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Development and optimisation of a generic micellar electrokinetic capillary chromatography method to support analysis of a wide range of pharmaceuticals and excipients

K.D. Altria [a,*], R. McLean [b]

[a] *Pharmaceutical Development Europe, GlaxoWellcome R&D, Ware, Hertfordshire, SG12 ODP, UK*
[b] *Department of Chemical and Life Sciences, University of Northumbria at Newcastle, Newcastle upon Tyne, NE1 8ST, UK*

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Abstract

A micellar electrokinetic capillary chromatography (MECC) method has been developed and validated to allow the analysis of a wide range of water soluble and insoluble acidic, basic and neutral drugs and excipients. An electronic database has been established to demonstrate the wide applicability of the method. The method has been validated and is now in routine use. In particular, acceptable injection precision is obtained through use of internal standards. Optimal sensitivity was obtained by using low UV wavelength detection. The method allows a number of cost and time saving benefits. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In CE and HPLC there are considerable cost and expense savings through the adoption of specific separation conditions, which can be directly applied to a wide range of drug compounds. These general application methods are termed generic methods. The use of a pH 2.5 phosphate buffer has been validated [1] as a generic buffer for the analysis of basic drugs by CE. This method has been shown to be useful for the analysis of a wide range of basic drugs and pharmaceutical excipients. Generic separations using a borate buffer (pH unadjusted) have been validated [2] for the analysis of a range of acidic drugs and excipients. In routine analysis the use of these generic methods is highly effective, as operating costs and both method validation and transfer issues are significantly reduced by adopting these standard methods. However these two generic methods cannot be applied to the analysis of neutral compounds or mixtures of charged and neutral compounds. Micellar electrokinetic capillary chromatography (MECC) is widely used to

* Corresponding author. E-mail: kda8029@ggr.co.uk.

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Table 1
Experimental conditions for the two instrument types

	Hewlett Packard	Beckman
Rinse 1	1 min with 0.1 M NaOH	1 min with 0.1 M NaOH
Rinse 2	1 min with electrolyte	1 min with electrolyte
Temperature (°C)	30	30
Injection	5 s pressure at 20 mbar from sample vial	1 s pressure from sample vial
Separation	12 kV (generates ~ 50 μA)	10 kV (generates ~40 μA)
Detection	200 nm (or specified wavelength)	200 nm with 4 nm bandwidth (or specified wavelength)
Capillary	34 cm × 50 μm (3 × bubble)	27 cm × 50 μm (800 μm slit)

separate neutral compounds through their partitioning with surfactant micelles. It was therefore decided to investigate the possibility of generating a generic MECC method.

The most commonly used anionic surfactant in MECC is sodium dodecyl sulphate (SDS). The lithium salt of dodecyl sulphate (LiDS) achieves similar separations but has the benefit [3] of a reduced operating current. Hydrophobic compounds have a high tendency to be incorporated into the micelle and therefore migrate late with poor resolution. The addition of organic solvents to the aqueous micellar can improve separations of hydrophobic solutes. However, evaporation and high UV absorption problems can occur with the use of solvents. An alternative approach is the addition of cyclodextrin (CD) to the buffer. These neutral CD migrate at the EOF velocity. Hydrophobic analytes can become incorporated into either the CD cavity or the micelle. The addition of CD enables the separation of highly hydrophobic analytes [4], which would otherwise be totally incorporated in the micelle. Effectively the addition of the CD establishes two pseudo stationary phases in the electrolyte, which can reduce analysis times and offer the possibility of improved separation. CDs have advantages over organic solvents as they are UV transparent and non-volatile.

This paper describes the optimisation and validation of a MECC method for the analysis of a wide range of pharmaceuticals and excipient materials. Standard generic MECC separation conditions have been devised and then applied to a broad range of drugs and drug classes. Validation aspects such as precision, linearity, method repeatability, accuracy, and method transfer between instrument types have been successfully evaluated.

2. Experimental

Analysis was performed using a number of Beckman (Fullerton, CA) and Hewlett Packard (Waldbronn, Germany) CE instruments. The method settings used for the two instrument types are given in Table 1. A bubble cell capillary arrangement was employed in the Hewlett Packard instrument to increase sensitivity. The particular bubble cell capillaries used increased the sensitivity and detection pathlength by a factor of three. A Hewlett Packard (Bracknell, Berks.) 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.).

All drug substances, samples and formulations were obtained from within GlaxoWellcome. The internal standard solution was prepared by initially dissolving the compounds in methanol at 1 mg ml^{-1} and subsequently diluting with water to yield a concentration of 0.1 mg ml^{-1}. The samples were prepared for analysis by dissolving the required concentration in the appropriate internal standard solution (benzoic acid or 4-hydroxyacetophenone). The buffer used was 20 mM borate containing both 75 mM LiDS and 15 mM β-cyclodextrin.

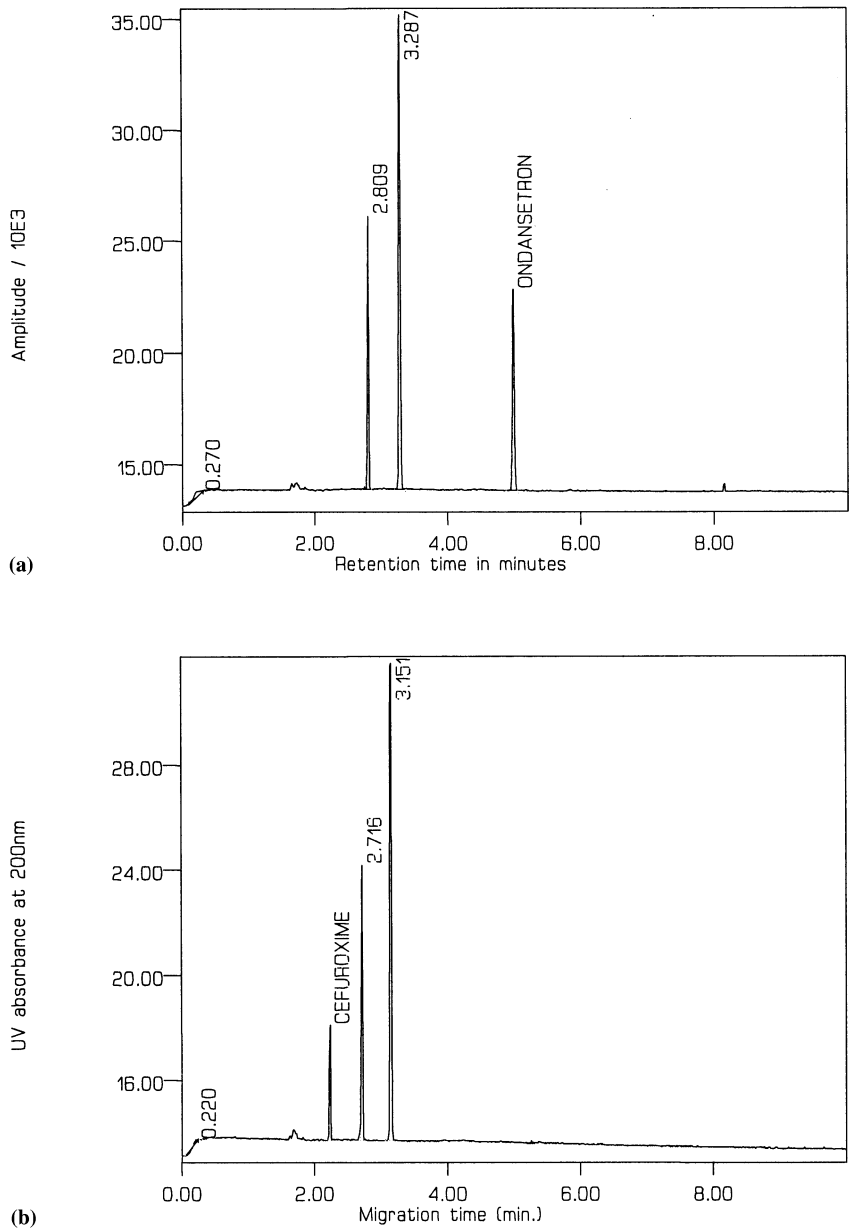

Fig. 1. Example separations. (a) Separation of a basic drug, Ondansetron, using generic MECC conditions. Separation conditions as in Table 1 for Beckman, peak at 2.8 min is 4-hydroxyacetophenone and peak at 3.3 min is benzoic acid. (b) Separation of an acidic drug, cefuroxime, using generic MECC conditions. Separation conditions as in Table 1 for Beckman, peak at 2.7 min is 4-hydroxyacetophenone and peak at 3.2 min is benzoic acid. (c) Separation of a neutral compound, ethylparahydroxybenzoate, using generic MECC conditions. Separation conditions as in Table 1 for Beckman, peak at 2.9 min is 4-hydroxyacetophenone and peak at 3.5 min is benzoic acid.

Best performance in terms of precision and consistent migration times was obtained by performing two blank injections prior to initiation of any analyses. These injections allowed the capil-

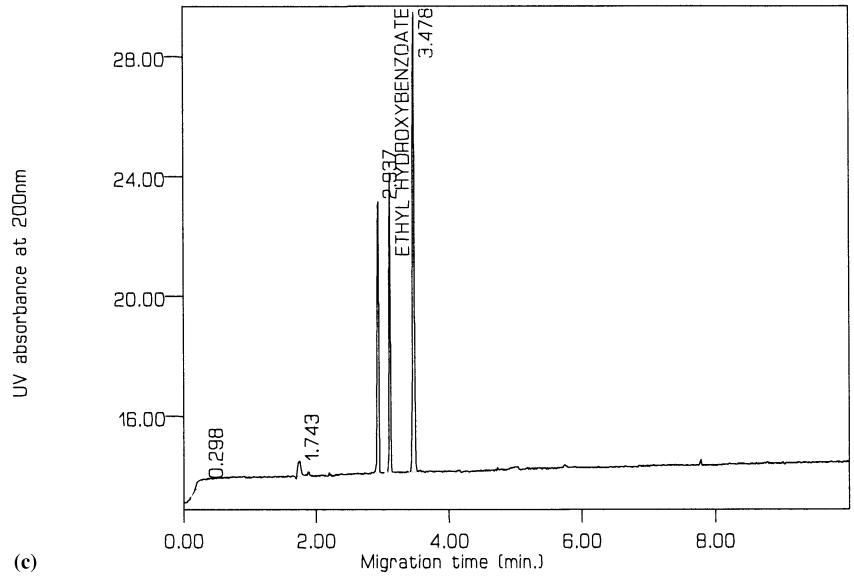

Fig. 1. (*Continued*)

lary wall surface to stabilise and the buffer and sample solutions to reach a consistent temperature on the autosampler tray. Each new capillary was preconditioned [5] prior to its first use by conducting a 20-min rinse with 0.1 M NaOH.

As the method is a general method, all development work was performed at 200 nm. However when the method is routinely applied to a particular compound, the wavelength is altered to a specific wavelength to obtain maximum sensitivity for that solute.

3. Results and discussion

The buffer composition was optimised by analysis of a test mixture of drugs. The influence of LiDS concentration was assessed and 75 mM was identified as optimal. Higher concentrations resulted in high retention times whilst lower LiDS concentrations gave poor separation of hydrophobic neutral compounds. The concentration of β-CD was chosen to be 15 mM as this acceptable resolution of hydrophobic compound. A borate concentration of 20 mM was selected as this gave a reasonable electrosmotic flow rate and did not generate excessive current.

3.1. Selectivity

The applicability of the method was assessed by analysing samples of a range of drug and excipient compounds. In each case identical separation conditions were used to allow comparison between results. Two internal standard reference compounds (benzoic acid and 4-hydroxyacetophenone) were included in each sample solution. These internal standards allowed identification of the tested compound by their migration times relative to the migration times of the marker peaks. Fig. 1 gives representative results from the samples tested and shows a neutral, basic and acidic sample.

The method was used to resolve a wide range of compounds including a range of cephalosporins, streptomycin, hydrocortisone sodium phosphate, nicotinic acid, a range of vitamins including riboflavine, caffeine, tetracyclines, and a range of basic drugs including salmeterol and AZT. Excipients including saccharin, benzalkonium chloride, parahydroxybenzoates, and dyes were also separated. Table 1 gives a listing of all the compounds resolved on this method and their migration positions relative to the two internal standards used. Cationic positively charged drugs can interact

with the negatively charged droplet through both partitioning and ion-pairing processes therefore they tend to produce (Table 2) long migration time. Acidic negatively charged solutes are charged repelled from the anionic droplet but are separated under normal operating conditions due to their electrophoretic mobilities. Therefore the RMT values of acidic compounds tend to be negative in Table 2 (i.e. they migrate before the internal reference peaks).

Table 2
Relative migration time data for a range of compounds

Compound	RMT1[a]	RMT2[b]
Acyclovir-Zovirax	−0.789	−1.302
Allopurinol	−0.673	−1.192
Amitriptyline HCl	2.637	2.112
Aspirin	−0.810	−1.339
Benzalkonium chloride	2.695	2.161
Caffeine	−0.564	−1.056
Ceftazidime Na pentahydrate	−0.705	−1.210
Cefuroxime Na	−0.497	−0.932
Cephalexin Na	−0.398	−0.838
Cephaloridine hydrate	1.250	0.759
Chlorpheniramine maleate	2.755	2.222
Clenbuterol HCl	2.661	2.114
Dequalinium chloride	2.712	2.188
Dihydrostreptomycin sulphate	2.234	1.696
Ephedrine HCl	2.250	1.722
Ethylhydroxybenzoate	0.014	−0.541
Griseofulvin	2.558	2.037
Hydrocortisone Na phosphate	−0.105	−0.647
Lamivudine	−0.722	−1.245
Lignocaine HCl	2.316	1.783
Naratriptan HCl	2.729	2.244
Nicotinic acid	−1.810	−2.343
Ondansetron HCl	2.171	1.693
Paracetamol	−0.714	−1.185
Patent blue violet	1.766	1.229
Phenoxymethylpenicillin	−0.136	−0.636
Propylhydroxybenzoate	0.621	0.077
Ranitidine HCl	1.665	1.207
Remifentanil	2.288	1.770
Riboflavin HCl	−0.336	−0.863
Saccharin Na	0.702	0.161
Salmeterol	2.786	2.233
Sumatriptan succinate	2.017	1.569
Terbutaline HCl	1.332	0.791
Troglitazone	2.088	1.533
Tryptophan	−0.529	−1.070
Tucaresol	0.137	−0.387
Uracil	0.223	−0.297
Valacyclovir HCl	−0.075	−0.614
Zidovudine-AZT	−0.629	−1.147

[a] RMT1, migration time of the compound relative to the migration time of 4-hydroxyacetophenone.

[b] RMT2, migration time of the compound relative to the migration time of benzoic acid.

Fig. 2 shows that the separation conditions can be used to simultaneously resolve a variety of neutral and charged compounds. This test mixture includes both water soluble and insoluble compounds. This ability to simultaneously analyse a range of compound types is important when analysing formulations containing a number of active ingredients and excipients.

3.2. Repeatability

The precision of injection for CE is improved by use of internal standards which eliminates injection volume related errors. High sample concentrations/injection volumes are also used to generate large peaks which reduces integration related imprecision. Acceptable injection precision was obtained (Table 3) when using an internal standard. Optimal precision was obtained when using a high concentration of both internal standard and drug compound. Typical assay concentrations of the drug and internal standard were therefore 0.5–1.0 mg ml^{-1}. Migration time precisions were typically 1–2% but this was improved to less than 1% when relative migration times were calculated. For example, ten replicate injections of a mixture of 4-hydroxyacetophenone and benzoic acid gave RSD values of 0.67 and 0.71%, respectively for migration time precision. This was improved to 0.06% RSD when relative migration time precision was calculated.

3.3. Sensitivity

A limit of quantitation (LOQ) of 5 μg ml^{-1} was determined for paracetamol by repeatedly injecting a solution at this concentration. A precision for peak area ratio was 6.51% RSD ($n = 10$) and a signal-to-noise ratio of 10 was obtained. A limit of detection (LOD) of 2 μg ml^{-1} was established as this produced a peak with a signal-to-noise ratio of 3. Improved sensitivity could be produced, if required for trace level monitoring,

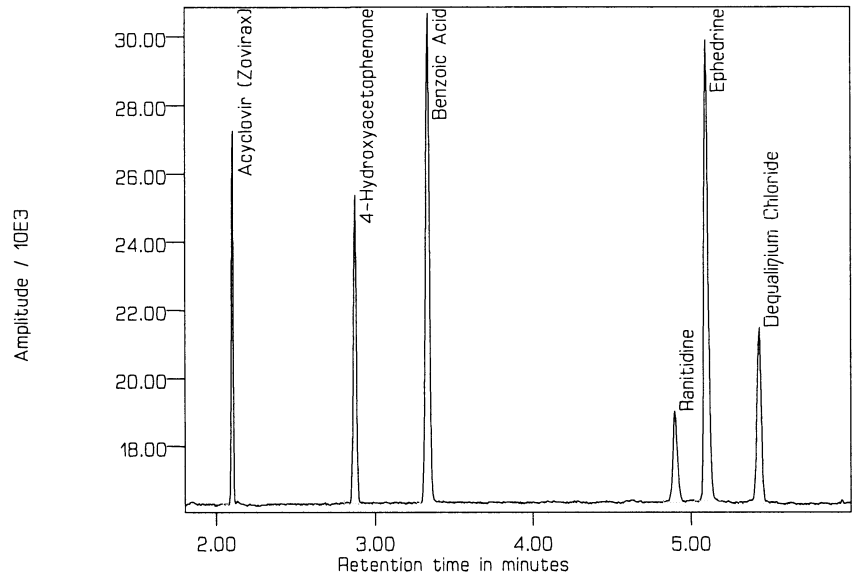

Fig. 2. Separation of a range of basic, acidic and neutral compounds using generic MECC conditions. Separation conditions as in Table 1 for Beckman.

using either longer injection times, wider bore capillaries, or modified capillary designs such as flow cells or bubble cells. Limits of detection of less than 0.1% were possible when the method was applied to the determination of drug related impurities in a number of drug substances.

3.4. Linearity

Acceptable detector linearity with sample concentration was shown for sumatriptan over the concentration range 0.05–0.15 mg ml^{-1}. Five standards were prepared and injected in duplicate. A blank of the internal standard solution (0.5 mg ml^{-1} benzoic acid in water) was also injected twice. A correlation coefficient of 0.9999 was obtained between peak area ratio and standard concentration. The intercept value was -0.5946% of the response for the 100% standard, which is within the acceptable 2% limit. A correlation coefficient of 0.9873 and an intercept value of -1.5936% were obtained for the same data when peak areas were plotted instead of peak area ratios. The improvement in data using the peak area ratio data strongly supports use of the internal standard approach.

Table 3
Results from precision studies

Sample (internal standard used)	Number of replicates	%RSD for peak area ratios
Sumatriptan (4-hydroxyacetophenone)	10	0.81
Terbutaline (benzoic acid)	10	1.32
Chlorpheniramine (benzoic acid)	8	0.98
Benzoate (4-hydroxyacetophenone)	10	0.74
Paracetamol (4-hydroxyacetophenone)	8	0.63

3.5. Accuracy

Piriton tablets containing the active ingredient chlorpheniramine maleate were assayed using the method. The results (3.997 mg $tablet^{-1}$) were in good agreement with the product label claim of 4 mg $tablet^{-1}$. A RSD of 0.98% ($n = 8$) was obtained for response factors of the calibration solution.

3.6. Repeatability of method

The method was successfully repeated on a number of instruments in different laboratories. The method was repeated using buffer prepared on several separate days and using a number of different capillaries. The method was also transferred to a Hewlett Packard instrument (the HP specific settings are given in Table 1). The terbutaline data in Table 3 was generated on a Hewlett Packard instrument which shows that inter-day reproducibility data is acceptable.

4. Conclusions

A set of generic MECC conditions has been optimised, which allow analysis of a wide range of drugs and excipients. Successful evaluation of a number of validation parameters has been demonstrated. The method is in routine use in our laboratories to support testing of both main peak assay and the determination of drug related impurities. The method supplements and supports the use of the generic CE methods for the analysis of basic and acidic drugs. A combination of the three methods allows separation of greater than 90% of the pharmaceuticals currently in test within our laboratories.

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