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Validated capillary electrophoresis method for the determination of atropine and scopolamine derivatives in pharmaceutical formulations

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Abstract

The simultaneous determination of atropine and scopolamine derivatives, which have similar structures, was investigated by using capillary zone electrophoresis. The effects of buffer pH, buffer concentration and hydroxypropyl-β-cyclodextrin concentration on migration time and resolution of the investigated compounds were systematically studied. The selected electrophoretic buffer consisted of a 80 mM sodium citrate pH 2.5, containing 2.5 mM hydroxypropyl-β-cyclodextrin as the complexing agent. Quantitative analysis was validated by testing the reproducibility of the method, giving a relative standard deviation less than 1 and 2% for the intermediate precision of migration times and peak area ratios, respectively. The linearity of the method was assessed between 50 and 150% of the theoretical content (coefficient of correlation greater than 0.99). The proposed method was found to be suitable and accurate for the determination of these basic drugs in pharmaceutical preparations.

Keywords: Atropine and scopolamine derivatives; Pharmaceuticals; Capillary electrophoresis; Method development and validation; Hydroxypropyl-β-cyclodextrin

1. Introduction

Atropine (($\pm$)-hyoscyamine) and scopolamine are alkaloids obtained from various solanaceous plants [1]. A number of analogs have been synthesized, the most common being homatropine, ipratropium, oxitropium, flutropium and *N*-butylscopolamine. These derivatives have similar structures and are generally used for anticholinergic purposes [2]. Analysis of these compounds in pharmaceutical preparations is, therefore, of special interest.

The analysis of tropane alkaloids has been studied using different separation techniques, including UV spectrophotometry [3,4], fluorimetry [5,6], gas chromatography [7,8], quantitative thin-layer chromatography [9] and, more recently, high-performance liquid chromatography [10–13]. However, few reports [14–17] were dedicated to

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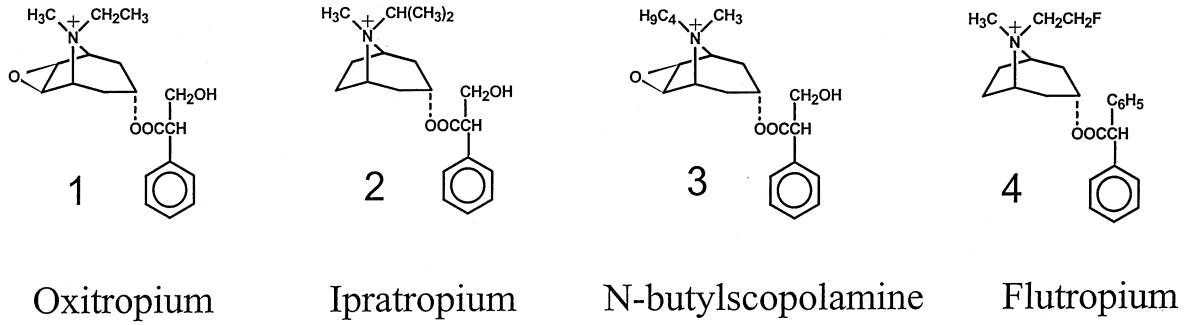

Fig. 1. Structure of the investigated alkaloids.

tropane alkaloid analysis by capillary electrophoresis (CE). Recently, the development of a validated capillary zone electrophoresis (CZE) method was described for the determination of atropine, scopolamine and homatropine in ophthalmic solutions [16]. The analysis of hyoscyamine and scopolamine in a plant extract using a micellar electrokinetic chromatography method was also reported [17].

The aim of this paper is the optimization of different electrophoretic parameters for the simultaneous analysis of four atropine and scopolamine derivatives, namely oxitropium, ipratropium, *N*-butylscopolamine and flutropium. The proposed method was validated and successfully applied to the determination of these basic drugs in different pharmaceutical formulations.

2. Experimental

2.1. Chemicals

Oxitropium bromide and flutropium bromide were kindly provided by Boehringer (Ingelheim, Germany). Ipratropium bromide and *N*-butylscopolamine bromide were purchased from Sigma (St-Louis, MO). Hydroxypropyl-β-cyclodextrin (HP-β-CD), with a degree of substitution of 0.6, was obtained from Roquette (Lestrem, France). Buscopan® (*N*-butylscopolamine) tablets, injections and suppositories, as well as Atrovent® (ipratropium) inhalation solution and Flubron® (flutropium) aerosol were purchased from a pharmacy. For Buscopan®, tablet and suppository are labeled as containing 10 mg *N*-butylscopolamine bromide, while each ml of injection solution contains 20 mg of the active principle. Each ml of Atrovent® solution is labeled as containing 0.25 mg ipratropium bromide while Flubron® aerosol contains 30 μg flutropium bromide per dose.

Sodium citrate and citric acid were obtained from Fluka (Buchs, Switzerland). Ultrapure water, provided by a Milli-Q RG unit from Millipore (Bedford, MA), was used for standard and sample preparation. Electrolyte solutions were filtered through a 0.20 μm microfilter (Supelco, Bellefonte, PA) before use.

2.2. Instrumentation and electrophoretic procedure

CE data were generated in an HP ^{3D}Capillary Electrophoresis system (Hewlett Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an automatic injector, an autosampler and a power supply able to deliver up to 30 kV. The total capillary (Hewlett Packard) was 64.5 cm, while the length to the detector was 56 cm, with a 50 μm internal diameter. An alignment interface, containing an optical slit matched to the internal diameter, was used and the detection wavelength was set at 191 nm with a bandwidth of 2 nm. A CE Chemstation (Hewlett Packard) was used for instrument control, data acquisition and data handling.

All experiments were carried out in cationic mode (anode at the inlet and cathode at the outlet). The capillary was thermostated at 25°C.

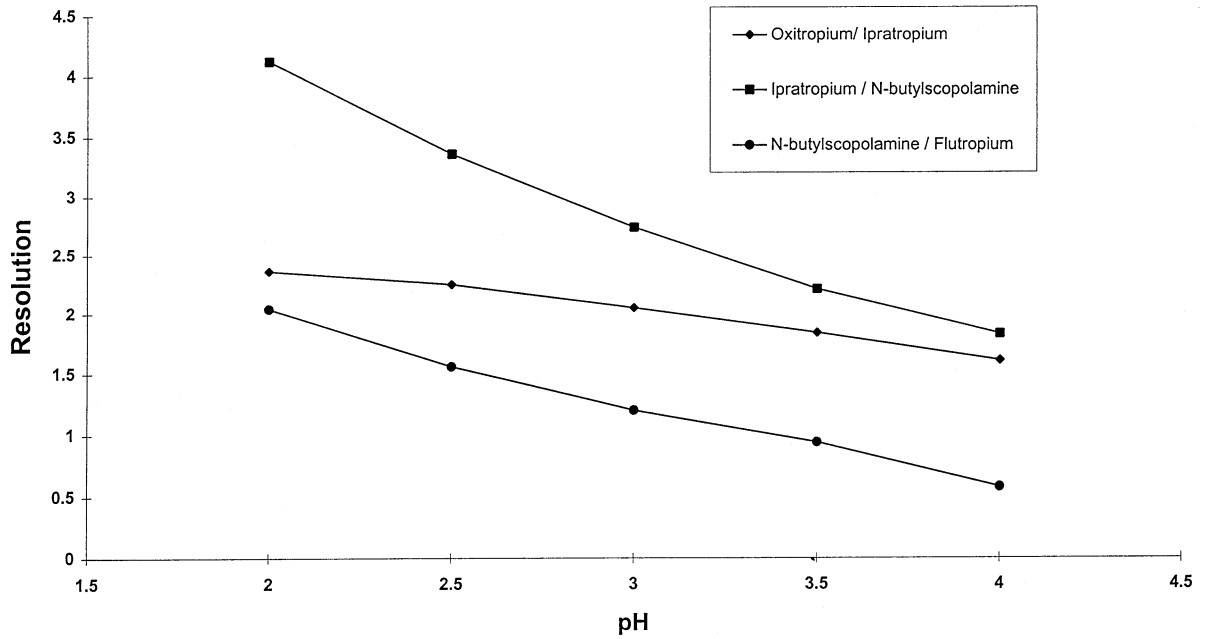

Fig. 2. Influence of citrate pH on the resolutions of the investigated compounds. Conditions: citrate concentration: 50 mM, applied voltage: 30 kV, temperature: 25°C. Other conditions are given in Section 2.2.

A constant voltage of 30 kV, with an initial ramping of 500 V s^{-1}, was applied during analysis. Sample injections (6 nl injection volume) were achieved using the pressure mode for 10 s at 25 mbar.

Before use, the capillary was washed with 0.1 M sodium hydroxide for 20 min, followed by water for 10 min. To achieve high migration time reproducibility and to avoid solute adsorption, the capillary was washed between analyses with 0.1 M sodium hydroxide for 2 min, followed by water for 2 min, then equilibrated with the running buffer for 3.5 min.

As electrolysis can alter the running buffer and subsequently change the electroosmotic flow (EOF), a replenishment system was also used to maintain a high reproducibility. Prior to each sequence, two blank injections were performed for stabilizing the capillary wall surface, and allowing the buffer and sample solutions to reach a constant temperature on the autosampler tray. Finally, triplicate injections were performed.

A mixture of citric acid and sodium citrate was used as running buffer in order to cover a pH range between 2 and 4.

2.3. Sample preparation

2.3.1. Standard solutions

Stock standard solutions of ipratropium, oxitropium, flutropium and *N*-butylscopolamine were prepared by dissolving each compound in methanol in order to give a concentration of 1 mg ml^{-1}. Working standard solutions were prepared by diluting the stock standard solution with water. The use of water as a dissolving agent allowed sample stacking which was effective in enhancing sensitivity by on-column preconcentration within the capillary.

A peak area calibration curve for ipratropium, flutropium and *N*-butylscopolamine was established over the sample concentration range of 50–150 μg ml^{-1} and in the presence of 100 μg ml^{-1} oxitropium, used as internal standard.

2.3.2. Pharmaceutical sample preparation

Liquids were diluted with water to obtain a 100 μg ml^{-1} final concentration of the analyte in the

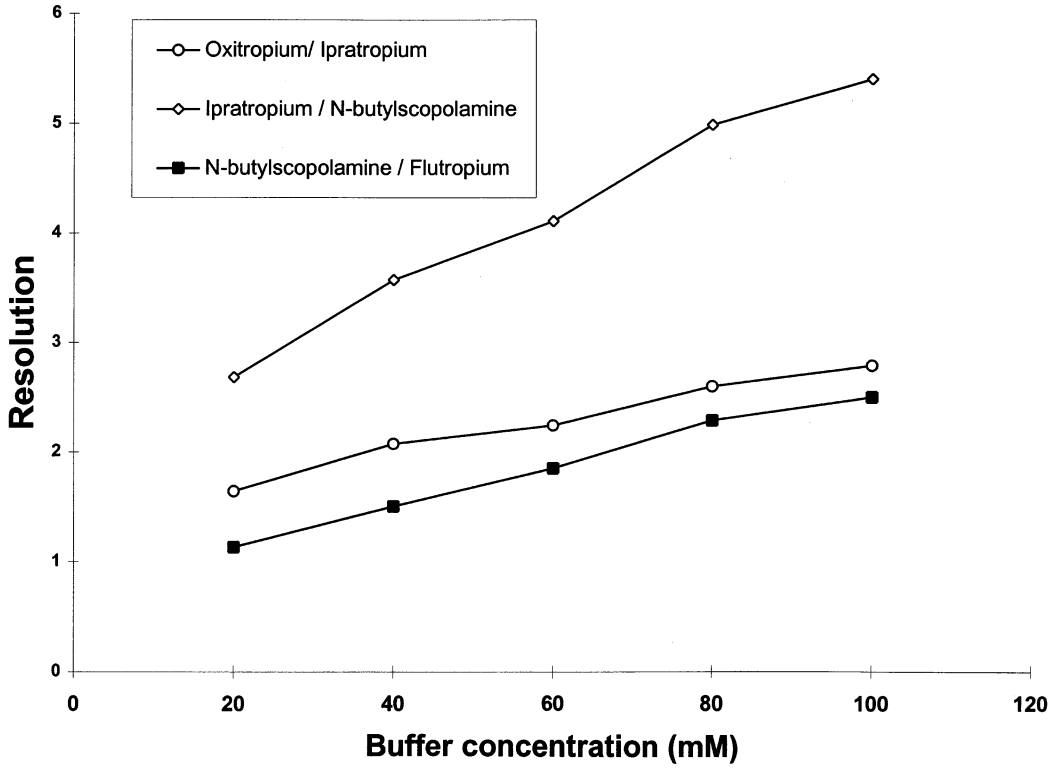

Fig. 3. Influence of citrate concentration on the resolutions of the investigated compounds. Conditions: buffer pH: 2.5, applied voltage: 30 kV, temperature: 25°C. Other conditions are given in Section 2.2.

solution. The internal standard was also present at a concentration of 100 μg ml^{-1}. The solution was homogenized by shaking, and was injected after filtering through 0.2 μm filters.

Three tablets were finely powdered and the equivalent of one tablet was accurately weighed and quantitatively extracted three times with 10 ml of water, with sonication for 15 min and vortex mixing at 5 min intervals to avoid aggregation of the powdered sample. After centrifugation ($2750 \times g$ for 5 min), supernatants were collected and diluted in a 100 ml volumetric flask. The internal standard was present at a concentration of 100 μg ml^{-1} and water was added in order to obtain a final concentration of 100 μg ml^{-1}. The aqueous solution was then filtered through a 0.2 μm filter and injected.

A suppository containing 10 mg of *N*-butylscopolamine was dissolved in 2 ml of dichloromethane and extracted three times with 10 ml of water, with sonication for 15 min and vortex mixing at 5 min intervals to homogenize the emulsion. After centrifugation ($2750 \times g$ for 5 min), supernatants were collected and diluted in a 100 ml volumetric flask. The internal standard was present at a concentration of 100 μg ml^{-1} and water was added in order to obtain a final concentration of 100 μg ml^{-1}. The aqueous solution was then filtered through a 0.2 μm filter and injected.

Five doses of the aerosol were collected in a 2 ml vial and 1.5 ml of water, containing 100 μg ml^{-1} of internal standard, was added. The aqueous solution was then filtered through a 0.2 μm filter and injected.

2.3.3. Preparation of the citrate buffer

The 80 mM citrate buffer (pH 2.5), used as a

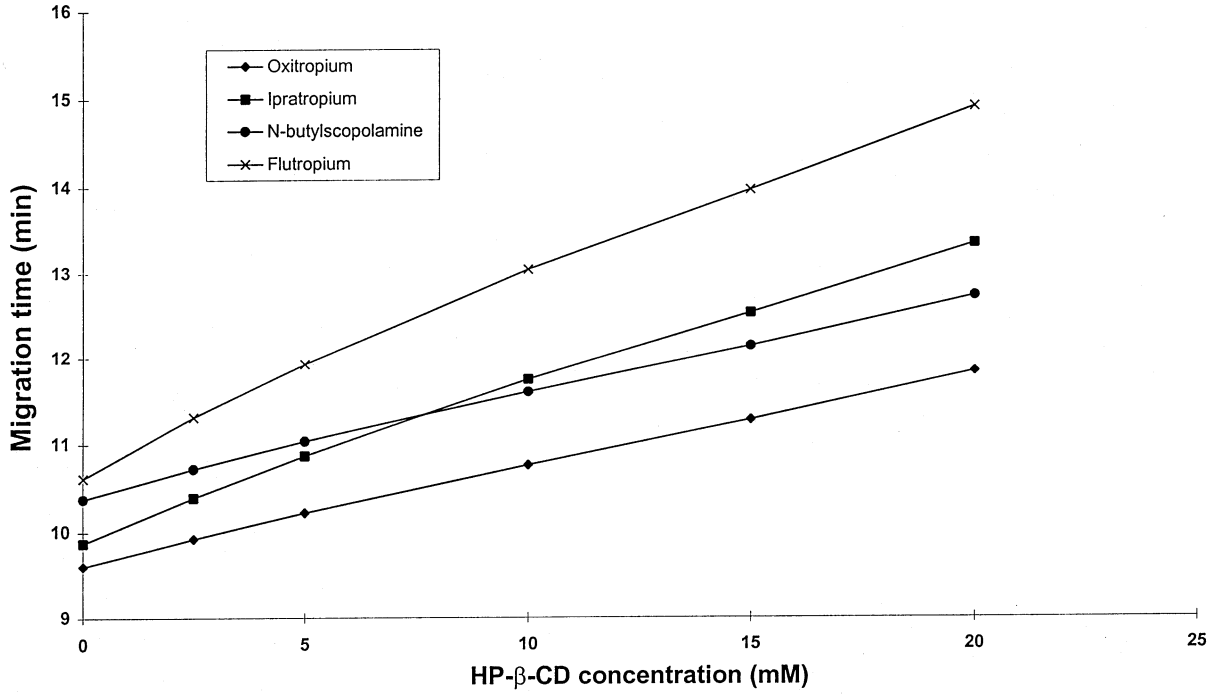

Fig. 4. Influence of HP-β-CD concentration on the migration times of the investigated compounds. Conditions: 50 mM citrate buffer at pH 2.5, applied voltage: 30 kV, temperature: 25°C. Other conditions are given in Section 2.2.

running electrolyte, was prepared using the Phoebus software 1.0 (Centre Analyse, Orleans, France). This buffer was prepared by transferring 15.78 ml of 2 M citric acid and 25.06 ml of 0.1 M sodium citrate in a 100 ml volumetric flask, and making up with water to the volume. Buffer solution was always freshly prepared and filtered immediately before use so as to remove particles.

3. Results and discussion

3.1. Method development

The optimization was performed using a synthetic mixture containing oxitropium, ipratropium, *N*-butylscopolamine and flutropium. The structure of these compounds is given in Fig. 1. The following parameters were consecutively optimized: buffer pH, buffer concentration and cyclodextrin concentration.

3.1.1. Buffer pH

The buffer pH is one of the most important parameters for improving selectivity in CE, especially for closely related compounds. In this study, the investigated pH range was restricted to the acidic region in order to reduce the negative charge on the fused silica capillary wall, thus minimizing interactions of the basic drugs with the silica surface. In addition, the investigated alkaloids are more stable at acidic pH [18] and the electroosmotic flow (EOF) is reduced, allowing higher resolutions of these alkaloids. The optimization was carried out with a sodium citrate buffer (50 mM) at a voltage of 30 kV and at a temperature of 25°C. The pH was varied between 2 and 4 with 0.5 units increment. Resolutions between these alkaloids versus pH are shown in Fig. 2. At acidic pH, the selected alkaloids are positively charged and their migration is mainly controlled by their electrophoretic mobility, as the EOF is negligible. Increasing the buffer pH results in lower resolutions. The best resolution was

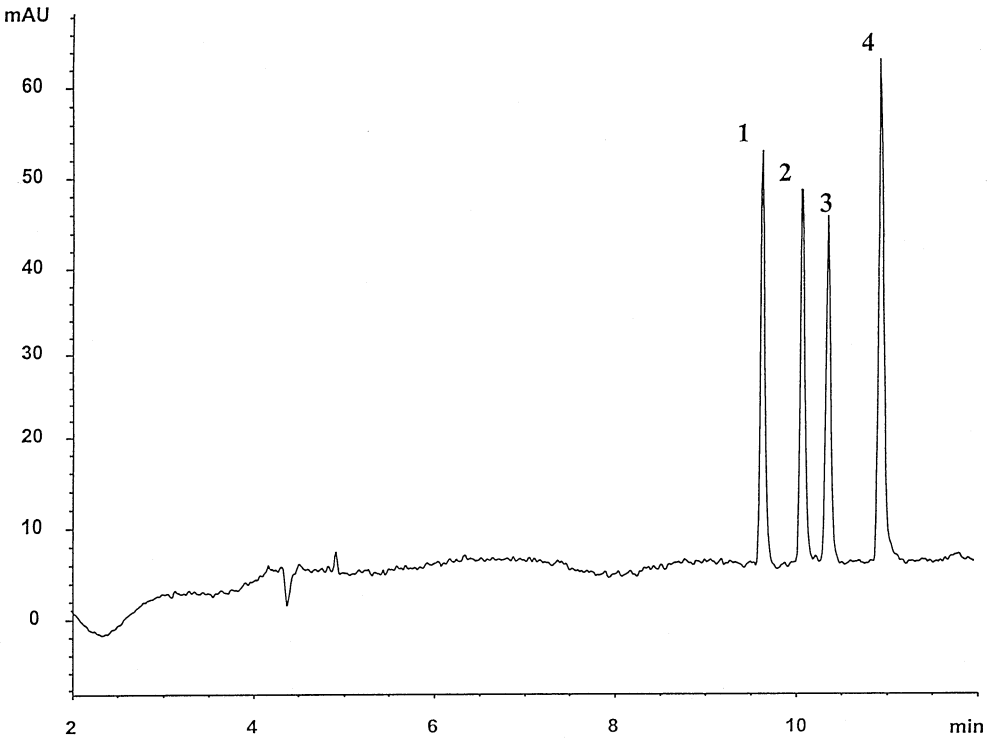

Fig. 5. Typical electropherogram of oxitropium (1), ipratropium (2), *N*-butylscopolamine (3) and flutropium (4) obtained by CZE, using 50 mM citrate buffer pH 2.5 in the presence of 2.5 mM HP-β-CD. Other operating conditions: uncoated fused-silica capillary $L = 64.5$ cm, $l = 56$ cm, I.D. = 50 μm; applied voltage 30 kV ($i = 45$ μA), temperature 25°C. Other conditions are given in Section 2.2.

achieved at pH 2, but working at such pH may have a dramatic effect on the capillary life-time. Thus, pH 2.5 was chosen for subsequent method development.

3.1.2. Buffer concentration

Buffer concentration has also a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. Keeping other parameters constant (pH 2.5, 30 kV, 25°C), the buffer concentration was varied from 20 to 100 mM. An increase in migration times and resolutions (Fig. 3) was obtained when the concentration of the buffer increased. This effect is related to the decrease of the zeta potential at the capillary-wall solution interface, reducing the EOF. Working at a high buffer concentration allows a better resolution and an improved sensitivity as a result of sample stacking during injection. However, high buffer concentration induces excessive heating caused by Joule effect. Consequently, a 80 mM citrate buffer was selected as a compromise between resolution, higher efficiency and short analysis time.

3.1.3. HP-β-CD concentration

Cyclodextrins (CDs), neutral oligomers of D-(+)-glucopyranose, have found several applications in CE. They are used as buffer additives to obtain better resolution [19] or as chiral selectors to achieve chiral separations [20]. CDs are also well known for their ability to encapsulate various molecules in the molecular state, conferring on them new physicochemical properties, including increased solubility and bioavailability, greater stability, and reduced incidence of side effects [21]. In addition, CDs have been reported to retard hydrolysis and racemization of the at-

Table 1
Precision of CE migration times and peak areas

	Ipratropium (%)	*N*-butylscopolamine (%)	Flutropium (%)
Repeatability			
Migration time	0.19	0.18	0.18
Peak area	3.89	3.36	4.49
Peak area ratio[a]	1.75	2.33	1.40
Intermediate precision			
Migration time	0.83	0.85	0.87
Peak area	3.87	4.41	4.88
Peak area ratio[a]	1.82	1.91	1.63

[a] The peak area ratio is defined as the analyte peak area divided by the internal standard (oxitropium) area.

ropine-like alkaloids [18,22]. Thus, hydroxypropyl-β-cyclodextrin was chosen in order to investigate its influence on resolution and separation selectivity.

As shown in Fig. 4, the concentration of HP-β-CD in the buffer influences the selectivity as well as the migration times. The separation principle is based upon the complexation differences of the studied alkaloids with the investigated cyclodextrin. Those complexes present reduced electrophoretic mobility due to their higher mass. The selectivity of *N*-butylscopolamine and ipratropium is particularly altered, which means that this latter exhibits a higher complexation constant.

Thus, for subsequent validation studies, a concentration of 2.5 mM was selected with respect to compound resolution and analysis time. From the above results, the optimized conditions, consisting of a solution made of 80 mM citrate buffer pH 2.5 in the presence of 2.5 mM HP-β-CD, were particularly suited for the separation of the investigated tropane alkaloids (Fig. 5). The method was therefore validated.

3.2. Method validation

The optimized method was validated for ipratropium, *N*-butylscopolamine and flutropium, using oxitropium as internal standard. The validation was carried out in a similar way to that generally adopted for HPLC and now employed to validate CE methods [23]. The procedure requires the assessment of migration time and peak area reproducibility, detector response linearity with sample concentration, sensitivity and accuracy.

3.2.1. Reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of migration times and peak areas for each alkaloid.

In order to determine the repeatability of the method, replicate injections ($n = 6$) of a 100 μg ml^{-1} solution containing oxitropium, ipratropium, *N*-butylscopolamine and flutropium were carried out. In Table 1, the relative standard deviation (RSD) values are given for migration time, peak area and peak area ratio. In all cases, repeatability was better than 0.2% for the migration time, 4.5% for the peak area and 2.4% for the peak area ratio. The use of an internal standard is necessary in order to compensate the poor precision observed with the hydrodynamic injection, and hence to achieve a good method precision [23].

The intermediate precision was also evaluated over 3 days by performing six successive injections each day. Results (Table 1) show that RSD values were in the same order of magnitude than those obtained for repeatability.

3.2.2. Linearity

Detector response linearities were assessed by preparing five calibration samples covering 50–150% range of the nominal sample concentration (50–150 μg ml^{-1}). Each sample was injected in

Table 2
Regression data for the calibration curves

	Range (μg ml^{-1})	r^2 No I.S.[a]	r^2 I.S.	Peak areas ratio		L.O.D. (μg ml^{-1})	L.O.Q. (μg ml^{-1})
				Slope	Intercept		
Ipratropium	50–150	0.947	0.997	0.011 ± 1.9E−4	−0.036 ± 0.020	0.6	1.8
N-butylscopolamine	50–150	0.948	0.998	0.010 ± 1.7E−4	−0.063 ± 0.018	0.8	2.4
Flutropium	50–150	0.932	0.991	0.018 ± 6.1E−4	−0.220 ± 0.064	0.5	1.5

[a] I.S. internal standard (oxitropium).

triplicate with the internal standard oxitropium. In all cases, correlation coefficient (Table 2) was improved by using peak area ratios. Regression curves were obtained by plotting peak area ratios (analyte peak area divided by internal standard area) versus concentration, using the least squares method.

3.2.3. Limits of detection (LOD) and quantification (LOQ)

The limit of detection, defined as the lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal to noise ratio. The LOD was determined by injecting test mixture solutions of various concentrations. The estimated limit of detection (Table 2) was determined as less than 1 μg ml^{-1}, giving a limit of quantitation (LOQ) value of less than 3 μg ml^{-1} for each of the examined drugs. As the investigated compounds possess an extremely low UV absorbance, performing detection at low wavelength (i.e. 191 nm) is of primordial importance.

3.2.4. Determination of alkaloids in pharmaceutical preparations

The determination of the alkaloids was performed on commercially available pharmaceutical preparations with the method described above. Fig. 6 shows a typical electropherogram for the determination of ipratropium in Atrovent® solution. The peak observed around 4 min is probably due to the presence of excipient or preservative. In all cases, the matrix did not interfere with the analyzed alkaloid.

Different types of commercial formulations, namely solutions, tablets, suppositories and aerosols, were assayed. Results generated by the developed CZE method were compared with those expected by the label claim. Table 3 shows that the results obtained were in good agreement with the labeled content, which demonstrates the good accuracy of the method. It is noteworthy, that in all cases, the recovery was quantitative (ca. 100%) and that the RSD values were low, attesting the performance of the method. These findings suggest that the described method may represent a valuable alternative for the analysis of atropine and scopolamine derivatives in pharmaceutical preparations.

4. Conclusion

The developed CZE method was found efficient for the rapid determination of atropine and scopolamine derivatives in pharmaceutical formulations. The presence of HP-β-CD in the buffer solution allows the manipulation of selectivity, depending on the complexation constant between the alkaloid and the cyclodextrin. In

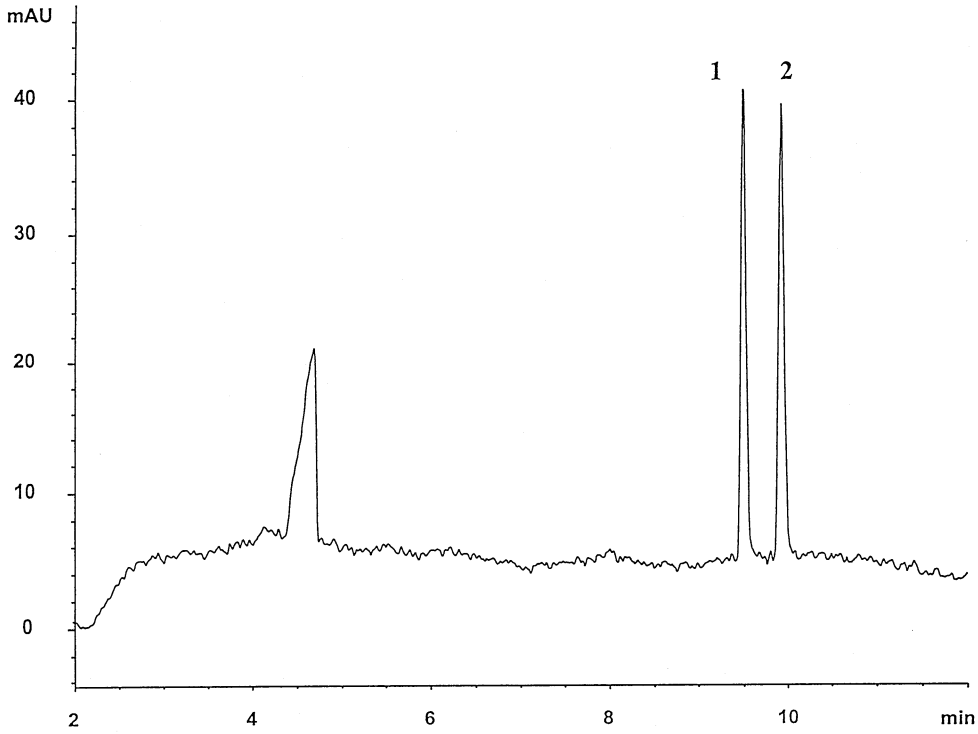

Fig. 6. Typical electropherogram of Atrovent® solution (2) in the presence of oxitropium (1) used as internal standard. Other conditions are the same as for Fig. 5.

Table 3
Results of CZE assay of ipratropium, *N*-butylscopolamine and flutropium in pharmaceutical preparations

Dosage formulation	Component[a]	Labeled claim	Amount found	Recovery (%)	R.S.D. (%)
Atrovent® (solution)	Ipratropium	0.25% (w/v)	0.251% (w/v)	100.52	1.93
Buscopan® (solution)	*N*-butylscopolamine	2% (w/v)	2.011% (w/v)	100.57	1.64
Buscopan® (tablets)	*N*-butylscopolamine	10 mg	10.244 mg	102.44	3.74
Buscopan® (suppository)	*N*-butylscopolamine	10 mg	10.122 mg	101.22	2.51
Flubron® (aerosol)	Flutropium	30 μg/dose	29.91 μg/dose	99.70	1.85

[a] Present as bromide salt in the tested pharmaceutical preparations.

addition, the migration times of ipratropium and *N*-butylscopolamine can be inverted as a function of HP-β-CD concentration. Finally, the optimized conditions were selected and the method was validated showing satisfactory validation data for selectivity, linearity, precision and accuracy. Results obtained for alkaloid determination in commercial pharmaceutical formulations attest the precision and the accuracy of the method.

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