Journal of Chromatography, 532 (1990) 351–361
Biomedical Applications
Elsevier Science Publishers B.V , Amsterdam

CHROMBIO. 5456

Determination of amineptine and its main metabolite in plasma by high-performance liquid chromatography after solid-phase extraction

POK PHAK ROP, J. SPINAZZOLA and M. BRESSON

Laboratoire Interrégional de Police Scientifique, 2 Rue du Commissaire Becker, 13224 Marseille Cédex 2 (France)

T CONQUY

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

and

A VIALA*

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

(First received March 14th, 1990; revised manuscript received May 21st, 1990)

ABSTRACT

Amineptine and its main metabolite were determined simultaneously in plasma by high-performance liquid chromatography using quinupramine as internal standard The method comprised adsorption on Extrelut® column from alkaline plasma, elution with diethyl ether–methylene chloride, evaporation in the presence of 0.01 *M* hydrochloric acid and injection of the acid solution onto a μBondapak C_{18} column, using acetonitrile–0 025 *M* potassium dihydrogenphosphate as mobile phase and ultraviolet detection at 210 nm. Average steady-state concentrations of the two compounds were determined in four patients under treatment regimen (two 100-mg doses of amineptine per day, at 8.00 and 12.00 h) The concentrations determined 20 h after the last dose were undetectable in all cases, whereas the concentrations determined 1 h after the second dose were found to be 780 ± 96 ng ml^{-1} for amineptine and 690 ± 137 ng ml^{-1} for its metabolite. The technique can also be applied to whole blood with, if necessary, identification on the basis of the ultraviolet spectrum obtained by photodiode-array detection.

INTRODUCTION

Amineptine (A), dihydro-10,11-dibenzo[*a*,*d*]cycloheptenyl-5-amino-7-heptanoic acid (Fig. 1), is a potent dopaminergic antidepressant [1] characterized by a stimulant clinical effect [2,3]. It is mainly metabolized by β-oxidation of the aliphatic chain with the loss of two CH_2 units [4]. Its metabolite (AM) (Fig. 1) shows also dopaminergic antidepressant activity [2]. In human plasma, both AM and A have been found. Conso and Garnier [5] demonstrated that the safety

0378-4347/90/$03.50

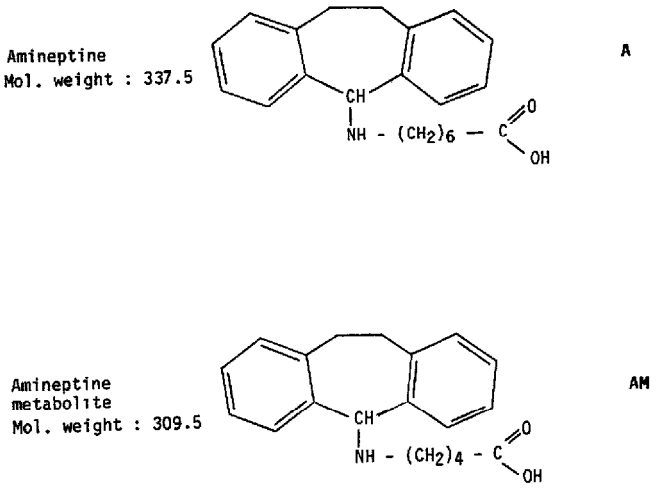

Fig. 1. Structures of amineptine (A) and its main metabolite (AM)

margin of A was very high, and no toxic signs were observed following ingestion of single doses of 4000 mg or higher.

Only a few methods using gas chromatography–mass spectrometry or liquid chromatography [4,6–9] have been described for the determination of A and AM in biological samples. Some pharmacokinetic parameters of the two compounds have also been established [6,9,10], but there was no obvious relation between plasma concentration and clinical activity or toxicity.

The kinetics of A is very rapid [6,9,10]. According to Lachâtre *et al.* [10], 10 h after the administration of a single 100-mg oral dose in tablet form to twelve young healthy adults, neither the drug nor its metabolite was detectable in plasma of any subject by the employed technique, the determination limit of which was 10 ng ml^{-1}. However, the peak plasma levels of A and AM were 772 ± 620 and 471 ± 281 ng ml^{-1}, respectively, at 1 and 1.50 h in the same subjects. According to Sbarra *et al.* [6] the peak blood concentrations of the compounds were 750 ± 150 and 1020 ± 170 ng ml^{-1} at 1 and 1.60 h for A and AM, respectively, after the administration of a single 200-mg oral dose (tablet form) to six volunteers.

This paper describes a high-performance liquid chromatographic (HPLC) procedure for the simultaneous determination of A and AM, in plasma and even in whole blood, with respect to clinical pharmacy.

EXPERIMENTAL

Reagents and glassware

All reagents were of analytical grade. Potassium dihydrogenphosphate (Normapur), sodium monohydrogencarbonate (Normapur) and hydrochloric acid

(Normapur) were obtained from Prolabo (Paris, France). Methanol (RS = Reagente Speciale), diethyl ether (RS), methylene chloride (RS), hexane (RS) acetonitrile (RS for HPLC) were from Carlo Erba (Milan, Italy). Extrelut® cartridges (3 ml) were from Merck (Nogent sur Marne, France).

All glassware was first washed with a 3% "RBS 25 biodegradable" alkaline solution (Biolyon, Dardilly, France), which contains a mixture of anionic and non-ionic detergents, and was then rinsed with distilled water and dried before use.

Standards

The standard compounds, A · HCl and AM, were supplied by Servier Labs. (Paris, France). Quinupramine · HCl (internal standard) was supplied by Pharmuka Labs. (Gennevilliers, France). Stock solutions of A, AM and quinupramine · HCl were prepared in methanol at a concentration of 1 $\mu g\ \mu l^{-1}$ and could be stored at 4°C for at least one month. The stock solutions were diluted to 100, 10, 1 and 0.1 ng μl^{-1} with methanol for the preparation of calibration standards.

Sample preparation

A 1-ml volume of plasma was pipetted into a 5-ml glass tube. A 50-μl volume of methanolic solution of quinupramine (internal standard; 10 ng μl^{-1}) was added and the solution was made up to 3 ml with a saturated aqueous sodium monohydrogencarbonate solution. After vortexing for 1 min, the mixture was transferred onto a 3-ml Extrelut cartridge. Elution was then carried out with 15 ml of diethyl ether–methylene chloride (70:30, v/v). The eluate was evaporated in the presence of 50 μl of 0.01 *M* hydrochloric acid under a stream of air in a 40°C water-bath. The acid solution[a] was washed by vortexing with 2 ml of hexane for 20 s. After centrifugation at 2800 *g* for 5 min, the hexane layer was removed and discarded. A 20-μl aliquot of the acid extract was injected into the chromatograph.

Apparatus and chromatographic parameters

Chromatographic analysis was performed on a Waters-Millipore (Saint-Quentin-en-Yvelines, France) system consisting of an M510 pump, a μBondapak C_{18} column (30 cm × 3.9 mm I.D., particle size 10 μm, ambient temperature) connected to a μBondapak C_{18}™ guard column (5 mm × 6 mm I.D.; two 2-μm filters, one at either end, holding the packing in place), an M481 multi-wavelength detector and an M380 control station. A 20-μl high-pressure loop injector (M7125 Rheodyne) was used to introduce the sample.

The mobile phase was a mixture of acetonitrile–0.025 *M* potassium dihydrogenphosphate (45:55, v/v) at a flow-rate of 0.8 ml min^{-1}. The detection wave-

[a] For whole blood analysis the acid extract must be purified (see *Applications*).

length was set at 210 nm. In addition, an M 990 photodiode-array detector was used to obtain spectra (between 200 and 350 nm) of the compounds eluted.

Calculation

The ratios of the peak heights of the compounds analyzed to the peak heights of the internal standard were calculated and plotted against the concentrations of the drugs tested after analysis of blank plasma spiked, respectively, with increasing concentrations (50, 100, 200, 500, 1000 and 2000 ng ml^{-1}) of A and AM and a constant amount (500 ng) of internal standard. In this concentration range a linear relationship for the two compounds was found. For lower concentrations (4, 8, 15, 30, 40 and 100 ng ml^{-1}) a lower amount of internal standard must be added, *e.g.* 50 ng. Under these conditions, a linear relationship was also observed.

The equations of the regression lines and their correlation coefficients (r) were as follows: 50–2000 ng ml^{-1} (internal standard, 500 ng): $y = 0.0040\,x - 0.0104$, $r = 0.997$ for A and $y = 0.0042\,x - 0.083$, $r = 0.997$ for AM; 4–100 ng ml^{-1} (internal standard, 50 ng): $y = 0.0038\,x - 0\,023$, $r = 0.996$ for A and $y = 0.0042\,x + 0.048$, $r = 0.996$ for AM, where y is the compound/internal standard peak-height ratio and x is the quantity of spiked compound.

RESULTS AND DISCUSSION

Under the conditions described above the capacity factor, k', and the selectivity factor, α, (relative to quinupramine) of the compounds analyzed were, respectively, 1.47 and 0.64 for A, 0.93 and 0.41 for AM and 2.27 and 1 for quinupramine (internal standard).

The main spectral data obtained in the mobile phase were characterized by a weak absorption maximum at 266 nm for the two compounds ($\varepsilon = 709$ for A and 898 for AM) and a strong maximum below 220 nm The detection wavelength was set at 210 nm on the basis of the ratio of the peak heights of both compounds to the noise level.

Fig. 2A shows a chromatogram of a blank plasma extract, and Fig. 2B shows a chromatogram of the same plasma spiked with quinupramine (internal standard), amineptine and its metabolite.

Recovery

The recovery of A and AM (4, 20 and 100 ng ml^{-1}) was measured under the extraction conditions described. The internal standard (50 or 500 ng) was added to the eluate just before evaporation in the presence of 50 μl of 0.01 M hydrochloric acid. Peak-height ratios of the extracts were compared with those obtained by direct injection of the residue of the same methanolic standard solutions after evaporation and dissolution of each residue in 50 μl of 0.01 M hydrochloric acid. The recoveries were *ca.* 90% for A and 78% for AM (Table I).

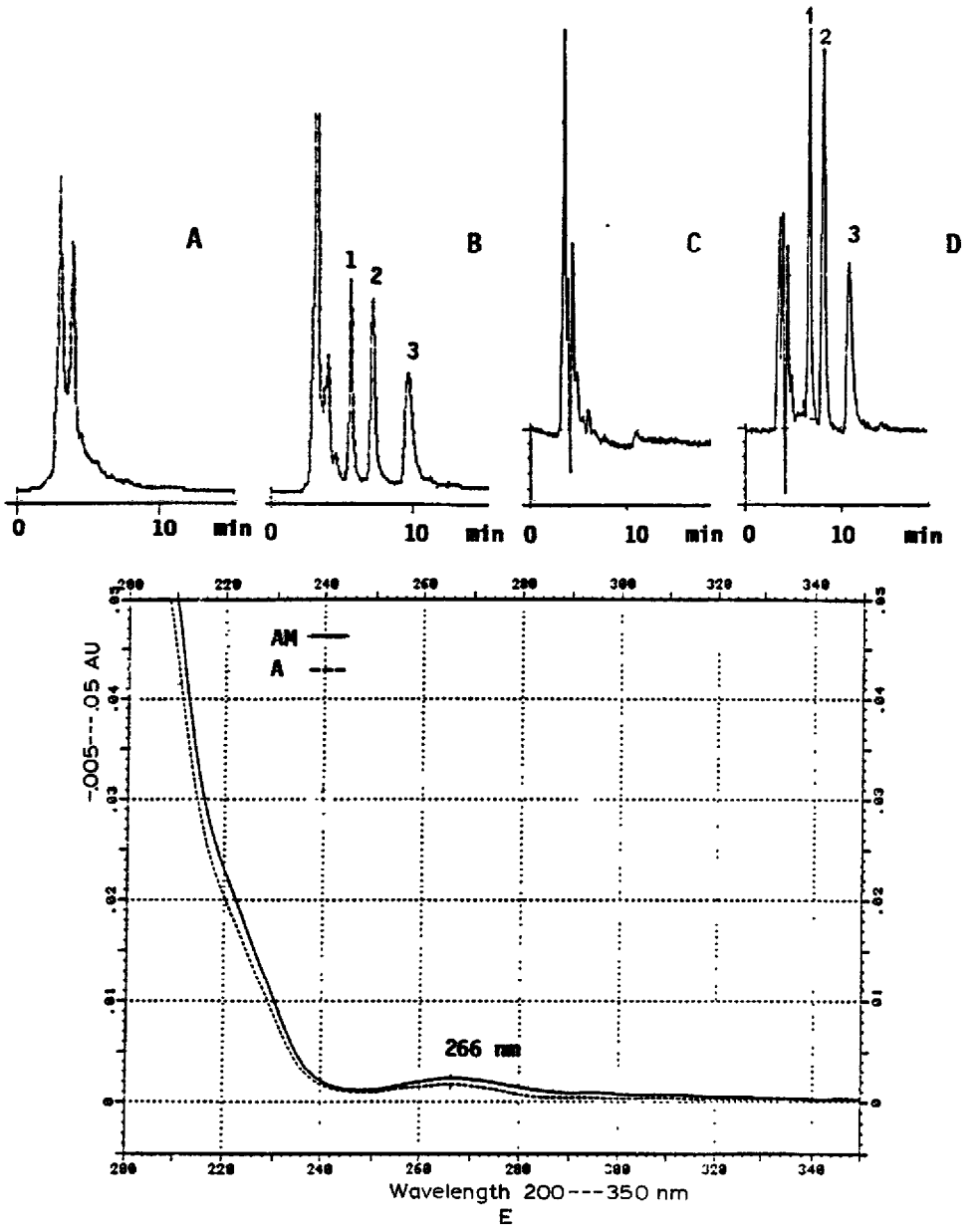

Fig. 2. (A) Chromatogram from a blank plasma (1 ml); M481 UV detector at 210 nm (B) Chromatogram from the same blank plasma (1 ml) spiked with 450 ng of AM (1) and A (2) and 500 ng of quinupramine (3) as internal standard; M481 UV detector at 210 nm. (C) Chromatogram from a blank whole blood (1 ml), M990 photodiode-array detector at 210 nm (D) Chromatogram from the same whole blood (1 ml) spiked with 570 ng of AM (1) and A (2) and 500 ng of quinupramine (3) as internal standard; M990 photodiode-array detector at 210 nm. (E) Spectra from the same whole blood extract; M990 photodiode-array detector (scanned wavelengths: 200–350 nm).

TABLE I

REPRODUCIBILITY AND RECOVERY

Drug	Added (ng ml^{-1})	Within-day		Day-to-day						Recovery (mean ± S D. n = 6) (%)
		Found (mean ± S.D., n = 9) (ng ml^{-1})	C.V. (%)	Found (ng ml^{-1})					C V (%)	
				Day 1	Day 7	Day 15	Day 30	Mean ± S.D , n = 10		
A	4	4 58 ± 0.54	11 80	4.30	3.95	3.05	3 90	3 80 ± 0 46	12.10	88.20 ± 5.70
	10	10 92 ± 0.76	6 95							90.80 ± 2 70
	20	19 60 ± 2.30	11.75	22.28	19.50	17 80	19.24	19 70 ± 1.62	8.23	
	100	100 90 ± 4.32	4.30							90 25 ± 3 10
AM	4	4 15 ± 0.50	12.05	4.40	3 85	4 40	3 20	3 96 ± 0 47	12.50	75 20 ± 6 70
	10	10 10 ± 1.06	10.50							78 50 ± 7 20
	20	21 50 ± 2.25	10.46	20 80	18 70	22 80	19 25	20 38 ± 1 60	7.80	
	100	100 55 ± 3.36	3.34							78 40 ± 7 50

Reproducibility

The within-day reproducibility was determined using a pool of blank plasma samples spiked with 4, 10, 30 and 100 ng ml^{-1} of each compound. Coefficients of variation (C.V.) obtained on the same day for A and AM were less than 11.80 and 12.05%, respectively (Table I).

The day-to-day coefficients of variation for concentrations of 4 and 20 ng ml^{-1} were less than 12.10% for A and 12.50% for AM (Table I).

Quantitative aspects

The lower limit of detection at 210 nm with the M481 UV–visible detector was found to be 0.8 ng (injected directly in the chromatograph). This amount gave a signal-to-noise ratio of 3 for A and 4 for AM at $5 \cdot 10^{-3}$ a.u.f.s., but in plasma (1 ml), after spiking with 0.8 ng of A or AM, no peak was observed. The determination limit with the same detector was found to be 4 ng for the two compounds in a 1-ml sample (signal-to-noise ratio of 3 for A and 4 for AM). If 2 ml of plasma were used it was possible to determine a concentration of 2 ng ml^{-1}. With the M990 photodiode-array detector, the determination limit was less than 50 ng ml^{-1} for A and AM.

Selectivity

The chromatogram of 1 ml of drug-free plasma from healthy subjects showed no background interference from endogenous constituents (Fig. 2A and C). Several drugs were also tested for possible interference (Table II). Bromazepam, carbamazepine, lorazepam, chlordiazepoxide, estazolam and temazepam might interfere with either A, AM or quinupramine (internal standard). Bromazepam, lorazepam and temazepam were eluted close to AM, A and quinupramine, respectively. However, under the extraction conditions described only very small amounts of these three compounds remained in the acid extract after washing with hexane.

Applications

Therapeutic application. Four patients, between 45 and 60 years (Table III), were treated for a period of at least one week with A · HCl (Survector) under standard regimen (two 100-mg doses per day at 8.00 and 12.00 h). A benzodiazepine (which did not interfere with the analysis) was also given in case a sedative was needed. Between the 7th and the 23rd day, when the plasma concentration was considered in the steady state, blood samples (on two successive days) were drawn from each patient at 8 h, just before the morning dose (20 h after the last ingestion), for determining the C_{20} concentration and at 13 h (1 h after the 12.00 h ingestion) for determining the C_1 concentration of the two compounds. After centrifugation plasma was analyzed as described above.

The results obtained are shown in Table III. No C_{20} concentration of A and AM was detectable (Fig. 3A) due to their short half-lifes: 0.8 and 2.5 h, respec-

TABLE II

CAPACITY FACTORS AND SELECTIVITY COEFFICIENT OF ANALYZED COMPOUNDS AND SOME DRUGS TESTED FOR POSSIBLE INTERFERENCE (AFTER INJECTION OF THEIR 0.01 *M* HCl SOLUTION)

Drug	Capacity factor (k')	Selective factor (α) relative to quinupramine (internal standard)
Caffeine	0.06	0.03
Amphetamine	0 50	0 22
Methylamphetamine	0 67	0.29
Amineptine metabolite[a]	0 93	0 41
Bromazepam	1.03	0 45
Carbamazepine	1 03	0 45
Dihydroergotamine	1 13	0.50
Norclobazam	1.17	0 51
Nitrazepam	1.20	0.53
Oxazepam	1 23	0.54
Clonazepam	1 27	0.56
Lorazepam	1.30	0.57
Amineptine[a]	1.47	0.64
Chlordiazepoxide	1 70	0.75
Estazolam	1 70	0 75
Opipramol	1 73	0.76
Doxepine	1.73	0.76
Flunitrazepam	1 73	0.76
Demexiptyline	1 83	0.80
Mianserine	1.87	0.82
Nordiazepam	2	0.88
Triazolam	2	0.88
Alprazolam	2.03	0.89
Nortriptyline	2.03	0.89
Desipramine	2 07	0.91
Clobazam	2 07	0.91
Temazepam	2 20	0.97
Quinupramine (internal standard)[a]	2.27	1
Imipramine	2 67	1.17
Amitriptyline	2 83	1.24
Alimemazine	2 87	1.26
Diazepam	2.90	1 28
Desmethylclomipramine	3 30	1.45
Demoxepine	3 37	1.48
Trimipramine	3 40	1.50
Carpipramine	3 50	1.54
Clomipramine	3 70	1.63
Clotiazepam	3 83	1.69
Tetrazepam	4.50	1 99
Medazepam	5	2.20
Prazepam	5 17	2.28

[a] Analyzed compound.

TABLE III

C_{20} AND C_1 CONCENTRATIONS OF A AND AM

Patient No.	Sex	Daily dose		Days of sampling	Concentration (ng ml^{-1})					
		mg	mg kg^{-1}		C_{20}			C_1		
					A	AM	A + AM	A	AM	A + AM
1	F	200	4.20	22–23	N D.[a]	N.D.	N.D	850	727	1577
2	F	–	3.80	15–16	N D	N.D.	N D	855	864	1719
3	M	–	3.20	10–11	N D.	N.D	N D	660	626	1276
4	M	–	2.90	7–8	N.D.	N.D	N D	762	545	1307
Mean ± S.D		200	3 52 ± 0 6		N.D.	N D	N D	780 ± 96	690 ± 137	1470 ± 214

[a] No response

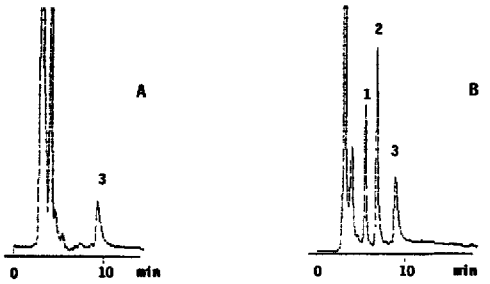

Fig. 3. Chromatograms from plasma samples of patient No 4 treated with amineptine hydrochloride (Survector) for seven days (two 100-mg doses per day). (A) C_{20} concentrations: A (2) and AM (1) were not detected, internal standard (3) at low range (50 ng), M481 UV detector at 210 nm (B) C_1 concentrations: A (2) = 762 ng ml^{-1}; AM (1) = 545 ng ml^{-1}, internal standard (3) at high range (500 ng); M481 UV detector at 210 nm.

tively [10]. Nevertheless the C_1 concentration was 660–855 ng ml^{-1} (mean ± S.D., 780 ± 96 ng ml^{-1}) for A and 545–864 ng ml^{-1} (mean ± S.D., 690 ± 137 ng ml^{-1}) for AM (Fig. 3B).

For monitoring the average steady-state level during antidepressant treatment, the measurement of the residual concentration has often been proposed. This is, however, not possible for A. Nevertheless, considering the fact that treatment with two 100-mg doses per day (100 mg at 8.00 h, 100 mg at 12.00 h) is an active posology and that there is no clear relation between plasma levels of several antidepressants and the therapeutic activity, sampling 1 h after the administration of A could be proposed as a method for the therapeutic monitoring of this drug. This study shows no accumulation of A or AM in the patients treated with A · HCl, even with a dosage regimen of two 100-mg doses per day at 8.00 and 12.00 for 7–23 days.

Whole blood analysis. If plasma is not available, whole blood analysis is also possible. A 1-ml volume of whole blood is first extracted according to the prior technique. After washing with hexane, the acid extract is made up to 3 ml with the saturated sodium monohydrogencarbonate solution, then transferred again onto an Extrelut cartridge and eluted as described above. After evaporation of the solvent, the residue is dissolved in 50 μl of mobile phase, and 20 μl of this solution are injected into the HPLC system. The M990 photodiode-array detector (Waters) can be used to scan and identify the spectral data of the drug and its metabolite (Fig. 2C, D and E). Quantitation is achieved by comparing the peak-height ratios of drug to internal standard with those from a drug-free whole blood sample spiked with amineptine, its metabolite and internal standard and carried out under the same conditions.

ACKNOWLEDGEMENTS

The authors express their gratitude to Servier Labs. (Paris, France) and to Dr. J. L. Girard and Dr. B. Fossat of E. Toulouse Hospital (Marseilles, France) for providing the standard compounds and the plasma samples, respectively.

REFERENCES

1 R. Samanin, A. Jori, S Bernasconi, E Morpugo and S Garattini, *J Pharm. Pharmacol.*, 20 (1977) 555
2 J. C Poignant, *Encéphale,* 5 (1979) 709.
3 P. Deniker, *Prat Méd*, 36 (1988) 29.
4 C. Sbarra, P. Negrini and R Fanelli, *J Chromatogr*, 162 (1979) 31
5 F Conso and R. Garnier, *Dépression et Suicide,* Pergamon Press, Paris, 1981, p 417
6 C. Sbarra, M. G Castelli, A Noseda and R. Fanelli, *Eur J Drug Metab Pharmarmacokin*, 6 (1981) 123.
7 C Tsaconas, P Padieu, P d'Athis, E Mocaer and N Bromet, *J Chromatogr.*, 487 (1989) 313
8 G. Nicot, G Lachâtre, C. Gonnet, J P. Valette, L. Merle, Y Nouaille and N Bromet, *J Chromatogr*, 396 (1984) 279
9 G. Nicot, G Lachâtre, J P. Valette, L. Merle, Y. Nouaille, N Bromet and E Mocaer, *J Psychiatr. Biol. Thér.*, 11 (1984) 31
10 G. Lachâtre, C. Piva, C. Riche, D. Dumont, R Defrance, E. Mocaer and G Nicot, *Fundam. Clin. Pharmacol.*, 3 (1989) 19.