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Validation of a capillary electrophoresis method for the determination of potassium counter-ion levels in an acidic drug salt

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Abstract: This paper describes the first report of the validation of a capillary electrophoresis methed for the quantitative determination of potassium levels in the potassium salt of an acidic drug. Validation criteria include precision, linearity, robustness and repeatability. The use of an internal standard enabled precision values of <1% RSD to be obtained for peak area ratios. Careful control of capillary conditioning and temperature enabled migration time precisions of <0.5% RSD. Results obtained by this method were in agreement with those generated by ion exchange chromatography and the theoretical potassium content of samples tested. Features of the method, compared to alternative analytical techniques, include simplicity, speed and accuracy. The method is now in routine use within our laboratories.

Keywords: *Capillary electrophoresis; pharmaceutical analysis; potassium; validation.*

Introduction

Several papers have appeared on the use of capillary electrophoresis (CE) to determine levels of inorganic metal ions [1–4]. Reported methods are generally simple to operate, and have been shown to generate rapid and accurate results for solutes such as sodium, potassium, calcium and magnesium. Detection of these ions is usually achieved by indirect UV detection [1, 2, 4] where a suitable compound is added, at millimolar levels, to provide the background UV signal. Alternatively, the separation and quantitation of alkaline earth metal ions, which are separated as EDTA complex, direct UV absorbance may be employed [3]. Quantitative applications of these methods have included the determination of cationic nutrients in foods [1], assessment of cation levels in multiple electrolyte solutions for parenteral use [2], levels of cations in drinking water and plasma [3], and levels of cations in mineral water [4]. The results obtained by CE were successfully cross-correlated with those generated by AAS [1], ion chromatography [1, 4] and EDTA titration [3].

There are a variety of application areas of CE to the analysis of pharmaceuticals. A recent review [5] shows that almost 100 papers have been published on determinations such as drug related impurity content, main component assay and chiral analysis. Increasingly, a number of recent papers are concerned with the aspects of validation of specific CE methods [5–8]. Often these methods are in routine use to complement or replace existing chromatographic methods. The CE methods often having advantages such as speed, simplicity and improved robustness.

Many drugs are manufactured as salts to optimise their pharmaceutical properties. The majority of drugs are bases in their cationic form, and typical salts include chlorides and sulphates. Potassium and sodium are typical counter-ion for acidic drugs. Synthetic processes are controlled to give predetermined ratios of drug–counter-ions. These ratios are termed the stoichiometry and are mainly 1:1 mixtures of drug–counter-ion, although other ratios are possible and are frequently employed. An analytical technique such as ion chromatography (IC) or titrimetry is often used to confirm the stoichiometry of each batch of drug manufactured. This testing is also possible by CE [9], and assay results obtained compared well to the theoretical stoichiometry and those results generated by other tech-

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niques. Acceptable performance criteria were reported [9] for measurements of linearity, precision and sensitivity.

Precision of peak areas in CE is generally poorer than for HPLC, largely due to variability in injection volumes. Use of an appropriate internal standard can [10] reduce this variability making precision data more equivalent to HPLC. Precision is also considerably improved [2] when employing higher sample loadings as area counts are accordingly higher and integration errors are minimized.

This report describes the successful validation of a CE method for the determination of potassium content in the potassium salt of an acidic drug. Good precision data is achieved by use of an internal standard with a relatively high sample concentration. This method is now in routine operation within our laboratories.

Experimental

The electrolyte composition is similar to that reported [4] for the determination of metal ions. The earlier paper employed a 5 mM imidazole solution pH adjusted to 4.5 with sulphuric acid. These conditions were initially successfully repeated but problems were encountered reproducibly preparing the electrolyte, as the titration of the imidazole with sulphuric acid was very sensitive in the pH 3–5 range. To control more accurately the electrolyte preparation formic acid was employed for pH adjustment. A weighted amount of formic acid was diluted to give a 40 mM solution. Sufficient imidazole was then accurately weighed and transferred to a volumetric flask along with a volume of 40 mM formic acid and the solution made to volume with water to produce a 6 mM imidazole/4 mM formic acid solution.

The capillary bore was increased from 75 to 100 μm [4] to improve sensitivity giving higher area counts. The length of capillary was minimized to reduce analysis time. Instruments from two CE manufacturers, Beckman (Fullerton, CA, USA) and ABI (San Jose, CA, USA) were used in the validation studies. Table 1 gives the settings for the specific instruments. The minimum capillary lengths possible on the two instruments were employed (Table 1) which necessitated the significant changes in applied voltage given in Table 1.

Sodium chloride, dissolved in purified water, was employed as an internal standard solution at a sodium concentration of 100 mg l^{-1}. Standards were prepared by diluting weighed amounts of AnalaR grade potassium chloride with internal standard solution. Samples were prepared by diluting weighed amounts of drug substance with internal standard solution. Sample weights and dilution volumes were adjusted to give equivalent potassium concentrations (nominally 10 mg l^{-1}) to those of the standards.

Careful control of the condition of the capillary is essential to both the repeatability and reproducibility of CE separations. All capillaries were initially conditioned with a 20 min rinse with 0.1 M NaOH prior to their initial use. This is essential to rehydrate surface silanols [11] which ensures a consistent electroendosmotic between capillaries. The capillary is rinsed with fresh electrolyte between injections (Step 1, Table 1). This repeated rinsing can cause siphoning effects when it is sufficient to significantly alter the level in the vials containing the electrolyte [8]. The presence of hydrodynamic flow due to siphoning, during a separation, will lead to drifts in migration times of the peaks. To avoid this problem specific autosampler vials are nominated for rinse and separation steps. In this way, the levels in the two vials used in the separation remain constant.

The cleanliness of glassware (including auto-

Table 1
Settings for different instruments

Step	Beckman P/ACE 2100	ABI 270A
Rinse electrolyte	0.5 min	0.5 min
Injection	2 s	1 s
Detection wavelength	Indirect at 214 nm	Indirect at 21.4 nm
Voltage	+5 kV	+15 kV
Temperature	Ambient	Ambient
Capillary fused silica	100 μm × 27 cm (20 cm to the detector)	100 μm × 50 cm (30 cm to the detector)
Potassium concentration	0.1 mg ml^{-1}	0.1 mg ml^{-1}

sampler vials) was maintained by appropriate rinsing with internal standard solution or purified water.

Inorganic chemicals were purchased from Aldrich (Poole, Dorset, UK). Samples of drug substance were obtained from within Glaxo. Capillaries were purchased from Composite Metal Services (Hallow, Worcs, UK). Purified water was obtained from a Waters MilliQ system (Watford, Herts, UK).

The CE instruments were linked to a Hewlett–Packard HP1000 (Bracknell, Berks, UK) computer system. All results were calculated using integrated peak areas. The polarity of the detector output was reversed to give apparently positive peaks, making automated integration easier. The majority of the analysis was performed on the Beckman CE instruments, with the ABI employed to demonstrate reproducibility between instruments.

A solution of the internal standard was analysed in duplicate at the beginning of each analytical sequence to show absence of potassium and to allow the instrument to stabilize.

Solution preparations

Internal standard was typically prepared by weighing 100 mg of NaCl into a 1 l volumetric flask and diluting to volume with purified water. Calibration solution were typically prepared by weighing 10 mg of KCl into a 100-ml volumetric flask and diluting to volume with internal standard solution. Sample solution was typically prepared by weighing 9 mg of drug substance (theoretical potassium content 6.0% w/w) into a 10-ml volumetric flask and diluting to volume with internal standard solution.

Results and Discussion

Figure 1(a) shows a typical separation achieved on the Beckman instrument employing the conditions described in Table 1. The non-Gaussian appearance of peaks is due [4] to their higher electrophoretic mobilities compared to imidazole.

All criteria commonly employed [12] during validation of either CE or HPLC methods were assessed. These criteria were precision of peak area and migration time, linearity, sensitivity, accuracy, ruggedness, robustness and repeatability.

Precision

Acceptable precision was obtained routinely using the specific conditions with typical values of 0.5–2% RSD for calibration response factors and <0.5% RSD for migration times. Table 2 shows precision values for peak areas repeated injections of standard solutions, with and without internal standard. It can be seen that peak area ratio (PAR) data is considerably better than for the potassium peak alone. The precision is also improved with higher sample concentration and injection time. The controlled nature of the method gave excellent consistency of migration times (Table 2).

Linearity

Eight individual calibrations (covering the range 5–150% of target concentration) were weighed and diluted with internal standard. Each solution was analysed in duplicate. Table 3 shows the correlation coefficients and intercept values obtained for the data reported for both peak area ratios, and for potassium alone. The linearity data for the peak area ratios is evidently better, demonstrating the improvement obtained using an appropriate internal standard. The intercept value for PAR data of 1.5% of the PAR value obtained for the 100 mg l^{-1} standard is considered acceptable as the figure of 2% is widely acknowledged as an acceptable deviation.

Accuracy

Results obtained by this method were in agreement [9] with those generated by an ion-exchange chromatography method. Results were also in close agreement with the theoretical potassium content of the tested samples.

Sensitivity

A limit of detection of 0.5 mg l^{-1} for potassium was obtained with a signal/noise ratio of <3. Figure 1(b) shows the limit of quantitation (LOQ) of 1.25 mg l^{-1}. Ten replicate injections at this LOQ value gave an acceptable 3.8% RSD value for peak area ratios. As in other separative techniques, poorer precision is obtained [13, 14] at lower sample concentrations. Sensitivity was considered acceptable since the potassium contents of both sample and standard solution is 100 mg l^{-1}.

Robustness/ruggedness

Few reports [15, 16] have considered the

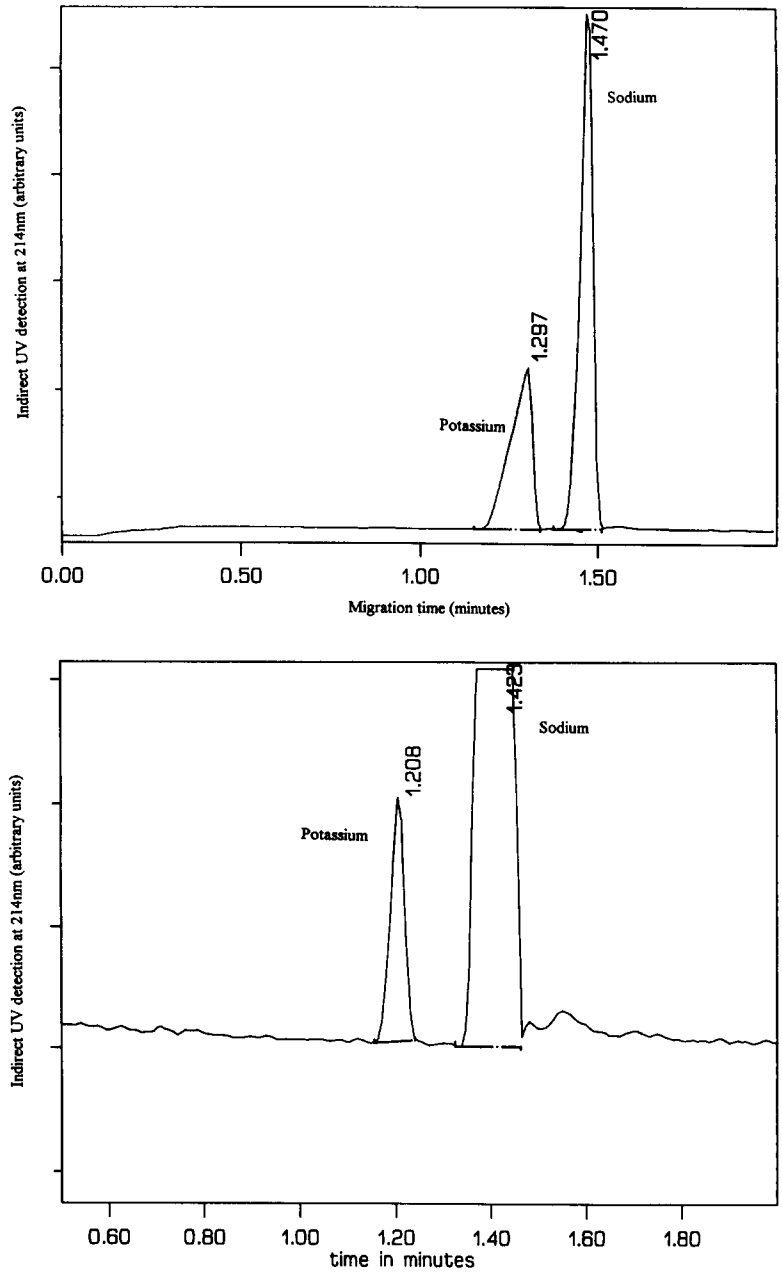

Figure 1
(a) Typical separation of a potassium standard (100 mg l^{-1}) in internal standard solution. (b) Limit of quantitation of 1.25 mg l^{-1} for potassium in internal standard solution. Separation conditions — 100 μm fused silica capillary, 6 mM imidazole in 4 mM formic acid, indirect UV detection at 214 nm (detector signal reversed), +5 kV, sodium concentration 100 mg l^{-1}, 2 s injection.

Table 2
Precision data obtained for different sample concentrations and injection times

Sample	Injection time (s)	No. of injections	Time range (min)	RSD (%) K	RSD (%) PAR
Potassium (100 mg l^{-1})	1	9	1.45–1.42	9.8	—
Potassium and sodium both at 100 mg l^{-1}	1	9	1.31–1.30	3.6	1.1
Potassium and sodium both at 100 mg l^{-1}	2	9	1.30–1.29	1.1	0.3
Potassium and sodium both at 100 mg l^{-1}	2	45	1.28–1.25	2.8	0.24
Potassium and sodium both at 50 mg l^{-1}	2	9	1.24–1.22	2.6	0.36

Note: PAR denotes peak area ratios.

Table 3
Linearity of detector response vs sample concentration (eight calibration points)

	Value
Potassium concentration	5–150% of sample loading (5–150 mg l^{-1})
Correlation coefficient using peak areas	0.9974
Intercept value using peak areas	3.7% of target concentation peak area
Correlation coefficient using peak area ratios	0.9998
Intercept value using peak area ratios	1.5% of target concentation peak area

Table 4
Repeatability of sample preparation. Results of % w/w potassium (calculated using peak area ratios)

Sample number	Injection 1	Injection 2	Average
1	5.69	5.71	5.70
2	5.74	5.73	5.74
3	5.80	5.78	5.79
4	5.73	5.73	5.73
5	5.72	5.72	5.72
6	5.66	5.67	5.67
7	5.74	5.75	5.75
8	5.71	5.79	5.75
9	5.70	5.72	5.71
10	5.77	5.76	5.77
Mean			5.73 (0.65% RSD)

repeatability of CE separations. A further report has shown the successful transfer of a CE method between seven independent pharmaceutical companies [17]. In this study, the separation conditions were shown to give reproducible performance on different capillaries, instruments, between analysts, and between both laboratories and sites. For example, 10 replicate injections on an ABI instrument gave an RSD of 0.5% for peak area ratios and 1.3% for potassium peak areas. All precision data obtained in the various repeatability studies gave acceptable RSD values of <2% for peak area ratio precision. The separation was shown to be repeatable on several different capillaries, this factor is similar to evaluating the repeatability of separation on different columns which would be assessed during robustness testing of HPLC methods.

A preliminary evaluation of the effect of variations in electrolyte composition upon the separation was performed. Levels of both formic acid and imidazole concentrations above and below the method settings were evaluated. Electrolytes containing both imidazole and formic acid at concentrations of 5:3, 7:3, 6:4, 5:5, 7:5 mM, respectively, were tested. A standard solution was analysed, in duplicate, using each of the electrolyte compositions. Separation selectivity and baseline resolution of potassium from sodium was maintained in each analysis showing that slight variation in the preparation of electrolyte to have no undue impact on performance. In all cases the migration times of the two peaks were maintained within the 2 min separation time of the method. A fuller, more extensive evaluation of the robustness of this method, employing appropriate designs has been performed [18].

Repeatability

The repeatability of response was measured for both sample and standard preparation, and the use of different electrolyte vials during an injection sequence.

Table 4 shows data which confirms the acceptable repeatability of sample preparation. Ten individual sample weighings were taken and each was analysed in duplicate. The average result confirmed previous results indicating that this batch contained a lower level of potassium than the theoretical level of 6.0% w/w. Identical separations in terms of resolution and migration times were obtained for all injections. In a similar fashion, 10 individual calibration solutions were prepared and analysed in duplicate. The calculated response factors for the calibrations produced a precision of 0.8% RSD for peak area ratios

whilst a poor precision of 5.7% RSD was obtained when calculated using the potassium peak areas only, which further endorses the importance of employing an internal standard in this method.

A single standard solution was analysed using five different sets of electrolyte vials. Nine analyses were performed using each set of electrolyte vials giving a total of 45 injections. Acceptable performance was obtained (Table 2) for precision of both migration time and peak area ratios.

Features of the method

The method enables simple, rugged, quick and accurate analysis compared to other techniques. Electrolyte preparation is simple and inexpensive. The method is rugged as the capillary does not require regeneration which may be required in IC. Compared to titrimetry the CE method is both quicker and produces auditable raw data. The method gives similar analysis times to IC, however, CE has advantages in terms of reduced set-up time and simplicity. The good agreement reported [9] between the CE results and those obtained by the other techniques confirms accuracy.

Conclusions

A CE method has been validated for the determination of potassium content in the potassium salt of an acidic drug. An internal standard was employed to ensure acceptable precision data. Good method performance was obtained for precision, linearity, sensitivity, ruggedness, robustness and repeatability.

This method is now in routine use within our laboratories. It is anticipated that similar methods will become an established widespread means of establishing drug stoichiometry.

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