Validation of a capillary electrophoresis procedure for the determination of calcium in calcium acamprosate

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Abstract

The quantitative aspects of a capillary electrophoresis (CE) technique for the determination of calcium in calcium acamprosate are reported. Separation was carried out on a 57-cm (50 cm to the detector)×75-μm I.D. fused-silica capillary at a potential of 10 kV and 25°C, using as electrolyte 10 mM imidazole containing 1 mM tetrabutylammonium sulfate adjusted to pH 4.5 with sulfuric acid. Standard solutions of calcium carbonate and test solutions of calcium acamprosate containing 10 ppm of Ca^{2+} and Mg^{2+} (internal standard) were injected hydrodynamically for 3 s. Indirect UV detection was carried out at 214 nm. A satisfactory agreement was found between CE, complexometry and theoretical content for determination in calcium acamprosate. Precision of CE on different capillaries using an internal standard complies with the requirements of quality control for the drug substance.

Keywords: Pharmaceutical analysis; Validation; Calcium; Calcium acamprosate

1. Introduction

Calcium acamprosate [CH$_3$-CO-NH-(CH$_2$)$_3$-SO$_3$]$_2$ Ca is an active drug which is administered as tablets to maintain abstinence in alcohol-dependent patients. EDTA titration is currently used for the assay of calcium both in the drug substance and drug formulation. However, a technique capable of being more specific and to give auditable raw data was desired by Lipha Laboratories. Preliminary investigations showed that capillary electrophoresis (CE) could be a potential technique for this purpose. In a wide range of areas (environmental, food, chemical analysis,...) small ion analysis by CE has shown a good correlation with other techniques, such as atomic absorption spectrometry (AAS) [1], AAS and ion chromatography (IC) [2], IC and gravimetry [3],

IC [4–6], inductively coupled plasma (ICP) [7] or ethylenediaminetetraacetic (EDTA) titration [8]. It has also proven to be a valuable option for the determination of counter-ions such as chloride, sulfate, potassium and sodium in drug substances [9–12]. This paper concerns the quantitative aspects of the calcium determination in calcium acamprosate.

2. Experimental

2.1. Chemicals

Calcium acamprosate (99.98% purity by EDTA titration) was kindly provided by Lipha laboratories (Lyon, France). Calcium carbonate (>99.5% purity by complexometry) from Merck (Darmstadt, Germany), magnesium sulfate heptahydrate (analytical grade) from Labosi (France), imidazole; (99% puri-

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ty) from Sigma (Buchs, Switzerland) and tetra-butylammonium sulfate (for synthesis) from Merck were used. Milli-Q distilled water was used through-out.

2.2. Solutions

2.2.1. Stock solutions

Stock standard solution of calcium (100 mg l^{-1} Ca^{2+}) was prepared by dissolving an appropriate amount of calcium carbonate in water acidified by a few drops of diluted hydrochloric acid (1:5, v/v).

A stock solution of calcium acamprosate (100 mg l^{-1}) was prepared by dissolving an appropriate amount of the salt in water. The theoretical content of Ca^{2+} calcium acamprosate is 10.01%.

Stock solution of magnesium sulfate was prepared by dissolving an appropriate amount to give a 100 mg l^{-1} Mg^{2+} solution in water.

2.2.2. Working solutions

Working solutions were prepared by adding a 10-ml aliquot of stock solution (calcium carbonate or acamprosate) to an equal volume of magnesium sulfate stock solution in a 100-ml volumetric flask and diluting to volume with water. These solutions were 10 mg l^{-1} Ca^{2+} and Mg^{2+}.

2.2.3. Electrolyte solutions

The electrolyte for separation was a 10 mM imidazole aqueous solution containing 1 mM tetra-butylammonium sulfate adjusted carefully to pH 4.5 with 0.3 M sulfuric acid. The electrolyte solution was stored at 4°C and used within 15 days of preparation.

2.3. Apparatus and operating conditions

CE separations were carried out on a Beckman (Palo Alto, CA, USA) P/ACE 5500 instrument equipped with a filter UV absorbance detector set at 214 nm. The system was controlled by a Dell Optiplex 466/L with Gold software. Separation was carried out on a 57-cm (50 cm to detector)×75-μm I.D. fused-silica capillary (Beckman, or Composite Metal Services, Hallow, UK) maintained in a car-tridge with a detection window of 800×100 μm. The capillary was conditioned prior to its first use by

rinsing with 0.1 M NaOH for 20 min and the water for 10 min. At the start of each sequence, the capillaries were washed with 0.1 M sodium hy-droxide followed by water for 5 min. All the separations were conducted using 4-ml vials. The rinse step was carried out using vials different from the separation vials in order to keep the level of buffer constant in the anodic separation vial. The capillary was filled for 2 min with the separation buffer, followed by a 3 s hydrodynamic sample injection. The separation was performed at +10 kV (11 kV for the TSP capillary) for 10 min (with a ramp voltage of 37.5 kV min^{-1}) at 25°C. The set of separation vials was changed after 10 runs.

Two or three blank injections were carried out at the beginning of each sequence for system equilibra-tion.

Standard and test solutions were placed in bracket-ing sequence and duplicate injections were used. The detector polarity output was reversed in order to get positive peaks for integration. Average relative peak areas (Ca^{2+}/Mg^{2+}) were used for calculations.

3. Result and discussion

3.1. Preliminary experiments

Initial conditions were based on papers published on cation separations (Refs. [5,13–20] and refer-ences therein), stating that imidazole was the most suitable visualization agent for calcium separation as its mobility (0.44 cm^2 kV s^{-1}) is similar to that of calcium (0.45 cm^2 kV s^{-1}). Using a 10 mM imida-zole solution, adjusted to pH 4.5, yielded a tailing peak for calcium which did not give a satisfactory precision for integration. Tetrabutyl ammonium sul-fate was added at low concentration to the elec-trolyte, as addition of an alkylammonium salt to the electrolyte separation gives it an improved peak shape [5] because it avoids adsorption to the silica wall. Under these conditions satisfactory peak shape was obtained together with an increase of the migration times due to the change in the electro-osmotic flow mobility. Because an internal standard could be suitable for quantitative analysis to compen-sate for variations in injection volume [21], different

cations were tested for this purpose (Fig. 1). It should be noted that sodium migrates before calcium and magnesium, in contrast to data reported in Ref. [5] where sodium migrates between calcium and magnesium with a very similar electrolyte. Among the different cations tested, magnesium was found to be the most appropriate internal standard as it migrates close to calcium with a satisfactory resolution of about 2 (Fig. 2) and an acceptable peak shape (tailing factor of about 2). Sodium was not selected, as contamination with this cation was previously noted in an inter-company cross-validation exercise [10].
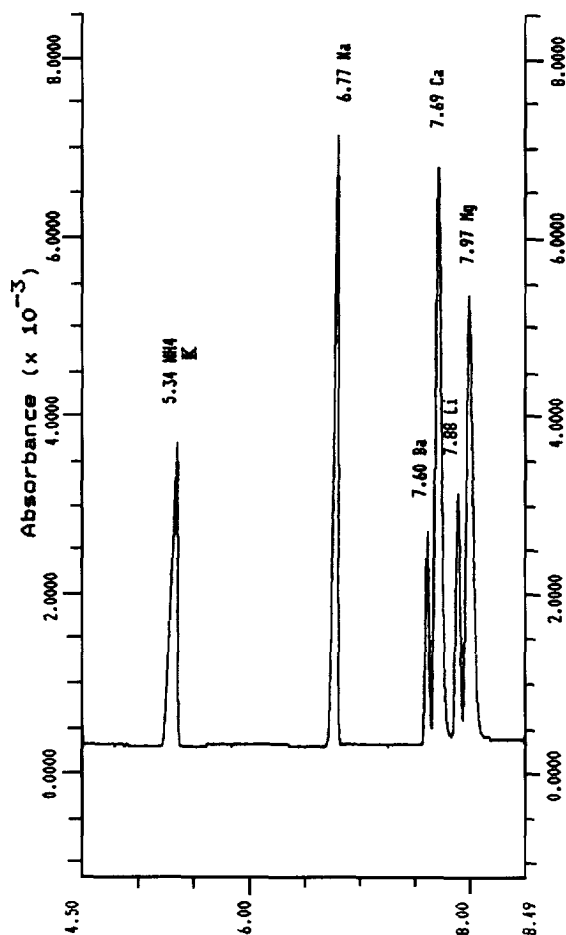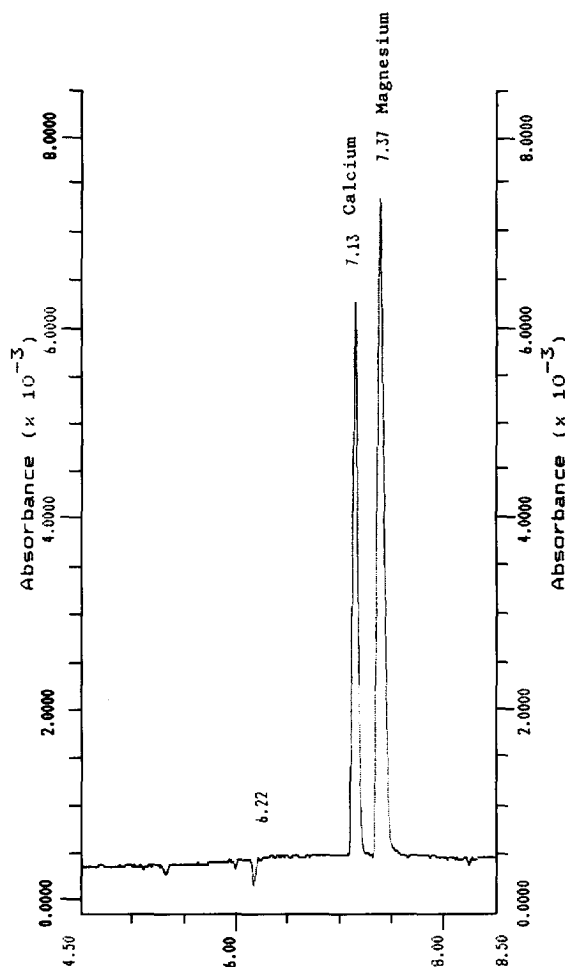

Fig. 2. Electropherogram of an acamprosate test solution (10 mg l^{-1} Ca^{2+}) spiked with Mg^{2+} (10 mg l^{-1}). For experimental conditions, Section 2.

3.2. Method validation for calcium in the drug substance

3.2.1. Specificity

Calcium and magnesium migrated typically between 7 and 8 min with relative migration times calcium/magnesium (RMT) of 0.97. Water injection did not give any interference at the migration time (t_m) of calcium and magnesium. Internal standard solution did not give any interfering peak at the t_m of calcium.

Fig. 1. Electropherogram of a mixed standard solution of NH$_4^+$ (5 mg l^{-1}), K$^+$ (5 mg l^{-1}), Na$^+$ (10 mg l^{-1}), Ba^{2+} (10 mg l^{-1}), Ca^{2+} (10 mg l^{-1}), Li$^+$ (1 mg l^{-1}), Mg^{2+} (10 mg l^{-1}). For experimental conditions, Section 2.

3.2.2. Linearity

The linearity of the response, relative peak areas Ca^{2+}/Mg^{2+} (RPA) vs. calcium concentration, was assessed with 5 standard solutions (5, 7.5, 10, 12.5 and 15 mg l^{-1} calcium) corresponding to 50, 75, 100, 125 and 150% of the target concentration of Ca^{2+} and spiked to a constant concentration of Mg (10 mg l^{-1}).

The regression equation was:

$$RPA = (0.0033 \pm 0.007) + (0.0537 \pm 0.0007)$$
$$\cdot \text{ calcium conc.}$$

with the confidence intervals calculated at $\alpha = 0.05$. The relationship was linear ($r^2 = 0.9999$) and went through the origin, which allowed the use of one calibration solution for routine operation.

3.2.3. Precision

Repeatability of successive injections was assessed by injecting the same standard solution ten times successively on capillaries from two different suppliers. The same set of electrolyte vials was used on each of capillaries. Although the electrolyte is not a buffered solution, 10 separations could be carried out with the same set of electrolyte vials with acceptable drift in the t_m of calcium and magnesium (typically 0.5–1.5% R.S.D.). Typical precision for peak areas was about 1–2% without internal standard but sometimes values as high as 6–7% were obtained.

Using an internal standard for calculation effectively reduced imprecision due to slight variations in injection volumes. Table 1 shows that within-capillary precision of both RMTs and RPAs on each of capillaries is satisfactory and that comparable selectivity and RPA are obtained on the two capillaries. Throughout this study, different batches of electrolyte solutions gave comparable t_m values and selectivity.

The repeatability of standard and sample solution preparation was assessed by preparing 5 solutions of each from different weighings. Each solution was injected in duplicate on both capillaries. The response factors obtained (Table 2) are very similar on the two capillaries. Precision between analysts was also found to be successful throughout the application of the method.

3.2.4. Accuracy

Two batches of calcium acamprosate were analysed for calcium content by CE (Beckman capillary) and complexometry. Duplicate preparations were carried out for each methods. The results are given in Table 3.

These results are in close agreement and compare well with the theoretical content of Ca^{2+} calcium acamprosate (10.01%).

3.2.5. Limit of detection and quantification

The limit of detection experimentally determined

Table 1
Repeatability of relative migration times calcium/magnesium (RMT) and relative peak areas calcium/magnesium (RPA), $n = 10$ injections of a standard solution of calcium carbonate, same set of electrolyte vials, two different capillaries

Injection	RMT		RPA	
	Beckman capillary	TSP capillary	Beckman capillary	TSP capillary
1	0.9704	0.9610	0.5443	0.5593
2	0.9703	0.9625	0.5436	0.55S6
3	0.9702	0.9629	0.5445	0.5566
4	0.9700	0.9629	0.5436	0.5568
5	0.9695	0.9627	0.5483	0.5594
6	0.9694	0.9625	0.5449	0.5569
7	0.9693	0.9621	0.5433	0.5S86
8	0.9693	0.9617	0.5438	0.5580
9	0.9690	0.9614	0.5441	0.5600
10	0.9688	0.9609	0.5446	0.5551
Average	0.96963	0.96207	0.5445	0.5576
S.D	0.00056	0.00077	0.0014	0.0017
R.S.D(%)	0.06	0.08	0.26	0.30

Table 2
Repeatability of sample and standard preparation

Preparation	RF Calcium carbonate		RF Calcium acamprosate	
	Beckman capillary	TSP capillary	Beckman capillary	TSP capillary
1	0.5564	0.5555	0.5588	0.5526
2	0.5545	0.5494	0.5582	0.5614
3	0.5656	0.5634	0.5656	0.5473
4	0.5570	0.5604	0.5525	0.5497
5	0.5622	0.5651	0.5600	0.5506
Average	0.55914	0.55876	0.55900	0.55232
S.D.	0.00460	0.00638	0.00468	0.00542
R.S.D.(%)	0.82	1.14	0.84	0.98

($n = 5$ preparations), duplicate injections of each solution. Results expressed as response factors (RF).

was about 0.7 mg l^{-1} Ca^{2+} ($S/N = 3$). A 2.5 mg l^{-1} calcium concentration (corresponding to a $S/N = 10$) gave a R.S.D. of RPA of 0.8%. Although no repeatability was carried out at lower concentrations, the limit of quantification is probably significantly lower than this concentration.

4. Conclusion

CE is a suitable method for the counter-ion determination of calcium acamprosate. Satisfactory precision data were obtained by use of an internal standard to improve injection precision and an electrolyte containing a cationic surfactant to reduce wall adsorption. Precision obtained complies with the requirements for the quality control of a drug substance. In comparison to EDTA titration currently used, CE presents as main advantages, traceability of the results and increased specificity. In addition, appreciation of the colour change at the end point titration in complexometry may be variable between operators.

Table 3
Results of calcium determination in calcium acamprosate

Batch	Theoretical content	CE	EDTA titration
C110	10.01% (m/m)	10.02% (m/m) (10.00, 10.04)	9.98% (m/m)
OTA 37	10.01% (m/m)	9.98% (m/m) (9.96, 10.01)	10.08% (m/m)

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