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Validation of a capillary electrophoresis method for the determination of a quinolone antibiotic and its related impurities

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ABSTRACT

Free solution capillary electrophoresis (FSCE) has been employed for the novel determination of a quinolone antibiotic which has limited solubility between pH values of 2 and 11. This limited pH range gave problems with HPLC methods that were attempted for quantitative analysis. The fused-silica capillaries utilised in CE are able to withstand pH extremes, therefore CE was used in preference to HPLC for determining both drug content and levels of related impurities present in drug substance.

A CE method operating at pH 1.5 was shown to be suitable for this analysis. The sample was prepared at 0.5 mg/ml in 0.1 *M* NaOH and injected utilising pH mediated sample stacking. This represents the first report describing the analytical performance of this stacking procedure.

Although several reports have shown CE to be suitable for pharmaceutical analysis this report is the first to provide validation details for an impurity determination method. Acceptable levels of precision, linearity, limits of detection and quantitation were achieved. Capillary electrophoresis of basic drug compounds at low pH offers a useful alternative and complement to HPLC.

INTRODUCTION

Renewed interest in electrophoresis was generated by the work of Jorgenson and Lukacs [1] in the early 1980's concerning capillary electrophoresis. Subsequently CE has been shown to be of use for the separation of a range of drug classes including antibiotics [2–5], non-steroidal anti-inflammatories [6], steroids [7] and analgesics [8]. Specific applications have included the determination drug content in formulations [9], clinical analysis [10], determination of related impurities content [11,12], and chiral separations [13,14]. Performance details have been described for the quantitative determination of the drug content in pharmaceutical formulations [9].

When analysing basic compounds by HPLC problems can occur [15] regarding peak tailing and the limited pH operating range of many columns. CE is well suited to this analysis as methods can be operated at pH extremes.

New drug classes are currently being developed to supplement the "traditional" antibiotics as many organisms have now become resistant. Such a group are the quinolones [16] of which Ciprofloxacin [17] is the most active. The structure of Ciprofloxacin is given in Fig. 1. To date CE has not been applied to this class of compounds.

A quinolone antibiotic with limited solubility between pH 2–11 presented considerable problems when analysed by HPLC. It may have been possible, after extensive method development, to have developed a suitable HPLC method. However, in this instance, suitable CE operating conditions were quickly developed and then validated for the separation and quantitation of this compound and its related impurities. Method validation including measures of precision,

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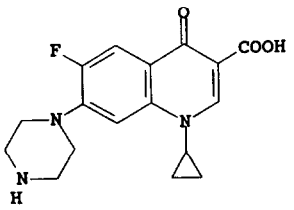

Fig. 1. Structure of Ciprofloxacin.

linearity, the determination of limits for both quantitation and detection. The results obtained are comparable to those expected from a standard HPLC method. Reliable HPLC data could not be obtained for comparison with that generated by CE.

EXPERIMENTAL

Chemicals were obtained from Aldrich (Poole, UK), and water was obtained from a Millipore Milli-Q system (Watford, UK).

The work was performed on an ABI 270HT CE instrument (San Jose, CA, USA) which was connected to a Hewlett-Packard (Bracknell, UK) data collection system. The fused-silica capillaries used in this study were purchased from Metal Composites (Hallow, UK).

The separation conditions employed are as follows: Pre-separation rinse 1:1 min with 0.1 *M* NaOH; pre-separation rinse 2:1 min with electrolyte; sampling: 10 s vacuum; separation: +20 kV applied for 20 min, UV at 272 nm, 30°C, 72 cm × 50 μm fused-silica capillary; electrolyte: 50 m*M* borax pH adjusted to 1.5 with concentrated H_3PO_4; sample concentration: (0.5 mg/ml) dissolved in 0.1 *M* NaOH.

RESULTS AND DISCUSSION

Method development

The principal method development options in CE [12,18] are variations in pH and the use of micellar based electrolytes. In this instance, given the pH solubility range of the solute, the choice was limited and the electrolyte ultimately chosen was 50 m*M* borax pH adjusted to 1.5 with concentrated orthophosphoric acid. The compound was soluble in this electrolyte. A typical separation achieved is given in Fig. 2. Minimum peak tailing was observed.

Low UV detection wavelengths (sub 220 nm) are often employed in CE [11,12] to compensate for the relative insensitivity of CE when directly compared to HPLC. However, in this example, the strong UV absorbance coefficient of the test solute at 272 nm enabled adequate response to be obtained at this wavelength.

Sample dissolving solvent. The choice of dissolving solvent in CE is critical as an incorrect choice can result in a severe loss of separation efficiency and resolution. Sample solutions containing either high ionic strength [19] or containing high percentage levels of organic solvents [20] cause particular problems. Given these limitations and the solubility problems encountered with this compound, the dissolving solvent options were either an acid or base. The latter option was selected for the reasons given below.

The ionic strength of the dissolving solvent is usually chosen to be lower than the electrolyte employed to achieve an on-column preconcentration [21] of sample within the capillary. This process has been termed "stacking" [21] and can improve method performance in terms of separation efficiency, resolution and sensitivity.

An alternative stacking procedure has been

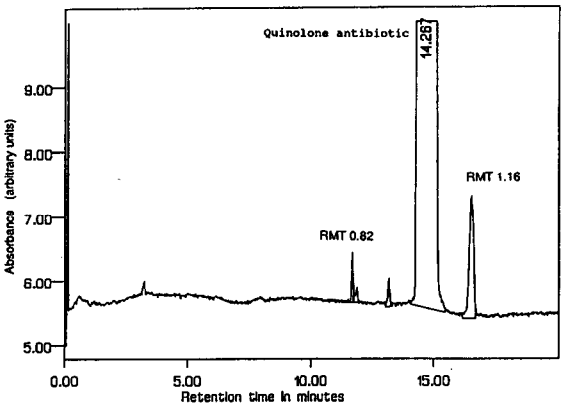

Fig. 2. Typical electropherogram. Separation conditions: 10 s vacuum sampling, +20 kV applied for 20 min, UV at 272 nm, 30°C, 72 cm × 50 μm fused-silica capillary, electrolyte: 50 m*M* borax pH adjusted to 1.5 with concentrated H_3PO_4, sample concentration: (0.5 mg/ml) dissolved in 0.1 *M* NaOH.

described which has been termed "pH-mediated sample introduction" [22]. In this procedure the pH of the dissolving solvent is selected such that the sample is present as an anion. A separation electrolyte is chosen in which the compound would be protonated. When a sample is introduced into the capillary and the separation voltage is applied the sample anions migrate to the back of the sample zone, become protonated, and are focussed at the boundary of the sample and electrolyte. The analytical performance of this procedure has not previously been studied.

It was appropriate in this instance to utilise pH-mediated stacking by employing a low-pH electrolyte for the separation, and by dissolving the sample in an alkaline solution.

METHOD VALIDATION

The validation undertaken for the CE method follows the general guidelines suggested for a HPLC method [23].

Linearity

It is usual practice to perform linearity determinations over a wide range of sample concentrations to fully assess the linear dynamic range of the detection system. For main peak assay purposes, linearity of the CE method was demonstrated (correlation coefficient = 0.9990) over the range 1–150% of the nominal target concentration (0.5 mg/ml). In a separate exercise the linearity of a narrower concentration range (20–150% of nominal) was assessed and a correlation coefficient of 0.9997 was obtained. Intercept values were less than 1% of the nominal concentration.

Precision of peak area and migration time

A single sample solution was injected 10 times and an acceptable R.S.D. of 0.6% was obtained for the main peak. The R.S.D. on the migration time of the main peak was 0.4%.

Reports [12,24,25] concerning the precision of peak area employing automated CE instruments indicate a precision level of 0.5–2% R.S.D. can be achieved. By employing an internal standard, variability can be reduced still further with typical R.S.D.s of below 1% being obtained [26].

Limit of quantitation (LOQ)

This figure can be defined [27] as the lowest concentration of sample that can be reproducibly quantified above the baseline signal. An R.S.D. of 4.9% was obtained for 10 injections of a solution equivalent to 0.3% of the nominal concentration. This is considered acceptable performance.

Limit of detection (LOD)

This figure may be defined as the lowest concentration of sample that can be clearly detected above the baseline signal. A solution equivalent to 0.1% of the nominal concentration gave a reproducible peak (Fig. 3) with a signal-to-noise ratio greater than 3. Swartz has reported [12] similar detection limits for salicylamide-related impurities by CE. This LOD represents a molar sensitivity of $1.6 \cdot 10^{-6}$ M which is comparable to that reported for other pharmaceuticals as determined by CE [5,9].

Levels as low as 0.02% (w/w) of selected dimeric impurities present in salbutamol drug substance have been reported using low UV wavelength detection and external standards of the impurities [11].

Consistency of impurity levels with sample concentration

A further part of method validation is to demonstrate that the impurity profile and con-

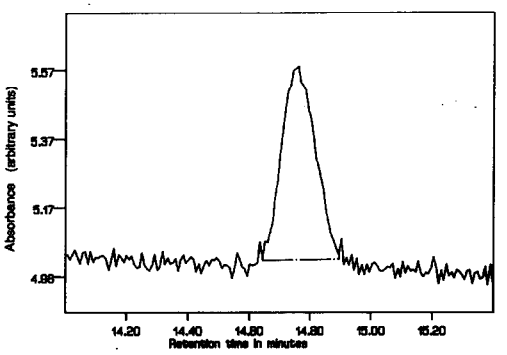

Fig. 3. Electropherogram of a solution equivalent to 0.1% (w/w) of the nominal sample concentration. Separation conditions: as given in Fig. 2.

TABLE I

CONSISTENCY OF IMPURITY PROFILE WITH SAMPLE DILUTION

	Concentration (% nominal)		
	100	75	50
Number of impurities	6	4	4
Total % impurities[a]	0.68	0.64	0.64
Greatest impurity (%)	0.45	0.47	0.46
RMT greatest impurity[b]	1.16	1.16	1.16
2nd greatest impurity (%)	0.11	0.10	0.11
RMT 2nd greatest impurity	0.82	0.82	0.82

[a] % = % total corrected area.
[b] RMT = Relative migration time.

tent do not vary with sample concentration. A single sample was diluted and aliquots of each solution were injected in duplicate and the impurity levels were determined (Table I). The peak area of each peak was divided by its migration time to compensate for the difference residence times of the peaks in the detector [28]. The corrected areas were used to calculate the impurity levels as %area/area. The need to normalise peak areas when quoted impurity levels has been reported [29]. Failure to normalise peak areas will result in incorrect %area/area data being reported [29].

The results (Table I) indicate that the impurity levels are consistent with dilution of the sample down to 50% of the nominal sample concentration. At the higher sample concentration two additional impurities, present at low levels (<0.05% area/area), could be detected.

Freedom from interference

A solution of the dissolving solvent (0.1 *M* NaOH) was injected onto the system, in duplicate, and no interfering peaks were observed.

CONCLUSIONS

A low-pH CE method has been validated for the determination of drug related impurities in a quinolone antibiotic drug substance. Analysis of this compound had presented a formidable challenge to HPLC due to the limited pH solubility range of the analyte. Therefore, in this instance, CE was used preferentially over HPLC due to the wide range of pH extremes that can be employed in CE.

To optimise method performance the sample dissolving solvent was selected to utilise pH mediated stacking of the sample. The performance of the method employing this sampling procedure indicates that this is a successful approach.

Method validation showed good levels of performance in terms of precision, linearity, LOD and LOQ. No interfering peaks were obtained from the dissolving solvent.

Capillary electrophoresis of basic drug compounds at low pH offers a useful alternative and complement to HPLC and has been demonstrated to give similar levels of method performance.

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