Journal of Chromatography A, 666 (1994) 275–282
Elsevier Science B.V., Amsterdam

CHROM. 25 711

Stereoselective high-performance liquid chromatographic assay of (±)-delmopinol in plasma using solid-phase extraction, a chiral derivatizing agent and electrochemical detection

Gabriele Egginger, Eva Blaschke and Wolfgang Lindner *

Institute of Pharmaceutical Chemistry, Karl-Franzens-University of Graz, A-8010 Graz (Austria)

Anne-Marie Olsson

Kabi Pharmacia AB, S-201 80 Malmö (Sweden)

ABSTRACT

An enantioselective HPLC bioanalytical method for (±)-delmopinol was established in order to elucidate the pharmacokinetic behaviour of this chiral drug. (±)-Delmopinol and (±)-M1652, a structurally related compound used as internal standard, were extracted from plasma by a solid-phase extraction procedure using CN cartridges. The enantiomers were derivatized with a chiral derivatizing agent (*R*,*R*)-O,O′-di-*p*-toluoyl tartaric acid anhydride yielding diastereomeric derivatives which were separated on a reversed-phase column with acetonitrile–0.1 *M* ammonium acetate buffer (65:35, v/v) pH 5.7 as mobile phase. The resolution values of the diastereomeric derivatives of (−)- and (+)-M1652 and of the derivatives of (−)- and (+)-delmopinol were 1.03 and 1.46, respectively. The limit of quantitation was approximately 3 pmol (1 ng)/enantiomer per 0.5 ml plasma using electrochemical detection (+0.75 V *versus* Pd/PdO reference electrode). The effectiveness of the derivatization was >98% and the total recovery of (±)-delmopinol and of (±)-M1652 from plasma or serum was found to be approximately 50%. The assay was applied to enantioselective pharmacokinetic investigations in humans, rats and dogs but showing here only one concentration time curve of the (+)- and (−)-delmopinol in a human subject after administering (±)-delmopinol in form of an aqueous mouth wash solution for 60 s.

INTRODUCTION

(±)-Delmopinol · HCl is a new substance active against plaque (see Fig. 1) which is currently undergoing clinical trials. Due to its surfactant nature the compound is capable of inhibiting plaque formation, of reducing bacterial acid production, and of dissolving established plaque, in contrast to chlorhexidine [1]. The drug is administered in the form of an aqueous mouthwash solution for *ca.* 60 s in the oral cavity, whereby a proportion of the compound will get absorbed via the mucosa. After 60 s the mouthwash solution was spat out. In order to elucidate the stereoselective pharmacokinetic characteristics of (+)- and (−)-delmopinol an enantioselective HPLC assay had to be developed. However, very low plasma concentrations were expected because the drug is only locally applied in the oral cavity for 60 s, and furthermore, it was known from animal and human studies that the substance undergoes extensive metabolism [2]. Therefore, a highly sensitive detection method had to be incorporated into this assay capable of detecting low pmol to fmol quantities of the enantiomers per ml plasma; it should be noticed

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1175-Y

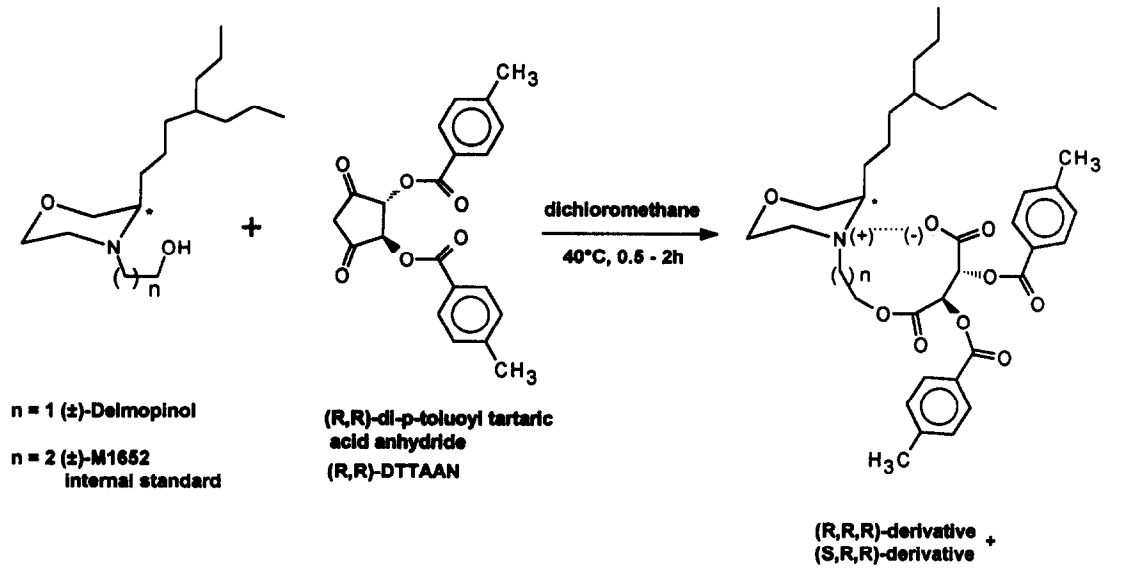

Fig. 1. Derivatization scheme of (±)delmopinol and (±)-M1652, used as internal standard, with (*R*,*R*)-O,O′-di-*p*-toluoyl tartaric acid anhydride [(*R*,*R*)-DTTAAN] leading to diastereomeric ester derivatives.

that the compound lacks any chromo- or fluorophore. Furthermore, the method should be easily transferable to other laboratories and applied to several hundreds of samples; the assay should be reliable, rugged and easy to perform.

This challenge was approached by establishing an enantioselective HPLC bioanalytical method based on the derivatization of (±)-delmopinol and (±)-internal standard (I.S.) with a chiral derivatizing agent (*R*,*R*)-O,O′-di-*p*-toluoyl tartaric acid anhydride [(*R*,*R*)-DTTAAN] resulting in resolvable pairs of diastereomers. The drug and the I.S. were extracted from plasma using a solid-phase extraction procedure prior to the derivatization procedure. The diastereomeric derivatives were separated by isocratic reversed-phase HPLC and electrochemically detected.

Finally, this HPLC assay was applied to enantioselective pharmacokinetic investigations of (±)-delmopinol administered to humans, rats and dogs in the course of the preclinical and clinical trials of this new drug.

EXPERIMENTAL

Instrumentation and chromatographic conditions

HPLC analyses were carried out on a Hewlett-Packard ChemStation 1050 with the corresponding DOS software. The system consisted of an autosampler 1050, a quaternary pump 1050, an interface 35900 and an electrochemical detector ESA Coulochem Model 5100A with a guard cell Model 5020 (+0.75 V) and an analytical cell Model 5010 (+0.75 V *versus* Pd/PdO reference electrode). A Vac-Elut Station from Supelco was used for the extraction procedure and for evaporations of the extracts we used a vacuum centrifuge Hetovac (Bartelt, Graz, Austria). The sonication bath was an Econo-Clean, 20 kHz, 500 W. Enantioseparations were carried out on a Hypersil ODS (5 μm) column 125 × 4 mm I.D. from Seibersdorf (Austria) together with a precolumn LiChrospher 10 × 4 mm I.D. RP-18 (5 μm) from Merck (Germany). The mobile phase consisted of 0.1 mol/l ammonium acetate buffer (membrane filtered 0.2 mm)–acetonitrile (35:65, v/v) at a flow-rate of 0.8 ml/min. The apparent pH of the mobile phase was adjusted to 5.7 with acetic acid.

Chemicals

(±)-, (+)-, (−)-Delmopinol · HCl (M_r = 307.91 g/mol) and (±)-M1652 · HBr (internal standard, I.S.; M_r = 366.39 g/mol) were supplied by Biosurface, Kabi Pharmacia (Malmö, Sweden). (*R*,*R*)-O,O′-Di-*p*-toluoyl tartaric acid

was obtained from Chemische Fabrik Uetikon (Uetikon, Switzerland). The optical purity was shown to be >99% (determined according to a procedure described earlier [3]). Acetyl chloride used for anhydride formation (see below) was purchased from Fluka (Buchs, Switzerland). Pooled serum used as "blank serum" in the course of method development was obtained from the local hospital, blank plasma of humans (containing citrate buffer as anticoagulant), rats and dogs were obtained from Kabi Pharmacia. Plasma samples were frozen and stored at −20°C until use. Dichloromethane, acetonitrile (LiChrosolv) and methanol (analytical-reagent grade) were obtained from Merck. Ammonium acetate buffer solutions and aqueous standard solutions were prepared with "HPLC water" purified with a Millipore apparatus. The citrate buffer was prepared by mixing 44.7 volume parts of an aqueous solution of citric acid monohydrate 0.1 mol/l with 55.3 volume parts of an aqueous solution of Na_2HPO_4 0.2 mol/l, pH 5.6. Analytichem Bond-Elut CN solid-phase extraction cartridges (100 mg), obtained from ICT (Vienna, Austria), were used for plasma or serum extractions.

Standard solutions

Concentrated stock solutions of (±)-delmopinol·HCl 12 μmol (3.7 mg)/25 ml and (±)-M1652·HBr 7.9 μmol (3.0 mg)/25 ml were prepared and diluted with water to a concentration of 3.83 nmol (1.16 ng)/ml and 3.24 nmol (1.18 ng)/ml, respectively.

The solutions of (*R,R*)-DTTAAN (10 mg ≈ 17 μmol/ml) were prepared in dichloromethane, which was dried via azeotropic distillation.

Synthesis of (R,R)-DTTAAN

The chiral derivatizing agent (*R,R*)-DTTAAN was synthesized according to the following procedure: 3 g of (*R,R*)-O,O′-di-*p*-toluoyl tartaric acid were suspended in 20 ml acetyl chloride and refluxed for 2 h. After 15 min the compound was completely dissolved and after 1.5 h a white precipitate was formed. After removing the reaction solvent by evaporation the remaining white crystals were washed and recrystallized twice in chloroform. Yield: 2.4 g, 80%. Melting point: 195°C. IR (KBr): 1890, 1810, 1750, 1710, 1610, 1340, 1280, 1270, 1250, 1230, 1180, 1120, 1090, 1060, 1020, 950, 850, 750/cm^{-1}. NMR (dimethyl sulphoxide): 2.6 m (6H), 6 s (2H), 7.4–8.2 aromate (8 H).

The reagent was stored under light petroleum (b.p. 40–60°C) at room temperature. Under these conditions it has shown to be stable for at least 2 months.

Sample extraction and derivatization procedure

Solid-phase extraction cartridges were conditioned by prewashing with 2 × 1 ml methanol followed by 1 ml water. A centrifuged plasma (serum) sample (0.5 ml) was spiked with 97.2 pmol (±)-I.S. (corresponds to 30 μl of the stock solution), mixed with 0.1 ml citric buffer and diluted to 1.2 ml with a saturated aqueous NaCl solution. This solutions were vortexed for 3 s, exposed to ultrasound in a sonication bath for 30 s, and loaded onto the prewashed CN cartridges using slight vacuum suction. After complete passage of the plasma, the column was washed with 2 × 1 ml water to remove the salt and the proteins. The retained (±)-delmopinol and (±)-I.S. were eluted with a total volume of 1.3 ml acetonitrile–water (80:20, v/v); the first 0.3 ml of the eluate were discarded. The remaining 1.0 ml eluate was collected in a disposable 5-ml glass tube and evaporated at 60°C on a rotovapor within 15 min. The residue was redissolved in 200 μl dichloromethane, vortexed and the solution transferred into a 200-μl microvial (inserted into a in 1.5-ml autosampler vial) via a disposable Pasteur pipette, and the solution was reevaporated again using a vacuum centrifuge. Finally, 40 μl of the reagent solution (*ca.* 0.4 mg reagent, corresponds to >3000 molar excess) were added and the tightly capped autosampler vial was put in an oven at 40°C for 1 h. After removing the solvent by vacuum centrifugation the residue was taken up in 100 μl acetonitrile–water (60:40, v/v), vortexed and 90 μl of this solution were injected onto the HPLC-column.

Validation of the assay

Calculations. Due to a frequently co-eluted peak under the second eluting peak of the I.S. (see Fig. 2) which was observed for blank plasma

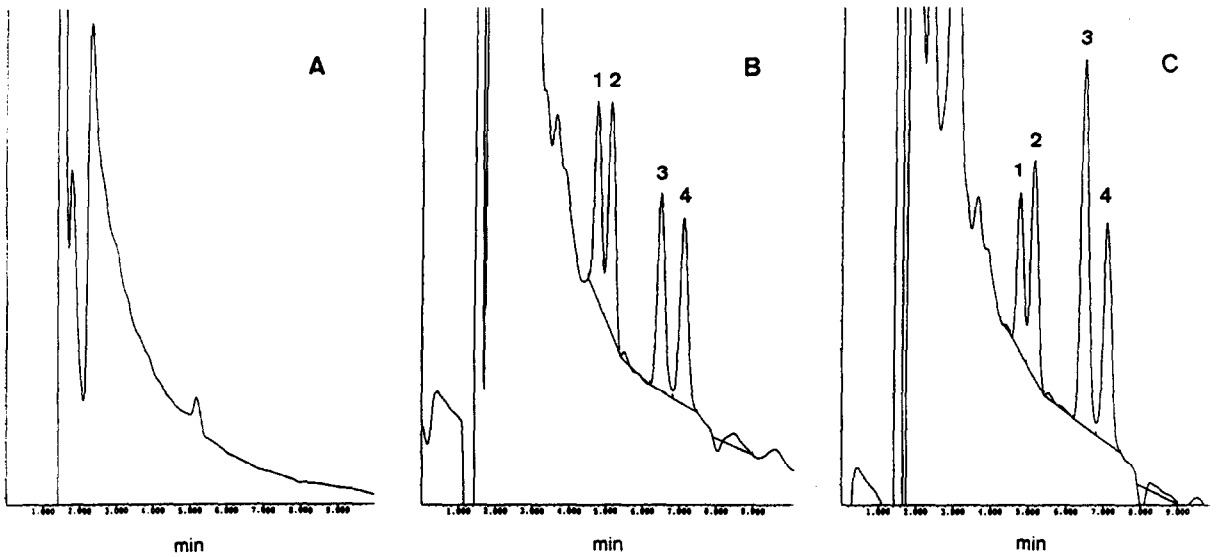

Fig. 2. Typical chromatograms of plasma extracts of (A) blank human plasma, (B) human plasma containing 19.5 pmol (6 ng) of each enantiomer and (C) authentic human sample. Peaks 1 and 2 correspond to (−)- (19.5 pmol) and (+)-M1652-derivatives (19.5 pmol), peaks 3 and 4 correspond to (−)- (27.8 pmol) and (+)-delmopinol derivatives (19.2 pmol).

but also for real-life samples, only the first peak, (−)-I.S derivative, was used for calculations of (−)- and (+)-delmopinol. To determine plasma concentrations of each enantiomer in unknown samples the peak height ratios were evaluated via multi-point calibration curves ($n = 6$) made together with a series (18–22 samples) of plasma samples.

Calibration curves. Aliquots (0.5 ml) of pooled plasma were spiked with 7.5, 18.8, 37.7, 75.7, 189.0, 340.0, 472.2 pmol (±)-delmopinol · HCl and 97.2 pmol (±)-I.S. · HBr ($n = 8$, including blank). Samples were extracted and derivatized as described above. Calibration curves were repeated 6 times on different days.

Day-to-day reproducibility. Day-to-day reproducibility was determined by comparing the peak height ratios at the concentration points of 18.8 and 189.0 pmol (±)-delmopinol · HCl of the calibration curves ($n = 6$) and calculating the relative standard deviation (R.S.D., %).

Within-day-reproducibility. Aliquots (0.5 ml) of pooled plasma were spiked with 97.2 pmol (±)-I.S. and 18.8 or 189.0 pmol (±)-delmopinol · HCl. Each concentration was repeated 6 times. Samples were extracted and derivatized as described above and the R.S.D. of the peak height ratios of (±)-delmopinol and (+)-I.S. was calculated at each concentration.

Accuracy. The estimated concentration of each enantiomer determined at two points of the calibration curve [18.8 pmol and 189.0 pmol (±)-delmopinol · HCl] was compared to the same nominal concentrations in plasma.

Recovery. Aliquots of 0.5 ml pooled serum were spiked with 162.4 pmol (±)-delmopinol · HCl and 136.5 pmol (±)-I.S. · HBr, or with 16.2 pmol (±)-delmopinol · HCl and 13.6 pmol (±)-I.S. · HBr. Samples were extracted and derivatized as described above. Each analysis was repeated 6 times. Lacking the isolated (±)-delmopinol- and (±)-M1652-DTTAAN derivatives as reference standards the relative recovery was calculated by comparing the peak heights of the derivatized enantiomers extracted from plasma with the peak heights of the derivatized enantiomers which had not undergone the extraction procedure.

RESULTS

Derivatization

The derivatization scheme is depicted in Fig. 1; the reaction of (±)-delmopinol and (±)-I.S. with (*R*,*R*)-DTTAAN was complete after 30 min at 40°C and the derivatization yield remained unchanged for more than 5 h (reaction kinetics data not shown). The derivatization yield was found to be >98%, judged by the analysis of underivatized delmopinol according to the method for the racemate described by Olsson *et al.* [4]. No differences in the derivatization kinetics of the single enantiomers of delmopinol as well as of the I.S. could be observed under the conditions chosen. With regard to automation of the method, it was important that the diastereomeric ester derivatives dissolved in the acidic mobile phase were stable for several hours or even days. To investigate degradation phenomena of the diastereomeric derivatives in the mobile phase, the derivatized samples containing 162 pmol of each enantiomer were taken up in 0.5 ml mobile phase, put in the autosampler at room temperature, and injected repetitively over a period of several days. It could be demonstrated that the derivatives of (±)-delmopinol and (±)-I.S. dissolved in mobile phase were stable over more than 1 week (data not shown).

Chromatography

Typical reversed-phase chromatograms of diastereomeric derivatives of (±)-I.S. and (±)-delmopinol are shown in Fig. 2. Retention times of the derivatives of (−)- and (+)-I.S. were 4.5 and 4.9 min ($k' = 2.21$ and 2.50, $\alpha = 1.13$, $R_s = 1.03$) and 6.1 and 6,7 min of (−)- and (+)-delmopinol ($k' = 3.35$ and 3.78, $\alpha = 1.12$, $R_s = 1.46$), respectively. It should be noticed at this point that the diastereomeric derivatives of the (±)-I.S. are eluting before those of (±)-delmopinol, despite the slightly higher lipophilicity of the M1652 molecule (see Fig. 1). This is in contrast to the results obtained when the compounds are chromatographed in their underivatized form as racemates under simple isocratic reversed-phase conditions without chiral derivatization [5]. The elution order of the diastereomeric delmopinol derivatives was verified via derivatization of the individual enantiomers and the elution order of the I.S. derivatives was assigned by analogy to (±)-delmopinol. In order to investigate differences between human serum, and human, rat and dog plasma in view of endogenous possibly co-eluting compounds, the different blank matrices were extracted and derivatized as described above. The chromatograms exhibited similar "peak-patterns", including the unknown but co-eluting peak with (+)-I.S. The derivatized pooled serum extract showed sometimes large interfering peaks, co-eluting in the time window of (±)-delmopinol and (±)-I.S. derivatives. This is most probably due to its randomly changing compositions and therefore only authentic blank plasma of controlled patients or volunteers could be used for the evaluation and validation of the method.

Statistical data of the HPLC assay

The statistical data of the method are summarized in Tables I–III.

Total recovery. At initial plasma concentrations of 136.5 pmol (50 ng) the recovery ranged from 58 to 63% and from 62 to 67% for (−)- and (+)-I.S.; the respective values for 162.4 pmol/l (50 ng) (−)- and (+)-delmopinol were 56–60% and 57–61%. The respective values at concentrations of 13.6 and 16.2 pmol/0.5 ml ranged from 50 to 54% and from 58 to 60% for (−)- and (+)-I.S. and from 50 to 53% and 51–53% for (−)- and (+)-delmopinol. The

TABLE I

STATISTICAL DATA OF THE HPLC ASSAY: RECOVERIES

S.D. = Standard deviation; R.S.D. = relative standard deviation; (±)-D = (±)-delmopinol · HCl; (±)-I.S. = (±)-internal standard · HBr, M1652. Spiked amounts of (±)-D/(±)-I.S. per 0.5 ml plasma: 162.4/136.5 pmol.

Recovery ± S.D. (%)			
(−)-D	(+)-D	(−)-I.S.	(+)-I.S.
58.4 ± 4.4	59.8 ± 4.1	60.1 ± 5.1	64.4 ± 4.9

TABLE II

STATISTICAL DATA OF THE HPLC ASSAY: REPRODUCIBILITY AND ACCURACY

Abbreviations as in Table I.

	Spiked amount: 18.8/97.2 pmol		Spiked amount:188.4/97.2 pmol	
	(−)-D	(+)-D	(−)-D	(+)-D
Within-day reproducibility, R.S.D. (%)	14.3	14.5	6.2	6.1
Day-to-day reproducibility, R.S.D. (%)	15.6	16.2	9.3	11.9
Accuracy (found pmol)	92% (8.54)	105% (9.97)	95% (89.6)	96% (90.6)

slightly higher recovery found for (−)-I.S. could be explained by a hidden but co-eluting peak, occurring after derivatization of plasma samples.

Calibration curves. Peak height ratios of the diastereomeric derivatives of (−)- and (+)-delmopinol and (−)-I.S. correlated linearly within the examined concentration range (7.5–472.2 pmol (±)-delmopinol/0.5 ml plasma). The mean correlation coefficients for (−)- and (+)-delmopinol were $r = 0.992$ and $r = 0.993$, respectively.

Within-day reproducibilities. Within-day reproducibilities at a concentration of 18.8 pmol (±)-delmopinol were approximately 14% for (−)- and (+)-delmopinol. The respective values at 189.0 pmol were *ca.* 6% ($n = 6$).

Day-to-day reproducibility. Day-to-day reproducibility was found to be *ca.* 16% for (−)- and (+)-delmopinol at a concentration of 18.8 pmol/0.5 ml plasma and *ca.* 10% at 189.0/0.5 ml plasma ($n = 6$).

Accuracy. The calculated concentrations of the single enantiomers ranged between 92 and 105% of the actual concentrations of 18.8 pmol and 189.0 pmol (±)-delmopinol · HCl initially present in 0.5 ml spiked plasma.

Limit of quantitation. The limit of quantitation was 2.3 pmol/0.5 ml for (−)- and 2.6 pmol/0.5 ml for (+)-delmopinol derivatives. Similar values were found for the derivatives of the I.S.

DISCUSSION ON METHOD DEVELOPMENT

Derivatives of tartaric acid anhydride (see Fig. 1), developed by Lindner *et al.* [3] have shown their usefulness as chiral derivatizing agents (CDAs) for several β-blockers and other compounds, possessing an amino alcohol structure. The reagent reacts with the hydroxy group of the analyte forming diastereomeric ester derivatives, whereas primary and secondary amines have to be protected by ion-pair formation with *e.g.* trifluoroacetic acid. Such (*R*,*R*)-tartaric acid anhydrides seemed also to be suitable as CDA to

TABLE III

STATISTICAL DATA OF THE HPLC ASSAY: LINEARITY AND LIMIT OF QUANTITATION

Abbreviations as in Table I. Spiked amounts: 7.5, 18.8, 37.7, 75.7, 189.0, 340.0 and 472.2 pmol per 0.5 ml.

Linearity (±S.D.)	(−)-D: $r = 0.992$ (±0.008)	$y = 0.095$ (±0.077) × −0.007 (±0.094)
	(+)-D: $r = 0.993$ (±0.006)	$y = 0.105$ (±0.097) × −0.009 (±0.094)
Limit of quantitation (*S*/*N* 6:1)	(−)-D: 2.2 ± 0.6 pmol/0.5 ml	(+)-D: 2.6 ± 0.6 pmol/0.5 ml

establish an enantioselective HPLC assay of (±)-delmopinol in order to investigate the stereopharmacokinetics of this chiral compound. The first established analysis for this drug was based on chiral derivatization together with fluorimetric detection. Due to the lack of any chromo- or fluorophore a fluorescent tag had to be introduced into the molecule via the CDA. For this purpose an (*R*,*R*)-tartaric acid derivative possessing fluorescent properties was developed: (*R*,*R*)-O,O′-bis-(3,4,5-trimethoxybenzoyl)tartaric acid anhydride, internally termed as (*R*,*R*)-BTMBTAAN [6].

However, during the process of optimizing the method handling and derivatizing plasma extracts with (*R*,*R*)-BTMBTAAN several problems appeared. Using fluorimetric detection the integration of the peaks of I.S. was critical as the derivatives eluted on the down slope of a large front peak —derived from the large excess of the fluorescent chiral reagent. Since fluorimetric detection of (*R*,*R*)-BTMBTAAN derivatives was not "compound specific" but "reagent specific" several co-derivatized and therefore co-detected peaks of co-extracted endogenous compounds occurred in the chromatograms.

Since it was known that (±)-delmopinol can be detected electrochemically [4,5] the possibility of electrochemical detection of the diastereomeric derivatives was also investigated revealing some advantages compared to fluorimetric detection: neither (*R*,*R*)-DTTAAN nor (*R*,*R*)-BTMBTAAN gave a strong electrochemical signal and were therefore "transparent" with respect to the detection; hence one could rely on analyte specificity. The absolute detection limit was found to be approximately 0.1 pmol (30 pg)/enantiomer, however, by switching the method of detection from fluorescence to electrochemical, the CDA was also changed for practical reasons. (*R*,*R*)-DTTAAN was used for further investigations because it was easier and faster to synthesize than the (*R*,*R*)-BTMBTAAN homologue.

The derivatization of β-blockers with (*R*,*R*)-O,O′-diacetyltartaric acid anhydride, used in stereopharmacokinetic investigation of (*R*,*S*)-propranolol, was reported to take about 4 h at 40°C [7]. However, it was demonstrated in the present case that the derivatization of (±)-delmopinol and (±)-I.S. with (*R*,*R*)-DTTAAN was already complete after 30 min, most probably because of the enhanced accessibility of the primary hydroxyl group of (±)-delmopinol compared to the secondary hydroxyl group within the β-blocker side chain.

To obtain a derivatization yield, the water content of the final sample extract prior to the derivatization must be minimized. This was achieved by performing an additional evaporation step of the dichloromethane solutions prior to the derivatization of the samples with (*R*,*R*)-DTTAAN (see Experimental).

Enantioseparations of diastereomeric ester derivatives with tartaric acid anhydrides are largely reliant on the formation of an intramolecular zwitterionic ring between the positively charged amino group and the negatively charged carboxyl group and thus enantioseparation of the diastereomeric derivatives is correlated to the pH of the mobile phase. Optimum separations of (±)-delmopinol and (±)-M1652-(*R*,*R*)-DTTAAN derivatives as well as of (*R*,*R*)-BTMBTAAN derivatives could be achieved within a pH range from 5.3 to 6.0.

In the course of the development of a satisfactory extraction protocol for (±)-delmopinol and (±)-I.S. various attempts, including liquid–liquid extraction, the use of laboratory made Extrelut extraction columns and ion-pair extraction using EDTA and HPF_6, were examined. Promising results were only achieved using the previously described solid-phase extraction procedure [4,5] but with slight modifications such as, for instance, ultrasonication of the samples prior to extraction. Even the use of CN cartridges obtained from different companies (*e.g.* Adsorbex CN cartridges, Merck) leads to irreproducible results. Extraction procedures via solid-phase extraction cartridges are usually quick and easy to perform using a multiport vacuum station. However, when human plasma samples were analyzed several of them could not be sucked through the extraction cartridges, most probably depending on the content of dispersed triglycerides, lipids and proteins. Therefore all fresh thawed plasma samples should be centrifuged (3000 *g*) prior to the extraction step.

It should also be mentioned that (±)-delmopinol and (±)-M1652 exhibit a distinct tendency to adsorb on several material *e.g.* on plastics but also on glass. Only silanized or later, only disposable tubes (for which the glass surface seems less activated compared to washed ones) were used throughout the whole procedure to overcome several problems such as carry-over effects of the compound, low recoveries and poor reproducibilities.

APPLICATION OF THE METHOD

The established enantioselective HPLC assay, based on solid-phase extraction via CN cartridges (Analytichem, ICT), chiral derivatization using (*R*,*R*)-DTTAAN, resolution of the diastereomers by reversed-phase chromatography and electrochemical detection of the analyte was employed in pharmacokinetic investigations of (±)-delmopinol in human and animals. Due to the lipophilic character of the drug it should be expected that biliary elimination of the compound is predominant and, furthermore, that the hepatic clearance of the drug is stereoselective. A plasma concentration time profile of one human subject after administration of 10 ml of a 0.2% solution of (±)-delmopinol for 60 s in the oral cavity is shown in Fig. 3. These preliminary data show that the plasma levels for (−)- and (+)-delmopinol are unequal. This is expected for a drug undergoing fast metabolism. However to check the overall selectivity of the total HPLC assay and the chromatographic system, respectively, and to assure that the quantitative results are not misleading due to unknown co-eluting compounds which could also arise from unknown metabolites, one would need a peak-purity test. Two known and available metabolites, (±)-delmopinol-glucuronide and (±)-ω-hydroxydelmopinol were shown not to interfere in the time window of the derivatives of (±)-delmopinol. A peak-purity test is not available for

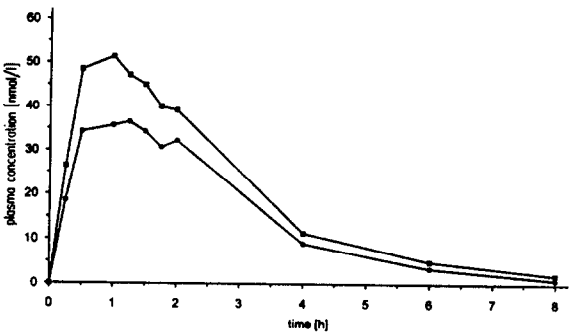

Fig. 3. Plasma concentration time curves of (●) (+)-delmopinol and (■) (−)-delmopinol in one human subject after rinsing with 10 ml of an 0.2% aqueous solution of (±)-delmopinol · HCl in the oral cavity for 60 s.

electrochemical detectors; however, the implementation of an LC–MS system could resolve the basic uncertainty inherent in analyses relying on non-compound specific detection principles.

ACKNOWLEDGEMENT

This study was generously supported by Biosurface, Kabi Pharmacia, Malmö, Sweden.

REFERENCES

1 T. Simonsson, E. Boudesson Hvid, J. Rundegren and S. Edwardsson, *Oral Microbiol. Immunol.*, 6 (1991) 305.
2 Kabi Pharmacia AB, unpublished results.
3 W. Lindner, C. Leitner and G. Uray, *J.Chromatogr.*, 316 (1984) 605.
4 A.-M. Olsson, U.-B. Bjelvebo, C.-G. Johansson and G.Hallström, presented at the *11th International Symposium on Column Liquid Chromatography, Amsterdam, 1987*, poster No. Mo-P-104.
5 C.-J. Trozell, U.-B. Bjelvebo, C.-G. Johansson and G. Hallström, presented at the *13th International Symposium on Column Liquid Chromatography, Stockholm, June 25–30, 1989*, poster No. TH/F-P-093.
6 G. Egginger, W. Lindner and A.-M. Olsson, presented at the *3rd Symposium on Chiral Discrimination, Tübingen, Oct. 1992*, poster 152.
7 W. Lindner, M. Rath, K. Stoschitzky and G. Uray, *J. Chromatogr.*, 487 (1989) 375.