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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ASSESSMENT OF THE PHARMACOKINETICS OF AMINEPTINE AND ITS MAIN METABOLITE IN VOLUNTEERS WITH LIVER IMPAIRMENT

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SUMMARY

A pharmacokinetic study of amineptine (Survector) and its C_5 metabolite, resulting from a β-oxidation of the heptanoic acid side-chain, was undertaken with ten human volunteers, who received a single 100-mg tablet of amineptine orally. They were affected with liver impairment in order to determine if this situation would alter greatly the pharmacokinetic parameters. The internal standard was the octanoic acid homologue. Analyses were carried out by gas chromatography (GC) and GC–mass spectrometry using TMS ester derivatives. Plasma samples were extracted using a C_{18} reversed-phase cartridge at pH 4.0. Mass fragmentographic measurements on the plasma samples were performed on the *m/z* ions $(M+H)^+$ and (base peak)$^+$ using ammonia chemical ionization. The global evaluation of precision was good and the coherence between the two modes of measurements, (base peak)$^+$ and $(M+H)^+$ ions, gave a regression factor *r* close to unity. For amineptine the total body clearance and mean residence time were accurate and precise with eight volunteers, but only four volunteers showed such coherent data for the slope of the elimination curve, β, and half-life. However, the β value, half-life and mean residence time of the C_5 metabolite were accurate and precise with seven, eight and ten volunteers, respectively. It is concluded that the drug was still detoxified at normal levels.

INTRODUCTION

Amineptine, dihydro-10,11-dibenzo[*a,d*]cycloheptenyl-5-amino-7-heptanoic acid, synthetized in 1972 by Malen and Poignant [1], is a tricyclic antidepressant

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drug (TAD) introduced into human therapeutics in 1978. The molecule is composed of an ω-animo acid moiety linked via the ω-amino group to a tricyclic ring. Human biotransformation leads to several blood and urine metabolites [2,3], some of which have been identified. The most important one, corresponding to 60–90% of all metabolites found in blood, urine and cerebrospinal fluid, is dihydro-10,11-dibenzo[*a,d*]cycloheptenyl-5-amino-5-pentanoic acid, which is the result of β-oxidation at the carboxylic end of the C_7 side-chain of the parent drug to a C_5 carboxylic side-chain. In this paper wescribe a method involving the extraction of amineptine and this main C_5 metabolite from plasma samples and conversion to the trimethylsilyl (TMS) derivative for identification by gas chromatography–mass spectrometry (GC–MS) and assessment by mass fragmentography (MF). The internal standard (I.S.) was a higher homologue of amineptine, dihydro-10,11-dibenzo[*a,d*]cycloheptenyl-5-amino-8-octanoic acid. This method was developed to establish the pharmacokinetics of amineptine (Survector) and its C_5 metabolite in each adult volunteer of a group of ten. For each volunteer the data are correlated with the liver function impairment.

EXPERIMENTAL

Products and reagents

Amineptine (S1694), its C_5 metabolite (S7981) and the I.S. (S1716) were a gift from Laboratoires Servier (Suresnes, France). Organic solvents and other reagents were obtained from Merck (Darmstadt, F.R.G.), except methanol, which was supplied by Prolabo (Paris, France). N,O-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was purchased from Sigma (St. Louis, MO, U.S.A.). The cartridges of Sep-Pak C_{18}-bonded reversed phase were from Waters Assoc. (Milford, MA, U.S.A.).

Derivatization

Amineptine, its C_5 metabolite and the I.S. were derivatized to the TMS esters. A known amount of BSTFA (50 or 100 μl) was added to the dry residue resulting from the evaporation of the mixture of the three drugs or of the extract from 1–2 ml of plasma (see below) under a stream of dry nitrogen and heated in a sand-bath at 60 °C for 30 min. The derivatives were stable for at least two months when kept at 4 °C in the dark.

Gas chromatography

The conditions of derivatization and the GC method were established using a Packard Model 427 gas chromatograph (Packard-Becker, Delft, The Netherlands) equipped with a fused-silica capillary column (25 m × 0.35 mm I.D.) coated with a film of OV-1 of thickness 0.20 μm (Spiral, Couternon-Dijon, France). The carier gas was dry nitrogen at a flow-rate of 20 ml/min in GC alone and helium at the same flow-rate in GC–MS. The derivatized sample was injected by means of an all-glass solid injector [4] and monitored using a flame ionization detector. Plasma elimination curves were established by GC–MF using a Ribermag Model GC–MS R10-10C quadrupole mass spectrometer with a SIDAR 150 computer

system (Delsi-Nermag, Rueil-Malmaison, France) coupled to a Varian Model 3700 gas chromatograph (Varian, Orsay, France) equipped with the same capillary column. The GC–MS analyses were carried out in electron-impact (EI) and ammonia chemical ionization (NH_3-CI) modes, the latter being used for quantitative drug analysis.

Plasma extraction

Extraction was effected by liquid–solid chromatography at pH 4.0 using a Sep-Pak C_{18} cartridge [5]. A known volume of plasma (up to 2 ml) was diluted with 5 ml of 10 mmol/l sodium acetate–acetic acid buffer (pH 4.0) containing a known amount of I.S. After thorough mixing, the sample was centrifuged at 800 *g* for 10 min at 4 °C. The Sep-Pak cartridge was washed with 6 ml of methanol delivered with a glass syringe and then equilibrated with 6 ml of buffer. The centrifuged supernatant liquid was passed through the cartridge at a rate of 2 ml/min. The cartridge was then washed with the buffer, dried under a stream of nitrogen and finally extracted with 8 ml of methanol at the same flow-rate using a glass syringe. The eluted fractions were evaporated at 60 °C under a stream of dry nitrogen and the dry residues were taken up in BSTFA.

Method of pharmacokinetic study

A single dose of 100 mg of amineptine (Survector) in tablet form (half of the daily dose) was administered in the morning to ten adult volunteers after overnight fasting. The volunteers were fasted for 4 h after drug intake. Thirteen blood samples were taken starting just after ingestion at $t=0$ and then at $t=15$, 30 and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. The blood was collected without any anticoagulating agent, centrifuged at 4 °C and the decanted plasma samples were kept at −20 °C until extraction and analysis. The calculation of the amount of amineptine and C_5 metabolite was made from at least two analyses per sample after verifying the linearity of the response. The response factor, k_i, was calculated from the peak areas of the selected ions, the *m/z* of the base peak ion $(BP)^+$ and the *m/z* of each adduct ion $(M+H)^+$, using the NH_3-CI mode. Calibration graphs and response coefficients were calculated from plasma samples spiked with equal or various amounts of either amineptine or C_5 metabolite (0, 10, 50, 100, 250, 500, 1000, 2500 and 5000 ng) versus a constant amount of I.S. (500 ng). Constancy of the response factor, which depends on the stability of the mass spectrometer, was monitored regularly.

RESULTS

Gas chromatography

Fig. 1 shows that the TMS derivatives of the mixture of the three authentic compounds, amineptine, its C_5 metabolite and the I.S., are eluted as unique peaks from the OV-1 fused-silia capillary column. Using the gas chromatograph alone the retention times were 16.2, 21.4 and 24.2 min, respectively, at a carrier gas (nitrogen) flow-rate of 20 ml/min and a temperature gradient at 1 °C/min starting at 230 °C.

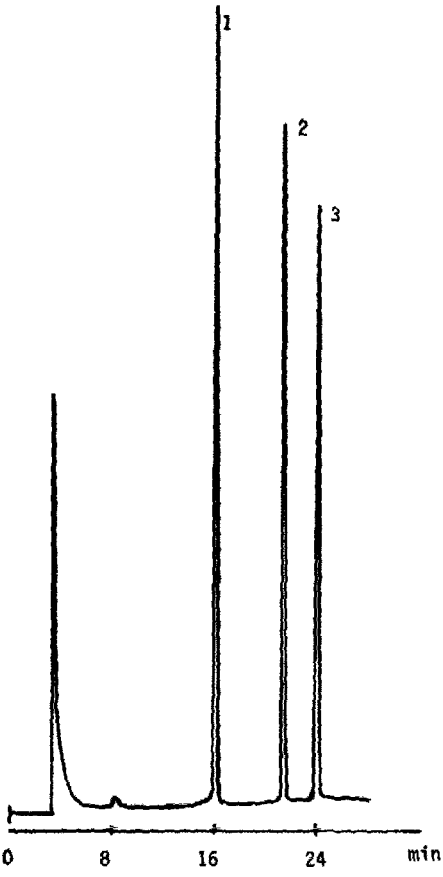

Fig. 1. Gas chromatogram of TMS derivatives of authentic amineptine (2), C_5 metabolite (1) and internal standard (I.S.) (3). For chromatographic conditions, see text.

Gas chromatography–mass spectrometry or mass fragmentography

Fig. 2 shows the principal mass fragments of amineptine and its homologues. Table I lists the main ions resulting from the two modes of operation, EI and NH_3-CI. In the EI mode, amineptine gives a molecular ion M^+ of $m/z=409$ with a low relative intensity ($RI=0.40\%$) at 35 eV and a source temperature of 240°C. This molecular ion is totally cleaved above 40 eV. In the NH_3-CI mode and at a source temperature of 140°C, the *RI* of the adduct ion $(M+H)^+$ of $m/z=410$ reached 21% at a pressure of 0.65 Torr. At 70 eV the *RI* dropped to 15%, but the loss of intensity was compensated for by a higher sensitivity. In both modes the C–N bond cleavage, leading to the common base peak and a specific side-chain ion, and the ring opening at the C_5 heptenyl bonds resulted from the same fragmentation (Fig. 2). Fragmentation *d* at the N–C bond on the carboxylic chain side was specific to NH_3-CI and ranged in intensity from 9 to 20%, depending on the compound. Table II gives the variations in the ratios of MF peak areas in the NH_3-CI mode between the ions $(M+H)^+$ and $(BP)^+$ of $m/z=193$.

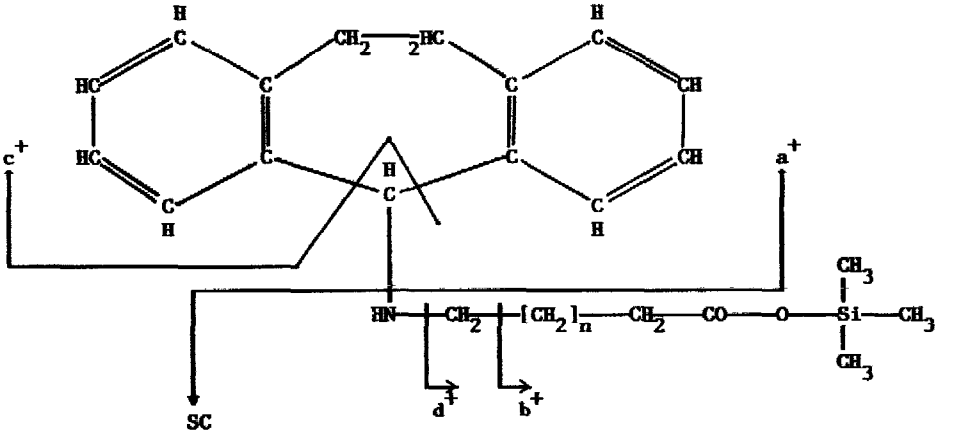

Fig. 2. Fragmentations of amineptine and its homologues as TMS derivatives: $n=2$, C_5 metabolite TMS; $n=4$, amineptine TMS; $n=5$, internal standard TMS.

TABLE I

MAIN FRAGMENTS OR ADDUCT IONS PRODUCED IN THE EI OR NH_3-CI MODE FROM TMS DERIVATIVES OF AMINEPTINE, C_5 METABOLITE AND INTERNAL STANDARD

EI[a]			NH_3-CI[b]		
m/z	Fragments[c]	*RI* (BP, %)	*m/z*	Fragments[c]	*RI* (BP, %)
Amineptine (TMS)					
178	$c^+=(180-2H)^+$	8	179	$c^+=(180-H)^+$	2
192	$a^+=(193-H)=(BP)^+$	100	193	$a^+=(BP)^+$	100
218	$(SC+2H)^+$	5	208	d^+	20
220	b^+	0.2	218	$(SC+2H)^+$	31
409	M^+	0.4	408	$(M-H)^+$	8
			410	$(M+H)^+$	21
C_5 metabolite (TMS)					
178	$c^+=(180-2H)^+$	15	179	c^+	2
190	$(SC+2H)^+$	11	190	$(SC+2H)^+$	34
192	$a^+=(193-H)^+=(BP)^+$	100	193	$a^+=(BP)^+$	100
220	b^+	0.6	208	d^+	9
381	M^+	0.5	380	$(M-H)^+$	13
			382	$(M+H)^+$	24
Internal standard (TMS)					
178	$c^+=(180-2H)^+$	8	179	$c^+=(180-H)^+$	3
192	$a^+=(193-H)^+=(BP)^+$	100	193	$a^+=(BP)^+$	100
220	b^+	0.6	208	d^+	24
232	$(SC+2H)^+$	3	232	$(SC+2H)^+$	28
423	M^+	0.2	422	$(M-H)^+$	9
			424	$(M+H)^+$	22

[a]In the EI mode the electron energy was 35 eV and the source temperature 240 °C.
[b]In the NH_3-CI mode the source parameters were 40 eV, 140 °C and 0.65 Torr ammonia pressure but for MF analysis in the NH_3-CI mode the electron energy was 70 eV (see text).
[c]Ions a^+ to d^+ refer to fragmentation shown in Fig. 2. BP = base peak; SC = side-chain.

TABLE II

VARIATION IN THE RATIOS OF PEAK AREAS BETWEEN THE MOLECULAR ADDUCT ION AND THE BASE PEAK, $(M+H)^+/(BP)^+$, OF MASS FRAGMENTOGRAMS IN THE NH_3-CI MODE FOR AMINEPTINE, C_5 METABOLITE AND INTERNAL STANDARD ($n=7$)

Compound	*m/z*		$(M+H)^+/(BP)^+$ peak-area ratio (mean ± S.D.)	C.V. (%)
	$(M+H)^+$	$(BP)^+$		
Amineptine	410.30	193.20	21.51 ± 1.77	8.23
C_5 metabolite	382.30	193.20	18.58 ± 1.48	7.96
Internal standard	424.30	193.20	15.80 ± 1.51	9.56

Human plasma extractions

Recoveries from human plasma, were assessed by MF. For this purpose the I.S. used for quantification was taken as an external standard and was therefore added just before derivatization. Recoveries were measured as a function of extraction pH for 0.10, 0.50, 1.25 and 2.50 μg/ml of plasma. At pH 4.0, the amineptine yield was 98.25 ± 2.36% ($n=4$), and similar values were found for the C_5 metabolite and I.S. Fig. 3 shows the mass fragmentograms of a solution of the three authentic compounds and of a routine plasma extract. To ensure a comparative check during the routine analysis in the NH_3-CI mode, two ions were monitored for each compound, a specific one [amineptine, $(M+H)^+$, $m/z=410.30$; C_5 metabolite, $(M+H)^+$, $m/z=382.30$; I.S. $(M+H)^+$, $m/z=424.30$] and a common one [$(BP)^+$, $m/z=193.20$]. Assays were performed in duplicate.

Response factor, k_i

Table III shows the response factors, k_{iD} (D = drug) for amineptine and k_{iM} (M = metabolite) for the C_5 metabolite. These k_i values were calculated for the same amount of each compound from peak-area ratios between the two monitored ions of amineptine or C_5 metabolite and the same ion of the I.S. The amount injected was 75 ng of each. For seven analyses the coefficients of variation (C.V.) were between 3.5 and 11.4%. These k_i values were checked regularly.

Analytical method

The specificity of MF assessment required the choice of the molecular ion or of a molecular adduct of the three compound ions, which implied the use of NH_3-CI mode (Table I). The accuracy and precision were increased when simultaneously monitoring a second common but less specific ion such as the base peak, $(BP)^+$ of $m/z=193$. The linearity of the mass spectrometer responses in the MF mode for the analysis of spiked plasma samples for both amineptine and the C_5 metabolite versus the I.S. was established from the peak-area ratios for each ion, either $(M+H)^+$ or $(BP)^+$: 410/424 or 193/193 for amineptine and either 382/424 or 193/193 for the C_5 metabolite. The I.S. was present at a level of 500 ng while the amounts of the two compounds varied from 10 ng to 5 μg in 2 ml of plasma. The precision of measurements was found to be between 3.50 and 11.40%

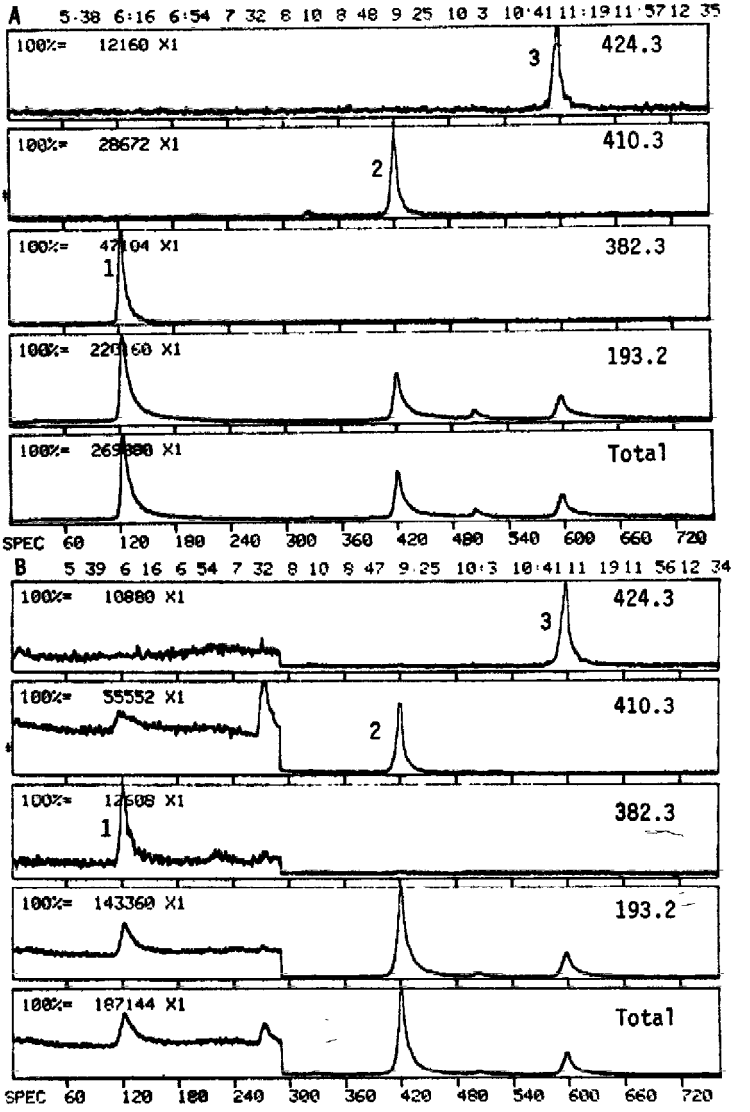

Fig. 3. Mass fragmentograms of a solution of the three authentic compounds (A) and of a plasma sample extract (B). For each compound two ions were monitored: first the $(M+H)^+$ ion, 1 = C_5 metabolite $(M+H)^+$, m/z = 382.30, 2 = amineptine $(M+H)^+$, m/z = 410.30, 3 = I.S. $(M+H)^+$, m/z = 424.30; and second the common base peak ion $(BP)^+$, m/z = 193.20.

for amineptine and the C_5 metabolite ($n=6$) for the same concentrations. The accuracy of the measurements was individually and then globally evaluated by the linear regression $y=a+bx$, where x and y are, respectively, the drug concentration calculated from the monitoring of $(BP)^+$ and $(M+H)^+$ of each assayed ion, m/z 193.2 and 410.3 for amineptine and 193.2 and 382.3 for the C_5 metabolite. Table IV gives the correlation coefficients r, which are close to 1 for each plasma elimination curve for the two compounds of the two series of ten curves and for the totality of each series of ten elimination curves. The regression curves showed

TABLE III

RESPONSE FACTORS FOR 75 ng OF AMINEPTINE (D), C_5 METABOLITE (M) OR INTERNAL STANDARD (I.S.) CALCULATED FROM THE PEAK-AREA RATIOS OF CORRESPONDING IONS, EITHER $(M+H)^+$ OR $(BP)^+$, FOR EACH COMPOUND

Monitored ions	$k_i \pm$ S.E.M. ($n = 7$)	C.V. (%)
$\frac{(M+H)^+_D}{(M+H)^+_{I.S.}} = \frac{410.30}{424.30}$	$k_{iD_1} = 2.24 \pm 0.13$	5.8
$\frac{(BP)^+_D}{(BP)^+_{I.S.}} = \frac{193.20}{193.20}$	$k_{iD_2} = 2.00 \pm 0.12$	6.0
$\frac{(M+H)^+_M}{(M+H)^+_{I.S.}} = \frac{382.30}{424.30}$	$k_{iM_1} = 4.53 \pm 0.51$	11.4
$\frac{(BP)^+_M}{(BP)^+_{I.S.}} = \frac{193.20}{193.20}$	$k_{iM_2} = 3.45 \pm 0.13$	3.5

TABLE IV

CORRELATION COEFFICIENT (r) FOR THIRTEEN MEASUREMENTS OF PLASMA CONCENTRATIONS OF AMINEPTINE AND C_5 METABOLITE DURING THE 24-h PERIOD OF DRUG ELIMINATION FOR EACH VOLUNTEER

Volunteer[a]	r	
	Amineptine	C_5 metabolite
1	0.998	0.998
2	0.999	0.998
3	1.000	0.995
4	1.000	0.999
5	0.999	0.992
6	1.000	0.998
7	1.000	0.999
8	1.000	0.999
9	0.997	0.989
10	0.861	0.997
Whole series of volunteers[b]	0.998	0.995

[a]For each plasma sample submitted to two sets of determinations per point and then in duplicate ($n=4$) and $n' = 13$ plasma samples per 24 h.
[b]Corresponds to the sum of the ten plasma elimination curves assayed twice ($n=20$), each established from the thirteen plasma sample analyses.

excellent linearity. The intercept on the ordinate, a, as close to zero, being 0.008 ± 0.006 for amineptine and 0.004 ± 0.003 for the C_5 metabolite, calculated from the total. The slope b was very close to unity, being 0.989 ± 0.007 for amineptine and 1.004 ± 0.010 for the C_5 metabolite. Therefore, the agreement between the two modes of measurement was excellent.

Clinical assays

Table V gives the values of the apparent clearance or total body clearance of amineptine in each volunteer, calculated for each of the ions $(M+H)^+$ or $(BP)^+$, and the mean C.V. for each plasma sample submitted to two sets of determinations per point and then in duplicate ($n=4$) or for the total of the elimination curves for the ten volunteers measured twice ($n=20$). Despite a high variation from one volunteer to another due to physiopathological differences, values calculated separately for each volunteer from the two independent ions $(BP)^+$ and $(M+H)^+$ were comparable, except for the fourth and fifth volunteers for amineptine. Table VI shows the kinetic profiles of the drug and its metabolite, giving peak time, peak concentration, elimination curve slope β or elimination constant, total body clearance for amineptine only, half-life and mean residence time, for a 100-mg tablet given orally. The data are compared with those of Sbarra et al. [6], the only published pharmacokinetic analysis of amineptine and its C_5 metabolite, and which were obtained after an oral administration of two 100-mg tablets of Survector to a group of six normal adult volunteers (Table VI) consisting of one woman and five men, 21–30 years old and weighing 55–83 kg. Later this group of volunteers was divided into two groups of three volunteers (Table VI) who were given orally three 100-mg tablets. In this work GC–MF relied on single-ion detection for the drug and I.S., viz. $(BP)^+$ of m/z 192. Table VII summarizes the correlation coefficient r of drug elimination parameters, giving the total body clearance for amineptine only, the elimination curve slope β, half-life and mean residence time in the plasma for amineptine and its C_5 metabolite.

TABLE V

TOTAL BODY CLEARANCE FOR TEN VOLUNTEERS CALCULATED SEPARATELY FROM THE TWO IONS OF AMINEPTINE, EITHER $(M+H)^+$, $m/z=410$ OR $(BP)^+$, $m/z=193$

Volunteer	Total body clearance (l/h)		
	$(BP)^+$	$(M+H)^+$	Mean ± C.V. (%) ($n=4$)[a]
1	19 262	19 375	19.32 ± 0.41
2	22.931	20.701	21.82 ± 7.23
3	40 131	39 935	40.03 ± 0.35
4	25.412	39.922	32.67 ± 31.41
5	35 355	29.521	32.44 ± 12.72
6	52.719	52.245	52.48 ± 0.64
7	29.871	28.489	29.18 ± 3.35
8	75.845	77 605	76.74 ± 1.59
9	22.054	21.270	21.66 ± 2.56
10	22.284	23.476	22.88 ± 3.68
Mean of total ± S.E.M.	34.598 ± 17.796 ($n=20$)[b]	35.254 ± 18.266 ($n=20$)[b]	

[a]See Table VI.
[b]See Table IV.

TABLE VI

KINETIC PROFILE OF AMINEPTINE AND C_5 METABOLITE AFTER A SINGLE DOSE OF A 100-mg TABLET GIVEN PER OS TO TEN ADULT VOLUNTEERS WITH LIVER IMPAIRMENT AND COMPARISON WITH THE DATA OF SBARRA ET AL. [6] AFTER A SINGLE DOSE OF EITHER 200 mg (2×100-mg TABLETS) GIVEN PER OS TO SIX NORMAL ADULT VOLUNTEERS OR 300 mg (3×100-mg TABLETS) TO TWO GROUPS OF THREE VOLUNTEERS

Parameter	This work				Sbarra et al. [6]	
	Amineptine		C_5 metabolite		Amineptine	C_5 metabolite
	$m/z=193.20$	$m/z=410.3$	$m/z=193.2$	$m/z=382.3$		
Peak time (min)	80 ± 61[c]		165 ± 132		62 ± 14[a] 70 ± 26[b] 100 ± 10[b]	97 ± 31[a] – –
Peak concentration (mg/l)	1.75 ± 0.91		0.50 ± 0.25		0.75 ± 0.15[a] 0.31 ± 0.03[b] 0.51 ± 0.06[b]	1.02 ± 0.17[a] – –
Elimination curve slope β (min^{-1})	0.0066 ± 0.0053	0.0097 ± 0.0056	0.0029 ± 0.0018	0.0028 ± 0.0014	0.012 ± 0.002[a] 0.015 ± 0.001[b] 0.006 ± 0.001[b]	0.07 ± 0.0004[a] – –
Total body clearance (1/min)	0.576 ± 0.297	0.587 ± 0.304	N.R.[d]		3.97 ± 0.61[b] 3.2 ± 0.26[b] –	N.R. N.R –
Half-life (min)	187 ± 133	125 ± 144	318 ± 211	353 ± 257	69 ± 20[a] 44 ± 2.96[b] 112 ± 26[b]	90 ± 7[a] – –
Mean residence time (min)	193 ± 171	312 ± 568	626 ± 837	634 ± 886	–	–

[a]200-mg dose (2×100-mg tablets) to six volunteers.
[b]300-mg dose (3×100-mg tablets) to two groups of three volunteers.
[c]Results are mean ± standard deviations.
[d]Not relevant.

TABLE VII

CORRELATION COEFFICIENTS OF DRUG ELIMINATION PARAMETERS (ELIMINATION CURVE SLOPE β, TOTAL BODY CLEARANCE, HALF-LIFE AND MEAN RESIDENCE TIME) OF AMINEPTINE AND C_5 METABOLITE AFTER A SINGLE DOSE OF A 100-mg TABLET GIVEN ORALLY TO THE TEN ADULT VOLUNTEERS

Drug elimination parameter	Amineptine	C_5 metabolite
Elimination curve slope β (h^{-1})	0.625	0.793
Total body clearance (1/h)	0.348	Not relevant
Half-life (h)	0.348	0 927
Mean residence time (h)	0.979	0.999

DISCUSSION AND CONCLUSION

Choice of the analytical method

In the last two decades almost all micro-scale methods for the assay of organic compounds in body fluids have been applied to the determination of TADs. These methods and the methods of extraction have been reviewed [7, 8]. Three methods were reported [7] to be accurate and precise: GC–MS, MF and double-isotope derivatization and dilution. GC–MS is the most sensitive method and MF is capable of assaying 0.10 μg/l TADs in plasma. In addition, GC–MS data afford the definite identification of a drug and its metabolites. Single-ion detection, proposed in 1961 [9], and automatic multiple-ion detection developed in 1966 by Sweeley et al. [10], were named MF by Hammar et al. [11] in the development of drug analysis in blood. Nortryptiline (NT) was first identified in human plasma by Hammar et al. in 1971 [12] by MF and then quantitatively assayed [13]. Almost all TADs bearing a side-chain with a secondary or tertiary amine have been assayed by GC–MF [14–17]. Most of these MF methods depended upon the EI mode until the introduction of the CI mode in 1977 for the quantitative assay of amitryptiline (AT) and NT [18] and for the simultaneous assessment up to six TADs [19,20]. GC with electron-capture detection, when TADs can be derivatized [21,22] or converted [23] into electron-donating compounds, was, however, found to be an order of magnitude less sensitive than MF [24]. Therefore, after a single oral dose of a TAD corresponding to a half-day treatment, the sensitivity of MF, especially using the CI mode, permits the specific, accurate and precise measurement of drug concentrations in plasma. High-performance liquid chromatography (HPLC) was introduced in 1975 [25] and many methods have been described since then [7]. Ion-pair reversed-phase chromatography [26] allowed the separation and quantitation of several TADs at levels down to 2 μg/l. A method for the HPLC assay of amineptine and its C_5 metabolite extracted by an ion-pair technique was described by Nicot et al. [27]. It was compared with MF on certain volunteer plasma samples in the course of our study. Using ion-pair extraction it allowed determinations at concentrations as low as 10 μg/l using 2 ml of plasma.

Choice of GC derivative

An MF method using a 1% OV-17 packed GC column for the assay of amineptine extracted from the blood and the brain of rats after receiving 20 mg/kg amineptine intraperitoneally was developed initially by Sbarra et al. [28]. Amineptine and added I.S. were derivatized on-column to their methyl esters. Because we used a quadrupole mass spectrometer with a direct transfer line from the capillary column to the source, samples were derivatized with diazomethane before injection. The gas chromatogram in Fig. 4 shows three peaks for amineptine. The structures of the N-methyl ester and the methyl ester derivatives were ascertained by GC–MS in the EI and NH_3-CI modes. Methylation was abandoned in favour of TMS derivatization. For the TMS esters, the yields of the ions $(BP)^+$ and $(M)^+$ or $(M+H)^+$ of amineptine, its C_5 metabolite and the I.S. in the NH_3-CI mode were compared with those in the EI mode. The results showed that (i) $(BP)^+$ gave *RI* of 19.5, 33.2 and 13.2%, respectively, compared with the EI mode, and (ii) $(M+H)^+$ had *RI* of 17, 10 and 22%, respectively, whereas in the EI mode the *RI* of the three $(M)^+$ ions were between 0.2 and 0.4%. The improved ionization yields of the C_5 metabolite $(BP)^+$ with respect to that of amineptine was advantageous as the level of the metabolite was as much as a third to a fifth of that of amineptine.

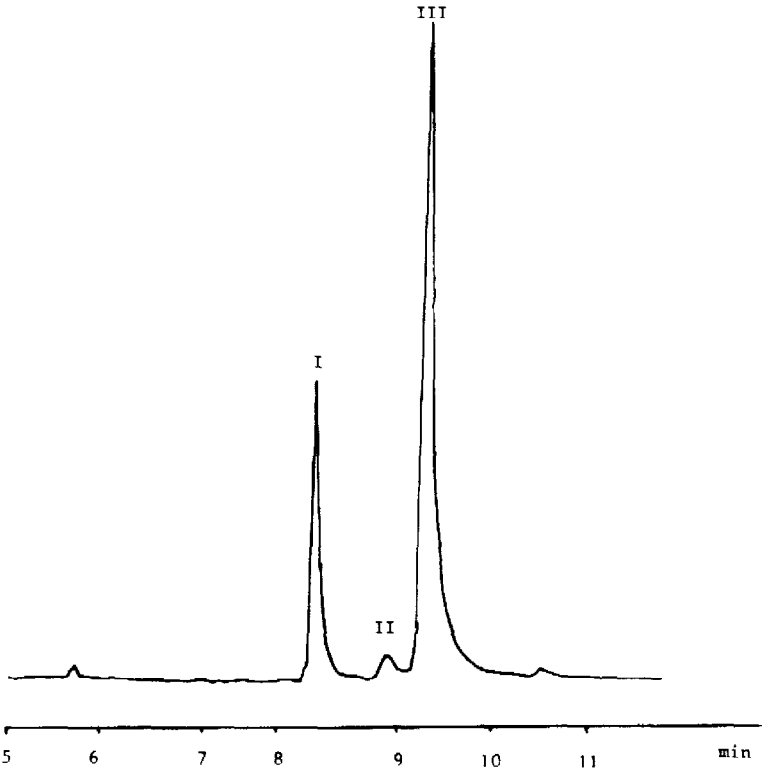

Fig. 4 Gas chromatogram of methyl ester derivatives of authentic amineptine. Peak I = N-methyl ester; peak II = not identified but the mass spectrum was similar to that of an amineptine derivative, peak III = methyl ester.

Method of extraction

Most TADs are strong lipophilic bases. Extraction at an alkaline pH using an organic solvent [6,7,27–30] must be followed by a clean-up step in which the organic phase is back-washed and re-extracted [6, 28–31]. Various extraction yields of between 50 and 100% were claimed for different TADs assayed independently of concentration range [30] and for six different TADs as functions of their concentrations [31]. This phenomenon was also found both for amineptine and its C_5 metabolite extracted by ion pairing and back-extracted [27]. The yield was about the same for the two drugs and ranged from 62 ± 8 to $73 \pm 3\%$ for concentrations of 0.1–1 mg/l [27]. However, a three-step organic solvent extraction of amineptine at different pH values gave recoveries of $91.7 \pm 5.2\%$ for the plasma and $83.1 \pm 4.8\%$ for brain [28]. These liquid–liquid extraction methods were unsatisfactory for GC–MF using a capillary column because of contaminant overload and mass spectrometer pollution. In addition, even after Sep-Pak extraction, the monitoring of selected ions was subject to interference from plasma contaminants, especially at the low mass of $(BP)^+$ but principally when the I.S. was eluted (Fig. 1). Therefore, the levels of amineptine and its C_5 metabolite were underestimated. As the three drugs were homologues differing only in the number of methylene groups in the side-chain, their recoveries were expected to be similar. Therefore, attempts were made to eliminate unwanted plasma contaminants which coeluted with the I.S. by modifying the pH of the plasma sample and the column during equilibration. The comparison of reconstructed chromatograms obtained with the monitored ions by MF and from the total ionization current proved that no plasma contaminants interfered in the region of the monitored peaks (Fig. 3) when extraction was carried out at pH 4.0.

Human pharmacokinetics

As amineptine may be administered to psychiatric patients with intercurrent liver malfunction, this study was undertaken with ten adult volunteers possessing chronic liver impairment with moderate cirrhosis as found in cases of mild depressive alcoholism. The study group consisted of two women and eight men ranging in age between 39 to 65 years, weighing between 48 and 100 kg and displaying relatively perturbed blood biological parameters of liver function: serum glutamo-oxaloacetate transaminase, 111 ± 102 I.U./l (range, 22–320 I.U./l, $n = 7$), normal 2–25 I.U./l; serum glutamo-pyruvate transaminase, 60 ± 49 I.U./l (13–158 I.U./l, $n = 10$), normal 2–29 I.U./l; γ-glutamyl-transpeptidase, 380 ± 338 U/l (19–1000 U/l, $n = 10$), normal 8–38 U/l; bilirubinaemia, 34 ± 17 μmol/l (2.6–69 μmol/l, $n = 10$), normal 0–10 μmol/l; however, total cholesterol, 3.8 ± 1.5 mmol/l (2.6–6.9 mmol/l), normal 2.5–6.5 mmol/l; and triglyceridemia, 0.78 ± 0.24 mmol/l (0.65–1.14 mmol/l), normal 0.8–1.4 mmol/l, were not impaired. The ten volunteers exhibited large individual physiopathological differences and therefore they would not constitute a normal distribution with respect to the normal population. Therefore, each volunteer would exhibit definitively informative kinetics should each plasma elimination curve be established from accurate and precise measurements. This principle guided the design of the method especially based on Sep-Pak extraction of plasma, capillary GC–MS in the NH_3-CI mode and the

use of a structural homologue as the I.S. MF was based on the stability of the mass spectrometer, which was verified by the constancy of both the peak-area ratios for standard ions (Table II) and standard response factors (Table III). Correlation coefficients (Table IV) and regression curves (Fig. 5) calculated from

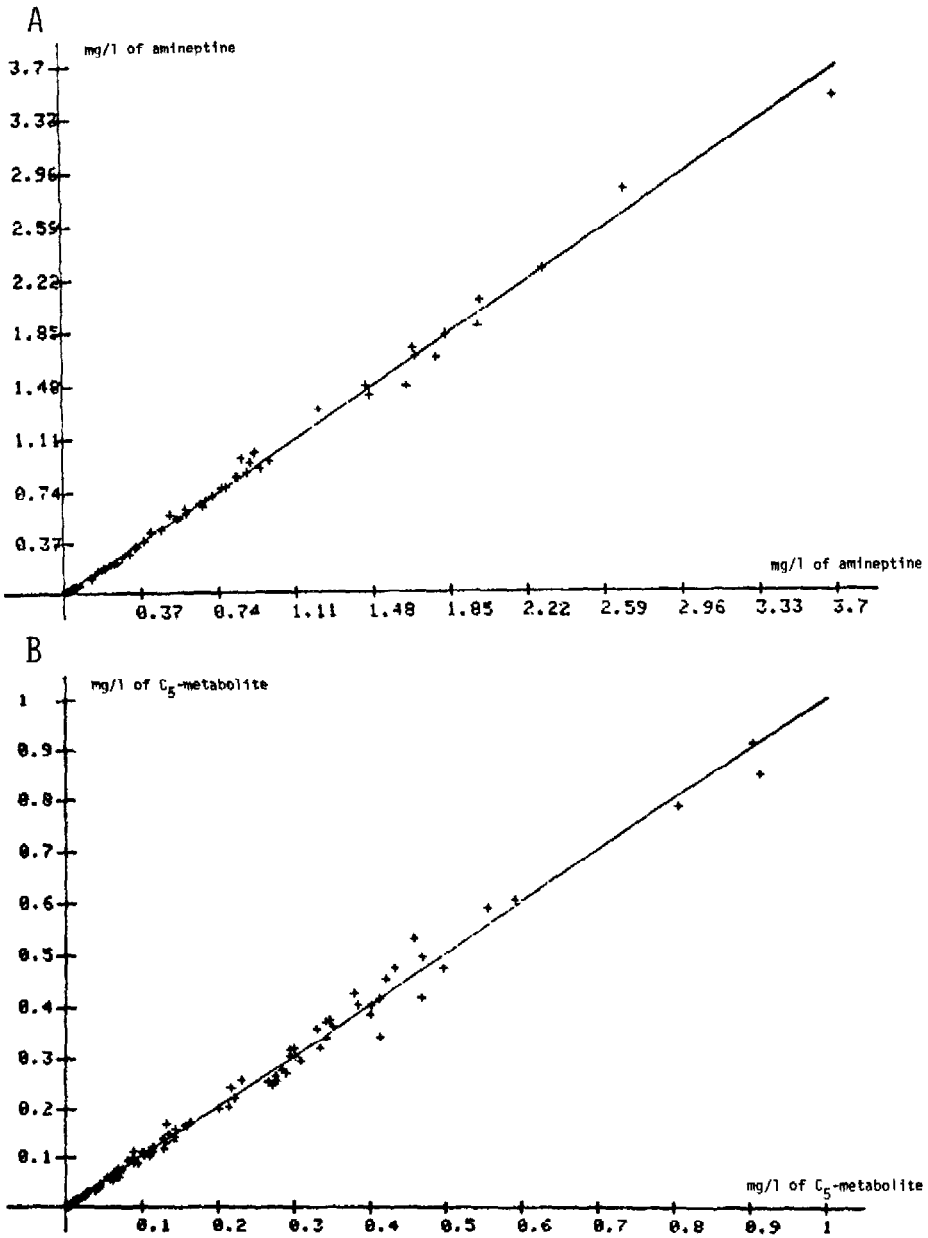

Fig. 5. Regression curves for (A) amineptine and (B) C_5 metabolite between concentrations calculated from the monitoring of two ions per drug, $x = (BP)^+$ and $y = (M+H)^+$.

measurements of drug plasma elimination correspond to ten plasma samples per volunteer, and for each sample four ion ratios were measured, two for each drug, at least in duplicate.

Global evaluation of parameters. Alterations of gastro-intestinal absorption, diffusion in the blood and liver metabolic function accounted for the large global variations of amineptine peak times [80 ± 61 min (± 69%)], peak concentrations [1.75 ± 0.91 mg (± 53%)] and half-lives (187 ± 133 min, 125 ± 114 min), which consequently affected the peak concentrations and peak times of the C_5 metabolites. Sbarra et al. [6] found that plasma concentrations of the C_5 metabolite were always higher than those of the parent drug. Liver defects may explain why, in this study, the plasma levels of amineptine were increased and why the amineptine metabolism was one third lower. The mean residence time of the C_5 metabolite was twice that for amineptine. However, this parameter had not been evaluated by Sbarra et al. [6]. Table VII gives the correlation coefficients for the last four parameters in Table VI. They are close to one for half-life and mean residence time, as was found for drug plasma concentrations (Table IV) with respect to the C_5 metabolite. These results, including an acceptable r value (0.793) for the elimination curve slope β, indicate that once the drug was taken up by the liver and eventually by other metabolizing tissues, the C_5 metabolite followed a normal fate in the body.Conversely, the poor global calculation of the elimination curve slope β, total body clearance and half-life of amineptine (Table VII) were the result of large individual variations in the physiopathological behaviour of the volunteers toward amineptine disposition.

Individual evaluation of parameters. The elimination curve slope β was calculated from each ion, either $(BP)^+$ or $(M+H)^+$, separately by extrapolation of the later values (after 12 h after ingestion of the drug) of the logarithmic plasma concentration to afford the best correlation. However, with amineptine, the later data for the elimination curves were more dispersed and it was difficult to match them with the hypothetical curve. Therefore, the same difficulties werre encountered when estimating the half-lives. Table VIII gives the number of volunteers

TABLE VIII

NUMBER OF VOLUNTEERS EXHIBITING ACCURATE AND PRECISE VALUES FOR ELIMINATION CURVE SLOPE β, HALF-LIFE, TOTAL BODY CLEARANCE AND MEAN RESIDENCE TIME CALCULATED SEPARATELY FROM THE MEASUREMENT OF EITHER $(BP)^+$ OR $(M+H)^+$

Parameter	Amineptine		C_5 metabolite	
	Range of C.V. (%)	n	Range of C.V. (%)	n
Elimination curve slope β	0 -9.07	4	0 -8.73	7
Half-life	0.93-9.06	4	0.22-6.69[a]	8
Total body clearance	0.35-6.88[a]	8	Not relevant	–
Mean residence time	0.08-9.08[a]	8	0.81-5.36	10

[a]For a ninth volunteer, the C V.s were 11.67, 12.30 an 13.55% for the half-life, total body clearance and mean residence time, respectively.

who exhibited accurate and precise data with a C.V. lower than 10% for these four main kinetic parameters. The elimination curve slope β and half-life for amineptine were accurate and precise in the range of C.V. between 0 and 9% in four volunteers only. However, the total body clearance and residence time of amineptine and the three parameters for the C_5 metabolite, as body clearance could not be measured in this instance, were found to be accurate and precise in seven to ten volunteers. For amineptine, such accurate and precise assessments of the elimination curve slope β and half-life data could have been obtained if the follow-up of drug elimination from the plasma had been realized on more blood samples during the second 12-h period. However, volunteers would have had to have been awakened several times. The study of the metabolism of AT in alcoholic depressive patients and in non-alcoholic volunteers demonstrated that plasma NT arising from AT demethylation was lower in the plasma from the former group than in that from the normal volunteers but the levels of conjugated AT, hydroxy-AT and conjugated hydroxy-AT wer higher [32]. However, that study was carried out in a steady-state condition without pharmacokinetic assays during the first 24 h after drug intake. That study, in addition to this one, shows that positive or negative variations in plasma levels of active and deactivated drugs may be of clinical and therapeutic relevance.

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