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DETERMINATION OF METAPRAMINE, IMIPRAMINE, TRIMIPRAMINE AND THEIR MAJOR METABOLITES IN PLASMA BY REVERSED-PHASE COLUMN LIQUID CHROMATOGRAPHY

POK PHAK ROP and T CONQUY

Centre Hospitalier Spécialisé Edouard Toulouse, 118 Chemin de Mimet, 13326 Marseille Cédex 5 (France)

and

F GOUEZO, A VIALA* and F GRIMALDI

Laboratoire de Toxicologie Générale et Biotoxicologie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cédex 5 (France)

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SUMMARY

The determination of metapramine, imipramine, trimipramine and their desmethyl metabolites after alkaline diethyl ether extraction from plasma is achieved by column liquid chromatography using two internal standards and a μBondapak C_{18} column Elution is carried out isocratically at 1 or 0 6 ml min^{-1} with two mixtures of acetonitrile—potassium dihydrogen phosphate—distilled water (45 55 10 for metapramine and its metabolites, 45 50 5 for imipramine, trimipramine and their metabolites) Detection is monitored by absorption at 254 nm The detection limit is < 5 ng ml^{-1} for each compound The coefficients of variation (within-day and day-to-day) for the eight compounds are < 11 3% Interference from several possible co-medications is discussed The technique can be used for routine therapeutic monitoring of the antidepressants as well as analytical toxicology However, the three antidepressants cannot be analysed simultaneously by this method because metapramine requires a different elution system and imipramine interferes with monodesmethyltrimipramine (retention times 8 90 and 8 60 min, respectively)

INTRODUCTION

The tricyclic antidepressants metapramine, imipramine and trimipramine

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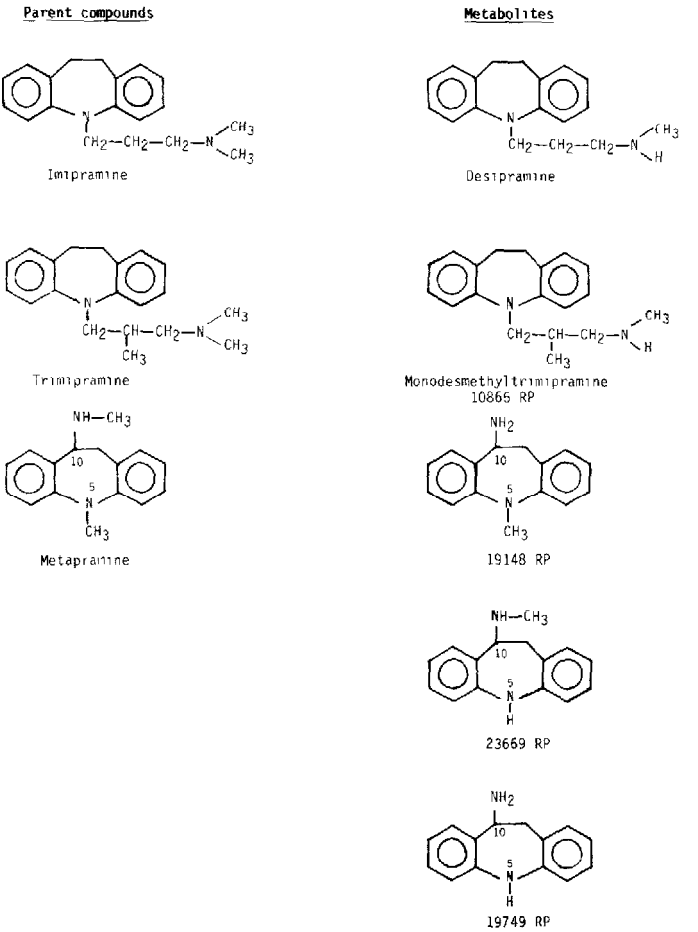

Fig 1 Chemical structures of compounds of interest

(Fig. 1) are often used for the treatment of depression. Since the major biotransformation products of tricyclic antidepressants are clinically active, the determination of these drugs and their primary metabolites is of great interest in clinical pharmacology [1–12]

Numerous procedures have been presented for the analysis of antidepressants [13- 30], some only quantitated the parent drug, some are specific for both the unchanged drug and its major metabolites The use of column liquid chromatography for clinical analysis of tricyclic antidepressants [22- 30] improved the separation of the unchanged drugs and their active metabolites.

Recently, we published an analytical procedure for the high-performance liquid chromatographic (HPLC) determination of three antidepressants (citalopram, amitriptyline and clomipramine) and their major metabolites [30] The present study was undertaken to develop this procedure for determining metapramine, imipramine, trimipramine and their metabolites in plasma For metapramine analysis, a different elution system was used The advantage of this method is the possibility of also quantitating three metapramine metabolites

EXPERIMENTAL

Reagents and glassware

All reagents were of analytical grade. Methanol (RS per HPLC), acetonitrile (RS per HPLC), diethyl ether (RPE) and 0.05 *M* phosphoric acid were from Carlo Erba (Milan, Italy), 0 025 *M* potassium dihydrogen phosphate was from Prolabo (Paris, France) and Titrisol buffer solution (pH 10) was from Merck (Darmstadt, F R G.)

All glassware was washed with sulphuric acid—potassium bichromate solution, then rinsed with distilled water and dried before use All glass centrifuge tubes were rinsed with acetone and ether

Standards

Metapramine fumarate, 5-methyl-10-amino-10,11-dihydrodibenzo[*b*,*f*]-azepine (19148 RP, I) base, 10-methylamino-10,11-dihydrodibenzo[*b*,*f*]-azepine (23669 RP, II) hydrochloride and 10-amino-10,11-dihydrodibenzo-[*b*,*f*]azepine (19749 RP, III) base, trimipramine maleate and monodesmethyl-trimipramine (10865 RP) maleate were supplied by Rhône-Poulenc (Vitry, France). Imipramine hydrochloride, desipramine hydrochloride and clomipramine hydrochloride were supplied by Ciba-Geigy (Rueil-Malmaison, France) Citalopram (Lu 10-171) hydrobromide was supplied by Lundbeck (Copenhagen, Denmark) For chemical structures of these compounds, see Fig. 1.

Stock solutions of each drug were prepared in methanol at a concentration of 1 mg ml^{-1}, stored at 4°C and protected from light They were diluted to 1 $\mu g\ ml^{-1}$ for preparation of calibration standards

Citalopram was used as internal standard for the analysis of metapramine and its metabolites Clomipramine was used as internal standard for the analysis of imipramine, trimipramine and their metabolites

Apparatus and chromatographic parameters

Chromatography was performed on a component system consisting of a Waters Model M45 delivery system, a Model U6K injector and a Model 441 UV detector monitored at 254 nm A μBondapak C_{18} column (30 cm × 3 9 mm I.D., particle size 10 μm, ambient temperature) was connected to the detector. The output of the detector was connected to an electronic integrator SP 4270 from Spectra-Physics (Lyon, France).

The mobile phase was acetonitrile—0.025 *M* potassium dihydrogen phosphate—distilled water. For the analysis of metapramine and its metabolites, the mixture proportions were 45 55 10 at a flow-rate of 0.6 ml min^{-1} For the analysis of imipramine, trimipramine and their metabolites, the mixture proportions were 45 50 5 at a flow-rate of 1 ml min^{-1} Retention times are indicated in Table I

Extraction procedure

Into a centrifuge tube add the internal standard 240 μl of citalopram solution (1 $\mu g\ ml^{-1}$) for analysis of metapramine and its metabolites or 200 μl of clomipramine solution (1 $\mu g\ ml^{-1}$) for analysis of imipramine, trimipramine

TABLE I

RETENTION TIMES OF DRUGS

Drug	Retention time (min)	
	Flow-rate 1 ml min $^{-1}$	Flow-rate 0 6 ml min $^{-1}$
Metapramine		8 60
I		7 50
II		6 75
III		6 00
Citalopram		10 80
Imipramine	8 90	
Desipramine	7 20	
Trimipramine	10 10	
Monodesmethyltrimipramine	8 60	
Clomipramine	13 00	

and their metabolites After evaporation of the solvent, add 1—2 ml of plasma, 2 ml of Titrisol buffer solution (pH 10) and 8 ml of diethyl ether Shake for 15 min and centrifuge for 5 min at 2800 *g*. Transfer the organic phase to another centrifuge tube and shake for 15 min with 100 µl of 0.05 *M* phosphoric acid Centrifuge for 5 min at 2800 *g* and discard the top layer Clean the residual extract by shaking with 2 ml of diethyl ether for 10 s on a Whirlimixer and centrifuge at 2800 *g* Discard the ether layer Inject 10—50 µl of the residual aqueous extract into the chromatograph for analysis.

Calibration

The ratio between the peak height 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 concentrations of each drug (5—500 ng ml $^{-1}$) and a constant amount of the appropriate internal standard (citalopram 240 ng, clomipramine 200 ng). The linear regression parameters for calibration curves were determined and the relations were linear between 5 and 300 ng ml $^{-1}$ for metapramine [$y = 10^{-3}(15.04x + 14\ 76)$, $n = 14$, $r = 0\ 999$], for I [$y = 10^{-3}(19\ 90x + 22.89)$, $n = 14$, $r = 0\ 999$], for II [$y = 10^{-3}(11\ 13x - 0\ 60)$, $n = 14$, $r = 0\ 997$] and for III [$y = 10^{-3}\ (16\ 68x - 37\ 85)$, $n = 14$, $r = 0\ 996$], 5 and 500 ng ml $^{-1}$ for imipramine [$y = 10^{-3}(10.40x - 0.94)$, $n = 14$, $r = 0\ 999$] and for desipramine [$y = 10^{-3}(10\ 60x - 1.28)$, $n = 14$, $r = 0\ 999$], 10 and 400 ng ml $^{-1}$ for trimipramine [$y = 10^{-3}(8\ 19x + 11\ 73)$, $n = 10$, $r = 0\ 999$] and for monodesmethyltrimipramine [$y = 10^{-3}(8\ 35x + 18.19)$, $n = 10$, $r = 0\ 999$]

RESULTS

Chromatograms of plasma extracts from psychiatric patients receiving 300 mg of metapramine, 200 mg of imipramine or 200 mg of trimipramine daily for three weeks are presented in Figs 2 and 3

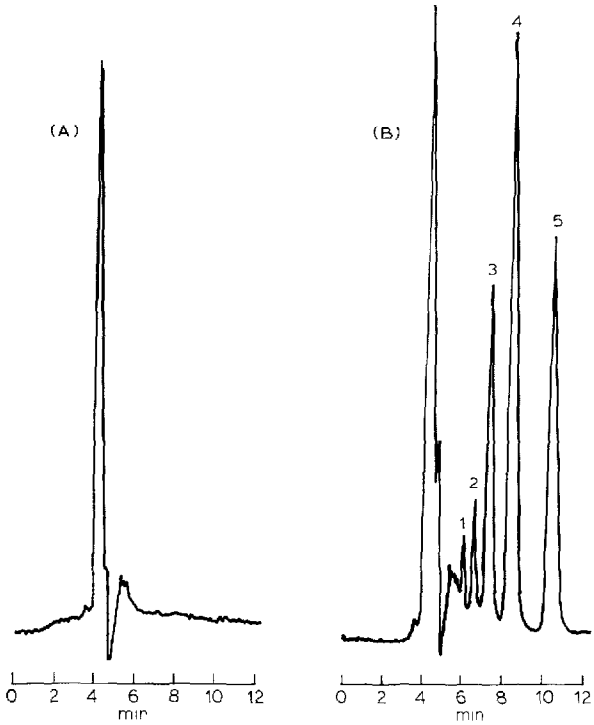

Fig 2 Chromatograms of (A) a 1-ml blank plasma extract and (B) a 1-ml plasma extract of a patient receiving 300 mg metapramine daily Mobile phase acetonitrile– 0 025 *M* potassium hydrogen phosphate—water (45 55 10), flow-rate 0 6 ml min $^{-1}$ Peaks 1 = III, 2 = II, 3 = I, 4 = metapramine, 5 = citalopram (internal standard)

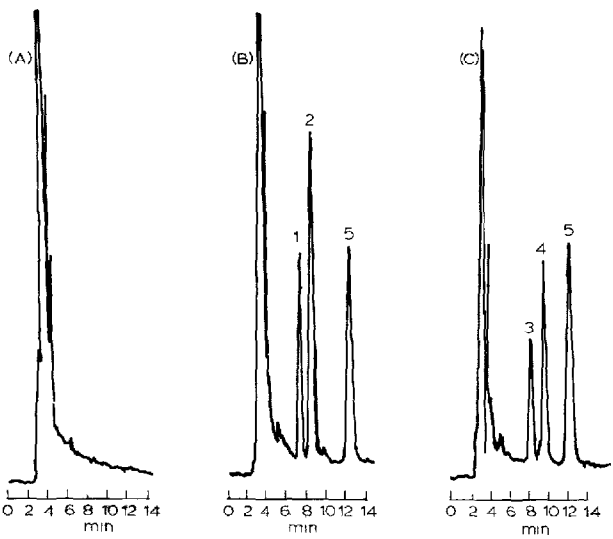

Fig 3 Chromatograms of (A) a 2-ml blank plasma extract, (B) a 1-ml plasma extract from a patient receiving 200 mg imipramine daily and (C) a 1-ml plasma extract from a patient receiving 200 mg trimipramine daily Mobile phase acetonitrile—0 025 *M* potassium dihydrogen phosphate—water (45 50 5), flow-rate 1 ml min $^{-1}$ Peaks 1 = desipramine, 2 = imipramine, 3 = monodesmethyltrimipramine, 4 = trimipramine, 5 = clomipramine (internal standard)

Recovery experiments

The percentage extraction of each drug (10—100 ng ml^{-1}) was measured using the extraction conditions described. For the assay, the tested drugs were added before the extraction procedure and the appropriate internal standard was added, after extraction, in the 0 05 *M* orthophosphoric acid solution (100

TABLE II

WITHIN-DAY REPRODUCIBILITY

Drug	Concentration (ng ml^{-1})	*n*	Peak-area ratio* (mean ± S D)	Coefficient of variation (%)
Metapramine	10	8	0 175 ± 0 015	8 50
	20	10	0 334 ± 0 019	5 79
	50	9	0 843 ± 0 055	6 57
	100	7	1 510 ± 0 023	1 52
	200	8	2 820 ± 0 077	2 71
I	10	8	0 238 ± 0 015	6 40
	20	10	0 453 ± 0 019	4 19
	50	9	1 017 ± 0 044	4 30
	100	7	2 110 ± 0 047	2 22
	200	8	3 717 ± 0 199	3 20
II	10	8	0 128 ± 0 013	10 58
	20	10	0 241 ± 0 018	7 50
	50	9	0 542 ± 0 037	6 80
	100	7	1 106 ± 0 046	4 13
	200	8	2 044 ± 0 107	5 25
III	10	8	0 176 ± 0 018	10 65
	20	10	0 321 ± 0 018	5 49
	50	9	0 733 ± 0 054	7 47
	100	7	1 606 ± 0 077	4 78
	200	8	2 836 ± 0 106	3 73
Imipramine	10	9	0 109 ± 0 027	11 00
	20	9	0 210 ± 0 015	7 17
	50	7	0 488 ± 0 012	2 54
	100	7	1 039 ± 0 065	6 30
	250	6	2 668 ± 0 071	2 66
Desipramine	10	9	0 115 ± 0 013	11 30
	20	9	0 209 ± 0 011	5 35
	50	7	0 556 ± 0 017	3 00
	100	7	1 044 ± 0 060	5 80
	250	6	2 753 ± 0.035	1 26
Trimipramine	10	7	0 094 ± 0 008	9 00
	40	7	0 030 ± 0 039	10 60
	100	7	0 826 ± 0 046	5 60
	200	7	1 660 ± 0 060	3 60
Monodesmethyltrimipramine	10	7	0 094 ± 0 010	11 00
	40	7	0 344 ± 0 015	4 40
	100	7	0 849 ± 0 060	7 00
	200	7	1 702 ± 0 046	2 70

*Ratio between peak area of the analysed drug and that of the internal standard

TABLE III

DAY TO DAY REPRODUCIBILITY AND ACCURACY

Drug	Added (ng ml $^{-1}$)	Found (n = 2) (ng ml $^{-1}$)					Mean ± S D (ng ml $^{-1}$)	Coefficient of variation (%)	Error (%)
		Day 1	Day 7	Day 15	Day 21	Day 30			
Metapramine	20	19 51	16 75	17 00	17 72	16 53	17 50 ± 1 08	6 17	12 50
	40	42 88	41 62	40 00	39 67	42 46	41 32 ± 1 28	3 10	3 30
I*	20	16 00	16 42	15 50	16 22	16 34	16 10 ± 0 33	2 05	19 50
	40	42 38	40 85	40 25	41 01	42 71	41 38 ± 1 30	3 15	3 45
II	20	22 82	18 25	16 65	20 32	18 06	19 22 ± 2 15	11 18	3 90
	40	42 75	40 40	38 75	41 17	43 21	41 25 ± 1 62	3 93	3 12
III	20	15 11	20 06	18 40	18 70	16 97	17 85 ± 1 68	9 41	10 75
	40	41 38	41 40	36 25	39 37	38 27	39 33 ± 1 95	4 95	1 67
Imipramine	50	50 30	40 40	49 75		48 85	49 57 ± 0 61	1 23	0 86
Desipramine	50	51 60	48 82	49 78		49 70	49 97 ± 1 36	2 72	0 06
Trimipramine	72	73 50	69 00	68 20		68 00	69 67 ± 2 58	3 70	3 23
Monodesmethyl trimipramine	36	34 80	33 50	32 00		30 80	32 77 ± 1 74	5 30	8 97

*The low values found for I (20 ng ml $^{-1}$) originate from operating conditions rather than from damage of this compound

TABLE IV

POSSIBLE INTERFERENCE FROM SEVERAL SUBSTANCES WITH THE DETERMINATION OF COMPOUNDS ANALYSED

For metapramine and compounds I, II and III the mobile phase was acetonitrile—0 025 M potassium dihydrogen phosphate—water (45 55 10), flow rate 0 6 ml min $^{-1}$, the internal standard was citalopram For imipramine, desipramine, trimipramine and monodesmethyltrimipramine the mobile phase was acetonitrile—0 025 M potassium dihydrogen phosphate (45 50 5), flow rate 1 ml min $^{-1}$, the internal standard was clomipramine + Possible interference with the analysed compound, x , possible interference with the internal standard

Substance tested	Metapramine	I	II	III	Imipramine	Desipramine	Trimipramine	Monodesmethyl trimipramine
Alimemazine								
Alprazolam								
Amineptine								
Amitriptyline							+	
Caffeine								
Carbamazepine	x	x	x	x				
Citalopram	x	x	x	x				
Clobazam							+	
Clomipramine					x	x	x	x
Desmethylfluni trazepam								
Diazepam					x	x	x	x
Dibenzepine								
Estazolam								
Ethyl loflazepate								
Flunitrazepam					+			+
Indalpine	+							
Levomepromazine					+		+	
Loprazolam								
Lorazepam								
Meprobamate								
Nitrazepam								
Norclobazam						+		
Nordiazepam								+
Nortriptyline								+
Oxazepam								
Triazolam						+		
Viloxazine			+					

μl) For the blank, drugs and internal standard were added together, after extraction, in the 0.05 *M* orthophosphoric acid solution (100 μl) Peak-height ratios of assay extracts were compared to peak-height ratios of blank extracts The recoveries were 90.27 ± 7 88% for metapramine, 91.88 ± 7.00% for I, 86.65 ± 6 18% for II, 92 10 ± 6.38% for III, 92 33 ± 4 64% for imipramine, 88 33 ± 8 95% for desipramine, 79 00 ± 5 54% for trimipramine and 75.00 ± 4 24% for monodesmethyltrimipramine (mean ± S D., $n = 4$—6).

Reproducibility and accuracy

The reproducibility of the analysis, within-day (six to ten determinations) and day-to-day (two determinations), is indicated in Tables II and III. The within-day coefficients of variation were between 6.4 and 11 3% for the lower concentrations (10 ng ml^{-1}) and 11% for the upper concentrations (20—250 ng ml^{-1}) The day-to-day coefficients of variation were between 1 23 and 11 18% for four to five determinations over a period of a month (the samples were frozen for 7—30 days) The percentage error is shown in Table III

Detection limits

The detection limits for quantitative determination from 2 ml of plasma (signal-to-noise ratio of 2.5—5 25) were 2.5 ng ml^{-1} for metapramine and I, 5 ng ml^{-1} for II and III, 2 ng ml^{-1} for imipramine and desipramine and 4—5 ng ml^{-1} for trimipramine and its desmethylmetabolite (injected volume 50 μl) If a plasma sample of 3 ml or more was used, it was possible to detect 1—2 ng ml^{-1} of each drug.

Selectivity

Chromatograms of a 2-ml plasma extract from healthy subjects showed no background interference from endogenous constituents (Figs. 2A and 3A)

Several drugs were also tested for possible interference (Table IV) For analysis of metapramine and its metabolites, it is not possible to use carbamazepine, indalpine, viloxazine and citalopram (internal standard) as co-medications, but no interference was noted with benzodiazepine derivatives For imipramine and desipramine determination, diazepam, flunitrazepam, levomepromazine, norclobazam (metabolite of clobazam) and triazolam were not resolved from either analysed compounds or internal standard (clomipramine) For trimipramine and monodesmethyltrimipramine analysis, interferences were noted from amitriptyline, nortriptyline, clobazam, diazepam, nordiazepam, flunitrazepam and levomepromazine.

CONCLUSIONS

The proposed method provides good sensitivity, reproducibility and accuracy for the HPLC analysis of metapramine, imipramine, trimipramine, and their desmethyl metabolites in plasma. Its selectivity could cause some inconvenience because of the possible interference of some drugs, especially benzodiazepine derivatives (except for metapramine and its metabolites), which could be administered as co-medications The procedure is suitable for therapeutic monitoring as well as for analytical purposes in cases of possible poisoning.

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REFERENCES

1 L F Gram, Clin Pharmacokin , 2 (1977) 237—251
2 L F Gram, Dan Med Bull , 24 (1977) 81- 89
3 L F Gram and J Christiansen, Clin Pharmacol Ther , 17 (1975) 555—563
4 A H Glassman and J M Perel, Arch Gen Psychiatry, 28 (1973) 649—653
5 A H Glassman, J M. Perel, M Shostak, S J Kantor and J L Fleiss, Arch Gen Psychiatry, 34 (1977) 197—204
6 C J S Walters, Proc R Soc Med , 61 (1971) 282—285
7 R R Levine, Prog Neuro-Psycho-Pharmacol , 3 (1979) 211—222
8 J P Moody, A C Tait and A Todrick, Br J Psychiatry, 113 (1967) 183—193
9 A Viala, J P Cano and A Durand, Encéphale, 6 (1980) 316- 332
10 A Viala, J P Cano, A Durand and S Monjanel, Sur le Métabolisme et l'Élimination de la Métaprimine, personal communication, 1980
11 A Viala, J P Cano, A Durand, S Monjanel and J P Rigault, Étude des Cinétiques Plasmatiques de la Métapramine chez l'Homme, personal communication, 1980
12 B Decouvelaere, B Terlain and A Bieder, Thérapie, 37 (1982) 249—257
13 A Viala, J P Cano, C Gola and F Gouezo, J Chromatogr , 59 (1971) 297—304
14 M Bertrand, C Dupuis, M Gagnon and R Dugal, Clin Biochem , 11 (1978) 117- 120
15 R G Jenkins and R O Friedel, J Pharm Sci , 67 (1976) 17—26
16 G Nyberg and E Martensson, J Chromatogr , 143 (1977) 491- 497
17 L A Gifford, P Turner and C M B Pare, J Chromatogr , 105 (1975) 107- 113
18 A Viala, J P Cano, A Durand and S Monjanel, J Chromatogr , 168 (1979) 195—201
19 A Kobayashi, S Sugita and K Nakazawa, J Chromatogr , 336 (1984) 410—414
20 R N Gupta and G Molna, Biopharm Drug Dispos , 1 (1980) 259—278
21 B A Scoggins, K P Maguire, T R Norman and G D Burrows, Clin Chem , 26 (1980) 5—17
22 F L Vandermak, R F Adams and G J Schmidt, Clin Chem , 26 (1978) 87- 91
23 D A Breutzmann and L D Bowers, Clin Chem , 27 (1981) 1907—1911
24 J T Streator, L S Eichmeier and M E Caplis, J Anal Toxicol , 4 (1980) 58- 62
25 I D Watson and M J Stewart, J Chromatogr , 134 (1977) 182—186
26 H F Proless, H J Lohmann and D G Miles, Clin Chem , 24 (1978) 1948—1953
27 S H Y Wong and T McCauley, J Liq Chromatogr , 4 (1981) 849—862
28 P M Kabra, N A Mar and L J Marton, Clin Chem , 111 (1981) 123—132
29 J P Somadossi, M Lemar, J Necciari, Y Sumirtapura and J P Cano, J Chromatogr , 228 (1982) 205—213
30 R Pok Phak, A Viala, A Durand and T Conquy, J Chromatogr , 338 (1985) 171—178