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Nonaqueous versus aqueous capillary electrophoresis for the dosage of *N*-butylscopolamine in various pharmaceutical formulations

Samir Cherkaoui, Lidia Mateus, Philippe Christen, Jean-Luc Veuthey *

Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva, Pavillon des Isotopes, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland

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Abstract

A simple nonaqueous capillary electrophoresis method is described for the separation of several atropine and scopolamine related drugs. The analysis of these pharmaceutical compounds was performed in a methanol-acetonitrile (25/75, v/v) mixture containing 25 mM ammonium acetate and 1 M acetic acid. The robustness was proved using a full factorial design at two levels. The method was validated and successfully applied for the determination of *N*-butylscopolamine in different pharmaceutical preparations. Results were compared to those obtained by a capillary electrophoresis method based on aqueous media. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Atropine and scopolamine derivatives; Pharmaceuticals; Capillary electrophoresis; Nonaqueous media

1. Introduction

Tropane alkaloids are widely distributed in the Solanaceae and related families. The principal alkaloids of medicinal interest in this group are (-)-hyoscyamine, its racemate atropine and scopolamine which possess anticholinergic properties. A number of atropine and scopolamine derivatives have been synthesized in order to improve their pharmacokinetic properties, their efficacy and to reduce their toxicity [1]. Among them, ipratropium, *N*-butylscopolamine, flutropium and oxitropium are present in various pharmaceutical preparations. Furthermore, littorine and apoatropine are also frequently encountered within the Solanaceae family. The chemical structure of these compounds is shown in Fig. 1. The analysis of these compounds in pharmaceutical preparations, or in complex matrices such as crude plant extracts, is of special interest. Therefore, several techniques such as gas chromatography [2,3] liquid chromatography [4–6] and immunoassay [7,8] have been reported concerning the determination of tropane alkaloids. Recently, capillary electrophoresis (CE) has evolved as a good alternative

* Corresponding author. Tel.: +41-22-702-63-36; fax: +41-22-781-51-93.

E-mail address: jean-luc.veuthey@pharm.unige.ch (J.-L. Veuthey)

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for the analysis of these compounds, both in pharmaceutical formulations [9–11] and in plant extracts [12–14]. However, due to their structural similarity, the simultaneous determination of such compounds in aqueous media requires different additives in the buffer solution, such as sodium dodecyl sulfate (SDS), organic solvents or cyclodextrin derivatives [10,13,14].

More recently, the use of nonaqueous media has gained considerable importance in the analysis of pharmaceuticals by CE and several applications have been reported [15,16]. Indeed, very high efficiency and resolution, short analysis time and the possibility to increase the analyte solubility have been demonstrated. To our knowledge, a few articles only have been devoted to the quantitation of pharmaceuticals [17–21]. In particular, a robustness study of nonaqueous capillary electrophoresis has never been reported. This parameter is of great importance because organic solvents are quick to evaporate which induces a lack of precision.

In this article, optimal nonaqueous conditions are described for the CE analysis of several atropine and scopolamine derivatives. The robustness of the nonaqueous CE method is presented using a full factorial design at two levels. The method is applied to the determination of *N*-butylscopolamine (BSC) in pharmaceutical formulations, including a solution, a tablet and a suppository. Quantitative data are compared to the results obtained by a previous method based on an aqueous buffer, containing hydroxypropyl-β-cyclodextrin (HP-β-CD) as additive.

2. Experimental

2.1. Chemicals

Oxitropium bromide and flutropium bromide were kindly provided by Boehringer (Ingelheim, Germany). Atropine sulfate, scopolamine hydrobromide, ipratropium bromide, and *N*-butylsco-

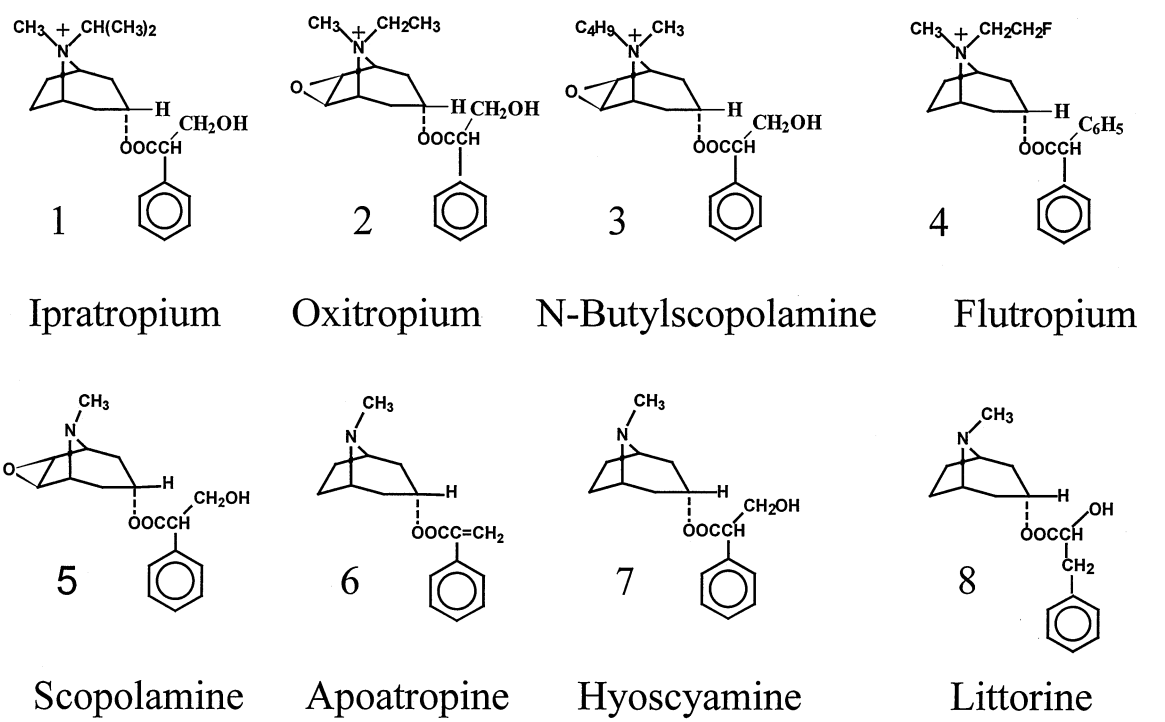

Fig. 1. Structure of the investigated alkaloids.

polamine bromide were purchased from Sigma (St Louis, MO). Apoatropine was obtained from Fluka (Buchs, Switzerland). Littorine was a gift from Dr K. Shimomura (Tsukuba Medicinal Plant Research Station, Japan). Acetic acid and ammonium acetate were obtained from Fluka (Buchs, Switzerland), while methanol and acetonitrile were provided by Romil (Kölliken, Switzerland). Buscopan® (*N*-butylscopolamine) tablets, injections and suppositories were purchased from a pharmacy. Tablets and suppositories are labeled as containing 10 mg *N*-butylscopolamine bromide, while each ml of solution contains 20 mg of the active principle.

2.2. Instrumentation and electrophoretic procedure

CE data was generated in an HP ^{3D}Capillary Electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. The total capillary length (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 200 nm with a bandwidth of 10 nm. A CE Chemstation (Hewlett-Packard) was chosen 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. 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. The non-aqueous buffer was 25 mM ammonium acetate and 1 M acetic acid in a methanol-acetonitrile (25/75, v/v) mixture.

The capillary was rinsed daily with 0.1 M sodium hydroxide, followed by water and acetonitrile for 5 min each. This flushing procedure is expected to remove any trace of water from the capillary [19]. Between analyses, the capillary was flushed with the running buffer for 3.5 min. When not in use, the capillary was washed with acetonitrile, water and then stored in air.

Robustness was tested with the NEMROD (LPRAI, Marseille, France) software package and response surfaces were drawn with Microsoft Excel (version 7.0).

2.3. Sample preparation

2.3.1. Standard solutions

Stock standard solutions of ipratropium, oxitropium, flutropium, *N*-butylscopolamine (BSC), apoatropine, scopolamine, atropine and littorine were prepared by dissolving each compound in methanol in order to give a concentration of 1 mg ml^{-1}. The alkaloid mixture was prepared by dissolution of individual compounds in methanol.

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

2.3.2. Pharmaceutical sample preparation

Injection solutions were evaporated to dryness under a nitrogen flow and methanol was added in order to obtain a 100 μg ml^{-1} final concentration of *N*-butylscopolamine. The internal standard (ipratropium) was added at a concentration of 100 μg ml^{-1}. The solution was homogenized by shaking and was injected after filtering through 0.2 μm filters.

The equivalent of one tablet was accurately weighed, from a sample of three tablets that had been finely powdered, and quantitatively extracted three times with 10 ml of methanol, with sonication for 15 min and vortex mixing at 5 min intervals to avoid aggregation of the powdered sample. After centrifugation (2750 × *g* for 5 min), supernatants were collected and diluted in a 100 ml volumetric flask. The internal standard was added at a concentration of 100 μg ml^{-1} and the volume was completed with methanol in order to obtain a final concentration of 100 μg ml^{-1}. The methanolic 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 hexane and extracted three times with 10 ml of methanol, 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 in a 100 ml volumetric flask. The internal standard was at a concentration of 100 μg ml^{-1} and methanol was added so as to obtain a final concentration of 100 μg ml^{-1}. The methanolic solution was then filtered through a 0.2 μm filter and injected.

3. Results and discussion

3.1. NACE method

In previous papers, the separation of several atropine and scopolamine derivatives, possessing similar charge to mass ratio, was reported using CE with aqueous buffers [9,10]. In order to enhance the selectivity, the addition of SDS, HP-β-CD, or of a low percentage of organic solvents was necessary [10,13,14]. Nonaqueous CE was also investigated as an alternative to aqueous systems for the separation of these compounds [22,23]. It was demonstrated that the selectivity, the migration time and the efficiency were critically affected by the composition of the methanol-acetonitrile mixture. Several electrophoretic parameters were considered for optimizing the method including the organic solvent composition, the acetic acid concentration, the ammonium acetate concentration, the temperature and the applied voltage. A methanol-acetonitrile mixture (25/75, v/v) containing 25 mM ammonium acetate and 1 M acetic acid yielded the best compromise in terms of analysis time, selectivity and separation efficiency. Fig. 2a and b show typical electropherograms of standard mixtures using optimized aqueous [10] and nonaqueous [23] CE methods. It is noteworthy that the complete resolution of the investigated compounds was achieved without the use of any additive in the nonaqueous media. As shown in Table 1, working at 25% methanol in the methanol–acetonitrile mixture results in rapid separation, higher efficiency and very low electric current, which prevents Joule effect. As already reported [23,24], this behavior is mainly attributed to the dielectric constant to viscosity ratio which presents a maximum value around 20% methanol.

Table 1
Comparison of some electrophoretic values obtained under aqueous and nonaqueous conditions[a]

	Aqueous medium	Nonaqueous medium
Separation time (min)	13.78	4.37
Efficiency for the BSC	213200	554800
Current (μA)	64	14

[a] Aqueous medium: 80 mM citrate (pH 2.5) containing 2.5 mM HP-β-CD [10]. Nonaqueous medium: MeOH–MeCN (25/75, v/v), 25 mM ammonium acetate and 1 M acetic acid. Uncoated fused silica capillary: $L = 64.5$ cm, $l = 56$ cm, I.D. = 50 μm. Applied voltage: 30 kV.

In addition, as tropane alkaloids are unstable in aqueous buffers, the use of organic solvents significantly reduces their rate of degradation.

3.2. Robustness

According to the ICH (International Conference on Harmonization) guidelines [25,26] and the USP (United States Pharmacopeia), robustness is defined as the capability of an analytical procedure to remain unaffected by small but deliberate variations in the method parameters. Within the pharmaceutical industry, the robustness of a method has become an important parameter to consider for method validation. Therefore, due to the risk of volatile solvents evaporation and its influence on the method reproducibility, the robustness of a nonaqueous system has to be assessed.

The most relevant electrophoretic parameters, which could affect separation performances, were examined:

1. methanol percentage
2. acetic acid concentration
3. ammonium acetate concentration and
4. temperature.

The values of the experimental factors are summarized in Table 2. The selection of four parameters suggests a 2^4 full factorial design, i.e. 16 experiments. In order to evaluate the standard deviation of the method, six points at the optimized experimental conditions (central values)

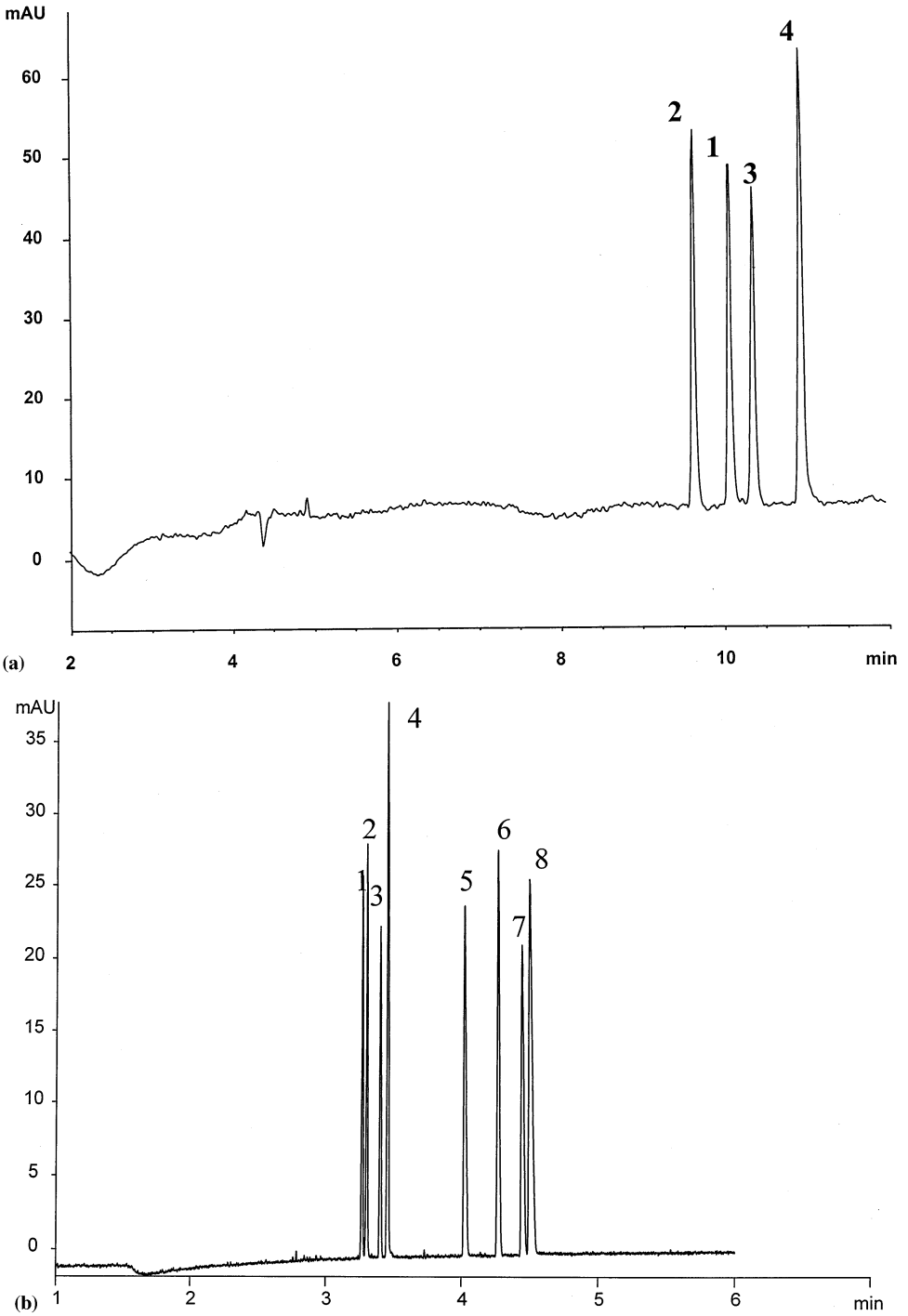

Fig. 2. Electropherograms obtained from (A) the aqueous method [10] and (B) the nonaqueous method [23]. Conditions: (A) 80 mM sodium citrate buffer, pH 2.5, 2.5 mM HP-β-CD. Applied voltage 30 kV ($i = 64$ μA). (B) methanol-acetonitrile (25/75, v/v), 25 mM ammonium acetate, 1 M acetic acid. Applied voltage 30 kV ($i = 14$ μA).Uncoated fused-silica capillary: $L = 64.5$ cm, $l = 56$ cm, I.D. = 50 μm. Peak assignment as in Fig. 1.

were added. A total of 22 experiments were randomly performed.

The measured responses were the resolution between BSC and ipratropium, used as an internal standard, their peak area ratio and the analysis time of BSC.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \quad (1)$$

Where Y represents the experimental response and Xi the independently evaluated factors (in coded values). The coefficients $b_{i\ (i=1-4)}$ of the equation (1) indicate the effect of methanol percentage, acetic acid concentration, ammonium acetate concentration and temperature on the studied responses, respectively. The coefficients (b_{ij}) reflect the interaction between two parameters. Higher order interactions were not considered. The estimated coefficients of the four electrophoretic parameters, with their confidence intervals ($P = 0.05$) for all the investigated responses, are summarized in Fig. 3 a, b and c.

A positive effect means an increase in the investigated response with an increase of the electrophoretic parameter while a negative effect means a decrease in the response with an increase in the electrophoretic parameter. When zero is included in the confidence interval range, it is concluded that the effect of the factor is not significantly different from zero. This means that the effect is not significant and can be attributed to experimental errors.

As shown in Fig. 3a, the zero is included in the confidence interval range for all coefficients (except for the acetic acid concentration). This means that none of the parameters have a significant effect on the resolution. The effect of the acetic acid concentration may be explained by the large variation chosen for this parameter (between 0.8 and 1.2 M). Therefore, the method can be considered as robust in the experimental domain.

Fig. 3b shows that both the methanol percentage and the acetic acid concentration have a significant influence on the BSC peak area ratio. Thus, careful attention has to be paid to methanol evaporation during the electrophoretic separation. The acetic acid effect is not critical for the same reasons as above.

It can be seen from Fig. 3c that, besides the acetic acid concentration, the ammonium acetate concentration has a significant positive influence and the temperature a negative influence on the BSC migration time.

In view of the linearity of all responses, response surface plots were constructed to determine the influence of the most important parameters. In Fig. 4a, the peak area ratio versus the acetic acid concentration and the methanol percentage is reported. The ammonium acetate and the temperature were kept constant at their optimal levels of 25 mM and 20°C, respectively. The figure shows that the peak area ratio increases at a high methanol percentage while the acetic acid concentration has almost no effect. Fig. 4b visualizes the evolution of the BSC migration time versus the temperature and the ammonium acetate concentration. As expected, an increase in temperature or ammonium acetate concentration results in a decrease of separation time. Regarding the response surface, the effect of

Table 2
Values of experimental factors

Coded values	Low value (−1)	Central value (0)	High value (+1)
Methanol percentage (%)	23	25	27
Acetic acid concentration (M)	0.8	1	1.2
Ammonium acetate concentration (mM)	23	25	27
Temperature (°C)	18	20	22

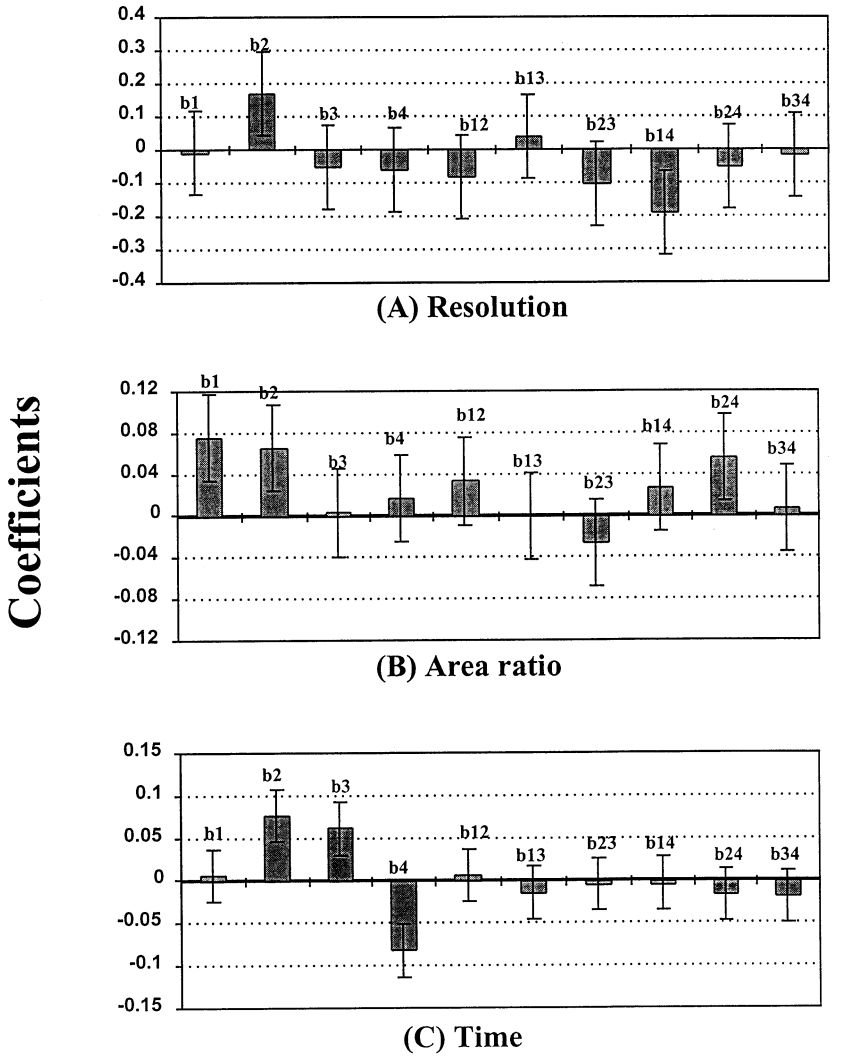

Fig. 3. Diagram plot for the estimated coefficients (b_i and b_{ij}) with their confidence intervals for (A) resolution (B) peak area ratio and (C) analysis time responses.

the latter parameters is not important within the studied range.

The results of this study show that the method is robust with respect to resolution, peak area ratio and migration time. Small changes in the main electrophoretic parameters do not drastically affect the separation. However, the methanol percentage in the methanol–acetonitrile mixture should be strictly controlled.

3.3. Quantitative performances

The quantitative aspects of this method were examined for BSC and the results are shown in

Table 3. The method validation was performed using ipratropium as an internal standard.

Method precision was determined by measuring repeatability and intermediate precision of migration times and corrected peak area (BSC peak area divided by internal standard peak area). The relative standard deviation values indicate that the method offers a good precision for the determination of BSC. Thus, the appropriate control of the separation temperature and the use of air-tight

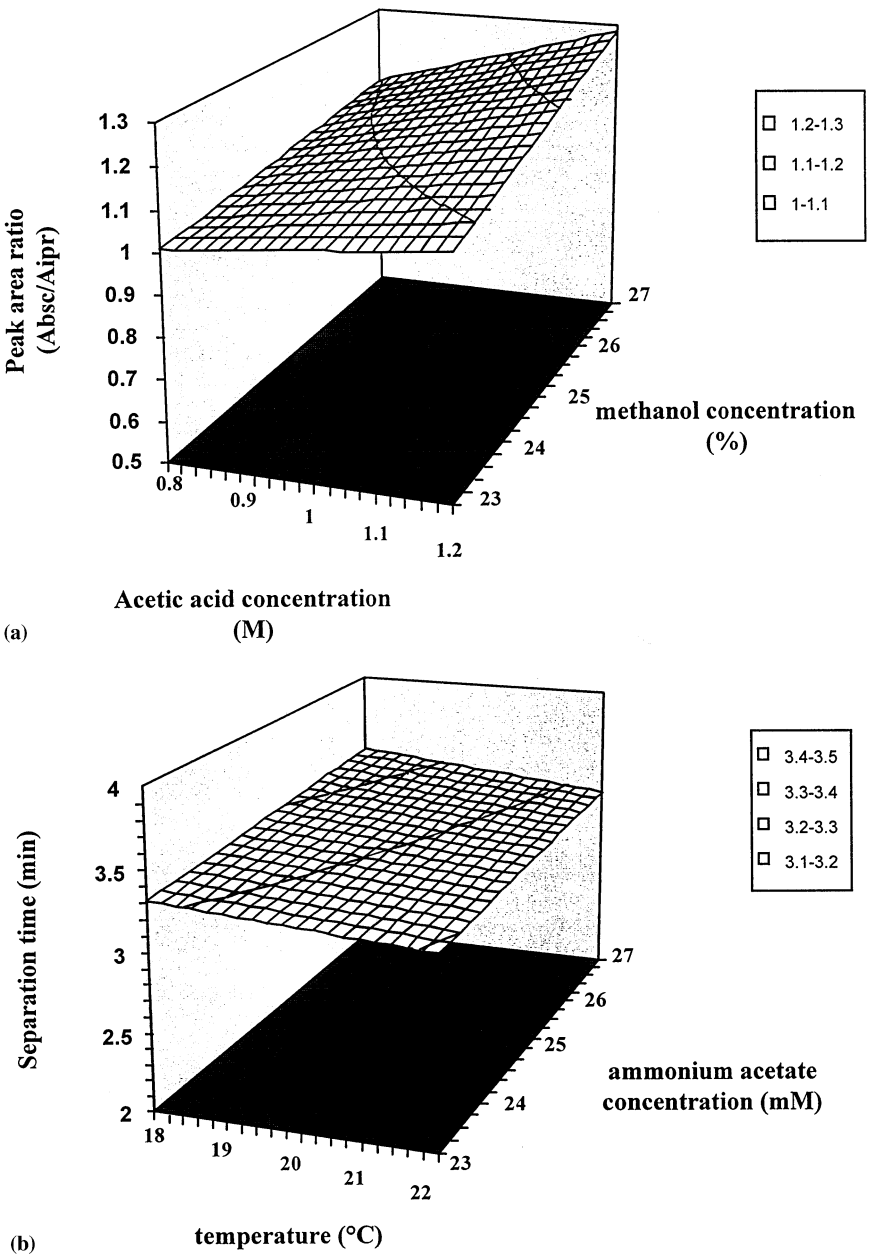

Fig. 4. Surface response plot (A) for the peak area ratio as a function of the methanol percentage and the acetic acid concentration (25 mM ammonium acetate and 20°C) and (B) for the analysis time as a function of the temperature and the ammonium acetate concentration (25% methanol and 1 M acetic acid).

Table 3
Quantitative performances

Parameter	*N*-Butylscopolamine
Repeatability	
Migration time	RSD = 0.25%
Corrected area	RSD = 0.72%
Intermediate precision	
Migration time	RSD = 2.82%
Corrected area	RSD = 1.04%
*Linearity**	
y = corrected area	y = 0.01.x − 0.03
x = BSC concentration	$r^2 = 0.9987$
range = 0.05-0.15 mg ml^{-1}	
LOD (S/N) = 3	0.40 μg ml^{-1}
LOQ (S/N) = 10	1.34 μg ml^{-1}

* Number of concentrations = 5, total number of analyses = 18.

vials to avoid solvent evaporation lead to good reproducibility data.

All other validation data (linearity and limits of detection and quantification) was determined following ICH guidelines and is reported in Table 3.

In order to assess the accuracy of the method, the determination of *N*-butylscopolamine in different pharmaceutical preparations, such as a liquid injection, a tablet and a suppository, was carried out. In addition, results were compared with those of a previously established method based on an aqueous media. A typical electropherogram for the analysis of BSC in Buscopan® suppositories is presented in Fig. 5. Accuracy was determined as the recovery, given in %, between the obtained and claimed values. As shown in Table 4, results generated by the two CE methods were in good agreement with the labeled claim. The low RSD values attest the good precision of the methods which are significantly equivalents (ANOVA).

4. Conclusion

A simple, efficient and specific nonaqueous CE method is reported for the qualitative separation of several tropane alkaloids and for the quantitative determination of *N*-butylscopolamine in several pharmaceutical preparations. The complete

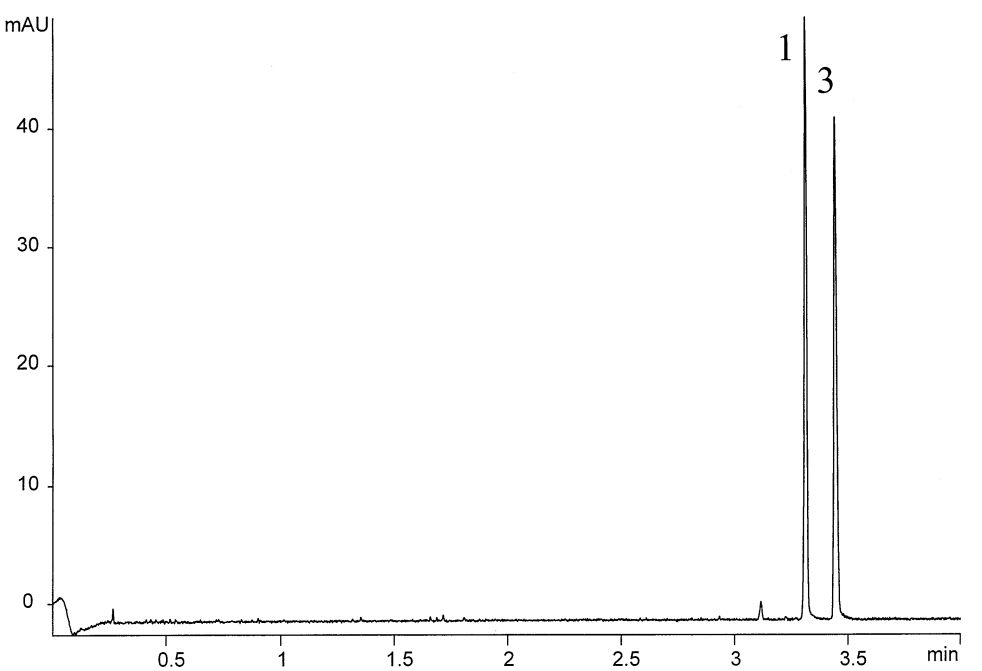

Fig. 5. Typical electropherogram of Buscopan® suppository (3) in the presence of ipratropium (1) used as internal standard. Other conditions are the same as for Fig. 2b.

Table 4
Determination of BSC in three different pharmaceutical preparations by aqueous and nonaqueous CE methods

Preparation	Claimed amount	Nonaqueous CE		Aqueous CE*	
		Assay amount (%)		Assay amount (%)	
		Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Buscopan® injection ($n = 6$)	2% (w/v)	100.6	1.59	100.2	1.44
Buscopan® tablet ($n = 6$)	10 mg	100.7	2.21	100.0	1.41
Buscopan® suppository ($n = 6$)	10 mg	101.9	1.21	101.4	1.04

* Electrophoretic conditions: Fused silica capillary, $L = 64.5$cm, $l = 56$ cm, I.D. 50 μm; background electrolyte, 50 mM citrate buffer pH 2.5 in the presence of 2.5 mM HP-β-CD; voltage, 30 kV; temperature, 25°C; hydrodynamic injection (6 nl injection volume).[10].

resolution of the investigated drugs was achieved without the addition of any expensive or non volatile additive. The robustness of the method was assessed using a full factorial design. Data achieved under the optimal nonaqueous conditions were in good agreement with those obtained by a previously established method based on aqueous buffer. Thus, nonaqueous CE can be a good alternative for the quality control of drugs in pharmaceutical preparations.

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