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Note

Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (–)-menthyl chloroformate

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Approximately 25% of the prescribed and dispensed drugs are racemates [1,2], which contain equal amounts of two enantiomers. Although similar in many aspects, the two enantiomers of a drug may elicit different pharmacologic and/or pharmacokinetic characteristics.

Most of the currently available β-adrenoceptor agents have at least one chiral center in their side-chain and are marketed as a racemic mixture [3]. β-Adrenoceptors, however, show a high degree of selectivity for enantiomers of exogenous drugs and endogenous neurotransmitters [4], the (–)-enantiomers being responsible for the activation or blockade of the receptors. Furthermore, enantioselectivity has also been shown in the pharmacokinetics of some of these drugs [5]. Therefore, it is necessary to develop analytical methods capable of separating enantiomers of these drugs.

Recently, utilizing a high-performance liquid chromatographic (HPLC) method, enantiomers of atenolol were separated as their (–)-menthyl chloroformate (MCF) derivatives [6]. In this paper, application of MCF to HPLC analysis of enantiomers of certain β-adrenoceptor agents (Fig. 1) is reported.

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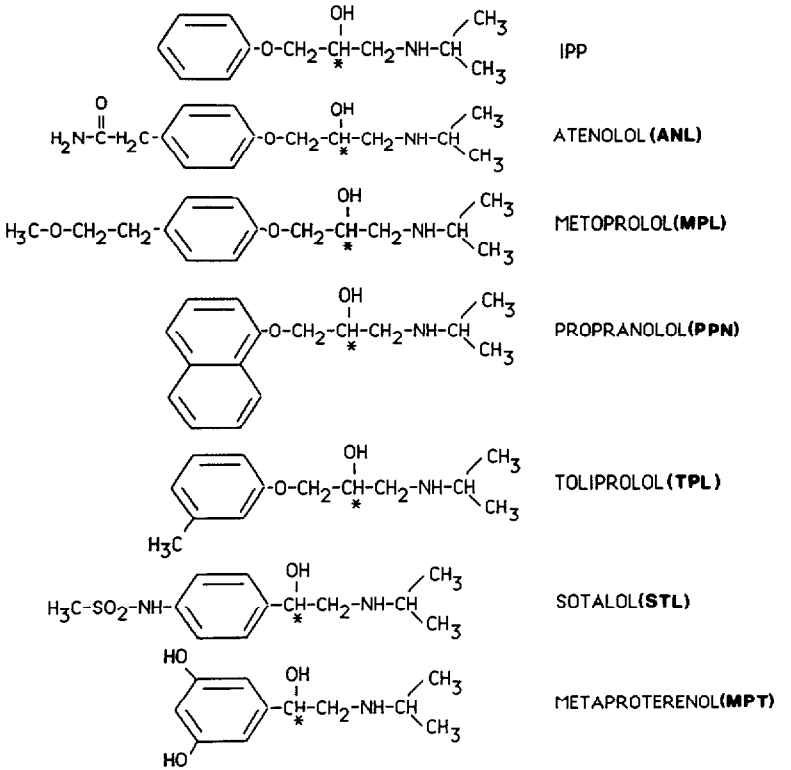

Fig. 1. Chemical structures of the adrenergic drugs investigated in this study. (*) Location of the asymmetric carbon; IPP = 1-isopropylamino-3-phenoxypropan-2-ol.

EXPERIMENTAL

Materials

(±)-Atenolol (ANL, Lot W2288) and (±)-propranolol hydrochloride (PPN, Lot C-24987D) were donated by Stuart Pharmaceuticals, Division of ICI Americas (Wilmington, DE, U.S.A.) and Ayerst Labs. (New York, NY, U.S.A.), respectively. Racemic powders of metaproterenol sulfate (MPT), metoprolol bitartrate (MPL), sotalol hydrochloride (STL), toliprolol hydrochloride (TPL), and 1-isopropylamino-3-phenoxypropan-2-ol hydrochloride (IPP) were generously provided by Dr. F.M. Pasutto of the University of Alberta, Faculty of Pharmacy (Edmonton, Canada). *R*(+)- and *S*(−)-PPN, MCF, and 4-hydroxy-L-proline were purchased from Aldrich (Milwaukee, WI, U.S.A.). (−)-MPL (Lot 2) and (+)-MPL (Lot 4) were gifts from Drs. H. Schroter and K. Scheibli of Ciba Geigy (Basle, Switzerland), while (+)-STL was donated by Bristol-Myers (Wallingford, CT, U.S.A.). Methanol, acetonitrile, and water were HPLC grade (American Scientific Products, Minneapolis, MN, U.S.A.). All other reagents and solvents were analytical reagent grade.

Analytical instrumentation

The HPLC instrument consisted of a 501 pump, a 712 Wisp autosampler, and a 745 data module (Waters, Milford, MA, U.S.A.) and an FD-200 variable-wavelength fluorescence detector (Spectrovision, Chelmsford, MA, U.S.A). Diastereomeric derivatives of the adrenergic drugs were analyzed at ambient temperature utilizing a 10 cm×4.6 mm analytical column containing 5-μm octadecylsilane packing material (Partisil 5 ODS3; Whatman, Clifton, NJ, U.S.A.) and a 5 cm×4.6 mm refillable guard column (Supelco, Bellefonte, PA, U.S.A.) packed with pellicular reversed-phase media (Pellicular ODS, Whatman).

The excitation wavelength of the detector was set at 200 nm for MPL, PPN, TPL, and IPP, and at 232 nm for ANL, MPT, and STL. No emission filter was used.

Mobile phase

Three different mobile phase systems were used (Table I).

Chromatographic characteristics of the assay

Capacity (k'), separation (α), and resolution (R) factors were calculated for the peaks of interest based upon the following equations:

$$k'_1 = (t_{R1} - t_{R0})/t_{R0}$$

$$k'_2 = (t_{R2} - t_{R0})/t_{R0}$$

$$\alpha = k'_2/k'_1$$

$$R = 2(t_{R2} - t_{R1})/(W_1 + W_2)$$

where t_R and W are retention time and band width, respectively. Subscripts 0, 1, and 2 refer to the solvent peak, and the first- and second-eluting isomers of the adrenergic drug, respectively.

Standard solutions

(−)-MCF solution was prepared in acetonitrile (1:25, v/v) and kept at −30°C; no apparent deterioration was observed in the reagent during a one-

TABLE I

CHARACTERISTICS OF THE MOBILE PHASE SYSTEMS

System	Acetonitrile (%)	Methanol (%)	Water (%)	Flow-rate (ml/min)	Drug
A	60	–	40	1	IPP, MPL, PPN, and TPL
B	–	60	40	1	MPT and STL
C	35	22	43	1.2	ANL

month period. Except for MPT and STL, stock solutions of the adrenergic drugs were prepared in acetonitrile; powders of MPT and STL were first dissolved in a small volume (~ 3 ml) of water and then diluted with acetonitrile.

Sample preparation

Stock solutions containing an enantiomeric amount of 62.5 ng of ANL, IPP, MPL, PPN, or TPL, or 625 ng of MPT or STL were evaporated under a nitrogen stream at room temperature. To the residue were added 200 μl of a saturated solution of sodium carbonate and 200 μl of the reagent, and the resultant mixture was vortex-mixed for 30 s. To remove excess of the chloroformate, an excess amount of 4-hydroxy-L-proline was then added, and the mixture was vortex-mixed for 30 s. After centrifugation (Model 235 C micro-centrifuge; Fisher Scientific, Springfield, NJ, U.S.A.) of the mixture for 3 min, 10–25 μl of the upper layer were injected into the HPLC system.

Order of elution of isomers

For MPL, PPN, and STL, the order of elution of the derivatized enantiomers was determined by subjecting the individual enantiomers to the procedure and comparing the respective retention times with those observed after analysis of the racemate.

RESULTS AND DISCUSSION

Racemic drugs constitute a significant portion of the dispensed medicines in both the United States and Europe [1,2]. Because of the difficulties in resolution of enantiomers, most of the earlier studies on the pharmacokinetics of racemic drugs have been conducted utilizing non-stereospecific analytical methods. Recently, however, a considerable attention has been paid to stereospecific methods capable of measuring individual enantiomers of the racemic drugs. These methods have been successfully applied to analysis of individual enantiomers of adrenergic drugs and, in particular, β-adrenoceptor blocking agents [7–17].

Presence of an alcohol and an amine group in the side-chain of the most adrenergic drugs renders them suitable for precolumn derivatization. Indeed, a majority of the reported stereospecific HPLC assays for β-adrenoceptor blocking agents have utilized precolumn derivatization with a homochiral isocyanate [8–12,14]. Some of these methods, however, have restrictions such as low sensitivity [9] and/or relatively long derivatization time [11]. (−)-MCF was first utilized in 1968 in stereospecific analysis of amino acids and some other amino compounds [18]. Our laboratory very recently reported [6] the application of MCF to the analysis of the individual enantiomers of ANL in human plasma and urine.

(−)-MCF-derivatized enantiomers of IPP, MPL, MPT, PPN, STL, and

TPL, in addition to those of ANL, were satisfactorily separated on a reversed-phase system (Fig. 2). Since these drugs cover a wide range of polarity, different mobile phase systems (Table I) had to be used for chromatography of their diastereomers. Capacity, separation, and resolution factors for the tested drugs are listed in the Table II. Among the tested drugs, the most and least satisfactory resolutions were obtained for the derivatized enantiomers of ANL and STL, respectively (Table II). In general complete baseline or near baseline resolution was obtained for most of the tested drugs (Fig. 2 and Table II). However, in some cases satisfactory resolution was associated with long retention time (Fig. 2). In the present study a 10-cm column was used. Application of a longer reversed-phase column (such as commercially available 25-cm columns) or normal-phase chromatography may allow a more satisfactory resolution in a shorter time.

After repeated injections of the samples into the HPLC system no interfer-

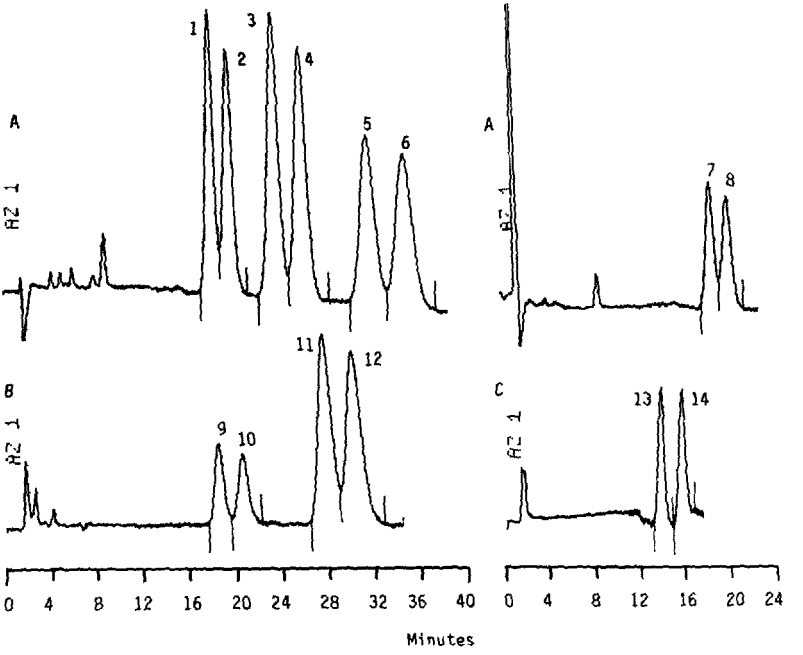

Fig. 2. Chromatograms of the diastereomeric derivatives of the adrenergic drugs metoprolol (peaks 1 and 2), toliprolol (peaks 3 and 4), propranolol (peaks 5 and 6), IPP (peaks 7 and 8), metaproterenol (peaks 9 and 10), sotalol (peaks 11 and 12), and atenolol (peaks 13 and 14). For propranolol, metoprolol, and sotalol the first- and second-eluting isomers correspond to the (−)- and (+)-enantiomers, respectively. The order of elution was not determined for the other drugs. The letters A, B, and C refer to the mobile phase systems utilized for the analysis of the drugs (Table I). The integrator attenuation was changed from 4 to 2 at 12 min.

TABLE II

CHROMATOGRAPHIC CHARACTERISTICS OF THE ADRENERGIC DRUGS

Abbreviations are explained in the text and Fig. 1.

Drug	k'_1	α	R
ANL	7.50	0.152	1.90
IPP	9.20	0.098	1.18
MPL	8.80	0.102	1.20
MPT	9.20	0.130	1.50
PPN	16.5	0.103	1.32
STL	14.4	0.097	1.10
TPL	11.9	0.109	1.39

ing peak was observed in the respective chromatograms, suggesting that there is no late-eluting by-product or reagent-related peak.

For MPL, PPN, and STL, the (−)-isomer eluted before the respective (+)-isomer. However, because of unavailability of the other drugs as their pure enantiomers, their order of elution could not be determined.

To optimize the derivatization reaction, different conditions were used for derivatization of ANL as a model compound. Interestingly, non-aqueous derivatization of ANL resulted in a decrease in the intensity of the observed peaks. Derivatization of the drug in an aqueous medium, on the other hand, increased the observed responses to a significant degree. The optimum condition was obtained by reacting the racemate with the reagent in the presence of saturated sodium carbonate. Since acetonitrile, the carrying vehicle of MCF, is not miscible with saturated sodium carbonate solution, a two-phase reaction system was obtained. Under these conditions, the derivatization reaction was complete within 30 s with an efficiency of close to 100% [6].

Although theoretically chloroformates may react with both amine and alcohol groups, under the stated conditions only the amine group in the side-chain of the tested drugs (Fig. 1) is expected to be derivatized [6]. Chloroformates may also react with phenolic groups [19], which are present in the structure of MPT (Fig. 1). However, a 1:2 or 1:3 derivative of MPT–MCF would be expected to elute with a much longer retention time. Nevertheless, possibility of the reaction of MCF with other reactive functional groups present in the structure of adrenergic drugs must be considered.

Since MCF precipitates when it is mixed with water in the mobile phase, it is necessary to remove excess of the reagent before injection of the acetonitrile layer into the HPLC system. Injection of the acetonitrile layer before removing the reagent resulted in a less satisfactory resolution. 4-Hydroxy-L-proline was, therefore, added to remove the reagent excess. This procedure allows injection of approximately 50 μl of the acetonitrile layer without a significant adverse effect on the resolution of the diastereomers. However, for application to bio-

logical samples, where higher sensitivities are needed, extraction of the derivatives into an organic solvent and subsequent injection into the HPLC system [6] is recommended.

Based upon the observed responses (Fig. 2) after derivatization of 62.5 ng of the adrenergic drugs (except for MPT and STL), the assay certainly has potential for application to analysis of these drugs in biological fluids. Furthermore, the sensitivity of the assay may be increased by substituting a highly fluorescent chloroformate, (+)-fluorenylethyl chloroformate [20], for MCF.

In conclusion a convenient and rapid derivatization method is developed for formation of diastereomers of chiral adrenergic drugs. These diastereomers are then resolved on a reversed-phase chromatographic system. The method has potential for application to other adrenergic drugs, as well as other groups of pharmaceuticals containing similar chemical structure.

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