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Electrokinetic behaviour of salbutamol and its decomposition products and determination of salbutamol by micellar electrokinetic capillary chromatography

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Abstract

Separation in capillary electrophoresis is governed by several factors, including type of buffer, buffer concentration, pH, temperature, voltage and micelles. Through proper adjustment of these parameters, salbutamol could be separated from its acidic and basic decomposition products. The determination of salbutamol in kinetic studies was achieved by micellar electrokinetic capillary chromatography (MECC) using a mixture of N-cetyl-N,N,N-trimethylammonium bromide (5 m*M*), disodium hydrogenphosphate (20 m*M*) and sodium tetraborate (20 m*M*) (pH 7.6) with phosphoric acid. The MECC results were in good agreement with results obtained by high-performance liquid chromatography.

Keywords: Micellar electrokinetic chromatography; Salbutamol

1. Introduction

Salbutamol is widely used in the treatment of asthma. It is administered as an aerosol, injection fluid, mixtures and tablets. Salbutamol sulphate decomposes in aqueous solutions at elevated temperatures. Many factors, such as buffer species, buffer concentration, pH and drug concentration, affect the stability [1,2]. Thus, salbutamol sulphate decomposes faster in citrate buffer than in acetate and phosphate buffers, and the decomposition is accelerated at higher concentrations of the initial drug and the buffer. Salbutamol sulphate shows maximum stability at pH ≈ 3.

High-performance liquid chromatography (HPLC) is a highly convenient technique for monitoring the decomposition of salbutamol [3]. Capillary electrophoresis (CE), which is an alternative to HPLC, combines high resolution and ease of automation with modest sample requirements and low solvent consumption. Capillary zone electrophoresis (CZE), with 0.01 *M* tris (hydroxymethyl)aminomethane (pH 5.0), has been used successfully to determine salbutamol in pharmaceutical dosage forms [4]. CZE has also been used in monitoring and determining two dimeric impurities in salbutamol drug sub-

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stance [5–7] and in resolving of chiral and achiral salbutamol impurities [8].

The aim of this work was to develop a CE method for the separation of salbutamol and its decomposition products. The factors affecting the separation and elution order were evaluated and the quality of the quantitative CE data and data obtained by HPLC was compared.

2. Experimental

2.1. Materials

Salbutamol sulphate was kindly supplied by Leiras (Turku, Finland) and orciprenaline sulphate was obtained from Sigma (St. Louis, MO, USA). All organic solvents and other chemicals were of analytical-reagent or chromatographic grade. Acetonitrile was purchased from Lab-Scan (Dublin, Ireland). 3-[3-Cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), Tris base and Tris hydrochloride were supplied by Sigma, triethylamine and benzyl alcohol by Fluka (Buchs, Switzerland), sodium metabisulphite by Baker (Phillipsburg, NJ, USA) and ammonium dihydrogenphosphate by Riedel-de-Haën (Seelze, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). Water was purified in an Alpha-Q water purification system (Millipore, Molsheim, France).

2.2. Samples

The samples used in the development of the CE method were decomposed solutions of salbutamol sulphate (18 m*M* with respect to salbutamol) in water or aqueous acetic acid (pH 2.2). The water samples were kept at 100°C for about 24 days and the acetic acid samples at 85°C for about 41 days. The samples in aqueous acetic acid were placed in a freeze-drier (Edwards, GWB, Crawley, Sussex, UK) to evaporate the acetic acid. The solid residue was dissolved in the same volume of water. Before analysis the samples were diluted fivefold with water, filtered (Gelman Nylon Bulk Acrodisk 13, 0.45 μm) and sonicated.

For the quantitative part of the study, salbutamol sulphate solutions [18 m*M* with respect to salbutamol in Sörensen citrate buffer (pH 5.2)] containing sodium metabisulphite and cysteine (0.05% of each) as antioxidants were kept in a preheated oven (85°C). At appropriate time intervals two or more samples were taken and their pH values were measured (PHM83 Autocal pH meter, Radiometer, Copenhagen, Denmark). In micellar electrokinetic capillary chromatography (MECC), 1 ml of the sample and 0.5 ml of orciprenaline sulphate (28.3 m*M* in water) as internal standard were diluted to 5 ml with water. In HPLC, 0.8 ml of the decomposed salbutamol solution was diluted to 10 ml with water, with no internal standard added. The samples were filtered before injection to the instrument.

Calibration graphs were constructed from salbutamol solutions of five different strengths (0.6–6.0 m*M* in MECC and 0.2–2.0 m*M* in HPLC). The highest and lowest concentrations were injected six times, the others twice. In MECC the graph was constructed by plotting corrected peak-area ratios of salbutamol to the internal standard against salbutamol concentration; in HPLC this was done without an internal standard.

2.3. CE instrumentation and conditions

The experiments were performed using a P/ACE 2200 CE instrument (Beckman, Fullerton, CA, USA), which was temperature controlled by liquid cooling. The wavelength was 265 nm, a compromise value for all compounds in the solutions. The separation conditions with the different uncoated fused-silica capillaries are given in Table 1. All samples were injected using pressure injection (0.5 p.s.i.). Quantitative data were obtained by using a 50 μm I.D. fused-silica capillary (57 cm long, 50 cm to the detector) and a mixture of N-cetyl-N,N,N-trimethylammonium bromide (CTAB, 5 m*M*), disodium hydrogenphosphate (20 m*M*) and sodium tetraborate (20 m*M*) (pH 7.6) with phosphoric acid. The operat-

Table 1
Conditions used in the development of the CE method for decomposed salbutamol sulphate solutions

Method	Capillary			Buffer	Concentration (m*M*)	pH[a]	Injection time (s)	Voltage (kV)	Temperature (°C)	Current (μA)
	Supplier	I.D. (μm)	Effective/total length (cm)							
1	Beckman	75	50/57	Na_2HPO_4	20–40	6.0–9.2	5	+14–30	18–30	50–240
2	Beckman	75	50/57	Na_2HPO_4	40	7.7	2.5–10	+14	20	69
	Beckman	50	50/57							29
	Beckman	50	20/27							76
	Isco	75	50/57							69
3	Beckman	75	50/57	$NH_4H_2PO_4$	40	7.7	5	+14	20	70
4	Beckman	75	50/57	KH_2PO_4	40	7.7	5	+14	20	85
5	Beckman	75	50/57	$(NH_4)_2HPO_4$	40	7.7	5	+14	20	98
6	Beckman	75	50/57	Tris	10 (50)	7.7 (7.8)	5	+14	20	9 (38)
7	Beckman	75	50/57	HEPES	40	7.7	5	+14	20	16
8	Beckman	75, 50	20/27, 50/57	Na_2HPO_4–$Na_2B_4O_7$–CTAB	20–40+ 20–40+ 0.5–5	7.5–7.9	5–7	−14–16	20–25	17–155

[a] pH was adjusted with phosphoric acid, ammonia solution or potassium or sodium hydroxide.

ing voltage was −16 kV and the operating temperature was 25°C. The samples were injected for 7 s.

The electroosmotic breakthrough time (t_0) was measured with benzyl alcohol or acetone and they were used as the reference peak in measuring relative migration times. New capillaries were successively purged with 0.1 *M* potassium hydroxide, water and buffer solution, each for 10 min. Before each injection the capillary was purged for 2 min with the buffer solution. At the beginning and end of each day the capillary was rinsed with potassium hydroxide solution and water.

2.4. HPLC instrumentation and conditions

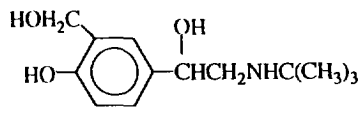
The Waters HPLC instrument consisted of two Model 501 pumps, an automated gradient controller and a photodiode array (PDA) system with a Model 5200 printer–plotter. The PDA system consisted of a Waters Model 991 diode-array detector, an NEC PowerMate 386/25 computer and PDA software (Waters, Milford, MA, USA). The analytical wavelength was 265 nm and the loop volume was 20 μl.

A LiChrosorb RP-18 (125 × 4 mm I.D., 5 μm) with an RP-18 precolumn was used as the column. The mobile phase was a mixture of acetonitrile, sodium dihydrogenphosphate (40 m*M*) and triethylamine (5.74 m*M*) (pH 3.0, adjusted with phosphoric acid). The acetonitrile content was increased from 4 to 9% after 6 min in one step, the flow-rate being 1.5 ml/min [3].

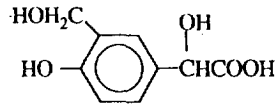
3. Results and discussion

3.1. Method development

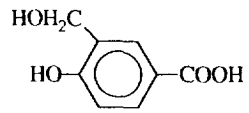
Salbutamol is an amphoteric compound with pK_a values of 9.3 and 10.3. It decomposes in aqueous solutions with the formation of several decomposition products (**I–VI**) [2]. Compounds **I** and **II** are phenolic acids, **III** is a phenolic aldehyde and **IV**, being a basic salicylaldehyde derivative, has retained the amphoteric character. Compounds **V** and **VI** are large molecules with dimeric structures. The pH of the solutions influenced product formation. Compound **I** was the primary product in strongly acidic solution; however, as the pH of the sample solution increased, the formation of **II** became dominant [1,2]. Fig. 1 shows the structures of salbutamol and its decomposition products **I**, **II** and **IV** as determined by liquid chromatography–mass spectrometry [9]. Despite the different charges and sizes of the studied compounds, the electro-



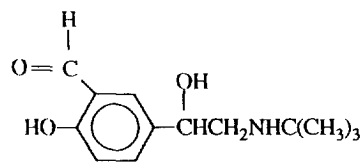
salbutamol



α,4-dihydroxy-3-hydroxymethylbenzene acetic acid (I)



4-hydroxy-3-hydroxymethylbenzoic acid (II)



2-hydroxy-5-(2-tert-butylamino-1-hydroxyethyl)benzaldehyde (IV)

Fig. 1. Structures of salbutamol and its decomposition products **I**, **II** and **IV**.

osmotic flow in CE transports all the compounds in the same direction and out of the capillary. Only three of the decomposition products (**I**, **II** and **IV**) were investigated, since the amounts of the others (**III**, **V** and **VI**) were always very small.

The solvent of the sample plays a very important role in CE. The salt concentration of the sample should be lower than that of the background electrolyte, since high concentrations broaden the peaks and make the resolution worse. In this part of the work the samples were prepared in water.

3.1.1. Capillary zone electrophoresis

Method development was started with 20 m*M* disodium hydrogenphosphate (pH 7.6) as electrolyte. At pH 7.6 all the compounds studied should be charged. The effect of electrolyte concentration on the mobility of salbutamol and its decomposition products was studied by increasing the disodium hydrogenphosphate concentration from 20 to 40 m*M* at 23 kV and 25°C (method 1 in Table 1). In general, an increase in ionic strength of the buffer decreases the electroosmotic flow and consequently increases the time for the compound to elute. On the other hand, the current also increases and the migration is faster [10]. These two factors seemed to counterbalance each other and the net effect of the buffer concentration on the mobility of salbutamol and its decomposition products was negligible. To limit the Joule heating inside the capillary, the maximum voltages were chosen from the Ohm's law plot (the plot of current vs.

voltage) and were 17, 15 and 14 kV for 20, 30 and 40 m*M* solutions, respectively. The best separation of salbutamol from the worst resolved small peaks (decomposition products **V** and **VI**) was achieved with 40 m*M* buffer at 14 kV.

The pH dependence of the relative migration time of salbutamol and compounds **I**, **II** and **IV** is illustrated in Fig. 2. In the pH range 6–9.2 (40 m*M* buffer, 14 kV, 25°C; method 1 in Table 1), positively charged compounds (salbutamol and decomposition products **IV–VI**) migrated from the capillary first. Then came the neutral solute, followed by the carboxylic acids **I** and **II**. Compound **II** eluted after **I** owing to its smaller molecular size and consequently stronger attraction towards the anode. At pH 9.2 the migration time of **II** increased sharply, suggesting that the phenolic OH group of **II** had begun to dissociate. Compound **I** was no longer observed in solutions at pH 9.2, presumably owing to its further decomposition. The best pH range for the separation of salbutamol and its decomposition products proved to be between 7.3–8 and this range was studied closely. Disodium hydrogenphosphate of 40 m*M* at pH 7.7 was chosen for further experiments. Acidic electrolytes (pH 2.2–2.5) were used in earlier investigations on CE separations of salbutamol and related impurities since the sample solutions then contained basic compounds [5–8].

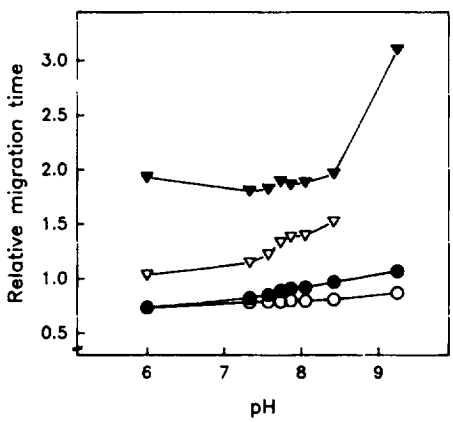


Fig. 2. Effect of buffer pH on the relative migration times of (○) salbutamol and its decomposition products (▽) **I**, (▼) **II** and (●) **IV**. Conditions: 40 m*M* disodium hydrogenphosphate, 14 kV, 25°C (method 1 in Table 1).

As the temperature was raised (18–30°C; method 1 in Table 1), the decrease in viscosity of the buffer solution and the increasing current inside the capillary decreased the migration times. To obtain reproducible results it is very important to keep the temperature constant during the analysis, e.g., by using capillary cooling, as was applied in this study. The resolution of salbutamol and the decomposition products was better at low temperature, so 20°C was chosen for further studies (Fig. 3).

The repeatabilities of the migration time and the corrected peak area of salbutamol were studied with three different uncoated fused-silica capillaries (Beckman 50 and 75 μm I.D., Isco 75 μm I.D.; method 2 in Table 1) and with different injection times (Table 2). A comparison of the repeatabilities showed that the smaller capillary inner diameter and an injection time of 5 s gave the best repeatability of the absolute migration time of salbutamol. However, no difference was seen between the capillaries when the migration time was related to the electroosmotic flow (measured with benzyl alcohol). Corrected peak-area values were used to compensate the differential peak velocities of the compounds [11]. The correction consisted of multiplying the area of the compound by its velocity (length to detector divided by migration time). The repeatability of the peak area of salbutamol was better with a capillary of larger inner diameter. Using a wider bore capillary the volume injected increased and larger peak sizes resulted. Better standard deviations of the peak area might be achieved by relating the peak area to that of the internal standard. With the short capillary (20/27 cm), the analysis time decreased considerably, but the resolution deteriorated. Also, the current increased with decrease in capillary length.

The biological buffers (methods 6 and 7 in Table 1) gave lower currents than the inorganic buffers (methods 2–5 in Table 1), but the separation of the compounds was not better than with disodium hydrogenphosphate.

3.1.2. Micellar electrokinetic capillary chromatography

To resolve salbutamol from the trace peaks, a MECC method was developed in which anionic,

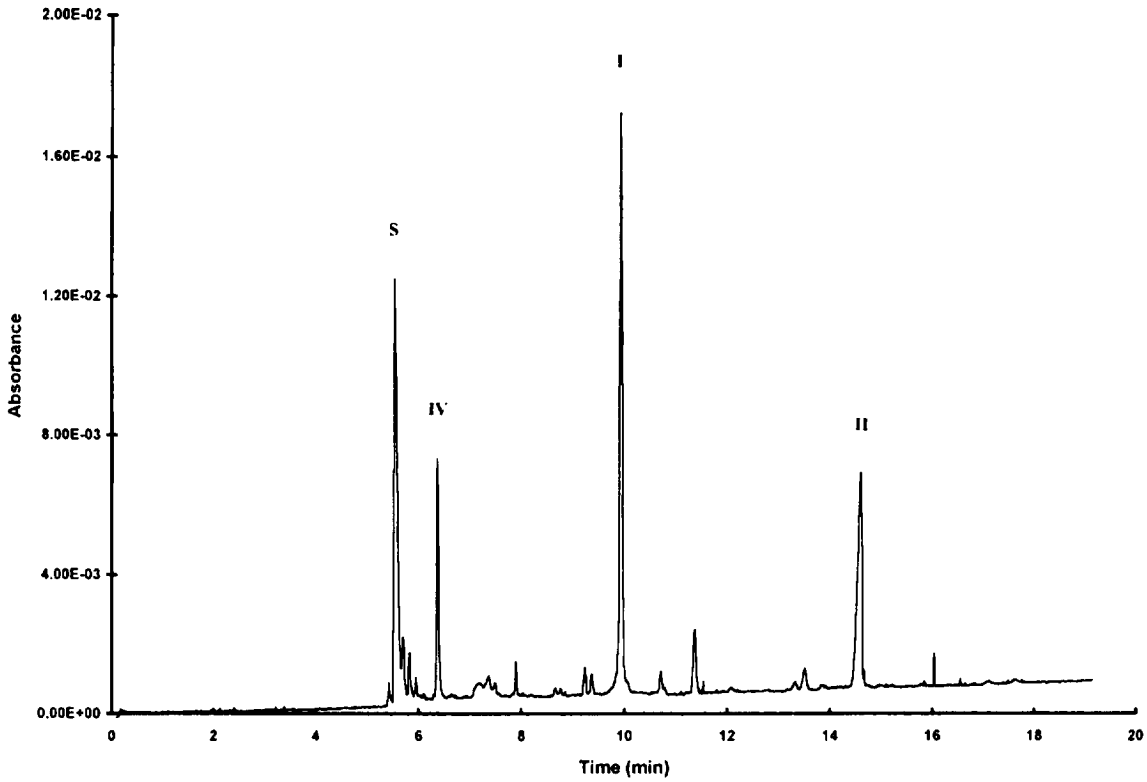


Fig. 3. Electropherogram of decomposed salbutamol sulphate solution recorded by using 40 m*M* phosphate buffer (pH 7.7) (method 1 in Table 1, 14 kV, 20°C). Sample: decomposed salbutamol sulphate in aqueous acetic acid (pH 2.2) (85°C). Peaks: S = salbutamol; I, II and IV = decomposition products **I**, **II** and **IV**, respectively.

cationic and zwitterionic surfactants [sodium dodecyl sulphate (20 m*M*), CTAB (20 m*M*) and CHAPS (20 m*M*)] in disodium hydrogenphosphate were studied. The heterogeneous character of the compounds required that all these surfactants were included. The most suitable surfactant for the analysis of decomposed salbutamol sulphate solution was CTAB, which reverses the

Table 2
Repeatability of the migration time and corrected peak area of salbutamol sulphate ($n = 6$) when three different capillaries and different injection times were used (method 2 in Table 1)

Capillary (50/57 cm)		Injection time	Migration time R.S.D. (%)		Area R.S.D.
Supplier	I.D. (μm)	(s)	Absolute	Relative	(%)
Beckman	50	2.5	0.34	0.30	12.18
		5	0.09	0.12	5.47
		10	0.38	0.25	9.40
Beckman	75	2.5	1.03	0.18	6.50
		5	0.65	0.26	4.33
Isco	75	2.5	4.04	0.96	8.90
		5	0.53	0.22	6.95

electroosmotic flow. The polarity of the power supply was reversed accordingly, causing the migration orders to be opposite to those in CZE, so that first the anionic and then the neutral and cationic compounds migrated (Fig. 4a). When sodium tetraborate was added to the buffer solution containing CTAB, salbutamol migrated before decomposition product **IV** and separated totally from the trace compounds (Fig. 4b). In the presence of sodium tetraborate, salbutamol as a salicyl alcohol derivative may have formed a neutral borate complex. As a salicylaldehyde derivative, compound **IV** would not do this. The concentration of CTAB had no effect on the migration order of the compounds. After all adjustments of buffer and surfactant concentration, pH, temperature, voltage, capillary length and inner diameter (method 8 in Table 1), 5 m*M* CTAB in 20 m*M* phosphate–borate buffer (pH 7.6) (−16 kV, 25°C) was chosen as electrolyte for the quantification studies (Fig. 5).

3.2. Determination of salbutamol

Although the electropherogram with phosphate buffer (Fig. 3) on the whole was superior in giving sharp peaks, the MECC method developed (Fig. 5) was better for the determination of salbutamol content in that it separated the parent drug from the trace compounds. A common disadvantage in CE has been the poor repeatability of the absolute migration times and peak areas. To improve the quantitative precision, an internal standard was used in MECC, as this compensated for any variability in injection volume. Peak area is better than peak height in CE quantification, because height increases are non-linear at high concentrations [12]. The peak-area precision can also be improved with high sample loadings.

The MECC method was compared with the HPLC method. Note that no internal standard was used in HPLC because the loop volume was the injection volume, and this effectively eliminated the changes in injection volumes. In MECC, the within-day precision of the relative migration time of salbutamol was better than 0.5% and the day-to-day precision was 0.55%. The same values for the retention time in HPLC were 1.96% and 1.44%, respectively. Relating the migration time to the electroosmotic flow decreased the R.S.D. relative to that of the absolute migration time.

The calibration graph for salbutamol sulphate showed excellent linearity for both methods. In MECC the equation was $y = 0.398x + 1.94 \cdot 10^{-2}$ ($r^2 = 1.000$) and the confidence intervals for the slope (95%) were 0.396–0.400, and in HPLC the equation was $y = 1.57 \cdot 10^{-2}x + 7.18 \cdot 10^{-4}$ ($r^2 = 0.999$) and the confidence intervals for the slope (95%) were 0.015–0.016 (in MECC y = corrected peak-area ratio of salbutamol and internal standard, in HPLC y = peak area; x = concentration in m*M*). The repeatability of the MECC method expressed as relative standard deviation ($n = 6$) was 0.33% for a 6.0 m*M* solution and 1.98% for a 0.6 m*M* solution. The corresponding values for HPLC were 0.75% for a 2.0 m*M* solution and 1.88% for a 0.2 m*M* solution.

To determine the suitability of the MECC method for the determination of salbutamol, salbutamol sulphate solutions containing two antioxidants were decomposed in the preheated oven. After appropriate time intervals, two or more samples were removed from the oven and the salbutamol content was determined by MECC and HPLC. The results are given in Table 3. The rate constants (k_{obs}) were determined from the slopes of the straight lines obtained by plotting the logarithm of the residual salbutamol concentration against time. MECC gave better results both for the recovery of salbutamol (100.5%) and for the deviations of parallel decomposed salbutamol solutions (R.S.D. < 2.3%). The corresponding values in HPLC were 103.0 and 4.1%. A correlation of the CE data (y) with the HPLC data (x) using salbutamol concentrations (m*M*) gave the equation $y = 0.999x - 0.568$ ($r^2 = 0.993$). A comparison of the two methods by least-squares fitting indicated that there was no significant difference between MECC and HPLC. Altria [13] applied the t-test in comparing impurity levels of salbutamol measured by CE and HPLC, and like-

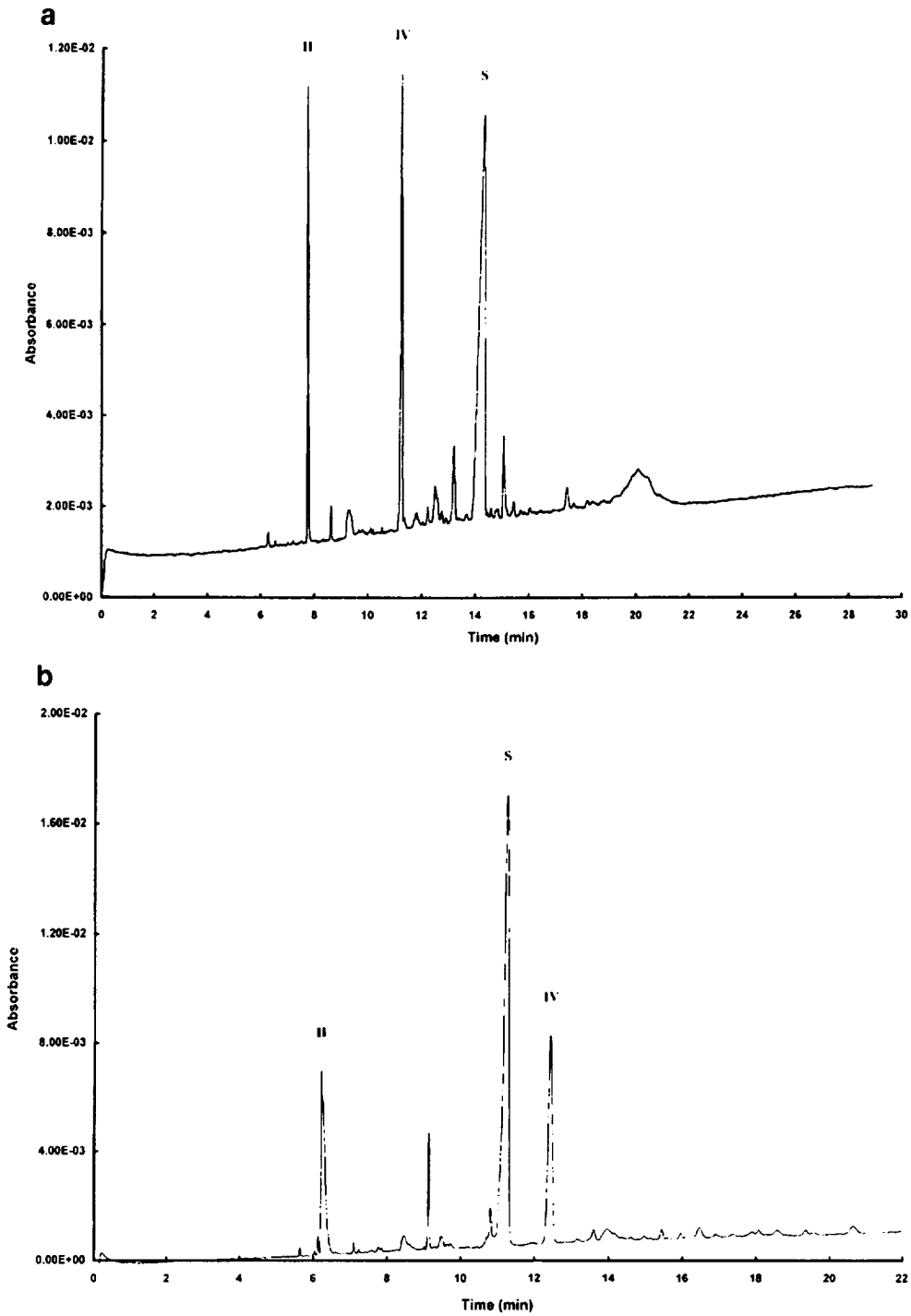


Fig. 4. Electropherogram of decomposed salbutamol sulphate solution obtained by MECC. Electrolyte: (a) 40 m*M* disodium hydrogenphosphate containing 20 m*M* CTAB (pH 7.7); (b) 40 m*M* disodium hydrogenphosphate containing 40 m*M* sodium tetraborate and 5 m*M* CTAB (pH 7.7). Voltage, −14 kV; other conditions as in Fig. 3. Sample: decomposed salbutamol sulphate in water (85°C). Peaks: S = salbutamol; II and IV = decomposition products **II** and **IV**, respectively.

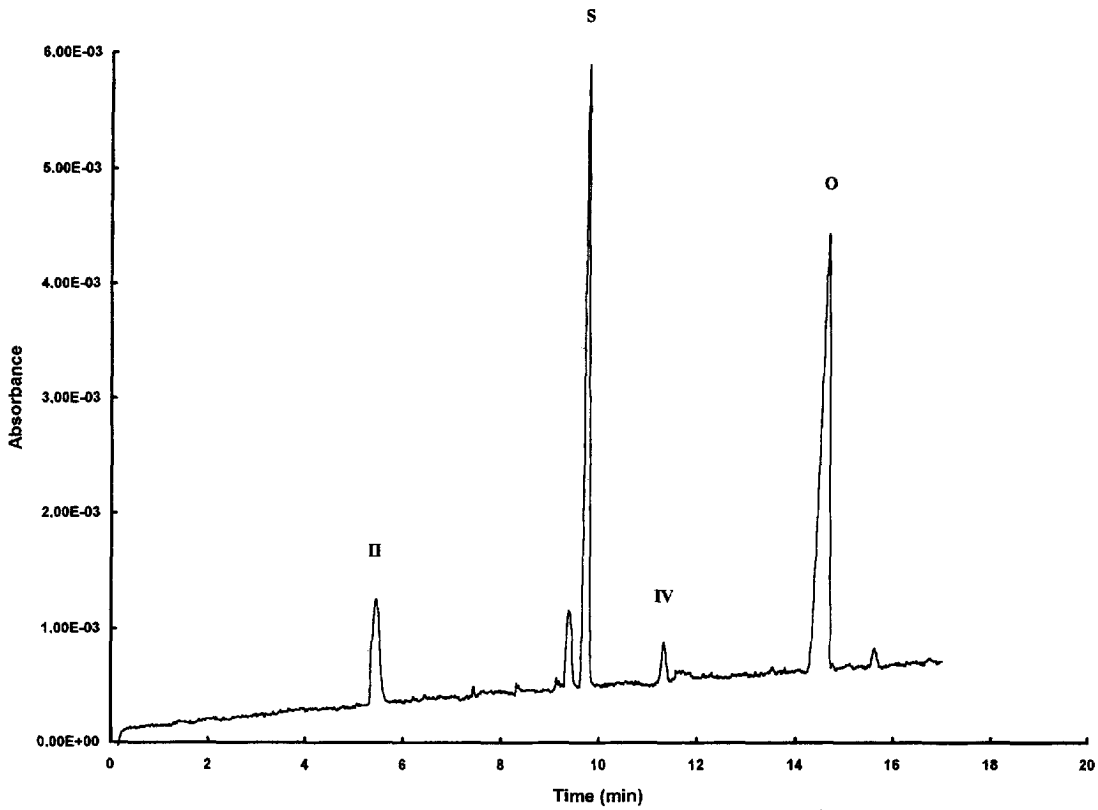


Fig. 5. Electropherogram of decomposed salbutamol sulphate in citrate buffer (pH 5.2). Electrolyte, 5 m*M* CTAB in 20 m*M* disodium hydrogenphosphate and sodium tetraborate solution (pH 7.6); voltage, −16 kV; temperature, 25°C; capillary, Beckman 50 μm (50/57 cm) uncoated fused silica. Peaks: S = salbutamol; O = orciprenaline (internal standard); II and IV = decomposition products **II** and **IV**, respectively.

Table 3
Determination of decomposed salbutamol sulphate by MECC and HPLC

Time (h)	% Salbutamol remaining	
	MECC	HPLC
0	100.0	100.0
24.1	73.9	73.5
114.2	65.5	66.6
168.5	58.2	63.1
210.0	52.1	52.3
271.2	43.7	44.3
441.0	38.0	40.0

k_{obs} ($h^{-1} \times 10^{-3}$) was 2.04 by MECC and 1.94 by HPLC.

wise found the two methods to give similar results.

4. Conclusions

This study has demonstrated that CE is suitable for separating salbutamol and both its acidic and basic decomposition products. MECC separated the similar compounds efficiently and provided a reliable determination of salbutamol. Good repeatability of both migration time and peak area was achieved. The correlation between the analytical data obtained by MECC and HPLC was good.

There are some advantages in using CE in studies of the decomposition of salbutamol: rela-

tive to HPLC, the overall method run time is short and solvent and sample consumption are low.

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