Fast Analysis of Terbutaline in Pharmaceuticals by CE-MS/MS

Application Note

Clinical Research

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Abstract

A capillary electrophoresis tandem mass spectrometry method (CE-MS/MS) for the determination of terbutaline in pharmaceutical products has been developed. The samples were diluted in background electrolyte, filtered, and injected, followed by electrophoretic separation in 25 mM propionic acid and 25 mM ammonium hydroxide as the background electrolyte (pH 7.0) using a fused silica capillary. The correlation coefficient of the calibration curve in the range of 0.05 to 50 µg/mL was 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were 0.01 and 0.03 µg/mL, respectively. The proposed method was successfully applied to the analyses of commercial syrup samples. The results were in accordance with the nominal value, and with those obtained using high performance liquid chromatography.

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Introduction

Terbutaline is a β2-adrenergic receptor agonist used as a reliever inhaler in the management of asthma symptoms. It is also used as an anticontraction medication to delay preterm labor for up to 48 hours[1]. Figure 1 shows the molecular structure of terbutaline.

It is important to develop methodologies to quantify terbutaline for the control of pharmaceutical products such as inhalants, aerosols, injectables, tablets, liquids, and syrups[2]. Different analytical methods for terbutaline determination have been reported in studies[3-5]. In this work, a capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method was developed. This CE-MS/MS method was evaluated in comparison to the official method described in British Pharmacopoeia[6] for the determination of terbutaline in drug samples.

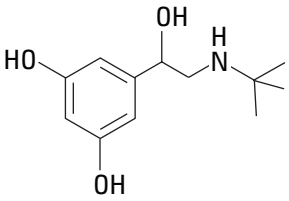

Figure 1. Structure of terbutaline.

Experimental

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	25 mM propionic and 25 mM NH_4OH in H_2O, pH 7.0
Applied voltage	25 kV
Capillary	Fused silica capillary 50 µm id × 60 cm total length
Injection	10 seconds at 50 mBar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 MS
Ion mode	ESI, positive ionization
Sheath liquid	BGE diluted four times in H_2O/methanol 1:1 (v/v)
Flow rate	5.0 µL/min
Capillary voltage	2,000 V
Drying gas flow (N_2)	8 L/min
Drying gas temperature	250 °C
Nebulizer pressure	10 psi

The stock solution of 0.1 M terbutaline sulfate was prepared by dissolving the solid salt in ultrapure water, and stored refrigerated in a dark flask. The standard solutions for the calibration curve were diluted with background electrolyte just before the measurements. All separations were performed at 25 °C using a 25 mM propionic acid and 25 mM NH_4OH in H_2O, pH 7.0, as background electrolyte (BGE). New fused silica capillaries were preconditioned by flushing 0.1 M NaOH for 3 minutes, followed by Milli-Q water and BGE for 3 minutes each.

Samples were introduced hydrodynamically in 10 seconds at 50 mbar, and analyzed with an applied voltage of 25 kV. The mass spectrometer was operated in positive multiple reaction monitoring (MRM) mode using two transitions. The most intense transition was used for quantification, and the other was used as a qualifying ion. Table 1 lists the monitored ions as well other MS/MS acquisition parameters.

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

Compound	t_M (min)	pKa[a]	Q1[b] (*m/z*)	Q3[c] (*m/z*)	CE[d] (V)	FE[e] (V)
Terbutaline	3.4	9.76	226.1	152*	12	100
				107	32	

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

Sample preparation

The pharmaceutical formulations of terbutaline were purchased from a local drugstore. All samples were homogenized and diluted 1:100 with the BGE. The syrup samples were then filtered through a 0.2-µm PVDF and PP membrane (Agilent Captiva filter cartridges p/n A5300002), and analyzed.

Results and Discussion

The BGE, sheath liquid composition, applied potential, and hydrodynamic injection were optimized for separation efficiency and sensitivity. Figure 2 shows the MRM electropherogram of a standard solution of terbutaline in BGE. The migration time (t_M) was only 3.5 minutes.

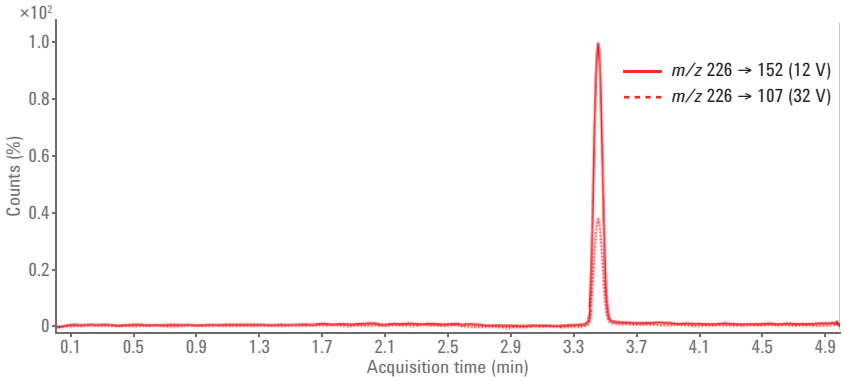

Figure 2. MRM electropherogram of 0.5 µg/mL terbutaline in BGE at optimum conditions.

The linearity of the analytical curve was studied in BGE at 10 different concentration levels, and analyzed in triplicate, ranging from 0.05 to 50 µg/mL. The correlation coefficient (R^2) calculated by linear regression was greater than 0.999, using Agilent MassHunter Workstation Quantitative Analysis software, as shown in Figure 3.

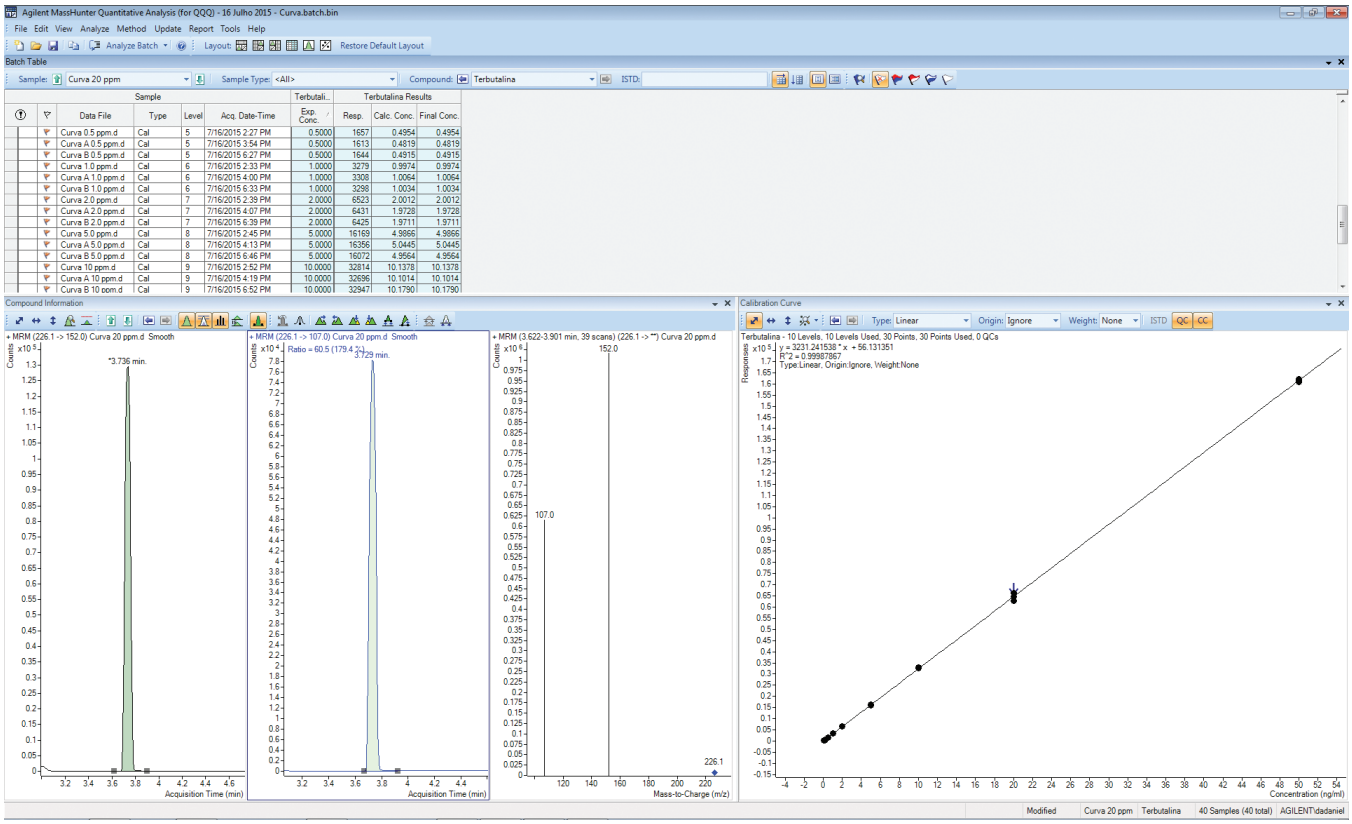

Figure 3. Calibration curve using Agilent MassHunter Quantitative Analysis software.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined as 0.01 and 0.03 µg/mL, respectively. Considering the LOD as three times the baseline noise, the LOQ was considered as the concentration that produced a signal 10 times the baseline noise, in a time close to the migration time of terbutaline.

An external standard calibration method was used in the determination of the terbutaline in commercial drugs sold at drugstores, and no interference from the formulation excipients was observed. Table 2 shows the results obtained for terbutaline in two different commercial samples of syrup by CE/MS/MS and the official HPLC method recommended by the British Pharmacopoeia[6]. Corresponding standard deviations were calculated from three independent measurements of each sample. The concentration obtained by the proposed CE-MS/MS method was very close to the labeled value (300 mg/mL), and with the results obtained by HPLC.

Table 2. Results obtained after analyses of terbutaline sulfate in two syrup samples by CE-MS/MS and HPLC[5]. Labeled value: 300 µg/mL.

Sample	CE-MS/MS (µg/mL)[a]	HPLC (µg/mL)[a]
S1	300.2 ± 5.6	301.0 ± 5.8
S2	299.0 ± 8.4	300.0 ± 5.6

[a] Average ± standard deviation for three determinations.

Conclusion

We have been able to show that CE-MS/MS is well suited for the analysis of terbutaline in pharmaceutical products. The proposed method presented a linear response to terbutaline sulfate in the concentration range from 0.05 to 50 µg/mL, with an LOD of 0.01 µg/mL. It uses a small amount of sample with low reagent consumption, and has easy solubilization and dilution procedures, without requiring an extra cleaning step. In addition, the method is fast, less than 5 minutes per sample, and presents linear calibration curves and excellent precision data for replicate injections. The sensitivity and specificity of the method demonstrates its potential for analysis of other pharmaceutical products.

References

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