ULTRA-FAST QUANTIFICATION OF SIXTEEN SULFONAMIDE RESIDUES IN DAIRY MILK USING LDTD-APCI-MS/MS

S. Sauvé[1*], P. Tremblay[2]; P. A. Segura[1], P. Picard[2], S. Fortier[3]; L. Gagnon[3]

[1] Département de Chimie, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, QC, Canada H3C 3J7, [2] Phytronix Technologies, 337, rue Saint-Joseph Est, Québec, QC, Canada G1K 3B3
[3] Ministère de l'Agriculutre, des Pecheries et de l'Alimentation du Québec (MAPAQ), Québec, QC, Canada G1R 4X6
*e-mail: sebastien.sauve@umontreal.ca

Université de Montréal

Overview

Sulfonamide antibacterial residues in dairy milk were analyzed in MS/MS using a novel ionization source based on Laser Diode Thermal Desorption (LDTD) combined with an APCI-type ionization. Sulfamerazine, sulfamethoxazole, sulfadimethoxine and sulfamethazine and other twelve sulfonamide antibacterials were purified using a liquid-liquid acetonitrile extraction. The optimized milk to acetonitrile ratio was found to be 1:4, which yielded the best S/N ratio. Paracetamol was used as the internal standard. The results showed excellent linearity (R^2 > 0.99, in most cases) over 1.5 to 500 µg L^{-1}. The run time using the LDTD-MS/MS system was 16 seconds per sample, which gave the method a high screening throughput capacity in combination with an excellent quantification.

Introduction

- Since the early 1950s, antibacterials have been used in agriculture to treat infections on food-producing animals. They are also utilized to prevent infections or as feed additives to reduce animal susceptibility to stress-related diseases and enhance growth [1].

- According to Khachatourians, non-therapeutic application of antibiotics amounts to about 90% of the total agricultural applications [2]. An independent report estimated that more than 1.6 millions of kilograms of antibacterials are used annually on cattle in the US alone [3].

- Sulfonamides are a class of antibacterial compounds widely used in the dairy industry. Improper use of these substances on lactating dairy cattle may result in sulfonamide residues in milk. This is of concern, as the occurrence of antibacterials in food products can contribute to the spread of antibacterial resistance in bacteria. Also it has been shown that sulfamethazine (also known as sulfadimidine) is carcinogenic [4].

- We have developed a high throughput method in order to meet the daily analysis of milk samples required to insure food safety and protection of consumers. Sixteen sulfonamides residues were detected and quantified in dairy milk (Figure 1) using a new Laser Diode Thermal Desorption (LDTD) ionization source coupled to a tandem mass spectrometer (Figure 2).

Materials and method

- **Sulfonamide standards preparation**

- Stock solution of sulfonamides (Figure 1): 1000 µg mL^{-1} of each sulfonamide in ACN.
- Internal standard solution: 10 µg mL^{-1} of paracetamol in water.
- Working solution of sulfonamides: 10 µL of stock solution in 1000 µL of water.

- **Milk samples preparation**

- A 800-µL volume of ACN was added to 200 µL of whole dairy milk.
- The samples were mixed with a vortex for 4 min and then centrifuged at 24 000 g for 5 min.
- We added 10 µL of internal standard solution and the sample was mixed again with a vortex.
- A 2-µL volume of the sample was then transferred to a LazWell plate well for LDTD-APCI-MS/MS analysis

- **Instrumentation**

- LDTD model T-960, Phytronix Technologies on a TSQ Quantum™ Ultra AM, Thermo Fisher Scientific.

- **LDTD-APCI parameters**

- Laser pattern: 0 % laser power for 2 s
  0 % to 35 % in 3 s
  35% to 0 % in 0.01 s
  0 % for 3 s
- Carrier gas: Flow rate: 3 L min^{-1}
- Spray current: 3 µA
- Ion sweep gas : 0.3 arbitrary units

- **MS/MS parameters**

- Quantification in the SRM mode (Table 1)
- Scan time: 0.05 s
- Scan width: 0.01 m/z
- Q1 peak width: 0.70 FWHM
- Q1 peak width: 0.70 FWHM
- Q2 gas pressure: 1.2 mTorr
- Chrom. filter: 3 s

Table 1. Sulfonamide SRM transtions, collision energry (CE) and tube lens values.

Compound	Precursor (m/z)	Product (m/z)	CE (V)	Tube Lens (V)
Sulfacetamide	213.0	171.0	21	50
Sulfachlorpyrazine	283.1	128.1	29	72
Sulfachlorpyridazine	283.1	107.0	21	54
Sulfadiazine	249.3	185.1	19	62
Sulfadimethoxine	309.3	131.0	34	67
Sulfadoxine	309.3	251.0	34	53
Sulfaethoxypyridazine	293.3	156.0	26	56
Sulfamerazine	263.3	199.1	19	65
Sulfamethazine	277.3	106.1	38	65
Sulfamethizole	269.0	196.1	23	47
Sulfamethoxypyridazine	279.1	156.0	26	56
Sulfamethoxazole	252.0	156.1	17	43
Sulfapyridine	248.3	184.1	20	62
Sulfaquinoxaline	299.1	144.1	29	72
Sulfathiazole	254.0	156.0	19	39
Sulfisoxazole	266.3	171.0	21	48

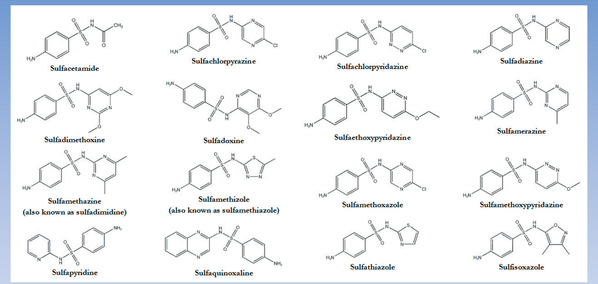

Figure 1. Molecular structures of the studied sulfonamides.

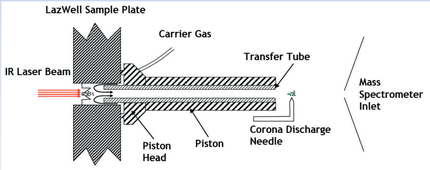

Figure 2. Diagram showing the Laser Diode Thermal Desorption (LDTD) ionization source.

- **Recovery study**

Spiked samples preparation

- A 10-µL volume of the sulfonamides working solution was added to 200 µL of whole dairy milk and then mixed using a vortex for 10 s.
- Spiked samples were then allowed to settle for a period of 12 h to be more representative of real milk samples. After the rest period, the standard milk preparation procedure was followed.

Preparation of spiked samples in a milk extract

- Following the standard milk preparation procedure, before we added the internal standard, 10 µL of the sulfonamide standards working solution was added into the milk extract and the sample mixed with a vortex for 10 s.

- Sulfonamide recovery was calculated using the following formula:

$$\text{Recovery (\%)} = \left[\frac{\left(\text{Sulfonamide Area} / \text{ISTD Area} \right)_{\text{spiked in milk}}}{\left(\text{Sulfonamide Area} / \text{ISTD Area} \right)_{\text{spiked milk extract}}} \right] \times 100\%$$

- Each test was performed three times and every solution was analyzed in triplicates. Spiked samples containing 50 and 500 µgL^{-1} of the sulfonamides were used.

- **Method validation**

Calibration curves. Calibration curves were done by spiking the sulfonamides in whole dairy milk, and each level was analyzed in triplicates.

Limit of quantification (LOQ). The limit of quantification was determined as the lowest concentration that could be quantified with a RSD% < 20 %.

Analysis of positive samples. Real dairy milk samples previously analyzed by LC-MS/MS at the MAPAQ laboratories [5] and containing sulfadiazine and sulfadimethoxine were used for comparison with our LDTD-MS/MS method.

Results

Table 2. Recovery of the studied sulfonamides.

Compound	% Recovery	RSD (%)
Sulfacetamide	101.2	9.4
Sulfachlorpyrazine	103.7	10.5
Sulfachlorpyridazine	80.2	7.3
Sulfadiazine	81.7	7.4
Sulfadimethoxine	100.2	9.5
Sulfadoxine	93.2	5.1
Sulfaethoxytpyridazine	82.4	1.6
Sulfamerazine	81.1	8.7
Sulfamethazine	101.5	8.9
Sulfamethizole	80.8	7.4
Sulfamethoxazole	95.6	9.9
Sulfamethoxypyridazine	77.5	8.6
Sulfapyridine	93.6	7.3
Sulfaquinoxaline	99.2	9.5
Sulfathiazole	93.4	8.7
Sulfisoxazole	88.6	8.9

Table 3. Calibration curves and limits of quantification of the sixteen sulfonamides.

Sulfonamide	Equation	R^2	LOQ (µg L^{-1})
Sulfacetamide	y = 0.00456 + 0.0010x	0.9898	1.6
Sulfachloropyridazine	y = -0.00080 + 0.0005x	0.9983	3.1
Sulfachloropyridazine	y = 0.00040 + 0.0006x	0.9989	1.6
Sulfadiazine	y = 0.00054 + 0.0018x	0.9930	1.6
Sulfadimethoxine	y = -0.00020 + 0.0001x	0.9612	13
Sulfadoxine	y = -0.00045 + 0.0007x	0.9818	1.6
Sulfaethoxytpyridazine	y = -0.00111 + 0.0028x	0.9917	1.6
Sulfamerazine	y = 0.00118 + 0.0018x	0.9954	1.6
Sulfamethazine	y = 0.00002 + 0.0009x	0.9863	1.6
Sulfamethizole	y = -0.00110 + 0.0011x	0.9996	1.6
Sulfamethoxazole	y = -0.00239 + 0.0017x	0.9931	1.6
Sulfamethoxypyridazine	y = -0.00349 + 0.0042x	0.9952	3.1
Sulfapyridine	y = -0.00186 + 0.0026x	0.9931	1.6
Sulfaquinoxaline	y = -0.00640 + 0.0007x	0.9963	6.3
Sulfathiazole	y = -0.00031 + 0.0002x	0.9761	13
Sulfisoxazole	y = -0.00185 + 0.0010x	0.9907	1.6

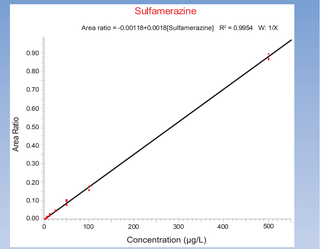

Figure 3. Calibration curve of sulfamerazine spiked in dairy milk.

Table 2. Results of the analysis of positive dairy milk samples and comparison to a LC-MS/MS method.

Sample identification	Sulfadiazine (µg L^{-1})		Sulfadimethoxine (µg L^{-1})	
	LDTD-MS/MS	LC-MS/MS	LDTD-MS/MS	LC-MS/MS
40573302	1.62	1.82	<LOQ	<LOQ
43381001	<LOQ	<LOQ	4.75	7.13

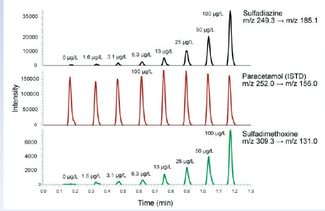

Figure 4. Internal calibration curve of sulfadiazine and sulfadimethoxine in dairy milk. Paracetamol was used as the internal standard.

Conclusion

- An ultra-fast and sensitive method of quantification of sixteen sulfonamide residues in whole dairy milk using LDTD-MS/MS was successfully developed and applied to real samples.

- Analysis of each sample was completed in only 16 seconds, 60 to 100 times faster than similar methods of determination based on LC-MS or GC-MS [6, 7].

- Recoveries from spiked milk samples ranged between 80.2 and 103.7 % with RSD% below 11%.Calibration curves were linear (R^2>0.99 in most cases) in the dynamic range (1.6 to 500 µg L^{-1}) and limits of quantification ranged between 1.6 and 13 µg L^{-1}. These LOQs are inferior or of the same magnitude as the maximum residue limits of sulfonamides in milk suggested by Health Canada (10 to 100 µg L^{-1})[8].

- Comparison of results obtained by LDTD-MS/MS and LC-MS/MS of whole dairy milk samples containing sulfadiazine and sulfadimethoxine showed a close agreement between the measured values.

- The proposed LDTD-APCI-MS/MS procedure is an efficient method for screening and monitoring sulfonamide residues in dairy milk with minimal sample preparation and without any HPLC separation.

Acknowledgements

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