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DETERMINATION OF CITALOPRAM, AMITRIPTYLINE AND CLOMIPRAMINE IN PLASMA BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The determination of citalopram, amitriptyline, clomipramine and their desmethyl metabolites after alkaline diethyl ether extraction from plasma is achieved by high-performance liquid chromatography using two internal standards and μBondapak C_{18} as stationary phase. Elution is carried out isocratically at 0.5 or 1 ml/min with a mixture of acetonitrile—potassium dihydrogen phosphate—distilled water (45:50:5). Detection is monitored by absorption at 254 nm. The detection limit is less than 5 ng/ml for each compound. The coefficients of variation are between 1.3% and 9.4% for 8—360 ng/ml. Interference from 22 possible co-medications is discussed. The technique can be used for therapeutic monitoring of these antidepressants as well as in analytical toxicology.

INTRODUCTION

The tricyclic antidepressants (Fig. 1) amitriptyline and clomipramine are widely prescribed for the treatment of depression [1, 2]. Citalopram (Lu 10-171) (Fig. 1), a new bicyclic antidepressant, is a specific potent serotonin re-uptake inhibitor [3—7]. The early onset of action and the rare side-effects of citalopram make the drug easy to apply and probably enhance compliance. The correlation between plasma levels of some antidepressants, even their major metabolites, and therapeutic effects suggests that measurement of plasma levels may provide valuable information for improving the clinical management of patients [8—16]. The use of high-performance liquid chromatography (HPLC) for clinical analyses has increased considerably during the past few years and the technique has been used routinely for many drug analyses [17].

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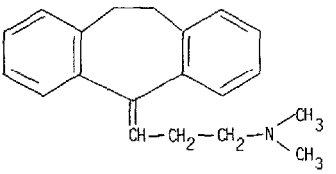
Tricyclics



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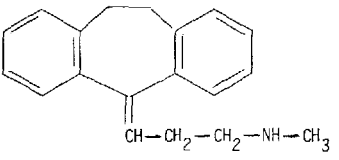

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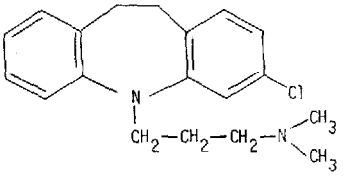
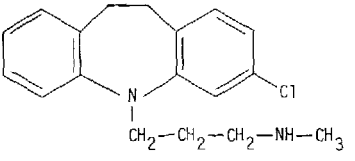
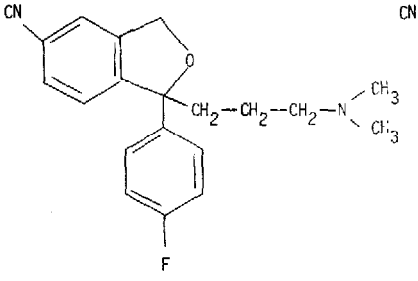


(CLOMIPRAMINE)

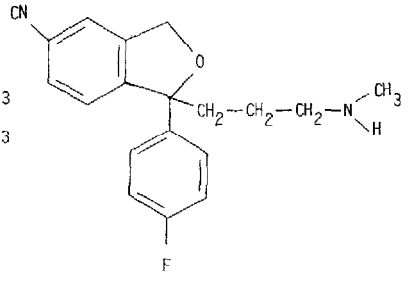


(DESMETHYLCLOMIPRAMINE)

Bicyclics



(CITALOPRAM)
(Lu 10-171)



(MONODESMETHYLCITALOPRAM)
(Lu 11-109)

Fig. 1. Chemical structures of compounds of interest.

HPLC procedures generally involve extraction of the tricyclic antidepressants from plasma or serum, followed by various modes of chromatographic separation and detection [18–24]. Recently, an analytical procedure for the determination of citalopram and its metabolites in plasma by HPLC was published [25].

The present study was undertaken to develop a common procedure for the routine clinical determination of citalopram, amitriptyline and clomipramine, as well as their desmethyl metabolites in plasma.

EXPERIMENTAL

Reagents

All reagents were of analytical grade. Methanol (RS per HPLC), acetonitrile (RS per HPLC), diethyl ether (RPE), 2 *M* sodium hydroxide (RPE) and acetone were from Carlo Erba. Potassium dihydrogen phosphate, 0.025 *M* was from Prolabo, and 0.5 *M* sulphuric acid from E. Merck.

Standards

Citalopram · HBr (Lu 10-171) and monodesmethylcitalopram · HCl (Lu 11-109) were supplied by Lundbeck. Amitriptyline · HCl was supplied by Roche. Nortriptyline · HCl was supplied by Squibb. Clomipramine · HCl, desmethylclomipramine · HCl and desipramine · HCl were supplied by Ciba-Geigy.

Stock solutions of each drug are prepared in methanol at a concentration of 1 μg/μl and stored at 4° C. They were diluted to 10 and 1 ng/μl for preparation of calibration standards.

Apparatus and chromatographic conditions

A Waters Model M45 pump fitted with a Waters U6K injector was used. A μBondapak C_{18} column, 10 μm particle size (30 cm × 3.9 mm I.D.) was connected to a Waters Model 441 detector monitored at 254 nm. The mobile phase was a mixture of acetonitrile—0.025 *M* potassium dihydrogen phosphate—distilled water (45:50:5) at a flow-rate of 0.5 ml/min for the bicyclic drug and 1 ml/min for the tricyclic compounds. Retention times are indicated in Table I. The increased flow-rate for tricyclic drugs allows retention times of less than 15 min.

TABLE I

RETENTION TIMES OF DRUGS

Drug	Retention time (min)	
	Flow-rate 0.5 ml/min	Flow-rate 1 ml/min
Citalopram	11.34	
Monodesmethylcitalopram	10	
Amitriptyline	18.66	10
Nortriptyline	15.66	8
Clomipramine	24	13
Monodesmethylclomipramine	19.84	10.20
Desipramine	13.85	7.20

Glassware

All glassware was washed with a mixture of sulphuric acid—potassium bichromate solution before use. All glass centrifuge tubes were rinsed with acetone and ether.

Extraction procedure

Into a centrifuge tube measure 100 μl of internal standard solution. Desipramine (1 ng/μl) is used as internal standard for citalopram and clomipramine analysis; clomipramine (1 ng/μl) is used for amitriptyline and nortriptyline analysis. Add 1—2 ml of plasma, 1 ml of 2 *M* sodium hydroxide and 10 ml of diethyl ether. Shake for 10 min and centrifuge for 5 min at 2800 *g*. Transfer the organic phase to another centrifuge tube and shake for 10 min with 2 ml of 0.5 *M* sulphuric acid. Centrifuge for 5 min at 2800 *g* and discard the top layer. To the aqueous layer add 3 ml of 2 *M* sodium hydroxide and re-

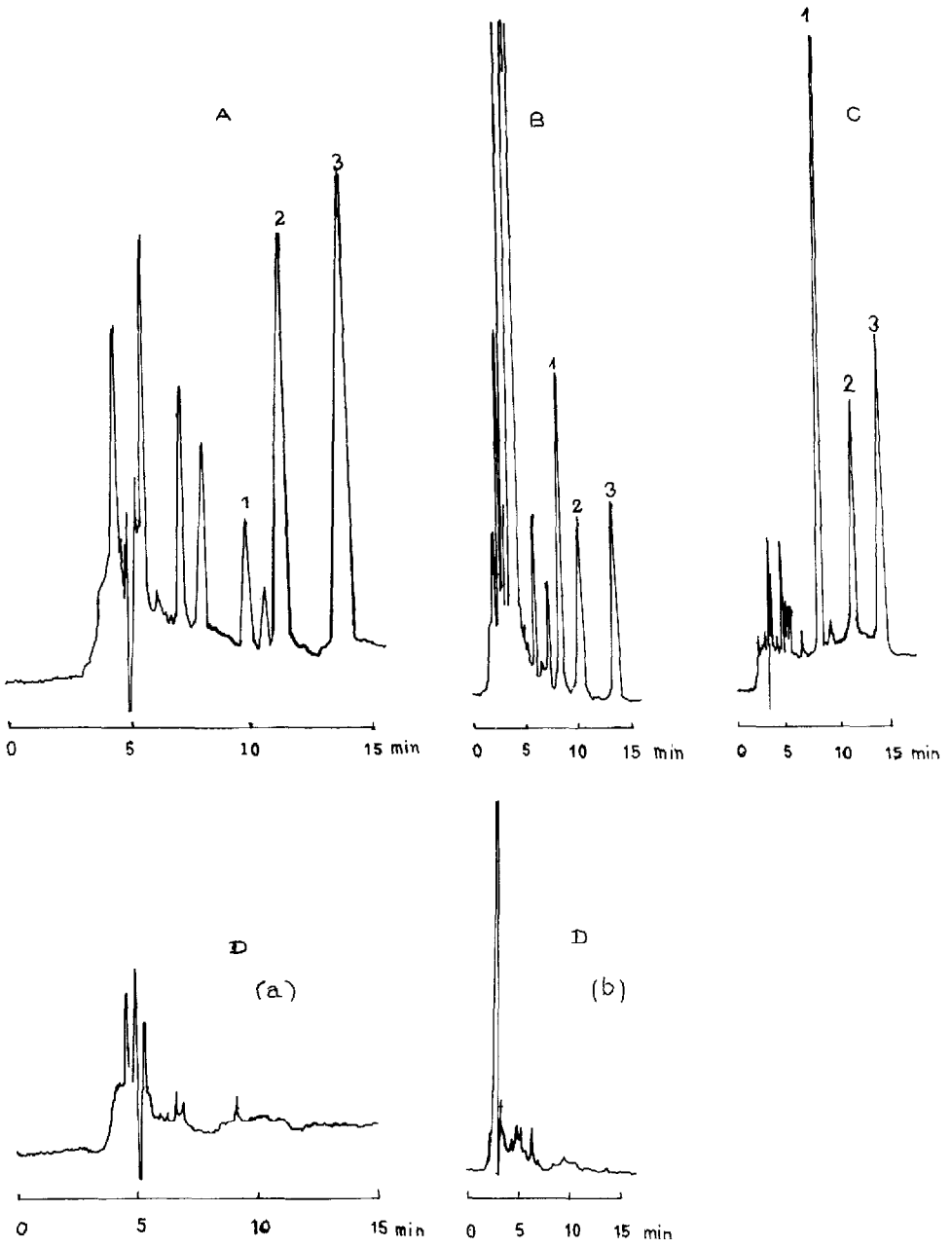


Fig. 2. (A) Chromatogram of a 1-ml plasma extract from a patient receiving daily 60 mg of citalopram · HBr for one month. Peaks: 1 = monodesmethylcitalopram, 2 = citalopram, 3 = desipramine (internal standard). (B) Chromatogram of a 1-ml plasma extract from a patient receiving daily 50 mg of amitriptyline · HCl intramuscularly for two weeks. Peaks: 1 = nortriptyline, 2 = amitriptyline, 3 = clomipramine (internal standard). (C) Chromatogram of a 1-ml plasma extract from a patient receiving daily 75 mg clomipramine · HCl for two months. Peaks: 1 = desipramine (internal standard), 2 = desmethylclomipramine, 3 = clomipramine. (D) Chromatogram of a 1-ml blank plasma extract; flow-rate 0.5 ml/min (a) or 1 ml/min (b).

extract with 10 ml of diethyl ether. After centrifugation, remove the organic phase and evaporate to dryness under nitrogen. Dissolve the residue in 100 µl of the mobile phase on a whirlmixer. Inject 20—50 µl of this solution into the chromatograph for analysis.

The ratio between the peak area of the analysed drug and that of the internal standard is calculated and plotted against the concentration of the tested drug after analysis of plasma samples spiked, respectively, with increasing amounts of each drug (10—400 ng/ml) and a constant amount of the appropriate internal standard (100 ng). The linear regression data for calibration curves were determined: the relations are linear between 10 and 300 ng/ml for bicyclics and between 10 and 400 ng/ml for tricyclics.

Chromatograms of plasma extracts from psychiatric patients receiving daily 60 mg of citalopram for one month, 50 mg of amitriptyline for two weeks, or 75 mg of clomipramine for two months are presented in Fig. 2.

RESULTS

Recovery experiments

The percentage extraction of each drug (10—400 ng/ml) was measured using the analytical conditions described. For the assay, the tested drugs are added before the extraction procedure and appropriate internal standard in the last organic phase. For the blank, drugs and internal standard are added together to the last organic phase. The recoveries were 84% and 80% for citalopram and its metabolite, 92% and 93% for amitriptyline and nortriptyline, 88% and 84% for clomipramine and monodesmethylclomipramine, respectively.

Detection limit

The detection limits for quantitative determination were 2—4 ng/ml for amitriptyline and nortriptyline, 2.5—5 ng/ml for clomipramine and desmethylclomipramine, 4—5 ng/ml for citalopram and monodesmethylcitalopram (the use of a UV—LC Philips detector at 239 nm for these bicyclic compounds allows a better limit, 1 ng/ml).

Reproducibility

The reproducibility of the analysis, within day (7—9 determinations) and day to day (three determinations), is indicated in Tables II and III. The within-day coefficients of variation are between 3.7% and 9.4% for the lower concentrations (8 or 9 ng/ml) and less than 6.5% for the upper concentrations (20—360 ng/ml). The day-to-day coefficient of variation is between 1.5% and 5.4% for four determinations over a period of a month (the samples were frozen for seven to thirty days).

Selectivity

Twenty-two drugs were tested for possible interference (Table IV). For analysis of bicyclics, carbamazepine, norclobazam and desmethylflunitrazepam, which might be partially extracted, were not resolved from either citalopram or desipramine (internal standard). For analysis of tricyclics, triazolam, nordiazepam, clobazam, trimipramine, diazepam, norclobazam and metabolites

TABLE II

WITHIN-DAY REPRODUCIBILITY

Drug	Concentration (ng/ml)	n	Mean $r_{S/IS}$* ± S.D.	C.V. (%)
Citalopram	8	9	0.028 ± 0.0016	5.7
	20		0.065 ± 0.0035	5.4
	40		0.146 ± 0.007	4.8
	80		0.295 ± 0.012	4
Monodesmethylcitalopram	9	9	0.040 ± 0.0015	3.7
	23		0.102 ± 0.0036	3.5
	45		0.209 ± 0.0038	2
	90		0.441 ± 0.010	2.3
Amitriptyline	9	7	0.110 ± 0.00818	8.4
	22		0.248 ± 0.01603	6.4
	44		0.490 ± 0.0196	4
	88		1.187 ± 0.057	4.8
	176		2.09 ± 0.0412	2
	352		4.284 ± 0.058	1.3
Nortriptyline	9	7	0.118 ± 0.0087	7.4
	22		0.33 ± 0.0085	2.6
	44		0.648 ± 0.0234	3.6
	88		1.308 ± 0.0850	6.5
	176		2.380 ± 0.122	5.1
	352		4.936 ± 0.1562	3.1
Clomipramine	9	7	0.059 ± 0.005	8.4
	45		0.009 ± 0.129	4.1
	90		0.567 ± 0.0256	4.5
	180		1.102 ± 0.0297	2.7
	340		2.007 ± 0.055	2.7
Desmethylclomipramine	9	7	0.068 ± 0.00642	9.4
	45		0.350 ± 0.014	4
	90		0.584 ± 0.018	3
	180		1.352 ± 0.034	2.5
	360		2.335 ± 0.066	2.8

*$r_{S/IS}$ = ratio between peak area of the analysed drug (S) and that of the internal standard (IS).

TABLE III

DAY-TO-DAY REPRODUCIBILITY

Drug	Added (ng/ml)	Found (ng/ml)				Mean ± S.D.	C.V. (%)
		Day 1 (n = 3)	Day 7 (n = 3)	Day 15 (n = 3)	Day 30 (n = 3)		
Citalopram	20	20.19	20.70	19.30	19.92	20.20 ± 0.63	3.15
Monodesmethylcitalopram	20	19.33	19.66	17.04	19.13	18.77 ± 1.016	5.40
Amitriptyline	176	182.40	176.80	174.20	174.40	177 ± 3.3	1.86
Nortriptyline	176	180.50	178.50	169	171.70	174 ± 2.36	1.5
Clomipramine	180	188	171.5	168	172	175 ± 7.6	4.4
Desmethylclomipramine	180	178	168.2	170.70	168.24	171.27 ± 3.8	2.22

TABLE IV

ELUTION OF CITALOPRAM, AMITRIPTYLINE, CLOMIPRAMINE AND THEIR METABOLITES, AND SOME SUBSTANCES TESTED FOR POSSIBLE INTERFERENCE

Flow-rate 0.5 ml/min.

Substance	Retention time (min)	Substance	Retention time (min)
Solvent front	4	*Desipramine*	13.85
Endogenous substances	4.10—7	7-Acetamidonitrazepam	15
Meprobamate	4.10	7-Aminonitrazepam	15.16
Caffeine	6.66	Triazolam	15.16
Viloxazine	6.70	*Nortriptyline*	15.66
Dibenzepine	7.84	Nordiazepam	16.66
Indalpine	8.10	Imipramine	16.90
Monodesmethylcitalopram	10.00	Flunitrazepam	17
Amineptine	11.20	Levomepromazine	17.66
Citalopram	11.34	Clobazam	18.34
Carbamazepine	11.66	*Amitriptyline*	18.66
Desmethylflunitrazepam	12.34	Trimipramine	18.90
Nitrazepam	12.50	*Desmethylclomipranine*	19.84
Doxepine	12.66	*Clomipramine*	24
Estazolam	13.34	Diazepam	24.50
Norclobazam	13.50		

of nitrazepam might interfere with either of these antidepressants or with desipramine (internal standard for clomipramine and its metabolite). Twelve drug-free plasmas from healthy subjects were extracted and analysed for possible interference by endogenous constituents, but no background interference was observed. Therefore citalopram cannot be quantitated in samples which also contain carbamazepine, clobazam and flunitrazepam. For analysis of tricyclics it is not possible to use diazepam (except for analysis of amitriptyline), triazolam, clobazam, or trimipramine as co-medications. These tests also indicate that the method can be extended to the quantitation of other antidepressants in plasma. We are now studying the possible applications for determining imipramine and desipramine, metapramine and its main metabolites.

CONCLUSIONS

The proposed method provides excellent sensitivity and reproducibility for the HPLC analysis of citalopram, amitriptyline, clomipramine and their desmethyl metabolites. Its selectivity could cause some inconvenience because of the possible interference from some drugs commonly administered as co-medications. The method is suitable for therapeutic monitoring and for analytical purposes in cases of possible intoxication.

ACKNOWLEDGEMENTS

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