Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 4/5, pp. 393–399, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Determination of FCE 23884, a new ergolene derivative, and its possible metabolite, the 6-nor-derivative in plasma by high-performance liquid chromatography with fluorescence detection

P. MARRARI, G. BASILEO, E. PIANEZZOLA* and M. STROLIN BENEDETTI

Farmitalia Carlo Erba, Research and Development, Erbamont Group, Via C. Imbonati 24, 20159 Milan, Italy

Abstract: A sensitive and selective high-performance liquid chromatographic method for the determination of FCE 23884 and its 6-nor-derivative (FCE 26506) in plasma has been developed. After buffering the plasma samples, the compounds and the internal standard were extracted with ethyl ether–*n*-octanol (9:1, v/v), back-extracted into 0.01 M phosphoric acid and then analysed by reversed-phase liquid chromatography. Quantification was achieved by fluorescence detection of the eluate. The linearity, precision and accuracy of the method were evaluated. No interference from the biological matrix was observed. The assay was adequate for the quantification of plasma levels of the two compounds after a single oral dose of 1 mg of FCE 23884 in humans.

Keywords: *Reversed-phase high-performance liquid chromatography; fluorimetric detection; dopamine agonists; plasma drug levels.*

Introduction

Recently, interest in ergot derivatives has been focused mainly on their potential to stimulate dopamine (DA) receptors. The dopaminomimetic properties of this class of compounds have had useful applications in the management of Parkinson's disease [1–3].

FCE 23884 [4-(9,10-didehydro-6-methylergolin-8β-yl)methylpiperazine-2,6-dione] is an ergolene derivative that behaves as a full DA antagonist in normal animals and as a powerful DA agonist after experimental procedures resulting in severe DA depletion [4, 5]. Phase I trials in healthy volunteers were conducted to evaluate tolerance (range of dose studied 0.2–1.5 mg). Hence a very sensitive analytical method was required to study the pharmacokinetics of this compound in humans. Since FCE 23884 had shown interesting fluorescence properties, efforts were directed towards the development of an HPLC method with fluorescence detection that would provide the necessary specificity and sensitivity.

A selective extraction step gave the opportunity to clean up the compound of interest from the constituents of the matrix as well as permitting concentration of the sample before the HPLC analysis. Furthermore, the identification among the structurally related compounds of a suitable internal standard allowed improvement of the reproducibility of the extraction step and hence of the whole method. This report describes in detail the development and validation of an HPLC assay for the determination of FCE 23884 and its possible metabolite, the 6-nor-derivative, in plasma.

Experimental

Materials and solutions

FCE 23884 [4-(9,10-didehydro-6-methylergolin-8β-yl)methylpiperazine-2,6-dione], FCE 26506 [4-(9,10-didehydroergolin-8β-yl)-methylpiperazine-2,6-dione] and FCE 22716 [1-(6-methylergolin-8β-yl)methyl-2,4-(3H, 5H)-imidazoledione] (internal standard, I.S.) were supplied by R.D/CNS Medicinal Chemistry and by the Chemical Development Department of Farmitalia Carlo Erba (see Fig. 1 for their structural formulae).

All other chemicals and solvents were analytical grade from Farmitalia Carlo Erba

* Author to whom correspondence should be addressed.

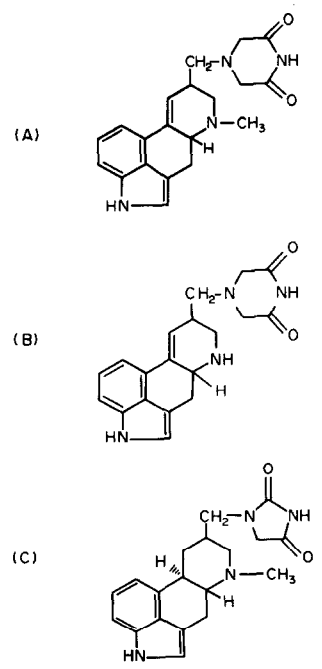

Figure 1
Structural formulae of (A) FCE 23884, (B) FCE 26506 and (C) FCE 22716.

(Milan, Italy), except *n*-octanol which was from Merck (Darmstadt, Germany).

Heparinized blank human plasma used to prepare spiked samples was purchased from AVIS (Milan, Italy).

Stock solutions were prepared by dissolving a weighed amount of each compound in 10 mM H_3PO_4. From these solutions (stable for at least 1 month if stored at 4°C in the dark) working solutions were prepared daily by dilution with 1 mM H_3PO_4.

Borate buffer (pH 8.5) was prepared according to the formula of Sörensen. A 12.404 g mass of H_3BO_3 was dissolved in 100 ml of 1 M NaOH and diluted to 1 l with water; 6.5 ml of this borate solution was then mixed with 3.5 ml of 0.1 M HCl.

All glassware was silanized before use by treatment with a 7% v/v solution of dimethyldichlorosilane in toluene followed by double rinsing with absolute ethanol and chloroform.

Equipment

The HPLC system comprised an Isochrom LC pump (Spectra Physics, Santa Clara, CA, USA) equipped with a Rheodyne Model 7125 sampling valve with a 200-μl loop, a model 821-FP fluorescence detector (Jasco, Hachioji, Japan) and a Chromjet recorder–integrator (Spectra Physics). A 1 V signal was sent from the detector to the integrator.

Chromatographic conditions

The chromatographic separation was performed by a 5-μm Hypersil ODS (Shandon) reversed-phase column (250 × 4.6 mm i.d.) with a Survival precolumn (70 × 2 mm i.d.) packed with 30–50 μm Pellicular ODS (Whatman).

The mobile phase was acetonitrile–0.05 M KH_2PO_4 (adjusted to pH 3 with 1 M H_3PO_4) (20:80, v/v).

The flow rate was set at 1.0 ml min^{-1} and the column was kept at room temperature (22 ± 1°C).

In order to optimize the fluorescent response of the three compounds, the excitation wavelength was automatically changed from 280 to 321 nm during the run (i.e. after the elution of the I.S.). However, since the emission characteristics of the three analytes are similar, it was possible to use the same emission wavelength (413 nm) for all three.

Extraction procedure

To 1 ml of plasma, 0.1 ml of working I.S. solution containing 14.70 ng of FCE 22716 was added and the resulting solution was placed in a 10-ml conical glass centrifuge tube. After the addition of 1 ml of borate buffer (pH 8.5) and 3 ml of ethyl ether–*n*-octanol (9:1, v/v) the tube was capped and immediately shaken using a rotary mixer for 1 min then centrifuged at 2000*g* for 5 min in order to clearly separate the two phases. The upper organic phase was transferred to another tube and the extraction step was repeated. The combined organic phases were extracted with 0.3 ml of 10 mM phosphoric acid by vortex-mixing for 1 min. After centrifugation as described above, the organic phase was discarded and the aqueous phase was washed twice with 1 ml of *n*-hexane by vortex-mixing. Finally the tube was centrifuged and the *n*-hexane removed. An aliquot (200 μl) of the aqueous solution was injected into the column. (Representative chromatograms are shown in Fig. 4.)

Determination of calibration and quality control samples

Analyses of blank plasma spiked with known amounts of FCE 23884, FCE 26506 and I.S. were carried out using the described method. Calibration curves in plasma were obtained in

the range 0.05–100 ng ml^{-1} for both FCE 23884 and FCE 26506. The precision and accuracy of the method were determined on different days by repeated analyses of spiked plasma samples in the whole concentration range of the calibration curves. All chromatograms obtained were evaluated by peak-area measurement. To increase the fit at low concentration, a weighted least squares linear regression equation (weighting factor = $1/y^2$) was used.

Chromatographic system suitability test

Before analysis of the biological samples the performance of the chromatographic system was monitored according to the recommendations of USP XXII (p. 1566). Four parameters were evaluated from the chromatograms obtained after injection of a standard solution of the three compounds.

System reproducibility. This chromatographic parameter was determined by five consecutive injections of a standard sample containing approximately 25 ng ml^{-1} of each compound. Their responses must give a relative standard deviation (RSD) not exceeding 5%.

Column efficiency. This was evaluated as the number (N) of theoretical plates of the column calculated by the equation $N = 5.54 (R_t/W_{0.5})^2$, where R_t is the retention time expressed in millimetres of the compound of interest and $W_{0.5}$ is the peak width, measured in millimetres at half peak height. The value of N must be at least 3500.

Peak symmetry. This was evaluated as the tailing factor T, calculated by the equation $T = W_{0.05}/2A$, where $W_{0.05}$ is the peak width at 5% peak height and A is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% peak height. T must be less than 1.3.

Resolution factor. The resolution factor (R_s) between the peak of FCE 23884 (b) and that of FCE 26506 (a) was calculated by the equation $R_s = 1.18 (R_{tb} - R_{ta})/W_{0.5a} + W_{0.5b}$, where R_{ta} and R_{tb} are the retention times, and $W_{0.5a}$ and $W_{0.5b}$ are the peak bandwidths (in mm) of the two compounds measured at half peak height. R_s must be higher than 1.0.

Results and Discussion

Under the chromatographic conditions used for the analysis, FCE 23884, FCE 26506 and FCE 22716 (I.S.) gave sharp peaks with retention times of about 10.9, 9.9 and 7.1 min, respectively (Fig. 2).

The possibility of changing the excitation wavelength during the analysis enabled optimization of the detection conditions of the structurally related compound FCE 22716 which could be advantageously used as I.S. thus giving the assay very good reproducibility.

The overall specificity of the assay, resulting from the combination of the extraction step and the selectivity of both chromatographic separation and fluorescence detection, enabled chromatograms free of interference to be obtained in the assay of blank rat, mouse, dog, monkey and human plasma samples (Figs 3–4). Plasma samples were then collected during a preliminary study on rats to which the test compound (2.5–22.5 mg kg^{-1}, p.o., single dose) was administered for the evaluation of the suitability of the analytical method in actual biological samples (results not shown). Another clearly drug-related peak (retention time identical to that of FCE 26506) appeared in plasma samples of treated animals and could be adequately separated from the parent drug peak and suitably quantitated. Although further studies are necessary to define un-

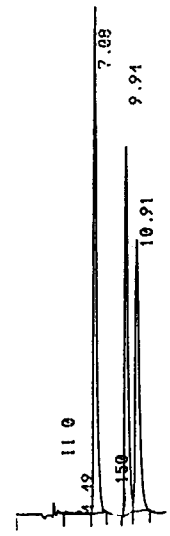

Figure 2
Chromatogram (HPLC) of a standard solution containing: 28.40 ng ml^{-1} of FCE 22716 (R_t = 7.08 min), 7.42 ng ml^{-1} of FCE 26506 (R_t = 9.91 min) and 6.93 ng ml^{-1} of FCE 23884 (R_t = 10.91 min).

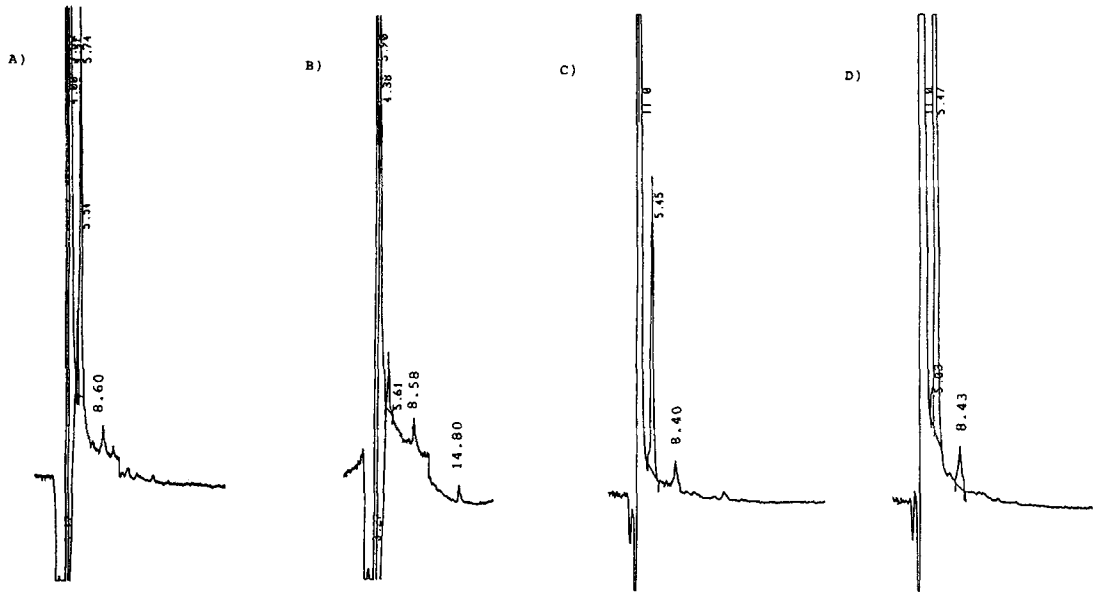

Figure 3
Typical chromatograms from 1 ml of blank plasma: (A) dog, (B) monkey, (C) mouse and (D) rat.

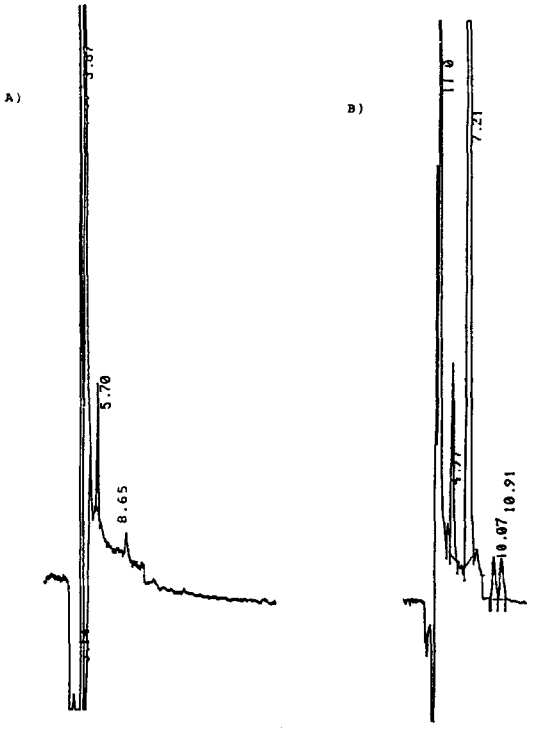

Figure 4
Chromatograms (HPLC) from 1 ml of blank human plasma (trace A) and blank human plasma spiked with 0.05 ng of FCE 23884 (R_t = 10.91 min) and FCE 26506 (R_t = 10.07 min) and 14.07 ng of FCE 22716 (R_t = 7.21 min) (trace B).

equivocally the chemical identity of this compound, there is strong evidence for attributing to it the structure of FCE 26506.

The linearity for the two compounds of interest was evaluated from calibration curves carried out on different days by plotting the area ratio (analyte peak area: I.S. peak area) versus the concentration ratio (analyte conc.: I.S. conc.) and analysing the data by applying a weighted ($1/y^2$) least squares linear regression equation. The mean ±SD parameters of the slope of the calibration curve obtained were: 5.094 ± 0.208 (RSD = 4.07%), $r > 0.9900$ ($n = 6$) for FCE 23884 and 4.738 ± 0.145 (RSD = 3.06%), $r > 0.9925$ ($n = 3$) for FCE 26506 (Tables 1 and 2). When analysed by Student's *t*-test, the intercept values were not significantly different from zero ($P > 0.05$).

The precision of replicate determinations of FCE 23884 and FCE 26506 was evaluated at five concentrations. Inter-day precision expressed as RSD ranged from 2.17 to 9.34% ($n = 18$) for concentrations of 0.07–81.72 ng ml^{-1} (FCE 23884) and from 1.16 to 11.52% ($n = 9$) for concentrations of 0.067–77.76 ng ml^{-1} (FCE 26506). Accuracy evaluated on the same samples and expressed as mean % found/added ±SD was 102.55 ± 8.20% for FCE 23884 and 98.45 ± 8.45% for FCE 26506. The results obtained on each day are reported in

Table 1
Calculated values for FCE 23884 in plasma

	Spiked sample (ng ml^{-1})						Regression estimates*		
Validation day	0.051	0.102	0.225	2.55	34.05	102.15	*m*	*b*	*r*
1	0.046	0.092	0.227	2.60	36.67	111.28	4.921	0.0	0.9944
2	0.050	0.083	0.256	2.69	35.78	107.43	4.900	0.0	0.9900
3	0.047	0.089	0.255	2.71	33.84	106.93	4.912	−0.001	0.9952
4	0.051	0.087	0.262	2.54	34.70	110.36	5.248	0.002	0.9930
5	0.044	0.100	0.248	2.43	38.98	99.01	5.211	0.001	0.9971
6	0.044	0.108	0.243	2.33	36.94	105.40	5.372	0.001	0.9977
Mean	0.047	0.093	0.248	2.55	36.15	106.74	5.094	0.0005	0.9946
SD	0.003	0.009	0.012	0.15	1.82	4.38	0.208	0.001	
RSD %	6.31	9.92	5.01	5.83	5.02	4.10	4.07		

*Estimates from weighted regression (weighting factor $1/y^2$): m = slope; b = intercept; and r = correlation coefficient.

Table 2
Calculated values for FCE 26506 in plasma

	Spiked sample (ng ml^{-1})						Regression estimates*		
Validation day	0.049	0.097	0.243	2.43	32.40	97.20	*m*	*b*	*r*
1	0.044	0.102	0.277	2.45	30.70	96.85	4.902	0.001	0.9985
2	0.041	0.119	0.238	2.33	35.89	91.85	4.626	0.005	0.9958
3	0.033	0.118	0.238	2.15	34.42	97.35	4.686	−0.001	0.9925
Mean	0.039	0.113	0.251	2.31	33.67	95.35	4.738	0.002	0.9956
SD	0.006	0.010	0.023	0.15	2.68	3.04	0.145	0.003	
RSD%	14.46	8.44	8.97	6.54	7.94	3.19	3.06		

*Estimates from weighted regression (weighting factor $1/y^2$): m = slope; b = intercept; and r = correlation coefficient.

Tables 3 and 4. The mean extraction recovery from plasma evaluated by comparison of the peak area obtained after extraction against the peak area obtained after direct injection of unextracted standard solution was 77.04% for FCE 23884, 66.88% for FCE 26506 and 60.66% for FCE 22716 (Table 5). The lower limit of quantification for both compounds was set at 0.05 ng ml^{-1}; at this concentration the signal-to-noise ratio was >5 and the RSD determined for replicated analyses was <15% for both compounds.

The method was applied to the determination of FCE 23884 plasma levels following a single oral dose of 1 mg in a first study aimed at evaluating the tolerability and preliminary pharmacokinetics and pharmacodynamics of the compound in healthy male volunteers. Blood was collected at 0, 1, 2, 4, 6, 8, 24 and 48 h after administration of a 1 mg dose to seven subjects under fasting conditions and the plasma levels were assayed. The curve of mean FCE 23884 plasma levels against time is shown in Fig. 5. The compound was rapidly absorbed and a mean peak plasma concentration of about 2.5 ng ml^{-1} was obtained within 1 h after administration. Plasma levels declined rapidly thereafter but were still measurable 48 h after administration.

FCE 26506 plasma levels were under the detection limit of the method at all tested times.

The analytical method described proved to be sensitive, specific, reproducible, precise, accurate and suitable for the analysis of plasma samples in clinical trials with the test compound.

Conclusions

Fluorescence detection coupled with HPLC has been used previously for the determination of ergot derivatives [6]. In the case of these compounds, however, sufficient sensitivity was not always achieved to enable the investigators to use chromatographic methods for their determination in biological fluids after administration of therapeutic doses in humans

Table 3
Accuracy and precision of the method for the determination of FCE 23884 in plasma

Spiked sample (ng ml^{-1})	Day	n	Accuracy: Mean found (ng ml^{-1})	Accuracy: % Found/added	Accuracy: SD	Precision: RSD % (intra-day)	Precision: Pooled RSD % (inter-day, n = 18)
0.070	1	3	0.081	116.15	0.009	11.13	
	2	3	0.071	100.85	0.010	13.56	
	3	3	0.081	115.67	0.002	2.66	
	4	3	0.060	86.37	0.010	16.96	
	5	3	0.066	94.62	0.005	7.84	
	6	3	0.074	106.15	0.003	3.92	9.34
0.204	1	3	0.215	105.49	0.017	7.71	
	2	3	0.211	103.48	0.009	4.37	
	3	3	0.203	99.27	0.003	1.60	
	4	3	0.191	93.77	0.006	3.16	
	5	3	0.205	100.29	0.013	6.28	
	6	3	0.194	95.09	0.012	6.05	4.77
2.04	1	3	2.21	108.26	0.05	2.24	
	2	3	2.05	100.62	0.02	1.21	
	3	3	2.06	101.11	0.05	2.19	
	4	3	1.92	94.07	0.06	3.33	
	5	3	2.03	99.46	0.07	3.36	
	6	3	1.91	93.71	0.01	0.70	2.17
27.24	1	3	29.99	110.08	0.89	2.96	
	2	3	27.84	102.19	0.22	0.79	
	3	3	28.82	105.81	1.65	5.74	
	4	3	28.23	103.63	1.79	6.34	
	5	3	28.67	105.26	1.48	5.16	
	6	3	29.21	107.24	0.38	1.29	3.71
81.72	1	3	88.84	108.22	1.36	1.54	
	2	3	85.48	104.60	0.39	0.46	
	3	3	89.51	109.53	1.08	1.20	
	4	3	84.03	102.83	4.25	5.06	
	5	3	86.31	105.62	5.74	6.65	
	6	3	79.22	96.94	3.70	4.67	3.26

Table 4
Accuracy and precision of the method for the determination of FCE 26506 in plasma

Spiked sample (ng ml^{-1})	Day	n	Accuracy: Mean found (ng ml^{-1})	Accuracy: % Found/added	Accuracy: SD	Precision: RSD % (intra-day)	Precision: Pooled RSD % (inter-day, n = 9)
0.067	1	3	0.061	91.15	0.007	10.92	
	2	3	0.068	102.16	0.011	15.63	
	3	3	0.075	111.45	0.006	8.01	11.52
0.194	1	3	0.196	100.97	0.016	8.32	
	2	3	0.192	98.79	0.027	14.00	
	3	3	0.190	98.01	0.012	6.22	9.51
1.94	1	3	1.77	91.03	0.03	1.93	
	2	3	1.83	94.28	0.01	0.69	
	3	3	1.81	93.27	0.02	0.85	1.16
25.92	1	3	25.55	98.57	1.58	6.20	
	2	3	26.58	102.53	1.89	7.10	
	3	3	27.22	105.03	0.28	1.04	4.78
77.76	1	3	73.88	95.02	4.14	5.60	
	2	3	78.06	100.39	3.70	4.75	
	3	3	73.06	93.96	4.64	6.35	5.57

Table 5
Absolute extraction recovery of the three analytes from plasma

Analyte	Label conc. in plasma (ng ml^{-1})	n	% Absolute recovery ±RSD %
FCE 23884	0.07	3	72.05 ± 7.80
	0.20	3	82.79 ± 8.20
	2.04	3	69.20 ± 8.17
	27.24	3	81.35 ± 1.60
	81.72	3	79.81 ± 1.20
FCE 26506	0.07	3	65.15 ± 10.70
	0.19	3	68.14 ± 8.00
	25.92	3	67.88 ± 2.30
	77.76	3	66.36 ± 3.00
FCE 22716	11.76	15	60.66 ± 6.00

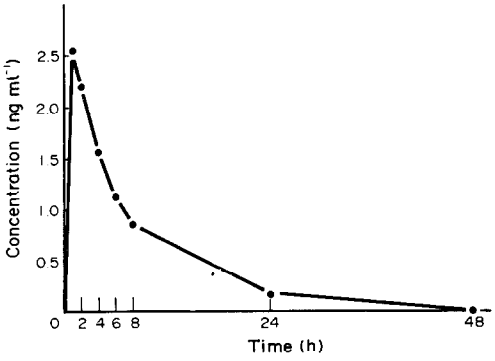

Figure 5
Mean plasma levels of FCE 23884 in man (n = 7) after a 1 mg single oral dose of the test compound.

[7]. Thus more sensitive but less specific RIA methods were employed for the ergot derivatives in biological fluids [8–11] and consequently pharmacokinetic data suffered from the imprecision of less specific analytical methods.

In the case of FCE 23884 the high quantum yield and the optimal chromatographic conditions used for analysis make it possible to quantitate the compound in plasma down to sensitivity levels typical of RIA methods with the specificity typical of the HPLC technique. This method will therefore be a very useful tool for investigation of the pharmacokinetics of the compound in further studies in humans.

Acknowledgements — The authors are thankful to Mrs S. Cozzi for typing the manuscript.

References

[1] A.N. Lieberman, G. Gopinathan, A. Neophytides, M. Leibowitz, R. Walker and E. Hiesiger, *Ann. Neur.* **13**, 44–47 (1983).
[2] M.L. Vance, W.S. Evans and M.O. Thorner, *Ann. Intern. Med.* **100**, 78–91 (1984).
[3] S.G. Diamond, C.H. Markham and L.J. Treciokas, *Neurology* **35**, 291–295 (1985).
[4] M. Buonamici, S. Mantegani, M.A. Cervini, R. Maj, A.C. Rossi, C. Caccia, N. Carfagna, P. Carminati and R.G. Fariello, *J. Pharmacol. Exp. Ther.* **259**, 345–355 (1991).
[5] N. Carfagna, C. Caccia, S. Mantegani, S. Cavanus, M.G. Fornaretto, M. Buonamici, A.C. Rossi, R. Roncucci and R.G. Fariello, *J. Pharmacol. Exp. Ther.* **259**, 356–364 (1991).
[6] P.O. Edlund, *J. Chromatogr.* **226**, 107–115 (1981).
[7] L. Zecca, L. Bonini and S.R. Bareggi, *J. Chromatogr.* **272**, 401–405 (1983).
[8] H.F. Schrau, H.J. Schwarz, K.C. Talbot and L.J. Loeffler, *Clin. Chem.* **25**, 1928–1933 (1979).
[9] M. Hümpel, B. Nieuweboer, S.H. Hasan and H. Wendt, *Eur. J. Clin. Pharmacol.* **20**, 47–51 (1981).
[10] W. Krause, R. Dorow, B. Nieuweboer and S.H. Hasan, *Eur. J. Clin. Pharmacol.* **27**, 335–339 (1984).
[11] S. Persiani, E. Pianezzola, F. Broutin, G. Fonte and M. Strolin Benedetti, *J. Immunoassay* **13**, 457–476 (1992).

[*Received for review 4 August 1992; revised manuscript received 26 October 1992*]