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Micellar electrokinetic chromatography of scopolamine-related anticholinergics

Hsin-Lung Wu*, Chiu-Hui Huang, Su-Hwei Chen, Shou-Mei Wu

Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical College, 100 Shih Chuen 1st Road, Kaohsiung 807, Taiwan

Abstract

A simple micellar electrokinetic chromatography (MEKC) method is described for the separation of scopolamine *N*-oxide hydrobromide (SO), scopolamine hydrobromide (SH), scopolamine *N*-methylbromide (SM) and scopolamine *N*-butylbromide (SB), and for the quantitation of SH, SM and SB (using SO as an internal standard). The analysis of these drugs was performed in a phosphate buffer (30 m*M*; pH 7.00) with sodium dodecyl sulfate (SDS) (30 m*M*) as an anionic surfactant. Several parameters affecting the separation of the drugs were studied, including the concentrations of the buffer and SDS. The stability of the drugs in the phosphate buffer (pH 7.00) was also examined. Partial application of the method to the determination of scopolamine *N*-butylbromide in tablets proved to be feasible. © 1998 Elsevier Science B.V.

Keywords: Pharmaceutical analysis; Scopolamine-related drugs; Alkaloids

1. Introduction

Scopolamine-related drugs possess a wide variety of biological activities and have been used for the treatment of many diseases; for example, scopolamine, a tropane alkaloid from solanaceous plants, is used as an anti-convulsant, anti-emetic, anti-parkinsonism and sedative. Scopolamine is also evaluated as a possible diagnostic agent for dementia.

Numerous approaches have been established for the determination of scopolamine hydrobromide (SH), scopolamine *N*-methylbromide (SM) or scopolamine *N*-butylbromide (SB). Among them, nonaqueous titrimetry [1,2], gas chromatography (GC) [1–4] and visible absorption spectrophotometry [2,5] are used for the determination of SH or SB in bulk and in pharmaceutical preparations. Nonaqueous titrimetry is simple but usually it lacks specificity. Absorption spectrophotometry and GC [1,2] require lengthy preparation of the drugs before analysis.

Highly sensitive methods including GC–mass spectrometry (MS) [6–8], radioreceptor assay [9] and enzyme-linked immunoassay [10] are used for the trace analysis of scopolamine in biosamples. The derivatization of scopolamine before analysis is required in the GC–MS method. A crossreaction with SM or SB is reported in the enzyme-linked immunosorbent assay of SH.

High-performance liquid chromatography (HPLC) [11–21] with various separation modes is widely used for the analysis of scopolamine or other tropane alkaloids in formulations and plants or in biosamples. But no available method is reported for the simultaneous analysis of scopolamine *N*-oxide hydrobromide (SO), SH, SM and SB. It will be useful

*Corresponding author.

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if simple and specific methods are available for qualitative and quantitative analyses of these drugs.

In this work, a simple micellar electrokinetic chromatography (MEKC) method is developed for the simultaneous separation and quantitation of SO, SH, SM and SB. The scopolamine-related drugs were analyzed on an uncoated capillary with phosphate buffer (30 m*M*; pH 7.00) and sodium dodecyl sulfate (SDS) (30 m*M*) as a background electrolyte (BGE). The quantitation range of each drug is 50–300 μ*M*. Application of the method to the determination of SB in tablets was demonstrated and proved to be satisfactory.

2. Experimental

2.1. Apparatus and capillary electrophoresis (CE) conditions

A Beckman P/ACE System 2200 (Fullerton, CA, USA) equipped with a filter UV detector and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 37 cm (effective length 30 cm)×50 μm I.D. Samples were injected by pressure for 2 s and the applied voltage was 24 kV. Separations were performed at about 25°C in phosphate buffer (30 m*M*; pH 7.00) with SDS (30 m*M*). The aforementioned CE conditions were used for the general study unless stated otherwise. A Beckman Gold software system was used for data processing.

2.2. Chemicals and buffer solutions

SO, SH, SM, SB, tropic acid (TA) and SDS (Sigma, St. Louis, MO, USA), disodium hydrogenphosphate (Na_2HPO_4) and phosphoric acid (H_3PO_4, 85%) (E. Merck, Darmstadt, Germany) were used without further treatment. Milli-Q (Millipore, MA, USA) treated water was used for the preparation of buffer and related aqueous solutions. Solutions of various phosphate buffers at pH 7.00 were prepared by neutralizing the related Na_2HPO_4 solution with H_3PO_4. Solutions of SDS at various levels were obtained by dissolving SDS in related phosphate buffer.

2.3. Reference and sample solutions

A reference solution of SO, SH, SM or SB at 300 μ*M* was prepared in phosphate buffer (30 m*M*; pH 7.00) for individual study. Mixed solutions of SH, SM and SB each at various concentrations (50–300 μ*M*) in phosphate buffer with a fixed concentration of SO (300 μ*M*) were prepared for the simultaneous determination. Sample solution for the content uniformity test of SB was prepared by dissolving each tablet of SB in water in a 50-ml volumetric flask with the aid of sonication for 5 min. A suitable amount of the resulting water extract was centrifuged at 130 *g* for 10 min. The supernatant was filtered (pore size, 0.5 μm) and the filtrate was used for MEKC of SB. Sample solution for the assay of SB was prepared as follows: 20 tablets of SB were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 3.0 mg of SB was transferred to a 25-ml volumetric flask. The dissolution of SB in water was effected by sonication for 5 min, and the solution was diluted to the volume with water. A suitable amount of the water extract was centrifuged and filtered as directed above (the preparation of SB solution for the content uniformity test). The resulting filtrate was used for the quantitation of SB.

3. Results and discussion

Separation of SO, SH, SM, and SB (see Fig. 1 for their structures) by capillary zone electrophoresis (CZE) was briefly studied at 24 kV with phosphate buffer (30 m*M*; pH 7.00) in the absence of SDS, resulting in overlapping of SH, SM and SB (see Section 3.2). This indicates that simple separation mode of CZE based mainly on the differences of charge to mass ratios of the analytes in the tested conditions is unable to resolve the scopolamine-related compounds. Therefore, MEKC with SDS as a micellar source was tried to separate the analytes in the expectation that additional factor contributed to the separation of the drugs could work such as ion interaction or chromatographic partition. As a consequence, simple parameters affecting the MEKC of these drugs were studied, including concentration of buffer and SDS. In addition, optimal absorption

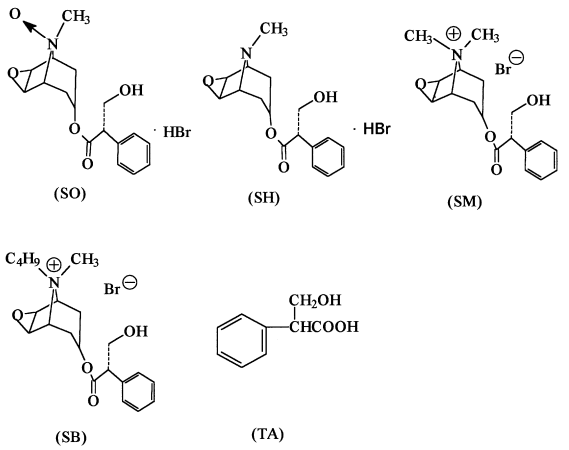

Fig. 1. Structures of scopolamine *N*-oxide hydrobromide (SO), scopolamine hydrobromide (SH), scopolamine *N*-methylbromide (SM), scopolamine *N*-butylbromide (SB) and tropic acid (TA).

wavelength for monitoring and the stability of the drugs at phosphate buffer (pH 7.00) were also briefly investigated.

3.1. Wavelength for detection

After MEKC separation of SO, SH, SM and SB, the eluted compounds were monitored at 200, 214 or 254 nm. The results in Fig. 2 indicate that a higher response and better sensitivity can be obtained by detecting the drugs at 200 nm and monitoring the drugs at 200 nm.

3.2. Concentration of SDS

The effects of SDS at concentration range 0–40 m*M* in phosphate buffer (30 m*M*; pH 7.00) on the separation are shown in Fig. 3. The results indicate that electrophoresis of the drugs in the absence of SDS results in no resolution of SH, SM and SB (Fig. 3A). MEKC of the drugs at lower levels of SDS (5–10 m*M*) gives resolved peaks, but the peaks are in extremely tailing, especially in the case of SDS concentration lower than its critical micellar concentration (CMC) (about 8 m*M*) (Fig. 3B). MEKC of the drugs at higher SDS concentration (20–40 m*M*) gives sharp and symmetric peaks (Fig. 3C). Therefore, MEKC of the drugs in the phosphate buffer (30 m*M*; pH 7.00) with SDS (30 m*M*) was adopted for the analysis.

3.3. Concentration of phosphate buffer

MEKC of the drugs in phosphate buffer (pH 7.00) in the concentration range 10–40 m*M* with constant

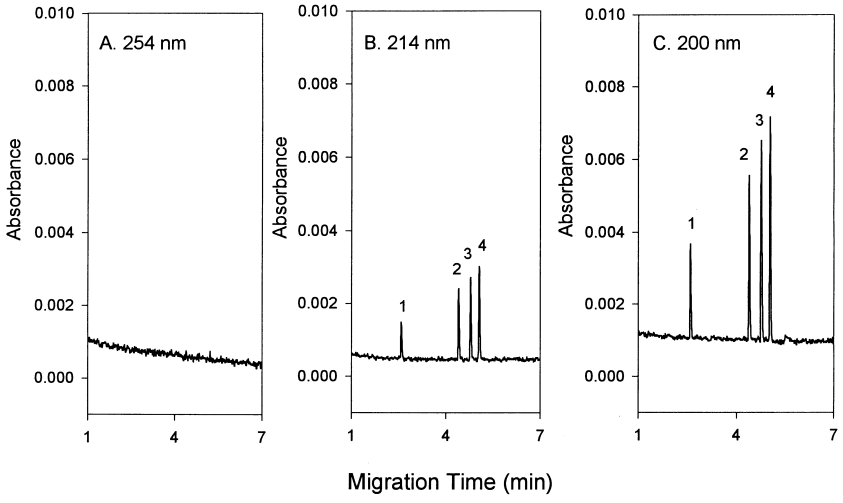

Fig. 2. Electropherograms of scopolamine related drugs each at 300 μ*M*, detected at (A) 254 nm, (B) 214 nm or (C) 200 nm. Peaks: 1=Scopolamine *N*-oxide; 2=scopolamine hydrobromide; 3=scopolamine *N*-methylbromide and 4=scopolamine *N*-butylbromide. MEKC conditions: buffer, 30 m*M* phosphate (pH 7.00) with 30 m*M* SDS; applied voltage, 24 kV (detector at cathode side); uncoated fused-silica capillary, 30 cm (effective length)×50 μm I.D.; sample size, 2 s by pressure.

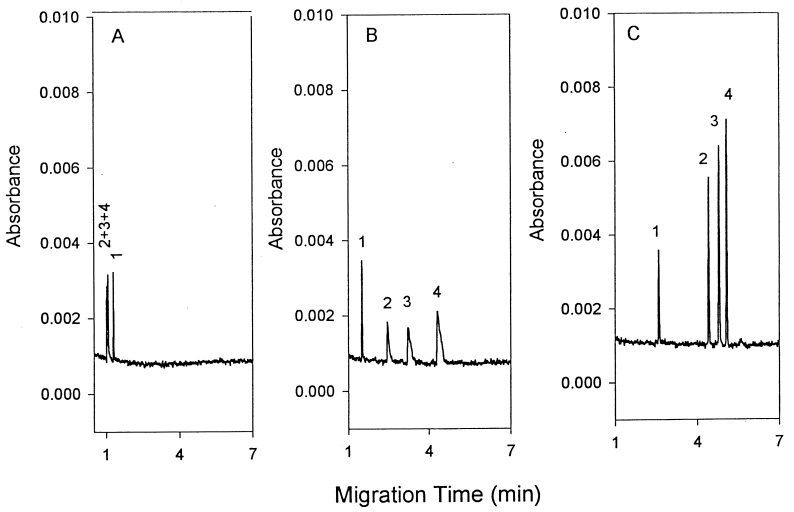

Fig. 3. Effects of SDS concentrations on the migration of scopolamine related drugs each at 300 μ*M* in phosphate buffer (30 m*M*; pH 7.00). Peaks: 1=Scopolamine *N*-oxide (SO); 2=scopolamine hydrobromide (SH); 3=scopolamine *N*-methylbromide (SM) and 4=scopolamine *N*-butylbromide (SB). Electropherograms: (A) overlapping peaks from separation in the absence of SDS, (B) tailing peaks from separation with SDS (5 m*M*) lower than its CMC and (C) sharp peaks from separation with suitable SDS (30 m*M*). See Fig. 2 for other CE conditions.

SDS level (30 m*M*) was studied (Fig. 4). The results indicate that baseline resolution of the drugs is unattainable at lower buffer concentration (≤20 m*M*) (Fig. 4B), but it can be favorably obtained at higher buffer concentration (30 m*M*). From the results of SDS and buffer effects on the MEKC of the drugs, optimization of the CE conditions were formulated as directed in Section 2.1. The method is simple, specific and rapid. Based on the short analytical time (about 5 min), sharp peak shape and well resolution of the drugs from present conditions, additional studies on other parameters such as or-

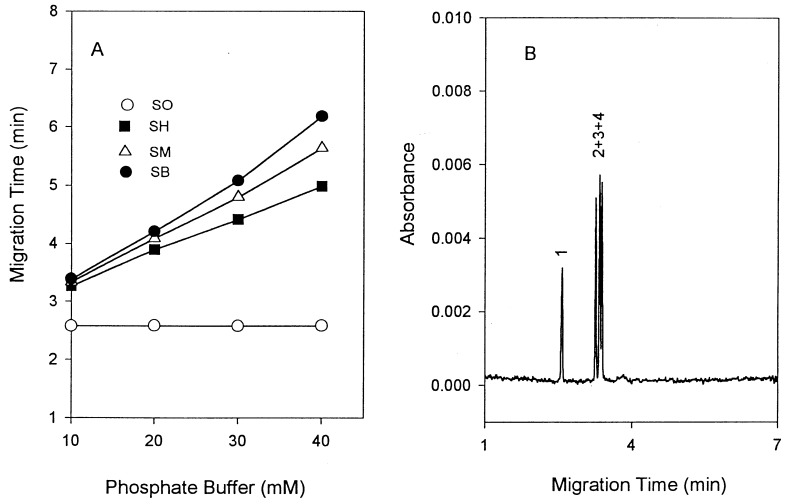

Fig. 4. Effects of buffer concentrations on the migration of scopolamine related drugs each at 300 μ*M* in phosphate buffer (10–40 m*M*) (pH 7.00) with constant SDS (30 m*M*). (A) SO, SH, SM and SB stand for scopolamine *N*-oxide, scopolamine hydrobromide, scopolamine *N*-methylbromide and scopolamine *N*-butylbromide, respectively. (B) Electropherogram with unresolved peaks obtained from separation in phosphate buffer at lower concentration (10 m*M*); peaks 1, 2, 3 and 4 for SO, SH, SM and SB, respectively.

ganic additive and alternative buffer seem to be unnecessary for practical purpose.

3.4. Stability of the drugs

Scopolamine, an ester, is reported [22,23] to be unstable in acidic and basic solutions. The stability of SH, SM, SB and SO each at 300 μ*M* in phosphate buffer (30 m*M*; pH 7.00) was cursorily studied, using TA (Fig. 1) as an identifying marker. TA is a degradation product from SO, SH, SM and SB. No observable peak of TA appears in 22 h following MEKC of the drugs in phosphate buffer (30 m*M*; pH 7.00) at room temperature (25°C). A significant peak was found from MEKC of the drugs in the phosphate buffer (30 m*M*; pH 7.00) after standing for 28 h at room temperature (Fig. 5B). Peak 0 in Fig. 5B corresponds to that of TA under the same CE conditions. This indicates that the drugs in phosphate buffer (30 m*M*; pH 7.00) are almost stable for daily run (<22 h).

3.5. Analytical calibration

For evaluating the quantitative applicability of the method, five different concentrations of SH, SM and SB each in the range 50–300 μ*M* were analyzed, using SO (300 μ*M*) as an internal standard (I.S.). The linearity between the peak-area ratios (y) of the related analyte to I.S. and the concentration (x, μ*M*) of analyte was investigated. The linear regression equation ($n=5$) obtained were $y=(0.0421\pm0.0207)+(0.0062\pm0.0002)x$ ($r=0.998$) for SH, $y=(0.0389\pm0.0272)+(0.0073\pm0.0002)x$ ($r=0.999$) for SM, $y=(0.0641\pm0.0261)+(0.0080\pm0.0002)x$ ($r=0.999$) for SB. The results indicate that high linearity between y and x is attainable over the range studied. The lower detection limits of SH, SM and SB are all about 10 μ*M* based on signal-to-noise ratio of 3. The relative standard deviation (R.S.D.) of the method based on the peak-area ratio for replicate determinations of SH, SM or SB each at 80, 160 and 280 μ*M* was studied. The results in Table 1 indicate that the intra-day R.S.D.s ($n=5$) of the three analytes each at 80 μ*M* were all below 4.2%, that at 160 μ*M* were all below 3.4% and that at 280 μ*M* were all below 2.5%; in parallel, the inter-day R.S.D.s ($n=5$) for the three analytes were all below 5.0% at 80 μ*M*, below 4.2% at 160 μ*M* and below 4.0% at 280 μ*M*. SO is selected as the I. S. for the quantitation because it is least used as an anti-cholinergic now; if in the case that the de-

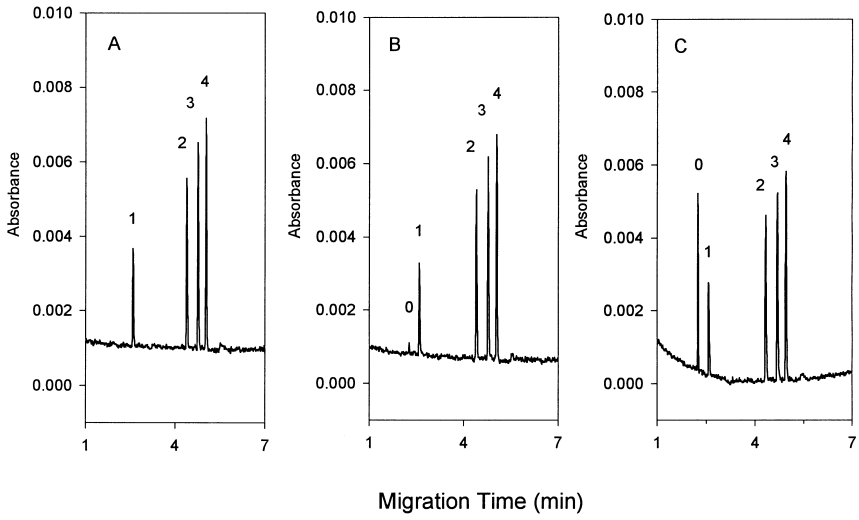

Fig. 5. Electropherograms for MEKC of scopolamine *N*-oxide (NO), scopolamine hydrobromide (SH), scopolamine *N*-methylbromide (SM) and scopolamine *N*-butylbromide (SB): (A) from a freshly prepared mixture of each at 300 μ*M* in phosphate buffer (30 m*M*; pH 7.00); (B) from the mixture (A) after standing at 25°C for 28 h and (C) from the mixture (A) spiked with tropic acid (TA) (300 μ*M*). Peaks: 0, 1, 2, 3 and 4, respectively, for TA, SO, SH, SM and SB.

Table 1
Precision for the determination of SH, SM and SB

Concentration known[a] (μM)	Concentration found (μM)	R.S.D. (%)
Intra-day analysis ($n=5$)		
SH		
80	79.24±3.30	4.16
160	160.44±4.14	2.58
280	276.64±5.99	2.16
SM		
80	82.29±3.39	4.12
160	159.80±5.27	3.30
280	283.76±6.96	2.45
SB		
80	82.22±2.31	2.81
160	157.55±5.26	3.34
280	282.31±6.54	2.32
Inter-day analysis ($n=5$)		
SH		
80	79.15±3.94	4.97
160	161.59±6.73	4.16
280	278.97±9.76	3.97
SM		
80	80.53±3.20	3.97
160	161.85±5.74	3.54
280	280.52±9.50	3.38
SB		
80	81.62±3.97	4.86
160	161.41±5.65	3.50
280	279.48±5.03	1.80

[a] SH, SM and SB are scopolamine hydrobromide, scopolamine *N*-methylbromide and scopolamine *N*-butylbromide, respectively.

termination of SO is required, SH, SM or SB can be used as an I.S.

3.6. Application

Application of the method to the analysis of SB in tablets was studied including both the content uniformity test and the assay usually required by an official Pharmacopoeia. The results are given in Tables 2 and 3, all the analytical values from 10 tablets (Table 1) fall within labeled amount of 85~115% required by the Chinese Pharmacopoeia (ChP) [2] for the content uniformity test; and those analytical results of the assay (Table 3) also pass the ChP requirement based on the content range 92.5~107.5% of labeled amount. A typical electropherogram for the analysis of SB in tablet is shown in Fig. 6. The recoveries of SB were simply studied, based on a known amount of reference SB added to the powder tablet of SB resulting in the preparation of three spiked levels of SB (50, 100 and 150 μM) for analysis. All the recoveries are above 96% (96.9~101.8%).

In conclusion, a simple and specific MEKC method has been developed for the qualitative separation of SO, SH, SM and SB, and for the quantitative analysis of SH, SM and SB using SO as the I.S. The method is simple and practical. It can be applicable to the quality control of these widely used drugs (SH, SM and SB) in bulk and in pharmaceutical preparations.

Table 2
Analytical results for content uniformity of scopolamine *N*-butylbromide in tablets obtained from a commercial source

Sample[a]	Amount found[b] (mg)	Percentage of claimed content[c] (%)
1	9.72±0.12	97.2
2	10.01±0.25	100.1
3	10.07±0.21	100.7
4	9.86±0.12	98.6
5	9.86±0.28	98.6
6	9.90±0.21	99.0
7	9.69±0.24	96.9
8	9.96±0.25	99.6
9	9.91±0.17	99.1
10	9.92±0.25	99.2

[a] Labeled amount of scopolamine *N*-butylbromide in each tablet is 10 mg.
[b] Mean±S.D. of five replicate analyses.
[c] Content uniformity test is used to check the variation of scopolamine *N*-butylbromide in each tablet.

Table 3
Assay results of scopolamine *N*-butylbromide in tablets obtained from a commercial source

Tablet[a]	Amount found[b] (mg)	Percentage of claimed content (%)
1	9.93±0.24	99.3
2	9.88±0.33	98.8
3	10.03±0.26	100.3
4	10.06±0.19	100.6
5	10.21±0.18	102.1
	Mean	100.2
	S.D.	1.28

[a] Labeled amount of scopolamine *N*-butylbromide in each tablet is 10 mg.
[b] Mean±S.D. of five replicate analyses.

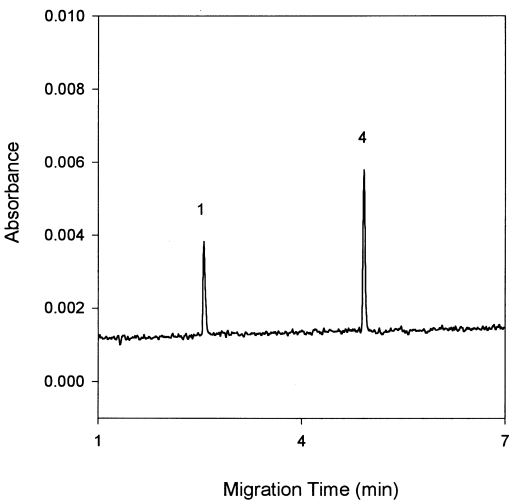

Fig. 6. Typical electropherogram for the analysis of scopolamine *N*-butylbromide (SB) in tablet. See Fig. 2 for MEKC conditions. Peaks 1 and 4, respectively, for NO and SB.

Acknowledgements

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