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Determination of opipramol in human plasma by high-performance liquid chromatography with photometric detection using a cyanopropyl column

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Abstract

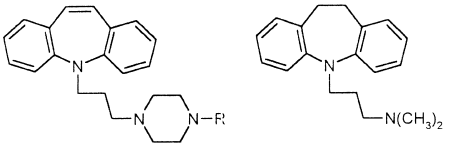
A high-performance liquid chromatographic method is described for the determination of opipramol in human plasma. Opipramol was extracted into *tert*.-butylmethyl ether, separated on a cyanopropyl silica column and detected at 254 nm. Imipramine was used as internal standard. The limit of quantitation was 250 pg/ml using 1.5 ml plasma. Precision was better than 9%, inaccuracy less than 8%. The assay is more sensitive than previously published methods, and it has been applied to the analysis of plasma samples from a pharmacokinetic study.

Keywords: Opipramol

1. Introduction

Opipramol is a tricyclic compound for therapy of anxious-depressive states, somatoform disorders, and general anxiety disorders. The drug is almost completely absorbed from the gastrointestinal tract, extensively metabolized by oxidation (Fig. 1) [1,2], and eliminated mainly via the kidneys.

Concentrations of opipramol in biological matrices were measured by photometric, fluorimetric [1], or radio-isotope techniques (Novartis Pharma, data on file). For drug monitoring purposes automatic immunoassay procedures or HPLC are applied [3]. All methods lack sensitivity and/or specificity for establishing single dose pharmacokinetics of opipramol in healthy volunteers. A sensitive HPLC method with electrochemical detection was published recently [4].



	R	
Opipramol	$-CH_2-CH_2-OH$	Imipramine
Deshydroxyethyl opipramol	-H	

Fig. 1. Chemical structure of opipramol, its inactive main metabolite deshydroxyethyl opipramol, and of imipramine (internal standard).

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In the present paper an improved and even more sensitive HPLC method with photometric detection is described using a cyanopropyl column.

2. Experimental

2.1. Chemicals

Opipramol·2HCl and imipramine·HCl (internal standard) were kindly supplied by Novartis, Nuremberg, Germany. *Tert*.-Butyl methyl ether (TBME) for HPLC was purchased from Fluka, Neu-Ulm, Germany, acetonitrile ultra gradient HPLC grade from Baker, Groβ-Gerau, Germany, and the other chemicals (p.a. grade) from E. Merck, Darmstadt, Germany. Water was purified by a Milli-Q water purification system (Millipore, Eschborn, Germany).

2.2. Stock and working solutions

Stock solutions of opipramol·2HCl and imipramine·HCl were prepared at 100 μg/ml in 10 m*M* HCl. The stock solutions were stable for at least 4 weeks in a refrigerator. Dilution down to 1 μg/ml was done in 20 m*M* HCl. Working solutions in the ng/ml range were prepared in blank plasma or eluent, because at low concentrations the content of opipramol decreased slowly with time in 20 m*M* HCl (presumably due to adsorption of opipramol onto the wall of the PPN tubes).

2.3. Sample treatment

The plasma samples were stored at −25°C and thawed in a water bath of room temperature. To 1.5 ml of plasma in a 100×17 mm polypropylene (PPN) tube were added 50 μl 1.0 *M* Na_2CO_3 and 50 μl internal standard solution (imipramine 1 μg/ml in 20 m*M* HCl). The calibration and quality control samples were spiked with additional 50 μl opipramol solution in plasma of 300, 900, 180 and 36 ng/ml to get samples of 10, 30, 6, 1.2 ng/ml (calibrator, and high, medium, low level control). The samples were extracted for 15 min with 3 ml TBME using a rotary extractor (REAX 2, Heidolph, Kelheim, Germany). After centrifugation (5 min, 2500 *g*) the tubes were transferred to a deep freezer (−70°C) for 10–15 min. Immediately upon removing the tubes, the organic layer was decanted from the frozen aqueous layer into 75×12 mm PPN tubes. The organic solvent was back-extracted with 300 μl of 20 m*M* HCl for other 15 min. After centrifugation and freezing, the organic layer was completely removed by aspiration. Extraction was repeated after adding 50 μl 1.0 *M* Na_2CO_3 and 2 ml TBME to the aqueous layer. Finally, the TBME was evaporated to dryness in a centrifugal vacuum concentrator at 40°C (RC 10-22, Jouan, Unterhaching, Germany). The samples were reconstituted with 150 μl mobile phase, and 50 μl were injected onto the column. The processed samples were stable in mobile phase at least for 17 h at room temperature and for 3 days in a refrigerator.

2.4. Instrumentation and chromatographic conditions

The chromatographic system comprised pump LC 10AS, autosampler SIL-10A, photometric detector SPD 10A (254 nm), system controller SCL-10A, integration software Class-LC10 (all from Shimadzu, Duisburg, Germany), and column oven ERC 125 (30°C, ERC, Alteglofsheim, Germany). Chromatography was carried out using a Phenomenex Luna CN (5 μm, 150×4.6 mm I.D., Phenomenex, Aschaffenburg, Germany). The mobile phase was 20 m*M* NH_4OAc20 m*M* HOAcacetonitrile (240:60:700, v/v/v). The retention time of opipramol was 8.7–8.9 min, and of imipramine (internal standard) 12.6–12.9 min at a flow-rate of 1.0 ml/min (column temperature 30°C). The peaks were quantitated by the peak height method, calibration was done by calibration curves from 0.67 to 33.3 ng/ml (equation $y=m*x$) or by one-point calibration at 10 ng/ml.

For comparison, several plasma samples were assayed by the present HPLC method and by HPLC with electrochemical detection (Novartis Pharma, data on file). An ESA Coulochem II detector (Bischoff, Leonberg, Germany) was used in combination with a pump model 2248 (Pharmacia, Freiburg, Germany), autoinjector SIL-9A, system controller SCL-6B, integrator C-R4A (Shimadzu, Duisburg, Germany) as described elsewhere [5]. The potential of the detector was set to 1.0 V (guard cell), 0.20 V (screening electrode E1), and 0.53 V (working electrode E2). Separation was performed on a Novapak

C_{18} 4 μm (150×4 mm I.D.) column (Waters, Eschborn, Germany) and 50 m*M* KH_2PO_4, 1.5 m*M* sodium octane sulfonate in water–acetonitrile (70:30, v/v) adjusted to pH 3.5 with 85% H_3PO_4 as eluent. The retention time of opipramol (flow-rate 1 ml/min, column temperature 30°C) was 3.9 min, and of imipramine 9.4 min.

3. Results and discussion

3.1. Sample treatment procedure

Tert.-Butylmethyl ether has been established as a standard extraction solvent for lipophilic basic drugs from biological matrices. In our laboratory we used an analogous sample treatment for the HPLC assay of thioridazine, dibenzepin, tropisetron, and macrolides [5]. The double extraction procedure provided somewhat cleaner samples. After single extraction into TBME and evaporation the reconstituted solution was sometimes cloudy, albeit the chromatographic background did not differ from that after double extraction.

3.2. Chromatography

Silica based C_{18} reversed phases are the most popular HPLC phases for the determination of drugs in biological matrices. However, for the separation of lipophilic amines such as phenothiazines, tricyclic antidepressants or macrolide antibiotics [5] cyano phases proved to be more efficient. The substances elute as sharp symmetrical peaks, and parent compounds and hydrophilic metabolites elute closer together compared to C_{18} phases, as has been also reported recently with clomipramine [6]. The present chromatographic system is based on published methods on the determination of phenothiazines by HPLC [7–9]. Nevertheless, the choice of a suitable cyano column may be crucial. Despite using a cyanopropyl column of the same manufacturer we were not able to reproduce the published chromatograms of thioridazine and mesoridazine [9]. Of the tested cyano phases such as LiChrospher, Nucleosil, Ultrasep, Zorbax, Spherisorb and Phenomenex Luna we selected Spherisorb 5 μm CN and Phenomenex Luna 5 μm CN, because of good peak shape, favourable retention factors and high plate number. For the determination of opipramol we used one single Luna 5 μm CN column for the analysis of about 1000 plasma samples without significant change in performance. A representative chromatogram is depicted in Fig. 2. The selectivity changed somewhat with time. The small peak eluting just before the internal standard imipramine was not more separated to baseline after 900 injections.

3.3. Validation data

The limit of detection ($S/N=3/1$) on column was calculated to 80 pg opipramol·2HCl by injecting 10 μl of a solution of opipramol·2HCl 20 ng/ml in eluent. The limit of quantitation was determined by processing 5 replicate spiked plasma samples at concentrations of 667, 500, 375, 250, and 167 pg/ml each. The lowest concentration could be assayed with acceptable precision and accuracy (deviation <15%, Table 1). The limit of quantitation was set to

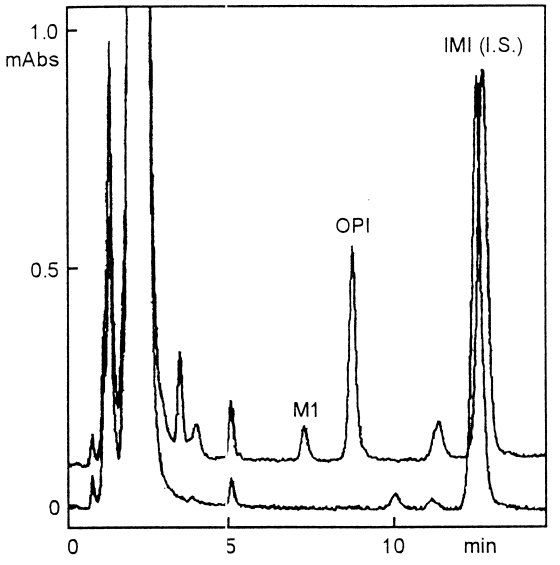

Fig. 2. Chromatograms of plasma of a volunteer before and 4 h (concentration of opipramol·2HCl 7.25 ng/ml) following an oral dose of opipramol·2HCl 50 mg. Abbr.: OPI, opipramol; IMI, imipramine (internal standard); M1, deshydroxyethyl opipramol. Column Phenomenex Luna CN 5 μm (150×4.6 mm I.D.), mobile phase 20 m*M* NH_4OAc–20 m*M* HOAc–acetonitrile (240:60:700, v/v/v), flow-rate 1.0 ml/min, column temperature 30°C.

Table 1
Limit of quantitation of opipramol in spiked plasma samples[a]

Added (pg/ml)	Found (pg/ml)	RSD (%)	Bias (%)
167	189	8.1	+ 14.0
250	260	6.5	+ 3.9
333	347	12	+ 4.0
500	486	7.8	+ 2.8
667	672	3.2	+ 0.8

[a] Mean, relative standard deviation (RSD), and accuracy are obtained from five replicate samples.

250 pg/ml in order compensate for somewhat less precision during routine work.

Eight separated calibration curves were constructed in duplicate from 0.667 to 33.3 ng/ml opipramol·2HCl. The mean coefficient for linear regression was 0.9992 (range 0.998–0.99996), precision and accuracy ranged from 1.5 to 5.6% and from 92.5 to 104.7%, respectively. One-point calibration at medium concentration (which saves plasma and reduces the number of samples to be analysed) can be applied with similar precision and accuracy compared to multi-point calibration constructing a standard curve (Table 2).

Precision and accuracy was monitored during the analysis of about 600 plasma samples from a bioequivalence study by the simultaneous assay of quality control samples. Precision was better than 9%, inaccuracy less than 8% (Table 3). Thirty plasma samples from the pharmacokinetic study were re-analysed to assess the precision during analysis of clinical samples. A good correlation could be demonstrated, the concentrations amounted to 94.9±9.1% of those of the first analysis (linear regression 2nd vs. 1st analysis: $y = 0.86x + 1.25$, $n = 30$, $r = 0.98$, Fig. 3). The recovery (mean±SD of 21 assays) of opipramol from the spiked quality control and calibration plasma samples was 60.7±6.3% for opipramol and 70.4±8.8% for the internal standard imipramine.

Table 3
Precision and accuracy of the determination of opipramol in plasma[a]

Added ng/ml	Found ng/ml	Precision (%)		Bias (%)
		Inter-assay	Intra-assay	
1.20	1.13	5.2	8.4	−6.3
6.00	5.82	5.9	6.9	− 4.7
30.0	31.8	6.8	5.6	+ 7.5

[a] The data were obtained from 20 assays.

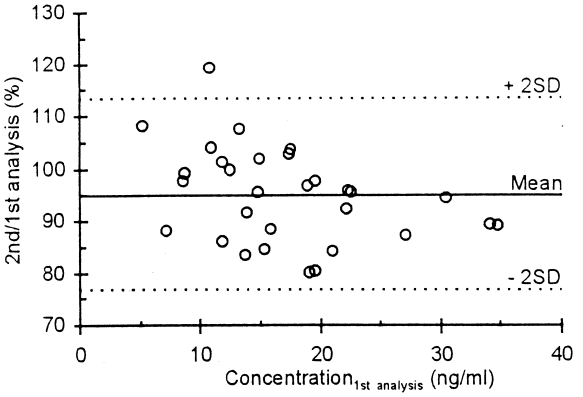

Fig. 3. Comparison of opipramol measurements in plasma by HPLC with photometric detection. The plot shows the ratio of 2nd/1st analysis vs. the concentrations obtained from the 1st analysis.

Table 2
Linearity of the assay of opipramol in plasma[a]

Added (ng/ml)	Calibration according to equation $y = m*x$			One-point calibration at 6.67 ng/ml		
	Found (ng/ml)	RSD (%)	Bias (%)	Found (ng/ml)	RSD (%)	Bias (%)
0.667	0.622	5.6	− 7.5	0.642	7.8	− 7.1
1.67	1.60	3.9	− 4.7	1.65	6.0	− 4.1
3.33	3.18	4.3	− 5.4	3.28	7.1	−5.3
6.67	6.49	6.5	− 5.8	calibrator	calibrator	calibrator
16.7	16.4	5.7	− 4.5	16.9	7.3	+ 5.4
33.3	33.5	1.5	+ 1.3	34.6	6.8	+ 6.4

[a] Mean, precision (relative standard deviation, RSD) and accuracy are obtained from eight calibration curves using equation $y = m*x$, and comparison of the results with one-point calibration at medium concentration.

3.4. Selectivity and specificity

Blank samples of fresh-frozen-plasma from six different patients and a plasma pool from healthy volunteers were assayed without detecting any interfering peak at the retention time of opipramol or imipramine. To ensure the identity of the opipramol peak samples from four different volunteers of the pharmacokinetic study were assayed by the present method, and by HPLC with electrochemical detection. The correlation between both methods was excellent, the concentrations determined by ECD amounted to 101±14% of those determined by UV detection (linear regression ECD vs. UV: $y = 0.94x + 0.275$, $n = 38$, $r = 0.96$, Fig. 4). The electrochemical method proved to be more selective as demonstrated by less peaks in the ECD chromatogramm (Fig. 5), but the UV method showed a better signal-to-noise ratio.

3.5. Application to a pharmacokinetic study

The method was applied to the determination of opipramol in plasma samples for the purpose of a bioequivalence study. A representative plasma concentration–time profile of a subject following oral administration of 50 mg opipramol·2HCl is depicted in Fig. 6. Obviously, the lower limit of quantitation (250 pg/ml) was quite adequate to monitor the plasma concentrations of opipramol up to 48 h. In addition, also the plasma concentration–time profile of deshydroxyethyl opipramol eluting at 7.2–7.4 min could be described. The peak was evaluated as

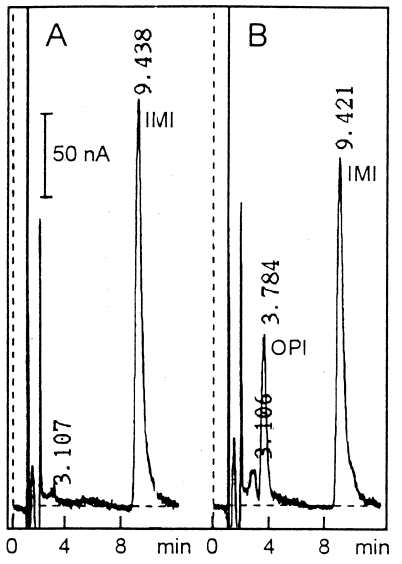

Fig. 5. Chromatograms of the plasma sample depicted in Fig. 2 analysed by HPLC with electrochemical detection. Deshydroxyethyl opipramol (retention time 3.10 min) is not separated from an endogenous peak. Abbr.: OPI, opipramol; IMI, imipramine (internal standard). Column Novapak C_{18} 4 μm (150×4 mm I.D.), mobile phase 50 m*M* KH_2PO_4, 1.5 m*M* sodium octane sulfonate in water–acetonitrile (70:30, v/v) adjusted to pH 3.5 with 85% H_3PO_4, flow-rate 1.0 ml/min, column temperature 30°C.

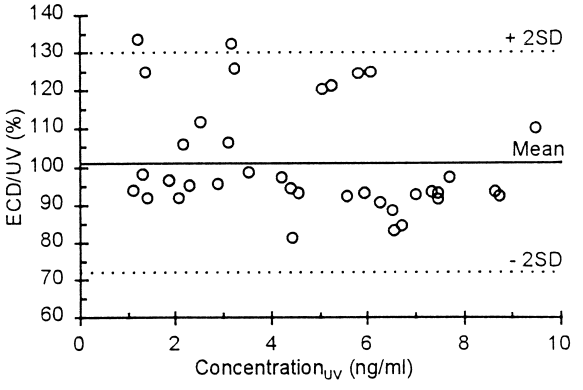

Fig. 4. Comparison of opipramol measurements in plasma by HPLC with electrochemical (ECD) vs. photometric detection (UV). The plot shows the ratio of ECD/UV vs. UV concentrations.

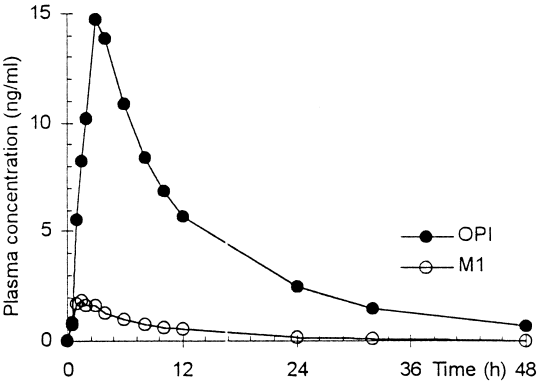

Fig. 6. Mean plasma concentration–time profile of opipramol (OPI) and deshydroxyethyl opipramol (M1, quantitated as opipramol) in 20 healthy volunteers following single oral dose of opipramol·2HCl 50 mg.

opipramol, because reference substance was not available.

4. Conclusions

The described method allows the determination of opipramol in human plasma down to the sub-ng/ml range using common photometric detection. Precision, accuracy and sensitivity permit the use of the method for single dose pharmacokinetic studies in man.

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