

Supplemental Table 1A. Ultra-Performance Liquid Chromatography (UPLC) gradient method used for the ion-pairing analysis.

Time (mins)	%A (0.5%HFBA in H₂O)	%B (0.75% HFBA in MeOH)
0.00	35	65
0.25	35	65
1.00	5	95
2.00	5	95
2.76	35	65
4.25	35	65

Supplemental Table 1B. Multiple Reaction Monitoring (MRM) transitions and specific mass spectrometry tuning parameters for the quantification of the analytes of interest.

Analyte	Parent Ion (amu)	Product Ion (amu)	Cone Energy (V)	Collision Energy (eV)	Quant/Qual Transition
Gentamicin C1	478.4 [M+H] ⁺	157.0	34	24	Quantifier
	478.4 [M+H] ⁺	322.1	34	15	Qualifier
Gentamicin C1a	450.3 [M+H] ⁺	160.0	32	24	Quantifier
	450.3 [M+H] ⁺	322.0	32	14	Qualifier
Gentamicin C2+C2a	464.4 [M+H] ⁺	160.0	28	25	Quantifier
	464.4 [M+H] ⁺	322.1	28	14	Qualifier
Gentamicin C1-dx (IS)	481.4 [M+H] ⁺	324.2	34	15	Quantifier
	480.4 [M+H] ⁺	160.2	34	25	Qualifier
Penicillin G	335.2 [M+H] ⁺	128.0	46	25	Quantifier
	335.2 [M+H] ⁺	289.2	52	24	Qualifier
Penicillin G-d7 (IS)	342.3 [M+H] ⁺	183.2	52	24	Quantifier
	342.3 [M+H] ⁺	128.0	52	25	Qualifier
Flunixin	297.2 [M+H] ⁺	279.1	46	22	Quantifier
	297.2 [M+H] ⁺	264.1	46	33	Qualifier
Flunixin-d3 (IS)	300.2 [M+H] ⁺	282.1	42	22	Quantifier
	300.2 [M+H] ⁺	112.0	42	48	Qualifier
5-hydroxyflunixin	313.1 [M+H] ⁺	295.1	46	22	Quantifier
	313.1 [M+H] ⁺	280.1	46	34	Qualifier
5-hydroxyflunixin-d3 (IS)	316.1 [M+H] ⁺	298.1	50	20	Quantifier
	316.1 [M+H] ⁺	280.1	50	33	Qualifier

Supplemental Table 1C. Mass spectrometer tuning parameters for the detection of the analytes of interest.

Parameter	Value
Capillary (kV)	0.90
Cone (V)	32
RF (V)	2.50
Extractor (V)	3.00
Source Temperature (°C)	150
Desolvation Temperature (°C)	500
Cone Gas Flow (L/Hr)	10
Desolvation Gas Flow (L/Hr)	500