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The use of microemulsion electrokinetic chromatography in pharmaceutical analysis

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Abstract

The use of a single set of microemulsion electrokinetic chromatography (MEEKC) separation conditions has been assessed for its applicability in the analysis of a range of pharmaceutical compounds. Particular emphasis was placed on neutral or very hydrophobic compounds, which can be difficult to analyse by conventional capillary electrophoresis. The microemulsion employed for the majority of separations consisted of 0.81% w/w octane, 6.61% w/w 1-butanol, 3.31% w/w sodium dodecyl sulphate and 89.27% w/w 10 mM sodium tetraborate buffer. Good separations of methyl, ethyl, butyl and propyl hydroxybenzoates, and a range of ionic and neutral water soluble and insoluble compounds was achieved using a single set of separation conditions. A number of novel applications of MEEKC were developed included the simultaneous determination of the active components and preservatives in liquid formulation and determination of drug related impurities. Improved performance was obtained through use of internal standards and preparation of the samples dissolved in the microemulsion solution. Validation aspects such as linearity, repeatability, accuracy, injection precision and sensitivity were successfully assessed.

Keywords: Microemulsion electrokinetic chromatography; Capillary electrophoresis; Pharmaceuticals; Hydrophobic compounds

1. Introduction

In free solution capillary electrophoresis, only charged solutes can be separated as they migrate at different rates depending on the sign and size of their electrophoretic mobility [1]. Uncharged solutes can be separated by Micellar electrokinetic chromatography (MEKC), which is able to separate both neutral and charged species by using micellar solutions of charged surfactants. MEKC separations are performed in a fused silica capillary filled with an ionic surfactant solution at a concentration of the surfactant which is higher than its critical micelle concentration (CMC), the point at which the surfactant forms micelles. Watarai [2] developed the use of oil in water (o/w) microemulsions as an alternative means of separating neutral species. This technique has been termed microemulsion electrokinetic chromatog-

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raphy (MEEKC) and is useful in the separation of hydrophobic and neutral compounds. Both micelles and microemulsions are thermodynamically stable aggregates of surfactant molecules in aqueous solutions.

Micelles are formed (Fig. 1) from agglomerates of surfactant molecules whilst microemulsion droplets are generally formed from an anionic surfactant (usually SDS), co-surfactant and a water immiscible organic solvent (oil) in an aqueous buffer solution. The microemulsions are prepared by mixing oil, water, surfactant and a co-surfactant such as a medium chain alkyl alcohol. An example would be heptane, 10 mM sodium tetraborate buffer, SDS and 1-butanol. The microemulsion has a core of minute droplets of oil (Fig. 1) with the surfactant and co-surfactant located on the outside to stabilise the oil drop.

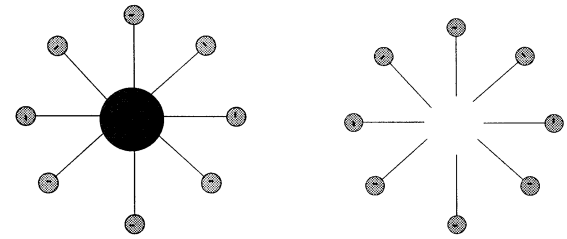


Fig. 1. Structure of micelle and microemulsion.

Both MEKC and MEEKC are normally performed at neutral or high pH conditions, where the electro-osmotic flow is greater than the electrophoretic mobility of the negatively charged micelle or microemulsion droplet. Fig. 2 shows a schematic of the MEEKC separation principle. When a neutral analyse is injected into the capillary, it will chromatographically distribute partition between the microemulsion droplet and the aqueous buffer phase. When the solute is dissolved in the droplet, it will attempt to migrate against EOF rate. Therefore, solutes which are strongly hydrophobic will have long retention times as they reside predominantly in the microemulsion droplet. Therefore, the analyse will migrate at a velocity between the two extremes, i.e. the electroosmotic velocity υ_{eo} and the velocity of the microemulsion υ_{mc} and migration time of the analyse, t_r will be between the migration time of the microemulsion droplet and the migration time of EOF solvent front. The velocity of the microemulsion droplet can be determined experimentally from the migration time of an extremely hydrophobic analyse such as dodecylbenzene. For electrically neutral solutes separated by MEKC, Terabe et al. [3] derived the following equation to describe its retention. This equation is also relevant to MEEKC.

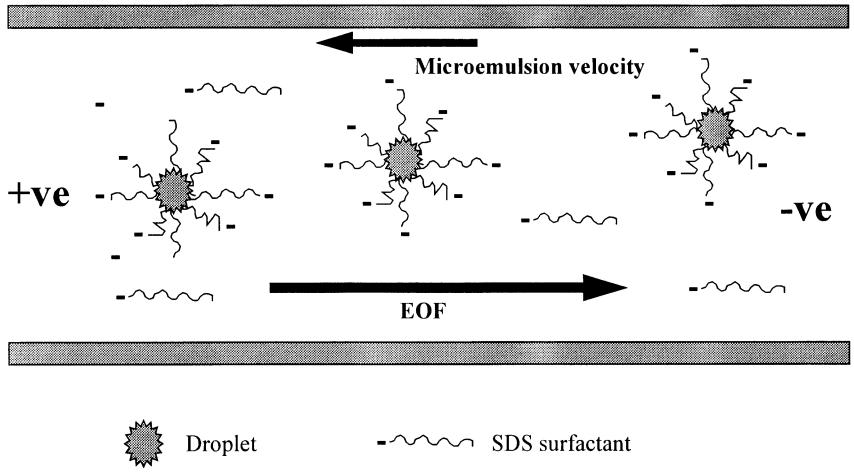


Fig. 2. Schematic of the separation principles of MEEKC.

where κ' is the capacity factor; t_r is the migration time of the solute; t_o is the migration time of the water; and t_{mc} is the migration time of the micelle or microemulsions

Cationic positively charged solutes could interact with the negatively charged droplet through both partitioning and ion-pairing processes. Acidic, negatively charged solutes are charged repelled from the anionic droplet but are separated under normal operating conditions due to their electrophoretic mobilities. When the solution pH is below 5, EOF is reduced because the silanol groups on the capillary walls are not fully ionized, thus the EOF is small. At this point, the electrophoretic mobility of the droplet is greater than the EOF and the micelles will migrate towards the anode.

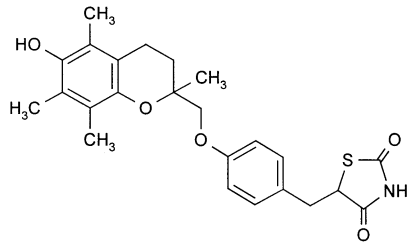
It is also possible to form microemulsions using cationic surfactants, e.g. tetradecyltrimethylammoniun bromide (TTAB). Under these conditions [4], the cationic surfactant molecules form an adsorbed bi-layer on the capillary wall, which reverses the direction of the EOF. Boso et al. [4] either use varied microemulsions of SDS or TTAB to alter the migration time and selectivity of the separation of water soluble and lipid soluble vitamins. Another possibility is the use of bile salt surfactants such as sodium cholate. These are useful for enantiometric separations because of their natural chirality.

To date, there have been only a limited [2–19] number of papers on the use of MEEKC. It has been used for the separation of mixtures of water soluble and insoluble vitamins [4], various analgesics [5], water insoluble steroids [6], and hop bitter acids [7], water insoluble pesticides [8], extremely hydrophobic bisdiphenylhydrazone derived sugars [9]. MEEKC has been used [10] for chiral separations where lipophilic chiral additives have been used to achieve chiral resolutions. The hydrophobicity ($\log P$) of a solute is an important physicochemical parameter and this is often measured experimentally. In MEEKC, the migration times are related to the $\log P$ as the more hydrophobic compounds are retained longer. Several reports [11–15] have reported correlation of MEEKC migration times to $\log P$ values and other measures of solute lipophilicity. Other reports [16–19] have included the separations of water-insoluble, neutral, polyaromatic hydrocarbons by MEEKC.

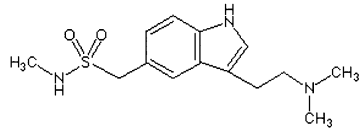
Therefore, this technique offers the possibility of widespread application in pharmaceutical analysis, however, the general applicability of this technique and its routine analytical performance has not been determined. Therefore, the focus of this paper has been to investigate the quantitative application of MEEKC to the analysis of a wide range of pharmaceutical compounds with a particular emphasis on water-insoluble compounds which are difficult to analyse by conventional CE. A single set of MEEKC operating conditions were successfully optimised for the separation of a variety of drugs and pharmaceutical excipients. These separation conditions were then applied to novel application areas such as main component assays and the determination of drug related impurities. The method gave good performance in a variety of analytical parameters such as injection precision, accuracy, linearity, sensitivity and method repeatability. In conclusion, it is suggested that MEEKC can be viewed as a routine alternative to conventional CE or MEKC and could offer distinct advantages in the separation of water insoluble neutral compounds.

2. Experimental

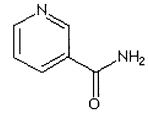
Analysis was performed using a number of Beckman (Fullerton, CA) and Hewlett-Packard (Waldbronn, Germany) CE instruments. A Hewlett-Packard (Bracknell, Berkshire) LAS 1000 data collection system was employed for integration and data handling. Inorganic chemicals were obtained from BDH (Poole, Dorset). Water was obtained from a Millipore Q system (Watford, Herts) and HPLC grade bottled water from Rathburn (Walkerburn, Scotland). Capillaries were purchased from Composite Metal Services (Hallow, Worcs.). 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



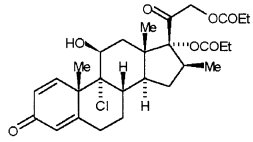
Troglitazone



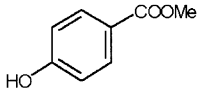
Sumatriptan



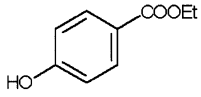
Nicotinamide



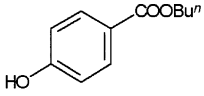
Beclomethasone dipropionate



Methyl hydroxybenzoate



Ethyl hydroxybenzoate

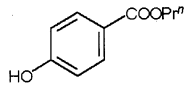


Butyl hydroxybenzoate

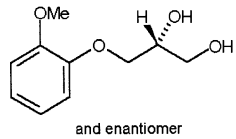
Fig. 3. Chemical structures of compounds separated in this study.

within GlaxoWellcome. The structures for a number of the test compounds are given in Fig. 3, which illustrate the structural diversity of the compounds. Separation were performed on Beckman instruments using 50 μm i.d × 27 cm (detection window at 20 cm) fused silica capillaries whilst 50 μm i.d × 32.5 cm (detection window at 24 cm) capillaries with a 150 μm detection bubble were used on the Hewlett-Packard instruments. The capillaries were rinsed between injections 0.1 M sodium hydroxide followed by the microemulsion.

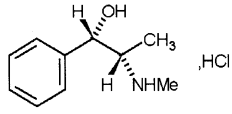
The composition of the microemulsion was similar to that of Watarai [2]. The microemulsions



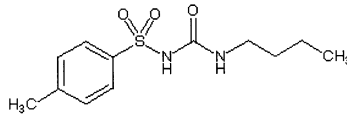
Propyl hydroxybenzoate



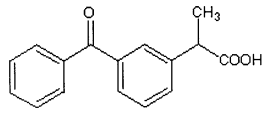
Guaiphenisin



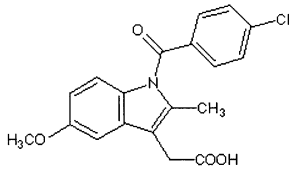
Pseudoephedrine



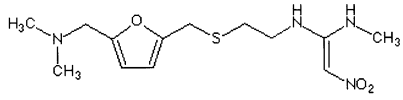
Tolbutamide



Ketoprofen



Indomethacin



Ranitidine Hydrochloride

Fig. 3. (*Continued*)

were prepared by weighing ~0.81 g of octane (or heptane), 6.61 g of 1-butanol and 3.31 g of SDS into a 100 ml volumetric flask. Approximately 90 ml of 10 mM aqueous sodium tetraborate, Capital HPLC (Broxburn, UK) was added and the flask sonicated for 20–30 min until all of the SDS had dissolved. This was allowed to settle and then made to volume with the borate buffer. Most compounds were made up in the microemulsion or another solvent at a concentration of ~0.1 mg ml^{-1}.

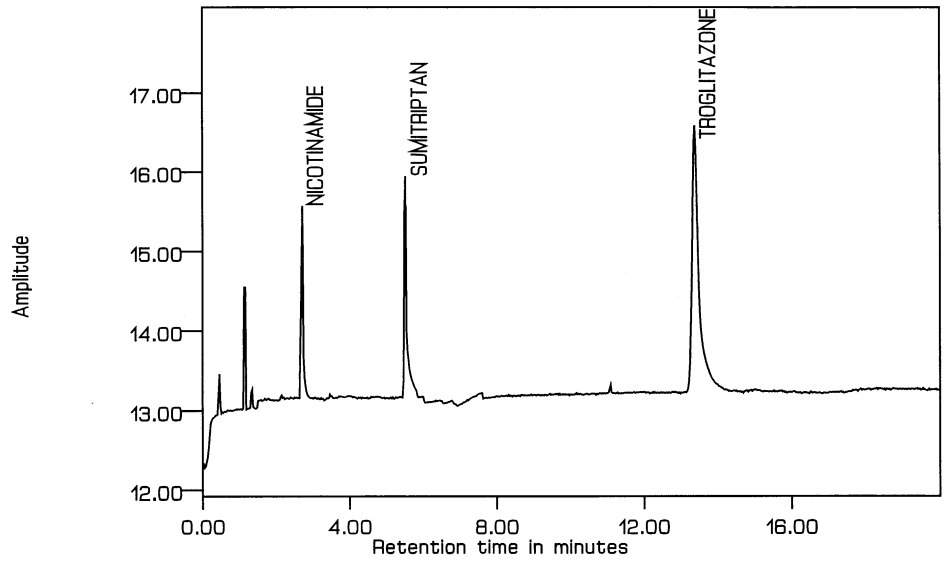


Fig. 4. A separation of a three component test mix run with an octane microemulsion.

3. Results and discussion

3.1. Method optimisation

3.1.1. Microemulsion composition

Some initial preliminary work was performed to assess previously reported [2–7] MEEKC conditions for the separation of acidic, neutral and basic drugs. These initial experiments were carried out using a microemulsion of 0.81% w/w heptane, 6.61% w/w, butanol, 3.31% w/w, SDS and 89.27% w/w 10 mM sodium tetraborate buffer. An initial test mix of three components, sumatriptan (a basic drug), nicotinamide (neutral vitamin) and troglitazone (an acidic drug) at a concentration of 0.1 mg ml^{-1} of each solute. An acceptable separation was obtained. Fu et al. [5] describe the effect of changing the core phase from heptane to octane. This has the effect of altering the retention time of some species. The same test mix is separated (Fig. 4) with an octane core phase microemulsion. The retention time of the most retained peak, troglitazone was shorter by two minutes. Fu also suggested the stability of the octane emulsions are greater because the number of carbon atoms in the surfactant, i.e. SDS equal the number of carbon atoms in the co-surfactant, i.e. octane plus the number of carbon atoms in the oil, i.e. butanol. The majority of work in this paper was therefore performed with the octane microemulsion.

3.1.2. SDS concentration

The concentration of surfactant will alter the retention time of the analyses as the number of charge density on the microemulsion will vary. At higher surfactant concentrations the number of SDS molecules associate with each droplet will increase. This effect was determined for the neutral solute nicotinamide. A concentration of 2% w/w produced a migration time of 5.7 min. This was increased to 8 min when the % w/w SDS was raised to 3% w/w and finally to 12.6 min with 5% w/w SDS. Therefore, in order to obtain reasonable analysis time and selectivity, a mid-concentration of 3.3% w/w SDS was adopted as the standard.

3.1.3. Dissolving solvent

The choice of dissolving solvent was found to have a significant impact on the quality of results obtained. Although it is possible to run samples dissolved in solvents other than the microemulsion, this can cause some baseline disturbance; this is probably due to the disruption of the microemulsion environment. This effect also oc-

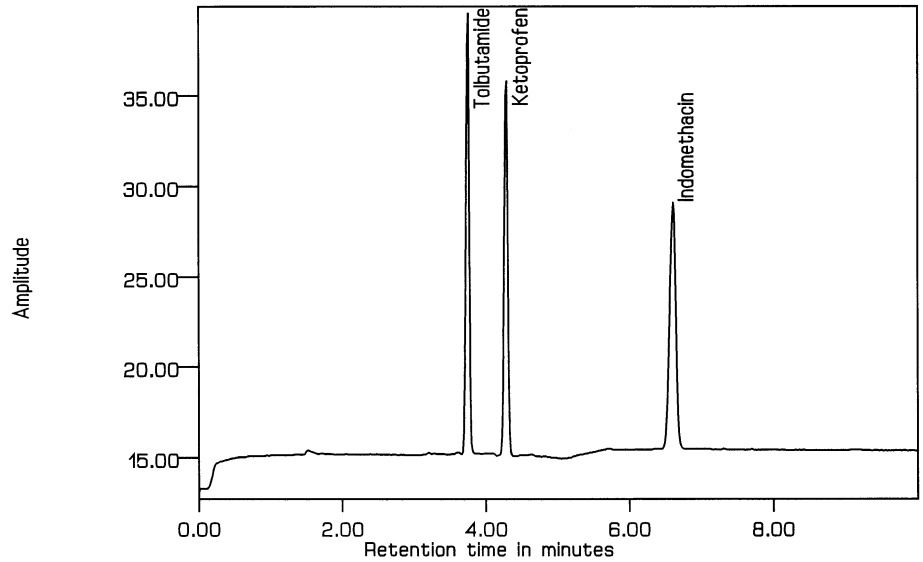


Fig. 5. Test mix of hydrophobic drugs dissolved in the microemulsion. Conditions: Beckman, 200 nm, 11 kV, 40°C, capillary 27 cm × 50 μm, injection 3 s, 80 μA, sample concentration, ~0. 1 mg ml^{-1}.

curs in MEKC, where the use of organic solvents to prepare samples can cause disturbances in the separation and loss of resolution. In this work, improved MEEKC injection reproducibility was evident when the water insoluble compound indomethacin was dissolved in microemulsion than in methanol. The %RSD of ten injections in methanol was 4.23% compared to 2.08% when indomethacin was dissolved in the microemulsion buffer. Peak shape was also generally poorer when the samples were dissolved in a solvent such as methanol. Therefore, it is recommended whenever possible to dissolve the sample in the MEEKC buffer used for the separation to avoid incompatibility issues.

3.1.4. Capillary dimensions

The 3.3% w/w SDS used to prepare the microemulsion is equivalent to 120 mM SDS ionic strength, which is relatively high. Therefore, to prevent the generation of excessive current inside the capillary short, narrow bore capillaries were used in-conjunction with relatively low voltages in the region of 10 kV. These conditions generated acceptable current values between 50 and 80 μA.

3.2. Analytical performance and application of MEEKC

3.2.1. Selectivity

The method was shown to be selective for a range of compounds shown in Fig. 1 which included (Fig. 5) the three hydrophobic drugs, namely ketoprofen, indomethacin and tolbutamide. The drug mixture was prepared by dissolving the compounds in the microemulsion. Fig. 6 shows the separation of the water-insoluble, neutral steroid beclomethasone dipropionate with the sample dissolved in methanol. MEEKC has previously been used [6] to resolve a number of related steroids.

3.2.2. Accuracy

Parabens (methyl, ethyl, butyl and propyl hydroxybenzoates) are commonly used in the pharmaceutical industry as preservatives to limit microbial growth. They are difficult to analyse by CE because they are strongly hydrophobic. The parabens are particularly water insoluble and require use of organic solvents or concentrated NaOH to prepare solutions. In MEEKC, they are readily soluble in the microemulsion buffer and

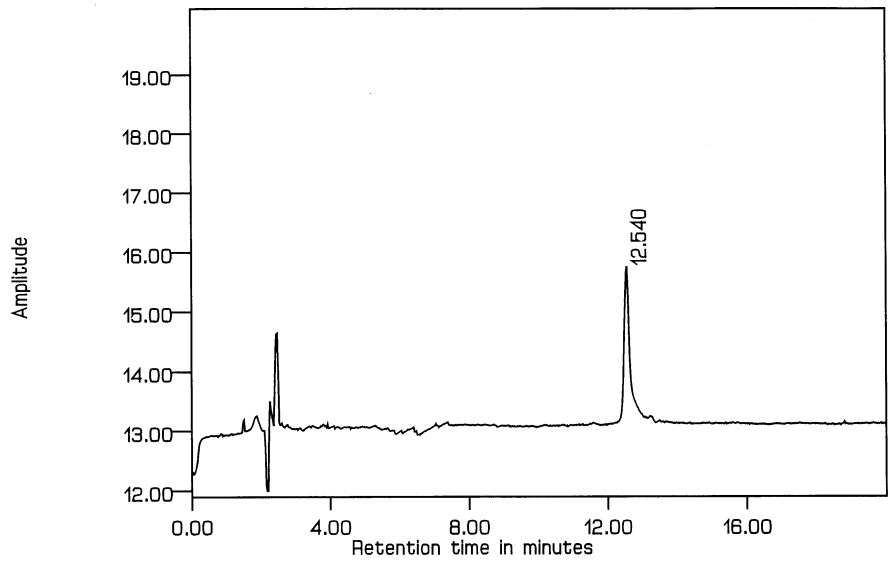


Fig. 6. Beclomethasone dipropionate dissolved in methanol. Conditions: Hewlett-Packard CE, 254 nm, 34 cm × 50 μm capillary, 10 kV, 30°C, Injection, 1 s at 50 mB, 63 μA, concentration, ~0.5 mg ml^{-1}.

can be injected onto the capillary where their different interactions with the core oil allows them to be separated. The propyl derivative has the highest hydrophobicity and is therefore retained the most. Although the microemulsion contains high levels of organic solvents, it is still possible to detect at 200 nm due to the small pathlength of the capillary. Fig. 7(a,b) show a separation of the active ingredients and preservatives present in a calibration solution and a sample of the liquid formulation Sudafed™ expectorant. This example shows that a main component assay of all the major constituents can be performed in one run using butyl hydroxybenzoate as an internal standard. The peaks seen at ~2 min in the sample solution are due to excipients such as colourants. Table 1 shows the results obtained with this method are well in line with the expected label claim for the content of both active ingredients and preservatives. The current reverse phase HPLC method for analysing Sudafed™ expectorant is time consuming and involves a sample preparation time of 45 min. Resolution of components is poor and column lifespan is short. Sample preparation for MEEKC involved dissolving the sample in the microemulsion buffer containing the internal standard and sonicating the solution to ensure it is clear. The resulting solution was directly injected onto the capillary.

3.2.3. Linearity

Using the Ketoprofen as an internal standard, a linearity of response was performed for both the tolbutamide and indomethacin over the range 0.05–0.1 5 mg ml^{-1}. Correlation coefficients of 0.9998 and 0.9993 were obtained for tolbutamide and indomethacin respectively with intercept values below 2% of the 100% assay value.

3.2.4. Sensitivity

An LOD of 0.001 mg ml^{-1} was obtained for tolbutamide and ketoprofen peaks. Improved sensitivity could be obtained using wider bore capillaries or modified capillary designs such as flow cells.

The method was applied to the novel analysis of drug related impurities for a number of compounds. For example, Fig. 8 shows a test mix of ranitidine hydrochloride and selected related impurities. Fig. 9 shows an electropherogram of the separation of tolbutamide and related impurities. Impurities could be detected at less than the de-

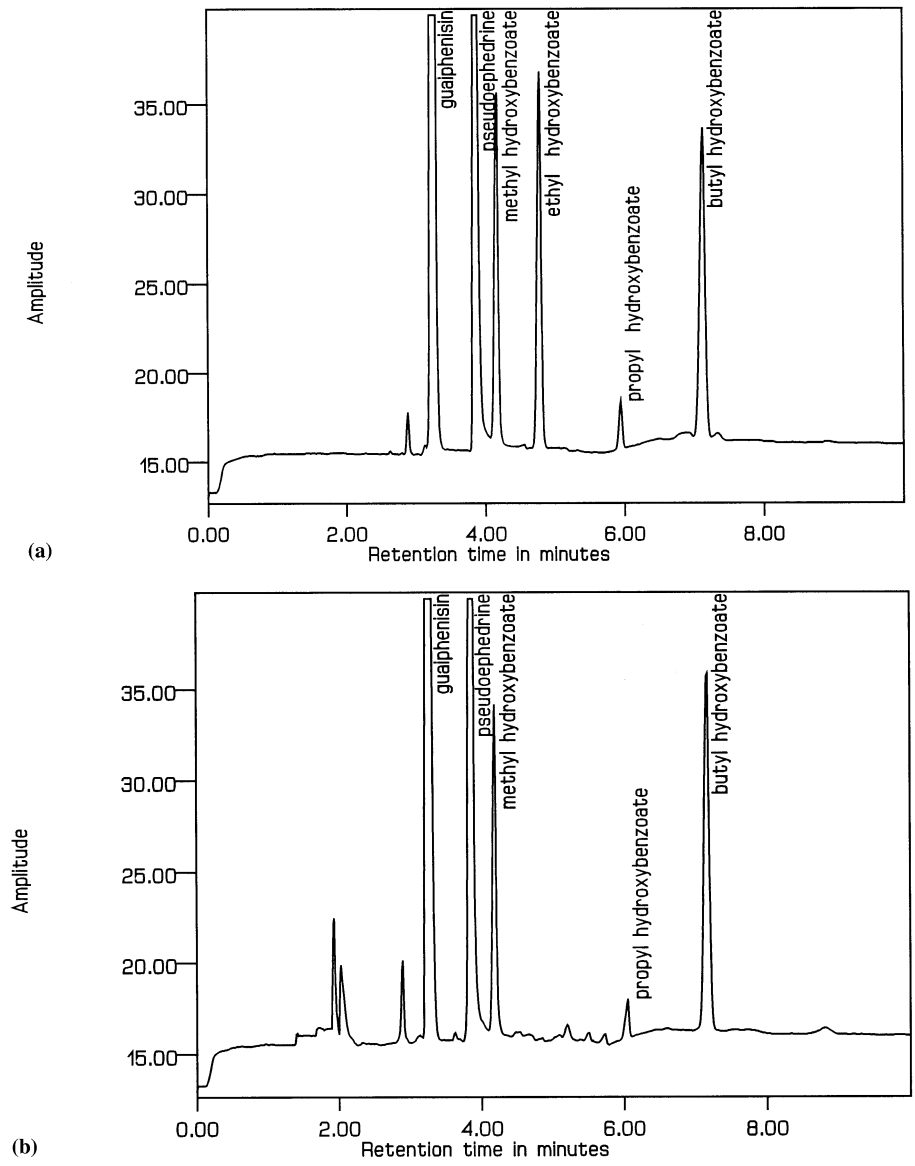


Fig. 7. Simultaneous determination of the active ingredients and excipients in a liquid formulation by MEEKC: (a) Calibration solution for Sudafed™ expectorant using Butyl hydroxybenzoate as an internal standard. Conditions: 200 nm, 11 kV, 40°C, capillary 27 cm × 50 μm, injection, 3 s, 80 μA; (b) Sudafed™ expectorant sample. Separation conditions as for (a).

sired sensitivity of 0.1% area/area of the main component peak. Since there are no stacking effects when the sample is prepared in the microemulsion solution, it is recommended wherever possible to prepare concentrated sample solutions and to perform short injection times in order to obtain maximum separation efficiency and sensitivity.

3.2.5. Injection volume reproducibility

The precision of injection for CE is improved by use of internal standards which eliminate in-

Table 1
Sudafed™ expectorant assay results

	Label claim	MEEKC assay results
Guaiphenisin	20 mg ml^{-1}	20.95 mg ml^{-1}
Pseudoephedrine	6 mg ml^{-1}	5.86 mg ml^{-1}
Methyl hydroxybenzoate	0.1 %w/v	0.096 %w/v
Propyl hydroxybenzoate	0.01 %w/v	0.01 %w/v

jection volume related errors. High sample concentrations/injection volumes are also used to generate large peaks which reduces integration related imprecision. For example, in this work the peak area %RSD was compared using three different injection times for a test mixture of parabens. The injection precision improved as a greater volume was injected onto the capillary which gave larger peaks. The peaks became wider rather than taller with the increased injection times as stacking effects are not possible if the sample is dissolved in the electrolyte. Table 2 shows that the precision of injection ($n = 10$) is improved when larger injection times are used and that this improvement is further enhanced when peak area ratios are calculated using an internal standard peak.

3.2.6. Method repeatability

The method conditions were repeated successfully on a number of different occasions using different batches of microemulsion and different capillaries in various laboratories. The method was successfully transferred between instrument types and analysis performed on both Beckman and Hewlett-Packard CE instruments. Once prepared, the microemulsion was highly stable and could be stored at room temperature on the bench for several months. Samples run with freshly prepared microemulsion and 1 month old microemulsion were compared and showed only slight differences in migration times with consistent selectivity. However, if the percentage of surfactant was only 2% or less, it was found that it became cloudy on standing as phase separation occurred.

3.3. Comparison of the use of MEEKC with MEKC

MEEKC and MEKC are useful complimentary techniques as they can both be utilised for the

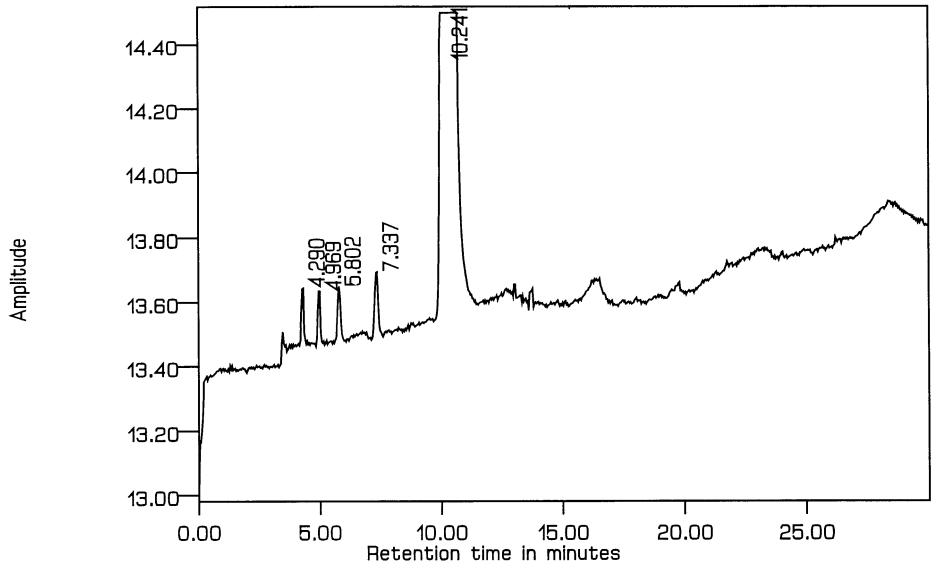


Fig. 8. Ranitidine hydrochloride and selected related impurities. Conditions: 214 nm, 7 kV, 30°C, 27 cm × 50 μm, injection, 5 s, 80 μA.

Table 2
Injection reproducibility for parabens[a]

	Injection time														
	1 s					2 s					3 s				
	1	IS	3	1/IS (PAR)	3/IS (PAR)	1	IS	3	1/IS (PAR)	3/IS (PAR)	1	IS	3	1/IS (PAR)	3/IS (PAR)
Mean area	2013 35	1885 53	1903 39	1.07	1.01	4099 24	3885 47	3917 37	1.06	1.01	6463 80	6094 37	6169 98	1.06	1.01
SD	6329	8345	5717	0.03	0.02	7573	7289	6194	0.02	0.01	10.43 9	1021 2	9577	0.00 3	0.01
%RSD	3.14	4.43	3.00	2.43	2.36	1.85	1.88	1.58	1.51	1.39	1.61	1.68	1.55	0.31	0.63

[a] 1, Methyl hydroxybenzoate; IS, Ethyl hydroxybenzoate; 3, Propyl hydroxybenzoate; PAR, peak area ratio.

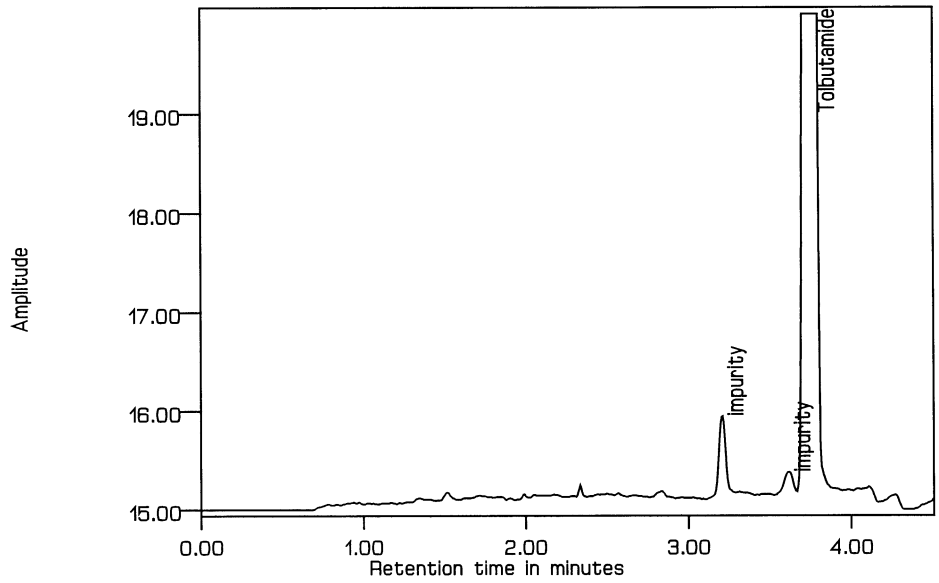


Fig. 9. Separation of tolbutamide and related impurities. Conditions, 200 nm, 11 kV, 40°C, capillary 27 cm × 50 μm, injection, 3 s, 80 μA.

separation of neutral solutes. Highly hydrophobic compounds are strongly retained and poorly resolved in MEKC and therefore organic solvents are often added to improve separation. These volatile organic solvents can cause evaporation problems, which are not frequently encountered in MEEKC. A disadvantage of MEEKC is that the separation is more strongly effected by choice of the dissolving solvent that in MEKC. Both techniques allow detection at low UV wavelengths to enhance sensitivity. MEEKC may be the technique of choice for strongly hydrophobic neutral compounds. MEKC has been more widely studied and selectivity options have been more widely explored than in MEEKC.

3.4. Comparison of the use of MEEKC with capillary electrochromatography (CEC)

As with MEEKC, CEC can be used to separate neutral compounds by using a chromatographic partition mechanism. However, in CEC, acidic compounds have an electrophoretic mobility which will oppose the EOF and therefore extend their analysis times or they may even be undetected. Basic compounds can interact unfavourably with the ionised silanols of the CEC stationary phase and peak tailing can occur. At present, there are still some operating problems to be solved with CEC such as fragile columns and air bubble formation. However, MEEKC can be performed on typical CE equipment with inexpensive fused silica capillaries.

4. Conclusions

The use of MEEKC has been found to be useful in the separation of hydrophobic compounds. The microemulsion itself is easily prepared and is stable at room temperature for up to six months. As with other CE methods, small volumes of solvents are required, thereby making it very cost effective and environmentally friendly.

It is possible to run samples dissolved in solvents other than the microemulsion, e.g. methanol, water and acetonitrile, although this can cause some baseline disturbance and poorer injection reproducibility is evident. This is probably due to the disruption of the microemulsion environment in the region of the sample plug. The migration time and separation selectivity can be

altered by changing the surfactant type and concentration and the organic solvents used to form the microemulsion droplets. Different core oils have been used in other work, e.g. heptane, hexane and octane to determine the effect on resolution of peaks. However, it has been suggested that the number of carbon atoms in the oil and co-surfactant should equal the number in the surfactant to give maximum stability. As octane and butanol equal the number of carbon atoms in SDS, these give the most stable microemulsion.

A single set of MEEKC operating conditions has been optimised, which allow analysis of a wide range of pharmaceuticals and excipients. These compounds included both water soluble and insoluble neutrals and ionic species. This wide selectivity range allowed the determination of both the active ingredients and preservatives in a liquid formulation with minimal sample preparation. The use of this general MEEKC method could greatly reduce the analysis time and may be potentially useful for QA laboratories.

The use of MEEKC is still relatively new and needs to be more fully investigated. In this work, methods have been shown to be both quantitative and reproducible. Therefore, the methods could be used for both main component assay and impurity determinations. Initial findings have shown it to be particularly useful in the analysis of very hydrophobic and neutral compounds, which are often difficult to separate by conventional CE.

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