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Determination of chiral pharmaceutical compounds, terbutaline, ketamine and propranolol, by on-line capillary electrophoresis–electrospray ionization mass spectrometry

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Abstract

On-line capillary electrophoresis–electrospray ionization mass spectrometry (CE–ESI-MS) has been employed for the determination of racemic mixtures of the chiral drugs, terbutaline, ketamine, and propranolol. Separation of the different chiral forms has been achieved by introducing cyclodextrins (CDs), which act as chiral selectors, into the CE operating electrolytes. Cyclodextrins function as chiral selectors in CE because of their ability to form host–guest complexes (inclusion complexes) of varying stability with an array of chiral drugs and other compounds. Derivatized forms of β-CD (i.e., dimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin) were used in this study due to their higher solubilities in the aqueous methanolic operating electrolyte than native β-CD. Addition of minor quantities of methanol to the aqueous-based CE operating electrolytes improved the stability of electrospray ionization conditions and further enhanced CE resolution of the enantiomeric pairs relative to purely aqueous systems. Introduction of the CDs into the CE operating electrolytes caused suppression of analyte signals in ESI-MS, and the dependence of analyte signal intensities on the solution concentrations of the derivatized β-CDs was examined. Under optimized conditions, the different enantiomeric forms of the compounds under investigation were successfully separated and detected by CE–ESI-MS.

Keywords: Enantiomer separation; Terbutaline; Ketamine; Propranolol

1. Introduction

Isolation and structural characterization of individual chiral forms of drugs are necessary steps for investigating the pharmaceutical and toxicological properties of each enantiomer. Many currently used drugs contain chiral centers and one isomer of a given drug may not have equivalent therapeutic value, or potentially could cause unwanted side effects, relative to another isomer [1,2]. Consequently, many commercial drugs are dispensed in a single chiral form. Because of the need to study properties of individual drug enantiomers, the development of methods to separate forms of different chirality has been a research focus for many separation scientists.

In recent years, as an alternative to HPLC, capillary electrophoresis (CE) has been used to separate chiral forms of pharmaceutical compounds. Compared to HPLC separations [3–5], CE offers the potential for higher resolution and faster analysis of chiral compounds owing to its unique flow profile and efficient heat dissipation (small diameter capillaries) [6]. Another advantage of using CE is that the total volume of solution eluted from the separation

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capillary is much less than that eluted from HPLC columns. When on-line electrospray ionization (ESI) mass spectrometry is used as a detector for CE, a very small volume of high concentration nonvolatile additives enters the electrospray source. The lower volume results in smaller quantities of electrolytes capable of suppressing analyte signals, hence, improved detection sensitivity as compared to HPLC.

There are two general strategies most frequently used for the separation of enantiomers. The first is manufacturing chiral stationary phase columns (the main drawbacks are the relatively limited lifetimes and the high cost). The second approach is adding chiral selectors to the mobile phase (electrolyte). If additives are employed in the mobile phase, an advantage of utilizing CE over HPLC for chiral drug separation is that smaller quantities of expensive chiral selectors are required owing to the very small capillary volumes characteristic of CE. Several kinds of cyclodextrins have been used as chiral selectors in CE buffers to separate enantiomeric forms of various drugs [7] including terbutaline [8–10], ketamine [9], and propranolol [8,9,11].

CE coupling to MS detection offers certain advantages over optical detection methods, such as access to specific molecular mass and structural information [12–15]. Since electrospray ionization efficiently creates gas phase ions out of nonvolatile compounds present in solution, it is considered as a preferred mass spectrometric ionization method for interfacing to CE systems. In a previous study [10] employing CE–MS for determination of the chiral drugs, terbutaline and ephedrine, a nonvolatile phosphate salt was used as background electrolyte. CE determinations can be accomplished using relatively high concentrations of chiral selectors (5–100 m*M*) and nonvolatile electrolytes in the CE buffers to achieve separation of the chiral compounds. When optical detection techniques are employed, such additives will not necessarily impede detection if they are not light-absorbing at the wavelength employed for detection of the analytes. However, high additive concentrations are usually not amenable to electrospray ionization mass spectrometry. Nonvolatile salts will rapidly accumulate on the surfaces of electrospray source parts and degrade the mass spectrometry detection sensitivity [16–18]. Furthermore, chiral selectors such as cyclodextrins present in high concentration, may compete with the analyte for available charge and thus suppress the analyte signal.

To date there are few examples showing the determination of different chiral drugs employing CE–ESI-MS. In this work we investigate the conditions for the separation of enantiomers of the chiral drugs ketamine, propranolol, and terbutaline using on-line CE–ESI-MS. Various substituted cyclodextrins and CE electrolytes were employed in view of optimizing CE separation, while enabling ESI-MS detection at adequate sensitivity.

2. Experimental

2.1. Chemicals

All solutions were prepared using HPLC-grade water and HPLC-grade methanol purchased from EM Science (Gibbstown, NJ, USA). Chiral drugs, ketamine and terbutaline were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and propranolol from Aldrich Chemical Co. (Milwaukee, WI, USA). These drugs were purchased as racemic mixtures. Chiral selectors, dimethyl-β-cyclodextrin (DM-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were purchased from Sigma and Aldrich, respectively. Glacial acetic acid purchased from J. T. Baker (Phillipsburg, NJ, USA) and ammonium acetate obtained from Aldrich, were used as the background electrolytes in capillary electrophoresis. All chemicals were used without further purification.

2.2. Capillary electrophoresis

CE separations were performed using a CE System I (Dionex Corp., Sunnyvale, CA, USA). Fused-silica capillaries (100 μm ID×245 μm OD×100 cm length) obtained from Polymicro Technologies (Phoenix, AZ, USA) were employed in all experiments. Before use, the new capillaries were washed with water for 20 min, followed by operating buffer for 20 min. Between successive runs the capillary was washed with operating buffer for 10 min. The electrolyte in the CE source vial was replaced every other run to minimize chemical contamination and concentration changes due to evaporation. The capillaries were stored overnight by placing both ends of

the capillary in water with a height differential to maintain electrolyte flow through the capillary.

2.3. Electrospray ionization mass spectrometry

A Vestec 201 electrospray mass spectrometer (Vestec Corp., formerly of Houston, TX, USA) was employed for on-line CE–ESI-MS experiments. The employed voltages at the electrospray needle and flat plate counter electrode were approximately 2.5 kV and 290 V, respectively. Collision-induced dissociation was minimized in all experiments by maintaining a low skimmer-collimator voltage difference (7 V). The mass spectrometer scan range for all experiments was from m/z 100 to 600 at a scan rate of 3 s/scan. A Sage syringe pump (Orion Research, Boston, MA, USA) was used for direct infusion experiments, as well as for the delivery of the sheath flow of liquid during CE–ESI-MS.

2.4. CE–ESI-MS interface

The CE–ESI-MS interface used in this study has been described previously [19]. The coaxial sheath flow [15] solution was 1% acetic acid in methanol, delivered at a rate of 2.5 μl min^{-1} through the 350 μm ID, 27.5 cm length, stainless-steel 'needle' surrounding the 245 μm OD CE capillary. Pneumatic assistance for ESI was not employed. The CE capillary tip was positioned about 0.1 mm outside the stainless-steel needle to maximize sensitivity [19]. MS operating conditions were optimized by adjusting the needle-counter electrode distance, chamber temperatures, liquid sheath flow-rates and applied electrospray potentials while a test analyte was continually introduced through the CE–ESI-MS interface. CE–ESI-MS runs were subsequently performed under these same optimized conditions.

3. Results and discussion

3.1. Effects of cyclodextrin concentration on the mass spectral signal intensities of the chiral drugs

The structures of the chiral compounds, terbutaline, ketamine and propranolol are shown in Fig.

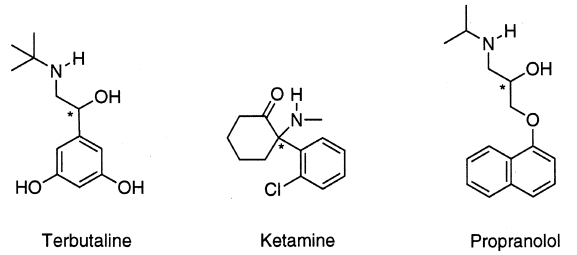

Fig. 1. Structures of chiral drugs: terbutaline, ketamine and propranolol; asterisks indicate chiral centers.

1. Cyclodextrins (CDs) and their derivatives are frequently used chiral selectors in enantiomeric separations. Cyclodextrins are commercially available in various ring sizes [e.g., α-CD (six glucose units), β-CD (seven glucose units), etc.] and types (e.g., derivatized CDs) that can be dissolved readily in separation buffers. Chiral recognition occurs because of the selective complexation of one enantiomer with the cyclodextrin molecule. The size of the cavity of the CD, which is determined by the number of glucose units constituting the cyclodextrin ring, plays an important role in stabilizing inclusion of the hydrophobic portion of the solute on the interior of the cavity. Derivatized CDs [i.e., dimethyl-β-cyclodextrin (DM-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD)] were used in this study mainly because they have higher solubilities in aqueous/organic solvents than the native CDs. The β-CD derivatives are synthesized by converting the secondary hydroxyls at the rim to various substituent groups. The two derivatized CDs, DM-β-CD and HP-β-CD, have been found to be suitable for forming inclusion complexes with compounds bearing one or two aromatic rings [11]. It is also known that the electrophoretic mobilities of inclusion complexes of substituted CDs are often lower than those of native CDs, which can increase the electrophoretic mobility difference between CD-analyte complexes and free enantiomeric analytes, and thus provide a better separation of a pair of enantiomers [20,21].

In a recent CE-ion spray mass spectrometry report describing chiral drug analysis [10], it was indicated that the presence of dimethyl-β-CD in the CE buffer up to 20 mM caused no apparent decrease in overall sensitivity of the mass spectrometer. However, under our experimental conditions, the addition of various

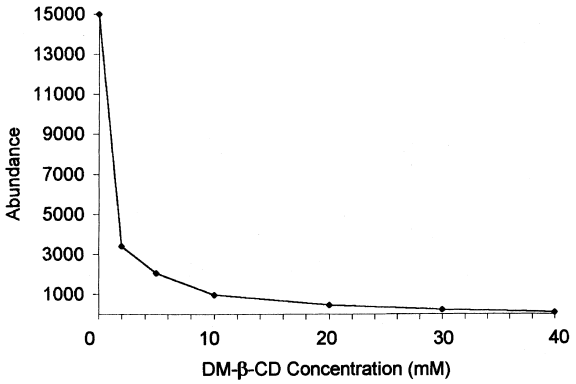

Fig. 2. Dependence of ESI-MS signal (arbitrary units) of protonated terbutaline (m/z 226) on the concentration of DM-β-CD in operating CE buffer consisting of 0.8 *M* acetic acid and 5 m*M* ammonium acetate in 80:20 methanol–water (v:v). The concentration of terbutaline is 10^{-4} *M*.

CDs to the CE electrolyte caused a degree of analyte signal suppression in the CE–ESI-MS analysis of chiral drugs. To evaluate the magnitude of the suppression effect of CD on the analyte signal, direct infusion ESI-MS of the three chiral drugs in the CE separation media containing varying levels of CD was performed. Figs. 2 and 3 show the dependences of ESI-MS signal intensities on the concentration of DM-β-CD for the chiral drugs, terbutaline and ketamine, respectively. Protonated molecules (MH^+) were detected by the electrospray mass spectrometer. It can be seen from Fig. 2 that the intensity of the terbutaline signal decreased sharply when CD concentration changed from 0 to 2 m*M*, with a continued steady decrease at higher concentrations. In these experiments, the terbutaline concentration was 10^{-4} *M*. When the CD concentration was 5 m*M*, the peak intensity of protonated terbutaline was about 10% of that observed in the absence of CD. For ketamine (Fig. 3), a similar sharp decrease in protonated analyte signal was observed upon addition of 2 m*M* DM-β-CD, followed by a similar leveling trend as that observed for terbutaline. Notably, the signal intensity of protonated ketamine was higher at each DM-β-CD level than the analogous sample solution containing terbutaline.

The analyte signal for another chiral drug, propranolol, was examined in the presence of increasing concentrations of two derivatized CDs, DM-β-CD and hydroxypropyl-β-CD (HP-β-CD). HP-β-CD was tested because DM-β-CD was found to not provide adequate CE separation of propranolol enantiomers, even at high concentration (40 m*M*), where very little analyte signal was visible. Fig. 4 shows the signal suppression effect of DM-β-CD and HP-β-CD on propranolol. While HP-β-CD suppresses propranolol signal to a slightly higher extent than DM-β-CD, suppression in either case is less than that observed for the other two drugs.

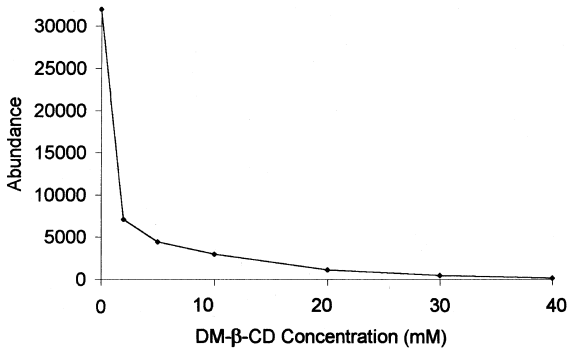

Fig. 3. Dependence of ESI-MS signal (arbitrary units) of protonated ketamine (m/z 238) on the concentration of DM-β-CD in the same operating CE buffer as shown in Fig. 2. Concentration of ketamine is 10^{-4} *M*.

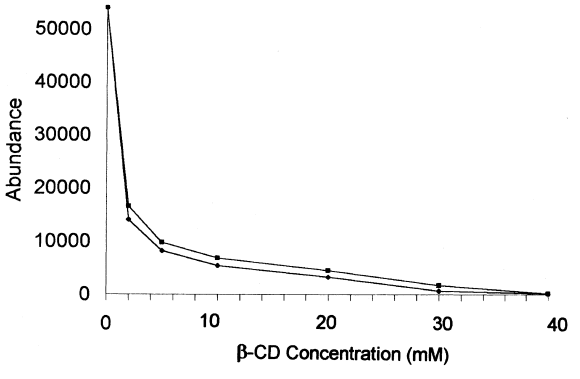

Fig. 4. Dependence of ESI-MS signal (arbitrary units) of protonated propranolol (m/z 260) on the concentration of (■) DM-β-CD and (♦) HP-β-CD in the same operating CE buffer as shown in Fig. 2. Concentration of propranolol is 10^{-4} *M*.

3.2. On-line CE–ESI-MS determination of chiral drugs in CD-containing electrolytes

Several investigators have reported that adding organic solvent into CE buffers containing chiral selectors will enhance the ability of CE to resolve enantiomers [3–5,11]. Furthermore, previous studies have shown that organic/water mixtures or nonaqueous CE–ESI-MS media provide more stable electrospray conditions and higher detection sensitivities than purely aqueous media. It is known that addition of methanol to purely aqueous systems permits droplet breakup at lower applied ESI voltages because the surface tension has been lowered [22]. Moreover, the relative ease of solvent evaporation in the methanol/water mixtures may result in an improved desorption efficiency of protonated analytes. For these reasons, mixed aqueous/organic media were used in this study.

Because selective host–guest interactions between analytes and CDs can occur for both charged and uncharged enantiomers [23], solution pH has a major effect on the ability to separate enantiomers. Furthermore, changing the pH of the CE buffer will change the charge state of the derivative CDs, and so change their electrophoretic mobilities [20,21]. In this study, the pH effect was briefly examined with addition of acetic acid to the CE operating electrolyte. It was observed that enantiomers of chiral drugs could be resolved when the acetic acid concentration was about 0.8 *M*. In these studies, the volatile buffer constituents ammonium acetate [24–26] and acetic acid [16,18] were used to preserve ESI-MS sensitivity and minimize ion source fouling problems which arise when nonvolatile electrolytes are used.

An electrolyte containing 5 m*M* DM-β-CD, 0.8 *M* acetic acid and 5 m*M* ammonium acetate made up in methanol–water (80:20, v/v) was used as the operating buffer for on-line CE–ESI-MS determinations of the chiral compounds. A sample solution was prepared with a racemic mixture of terbutaline at a concentration of 0.1 m*M* in operating buffer. With the CE 'source vial' voltage set at 25 kV, the CE separation current was stable at about 13 μA. Fig. 5 shows the on-line CE–ESI-MS electropherogram obtained from a single run of the chiral drug terbutaline in the operating buffer. This selected ion electropherogram (m/z 226) shows the detection and

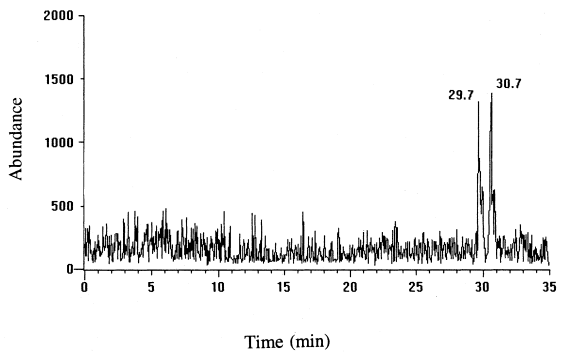

Fig. 5. Selected-ion electropherogram showing raw data from on-line CE–ESI-MS analysis of terbutaline (MH^+, m/z 226). Experimental conditions: sample concentration: $10^{-4}M$; CE voltage: 25 kV; capillary: 100 μm ID×100 cm; buffer: 5 m*M* DM-β-CD, 5 m*M* ammonium acetate and 0.8 *M* acetic acid in methanol–water (80:20, v/v); gravity injection: 15 cm for 2 s; ES voltage: 2.48 kV; MS scan range: m/z 100–600; MS scan rate: 3 s/scan.

baseline resolution of two protonated enantiomeric components representing enantiomeric forms of terbutaline whose migration times differed by about one min (29.7 vs 30.7 min). It has been reported that (+)-terbutaline migrates out of the CE capillary with a lower velocity than the (−)-isomer under similar separation conditions [8]. Baseline resolution was not achieved when the DM-β-CD concentration was less than 5 m*M*, although the MS signal intensities improved at lower concentrations of DM-β-CD. For a detection limit defined as $S/N=3$ the lower limit of detection of terbutaline in 5 m*M* CD was about 5 x 10^{-5} *M* employing the full scan (m/z 100–600) mode. If data had been acquired in the selected ion monitoring (SIM) mode, signal improvement (e.g., $(500)^{1/2}=22$-fold) is possible, although SIM is only useful for determinations of known compounds.

A racemic mixture of ketamine was analyzed by on-line CE–ESI-MS, again using 0.8 *M* acetic acid and 5 m*M* ammonium acetate in 20% methanol containing DM-β-CD. The enantiomeric components of ketamine could not be adequately separated at DM-β-CD concentrations below 15 m*M*. The CE–ESI-MS selected-ion electrophoregram (m/z 238) representing protonated molecules (MH^+) of the chiral drug ketamine is shown in Fig. 6. The detected enantiomeric components were baseline resolved

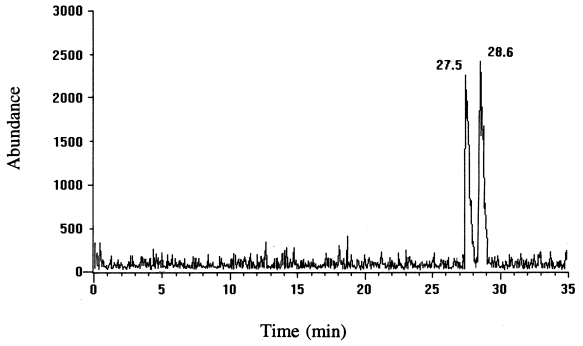

Fig. 6. Selected-ion electropherogram showing raw data from on-line CE–ESI-MS analysis of ketamine (MH^+, m/z 238). DM-β-CD concentration was 15 m*M*; other experimental conditions were the same as in Fig. 5.

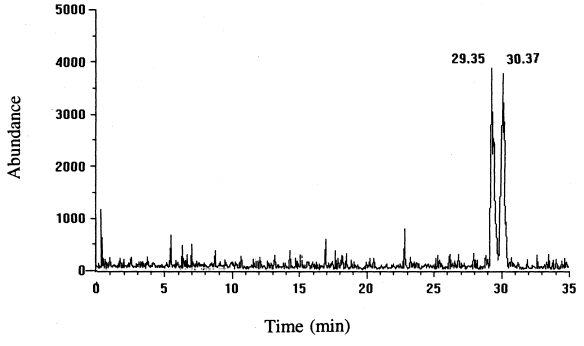

Fig. 7. Selected-ion electropherogram showing raw data from on-line CE–ESI-MS analysis of propranolol (MH^+, m/z 260). HP-β-CD concentration was 20 m*M*; other experimental conditions were the same as in Fig. 5.

with migration times of 27.5 and 28.6 min. With a starting sample concentration of 0.1 m*M*, the detection limit was about 10^{-5} *M* for ketamine employing mass spectrometer scans from m/z 100 to 600. The CE current was 16 μA compared to 13 μA for the determination of terbutaline, due to the higher concentration of DM-β-CD. This CE current permitted stable operation of the ESI-MS. However, slightly higher CE currents can cause CE–ESI-MS operation to become unstable because of the decreasing ability to maintain distinct voltages at the CE source vial and the electrospray needle [18,19].

An initial test for the on-line CE–ESI-MS analysis of the chiral drug, propranolol, was carried out with the same operating buffer as employed for analysis of terbutaline and ketamine. However, the enantiomeric components of the chiral drug could not be adequately resolved at any DM-β-CD concentration up to 40 m*M*, where the mass spectrometer signal for protonated propranolol was barely detectable. Consequently, another derivatized β-CD, HP-β-CD was chosen as the chiral selector because it showed less signal suppression (Fig. 4) and could adequately separate the enantiomeric components of propranolol [11]. The concentration of the HP-β-CD was gradually increased in the CE operating buffer until the enantiomers of propranolol were well resolved at a concentration of 20 m*M* of HP-β-CD. The selected-ion electropherogram (m/z 260, Fig. 7) shows the detection of two protonated enantiomeric components whose migration times were 29.4 min and 30.4 min, representing (*S*)-(−)-propranolol and (*R*)-(+)-propranolol, respectively [8,9]. Because propranolol showed a higher signal response than the previous two drugs, even though a relatively high concentration of HP-β-CD (20 m*M*) was used, the CE–ESI-MS detection limit was still similar to that of ketamine.

4. Conclusion

Derivatized β-CDs contained in water/methanol electrolyte mixtures have proven to be useful for CE–ESI-MS determinations of individual enantiomers of chiral drugs. The combination of both derivatized β-CDs and organic solvent seem to be vital for sufficient resolution of enantiomers and adequate signal response. Even though the presence of CDs tends to lower ESI-MS detection sensitivity, a compromise can be reached to offer acceptable detection ability and adequate separation from a single CE–ESI-MS condition. While single-stage mass spectrometric detection cannot distinguish between different chiral forms (or inclusion complexes of enantiomeric pairs) exhibiting zero mass difference, it is still highly useful for confirming that observed peaks do indeed correspond to an enantiomeric pair. It can also differentiate these peaks from any extraneous peaks arising from contaminants, background interferences, or other co-eluting compounds that may be present in the sample matrix.

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