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Validated capillary electrophoresis method for the assay of a range of basic drugs

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Abstract

A capillary electrophoresis method has been developed and validated for the analysis of a wide range of basic drugs. Acceptable precision was obtained by employing an internal standard. Optimal sensitivity was obtained using low UV wavelengths. An experimentally designed study showed the method to be robust. The method has advantages over HPLC in terms of simplicity, speed and cost. The method is now in routine use for identity confirmation and assay of both drug substance and formulations.

Keywords: Basic drugs; Capillary electrophoresis; Drug formulations; Internal standard; Validation

1. Introduction

Capillary electrophoresis (CE) is becoming widely accepted as a routine technique within the pharmaceutical industry. A recent survey of major US and UK pharmaceutical companies [1] confirmed that methods have been successfully accepted by regulatory authorities, and that acceptable method validation and transfer can be achieved. The survey also indicated that CE is being applied to assay, impurity determinations, chiral separations and confirmation of the identity of test samples.

The application of CE by the pharmaceutical industry is due mainly to the wide range of possible benefits that may be obtained, when compared to the well established and widely used technique of HPLC. Principal advantages [2] which are likely to be obtained include improvements in cost efficiencies, avoidance of solvent purchase and disposal, method robustness and simplicity. These benefits are obtained in particular when performing simple assay or identity confirmation testing, as many compounds can often be analyzed using a single set of operating conditions. For example, 17 basic drugs were resolved using a low pH phosphate buffer [3], and a similar buffer was employed [4] to separate 12 different basic drugs. Another report described the separation of 16 sulphonamides at pH 7 [5].

The separation of bases at low pH is the simplest CE separation process to control. Smaller, more highly charged solutes migrate faster along the capillary and are therefore detected first. If the separation is performed at pH 2–3, the majority of basic compounds will be fully protonated. Under these conditions, the charge-to-mass ratio is a characteristic of the molecule and is therefore independent of the capillary. This feature ensures that the selectivity of methods operating at low pH is highly consistent. This consistency is very attractive in quality control (QC) type analysis by CE [6], where a large number of samples are analysed.

The combined assay and identity confirmation of drugs as drug substance, or in formulations, is typically performed by HPLC. Assay is

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Table 1
Experimental conditions

Rinse 1	1 min with 0.1 M NaOH
Rinse 2	1 min with electrolyte (see below)
Set temperature	30 °C
Injection	5 s pressure
Separation	+13 kV for 10 min [a]
Sample	Aqueous solutions containing trace levels of drug
Electrolyte	25 mM NaH_2PO_4, pH 2.3 adjusted with conc. H_3PO_4
Detection	200 nm (or specified wavelength) [a]
Capillary dimensions	37 cm × 75 μm
Instrument	Beckman P/ACE 2200 or 5100

[a] Wavelength and run-time may be varied for determinations of specific drugs to optimise performance.

usually performed by relating the peak area to a response factor, obtained through analysis of a standard. Identity confirmation can also be obtained through an agreement between the retention times of the standard and sample peaks. Additional confirmation may be obtained by comparing UV spectra through the use of HPLC diode array detectors. The use of CE to confirm identity through concordance of migration times has been reported [7], and CE diode array detectors [3,8] are available from commercial instrument suppliers.

There are several possible benefits to adopting a low pH CE method for separating a range of basic drugs. The major benefit is the reduced cost of reagents and HPLC columns. The electrolyte is water-based and approximately only 20 ml are used per analysis day. Thus, the electrolyte can be prepared and stored ready for use. This avoidance of the need to purchase and dispose of organic solvents has been identified as a major advantage of CE over HPLC [9]. Other considerable cost efficient benefits include reduced method validation and transfer time, and reductions in staff training requirements. Given these obvious attractions, it was decided to validate a method for the analysis of both basic drugs and appropriate excipients. A range of drugs obtained from within Glaxo and from external suppliers were employed as test compounds. Two appropriate internal standards were identified. The validation was successful, and this method is now in routine use within our laboratories for both identity confirmation and assay purposes.

2. Experimental

Analysis was performed using Beckman P/ACE 2200 and P/ACE 5100 (Fullerton, CA) CE instruments. The method settings are given in Table 1.

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 Inc., Minneapolis).

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).

The 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 [10] prior to initial use by conducting a 20 min rinse with 0.1 M NaOH.

All drug substances, samples and formulations were obtained within Glaxo. The compound denoted as GRD1 has several structural similarities to Sumatriptan. The structures for a number of the test compounds are given in Fig. 1.

3. Results and discussion

The separation conditions listed in Table 1 are modifications of those previously employed [3,4] for the analysis of basic drugs at low pH using a phosphate buffer. The validation crite-

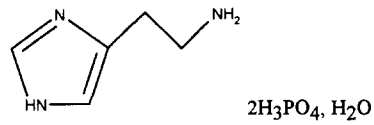
ria employed when assessing a CE method are similar to those used when evaluating the performance of HPLC methods [11,12].

3.1. Selectivity

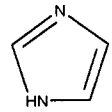
The conditions of the method were shown to be suitable for the analysis of a range of raw materials and basic drugs. The selectivity was demonstrated for a range of basic compounds including histamine acid phosphate, aspartame, cimetidine, salbutamol, sumatriptan, ranitidine, 3TC and GRD1.

Fig. 2 shows a typical test mixture separated under these conditions.

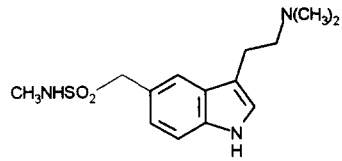
Histamine acid phosphate



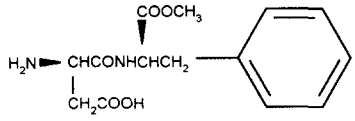
Imidazole



Sumatriptan



Aspartame



Salbutamol

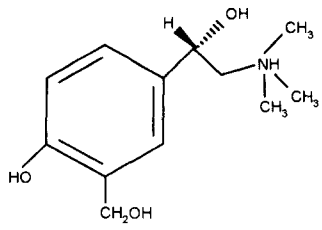
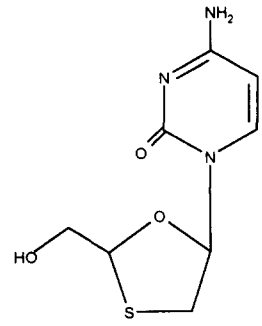


Fig. 1.

3TC



Cimetidine

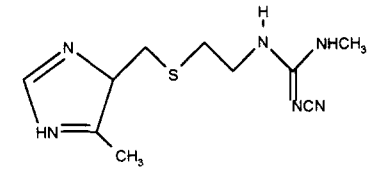


Fig. 1 (contd.)

Fig. 1. Structures of test compounds.

3.2. Precision

Imidazole or aminobenzoic acid was employed as an internal standard to improve peak area precision. Concentrations in the range of 50–100 ppm ($\mu g\ ml^{-1}$) were used for test solutions to generate relatively large peak areas, thereby minimising errors due to integration. The precision was assessed for several compounds on different days and different capillaries. Table 2 shows that precision values of 0.7–1.1% RSD could be obtained for peak area ratios. The relative migration time precision was better and ranged between 0.3 and 0.9% RSD. In CE, peak areas are related to

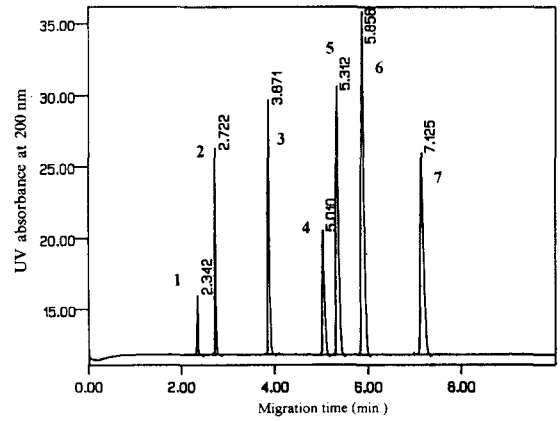


Fig. 2. Typical separation of a test mixture. Peak 1, HAP; peak 2, imidazole; peak 3, GRD1; peak 4, 3TC; peak 5, cimetidine; peak 6, salbutamol; peak 7, aspartame.

Table 2
RSD values for various parameters ($n = 10$) [a]

Compound	Peak area	Peak area ratio	Migration time (s)	Relative migration time (s)
Histamine acid phosphate	2.0 (2.2)	0.9 (1.2)	0.4	0.5
GRD1	1.2 (1.2)	1.1 (0.9)	0.3	0.3
3TC	1.0 (0.4)	1.1 (1.4)	0.7	0.4
Cimetidine	1.2 (0.5)	1.0 (1.3)	0.8	0.5
Salbutamol	1.4 (0.7)	1.1 (1.3)	0.9	0.6
Aspartame	1.7 (0.6)	0.7 (1.2)	1.2	0.9

[a] Peak area ratio compared to imidazole; samples and imidazole were each at 100 ppm, and dissolved in water. Results in brackets denote calculations using spatial (normalised) peak areas and ratios.

both sample concentration and migration time. Variations in migration time can be reflected as increased imprecision. Therefore, it is possible to calculate spatial (or normalised) areas by dividing the measured area of each peak by its corresponding migration time [13]. In some cases, use of spatial areas can produce improved precision (Table 2), especially for later migrating peaks.

The method was repeated on a new capillary, which gave similar precision and selectivity to the previously employed capillary. Table 3 shows precision data obtained for 3TC on the two capillaries.

Sample preparation repeatability was shown for 3TC drug substance. Five samples of a batch were prepared and each sample solution injected in duplicate. The assay results ($n = 10$) gave a mean of 101.8% w/w with an RSD of 1.1%.

In routine operations this method has given precision values of between 0.5–1.5% RSD for peak area ratio precision and for calibration response factors.

3.3. Accuracy

Results generated by the CE method were compared with those previously obtained by HPLC for testing the contents of two sets of tablets with a label claim of 100 and 150 mg of 3TC. The CE results in Table 4 were in accordance with the label claim and HPLC results. In addition, two samples of a previously assayed batch of histamine acid phosphate (HAP) were analysed. The assay results (Table 4) show excellent recovery.

3.4. Repeatability

During the 12 months of daily routine use of this method within our laboratory, the method has been satisfactorily repeated by several different analysts on several instruments using different capillaries.

3.5. Sensitivity

Histamine acid phosphate (HAP) has a low

Table 3
Precision data obtained for 3TC on the two capillaries

	Precision RSD (%) ($n = 10$)		
	Capillary 1 (10 s)	Capillary 1 (5 s)	Capillary 2 (5 s)
Imidazole migration time	0.42	1.88	0.79
Imidazole peak area	0.78	0.58	0.48
3TC migration time	0.41	0.72	0.71
3TC peak area	0.72	1.79	0.97
3TC RMT [a]	0.47	0.24	0.29
3TC PAR [b]	0.14	0.63	0.78

[a] RMT = relative migration time.
[b] PAR = peak area ratio.

Table 4
Assay results for CE for tablets and drug substance

3TC content (mg per tablet)	
Label claim	150
HPLC	152.2
CE	155.6
Label claim	100
CE	104.0
Histamine acid content (% w/w)	
CE Sample 1	100.3, 100.2
Sample 2	100.8, 100.7
Average	100.5

UV absorbance coefficient and therefore is not analysed by HPLC. The most frequent method of analysis is by preparation of a fluorescent derivative followed by fluorescence measurements. However, HAP has sufficient UV absorbance at 214 nm to allow adequate detection by CE.

Separations were performed over a range of operating wavelengths. The highest sensitivity was obtained at 200 nm for cimetidine, salbutamol, GRD1, imidazole and aspartame. Improved sensitivity was achieved at 214 nm for 3TC and HAP.

A limit of detection (LOD) of 0.4 ppm ($\mu g\ ml^{-1}$) was obtained for cimetidine, salbutamol, GRD1, imidazole, 3TC and aspartame at 200 nm. HAP gave a poorer LOD of 2.0 ppm. A limit of quantification (LOQ) of 1.0 ppm was obtained for all compounds (except histamine acid phosphate). Ten replicate injections at the LOQ level gave acceptable RSD values ranging from 6.6 to 11.2% for peak area precision.

Fig. 3 shows a typical separation at the detection limit of 0.4 ppm.

3.6. Linearity

Detector linearity for both aspartame and 3TC were assessed over the range 50–150 ppm (equivalent to 50–150% of target concentration). The linearities for both peak area ratios (relative to imidazole) were calculated and are given in Table 5. All correlation coefficients are greater than 0.995. It should be noted that the relatively high positive intercept values for peak area in Table 5 are due [14] to a 'spontaneous' injection of sample solution into the capillary immediately after it is inserted into the sample solution.

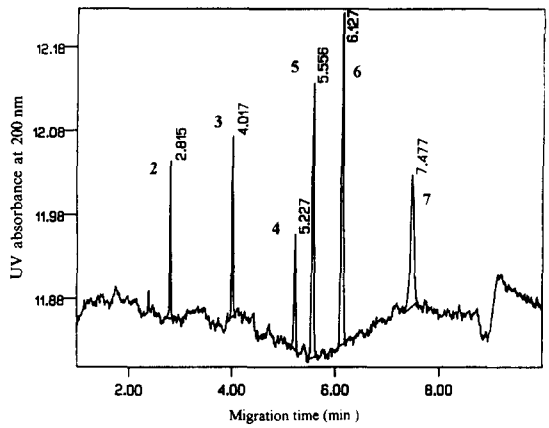


Fig. 3. Separation of a test mixture with components at the detection limit of 0.4 ppm.

3.7. Stability of solutions

An aqueous test mixture containing HAP, cimetidine, salbutamol, GRD1, aspartame, imidazole and 3TC was prepared. The test mixture was stored in a refrigerator for 8 days. The test mixture was then 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, indicating good solution stability.

A 3 month shelf-life for this electrolyte has been assigned when stored in high density polyethylenene plastic containers at RT, unprotected from light. Similar sensitivity and selectivity was obtained for separation of a test mixture using freshly prepared electrolyte or one stored for 3 months as described above.

3.8. Robustness

The beneficial use of experimental designs in the robustness testing of CE methods has been

Table 5
Linearity data

Response	3TC	Aspartame
Correlation (areas)	0.9963	0.9971
Correlation (area ratios)	0.9965	0.9957
Slope (areas)	16694	30702
Slope (area ratios)	0.0123	0.0228
PI [a] (areas)	4.4%	5.6%
PI (area ratios)	0.6%	0.9%

[a] PI denotes intercept value calculated as a percentage of the value of the area produced at the nominal sample concentration.

shown [15]. Fractional factorial designs have been utilised to screen simultaneously the impact of varying several operating parameters within a single sequence. The factors having a significant effect upon system performance have then been evaluated using an appropriate central composite design. A similar approach was adopted during robustness testing of this method. A fractional factorial design was used to investigate the impact of varying electrolyte concentration (25 ± 5 mM), electrolyte pH (pH 2.3 ± 0.2), rinse time with NaOH (1 ± 0.2 min), rinse time with electrolyte (1 ± 0.2 min), temperature (30 ± 5 °C), injection time (5 ± 1 s), and applied voltage (15 ± 2 kV). The system suitability criteria for this exercise were set at a minimum of baseline resolution within 10 min of a test mixture comprising HAP, cimetidine, salbutamol, GRD1, 3TC, imidazole and aspartame (each at 10 mg l^{-1}). The seven factors were evaluated in a 40 injection sequence, each of the 20 combinations of parameters being performed in duplicate. The exact method settings were repeated eight times to assess repeatability. In all instances, baseline resolution (greater than 1.5) of all peaks was obtained within the specified 10 min. Consistent selectivity was maintained in all injections. The resolution between 3TC and cimetidine was identified as the critical resolution, with a reduced resolution being obtained at higher injection times. It was concluded that the method was robust within the limits given above.

3.9. Benefits of the method

A single, inexpensive capillary can be used to replace a range of different HPLC columns. A volume of electrolyte can be prepared and stored which eliminates the need to prepare a variety of HPLC mobile phases for each analyte tested. The method validation covers use of the method for the basic compounds tested; additional validation assessments such as precision, sensitivity and linearity measurements would be conducted when the method is employed using a previously untested solute. Similar efficiency benefits are obtained in method transfer exercises where the method is initially formally transferred, and only limited validation aspects would need to be assessed when the method is applied to a previously unanalysed solute. Staff training requirements would be reduced as the need to be familiar with a variety of HPLC methods would be avoided.

Overall analysis time for a set of samples would be reduced compared to HPLC analysis, as the time taken to prepare the mobile phase, change HPLC columns and allow the HPLC system to equilibrate would all be avoided. Cost savings include the elimination of purchase and disposal of organic solvents and the requirement for a range of HPLC columns. Additional benefits may include the ability to assay simultaneously several components in a combination product, avoidance of the need to derivatise samples by employing low UV wavelengths and the ability to monitor possible cross-contamination of products.

4. Conclusions

A set of CE operating conditions has been demonstrated to be suitable for the identity confirmation and assay of a range of basic drugs and excipients. The use of an appropriate internal standard was shown to improve the precision of injection. Successful validation included selectivity, precision, accuracy, linearity, sensitivity and robustness. This method is now in routine use within our laboratories for both identity confirmation and assay purposes.

Acknowledgement

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References

[1] K.D. Altria and M. Kersey, LC-GC, 13 (1995) 40–46.
[2] K.D. Altria and M.M. Rogan, Chromatogr. Anal., April/May (1994) 3–8.
[3] G.L. Chee and T.S.M. Wan, J. Chromatogr., 612 (1993) 172–177.
[4] K.D. Altria, LG-GC. Int., 6 (1993) 164–172.
[5] M.T. Ackermans, J.L. Beckers, F.M. Everaerts, H. Hoogland and M.J.H. Tomassen, J. Chromatogr., 596 (1992) 101–109.
[6] B.R. Thomas, X.G. Fang, X. Chen, R.J. Tyrell and S. Ghodbane, J. Chromatogr., 657 (1994) 383–394.
[7] K.D. Altria and D.C.M. Luscombe, J. Pharm. Biomed. Anal., 11 (1993) 415–420.
[8] W. Beck, R. van Hoek and H. Engelhardt, Electrophoresis, 14 (1993) 540–546.
[9] E.M. Cohen and R.G. Bell, Pharm. Form., 20 (1994) 7870–7871.

[10] P. Coufal, K. Stulik, H.A. Claessens and C.A. Cramers, JHRCC, 17 (1994) 325–334.
[11] G.S. Clarke, J. Pharm. Biomed. Analy., 12 (1994) 643–652.
[12] E.C. Rickard and R.J. Bopp, J. Chromatogr., 680 (1994) 609–621.
[13] K.D. Altria, Chromatographia, 35 (1993) 177–182.
[14] P. Coufal, H.A. Claessens and C.A. Cramers, J. Liq. Chromatogr., 16 (1993) 3623–3652.
[15] K.D. Altria and S.D. Filbey, Chromatographia, 39 (1994) 306–310.