Journal of Chromatography, 532 (1990) 193–202
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5431

Note

High-performance liquid chromatographic analysis of haloperidol and hydroxyhaloperidol in plasma after solid-phase extraction

C. CAHARD, POK PHAK ROP and T. CONQUY

Hôpital Edouard Toulouse, 118 Chemin de Mimet, 13326 Marseille Cedex 5 (France)

and

A. VIALA*

Laboratoire de Toxicologie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5 (France)

(First received February 26th, 1990; revised manuscript received May 30th, 1990)

Haloperidol (H) (Fig. 1) is a neuroleptic of the butyrophenone group. After oral administration of the drug itself or intramuscular injection of its decanoic ester, H is the main active compound. It can be reduced in humans into a more hydrophilic alcohol metabolite (Fig. 1), reduced haloperidol (RH) [1] which also possesses some biological activity [2]. In volunteers, a good relationship was observed between doses of H and peak plasma concentrations or area under the curve values [3].

A correlation was also found between the administered dose of the decanoic ester and the plasma H concentrations [4,5]. According to several investigations cited in the bibliographic study of Dahl [6] and to Vatassery *et al.* [7], therapeutic steady-state plasma levels of H in cases of normal dosing would be in the range 3–25 ng ml^{-1}. RH was occasionally measured after ingestion of haloperidol: the steady-state RH plasma concentrations were sometimes higher, sometimes lower than those of H and, for the same dosage, inter-individual variations in both H and RH levels were observed. RH is more concentrated in red blood cells than in plasma [8]. High levels of RH could be associated with a poor therapeutic response [9,10]. Thus the simultaneous determination of haloperidol and its reduced metabolite may be clinically significant.

Several techniques have been used for the determination of H in human plasma, involving gas chromatography [11–14], gas chromatography–mass spectrometry [15,16], radioimmunoassay [17,18], radioreceptor assay [19] or high-performance liquid chromatography (HPLC) [20–28]. The determination of both H and RH was also performed, but only on patients under oral treatment with haloperi-

0378-4347/90/$03.50

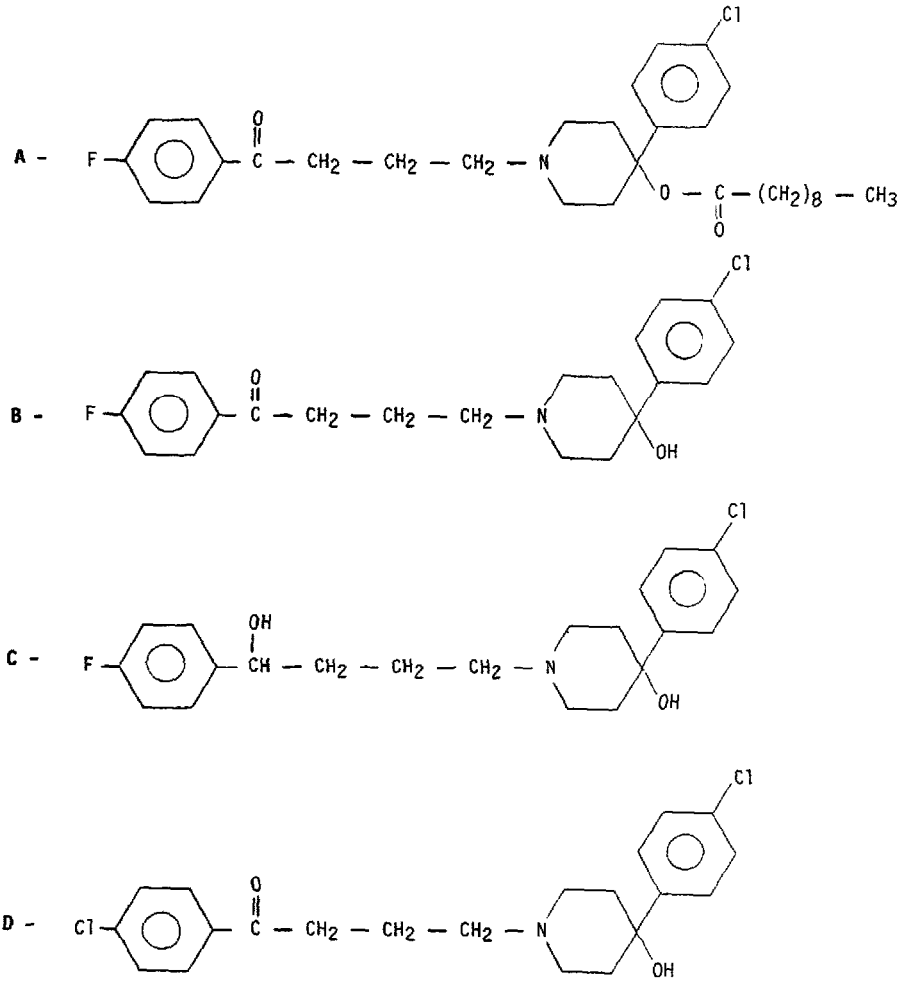

Fig. 1. Structures of (A) haloperidol decanoate, (B) haloperidol (H), (C) reduced haloperidol (RH) and (D) chlorohaloperidol (CH) (internal standard)

dol. The applied techniques were radioimmunoassay [29], with a two-step subtraction technique not easily adapted for routine clinical analysis, radioreceptor assay [30] or HPLC with electrochemical or UV detection [7,8,31–35].

The present study was therefore undertaken to develop a rapid HPLC procedure using a combination of a solid-phase extraction and a liquid cleaning step for the simultaneous routine determination of H and RH in plasma from patients on oral haloperidol or intramuscular haloperidol decanoate treatment.

EXPERIMENTAL

Reagents and glassware

All reagents were of analytical grade. Potassium dihydrogenphosphate (Normapur) and 0.01 *M* hydrochloric acid were from Prolabo (Paris, France). Methanol (RS, Reagente Speciale), acetonitrile (RS), *n*-hexane (RS), diethyl ether (RS) and Normex® buffer (pH 11) were from Carlo Erba (Milan, Italy). Extrelut® cartridges (3 ml) were 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 diethyl ether.

Standards

H, RH and chlorohaloperidol (internal standard, I.S.) were kindly supplied by Janssen Labs. Stock solutions of each compound were prepared in methanol at a concentration of 1 mg ml^{-1} and stored at 4°C. The stock solutions were stable for at least one month. They were diluted to 10 and 1 ng μl^{-1} with methanol before use.

Procedure

Plasma samples (2 ml) were pipetted into 5-ml glass centrifuge tubes, and 40 μl of I.S. in methanol (1 ng μl^{-1}) and 2 ml of Normex buffer solution (pH 11) were added. After vortex-mixing for 1 min, the mixture was passed onto a 3-ml Extrelut cartridge. Elution was carried out with diethyl ether. The eluate was evaporated to dryness under a stream of filtered air in a 40°C water-bath. The residue was dissolved by vortex-mixing in 100 μl of 0.01 *M* hydrochloric acid. The acid extract was cleaned by shaking with 2 ml of hexane for 20 s on a whirlmixer and centrifuged for 5 min at 2800 *g*. The hexane layer was then discarded, and 20–40 μl of the acid extract were injected into the chromatograph.

Apparatus and chromatographic conditions

Chromatographic analysis was performed on a Waters–Millipore system (Saint-Quentin en Yvelines, France) consisting of an M 45 pump, a U6K injector and a μBondapak C_{18} column (30 cm × 3.9 mm I.D., particle size 10 μm, ambient temperature), an M481 multi-wavelength detector monitored at 220 nm and connected to an SP 4270 electronic integrator from Spectra Physics (Lyon, France). The mobile phase was acetonitrile–0.025 *M* potassium dihydrogenphosphate–water (45:50:5, v/v) at a flow-rate of 0.8 ml min^{-1}.

Under these conditions, the capacity factors (k') and the selectivity coefficients (α) were 1.33 and 0.60 for RH, 1.77 and 0.80 for H and 2.20 and 1 for I.S.

Calibration

The ratios between the peak heights of the drugs and that of the I.S. were

calculated for the analysed plasma and plotted against the concentrations of the tested drugs added to blank samples at increasing concentration (H = 1, 5, 10, 20, 50 ng ml^{-1}; RH = 2.5, 5, 10, 20, 50 ng ml^{-1}) with a constant amount of I.S. (40 ng ml^{-1}). The linear regression parameters for the calibration curves were determined: the relations were linear between 1 and 50 ng ml^{-1} for H ($y = 0.042x - 0.039$; $r = 0.999$) and between 2.5 and 50 ng ml^{-1} for RH ($y = 0.050x - 0.0147$; $r = 0.999$), where y is the ratio of the analysed compound to the I.S. and x is the amount of spiked compound.

RESULTS

Fig. 2 shows the chromatograms obtained from a blank plasma (A), from the same plasma spiked with H, RH and I.S. (B) and from the plasma of a patient under treatment with haloperidol decanoate (C).

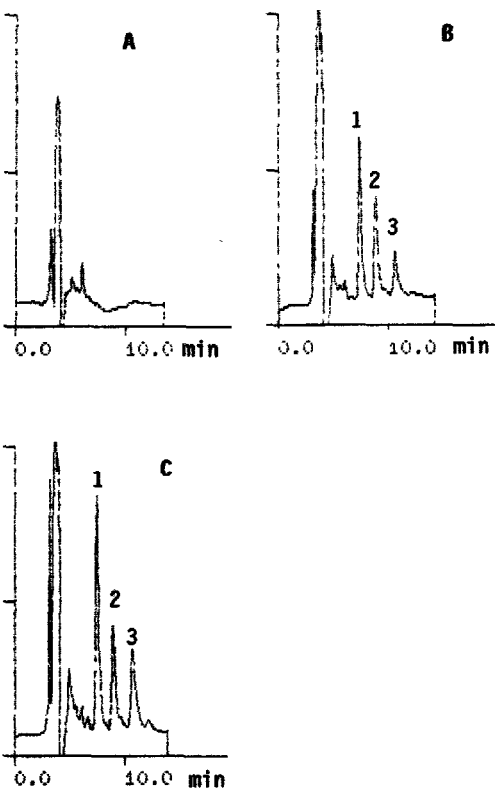

Fig. 2. Chromatograms obtained from (A) a blank plasma, (B) the same plasma spiked with H, RH and CH (20, 40, and 40 ng ml^{-1}, respectively) and (C) a plasma collected from a patient at the 28th day following 150 mg haloperidol decanoate intramuscularly. Peaks: 1 = RH; 2 = H; 3 = CH.

Recovery experiments

The percentage extractions of the two drugs and the I S. were measured. For the assay, the drugs (H and RH) were added (5, 10, 20 ng ml^{-1}) to the plasma before extraction and the I.S. was added after extraction. For the control, the drugs and the I.S. were added together, after the extraction. Haloperidol was used as internal standard to measure the percentage extraction of chlorohaloperidol. Peak-height ratios of the assay extracts were compared with those of the control extracts. The recoveries are shown in Table I. According to these results the highest recovery was observed at 20 ng ml^{-1} for all compounds: H, RH and I.S. There is certainly a small population of sites with irreversible binding on the Extrelut column.

Reproducibility

Within-day (four to seven determinations) and day-to-day (two determinations for each concentration at day 1, day 7 and day 15) reproducibility results are indicated in Table II. The within-day coefficients of variation (C.V.) were between 4.6 and 14.7% for H and between 3.8 and 12.3% for RH. The day-to-day C V. were between between 5.9 and 14.7% for H and between 2.8% and 11% for RH, over a period of two weeks (the samples were frozen for fifteen days).

Sensitivity

The limits of quantitation from 2 ml of plasma (signal-to-noise ratio of 3 for H and 3.5 for RH at 0.005 a.u.f.s.) were 1 ng ml^{-1} (H) and 2.5 ng ml^{-1} (RH).

Selectivity

Chromatograms of plasma extracts from healthy subjects showed no back-

TABLE I

PERCENTAGE EXTRACTION

n = 6 for each concentration.

	Concentration (ng ml^{-1})	Recovery (mean ± S.D) (%)	C V (%)
Haloperidol	5	73 ± 5	6.8
	10	73 ± 4	5 5
	20	88 ± 3	3.4
Reduced haloperidol	5	79 ± 6	7 6
	10	77 ± 4	5.2
	20	95 ± 3	3 1
Chlorohaloperidol (I.S.)	5	75 ± 4	5 3
	10	76 ± 4	5 3
	20	90 ± 3	3.3

TABLE II

WITHIN-DAY AND DAY-TO-DAY REPRODUCIBILITY

Compound	*n*	Added (ng ml^{-1})	Found (mean ± S.D.) (ng ml^{-1})	C.V. (%)
Within-day				
Haloperidol	4	1	1.2 ± 0.15	12.0
	6	2.5	2.5 ± 0.2	12.5
	7	5	4.8 ± 0.7	14.7
	4	10	8.5 ± 1	11.5
	5	20	19.3 ± 1.4	7.3
	4	25	22.6 ± 1	4.6
	6	50	52.4 ± 3	5.8
Reduced haloperidol	4	2.5	3.0 ± 0.1	3.8
	5	5	4.6 ± 0.5	11.2
	4	10	8.9 ± 1.1	12.3
	5	20	19.3 ± 1.6	8.5
	4	25	22.6 ± 1.2	5.5
	6	50	48.7 ± 2.9	5.9
Day-to-day				
Haloperidol	2	5	5.4 ± 0.8	14.7
	2	10	9.3 ± 1.0	10.5
	2	20	22.0 ± 1.3	5.9
Reduced haloperidol	2	5	6.5 ± 0.6	11
	2	10	10.0 ± 0.4	4.3
	2	20	20.6 ± 0.6	2.8

ground interferences from endogenous constituents (Fig. 2A). Several drugs were also tested for possible interference. As seen in Table III no interferences were noted with caffeine, meprobamate, heptaminol, several neuroleptics, anticonvulsants, antiparkinsonians, benzodiazepines and several antidepressants. Desipramine, lorazepam, nortriptyline, desmethylclomipramine, cyamemazine, trihexyphenidyle, amitriptyline and imipramine were not well resolved from either analysed compounds or I.S. However, concerning lorazepam, only a small amount remained in the acid extract.

Application

Therapeutic applications included seven psychiatric patients treated with haloperidol followed by haloperidol decanoate. Information regarding the patients is listed in Table IV.

Haloperidol was administered orally: 5–20 mg (0.07–0.32 mg kg^{-1} body weight) every day. After at least fifteen days of treatment, haloperidol decanoate

TABLE III

CAPACITY FACTORS AND SELECTIVITY COEFFICIENTS OF ANALYSED COMPOUNDS AND SOME DRUGS TESTED FOR POSSIBLE INTERFERENCE

Drug	Capacity factor (k')	Selectivity coefficient (α) (relative to I S)
Meprobamate	–	–
Heptaminol	–	–
Sultopride	0.23	0.10
Caffeine	0.23	0.10
Viloxazine	0.33	0.15
Carbamazepine	0 90	0.41
Biperidene	1 11	0.50
Oxazepam	1 16	0.53
Ethyl-loflazepate	1.20	0 54
Desipramine	1.26	0 57
Lorazepam	1.30	0 59
Reduced haloperidol	1.33	0 60
Triazolam	1.71	0.77
Nortriptyline	1.73	0.77
Haloperidol	1.77	0.80
Clorazepate dipotassium	2 01	0.91
Nordiazepam	2 03	0 92
Desmethylclomipramine	2.15	0.98
Cyamemazine	2.15	0 98
Chlorohaloperidol	2.20	1
Trihexyphenidyle	2.20	1
Amitriptyline	2.23	1.01
Imipramine	2.23	1.01
Clobazam	2.30	1.04
Trimipramine	2.49	1.13
Flunitrazepam	2.50	1.13
Clomipramine	2.60	1.18
Alimemazine	2.60	1 18
Levomepromazine	2.80	1.27
Tropazepine	2.80	1 27
Ethybenztropine	3.20	1.45
Diazepam	3.37	1 53
Amisulpride	4.17	1.89
Tiapride	4.28	1.89
Sulpiride	4.53	2.05

was administered in relay intramuscularly: 141–352.5 mg (2–5.64 mg kg^{-1} body weight) every four weeks over twenty weeks (*i.e.* five periods). The patients were also given other drugs (antiparkinsonians, night sedatives, oral neuroleptics, anti-epileptics, etc.). None of these drugs interfered with the determination of the

TABLE IV

DATA CONCERNING PATIENTS, DRUG ADMINISTRATION AND SAMPLING TIME OF PLASMA

Patient No.	Sex	Age (year)	Weight (kg)	Dose				Sampling time[a]		
				Haloperidol		Haloperidol decanoate		So	Sw	Sp
				mg	mg kg^{-1}	mg	mg kg^{-1}			
1	M	26	72	15	0.21	282	3.90	+	−	−
2	F	29	65	15	0.24	282	4.34	+	−	−
3	M	25	63	20	0 32	352.5	5.60	+	+	−
4	M	44	62	10	0 16	211.5	3.41	+	+	−
5	F	54	50	15	0.30	282	5 64	+	+	−
6	M	52	70	5	0.07	141	2.00	+	−	+
7	M	38	103	20	0.20	352.5	3.42	+	−	+

[a] So: just before injection of haloperidol decanoate after oral haloperidol treatment, Sw: weekly for two periods of haloperidol decanoate treatment; Sp just before each injection of haloperidol decanoate over five periods of treatment.

analysed compounds. Blood samples (10 ml each) were drawn in oxalate tubes, according to the scheme given in Table IV during two to five periods of haloperidol decanoate treatment, after at least fifteen days of normal treatment. Plasma,

TABLE V

CONCENTRATIONS OF H AND RH IN SEVEN PATIENTS

No = No response for RH (<2.5 ng ml^{-1}) or for H (<1 ng ml^{-1}), C_0 = concentration measured just before injection of haloperidol decanoate after oral administration, C_w = concentration measured weekly between two injections of haloperidol decanoate (one injection every four weeks)

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7	
	H	RH	H	RH	H	RH	H	RH	H	RH	H	RH	H	RH
C_0	16	3.5	5	5.5	4	No	2	No	3 8	8	2	No	38	2 5
C_{w1}					6	No	5	No	11	10				
C_{w2}					−	−	4	No	13	6				
C_{w3}					−	−	1.2	4.5	9.5	6				
C_{w4}					3	No	No	No	2.2	4.5	1 5	No	33	2.5
C_{w5}					10	3	10	4	9.5	6				
C_{w6}					5	No	14	3.5	−	−				
C_{w7}					−	−	57	11	−	−				
C_{w8}					2.2	4	21	2.5	3.5	−	−	−	−	−
C_{w12}											3.4	No	27	3
C_{w16}											−	−	28	3 2
C_{w20}											1 6	No	25	3

obtained after immediate centrifugation, was stored frozen at −10°C till the analysis took place.

The plasma steady-state concentrations of H and RH in seven patients under treatment (C_0) were found between 2 and 38 ng ml^{-1} for H and 2.5 to 8 ng ml^{-1} for RH, but in some patients (Nos. 3, 4 and 6) RH was not detected or below the limit of quantitation (Table V).

The concentrations of H and RH (C_w) measured one week after injection of haloperidol decanoate (C_{w1}, C_{w5}) were slightly higher than those found before injection. As seen in Table V, the weekly plasma concentrations of H and RH on the patients (Nos. 3, 4 and 5) showed inter-individual and intra-individual variations. The residual concentrations of H (in patients 6 and 7) and even RH (in patient 7) determined just before each injection of haloperidol decanoate showed a plateau, as measured every four weeks, and the levels were in the same range as those found after normal haloperidol treatment (C_0). The concentrations of RH in patient 6 were below the limit of quantitation in all cases.

CONCLUSION

The proposed HPLC procedure uses a combination of solid–liquid extraction, cleaning and UV detection, giving acceptable sensitivity for H and RH. Owing to its relative simplicity, the method can be applied for routine determination of H and RH in plasma from patients on oral haloperidol or intramuscular haloperidol decanoate treatment, as well as for the diagnosis of possible overdose. Results of determinations on seven psychiatric patients showed inter-individual and intra-individual variations.

ACKNOWLEDGEMENTS

The authors thank Janssen Laboratories and Dr. Castano, Dr. Heiselbec and Dr. Dirruza of E. Toulouse Hospital in Marseilles for providing the standard compounds and the blood samples, respectively.

REFERENCES

1 A. Forsman and M Larsson, *Curr. Ther. Res.*, 24 (1978) 567
2 R. T. Rubin and R E Poland, in S. Dahl and E. Usdin (Editors), *Clinical Pharmacology in Psychiatry, Neuroleptic and Antidepressant Research*, McMillan, London, 1981, p. 217
3 A Forsman and R. Ohman, in B. Saletu, P Berner and L Hollister (Editors), *Neuropsychopharmacology*, Pergamon, Oxford, 1979, p. 559.
4 M. Parent, Ch Toussaint, F. Driesens and Y. Gelders, *Acta Psychiatr. Belg.*, 81 (1981) 399.
5 A J. M Reyntjens, J. J. P Heykants, R. J. H. Woestenborghs, Y G. Gelders and T. J. L. Aerts, *Int. Pharmacopsychiatry*, 17 (1982) 238.
6 S G Dahl, *Clin Pharmacokinetics*, 11 (1986) 36.
7 G T Vatassery, L. A. Herzan and M W Dysken, *J. Chromatogr.*, 433 (1988) 312.
8 G T. Vatassery, L A Herzan and M. W. Dysken, *J Anal. Toxicol*, 14 (1990) 25

9 L. Ereshefsky, C M Davis, C A. Harrington, M. W Jann, J L Browning, S R Saklad and N R. Burch, *J. Clin Psychopharmacol.*, 4 (1984) 138
10 A C Altamura, M C. Mauri, R Cavallaro and A. Gorni, *Lancet*, i (1987) 814.
11 A. Forsman, E. Märtensson, G. Nyberg and T. Öhman, *Arch Pharmacol*, 286 (1974) 113.
12 G. Bianchetti and P. L. Morselli, *J. Chromatogr.*, 153 (1978) 203.
13 M Franklin, *Clin Chem*, 26 (1980) 1367.
14 D R. Abernethy, D J Greenblatt, H. R. Ochs, C. R. Willis, D. D. Miller and R. L. Shader, *J. Chromatogr.*, 307 (1984) 194.
15 N Haring, Z. Salama, L Todesko and H Jaeger, *Arzneim. Forsch.*, 37 (1987) 1402.
16 M A. Moulin, R. Camsonne, J P Davy, E Poilpre, P. Morel, D. Debrugne, M. C. Bigot, M. Dedieu and M Hardy, *J. Chromatogr.*, 178 (1979) 324.
17 R. E. Poland and R T. Rubin, *Life Sci*, 29 (1981) 1837
18 R. T. Rubin, B. B Tower, S E Hays and R. E. Poland, *Methods Enzymol.*, 84 (1982) 532.
19 M A Javors and C L Bowden, *Clin. Pharmacol. Ther.*, 37 (1985) 203.
20 K. Miyazaki, T. Arita, I. Oka, T. Koyama and I Yamashita, *J Chromatogr.*, 223 (1981) 449
21 P. I. Jatlow, R Miller and M. Swigar, *J Chromatogr*, 227 (1982) 233.
22 M J. Kogan, D Pierson and K Verebey, *Ther. Drug Monitor.*, 5 (1983) 485.
23 A. K. Dhar and H Kutt, *Clin. Chem.*, 30 (1984) 1228.
24 A. Mc Burney and S. George, *J. Chromatogr.*, 308 (1984) 387
25 D. Hequet, C. Jarry, C. Rouquette and A Brachet-Liermain, *Ann. Biol. Clin*, 43 (1985) 739.
26 Ph Puig, J. Poey and P Bourbon, *Analusis*, 14 (1986) 242.
27 M Hayakari, Y Hashimoto, T Kita and S. Murakami, *Forensic Sci. Int.*, 35 (1987) 73.
28 L. B. Nilsson, *J. Chromatogr.*, 431 (1988) 113.
29 J. L. Browning, C. A. Harrington and C M Davis, *J. Immunoassay*, 6 (1985) 45
30 E. R Korpi, G. N Ko, B H Phelps and R. J. Wyatt, *J. Clin. Psychopharmacol*, 4 (1984) 332
31 M Larsson, A. Forsman and R. Öhman, *Curr. Ther Res*, 84 (1983) 999.
32 E. R. Korpi, B. H. Phelps, H. Granger, W. J Chang, M Linnoila, J. L. Meek and R. J. Wyatt, *Clin. Chem.*, 29 (1983) 624
33 R L Miller and C. L. De Vane, *J. Chromatogr.*, 374 (1986) 405
34 K. K. Midha, J. K. Cooper, E. M. Hawes, J W. Hubbard, E D Korchinski and G. McKay, *Ther. Drug Monitor.*, 10 (1988) 177
35 N. Eddington and D. Young, *J. Pharm. Sci*, 77 (1988) 541.