Rapid Simultaneous Capillary Electrophoretic Determination of (*R*)- and (*S*)-Secobarbital from Serum and Prediction of Hydroxypropyl-γ-cyclodextrin–Secobarbital Stereoselective Interaction Using Molecular Mechanics Simulation

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Abstract

Secobarbital is a hypnotic sedative that is used in the treatment of preoperative anxiety and to manage elevated intracranial pressures and cerebral ischemia due to neurosurgical procedures. Stereoselective resolution is first predicted using hydroxypropyl-γ-cyclodextrin (HPGCD)–(*R*)- and (*S*)-secobarbital transient complex energy calculations using SYBYL 6.01 molecular modeling software. Separation is accomplished as predicted using 40mM HPGCD in 50mM phosphate buffer (pH 9.0), resulting in a rapid, sensitive method for the determination of (*S*)- and (*R*)-enantiomers of secobarbital from serum using solid-phase extraction, capillary electrophoresis (CE), and ultraviolet detection. The method involves extraction of both enantiomers and the internal standard, aprobarbital, from serum using C_{18} Bond-Elut solid-phase cartridges. The CE system consists of fused-silica capillary (52 cm × 75-μm i.d.) maintained at a run voltage of 15 kV with detection performed at a wavelength of 254 nm. The detection and quantitation limits from serum for (*S*)- and (*R*)-secobarbital are 1 μg/mL. Linear calibration curves from 1 to 60 μg of both (*S*)- and (*R*)-secobarbital show a coefficient of determination of more than 0.999. The precision and accuracy of the method, calculated as relative standard deviation (%) and percent error, are 0.35–4.48% and 0.71–8.67%, respectively, for (*R*)-secobarbital and 0.39–4.08% and 2.16–3.70%, respectively, for (*S*)-secobarbital.

Introduction

Chirality of large biomolecules such as proteins plays a profound role in the regulation of the biological systems of which they constitute an important part. Enantiomers of smaller chiral drug molecules that target these proteins (receptors) and peptides may differ substantially in the way they interact with these biomolecules, thereby leading to different pathways of their metabolism, disposition, and physiological effects (1). Active processes like enzymatic metabolism and protein binding show a higher degree of stereoselectivity than general passive processes such as distribution and excretion (2).

Chiral chromatography was initially the primary technique for the analytical separation of enantiomers. However, capillary electrophoresis (CE) has become a powerful alternative due to its inherent separation efficiency, high speed, low cost, and lack of pre- or postcolumn derivatization steps (3).

Cyclodextrin (CD) -mediated CE is able to give high enantio-resolution efficiency (4). Different natural and derivatized CDs also provide a broad spectrum of selectivities at a relatively low cost. The selection of an appropriate chiral complexing agent involves screening of native and derivatized CDs. This process is time-consuming because it is very difficult to a priori predict chiral selectivity of a complexing agent based simply on the structure of the analyte and CD. Calculating the energy of the complex formed between the CD and the two enantiomers individually using tools of molecular modeling can give one an idea about the stabilities of these transient complexes and aid in the search for an appropriate complexing agent (5–8). The correlation between the structure of an analyte and its enantio-resolution and retention behavior has been studied previously (9–13). Empirical methods based on available crystal structures of drugs and CDs may not give a foolproof prediction because there have not been any consistent methodologies developed or modes of comparison with successful chiral separations. Resolution of enantiomers using CE have also been performed using other chemically diverse agents like macrocyclic antibiotics (vancomycin, teicoplanin) (14), bile salts (taurocholic acid, glycocholic acid) (15), bovine serum albumin (16), and ergot alkaloids (17).

CDs separate enantiomers using the phenomenon of host–guest complexation in which a transient diasteriomeric complex is formed between the CD and the analyte. The affinity of the CD for the analyte is due to hydrophobic interactions between the CD cavity and analyte as well as hydrogen bonding between the functional groups of the analyte with the hydroxyl groups on the CD ring (18).

Hydroxypropyl-γ-cyclodextrin is one of the most soluble CDs;

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its solubility is about 45 g/100 mL. A solution of this CD may be stored for several weeks at room temperature. Its cavity size is 8.3 Å, and as such, it accommodates slightly larger substrates.

Barbiturates enhance the binding of γ-aminobutyric acid (GABA) to the $GABA_A$ receptor and cause the channel to open in prolonged bursts, hence producing a general inhibitory effect on neurotransmission (19); in this respect, they are usually sedative depressants. Some barbiturates can cause convulsions; this diversely varying effect has been linked to the stereochemistry of the drug. Therefore one of the major side effects of barbiturates is strongly linked to one of its stereoisomers. In addition, the sedative effect of barbiturates is itself dependent on the chirality of the five-position substituent. In secobarbital, the (*S*)-enantiomer is more active in prolonging sleeping times (sedative effect) than the (*R*)-enantiomer (20).

Thus it becomes imperative to develop methods that rapidly and selectively measure the levels of these enantiomers in biological fluids such as serum in order to identify and treat addicts, overdose patients, and unwary children of addicts because secobarbital is also a drug of abuse.

Experimental

Materials and reagents

Racemic secobarbital, (*S*)- and (*R*)- enantiomers, and the two metabolites of secobarbital, (1'*RS*,3'*RS*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl)barbituric acid and (1'*RS*,3'*SR*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl)barbituric acid, were obtained from the National Institute for Drug Abuse. Phosphoric acid (85%), sodium dihydrogen phosphate monohydrate, and ammonia solution were obtained from J. T. Baker (Phillipsburg, NJ). Hydroxypropyl-γ-cyclodextrin was obtained from Research Biochemicals International (Natick, MA). Aprobarbital, β-CD, and dimethyl-β-CD were obtained from Sigma (St. Louis, MO). Drug-free human serum was obtained from Biological Specialty (Colmar, PA). C_{18} solid-phase extraction columns of 1 mL capacity (100 mg/mL) were obtained from Varian Sample Preparation Products (Harbor City, CA). All solutions were filtered through a 0.2-mm nylon filter (Acrodisc 13, Gelman Sciences, Ann Arbor, MI).

Molecular modeling software

Sybyl (Tripos, St. Louis, MO) was used for the graphic display of structures, docking studies, energy minimization, and grid conformation searches. The Quest Program of Cambridge Crystallographic Structural Database (CCSD) was used to obtain three-dimensional crystal structures of secobarbital and γ-CD. Software was run on a silicon graphics (SGI, Mountain View, CA) work station with an IRIX operating system (version 5.3).

Molecular modeling protocol

The chemical structures that were obtained from CCSD were further refined to meet the requirements of our experiment. Hydroxypropyl side chains in HPGCD were constructed using the crystal structure of γ-CD as a template and using the structure of hydroxypropyl-β-CD as a reference. The macromolecule was then minimized using the MAXIMIN option of Sybyl. Default parameters of MAXIMIN were used, and 1000 iterations of minimization were performed assigning charges using a Gasteiger-Marsele charge set. The (*R*)- and (*S*)- conformations of secobarbital were obtained by modifying the crystal structure of the available closely related barbiturates. Further optimization of the chemical structures built were obtained using a grid search on all the rotatable bonds using a Kollman forcefield to find the minimum energy conformer. A grid search is a torsion angle driver combined with energy minimization that searches for energy distribution for various torsional states of a molecule. An angle increment of 10 degrees was used for each rotatible bond through 360 degrees. The set of torsional angles giving the lowest energy for rigid rotations was obtained and was referred to as the global energy minimum conformation. At every step of the construction process of HPGCD, it was correlated with data from previously conducted nuclear magnetic resonance (NMR) experiments on CDs (20) and other molecular mechanic investigations such as symmetry-breaking in CDs (21) in order to build a structure that truly reflected the solution state conformation of this molecule.

The (*R*)- and (*S*)-secobarbital were individually docked inside the HPGCD cavity. The DOCK command provided a real-time estimate of the intermolecular energy of interaction between a ligand and the rest of a molecule such as (in this case) the chiral analyte and CD. In order to simulate a real solution-state docking phenomenon, reported crystal structures of CD drug complexes in the Cambridge Crystallographic Structural Database were investigated. It was found that creating a "dummy atom" at the approximate center of the CD cavity using the CD backbone helped in identifying a locus where the molecule could be centered. The enantiomers were rotated and moved slowly inside the cavity to identify possible low-energy interaction states until the total energy of hydrogen bonding and steric and electrostatic interactions reached the minimum. This method was identified as giving the best real-time simulation of solution-state interactions by analyzing the crystal structures of CD complexes in CCSD.

The guiding parameter in the search for a position of minimum energy interaction was the inclusion of enantiomers in the cavity with maximum interaction between the chiral center containing the side-chain of secobarbital and the CD. This interaction was suspected to be the driving force in enantio-resolution. After the minimum energy state of interaction was located, the SYBYL merge command was used to merge an enantiomer into HPGCD to form a complex in one molecular area. This was then frozen to form an interactive complex and then minimized as one entity. The energy calculations were based on steric contributions from the TRIPOS forcefield and electrostatic contributions from the Gasteiger-Marsele charge set. The total potential energy of the system (E_{total}) was calculated using the following formula:

$$E_{total} = \Sigma E_{stretch} + \Sigma E_{bend} + \Sigma E_{out\ of\ plane} + \Sigma E_{torsion} + \Sigma E_{van\ der\ Waals} + \Sigma E_{electrostatic} + \Sigma E_{distance\ constraints} + \Sigma E_{range\ constraints} + \Sigma E_{torsional\ constraints} + \Sigma E_{multifit} + \Sigma E_{field\text{-}fit}$$

where each energy value was calculated as the difference between the actual and natural states or as a value arising from interactions or constraints.

Preparation of stock and standard solutions

Individual stock solutions were prepared in 30% methanol water to give a concentration of 1 mg/mL of (*S*)- and (*R*)-secobarbital and the internal standard, aprobarbital. Appropriate volumes of the individual (*S*)- and (*R*)-secobarbitals and internal standard were pipetted, and 1 mL serum was added and mixed well. A stock solution of sodium dihydrogen phosphate was prepared in double-distilled, deionized water; the pH was adjusted to 9.0 using 100mM sodium hydroxide and concentrated phosphoric acid.

CE system

All CE experiments were performed using a P/ACE System 5000 (Beckman, Fullerton, CA) equipped with an ultraviolet (UV) detector. An uncoated fused-silica capillary (total length, 62 cm; effective length, 52 cm; 75-µm i.d.) (Polymicro Technologies, Phoenix, AZ) was used for analysis. The capillary was temperature-controlled at 25°C, and the applied voltage was 15 kV. The typical running current was about 100 µA. A 0.5-cm detection window was created by stripping the polyamide coating of the capillary. Detection was toward the cathodic end. The run buffer consisted of an aqueous solution of 50mM phosphate buffer (adjusted to pH 9.0 with 100mM sodium hydroxide) containing 40mM hydroxypropyl-γ-CD. The analytes were monitored at a wavelength of 254 nm.

CE conditions

New capillaries were conditioned by rinsing with 1M sodium hydroxide for 10 min followed by 10 min each with water, 0.1M hydrochloric acid, and running buffer solutions. Sample introduction was performed using vacuum injection (0.5 psi) for 10 s. Before each analysis, the capillary was rinsed first with 0.1M sodium hydroxide for 2 min and then with the running buffer solution for 2 min.

Assay procedure from serum

Sample cleanup was performed using Bond Elut C_{18} solid-phase extraction cartridges. The cartridge was pretreated using 3 mL of methanol and then 3 mL of pH 9.0 phosphate buffer. The sample that contained (*R*)- and (*S*)-secobarbital and the internal standard aprobarbital in 1 mL of serum was added to the cartridge and allowed to flow down under low vacuum. The cartridge was not allowed to dry between the pretreatment and sample application steps. The column was then washed with 3 mL of the buffer and allowed to dry for 5 min. The analytes were then eluted with 3 mL of methylene chloride. The eluant was passed through a nylon filter prior to evaporation using a nitrogen stream. The samples were reconstituted in 1 mL of 30% methanol in water mixture and vacuum-injected into the capillary for 10 s. Absolute recoveries were calculated by comparing the drug peak

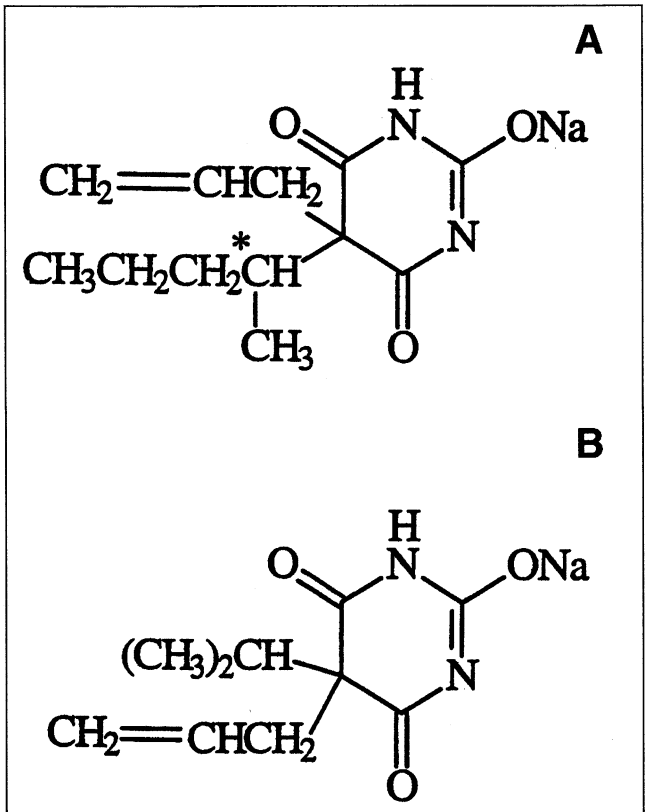

Figure 1. Chemical structures of (A) secobarbital (* denotes the chiral carbon) and (B) the internal standard aprobarbital.

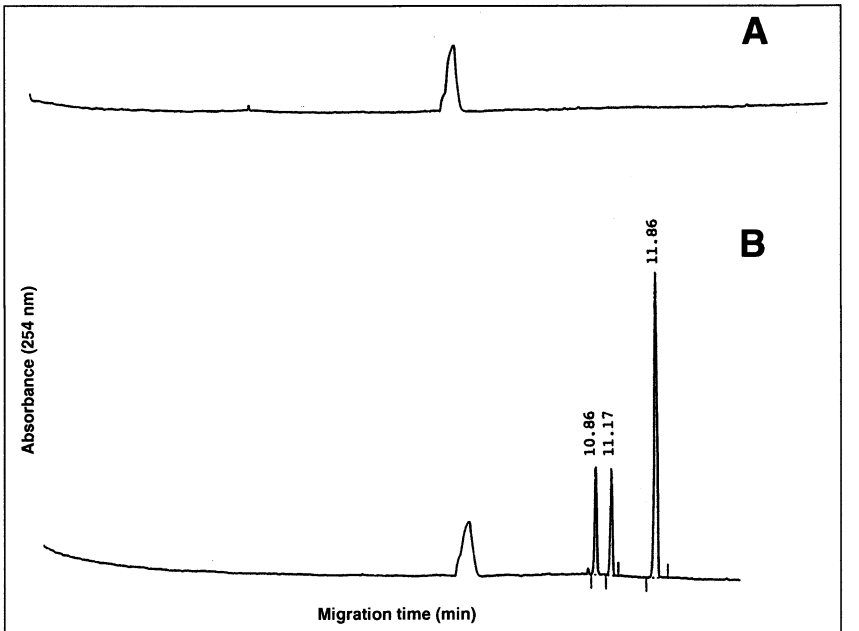

Figure 2. Typical electropherogram of (A) blank serum and (B) serum spiked with (*R*)-secobarbital (10.86 min), (*S*)-secobarbital (11.17 min), and the internal standard aprobarbital (11.86 min) on a 57-cm x 75-mm fused-silica capillary. The run buffer contained 40mM HPGCD in 50mM phosphate buffer (pH 9.0); detection was at 254 nm. The capillary was temperature-controlled at 25°C, and the run voltage was 15 kV.

height in the spiked serum analyte sample to the unextracted stock solutions, which had been injected directly into the CE system. Solid-phase extraction with Bond Elut C_{18} cartridges gave absolute recoveries of 98–99% for both analytes and the internal standard.

Calibration curves were constructed using 1–60 µg/mL. Linear regression analysis of concentration versus drug–internal standard peak height ratios gave slope and intercept data for each analyte, which were used to calculate the concentration of unknown analytes in serum samples.

Results and Discussion

The logic behind the molecular dynamics study was to simulate the transient complex formed between CD and the two enantiomers. The energy of these individual transient complexes is crucial in determining how stable these complexes are and how long the complex will remain intact. Previous reports have speculated the existence of a correlation between chiral resolution and the cavity size of the complexing agent, but we find contradictory evidence to this; enantiomers of drugs like bupivacaine, isothipendyl, mefloquine, nicardipine, tetryzoline, tropicamide, zopiclone, are separated by both hydroxypropyl-β-CD and hydroxypropyl-γ-CD (22). The interactions are clearly more complicated than a simple drug–CD steric interaction and simple fitting into a cavity. The total energy for the (*R*)-secobarbital–HPGCD complex was 166.030 kcals/mol, and the corresponding energy for the (*S*)-secobarbital–HPGCD complex was 155.892 kcals/mol. This suggests a clear difference in the stabilities of the two complexes; based on empirical molecular mechanics calculations, (*R*)-secobarbital would be expected to elute before (*S*)-secobarbital.

The chemical structures of secobarbital and the internal standard aprobarbital are shown in Figure 1. A run buffer of pH 9.0 was used because barbiturates are acidic in nature and it was suspected that ionoselective interactions between CD and secobarbital enantiomers were responsible for enantio-resolution. Baseline resolution was achieved as expected from the energy calculations at HPGCD concentrations of 25, 30, 35, and 40mM, but depletion of HPGCD on repeated runs lead to a decrease in resolution at concentrations less than 40mM. The order of elution was also verified and correlated with the results obtained with molecular modeling calculations. This was utilized in the stereoselective analysis of secobarbital from the biological fluid, serum. Baseline separation (resolution [R_s], 3.643) of secobarbital was achieved using 40mM HPGCD as a chiral complexing agent. The theoretical column plate number of 236,000 was achieved in average for all component peaks in the assay. The separation efficiency was also studied at pH 7.5, 8.0, and 9.0 and was found to be best at pH 9.0, as suspected. Figure 2A shows the electropherogram of blank serum, and Figure 2B shows the electropherogram of spiked (*R*)- and (*S*)-secobarbital with the internal standard aprobarbital. Retention times were not significantly affected by increasing the concentration of the run buffer. The analyte peak shape symmetries were found to optimize in 50mM phosphate buffer with a migration time of approximately 10 min. Migration time reproducibility was high; RSDs were less than 0.5% (nine replicates) for all peaks.

A lower detection limit and reduced band-broadening was achieved by using the principle of analyte-stacking. The sample was prepared in a solvent with lower conductivity (methanol–water) than the electrolyte solution. When a voltage was applied across the capillary, a greater field developed across the sample plug, causing the ions to move faster. When the ions reached the buffer, they slowed down due to the reduced field to which they were subjected, thereby resulting in analyte-stacking within a narrow zone of the capillary (23,24). Significant improvement in the resolution was achieved by effecting coun-

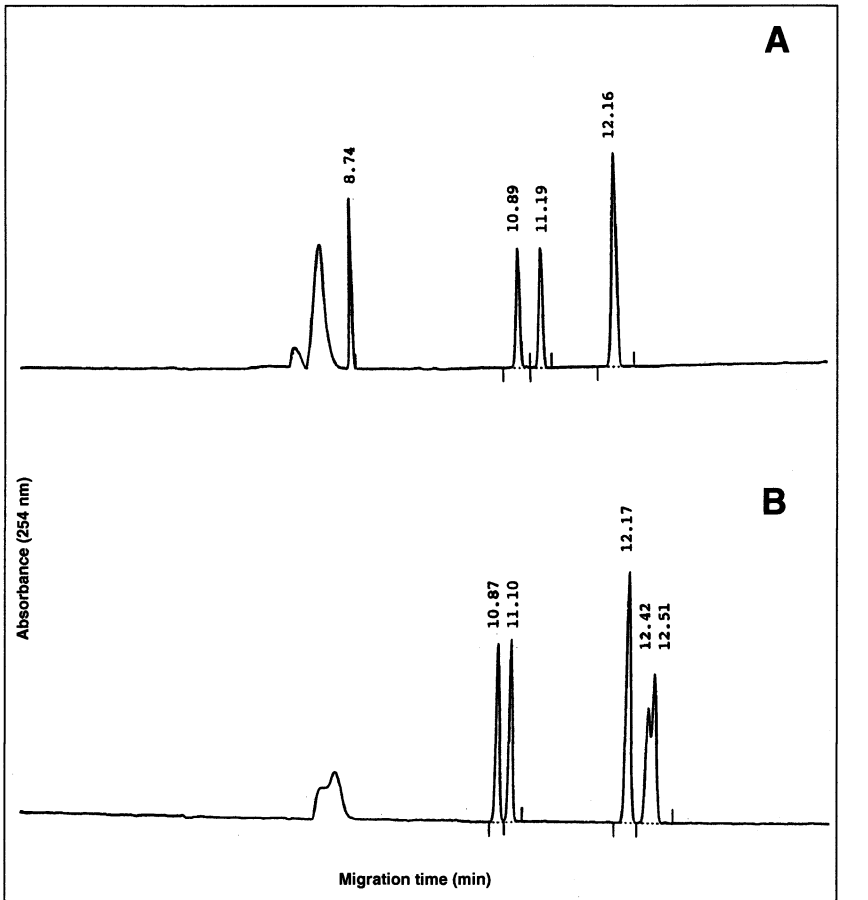

Figure 3. Electropherogram showing separation of (A) clonazepam (8.74 min), (*R*)-secobarbital (10.89 min), (*S*)-secobarbital (11.19 min), and the internal standard aprobarbital (12.16 min) and (B) (*R*)-secobarbital (10.87 min), (*S*)-secobarbital (11.10 min), and the internal standard aprobarbital (12.17 min). The two unresolved peaks (12.42 and 12.51 min) are the two metabolites of secobarbital, (1'*RS*,3'*RS*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl) barbituric acid and (1'*RS*,3'*SR*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl) barbituric acid. The separation conditions were the same as in Figure 2.

tercurrent flow of the analyte and CD by negatively charging the analyte and moving it against the flow (25).

Secobarbital is sometimes administered in combination with the anticonvulsant clonazepam. Figure 3A shows the separation of the secobarbital enantiomers aprobarbital and clonazepam. In any biological method development, it is imperative to show that metabolites of the drug that might be present in the assayed biological fluid do not interfere with the determination of the drug. The two metabolites of secobarbital, (1'*RS*,3'*RS*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl) barbituric acid and (1'*RS*,3'*SR*)-5-allyl-5-(3'-hydroxy-1'-methylbutyl) barbituric acid, were included in the runs and eluted after the drug and internal standard, thus not affecting the assay, as shown in Figure 3B.

The calibration curve showed good linearity over the range covering therapeutic serum levels of 1–60 µg/mL for both (*R*)- and (*S*)-secobarbital. The coefficient of determination was 0.999 (three replicates). Representative linear regression equations obtained for (*R*)- and (*S*)-secobarbital were $y = 58.09x - 8.25$ and $y = 50.55x - 7.31$, where y and x are concentration and drug-to-internal-standard peak height ratios, respectively. The intraday precision and accuracy (three replicates) as expressed by the RSD and percent error were 4.47–4.48% and 6.80–8.67%, respectively, for (*R*)-secobarbital and 0.39–1.90% and 3.05–3.70%, respectively, for (*S*)-secobarbital. The interday precision and accuracy over three days (nine replicates) expressed by the RSD and percent error were 0.35–2.59% and 0.71–4.75%, respectively, for (*R*)-secobarbital and 1.30–4.08% and 2.16–3.13%, respectively, for (*S*)-secobarbital. Table I gives the inter- and intraday precision and accuracy values of this method. Table II gives the values of the various energies involved in the individual (*R*)-secobarbital–HPGCD and (*S*)-secobarbital–HPGCD complexes. The method's limit of quantitation was found to be 1 µg/mL at a signal-to-noise ratio of 3:1.

Table I. Accuracy and Precision of Secobarbital Enantiomers Added to Serum

	Concentration added (µg/mL)	Concentration found (µg/mL)	Error (%)*	RSD (%)
Intraday (three replicates)				
(*R*)-Secobarbital	10	10.95 ± 0.49	8.67	4.47
	30	32.19 ± 1.44	6.80	4.48
(*S*)-Secobarbital	10	9.69 ± 0.19	3.05	1.90
	30	31.12 ± 0.12	3.70	0.39
Interday (nine replicates)				
(*R*)-Secobarbital	10	9.53 ± 0.25	4.75	2.59
	30	30.21 ± 0.11	0.71	0.36
(*S*)-Secobarbital	10	9.79 ± 0.13	2.16	1.30
	30	29.06 ± 1.18	3.13	4.08

* Mean ± SD.

Table II. Various Energies of Interaction between (*R*)- and (*S*)-Secobarbital and Hydroxypropyl-γ-CD Based on Empirical Forcefield Energy Calculations

Description	(*R*)-Secobarbital (kcals/mol)	(*S*)-Secobarbital (kcals/mol)
Bond stretching energy*	12.154	11.569
Angle bending energy†	43.000	42.582
Torsional energy‡	67.903	59.458
Out of plane bending energy§	0.336	0.080
van der Waals energy**	–35.668	–37.945
Electrostatic energy††	78.306	80.147
Total energy	166.031	155.891

* Energy associated with the stretching of bonds.
† Energy associated with the bonds as they are bent from their natural state.
‡ Energy associated with torsional strains induced into the molecule.
§ Energy of bonds between atoms in a plane as they are bent above or below the plane.
** Energy associated with non-bonding van der Waals interactions in the analyte–CD complex.
†† Energy associated with the natural electrostatic states in the analyte–CD complex.

Conclusion

In conclusion, this paper clearly demonstrates that theoretical molecular mechanics simulations can aid in the prediction of enantio-resolution and elution order of drugs. The biological assay developed in this paper is sensitive and independent of changes in laboratory conditions; the applicability of this method was tested over a period of 2 weeks. The solid-phase extraction method used provides excellent sample cleanup, and recovery is around 98–99%. The inter- and intraday precision and accuracy data show that the method is very dependable and accurately reflects serum levels of the enantiomers. This method provides a fast and cost-effective alternative to existing chromatographic methodologies.

References

1. B. Holmstedt, H. Frank, and B. Testa. *Chirality and Biological Activity.* Alan R. Liss, New York, NY, 1990, pp. 191–98.
2. J. Cadwell. Stereochemical determinants of the nature and consequences of drug metabolism. *J. Chromatogr. A* **694:** 39–48 (1995).
3. R. Velspac and P. Bocek. Chiral Separations by CE: Present State of the Art. *Electrophoresis* **15:** 755–62 (1994).
4. A. Guttman, S. Brunet, and N. Cooke. Capillary electrophoresis separation of enantiomers by cyclodextrin array chiral analysis. *LC–GC* **14:** 32–42 (1996).

5. K.B. Lipkowitz, S. Raghothama, and J. Yang. Enantioselective binding of tryptophan by α-cyclodextrin. *J. Am. Chem. Soc.* **114:** 1554–62 (1992).
6. D.G. Durham and H. Liang. Molecular modeling of inclusion complexes between β-cyclodextrin and (R)/(S)-methylphenobarbitone and its application to HPLC. *Chirality* **6:** 239–44 (1994).
7. K.B. Lipkowitz, K.M. Green, J. Yang, G. Pearl, and M.A. Peterson. Theoretical study of enantiodifferentiation in solid state cyclodextrins. *Chirality* **5:** 51–57 (1993).
8. F. Kobor, K. Angermund, and G. Schromburg. Molecular modeling experiments on chiral recognition in GC with specially derivatized cyclodextrins as selectors. *J. High. Res. Chromatogr.* **16:** 299–311 (1993).
9. E. Tesarova, M. Gilar, P. Hobza, K. Akbelac, Z. Deyl, and E. Smolkova-Keulmansova. Correlation between structure of dihydropyridine calcium antagonist and their retention behaviour and enantio-separation on the β-cyclodextrin stationary phases in HPLC. *J. High Res. Chromatogr.* **18:** 597–601 (1995).
10. M.E. Amato, F. Djedaini, G.C. Papalardo, B. Perly, and G. Scarlata. Molecular modeling of β-cyclodextrin with nootropic drugs. *J. Pharm. Sci.* **81:** 1157–61 (1992).
11. T. Lu, D. Zhang, and S. Dong. Molecular–mechanical study of cyclodextrin and its inclusion complexes. J. *Chem. Soc., Faraday Trans.* **85(9):** 1439–45 (1989).
12. K.B. Lipkowitz, K. Green, and J. Yang. Structural characterization of cyclodextrins in solid state. *Chirality* **4:** 205–15 (1992).
13. A. Berthod, W. Li, and D.W. Armstrong. Multiple enantio-selective retention mechanisms on derivatized cyclodextrin gas chromatographic chiral stationary phases. *Anal. Chem.* **64:** 873–79 (1992).
14. D.W. Armstrong and K.L. Rundlett. CE resolution of neutral and anionic racemates with glycopeptide antibiotics and micelles. *J. Liq. Chromatogr.* **18:** 3659–74 (1995).
15. S. Terabe, K. Otsuka, and H. Nishi. Separation of enantiomers by capillary electrophoretic technique. *J. Chromatogr. A* **666:** 295–319 (1994).
16. D. Eberle, R.P. Hummel, and R. Kuhn. Chiral resolution of pantoprazole sodium and related sulfoxides by complex formation with BSA in capillary electrophoresis. *J. Chromatogr. A* **759:** 185–92 (1996).
17. B.A. Ingelse, M. Flieger, H.A. Claessens, and F.M. Everaerts. Ergot alkaloid as chiral selector in CE, determination of separation mechanism. *J. Chromatogr. A* **755:** 251–59 (1996).
18. M. Siluveru and J.T. Stewart. Enantioselective determination of *S*-(+)- and (*R*)-(-)- ondansetron in human serum using derivatized cyclodextrin-modified capillary electrophoresis and solid-phase extraction. *J. Chromatogr. B* **691:** 217–22 (1997).
19. R. Crossley. *Chirality and the Biological Activity of Drugs.* CRC Press, New York, NY, 1995, pp. 57–60.
20. A.F. Casy, A.D. Cooper, T.M. Jefferies, R.M. Gaskell, D. Greatbanks, and R. Pickford. HPLC and ^{1}H-NMR study of chiral recognition in some thromboxane antagonists induced by β-cyclodextrin. *J. Pharm. Biomed. Anal.* **9:** 787–92 (1991).
21. K.B. Lipkowitz. Symmetry breaking in cyclodextrins: A molecular mechanics investigation. *J. Org. Chem.* **56:** 6357–67 (1991).
22. B. Lin, X. Zhu, B. Koppenhoefer, and U. Epperlein. Investigation of 123 chiral drugs by cyclodextrin-modified capillary electrophoresis. *LC–GC* **15:** 40–46 (1997).
23. D.S. Burgi and R.L. Chien. Application and limits of sample stacking in CE. *Methods Mol. Biol.* **52:** 211–26 (1996).
24. D.S. Burgi and R.L. Chien. Improvement in the method of sample stacking for greater injection in CZE. *Anal. Biochem.* **202(2):** 306–309 (1992).
25. B. Chankvetadze, G. Endresz, and G. Blaschke. About some aspects of the use of charged cyclodextrins for the capillary electrophoresis enantio separation. *Electrophoresis* **15:** 804–809 (1994).

Manuscript accepted October 22, 1997.