Sulfonamide Residues in Milk : High Throughput Analysis Using LDTD-MS/MS

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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 bacteria strain of 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 (**Fig. 1**) using a new Laser Diode Thermal Desorption (LDTD) source coupled to MS/MS.

Goals

- Illustrate the efficiency of the LDTD-APCI source for highly charged matrices such as milk;

- Develop a rapid multi-residues high throughput LDTD-APCI MS/MS screening and quantifying method for 16 sulfonamide residues in milk using a thermal desorption process of 26 seconds.

Instrumentation

- Phytronix Technologies LDTD ionization source (model T-960);

- Thermo Scientific Corporation TSQ® Quantum™ Ultra AM mass spectrometer.

LDTD Ionization Process

The LDTD source uses an infrared laser diode to desorb samples that have been dried onto a well of the LazWell™ (96-well plate) without photon interactions with the sample. The desorbed gas-phase molecules are carried over by a carrier gas into a corona discharge region to undergo APCI and then transferred directly into the mass spectrometer.

Sample Preparation

Whole milk was spiked with in a concentration range from 2 ng/mL to 1000 ng/mL. A ratio of 1:5 of acetonitrile was added to the milk solution to precipitate mainly milk proteins. Indapamide, used as the internal standard, was added to the acetonitrile to yield a concentration of 150 ng/mL in the final sample. Following sample centrifugation, the sample was filtered on Nanosep 0.2 µm and a volume of 2 µL of the filtrated liquid was directly deposited onto a well of the LazWell™ plate and was allowed to dry at room temperature.

LDTD Parameters

Stabilization time	3 s
Laser power pattern	Ramp 0 to 19 % in 11 s
	Hold at 19 % for 9 s
	Hold at 0 % for 3
Carrier gas flow	4.0 L/min (Air)
Carrier gas temperature	20 ^{o}C

MS Parameters

Collision gas pressure	1.5 mTorr (Argon)
Scan time	0.02 s
Q1 and Q3 width	0.7 amu
Needle voltage	-5 kV
Capillary temperature	100 ^{o}C
Sweep gas flow	1 arbitrary unit

MRM Scan Parameters

| Compound	Q1	Q3	Collision Energy (V)
	(m/z)		
Sulfacetamide	213	170	25
Sulfadiazine	249	185	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
Sulfachlorpyridazine	283	107	34
Sulfaquinoxaline	299	144	28
Sulfisoxazole	266	171	28
Sulfadimethoxine	309	131	34
Sulfadoxine	309	251	34
Sulfamethoxypyridazine	279	156	25
Sulfaethoxypyridazine	293	156	25
Indapamide (internal standard)	364	190	26
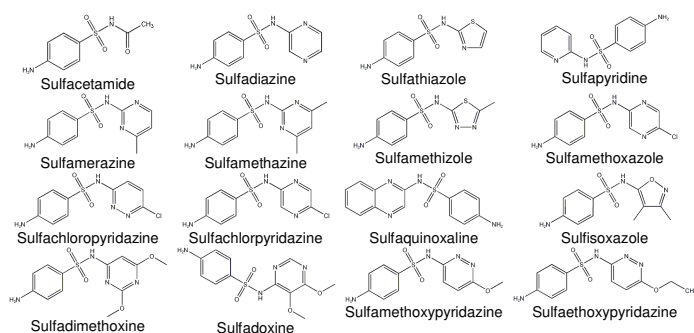
Figure 1 Chemical structure of the studied sulfonamides

Results and Discussion

All sulfonamide MS/MS spectra show typical fragmentation patterns and allow the identification of sulfonamide residues in milk using two MS/MS fragments (e.g. **Fig. 2**).
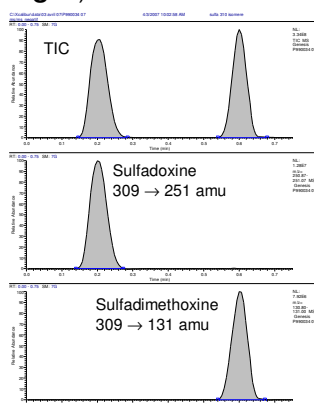

Figure 2 Method selectivity for sulfonamide isomers detection

Linearity range and limit of detection

The LDTD pattern was optimized to quantify each sulfonamide residue in milk. The fast thermal desorption process (26 seconds) yields the same profile over the concentration range under investigation. The linearity was tested from 2 ng/mL to 1000 ng/mL and the linearity ranges found are reported in **Table 1**. Reported range was found to be between 20 ng/mL to 5000 ng/mL[1] which correspond to the linearity range found for 7 sulfonamides tested. Lower linearity ranges (from 40 to 1000 ng/mL) were found and are associated to the combination of recovery variation and less intense daughter ions selected 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 1 Linearity range for sulfonamide residues detection in milk

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

The limit of detection was evaluated to be 2 ng/mL (0.66 pg deposited into well) for all sulfonamides as the background signal recorded for blank samples was low.

Conclusion

This application note shows the identification and quantification of 16 sulfonamides residues in whole milk by LDTD-APCI-MS/MS. The limit of detection for each compound is 2 ng/mL. A linearity range of 2 order magnitude is achieved for all sulfonamides.

References

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