RAPID ANALYSIS OF SULFONAMIDE RESIDUES IN FOOD MATRICES USING ACQUITY UPLC ™

Waters

Mark E. Benvenuti, Michael S. Young, Eric S. Grumbach, Joseph P. Romano
Waters Corporation, 34 maple St., Milford, MA. 01757

INTRODUCTION

The screening of foodstuffs for antibiotic residues is an important aspect of food safety both from a quality control standpoint, (insuring the integrity of " organic " products) and to detect regulated or banned substances in imported foods. Current LC-MS methods are quite costly and require experienced personnel.

An inexpensive, high resolution rapid screening technique has been developed for several sulfonamide antibiotics, including sulfamethazine, probably the single most popular vetinary drug.

Using ACQUITY UPLC™ with UV detection, required detection limits for several sulfonamide compounds have been achieved in milk after solid phase extraction with run times less than two minutes.

UPLC™ METHOD FOR SULFONAMIDES

Chromatographic Conditions:

System: Waters ACQUITY UPLC™
Column:Waters ACQUITY UPLC™ BEH C_{18}, 2.1 X 50 mm, 1.7 µm @ 47° C
Eluent A– 0.1% Formic Acid in water
Eluent B– 0.1% Formic Acid in methanol
Flow rate: 0.65 ml / min
Injection Volume– 5 µl
Gradient Elution (see below)
Detection– UV @ 270 nm
Data– Waters Empower™ Chromatography Software

Step	Time	Flow	%A	%B	Curve
1	Initial	0.65	90.0	10.0	Initial
2	2.0	0.65	80.0	20.0	6
3	2.01	0.65	90.0	10.0	6

Table 1. Gradient Profile for Sulfonamide separation.

EXPERIMENTAL

A stock sulfonamide 1000 mg / L mixture was prepared by dissolving 0.1 g of each compound listed below in water and diluting to 100 ml. Dropwise addition of concentrated ammonium hydroxide was used to effect complete dissolution. From this stock, the following seven levels were prepared and injected, 50, 100, 200, 400, 600, 800, and 1000 µg / L, along with an aqueous blank.

Seven aliquots of whole milk were then spiked at the same levels described above. These along with an unspiked whole milk blank were prepared according to the procedure found in Table 3, then injected

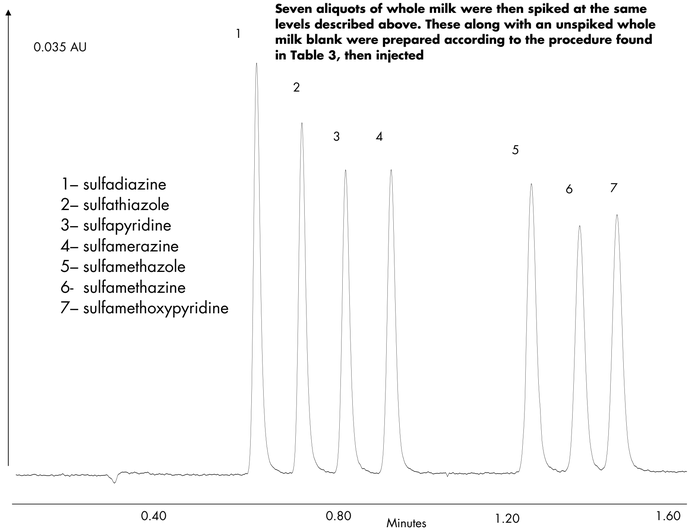

Figure1. Sulfonamide Separation 1 mg / L level

RESULTS

Figure 2 is an overlay of the aqueous blank and the 50 µg / L mixture. Note the excellent signal to noise response at this level. The Linearity(R*2)of these seven compounds from 50 to 1000 µg / L exceeds 0.995 . Figure 3 is an overlay of the unspiked whole milk sample aand the 50 µg / L spiked milk analogous to figure 2. Note the minimal interferences from the blank. Table 4 lists the recoveries of the sulfonamide analytes for each level spiked which were greater than 60% in all cases.

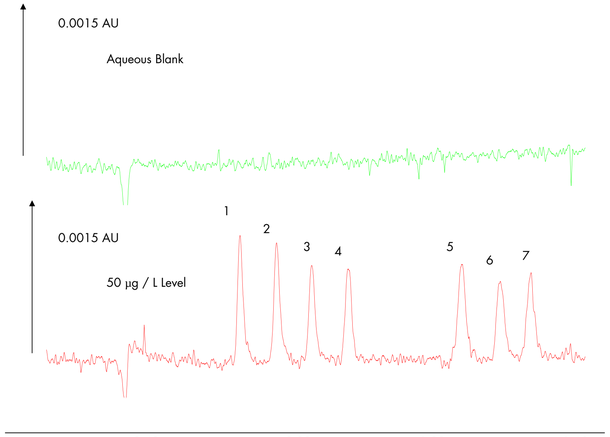

Figure2. Overlay of aqueous blank and 50 µg / L Level

Compound	Linearity (R*2)
Sulfadiazine	**0.999**
Sulfathiazole	**0.998**
Sulfapyridine	**0.996**
Sulfamerazine	**0.997**
Sulfamethazole	**0.996**
Sulfamethazine	**0.998**
Sulfamethoxypyridine	**0.999**

Table 2. Linearity of Sulfonamide analytes from 50 to 1000 µg / L.

Cartridge	Oasis® MCX 3cc, 60 mg
Condition	**2 ml methanol, 2 ml water**
Load	**5 ml whole homogenized milk**
Wash 1	**1 ml water**
Wash 2	**1 ml 0.5M HCl (aqueous)**
Wash 3	**1 ml methanol**
Elute	**2.5 ml 5% ammonium hydroxide in methanol, evaporate and reconstitute in 0.5ml mobile phase (10X), vortex and filter through a 0.45µ PTFE**

Table 3. SPE procedure for whole milk

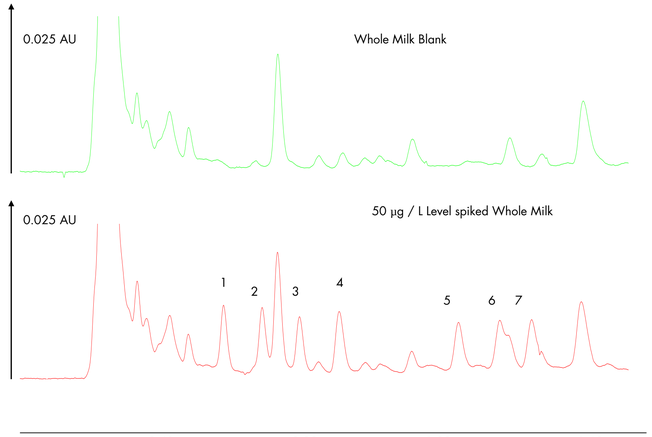

Figure 3. Overlay of whole milk blank nd 50 µg / L Level spiked whole milk after SPE

Analyte	50 ppb spike	100 ppb spike	200 ppb spike	400 ppb spike	600 ppb spike	800 ppb spike	1000 ppb spike
Sulfadiazine	**66.1**	**63.7**	**65.9**	**69.5**	**62.7**	**61.8**	**64.6**
Sulfathiazole	**72.5**	**71.3**	**76.4**	**82.1**	**78.8**	**80.1**	**78.9**
Sulfapyridine	**69.8**	**72.2**	**77.5**	**85.5**	**85.8**	**86.63**	**83.4**
Sulfamerazine	**92.2**	**82.7**	**79.2**	**80.6**	**74.0**	**74.2**	**73.5**
Sulfamethazole	**60.0**	**61.7**	**63.9**	**67.3**	**62.2**	**62.7**	**64.0**
Sulfamethazine	**82.9**	**101.0**	**91.4**	**91.6**	**84.7**	**84.2**	**81.2**
Sulfamethoxypyridine	**77.9**	**81.0**	**82.2**	**86.0**	**84.5**	**85.1**	**80.2**

Table 4. % Recoveries of analytes per spike level

CONCLUSION

The efficacy of this method for the rapid screening of sulfonamide residues in milk is evident and can readily meet the 100 µg /kg residue limit for sulfonamides in animal tissue imposed by the European Union[1] and the 10 µg / L tolerance mandated by the US FDA for milk.[2]

Acknowledgement

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References

1- Hela et. Al. " Determination of Sulfonamides in Animal Tissue using Cation Exchange Reversed Phase Sorbent for Sample Cleanup and HPLC-DAD for Detection" Food Chemistry, 83 (2003) pp.601-608

2– US FDA, Center for Food Safety and Applied Nutriton " Tolerance and/or Safe Levels of Animal Residues in Milk " September 27, 2005

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