Journal of Chromatography, 633 (1993) 221–225
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 794

Peak homogeneity determination and micro-preparative fraction collection by capillary electrophoresis for pharmaceutical analysis

K. D. Altria* and Y. K. Dave

Pharmaceutical Analysis, Glaxo Group Research, Park Road, Ware, Herts. (UK)

(First received April 7th, 1992; revised manuscript received October 28th, 1992)

ABSTRACT

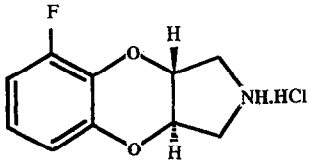
This paper described the novel employment of micropreparative CE to a pharmaceutical analysis problem. Capillary zone electrophoresis (CZE) and HPLC are used separately to quantify drug related impurity levels. Good agreement was obtained between the two techniques. Peak homogeneity was determined for both fractions obtained by HPLC and CZE. This peak purity determination was achieved by analysing the appropriate fraction by the alternative technique. This work demonstrates that CZE and HPLC, used together, are a powerful analytical combination.

INTRODUCTION

The capillary electrophoretic (CE) methods of capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) are finding increasing application [1–6] within the area of pharmaceutical analysis. Particular areas include the quantitative determination of drug content, drug related impurities and chiral analysis [5,6]. Due to the low sample volumes introduced into the capillary for analysis, typically of the order of a few nanolitres, CE is not routinely considered for preparative purposes.

Fluparoxan is a compound currently under development within Glaxo Group Research. The structure is shown in Fig. 1. Previously, we have reported [7] the use of CZE to separate fluparoxan from one of its related impurities. Having established the applicability of CZE for determination of fluparoxan related impurities the work was extended to the determination of other specific related impurites. For this purpose it was appropriate to employ micro-preparative CZE facilities.

In this report we demonstrate the micro-preparative use of CZE to collect fractions and to verify peak purity of fractions prepared by HPLC. This is the first report of the use of micro-preparative CZE for pharmaceutical analysis. Previous reports have centred on protein and nucleotide separations [8–12]. In addition, the complimentary nature of applying CZE and HPLC in combination is shown, and this novel parallel approach to method validation is discussed.



* Corresponding author.

0021-9673/93/$06.00

EXPERIMENTAL

Materials and instruments

Electrolyte reagents were obtained from Aldrich (Poole, UK). Water was obtained from a Millipore Milli-Q system (Watford, UK). CE was performed on a P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, USA) which was connected to a Hewlett-Packard (Bracknell, UK) data collection system. Samples of fluparoxan (GR50360) were obtained from within Glaxo Group Research.

The fused-silica capillaries used in this study were purchased from Beckman.

Procedure

Automated fraction collection is possible with the CZE instrument available within our laboratory (Beckman P/ACE 2000). This collection is possible by suitable programming of a separation method into the personal computer controlling the CE instrument.

The separation method is a user-defined, stepwise control programme for the instrument. The separation method used for the micro-preparative CZE is given in Table I.

In step V a voltage is applied across the capillary causing migration of solutes through the detector. The low field end of the capillary is dipped into a vial containing electrolyte. When performing the micro-preparative CZE fraction collection step (step VI) this reservoir is replaced, at the selected time, by a microvial containing a few microlites of water. This switch is timed to be immediately prior to the elution of the solute peak from the capillary end. Following the migration of the selected peak from the capillary into the collection microvial the separation is stopped and the capillary rinsed.

The exact timing of the reservoir switch to the collection microvial in the selected example was calculated as given below. The migration time, to the detector, of the peak of interest was 7.8 min. The total length of the capillary used was 57 cm long; 50 cm to the detector. Therefore, the time taken for the peak to reach the tip of the capillary is calculated by:

$$57/50 \cdot 7.8 = 8.9 \text{ min}$$

In the separation method employed (step VI, Table I) the switch to the collection vial is timed to be after 8.3 min. The material eluted from the capillary after the switch contains the required impurity is collected for 30 s into the collection vial (step VI, Table I).

The volume of sample injected in CZE is in the order of a few nanolitres. Therefore the sample concentration was increased from 0.5 mg/ml, for the analytical separation, to 25 mg/ml for the micro-preparative separation. This was the maximum concentration that could be analysed whilst still obtaining suitable resolution of impurity I from the fluparoxan peak. In CZE (as in HPLC) there is a loss in both resolution and peak efficiency with increased sample loading.

TABLE I

CAPILLARY ELECTROPHORESIS SEPARATION METHOD

Step I	Rinse cycle 1: 0.5 *M* NaOH, 2 min
Step II	Rinse cycle 2: run buffer, 4 min
Step III	Set detector: 0.02 AUFS, 210 nm
Step IV	Sampling: 5.0 s pressure
Step V	Operating voltage: 30 kV Operating temperature: 25°C Run time: 8.3 min
Step VI	Operating voltage: 30 kV Operating temperature: 25 °C Run time: 0.5 min
	Electrolyte: 50 m*M* borax pH adjusted to 2.5 with conc. H_3PO_4 Capillary dimensions: 57 cm × 75 μm

RESULTS AND DISCUSSION

Determination of related impurities by CZE and HPLC

A fluparoxan drug substance sample, prior to purification (batch 1, Table II) containing high levels of related impurities was analysed by both CZE and HPLC. In addition, more purified drug substance batches (batches 2 and 3) were also analysed. Table II compares impurity levels as determined by both methods. The reasonable agreement achieved by two entirely different separative techniques suggests that all principal impurities are being quantified by both techniques.

Fig. 2 shows two replicate CZE separations of batch 1. The migration position of a selected syn-

TABLE II

IMPURITY LEVELS (%AREA/AREA) BY CZE AND HPLC

	Batch 1	Batch 2	Batch 3
HPLC total impurities	13.4	1.8	0.3
CZE total impurities	11.2	1.8	0.3
Impurity 1 content by HPLC	2.74	0.08	ND[a]
Impurity 1 content by CZE	2.41	0.09	ND

[a] ND = Not determined.

thetic impurity, termed "impurity 1" is indicated. The selectivity of the CE separation was markedly different to that of the HPLC analysis (Fig. 3). For example, impurity 1 is a late running peak by HPLC whilst it migrates before the main peak in CZE.

Determination of peak purity by CZE and HPLC

Peak purity is of concern when using electrophoretic or chromatographic techniques since it is possible that a minor component may be co-eluting/co-migrating with the principal component. This situation would lead to an underestimation of impurity levels. Accordingly, spectral purity of HPLC peaks are often determined to confirm peak purity using a diode array detector and comparing the spectra with an authentic sample. This method is however incapable of detecting minor component levels of less than about 1%. Since impurities are often present at lower levels a further means of peak homogenity testing should be considered. This is often the collection of main peak fractions and their subsequent analysis under different operating conditions, or by employing an alternative orthogonal analytical technique. In this example the orthogonal techniques of CZE and HPLC are employed to confirm peak homogeneity of fractions taken from preparative HPLC and CZE analysis.

A HPLC fraction was prepared containing impurity 1. This solution was analysed by CZE to confirm the presence of a major component. The CZE separation (Fig. 4) showed the sample to be a single component.

A sample of fluparoxan was spiked with the HPLC fraction to identify the CZE migration time of impurity 1. Levels of impurity 1 were quantified in batches 1–3 by both CZE and HPLC. Table II shows there to be good agreement between the levels quantified by HPLC and CZE.

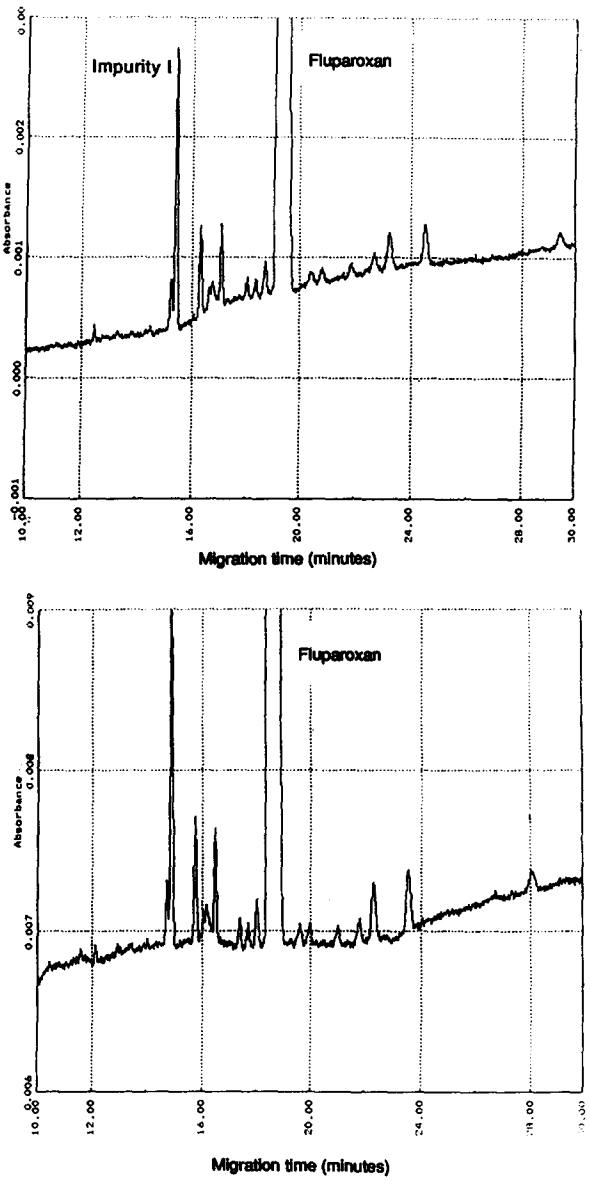


Fig. 2. Replicate CE analyses of a sample of batch 1. Separation conditions: 50 m*M* borax pH adjusted to 2.5 with conc. H_3PO_4, 5.0 s pressure sampling, +30 kV, 25°C, 57 cm (50 cm to detector) × 75 μm.

Fraction collection by CZE

Micro-preparative CZE has advantages over the preparative use of conventional electrophoresis. These are principally the ease of sample handling and the full automation of preparative CZE. In addition, the standard equipment can be used for both

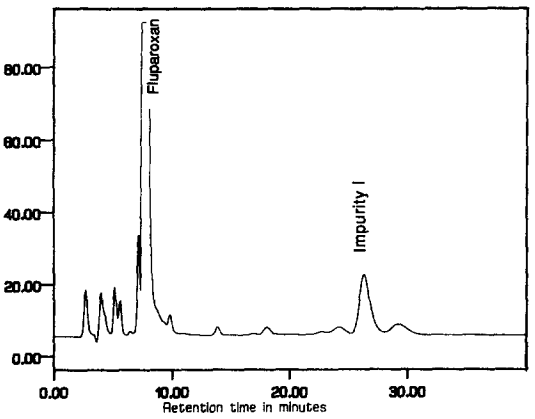


Fig. 3. HPLC analysis of batch 1. Separation conditions: 15 cm × 4.6 mm I.D. Spherisorb CN 5 μm, acetonitrile–0.005 *M* $NH_4H_2PO_4$–methanol (6:5:3, v/v/v), 0.8 ml min^{-1}, UV at 210 nm, 20 μl injection volume. Y-axis represents absorbance (arbitrary units).

analytical and micro-preparative CZE. The major disadvantage is that the sample volumes involved with CZE are relatively small.

Fraction collection by HPLC is an established technique and several automated fraction collection systems are commercially available. The operation of micro-preparative CZE is less common. Therefore, it is appropriate that the operation of CZE in a preparative mode should be discussed.

Using the method given in Table I, 27 fractions were collected overnight into the collection vial. This produced a 10 μg/ml solution of the impurity, the concentration was calculated as follows:

2 s pressure injection on a 57 cm × 75 μm capillary = 11.8 nl injection volume [13];
25 mg/ml solution, therefore injected 0.295 μg material;
impurity present at 2.5 % level therefore injection is 7.4 ng;
27 replicate injections = 198 ng collected into 20 μl of water;
concentration = 10 μg/ml in water.

Sample solutions of this concentration are suitable for submission for identification by mass spectrometry.

The CZE generated fraction was analysed by HPLC (Fig. 5) and the principal component of the chromatogram was confirmed as impurity 1 by retention time.

FUTURE DEVELOPMENTS

The small volumes injected in CZE, typically in the order of a few nanolitres remains the greatest limitation to development in this area. The two solutions that have been suggested are the use of wider bore capillaries [8] or to use bundles of capillaries [12,14]. The first approach is somewhat limited in that heat dissipation is significantly reduced with wider bore tubes making band broadening effects more appreciable. The alignment of capillary bundles is essential to retain the high efficiencies of a CE separation. This alignment would represent a serious mechanical challenge.

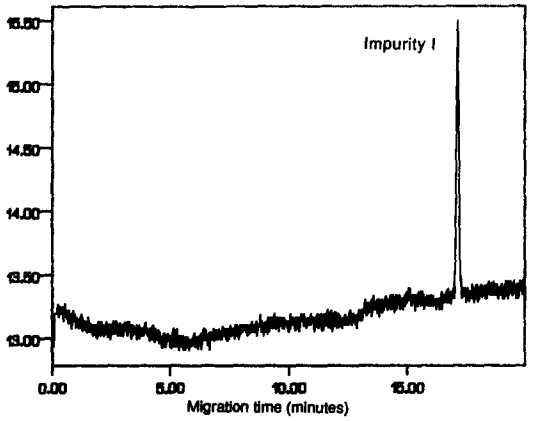


Fig. 4. CZE analysis of HPLC fraction containing impurity 1. Separation conditions as in Fig. 2. Y-axis represents absorbance (arbitrary units).

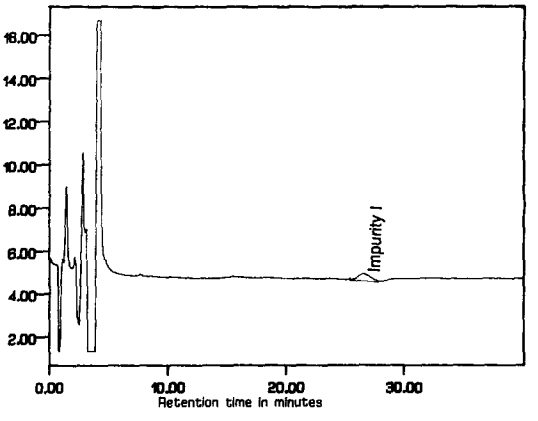


Fig. 5. HPLC analysis of CZE fraction containing impurity 1. Separation conditions as in Fig. 3. Y-axis represents absorbance (arbitrary units).

CONCLUSIONS

CZE has been shown to be useful for micro-preparative fraction collection of a drug related impurity. This facility allows the determination of the purity of CZE peaks by analysing appropriate fractions by an orthogonal separative method. In addition CZE has been used for the determination of HPLC peak homogeneity.

Good cross-correlation with results generated by both CZE and HPLC has also been shown with respect to total impurity content and for an individual impurity. This information could form part of the validation of either the HPLC or CZE method. This work demonstrates that CZE and HPLC, used together, are a powerful analytical combination.

Micro-preparative CZE is a viable option and should be considered for suitable applications.

REFERENCES

1 K. D. Altria and C. F. Simpson, *J. Pharm. Biomed. Anal.*, 6 (1988) 801.
2 K. D. Altria and M. M. Rogan, *J. Pharm Biomed. Anal.*, 8 (1990) 1005.
3 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Pharm. Sci.*, 79 (1990) 519.
4 H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
5 K. D. Altria, D. M. Goodall and M. M. Rogan, *Chromatographia*, 34 (1992) 19.
6 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
7 K. D. Altria and N. W. Smith, *J. Chromatogr.*, 538 (1991) 506.
8 A. Guttman, A. S. Cohen, D. N. Heiger and B. L. Karger, *Anal. Chem.*, 62 (1990) 137.
9 A. S. Cohen, D. R. Najarian, A. Paulus, A. Guttman, J. A. Smith and B. L. Karger, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 9660.
10 D. J. Rose and J. W. Jorgenson, *J. Chromatogr.*, 438 (1988) 23.
11 P. Camilleri, G. N. Okafo, C. Southan and R. Brown, *Anal. Biochem.*, 198 (1991) 36.
12 N. A. Guzman, L. Hernadez and B. G. Hoebel, *Biopharm.*, 2 (1989) 22.
13 G. McLaughlin, R. Biehler and H. E. Schwartz, *Application Datasheet TIBC-103*, Beckman, Palo Alto, CA, 1991.
14 K. D. Altria and C. F. Simpson, *Chromatographia*, 24 (1987) 527.