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Application of microemulsion electrokinetic chromatography to the analysis of a wide range of pharmaceuticals and excipients

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Abstract

Microemulsion electrokinetic chromatography (MEEKC) is a capillary electrophoresis (CE) technique in which solutes partition with moving oil droplets present in a microemulsion buffer. Ionised species will also separate by electrophoresis. In this paper MEEKC is shown to give highly efficient and relatively rapid separations for a wide range of pharmaceuticals, vitamins and excipients. A single set of operating conditions was used to resolve both water-soluble and insoluble compounds. The method was also used to separate both ionic and neutral compounds. The method was especially useful in the analysis of water-insoluble neutral compounds such as steroids and lecithin, which are difficult to analyse by CE. The method was found to be both quantitative and highly repeatable. The quality of the separation was found to be dependent upon the sample diluent used if large injection volumes are employed. The use of MEEKC for the determination of complex mixtures such as multi-ingredient formulations and drug-related impurities was successfully demonstrated. MEEKC offers significant advantages over many forms of CE and capillary electrochromatography (CEC) and should be considered as an extremely useful option in pharmaceutical analysis.

Keywords: Electrokinetic chromatography; Microemulsion electrokinetic chromatography; Pharmaceutical analysis; Vitamins; Steroids; Antibiotics

1. Introduction

The use of capillary electrophoresis (CE) methods for the analysis of pharmaceuticals has become increasingly popular in recent years. The application range is similar to HPLC and includes [1] assay of drugs, determination of drug-related impurities, and the analysis of vitamins and pharmaceutical excipients. The attractions of the use of CE in pharmaceutical analysis include speed and cost of analysis, reductions in solvent consumption and disposal, and the possibility of rapid method development.

The majority of drugs are basic and are therefore ionised at low pH. A number of reports have shown the separation of a range of basic drugs by CE using a variety of low pH buffer systems. For example a low pH phosphate buffer has been used [2] to analyse 550 different basic drugs. A similar low pH phosphate buffer has been validated [3] for analysis of a variety of basic drugs, excipients and raw materials. The separations are based on the size of the compounds and the number of positive charges. Neutral components are not detected. Water-insoluble basic drugs may present difficulties, although the use of non-aqueous CE systems [4] can be applied for particularly water-insoluble drugs.

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Acidic drugs such as cephalosporins [5] and acidic vitamins [6] have been separated in CE using high pH borate or phosphate buffers. Use of high pH generates an electroendosmotic flow (EOF) in the capillary which forces the negatively charged acids to the detector despite their attempt at electrophoretic migration in the opposite direction. Neutral solutes are unresolved and are swept along the capillary by the EOF. The problems of selectivity are similar to those of basic drugs where similar sized and charged acidic solutes may be poorly resolved. Use of non-aqueous CE systems has been shown [7] to be useful for the analysis of water-insoluble acidic drugs. Selectivity can be adjusted [7] in non-aqueous CE by the choice, and ratios, of solvent mixtures used.

Use of micellar electrokinetic chromatography (MEKC) [8] addresses many of the difficulties described earlier. Typically MEKC is performed using high pH buffers containing sodium dodecyl sulphate (SDS). The SDS is usually present in the range of 20–100 m*M* where the SDS surfactant molecules aggregate to form negatively charged micelles. Neutral solutes partition into the micelle depending upon their hydrophobicity (solubility). The negatively charged SDS micelles attempt to migrate against the BOF and therefore hydrophobic solutes are retained by the micelle and are detected late. Ionised solutes will also electrophoretically migrate due to their charge. Mixtures of charged and neutral components can therefore be resolved by MEKC methods. For example a range of charged and neutral cold medicine ingredients were [9] resolved by MEKC. A single set of MEKC operating conditions has been validated [10] for the analysis of uncharged drugs or mixtures of charged and uncharged drugs and excipients.

Highly hydrophobic compounds tend to be strongly retained by the micelle and are highly retained with poor resolution. Organic solvents are therefore often added to reduce retention times and improve resolution. For example methanol was added to the buffer [11] to improve resolution of water-insoluble cardiovasculars. Use of these solvents can however present difficulties [12] with solvent evaporation problems. Cyclodextrins can be added to the buffer [10] instead of organic solvents to improve the solubility of solutes. The use of cyclodextrins as an alternative to organic solvents eliminates [10] evaporation problems, but the analysis of highly hydrophobic solutes remains problematic.

The use of capillary electrochromatography (CEC) has recently [13] been demonstrated to give highly efficient separations of pharmaceuticals. CEC is particularly useful [13,14] for separation of water-insoluble neutral drugs such as steroids. The analysis of charged components can, however [13], cause problems in CEC. Acidic solutes can migrate against the reduced EOF flow and be undetected. Therefore, CEC methods can be operated [15] at low pH where the acids are unionised. Positively charged basic drugs can tail significantly due to interactions with silanols on the stationary phase packing and electrolyte additives such as triethylamine can be employed [16] to reduce peak tailing.

The separation of drug-related impurities can often be difficult in CE as the impurities tend to have similar charge-to-mass ratios to each other, and to that of the main drug component. For example positional isomers are common impurities which have identical size and mass and are therefore difficult to separate by CE. The optimisation of a CE method for related impurities determination may involve [17] extensive method development and include electrolyte optimisation and use of cyclodextrins and ion-pair reagents to establish simultaneous chromatographic and electrophoretic processes to optimise selectivity. If the impurity of a basic drug does not possess a positively charged group then no peak will be obtained. Similarly, the loss of the acidic functionality in a acidic drugs related impurities causes the impurity to migrate unresolved at the solvent front. Improved selectivity can be obtained in MEKC as the related impurities can be resolved based on both their varying hydrophobicities and ionic charges.

Microemulsion electrokinetic chromatography (MEEKC) [18,19] is another variant of CE which has received relatively little attention compared to the other modes of CE. The separation principle in MEEKC for neutral compounds is chromatographic and involves solute partitioning with a moving oil droplet. The background to microemulsions and their use in analytical chemistry has recently [20] been reviewed. In MEEKC the microemulsion droplets are generally formed by mixing an immiscible oil such as heptane or octane with water. SDS is added at

relatively high concentrations to stablise the emulsion by coating the outside of the droplet. A co-solvent such as butan-l-ol is also added which further stabilises the microemulsion. High pH buffers such as borate and phosphate are generally used in MEEKC as these generate a substantial EOF flow. The droplet is negatively charged due to the SDS coating. The electrophoretic migration of the droplet therefore attempts to oppose the EOF. Hydrophobic solutes favour partitioning into the oil droplet and therefore are more highly retained than water-soluble solutes which have a low partitioning tendency into the oil droplet. The migration time of a neutral solute in a MEEKC separation is therefore directly proportional to its hydrophobicity (log P) and MEEKC has been used by a number of groups [21,22] to calculate log P values.

The application range of MEEKC has largely been limited to highly hydrophobic solutes such as fatty acid esters [23], hop bitter acids [24], herbicides [25] and neutral aromatics [19]. Pharmaceutical applications have also focused on hydrophobic species such as steroids [26], fat-soluble vitamins [27] and water-insoluble drugs [28]. These applications have demonstrated that MEEKC offered great potential in the analysis of these hydrophobic solutes. Many of these papers compared separations obtained by MEEKC with those obtained by MEKC. A general conclusion was that MEEKC offered a greater separation capability for water-insoluble compounds than MEKC. The microemulsions employed possessed significant attractions including reduction of evaporation losses, as the microemulsion components are relatively involatile. The UV absorbance of the microemulsion is also sufficiently low to enable operation at low UV wavelengths such as 200 nm. The solvating properties of the microemulsion enables resolution of a wide range of solutes of differing hydrophobicity. For example, MEEKC has been applied [29] to dyes, urine, and acids.

In this study a standard set of MEEKC operating parameters were devised which gave rapid and highly efficient separations of a range of pharmaceuticals, including both water-soluble and insoluble drugs. The method was also used to separate both neutral and ionised drugs. The method was found to be applicable to a wide range of drug classes, vitamins and excipients. The method was also used to separate complex mixtures of drug-related impurities and multi-analyte formulations.

The method has been established as a general analysis method which gives a number of significant cost, and time advantages. The method conditions can be directly applied to a new compound which greatly reduces method development activities. This reduction is of great benefit in new chemical entity development laboratories where the throughput of new compounds is high. The method also has advantages in quality control laboratories in terms of reduced analytical set-up time and operating costs. Savings can also be accrued [3,10] in method transfer and validation exercises when a general method is applied to a range of solutes.

It is concluded that microemulsion CE is a highly efficient technique for the analysis of pharmaceuticals and related analytes. A single set of operating parameters could be successfully applied to a wide range of pharmaceuticals and excipients. The technique offers advantages over MEKC for the simultaneous separation of compounds of a wide range of solubilities. The ability to resolve charged and neutral solutes simultaneously and the use of inexpensive durable capillaries offers advantages over CEC. Considerable cost and time savings can be accrued when implementing this single set of MEEKC operating conditions. The uptake of CE within the pharmaceutical industry will undoubtedly improve with the increased use of MEEKC as it addresses a number of areas of difficulty that exist with current CE technologies.

2. Experimental

Analysis was performed using Beckman (Fullerton, CA, USA) MDQ CE instruments. A Hewlett-Packard (Bracknell, UK) LAS 1000 data collection system was employed for integration and data handling. Inorganic chemicals were obtained from BDH (Poole, UK). Buffer and NaOH solutions were obtained from Capital HPLC (Broxburn, UK). Water was obtained from a Milli-Q system (Millipore, Watford, UK). Capillaries were purchased from Composite Metal Services (Hallow, UK). Detection windows were prepared using an electrical burner device from Capital HPLC. Each new capillary was

pre-conditioned prior to its first use by conducting a 20 min rinse with 0.1 *M* NaOH. All drug substances samples and formulations were obtained from within GlaxoWellcome. Separations were performed on 30 cm×50 μm I.D. fused-silica capillaries (detection window at 22 cm). The capillaries were rinsed between injections with 0.1 *M* NaOH (1 min) followed by the microemulsion solution (1 min).

The microemulsions were prepared by weighing 0.81 g octane, 6.61 g butan-1-ol, 3.31 g SDS and 89.27 g of 10 m*M* sodium tetraborate buffer to a 10 ml volumetric flask. This was then sonicated for 30 min until all of the SDS had dissolved. After this time an optically transparent microemulsion had formed which was stable for several months. The microemulsion was filtered through a 0.45 μm filter to remove particulate matter, which may otherwise cause baseline spikes.

3. Results and discussion

3.1. Method optimisation

The requirements for use of an analytical method in a routine pharmaceutical analysis include a relatively rapid separation and acceptable separation repeatability. Previously a number of papers [23–26] describing the application of MEEKC had shown 40–60 min analysis time which are generally considered too excessive for routine analysis. In this study the shortest capillaries possible were used to minimise the analysis times. A moderate voltage (15 kV) was used to reduce analysis time and limit the generation of excessive operating current. A narrow bore, 50 μm, capillary was also used to limit operating current which were typically in the range of 80–90 μA. A temperature of 40°C was adopted as this gave reasonable analysis times and resolution. Higher temperatures gave faster separations, but with reduced resolution. Lower temperatures improved resolution of early migrating peaks, but unduly extended analysis times.

The selection of sample diluent was previously [30] found to have a significant effect on the quality of separation achieved in MEKC. When the sample was dissolved in an inappropriate solvent the micelles adjacent to the sample zone were disrupted which lead to loss of retention, separation efficiency and resolution. In particular [30] the use of organic solvent diluents disrupts the micelles and leads to loss of resolution and separation efficiency. A similar effect was found to occur in MEEKC where the presence of the solvents disrupts the microemulsion environment adjacent to the sample zone.

Use of short injection times (less than 3 s at 0.1 p.s.i., 1 p.s.i.=6894.76 Pa) in MEEKC allowed successful injections of sample solution containing high levels of organic solvent. However, longer injection times or higher pressures resulted in poor separation in terms of peak shape and reduced migration times and lower resolution.

Fig. 1 shows the effect of the choice of sample dissolving solvent upon the quality of the separation of a number of neutral solutes achieved by MEEKC. Samples were prepared at the same concentration (0.5 mg/ml) in either the microemulsion buffer or methanol. Short injections (1 s at 0.1 p.s.i.) of either sample gave equivalent separations (Fig. 1(a) and (b)). However longer injections of the methanolic sample (5 s at 0.1 p.s.i.) caused loss of retention and peak tailing (Fig. 1(c)). Acceptable method performance was maintained for longer injections of the sample dissolved in microemulsion buffer. Fig. 1(d) shows the separation obtained for a 10 s injection at 0.1 p.s.i.. Extensive peak broadening eventually lead to loss of resolution for a 20 s injection at 0.1 p.s.i. (Fig. 1(e)).

3.2. Selectivity assessment

It was considered important that the general applicability of the method was evaluated. This was performed by analysing a wide range of compounds dissolved in a solution containing three migration time reference markers. These markers covered a range of hydrophobicities and were used to calculate the relative migration times for each test solute. Calculation of the relative migration time in CE improves precision data and transfer of data between instrument type. Fig. 2 shows a typical separation; in particular the analysis of 5,5-diethylbarbituric acid (barbitone). Table 1 shows the relative migration time data obtained for a range of drugs, vitamins, raw materials and excipients. These solutes include both neutral and charged compounds. The listing

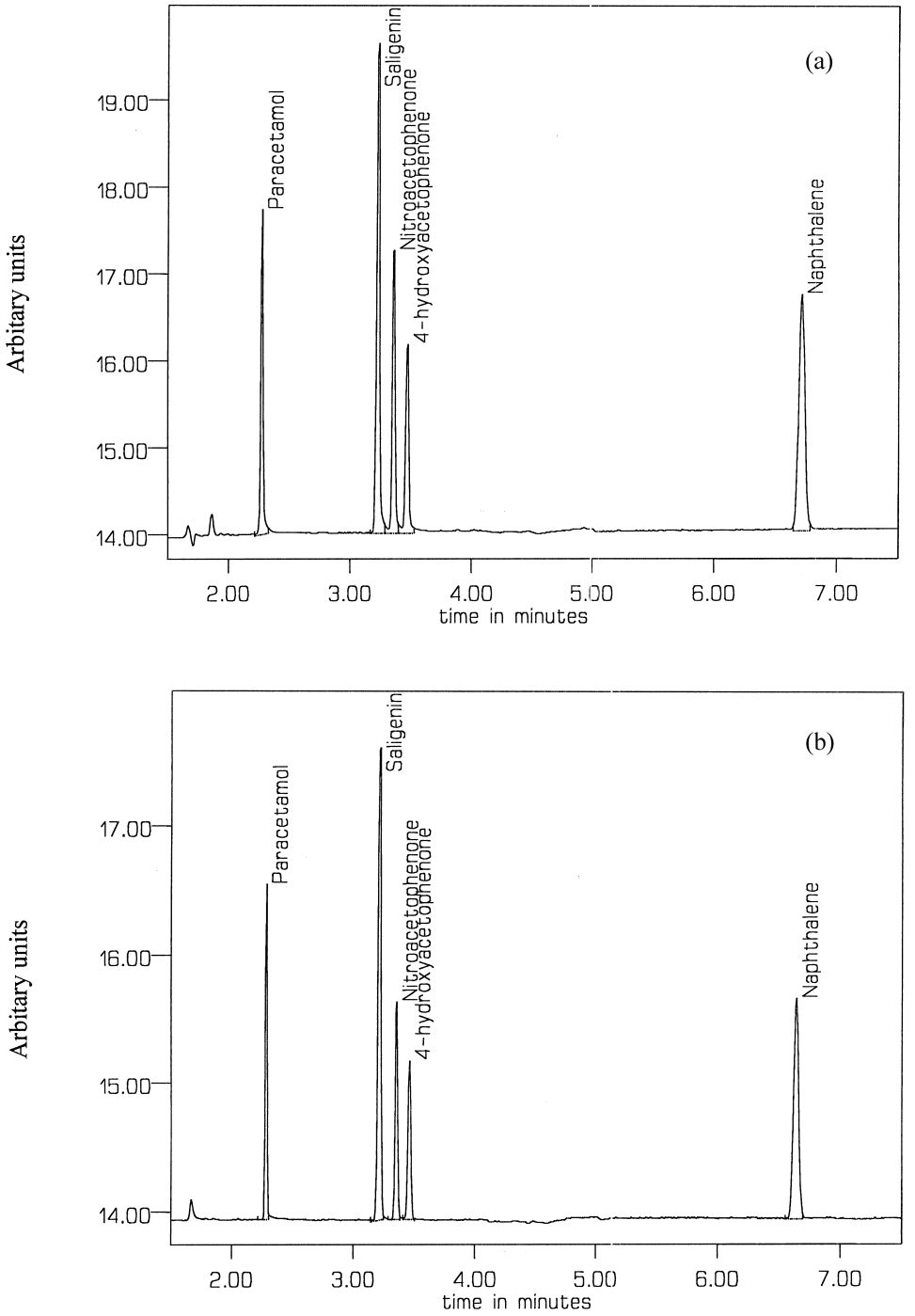


Fig. 1. Effect of sample diluent and injection time on separation of a range of neutral solutes by MEEKC. (a) Sample dissolved in methanol injected for 1 s at 0.1 p.s.i. (b) Sample dissolved in buffer injected for 1 s at 0.1 p.s.i. (c) Sample dissolved in methanol injected for 5 s at 0.1 p.s.i. (d) Sample dissolved in buffer injected for 10 s at 0.1 p.s.i. (e) Sample dissolved in buffer injected for 20 s at 0.1 p.s.i. Separation conditions: 0.81 (w/w) octane, 6.61% (w/w) butan-1-ol, 3.31% (w/w) sodium dodecyl sulphate and 89.27% (w/w) 10 m*M* sodium tetraborate buffer, 15 kV, 30 cm×50 μm I.D. capillary (detection window at 22 cm), 40°C, 200 nm.

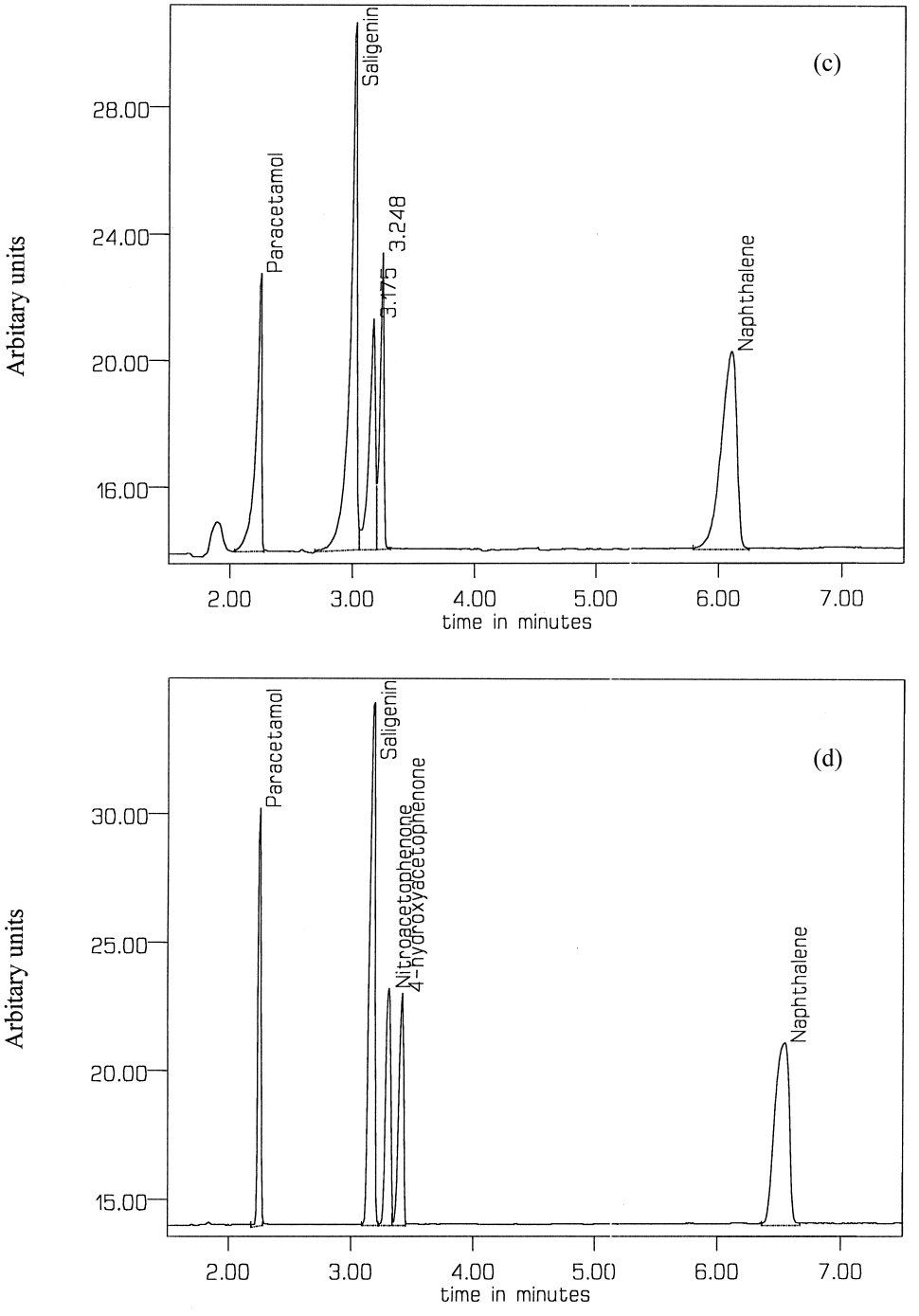
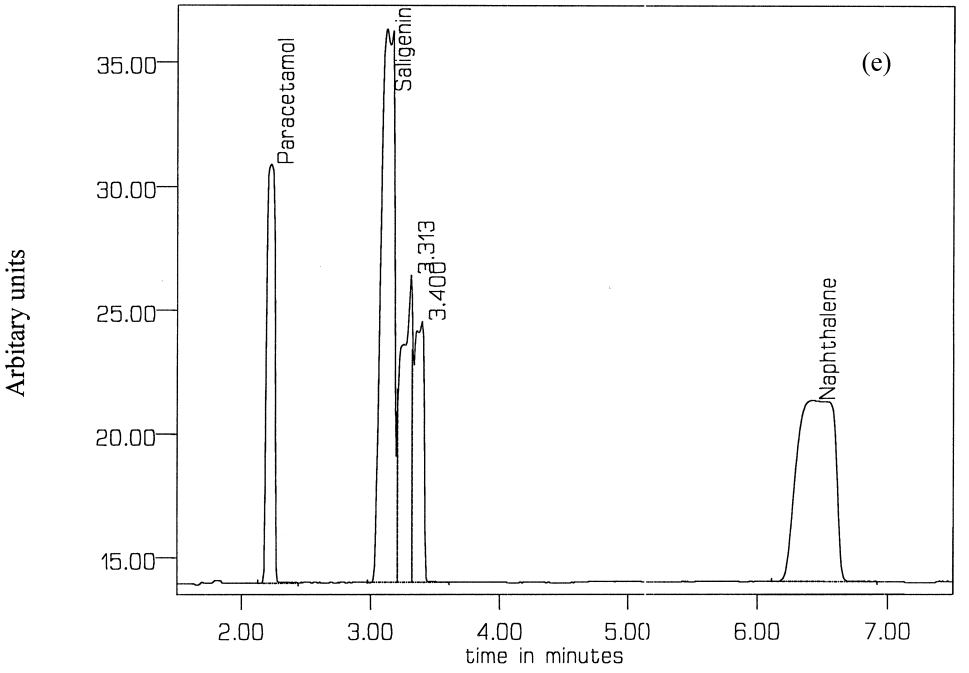


Fig. 1. (*continued*)



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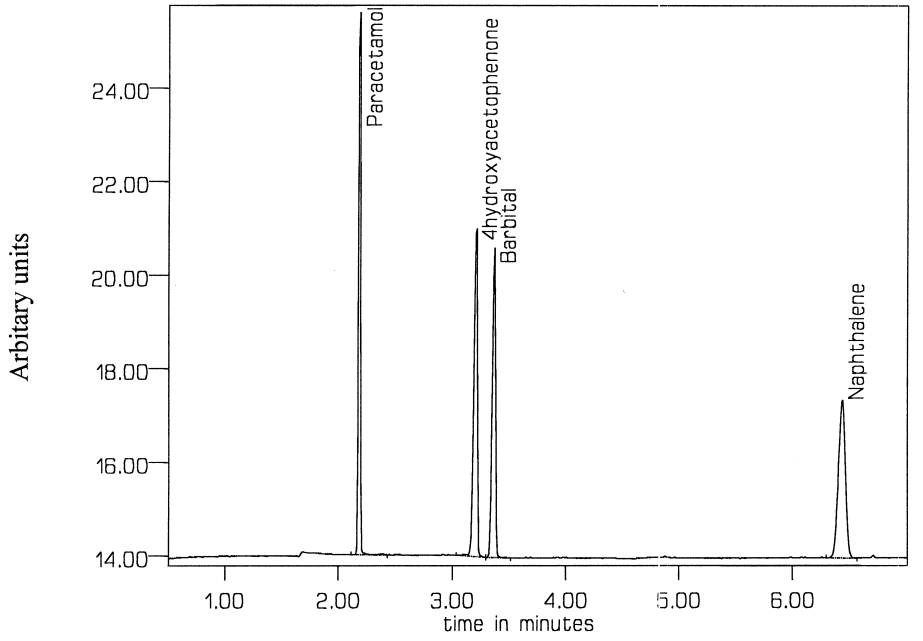

Fig. 2. Separation of 5,5-diethylbarbituric acid (barbitone) and three marker compounds. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

Table 1
Data from screening of MEEKC method

Compound	RMT1[a]	RMT2	RMT3
Acetylsalicyclic acid	1.54	1.02	0.55
Allopurinol	1.18	0.78	0.47
Aminobenzoic acid	1.55	0.91	0.51
Aniline	1.15	0.76	0.40
Anisole	1.92	1.28	0.68
Antipyrine	1.09	0.73	0.37
Ascorbic acid	1.50	0.98	0.51
Aspartame	2.05	1.35	0.72
Atenolol	1.25	0.81	0.43
Barbital	1.54	1.05	0.52
Benzamide	1.10	0.74	0.39
Benzoic acid	2.00	1.31	0.68
Budenoside	2.45	1.62	0.97
Bupivacaine	2.73	1.81	0.99
Caffeine	0.92	0.62	0.37
Cephalothin	1.28	0.85	0.46
Ceftazidime	1.03	0.68	0.36
Cefuroxime	1.14	0.76	0.41
Cephaloridine	1.20	0.79	0.42
Cephalexin	1.22	0.81	0.43
Chlorpheniramine	2.72	1.82	1.00
Cholecalciferol	2.87	1.89	1.13
Clenbuterol	2.17	1.45	0.80
Diaminobenzoic acid	1.49	1.00	0.53
Ethylparaben	1.82	1.19	0.62
Guaiphensin	1.21	0.80	0.48
Hydrocortisone	1.50	0.91	0.53
Hydrochlorothiazide	1.17	0.78	0.42
Hydroquinone	0.95	0.63	0.34
Ibuprofen	2.31	1.54	0.82
Indomethacin	1.79	1.14	0.60
Lamotrigine	1.67	1.12	0.59
Leucovorin	1.48	1.00	0.51
Lignocaine	2.27	1.51	0.83
p-Methoxybenzoic acid	1.61	1.08	0.57
Methylparaben	1.59	1.04	0.54
Naphthalenedicarboxylic acid	2.74	1.85	0.97
β-Naphthoxyacetic acid	1.88	1.23	0.64
Nicotinamide	0.87	0.58	0.35
Nicotinic acid	1.82	1.20	0.64
Nitroacetophenone	1.47	0.98	0.52
Octopamine	0.89	0.60	0.32
Oxicanazole	3.06	2.04	1.08
Pantothenate	1.56	1.02	0.53
Penicillin G	1.43	0.96	0.51
Phenoxymethylpenicillin	1.67	1.09	0.57
Phenylglycine	1.24	0.82	0.44
Propylparaben	2.70	1.77	0.92
Pseudoephedrine	1.49	0.97	0.51
Pyridoxine	1.22	0.80	0.43
Ranitidine	1.38	0.93	0.51
Riboflavin-*S*-phosphate	1.44	0.94	0.49
Saccharin	1.86	1.25	0.65
Salbutamol	0.96	0.64	0.35
Salmeterol	2.70	1.77	1.04
Salicyclic acid	2.03	1.36	0.72
Sorbic acid	1.77	1.19	0.61
Terbutaline	1.05	0.71	0.39
Theobromide	0.93	0.63	0.32
Theophylline	1.28	0.86	0.43
Triprolidine	2.84	1.89	1.03
Tryptophan	1.15	0.77	0.41
Tryptophan methyl ester	1.64	1.08	0.58
Verapamil	2.78	1.87	1.03
Warfarin	1.35	0.90	0.48

[a] RMT1 is the migration time of the solute relative to that of paracetamol; RMT2 is the migration time of the solute relative to that of 4-hydroxyacetophenone; RMT3 is the migration time of the solute relative to that of naphthalene.

also contains both water-soluble and insoluble compounds. Concentrated solutions (typically 10–20 mg/ml) of the water-insoluble compounds were prepared in an appropriate solvent (methanol, acetonitrile or acetone) and this solution was then diluted with the microemulsion solution to give a final concentration of 0.5 mg/ml. The method was also successfully applied to a number of confidential research compounds currently under development within GlaxoWellcome. The method was assessed for applicability to over 200 test solutes and was unsuccessful on only three solutes, including the drug Busulphan, which were highly water-insoluble and where dilution with the microemulsion caused immediate sample precipitation.

3.3. Analysis of analgesics and cold medicine ingredients

Fig. 3 shows the efficient separation of a range of analgesics and cold medicine ingredients using the MEEKC method. These include basic drugs such as chlorpheniramine, neutral solutes such as paracetamol and acidic analytes such as salicyclic acid. Pharmaceutical preparations often involve use of mixtures of these ingredients and the ability to resolve charged and neutral species simultaneously is therefore a key requirement in this type of analysis. MEKC has previously [8] been used in this area as

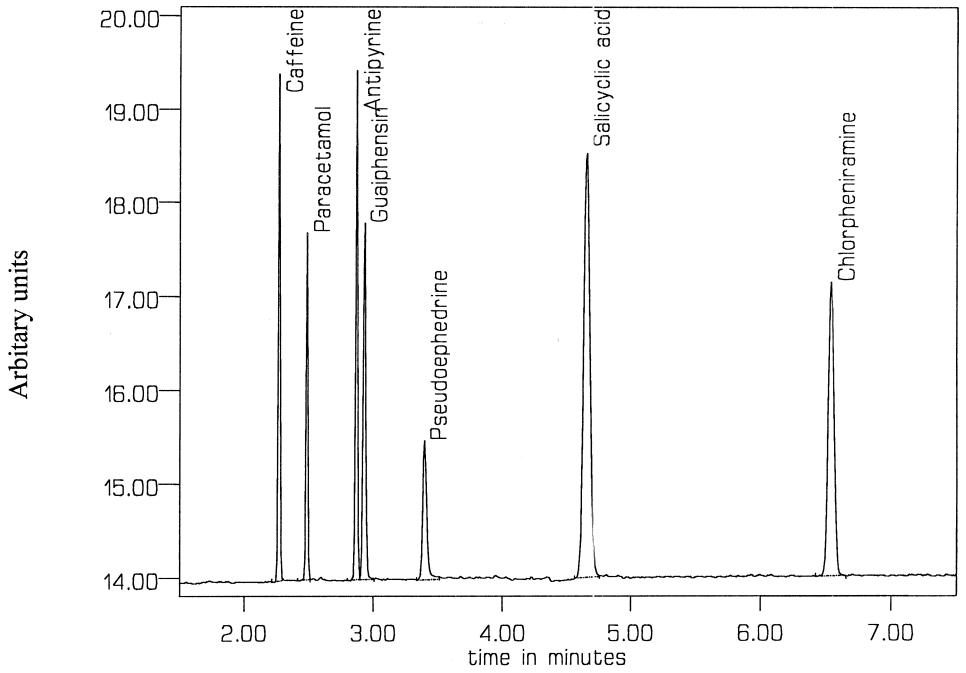

Fig. 3. Separation of a range of cold medicine ingredients. Separation conditions: as Fig. 2 except 1 s injection at 0.1 p.s.i.

simultaneous analysis of neutral and charged solutes is also possible in MEKC. The method was also used to analyse cold medicine ingredients in a liquid formulation. The components of this formulation include both charged and neutral components. Table 2 gives some quantitative data [28] from this analysis.

3.4. Vitamin analysis

Many vitamins such as ascorbic acid (vitamin C) are water-soluble and acidic and can [6] be analysed by CE using high pH buffers. However, there are also a number of vitamins such as nicotinamide (vitamin B_{12}) which are neutral and water-soluble.

Table 2
Quantitative application and repeatability of the MEEKC method

Sudafed™ expectorant assay results	Label claim	MEEKC assay results	
Guaiphenisin	20 mg/ml	20.95 mg/ml	
Pseudoephedrine	6 mg/ml	5.86 mg/ml	
Ethylhydroxybenzoate	0.1% w/v	0.096% (w/v)	
Propylhydroxybenzoate	0.01% w/v	0.01% (w/v)	
Trogliazatone tablets	200 mg/tablet	199.4 mg/tablet	
Injection precision measurements			
Injection precision	Internal standard	No. injections	PAR[a] (% RSD)
Methylparaben	Ethylparaben	10	0.31%
Propylparaben	Ethylparaben	10	0.63%

[a] PAR is peak area ratio of the solute peak area compared to the area of the internal standard peak.

There are also a number of fat-soluble vitamins. MEEKC has been applied [27] to the analysis of fat-soluble vitamins.

Formulations often contain a number of both water-soluble and fat-soluble vitamins which represent a significant analytical challenge. Fig. 4 shows the successful separation of a liquid multi-vitamin preparation containing a range of water and fat-soluble vitamin components. The other peaks in this separation are due to excipients such as flavourings. The late migrating peaks at 9 min are the water-insoluble vitamins A and D.

3.5. Basic drugs

Generally basic drugs are resolved in CE using low pH buffers [2,3] and separation occurs due to differences in charge-to-mass ratios. Fig. 5 shows the highly efficient separation of a range of basic drugs using MEEKC. The separation order obtained is based on both electrophoretic mobility and partitioning. The borate buffer used in this method gives a pH 9.5 which will limit the ionisation of basic drugs depending upon their pK_a values. However, some basic drugs will remain ionised at this high pH and these will interact with the droplet through both partitioning and ion-pair interactions. It is possible [21] to use pH 13 microemulsions where basic drugs will be unionised and the separations occur purely on partitioning effects.

3.6. Acidic drugs

MEEKC has previously been used [28] to separate water-insoluble non-steroidal antiinflammatories such as indomethacin and ketoprofen. Fig. 6 shows separation of a range of both water-soluble and insoluble acidic drugs. These drugs are ionised at this high pH (pH 9.5) and therefore the separation is obtained due to a combination of both partitioning and electrophoresis. The extent of partitioning will be limited as the negatively charged acidic drugs will be, to some extent, charge repelled by the negative charges on the droplets. The more water-insoluble drugs such as ibuprofen and trogliatazone partition more strongly with the oil droplet and are therefore

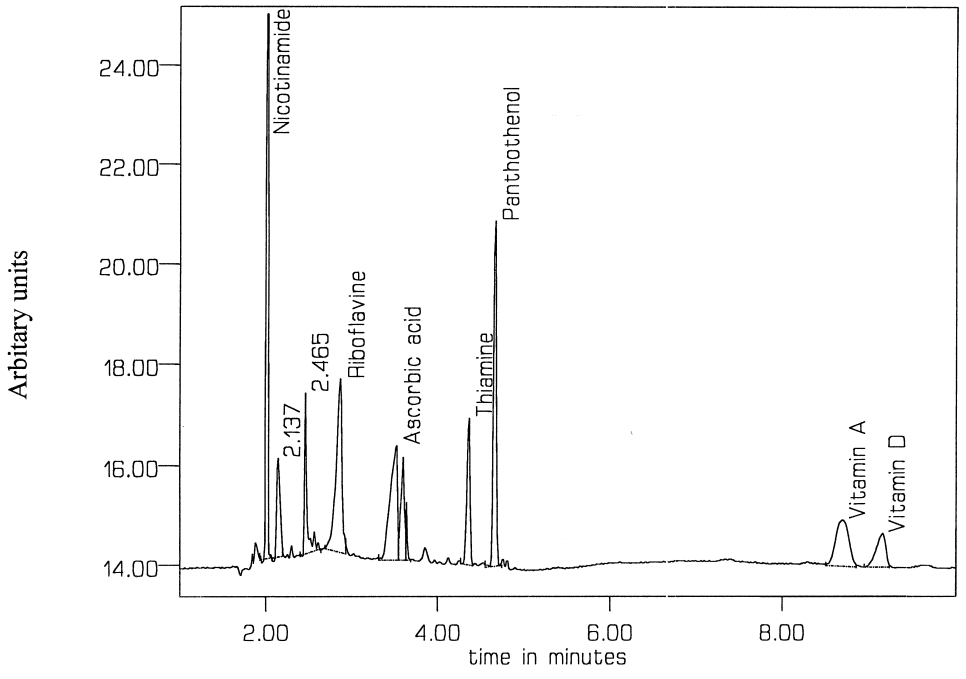

Fig. 4. Separation of vitamins and excipient components in a liquid formulation. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

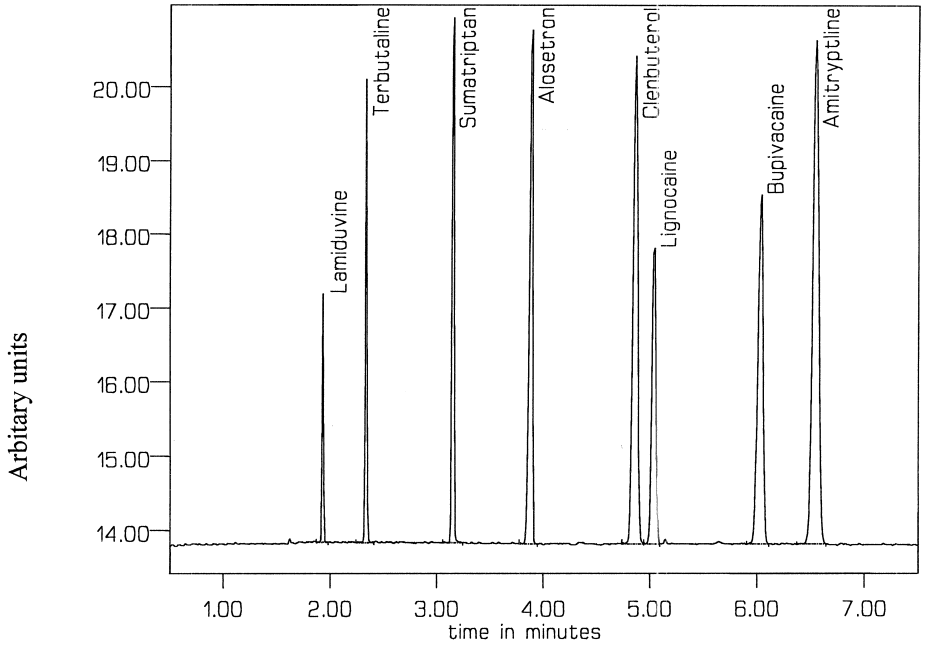

Fig. 5. Separation of basic drugs. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

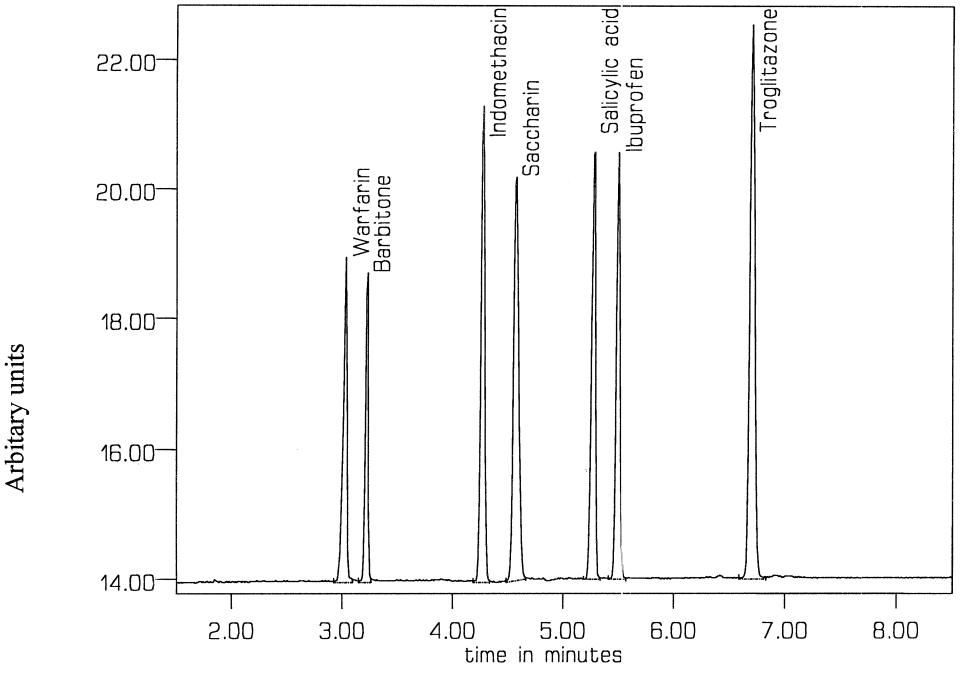

Fig. 6. Separation of acidic drugs. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

detected later in Fig. 6. It is possible to use a microemulsion buffer at a low pH where the acids will be unionised and separate by partitioning only. For example a pH 1.2 microemulsion was used [21] to determine the log *P* value of acidic solutes in their unionised form.

3.7. Raw materials and excipients

A variety of CE methods have been used [31] to analyse excipients and raw materials. For example acidic flavourings and dyes can be analysed using high pH buffer or water-insoluble neutral excipients can be analysed [31] using MEKC with the addition of an appropriate solvent.

Fig. 7 shows separation of a range of excipients and raw materials using MEEKC. The solutes include mixtures of charged and neutral water-soluble or insoluble compounds. The *p*-hydroxybenzoate preservatives are neutral and are separated chromatographically, therefore propylparaben is detected last as this is the least water-soluble. Acid blue is a water-insoluble acidic dye which strongly interacts with droplet and is therefore well retained.

Lecithin is a natural product which can be used as a lubricant in pharmaceutical formulations. This material is difficult to analyse as it is highly water-insoluble and has very limited UV absorbance. The analysis can be performed [31] by MEKC with addition of propan-2-ol to a bile salt buffer with detection at 200 nm. The optimised MEEKC method was applied to an input batch of lecithin and shows resolution of the lecithin components. The lecithin sample was initially prepared in methanol and then diluted 1 ml to 10 ml with microemulsion buffer.

3.8. Related impurities determinations

The separations obtained in MEEKC are based on partitioning and electrophoretic migration. This therefore allows resolution of both neutral and charged drug-related compounds. Resolution of achiral isomers is difficult in CE as they have near identical electrophoretic mobilities. However, isomers can be resolved in MEEKC as they have different solubilities and will therefore have different k' values. Cefuroxime axetil is a water-insoluble neutral cephalosporin which is produced as a mixture

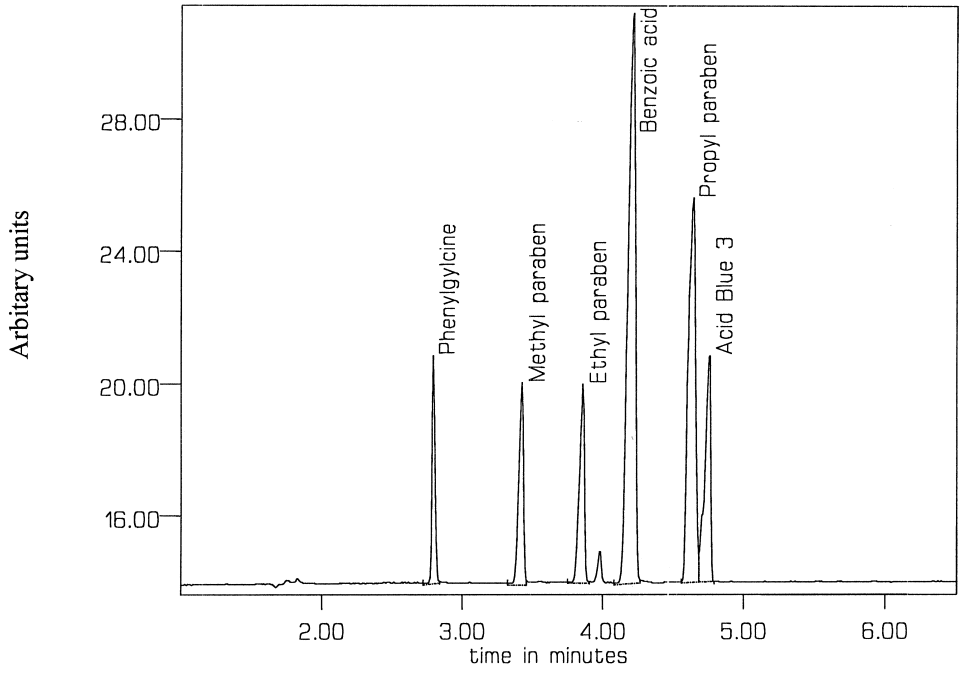

Fig. 7. Separation of excipients and raw materials. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

of two diastereoisomers. The principal hydrolysis product of cefuroxime axetil is cefuroxime which is acidic and water soluble. The isomers of cefuroxime axetil have been resolved by CEC [32], however the acidic cefuroxime migrated against the EOF and was therefore undetected. Fig. 8 shows separation of a heavily degraded solution of cefuroxime axetil obtained by the MEEKC. Detection was performed at 280 nm which is the UV maximum for these compounds. The neutral diastereoisomers are chromatographically resolved in MEEKC as they have different solubilities. Cefuroxime is resolved by a mixture of its electrophoretic mobility and limited partitioning with the microemulsion.

Fig. 9 shows resolution of a heavily degraded solution of phenoxymethylpenicillin. The degradation impurities include both charged and neutral impurities and therefore would require use of MEKC or MEEKC to obtain adequate resolution.

3.9. Antibiotics

Many antibiotics are acidic and are often manufactured as sodium or potassium salts. High pH buffers have been used [1] to separate acidic cephalosporins and penicillins. MEKC has also been successfully applied [8] to this area. Fig. 10 shows that MEEKC can be applied to the analysis of a range of water-soluble and insoluble cephalosporin and penicillin antibiotics. Particular advantages in the use of MEEKC for this analysis include the ability to determine additional charged or neutral drugs simultaneously in multi-component formulations and the ability to quantify both the antibiotic and excipient/preservative in a formulation.

3.10. Steroids

Steroids are generally uncharged and highly water-insoluble. Therefore, CE is not widely used in this type of analysis. MEKC has been used [33] for steroid analysis and required addition of organic solvents to the separation buffers due to the highly hydrophobic nature of steroids. CEC is highly efficient in the analysis of water-insoluble neutral solutes and has been shown [14] to be useful for the

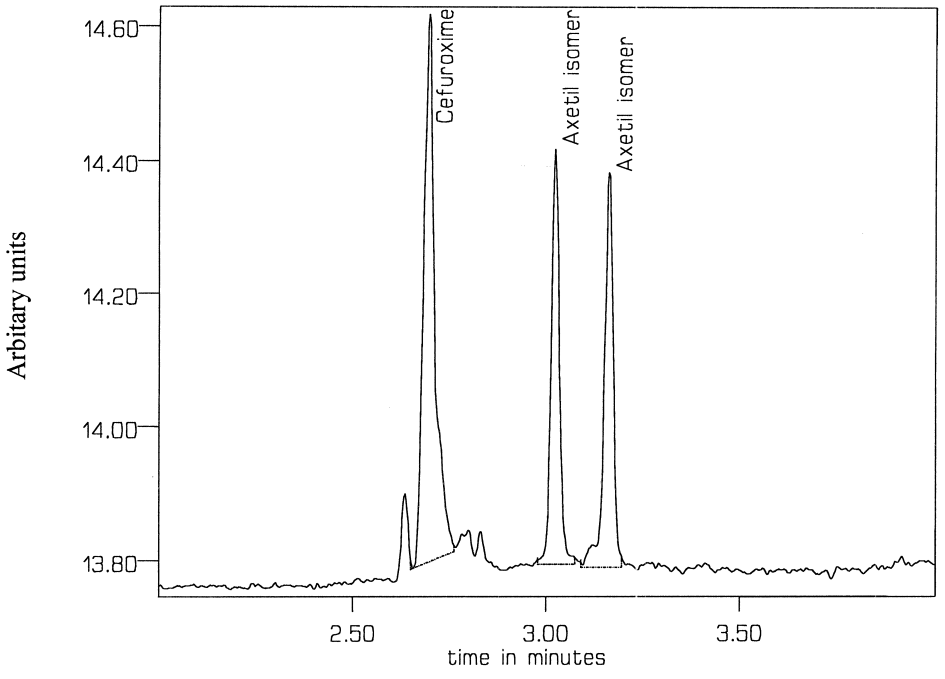

Fig. 8. Separation of degraded cefuroxime axetil solution. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i., detection at 280 nm.

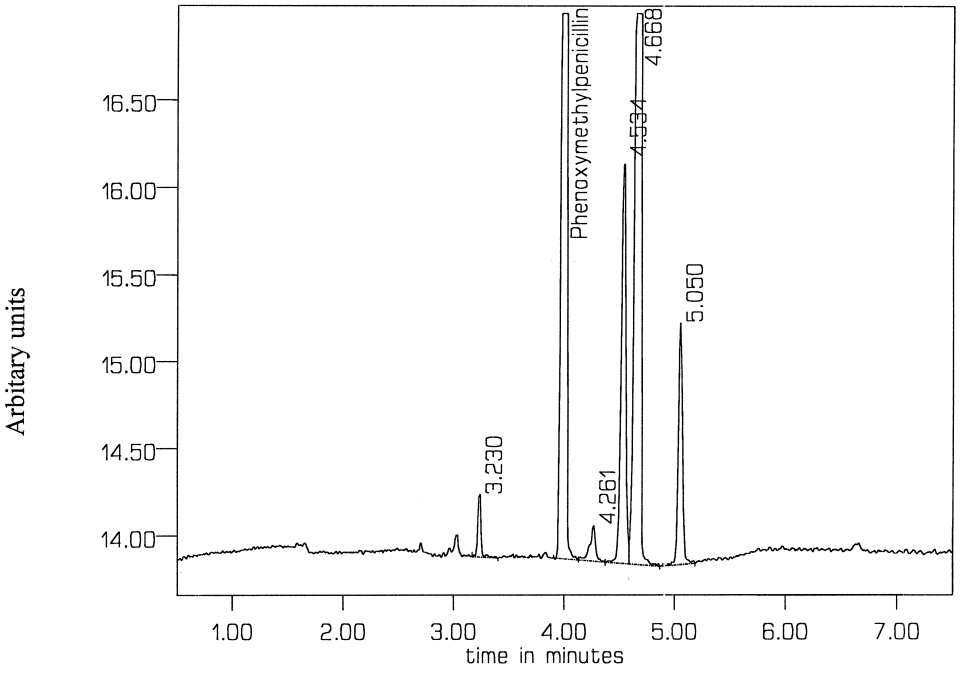

Fig. 9. Separation of degraded phenoxymethylpenicillin solution. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i.

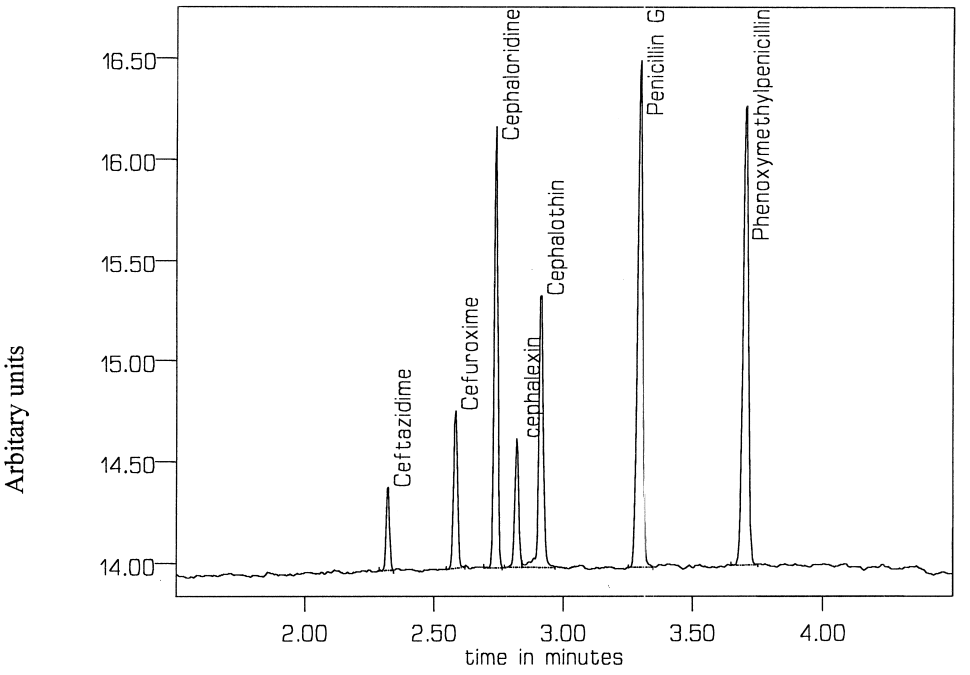

Fig. 10. Separation of a mixture of antibiotics. Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i., detection at 280 nm.

analysis of neutral steroids. However, acidic steroids migrate against the EOF and are undetected in CEC [15] unless the separation is performed at low pH where the acidic steroids are unionised.

MEEKC has previously been shown [26] to separate nine neutral steroids within 30 min using a similar buffer composition that that employed in this study. Fig. 11 shows application of the optimised MEEKC method to separation of two water-soluble acidic steroids and three insoluble neutral steroids within 9 min. The water-soluble acidic steroids, hydrocortisone sodium phosphate and beclomethasone sodium phosphate, are detected earlier than the highly retained hydrophobic neutral steroids. Budesonide is manufactured as a mixture of isomers which are partially resolved in Fig. 11. Detection of the steroids was performed at 254 nm which is a UV maximum for many steroids.

3.11. Method precision

The injection repeatability typically with this MEEKC method was equivalent to standard CE methods. Use of internal standards and the optimised methods allowed [28] RSD values of less than 1% for both peak area ratios and migration time within injection sequences. Preparation of repeatable microemulsion buffers has been identified [34] as a potential problem. Other workers [35] have reported repeatable production of microemulsion batches. In our work a large number of batches of microemulsion were repeatably prepared. The buffer was strictly prepared by 30 min of sonication, which ensures generation of a uniform droplet size. Segregation of the microemulsion into layers can occur if the surfactant content is too low to maintain a stable microemulsion. In this example 3.31% (w/w) SDS was employed. If the SDS content was lowered to below 2% (w/w) then segregation occurred within a few hours.

4. Conclusions

Microemulsion electrokinetic chromatography has been shown to be a highly applicable technique for

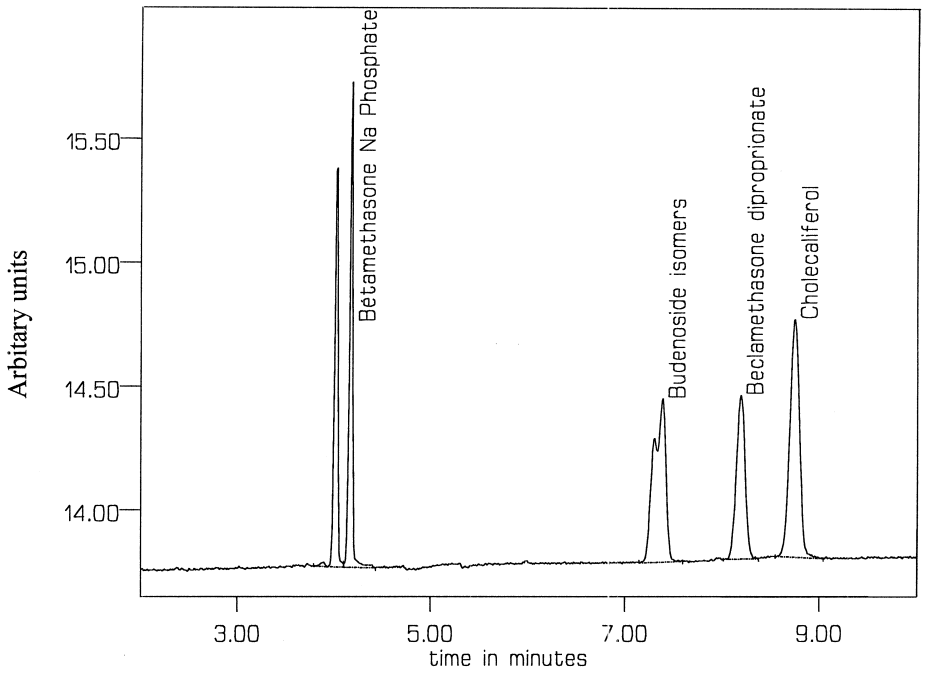

Fig. 11. Separation of water-soluble and insoluble steroids (peak at 4.1 min is hydrocortisone sodium phosphate). Separation conditions: as Fig. 2 except 3 s injection at 0.1 p.s.i., detection at 280 nm.

the analysis of a wide range of drugs, vitamins and excipients. The technique is also useful for the analysis of complex mixtures such as multi-component formulations and drug-related impurities. The technique is equally applicable to the analysis of water-insoluble neutral species such as steroids, which are normally difficult to analyse by CE. Use of MEEKC can offer advantages over conventional forms of CE, MEKC and CEC. It is therefore likely that MEEKC methods will be increasingly applied for pharmaceutical analysis in forthcoming years.

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