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Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase inhibitor tranylcypromine and its applicability in pharmacokinetic studies

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ABSTRACT

In order to be able to measure low concentrations of tranylcypromine enantiomers in biological material, chiral fluorescent derivatization and high-performance liquid chromatography (HPLC) were employed. The internal standard *S*-(+)-amphetamine and borate–sodium hydroxide buffer pH 11 were added to plasma or urine sample aliquots. *o*-Phthaldialdehyde was used for precolumn derivatization in combination with the chiral mercaptan N-acetylcysteine. HPLC resolution of the diastereoisomeric derivatives was possible on an octadecylsilane column. The mobile phase consisted of sodium phosphate buffer solution pH 6.5, methanol and tetrahydrofuran. The fluorescence of the eluate was monitored at 344/442 nm. The intra-day coefficients of variation were below 10%, the limit of determination was 0.5 ng/ml. The assay was found to be applicable for routine analyses in a preliminary pharmacokinetic study, in which an oral dose of 20 mg racemic tranylcypromine sulfate was administered to three healthy volunteers. The plasma concentrations were generally low, and those of *S*-(−)-tranylcypromine significantly exceeded those of the *R*-(+)-enantiomer. Average maximum concentrations were 57.5 and 6.3 ng/ml for *S*- and *R*-tranylcypromine, respectively. While *S*-tranylcypromine was well detectable within the whole study period (8 h), *R*-tranylcypromine concentrations fell below the detection limit after 4 h in two out of the three studied volunteers.

INTRODUCTION

R-Tranylcypromine (*R*-TCP) and *S*-TCP are the two optically active *trans*-2-phenylcyclopropylamines (*R* = 1*S*,2*R*-, *S* = 1*R*,2*S*-*trans*-2-phenylcyclopropylamine, Fig. 1a). The racemically administered compound acts as a monoamine oxidase (MAO) inhibitor mainly at MAO-B [1,2] with the *R*-(+)-enantiomer exhibiting a significantly higher inhibitory potency than its optical antipode [3].

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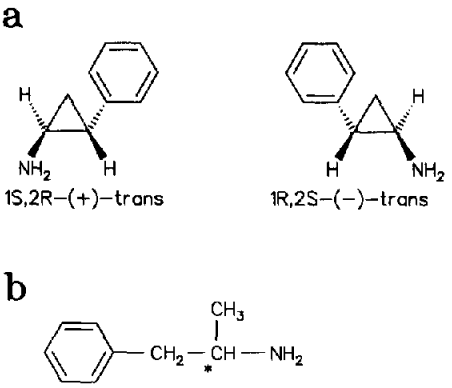

Fig. 1. Chemical structures of (a) tranylcypromine enantiomers and (b) amphetamine.

Present in plasma in low concentrations only — according to a previous study from our group with a maximum concentration of 25 ng/ml after a 20-mg dose of racemic drug [4] — the detection of TCP needs a very sensitive analytical technique. Therefore, the strongly fluorescent benoxaprofen and flunoxaprofen were chosen as potential coupling components. One method has been developed based on derivatization with benoxaprofen chloride [5]. Although very sensitive and precise with a good resolution of the diastereomeric products, problems with interfering peaks and high background fluorescence occurred from time to time during routine employment of this procedure. Thus benoxaprofen chloride (BOP-Cl) was substituted by the less lipophilic *S*-flunoxaprofen chloride (FLOP-Cl) [6] or *S*-flunoxaprofen isocyanate (FLOPIC) [7], respectively, under the assumption that the resulting more polar derivatives would be characterized by a higher retention on silica gel columns, a property that could facilitate the separation of the products from interfering compounds. Both procedures were basically applicable in routine analyses of biological material [8]. Yet frequent column washing was usually still necessary to ensure long-term applicability.

Hence the purpose of our recent studies was the development of a more specific derivatization procedure for the non-fluorescent TCP that would nevertheless permit highly sensitive detection of traces of TCP enantiomers. *o*-Phthaldialdehyde (OPA) represents a well known derivatization reagent that selectively reacts with primary amines in the presence of an SH donor and itself is non-fluorescent. In combination with chiral or achiral mercaptans it can be used as derivatizing agent leading to highly fluorescent isoindole derivatives [9]. This procedure had previously been applied for the assay of a novel prodrug of L-DOPA in biological samples [10], for the enantiospecific assay of amino acids in peptide hydrolysates [11] as well as for the enantiospecific assay of baclofen [12]. We describe here an assay method for TCP enantiomers on the basis of OPA using *S*-(+)-amphetamine (Fig. 1b) as internal standard.

EXPERIMENTAL

Compounds, reagents and solvents

Tranylcypromine sulfate racemate and enantiomers (as sulfates) were obtained from Röhm Pharma (Weiterstadt, Germany). *S*-(+)-Amphetamine was kindly donated by Merck (Darmstadt, Germany). Norephedrine enantiomers, norpseudoephedrine enantiomers, α- and β-phenylethylamine(s), norepinephrine, tyramine and L-amino acids were purchased from Sigma (Deisenhofen, Germany). All organic solvents were of analytical or LiChrosolv grade and obtained from Merck, as well as boric acid, potassium dihydrogenphosphate and disodium hydrogenphosphate dihydrate. OPA, N-acetyl-L-cysteine (N-AC) and N-acetyl-D-penicillamine (N-AP) were purchased from Fluka (Buchs, Switzerland).

Buffer solutions

Solution A: 12.37 g of boric acid + 100 ml of 1 *M* sodium hydroxide up to 500 ml with deionized water; solution B: 0.1 *M* sodium hydroxide; solution C: 1/15 *M* potassium dihydrogenphosphate (9.07 g/l); solution D: 1/15 *M* disodium hydrogenphosphate dihydrate (11.87 g/l). Sodium borate buffer (0.4 *M*, pH 10) was prepared by mixing 59.6 parts of solution A with 40.4 parts of solution B. Sodium phosphate buffer (0.05 *M*, pH 6.5) was composed of 65.3 parts of solution C

and 34.7 parts of solution D. Borate–sodium hydroxide buffer (0.1 *M*, pH 11) was purchased from Merck.

Reagent solutions

S-(+)-Amphetamine (internal standard) was dissolved in methanol giving final concentrations of 1 μg/ml (solution I) and 10 μg/ml (solution II).

Methanolic standard solutions were used for TCP as well. OPA–N-AC or OPA–N-AP reagent solution was always freshly prepared once a day and consisted of OPA (10 mg), ethanol (0.5 ml), 0.4 *M* sodium borate solution (5 ml) and N-AC (40 mg) or N-AP, respectively. The extraction solvent consisted of 100 parts of diethyl ether and 1.5 parts of ethanol (= 1.48% ethanol).

Chromatography

Fluorescence assay. A Zorbax ODS column (250 mm × 4.6 mm, 5 μm particle size, DuPont, Wilmington, DE, USA) was used as stationary phase. The mobile phase for the analysis of plasma and urine samples consisted of 0.05 *M* sodium phosphate solution pH 6.5 (see above)–methanol–tetrahydrofuran (50:60:1, v/v). A Knauer Model 60 pump (Berlin, Germany) was used. A flow-rate of 1.2 ml/min was applied at ambient temperature resulting in an average pressure of 19.5 MPa.

The eluate was monitored at 344/442 nm (excitation/emission) with a Shimadzu RF-535 fluorescence monitor. The time constant was set to medium, the excitation and emission band widths were 13 and 15 nm, respectively, and the sensitivity was set to high at an attenuation of 32. For routine analysis a Merck Hitachi AS 4000 autosampler (Darmstadt, Germany) may be used.

UV assay system (used to determine the derivatization yields). Fractions remaining underivatized were determined by non-enantiospecific reversed-phase chromatography on a LiChrosorb RP-8 column (120 mm × 4 mm, 5 μm particle size, Knauer) using a mixture of methanol and aqueous ammonium carbonate 0.1% (55:45, v/v) at a flow-rate of 1 ml/min and ambient temperature. The absorption of the eluate was monitored at 225 nm with a Shimadzu SPD-6A UV spectrophotometric detector. The retention time of TCP was 6.8 min and that of amphetamine 8.4 min.

Comparison of N-AC and N-AP as chiral mercaptans

Racemic TCP (100 ng) and 50 ng of each enantiomer, respectively, were made to react with either N-AC or N-AP reagent solution. In order to evaluate the resolution of the diastereomeric products on an ODS stationary phase, a 35:65:1 mixture of pH 6.5 buffer (see above)–methanol–tetrahydrofuran was used. For the derivatives with a higher separation and resolution factor, the mobile phase was then further optimized for biological material.

Evaluation of the reaction time for OPA–N-AC

Aliquots of methanol solutions of (+)-amphetamine (20 ng) and racemic TCP (50 ng) were pipetted into reaction tubes, and the solvent was evaporated. Reagent solution (100 μl) was added. Reaction times were 0.5, 1, 2, 3, 5, 10 and 20 min. After the corresponding reaction time, the solution was immediately injected and the derivatization products were quantified. Peak heights were measured and used to study the fluorescence yield for both TCP enantiomers and *S*-(+)-amphetamine *versus* time.

Extraction yields from plasma and urine

To blank plasma and urine different amounts of racemic TCP were added (20, 100 and 200 ng/ml) and the extraction yields estimated by repetitive extraction with diethyl ether–ethanol. After evaporation of the solvents, derivatization with OPA–N-AC and measurement of the peak heights, the amount extracted was determined by comparison with the peak heights obtained when derivatizing defined amounts of TCP sulfate reference compound.

Determination of TCP enantiomers in plasma and urine

Plasma. To a 1 ml-aliquot of plasma 1 ml of borate–sodium hydroxide buffer pH 11, 20 μl of internal standard solution I (= 20 ng of internal

standard) and 5 ml of extraction solvent (diethyl ether with *ca.* 1.5% ethanol) were added in a screw-capped tube. After mixing for 10 min and centrifugation (10 min at 1500 *g*) the organic layer was transferred into a second tube and kept at room temperature until HPLC analysis was performed. Prior to injection the solvent was evaporated and 100 μl reagent solution were added. After brief vortex-mixing the mixture was allowed to stand at room temperature under protection from light. Total time between addition of reagent and injection should be 5.0 min. The resulting solution was directly injected onto the column.

Urine. To a 0.5-ml aliquot of urine 1 ml of pH 11 buffer, 20 μl of internal standard solution II (= 200 ng internal standard) and 5 ml of diethyl ether with ethanol were added and the procedure continued as described for plasma.

(+)-Amphetamine as potential TCP metabolite in biological samples

In order to ensure that biological samples obtained after TCP dosage do not contain (+)-amphetamine in concentrations that may interfere with the assay, plasma and urine samples of three healthy TCP-dosed (20 mg *R/S*-TCP sulfate) volunteers obtained at different times were randomly screened for amphetamine using the TCP procedure, yet without addition of internal standard.

Interferences with other TCP metabolites, other primary amine drugs or drug metabolites and endogenous compounds, respectively

The TCP metabolite *p*-hydroxytranylcypromine [13] as well as phenylethylamines, norephedrine, norpseudoephedrine, norepinephrine, tyramine and amino acids were tested for potential interference with the assay. For this purpose, the compounds were added to blank plasma samples and extraction and derivatization performed as decribed for TCP.

No further studies were performed with drugs which form metabolites that are primary amines.

Short- and long-term stability of TCP in biological samples upon storage

Biological samples that contained TCP were assayed immediately after sampling, after a 3-month as well as after a 24-month storage period at −20 and −80°C.

Comparison with an alternative enantiospecific procedure for TCP

The method described by Weber [14] was used to prove the reliability of the proposed new procedure. The plasma extraction procedure was similar to the one used in the OPA–N-AC assay. However, no internal standard was added, diethyl ether was replaced by diisopropyl ether and the extraction was performed twice. Urine (1 ml) was alkalized by addition of 1 ml of 0.05 *M* sodium hydroxide solution and the extraction performed as for plasma. The ether layers were transferred into reaction tubes and the organic solvents evaporated under nitrogen. *S*-Benoxaprofen chloride solution [100 μl, concentration (in dichloromethane): 1 mg/ml] was added and kept at ambient temperature for 60 min. The solution (25 μl) was injected onto a Zorbax-Sil stationary phase that was run with a mixture of cyclohexane–dichloromethane–tetrahydrofuran [composition (v/v) 5:1:1 for plasma and 7:1:1 for urine] at a flow-rate of 1 ml/min (8.5 MPa). The fluorescence of the eluate was monitored at 312 nm for excitation and 365 nm for emission.

Pharmacokinetic study

Three healthy volunteers were administered 20 mg of racemic TCP sulfate as commercially available tablets with 200 ml of tap water (2 h before breakfast). Ethics committee approval had been obtained for the study. The volunteers received a standardized diet throughout the study period. Venous blood samples (10 ml) were taken prior to dosage and 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h afterwards. They were collected in heparinized tubes. Plasma was prepared by centrifugation at 1000 *g* for 20 min. Urine was collected up to 8 h post-dose. Samples were stored frozen (−80°C) in glass tubes until analysis.

Definitions of pharmacokinetic parameters and data analysis

Pharmacokinetic parameters were determined via non-compartmental procedures. C_{max} represents the maximum concentration that was detected in plasma, t_{max} the corresponding time. $t_{1/2}$ is defined as the half-life of the terminal log-linear phase of the concentration *versus* time curve and was calculated from the slope of the terminal log-linear phase of the curve as $\ln 2/\lambda_z$, where λ_z is the respective rate constant. The area under the concentration–time curve, AUC, was calculated using the lin-trapezoidal rule for the ascending and plateau portion and the log-trapezoidal rule for the descending portion of the curve. The apparent oral clearance (Cl/F) was calculated from the dose and the AUC that was extrapolated to infinite time. The mean residence time (MRT) for an enantiomer was calculated as AUMC/AUC as proposed by Benet and Galeazzi [15], where AUMC is the area under the first-moment curve. The renal clearance, Cl_R, was determined as the ratio of the amount excreted into urine until infinite time, $Ae_{0-\infty}$, and of the AUC until infinite time. $Ae_{0-\infty}$ was extrapolated on the basis of the terminal rate constant that was obtained from the plasma data:

$$Ae_{0-\infty} = Ae_{0-t}/(1 - e^{-\lambda_z t})$$

RESULTS AND DISCUSSION

Derivatization and chromatographic properties of the products

In general, both tested chiral mercaptans were applicable for the determination of TCP. A direct comparison of N-AC and N-AP derivatization products using a different mobile phase than finally applied for the TCP assay in biological material resulted in broader peaks and a longer retention on the stationary phase for the N-AP derivatives. Separation and resolution factors for the N-AC derivatives were 1.14 and 1.2 [capacity factors (k'): 1.23 for (−)-TCP, 1.41 for (+)-TCP], while those for the N-AP derivatives amounted to 1.08 and 1.09, respectively [k': 5.41 for (−)-TCP, 5.82 for (+)-TCP]. Since the N-AC derivatives showed better resolution at more reasonable analysis times, all subsequent experiments were done with OPA–N-AC.

For *R*- and *S*-TCP, assayed with OPA–N-AC after extraction from plasma and urine samples, no difference was detected with respect to extraction yields. The plasma and urine extracts (prior to derivatization) were found to be storable at 2–4°C for 4–6 days without affecting the accuracy of the assay. (+)-Amphetamine was found to be a suitable internal standard with similar extraction yields as for TCP (for a single extraction from plasma: approximately 92% for *S*-TCP and for *R*-TCP, 97% for amphetamine; from urine: *ca.* 82% for both TCP enantiomers, 89% for amphetamine). For the assay of TCP average *S*-(−)/(R)-(+) peak-area ratios of 1.02 (plasma) and 0.95 (urine) were calculated.

Relative fluorescence (= product) yields within 20 min are shown in Fig. 2 for TCP enantiomers and amphetamine. For both compounds highest yields were detected 5 min after addition of the reagent. Then the peak heights decrease again, yet, the peak-area ratios for TCP and internal standard remain constant for at least 5 min and change slightly thereafter. There was no significant difference between the formation and stability of the two TCP products.

Due to the instability of the isoindole product, its isolation and purification was not possible.

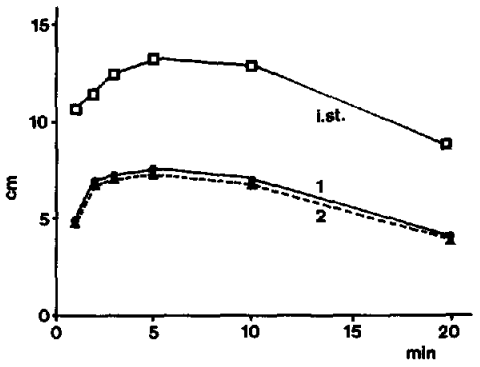

Fig. 2. Relative fluorescence yields (peak heights) for the derivatization of TCP enantiomers and (+)-amphetamine with OPA–N-AC within 20 min after addition of the reagent solution: 1 = *S*-(−)-TCP (25 ng); 2 = *R*-(+)-TCP (25 ng); i.st. = internal standard, amphetamine (20 ng).

Hence, the derivatization yields were determined by quantification of underivatized drug. Residual fractions of substrate were already negligible for TCP after 1 min indicating a fast and complete derivatization. After 2 min the peak-area ratios for derivatized TCP and internal standard remained constant for at least 5 min and changed slightly thereafter.

Amphetamine and TCP as well as both TCP enantiomers were well separated. The capacity factors for *S*-TCP, *R*-TCP and (+)-amphetamine were 16.1, 17.3 and 12.1. The separation and resolution factors for the TCP enantiomers amounted to 1.07 and 1.38, respectively. Chromatograms obtained from plasma standards and a plasma sample after racemate dosage are depicted in Fig. 3.

Various alternative potential internal standards, including amino acid derivatives, had been tested during assay development. However, amphetamine was found to be superior to any other compound with respect to the chromatographic properties of the derivative and most similar to TCP as far as extraction yield and product stability are concerned. This outweighs problems which may occur due to the restrictions of drug control authorities for the use of this compound.

Potential interferences with TCP metabolites or endogenous compounds

As can be seen from the chromatograms for blank plasma (Fig. 3), there were no interferences from plasma constituents. Similar chromatograms were obtained for blank urine samples. In accordance with Riederer *et al.* [16], no amphetamine was detected in TCP samples when assaying plasma and urine from healthy volunteers dosed with 20 mg of racemic TCP. The detection limit for (+)-amphetamine was 1 ng/ml. Furthermore, neither the more hydrophilic metabolite *p*-hydroxy-TCP interfered with the assay, nor did norephedrine, norpseudoephedrine and tyramine.

Since TCP inhibits the metabolism of biogenic amines by inhibiting the monoamine oxidases, the concentration of these amines, such as β-phenylethylamine (β-PEA), may be enhanced after TCP dosage. Hence, it may be possible that interferences with endogenous compounds occur under TCP therapy. Norepinephrine (and α-PEA) do not interfere, yet the derivative of β-PEA elutes at a time that is close to those of the TCP enantiomers.

Although not completely separated from TCP, β-PEA can be identified and does not interfere

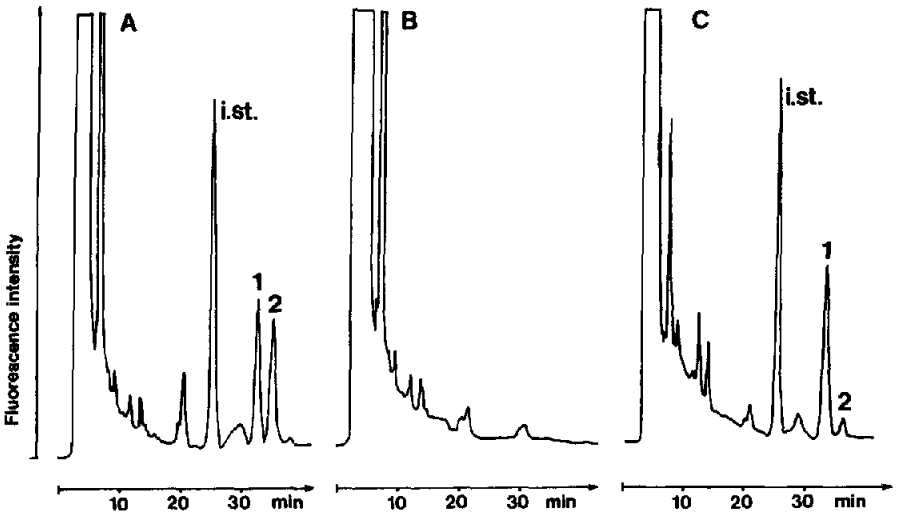

Fig. 3. Chromatograms obtained for plasma standard samples and a volunteer's sample. (A) Plasma containing 50 ng/ml racemic TCP, *i.e.* 25 ng/ml of each enantiomer; (B) blank plasma; (C) plasma sample 4.0 h after oral dosage of 20 mg racemic TCP sulfate. Peaks: 1 = *S*-(−)-TCP; 2 = *R*-(+)-TCP; i.st. = internal standard, amphetamine.

with the assay. In the samples from a single oral dosage of 20 mg racemate significant amounts of β-PEA were not found.

Since no further studies were performed with other drugs that are primary amines or form primary amine metabolites, such compounds should be tested for interference when coadministered to volunteers or patients, who are participating in pharmacokinetic studies with TCP.

Detection limit, linearity, accuracy and intra-day variability

The limit of determination for plasma was 0.5 ng/ml for each of the TCP enantiomers, while for urine it amounted to 2 ng/ml for *S*-(−)- or *R*-(+)-TCP. It may be improved by increasing the sample volume.

The peak-area ratio *versus* concentration relationship was found to be linear for both TCP enantiomers in plasma and urine in the investigated concentration ranges [plasma: 1–10 ng enantiomer per ml (n = 5); *S*-(−)-TCP, $y = 0.0176x + 0.00091$, $r = 0.9955$; *R*-(+)-TCP, $y = 0.0164x + 0.00080$, $r = 0.9981$; 10–250 ng enantiomer per ml (n = 5); *S*-(−)-TCP, $y = 0.0187x + 0.00101$, $r = 0.9996$; *R*-(+)-TCP, $y = 0.0172x - 0.00042$, $r = 0.9990$; urine: 50–400 ng enantiomer per ml (n = 6); *S*-(−)-TCP $y = 0.0859x + 0.00836$, $r = 0.9998$; *R*-(+)-TCP, $y = 0.0888x - 0.00522$ $r = 0.9993$].

The intra-day assay variability is shown in Table I. Coefficients of variation were always below 10%. A comparison of the new procedure with that used in our earlier studies with TCP [8,14] did not show any significant difference in the concentrations.

Stability of TCP in biological samples

Neither total TCP concentrations nor the enantiomeric composition were affected by long-term storage (up to 24 months) of biological samples at −20 or −80°C.

Application of the assay in a preliminary pharmacokinetic study

Plasma concentration *versus* time curves after dosage of racemic TCP sulfate are depicted in Fig. 4. All plasma concentration *versus* time curves can be described by a one-compartment open model with first-order absorption. The individual

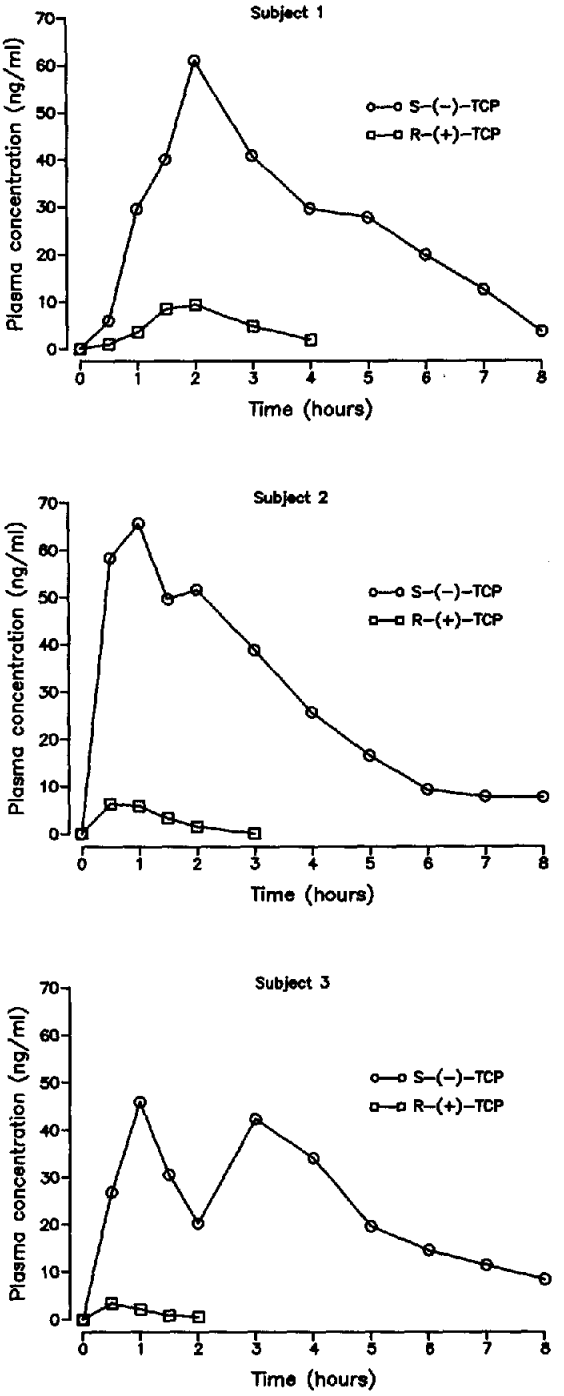

Fig. 4. Plasma concentration *versus* time curves of *R*- and *S*-TCP after a single oral dose of 20 mg racemic TCP sulfate in three healthy subjects.

TABLE I

COEFFICIENTS OF VARIATION OBTAINED FOR PLASMA AND URINE SAMPLES AT DIFFERENT CONCENTRATIONS (n = 9)

TCP enantiomer concentration (ng/ml)	Coefficient of variation (%)	
	S-(−)-TCP	*R*-(+)-TCP
Plasma		
50.0	5.39	5.87
25.0	5.25	5.24
12.5	6.81	5.61
2.5	8.78	9.14
0.5	4.08	5.30
Urine		
200	6.91	7.60
100	4.26	4.11
50	3.69	3.52

pharmacokinetic parameters are given in Table II. Average C_{max} values were 57.5 ng/ml for *S*-(−)-TCP and 6.3 ng/ml for *R*-(+)-TCP, respectively. Terminal plasma half-lives were different for both enantiomers, the average $t_{1/2}$ for the levorotatory enantiomer amounted to 1.66 h, that for the dextrorotatory enantiomer to 0.67 h. The average MRTs were 3.53 h for *S*-(−)-TCP and 1.59 h for *R*-(+)-TCP. The apparent oral clearance of the *R*-(+) enantiomer significantly exceeded that of the *S*-(−) enantiomer, with arithmetical means of 25.2 l/min for *S*-(−)-TCP and 0.76 l/min for *R*-(+)-TCP. One possible explanation is a highly stereoselective first-pass effect. Significantly more *S*-(−)- than *R*-(+)-TCP was excreted into urine (133.4 μg S *versus* 17.9 μg *R*). In one of the volunteers (volunteer 3) no *R*-(+)-TCP was detected at all in urine. The unchanged renal clearance was low: an average of 12.0 ml/min was calculated for *S*-(−)-TCP and 17.1 ml/min for *R*-(+)-TCP.

Overall, the present investigations show a good applicability of the OPA–N-AC procedure to pharmacokinetic studies and confirm the distinct differences between the two TCP enantiomers after racemate dosage. The range of the obtained pharmacokinetic parameters (*e.g.* $t_{1/2}$) is in good agreement with that found previously for racemic drugs [17,18]. More detailed pharmacokinetic studies including dosage of the single enantiomers will be subject of a separate communication [19].

TABLE II

PHARMACOKINETIC PARAMETERS OF *S*-(−)- and *R*-(+)-TCP FOR THREE HEALTHY VOLUNTEERS AFTER A 20-mg DOSE OF RACEMIC TCP SULFATE

Parameter	Volunteer 1		Volunteer 2		Volunteer 3	
	S	*R*	*S*	*R*	*S*	*R*
Cl_0 (ml/min)	754	7788	671	18376	869	49603
C_{max} (ng/ml)	60.9	9.3	65.6	6.4	45.9	3.4
t_{max} (h)	2	2	1	0.5	1	0.5
$AUC_{0-\infty}$ (ng ml^{-1} h)	221.0	21.4	248.4	9.1	191.7	3.4
$AUMC_{0-\infty}$ (ng ml^{-1} h^2)	818.0	55.2	855.7	10.8	658.3	3.3
MRT (h)	3.70	2.58	3.44	1.19	3.43	1.00
λ_z (h^{-1})	0.439	0.666	0.338	1.333	0.517	1.475
$t_{1/2}$ (h)	1.58	1.04	2.05	0.52	1.34	0.47
$Ae_{0-\infty}$ (μg)	258.0	29.6	66.6	6.1	75.5	–
Cl_R (ml/min)	18.8	22.9	4.2	11.2	6.6	–

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REFERENCES

1 H. Nohta, K. Zaitsu, Y. Tsuruta and Y. Ohkura, *Anal. Chim. Acta*, 156 (1983) 253.
2 V. J. Nickolson and R. M. Pinder, in D. F. Smith (Editor), *CRC Handbook of Stereoisomers: Drugs in Psychopharmacology*, CRC Press, Boca Raton, FL, 1983, pp. 215–240.
3 G. P. Reynolds, W. D. Rausch, and P. Riederer, *Br. J. Clin. Pharmacol.*, 9 (1980) 521.
4 A. Lang, H. E. Geissler and E. Mutschler, *Arzneim.-Forsch.*, 28 (1978) 575.
5 H. Weber, H. Spahn, E. Mutschler and W. Möhrke, *J. Chromatogr.*, 307 (1984) 167.
6 H. Spahn, *J. Chromatogr.*, 427 (1988) 131.
7 E. Martin, K. Quinke, H. Spahn and E. Mutschler, *Chirality*, 1 (1989) 223.
8 H. Weber, H. Spahn, W. Möhrke and E. Mutschler, *J. Pharm. Pharmacol.*, 36 (Suppl.) (1984) 50W.
9 P. Lindroth and K. Mopper, *Anal. Chem.*, 51 (1979) 1667.
10 A. Hisaka, S. Kasamatsu, N.Takenaga and M. Ohtawa, *J. Chromatogr.*, 494 (1989) 183.
11 R. H. Buck and K. Krummen, *J. Chromatogr.*, 387 (1987) 255.
12 E. W. Wuis, E. W. J. Beneken Kolmer, L. E. C. van Beijsterveldt, R. C. M. Burgers, T. B. Vree and E. van der Kleyn, *J. Chromatogr.*, 415 (1987) 419.
13 G. B. Baker, D. R. Hampson, R. T. Coutts, R. G. Micetich, T. W. Hall and T. S. Rao, *J. Neural Transm.*, 65 (1986) 233.
14 H. Weber, *Ph. D. Thesis*, Department of Pharmacology, Johann Wolfgang Goethe-University Frankfurt/Main, 1984.
15 L. Z. Benet, and R. L. Galeazzi, *J. Pharm. Sci.*, 68 (1979) 1071.
16 P. Riederer, G. P. Reynolds and M. B. H. Youdim, in M. B. H. Youdim and E. S. Paykel (Editors), *Monoamine Oxidase Inhibitors — The State of the Art*, Wiley, London, 1981, pp. 63–76.
17 A. G. Mallinger, D. J. Edwards, J. M. Himmelhoch, S. Knopf and J.Ehler, *Clin. Pharmacol. Ther.*, 40 (1986) 444.
18 A. G. Mallinger, J. M. Himmelhoch, M. E. Thase, D. J. Edwards and S. Knopf, *J. Clin. Psychopharmacol.*, 10 (1990) 176.
19 H. Weber-Grandtke, G. Hahn, E. Mutschler, W. Möhrke and H. Spahn-Langguth, *Br. J. Clin. Pharmacol.*, in press.