Forensic Screening of Animal Metabolites - Determination of Sulfonamide Antibiotics in Bovine Liver by LC-MS/MS

Instrumentation: Agilent 1200 Series HPLC with diode array detector (DAD) **with** AB Sciex LC/MS/MS System
Column: Agilent ZORBAX Solvent Saver HT Eclipse Plus C18, 50 x 3.0 mm, 1.8-µm particle size
Elution Type: Gradient
Needle wash: 1:1:1:1 ACN/MeOH/IPA/H2O w/0.2% FA.
Mobile Phase A: 5mM Ammonium acetate, pH 3.0 in water
Mobile Phase B: Methanol / acetonitrile (1:1 v/v)
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	85	15
2	0.2	85	15
3	6.0	40	60
4	6.01	0	100
4	7.0	STOP	

Post run: 3.5 min
Total cycle time: 11 min
Flow Rate: 0.3 mL/min
Col. Temp: 30 °C
Inj. Vol.: 10 µL
Detection: Tandem Mass Spec (MS-MS) **@ amu (550 °C)** (Ctrl + Click to follow link)
Detector Info: AB Sciex API 4000 (Ctrl + Click to follow link)
MS Conditions
Source: Positive ESI
Nebulizer: 50 psi
Gas flow: 8 L/min
Gas temp: 325 °C
Vcap: 4000 V
MRM: Instrument Acquisition Data Used for the Analysis of Sulfonamide Antibiotics:

Analyte	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)
Sulfadizine	1) 251.1 → 108.0	100	25	2.1
	2) 251.1 → 156.0		13	
Sulfathiazole	1) 256.0 → 156.0	94	13	2.3
	2) 256.0 → 92.1		29	
Sulfamerazine	1) 265.1 → 92.1	125	29	2.9
	2) 265.1 → 108.1		25	
Sulfamethizole	1) 271.0 → 156.0	112	9	3.7
	2) 271.0 → 92.1		29	
Sulfamethazine	1) 279.1 → 124.0	116	21	3.8
	2) 279.1 → 92.1		33	
Sulfamethoxypyridazine	1) 281.1 → 156.0	128	13	3.9
	2) 281.1 → 92.1		29	
Sulfachloropyridazine	1) 285.0 → 156.0	106	9	4.5
	2) 285.0 → 92.1		29	
Sulfamethoxazole	1) 254.1 → 92.1	113	25	4.8
	2) 254.1 → 108.0		21	
Sulfadimethoxin	1) 311.1 → 156.0	141	17	6.0
	2) 311.1 → 92.1		37	
Sulfapyridine (IS)	250.1 → 92.1	113	29	2.7

1) Quantifier transition channel 2) Qualifier transition channel

Sample Preparation:

The procedure involves a rapid and efficient pretreatment with SampliQ QuEChERS kits. The homogenized liver sample was initially extracted in a buffered aqueous/1% acetic acid acetonitrile system with an extraction and partitioning step after the addition of salts. Finally, the sample was cleaned up using dispersive solid-phase extraction (dispersive-SPE). The final extracts were analyzed by the sensitive and selective determination of all compounds in a single run using LC-ESI-MS-MS operating in positive ion multiple reaction monitoring (MRM) mode. Sulfapyridine was selected as the internal standard.

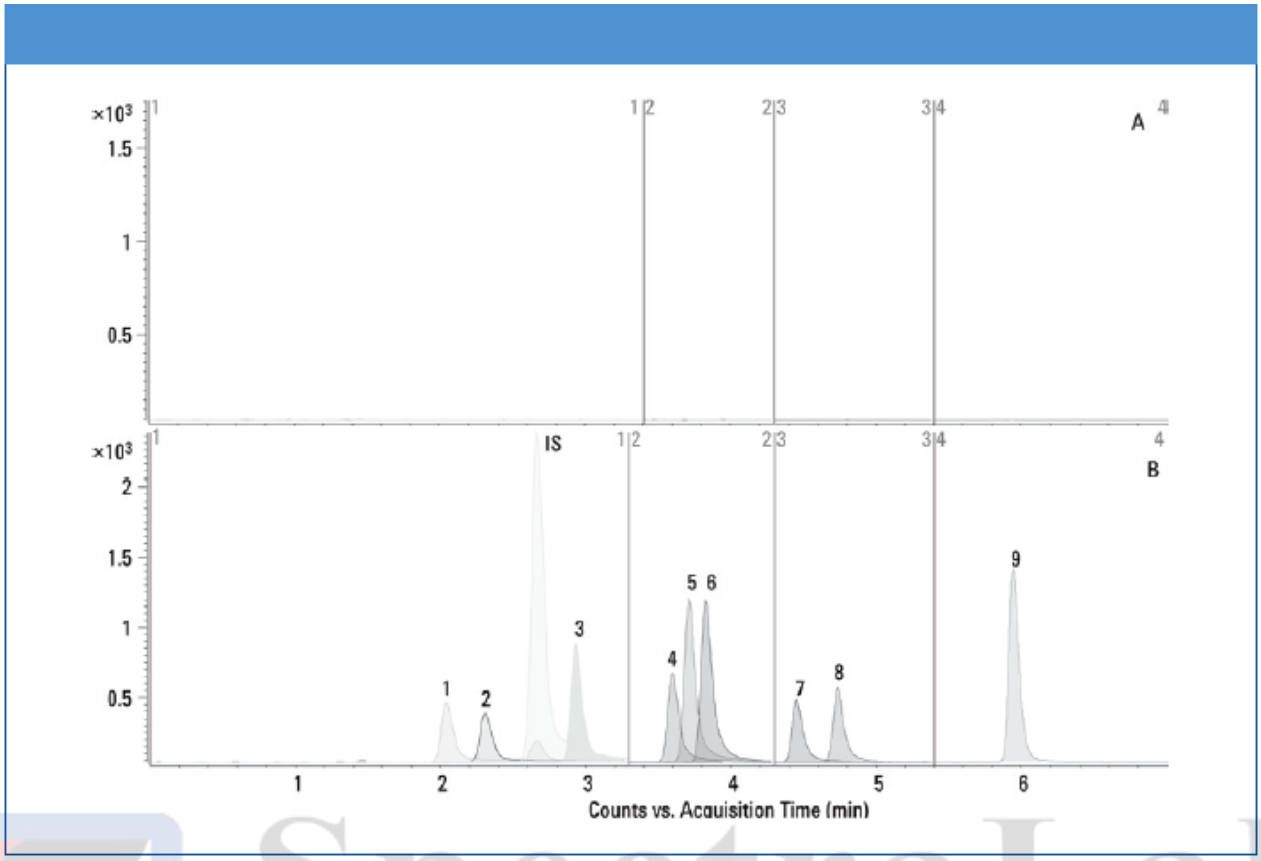

Figure 1: LC–MS-MS Chromatograms of A) liver blank extract, and B) 100 ng/g fortified liver extract. Peaks identification: 1. sulfadizine, 2. sulfathiazole, 3. sulfamerazine, 4. sulfamethizole, 5. sulfamethazine, 6. sulfamethoxypyridazine, 7. sulfachloropyridazine, 8. sulfamethoxazole, 9. sulfadimethoxin, IS (internal standard), sulfapyridine.

Results:

The accuracy of the method, expressed as recovery, is between 53 and 93%, with an acceptable recovery average of 77.8%. The precision, expressed as RSD, is between 2.1 and 16.8%. The established 5 ng/g limits of quantification (LOQ) is much lower than the respective Maximum Residue Limit (MRL) for sulfonamide in animal food products (20-100 ng/g).

References:

https://www.chem.agilent.com/Library/applications/5990-5086EN.pdf
http://www.chromatographyonline.com/lcgc/Application+Notes/Determination-of-Sulfonamide-Antibiotics-in-Bovine/ArticleStandard/Article/detail/655617