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Report

Measurement of Underivatized Propranolol Enantiomers in Serum Using a Cellulose–Tris(3,5-Dimethylphenylcarbamate) High-Performance Liquid Chromatographic (HPLC) Chiral Stationary Phase

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A commercially available high-performance liquid chromatographic (HPLC) chiral stationary phase (HPLC-CSP) has been used to measure serum levels of *d*- and *l*-propranolol. The HPLC-CSP is based upon cellulose–tris(3,5-dimethylcarbamate) and is able to stereochemically resolve *d*- and *l*-propranolol without precolumn derivatization using a mobile phase composed of hexane:2-propranol:*N*,*N*-dimethyloctylamine (92:8:0.01, v/v/v). Under these conditions the observed stereochemical resolution (α) of the two enantiomers was $\alpha = 2.2$. A subject's concentration–time curve of the two isomers was determined following the ingestion of 160 mg racemic propranolol.

KEY WORDS: propanolol enantiomers; enantioselective high-performance liquid chromatography (HPLC); HPLC chiral stationary phase; serum levels.

INTRODUCTION

The beta-blocking agent, propranolol, is an enantiomeric molecule which is commercially available as a racemic mixture. Earlier studies have demonstrated that *l*-propranolol is about 100 times as potent as *d*-propranolol (1). In addition, Olanoff *et al.* (2) have recently reported that the oral clearance of *d*-propranolol was 40 to 50% higher than that of *l*-propranolol, thus establishing that propranolol's hepatic metabolism is stereospecific. Thus, in order to understand better the relationship between serum drug concentrations and pharmacologic effect, the characterization of the pharmacokinetics of each enantiomer is critically important.

A number of different high-performance liquid chromatographic (HPLC) approaches have been used to resolve and quantify the enantiomers of propranolol. The most common approach had been the conversion of the propranolol enantiomers into diastereomers using a homochiral (i.e., enantiomerically pure) derivatizing agent (HDA) followed by chromatography on an achiral HPLC system. For example, Silber and Riegelman (3) and Hermansson and von Bahr (4) have used (−)-*N*-trifluoroacetyl-*L*-prolyl chloride [(−)-TPC] as the HDA, while Hermansson (5) has used t-butoxycarbonyl-*L*-alanine and t-butoxy-*L*-leucine. In both methods, the resulting diastereomers were resolved on achiral octadecylsilane stationary phases. The same type of chromatographic phase was used by Walle *et al.* (6) to resolve the diastereomeric (+)-l-phenethyl isocyanate derivatives of *d*- and *1*-4′-hydroxypropranolol sulfate.

The determination of enantiomeric purity through the synthesis and separation of diastereomeric derivatives inherently contains the danger of inaccurate results due to possible enantiomeric contamination of the HDA. This danger was pointed out by Silber and Riegelman (3), who found that commercial (−)-TPC was contaminated with from 4 to 15% of the (+)-enantiomer and that the reagent rapidly racemized during storage. An additional complication is that enantiomers may have quite different rates and/or equilibrium constants when they react with the HDA (7). If the derivatization reaction is not carried out to completion, this will result in the generation of diastereomeric products differing in proportions from the starting enantiomeric composition.

These problems can be avoided by resolving the enantiomers as enantiomers using enantioselective chromatography. This can be achieved using either chiral mobile phases or chiral stationary phases. Pettersson and Schill (8) have resolved propranolol using chiral ion-pair chromatography with (+)-10-champhorsulfonate as the chiral counter ion.

The enantiomers of propranolol have also been resolved using the HPLC chiral stationary phases (HPLC-CSP). Pirkle *et al.* (9) and Wainer *et al.* (10) have used an HPLC-CSP based on (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine (DNPG-CSP) and Hermansson (11) and Schill *et al.* (12) have used an HPLC-CSP based on α1-acid glycoprotein (AGP-CSP). The first three approaches required derivatiza-

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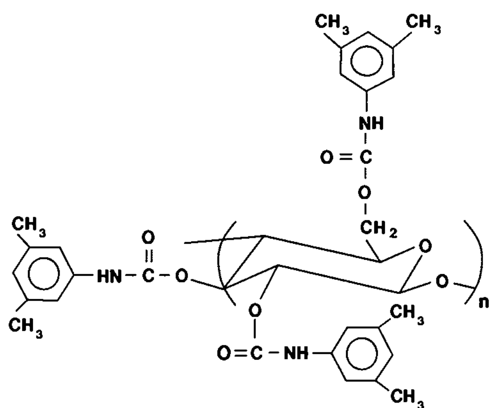

Fig. 1. The structure of the chiral stationary phase (OD-CSP) used in this study.

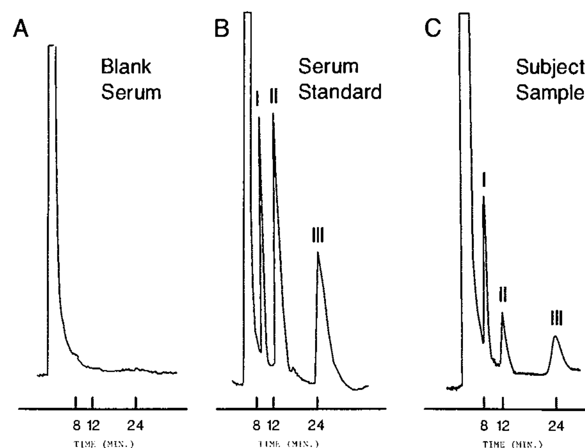

Fig. 2. Representative chromatograms for (A) an extracted blank serum sample, (B) a serum sample spiked with 300 ng of verapamil and 150 ng of racemic propranolol per ml, and (C) a subject's serum sample 12 hr after ingestion of a 160-mg dose of racemic propranolol. I, Verapamil; II, *d*-propranolol; III, *l*-propranolol.

tion with an archiral reagent, while the last method resolved the underivatized compound. Only one of the direct methods has been used in pharmacokinetic and pharmacological studies (10,13).

Okamoto *et al.* (14) have recently reported the development of a new HPLC-CSP based upon cellulose–tris(3,5-dimethylphenylcarbamate) which has been coated on macroporous silica (OD-CSP) (Fig. 1). Okamoto *et al.* (15) have also demonstrated that the underivatized enantiomers of propranolol can be resolved on the OD-CSP with a relatively high efficiency and short retention times. This is a marked improvement over previous HPLC-CSP resolutions.

The present paper describes the development of an analytical method for the determination of the enantiomeric composition of propranolol in serum using the OD-CSP. The method is rapid, accurate, and applicable to pharmacokinetic studies.

EXPERIMENTAL

Apparatus. The chromatography was performed with a Constametric III pump (Laboratory Control Data/Milton Roy, Riviera Beach, Fla.), Spectroflow 980 fluorescence detector (Kratos Analytical, Ramsey, N.J.) Rheodyne Model 7125 sample injection valve equiped with a 50-μl loop (Alltech Associates, Deerfield, Ill.), and Series 5000 Fisher Redcordall strip chart recorder (Fischer Scientific, Lexington, Mass.). The CSP was a cellulose–tris(3,5-dimethylphenycarbamate) polymer absorbed on macroporous silica and commercially prepacked in a (25 cm × 4.6-mm i.d.) stainless-steel column (Daicel Chemical Industries, New York). The column temperature was maintained within ± 0.1°C of the desired setting with a LC-22A temperature controller (Bioanalytical Systems Incorporated, West Lafayette, Ind.).

Materials. Racemic propranolol HCl, *d*-propranolol HCl, *l*-propranolol HCl, and 80-mg tablets of racemic propranolol HCl (Inderal) were obtained from Ayerst Laboratories (New York), and the internal standard, verapamil HCl, from Knoll Pharmaceuticals (Whippany, N.J.). HPLC-grade hexane and 2-propranol were obtained from American Burdick and Jackson (Muskegon, Mich.) and the *N,N*-dimethyloctylamine was obtained from Aldrich Chemical Company, Inc. (Milwaukee, Wis.). The other chemicals were reagent grade and were used as purchased.

Sample Preparation. A 30-μl aliquot of the internal standard (verapamil, 10 ng/μl in methanol), was added to a

Table I. Precision and Accuracy

	d-Propranolol, sample[a]		*l*-Propranolol, sample[a]	
	A	B	A	B
Within day (N = 7)				
Spiked concentration (ng/ml)	30.0	80.0	30.0	80.0
Mean determined concentration	30.5	77.1	29.8	76.4
CV (%)	4.1	3.4	2.7	3.2
Between day (N = 6)				
Spiked concentration (ng/ml)	30.0	80.0	30.0	80.0
Mean determined concentration	30.3	77.4	31.0	79.2
CV (%)	9.7	6.6	8.8	6.5

[a] Samples prepared from racemic propranolol and drug-free serum. Sample A, 60 ng/ml racemic propranolol; sample B, 160 ng/ml racemic propranolol.

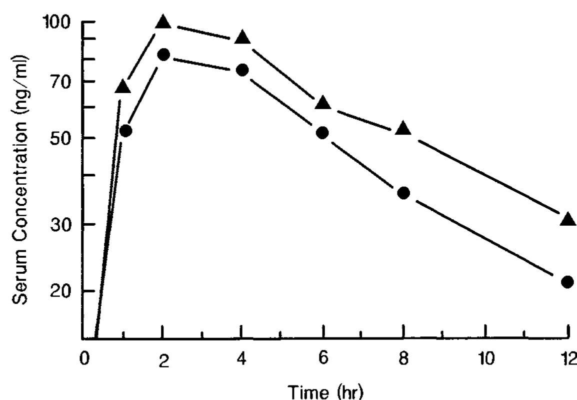

Fig. 3. Serum concentration–time curve for a healthy subject after ingestion of a 160-mg dose of racemic propranolol. (●) *d*-Propranolol; (▲) *l*-propranolol.

1-ml serum sample. This was followed by the addition of 0.5 ml of carbonate buffer (1 *M*, pH 10.0) and 7 ml of diethylether. The tubes were vortexed for 2 min, then centrifuged for 10 min, and the ether layer was collected and evaporated to dryness under nitrogen. Each sample was reconstituted by adding 100 μl of mobile phase immediately prior to injection.

Chromatographic Conditions. The mobile phase consisted of hexane:2-propanol:*N,N*-dimethyloctylamine (92:8:0.01, v/v/v). A column temperature of 37°C and a mobile phase flow rate of 1 ml/min were maintained throughout the study. The propranolol was monitored using an excitation wavelength of 290 nm and a 340-nm emission cutoff filter.

Standard Curves. Standard curves were constructed using serum samples containing 20, 50, 100, 150, 200, and 300 ng of racemic propranolol per ml of serum. The samples were run every day during the validation of the assay.

Pharmacokinetic Study. One healthy fasting male subject received a single oral dose of 160 mg racemic propranolol HCl according to a previously described protocol (16). Blood samples were collected by separate venipunctures at 0, 1, 2, 4, 6, 8, and 12 hr after dosing. The blood was allowed to clot, and the serum collected and stored in glass tubes with Teflon screw caps at −20°C until analysis.

RESULTS

Representative chromatograms of 1 ml of blank serum and 1 ml of serum spiked with verapamil (300 ng) and racemic propranolol (150 ng) are presented in Figs. 2A and B, respectively. The capacity factor (k') for verapamil was 1.6. Under these chromatographic conditions, racemic verapamil was not resolved into its enantiomeric forms. The elution order of the propranolol enantiomers on this CSP was established by injecting the pure *d*- and *l*-enantiomers and was the same as previously reported (15). Under these chromatographic conditions *d*-propranolol eluted with a $k' = 2.9$, and *l*-propranolol with a $k' = 6.5$. The stereochemical selectivity (α) for *d*- and *l*-propranolol was 2.2 and the stereochemical resolution factor (R_s) was 3.7.

The standard curves for the *d*- and *l*-propranolol were linear over the range investigated. The equation describing the *d*-propranolol standard curve was $y = 0.016\,x - 0.059$, with a correlation coefficient of 0.999. The equation describing the *l*-propranolol standard curve was $y = 0.009\,x - 0.039$, with a correlation coefficient of 0.999.

Within-day and between-day measurements were done using samples spiked with 60 and 160 ng of racemic propranolol per ml of serum (30 and 80 ng/ml of each isomer, respectively). The precision and accuracy are summarized in Table I. Based on a signal-to-noise ratio of 4, the lower limit of detection was 7.5 ng/ml of each enantiomer. The extraction efficiency at a concentration of 160 ng of racemic propranolol per ml of serum was 89 and 85% for the *d*- and *l*-enantiomers, respectively.

A subject's concentration–time curve of the two enantiomers of propranolol following the ingestion of 160 mg of racemic propranolol is presented in Fig. 3. The apparent oral clearances, calculated as dose/area under the curve from time 0 to infinity, were 19.4 and 15.0 ml/min/kg for *d*- and *l*-propranolol, respectively. The terminal half-lives were 4.4 hr for *d*-propranolol and 5.4 hr for *l*-propranolol. These results are consistent with previously reported values (2).

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