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Determination of salbutamol-related impurities by capillary electrophoresis

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ABSTRACT

This paper describes the use and capabilities of capillary electrophoresis (CE) in the determination of two dimeric impurities present in salbutamol sulphate drug substance. Acceptable measures of detector linearity of response over the typical impurity range, detection limits, precision of peak area and migration times were obtained. The results obtained by CE were directly compared to those obtained by HPLC and TLC. The data shows agreement between the three techniques.

INTRODUCTION

Capillary electrophoresis (CE) has previously been used for the determination of drug-related impurities [1–5]. In addition there have also been a number of reports [5,6–9] of the separation of pharmaceuticals by the associated technique of micellar electrokinetic capillary chromatography (MECC) . Elegant separations have been published showing the resolution of relatively low-concentration test mixtures of specific drug related compounds. However, there has been little emphasis on showing that these methods are capable of use in working analytical environments.

Salbutamol sulphate is a bronchodilator widely used for the treatment of asthma, which is sold under the Glaxo tradename of Ventolin. There are a range of well characterised potential, and actual, synthetic and degradative impurities possible [10]. HPLC methods have been developed and reported for the determination of these impurities [10,11]. There are several dimeric impurities, largely arising from degradation. These impurities are late eluters (>30 min) using the HPLC methods employed which makes quantitation of trace levels difficult. Two of these late eluting dimeric compounds of particular interest are the "bis ether" impurity and "side-by-side" impurity. The structures of these impurities and salbutamol itself are given in Fig. 1.

This paper describes the preliminary validation experiments, and application of a free zone CE method, for the determination of selected salbutamol-related impurities present at low levels in drug substance.

EXPERIMENTAL

Sodium citrate (20 m*M*, pH 2.5) was obtained from Applied Biosystems (San Jose, CA, USA). Water was obtained from a Milli-Q system (Millipore, Watford, UK). A P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, USA) which was connected to a Hewlett-Packard (Bracknell, UK) data collection system was used for CE analysis. The fused-silica capillaries used in this study were purchased from Beckman. Samples were obtained from within Glaxo.

Drug substance sample solutions were prepared by accurately weighing 12 ± 1.2 mg into 10 ml of distilled water. Bis ether standards were prepared by diluting accurately weighed amounts of bis ether standard to 10.0 ml of distilled

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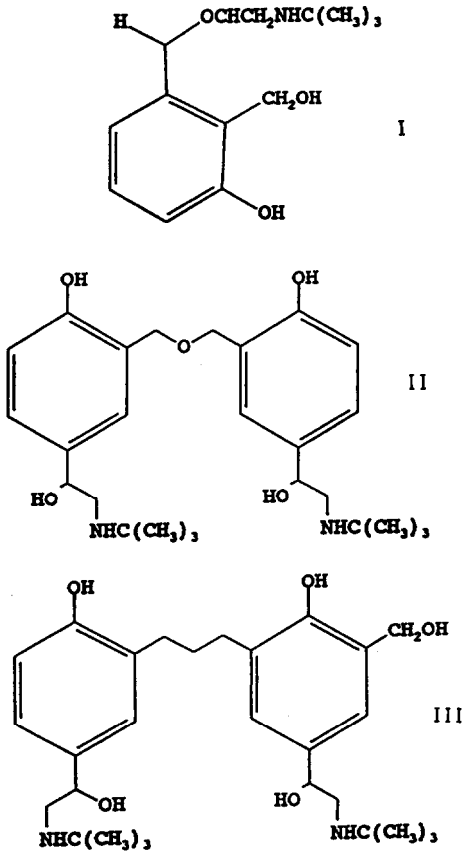

Fig. 1. Structures of salbutamol and dimeric salbutamol impurities. I = Salbutamol; II = bis ether; III = side-by-side.

water. Both samples and standards were prepared in duplicate.

The separation conditions are described in Table I, the method consisting of five automated steps.

TABLE I

CAPILLARY ELECTROPHORESIS SEPARATION METHOD

Step No.	Conditions
I	Rinse cycle 1: 0.5 *M* NaOH, 2 min
II	Rinse cycle 2: run buffer, 4 min
I I I	Set detector 0.02 AUFS
IV	5.0-s hydrodynamic sampling
V	Operating voltage: +30 kV
	Operating temperature: 25°C
	Capillary dimensions: 57 cm x 7.5 μm fused silica
	Run time: 10 min
	Wavelength: 200 nm

RESULTS AND DISCUSSION

Optimization of the CE separation procedure

The principal CE variable is carrier electrolyte pH as this affects both solute mobility and electroendosmotic flow (EOF) velocity [12]. An electrolyte pH range of 2.5 to 11.0 was evaluated and a low pH (pH 2.5) electrolyte was selected which gave adequate separation with a run-time of 10 min.

To minimise the analysis time the maximum appliable voltage of +30 kV was used to perform the separation. This presented no problem in terms of joule heating whilst employing the relatively low electrolyte concentration (20 m*M*).

The limits of detection are generally poorer in CE compared to those obtained in I-IPLC. However, it is possible with CE to employ detection wavelengths as low as 190 nm where many solutes have an enhanced absorptivity. For example salbutamol has an eight-fold increase in signal when monitoring at 200 nm compared to the HPLC wavelength of 276 nm.

Viscosity influences the amount of solute introduced in both electrokinetic and hydrodynamic sampling [13,14]. Therefore, both samples and calibrations solutions were prepared in water to match the viscosities of the solutions. Injection of aqueous samples and calibrations also gives rise to a focussing, or pre-separation concentration [15] of sample ions in the initial portion of the separation capillary.

The use of rinse cycles is strongly advocated by CE instrument manufacturers as these cycles have been shown [16] to improve peak area and migration time reproducibility. Standard cycles used are an alkaline or acid wash followed by a pre-separation rinse with the carrier electrolyte.

System performance assessment

Selectivity. Test mixtures containing known amounts of authentic working standards for both salbutamol and the related impurities were prepared and analysed under the conditions given in

Table I. Good resolution between the bis ether impurity and side-by-side impurity from each other and salbutamol was obtained within a run-time of 10 min. Fig. 2 shows the migration order and relative impurity levels in a typical salbutamol sample. The order of elution being side-by-side, bis ether and lastly salbutamol.

This migration order can be explained in terms of the charges and sizes of the individual solutes. Electrophoretic mobility is related to the ratio of the charge and hydrated ionic radius (HIR) of an ion [17]. In this separation the dimeric species have twice the charge of the salbutamol ion but less than double its HIR. This gives them a higher charge/HIR ratio and they therefore elute before the salbutamol. The spatial orientation of the side-by-side in solution may be such that its HIR is smaller than bis ether which may explain the separation observed between these dimeric species.

It is anticipated that variation in the method parameters such as capillary type and coatings, addition of organic modifier, additives, temperature increase, and the electrolyte nature and concentration may have a beneficial effect upon the separation. Nielen [18] has reported the effect of varying such parameters upon the separation of aminobenzoic acid positional isomers. However, the separation conditions given above gave a robust, selective, relatively uncomplicated analytical method with adequate resolution, within an acceptable analysis time.

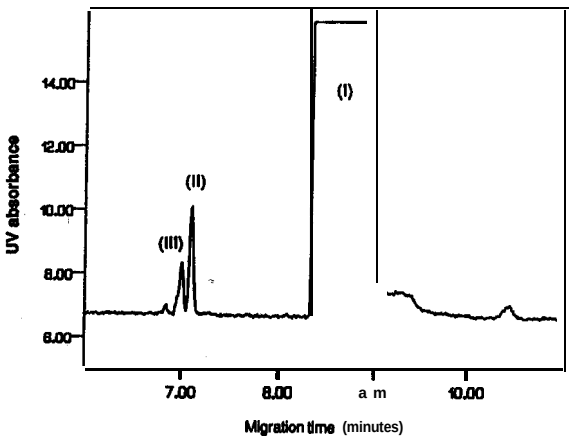

Fig. 2. Typical electropherogram of a salbutamol sample. Separation conditions: 20 m*M* sodium citrate pH 2.5, +30 kV, 25°C, 57 cm × 75 μm fused silica (50 cm to detector), 200 nm, sample concentration 1 mg/ml in water, injection time 5 s. Peaks: I = salbutamol; II = bis ether; III = side-by-side.

Sensitivity. A limit of detector (LOD) of 0.02% (w/w) of the salbutamol loading (1 mg/ml) was obtained with a signal-to-noise ratio of greater than 3. This is equivalent to an LOD of 200 ng /ml in solution. This figure is in line with those reported previously [19].

Precision. To measure the performance of the system in terms of reproducibility a variety of tests were performed. Initially a single sample solution was analysed sequentially six times. The acceptable data obtained for the various measurements of operating performance are given in Table II.

External standardisation was also used to quantify bis ether levels. This method consists of preparing bis ether calibration solutions and obtaining appropriate response factors. To assess the repeatability of this method, four individual calibration solutions were prepared, and injected in duplicate, obtaining a mean overall R.S.D. of 4.0% for response factors. One of these calibration solutions was then injected seven times and a R.S.D. figure of 2.45% was obtained for response factor. This performance was considered acceptable given the low levels being quantified.

Separation efficiencies. Separation efficiencies as measured by theoretical plate count values can be exceptional in CE. However, there is a marked reduction in peak efficiency with increased sample concentration. In this specific example, salbutamol at a concentration of 1 mg/ml gave an average plate count of 1600 whilst the bis ether at ***ca.*** 0.3% (w/w) of the salbutamol loading (equivalent to 0.3 μg/ml) gave an average plate count of 163 284.

This reduction of separation efficiency with sample loading is largely due to increased distortion of the conductivity profile along the capillary with increased sample loading [20].

Migration times. Table II shows relative migration times to have good reproducibility. The larger variation for the impurity peak times relative to salbutamol can be explained by the poorer peak shape of the salbutamol peak giving increased variability in measuring the peak apex.

TABLE II

ANALYSIS OF SIX REPLICATE INJECTIONS OF A SALBUTAMOL DRUG SUBSTANCE SAMPLE

	Peak area data		
	Salbutamol	Dimer	Bis ether
Maxima	583658	3056	2347
Minima	571094	2889	2238
Mean	578520	2967	2293
R.S.D. (%)	0.76	2.2	2.0
	%area/area with respect to salbutamol		
	Dimer	Bis ether	
Maxima	0.525	0.406	
Minima	0.502	0.387	
R.S.D. (%)	1.6	1.8	
	Relative retention time data		
	Dimer wrt salbutamol[a]	Bis ether wrt salbutamol[a]	Bis ether wrt dimer[a]
Maxima	0.890	0.903	0.987
Minima	0.884	0.898	0.985
Mean	0.888	0.901	0.986
R.S.D. (%)	0.30	0.32	0.07
	Theoretical plates count data		
	Salbutamol	Dimer	Bis ether
Maxima	1830	172530	195061
Minima	1319	148214	173506
Mean	1594	163284	185350
R.S.D. (%)	12.1	5.2	4.5

[a] wrt = with reference to.

This variability can be reduced by calculating the effective mobility [21] of the solute peaks.

Quantitation. There have been several reports [22–26] concerning the reproducibility of peak areas on automated instruments. Instrument manufacturers typically quote that R.S.D.s of less than 2% can be routinely obtained. Use of rinsing routines can assist in reducing levels of error to a level comparable to HPLC. By employing an internal standard, variability can be reduced still further with typical R.S.D.s of below 1% being obtained [26].

The %area/area data for both the bis ether and side-by-side (Table II) indicates good reproducibility with R.S.D.s below 2%. The results obtained for the salbutamol peak area are comparable to HPLC system performance. The R.S.D.s obtained for the impurity peaks acceptable given the variations obtained in measuring such small peaks.

It is recognised [27] that in CE, peak areas are directly proportional to both the sample concentration and migration time. The latter is related to the residence time that the peak spends in the detection window *i.e.* for a sample injection containing two solutes with identical UV response and concentration the slower moving peak will give a larger peak area. The extent of the peak area increase is directly related to the ratio of the two migration times.

Therefore it is necessary to normalise peak areas to their migration times to quote %area/area ratios. This normalisation simply consists of dividing the peak area obtained by the migration time of the peak [28].

The bis ether content was calculated for a particular batch of salbutamol drug substance using both the external standard calibration and standard addition approaches. The result obtained from the standard addition method (0.34%, w/w) was in good agreement with that calculated by external calibration (0.32%, w/w).

Linearity. Standard solutions of bis ether equivalent to between 0.05 and 1.4% (w/w) of a 1 mg/ml salbutamol sample were prepared and analysed in duplicate. A linear detector response (peak area) with bis ether content was obtained with a correlation coefficient of 0.999 and intercept of less than 1% of typical values, the gradient of the line was 7972. Typical levels of these impurities in samples are in the region of 0.1 to 0.5 (as determined by TLC).

In addition a 1 mg/ml salbutamol sample was spiked with known amounts of bis ether [between 0.1 and 1.4% (w/w) of the salbutamol loading] using a standard addition type method. A linear increase in detector response (peak area), with'bis ether content, was obtained using this approach with a correlation coefficient of 0.993. The slope of this line was 7581. An intercept value of 3120 was obtained as the batch

of salbutamol used contained a residual amount of dimeric impurities.

QUANTITATIVE ANALYSIS

Comparison of CE with HPLC and TLC

Bis ether levels were quantified (in duplicate) in a range of batches by both HPLC and CE employing external standards of the bis ether compound. The results obtained (Table III) agree well. A paired t-test at a 95% confidence interval indicates than no significant difference exists between the CE and HPLC bis ether results.

Side-by-side levels were quantified by CE employing response factors from the bis ether standards. Side-by-side levels were generated by HPLC using side-by-side external standards.

TABLE III

COMPARISON OF BIS ETHER AND SIDE-BY-SIDE LEVELS IN EXPERIMENTAL SALBUTAMOL SULPHATE DRUG SUBSTANCE BATCHES AS DETERMINED BY CE, HPLC AND TLC

Batch	Bis ether (%, w/w)			Dimer (%, w/w)		
	CE	HPLC	TLC	CE	HPLC	TLC
1	0.14	0.16	0.18	0.08	0.08	0.09
	0.14	0.16		0.08	0.08	
2	0.10	0.11	0.19	0.06	0.07	0.12
	0.10	0.11		0.07	0.06	
3	0.20	0.19	0.24	0.13	0.11	0.16
	0.20	0.19		0.14	0.10	
4	0.12	0.13	0.20	0.07	0.06	0.13
	0.15	0.14		0.08	0.05	
5	0.13	0.14	0.12	0.08	0.06	0.08
	0.12	0.13		0.07	0.05	
6	0.31	0.28	0.32	0.18	0.17	0.23
	0.31	0.26		0.19	0.15	
7	0.07	0.09	0.12	0.05	0.04	0.06
	0.08	0.10		0.06	0.03	
8	0.38	0.38	0.45	0.20	0.18	0.28
	0.44	0.38		0.22	0.19	
9	0.37	0.38	0.36	0.19	0.17	0.30
	0.37	0.35		0.19	0.17	
10	0.75	0.66	0.69	0.39	0.33	0.45
	0.77	0.67		0.40	0.35	

Analysis of residuals indicates that the CE side-by-side data is typically 0.02 higher than that obtained by HPLC. This is explained by the different calibration procedures employed and the level of discrepancy does not unduly impact on the experimental results given the relatively low levels of impurities being determined.

TLC results are the mean of four individual analyses. TLC impurity levels were compared against salbutamol standards as detected at 254 nm. This procedure may explain significant differences between the results obtained by TLC and those generated by CE and HPLC. However the TLC results do serve to confirm the ranking of the batches in terms of relative impurity levels within this sample set.

CONCLUSIONS

This report demonstrates the use of a CE based method for the determination of drug related impurities in a working analytical environment. Acceptable levels of precision in terms of both migration time and peak area were obtained. The limit of detection for the related impurities was found to be 0.02% of the salbutamol loading (equivalent to 200 ng/ml of the impurity in solution) which is at least comparable to that achieved by HPLC. Linearity of the method was demonstrated by the use of both external standardisation and a standard addition method. Good cross-correlation for related impurity levels was obtained between HPLC, TLC and CE. It is strongly suggested that the complementary nature of CE based methods to HPLC will increase their application in pharmaceutical analysis for the quantitative determination of drug related impurities.

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