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Quantitative determination of perphenazine and its metabolites in plasma by high-performance liquid chromatography and coulometric detection

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Abstract

An accurate, reliable method has been developed for the therapeutic monitoring of perphenazine (PPZ) and its major metabolites in human plasma samples. Steady-state plasma levels of PPZ and its metabolites were quantitated for 30 elderly patients (mean age: 75) undergoing concurrent treatment with nortriptyline (NT) and PPZ, doses ranging from 4 to 32 mg/day for PPZ. The assay was suitable with patients on concurrent medications, and smaller patient plasma volumes (1 ml) were used indicating sufficient sensitivity and specificity. After plasma extraction and separation on a Nucleosil 5-μm C_{18} column, the recoveries (mean ±S.D.) of PPZ and its metabolites were determined; perphenazine 92 ± 7.5%, deshydroxyethylperphenazine 81 ± 7.2%, perphenazine sulfoxide 68 ± 6.4%, and 7-hydroxyperphenazine 45 ± 5.5%. The assay also had limits of quantitative detectability for PPZ and its metabolites as follows: perphenazine 0.5 ng/ml, deshydroxyethylperphenazine 1.0 ng/ml, perphenazine sulfoxide 0.5 ng/ml, and 7-hydroxyperphenazine 5 ng/ml. Inter-assay reproducibility (C.V.) for the quality controls and patient samples ranged from 18.8 to 2.4%. The sensitivity and reproducibility of this method should improve PPZ therapeutic drug monitoring and research on interactions in depressed geriatric patients.

1. Introduction

Therapeutic drug monitoring (TDM) plays an increasingly important role in the clinical use of tricyclic antidepressants and other psychiatric drugs [1]. By contrast, TDM for antipsychotic medications remains controversial and limited [2]. In part, this has been due to technical limitations in the measurement of plasma neuroleptic levels. Typically, neuroleptic plasma levels (1 to 15 ng/ml) in patients receiving clinical doses [3] are 10 to 20 times lower than the plasma levels of tricyclic antidepressants (50–300 ng/ml) [1].

Techniques available for monitoring perphenazine and other neuroleptics include gas chromatography and high-performance liquid chromatography (HPLC) [4–15]. These techniques have been cumbersome in design and usually required large sample volumes to achieve required sensitivity and specificity for per-

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phenazine and its metabolites. In the procedure by Larsson and Forsman [4], 3 ml of serum was necessary to identify perphenazine and its dealkylated metabolite and in another HPLC procedure, 2–2.5 ml of either plasma or serum was assayed to obtain the required sensitivity for perphenazine and its metabolites.

The present method measures perphenazine, its metabolites, and a structurally related internal standard (Fig. 1) with minimal sample volume and is sensitive enough to measure the therapeutic levels of perphenazine in plasma. The method was used to study a group of geriatric patients with psychosis that was treated with a flexible dose regimen of perphenazine (4 to 32 mg per day), with or without nortriptyline (NT).

2. Experimental

2.1. Materials

Ethyl acetate, *n*-hexane, acetonitrile and methanol were obtained from Burdick and Jackson (Obetz, OH, USA). Potassium phosphate and 85% phosphoric acid, HPLC grade, were obtained from Fisher Scientific (Pittsburgh, PA, USA) and tetramethylammonium chloride was

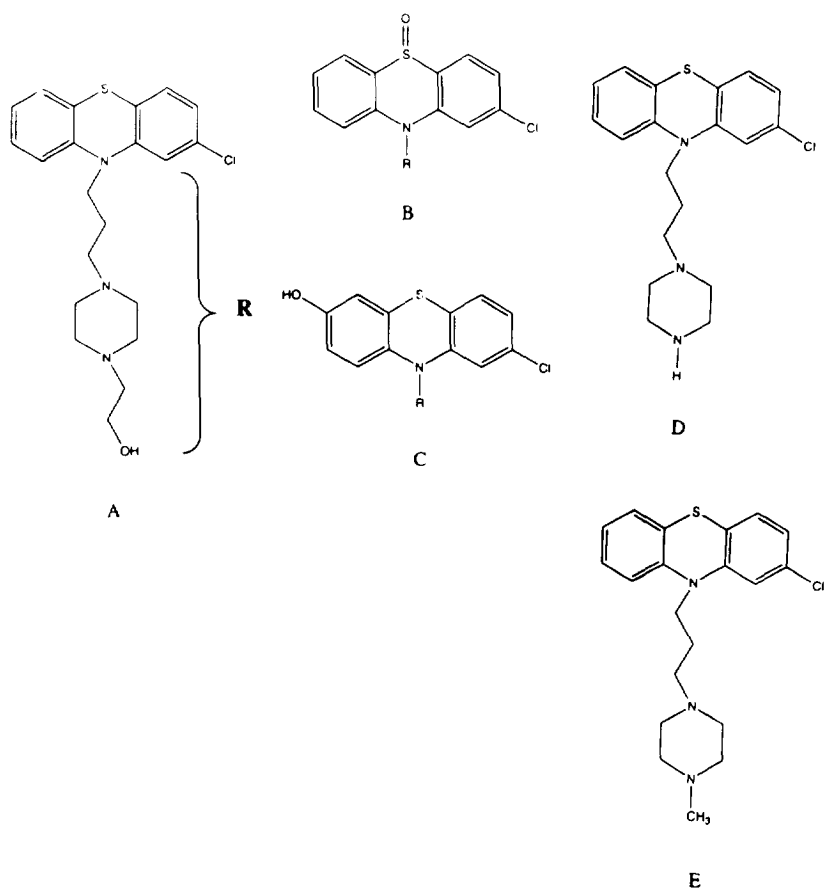

Fig. 1. Perphenazine (A), perphenazine sulfoxide (B), 7-hydroxyperphenazine (C), deshydroxyethylperphenazine (D), and prochlorperazine dimaleate (E) the internal standard.

purchased from Fluka (Ronkonkoma, NY, USA). The remaining chemicals used were analytical grade. The prochlorperazine dimaleate (PRO), the internal standard, is from Research Biochemicals (Natick, MA, USA), perphenazine sulfoxide (PPZSO), 7-hydroxyperphenazine (7OH), *n*-deshydroxyethylperphenazine (DAPPZ), and perphenazine (PPZ) were graciously supplied by Dr. Mitchell Cayen, Dr. Josephine Calabresi, Dr. Martin Steinman, and Dr. Arthur DeSilva from Schering-Plough Research Institute, Kenilworth, NJ, USA.

2.2. Apparatus

The analysis was performed on an EAS Coulochem Model 5100A electrochemical detector (ESA, Bedford, MA, USA) with a LKB 2150 HPLC pump (LKB, Pharmacia, Piscataway, NY, USA). The settings for the electrochemical unit were as follows: detector 1: +0.20 V, detector 2: +0.73 V, and guard cell: 0.75 V with a gain of 7×10 and response time 0.4 s. A straight, stainless-steel column (120 mm × 4.6 mm I.D.) packed with 5-μm Nucleosil C_{18} (Knauer, Berlin, Germany) was used at room temperature. The autosampler was a Perkin Elmer ISS-100 (Perkin Elmer, Pittsburgh, PA, USA) which was fitted with a 7126 Rheodyne injector with peak tubing and a 50-μl loop (Rainin Instruments, Woburn, MA, USA). A Spectra-Physics 4270 integrator was used for data gathering and reduction.

2.3. Human plasma samples

Human plasma samples were obtained from elderly patients with psychotic depression who were treated with a combination of perphenazine with or without nortriptyline. Patients were first started on nortriptyline and the dose was adjusted to yield a plasma level between 50 to 150 ng/ml. Perphenazine was added and titrated upwards until the emergence of Parkinsonian symptoms. Perphenazine doses ranged from 4 to 32 mg/day. Perphenazine was given as one dose at bedtime (9:00 pm) and a sample was drawn the following morning (7:00 am). Samples were obtained 5 days after a dosage adjustment. Plasma was separated by centrifugation in an EDTA tube for 10 min at 3000 *g* and stored at −20°C.

2.4. Extraction procedure for plasma samples

The analytical method for HPLC determination of perphenazine and each of its metabolites is as follows: 1 ml of plasma is added to a 10-ml screw-capped polypropylene tube (Fred Morrow Scientific, New Milford, NJ, USA) containing 10 μl of a 1 mg/ml stock of prochlorperazine as the internal standard. The stock standards were stored in methanol at −20°C, then diluted in 0.025 *M* KH_2PO_4 at a pH of 2.4 for the addition to the standard curve. The internal standard, prochlorperazine, and 7-hydroxyperphenazine must be sonicated in methanol to dissolve. To each sample, 1 M NaOH (filtered) was added to pH 9.0 and 6 ml ethylacetate–*n*-hexane (4:2). This mixture is shaken for 30 s and centrifuged at 3000 *g* for 3 min. The organic layer is transferred to another 15-ml polypropylene vial containing 100 μl of 0.025 *M* KH_2PO_4 at a pH of 2.4. This mixture is again shaken for 30 s and centrifuged for 3 min at 3000 *g*. The top organic layer is discarded by aspiration and the remaining aqueous phosphate layer is placed in autosampler vials under the hood for 15 min. The vials are then placed in the autosampler for 50-μl injections into the chromatograph. The entire assay and injections are done under subdued incandescent lighting to preserve the chemical stability of the drugs.

2.5. Chromatography conditions

The mobile phase for the separation of perphenazine and its metabolites was 0.01 *M* potassium dihydrogen phosphate buffer and 5 mM tetramethylammonium chloride, [adjusted to pH 2.4 with 85% phosphoric acid (HPLC grade), filtered and degassed]–acetonitrile–methanol (70:26:4, v/v/v). The flow-rate was maintained at 1 ml/min. The column was maintained at

room temperature. The retention times (min) of the compounds were as follows: PPZSO 2.80, 7OH 5.54, DAPPZ 10.94, PPZ 15.01, and PRO 17.80.

2.6. Recovery and linearity

Recoveries for the electrochemical method were performed with spiked stripped plasma (Scantibodies, Santee, CA, USA) at three different concentrations of perphenazine and its metabolites with 10 μl of 1 mg/ml solutions of prochlorperazine. The linearity of the electrochemical assay was determined between 0.5 and 10 ng/ml for PPZSO and PPZ, 1 and 10 ng/ml for DAPPZ, and 5 and 25 ng/ml for 7OH.

3. Results

Electrochemical detection of perphenazine and its metabolites by this method is sensitive enough for therapeutic monitoring of patient plasma samples. The limit of quantitation for PPZ was 0.5 ng/ml with signal-to-noise ratio of 6:1, PPZSO was 0.5 ng/ml with signal-to-noise ratio of 7:1, PPZD was 1 ng/ml with signal-to-noise ratio of 12:1, and 7OH was 5 ng/ml with signal-to-noise ratio of 18:1. Even though the signal-to-noise ratio for 7OH might suggest a lower limit of quantitation of 1 ng/ml, unreliable results between 1 and 5 ng/ml occurred. Adsorption was suspected; thus a limit of quantitation of 5 ng/ml was selected. The recoveries (Table 1) at three different concentrations had an average of 68.0% ± 6.4 for PPZSO, 45.0% ± 5.5 for 7OH, 81.3% ± 7.2 for DAPPZ, 91.7% ± 7.5 for PPZ, and 83.2% ± 5.5 for the internal standard. The linearity of the electrochemical assay over a range of 0.5–10 ng/ml for PPZSO was shown with a correlation coefficient of 0.9991 ($y = 2.856x + 0.0361$), for 7OH over a range of 5–25 ng/ml of 0.9933 ($y = 3.268x - 0.652$), for DAPPZ over a range of 1–10 ng/ml of 0.9964 ($y = 1.338 \times -0.0409$), and for PPZ over a range of 0.5–10 ng/ml of 0.9995) $y = 2.299x - 0.0367$). The inter-assay reproducibility of spiked plasma from the standard curve (Table 2) C.V. range for PPZSO was 8.3 to 4.2%, 7OH was 17.2 to 4.2%, DAPPZ was 8.8 to 3.0%, and PPZ was 10.0 to 4.1%. The reproducibility of controls is shown in Table 3, where the C.V. range for PPZSO was 18.8 to 9.9%, 7OH was

Table 1
Assay recovery study for perphenazine and its metabolites from spiked plasma ($n = 5$)

Drug	Amount (ng/ml)	Recovery (mean ± S.D.) (%)	C.V. (%)
PPZSO	1	67 ± 8.4	12.6
	5	82 ± 8.8	10.8
	10	55 ± 1.9	3.5
7OH	5	31 ± 3.9	12.6
	10	43 ± 4.4	10.2
	20	61 ± 8.1	13.3
DAPPZ	1	76 ± 6.0	7.9
	5	88 ± 7.3	8.3
	10	80 ± 8.3	10.3
PPZ	1	93 ± 7.5	8.0
	5	93 ± 8.0	8.7
	10	89 ± 7.1	7.9

Table 2
Inter-assay reproducibility of perphenazine and its metabolites from spiked plasma ($n = 4$)

Drug	Concentration (ng/ml)	Peak-height ratio (mean)	C.V. (%)
PPZSO	0.5	0.1745	6.6
	1	0.3540	4.2
	2	0.7068	8.3
	5	1.7200	8.2
	10	2.7600	7.2
7OH	5	1.3650	16.8
	10	3.7000	5.6
	15	5.0200	7.9
	20	7.0800	17.2
	25	8.6700	4.2
DAPPZ	1	0.0985	8.6
	2	0.2240	4.3
	5	0.6240	3.0
	7	0.9700	8.8
	10	1.2340	4.7
PPZ	0.5	0.0878	10.0
	1	0.1845	6.4
	2	0.3395	4.1
	5	0.9700	6.1
	10	1.9080	8.2

Table 3
Inter-assay reproducibility of quality control spiked plasma at three different concentrations ($n = 7$)

Drug	Actual (ng/ml)	Mean (ng/ml)	C.V. (%)
PPZSO	0.75	0.88	18.8
	1.50	1.42	9.9
	7.00	6.96	10.8
7OH	7.00	6.05	18.3
	12.00	11.76	8.1
	22.00	23.66	10.9
DAPPZ	1.50	1.48	10.7
	2.50	2.41	11.8
	8.00	8.29	6.0
PPZ	0.75	0.75	8.2
	1.50	1.41	9.1
	7.00	7.25	3.4

18.3 to 8.11%, DAPPZ was 11.76 to 6.00%, and PPZ was 9.13 to 3.37%. Quality controls are a crucial factor in determining the stability and predictability of perphenazine and its metabolites in every assay performed because degradation of the chemicals over time or any unforeseen daily experimental or mechanical problems is documented and recognized immediately.

The inter-assay reproducibility of patient plasma levels is described in Table 4, where the C.V. range for PPZSO was 7.8 to 4.2%, DAPPZ was 3.9 to 2.4%, and PPZ was 13.7 to 4.8%. The metabolites PPZSO and DAPPZ along with PPZ were very consistent from day to day; however, since the 7OH was always <5 ng/ml, it is difficult to say in these samples how reproducible this one compound is. Values greater than 5 ng/ml of 7OH were measured mostly in patients receiving more than 16 mg/day of PPZ. The lowest dose of PPZ given to a patient that was analyzed was 4 mg/day. Several patient profiles of PPZ and its metabolites with doses ranging from 4 to 32 mg/day, with or without nortriptyline, reveal the variance between the parent drug and metabolites levels from one patient to another in Table 5. Potential drug interferences were investigated and Table 6 lists those drugs that had retention times and peak heights that could distort the chromatography of this assay. Also assayed were drugs which, when injected under these conditions, did not produce any detectable peak heights to interfere with perphenazine or its metabolites. These drugs were doxepin, nordoxepin, verapamil, propranolol, theophylline, nortriptyline, metoprolol, trihexyphenidyl, and trifluoperazine. Figs. 2 and 3

Table 4
Interassay reproducibility of perphenazine and its metabolites with patient samples

Patent code	PPZI metabolite	Assay 1	Assay 2	C.V. (%)
B2	PPZSO	0.5	0.5	4.2
dose given	7OH	<5	<5	–
16 mg/day	DAPPZ	7.0	6.7	2.5
	PPZ	7.4	8.7	8.5
B3	PPZSO	0.67	0.67	0.0
dose given	7OH	5.0	<5	–
16 mg/day	DAPPZ	5.6	5.2	3.8
	PPZ	4.3	4.9	7.6
S1	PPZSO	8.9	9.9	4.9
dise given	7OH	<5	<5	–
12 mg/day	DAPPZ	19.9	18.4	3.9
	PPZ	21.5	16.3	13.7
S5	PPZSO	2.9	2.5	7.8
dose given	7OH	<5	<5	–
12 mg/day	DAPPZ	4.6	4.8	2.4
	PPZ	5.0	4.6	4.8

Table 5
Examples of different patient plasma levels of perphenazine and its metabolites, with doses ranging from 4 to 32 mg per day with or without nortriptyline

Sample	Dose (mg/day)	PPZSO (ng/ml)	7OH (ng/ml)	DAPPZ (ng/ml)	PPZ (ng/ml)	With nortriptyline
1	4	<0.5	0	1.5	1.8	Yes
2	6	1.7	<5	3.1	2.2	No
3	8	1.2	0	1.0	1.5	Yes
4	12	3.1	<5	4.9	1.9	Yes
5	16	3.8	5.7	2.8	2.6	No
6	24	7.2	6.3	3.2	3.6	Yes
7	32	3.1	8.0	10.4	6.5	Yes

show the chromatograms of an extracted blank and a patient receiving 12 mg/day to demonstrate the resolution of perphenazine and its metabolites for this analytical procedure.

4. Discussion

The monitoring of perphenazine and its metabolites in psychotic depressed geriatric patients is important for interpretation of issues concerning optimal therapeutic response, toxicity, occurrence of side-effects, compliance, and neuropharmacology. The reproducibility of this assay is highly advantageous for analyzing clinical samples to measure therapeutic response. Recoveries for the compounds of interest were as follows: PPZ 92%, PPZSO 68%, 7OH 45%, DAPPZ 81%, and PRO 83%. The limits of quantitation were low enough for the doses (4 to 32 mg/day) of perphenazine administered, which represents typical clinical situations. The day-to-

Table 6
Potential drug interferences for electrochemical detection

Drug	Retention time (min)
Diltizem	3.42
Chlorpheniramine	3.63
Alprazolam	3.65
Rantidine	3.65
Lorazepam	3.66
Nifedipine	3.67
Mesoridazine	8.52

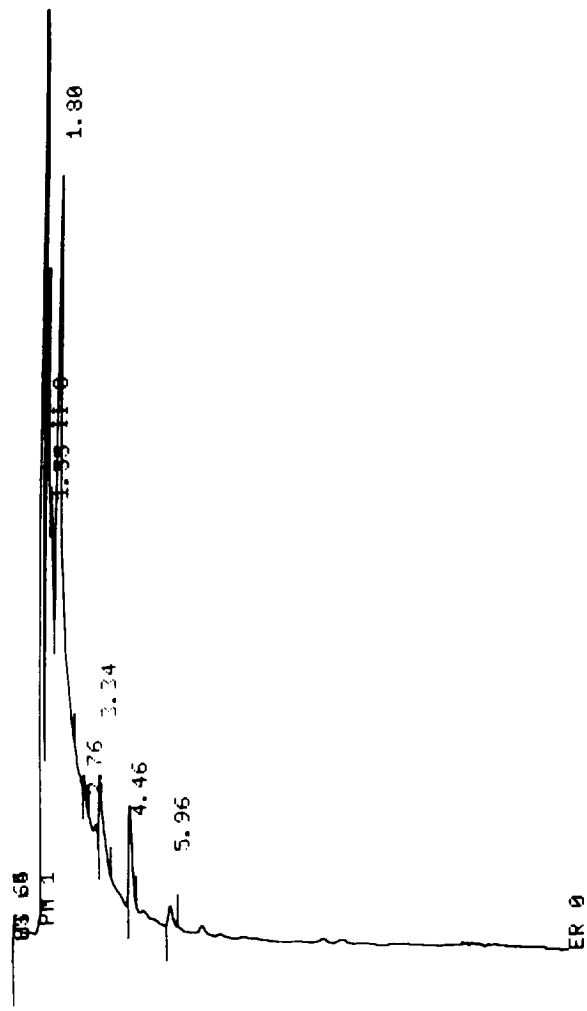

Fig. 2. Blank plasma extract.

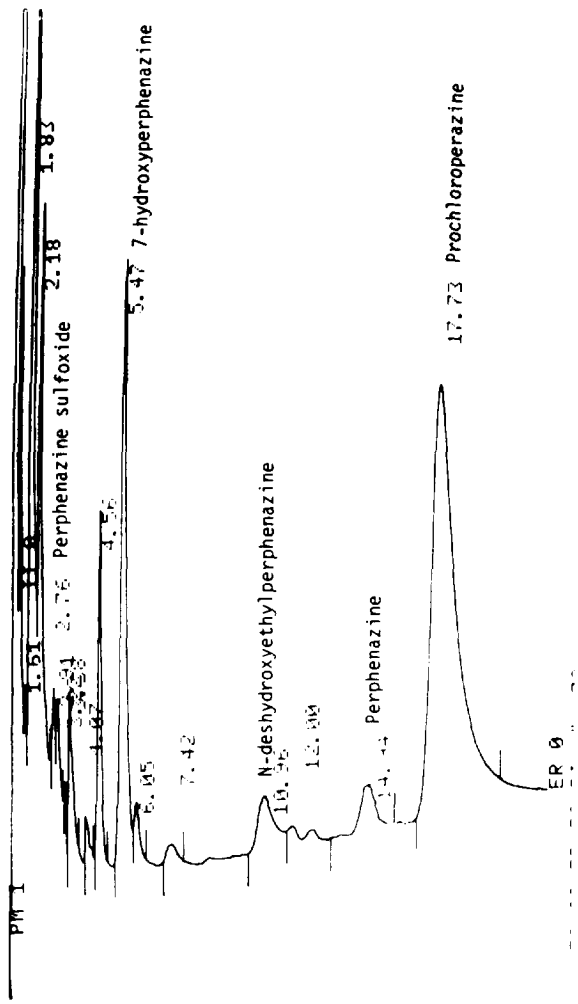

Fig. 3. Plasma extract of patient given a 12-mg dose of perphenazine/day. Detected levels were 3.2 ng/ml of PPZSO, <5 (4.8) ng/ml of 7OH, 7.7 ng/ml of DAPPZ, 5.8 ng/ml of PPZ, and 10 ng/ml of PRO.

day reproducibility for this method is consistent, so that the pharmacodynamic changes may be assessed and related to the plasma levels of the parent drug and/or the metabolites for each patient. The plasma sample volumes of the assay were low (1 ml) for steady-state conditions, while other techniques used larger volumes of plasma or serum to obtain suitable analytical sensitivity. When studying a geriatric population, the amount of plasma drawn from the patient is an important factor in their comfort and compliance. The specificity of this assay is high, even when patients are given numerous medications. In a geriatric population, where TDM may be performed on multiple medications, developing a procedure that utilizes a small plasma volume is very advantageous.

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References

[1] S.H. Preskorn and G.A. Fast, J. Clin. Psychiatry, 52 (Suppl.) (1991) 6.
[2] T. Van Putten, S.R. Marder, W.C. Wirshing, M. Aravagiri and N. Chabert, Schizophren. Bull., 17 (1991) 197–216.
[3] T. Van Putten, S.R. Marder, J. Mintz and R.E. Poland, Am. J. Psychiatry, 149 (1992) 500–505.
[4] M. Larsson and A. Forsman, Ther. Drug Monit. 5 (1983) 225–228.
[5] U.R. Tjaden, J. Lankelma, H. Poppe and R.G. Munsze, J. Chromatogr., 125 (1976) 275–286.
[6] D.F. Levinson, G.M. Simpsin and H. Singh, Arch. Gen. Psychiatry, 45 (1988) 878–879.
[7] T.B. Cooper, Clin. Pharmacokin., 3 (1978) 14.
[8] N.E. Larsen, in E. Usdin (Editor), Clinical Pharmacology in Psychiatry, Macmillan, London, 1981, p. 3.
[9] L.B. Hansen, N.E. Larsen and N. Gulmann, Psychopharmacology, 78 (1982) 112–115.
[10] N.E. Larsen and J. Naestoft, J. Chromatogr., 109 (1975) 259–264.
[11] N.E. Larsen, L.B. Hansen and P. Knudsen, J. Chromatogr., 341 (1985) 244–250.
[12] L.B. Hansen, J. Elley, T.R. Christensen, N.E. Larsen, J. Naestoft and E.F. Hvidberg, Br. J. Clin. Pharmacol., 7 (1979) 75–80.
[13] L.B. Hansen and N.E. Larsen, Psychopharmacologia, 37 (1974) 31–36.
[14] C.M. Mazure, J.C. Nelson, P.I. Jatlow, P. Kinaire and M.B. Bowers, Jr., J. Clin. Psychatiry, 51 (1990) 330–334.
[15] P.A. Hals and S.G. Dahl, Nord. Psykiat. Tidssk., 10 (Suppl.) (1984) 17.