Development of an Analytical Method for Simultaneous Determination of Amphenicols and Sulphonamides Residues in Milk Using QuEChERS and Analysis by CE-MS/MS

Application Note

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Abstract

This application note describes a fast analytical method for simultaneous determination of amphenicols and sulphonamides residues in milk using AOAC QuEChERS and analysis by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS).

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Introduction

The indiscriminate use of antibiotics by farmers in the treatment of infectious diseases of dairy cows, particularly mastitis, has caused adverse effects to humans, such as hypersensitivity, anaphylactic shock, and imbalance of intestinal flora. This is primarily due to indirect consumption of these compounds from milk and its derivatives [1]. In Brazil, the National Health Surveillance Agency (ANVISA) sets the maximum residue levels (MRLs) allowed for these compounds in milk [2]. The monitoring of antibiotic residues occurs through the National Residue Control Plan conducted by the Ministry of Agriculture, Livestock, and Supply.

The primary technique for the determination of veterinary drugs in food is liquid chromatography and immunoassays. This application note presents a method for the simultaneous quantification of chloramphenicol (CAP), florfenicol (FF), sulfamethazine (SMZ), sulfadimethoxine (SDM), and sulfathiazole (STZ) in milk samples using capillary electrophoresis coupled to Tandem mass spectrometry (CE-MS/MS).

Experimental

CE conditions

Instrument	Agilent 7100 CE System
Background electrolyte	100 mM NH_4OH, pH 10.8
Applied voltage	28 kV
Fused-silica capillary	PVA coated 50 µm × 85 cm total length
Injection	30 seconds at 100 mBar
Temperature	25 °C

MS conditions

Instrument	Agilent 6430 Triple Quadrupole LC/MS System
Ion mode	ESI, negative ionization
Sheath liquid	25 mM NH_4OH/ACN (50:50 v/v), pH 9.0
Flow rate	4.0 µL/min
Capillary voltage	4,500 V
Drying gas (N_2)	6 L/min
Drying gas temperature	250 °C
Nebulizer	6 psi

A CE-MS/MS method was developed for CAP, FF, SMZ, SDM, and STZ analysis using two MRM transitions for each compound, as shown in Table 1.

Table 1. MRM Conditions for the Analysis of CAP, FF, SMZ, SDM, and STZ

Analyte	RT (min)	Transition (*m/z*)	CE (eV)	Dwell time (ms)
Florfenicol	3.92	356 > 336	4	200
		356 > 185	12	
Chloramphenicol	4.80	321 > 257	4	200
		321 > 152	8	
Sulfadimethoxine	5.10	309 > 195	4	200
		309 > 66.0	36	
Sulfamethazine	5.30	277 > 122	28	200
		277 > 106	32	
Sulfathiazole	5.60	254 > 156	0	200
		254 > 98	12	

Sample preparation

Extraction of the veterinary drugs from milk was performed using the AOAC QuEChERS method. A 10-mL aliquot of homogenized sample was placed into a 50-mL centrifuge tube. After vortexing the sample for 30 seconds, 10 mL of acetonitrile was added to each tube using the dispenser. To each tube, an Agilent Bond Elut QuEChERS AOAC extraction salt packet (p/n 5982-5755) was directly added. The sample tubes were capped tightly, and hand-shaken vigorously for 1 minute. Then the tubes were centrifuged at 4,000 rpm for 5 minutes.

For the cleanup step, 8 mL of the supernatant was placed into an Agilent Bond Elut QuEChERS dispersive-SPE 15-mL tube (p/n 5982-5058). The tube contained 400 mg of PSA sorbent and 1,200 mg of anhydrous $MgSO_4$. The tubes were tightly capped and vortexed for 1 minute, and then centrifuged at 4,000 rpm for 5 minutes. In sequence, 0.5 mL of the extracts were transferred to vials and diluted with 0.5 mL of background electrolyte (1:1, v/v). The samples were then ready for CE-MS/MS analysis.

Results and Discussion

Figure 1 shows the MRM electropherograms obtained for the mixture of standards of CAP, FF, SMZ, SDM, and STZ at 100.0 µg/L.

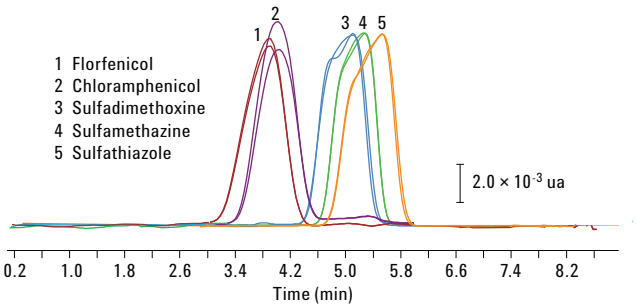

Figure 1. CE-MS/MS electropherograms at optimum conditions of 100.0 µg/L CAP, FF, SMZ, SDM, and STZ standard solutions in milk sample extract spiked.

The linearity of the analytical curve was studied using matrix-matched antibiotic standard solutions in six concentrations ranging from 5.0 to 200.0 µg/L (ppb). For all compounds, the correlation coefficients (R^2) calculated by linear regression was higher than 0.99. The example of the response for amphenicols and sulfonamides in milk extract obtained by QuEChERS methodology is shown in Figures 2 and 3.

The linear range in the matrix extract, Limits of Detection (LOD) calculated by standard deviation of the response and the slope of the calibration curve and the maximum residue levels (MRLs) allowed by ANVISA [2] are summarized in Table 2.

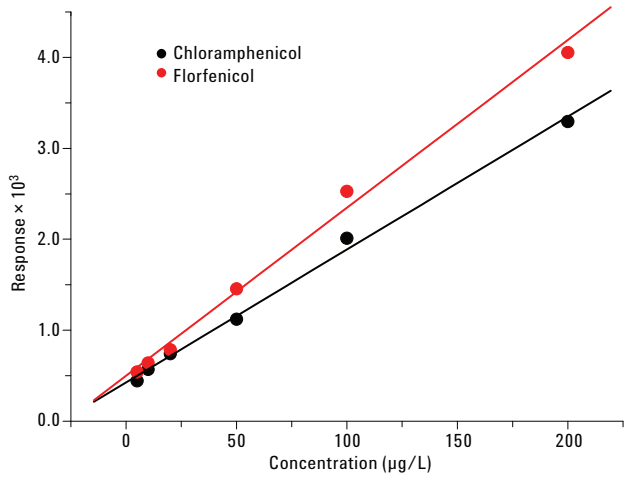

Figure 2. Calibration curves of FF and CAP obtained in milk sample extract spiked (from 5 to 200.0 µg/L).

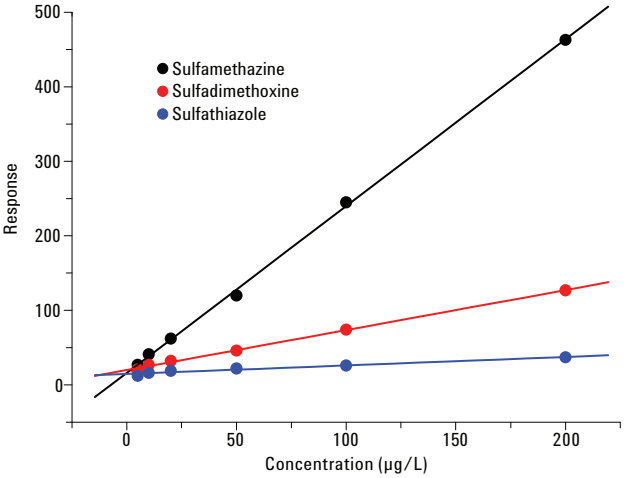

Figure 3. Calibration curves of SMZ, SDM, and STZ in milk sample extract spiked (from 5 to 200.0 µg/L).

Table 2. Linear Range, LOD (µg/L), and MRLs (µg/L) Recommended by ANVISA

Analyte	Linear range (µg/L)	LOD (µg/L)	MRL (µg/L)
Florfenicol	5–200	10.6	ND*
Chloramphenicol	5–200	5.7	0
Sulfamethazine	5–200	1.6	100**
Sulfadimethoxine	5–200	8.8	100**
Sulfathiazole	5–200	1.1	100**

* Not defined.
** Sum of all compounds.

Conclusion

The proposed method is fast (< 6.0 minutes for simultaneous analysis), and has been successfully applied to the determination of amphenicols and sulfonamides in samples of milk and dairy products. The sensitivity and specificity of the method are suitable to meet the residue limits established in most countries. The proposed methodology is simple, quick, and presents linear calibration curves and excellent precision data for replicate injections. This shows that the method should be a good alternative for classical methods of analysis.

References

1. M. Castro-Puyana, A.L. Crego, and M.L. Marina *Electrophoresis* 2008, **29**, 274.
2. National Health Surveillance Agency (ANVISA) at http://www.anvisa.gov.br

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