Journal of Chromatography, 641 (1993) 147–153
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 124

Inter-company cross-validation exercise on capillary electrophoresis

I. Chiral analysis of clenbuterol

K.D. Altria*
Pharmaceutical Analysis, Analytical Evaluation Group, Glaxo Group Research, Park Road, Ware, Herts. SG12 0DP (UK)

R.C. Harden
Lilly Research Centre Ltd., Windlesham (UK)

M. Hart
Rhone-Poulenc-Rorer Ltd., Dagenham (UK)

J. Hevizi
SmithKline Beecham, Welwyn Garden City (UK)

P.A. Hailey
Pfizer Central Research, Sandwich (UK)

J.V. Makwana
Boots Company, Nottingham (UK)

M.J. Portsmouth
Fisons Pharmaceuticals, Loughborough (UK)

(First received January 19th, 1993; revised manuscript received March 16th, 1993)

ABSTRACT

In order to assess the repeatability of capillary electrophoresis (CE) and to demonstrate the successful transfer of CE methods between independent laboratories a working party comprising seven pharmaceutical companies was established. Three cross-validation exercises are scheduled. The first is the chiral analysis of clenbuterol. This paper gives the results from this initial study. The method was successfully transferred between all the participating companies. In each case baseline separation or better of the enantiomers was obtained. Good performance in terms of precision for both peak area and migration time, linearity and accuracy was obtained. This exercise clearly demonstrates that the CE method investigated is capable of generating accurate and precise data.

* Corresponding author.

0021-9673/93/$06.00

INTRODUCTION

Capillary electrophoresis (CE) is rapidly becoming established as an alternative and complementary analytical technique to HPLC for the quantitative analysis of pharmaceuticals. CE has been employed for the determination of the active ingredients in formulations [1–3], and for the determination of drug-related impurities [4–6]. Several groups have also demonstrated the use of CE for chiral analysis of pharmaceuticals [7–9]. Good cross-correlation of CE data with those generated by chromatographic methods has been shown [1,2,5,6]. However, as with all developing techniques performance is rightfully being questioned. Several reports [1–6] have shown CE to be capable of generating high-quality data with good performance in terms of precision, accuracy and linearity. However, repeatability and the transfer of methods has yet to be reported.

Given the increased use of CE for pharmaceutical analysis a working group of seven pharmaceutical companies was established with the objective of assessing the ability of CE methods to be transferred successfully between independent laboratories. The working group was established through their involvement in the UK Pharmaceutical Analysis Science Group, an organisation of research based pharmaceutical companies established to mutually develop areas of common interest in the field of pharmaceutical analysis.

It was agreed that selected CE methods would be validated by each of the participating companies using a common validation protocol. Given the principal application areas of CE, three studies were considered: (1) separation of the enantiomers of a chiral drug substance, (2) determination of the active ingredients in a formulation, and (3) quantitative analysis of drug-related impurities.

This paper is the first in a series of 3 and details the inter-company cross-validation of a CE method for the separation of the enantiomers of a chiral drug substance.

A CE method for the separation of the enantiomers of the bronchodilator, clenbuterol has been published [9]. These reported operating conditions were modified in terms of the selection of electrolyte and cyclodextrin. Previously a citrate–phosphate electrolyte with β-cyclodextrin had been employed for the separation of clenbuterol enantiomers with a run-time of 33 min. In this study a borate–phosphate electrolyte was employed incorporating hydroxypropyl-β-cyclodextrin which has a higher enantioselectivity for clenbuterol. This increase in selectivity permitted a typical reduction in analysis time to *ca.* 12 min. In both instances chiral selectivity was achieved through incorporation of a cyclodextrin into a low-pH carrier electrolyte. This is the most common mode of chiral analysis in CE and has been successfully applied to a number of basic compounds [10].

Future papers will cover the quantitative assay of the active ingredients in a formulation and the determination of drug related impurities.

EXPERIMENTAL

The compounds and reagents selected for this exercise are commercially available.

CE instrumentation from three suppliers, Applied Biosystems (San Jose, CA, USA), Beckman (Palo Alto, CA, USA) and Spectra-Physics (Freemont, CA, USA) was employed. Standard capillary lengths and bores were adopted. Sampling times for the various instruments were set to produce an injection volume of 8 nl. Hydroxypropyl-β-cyclodextrin was purchased from Aldrich (Gillingham, UK). The method details are as follows:

Rinse 1	0.1 *M* NaOH 1 min
Rinse 2	Electrolyte 2 min
Injection	8 nl
Detection	UV absorbance at 214 nm
Voltage	+30 kV
Temperature	Ambient
Capillary	Fused silica 57 cm × 50 μm
Sample	0.15 mg/ml in water

Electrolyte: 30 m*M* hydroxypropyl-β-cyclodextrin (typically 0.83 g per 20 ml) in 50 m*M* disodium tetraborate (typically 1.91 g $Na_2B_4O_7 \cdot 10H_2O$ per 100 ml), pH adjusted to 2.2 with concentrated orthophosphoric acid.

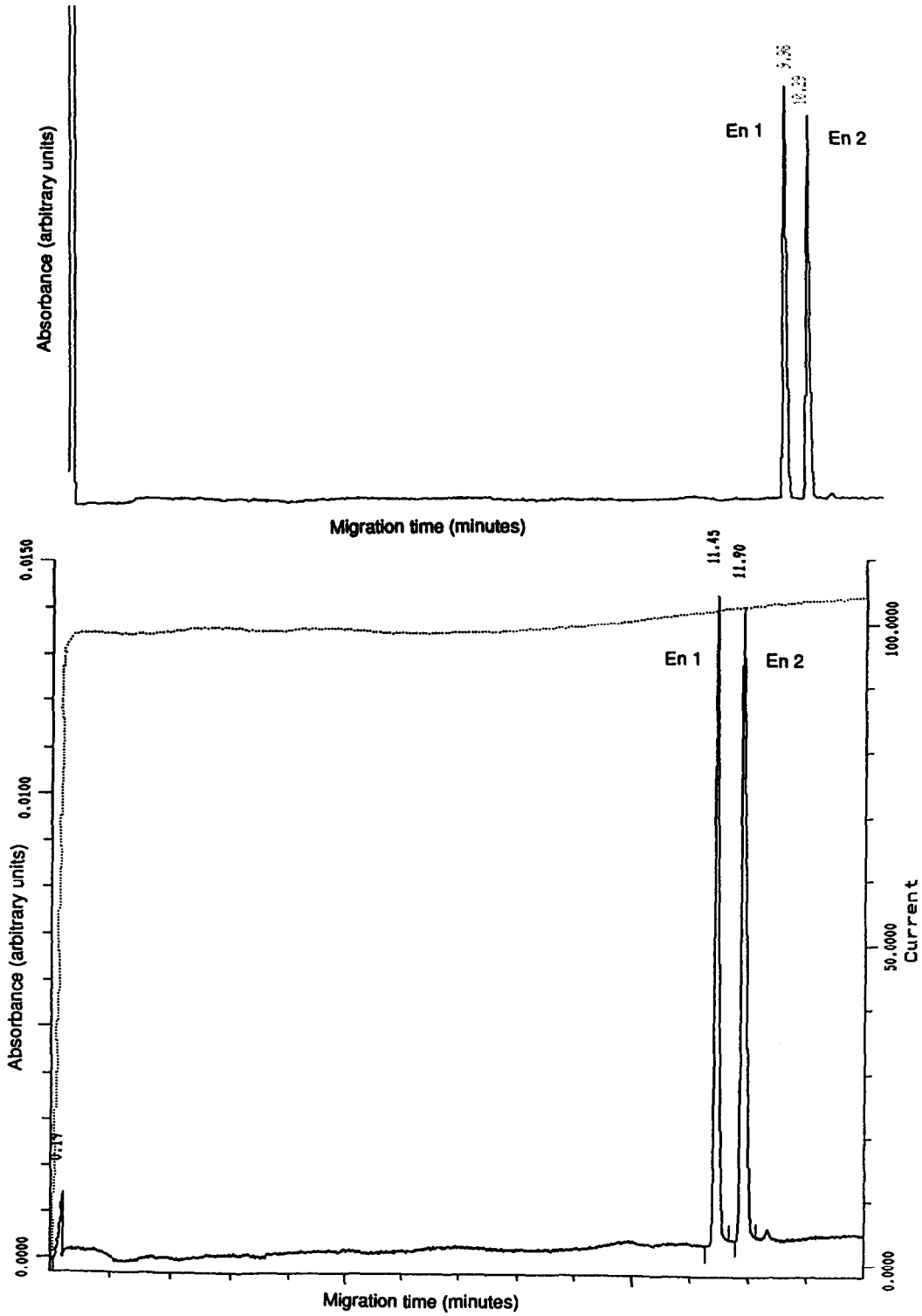

Fig. 1.

(Continued on p. 150)

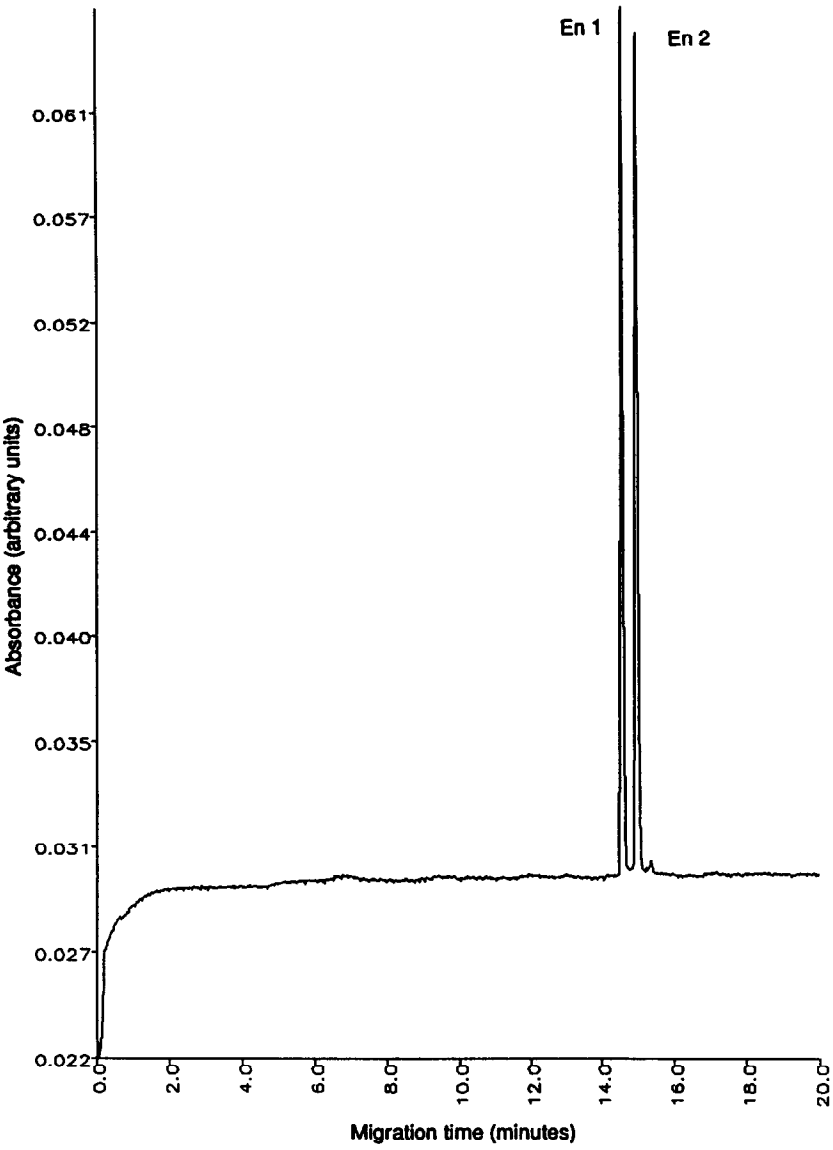

Fig. 1. Chiral CE separations of clenbuterol. Separation conditions as given in Experimental. Current measured in μA.

A clenbuterol sample was purchased from Sigma (Poole, UK) and subsamples were dispatched to each of the companies for testing.

The following experiments were performed:

(i) Repeated injections of a clenbuterol sample solution (target concentration 0.15 mg/ml) were used to measure precision.

(ii) Detector linearity over a range of 10–150% of clenbuterol target concentration was measured. Two samples were prepared and serially diluted to give appropriate standards of 10, 25, 50, 75, 100 and 150% of target concentration.

RESULTS AND DISCUSSION

The following measures of method performance were assessed: (i) selectivity, (ii) migration time precision, (iii) peak area and peak height precision, (iv) detector response linearity with sample concentration, and (v) %area/area accuracy in comparison with theoretical value.

Selectivity

Using the separation conditions given in the Experimental section all companies were able to achieve baseline separation or greater of the clenbuterol enantiomers. Fig. 1 shows three representative electropherograms. An impurity was present in the clenbuterol sample which consistently migrated after the second enantiomer in all separations. Variations in actual migration times were mainly due to differences in the distances along the capillary to the detector in the various instruments employed, and to variations in operating temperature.

Migration time precision

Table I shows the average relative standard deviation (R.S.D.) for the precision of migration time for the first migrating enantiomer to be under 1%. In all cases precision of less than 0.05% R.S.D. was obtained for the relative migration time (RMT) of enantiomer 1, relative to enantiomer 2. These data indicate that excellent consistency of selectivity was obtained during the injection sequences.

Linearity data

The linearity of detector response for peak area over a range of 10–150% of clenbuterol target concentration was measured. Acceptable linearity data (Table II) were obtained by all companies with an average correlation coefficient of 0.995. The average % intercept was +1.2% of detector response for target clenbuterol concentration.

TABLE II

PEAK AREA LINEARITY DATA (CORRELATION COEFFICIENT)

Company	En1	
	Linearity	Intercept
1	0.999	0.98
2	0.997	0.31
3	0.999	−0.33
4	0.992	5.10
5	0.996	0.33
6	0.990	−1.34
7	0.994	3.53

Peak area precision

Peak area precision improves in CE with increased sample concentration [11]. When employing high sample concentrations [1,2,12,13] typical CE peak area precision in the order of 1–2% R.S.D. can be obtained. This is in contrast to precision of 5% R.S.D. which has been reported for low-concentration test mixtures [14,15].

In CE, peak areas are related to both sample concentration and migration time. Later migrating peaks pass more slowly through the detector giving a higher apparent peak area response. This effect can be compensated for by the division of the area of each peak by its corresponding migration time [16]. The impact of not normalising areas upon %area/area impurity data and chiral separations has been reported [17].

TABLE I

MIGRATION TIME PRECISION (ENANTIOMER 1)

Company	Instrument supplier	Migration time (%R.S.D.) ($n = 10$)	RMT	RMT En1 (%R.S.D.) ($n = 10$)
1	Beckman	1.3	0.98	<0.05
2	Beckman	0.3	0.94	<0.05
3	Beckman	0.8	0.96	<0.05
4	Spectra-Physics	0.4	0.96	<0.05
5	ABI	0.6	0.97	<0.05
6	Beckman	0.5	0.96	<0.05
7	ABI	0.2	0.97	<0.05

TABLE III

PEAK AREA PRECISION

Company	Precision (R.S.D.) of peak area En1		Precision (R.S.D.) of peak area En2		Peak area ratio En1/En2 (PAR)	
	Actual	Normalised	Actual	Normalised	PAR	R.S.D. ($n = 10$)
1	1.2	0.8	1.4	0.8	0.974	0.2
2	2.6	2.5	1.9	1.8	1.004	0.5
3	0.6	1.0	0.4	1.1	0.993	0.4
4	1.3	1.2	1.7	1.6	0.992	0.9
5	2.2	2.1	2.2	2.0	0.998	0.3
6	2.5	1.1	2.9	1.8	1.000	0.6
7	1.7	1.7	1.5	1.5	1.000	0.9

Peak area precision was measured (Table III) for the repeated injections of the clenbuterol sample. Table III shows that normalisation can improve peak area precision, the improvement was more pronounced when a higher variation in migration times was observed.

Peak area ratios were calculated using the ratio of enantiomer 2 (En2) unnormalised peak area divided by the un-normalised peak area of enantiomer 1 (En1). The precision was clearly improved with all R.S.D. values below 1% (Table III), as variations in sample injection volumes and migration times are internally compensated for. Use of internal standards has been suggested to improve quantitative analysis in CE [18].

Peak height precision

The precision for peak height was measured by several companies and R.S.D. values of 1–2% were obtained (Table IV) indicating reproducible data could be obtained employing these measurements. However, non-linear increases in peak height with increased sample loadings, at higher sample concentrations, have been observed [19].

TABLE IV

PEAK HEIGHT PRECISION

Company	Peak height En1 (%R.S.D.) ($n = 10$)
1	1.0
2	2.3
3	2.1
4	Not recorded
5	1.7
6	1.4
7	1.7

% Peak area data

Clenbuterol is a racemic compound and should therefore give a %area/area result of 50:50 for the two enantiomers. Table V shows the average %area/area to be 50:50 with an acceptable precision of 0.6% R.S.D. for the seven results.

TABLE V

% NORMALISED PEAK AREA DATA ($n = 10$)

Company	% Peak area		
	En1	En2	
1	49.4	50.6	
2	50.2	49.8	
3	49.6	50.4	
4	49.6	50.4	
5	49.9	50.1	
6	50.0	50.0	
7	50.0	50.0	
Mean	49.8	50.2	R.S.D. = 0.6%

CONCLUSIONS

This first inter-company cross-validation exercise has confirmed that a CE method was able to be transferred between seven companies. Instruments from three manufacturers were employed

in this study. In all instances baseline resolution or greater of the two enantiomers was achieved. Acceptable measures of linearity and precision of both migration time and peak area were obtained. The %area/area results clearly indicate that this chiral CE method is capable of generating precise data.

REFERENCES

1 M.T. Ackermans, J.L. Beckers, F.M. Everaerts and I.G.J.A. Seelen, *J. Chromatogr.*, 590 (1992) 341.
2 E.W. Tsai, M.M. Singh, H.H. Lu, D.P. Ip and M.A. Brooks, *J. Chromatogr.*, 626 (1992) 24.
3 R. Weinberger and M. Albin, *J. Liq. Chromatogr.*, 14 (1991) 953.
4 M. Swartz, *J. Liq. Chromatogr.*, 14 (1991) 923.
5 K.D. Altria, *J. Chromatogr.*, 634 (1993) 323.
6 A. Pluym, W. Van Ael and M. De Smet, *Trends Anal. Chem.*, 11 (1992) 27.
7 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
8 M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chromatogr.*, 15 (1992) 1023.
9 K.D. Altria, D.M. Goodall and M.M. Rogan, *Chromatographia*, 34 (1992) 19.
10 R. Kuhn and S. Hoffstetter-Kuhn, *Chromatographia*, 34 (1992) 505.
11 H. Wätzig and C. Dette, *J. Chromatogr.*, 636 (1993) 31.
12 G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, J.A. Morrison and T.J. Bronzert, *J. Liq. Chromatogr.*, 15 (1992) 961.
13 S.E. Moring, J.C. Colburn, P.D. Grossman and H.H. Lauer, *LC·GC Int.*, 3 (1990) 46.
14 Q. Dang, L. Yan, Z. Sun and D. Ling, *J. Chromatogr.*, 630 (1993) 363.
15 T. Nagawaka, Y. Oda, A. Shibukawa, H. Fukuda and H. Tanaka, *Chem. Pharm. Bull.*, 37 (1989) 707.
16 M.W.F. Nielen, *J. Chromatogr.*, 588 (1991) 321.
17 K.D. Altria, *Chromatographia*, 35 (1993) 177.
18 E.V. Dose and G.A. Guiochon, *Anal. Chem.*, 63 (1991) 1154.
19 D.M. Goodall, S.J. Williams and D.K. Lloyd, *Trends Anal. Chem.*, 10 (1991) 272.