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Journal of Chromatography A, 680 (1994) 579–586

JOURNAL OF
CHROMATOGRAPHY A

Determination of pharmaceuticals and related impurities by capillary electrophoresis

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Abstract

In the first part of this work, the use of capillary electrophoresis (CE) for the separation of two groups of pharmaceuticals, namely a metabolite of tamoxifen and a basic drug substance, DS1, was investigated. The effects of pH and types of modifiers, *e.g.* surfactant, bile salt, γ-cyclodextrin and hydroxypropyl-β-cyclodextrin on selectivity, separation and peak shape were studied. Besides achieving complete separation of the compounds, the CE system was capable of providing separation with significant improvements in overall peak shape of the compounds compared with HPLC. In the case of the basic drug substance DS1, validation of the CE system developed in terms of linearity, selectivity, sensitivity and reproducibility was satisfactorily performed. At the same time, a study of the sample solvent matrix effects on the separation of this group of compounds was examined. The system was successfully applied to the analysis of laboratory-synthesized samples. Good correlation was observed between CE and HPLC, although higher efficiency and faster speed of separation were obtained using the CE system developed. For the tamoxifen metabolite, special emphasis was placed on the use of CE for the separation of the pair of isomers. This was readily achieved through the introduction of γ-cyclodextrin in the electrolyte. Resolution of at least 1.5 was obtained for the isomers using the CE method.

1. Introduction

Pharmaceutical drug analyses are often conducted using HPLC, as it offers excellent reproducibility and precision. However, basic drug analysis by HPLC usually suffers from the problem of peak tailing and long retention times [1]. This would be a disadvantage if high sample throughput is needed in routine laboratories where fast analyses with high precision are required. Capillary electrophoresis (CE), with features such as high efficiencies, high separatory power and rapid analysis may be a viable alternative and hence recently there has been a noticeable increase in the use of CE for pharmaceutical analysis [2–11]. The areas that have been covered include chiral separation of drugs [2–4], main drug assay [5,6], determination of drug-related impurities [7–9] and therapeutic drug monitoring [10,11]. In these works, parameters such as precision, linearity, accuracy and reproducibility were reported to be as good as for HPLC. Quantitative results obtained by CE are also often compared with those of HPLC.

In this work, the use of CE for the analysis of basic and polar drugs was investigated. In the first part of the work, the separation of two groups of pharmaceuticals, namely a metabolite

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SSDI 0021-9673(94)00326-5

of tamoxifen [1-(4-hydroxyphenyl)1,2-diphenylbut-1-ene, abbreviated as T10], and a basic drug substance with structure related to ondansetron [9] (abbreviated as DS1) were examined. As part of method development, the effects of pH and modifiers on the separation and peak shapes were investigated. Modifier systems were used mainly to overcome the chromatographic problems often associated with peak tailing observed during the analysis of these basic compounds. The CE system was subsequently employed to analyse laboratory-synthesized samples. A study of sample solvent matrix effects on the separation was also made. Correlation between the results obtained using CE and those obtained by HPLC was investigated. In the case of the tamoxifen metabolite, special emphasis was placed on the use of modifiers to enhance the CE separation of the pair of isomers present in the sample.

2. Experimental

2.1. Instrumental

CE was conducted with a laboratory-built system. A Spellman Model RM15P10KD (Plainview, NY, USA) high-voltage power supply, capable of delivering up to 15 kV, was used. Detection of peaks was accomplished through the use of a Carlo Erba Model microUVis20 UV-detector (Milan, Italy) with wavelength set at 220 nm for DS1 and its related impurities and 254 nm for the metabolite of tamoxifen (T10). The untreated fused-silica capillaries (50 μm I.D.) used in this work were obtained from Polymicro Technologies (Phoenix, AZ, USA). Chromatographic data were recorded on a Shimadzu Model R6A (Kyoto, Japan) integrator or a Perkin Elmer Model R100 (Oak Brook, IL, USA) recorder.

2.2. Reagents and materials

Sodium dihydrogen phosphate and sodium tetraborate used for preparing the buffer solutions were purchased from Fluka (Buchs, Switzerland). Hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin were obtained from Aldrich (Milwaukee, WI, USA). Standards of DS1 and its related impurities GDL1, GDL2 and GDL3 were obtained from Glaxo Development (Singapore) and T10 was synthesized by colleagues in another laboratory.

2.3. Sample preparation

Standard solutions in the range of 40–160 μg/ml were prepared for calibration. As a result of sample matrix effects (to be discussed), samples were first dissolved in methanol (concentration of 0.36 mg/ml) and were subsequently diluted to the required concentration with working buffer. Samples were hydrodynamically injected at a height of 8 cm for 10 s. The amount injected was approximately 3 nl.

3. Results and discussion

3.1. DS1

DS1 was first chromatographed using reversed-phase HPLC with a C_{18} column. Though most of the peaks were satisfactorily separated, peak tailing was rather prevalent for some of the peaks. Fig. 1 shows a typical chromatogram of DS1 obtained using HPLC. Significant peak tailing was observed for all the late eluting peaks. This is largely attributed to the basicity of

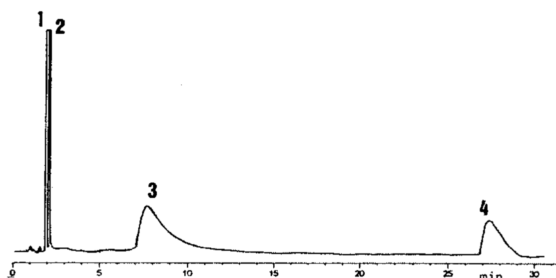

Fig. 1. A typical HPLC chromatogram of the separation of DS1 and impurities. Column: 15 cm × 4.6 mm C_{18}; mobile phase: acetonitrile–methanol–isopropanol–0.02 *M* NaH_2PO_4 (50:15:5:30%, v/v); flow-rate: 1.0 ml/min. Peak identification: 1 = GDL2, 2 = GDL3, 3 = DS1, 4 = GDL1.

DS1 [9]. In view of the fact that CE has shown great potential in the separation of basic compounds [12,13], an attempt was made to study the feasibility of using CE for the separation of DS1.

Method development

Effect of pH on separation

It was found that DS1 and one of its impurities, GDL1, migrated before the neutral marker (methanol), indicating that they were positively charged at the pH values investigated (pH 4–7). The rest of the peaks either co-migrated with or migrated later than methanol depending on whether the compounds were neutral or negatively charged in the pH range investigated (pH 4–7). These observations are typical of CZE where both charged and neutral compounds can be eluted under the influence of electroosmotic flow. The effect of pH on the separation of DS1 and related impurities is shown in Fig. 2. It was noted that the migration times for all solutes decreased with increase in pH. This was in response to an increase in the electroosmotic flow as the electrolyte solution became less acidic. CZE at pH 4 was found to offer higher selectivity and the best overall peak shape for most of the peaks, although the resolution between GDL2 and GDL3 was not satisfactory. These two peaks co-migrated with the neutral marker which meant that subsequent quantitation work would be problematic for these compounds. In order to overcome this problem, the use of modifiers to improve the resolution was investigated.

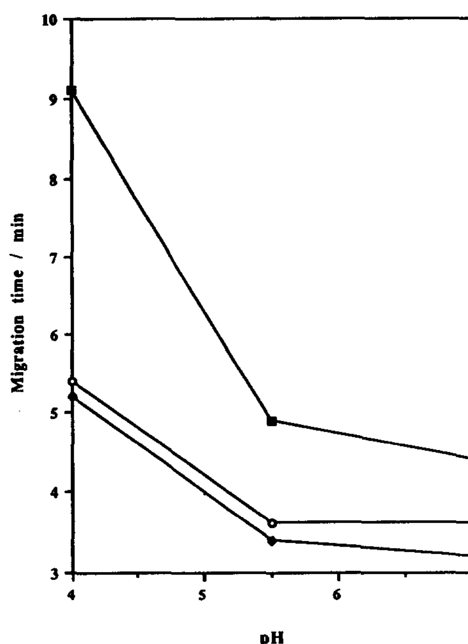

Fig. 2. Plot of migration times of DS1 and impurities against pH of buffer. Electrophoretic conditions: 25 mM phosphate–borate buffer at various pH values; voltage: 15 kV; detection wavelength: 220 nm; column: 50 μm I.D. fused silica, 45 cm effective length. Legend: ◆ = GDL1; ○ = DS1; △ = GDL2; ■ = GDL3 (curves for GDL2 and GDL3 overlapped).

Effect of bile salts on the selectivity

Bile salts are characterized by their high solubility in water and their ability to form micelles consisting of fewer monomers compared to sodium dodecylsulphate (SDS). Fig. 3 shows the electropherogram obtained when 30 mM of sodium cholate was added to the electrophoretic buffer at pH 7. Under this condition, all peaks were separated with a resolution of at least 1.5. However, a broad peak was observed for DS1, which could be attributed to the formation of an ion pair between the positively charged DS1 and the negatively charged cholate micelles. The negatively charged cholate micelles, due to electrophoretic flow, would be attracted to the anode. DS1, being the largest of the three positively charged species, was affected to the greatest extent and consequently experienced the most severe peak broadening. To eliminate such peak broadening, the addition of HP-β-CD as modifier was investigated.

Effect of concentration of HP-β-CD

The effect of the concentration of HP-β-CD on the selectivity and migration time is shown in Fig. 4. The addition of the HP-β-CD to an electrolyte consisting of 30 mM sodium cholate significantly reduced the migration times for all the solutes. This is attributed to the formation of complexes between DS1 with HP-β-CD. Since HP-β-CD is neutral, its apparent velocity is

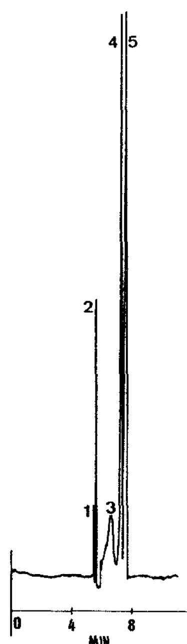

Fig. 3. A typical electropherogram of DS1 and its related impurities. Electrophoretic conditions: 30 mM sodium cholate in 25 mM phosphate–borate buffer, pH 7.0; voltage: 15 kV; detection wavelength: 220 nm; column: 50 μm I.D. fused silica, 45 cm effective length. Peak identification: 1 = GDL1, 2 = DS1, 3 = methanol, 4 = GDL2, 5 = GDL3.

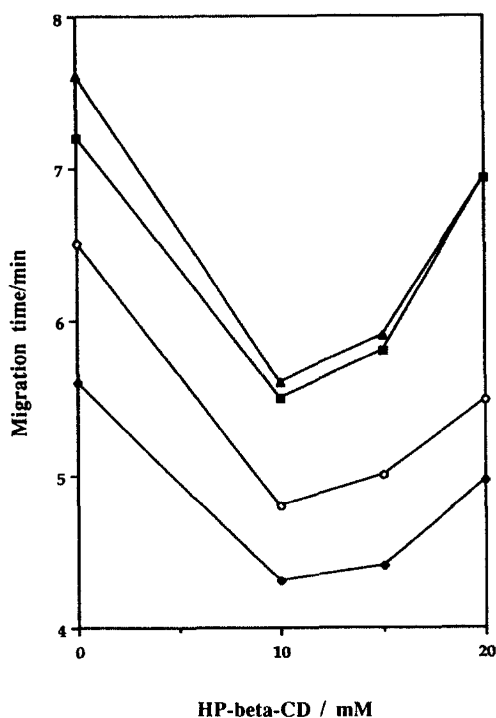

Fig. 4. Plot of migration time against concentration of hydoxypropyl-β-cyclodextrin. Electrophoretic conditions: 30 mM sodium cholate in 25 mM phosphate–borate, pH 6 with varying concentrations of HP-β-CD; voltage: 15 kV; detection wavelength: 220 nm; column: 50 μm I.D. fused silica, 45 cm effective length. Legend: ◆ = GDL1; ○ = DS1; △ = GDL2; ■ = GDL3.

greater than the negatively charged cholate micelles. This would account for the decrease in the migration times observed. However, when more than 10 mM of HP-β-CD was added into the electrolyte, a significant increase in the viscosity of the electrolyte resulted. Consequently, an increase in migration times for the solutes at higher concentrations of HP-β-CD was obtained (Fig. 4). Satisfactory separation of ondansetron and its drug-related impurities was obtained with a 25 mM phosphate–borate buffer at pH 6.3, containing 30 mM sodium cholate and 15 mM HP-β-CD. A typical electropherogram is shown in Fig. 5. In this figure, it was noted that sharp peaks were obtained for DS1 and its related impurities.

From Fig. 5 it can be seen that all the peaks migrated out within 8 min and resolutions were greater than 1.5 for all the peaks. It should be noted that the short analysis time obtained in this system is certainly a very attractive feature as this, if implemented for routine work, would mean that faster sample turnaround times and high efficiencies are possible. Furthermore, the small sample solution requirement of the CE method provides another advantage for it to be considered as an alternative analytical tool.

Another observation is that chiral separation of DS1 was achieved partially (Fig. 5), which further demonstrated the high efficiency and selectivity of the CE system developed. This observation is in agreement with previous investigations where bile salts were found to provide the selectivity required for chiral separations [14–18].

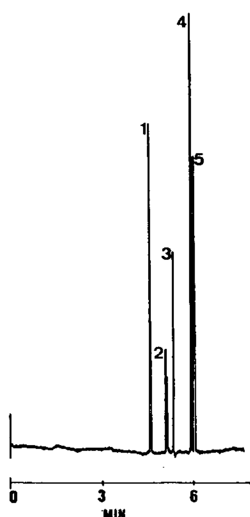

Fig. 5. A typical electropherogram obtained using optimum conditions for the separation of DS1 and its related impurities. Electrophoretic conditions: 30 mM sodium cholate and 15 mM HP-β-cyclodextrin in 25 mM phosphate–borate, pH 6.3; voltage: 15 kV; detection wavelength: 220 nm; column: 50 μm I.D. fused silica, 45 cm effective length. Peak identification: 1 = GDL1, 2 = DS1, 3 = methanol, 4 = GDL2, 5 = GDL3.

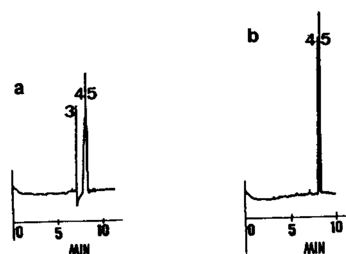

Fig. 6. Typical electropherograms of GDL2 (80 μg/ml) and GDL3 (90 μg/ml) in (a) methanol and (b) methanol–working buffer (50:50). Electrophoretic conditions: 30 mM sodium cholate and 15 mM HP-β-cyclodextrin in 25 mM phosphate–borate, pH 6.3; voltage: 15 kV; detection wavelength: 220 nm; column: 50 μm I.D. fused silica, 45 cm effective length.

Effect of sample matrix

It was known that in certain type of analysis, the nature of the solvent used to dissolve the sample has a great impact on the peak shape and separation efficiency. To study the effect of sample matrix, the impurities of DS1, *i.e.* GDL2 and GDL3, were examined. When the sample containing GDL2 and GDL3 was dissolved in a totally organic solvent such as methanol, poor separation and peak broadening was observed for these two solutes (Fig. 6a). On the other hand, when the sample was dissolved in a solvent consisting of working buffer–methanol (50:50), the two impurities were totally resolved with high efficiencies (Fig. 6b). The reason for such differences in migration behaviour could be that owing to strong solubility effects, the analytes tend to remain in the methanol plug for a longer time. In addition, the presence of the methanol plug reduced the interaction of these two impurities with the micelles. Consequently, a reduction in separation efficiency was observed. On the other hand, when a mixture of working buffer and methanol was used as the solvent, the presence of the micelles in the sample helped to solvate these two solutes, resulting in a sample plug that was more compatible with the working electrolyte [19]. In addition, sample stacking may have occurred, resulting in sharper peaks [20]. Therefore, the choice of an appropriate sample matrix is very important, particularly during impurity determination as it may affect the actual quantitation.

Method validation

As part of method validation for the CE system, linearity, selectivity and reproducibility of the system were evaluated. Columns of similar dimensions were used and the corresponding electropherograms were found to exhibit similar migration profiles and separation as compared to Fig. 5. Calibration plots in the range up to 160 μg/ml were obtained for DS1 and its impurities. The reproducibilities of migration times, peak areas and detection limits are illustrated in Table 1. Excellent linearity was obtained for all the drug-related impurities, with correlation coefficients greater than 0.999. On the other hand, absolute mass limits of detection were found to be lower than those obtained by HPLC, although the concentration limits of detection were higher.

Table 1
Reproducibility data (R.S.D.) and detection limits of DS1 and related impurities.

Compound	R.S.D. (%) ($n = 4$)		Detection limits			
	Area	Migration time	CE[a]		HPLC[b]	
			μg/ml	pg	μg/ml	pg
GDL1	2.9	0.8	5	15	0.005	250
DS1	4.4	0.8	4	12	0.005	250
GDL2	1.8	0.7	3	9	0.005	250
GDL3	2.4	0.6	2	6	0.005	250

[a] Injection volume = 3 nl.
[b] Injection volume = 50 μl.

Applications

Determination of impurities

DS1 samples synthesized in the laboratory were analysed. The results are illustrated in Table 2. From this table, it can be seen that good correlation between HPLC and CE was obtained. It should be noted that the small difference is within analytical variability as well as slight differences in the two methodologies. Typical electropherograms are shown in Fig. 7.

3.2. T10

Tamoxifens are triphenylethylene derivatives which are non-steroidal anti-estrogens used for the treatment of hormone-dependent breast cancer [21]. Analysis of these compounds by HPLC methods has been investigated previously [22,23]. It has been reported that the separation

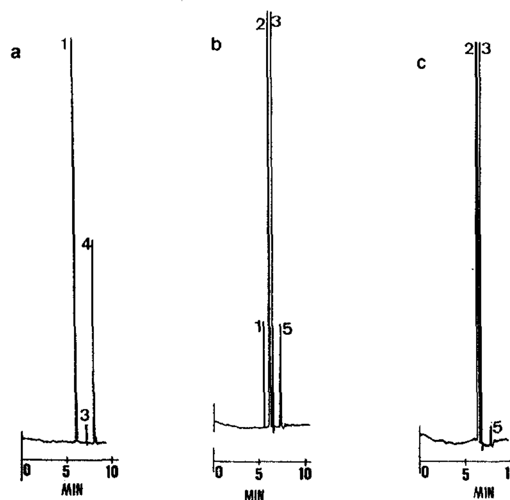

Fig. 7. Typical electropherograms of (a) sample 465, (b) sample 369 and (c) sample 466. Electrophoretic conditions as in Fig. 5. Peak identification: 1 = GDL1; 2 = DS1; 3 = methanol; 4 = GDL2; 5 = GDL3.

Table 2
Comparison of results of impurity level for laboratory samples by HPLC and CE

Sample No.	GDL2 (%, w/w)		GDL3 (%, w/w)		GDL1 (%, w/w)	
	CE	HPLC	CE	HPLC	CE	HPLC
465	11.33	13.66	ND[a]	ND	NQ[b]	NQ
369	ND	ND	4.26	4.76	3.00	2.31
466	ND	ND	0.36	0.45	ND	ND

[a] ND = not detected.
[b] NQ = not quantified.

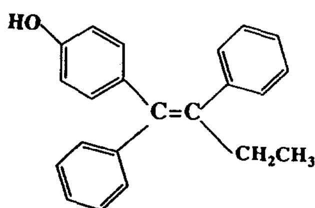

Fig. 8. Structure of T10.

of the isomers could only be achieved using either phenyl or cyclodextrin columns [22]. In this part of the work, the separation of *cis* and *trans* isomers of a metabolite of tamoxifen, abbreviated as T10, using CE was examined. The structure of T10 is given in Fig. 8 where it is expected to possess *E* and *Z* isomers.

T10 was first chromatographed using micellar electrokinetic chromatographic (MEKC) conditions with SDS in the buffer. The corresponding electropherogram is shown in Fig. 9 where a single peak was observed. Since T10 is rather polar, interaction with the SDS micelles did not provide sufficient selectivity. As a result, separation of the isomers was not achieved using the MEKC system. Nevertheless, it was noted that sharper peaks compared to those of HPLC were obtained [22,23].

γ-Cyclodextrin was employed subsequently to enhance the separation of the *E* and *Z* isomers of T10. Fig. 10 shows the electropherogram obtained with the addition of γ-cyclodextrin to the buffer. It can be seen that complete separation of *E* and *Z* isomers of T10 was achieved. The enhancement in selectivity may be attributed to the capability of γ-cyclodextrin to form inclusion complexes [24,25]. A unique feature of cyclodextrin is that both lipophilic as well as hydrophilic types of interaction are possible. As T10 possesses polar functional groups, hydrophilic interaction with the cyclodextrin present in the buffer would occur. Since the *cis* and *trans* isomers differ from each other by the orientation of the substituent groups, the extent of interaction with cyclodextrin would therefore be different for these two isomers. As a result, by optimizing the amount of the γ-cyclodextrin in the buffer, separation of the *cis* and *trans* isomers of T10 could be achieved. It should be noted that in the CE system, unlike in HPLC where expensive stationary phases are needed for chiral separations, enhancement in the selectivity for chiral separation can be easily obtained by the addition of suitable modifiers.

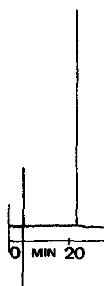

Fig. 9. A typical electropherogram of T10 using MEKC conditions. Electrophoretic conditions: 30 m*M* SDS in 25 m*M* phosphate–borate, pH 7.5; voltage: 15 kV; detection wavelength: 254 nm; column: 50 μm I.D. fused silica, 50 cm effective length.

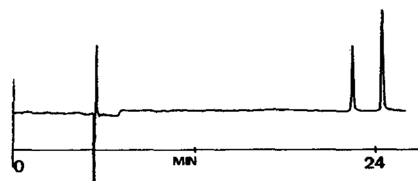

Fig. 10. A typical electropherogram of T10 with γ-cyclodextrin showing separation of the isomers. Electrophoretic conditions: 50 m*M* SDS and 20 m*M* γ-cyclodextrin in 25 m*M* phosphate–borate, pH 7.5; voltage: 15 kV; detection wavelength: 254 nm; column: 50 μm I.D. fused silica, 50 cm effective length.

4. Conclusion

In this paper, the applications of CE in different aspects of pharmaceutical analysis were investigated. From the results obtained, it was found that satisfactory separation of DS1 with significant improvement in the peak shape over HPLC was achieved using CE. Good reproducibility and linearity were obtained for all the solutes with detection limits in the picogram range. The levels of impurities determined using CE were found to be comparable to those by HPLC. The use of CE with γ-cyclodextrin to separate *E* and *Z* isomers of the metabolite of tamoxifen further demonstrated the high resolv-

ing power of CE. There should be great potential in the use of CE as an alternative analytical tool for the analysis of complex mixtures of pharmaceuticals and impurities.

Acknowledgements

The authors thank the National University of Singapore for financial support and Associate Professor K.Y. Sim for the supply of T10.

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