ASMS 2013

MP-133

Determination of Sulfonamide Residues in Whole Milk Using a Novel Lipid-Stripping Filtration Cartridge and LC/MS/MS

Irina Dioumaeva, Agilent Technologies Inc., Lake Forest, CA

Introduction

Removal of proteins and lipids from a sample matrix is one of the major goals of sample preparation. Recently introduced Captiva ND Lipids filtration cartridges (Agilent Technologies Inc., Santa Clara, CA) allow a one-step in-tube protein/lipid removal due to a unique combination of a non-drip membrane and a lipid-stripping sorbent. We tested performance of these cartridges while developing a method for determination of sulfonamide residues in whole milk.

According to US federal standards, whole milk contains not less than 3.25% milk fat and 8.25% solids-not-fat. Organic whole milk used in this method contained 33 mg fat per 1 mL and an equal amount of proteins. At these protein and lipid concentrations, sample cleanup is essential as these matrix components will contaminate LC columns and mass spectrometers and complicate MS detection.

Sulfonamides are common antimicrobials administered to dairy cattle and excreted in milk in amounts that may be of serious concern for human health. The maximum residue limit for sulfonamides in milk in the US is 10 ng/mL (established for sulfaquinoxaline) and 100 ng/mL in the EU.

Efficient sample cleanup with Captiva ND Lipids allowed detection of very polar (sulfaguanidine) to nonpolar (sulfaquinoxaline) compounds at initial concentrations in whole milk below 5 ng/mL, with good method linearity, accuracy and precision. Screening of milk samples processed by Captiva ND Lipids for four classes of phospholipids revealed almost complete removal of phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE) and phosphatidylserine (PS). To assess the efficiency of lipid removal, chromatograms of residual phospholipids in Captiva ND Lipids milk extracts are shown along with the chromatograms of phospholipids in milk samples processed by protein precipitation.

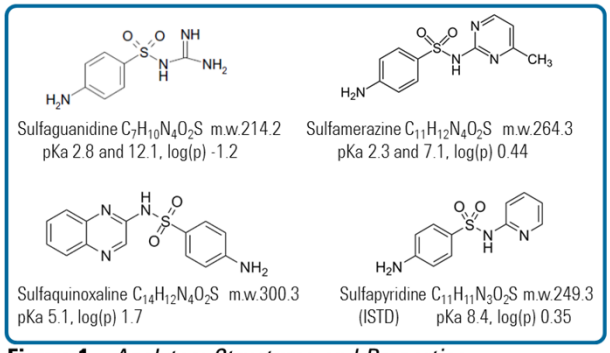

Figure 1. *Analytes: Structures and Properties*

Experimental

Materials and instrumentation

SPP: Agilent Captiva ND Lipids filter cartridges, 3 mL

LC: Agilent Poroshell 120 EC-C18, 3 x50 mm 2.7 µm column
Agilent Poroshell Phenyl-Hexyl, 2x50 mm 2.7 µm column
Agilent 1260 Infinity LC

MS: Agilent 6460A Triple Quadrupole with AJS source

Sample Preparation

1. Load 1.3 mL acetonitrile into Captiva ND Lipids cartridge
2. Add 50 µL ISTD (0.5 µg/mL working solution of sulfapyridine)
3. Load sample: 0.25 mL spiked whole milk
4. Mix contents of each cartridge with a 1 mL pipette (five aspiration-dispensing cycles)
5. Apply vacuum (~15 in Hg) and collect eluate
6. Evaporate under nitrogen to dryness at 40 °C
7. Reconstitute in 0.125 mL initial mobile phase (5% acetonitrile, 95% water)

Instrumental methods - Sulfonamides

LC Conditions
Poroshell 120 EC-C18, 3 x 50 mm, 2.7 µm column
Eluent: A: 0.1% formic acid in water
B: 0.1% formic acid in ACN
Injection volume: 10 µL

Time (min)	% B	Flow rate, mL/min
0.0	5	0.5
0.5	5	0.5
1.0	40	0.5
1.5	60	0.5
2.5	95	0.5
6.0	95	0.5
6.1	5	0.5

AJS source parameters
Ionization mode: Positive
Capillary voltage: 3,600 V
Drying gas flow: 7 L/min
Drying gas temp.: 350 °C
Nebulizer gas: 40 psi
Sheath gas flow: 9 L/min
Sheath gas temp.: 350 °C
Nozzle voltage: 0 V

MS parameters
Scan type: MRM
Delta EMV: (+) 300 V

Instrumental methods - Phospholipids

LC Conditions
Poroshell 120 Phenyl-Hexyl 2 x 50 mm, 2.7 µm column
Eluent: A: 0.1% formic acid in water
B: 0.1% formic acid in methanol
Injection volume: 10 µL

Time (min)	% B	Flow rate, mL/min
0.0	80	0.5
1.0	80	0.5
4.0	100	0.5
6.0	100	0.5
6.1	100	1.0
12.0	100	1.0
12.1	80	0.5

AJS source parameters
Ionization mode: Positive
Capillary voltage: 4, 000 V
Drying gas flow: 12 L/min
Drying gas temp.: 350 °C
Nebulizer gas: 45 psi
Sheath gas flow: 10 L/min
Sheath gas temp.: 350 °C
Nozzle voltage: 500 V

MS parameters
Scan type: Precursor Ion
Neutral Loss
Delta EMV: (+) 250 V

Results and Discussion

Table 1. *MRM Transitions for Sulfa Drugs*

Compound	ISTD?	Prec ion	MS1 res	Prod ion	MS2 res	Fragm (V)	CE (V)	Polarity
Sulfaquinoxaline	no	301.1	Unit	156*	Wide	110	15	Positive
Sulfaquinoxaline	no	301.1	Unit	108	Wide	110	28	Positive
Sulfamerazine	no	265.1	Unit	156*	Wide	105	15	Positive
Sulfamerazine	no	265.1	Unit	108	Wide	105	28	Positive
Sulfaguanidine	no	215.1	Unit	156*	Wide	85	12	Positive
Sulfaguanidine	no	215.1	Unit	108	Wide	85	24	Positive
Sulfapyridine	yes	250.1	Unit	156*	Wide	105	14	Positive
Sulfapyridine	yes	250.1	Unit	108	Wide	105	27	Positive

* *Asterisk denotes a quantifier product ion*

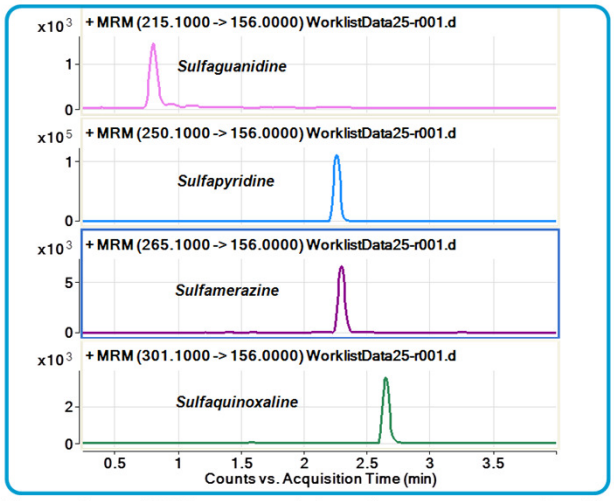

Figure 2. *MRM extracted ion chromatograms of sulfa drugs in milk extract, down from the top: 10 ng/mL sulfaguanidine; 100 ng/mL sulfapyridine (ISTD); 10 ng/mL sulfamerazine; 10 ng/mL sulfaquinoxaline*

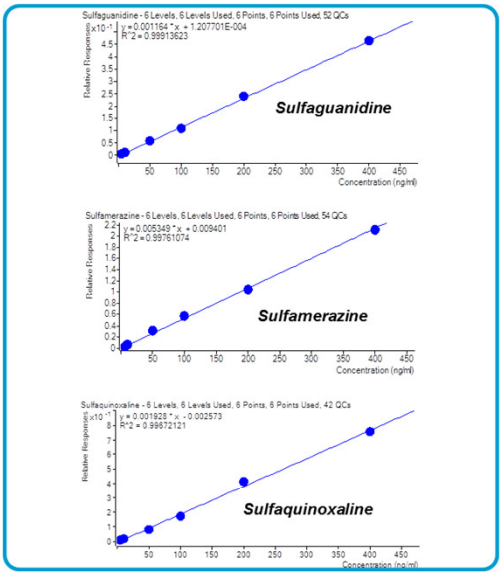

Figure 3. *Example calibration curves for three sulfa drugs in milk extract, calibration range 5 to 400 ng/mL, linear fits $R^2 \geq 0.997$*

Table 2. *Captiva ND Lipids Method Performance for Sulfa Drug Residues in Milk Extract, n = 5*

Compound	R^2	10 ng/mL		50 ng/mL		200 ng/mL	
		Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Sulfaguanidine	0.999	96.0	6.9	94.8	4.0	104.1	5.0
Sulfamerazine	0.998	98.7	3.8	107.3	3.8	98.6	5.0
Sulfaquinoxaline	0.997	105.2	3.1	91.3	9.4	94.4	14.5

Results and Discussion

Table 3. *MS/MS Acquisition Methods for Residual Phospholipids (for molecular ions with the highest signal)*

PC, SM and PE Precursor Ion Mode	Prod Ion	MS1 From	MS1 To	Scan Time	Fragm. Mode	Fragm. (V)	CE (V)	Polarity
PC, Lyso PC, SM as M+H	184	700	840	100	Fixed	160	35	Positive
PE as M+Na	164	720	820	100	Fixed	160	27	Positive
PS and LysoPS Neutral Loss Mode	**Neutral Loss**	**MS1 From**	**MS1 To**	**Scan Time**	**Fragm. Mode**	**Fragm. (V)**	**CE (V)**	**Polarity**
PS, Lyso PS as M+Na	87	540	840	100	Fixed	180	25	Positive

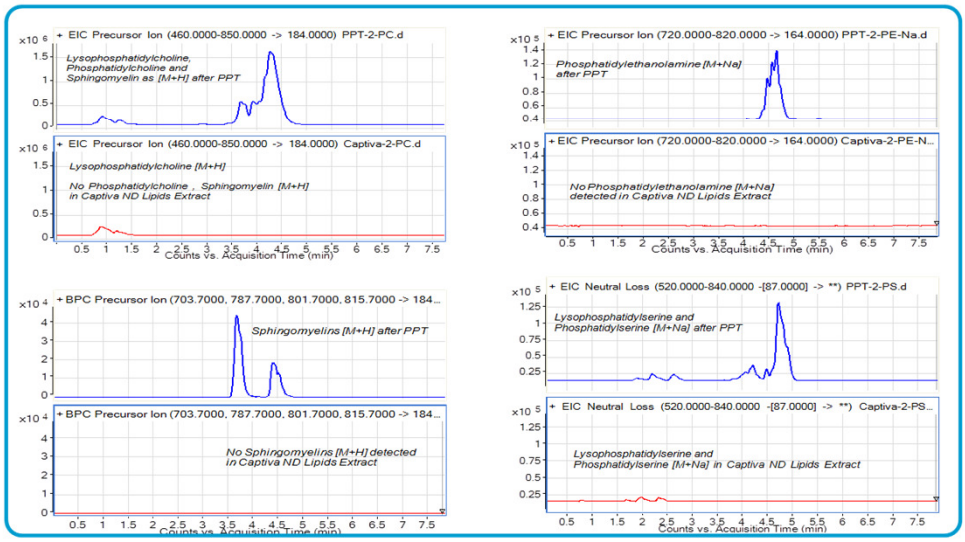

Figure 4. *Chromatograms of residual phospholipids in milk samples after protein precipitation and Captiva ND Lipids, with 0.25 mL milk and 1.3 mL ACN used in each method*

On each panel of two chromatograms, ***blue line stands for PPT, red line – for Captiva ND Lipids*** *cleanup method*

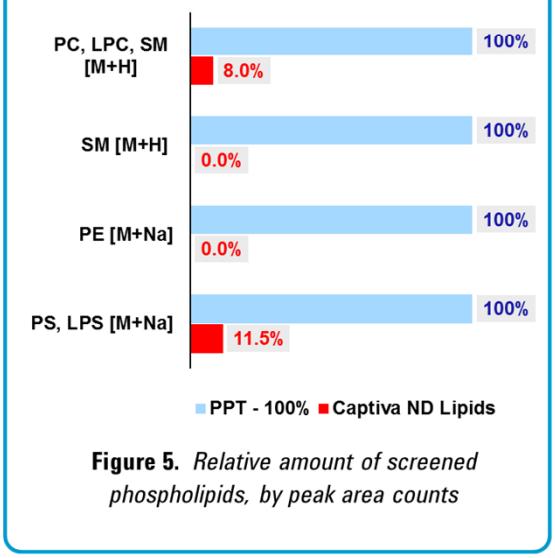

Figure 5. *Relative amount of screened phospholipids, by peak area counts*

Conclusions

- Captiva ND Lipids filter cartridges is an easy and sufficient sample cleanup technique for LC/MS/MS detection of sulfonamide drug residues in whole milk.
- Compared to conventional protein precipitation-centrifugation, the Captiva ND Lipids sample cleanup method described here provides significantly cleaner extracts for the each of four major phospholipids in milk.
- Screening of residual phospholipids in milk samples conducted with optimized, highly sensitive MS detection methods shows excellent removal of phosphatidylcholine, sphingomyelin, phosphatidylethanolamine and phosphatidylserine by Captiva ND Lipids.
- With a milk sample size as low as 0.25 mL, this method meets the requirements of the US and EU for the limit of detection, method linearity, regression coefficients, accuracy and precision.