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Investigation and optimisation of the use of micellar electrokinetic chromatography for the analysis of six cardiovascular drugs

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Abstract

A micellar electrokinetic chromatography method was optimised for the separation of the six cardiovascular drugs atenolol, nicardipine, nifedipine, diltiazem, verapamil, and amlodipine by investigating the effects of pH, sodium dodecyl sulphate (SDS) concentration, selection and concentration of organic modifier. An electrophoresis buffer of 100 m*M* borate pH 8.1 containing 50 m*M* SDS and 15% (v/v) acetone was found to provide the optimum separation with respect to resolution and migration time.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is an adaptation of capillary electrophoresis originally reported by Terabe et al. [1,2]. It was originally used for the separation of electrically neutral compounds but is also effective for separating ionic compounds which because of their similar electrophoretic mobilities are not adequately resolved by capillary zone electrophoresis.

MEKC requires the addition of a charged surfactant to the background electrolyte at a level greater than its critical micelle concentration. The micelles formed have their own electrophoretic mobility which is different from the surrounding aqueous phase. Analytes may differentially partition themselves between the micellar and aqueous phases thus promoting selectivity. This selectivity can be further manipulated by the addition of an organic modifier [3] such as methanol, acetonitrile or acetone [4] to increase the elution range.

In this work, the separation of six cardiovascular drugs was optimised by the addition of an organic modifier and assessment of the effect of pH and concentration of sodium dodecyl sulphate (SDS) in the borate buffer.

2. Experimental

2.1. Reagents

Purified water was provided by a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). SDS, sodium tetraborate, boric acid and sodium hydroxide were obtained from BDH (Poole, UK). Acetone (Analar grade) and acetonitrile (Hipersolv grade) were also purchased

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from BDH and methanol (HPLC grade) was purchased from Fisons Scientific Equipment (Loughborough, UK). Atenolol, nifedipine, amlodipine, diltiazem, verapamil and nicardipine were supplied from within Bristol-Myers Squibb.

2.2. Apparatus and method

A capillary electrophoresis P/ACE system 5510 (Beckman Instruments, Palo Alto, CA, USA) equipped with diode array UV detector, an automatic injector, a fluid cooled cartridge and a System Gold data station was used in this study. All electrophoresis was carried out at 30°C, with an applied voltage of +25 kV and UV detection wavelength of 200 nm. Sample introduction was performed using the pressure option for 5 s. The capillary was a 57 cm × 75 μm I.D. (50 cm to detector) fused-silica capillary tube (Beckman Instruments), and was rinsed with 0.1 *M* sodium hydroxide and the electrophoresis buffer before each electrophoretic separation

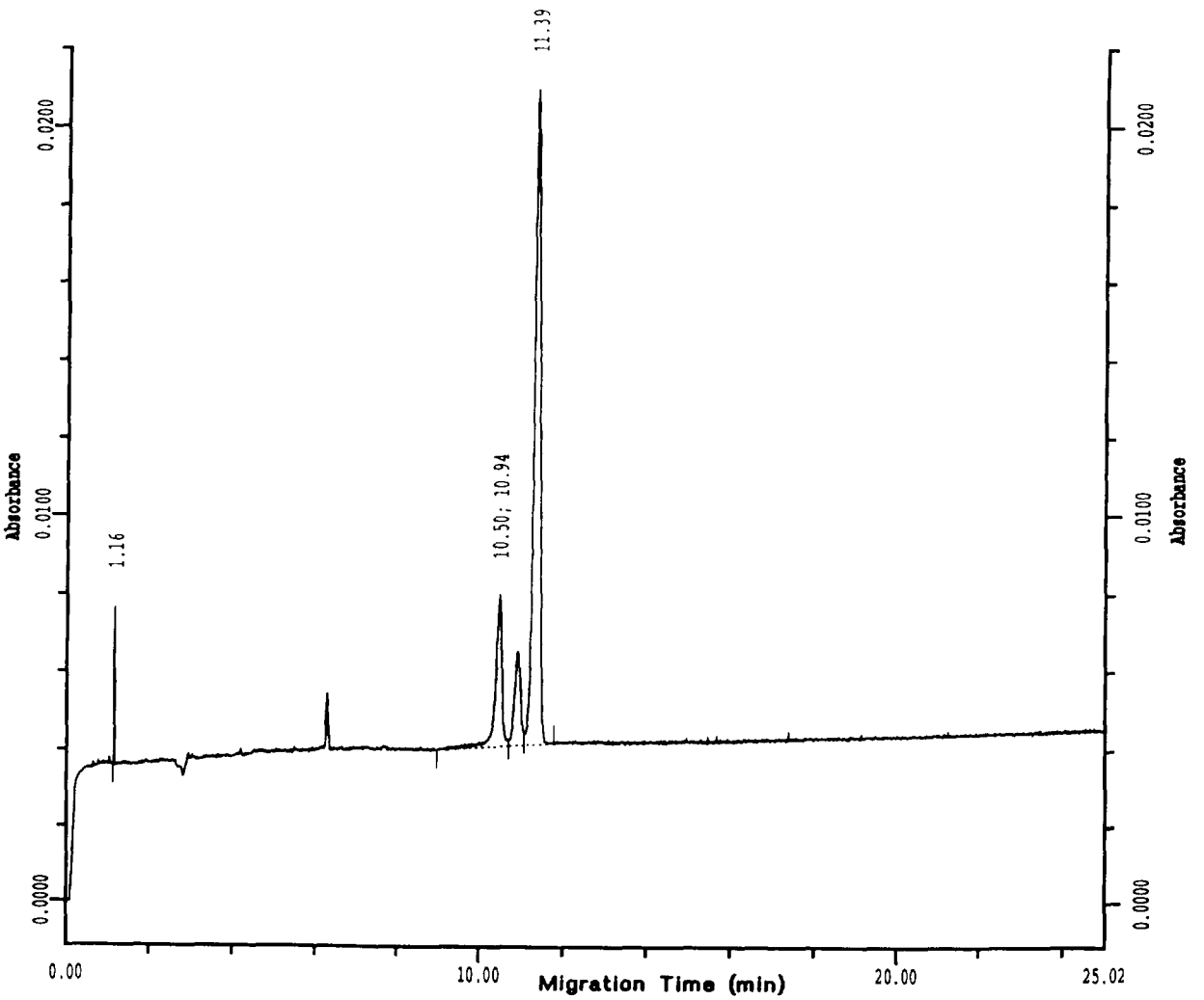

Fig. 1. Electropherogram of six cardiovascular drugs using an electrophoresis buffer of 100 m*M* borate buffer pH 8.1 containing 50 m*M* SDS. The identities of the peaks are as follows: atenolol 10.5 min, nifedipine 10.9 min and nicardipine, diltiazem, amlodipine and verapamil co-migrating at 11.4 min. Separation conditions are given under experimental.

was performed. For each separation performed for the mixture of the six cardiovascular drugs the individual standard solutions were injected under the same conditions to confirm the migration times.

2.3. Standard solutions

Stock solutions of each individual cardiovascular drug and a mixture of all six were prepared by dissolving the compounds in methanol–acetonitrile–water (1:1:2, v/v/v) at a concentration of 0.6 mg/ml. Dilution of the stock solutions was performed in 100 m*M* borate buffer pH 8.1 containing 50 m*M* SDS to give a final analyte concentration of 0.15 mg/ml.

3. Results and discussion

The separation of the six cardiovascular drugs was initially attempted using an electrophoresis buffer of 100 m*M* borate pH 8.1 containing 50 m*M* SDS. Fig. 1 shows the electropherogram, where the resolution of atenolol and nifedipine at 10.5 and 10.9 min was obtained but the remaining four compounds, nicardipine, diltiazem, amlodipine and verapamil co-migrated at 11.4 min.

In an attempt to achieve resolution of these four compounds the initial buffer of 100 m*M* borate pH 8.1 containing 50 m*M* SDS was modified by the addition of acetonitrile, methanol and acetone at concentrations of 5 and 10% (v/v). Fig. 2 shows the migration times of the six

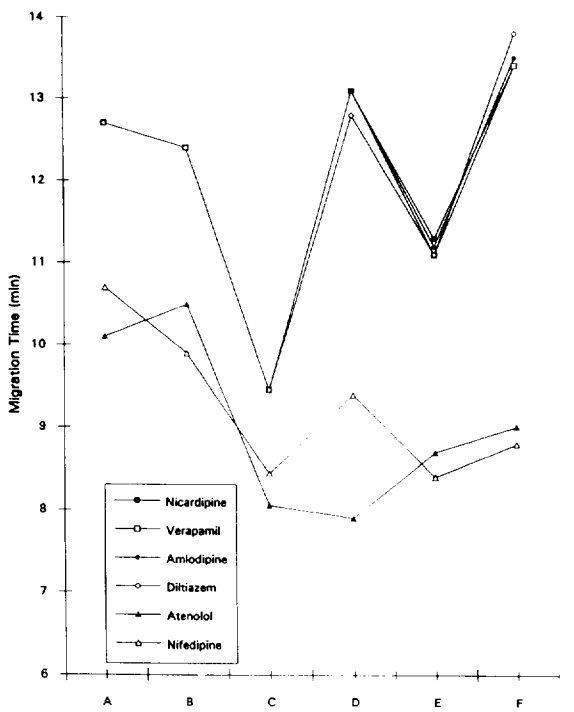

Fig. 2. The effect of organic modifiers on the migration times and separation of six cardiovascular drugs using electrophoresis buffers of 100 m*M* borate pH 8.1 containing 50 m*M* SDS with: A = 5% (v/v) methanol; B = 10% (v/v) methanol; C = 5% (v/v) acetonitrile; D = 10% (v/v) acetonitrile; E = 5% (v/v) acetone; F = 10% (v/v) acetone. All the separations were performed using the conditions described under Experimental.

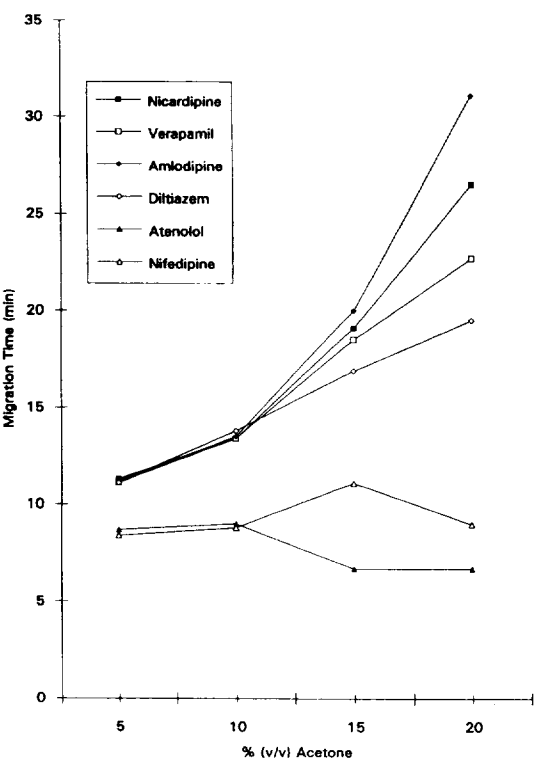

Fig. 3. Migration times of six cardiovascular drugs using electrophoresis buffers of 100 m*M* borate pH 8.1 containing 50 m*M* SDS and varying concentrations of acetone. All separations were performed using the conditions described under Experimental.

cardiovascular drugs when the buffers containing the organic modifiers were used.

Methanol has no effect on the electroosmotic flow showing migration times similar to the initial buffer system. Acetonitrile at 10% (v/v) increases the electroosmotic flow causing diltiazem to be resolved from the other three compounds. Acetone shows the greatest effect; at 5% (v/v) the electroosmotic flow has decreased but the separation capacity has increased showing resolution of amlodipine and nicardipine at 11.2 and 11.3 min but co-migration of verapamil and diltiazem at 11.1 min. At 10% (v/v) amlodipine and diltiazem at 13.5 and 13.8 min are resolved whereas the verapamil and nicardipine now co-migrate at 13.4 min.

Of the three organic modifiers acetone showed the greatest effect and so further electrophoresis buffers were prepared containing 15 and 20% (v/v) acetone.

Fig. 3 shows the effect on the migration times when increasing the acetone concentration from 5 to 20% (v/v). The optimum separation with respect to resolution and migration time was achieved with 15% (v/v) where the migration times are atenolol 6.7, nifedipine 11.1, diltiazem 16.9, verapamil 18.5, nicardipine 19.1 and amlodipine 20.0 mins.

Raising the acetone concentration to 20% (v/v) achieves increased resolution of the six drugs but with migration times in excess of 20 min for verapamil, nicardipine and amlodipine, this being considered an unacceptably long run time. The effect of varying the pH of the borate buffer and the concentration of SDS were then investigated.

Electrophoresis buffers were prepared containing 12.5, 25, 75 and 100 m*M* SDS with 100 m*M* borate buffer pH 8.1 and 15% (v/v) ace-

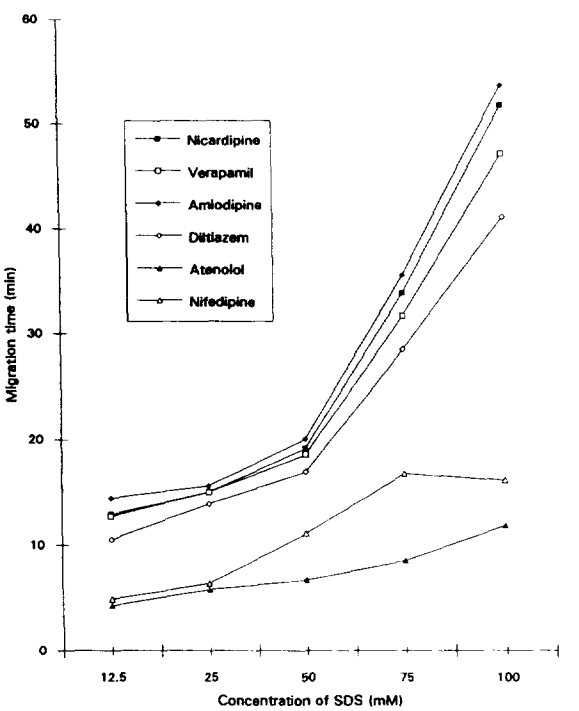

Fig. 4. The effect of varying the concentration of SDS using an electrophoresis buffer of 100 m*M* borate containing SDS and 15% (v/v) acetone. All separations were performed using the conditions described under Experimental.

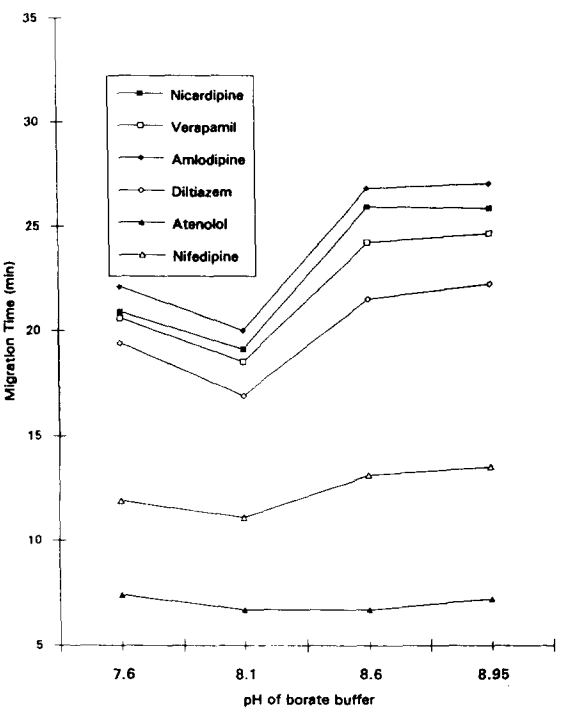

Fig. 5. Migration times of six cardiovascular drugs using an electrophoresis buffer of 100 m*M* borate buffer of varying pH containing 50 m*M* SDS and 15% (v/v) acetone. All separations were performed using the conditions described under Experimental.

tone. Fig. 4 shows the effect on the migration times of the six cardiovascular drugs. As the concentration of SDS increases the electroosmotic flow increases causing loss of peak shape due to band broadening and causing migration times to become greater than 25 min for diltiazem, nicardipine, verapamil and amlodipine.

To assess the effect of pH, electrophoresis buffers were prepared where the pH of the 100 m*M* borate buffer was 7.6, 8.1, 8.6 and 8.95 contained in 50 m*M* SDS and 15% (v/v) acetone. Fig. 5 shows the effect of pH on the migration times of the six cardiovascular drugs. As the pH of the borate buffer increases from 7.6 to 8.95, the migration times first decrease then after pH 8.1 show an increase, the separation being largely unchanged. Hence, pH 8.1 was considered to offer the optimum separation with respect to resolution and migration time.

4. Conclusions

Complete separation of the six analytes atenolol, verapamil, nifedipine, nicardipine, diltiazem and amlodipine proved difficult using MEKC with either of the commonly used organic modifiers acetonitrile or methanol. However, the use of acetone gave an increased selectivity emphasising the utility of this solvent as an organic modifier for MEKC.

By optimising the other operational parameters (the pH of the borate buffer, the SDS concentration and the concentration of organic modifier) an excellent separation of the six analytes was obtained (Fig. 6).

The separation illustrates the applicability of MEKC for the separation of a wide variety of low-molecular-mass analytes and should be considered as an alternative approach to HPLC.

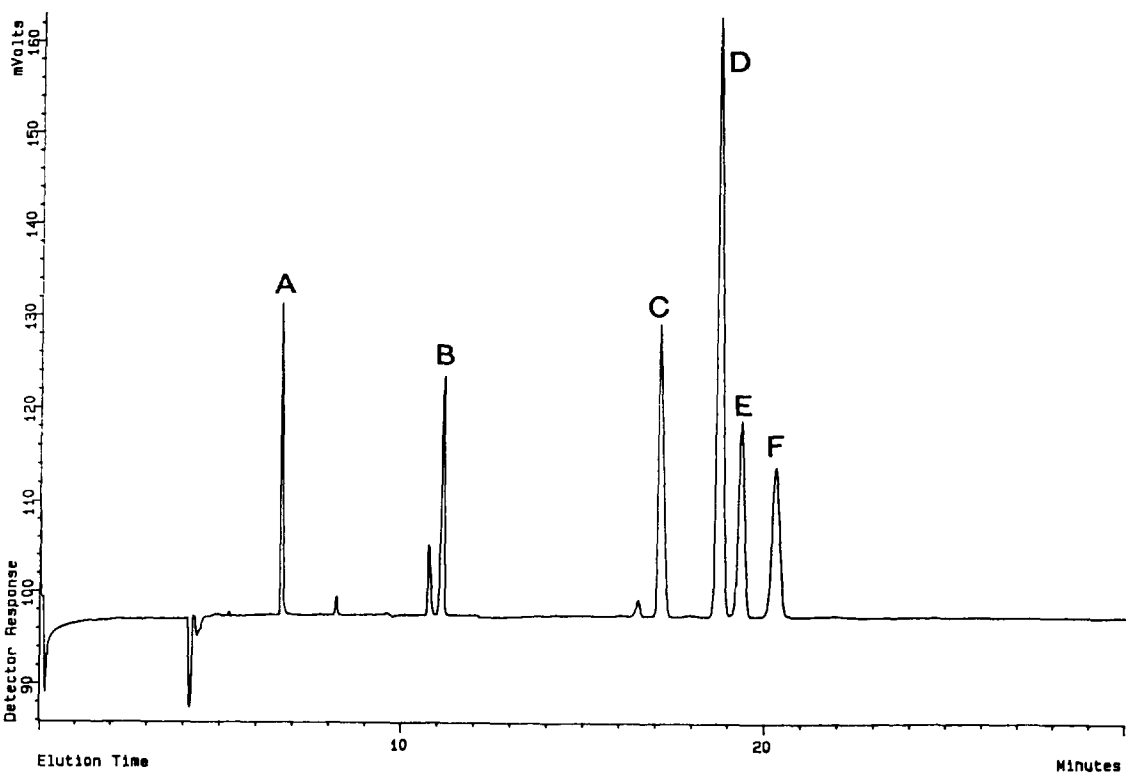

Fig. 6. An electropherogram showing the optimum separation of the six cardiovascular drugs using an electrophoresis buffer of 100 m*M* borate buffer pH 8.1 containing 50 m*M* SDS and 15% (v/v) acetone. A = atenolol; B = nifedipine; C = diltiazem; D = verapamil; E = nicardipine; F = amlodipine. The remaining separation conditions as described under Experimental.

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