Determination of Sulfonamide Residues in Milk with Agilent Captiva ND Lipids Filtration and LC/MS/MS

Application Note

Food Testing & Agriculture

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Abstract

This application note describes the analysis of sulfa drug residues in whole milk using a simple extraction procedure coupled to LC/MS/MS detection. Captiva ND Lipids is a 3 mL filtration cartridge with a 0.22 µm nondrip membrane and a lipid-stripping sorbent for quick one-step, in-tube protein precipitation/sample cleanup. With Captiva ND Lipids, centrifugation and supernatant transfer are eliminated, reducing the chance of sample loss and saving analyst time and effort. Extracts obtained with Captiva ND Lipids from the whole milk are transparent and can be introduced into the LC/MS/MS system without fear of contamination. Excellent method linearity is demonstrated for each analyte from 5 to 400 ng/mL, together with good accuracy (% recovery) and precision (% RSD) data at three concentration levels.

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Introduction

Milk is a very important component of the human diet. Veterinary drugs from animal feed found in milk are of serious concern for human health, and, therefore, subject to regulation in many countries. Sulfonamides are common antimicrobials administered to dairy cattle. 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 [1,2].

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.

With Captiva ND Lipids, sample preparation becomes extremely simple. All that is required is in-cartridge sample mixing with acetonitrile and a brief application of vacuum to pull a sample through the non-drip membrane. A protein pellet is left behind on top of the filter and the majority of lipids is retained on the sorbent bed. The collected eluates are transparent and can be evaporated and reconstituted or simply diluted with water and analyzed by LC/MS/MS.

Efficient sample cleanup allowed detection of very polar (sulfaguanidine) to nonpolar (sulfaquinoxaline) analytes at initial concentrations in whole milk below 5 ng/mL. This was achieved with an injection of only 10 µL of preconcentrated (2x) milk filtrate. A structurally similar sulfapyridine was used as an internal standard (ISTD).

A previous Agilent method for sulfonamide residues analysis in milk used Agilent Bond Elut SampliQ polymeric strong cation exchange (SCX) cartridges, an Agilent ZORBAX Eclipse Plus C18, 3.0 × 50 mm column with 1.8 µm particles, and an Agilent 6410 Triple Quadrupole LC/MS System [3].

Experimental

The LC portion of the method employs a superficially porous Agilent Poroshell 120 EC-C18, 3.0 × 50 mm column with 2.7 µm particles, which has similar efficiency to sub-2 µm UHPLC columns, but with approximately 40% less backpressure. During analysis, the actual pressure in the LC system was significantly below 400 bar. MS detection was carried out using a 6460 Triple Quadrupole LC/MS system with an AJST electrospray source. Sample preparation was done using Captiva ND Lipids. Agilent vials, caps, and inserts, stopcock valves and vacuum manifold were also used.

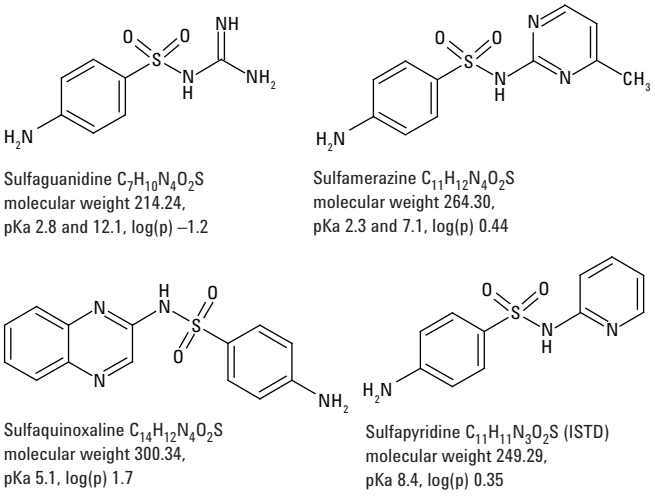

Figure 1. Analyte structures and properties.

Analytes

Sulfonamide standards, including internal standard, were purchased from Sigma-Aldrich Corp., St Louis, MO, USA. Original individual stock solutions (1 mg/mL) for three analytes and ISTD were prepared in methanol and further diluted to a combined 10 µg/mL working solution of three sulfa drugs in water. The ISTD working solution was 0.5 µg/mL sulfapyridine in water. All stock solutions and working solutions were stored at –20 °C. Homogenized pasteurized organic whole milk was purchased from the local supermarket.

Materials and instrumentation

SPE: Agilent Captiva ND Lipids filter cartridges, 3 mL, 100/pk (p/n A5300635)

Agilent VacElut 20 vacuum manifold (p/n 12234100)

Agilent stopcock valves (p/n 12234520)

Agilent autosampler vials 2 mL (p/n 5182-0716)

Agilent vial inserts with polymer feet, 250 µL, deactivated glass (p/n 5181-8872)

Agilent screw caps for AS vials (p/n 5182-0717)

LC: Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm column (p/n 699775-302)

Agilent 1260 Infinity LC (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, G1330B thermostat)

MS: Agilent 6460A Triple Quadrupole LC/MS with AJST electrospray ionization source

Sample preparation

No sample pretreatment was required.

Extraction

1. Load 1.3 mL acetonitrile into a 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 (approximately 15 in Hg), and collect eluate.
6. Evaporate eluate under nitrogen to dryness at 40 °C.
7. Reconstitute in 0.125 mL initial mobile phase (5% acetonitrile, 95% water).

LC conditions

Eluent:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	
Injection volume:	10 µL	
Flow rate:	0.5 mL/min	
Gradient:	Time (min)	% B
	0.0	5
	0.5	5
	1.0	40
	1.5	60
	2.5	95
	6.0	95
	6.1	10
Stop time:	6.2 min	
Post run:	2.5 min	
Max pump pressure:	400 bar	
Needle wash:	Flush port with 75:25 acetonitrile:water for 10 s	
Overlapped injection:	Disabled	
Automatic delay volume reduction:	No	

ES source parameters

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

MS parameters

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

Results and Discussion

A quick and easy Captiva ND Lipids filtration with in-tube protein precipitation and lipid removal provides visibly cleaner extracts than protein precipitation/centrifugation. With the same milk sample size and amount of acetonitrile used, a signal loss due to matrix effect for sulfaquinoxaline (the least polar of three analytes) after Captiva ND Lipids filtration was half of the signal loss due to matrix effect after protein precipitation/centrifugation (27% and 60%, respectively). Sufficient cleanup of milk matrix permitted reliable quantitation of sulfonamides in a broad range of polarities with log(p) from -1.2 (sulfaguanidine) to 1.7 (sulfaquinoxaline).

Table 1 gives a list of MRM transitions for sulfonamides showing first a quantifier, and secondly a qualifier transition.

Separation on the Agilent Poroshell 120 EC-C18 column occurred within 3 minutes, with the remaining portion of the run designated for column cleanup and re-equilibration. Figure 2 shows chromatograms of quantifier transitions of three sulfonamides in milk spiked at 10 ng/mL, with sulfapyridine as an internal standard at 100 ng/mL.

Table 1. MRM transitions.

Compound	ISTD?	Prec ion	MS1 res	Prod ion	MS2 res	Frag (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

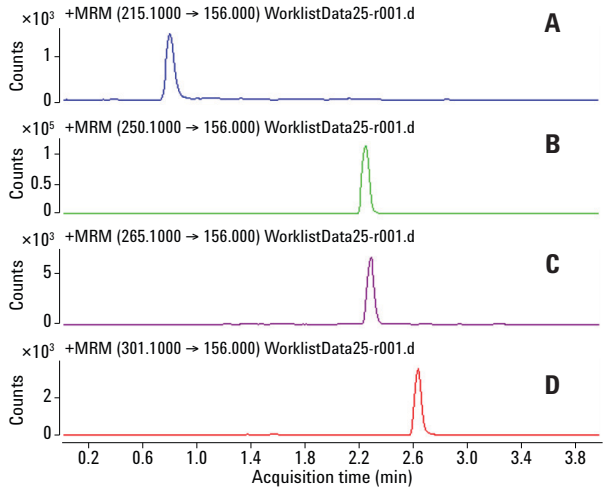

Figure 2. MRM extracted ion chromatograms of sulfa drugs in milk extract; A, 10 ng/mL sulfaguanidine; B, 100 ng/mL sulfapyridine (ISTD); C, 10 ng/mL sulfamerazine; D, 10 ng/mL sulfaquinoxaline.

Calibration was performed using whole homogenized milk spiked pre-extraction with a working solution of three sulfa drugs in water to obtain six concentration levels, 5, 10, 50, 100, 200, and 400 ng/mL (Figure 3). QC standards for method evaluation were prepared at three levels separately from the calibration standards (Table 2).

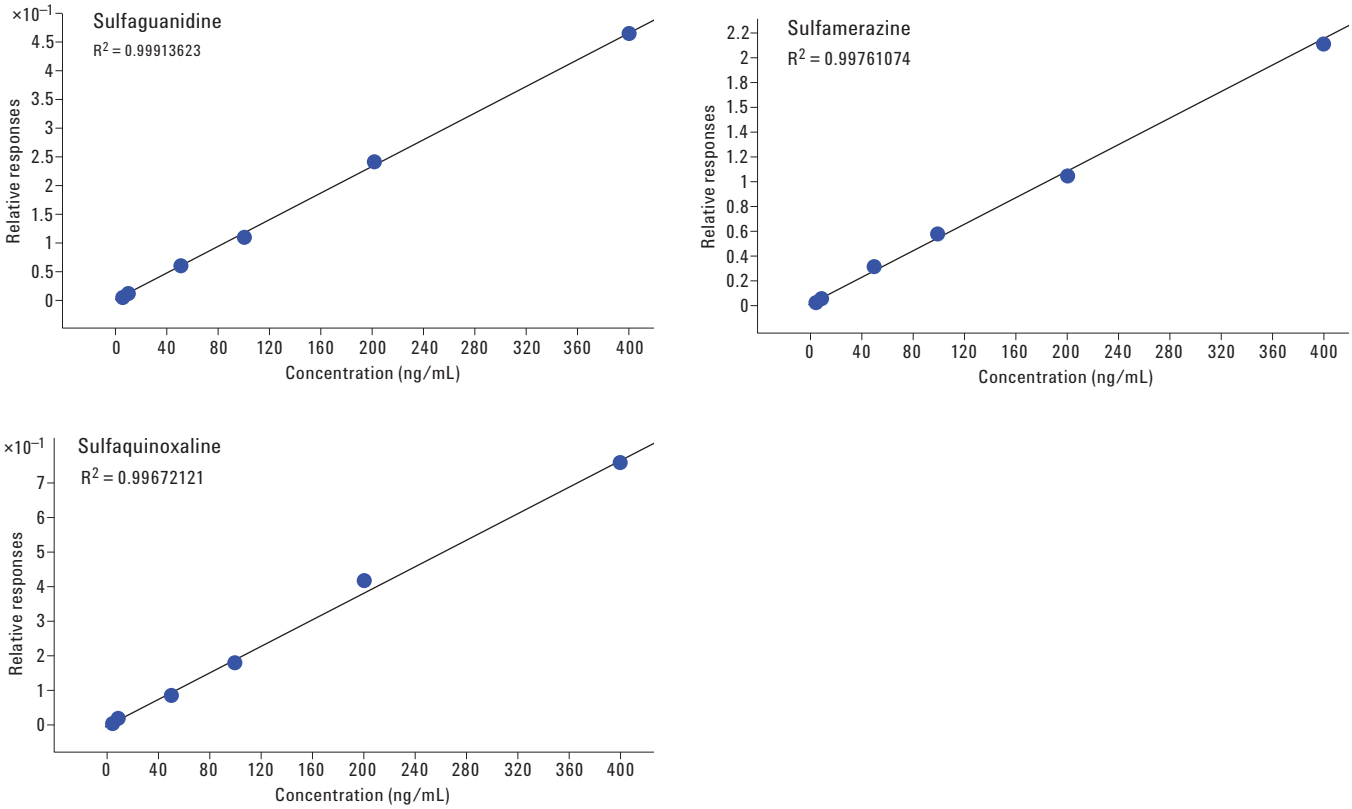

Figure 3. Example calibration curves for three sulfonamide drugs in milk extracts, calibration range 5 to 400 ng/mL, linear fits $R^2 \geq 0.997$.

Table 2. Method performance for sulfonamide drug residues in milk, n = 5.

		10 ng/mL		50 ng/mL		200 ng/mL	
Compound	**R^2**	**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

Conclusions

Captiva ND Lipids filter cartridges simplify workflows and make particulate removal, protein precipitation, and lipid removal more user-friendly. This 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 method provides cleaner extracts. A dual-depth filter construction enables a fast, reproducible flow, leading to reliable quantitation with good accuracy and precision. The benefits of this improved sample cleanup include extended column life, reduced instrument downtime, and lower matrix effects.

Separation of sulfonamides in whole milk was completed within 3 minutes and could be performed on most Agilent LC systems. MS source parameters developed for the Agilent 6460 Triple Quadrupole LC/MS system could be easily modified for use with other Agilent triple quadrupole systems.

References

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3. C. A. Gonzales, K. M. Usher, A. E. Brooks, R. E. Majors. Determination of Sulfonamides in Milk using Solid Phase Extraction and Liquid Chromatography - Tandem Mass Spectrometry. Application note, Agilent Technologies, Inc., Publication number 5990-3713EN (2009).

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