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GAS CHROMATOGRAPHIC–MASS FRAGMENTOGRAPHIC DETERMINATION OF "STEADY-STATE" PLASMA LEVELS OF IMIPRAMINE AND DESIPRAMINE IN CHRONICALLY TREATED PATIENTS

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SUMMARY

A sensitive and specific method for the simultaneous determination of imipramine and desipramine in the blood plasma of depressed patients under treatment that involves separation by gas chromatography and detection by mass fragmentography is described. Concentrations were determined by focusing the mass spectrometer upon the ions at *m/e* 280 and 235 for imipramine and 308, 236 and 114 for desipramine (N-acetyl derivative), while promazine was used as the internal standard (ions at *m/e* 284 and 238). Determinations are possible at levels as low as 10 ng/ml in plasma.

INTRODUCTION

In recent years, the determination of drug levels in plasma after a single administration or during chronic treatment in humans has permitted the accumulation of valuable information concerning the bioavailability of the drug and its therapeutic and toxic thresholds, thus forming the basis for more rational therapy[1–5].

In work on tricyclic antidepressant drugs, the pharmacokinetics of secondary amines, *i.e.*, monomethylated compounds such as desipramine and nortriptyline, have been the subject of extensive studies[6–12]. However, little information is available on plasma level correlations between tertiary amines of tricyclic antidepressants and their major metabolites, the demethylated secondary amines[13–16]. Several methods have been described for the simultaneous determination of imipramine and desipramine in biological fluids, but they lack specificity and/or sensitivity, require large amounts of blood or necessitate very long procedures[13,16,17–22].

In the clinical situation, the determination of drug plasma levels is often more complicated because of interactions with other drugs or because their metabolic products may cause changes in the metabolism of the drug under study. Therefore, sensitivity and specificity are important characteristics for the determination of tri-

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cyclic antidepressants, especially because these drugs are pharmacologically active at very low levels.

In the work described here, a simple mass fragmentographic method has been devised for the separation and specific simultaneous measurement of imipramine and desipramine with a sensitivity of about 4 ng/ml in plasma.

EXPERIMENTAL

Chemicals

Imipramine and desipramine were obtained from Ciba Geigy (Basel, Switzerland) and promazine (used as an internal standard) from Pierrel (Milan, Italy).

The following reagents were used: acetic anhydride and sodium hydroxide (Merck, Darmstadt, G.F.R.), pyridine, dichlorodimethylsilane, *n*-hexane, toluene, absolute ethanol and isoamyl alcohol (Carlo Erba, Milan, Italy).

Glassware

All glassware was silanized by soaking for 6 h in a 5% solution of dichlorodimethylsilane in toluene and dried in an oven at 80°, then rinsed with ethanol prior to use.

Gas chromatography–mass fragmentography

A Finnigan Model 3100 quadrupole mass spectrometer equipped with a gas chromatograph and a Model 6000 computer system was used.

The chromatographic conditions were as follows: column, glass tube, 1 m long and 2 mm I.D., packed with 3% OV-1 on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, Pa., U.S.A.); oven temperature, 240°; injection port temperature, 260°; and carrier gas (helium) flow-rate, 20 ml/min. The mass spectrometer was set at the following conditions: separator temperature, 250°; ion source temperature, 100°; energy of ionization beam, 70 eV; and ionization current, 200 μA.

Measurements were performed by multiple ion detection focusing the mass spectrometer upon the ions at *m/e* 280 and 235 for imipramine, *m/e* 308, 235 and 114 for the N-acetyl derivative of desipramine and *m/e* 284 and 238 for promazine.

Extraction procedure and derivative formation

A 1-ml volume of heparin-treated plasma, previously acidified with 0.5 ml of 0.1 *N* hydrochloric acid and stored at −20°, was made alkaline by adding 0.5 ml of 1 *N* sodium hydroxide solution and extracted with 3 ml of *n*-hexane by shaking for 30 min on an automatic shaker.

After centrifuging at 1700 *g* for 10 min, 2 ml of the organic layer were carefully transferred into conical glass-stoppered tubes with pipettes that had been rinsed with isoamyl alcohol just before use. Then 50 μl of pyridine and 100 μl of acetic anhydride were added and the stoppered tubes allowed to stand for 30 min in a sand-bath at 70°.

The reaction kinetics of the formation of the N-acetyl derivative of desipramine at room temperature (25°) and at 70° are reported in Fig. 1. The reaction yield was 100% and the derivative was also found to be very stable in acidic or basic aqueous solutions.

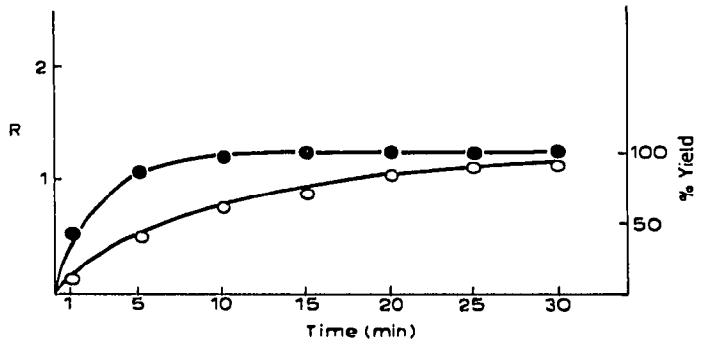

Fig. 1. Rates and yields of desipramine N-acetylation reaction at 25° (○—○) and 70° (●—●).

The stopper was then removed and the reaction mixture dried under a gentle stream of nitrogen at 70°. After cooling to room temperature, the dry residue was re-dissolved in 100 μl of absolute ethanol containing promazine (1 μg/ml) and 1, 2 or 3 μl of this solution were injected on to the gas chromatographic column.

Measurement of imipramine

A range of standard solutions, each containing 100 ng of promazine and 5–200 ng of imipramine (free base), were made up in 100 μl of absolute ethanol. A standard calibration graph was prepared by injecting 1-μl aliquots of these solutions prior to determining imipramine in the test samples (Fig. 2). The ratio of the peak area of imipramine to that of promazine was linear over the range 50 pg to 2 ng of imipramine on injection. The standard solutions were found to be stable over a period of several months when stored at 4° in a refrigerator.

Measurement of desipramine

As this drug is determined as its N-acetyl derivative, standard solutions were prepared as follows. To a range of samples, each containing 5–200 ng of desipramine, 50 μl of pyridine and 100 μl of acetic anhydride were added. After reaction, as previously described, 100 μl of a 1 μg/ml solution of promazine in ethanol were added to the dry residue. A standard calibration graph (reaction yield 100%) was prepared by injecting 1- or 2-μl aliquots of these solutions (Fig. 2). The ratio of peak area of

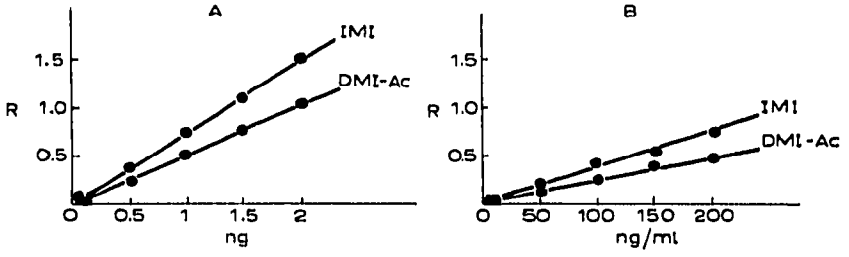

Fig. 2. (A) External calibration graphs for imipramine (IMI) and desipramine (N-acetyl derivative) (DMI-Ac). (B) Calibration graphs obtained by adding known amounts of imipramine and desipramine to 1 ml of human plasma and processing them as described under *Extraction procedure and derivative formation*. R = ratio of the peak areas of imipramine and desipramine (N-acetyl derivative) and the internal standard promazine.

desipramine to that of promazine was linear over the range of 100 pg to 2 ng of desipramine on injection. The standard solutions were found to be stable for several days when stored in a refrigerator at 4°.

Recovery studies

The separate and combined addition of imipramine and desipramine hydrochloride to drug-free plasma at concentrations ranging from 5 to 200 ng/ml for each drug resulted in over-all recoveries of 80 ± 3% for imipramine and 76 ± 4% for desipramine. A linear response was found over the range 4–200 ng/ml in plasma (Fig. 2).

Stability studies

The stability of plasma samples on storage was investigated as follows. Plasma samples were divided into two 1-ml aliquots, one being stored at —20° and the other analyzed for imipramine and desipramine content by the method described, just after blood samples were withdrawn. No differences in the samples stored at —20° for 2 months were found, indicating that no decomposition of either drug had taken place.

RESULTS AND DISCUSSION

The gas chromatogram of imipramine, the N-acetyl derivative of desipramine and the internal standard promazine is shown in Fig. 3.

The acetylation reaction was chosen because of the suitable retention time of

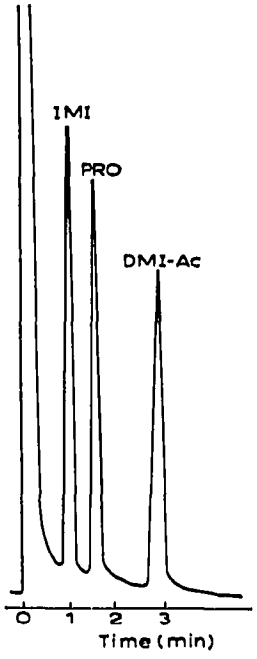

Fig. 3. Gas chromatogram of imipramine (IMI), desipramine (N-acetyl derivative) (DMI-Ac) and the internal standard promazine (PRO).

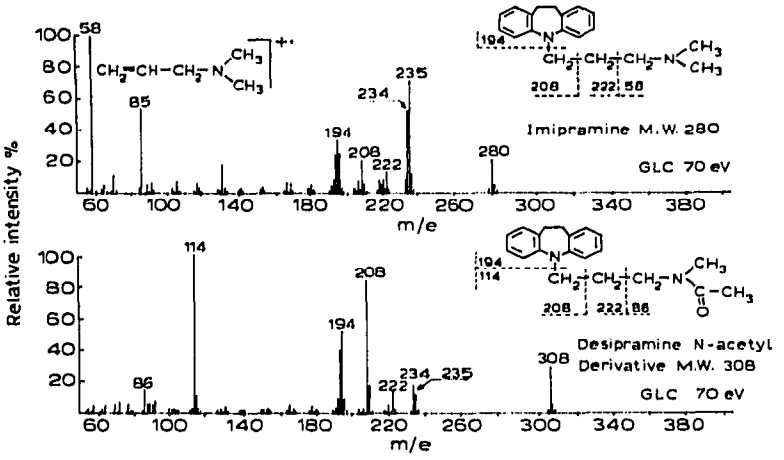

Fig. 4. Mass spectra of imipramine (above) and desipramine (N-acetyl derivative) (below).

the derivative formed and in order to avoid absorption of desipramine on the stationary phase of the gas chromatographic column. The mass spectrum of imipramine (Fig. 4) shows the base peak at *m/e* 58, corresponding to a β-bond fission with respect to the nitrogen atom of the side-chain, giving rise to N,N-dimethylformimine with retention of the positive charge. Other intense peaks are present at *m/e* 194, 208 and 222, arising as shown in Fig. 4. The peak at *m/e* 85 is due to a cleavage of the bond between the ring nitrogen and the first carbon of the side chain, with a rearrangement of a hydrogen atom onto the ring nitrogen.

Desipramine (N-acetyl derivative) (Fig. 4) and promazine (Fig. 5) showed analogous behaviour.

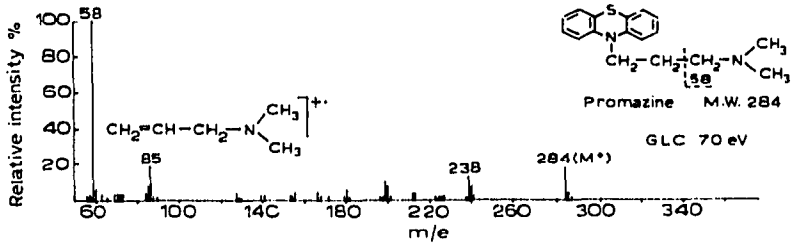

Fig. 5. Mass spectrum of promazine.

The origins of the peaks at *m/e* 235 and at *m/e* 238 are explained in Fig. 6. Imipramine and desipramine (N-acetyl derivative) undergo a 1,4-rearrangement and, by losing N,N-dimethylamine and N-methylacetamide, respectively, give rise to the fragment ions at *m/e* 235 with subsequent loss of a hydrogen radical to form the fragments at *m/e* 234.

Promazine, after a similar 1,4-rearrangement, loses N,N-dimethylamine, giving rise to the fragment at *m/e* 239 and, with the subsequent loss of a hydrogen radical, the fragment at *m/e* 238.

According to the mass fragmentographic technique[23], the mass spectrometer

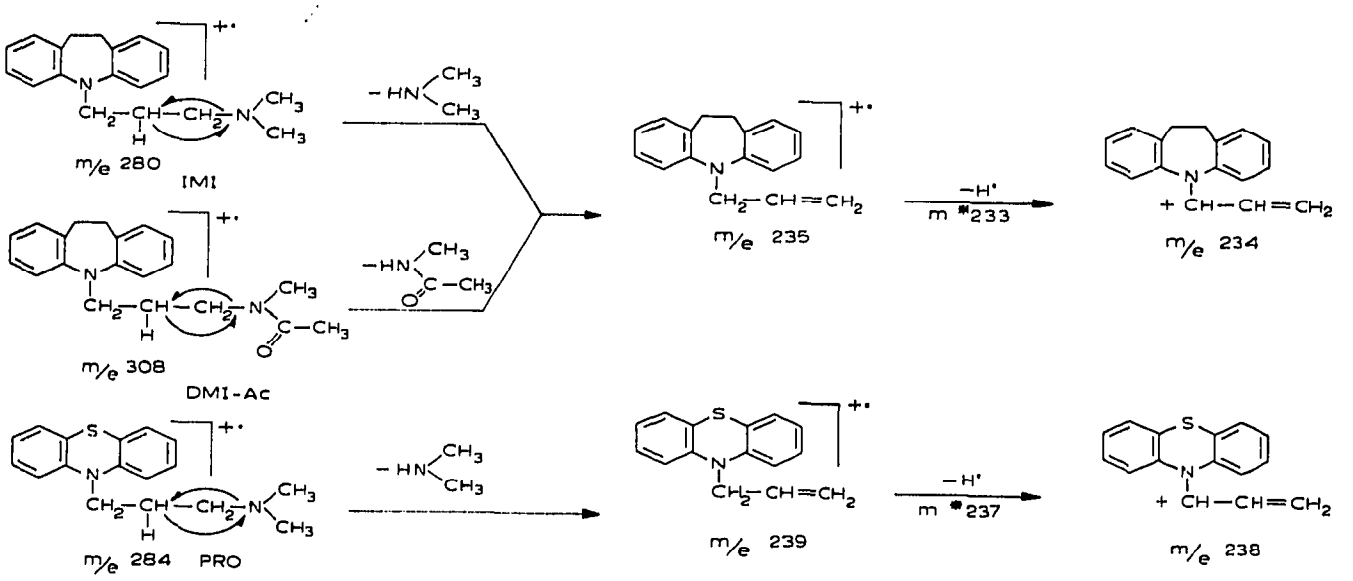

Fig. 6. Fragmentation pathways for imipramine (IMI), desipramine (N-acetyl derivative) (DMI-Ac) and promazine (PRO).

is used as a detector for the effluent from the gas chromatographic column. The mass spectrometer can be set to detect one or more characteristic fragment ions of the compounds under study, thereby introducing a further parameter of identification in addition to the retention time in the gas chromatograph. Thus the method combines the high resolving power of the gas chromatograph with the high sensitivity and specificity of identification provided by the mass spectrometer[24,25].

A typical mass fragmentogram of a plasma sample taken from a patient receiving chronic treatment with imipramine is reported in Fig. 7. Quantitation was

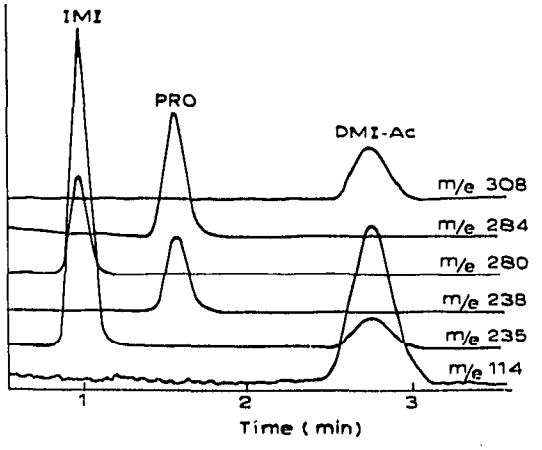

Fig. 7. Mass fragmentogram obtained from plasma of a patient undergoing chronic treatment with imipramine. IMI, DMI-Ac and PRO as in Fig. 6.

performed by focusing the instrument (at 70 eV) first upon the ions at *m/e* 308, 284, 280 and 235 and then upon the ions at *m/e* 280, 238, 235 and 114.

Endogenous compounds were found not to interfere in the analysis, and no interference was observed from concomitant treatment with benzodiazepines or barbiturates.

The validity of this analytical procedure was tested in two different experimental situations involving acute and chronic treatment with imipramine, where it was possible to follow plasma levels of both imipramine and desipramine (Figs. 8–12). The clinical significance of these results will be discussed elsewhere.

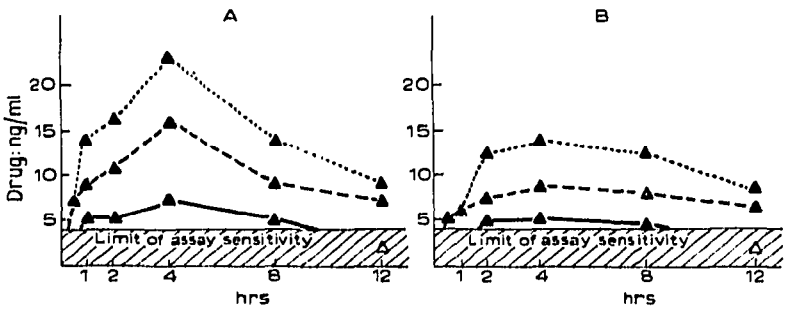

Fig. 8. Plasma levels of imipramine (IMI) and desipramine (DMI) in two healthy volunteers after a single oral dose of imipramine. ▲---▲, IMI; ▲—▲, DMI; ▲···▲, IMI + DMI. (A) Subject: P.I., female, age 31, weight 51 kg; dose, 75 mg (1.47 mg/kg). (B) Subject: P.C., male, age 23, weight 55 kg; dose, 75 mg (1.36 mg/kg).

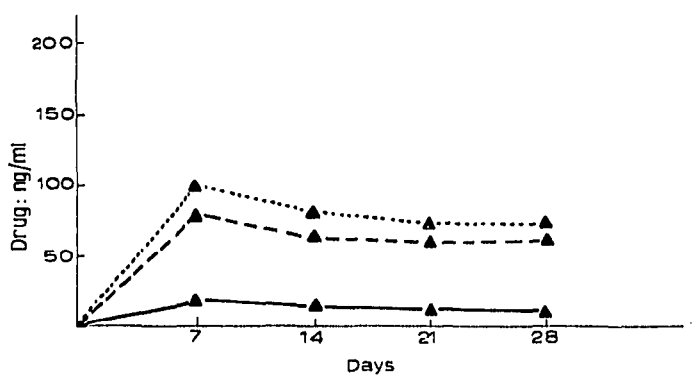

Fig. 9. Plasma levels of imipramine (IMI) and desipramine (DMI) of one subject during chronic treatment with imipramine. Curves as in Fig. 8. Subject: P.M.G., female, age 27, weight 47 kg; dose, 75 mg/day (1.59 mg/kg); diagnosis, reactive depression; clinical judgement, good improvement.

However, from the results obtained, this work has demonstrated not only that the technique described seems to satisfy the requirements for its applicability to monitoring imipramine and desipramine plasma levels in depressed patients undergoing chronic treatment, but also that, with four subjects, repeated oral dosing with imipramine hydrochloride led to a gradual build-up of both imipramine and desipramine plasma contents until a "steady-state" level was established.

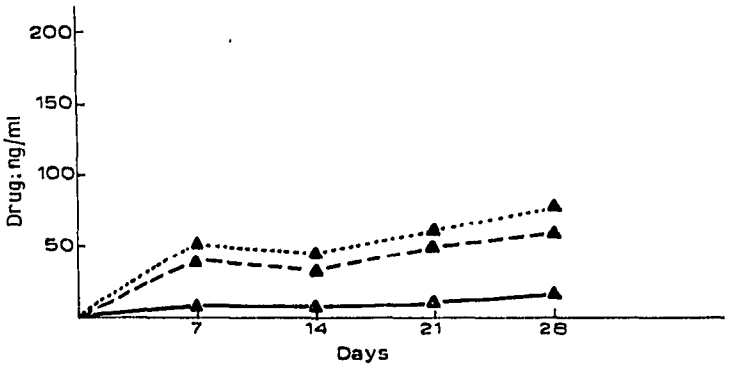

Fig. 10. As Fig. 9. Subject: C.I., female, age 48; weight 62 kg; dose, 75 mg/day (1.21 mg/kg); diagnosis, depressive neurosis; clinical judgement, good improvement.

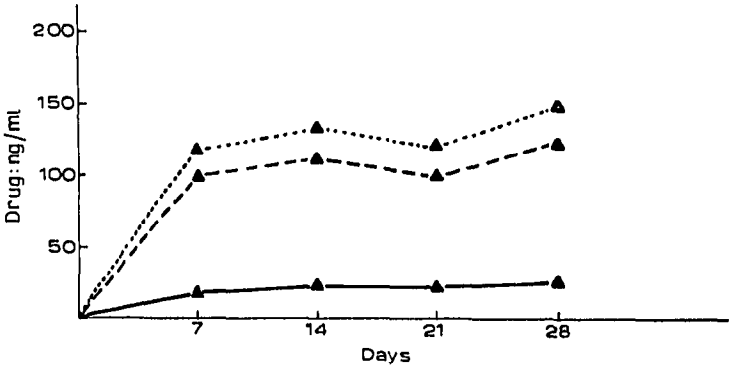

Fig. 11. As Fig. 9. Subject: B.A., female, age 63, weight 75 kg; dose, 100 mg/day (1.33 mg/kg); diagnosis, endogenous depression; clinical judgement, moderate improvement.

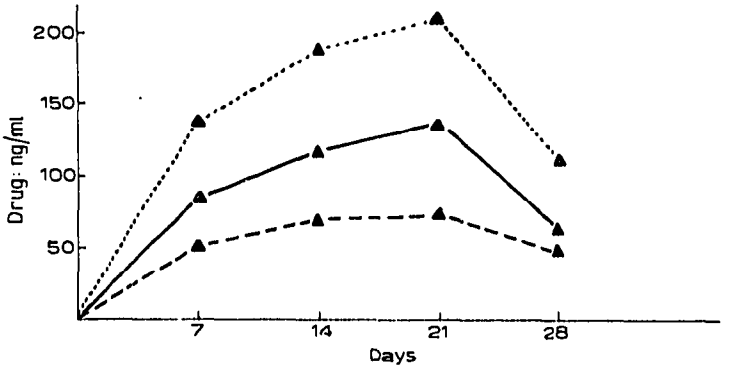

Fig. 12. As Fig. 9. Subject: A.T., female, age 45, weight 56 kg; dose, 100 mg/day (1.78 mg/kg); diagnosis, depressive neurosis; clinical judgement, good improvement.

Contrary to common belief, in three out of four patients, imipramine plasma levels were significantly higher than those of desipramine. As imipramine and desipramine show different inhibitory activities on the presynaptic uptake of serotonin and noradrenaline in the brain[26–28], their ratio in the brain may necessitate therapeutic alterations or produce different side-effects.

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REFERENCES

1 D. A. P. Evans, *Ann. N.Y. Acad. Sci.*, 123 (1965) 178.
2 W. Hammer, C.-M. Ideström and F. Sjöqvist, in S. Garattini and M. N. G. Dukes (Editors), *Antidepressant Drugs*, Excerpta Medica, Amsterdam, 1967, p. 301.
3 P. L. Morselli, M. Rizzo and S. Garattini, *Ann. N.Y. Acad. Sci.*, 179 (1971) 88.
4 J. Koch-Weser and S. W. Klein, *J. Amer. Med. Ass.*, 215 (1971) 1454.
5 E. S. Vesell and G. T. Passananti, *Clin. Chem.*, 17 (1971) 851.
6 W. Hammer and F. Sjöqvist, *Life Sci.*, 6 (1967) 1895.
7 F. Sjöqvist, W. Hammer, C.-M. Ideström, M. Lind, D. Tuck and M. Åsberg, in S. B. de C. Baker, J. R. Boissier and W. Koll (Editors), *Proceedings of the European Society for the Study of Drug Toxicity, Vol. 9, Toxicity and Side-Effects of Psychotropic Drugs*, Excerpta Medica, Amsterdam, 1968, p. 246.
8 F. Sjöqvist, W. Hammer, O. Borgå and D. L. Azarnoff, in A. Cerletti and F. J. Bové (Editors), *The Present Status of Psychotropic Drugs*, Excerpta Medica, Amsterdam, 1969, p. 128.
9 C.-G. Hammar, B. Alexanderson, B. Holmstedt and F. Sjöqvist, *Clin. Pharmacol. Ther.*, 12 (1971) 496.
10 B. Alexanderson, *Eur. J. Clin. Pharmacol.*, 4 (1972) 82.
11 B. Alexanderson, *Eur. J. Clin. Pharmacol.*, 5 (1972) 1.
12 M. Rizzo, E. Jogan, A. Giannelli, S. M. Standen and P. L. Morselli, *Riv. Sper. Freniatr. Med. Leg. Alienazioni Ment.*, 97 (1973) 1.
13 J. P. Moody, A. C. Tait and A. Todrick, *Brit. J. Psychiat.*, 113 (1967) 183.
14 O. J. Rafaelsen and J. Christiansen, in A. Cerletti and F. J. Bové (Editors), *The Present Status of Psychotropic Drugs*, Excerpta Medica, Amsterdam, 1969, p. 118.
15 R. A. Braithwaite and B. Widdop, *Clin. Chim. Acta*, 35 (1971) 461.
16 A. Frigerio, G. Belvedere, F. de Nadai, R. Fanelli, C. Pantarotto, E. Riva and P. L. Morselli, *J. Chromatogr.*, 74 (1972) 201.
17 B. Herrmann and R. Pulver, *Arch. Int. Pharmacodyn.*, 126 (1960) 454.
18 J. V. Dingell, F. Sulser and J. R. Gillette, *J. Pharmacol. Exp. Ther.*, 143 (1964) 14.
19 W. M. Hammer and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 157 (1967) 503.
20 H. J. Weder and M. H. Bickel, *J. Chromatogr.*, 37 (1968) 181.
21 S. R. Harris, L. E. Gaudette, D. H. Efron and A. A. Manian, *Life Sci.*, 9, pt. I (1970) 781.
22 T. Walle and H. Ehrsson, *Acta Pharm. Suecica*, 8 (1971) 27.
23 C.-G. Hammar, B. Holmstedt and R. Ryhage, *Anal. Biochem.*, 25 (1968) 532.
24 C.-G. Hammar, B. Holmstedt, J.-E. Lindgreen and R. Tham, *Advan. Pharmacol. Chemother.*, 7 (1969) 53.
25 A. E. Gordon and A. Frigerio, *J. Chromatogr.*, 73 (1972) 401.
26 A. Carlsson, H. Corrodi, K. Fuxe and T. Hokfelt, *Eur. J. Pharmacol.*, 5 (1969) 357.
27 A. Carlsson, H. Corrodi, K. Fuxe and T. Hokfelt, *Eur. J. Pharmacol.*, 5 (1969) 367.
28 R. Samanin, D. Ghezzi and S. Garattini, *Eur. J. Pharmacol.*, 20 (1972) 281.