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Note

Determination of chlorimipramine and desmethylchlorimipramine in human plasma by ion-pair partition chromatography

BRITT MELLSTRÖM*

Department of Clinical Pharmacology, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge (Sweden)

and

STAFFAN EKSBORG

Department of Analytical Pharmaceutical Chemistry, University of Uppsala, Box 574, S-751 23 Uppsala (Sweden)

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There is now substantial evidence that the utilization of tricyclic antidepressant drugs can be improved by monitoring their steady-state plasma concentrations in individual patients[1]. Good analytical methods are available for some of these drugs but not for chlorimipramine. Like the other tertiary tricyclic antidepressants, it has an active demethylated metabolite which therefore also has to be measured in combined pharmacokinetic and pharmacodynamic studies of chlorimipramine. This paper presents a method for the simultaneous determination of chlorimipramine and desmethylchlorimipramine in human plasma based upon ion-pair partition chromatography. The potential usefulness of ion-pair chromatography has been pointed out earlier[2–4], but there have so far been only a few applications of this technique to the determination of compounds in biological fluids[5–7].

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of an LDC 711-26 Solvent Delivering System as the pumping unit. A Perkin-Elmer LC-55 spectrophotometer (cell volume 8 μl, path-length 6 mm) was used as a UV detector and operated at 255 nm. The separation column was a silanized borosilicate glass column (300 mm × 2.7 mm I.D.). For equilibration of the mobile phase, a pre-column was used (300 mm × 9 mm I.D.). All connections and the septum injector were made of stainless steel (modified Swagelok unions).

Chromatographic system

DiaChrom, 37–44 μm, was used as the support, coated with 25% (w/v) of a mixture of 0.1 *M* hydrochloric acid and 0.1 *M* tetraethylammonium chloride as the

* Present address: Karolinska Pharmacy, Fack. S 104 01 Stockholm 60, Sweden.

stationary phase. The mobile phase was *n*-hexane–isobutanol (9:1) with a flow-rate of 0.5 ml/min. The phases were carefully equilibrated with each other at the experimental temperature (22°). The system was operated under thermostatically-controlled conditions[8]. The columns were packed by a slurry technique described by Eksborg and Schill[8]. The same filling was used in the pre-column and the separation column.

Chemicals and materials

DiaChrom, 37-44 μm (Applied Science Labs., State College, Pa., U.S.A.), was purified by boiling it with 2 *M* perchloric acid and water. *n*-Hexane of certified grade H 291 (Fischer Scientific, Pittsburgh, Pa., U.S.A.) was used. All other chemicals used were of analytical-reagent grade. Chlorimipramine hydrochloride (CIM·HCl) and desmethylchlorimipramine hydrochloride (DCIM·HCl) were supplied by Ciba-Geigy (Basle, Switzerland) and trimipramine (TRIM) maleate was supplied by AB Leo (Helsingborg, Sweden). The following standard solutions were prepared: $1.13 \cdot 10^{-5}$ *M* CIM·HCl, $1.23 \cdot 10^{-5}$ *M* DCIM·HCl and $2.86 \cdot 10^{-5}$ *M* TRIM maleate in 0.1 *M* orthophosphoric acid.

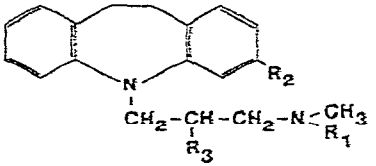
Analytical method

A 2.0-ml plasma sample is mixed with 50 μl of TRIM standard solution (internal standard), 200 μl of 2.5 *M* sodium hydroxide solution are added and the plasma sample is extracted with 5 ml of diethyl ether for 90 min. After centrifugation, the ether layer (*ca.* 4.5 ml) is transferred into 1.0 ml 0.25 *N* sulphuric acid in another tube. After shaking for 20 min and centrifugation, the ether is discharged and the aqueous phase is transferred into a narrow 2-ml tube (length 10 cm, I.D. 5 mm). The sample is made alkaline with 500 μl of 2.5 *M* sodium hydroxide solution, 150 μl of the mobile phase are added and the tubes are rotated slowly for 90 min and then centrifuged. The organic (upper) phase is injected into the separation column.

Standard curves are obtained by the addition of known amounts of CIM and DCIM standard solutions to drug-free human plasma, and carried through the analytical method. Quantitation is based on peak-height measurements and plotting the peak-height ratios *versus* drug concentration expressed as nanograms per millilitre of the hydrochloride.

RESULTS AND DISCUSSION

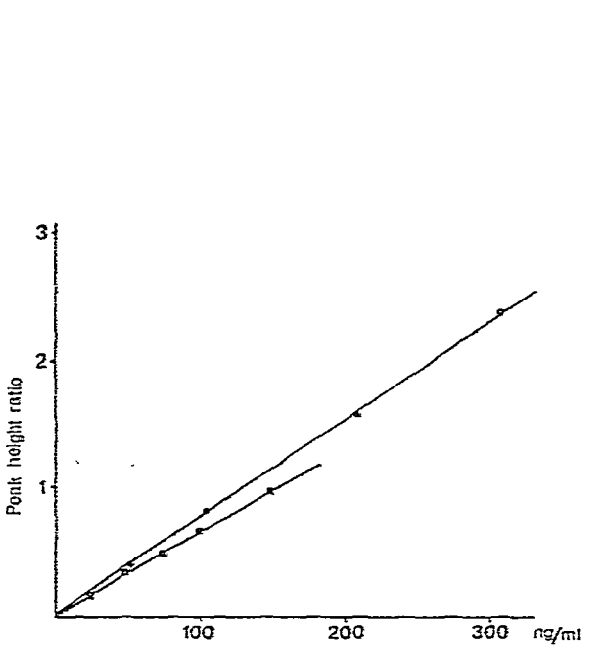
The theoretical background of ion-pair extractions has been reviewed by Schill[9] and its application to liquid–liquid chromatography has been studied by Eksborg[10]. High selectivity permits separations to be achieved with short columns, resulting in a high detection sensitivity. A sufficiently high separating efficiency is obtained by the use of large particle diameters (37–44 μm), which produce a very low pressure drop over the column. The structures of the drugs are shown in Fig. 1. These molecules are lipophilic and the low extraction degree obtained by use of chloride as the counter ion gave a suitable partition ratio in the chromatographic system. The stationary phase was modified with tetraethylammonium chloride in order to increase the separating efficiency[11]. The extraction from alkalinized plasma was found to be slow and shaking for 90 min with diethyl ether was necessary in order to achieve quantitative extraction. A re-extraction step was introduced in order to reduce the



	R_1	R_2	R_3
CIM	CH_3	Cl	H
DCIM	H	Cl	H
TRIM	CH_3	H	CH_3

Fig. 1. Structural formulae of chlorimipramine (CIM), desmethylchlorimipramine (DCIM) and the internal standard trimipramine (TRIM).

volume of the extract and to bring the compounds into the mobile phase. The final extraction was carried out in special narrow tubes that made it possible to remove a larger fraction of the upper organic phase for injection into the column so as to achieve a higher detection sensitivity. Standard curves for CIM and DCIM are shown in Fig. 2 and a chromatogram from a plasma sample in Fig. 3. No interferences were found in the blank plasma chromatograms. The relative standard deviation was 4.3% for CIM and 6.3% for DCIM (n = 20, range 25–150 ng/ml for CIM and 50–300 ng/ml for DCIM as the hydrochlorides). The method was applied to the determination



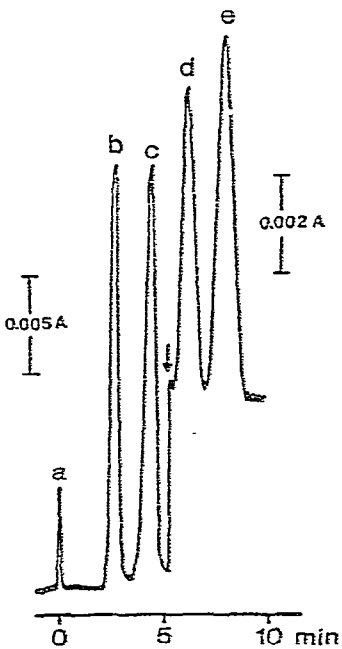
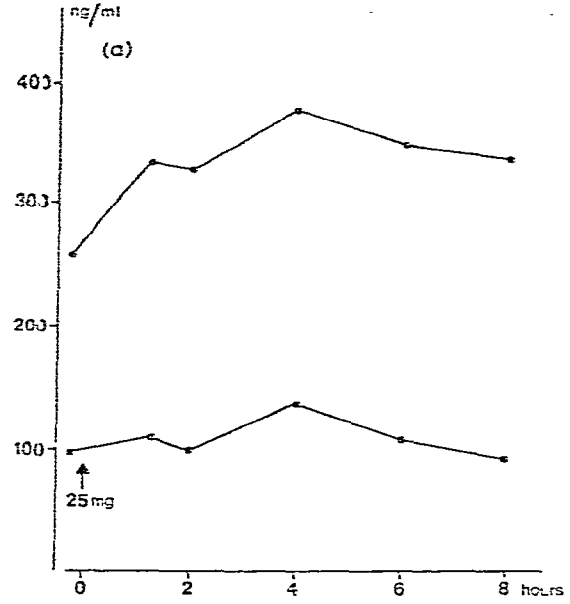
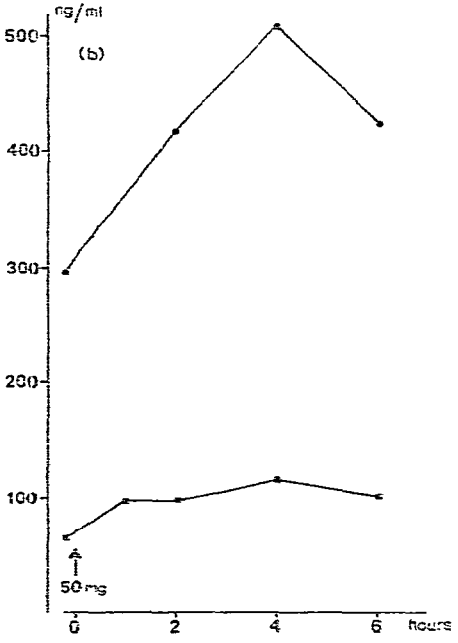


Fig. 2. Standard curves for CIM (■) and DCIM (●). Trimipramine was used as the internal standard. Concentrations refer to the hydrochlorides.

Fig. 3. Chromatogram of a plasma sample from a patient receiving chlorimipramine hydrochloride. a = Sample injection; b = solvent front; c = DCIM; d = CIM; e = TRIM (internal standard). The amplification was increased 2.5-fold after 5 min (arrow).





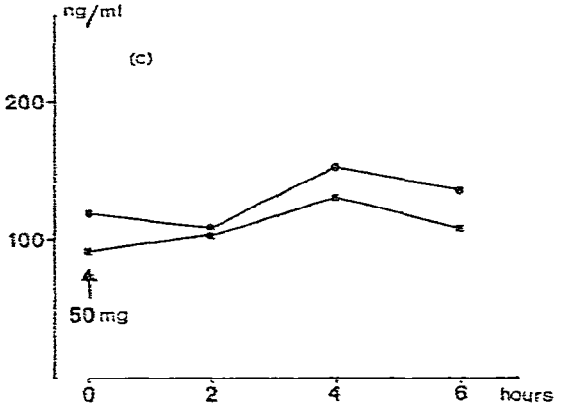


Fig. 4. Plasma concentrations of CIM (▣) and DCIM (●) (as hydrochlorides) during the first dosage interval of the day in patients treated with chlorimipramine hydrochloride for at least 2 weeks. The daily doses were (a) patient S.F., 25 + 25 + 50 mg; (b) patient M.K., 50 + 50 + 75 mg; (c) patient G.H., 50 + 50 + 50 mg of chlorimipramine hydrochloride.

of plasma concentrations of patients treated with chlorimipramine hydrochloride. Blood samples were drawn during a dosage interval so as to establish plasma levels and variations during the interval. As expected[1], the plasma concentrations of both CIM and DCIM and the ratio between them varied markedly between the three patients studied (Fig. 4). It is remarkable that the plasma concentrations of the metabolite were higher than those of the parent drug. Other tricyclic antidepressant drugs (*e.g.*, amitriptyline and nortriptyline) can be chromatographed with the same system and there is a possibility of interference if the patient, contrary to accepted practice, receives more than one compound of this class of drugs at the same time.

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