ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 1091–1101

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Validated capillary electrophoresis method for the analysis of a range of acidic drugs and excipients

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Accepted 20 June 1996

Abstract

A capillary electrophoresis (CE) method employing a high pH borate buffer has been validated to allow analysis of a wide range of acidic compounds including active drugs, pharmaceutical formulations, excipients, starting materials and intermediates. An internal database has been established to demonstrate the wide applicability of the method. The method has been extensively validated and is in routine use in a number of our laboratories worldwide. In particular, acceptable injection precision is obtained through the use of internal standards and the method robustness was evaluated using an experimental design. The method allows a number of cost and time saving benefits.

Keywords: Capillary electrophoresis; Pharmaceuticals; Validation; Excipients; Acidic drugs

1. Introduction

The use of a single set of operating parameters to analyse a wide range of compounds is one of the features of capillary electrophoresis (CE). For example separation conditions have been validated for the analysis of a wide range of basic drugs and excipients [1]. The simple low pH phosphate buffer used allowed accurate quantitation of the analytes present as input materials or as active components in formulations. Similar separation conditions have been shown [2] to be applicable to the separation of 17 other basic drugs.

Considerable benefits are obtained when using these generally applicable separation conditions. These benefits include reduction or elimination of method development time as the method can generally be immediately applied to a new test compound. Operating costs are also reduced as an inexpensive combination of a uncoated fused silica capillary and an aqueous buffer compare favourably with maintenance of a variety of specific HPLC columns and assorted mobile phases. The generic CE method for basic drugs has been found to be highly robust [1] as the separation is dependent on the solute physicochemical properties of ionic charge and size. The ability to apply the method to a range of compounds also leads to productivity gains as validation, training and method transfer exercises are minimised when the method is applied to a previously unseparated compound.

Given the attractive benefits associated with generic type methods, it was decided to investigate

0731-7085/97/$17.00
PII S0731-7085(96)02000-6

Table 1
Selection of acidic compounds separated by CE

Solute(s)	Electrolyte	Comments	Reference
Adenosine and other polyhydroxyl species	Borate pH10	Determinations in urine	[3]
Aminogylcoside antibiotics	Borate 9.4	Alternative to USP method, good validation data	[4]
Anionic dyes	Phosphate pH 9	Separation of 4 component test mixture	[5]
Antraquinone sulphonates	Borate pH 10	Increased precision using internal standard	[6]
Cephalosporins and penicillins	Borate–phosphate, pH 7	9 component test mixture resolved	[7]
Cefotaxime	Phosphate pH 8	Comparison of CE and HPLC data	[8]
Diol compounds including DOPA	Borate pH 8.5	Borate complexes resolved	[9]
Enalapril maleate	Phosphate 9.5	Assay of tablet content, validation details	[10]
Fosinopril sodium	Borate pH 10	Data comparison with HPLC	[11]
NSAID's	Glycine–triethanolamine, pH 9.1	Validation study, assessment of buffers	[12]
Parahydroxybenzoates	Borate pH10	Determination in cosmetic preparations	[13]
Phenol pollutants	Borate pH10	LOD of 1 ppm	[14]
Sugars	Borate. pH 9.5	Detection at 195nm, high temperature operation	[15]
Surfactant (dodeyclbenzenesulphonate)	Borate–phosphate pH 9	Trace level detection, 200 nm	[16]
Thymidine isomers and anomers	Borate pH 9.5	High temperature operation	[17]
Vitamins	Borate pH 9	Cross-correlation with HPLC method	[18]
Vitamins—water soluble	Phosphate pH 7	Vitamin content in tablets determined	[19]
X-ray contrast agents	Borate pH 10	Borate complexes separated	[20]

the possibility of obtaining a set of operating conditions applicable to the separation of a range of acidic drugs and excipients. Acidic compounds have generally been analysed by CE using phosphate, borate, or phosphate–borate buffer combinations in the pH range 7–10. Table 1 shows that these buffers have been used [3–20] in the separation of a range of acidic solutes including drugs, dyes, vitamins, sugars and surfactants. These electrolytes have minimal background UV absorbances which allows the use of low UV wavelengths such as 195–215 nm where many compounds have significantly higher responses than at the higher wavelengths commonly used in HPLC. Use of these low wavelengths can allow direct detection of compounds which have extremely poor UV activity such as sugars [15] and aminoglycoside antibiotics [4].

A borate buffer was selected for this study as it was considered to have several interesting features. Borate has a natural pH of 9.4 which would be sufficiently high for it to be applicable to the separation of the majority of acids. The natural pH represents a plateau in the titration curve of boric acid which ensures maximised buffering capacity which improves the method robustness and method simplicity as pH adjustment is not required. The electro-osmotic flow (EOF) characteristics of borate have been studied [21] and it was reported to give a substantial and consistent EOF. The buffer is prepared using borax (Na tetraborate), therefore 1 mol borax generates 4 mol borate ions; this maximises the concentration of buffering ions whilst minimising counter-ion (Na) concentrations. This is an advantage when compared to Na_2HPO_4 which generates 2 mol Na^+ and only 1 mol phosphate. The higher relative number of borate ions also reduces any buffer depletion effects [22] without generation of excessive currents. Complexation of borate ions with neutral compounds containing diol functions is possible. The borate-compound complex is negatively charged and can be therefore be separated. These compounds include sugars [15], aminogly-

cosides [4] and diol-containing drugs [3,9]. Another minor advantage is that the natural pH of the buffer ensures that pH adjustment is avoided which improves method robustness and increases the simplicity of the method.

As the method is intended for quantitative analysis of a drug in formulations, an acceptable precision is required. Several factors, including sample solution viscosity, influence the volume of sample solution injected into the capillary [23]. The factors affecting precision have been studied extensively [23] using a model separation of a mixture of two aromatic acids (β-naphthoxyacetic acid and aminobenzoic acid) with a borate buffer. It was recommended [23] that use of an internal standard and high sample concentrations improved precision. The borate buffer used in this previous study gave highly consistent EOF which was exhibited by the sub 1% R.S.D. values obtained routinely for migration times. Calculation of migration times relative to an internal standard generally give improved precision data [23] which is useful for performing peak identification.

In this study the applicability of the method to the separation of a range of water soluble and insoluble drugs was assessed using a variety of acidic test compounds. The method was applied to acidic excipients such as the preservatives sorbic acid and *para*hydroxybenzoates, methyl red (dye) and aspartame (an artificial sweetener). The method was also applied to the analysis of placebo formulations and therefore sufficient sensitivity was required to monitor low levels of drug. This was achieved through use of low UV wavelength detection.

Optimisation of the sample diluent was appropriate for poorly soluble compounds as the incorrect choice of solvent can have a severely detrimental effect on the quality of the separation obtained. For example [24] the presence of methanol in the sample solution disrupts the sodium dodecyl sulphate (SDS) micelles widely

Table 2
Experimental conditions for the two instrument types

	Hewlett Packard	Beckman
Rinse 1	0.5 min with 0.1 M NaOH	0.5 min with 0.1 M NaOH
Rinse 2	0.5 min with electrolyte	0.5 min with electrolyte
Temperature	30°C	30°C
Injection 1	3 second pressure at 25 mbar from sample	1 second pressure from sample
Injection 2	1 second pressure at 25 mbar from buffer	1 second pressure from buffer
Separation	7 kV	6.5 kV
Electrolyte	15 mM $Na_2B4O_7 \cdot 10H_2O$	15 mM $Na_2B4O_7 \cdot 10H_2O$
Detection	200 nm (or specified wavelength)	200 nm (or specified wavelength)
Capillary	34 cm × 75 mm (bubble −3)	27 cm × 75 mm

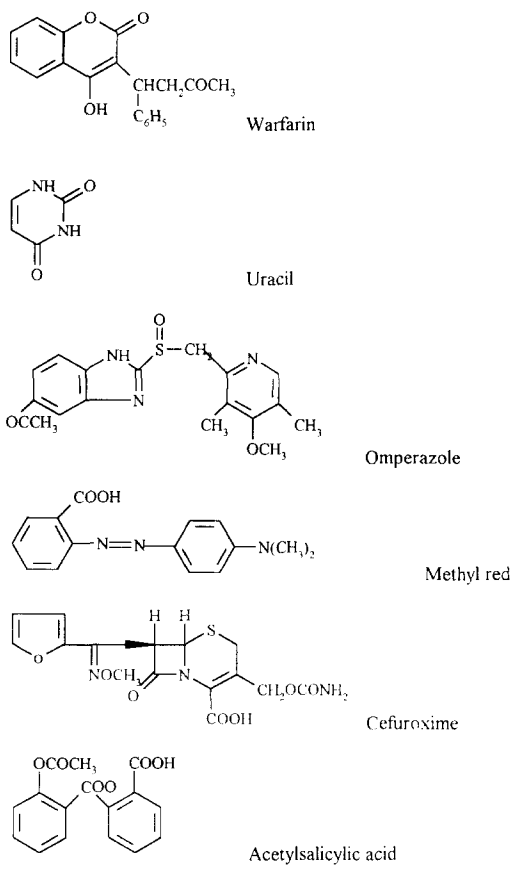

Fig. 1. Chemical structures of a selection of the compounds resolved warfarin, uracil, omperazole, methyl red, cefuroxime and acetylsalicylic acid.

used in micellular electrokinetic capillary chromatography (MECC) separations. This method was applied to a range of water soluble and insoluble compounds. Soluble compounds were dissolved in water containing the appropriate internal standard to maximise stacking effects. Water insoluble compounds were generally dissolved in dilute NaOH solutions. However this led to solution degradation in many cases. If degradation was a problem then samples were dissolved in organic solvents or aqueous–organic solvent mixtures.

The optimal method conditions were validated by assessing a variety of performance criteria. The criteria evaluated included precision, linearity, accuracy, solution stability, repeatability and method robustness. An experimental design statistical evaluation of method robustness was performed as this approach has previously been shown to be highly appropriate [25] when simultaneously assessing the impact of a number of factors upon a separation.

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 2.

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 path length by a factor of three.

A Hewlett Packard (Bracknell, Berks) LAS 1000 data collection system was employed for integration and data handling. The experimental designs and statistical analysis of the experimental data generated during robustness testing were performed using Design Ease (version 2.07) and Design Expert (version 3.05) software (Stat-Ease, MN).

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).

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 capillary wall surface to stabilise and the buffer and sample solutions to reach a consistent temperature on the autosampler tray. Each new capillary was pre-conditioned [26] prior to its first use by conducting a 20 min rinse with 0.1 M NaOH.

All drug substances samples and formulations were obtained within GlaxoWellcome. The structures for a number of the test compounds are given in Fig. 1.

Table 3
Relative migration time data for a range of compounds

Compound	RMT1	RMT2
Acetylsalicylic acid	1.02	0.93
Bacitracin	0.72	0.65
Benzoate salt	1.19	1.10
Beclomethasone ($NaPO_4$)	1.05	0.96
Cefuroxime (Na)	0.84	0.77
Ceftizoxime	0.89	0.78
Ceftriaxone	1.00	0.92
Cephalothin	0.85	0.77
Cromoglycate (Na)	1.17	1.06
Embonic Acid	1.29	1.17
Epoprostenol (Na)	0.85	0.77
Ethyl-*para*hydroxybenzoate	0.92	0.84
Glibenclamide	0.78	0.71
Methyl Red	0.91	0.78
Nedocromil (Na)	1.25	1.13
Nystatin	0.88	0.80
Omeprazole	0.79	0.72
Phenylglycine	0.83	0.76
Phthalate	1.09	1.00
Prednisolone (Na)	1.05	0.96
Sorbic acid	1.09	1.00
Thyroxine (Na)	1.00	0.89
Tryptophan	0.74	0.67
Uracil	0.80	0.72
Warfarin (Na)	0.89	0.82
Zidovudine (AZT)	0.70	0.63

RMT 1 is the migration time relative to the migration time of β-naphthoxy acetic acid.
RMT 2 is the migration time relative to the migration time of aminobenzoic acid.

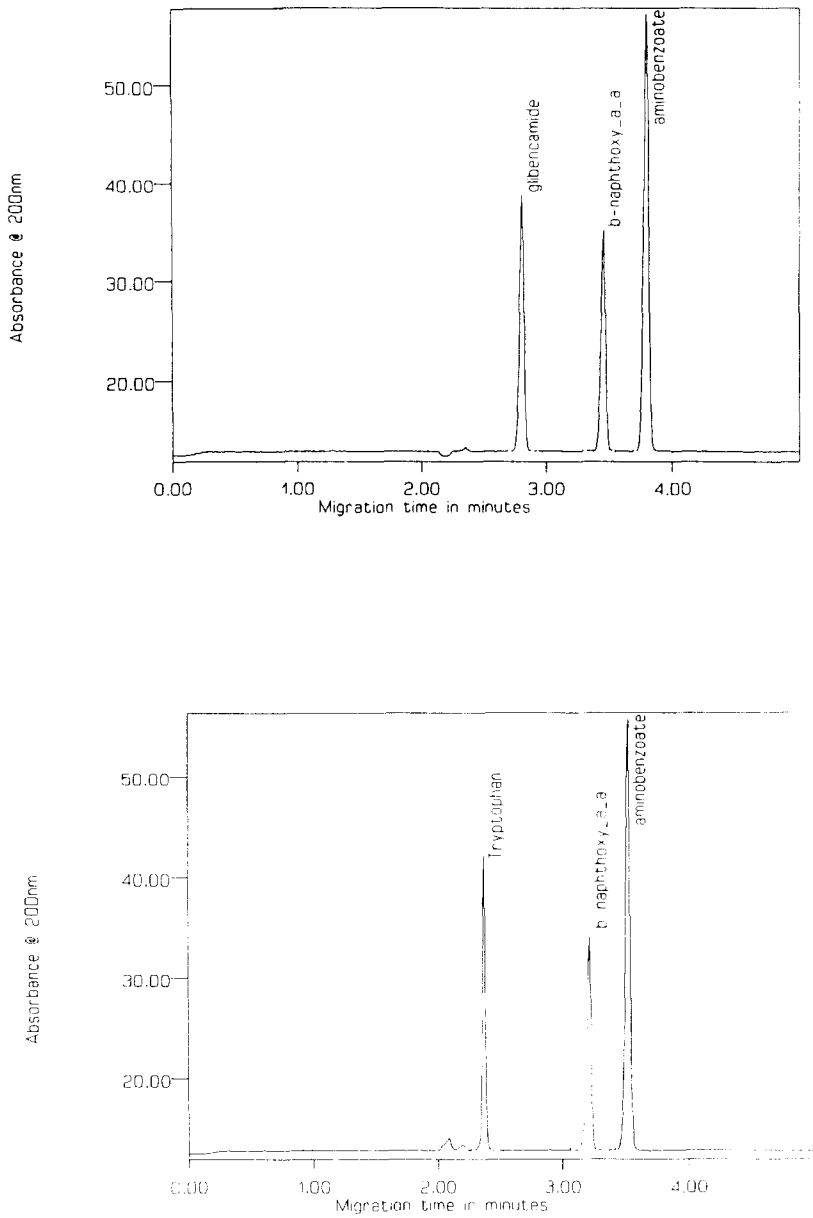

Fig. 2. Representative separations from the database. Separation conditions as in Table 2 for the Beckman instrument.

3. Results and discussion

The validation criteria employed when assessing a CE method are similar to those used when evaluating the performance of HPLC methods [1]. The method was applied to a range of sample types including tablets, capsules, raw drugs and liquid pharmaceutical preparations.

3.1. Selectivity

The conditions of the method were shown to be suitable for the analysis of a range of raw materials and acidic drugs. The selectivity was demon-

Table 4
Precision of injection for a range of acidic drugs

Solute	RMT	PAR
GW1 (calibration)	0.23	0.34
GW1 (sample)	0.19	0.56
GW2 (calibration) Beckman	0.21	0.76
GW2 (calibration) Hewlett-Packard	0.13	1.31
GW2 (sample)	0.34	0.89
Levothyroxine	0.32	0.58
Omperazole	0.31	0.89

Precision of injection %R.S.D. ($n = 10$).

strated for a range of acidic compounds including acetylsalicylic acid, omeprazole, glibenclamide, warfarin and prednisolone (sodium salt). A database has been constructed in which a wide range of compounds have been screened using this general method. In all circumstances the compound was diluted with a solution containing the two internal standards, aminobenzoic acid and β-naphthoxy acetic acid. The relative migration times (RMT) of the solutes were calculated compared to these two internal standards. Table 3 shows a small selection of the compounds incorporated into the database together with their RMT values. Fig. 2 shows two representative separations from the database. Fig. 1 shows that the structures of the compounds resolved are diverse. The polarity and solubility of the compounds separated are equally diverse. It is therefore inconceivable that a single HPLC method could be established to resolve such a wide range of acidic compounds.

The method has also been applied to a wide range of water soluble and insoluble acid drug candidates and intermediate compounds currently under development within GlaxoWellcome. In particular the method has been applied to GW1, a water soluble acidic drug and GW2, a water insoluble acidic drug which is soluble in acetonitrile–water mixtures.

The method has also been shown to be of use for the rapid screening of the impurity profile of acidic drug candidates.

3.2. Precision

Two internal standards, β-naphthoxyacetic acid (Na salt) and aminobenzoic acid (HCl salt), were used to improve peak area precision. Sample and internal standard concentrations of 100 ppm (0.1 mg ml^{-1}) were used to generate relatively large peak areas which minimised integration errors. Precision was assessed for several compounds on different days and with different capillaries (Table 4). Peak area ratios (PAR) were employed as these gave improved precision values compared to use of peak areas. For example the precision data for Levothyroxine was 1.20% calculated using peak areas and 0.58% for peak area ratios.

Repeatability of sample and calibration preparation are important validation issues. Ten calibration solutions were prepared and analysed in duplicate for both GW1 and GW2. The % R.S.D. for the response factors were 0.22 and 0.78% respectively. Ten samples of GW1 and GW2 tablets were prepared and each solution injected in duplicate. The %R.S.D. on the assay results were 1.22 and 1.13% respectively. The higher R.S.D. values for the sample results compared to injection of calibration solutions reflects the variability of the drug content in the tablets.

As the method has been in routine use within a number of our laboratories on several sites for

Table 5
Assay results for CE against label claim

Tablet	Label claim (mg per tablet)	CE result (mg)	Label claim (%)
GW1	50	50.2	100.4
GW2	200	198	99
GW2	400	402	100.5
Levothyroxine	0.1	0.103	103
Omeprazole	20	20.7	103

over 2 years, the method has been repeated on several instruments, different instrument types, and by several different analysts at various sites. The reagents and capillaries have been locally sourced by each laboratory which again demonstrates the method repeatability.

3.3. Accuracy

Results generated by the CE method were compared with those expected by the label claim. Table 5 shows that the CE results obtained were in accordance with the label claim.

Additional verification of the identity of the sample could be accomplished using the diode array facilities available on the CE instruments used. Fig. 3 shows the separation of GW2 and the spectrum obtained for the GW2 peak which is used for additional identity verification. The spectrum also clearly shows the improved detection sensitivity possible when using low UV wavelengths.

3.4. Sensitivity

The limits of detection (peak with 3 × signal-to-noise) obtained are specific for each of the analytes assessed and are dependent on the UV absorbance of the compound at 200 nm, the common wavelength employed. For instance a limit of detection of 0.4 ppm (mg l^{-1}) was established for GW1, warfarin and glibenclamide.

Limits of quantitation (LOQ) were assessed by ten replicate injections of low level concentrations. Satisfactory precision data of less than 10% R.S.D. were required. Typical LOQ values obtained were in the region of 1.2–1.7 mg l^{-1}.

3.5. Linearity

Detector response linearities were assessed (Table 6) by preparing five calibration samples covering the range 50–150% of the nominal sample concentration (50–150 ppm mg l^{-1}). Each sample was injected in duplicate together with duplicate injections of the internal standard diluent. Linearity data was always improved when calculated using peak area ratios as the precision of the measurements was better, resulting in less scatter on the linearity plot.

3.6. Stability of solutions

An aqueous test mixture containing aminobenzoate, bitrex and warfarin was prepared and stored in a refrigerator for 20 days. The test

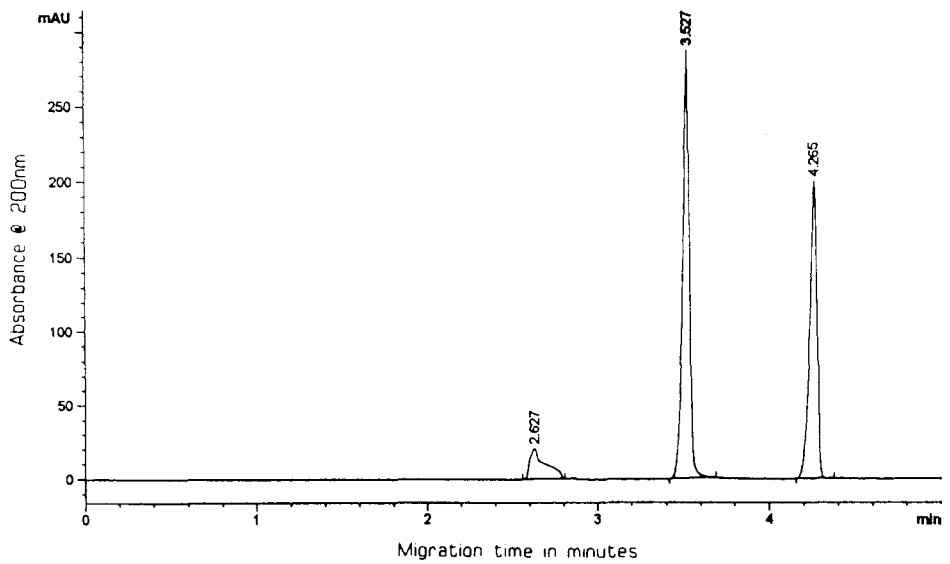

Fig. 3.

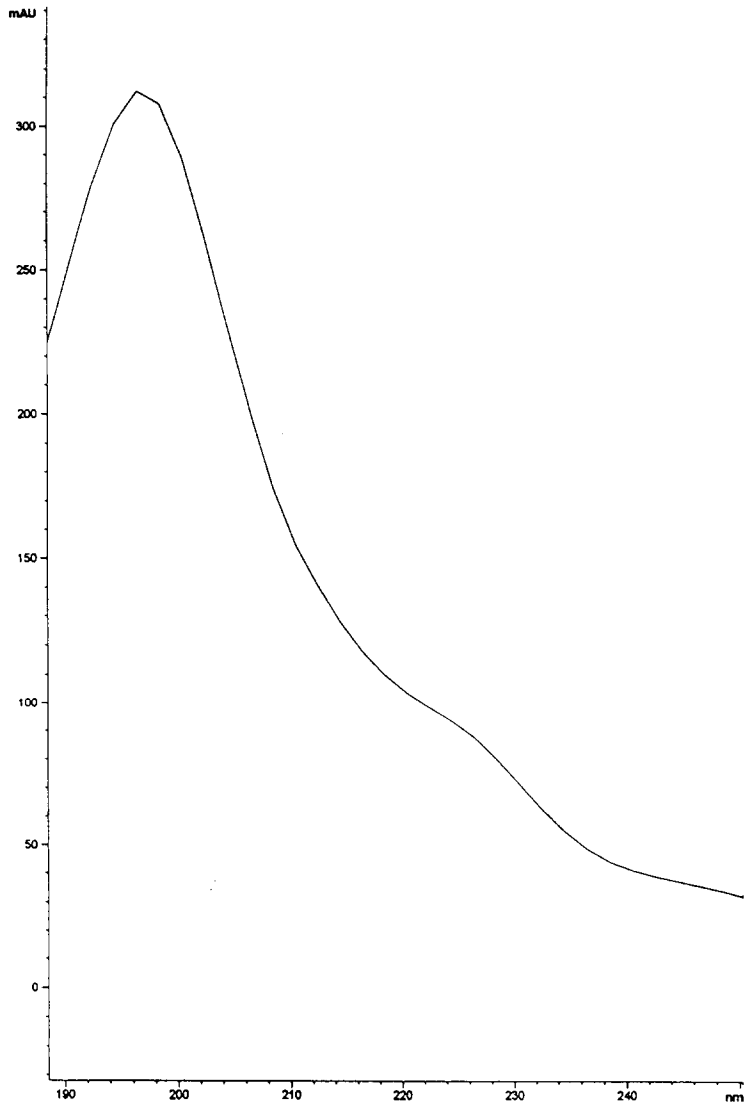

Fig. 3. Separation of GW2 and associated diode array spectra of GW2. Separation conditions as in Table 2 for the Hewlett Packard instrument.

mixture was re-analysed together with a freshly prepared test mixture of similar composition. Consistent selectivity and peak area ratios were obtained for both the 'fresh' and 'stored' test mixtures. No additional peaks were obtained in the 'stored' sample and this indicates good solution stability. This solution shelf-life was not possible for all solutes. For example GW2 solutions were unstable for periods longer than a day. Therefore, these solutions are freshly prepared on the day of analysis. Accordingly the stability of each compound in the appropriate diluent and storage conditions is specifically determined.

Similar sensitivity and selectivity was obtained for separation of a test mixture using both a freshly prepared electrolyte and an electrolyte stored for 3 months in high density polyethylene plastic containers at room temperature, unprotected from light. Therefore a 3 month shelf-life for this electrolyte has been assigned.

Table 6
Detector linearity studies

Correlation coefficient over the range 50–150 ppm		
Solute	Peak areas	Peak area ratios
Glibenclamide	0.9168	0.9989
GW1	0.9933	0.9998
GW2	0.9715	0.9979
Omeprazole	0.9958	0.9978
Levothyroxine	0.9986	0.9999

3.7. Robustness

The benefits of using experimental designs to determine the robustness of CE methods have been demonstrated [25]. Fractional factorial designs have been utilised to screen simultaneously the impact of varying several operating parameters within a single sequence. A similar approach was used during the robustness testing of this method.

The objective was to identify the method parameters upon which the method responses are significantly dependent and determine the ranges over which they can be varied, without unduly affecting the method performance characteristics. Table 7 shows the ranges over which the key operating parameters were varied in the robustness study. The method was assessed using test mixtures containing GW1, aminobenzoate, prednisolone and benzoate. Fig. 4 shows a typical separation under the method conditions.

Table 7
Ranges of method parameters evaluated in method robustness study

Parameter	Low level	Method level	High level
Rinse 1, 0.1 M NaOH (min)	0.4	0.5	0.6
Rinse 2, buffer (min)	0.4	0.5	0.6
Buffer (mM)	12	15	18
Temperature (°C)	25	30	35
Injection time (s)	1	1[a]	1.1
Voltage (kV)	6	6.5	7

[a] One second is the lowest injection time possible on the Beckman instrument used in the robustness study.

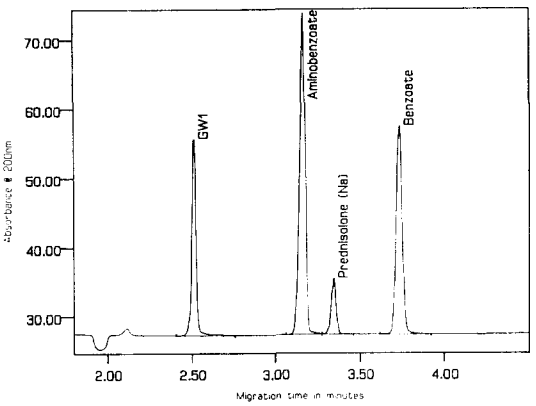

Fig. 4. Separation of the test mixture used in the robustness studies under method conditions. Separation conditions as in Table 2 for the Beckman instrument.

Consistent baseline selectivity and migration times, within the time window set for the system suitability, were maintained in all injections covering the ranges given in Table 7. The method was therefore considered to be robust with respect to resolution and migration time.

3.8. Recovery

Levels of drug were spiked into solutions containing placebo formulations. Table 8 shows that acceptable recovery data was obtained over the required sample concentration range. The spiking levels covered 50–150% of the nominal sample concentration.

3.9. Application range

The method has been applied to support both quality control and research/development purposes. These applications include assay of active content in solid and liquid pharmaceutical formulations, analysis of pharmaceutical intermediates such as tablet blends, analysis of excipients, assay of placebo formulations for absence of active drug, impurity screening of drug substance, confirmation of sample identity by concordance of sample RMT with the RMT obtained for a standard solution, and also the determination of

Table 8
Recovery from excipients

Recovery from placebos	50%	75%	100%	125%	150%
GW1	100.2	99.5	100.1	99.6	99.4
GW2	99.7	100.9	99.4	99.5	100.4

acidic drug salt counter-ions such as benzoate, tosylate and hydroxynaphthoate.

4. Advantages and disadvantages of the method

The method has become established as a useful alternative, and addition, to the existing HPLC methods for the separation of acidic drugs. The major benefit is that no method development is required for new, previously unanalysed solutes. This is especially important in research areas where many new drug candidates may be analysed on only one or two occasions. The method optimisation consists merely of selection of the appropriate internal standard, analysis time and detection wavelength.

The method initially undergoes a comprehensive method validation. Therefore subsequent validation requirements are minimal for each new compound as core validation criteria such as method repeatability, robustness and electrolyte solution stability have been fully assessed. The compound-specific validation requirements include assessments of sensitivity, detector linearity, injection precision and solution stability. These compound-specific validation aspects can generally be assessed in two injection sequences. The initial method transfer exercise between laboratories can be comprehensive. This means that subsequent method transfer exercises to support testing of further compounds are highly efficient as the subsequent method transfer can be more limited.

Detection at 200 nm allows quantitation of compounds which have only limited UV activity and would require derivitisation to be detected by UV absorbance in HPLC. This ability can significantly reduce sample pre-treatment needs. Sample solutions can often be directly injected into the capillary due to the rugged nature of the capillary and the use of rinse cycles between injections. Direct injections of these solutions would often cause fouling of HPLC columns. Direct injection of sample solutions reduces both analysis time and costs of consumables such as filter units. Other savings are accrued due to savings related to reduced organic solvent purchase and disposal and the need to retain a number of compound specific HPLC columns.

The principal disadvantages are that the method is less sensitive than HPLC equivalents which may be an issue in the testing of placebo products and that the use of internal standards is required to obtain sufficiently good injection precision.

5. Conclusions

A general capillary electrophoresis method is described for the efficient analysis of a range of water soluble and insoluble acidic drugs and excipients. The method employs a simple borate buffer with low UV wavelength detection. The method has been validated by assessments of a range of factors such as selectivity, precision, linearity, sensitivity, accuracy, and robustness. Internal standards are used to obtain acceptable injection precision. The method has been applied to a number of applications including analysis of active and placebo formulations.

Over 80% of the compounds currently under test within our laboratories can be quantified by the use of a combination of this method and a previously validated method [1] for analysis of basic drugs using a low pH phosphate buffer.

Considerable operating benefits can be obtained by adopting these methods in terms of the elimination of method development for new compounds, more efficient operating procedures,

reduced operating costs and method validation/transfer exercises. The disadvantages are reduced sensitivity compared to HPLC and the need to employ an internal standard.

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