Determination of Sulfonamides in Commercial Veterinary Formulations by CE-MS/MS

Application Note

Pharma & Biopharma

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Abstract

A capillary electrophoresis tandem mass spectrometry (CE-MS/MS) method for the determination of four different sulfonamide antibiotics in commercial veterinary formulations has been developed. The samples were homogenized, solubilized in methanol, diluted in H_2O/methanol (50:50, v/v), filtered, and injected, followed by electrophoretic separation in a polyvinyl alcohol (PVA)-coated capillary using a solution of 0.5 M acetic acid, pH 2.5 as background electrolyte (BGE). Calibration curves were constructed at six different concentrations, from 50 to 500 µg/L, and exhibited determination coefficients higher than 0.995 with limits of detection lower than 2.7 µg/L for all analytes. The proposed method was successfully applied to the determination of sulfamethazine, sulfamethizole, sulfachloropyridazine, and sulfadimethoxine concentration in commercial samples of veterinary formulations, with a separation time of less than 6.0 minutes. This high level of separation performance with sulfonamides is largely attributable to the powerful selective capability of the tandem mass detection (MS/MS) and PVA-coated capillary used, considering the similar electrophoretic mobility of these molecules.

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Introduction

Sulfonamides are amides generated from sulfonic acids, and include a group of wide-spectrum antibiotics widely used in veterinary medicine for therapeutic and prophylactic purposes. These sulfonamides are analogues of *para*-aminobenzoic acid (PABA), and act to inhibit folic acid synthesis in susceptible microorganisms[1,2].

New methodologies are needed to quantify sulfonamides for the control of veterinary pharmaceutical products such as tablets, liquids, ointments, syrups, and other matrices. Refined analytical methods for sulfonamide determination have been reported; the most widely applied analytical technique for sulfonamides is liquid chromatography with ultraviolet or fluorescence detection (LC-UV or LC-FD) followed by gas chromatography with atomic emission spectrometry (GC-AES) and capillary electrophoresis[3-5] with UV or fluorescence detection (CE-UV or CE-FD). In this context, capillary electrophoresis coupled to tandem-mass spectrometry (CE-MS/MS) is a promising technique for the analysis of these compounds due to the high resolution that can be achieved in a relatively short analysis time, combined with low reagent consumption and low detection limits. Sulfonamides are cations at low pH values, making it possible to achieve separation by CE, and quantification by MS.

This Application Note presents a sensitive, selective, and fast CE-MS/MS method for the simultaneous quantification of sulfamethazine (SMZ), sulfadimethoxine (SDM), sulfachloropyridazine (SCP), and sulfamethizole (SMT) in veterinary drugs, using sulfathiazole (STZ) as internal standard. Figure 1 shows the chemical structures of the sulfonamides analyzed.

Experimental

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	0.5 M acetic acid, pH 2.5
Applied voltage	28 kV
Capillary	PVA capillary 75 µm id with 55 cm total length (p/n G1600-67319, 125 cm length, cut to 55 cm)
Injection	12 seconds at 50 mBar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 MS
Ion mode	ESI, positive ionization
Sheath liquid	BGE solution diluted 10x with H_2O/methanol (50:50 v/v)
Flow rate	5.0 µL/min
Capillary voltage	1,000 V
Drying gas flow (N_2)	5 L/min
Drying gas temperature	180 °C
Nebulizer pressure	12 psi

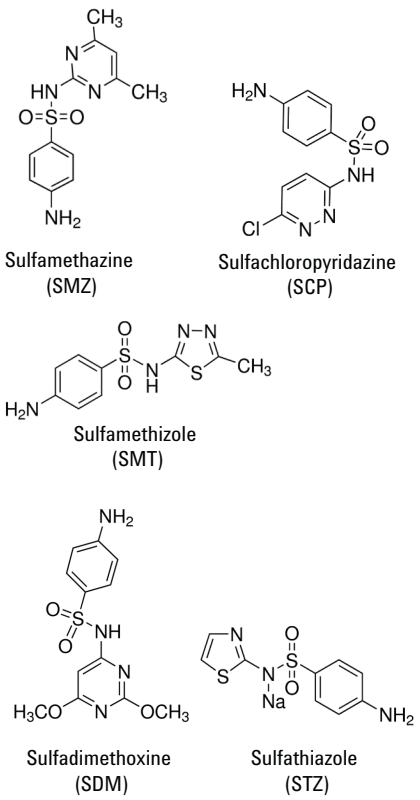

Figure 1. Chemical structures of sulfonamides.

All separations were performed at 25 °C using 0.5 M acetic acid, pH 2.5, as background electrolyte (BGE). The sheath liquid was prepared by diluting the background electrolyte 10-fold with H_2O/methanol 50:50 (v/v). The PVA-coated capillaries were preconditioned by flushing with Milli-Q water for 3 minutes followed by BGE for 5 minutes. Samples were introduced hydrodynamically over 12 seconds at 50 mbar, and analyzed with an applied voltage of 28 kV. The mass spectrometer was operated in positive ionization mode, using multiple reaction monitoring (MRM) mode for two specific transitions for each compound. Table 1 lists the monitored ions as well as other MS/MS acquisition parameters. A 250 µg/L sulfathiazole solution was used as internal standard. Sulfonamide standards were prepared by dilution from a more concentrated standard kit (Agilent Sulfa Drug LCMS OQPV Standard kit, p/n 5188-6523). Samples were purchased from veterinary stores, and were homogenized, solubilized with methanol, appropriately diluted with H_2O/methanol (50:50, v/v) and filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges, p/n A5300002).

It is worth noting that all sulfonamides except sulfamethazine exhibit good abundance of the fragment ion *m/z* 156, which is a typical sulfonamide fragment (Figure 2).

Results and Discussion

Background electrolyte and sheath liquid composition, applied potential, and hydrodynamic injection were optimized to achieve a good compromise between separation efficiency, sensitivity, and analysis time. Figure 3 shows the normalized MRM electropherogram of sulfonamide antibiotics standards in BGE. The migration time (t_M) for all sulfonamides was less than 6.0 minutes.

Figure 3 shows that the coupling between CE and MS/MS was satisfactory once the separation time was less than 6.0 minutes, and all sulfonamides were selectively identified due to the different fragment ions, despite the similar electrophoretic mobility of the intact molecules.

Table 1. Migration time (t_M), MS/MS acquisition parameters used for the identification and quantification of sulfonamides in veterinary pharmaceutical formulations.

Compound	t_M (min)	pKa[a]	Q1[b] (*m/z*)	Q3[c] (*m/z*)	CE[d] (V)	FE[e] (V)
Sulfamethazine	5.17	2.00	279.1	186.1* 124.1	12 24	132
Sulfathiazole (IS)	5.32	2.04	256.0	156.0* 92.1	12 28	102
Sulfamethizole	5.54	1.95	271.0	156.0* 108.0	10 20	150
Sulfachloropyridazine	5.65	2.02	285.0	156.0* 92.1	12 24	108
Sulfadimethoxine	5.71	1.95	311.1	156.0* 108.1	16 28	128

[a] pKa values were calculated at www.chemicalize.org (accessed October, 2016)
[b] Precursor ion (Q1)
[c] Fragment ions (Q3)
[d] Collision energy
[e] Fragmentor energy
* Transition used for quantification

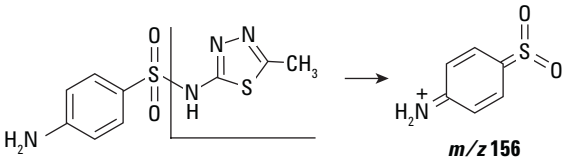

Figure 2. Fragmentation of sulfonamides.

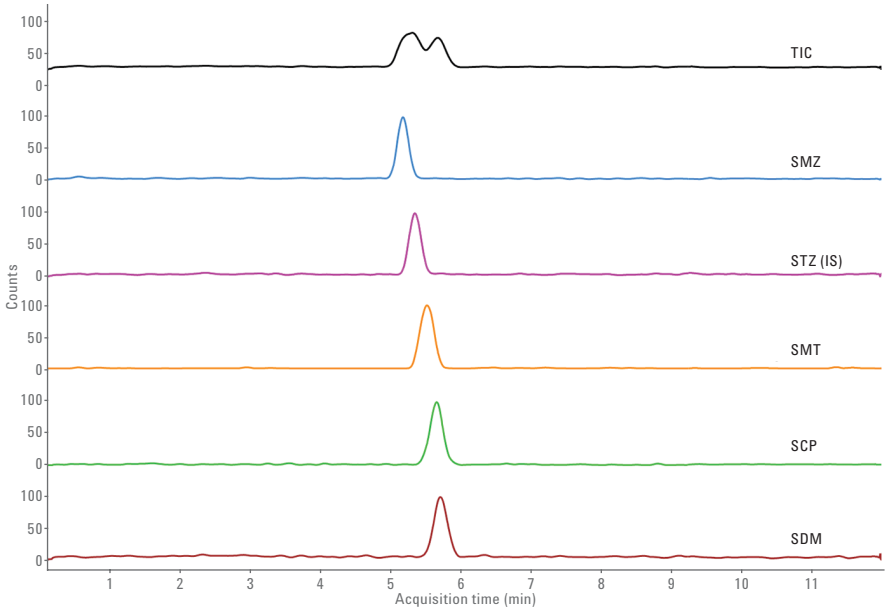

Figure 3. Normalized MRM electropherogram at optimum conditions of a mix of sulfonamide antibiotics at 500 µg/L each in BGE. TIC: total ion chromatogram; SMZ: sulfamethazine; STZ: sulfathiazole, used as internal standard at 250 µg/L; SMT: sulfamethizole; SCP: sulfachloropyridazine; SDM: sulfadimethoxine.

The linearity of the analytical curves was determined in BGE at six different concentration levels ranging from 50 to 500 µg/mL using Agilent MassHunter Quantitative Software (Figure 4). Each concentration was analyzed in triplicate; the run-to-run relative standard deviations (RSDs) ranged from 1.1 to 5.3 %. The limits of detection (LODs) and limits of quantification (LOQs) were determined considering the corresponding concentration to produce signal-to-noise ratios (S/N) of 3 and 10, respectively, using the baseline noise in a region close to the migration time of each sulfonamide. Table 2 shows the characteristic parameters for the developed method. Standard deviations of residuals were obtained by analysis of variance (ANOVA).

Table 2. Analysis of results from the proposed method for the determination of sulfonamides in commercial veterinary formulations.

Compound	Slope	Intercept	R^2	$S_{y/x}$	LOD (µg/L)	LOQ (µg/L)
Sulfamethazine	0.4705	0.0144	0.998	0.015	2.7	9.0
Sulfamethizole	0.3075	0.0041	0.998	0.009	2.4	7.9
Sulfachloropyridazine	0.3061	-0.0183	0.998	0.009	2.2	7.4
Sulfadimethoxine	0.5888	-0.0094	0.995	0.025	2.4	7.9

R^2 = determination coefficient; $S_{y/x}$ = standard deviation of residuals; LOD = limit of detection; LOQ = limit of quantitation. Results obtained by analysis of variance (ANOVA).

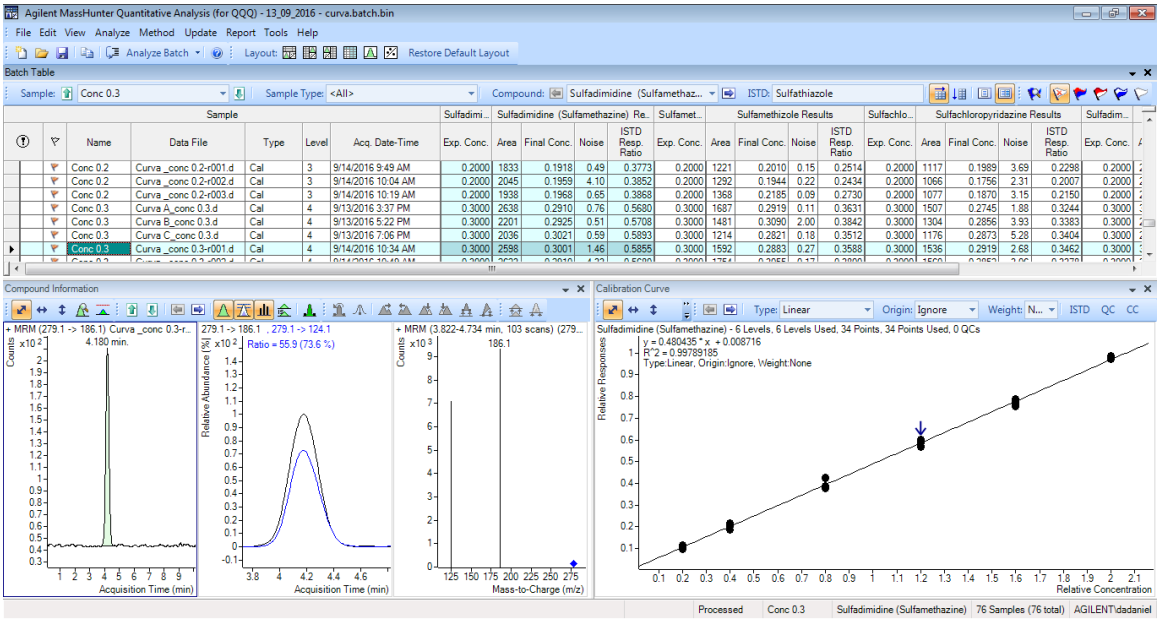

Figure 4. Agilent MassHunter software quantitative window used for the determination of sulfonamide antibiotics in commercial veterinary formulations.

The external standard calibration method was used in the analysis of sulfonamide antibiotics in commercial veterinary formulations; interferences from the formulation excipients were not observed. Table 3 shows the results obtained for sulfonamide antibiotics in seven different commercial samples by CE-MS/MS. Corresponding standard deviations were calculated from three independent measurements of each sample. The concentrations obtained by the described CE-MS/MS method were very close to the labeled values.

According to the presented results, CE-MS/MS offers a precise and accurate method for the analysis of sulfonamides in veterinary samples. In fact, the calculated t-test values were lower than T_{critic} at a 95 % confidence level.

Conclusion

We have shown that CE-MS/MS is well suited for the analysis of sulfonamide antibiotics in commercial veterinary formulations. The proposed method presented a linear response to sulfonamides in the concentration range from 50 to 500 µg/L, with the LOD below 2.7 µg/L. It uses a small amount of sample with low reagent consumption, and features easy sample treatment, without requiring an extra cleanup step. In addition, the method is fast, at less than 6 minutes per analysis, and delivers linear calibration curves and excellent precision for replicate injections. The sensitivity and specificity of the method demonstrate its potential for use in the analysis of other pharmaceutical products.

Table 3. Results obtained for sulfonamide analysis in veterinary formulation samples by CE-MS/MS.

Sample	Active ingredient	Label	Found*	RSD (%)	**t-test
Giarcid	Sulfadimethoxine	50 mg/comp	49.8 ± 1.8	3.6	0.192
Vetococ	Sulfamethazine	12.5 g/100 g	13.0 ± 0.6	4.6	1.443
Trissulfin	Sulfadimethoxine	115 mg/400 mg	113.1 ± 3.6	3.2	0.914
Otolin	Sulfamethazine	4 g/100 mL	4.04 ± 0.20	4.9	0.346
Sulfamicina	Sulfadimethoxine	1.25 g/100 mL	1.27 ± 0.02	1.6	1.732
Avemetazina	Sulfamethazine	10 g/100 mL	10.20 ± 0.47	4.6	0.737
Cosumix	Sulfachloropyridazine	62.5 g/100 g	64.9 ± 2.6	4.0	1.599

* For n = 3

** Unpaired t-test at 95 % confidence. T_{critic} = 4.303.

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Published in the USA, November 30, 2016
5991-7682EN

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