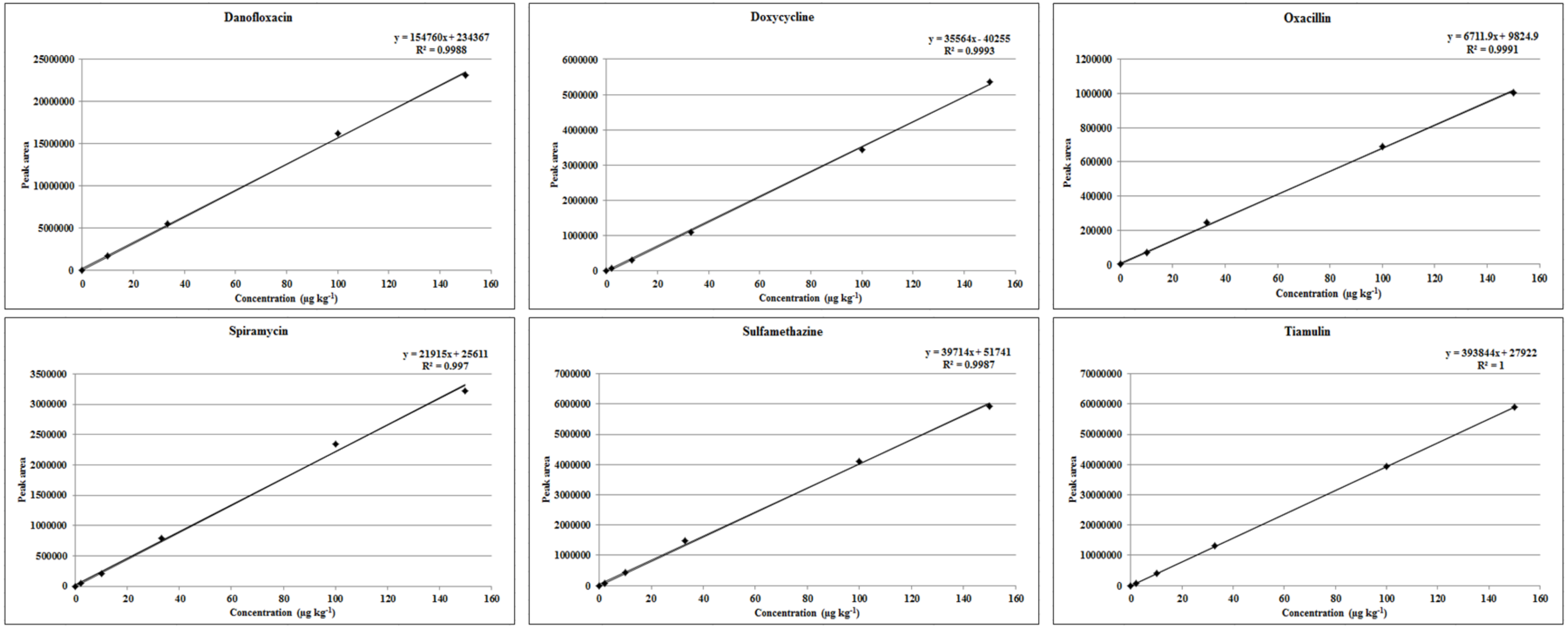

Figure S3 – Matrix-matched calibration curves of of danofloxacin, doxycycline, oxacillin, spiramycin, sulfamethazine and tiamulin in milk

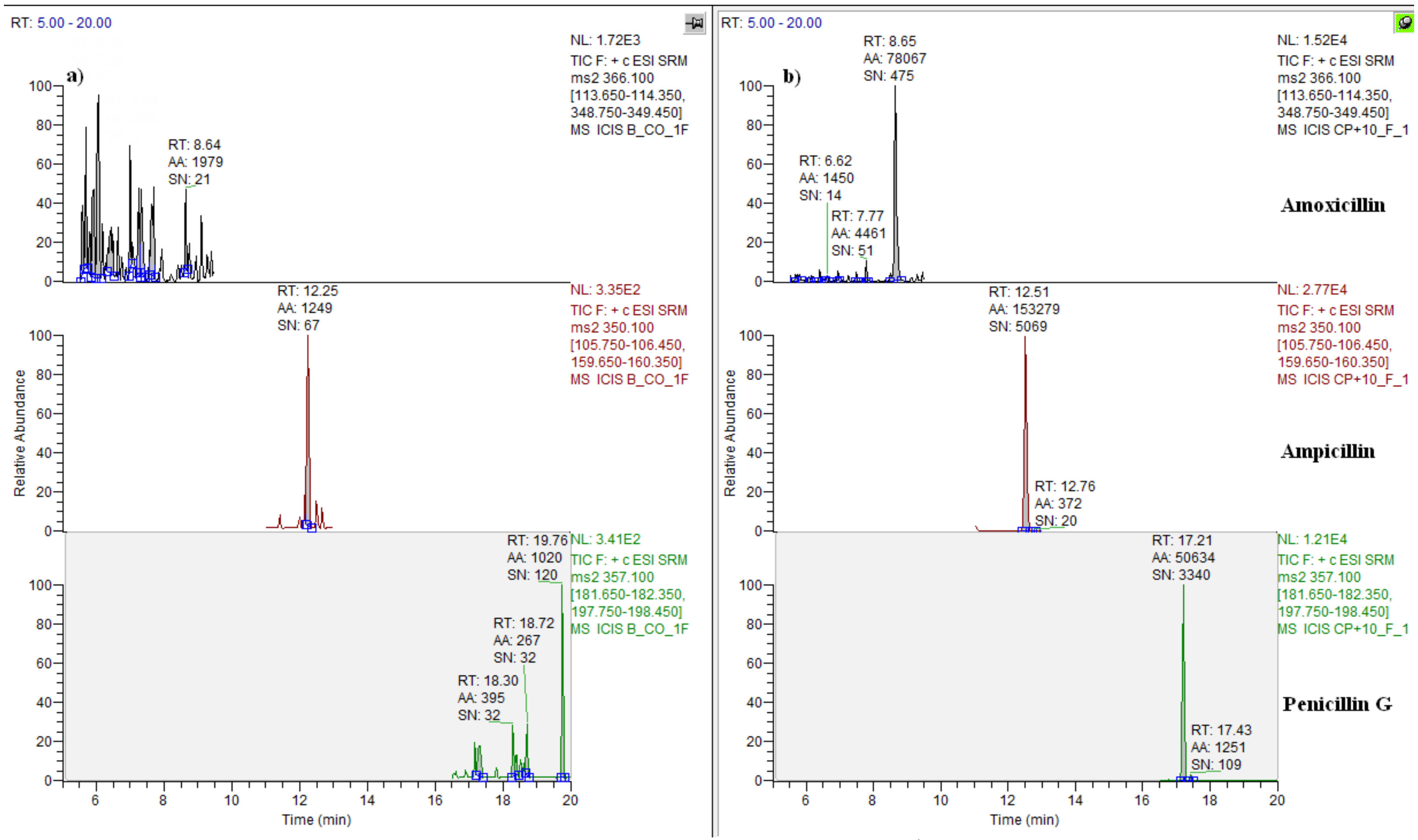

Figure S4- LC-QqQ chromatograms of a blank (a) and a milk sample spiked at 2 μg kg^{-1} (b) of the three penicillins with MRL at 4 μg kg^{-1}

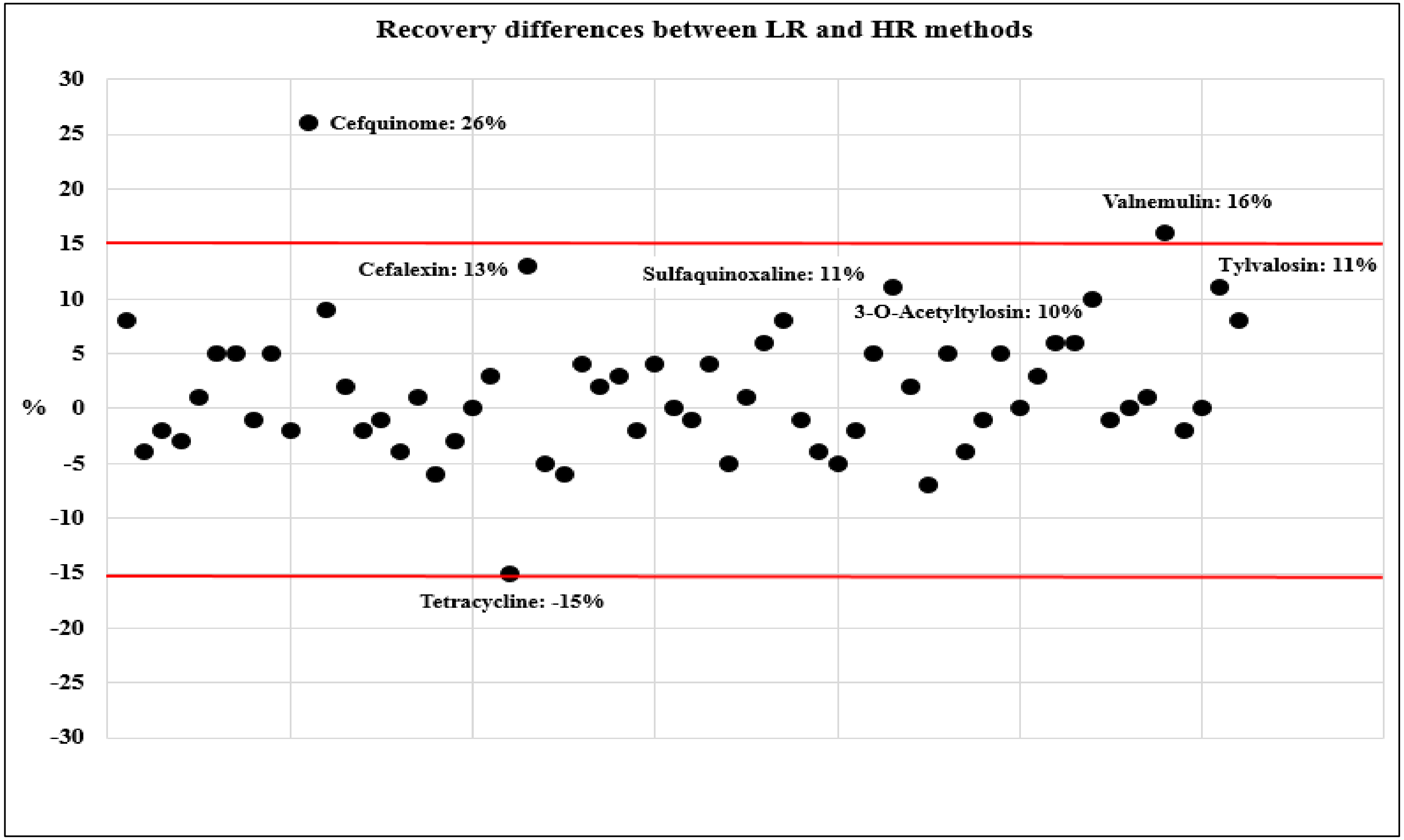

Figure S5 – Recovery differences between the LC-QqQ and LC-Q-Orbitrap methods in meat. Positive values (%) indicate better recovery of LC-QqQ procedure and vice versa

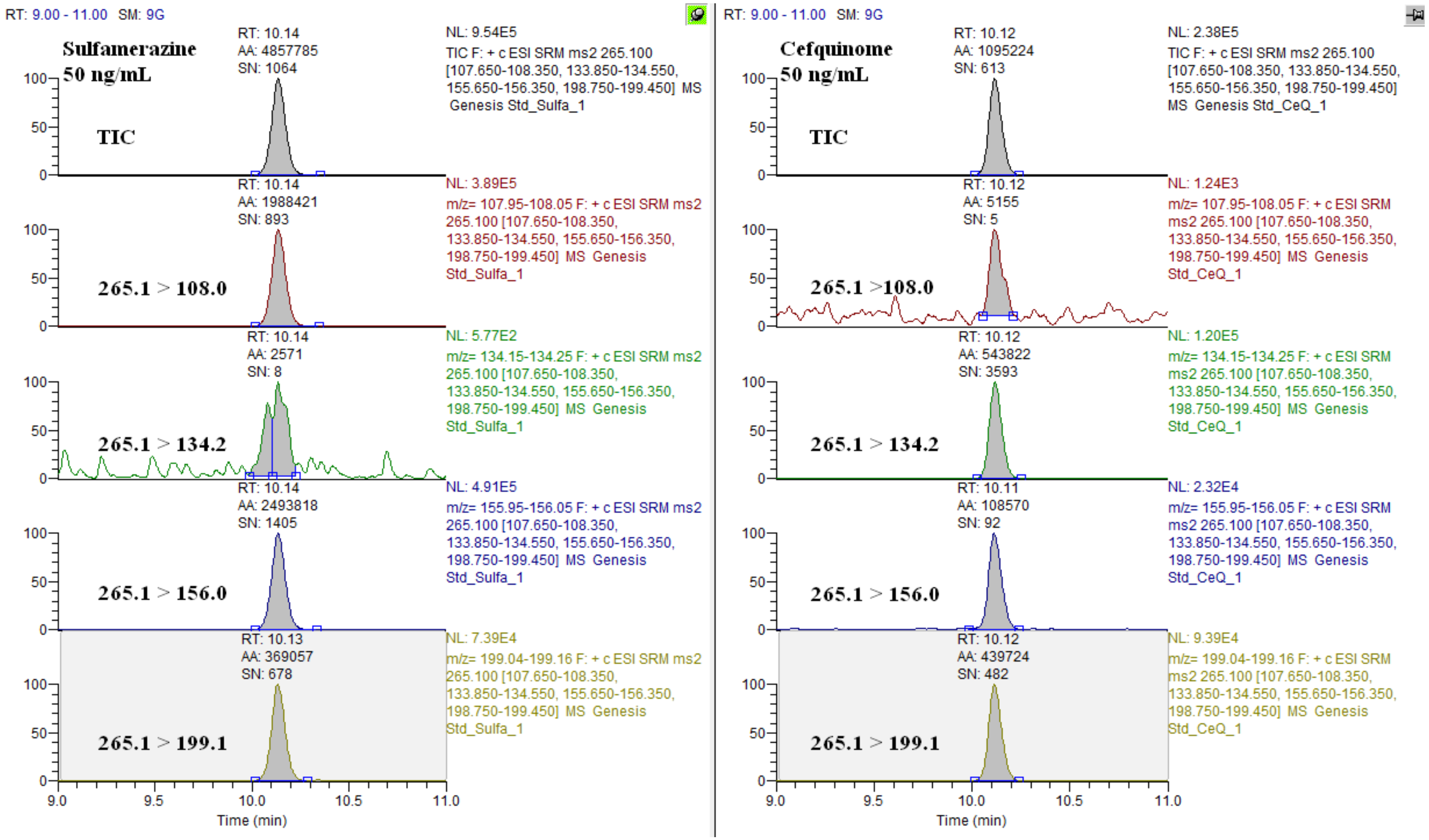

Figure S6 – LC-QqQ chromatograms of individual solutions (50 ng mL^{-1}) of sulfamerazine (left) and cefquinome (right)

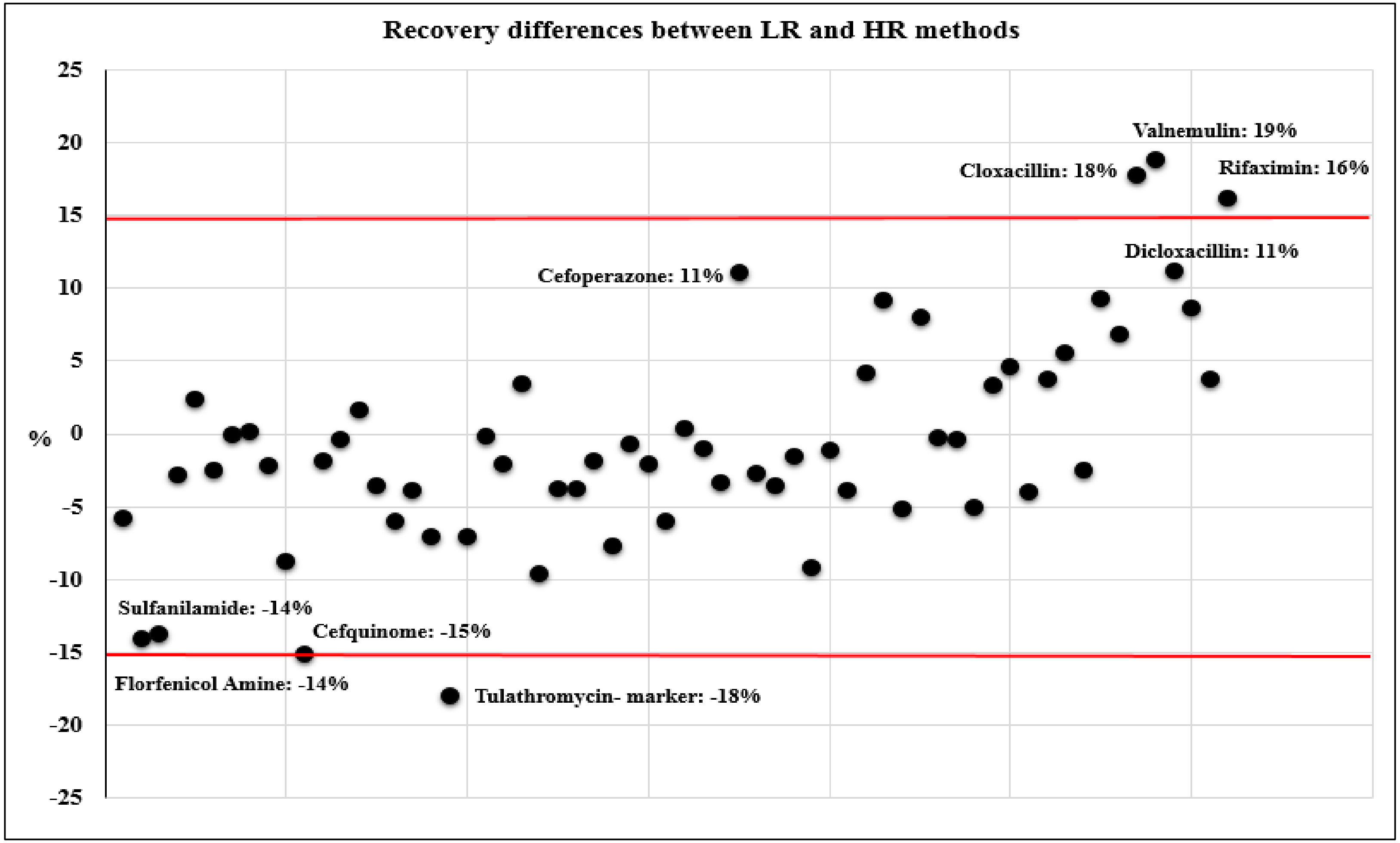

Figure S7 – Recovery differences between the LC-QqQ and LC-Q-Orbitrap methods in bovine milk. Positive values (%) indicate better recovery of LC-QqQ procedure and vice versa

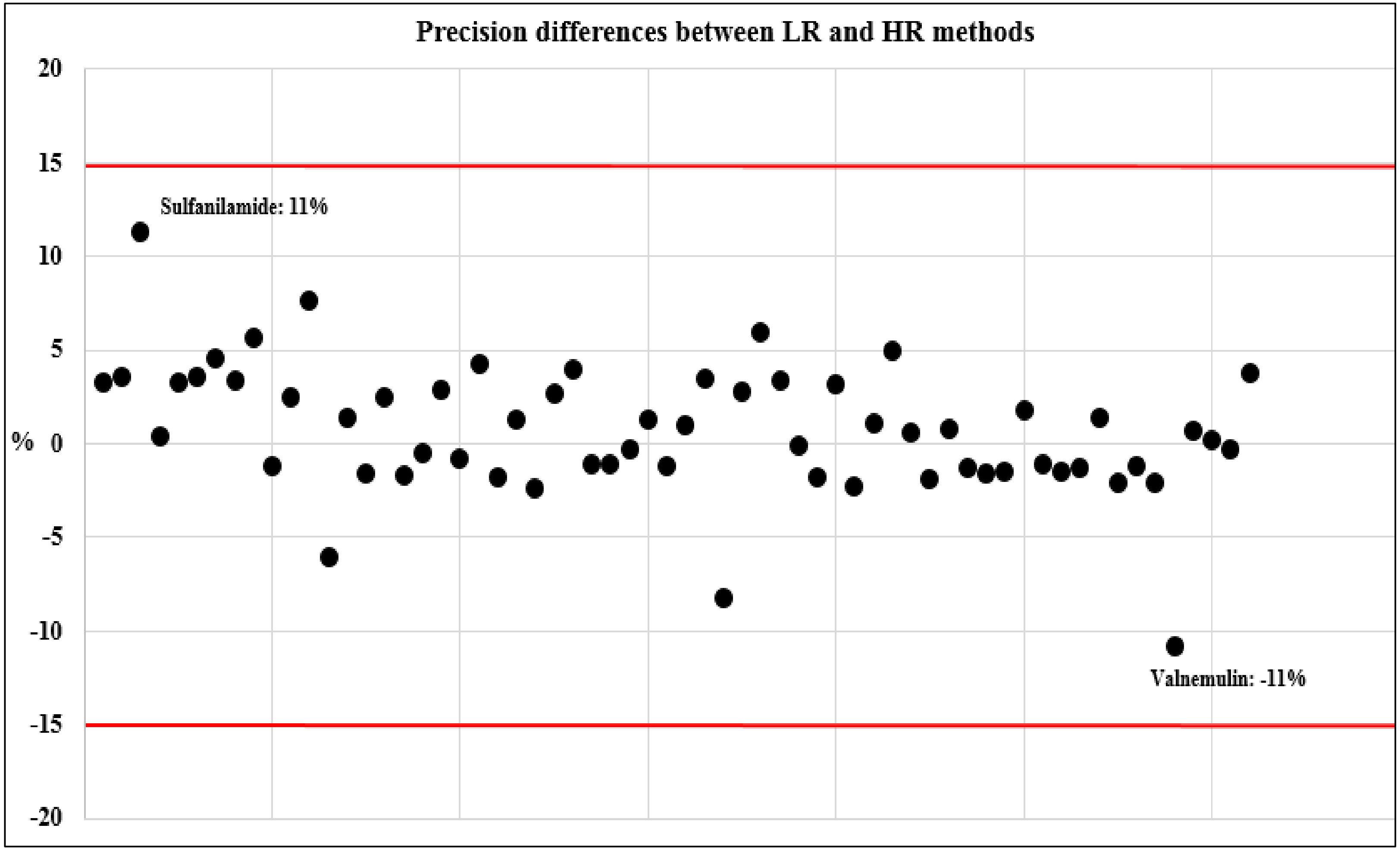

Figure S8 – Precision differences between the LC-QqQ and LC-Q-Orbitrap methods in meat. Positive values (%) indicate better precision of LC-QqQ procedure and vice versa

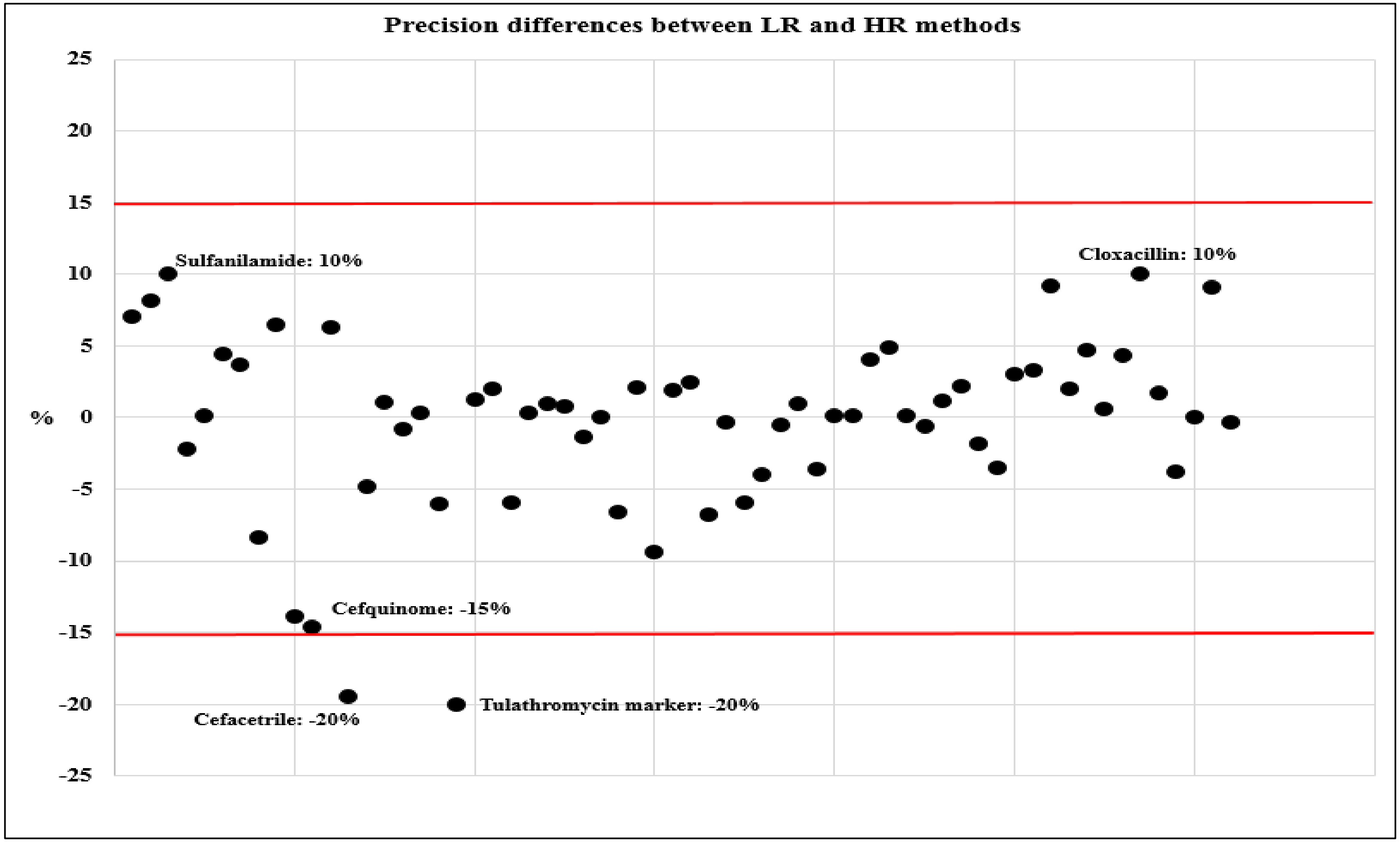

Figure S9 – Precision differences between the LC-QqQ and LC-Q-Orbitrap methods in bovine milk. Positive values (%) indicate better precision of LC-QqQ procedure and vice versa