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Selectivity of capillary electrophoresis for the analysis of cardiovascular drugs

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Abstract

Examples of several classes of cardiovascular drugs have been separated using capillary electrophoresis and its associated application, micellar electrokinetic chromatography. The therapeutic classes of drug investigated include β-blockers, antiarrhythmic, calcium channel antagonists and angiotensin converting enzyme inhibitors. The exceptional selectivity of capillary electrophoresis is demonstrated using conditions that separate some twenty cardiovascular drugs in a single mixture, including all the aforementioned classes of drugs. The compounds have unrelated structures and varying molecular masses and yet are still resolved using a single set of conditions. The application of such selectivity is discussed together with a comparison with high-performance liquid chromatography.

Keywords: Pharmaceutical analysis; Drugs, cardiovascular; Calcium channel antagonists; Beta blockers

1. Introduction

Since the early reports using capillary electrophoresis (CE) [1,2], the number of applications for the separation of pharmaceutical compounds has steadily increased. Many papers on capillary electrophoresis and its various forms include comments that compare the technique to other separation techniques, and in particular high-performance liquid chromatography (HPLC) [3], which is accepted as the most universally applied method in pharmaceutical analysis. It is now becoming recognised that the separating power of capillary electrophoresis is very often superior to HPLC and small differences in drug molecules can be exploited to effect a complete separation. Capillary electrophoresis and its derivative forms, such as micellar electrokinetic chromatography (MEKC) [4] and MEKC using organic modifiers [5–7], offer an alternate separation mechanism and, together with the high efficiency of the separation can offer an alternative specificity, compared with HPLC. Within therapeutic areas, different combinations of drugs are often prescribed which may exhibit very different polarities or hydroprobicities that make a separation on HPLC very difficult. Cardiovascular drugs are an example of where multiple therapies are often given simultaneously, and the authors' laboratories have developed CE separations of various classes of compounds with cardiovascular activity. This paper reviews some previously reported applications of CE to the analysis of cardiovascular drugs and includes additional novel examples, further demonstrating the selectivity of the technique.

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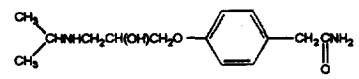

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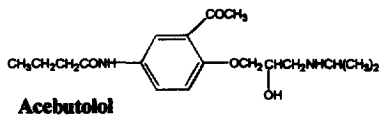

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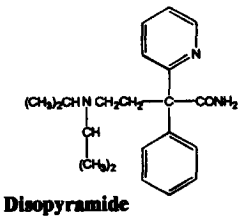

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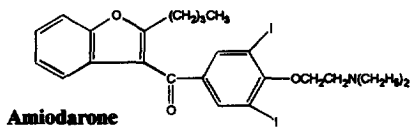

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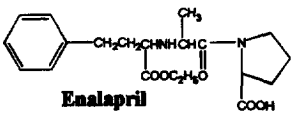

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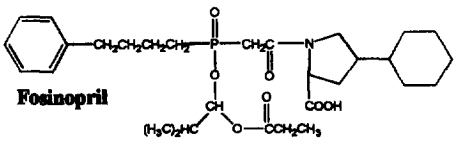

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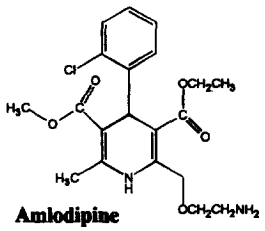

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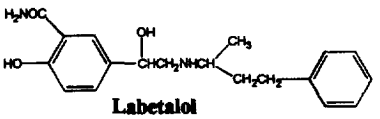

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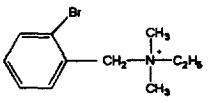

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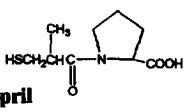

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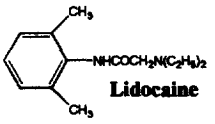

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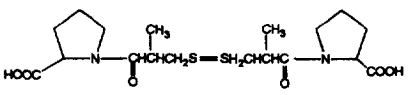
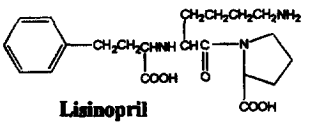


Captopril Disulphide



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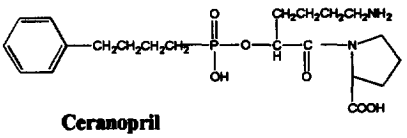

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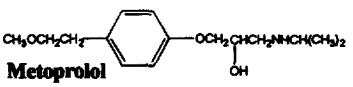

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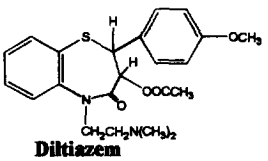
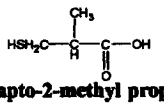


Diltiazem



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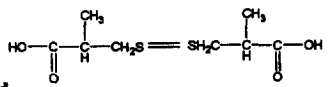

3-mercapto-2-methyl propionic acid disulphide

Fig. 1.

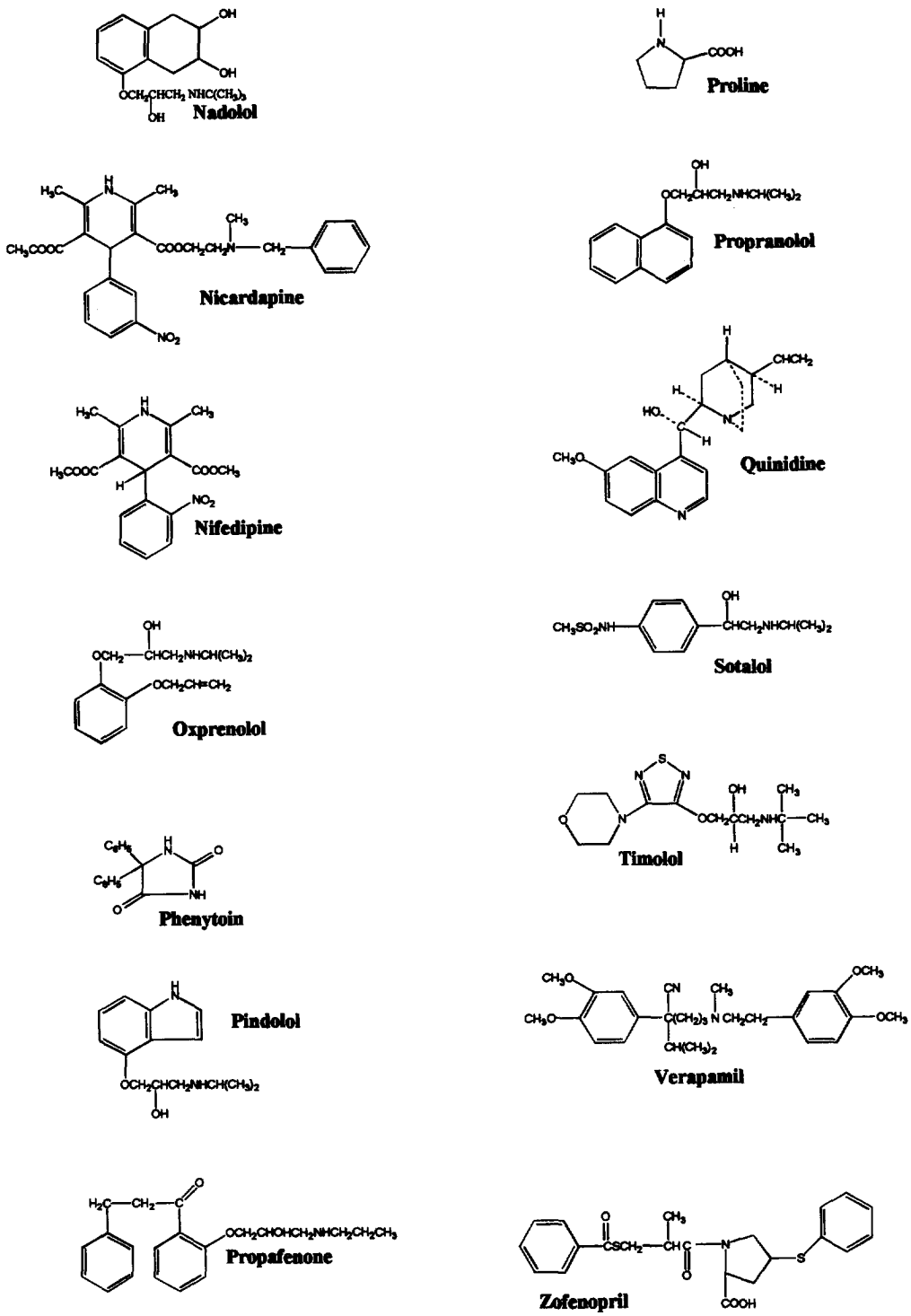

Fig. 1. Structures of cardiovascular drugs used in electrophoretic studies.

2. Experimental

2.1. Reagents

The drugs which have been studied were obtained from Sigma (Poole, Dorset, UK), the US Pharmiacopoeial Commission as USP reference standards or as Bristol-Myers Squibb "in-house" reference standards. Structures of all drugs are given in Fig. 1. Purified water was provided by a Milli-Q Plus water purification system (Millipore, Watford, UK). Sodium dodecyl sulphate (SDS), sodium tetraborate, boric acid, sodium hydroxide, acetone, ethanol 96%, butan-1-ol, butan-2-ol, methyl ethyl ketone (AnalaR grade) and acetonitrile (Hipersolv grade) were obtained from BDH (Poole, UK). Methanol and propan-2-ol (High Purity Grade) were purchased from Fisons Scientific Equipment (Loughborough, UK).

2.2. Apparatus

A capillary electrophoresis P/ACE system 5510 (Beckman Instruments, High Wycombe, UK), equipped with a diode array UV detector, an automatic injector, a fluid cooled cartridge and a System Gold data station were used in this study. All electrophoresis was carried out at 30°C, with an applied voltage of +25 kV and UV detection. Sample introduction was performed using the pressure option for 5 or 10 s. Electrophoresis was performed in a 57 cm×75 μm I.D. (50 cm to detector) fused-silica capillary (Beckman Instruments), and was rinsed with 0.1 *M* sodium hydroxide and the electrophoresis

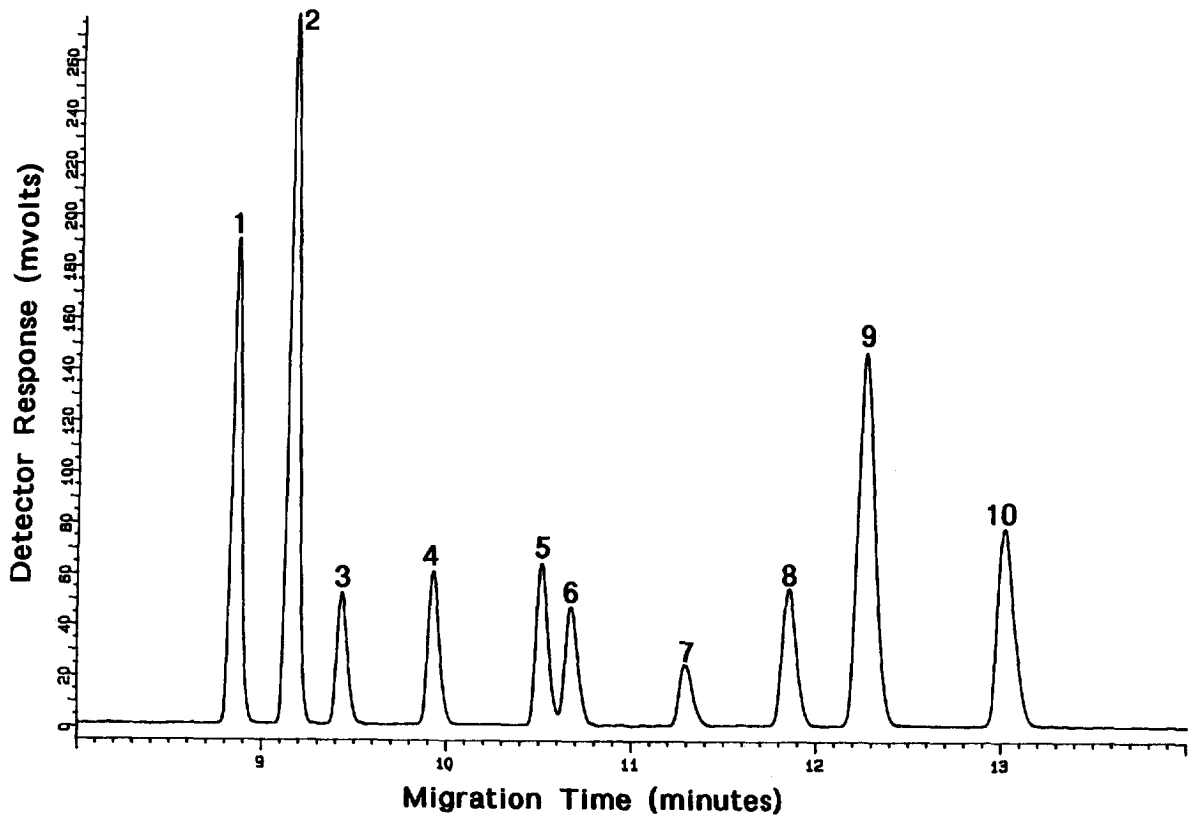

Fig. 2. Electropherogram of β-blocker CE separation using a buffer consisting of 0.1 *M* phosphoric acid adjusted to pH 3.1 using triethanolamine. Peaks: 1=pindolol, 2=oxprenolol, 3=propranolol, 4=sotalol, 5=ateinolol, 6=metoprolol, 7=timolol, 8=nadolol, 9=labetolol, 10=acebutolol.

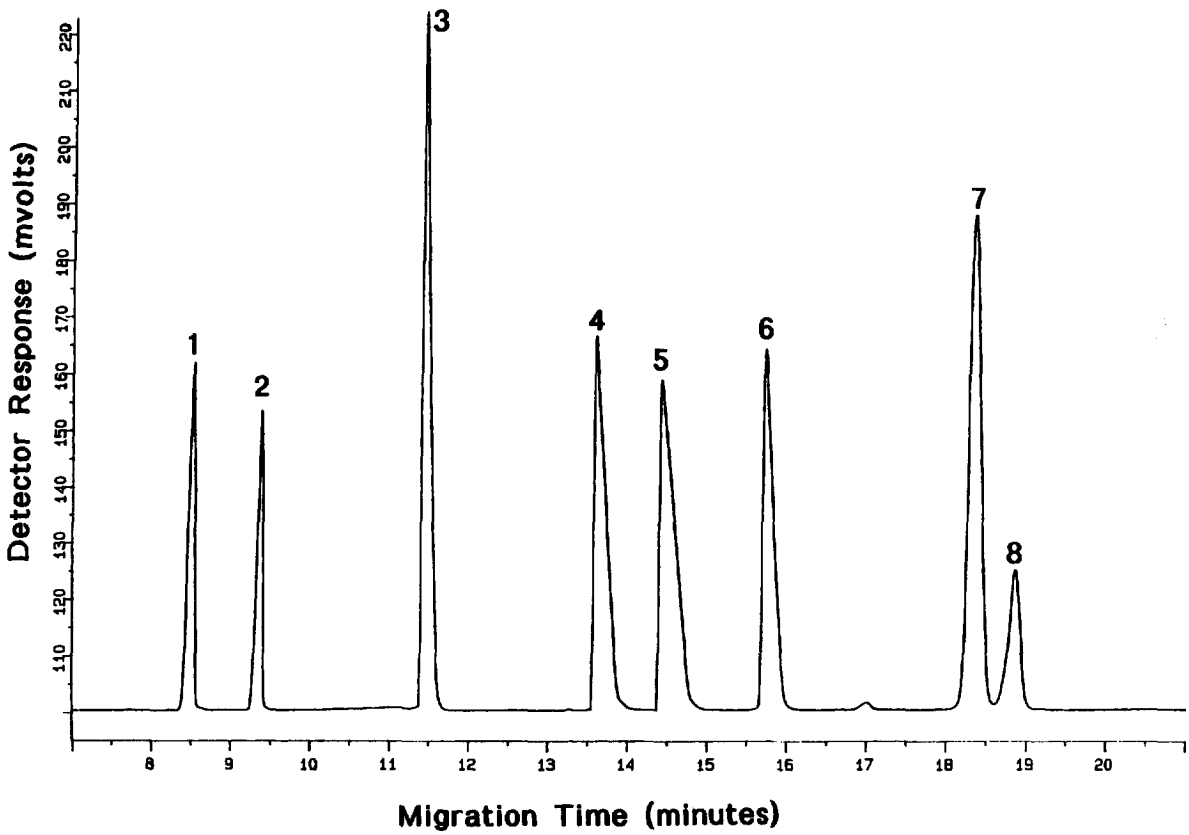

Fig. 3. Electropherogram of drugs with antiarrhythmic activity, separated using a MEKC buffer consisting of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1, containing 10% (v/v) propan-2-ol. Peaks: 1=phenytoin, 2=tosylate ion, 3=lidocaine, 4=bretylium, 5=disopyramide, 6=quinidine, 7=propafenone, 8=amiodarone.

buffer before each electrophoretic separation was performed. For all buffer systems used, individual solutions of each model analyte were injected to confirm the migration times.

3. Results and discussion

3.1. β-Blocker drugs

The separation of ten beta-blockers was achieved using conventional CE conditions previously reported for the separation of a smaller number of beta-blockers. The ten drugs separated were pindolol, oxprenolol, propranolol, sotalol, atenolol, metoprolol, timolol, nadolol, labetolol and acebutolol, each present at a concentration of 0.01 mg/ml in water. A run buffer consisting of 0.1 *M* phosphoric acid adjusted to pH 3.1 using triethanolamine was detected by UV absorbance at 244 nm [8]. Fig. 2 shows that baseline resolution was achieved for each of the beta-blockers within a time window of less than 5 min. The length of time taken for the onset of migration past the detector window, and therefore the time of analysis, could be shortened by reducing the length of the capillary.

3.2. Drugs with antiarrhythmic activity

The separation of seven drugs that exhibit antiarrhythmic activity was optimised using an MEKC system containing organic solvent as a modifier. The seven drugs studied were amiodarone hydrochloride, bretylium tosylate, disopyramide, lidocaine, pheny-

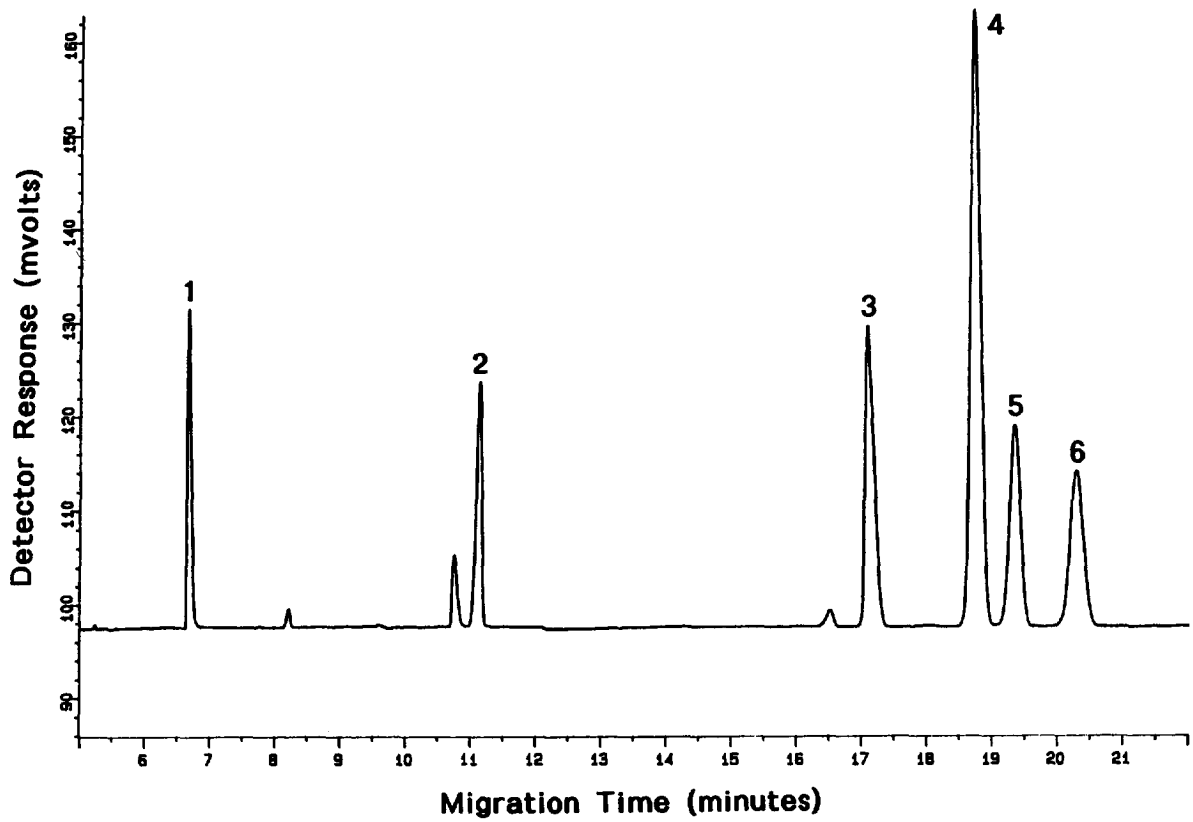

Fig. 4. Electropherogram of drugs with calcium crannel antagonist activity separated using a MEKC buffer consisting of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1, containing 15% (v/v) acetone. Peaks: 1=atenolol, 2=nifedipine, 3=diltiazem, 4=verapamil, 5=nicardipine, 6=amlodipine.

toin, propafenone and quinidine. The electrophoretic buffer consisted of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1, containing 10% (v/v) propan-2-ol. Detection was by UV absorbance at 200 nm. Complete resolution (Fig. 3) and excellent repeatability (R.S.D.) of both the migration time and the corrected peak area over five replicate injections was demonstrated. Similar electrophoretic performance was also obtained using other low-molecular-mass ketones e.g. 15% (v/v) acetone and 15% (v/v) methyl ethyl ketone [6].

3.3. Calcium channel antagonists

The use of MEKC buffers containing organic modifiers was further applied to the separation of a series of drugs with calcium channel antagonist activity. This series included amlodipine, atenolol, diltiazem, nicardipine, nifedipine and verapamil and were prepared in a solution of methanol–acetonitrile–water (1:1:2, v/v/v) at a concentration of 0.6 mg/ml. The electrophoretic MEKC buffer consisted of 50 m*M* SDS in 100 m*M* borate buffer pH 8.1, containing 15% (v/v) acetone, with detection by UV absorbance at 200 m. The addition of organic modifier was required to achieve resolution between nicardipine, diltiazem, amlodipine and verapamil, which were all found to co-migrate in the absence of a modifier. Conditions for the separation were optimum with the addition of 15% (v/v) acetone [5] (Fig. 4).

3.4. Angiotensin converting enzyme inhibitors

A separation of six angiotensin converting enzyme (ACE) inhibitors was achieved using MEKC con-

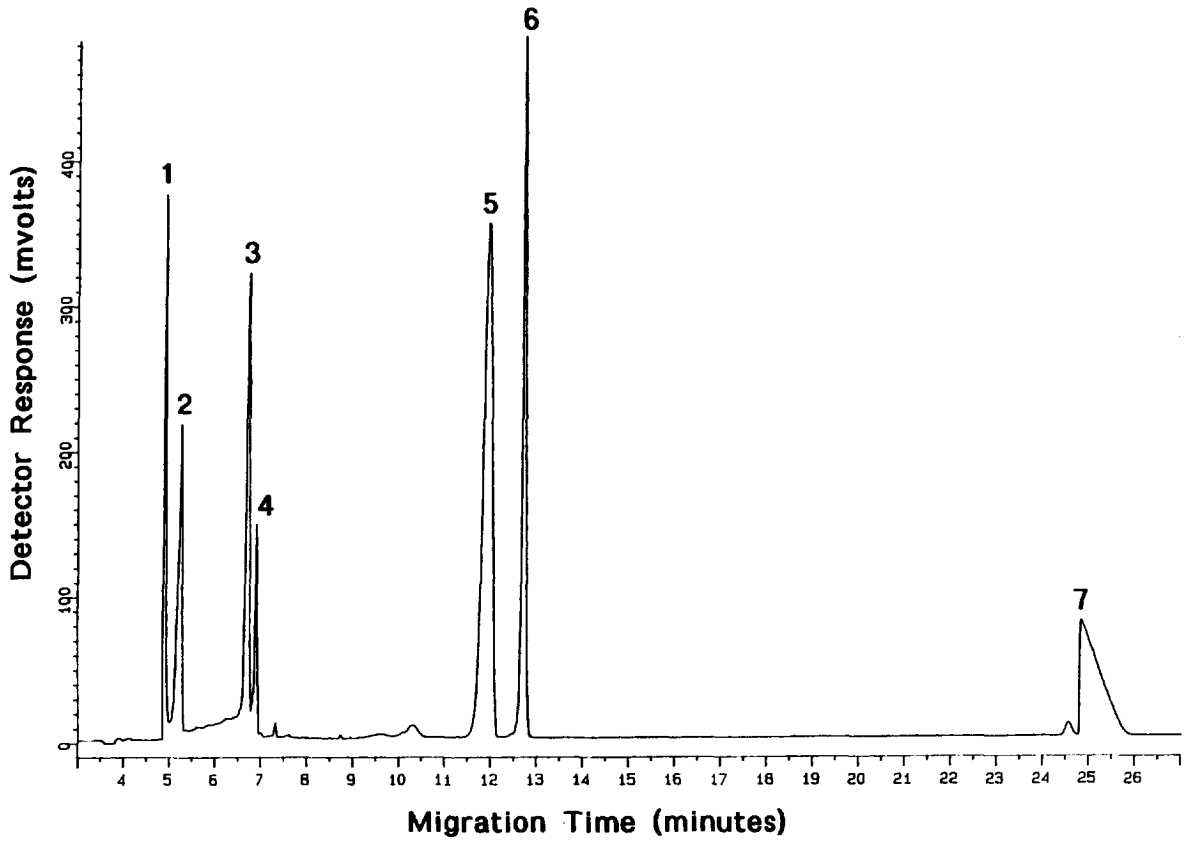

Fig. 5. Electropherogram of ACE inhibitors separated using a buffer consisting of 100 m*M* SDS in 50 m*M* borate buffer, pH 8.2. Peaks: 1=lisinopril, 2=ceronapril, 3=captopril, 4=enalapril, 5=zofenopril, 6=fosinopril, 7=maleic acid (salt of enalapril).

ditions. The compounds separated included captopril, enalapril, zofenopril, lisinopril, ceronapril and fosinopril, each at a concentration of 0.16 mg/ml. The electrophoretic buffer consisted of 100 m*M* SDS in 50 m*M* borate buffer, pH 8.2, with UV detection at 200 nm. All six compounds migrated within a window of 8 min, although the maleic acid from the enalapril maleate salt migrated somewhat later. Fig. 5 shows an electropherogram of the separation. Enalapril maleate exhibits band broadening which is thought to be due to the interconversion of the *cis* and *trans* rotamers of the enalapril. This has previously been observed by Qin et al. [9], who demonstrated the effect of temperature on the rotamer interconversion using CE, showing that at 20°C, a stable separation was observed, whereas at above 40°C the rotamers coalesced and eluted as a broad peak.

3.5. Cardiovascular screening

The conditions optimised for the series of calcium channel antagonist drugs with 15% acetone in the run buffer were optimum for the separation of a considerable number of the cardiovascular drugs described in the previous applications. Altogether, some twenty cardiovascular drugs migrate with excellent efficiency and resolution (Fig. 6). The separation also includes the tosylate counter ion of bretylium which could be quantitatively determined. Such is the electrophoretic selectivity of this system, that it is unlikely that other routinely used analytical techniques could separate such a wide range of drugs with such diverse structures under a single set of conditions.

The usefulness of such a separation would be in identity testing such as a forensic or clinical labora-

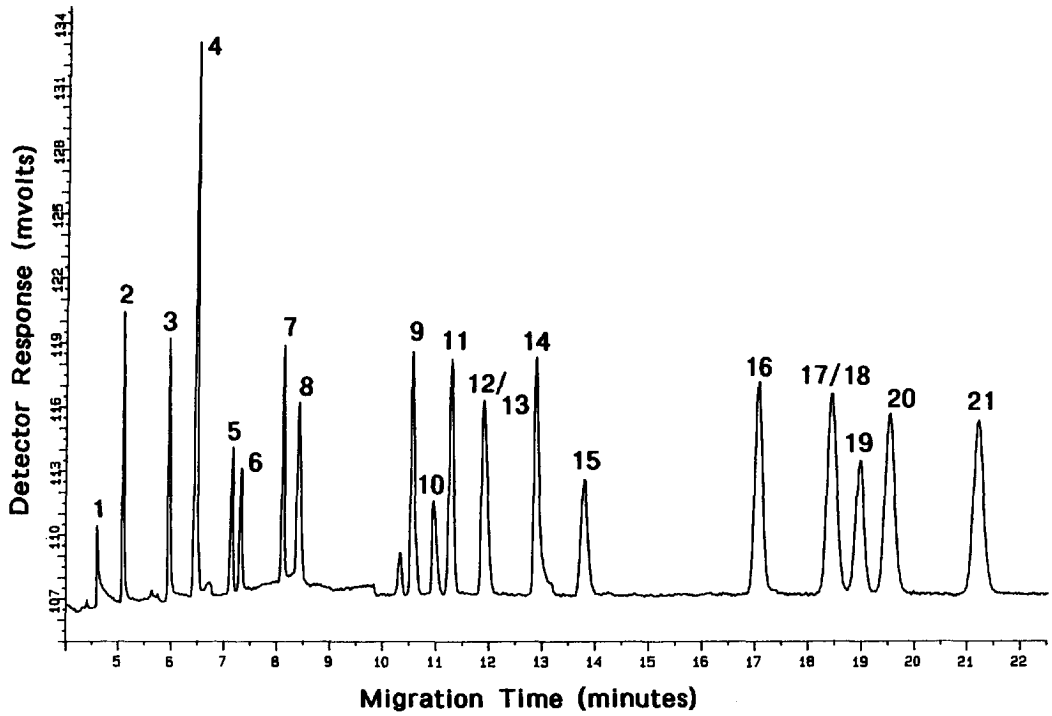

Fig. 6. Electropherogram of cardiovascular drug screen using a buffer consisting of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1, containing 15% (v/v) acetone. Peaks: 1=lisinopril, 2=sotalol, 3=atenolol, 4=phenytoin, 5=captopril, 6=nifedipine, 7=lidocaine, 8=bretylium, 9=pindolol, 10=acebutolol, 11=tosylate salt of bretylium, 12=metoprolol, 13=timolol, 14=disopyramide, 15=quinidine, 16=diltiazem, 17=verapamil, 18=propranolol, 19=nicardipine, 20=propafenone, 21=amiodarone.

tory where rapid identification of unknown drugs is required. In this instance MEKC is providing a "universal method" which would also be useful in a pharmaceutical quality control laboratory where multiple drugs may be identified using one set of conditions. In combination with a diode-array scanning detector, where UV spectra of migrating peaks may be taken, this separation would provide an excellent analytical approach to identification, which could be confirmed by the co-migration of authentic standard materials. A further use would be in the analysis of samples that were being used to validate the cleaning of pharmaceutical manufacturing equipment [10]. Such a separation would not only give information on residues from the previous product being manufactured, but also information on any other previously manufactured products that had left a residue on the equipment.

3.6. Cardiovascular degradation products

The application of CE to resolve a drug component from its related degradants has been demonstrated using the ACE inhibitor captopril. Captopril can degrade by hydrolysis to form proline and 3-mercapto-2-methyl propanoic acid (MMPA), and by oxidation to form captopril disulphide. Degradation fragments such as MMPA may further oxidise to form MMPA disulphide. These degradants have been separated from captopril using an electrophoretic buffer consisting of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1, and detected by UV absorbance at 192 nm (Fig. 7). Captopril and its degradants have very weak absorbance spectra above 210 nm, which increases significantly below this wavelength. HPLC separations with mobile phases containing organic solvents which have a high UV absorbance cutoff at

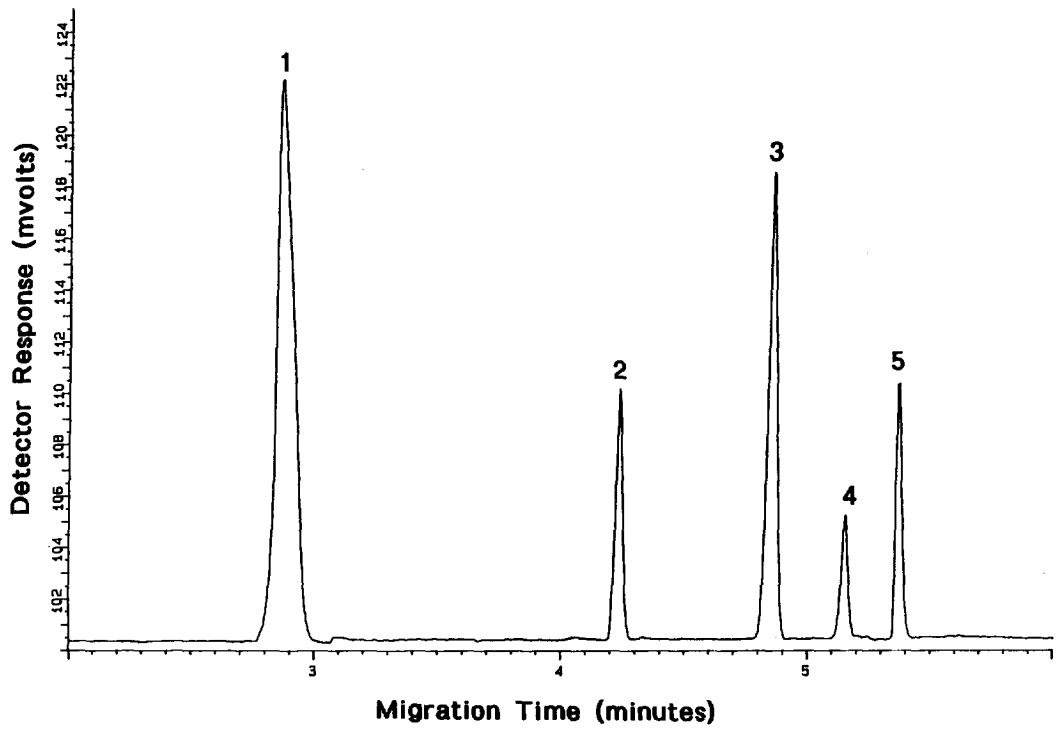

Fig. 7. Electropherogram of captopril and its degradants separated using an electrophoretic buffer consisting of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1. Peaks: 1=proline, 2=captopril, 3=captopril disulphide, 4=MMPA disulphide, 5=MMPA.

about 210 nm severely restrict the detection limit of captopril degradants. The CE separation described above does not use organic modifiers and does not suffer such limitations, giving an advantage over HPLC analyses of captopril degradants. A similar separation was also demonstrated with the ACE inhibitor fosinopril sodium [11]. Although separation of three known degradants from the drug was achievable using HPLC, separation of the same analytes by CE had improved peak efficiency giving a much shorter analysis time.

4. Conclusions

The separation of drugs using the cardiovascular screen described above demonstrates the exceptional selectivity of CE, particularly using MEKC mode. The electrophoretic conditions are typical of many MEKC systems which have been demonstrated as being robust in their application. To perform such a separation using HPLC, it is likely that complex gradient conditions would have to be used, and even then, considerable time would be required to optimise the separation. It is unlikely that such large numbers of compounds with differing structures could be resolved with a single set of conditions using HPLC. This demonstrates the ease of development of complex separations using CE. The separation and detection of captopril degradants show that the use of CE without organic modifiers using UV absorbance detection wavelengths below 200 nm can offer sensitivity advantages over HPLC.

Although the conditions described for the above applications do not separate the enantiomers of the drugs, previous workers [12] have shown the ability of CE to perform chiral separations of cardiovascular drugs, often by the simple addition of a chiral

modifier such as substituted β-cyclodextrins [13–16]. Such separations do not require expensive columns as in HPLC and chiral-resolving modifiers added to electrophoretic buffers can be rapidly evaluated leading to fast chiral method development.

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