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Research paper

Development and validation of a capillary electrophoresis method for the determination of sulfate in effervescent tablets

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Abstract

A suitable capillary electrophoresis (CE) method was developed and validated for sulfate anion determination in effervescent tablets of Digedryl®. The large excess of other ions in the matrix (i.e. excipients) constituted the main difficulty of this method's development. So an original analytical procedure for both the conditioning and rinsing of the capillary was purposed including a running electrolyte constituted by boric acid 20 mM and hexamethonium dibromide 0.75 mM at pH 8.00. Separation was carried out on a 60.2 cm (50 cm to the detector) × 0.75 μm i.d. fused-silica capillary at a potential of −29 kV and 35 °C. Indirect UV detection was performed at a wavelength of 254 nm using a background electrolyte containing potassium chromate. Nitrate anion was used as an internal standard for quantification. This CE method was validated in terms of selectivity, linearity, accuracy and precision.

Keywords: Capillary electrophoresis; Sulfate anion; Validation; Pharmaceutical analysis; Quality control

1. Introduction

Sodium sulfate is an active drug which is used as saline laxative. Its association with monosodium phosphate in Digedryl® is prescribed to prevent dyspepsia. The sulfate anion only represents 1.5% (w/w) of the total mass of effervescent tablet. The remaining part of the tablets is composed by a mixture of excipients including anhydrous sodium hydrogen carbonate, sodium benzoate, anhydrous citric acid, anhydrous tartaric acid and anise oil. Clearly the large excess of other ions in the matrix is a critical analytical problem in order to perfect a quantification method for sulfate anion.

The turbidimetric determination of sulfate in different matrices has been often reported [1–3] and presents the advantage of being simple. However it suffers from a lack of specificity which prevents its use for this pharmaceutical formulation. Ion chromatography (IC) has been usually applied for the simultaneous determination of various anions including sulfate [4–7]. However the massive amount of other tablet anions compared with sulfate quantity could adversely affect the peak shapes resulting in a rather poor separation.

CE with indirect UV detection constitutes a suitable method since this technique has been successfully applied to the analysis of many organic and inorganic anions [8–10]. CE presents some advantages in terms of short-time analysis and small solvent consumption. Furthermore, it is well established as an analytical technique for the assessment of small molecules in pharmaceuticals [11–13]. But the striking performance of CE compared with that of IC is the separation efficiency which could allow a convenient separation and quantification of sulfate in spite of the large amount of interfering anions. However, a disturbance of the CE separation could occur because of the high ionic concentration of sample. The difference of both the viscosity and conductivity between the sample zone and

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the running electrolyte could increase diffusion effects and disturb the baseline.

The aim of this work is the development and the optimisation of a simple electrophoretic method for the quantification of sulfate anion in Digedryl® despite a highly ionic matrix. This method is validated according to the International Conference on Harmonisation (ICH) guidelines [14].

2. Experimental

2.1. Chemicals and solutions

All used chemicals were of analytical grade and purchased from Fluka-Riedel-deHaën (St. Quentin Fallavier – France). Effervescent tablets were obtained from commercial source. Digedryl® is a mixture of two active principles (anhydrous sodium sulfate, anhydrous monosodium phosphate) and different excipients (anhydrous sodium hydrogen carbonate, sodium benzoate, anhydrous citric acid, anhydrous tartaric acid and anise oil). Deionized water was obtained from a Milli-Q system.

The running electrolyte (E) was constituted by boric acid (BA) at 20 mM and hexamethonium dibromide (HMB) at 0.75 mM. The background electrolyte (BGE) was obtained by adding potassium chromate at 5 mM to the previous solution. In each case the pH was adjusted to 8.00 with 0.1 M sodium hydroxide. E and BGE solutions were stored at 4 °C and prepared daily by diluting stock solutions in order to obtain the required final concentration. They were filtered through 0.45 μm nylon syringe filters before use.

2.2. Instrumentation

CE experiments were performed using a Beckman P/ACE MDQ instrument (Beckman-Coulter, Fullerton, CA) equipped with a diode array detector. A P/ACE system MDQ software was used for CE control, data acquisition and data handling. The operating conditions for CE are listed in Table 1.

Before its first use, the capillary was conditioned by rinsing with 0.1 M sodium hydroxide for 6 min followed by pure water for 1 min and finally with the running electrolyte (E) for 20 min. The capillary was conditioned daily with E (5 min, 20 psi) before use. Because of the slight solubility of one ingredient of the tablets each analysis needed many rinsing precautions as discussed in the next part. For the same reason the capillary was subjected to a rigorous rinsing procedure after daily analysis. It was kept full of methanol for 5 min then washed with water (5 min, 20 psi). The capillary was left filled with water between analysis and when not in use.

Table 1
Operating conditions for CE

Capillary	50 cm effective length × 75 μm i.d., total length 60.2 cm, uncoated
Voltage	29 kV (negative polarity)
Detection	UV detection, 254 nm (indirect detection)
Injection	Hydrodynamic, 0.5 psi for 5 s
Capillary temperature	35 °C
Electrolyte	20 mM BA and 0.75 mM HMB, pH 8.00
Background electrolyte	20 mM BA, 0.75 mM HMB and 5 mM K_2CrO_4, pH 8.00

2.3. Calibration curves

The majority of imprecision in CE could be effectively eliminated by use of an appropriate internal standard (IS) [15]. Nitrate was found to be suitable for the analysis. Potassium nitrate was used.

Regression curves were obtained by plotting the area ratio between analyte and IS vs. analyte concentration. The linearity for both standard (std) and reconstituted dosage formulation (rdf) solutions was evaluated across the 80–120% range. The 100% corresponded to a concentration equal to 73.04 mg L^{-1}. Fixed nitrate concentration was chosen to allow adequate quantification (69.91 mg L^{-1}).

For each calibration curve five separate weighings of analyte or synthetic mixture (i.e. rdf) were used. Each solution was prepared in water and injected in triplicate. Before injection the solutions were filtered through a 0.22 μm membrane and sonicated.

2.4. Tablet assay

Twenty effervescent tablets were weighted and the average mass value was calculated. For each determination, an independent tablet was accurately weighed and put into a beaker with about 70 mL H_2O. The solution was stirred then sonicated until effervescent tablet particles were completely dissolved. The content was transferred into a 100 mL volumetric flask and completed to volume with water. The final solution and IS solution were diluted with water in order to prepare the working concentration range. Before injection the solutions were filtered through a 0.22 μm membrane and sonicated.

3. Results and discussion

3.1. Preliminary experiments

A running electrolyte constituted by 20 mM BA and 0.75 mM HMB was used in the first step of the analytical procedure to achieve the conditioning of capillary wall (Table 2).

The optimum pH value for a good separation and a correct baseline was 8.00. Higher pH values increased electroosmotic flow (EOF) which led to the analysis time decrease. Due to the important concentrations of excipient anions, the separation between anions of interest (i.e. sulfate and nitrate) and other anions included in the tablets (i.e. namely phosphate, citrate and tartrate) was lost.

Table 2
Analysis procedure

Event	Running solutions	Conditions
Rinse/conditioning	E	30 s, 20 psi
Rinse	BGE	30 s, 20 psi
Injection (sample)		5 s, 0.5 psi
Separation	BGE	4 min, −29 kV, 35 °C
Rinse	Methanol	1 min, 40 psi (reverse mode)
Rinse	H_2O	30 s, 20 psi
Rinse	NaOH 0.1 M	30 s, 20 psi
Rinse	H_2O	30 s, 20 psi

Therefore at pH 8.00, different concentrations of BA (5–40 mM) were tested which is commonly used as electrolyte in CE [16–18]. With BA concentration at 20 mM, the peak areas were found to be greater and the reproducibility of both migration times and areas was better, with respect to an acceptable analysis time. Furthermore with this electrolyte concentration, the current was low and constant (−45 μA).

By introducing HMB for reversing EOF [19], the run time of the analysis was decreased. HMB with its small hydrophobic moiety and double charge was preferred to other surfactants more commonly used because of its better water-solubility [20–23]. As expected in this condition, the order of migration was sulfate and then nitrate anion.

Due to the lack of absorbance properties of the sulfate anions, indirect UV detection was performed using a background electrolyte with a strong UV absorption. Previous works devoted to anion separation suggest the use of chromate ion for sulfate detection [24,25] since their electrophoretic mobilities are similar (i.e. 0.59 and 0.52 $cm^2\,K\,V^{-1}\,s^{-1}$ for chromate and sulfate anions, respectively) [8]. A 5 mM chromate concentration was selected. Lower concentrations produced a sensitivity problem while higher concentrations caused more baseline drift. Finally BGE was prepared by adding 5 mM potassium chromate to the running electrolyte E. We noticed that using BGE during the conditioning step decreased the capillary performances. So this BGE was used after the conditioning rinsing and constituted the second step of the analytical procedure.

Poor migration time repeatability and blocked capillary problem were observed due to deposition of the anise oil (i.e. excipient of Digedryl®) onto the inner surface of the capillary. If it was not removed after each run, analyte migration time was considerably altered from run to run. A cleaning procedure with methanol after each separation was used (i.e. vial in outlet position). Then 3 steps of rinsing with water, 0.1 M NaOH and water again were implemented at the end of each run. Thus methanol was eliminated and "HMB coating" removed.

As described in Table 2, an original analytical procedure was purposed to avoid capillary contamination and improved its performances. Using this rinsing procedure, more than 500 injections were performed on the same capillary.

Different anions migrating close to sulfate ions were tested as IS. Nitrate was found to be the most appropriate because it migrates between sulfate and citrate anion (Fig. 1) which is one of the excipients.

Under all these conditions, a satisfactory resolution (about 2.3) and good relative migration times sulfate/nitrate (a mean value of 0.93 was founded with a RSD of 0.17) were obtained from 20 determinations. The asymmetry factor of sulfate peak (about 1.4) was acceptable considering both indirect UV detection method and low sulfate content in a tablet (1.5% w/w).

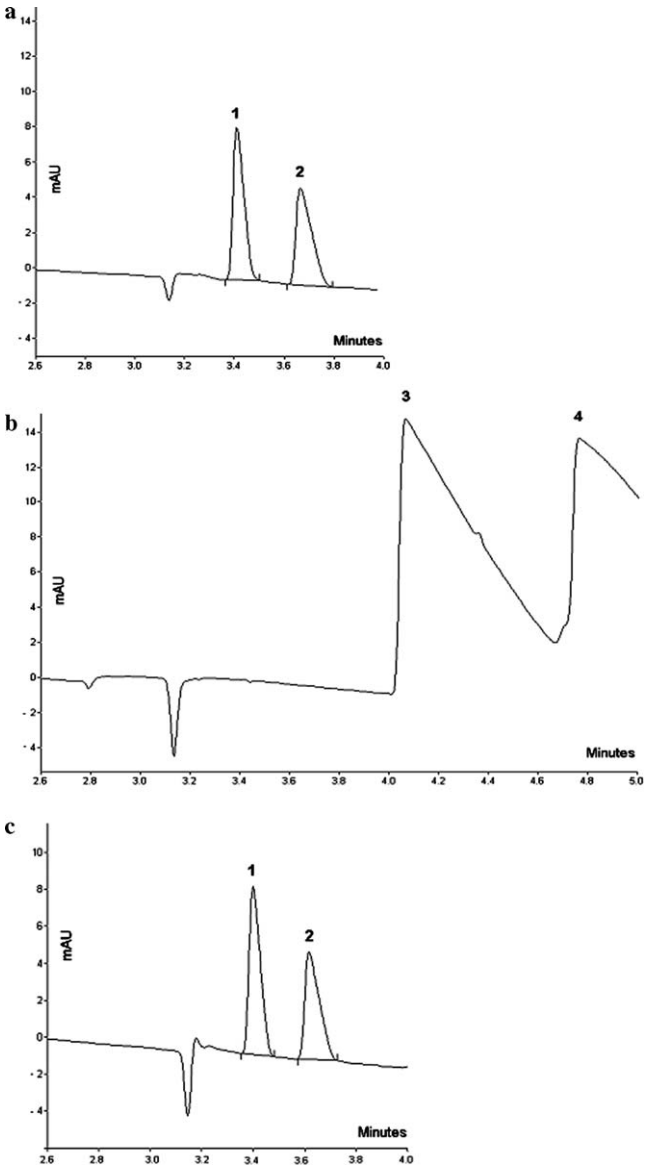

Fig. 1. Electropherograms: (a) standard solution: sulfate 73 mg L^{-1} (1), nitrate 70 mg L^{-1} (2); (b) reconstituted dosage formulation solution without sulfate: citrate 732 mg L^{-1} (3), tartrate 731 mg L^{-1} (4); (c) Digedryl solution containing sulfate 73 mg L^{-1} (1) and spiked with nitrate 70 mg L^{-1} (2). All conditions are described in Table 1.

3.2. Validation

The developed CE method was validated with respect to specificity, linearity, accuracy, injection repeatability, precision and limit of detection (LOD) and quantification (LOQ).

3.2.1. Selectivity

The selectivity of the method was assessed by analysing a solution containing all the components tablet except sulfate anion. The electropherogram (Fig. 1(b)) was free of any peak between 3.25 and 4 min. Sulfate and nitrate ions migrated in this interval (Fig. 1(a)) with relative migration time sulfate/nitrate of 0.93. Citrate and tartrate ions which are present in high amount in the tablets presented strong peak tailing right after the nitrate. A "peak system" was observed at 3.15 min because of the presence of bromide as anion of hexamethonium. Consequently no compound interferes with analytes of interest and the CE method was applicable for analysis of sulfate in Digedryl® as shown in Fig. 1(c).

3.2.2. CE system repeatability

CE system repeatability was determined by injecting 10 times the same standard solution. The precision for both the relative peak areas and relative migration times expressed as RSD was 0.58% and 0.07%, respectively. Above 10 injections with the same electrolyte vial, the migration times increased significantly (more than 5%). The migration time alteration may be due to the electrolyte which was not a buffer solution. Therefore the electrolyte vial must be changed each 10 injections.

3.2.3. Linearity

According to SFSTP guideline validation [26], linearity was tested on 3 days at 5 concentration levels in the 80–120% range of the nominal concentration on std and rdf solutions. E, BGE and various solutions were prepared each day. In order to confirm linearity different statistical tests were performed. They are reported in Table 3.

The relationships were linear. The calculated curves went through the origin. The t-test was calculated to statistically determine if the difference between the slopes and the intercepts of std and rdf calibration curves was significant. The t-values (0.17 and 0.08, respectively) were found to be less than the critical t-value at 95% confidence level (2.06). In spite of the high concentration of other ions in rdf solutions, inducing different electrical fields in sample and IS zones, no matrix effect was noticed. Therefore, the determination of sulfate anion content in tablets can be performed using std calibration curve.

Table 3
Linearity study parameters and regression results

	std value	rdf value
Linearity		
Nominal concentration (mg L^{-1})	73.04	73.04
Concentration range of nominal concentration (%)	80–120	80–120
Number of concentration levels	5	5
Regression results		
Determination coefficient (r^2)	0.997	0.991
y-intercept	0.0058 ± 0.0187	0.0087 ± 0.0320
Slope	0.0163 ± 0.0003	0.0164 ± 0.0004

3.2.4. Accuracy

Accuracy was assessed over the same concentration range that was investigated in the linearity study. Table 4 shows mean recoveries and confidence intervals calculated by using all 15 points of the rdf solutions. As all confidence intervals are included in the range 98–102%, the CE method is accurate.

3.2.5. Precision

Precision of the method was tested on 15 rdf samples that covered the specified range of the procedure (5 concentrations/3 replicates each at 3 different days). Moreover the third day, the capillary was replaced by a new one. The RSD values were 2.34% and 3.34% for repeatability and intermediate precision, respectively. They were within the acceptance criteria of 5% and showed that the method is precise.

3.2.6. Limit of detection and quantification

For determining both LOD and LOQ several approaches are possible. The selected determination was based on the standard deviation (σ) and slope (S) evaluated from the calibration curve of analyte.

$$\mathrm{LOD} = 3\sigma/S \qquad \mathrm{LOQ} = 10\sigma/S.$$

In these conditions LOD and LOQ were estimated at 5.9 and 19.5 mg L^{-1}, respectively.

3.2.7. Analysis of pharmaceutical tablets

Three batches of Digedryl® were analysed for sulfate content with two different capillaries (CapI and CapII). Three tablets were evaluated for each batch. Triplicate injections were carried out for each analysed tablet. The results are reported in Table 5.

These results are in close agreement with the theoretical content of SO_4^{2-} in Digedryl®.

Table 4
Accuracy results

Concentration levels (%)	Mean recovery (%)[a]	Confidence interval
80	101.50 ± 0.16	101.34–101.66
90	99.97 ± 0.66	99.31–100.63
100	100.77 ± 0.72	100.05–101.49
110	100.81 ± 1.11	99.7–101.92
120	100.82 ± 0.43	100.39–101.25

[a] 95% confidence level.

Table 5
Results of sulfate determination in Digedryl®

Batch	Theoretical concentration (mg/tablet)	Found concentration[a] (mg/tablet)		Recovery[a] (%)		RSD[a] (%)	
		CapI	CapII	CapI	CapII	CapI	CapII
3003	36.52	36.85	37.21	100.90	101.87	1.47	4.55
3004	36.52	37.84	36.99	103.61	101.28	2.85	2.84
3005	36.52	37.54	36.5	102.78	99.97	1.91	4.74

[a] Mean of nine determinations.

4. Conclusion

A CE method with indirect UV detection was successfully developed for the quantification of sulfate anion in a pharmaceutical formulation. A specific rinsing procedure was purposed that provided a stable and efficient response of the capillary and prevented its blockage because of the weak solubility of anise oil. Satisfactory precision data were obtained using nitrate as internal standard. Hexamethonium bromide was found to be the most suitable flow modifier since it is widely water-soluble.

The method was validated according to ICH guidelines and SFSTP recommendations and fulfilled all requirements. Consequently, the proposed procedure allows the determination of sulfate in Digedryl® despite the presence of large amounts of interfering anions in the tablets. Once again CE seems to be a convenient method for pharmaceutical quality control and may be considered for the routine analysis of drug substances.

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