キャピラリー電気泳動法による整腸薬の分離分析

Capillary electrophoretic separation and analysis of antidiarrheal drugs

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Abstract Atropine, berberine and acrinol were separated and analysed by capillary electrophoresis. Several modifiers such as sodium dodecylsulfate and ethanol were added to the electrophoretic buffer solution (20 mM sodium borate, pH 9.2) to improve the separation of the three compounds. These compounds were successfully separated within 13 min by using a 72 cm × 50 μm i.d. fused-silica capillary at 30 kV. The detection was done at 200 nm. The calibration graphs were straight lines over the range 0.5 – 2.0 × 10^{-4} mol l^{-1} for each atropine, berberine and acrinol. RSDs were 1.7%, 1.9% and 1.3% for 1 × 10^{-4} mol l^{-1} atropine, berberine and acrinol, respectively.

1. Introduction

Acrinol (2-ethoxy-6,9-diaminoacridine monolactate monohydrate) and berberine are combined together in the antidiarrheal drugs, and also a medicine for stomach involving atropine is taken for loosening of the bowels. It is very important to analyse these compounds in the pharmaceutical preparations and urine medically and pharmacologically. We found that extraction-spectrophotometry with Tetrabromophenolphthalein ethyl ester and Bromocresol Green dye anions were useful for the determination of quaternary ammonium compounds at the level of 10^{-7} – 10^{-6} mol l^{-1}.[1)-3)] However, the selectivity of the methods was poor it was difficult to separate only quaternary ammonium compounds.

To enhance selectivity among quaternary ammonium salts, high-performance liquid chromatography (HPLC)[4)], pyrolysis gas chromatography-mass spectrometry[5)], supercritical fluid extraction and liquid chromatography[6)] have been used.

Recently, capillary electrophoresis (CE) has been used to separate multi-component pharmaceuticals in human plasma and cold preparations.[7),8)] Lin *et al.* have reported the difficulties to separate quaternary ammonium compounds by capillary zone electrophoresis (CZE),[9),10)] whereas the characterization of the cationic surfactant in the electroosmotic flow has been studied by CZE.[11)] However, CE is scarcely applied to the analysis of antidiarrheal drugs involving quaternary ammonium salts.

This paper deals with the rapid and simple separation and simultaneous determinations of atropine, berberine and acrinol as antidiarrheal drugs.

2. Experimental

2・1 Reagents

Standard acrinol and berberine solutions (2.5 × 10^{-3} mol l^{-1}) and atropine (1.25 × 10^{-3} mol l^{-1}) solution were prepared by dissolving a proper amount of the salt in 100 ml of distilled water.

Pentanesulfonate (0.25 mol l^{-1}) (SPS) and dodecylsulfate solutions (0.1 mol l^{-1}) (SDS) were prepared.

Acetate buffers (pH 3.0 – 6.0), phosphate buffers (pH 7,8) and borate buffers (pH 9.2) were prepared.

2・2 Apparatus

Electrophoretic experiments were carried out using Perkin Elmer Applied Biosystems 270A-HT (U.S.A) with a spectrophotometric detection. An uncoated fused-silica capillary was 50 μm i.d. and 375 μm o.d.

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(GL sciences, Japan) with an end-to-end length of 72 cm and an end-to-detection length of 50 cm. Electropherograms, as well as migration times, peak heights and peak areas, were recorded with a Hitachi D-2500 Chromato-Integrator.

2·3 Procedure

The capillary used was made by scraping off polymide coating (*ca.* 5mm) before mounting in the cassette. Samples were injected by a vacuum technique from the cathodic end for 1 s. The rate of the injection volume was about 3 nl/s. The temperature and applied voltage were held at 35 ℃ and 30 kV, unless otherwise specified. Before each run, the capillary was rinsed successively for 30 min with 0.1 mol l^{-1} NaOH and washed for another 30 min with the electrophoretic running buffer. On-column detection of separated analytes was performed at 200 nm.

3. Results and discussion

3·1 Absorption spectra

Absorption spectra of atropine, berberine and acrinol from 200 nm to 400 nm were measured. Berberine had absorptions at 228 nm, 263 nm and 345 nm and the absorptions of acrinol were at 269 nm and 358 nm. Atropine had absorption at low UV wavelength. For the simultaneous determination of these drugs, the detection was done at 200 nm.

3·2 Effect of pH

The apparent mobilities of acrinol, atropine and berberine were studied in the range of pH 3.0 – 6.0. The mobilities of berberine and atropine slightly decreased with increasing pH and that of acrinol decreased remarkably above pH 3.4 and its peak got out of shape. The separation behaviour was also studied at pH 7-9.2. Mobilities of atropine, berberine and acrinol at pH9.2 with 20 mM sodium borate increased and separation of atropine and berberine was improved compared with that at lower pH range. However, the overlap of the peaks of acrinol, atropine or berberine could not be solved.

3·3 Effect of surfactants

Terabe *et al.*[12] and Nakagawa *et al.*[13] have proposed the micellar electrokinetic chromatography using SDS for the efficient separation of drugs. In this work, the effects of SPS and SDS, which are expected to give both the micellar effect and the ion-pairing effect and to improve the separation, were also investigated. The migration time increased with increasing SPS and SDS concentrations. The peak of acrinol with 100 mM SPS (Fig. 1B) was sharp compared with that of 50 mM SPS (Fig. 1A). However, addition of 10 mM SDS gave sharper peak as shown in Fig. 1C and also the peak height in Fig. 1B was obtained for the concentration of 5×10^{-4} mol l^{-1} acrinol and that in Fig. 1C was obtained for the concentration of 1×10^{-4} mol l^{-1}. As a result, the sensitivity was improved by adding 10 mM SDS. However, the separation among three compounds was not complete under such conditions.

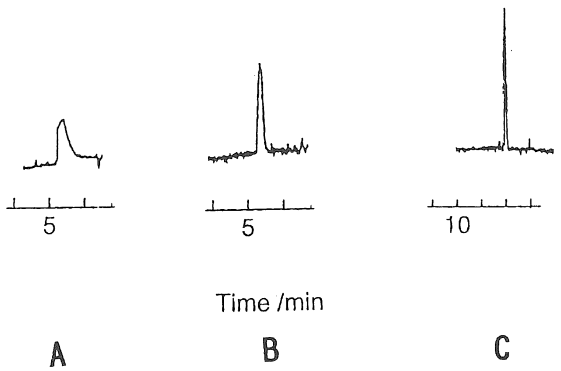

Fig. 1 Peak profiles of acrinol by addition of SPS and SDS at pH 9.2. A: 20 mM sodium borate + 50 mM SPS, [acrinol] = 5×10^{-4} mol l^{-1}; B: 20 mM sodium borate + 100 mM SPS, [acrinol] = 5×10^{-4} mol l^{-1}; C: 20 mM sodium borate + 10 mM SDS, [acrinol] = 1×10^{-4} mol l^{-1}.

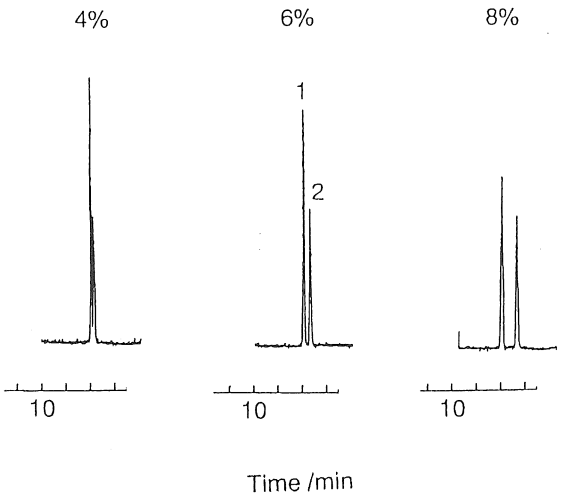

Fig. 2 Electropherograms of berberine and acrinol with different ethanol concentration. Buffer, 20 mM sodium borate + 10 mM SDS + ethanol (pH 9.2); [berberine] = 1×10^{-4} mol l^{-1}; [acrinol] = 1×10^{-4} mol l^{-1}.

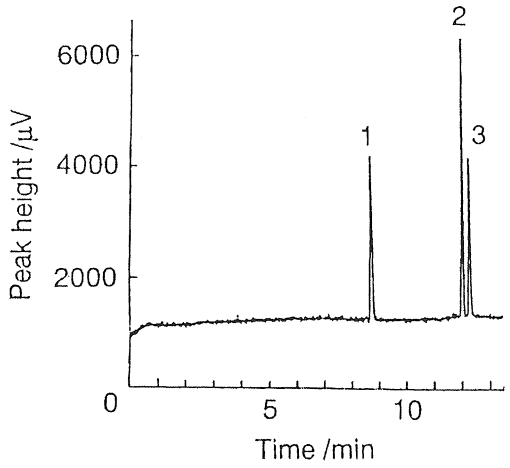

Fig. 3 Electropherogram of standard atropine, berberine and acrinol solutions. Buffer, 20 mM sodium borate + 10 mM SDS + 6% ethanol (pH 9.2). 1: 1 × 10^{-4} mol l^{-1} atropine; 2: 1 × 10^{-4} mol l^{-1} berberine; 3: 1 × 10^{-4} mol l^{-1} acrinol; conditions: 30kV, 35℃, 200nm, 1V = 1 Abs. unit.

3・4 Effect of ethanol concentration

The effect of organic solvents miscible with water to separate quaternary ammonium compounds has already been studied to separate quaternary ammonium compounds.[9),14)–16)] In this study, ethanol was added to the buffer containing 20 mM sodium borate and 10 mM SDS and its effect was investigated. Ethanol concentration was varied from 4% to 8%. As shown in Fig. 2, the separation between berberine and acrinol was complete over 6% ethanol and the reproducibility of the peak was improved. However, 8% ethanol gave smaller peak height for berberine. It seems that the formation of micelles was disrupted by adding 8% ethanol and the interaction between SDS-quaternary ammonium cations was changed. As a result, it was found that three compounds were completely separated at pH 9.2 by adding 20 mM sodium borate, 10mM SDS and 6% ethanol (Fig. 3). Linear graphs of peak area *vs.* the concentration of atropine, berberine and acrinol were obtained in the concentration ranges from 5 × 10^{-5} mol l^{-1} to 2 × 10^{-4} mol l^{-1} for about 3 nl injection, and the relative standard deviations (RSD) were 1.7%, 1.9% and 1.3% for 1 × 10^{-4} mol l^{-1} of atropine, berberine and acrinol, respectivery. Determination limit for three compounds were about 2 × 10^{-5} mol l^{-1}.

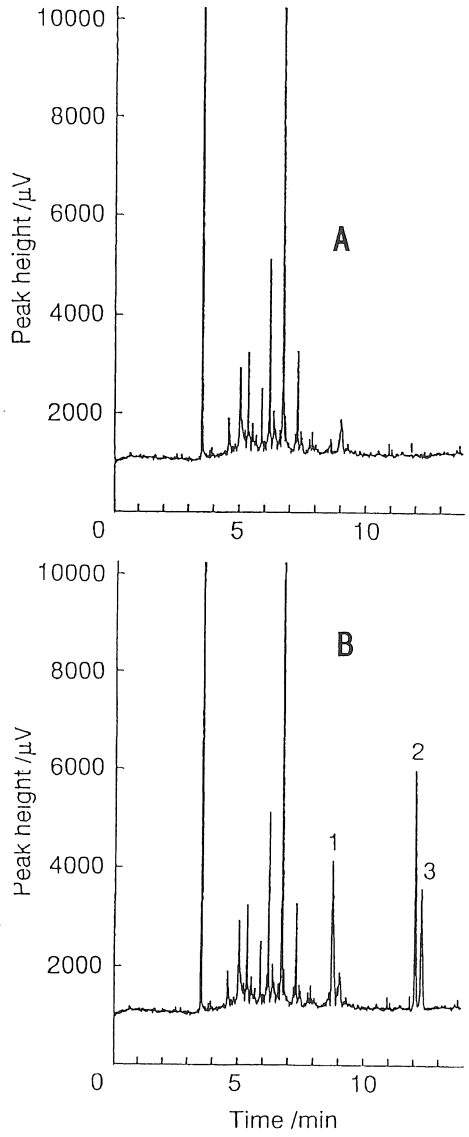

Fig. 4 Electropergrams of only urine (A) and the mixture in urine(B). Conditions are the same described above.

3・5 Recovery test of atropine, berberine and acrinol

Berberine and acrinol are combained together in antidiarrheal drugs and atropine is contained in the pharmaceuticals for stomach and bowels. About 3% of acrinol and berberine taken is excreted in urine and that of atropine excreted is about 50 – 85 %.[17)] When we have loose bowels, antidiarrheal drugs containing acrinol and berberine is taken and atropine is used at the different time. In this work, the possibility of detection of three compounds was investigated. To human urine three standard solutions(1 × 10^{-3} mol l^{-1})were added. After 10-fold dilution of the artificial urine sample, the

Table 1 Recovery test of atropine, berberine and acrinol in a synthesized urine sample

Compounds	Added/mol l^{-1}	Found(%)	RSD(%)†
Atropine	1×10^{-4}	98	6.0
Berberine	1×10^{-4}	97	1.9
Acrinol	1×10^{-4}	95	1.5

†By 3 determinations

electropherograms were observed and the recoveries were estimated. The results are shown in Fig. 4 and Table 1. Each recovery was almost 100%. But, the RSD for atropine was large compared with others. It seems that the peak of atropine and the peak-skirt resulting from urine overlapped slightly.

4. Conclusion

Capillary electrophoresis with 10 mM SDS and 6% ethanol at pH 9.2 was useful for the assay of atropine, berberine and acrinol as antidiarrheal drugs. The method has advantages of simplicity and rapidity for complicated samples such as commercial drugs and urine samples.

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