



Determination of Pharmaceutical Residues in Bovine Milk via LC-MS/MS Following Solid Phase Extraction

Application Note FB0113

Keywords

SPE (Solid Phase Extraction), Bovine Milk, JECFA (Joint FAO/WHO Expert Committee on Food Additives), Food, Beverage, Pharmaceutical Residues, LC-MS/MS (Liquid Chromatography Tandem Mass Spectroscopy)

Introduction

Quantifying the levels of pharmaceutical residues in foods and beverages is a standard process to evaluate the results against the ADI (Acceptable Daily Intake) as determined by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). The JECFA determines if an ADI and MRLs (Maximum Residue Limits) can be recommended for veterinary drugs, determining daily safe consumption limits.¹ This application note focuses on comparison LC-MS/MS pharmaceutical residue results detected in bovine milk using four different SPE cartridge manufacturers to identify the cartridge producing consistent recoveries at the lowest detection level, as well as consistent recoveries at 10x the lowest detection level with associated ion suppression values.

Materials & Methods

Materials

- Solid Phase Extraction Cartridges:
 - ASPEC™ HLB 3 mL/60 mg
 - Gilson Part Number: 54350562

Sample Preparation

1. Combine 600 μ L of bovine milk with 250 μ L of 20% TCA in H₂O (w/v)
2. Vortex for 2 minutes
3. Add 2.5 mL of McIlvain buffer
4. Vortex for 3 minutes
5. Adjust the pH to 5.5 with 1.0 M NaOH
6. Centrifuge at 3000 rpm for 5 minutes



Solid Phase Extraction Steps

1. **Condition 1:** 3 mL of MeOH @ 8 mL/min
2. **Condition 2:** 3 mL of H₂O @ 8 mL/min
3. **Load:** 1 mL of the prepared milk sample @ 2.5 mL/min
4. **Wash 1:** 3 mL (10/90) MeOH/ ammonium acetate pH 5.5 (v/v) @ 6 mL/min
5. **Wash 2:** 3 mL (10/90) MeOH/ ammonium acetate pH 5.5 (v/v) @ 6 mL/min
6. **Dry:** Remove any water held on the cartridge packing
7. **Elution Fraction:** 3 mL MeOH @ 3.0 mL/min

Sample Reconstitution

- Sample fractions were evaporated at 40°C for 40 minutes
- Evaporated fractions were reconstituted with 300 µL of (90/10) MeOH/H₂O (v/v)

Chromatographic Conditions

- **Mobile Phase:** 1.000 mL/min
 - **A:** 1 mM ammonium formate in (90/10) MeOH/H₂O, 0.1% NaOH (v/v)
 - **B:** 1 mM ammonium formate in (10/90) MeOH/H₂O, 0.1% NaOH (v/v)

GRADIENT		
Time (min)	MPA (%)	MPB (%)
0	90	10
2.00	90	10
2.01	55	45
4.00	55	45
4.01	0	100
5.00	0	100
5.01	90	10
8.00	90	10

- **Column:** 3.0 x 30 mm C18, 2.5 µm @23°C
- **Detector:** Sciex API 3000
 - **Turbo Ion Spray Heater Gas Flow:** 8000 cc/min
 - **Turbo Ion Spray Heater Temperature:** 375°C, ESI⁺, MRM SCAN
 - **MRM Transition:**
 - Sulfathiazole 256.2 → 156.0
 - Sulfadiazine 251.3 → 156.1
 - Sulfadoxypyridazine 281.3 → 156.1
 - Sulfamethazole 254.3 → 156.1
 - Sulfamethazine 279.3 → 186.1
 - Pyrimethamine 249.3 → 177.1
 - Tetracycline 445.3 → 410.2
 - Oxytetracycline 461.3 → 426.2
- **Injection Volume:** 10 µL



Results

Analysis results from LC-MS/MS at the lower level of detection (10 ng/mL) yielded acceptable recovery values on the suite of eight pharmaceutical compounds at > 80% for all four SPE cartridge manufacturers (Figure 1). Competitor C and A recovery ranges for specific compounds (n=3) were larger than the ranges for the same compound recoveries using ASPEC HLB and competitor B cartridges.

Analysis results from LC-MS/MS at 10x the lower level of detection (100 ng/mL) yielded acceptable recovery values on the suite of eight pharmaceutical compounds at > 80% for all four SPE cartridge manufacturers (Figure 2). Ion suppression values were acceptable for all cartridge manufacturers at 100 ng/mL (Table 1).

Figure 1: Recovery Results at 10 ng/mL

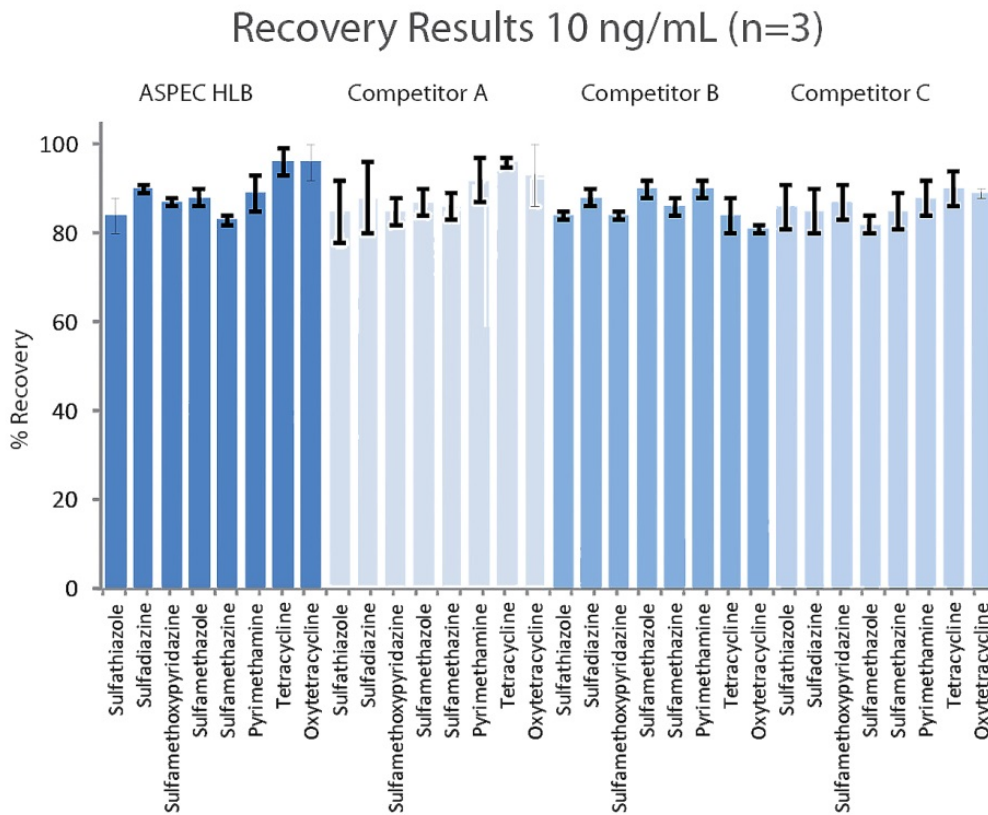




Figure 2: Recovery Results at 100 ng/mL

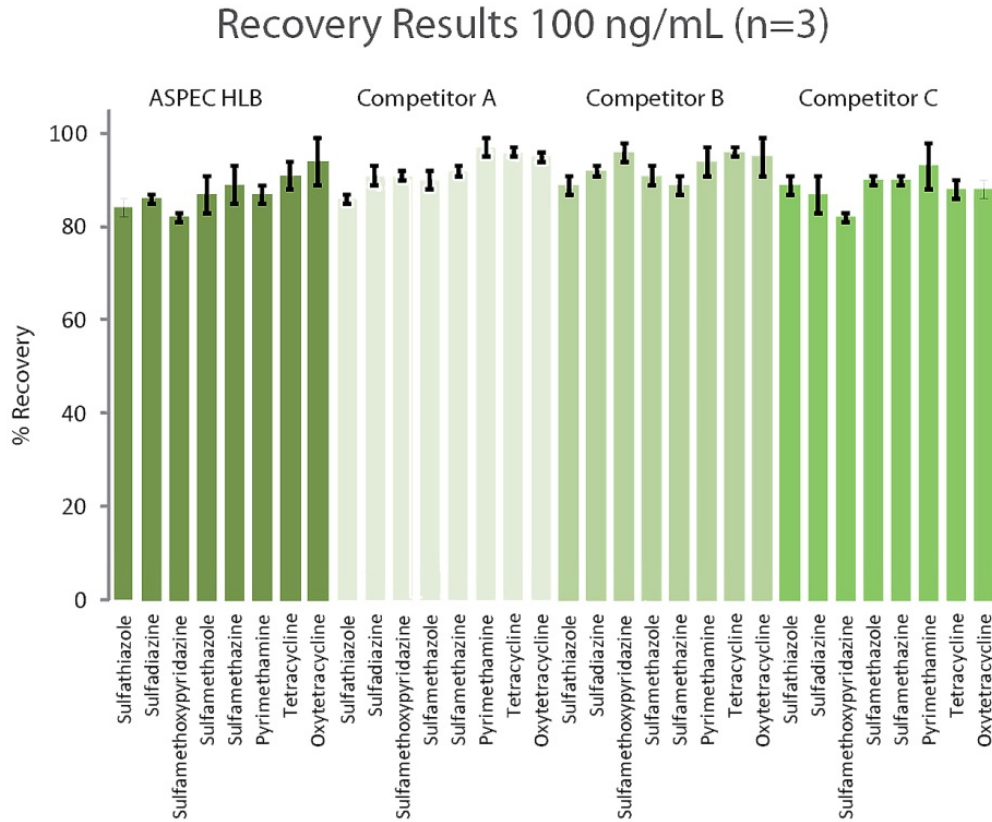


Table 1: Ion Suppression Results Expressed as a Percentage at 100 ng/mL

ION SUPPRESSION MEASURED AT 100 NG/ML (N=3)					
Compounds	ASPEC HLB (%)	ASPEC DVB (%)	Competitor A (%)	Competitor B (%)	Competitor C (%)
Sulfathiazole	-1	-1	1	-2	-2
Sulfadiazine	-1	-2	-2	-3	2
Sulfamethoxy-pyridazine	-6	-3	-5	-13	-6
Sulfamethazole	7	8	1	7	7
Sulfamethazine	5	6	1	5	7
Pyrimethamine	-12	-9	-4	-7	-7
Tetracycline	-5	-1	-1	-5	-4
Oxytetracycline	1	-5	-1	-3	2



Conclusion

The LC-MS/MS analysis provided data at the lower level of detection and at higher levels (10x) to determine the acceptability of cartridge manufacturer based on recoveries and ion suppression values for pharmaceutical residues in a complex bovine milk matrix. Recovery results from the SPE method were acceptable for compound selectivity, which is a direct result of obtaining a clean SPE extract to inject. Milk is a difficult matrix due to high levels of protein and fat content, so sample cleanup to eliminate interferences in analysis is critical to low level compound detection. Ion suppression results were acceptable, confirming the clean extract was obtained prior to LC-MS/MS analysis.

References

1. Codex Alimentarius Commission (2012). Maximum Residue Limits for Veterinary Drugs in Foods:
http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=6&ved=0CFMQFjAF&url=ftp%3A%2F%2Fftp.fao.org%2Fcodex%2Fweblinks%2FMRL2_e_2012.pdf&ei=qKTxUYutKsTIqQH-4G4AQ&usg=AFQjCNFYI39xvERzbZDqAVClcLpUdj3xeQ&bvm=bv.49784469,d.aWM&cad=rja