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Quantitative bioanalysis of enantiomeric drugs using capillary electrophoresis and electrospray mass spectrometry

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Abstract

A novel assay method for an enantiomeric pair of drugs has been developed using a combination of capillary electrophoresis and electrospray tandem mass spectrometry connected with a homemade interface. Accurate quantification was demonstrated in plasma from 0.25 to 50 μg/ml. A liquid–liquid sample preparation technique allowed improvement in the quantitation limit to 10 ng/ml. Variables for the enantiomeric separation, including chiral selective reagent, organic solvents, buffer and acid concentration as well as injection technique, were optimized. This assay proved adequate for analysis of neat, spiked plasma, and plasma from a pharmacological study of the drug enantiomers.

Keywords: Tandem mass spectrometry; Electrospray ionization; Biological fluids; Chiral

1. Introduction

Two important trends in the area of bioanalysis for therapeutic drugs have recently begun to converge. One of these trends is a persistent need for chiral assays to characterize the potential for in vivo racemization and/or enantioselective clearance of chiral compounds [1–6]. More recently, many advances in techniques, such as capillary electrophoresis and capillary electrochromatography, have begun to impact chiral separations [7–12].

Electrospray ionization mass spectrometry (ESI/MS) has also made a dramatic impact on the practice of bioanalytical chemistry by providing great selectivity and, in some cases, more sensitivity than many older approaches [13]. As a result, the time needed to develop analytical methods to quantify drugs in biological samples has decreased dramatically, from months or weeks to days.

Abbreviations: α, selectivity; CD, cyclodextrin; HS, highly sulfonated; k', capacity factor; N, separation efficiency.

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Although the bioanalytical capabilities of ESI/MS are impressive, the approach is still restrictive in terms of compatibility with many analytical separations. For example, although many reverse-phase separations work well, ion-exchange separations are of limited applicability due to the incompatibility of many buffers with ESI/MS. Due to the need for nonvolatile reagents, ion pair reagents, mainstays of liquid chromatographic separations over the last three decades [14], are of limited utility in LC/MS. While a wide variety of chiral HPLC separations are practical in both normal- and reverse-phase separation modes, it appears that the most consistent of these are normal-phase separations on modified cellulose sorbent [15,16]. While both normal- and reverse-phase separations lend themselves to atmospheric pressure ionization mass spectrometry (API/MS), in our experience, normal-phase separations are much less convenient to apply routinely and cannot readily be used with electrospray if the mobile phase is highly flammable.

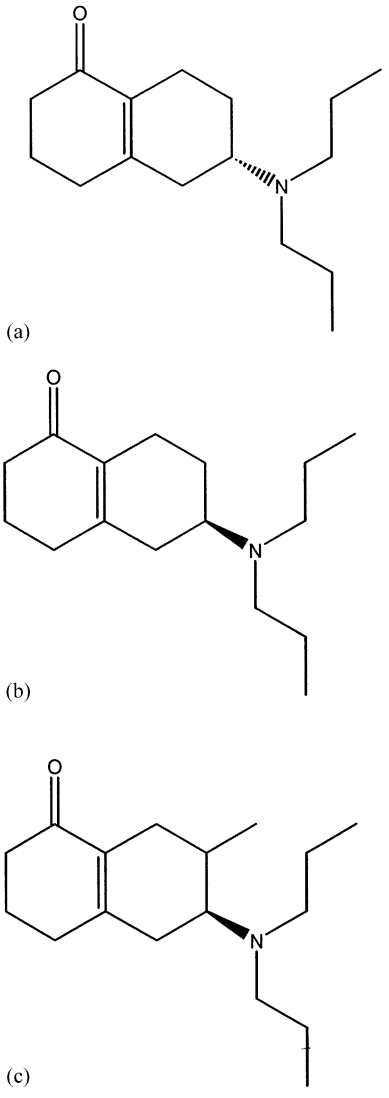

Fig. 1. Chemical structures for compounds 1 (a), 2 (b) and 3 (c) used in these studies.

In this paper, we investigate the potential of capillary electrophoresis/electrospray ionization mass spectrometry (CE/ESI-MS) as a bioanalytical tool for detecting and quantifying enantiomers of chiral drugs in vivo. The instrumentation used here has been constructed from a commercially available electrophoresis instrument, as well as a mass spectrometer with homemade nanoflow ion-source components. We have applied this approach to the quantitative determination of the enantiomer of an illustrative novel drug compound in plasma to determine its potential for chiral interconversion. Those parameters that we found to be important for the proper performance of the method were investigated in some detail. Technique performance, relative to well-established bioanalytical method performance criteria [17], is discussed.

2. Materials and methods

2.1. Materials

2.1.1. Structures

Test compounds (Fig. 1) were obtained from Pfizer (Ann Arbor, MI). They are designated as PD0217015 (compound 1), and PD0217016 (compound 2). PD0149388 (compound 3) was chosen as an internal standard. Acetic acid, formic acid, methanol, acetonitrile, hydrochloric acid and water were obtained from Mallinckrodt Baker (Paris, Kentucky). Highly sulfonated (HS)-β-cyclodextrins (20% w/v) were obtained from Beckman Coulter (Fullerton, CA). The β-cyclodextrins and HS-β-cyclodextrins were obtained from Sigma

(St. Louis, MO). Ammonium acetate was purchased from EM Science (Gibbstown, NJ). Monkey plasma (heparinized) was harvested from in-house animal colonies. All reagents were used as received, without further purification.

2.2. Instrumentation

2.2.1. Capillary electrophoresis

Compounds 1 and 2 were separated by electrokinetic migration in neat and biological sample extracts, using 25% methanol, 75% 5 mM ammonium acetate (~ pH 6), 1.0% acetic acid, and 0.3% (w/v) HS-β-cyclodextrins as the background electrolyte (BGE). Studies were conducted at room temperature with a +25 kV potential (30 kV at the CE and +5 kV at the MS) and a 1 psi push of (BGE) from the CE. The separation capillary was bare-fused silica (180 OD × 50 I.D., Polymicro Technologies, Phoenix, AZ). Electrophoretic separations were performed using a Beckman P/ACE MDQ (Beckman-Coulter, Fullerton, CA). Sample was introduced into the capillary by the electrokinetic injection. A 10 kV potential was applied for 30 s to the sample vial while the electropherograph end of the capillary was placed inside the vial.

2.2.2. Mass spectrometry–quadrupole experiments

A Quattro-LC LC/MS/MS system (Micromass, Manchester, UK) operating under MASSLYNX 3.4 software, was used for the quadrupole MS/MS experiments. The ion source was a Z-spray® configuration with the capillary mount adapted for our homemade nanospray electrospray interface. The source conditions for positive ion experiments were typically: capillary 5.0 kV, cone 30 V, extractor 3 V, RF lens 0.2 V, and source temperature 100 °C. Desolvation temperature control was not applicable to these experiments. Quadrupole 1 (Q1) parameters were as follows: LM resolution 13.0, HM resolution 13.0, and ion energy 1.0. Collision cell parameters were as follows: entrance 1 V, collision 1 V, and exit 1 V. Quadrupole 2 (Q2) parameters were LM resolution 13.0, HM resolution 13.0, and ion energy 1.0. The multiplier was set at 650 V. The precursor and daughter ion combinations for the compounds were: Compound (1) and (2) 250 → 102 (collision energy: 20 eV), and compound (3) 264 → 102 (collision energy: 25 eV). Dwell time for each channel was 200 ms. Inter channel delay was 20 ms.

2.2.3. Mass spectrometry–TOF experiments

All API–TOF–MS experiments were performed on a Perseptive Biosystems Mariner instrument (Framingham, MA) configured with a nanospray source adapted for the homemade electrospray interface. Source conditions were as follows: Positive-ion electrospray, capillary voltage = 5.0 kV, sample cone voltage = 35 V, extraction cone voltage = 10 V, source temperature 100 °C, and desolvation temperature 250 °C. Transfer optics settings were as follows: RF lens = 200 V, RF DC offset 1 = 3.0 V, RF DC offset 2 = 1.0 V, aperture = 10.0 V, acceleration = 200.0 V, focus = 1.0 V, and steering = 0.0 V. Analyzer settings were as follows: MCP detector 2700 V, ion energy = 38.0 V, tube lens = 5.0 V, grid 2 = 55.0 V, flight tube = 4660 V, and reflectron = 1782 V. The pusher cycle time was 55 μs. Data files were acquired in continuum (profile) mode and spectra were stored from 100–600 m/z with a 500 ms accumulation time per averaged spectrum. Each averaged spectrum was stored to the data system, therefore, contained approximately 9091 individual spectra (55 μs per spectra averaged over 500 ms).

2.2.4. CE/MS interface—mariner and micromass

The CE/MS interface was designed and assembled in-house. The mount adapter that held the interface to the Micromass CE/MS was machined by the Pfizer Ann Arbor Engineering Department. The interface is a coaxial liquid sheath flow design (Fig. 2). This design is relatively simple and was easy to adjust for spray optimization.

2.2.5. Make-up flow

The make-up, or sheath, flow solvent consisted of 75/25/0.1 acetonitrile/5 mM ammonium acetate/formic acid (v/v) and was introduced into the CE/MS interface at a flow of 2 μl/min using a Shimadzu LC-10AD pump. Optimization of the

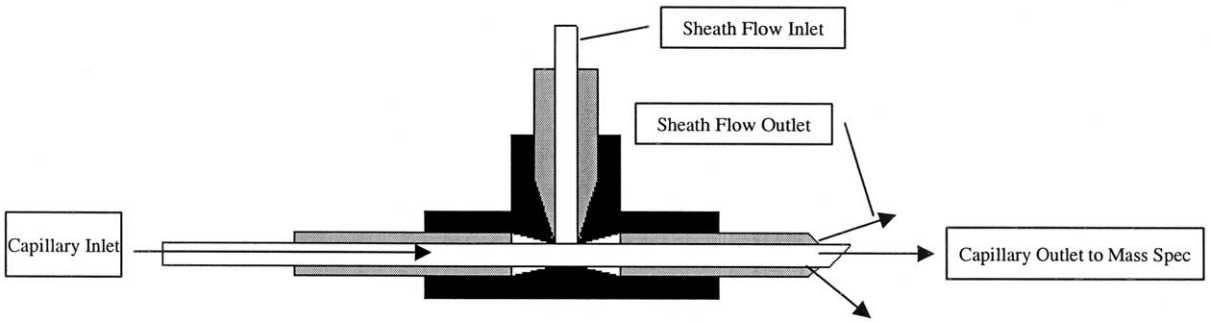

Fig. 2. Schematic representation of coaxial liquid sheath flow CE/MS interface.

make-up solvent centered on two key parameters—consistency of the nebulizing (or nano) spray, and maximum signal. No nebulizing or drying gas was used, allowing better analyte sensitivity. The CE running buffer contained nonvolatile components. A high organic solvent was, therefore, infused at a low flow rate to facilitate the transition of ions into the gas phase more readily. A 75% acetonitrile solution provided the necessary volatility and the most consistent spray, while formic acid gave adequate analyte ionization in electrospray positive ionization mode. Ammonium acetate was also included in the makeup flow to ensure sufficient electrical contact between the electrophoretic capillary and the surrounding stainless steel capillary of the interface.

2.3. Separation optimization experiments

2.3.1. Percent organic

The ratio between 5 mM ammonium acetate (in water) and methanol in the BGE was changed accordingly, while the amount of acetic acid and highly sulfonated-β-cyclodextrins (HS-β-CD) were held constant. Ratios of 10:1, 5:1, 3:1, and 1:1 were evaluated.

2.3.2. Buffer concentration

The effect of ammonium acetate concentration on the separation was checked at the following levels: 1, 5, 10, and 20 mM.

2.3.3. Acid concentration

The percent of acetic acid in the BGE was tested at the following levels: 0.1, 0.5, 1.0, and 2.0% (v/v). The acid component was switched to formic acid and the percentage in the BGE was tested at 0.1, 0.5, 1.0, and 2.0% (v/v).

2.3.4. Chiral selector selectivity and concentration

The effects of different chiral selectors, including hydroxypropyl-β-cyclodextrins, β-cyclodextrins, highly-sulfonated-αβ-cyclodextrins, and highly-sulfonated-γ-cyclodextrins on chiral resolution were investigated. Starting with a 20% (w/v) solution of the cyclodextrins, the first three were tested at 0.1, 0.2, 0.3, 0.4, and 0.7% (v/v) of the total running buffer. The other two cyclodextrins were only tried at 0.3 and 1.0%. Vancomycin was also evaluated as a chiral selector. Starting with a 100% (w/v) solution of vancomycin in water, concentrations of 1.0, 5.0, 10.0, and 20.0% (v/v) in the running buffer were evaluated.

2.4. Selection of internal standard

The selection of an internal standard was imperative in order provide an assay at the desired lower limits of quantification. With only the difference of a methyl group from the targeted compounds, the internal standard was ideal to provide correction for variability associated with extraction, sample preparation, analyte electrokinetic mobility, and analyte electrospray ionization efficiency.

2.5. Standard preparation and standard curve construction

Starting with a 1 mg/ml neat solution of compounds 1 and 2 (racemic mixture) in water, serial dilutions in plasma were performed at the following concentrations: 50, 25, 10, 5, 2.5, 1.0, 0.5, and 0.25 μg/ml. A 20 μl aliquot of a 5 μg/ml solution of compound 3 (internal standard) was mixed with 100 μl aliquot of each standard.

2.6. Biological sample preparation

A semi-automated liquid–liquid extraction method was used for sample preparation [18,19]. An automatic liquid handling workstation (Quadra-96 model 320, Tomtec, Hamden, CT) was used for all liquid transfers. In an effort to optimize the efficiency of injecting samples onto the CE/MS/MS electokinetically, maximized sample cleanup and reduced biological salt content were desirable. Therefore, a liquid–liquid extraction method was used for sample preparation. Briefly, to 1.5 ml polypropylene microcentrifuge tubes, the following was added: (1) 100 μl of plasma sample or standard, (2) 20 μl internal standard solution (5 μg/ml compound 3 in acetonitrile), (3) 200 μl of buffer solution (0.5 M K_2HPO_4, pH 12), and (4) 1 ml of methyl *t*-butyl ether. Tubes were shaken vigorously for 15 min and centrifuged at high speed for 5 min. The upper (organic) layer was transferred to another set of tubes and evaporated to dryness under nitrogen at 36 °C. The samples were reconstituted in 100 μl of running buffer, vortexed briefly ($\sim$30 s), and transferred to autosampler vials for injection.

2.7. Study sample collection and preparation

Compound 1 was administered to each monkey as a single 3 mg/kg dose by oral gavage. Heparinized plasma samples were collected at pre-dose (0 min), 0.5, 1, 2, 4, 6, 7, and 8 h. Aliquots (200 μl) of the plasma samples were transferred to a 96-tube rack according to established procedures [8,19].

3. Results and discussion

3.1. Separation buffer composition

3.1.1. Representative electropherogram in neat solution

Fig. 3 shows a representative electropherogram for a neat solution containing 2500 ng/ml each, of compounds 1 and 2. The parameters describing this separation, shown in Table 1, suggest an efficient separation ($N > 40\,000$) with adequate resolution ($R_s > 1$) between the enantiomers. Those variables that were important for control of the separation are described in some detail below.

3.1.2. Percent methanol in separation buffer

The effect of methanol in the separation buffer on the electrophoretic capacity factor ($k' = (t_r - t_o)/t_o$) and the separation of the enantiomers are shown in Fig. 4a. The capacity factor for the compounds increases in a sigmoid function as the methanol percentage increases, with the greatest capacity increase as methanol goes from 20 to 30%. The highest resolution was obtained at $\sim$30% methanol. Above this composition, peak broadening neutralized any improvements in the resolution due to greater capacity.

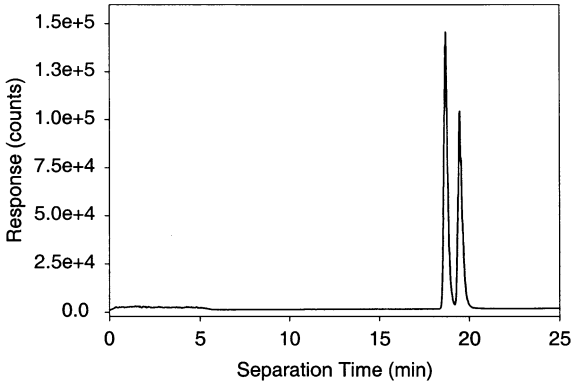

Fig. 3. Electropherograms for a standard containing 1250 ng/ml of compound 1 and its enantiomer from neat solution. An electrokinetic injection of 10 kV for 60 s was used.

Table 1
A comparison of electrophoretic separation parameters for neat and plasma samples containing 2500 ng/ml of compounds 1 and 2, respectively

Matrix	Concentration (ng/ml)	Injection time (s)	Efficiency (N_1)	Efficiency (N_2)	Capacity factor (k'_1)	Capacity factor (k'_2)	Resolution (R_s)
Neat	2500	30	44 000	43 000	2.5	2.6	3.5
Plasma	2500	60	20 700	13 800	1.9	2.1	2.6

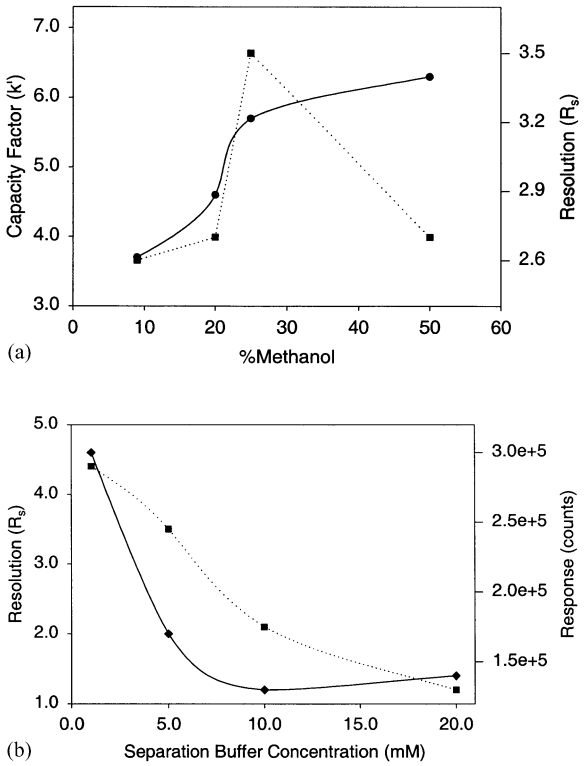

Fig. 4. (a) Capacity factor (–●–, k') and resolution (. . .■. . ., R_s) for compound 1 and enantiomer as functions of methanol percentage in the separation buffer. (b) Resolution (. . .■. . ., R_s) and mass spectral response (–◆–) as functions of separation buffer concentration for compound 1 and its enantiomer.

3.1.3. Concentration of the separation buffer

The effect of running buffer concentration on resolution and response is shown in Fig. 4b. Both the resolution and the mass spectral response decreased as the separation buffer concentration increased. These observations are as expected from the capillary electrophoresis and mass spectrometry perspectives. For capillary electrophoresis, an increase in the buffer concentration contributed to higher conductivity and higher Joule heating, thereby decreasing the separation efficiency [20]. For mass spectrometry, the greater the concentration of buffer introduced into the ion source, the more the analyte molecules must compete for ionization. Decreased sensitivity is the result of this ion suppression [21]. It is, therefore, desirable to keep the concentration of the separation buffer as low as possible, to maximize both enantiomeric resolution and sensitivity.

3.1.4. Acid concentration

Fig. 5a and b show the effect of formic and acetic acid concentration, respectively, on enantiomeric resolution and mass spectral response. Although the resolution at very low formic acid concentration was clearly the best, acceptable resolution was also obtained using higher concentrations (0.5–1%) of acetic acid. Mass spectral response was modestly higher for these acetic acid levels and for this reason acetic acid was selected as the acid modifier for the separation buffer.

3.1.5. Cyclodextrin selectivity and concentration

Five varieties of cyclodextrins were evaluated for the enantiomeric separation of compounds 1 and 2. No separation was detected for the highly sulfonated (HS) α- or γ-cyclodextrins and these were not pursued. Similar lack of resolution was noted for β-cyclodextrin and Hydroxypropyl β-cyclodextrins. For the analytes under consideration here, the only enantiomeric resolution was obtained using HS β-cyclodextrin at concentrations varying from 0.1 to 0.7% (w/v). Higher concentrations of this cyclodextrin resulted in increased variability of the peak ratio response of the enantiomers. This previously reported phe-

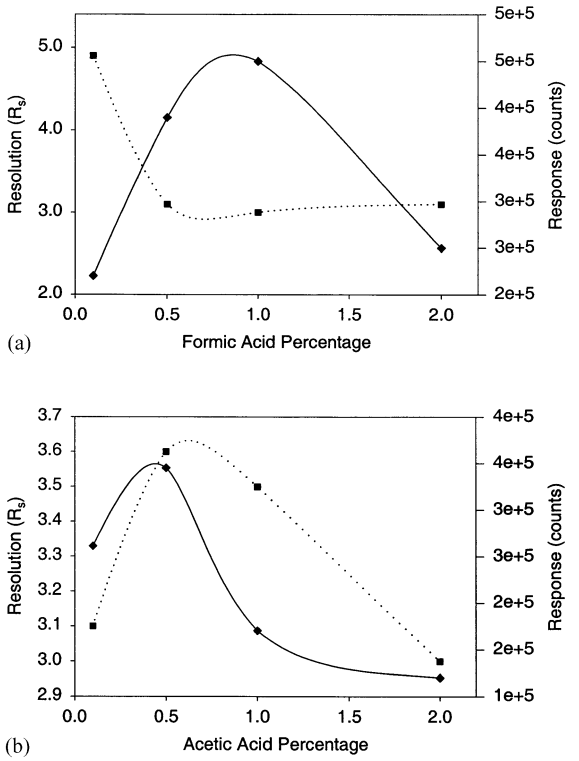

Fig. 5. Resolution (...■..., R_s) and mass spectral response (–◆–) for compound 1 and its enantiomer as functions of, (a) percent formic or; (b) percent acetic acid in the separation buffer.

nomena [22] will be discussed below. To minimize this variability, a lower concentration of HS β-cyclodextrin (0.3%) was selected for routine use.

3.1.6. Vancomycin selectivity

Vancomycin was evaluated as an alternative chiral selector [23], replacing the highly sulfonated cyclodextrin. Unfortunately, although a range of vancomycin concentrations (1, 5, 10 and 20% (w/v)) were evaluated, with vancomycin replacing the cyclodextrin in the normal running buffer, no chiral resolution was observed for the test compounds evaluated here. Although the chiral separation was not effective in this example, the peak shapes and efficiencies for the analyte were comparable to those observed with cyclodextrins. At 10 and 20% vancomycin, the electrophoretic peaks broadened significantly, possibly due to Joule heating in the capillary. In other situations where effective chiral separations can be obtained, vancomycin appears to be a viable alternative chiral selector for bioanalysis.

3.1.7. Pressure injection versus electrokinetic injection

Both types of sample introduction were investigated for use with this quantitative assay. Both techniques showed that sample was injected. With the increasing injection volume, pressure injections were not able to provide the adequate separation of the two compounds. The volume of injection was decreased in order to provide resolution, but the desired limit of quantitation was unattainable. The electrokinetic injection offered both adequate quantitation and resolution and was not complicated by injection volume. It is possible that greater sensitivity could be obtained using a peak-stacking technique [24], but this was not evaluated.

3.2. Variability of enantiomeric peak intensity

3.2.1. Internal standard selection and data treatment rationale

In this work, compound 3 was chosen as an internal standard. This compound, being a derivative of the same series as the analytes, required minimal additional optimization for sample preparation, or CE/MS/MS parameters. It yielded reasonable extraction efficiency following the liquid–liquid method. Electrophoretic running conditions optimized for compounds 1 and 2, provided reasonable retention time for the internal standard as well. Compound 3 not only exhibits structural similarity to the enantiomers (compounds 1 and 2), but also is itself a racemic mixture of a pair of stereoisomers due to the fact that it contains a related chiral center. Under the CE/MS/MS conditions developed for the determination of compounds 1 and 2, compound 3 was also resolved into its enantiomers. This significant revelation demonstrates that the CE/MS/MS conditions for this chiral assay could also be applied to similar compounds of interest without significant modification.

Some of the analytical problems encountered during the method development for compounds 1

and 2 were also observed with compound 3, particularly inconsistent peak ratios and resolution between the enantiomers. These problems contributed to variability in the precision and accuracy of our results whenever one of the internal standard enantiomers was used in quantification. Although either of the enantiomers of compound 3 could occasionally be used to quantify an acceptable run for compound 1 or 2, we chose not to further modify our running conditions to correct this situation. Instead, modifying the post-run integration parameters for the internal standard provided the simplest solution. By increasing the number of smoothes and window size (scans) within the chromatographic smoothing method, the two enantiomers of compound 3 could be blended into a single peak. Therefore, any inconsistencies in peak ratio or resolution for one enantiomer within the internal standard electropherogram were compensated for by the inclusion of the other enantiomer and calculated analyte variability was subsequently reduced.

Derivatized cyclodextrins are chiral, neutral oligosaccharides with a hydrophilic exterior and a relatively hydrophobic interior. Their capability of forming complexes with analytes is strongly influenced by the hydrophobic interaction of the interior cavity, as well as the interaction between the analyte and substituents at the perimeter of the CD. The higher affinity for one of the enantiomers for the CD is a result of better steric orientation in the CD-analyte inclusion complex [25]. Although the free drug may be in dynamic equilibrium with the cyclodextrin as it migrates through the capillary, the amount of free drug detected by the mass spectrometer may be influenced by the dissociation of the complex during nebulization [22]. Therefore, inconsistencies in abundance between each free enantiomer may be due to differences in their effective binding constant for the drug–cyclodextrin complex, as well as inconsistencies in the source fragmentation of each enantiomer from the CD.

In the development of this method, an effort was made to detect the CD inclusion complex on the mass spectrometer by adding the expected m/z ion current profile, as well as scanning at a higher mass range. This effort was unsuccessful, mostly likely because HS-β-CD are negatively charged over the entire pH range which may cause the complex to ionize in the opposite electrospray mode. Time-of-flight mass spectrometry was also used in attempts to observe the analyte: HS-β-cyclodextrin inclusion complex. The approximate molecular weight of this complex was thought to be 2540 Da. Although both positive and negative, as well as single and multiply charged, ions were considered, the inclusion complex was not evident.

It was observed that decreasing concentrations of HS-β-CD in the running buffer deteriorated the resolution between the enantiomers (Fig. 6), while response remained relatively constant and the variability in their relative abundance was reduced. Therefore, the concentration of HS-β-CD that we selected for the running buffer (0.3%) allowed the minimal acceptable separation between the enantiomers, but increased the reproducibility in their relative abundance by more than 15%. Also, when comparing electropherograms, the variability in intensity of both enantiomers resulting from the formation of the CD inclusion complex appeared to be compensated for by a corresponding fluctuation in internal standard intensity.

3.3. Performance with plasma extracts

3.3.1. Representative plasma electropherograms

Fig. 7 shows three electropherograms representing (a) a spiked sample containing 2500 ng/ml,

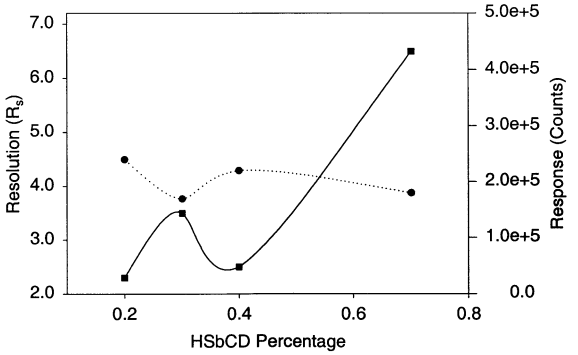

Fig. 6. Resolution (. . .■. . ., R_s) and mass spectral response (–◆–) for compound 1 and its enantiomer as a function of percent highly sulfonated β-cyclodextrans in the separation buffer.

each, of compounds 1 and 2 extracted from rat plasma, (b) the separation of the two internal standard enantiomers and (c) the two internal standards smoothed to form a single homogeneous peak.

Of note in this electropherogram is that the separation parameters for the plasma sample (Table 1) are significantly different from those for the neat electropherogram (Fig. 3). The efficiency (N) for the plasma separation is about half of that for the neat sample. Some peak broadening has occurred with the plasma sample, and this is likely due to the high ionic strength of the plasma extract relative to clean solution. Relative capacity factors for the analytes changed slightly, so that the selectivity (α) increased from 1.04 to 1.11 and this helped to offset the decrease in resolution brought on by higher sample ionic strength. Although the resolution for the plasma work has decreased to 2.6, the approach can distinguish between enantiomers to within 5%.

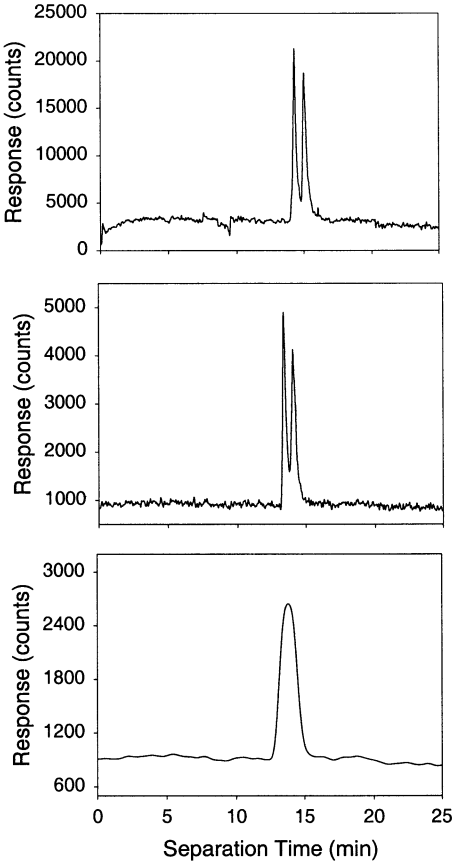

Fig. 7. Electropherograms for (a) a standard containing 2500 ng/ml of compound 1 and its enantiomer, (b) the internal standard at critical smoothing and (c) the internal standard over smoothed after extraction from plasma.

3.3.2. Effects of reconstitution volume on separation

It was apparent that the efficiency of the electrophoretic separation was dependent upon the volume of running buffer used to reconstitute the dried extract. This phenomenon, then, presents a direct trade-off between assay sensitivity and separation quality. Smaller (<100 μl) reconstitution volumes allow better sample utilization and can improve the detector response by introducing more mass on capillary. Unfortunately, small reconstitution volumes allow for a higher sample ionic strength and lead to increases in peak tailing, loss of efficiency and decreased resolution. In this work, 200 μl of plasma, extracted and brought up in 100 μl of running buffer, appeared offer the best compromise between separation performance and sensitivity.

3.3.3. Performance of standard curves

Table 2 displays results from a representative standard curve used for assessing quantitative performance. For calibration curves ranging from 0.250 to 50.0 μg/ml of each enantiomer in plasma (200 μl of plasma extracted, dried down and reconstituted in 100 μl of running buffer), the average percent deviation in the back-calculated concentrations was less than 15.5%, thereby indicating quantitative performance. The signal-to-noise (S/N) ratio at the lowest level of these curves was approximately 10, suggesting readily detectable peaks. At levels below 0.10 μg/ml, the peaks became indistinguishable from baseline noise.

3.3.4. Limit of quantitation

Fig. 8 shows an electropherogram for a plasma sample spiked with compounds 1 and 2 at the 0.01 μg/ml level (i.e. 5 ng/ml of each component). In order to achieve detectability at this low concentration level, 1.0 ml of plasma was extracted, the extract evaporated to dryness and reconstituted in

Table 2
Plasma standard curve summary for compounds 1 and 2, respectively, over the calibration range from 250 to 50 000 ng/ml

Compound	Calibration type	Weighting	Regression coefficient a[a]	Regression coefficient b[a]	Regression coefficient c[a]	Coefficient of determination (r^2)	Maximum % deviation[b]	Average % deviation[b]
1	Quadratic	$1/x^2$	$1.81e-5$	0.0196	0.00911	0.9821	±19.1	±9.1
2	Quadratic	$1/x^2$	$5.63e-5$	0.0172	0.0206	0.9966	±46.5	±15.5

[a] For a quadratic regression equation defined as $Y = aX^2 + bX + c$.
[b] Percent deviation in back-calculated standard curve concentrations.

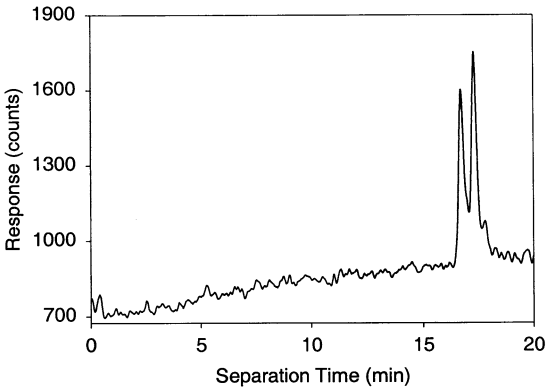

Fig. 8. Electropherogram for a standard contains 5 ng/ml of each analyte. To achieve this detection limit, 1 ml of plasma was extracted and concentrated to a final volume of 100 μl. An electrokinetic injection of 10 kV for 60 s was used.

100 μl of running buffer. This electropherogram clearly shows resolution of the two enantiomers at a reasonable S/N ratio. Again, some loss of resolution is present due to the concentration of matrix components in the sample. If the need for high concentration sensitivity does arise, concentrations that are competitive with other techniques can be achieved. A tradeoff with the performance of the separation has been noted.

3.3.5. Ruggedness of the approach

There were two important observations involving the ruggedness of this approach, including the reproducibility of the capillary separation and the cleanliness of the ion-source sample cone upon extended use. The separation appeared to be reproducible from day-to-day, with consistent chiral resolution being observed over time. A small decrease (from $\sim$2.5 to 2.0) in analyte capacity factor was noted over a period of weeks. It is likely that this decrease in capacity resulted from ionic binding of matrix components to silanol sites in the capillary. Although we felt it to be unnecessary, the original performance of the capillary could have been restored by stripping the surface of the capillary with sodium hydroxide. The inter-injection variability in retention time was 6% for 16 consecutive plasma injections and the retention difference between initial and final injections was

1.3 min (8%), indicating adequate inter-injection ruggedness.

The fouling of the ion source cone can be a significant detriment to long term operation of API/MS systems. When the correct mobile phase components are selected, modern commercial instrumentation allows at least several hundred injections before source cone cleaning is required. With the running buffer and make-up flow components selected here, the ion source cone did not need any additional cleaning or maintenance relative to usual operation as a LC/MS system.

4. Study results

Fig. 9 shows an electropherogram of a plasma sample from a monkey drawn 1 h after being dosed with 3 mg/kg of compound 1. Due to the high selectivity associated with this technique, the electropherogram is very clean, showing only the dosed compound and a small peak corresponding to its enantiomer. The concentration of the dosed enantiomer in this sample was found to be ~ 0.3 μg/ml. This electropherogram suggests that the extent of in vivo enantiomeric interconversion for compound 1 is about 8% over this time frame. A larger or smaller degree of enantiomeric interconversion could be monitored as necessary.

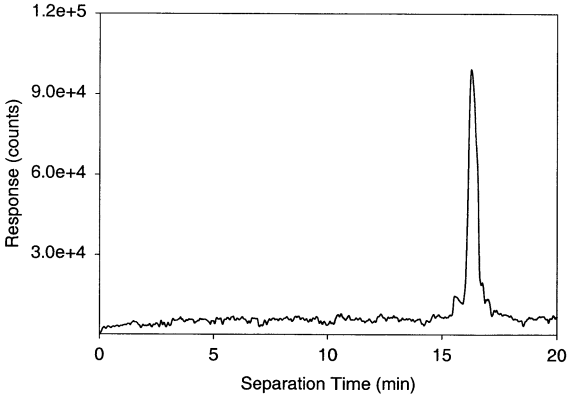

Fig. 9. Electropherogram for a study sample drawn at 1 h from a monkey dosed with 3 mg/kg of compound 1.

5. Concluding remarks

Capillary electrophoresis/electrospray mass spectrometry appears to have utility in bioanalysis as a technique for chiral methods. This approach could be successfully applied to study the biological interconversion of a pure, dosed enantiomer or the clearance properties of racemic drugs. Some of the difficulties that persist with this technique include low sample utilization leading to poor concentration sensitivity, higher variability than LC/MS, and sensitivity of the separation due to the ionic strength of the matrix. To some extent, these problems can be compensated for by inclusion of an appropriate internal standard and by careful selection of the operating parameters for the separation. Additional advances in sensitivity are still needed and the availability of these could improve the acceptability of the technique in the future. Capillary electrophoresis/mass spectrometry appears to have a convenience advantage over normal-phase LC/MS separations for pharmacological characterization of chiral drugs.

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