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HPCE determination of $R(+)$ and $S(-)$ mepivacaine in human serum using a derivatized cyclodextrin and ultraviolet detection

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Abstract

A high performance capillary electrophoresis assay for the quantitative determination of $R(+)$ and $S(-)$ mepivacaine in human serum is reported using heptakis (2,6-di-*O*-methyl) β-cyclodextrin as the chiral selector for the separation of the enantiomers. The background electrolyte was a 100 mM phosphate buffer (pH 2.5) containing 20 mM heptakis (2,6-di-*O*-methyl) β-cyclodextrin and 30 nM hexadecyltrimethylammonium bromide (HTAB). A 72 cm uncoated fused silica capillary was used for the analysis. HTAB was used as the buffer additive to decrease the adsorption of endogenous substances onto the silica wall. To separate the analytes of interest from the endogenous serum substances, a liquid–liquid extraction procedure was used. The extraction recoveries were greater than 70% for both $R(+)$ and $S(-)$ mepivacaine. The detection limits were around 150 ng ml^{-1} using 1 ml of serum and the limits of quantitation were 200 ng ml^{-1}. The calibration curves were linear over a range of 200–2000 ng ml^{-1} with $R(-)$ prilocaine as internal standard (IS) and coefficients of determination were greater than 0.999 ($n=3$). Precision and accuracy of the method were 4.1–7.2 and 2.6–5.9%, respectively, for $R(+)$ mepivacaine and 4.0–7.4 and 3.2–7.4% for respectively, for $S(-)$ mepivacaine. The HPCE method was compared to an existing HPLC method in terms of sensitivity and selectivity for the routine analysis of the drugs. © 1997 Elsevier Science B.V.

Keywords: HPCE; Chiral; Heptakis (2,6-di-*O*-methyl) β-cyclodextrin; Quantitation; Human serum

1. Introduction

Recently, high performance capillary electrophoresis (HPCE) has evolved to be the technique of choice for the resolution of enantiomers. The principal advantages of capillary electrophoresis are its simplicity, high efficiency and low sample consumption [1–6]. Chiral separations in HPCE have been achieved using various background electrolyte additives as chiral selectors. Some of the chiral selectors used to date are cyclodextrins (CD) and their derivatives, crown ethers, optically active micelles, macrocyclic antibiotics and carbohydrates [7–13]. The most commonly used chiral selectors are native and derivatized cyclodextrins. Native CDs such as α, β and γ cyclodextrins contain 6,7, and 8 D(+)

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glucopyranose units linked together by 1–4 glycosidic bonds, are inherently chiral, and undergo chiral interactions with analytes. Enantio-recognition is a combination of inclusion phenomenon and additional interactions with the functional groups on the rim of a cyclodextrin. The various derivatized CD have increased solubility compared to the native ones and undergo additional interactions with the analytes, thereby enhancing chiral recognition.

Mepivacaine is a local anesthetic of the amide type which is used for all types of infiltration and regional nerve block anesthesia. It is marketed as a racemic mixture of $R(+)$ and $S(-)$ mepivacaine. Both enantiomers differ in their biological activity with the $S(-)$ being more biologically active than the $R(+)$ mepivacaine and the plasma levels of $S(-)$ being higher than $R(+)$. HPLC resolution of $R(+)$ and $S(-)$ mepivacaine has been reported by this laboratory and others using a chiral stationary phase along with the quantitation of enantiomers in human serum [14–16].

This paper reports an HPCE method for the quantitation of $R(+)$ and $S(-)$ mepivacaine in human serum using heptakis (2,6 di-*O*-methyl)-β-cyclodextrin (DM-β-CD) as the chiral selector. The typical blood levels are between 0.36–1.46 μg ml^{-1} for $R(+)$ and 0.76–2.2 μg ml^{-1} for $S(-)$ mepivacaine [15]. The method is linear in the range of 200–2000 ng ml^{-1} for each enantiomer and provides the necessary sensitivity and selectivity for monitoring the blood levels of $R(+)$ and $S(-)$ mepivacaine.

2. Experimental

2.1. Reagents and chemicals

Racemic mepivacaine HCl, $R(+)$, $S(-)$ mepivacaine HCl and $R(-)$ prilocaine HCl were gifts from Astra (Westborough, MA). Hexadecyltrimethylammonium bromide (HTAB) was obtained from Sigma (St. Louis, MO). Phosphoric acid (85%), sodium dihydrogen phosphate monohydrate, diethyl ether and ethyl acetate were obtained from J.T.Baker (Phillipsburg, NJ). α, β And γ cyclodextrins, heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) were also obtained from Sigma. Hydroxypropyl β-cyclodextrin (HP-β-CD), hydroxypropyl α-cyclodextrin (HP-α-CD) and hydroxypropyl γ-cyclodextrin (HP-γ-CD) were obtained from Aldrich (Milwaukee, WI). Hydroxyethyl β-cyclodextrin (HE-β-CD), carboxymethyl-β-cyclodextrin (CM-β-CD) and amino β-cyclodextrin were obtained from Advanced Separation Technologies, (Whippany, NJ). Sulfated-β-cyclodextrin was a gift from American Maize Products (Hammond, IN). Drug free human serum was obtained from Biological Specialty (Colmar, PA). All solutions were filtered through a 0.2 μm nylon filter (Acrodisc 13, Gelman Sciences, Ann Arbor, MI).

2.2. Preparation of stock and standard solutions

Individual stock solutions were prepared in absolute methanol to give concentrations of 100 μg ml^{-1} of $R(+)$ and $S(-)$ mepivacaine HCl calculated as free base and 10 μg ml^{-1} of *R* prilocaine HCl (IS). For the preparation of the standard curve and spiked solutions, appropriate volumes of the individual $R(+)$ and $S(-)$ mepivacaine stock solution were pipetted into 10 ml volumetric tubes and evaporated with the aid of a nitrogen stream. Then 1 ml of serum, 40 μl of the IS solution and 100 μl of 1M NaOH were added to the tubes and mixed well. A stock solution of 100 mM sodium dihydrogen phosphate was prepared in double distilled, deionized water and the pH adjusted to 2.5 using 100 mM phosphoric acid.

2.3. Instrumentation

The electrophoretic system consisted of an ABI model 270A capillary electrophoresis (Applied Biosystems, Foster City, CA) equipped with a UV detector. An uncoated fused silica capillary (L = 72 cm, l = 50 cm, 50 μm i.d, Polymicro Technologies, Phoenix, AZ) was used for the analysis. The polyimide coating of capillary was stripped to create a 0.5 cm detection window. The detection was towards the cathodic end.

2.4. Electrophoretic conditions

The background electrolyte consisted of an aqueous solution of 100 mM sodium dihydrogen phosphate, pH 2.5 (adjusted with 100 mM phosphoric acid) containing 20 mM DM-β-CD and 30 nM HTAB. The analytes were monitored at 215 nm.

New capillaries were conditioned by rinsing with 1 M NaOH and water for 10 min each. The capillary temperature was kept at 35°C and the applied voltage was 25 kV. The sample was introduced using vacuum injection for 20 s. After each analysis, the capillary was rinsed for 2 min with 0.1 M NaOH and 3 min with background electrolyte solution.

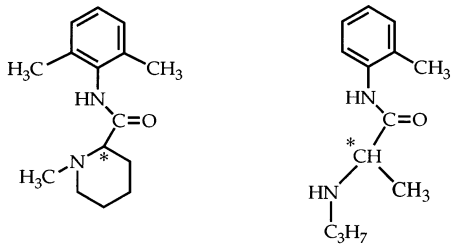
2.5. Assay procedure

To 1 ml of human serum containing $R(+)$ and $S(-)$ mepivacaine were added 40 μl of the IS and 100 μl of 1 M NaOH solution. Then 6 ml of 50:50 (v/v) diethyl ether–ethyl acetate were added and vortexed for 2 min. The solution was then centrifuged for 5 min at 4000 rpm and the supernatant filtered through a 0.2 μm nylon syringe filter prior to evaporation under a nitrogen stream. The samples were reconstituted in 100 μl of distilled water and injected into the capillary using vacuum injection for 20 s. Linear calibration curves were constructed in the range 200–2000 ng ml^{-1}. Linear regression analysis of drug to IS peak area ratios versus concentration gave slope and intercept data for each analyte which were used to calculate the concentration of each analyte in the serum samples. Absolute recoveries were calculated by comparing drug peak area of the spiked analyte samples to unextracted stock solutions injected into the electrophoretic system.

3. Results and discussion

The chemical structures of $R(+)$ and $S(-)$ mepivacaine and $R(-)$ prilocaine are shown in Fig. 1. Various native and derivatized CD were evaluated for their capacity to separate the mepivacaine enantiomers. The neutral CD investigated were the native α, β and γ DM-β-CD, TM-β-CD, HP-β-CD, HP-α-CD, HP-γ-CD and HE-β-CD. The charged cyclodextrins investigated were CM-β-CD, amino β-cyclodextrin and sulfated-β-cyclodextrin. No separations were obtained using any of the cyclodextrins studied except the neutral DM-β-CD, which gave baseline resolution of the enantiomers. CD separate enantiomers utilizing the phenomenon of host–guest complexation along with other interactions where transient diastereomeric complexes are formed between the CD and the analytes. From a mechanistic point of view, it is clear that substitution of two secondary hydroxyl groups (per glucose unit) on the rim of the β-CD to give DM-β-CD decreases the polarity of the rim leading to increased hydrophobicity of the CD. It also leads to an increase in size of the host molecule which may possibly contribute to the host–guest complexation [17]. Molecular modeling studies are underway in this laboratory to explain how DM-β-CD separates mepivacaine enantiomers when α, β and γ CD were unable to resolve them.

Optimization of the background electrolyte was performed by studying the effects of phosphate buffer and DM-β-CD concentrations, pH of the buffer and applied voltage. It was found that the analyte peak shapes improved in symmetry as the phosphate buffer concentration increased with no significant effect on the migration times and resolution. An increase in DM-β-CD concentration increased migration times of both enantiomers without a significant increase in resolution. However significant broadening of the peaks was observed at concentrations 50 mM. The



MEPIVACAINE PRILOCAINE

Fig. 1. Chemical structures of mepivacaine and prilocaine (IS).

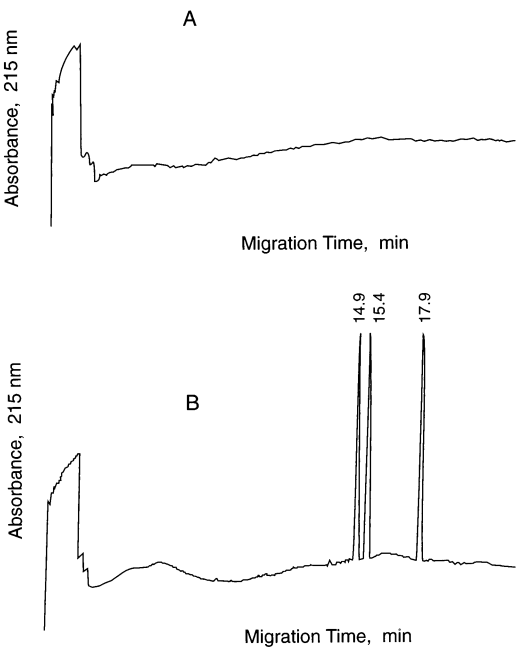


Fig. 2. Electropherograms of (A) blank human serum and (B) human serum spiked with $R(+)$ mepivacaine (1.2 μg ml^{-1}, 14.9 min), $S(-)$ mepivacaine (1.2 μg ml^{-1}, 15.4 min) and $R(-)$ prilocaine (400 ng ml^{-1}, 17.9 min).

phosphate buffer pH had a profound effect on the resolution. As the pH was increased above 6.5, there was a complete loss of enantiomer resolution accompanied by a decrease in migration times. As the voltage increased above 25 kV, there was a decrease in migration times due to the deleterious effects of joule heating the efficiency decreased and at voltages <15 kV, broad peaks were obtained with long migration times. The effect of cationic detergents such as HTAB in reducing the adsorption of endogenous substances in serum with the silanol groups on the wall of the capillary has been well documented [18,19]. A 30 nM HTAB concentration added to the background electrolyte was shown to provide good peak shapes and stable migration times. Based upon these studies, the optimized conditions for the assay were a voltage of 25 kV, and a background electrolyte of 100 mM sodium dihydrogen phosphate pH 2.5 containing 20 mM DM-β-CD and 30 nM HTAB. Fig. 2A and B show typical electropherograms of blank serum and serum spiked with $R(+)$ and $S(-)$ mepivacaine and the IS $R(-)$ prilocaine.

Extractions of the enantiomers and IS using an octadecylsilane solid phase cartridge gave absolute recoveries greater than 85%, but there was an interfering peak from endogenous substance eluting at the same time as R mepivacaine. Other solid phase cartridges such as octylsilane, ethylsilane, methylsilane and phenylsilane were investigated along with various wash and elution steps. Good recoveries ($>80\%$) were obtained with octylsilane and ethylsilane solid phase cartridges, but there were also interfering peaks from endogenous substances in the electropherogram. Increased organic wash steps led to cleaner extracts, but analyte recoveries were less than 50% for all the solid phase cartridges. A protein precipitation step was performed prior to solid phase extraction to see if cleaner extracts were obtained, but this procedure did not produce an acceptable sample cleanup. Therefore it was decided to investigate liquid–liquid extraction. Various solvents were studied to achieve a cleaner extract and satisfactory recoveries. It was found that a mixture of diethyl ether–ethyl acetate (50:50, v/v) gave good sample cleanup with satisfactory recovery. Mean absolute recoveries were $73.4 \pm 3.9\%$ for $R(+)$ mepivacaine, $72.1 \pm 4.7\%$ for $S(-)$ mepivacaine and $68.6 \pm 4.3\%$ for $R(-)$ prilocaine ($n=3$).

The linear regression curves showed good linearity in the range 200–2000 ng ml^{-1} for both $R(+)$ and $S(-)$ mepivacaine with coefficients of determination greater than 0.999 ($n=3$). Representative linear regression equations obtained for $R(+)$ and $S(-)$ mepivacaine were $y = 0.001763x - 0.03178$ and $y = 0.001823x - 0.01668$, respectively, where y and x are the drug to IS peak area ratios and concentration of each analyte, respectively. The intra-day precision and accuracy ($n=3$) as expressed by % R.S.D. and % error were 4.1–5.8 and 2.7–5.1%, respectively, for $R(+)$ mepivacaine and 4.0–6.0 and 4.2–6.0% for $S(-)$ mepivacaine, respectively. The inter-day precision and accuracy ($n=9$, over three days) expressed by % R.S.D. and % error were 4.6–7.2 and 2.6–5.9%, respectively, for $R(+)$ mepivacaine and 5.5–7.4 and 3.2–7.4%, respectively, for $S(-)$ mepivacaine. The detailed analytical data are shown in Table 1.

Table 1
Accuracy and precision of serum samples with added $R(+)$ and $S(-)$ mepivacaine

	Concentration added (ng ml^{-1})	Concentration found (ng ml^{-1})[a,b]	R.S.D. (%)	Error (%)
Intra day				
R	225	204.0 ± 11.78	5.8	5.1
	1975	2028.3 ± 83.32	4.1	2.7
S	225	224.0 ± 13.36	6.0	4.2
	1975	2093.0 ± 84.36	4.0	6.0
Inter day				
R	225	227.7 ± 16.35	7.2	5.9
	1975	2026.0 ± 93.16	4.6	2.6
S	225	199.0 ± 14.72	7.4	7.4
	1975	1911.0 ± 105.22	5.5	3.2

[a] Based on $n = 3$, for intraday assay.
[b] Based on $n = 9$, for interday assay.

Table 2
Comparison of HPCE and HPLC assays for $R(+)$ and $S(-)$ mepivacaine in serum

Mepivacaine	HPCE		HPLC	
	R	*S*	*R*	*S*
Selectivity (α)	1.03		1.23	
Resolution	1.75		2.80	
Theoretical plates (N)	42 238	52 056	4245	2164
Migration times (t_m, min) or Retention times (t_r, min)	14.9	15.4	9.3	11.4
Linear concentration range (ng ml^{-1})	200–2000		150–2400	
r^2 ($n = 3$)	>0.999		>0.999	
Limit of detection (ng ml^{-1})[b]	150	150	100	100
Limit of quantitation (ng ml^{-1})[b]	200	200	150	150

[a] See [14] for method.
[b] S/N = 3.

In comparison to existing chiral HPLC methods for this drug, the HPCE method provides good sensitivity and selectivity. Table 2 shows a comparison of this HPCE assay with an HPLC assay for mepivacaine previously reported by our laboratory [14]. Even though selectivity is lower, complete baseline resolution is obtained with the HPCE method because the high plate numbers are ten orders of magnitude greater than those obtained with the HPLC method. The HPCE migration times are longer than HPLC retention times, but they can be decreased by shortening the length of the capillary. The HPCE detection limits are slightly higher, but can be improved by using a bubble shaped detection window or by decreasing the reconstitution volume after liquid–liquid extraction.

In conclusion, a sensitive and selective chiral HPCE assay has been developed for the analysis of $R(+)$ and $S(-)$ mepivacaine in human serum. The method is sensitive to 150 ng ml^{-1} of each enantiomer with a total runtime of 18 min. The method is comparable to an existing chiral HPLC method and could also be used for routine analysis of mepivacaine enantiomers.

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