

OVERVIEW

Purpose

- Simultaneous identification and quantification of 16 sulfonamides residues in whole milk
- New Laser Diode Thermal Desorption (LDTD) ionization source
- Negative APCI combined to tandem mass spectrometry
- Low sample preparation combined with fast sample-to-sample run time

Method

- Sulfonamides spiked in whole milk
- Protein precipitation using acetonitrile (1:5)
- Concentration range : 2 ng/mL to 1000 ng/mL
- LDTD-APCI-MS/MS analysis : Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer

Results

- Excellent linearity ($R^2 > 0.99$)
- Sample-to-sample run time of 57 seconds
- No matrix effect
- No carryover
- Excellent selectivity
- Negative APCI allows sulfonamide isomers identification

INTRODUCTION

Sulfonamides represent a class of antibacterial compounds widely used in food-producing animals for therapeutic, prophylactic and growth-promoting purposes. Improper use of sulfonamides in the dairy industry, such as excessive administration and inappropriate withdrawal period, may result in sulfonamide residues in milk. The presence of Sulfonamide residues in milk is of great concern, as some sulfonamides such as sulfamethazine are carcinogenic and all of them can promote growth of antibiotic-resistant strain of bacteria leading to inefficiency of this type of drug for therapeutic use.

We have developed a high throughput method in order to meet daily analysis of milk samples required to insure food safety and protect population. 16 sulfonamides residues are detected and quantified in milk (Figure 1) using a new Laser Diode Thermal Desorption (LDTD) source coupled to MS/MS.

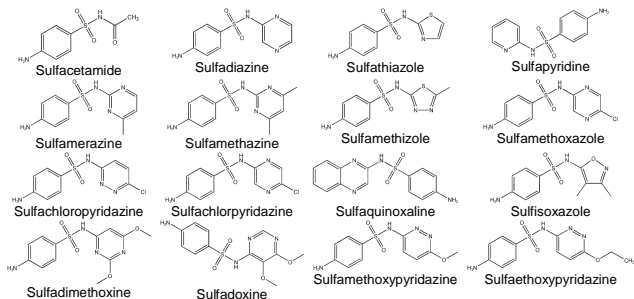


Figure 1 Sulfonamides studied

METHODS

Instrumentation

- LDTD model T-960, Phytronix Technologies (Figure 2)
- Thermo Scientific TSO² Quantum™ Ultra AM

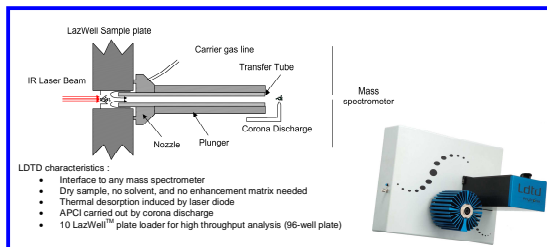


Figure 2 LDTD ionization source for mass spectrometry

LDTD Parameters

- Laser power Pattern
- Hold at 0 % for 3 s (stabilization)
- Increase to 19 % in 11 s
- Hold at 19 % for 9 s
- Decrease to 0 % in 0.05 s
- Hold at 0 % for 3 s
- Carrier gas flow : 4.0 L/min (Air)
- Carrier gas temp. : 20 °C
- Corona voltage value : 5 kV
- Deposited sample volume: 2 µL

MS Parameters

- Collision gas pressure : 1.5 mTorr (Argon)
- Scan time : 0.02 sec
- Scan width : 0.7 amu
- Q1 width : 0.7 amu
- Q3 width : 0.7 amu

Table 1 MS MRM Parameters

Compound	Q1 (m/z)	Q3 (m/z)	Collision Energy (eV)
Sulfacetamide	213	170	25
Sulfadiazine	249	165	25
Sulfathiazole	254	156	22
Sulfapyridine	248	184	25
Sulfamerazine	263	199	26
Sulfamethazine	277	122	28
Sulfamethizole	269	196	28
Sulfamethoxazole	252	156	28
Sulfachloropyridazine	283	128	34
Sulfachloropyridazine	283	107	34
Sulfamethoxypyridazine	299	144	28
Sulfisoxazole	266	171	28
Sulfadimethoxine	309	131	34
Sulfadoxine	309	251	34
Sulfamethoxyypyridazine	279	156	25
Sulfathoxyypyridazine	293	156	25
Indapamide (internal standard)	364	190	26

Sample preparation

- 0.50 mL of whole milk
- Centrifuge at 14000 RPM for 10 min
- 2.50 mL of acetonitrile (precipitation agent) with indapamide at 0.24 g/mL (Internal Standard)
- Vortex for 30 sec.
- Centrifuge at 14000 RPM for 10 min
- Filtrate on NanoSep 2 µm (centrifugation at 14000 RPM for 1 min)
- Transfer 2.0 µL onto LazWell™ to perform LDTD-MS/MS analysis

RESULTS

Method Specificity

Sulfonamide isomers like sulfadimethoxine and sulfadoxine (Figure 3) can be identified and quantified using LDTD ionization operated in negative APCI mode. Specific MS/MS transitions allow isomers analysis without the need for chromatographic separation prior to the MS detection as shown in Figure 4. As no LC mobile phase is present during the ionization process and no external matrix is needed, the LDTD ionization does not suffer from matrix effect as shown by the low background signal. Moreover, no carryover is observed.

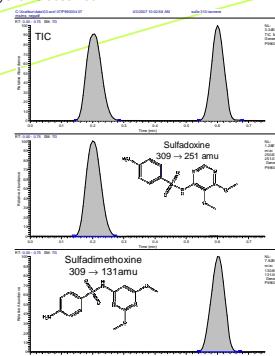
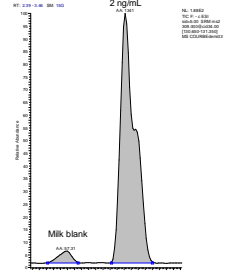


Figure 3 Method selectivity for sulfonamide isomers detection.

Detection Limit

The thermal desorption process induced by laser diode produces a low background signal (Figure 4) which allows the detection of all tested sulfonamides in milk simultaneously at a concentration corresponding to 2 ng/mL (0.66 pg deposited into well).

Figure 4 Signal for milk blank and milk sample spiked with sulfadimethoxine at 2 ng/mL



Recovery and Quantification Limits

The mean extraction recovery of sulfonamides in milk using acetonitrile as protein precipitation agent is evaluated to be 42.1 % ± 8.2 % (Table 1) which is usually found in literature¹ and may be attributed to the different phases present in milk (globular protein, lipoproteins and casein micelles).

Table 1 Sulfonamides extraction recovery from whole milk

Compound	Recovery (%) (at 500 ng/mL)
Sulfacetamide	46.1
Sulfadiazine	49.7
Sulfisoxazole	37.5
Sulfapyridine	55.7
Sulfamerazine	39.1
Sulfamethazine	48.2
Sulfamethizole	45.8
Sulfamethoxazole	38.4
Sulfachloropyridazine	33.2
Sulfachloropyridazine	68.9
Sulfamethoxypyridazine	48.7
Sulfisoxazole	43.2
Sulfadimethoxine	43.5
Sulfadoxine	52.9
Sulfamethoxyypyridazine	48.0
Sulfathoxyypyridazine	45.0

Linearity Range

The linearity was tested from 2 ng/mL to 1000 ng/mL and the linearity range found is reported in Table 2. Reported linearity range for 7 sulfonamides analyte are between 20 ng/mL to 5000 ng/mL. The lower linearity range (from 40 to 1000 ng/mL) is explained by the combination of recovery variation and the use of a less intense daughter ions selection in order to improve the method selectivity. To reach lower quantification limits and higher linearity range, the sulfonamide extraction procedure could be improved to lower the variability affecting the quantification.

Table 2 Sulfonamides linearity range for quantification in milk

Compound	Linearity Range (ng/mL)	R ²
Sulfamerazine	10 - 1000	0.99
Sulfamethazine		
Sulfadiazine		
Sulfamethoxazole		
Sulfadoxine	20 - 1000	0.99
Sulfamethoxyypyridazine		
Sulfachloropyridazine		
Sulfacetamide		
Sulfapyridine		
Sulfamethoxazole	40 - 1000	0.99
Sulfisoxazole		
Sulfadimethoxine		

High Throughput Analysis

The LDTD-MS/MS method allows to detect and quantify 16 sulfonamides in 57 seconds compared to 20 minutes run time obtained in traditional LC/MS/MS method.

Table 3 Reported run time for sulfonamides analysis in water, urine and milk samples

Study	Matrix*	Run time (min)	Nb. SA*
Talanta 64 (2004) 87-100	Water	80	11
	Urine	40	n.d. [†]
	Milk	n.d. [†]	
J. Agric. Food Chem. 53 (2005) 8468-8473	Water	22	5
	Milk	20	
LDTD-MS/MS	Milk	1	18

* Matrix from which Sulfonamides were extracted
† Number of Sulfonamides tested
- No data available

CONCLUSION

- 16 sulfonamides residues identification and quantification in whole milk is 20 times faster than LC/MS/MS methods
- Detection limit of 2 ng/mL for each sulfonamide
- Excellent linearity range for most sulfonamides (10 ng/mL)

REFERENCE

1. Msagati T.A.M. and Nindi M.M., Talanta. 2004, 64, 87-100