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High-performance liquid chromatographic determination of tianeptine in plasma applied to pharmacokinetic studies

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Abstract

An improved analytical method for the quantitative measurement of tianeptine and its main metabolite MC_5 in human plasma was designed. Extraction involved ion-paired liquid–liquid extraction of the compounds from 1.0 ml of human plasma adjusted to pH 7.0. HPLC separation was performed using a Nucleosil C_{18}, 5 μm column (150×4.6 mm I.D.) and a mixture of acetonitrile and pH 3, 2.7 g l^{-1} solution of sodium heptanesulfonate in distilled water (40:60, v/v) as mobile phase. UV detection was performed using a diode array detector in the 200–400 nm passband, and quantification of the analytes was made at 220 nm. For both tianeptine and MC_5 metabolite, the limit of quantitation was 5 μg l^{-1} and the calibration curves were linear from 5 to 500 μg l^{-1}. Intra- and inter-assay precision and accuracy fulfilled the international requirements. The recovery of tianeptine and its metabolite from plasma was, respectively, 71.5 and 74.3% at 20 μg l^{-1}, 71.2 and 70.8% at 400 μg l^{-1}. The selectivity of the method was checked by verifying the absence of chromatographic interference from pure solutions of the most commonly associated therapeutic drugs. This method, validated according to the criteria established by the Journal of Chromatography B, was applied to the determination of tianeptine and MC_5-metabolite in human plasma in pharmacokinetic studies.

Keywords: Tianeptine

1. Introduction

Tianeptine, which has been available in France and other countries since 1988, is still received with both interest and scepticism by clinicians [1–6]. This tricyclic compound really exhibits an unusual chemical structure (a substituted dibenzothiazepin nucleus with a long amino acid lateral chain): 7-[(3-chloro-6,11-dihydro-6-methyl-dibenzo [c, f] [1,2] thiazepin-11-yl) amino]heptamino acid *S*,*S*-dioxide (Fig. 1). Its major metabolic pathway is β-oxidation of the heptanoic acid side chain to form short chain derivatives: MC_5 with a pentanoic acid side chain (Fig. 1) and MC_3 with a propionic acid side chain. MC_5 metabolite, which possesses an antidepressant activity, is the major metabolite of tianeptine in plasma, whereas MC_3 is the major one in urine [7]. The biochemical and pharmacological properties of tianeptine on animal behaviour are genuinely different from those of classical antidepressants. Indeed, tianeptine stimulates 5-hydroxytryptamine uptake (5-

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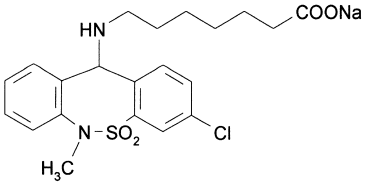
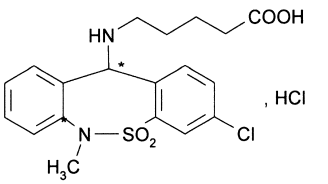
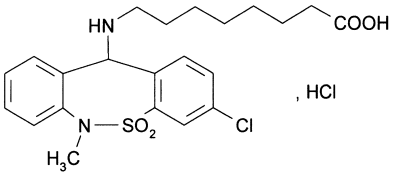


S 1574: tianeptine, sodium salt
(formula: $C_{21}H_{24}ClN_2O_4S$, Na ;
molar mass: 458.9)



S 8849-1: metabolite MC_5, hydrochloride
(formula : $C_{19}H_{21}ClN_2O_4S$, HCl ;
molar mass: 445.4)



S 1719-1: internal standard, hydrochloride
(formula: $C_{22}H_{27}ClN_2O_4S$, HCl ;
molar mass: 487.5)

Fig. 1. Chemical structure compounds of interest and internal standard.

HT) in rat brain synaptosomes and rat and human platelets, increases 5-hydroxyindoleacetic acid (5-HIAA) concentrations in cerebral tissue and plasma, and reduces serotonergic-induced behaviour [8,9]. As its therapeutic profile appears to be neither stimulating nor sedative, tianeptine can be placed in a middle position in the bipolar classification of antidepressants [10].

Consequently, this atypical and paradoxical antidepressant remains the topic of many investigations [7]. An analytical method for the simultaneous determination of tianeptine and its MC_5-metabolite in human plasma was developed in our department long ago [11]. This method was adopted in most of the studies involving investigation of the kinetic behaviour of tianeptine [7]. It included an ion-paired liquid–liquid extraction followed by reversed-phase high-performance liquid chromatography (RP-HPLC) and a single-wavelength UV detection of the compounds, and showed a limit of quantitation close to 5 $\mu g\,l^{-1}$ using a 2-ml sample volume.

The aim of this work was to improve our previous method using a smaller sample volume and a more sensitive and specific diode-array UV detector, and to validate the whole analytical method, according to international guidelines in order to obtain an efficient tool for further pharmacokinetic studies.

2. Experimental

2.1. Chemical and reagents

Acetic acid (Rectapur), orthophosphoric acid, purified octanol, sodium and di-sodium dihydrogenophosphate were provided by Prolabo (Fontenay-sous-bois, France). Sodium heptane sulfonate and tetraheptyl ammonium bromide were obtained from Eastman Kodak (Rochester, NY, USA). Methanol, acetonitrile, *N*-heptane and *N*-hexane (all HPLC grade) were purchased from Carlo Erba Reagenti (Val de Reuil, France).

Tianeptine, MC_5-metabolite and internal standard (S 1719-1) (Fig. 1) were kindly provided by Servier Laboratories (Courbevoie, France).

Deionised and purified water was obtained using a Milli-Q water system (Millipore, Saint Quentin-Yvelines, France). All glassware used (flasks and tubes) was borosilicated.

2.2. Apparatus

The HPLC system consisted of LC10 AVP chromatographic pumps, a SIL 10 AXL automatic sampler and injector, a SPD M10 AVP diode array detector (Shimadzu, Touzart et Matignon, Cour-

taboeuf, France) and an Optiplex® GS microcomputer (DELL, France) equipped with a Shimadzu CLASS® software.

The mobile phase, delivered at a flow-rate of 1.3 ml min^{-1}, consisted of a mixture of acetonitrile and sodium heptane sulfonate buffer (1.5 g l^{-1}) adjusted to pH 3 with phosphoric acid (40:60, v/v), filtered then degassed with helium. The chromatographic separation was performed at room temperature on a Nucleosil C_{18}, 5-μm particle size (150×4.6 mm I.D.) column (Touzart et Matignon, Courtaboeuf, France). The UV spectrum was continuously recorded by the diode array detector from 200 to 400 nm, with a resolution of 1 nm. The quantitation wavelength was 220 nm.

2.3. Standards

Stock solutions of tianeptine and MC_5-metabolite were separately prepared at 1 g l^{-1} in methanol (concentration expressed as base compound), and were stored at −20°C for a maximum of 1 month. These solutions were diluted immediately before use with purified water, so as to obtain working solutions at 20, 5, 4, 2, 1, 0.5, 0.2, 0.1, and 0.05 mg l^{-1} for each analyte. In the same way, a stock solution of internal standard S 1719-1 was prepared at 1 g l^{-1} in methanol (concentration expressed as base compound), and was stored at –20°C for a maximum of 1 month. A working solution at 5 mg l^{-1} was prepared by dilution 1:200 in purified water and was stored at 4°C for a maximum of 1 week.

2.4. Calibration curves and quality control samples

The calibration curve was constructed in the 5–500 μg l^{-1} range (to encompass the usual concentrations measured in samples from pharmacokinetic studies), using free human plasma from heparinized blood, supplied by Western States (Fallbrook, CA, USA) and distributed in France by Valbiotech (Paris, France). The calibrating samples were prepared immediately prior to use by spiking 1000 μl free human plasma with 100 μl of a convenient working solution in purified water, in order to add the following amount of both tianeptine and MC_5-metabolite: 5, 10, 50, 100, 200 and 500 ng. Two samples (free plasma and free plasma spiked with the internal standard alone) were additionally analyzed (but not used for the calculation of the calibration equation) with each calibration curves to check for the absence of the interfering peak caused by the biological matrix. Standard curves were constructed by plotting the peak area ratio of the analyte to the internal standard as a function of the added concentration. The weighting procedure giving the best weighted least-square linear regression was chosen. Finally, the calibration equation was validated if the relative difference between the theoretical and the back-calculated concentrations of each sample of the calibration set did not exceed 20% at the lowest concentration and 15% at the other concentrations.

Quality control samples (three different levels: 10, 200 and 400 μg l^{-1}), used for the determination of intra-assay and inter-assay precision and accuracy of the method, were obtained by spiking 9-ml volumes of free human plasma with 1 ml of an appropriate working solution. A fourth quality control (2000 μg l^{-1}), used for the dilution test was obtained in the same way. One-milliliter aliquots of these mixtures were then stored in glass tubes at 4°C for a maximum of 1 week.

2.5. Sample extraction procedure

A 1 ml volume of plasma was placed into a 15-ml screw-capped glass tube to which were successively added: 100 μl of water (for quality controls and unknowns) or of an appropriate working solution (for calibrating standards), 50 μl of a 5 mg l^{-1} working solution of internal standard, 1 ml of pH 7.0, 0.5 *M* Na_2HPO_4–0.5 *M* NaH_2PO_4 buffer, and 8 ml of an heptane–octanol–tetraheptyl ammonium bromide (98:2:0.005, v/v/w) mixture as the extraction solvant. The tube was capped, tumble mixed on an Agitelec reciprocating shaker (Toulemonde, Paris, France) for 10 min and centrifuged at 3000 rpm (1600 *g*) for 10 min using a G-412 centrifuge (Jouan, Saint Nazaire, France). The organic layer was decanted in a 10-ml screw-capped glass tube and subsequently back-extracted with 200 μl of a methanol–0.17 *M* acetic acid mixture (10:90, v/v), by means of another 10-min agitation and 5-min centrifugation (1600 *g*). The collected acidic phase

was afterwards washed with 2-ml hexane by 30 s vortex mixing and 5 min centrifugation (1600 *g*). Finally, the organic layer was removed by aspiration and 50 μl of the lower phase was injected into the chromatograph.

2.6. Validation procedure

2.6.1. Linearity

For each calibration curve generated, the concentrations of tianeptine and MC_5 metabolite of each calibrating sample were back calculated. The slope, intercept and coefficient of determination (r^2) of each calibration curve together with the mean, standard deviation, precision coefficient of variation (C.V.) and mean relative error (MRE) for each calibrating level were determined. Precision C.V. was calculated according to the relation:

$$\text{C.V.} = \frac{\text{Standard Deviation (SD)}}{\text{Mean value}} \times 100$$

The MRE, expressed as a percentage, was calculated as follows:

$$\text{MRE} = \frac{(\text{Mean measured value} - \text{Theoretical value})}{\text{Theoretical value}} \times 100$$

2.6.2. Intra- and inter-assay precision and accuracy

The intra- and inter-assay precision and accuracy study was carried out using quality control samples at three concentration levels: 10, 200 and 400 $\mu g\,l^{-1}$. For the intra-assay study, six replicates for each concentration level were analyzed on the same day. For the inter-assay study, duplicates of the three concentration levels were analyzed each day for 5 days. For each concentration level, the mean and standard deviation was calculated and the intra- and inter-assay precision and accuracy were determined. Precision was expressed as the coefficient of variation (C.V.), and MRE was calculated from the mean of duplicates. The acceptance criteria for precision and accuracy were the same for both intra- and inter assay-studies: the precision C.V. and the MRE had to be within ±20% for the lowest concentration and ±15% for the two upper levels.

2.6.3. Limit of quantitation

The limit of quantitation (defined as the lowest concentration yielding a precision C.V. and a MRE lower than 20%) was expected to be 5.0 $\mu g\,l^{-1}$ for both compounds using a 1 ml volume of plasma. It was determined using inter-batch tests, determined in duplicate each day for 7 days.

2.6.4. Recovery

The extraction recovery of tianeptine and its MC_5-metabolite from human plasma were determined at 20 and 400 $\mu g\,l^{-1}$, by comparison of the results from extracted quality control samples where S 1719-1 was added after the extraction (external standard) and from unextracted pure solutions directly injected into the chromatographic system. The recovery of the internal standard itself was determined in triplicate at 500 $\mu g\,l^{-1}$, using tianeptine as the external standard.

2.6.5. Dilution test

The applicability of the method to the determination of concentrations exceeding the highest level of the calibration range, using a dilution step, was assessed. Six replicates of a quality control at 2000 $\mu g\,l^{-1}$ were diluted to 1:100 with free human plasma. The difference between the theoretical value and that back-calculated after dilution had to be within ±15%. This individual accuracy was evaluated by means of the relative error (RE), expressed as a percentage and calculated according to the following expression:

$$\text{RE} = \frac{(\text{Measured value} - \text{Theoretical value})}{\text{Theoretical value}} \times 100$$

2.6.6. Selectivity

The selectivity of the present method was tested by analyzing one-by-one pure solutions of therapeutic drugs (Table 5), frequently co-prescribed with tianeptine in psychiatric or depressed patients. Then, all those presenting a retention time close (±1 min) to those of tianeptine, MC_5-metabolite or the internal standard, were added to free human plasma, extracted and assayed following the present method.

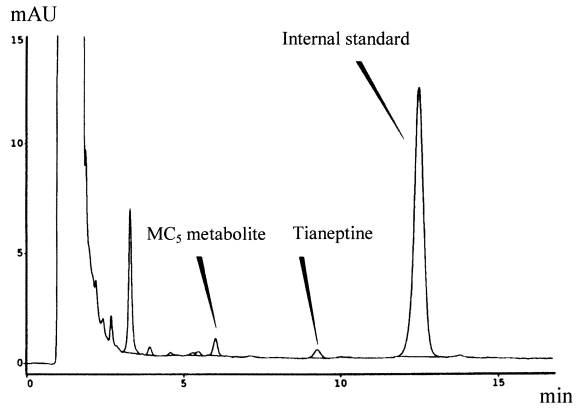


Fig. 2. Chromatogram from extract of free human plasma together spiked with the internal standard with 10 μg l^{-1} of tianeptine and its MC_5 metabolite.

2.6.7. Application to pharmacokinetic studies

The concentrations of tianeptine and MC_5 metabolite were determined in human plasma samples collected in 20 elderly depressed patients participating in a 6-month open study of the efficacy and acceptability of tianeptine as a 50-mg prolonged release form (PR), in order to document the pharmacokinetics of PR-tianeptine in this population.

Blood samples were collected by direct venous puncture in 5-ml heparinized tubes, between day 70 and month 8, at the following times: 0 (before administration) and 1, 2, 4, 6, 8 and 10 h post-dose. These samples were immediately centrifuged at 3000 rpm (1600 *g*) for 10 min at 4°C and the plasma was stored at −20°C until analysis.

3. Results and discussion

3.1. Standard curves

The chromatogram of an extract of free human plasma spiked with tianeptine and metabolite at low concentration (10 μg l^{-1}) is given in Fig. 2. The mean retention times of internal standard, tianeptine and its MC_5 metabolite were 13.0, 9.5, and 6.2 min, respectively. Standard curves were constructed in the range 5–500 μg l^{-1}, using a 1/C weighted, least-squares regression. The mean, standard deviation, precision C.V. and MRE calculated for each calibrating level of tianeptine and metabolite used in this study are reported in Table 1, together with the slope, intercept and coefficient of determination (r^2) of each calibration curve. The precision C.V. values were always lower than 12% and the mean relative error lower than 6%. The coefficient of determination r^2 was always higher than 0.997.

3.2. Intra- and inter-assay precision and accuracy

The results of the intra-assay precision and accuracy study are given in Table 2. The precision C.V. values were between 1.6 and 4.4% for tianeptine, 1.5 and 6.7% for its MC_5 metabolite, while the MRE was between 0.1 and 5.8%. The method was thus shown to be precise and accurate, on an intra-assay basis. The inter-assay precision and accuracy were studied by means of the C.V. and MRE values obtained in two different ways: from the five calibration curves (5–500 μg l^{-1}) used for the linearity

Table 1
Calibration curves of tianeptine and MC_5-metabolite ($n=6$)

	Theoretical concentration (μg l^{-1})						r^2	Slope	Intercept
	5	10	50	100	200	500			
Tianeptine									
Mean	5.3	10.2	48.6	100.8	197.6	502.0	0.9990	392.60	0.8972
C.V. (%)	5.6	6.1	6.7	4.3	2.7	1.6	0.03	4.21	102.73
MRE (%)	5.7	2.1	− 3.0	0.8	−1.2	0.4	–	–	–
MC_5-metabolite									
Mean	4.8	10.0	49.5	101.6	199.9	497.9	0.9988	314.34	0.7980
C.V. (%)	11.9	4.8	4.9	3.5	4.3	1.9	0.09	4.96	119.05
MRE (%)	−4.9	0.3	−1.0	1.6	−0.1	−0.4	–	–	–

Table 2
Intra-assay precision and accuracy ($n=6$)

Theoretical concentration ($\mu g\,l^{-1}$)	Concentration found (mean) ($\mu g\,l^{-1}$)	C.V. (%)	MRE (%)
Tianeptine			
10	10.6	4.4	5.8
200	199.2	1.6	−0.4
400	421.3	1.9	5.1
MC_5-metabolite			
10	10.0	6.7	0.1
200	191.7	2.4	−4.4
400	410.9	1.5	2.6

study (Table 1), as already mentioned; and from the average value of quality controls at 10, 20 and 400 $\mu g\,l^{-1}$ analyzed in duplicates each day for 5 days (Table 3); the precision C.V. values calculated in this latter case were lower than 10% at 10 $\mu g\,l^{-1}$ and the MRE lower than 2%.

3.3. Limit of quantitation

The detailed results of the inter-assay precision and accuracy at the targeted limit of quantitation of 5 $\mu g\,l^{-1}$ are given in Table 4. The precision C.V. values for tianeptine and MC_5 metabolite were 8.7% and 10.9% and the MRE −1.8% and −10.3%, respectively, thus validating 5 $\mu g\,l^{-1}$ as a precise and accurate the limit of quantitation.

Table 3
Inter-assay precision and accuracy ($n=6$ duplicates)

Theoretical concentration ($\mu g\,l^{-1}$)	Concentration found (mean) ($\mu g\,l^{-1}$)	C.V. (%)[a]	MRE (%)[a]
Tianeptine			
10	10.0	9.2	0.4
200	205.0	4.1	2.5
400	407.0	2.9	1.7
MC_5-metabolite			
10	10.2	5.8	1.8
200	196.4	3.3	−1.9
400	394.0	3.8	−1.5

[a] C.V. and MRE were calculated from the average value of duplicates.

Table 4
Validation of the targeted limit of quantitation (5 $\mu g\,l^{-1}$) ($n=7$ duplicates)

	Concentration found (mean) ($\mu g\,l^{-1}$)	C.V. (%)[a]	MRE (%)[a]
Tianeptine	4.9	8.6	−1.8
MC_5-metabolite	4.5	10.9	−10.3

[a] C.V. and MRE were calculated from the average value of duplicates.

3.4. Recovery

The extraction recoveries of tianeptine were 71.5 and 71.8% at 20 and 400 $\mu g\,l^{-1}$, respectively, and those of the MC_5 metabolite were 71.5 and 74.3%. The recovery of the internal standard was 54%.

3.5. Dilution test

Free human plasma samples spiked at a theoretical concentration of 2000 $\mu g\,l^{-1}$ of tianeptine and metabolite, after dilution at 1:100 with blank human plasma, yielded mean measured concentrations ($n=6$) of 2167 (MRE = 7.7%) and 2096 (MRE = 4.6%), respectively. The maximal individual RE was 12.0% for tianeptine and 9.5% for MC_5.

3.6. Selectivity

The injection of pure solutions of 44 therapeutic drugs often associated with tianeptine showed that 18 of them had a retention time within 1 min of the retention time of either analytes or internal standard (Table 5). After submitting these potentially interfering compounds to the extraction procedure described above, only 12 were still detected: buspirone, risperidone, carbamazepine and carbamazepine-10,11-epoxide, were totally resolved from either analytes or internal standard when injected together; hydroxyzine and haloperidol (with internal standard), citalopram (with tianeptine) and nitrazepam (with MC_5 metabolite) were partially resolved; amineptine and acepromazine co-eluted with tianeptine, MC_5-amineptine and clozapine co-eluted with MC_5 metabolite. However, the presence of each of them could be seen by the change in the UV spectrum brought about by the mixture of the analyte and the

Table 5
Interferences of psychotropic drugs with the determination of tianeptine and MC_5-metabolite

Drug tested (ICD)	Retention time (min)	Retention time difference[a] (min)	Extraction recovery
Tianeptine	9.4		
MC_5-metabolite	6.0		
E.I. (S1719)	12.7		
Alprazolam	9.93	<0.5	
Bromazepam	3.88	>1	
Chlorazepate di K	8.42	>1	
Clobazam	10.71	>1	
Clotiazepam	16.66	>1	
Desmethyldiazepam	8.41	>1	
Flunitrazepam	9.73	<0.5	
Loprazolam	5.76	Between 0.5 and 1	+
Lorazepam	6.39	<0.5	
Lormetazepam	10.94	>1	
Nitrazepam	6.37	<0.5	
Oxazepam	5.67	Between 0.5 and 1	
Prazepam	>20	>1	
Temazepam	9.25	Between 0.5 and 1	
Triazolam	10.55	>1	
Diazepam	15.29	>1	
Desmethylclobazam	6.63	Between 0.5 and 1	+
Acepromazine	9.87	<0.5	+
Chlorpromazine	23.82	>1	
Clozapine	5.97	<0.5	+
Haloperidol	12.20	Between 0.5 and 1	+
Sulpiride	1.96	>1	
Risperidone	5.39	Between 0.5 and 1	+
MC_5-amineptine	6.14	<0.5	+
Amineptine	9.91	<0.5	+
Amitryptilline	17.14	>1	
Citalopram	8.89	Between 0.5 and 1	+
Clomipramine	26.63	>1	
Fluoxetine	19.62	>1	
Imipramine	14.51	>1	
Mianserine	8.82	Between 0.5 and 1	
Sertraline	22.31	>1	
Carbamazepine	5.32	>1	
CBZ-10,11-epoxide	3.14	>1	
Depamide	ND		
Lithium	ND		
Buspirone	5.41	>1	+
Difebarbamate	ND		
Hydroxyzine	13.72	<0.5	+
Meprobamate	ND		
Phenobarbital	3.18	>1	
Febarbamate	13.66	<0.5	+

[a] Difference between the retention time of the drug tested and that of the nearest analyte; ND, not detected.

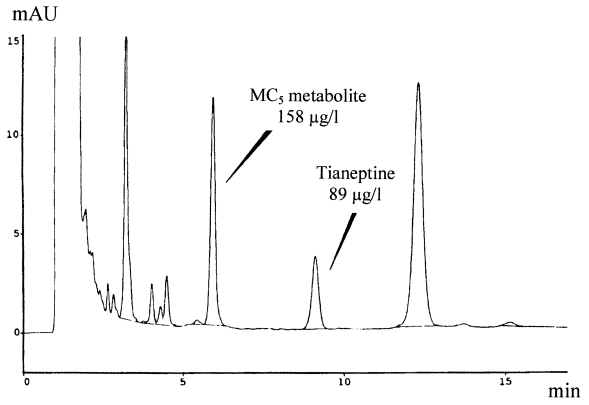


Fig. 3. Chromatogram of a typical patient's plasma sample.

interfering compound, which is the main advantage of using a diode array detector. Moreover, amineptine has not been commercialized in France since 1999.

3.7. Application to pharmacokinetic studies

Our method was applied to the pharmacokinetic study of a 50-mg prolonged release form in depressed elderly patients, during chronic treatment. A chromatogram of a typical patient's plasma is given in Fig. 3 and a concentration vs. time profile of tianeptine and MC_5-metabolite is presented in Fig. 4.

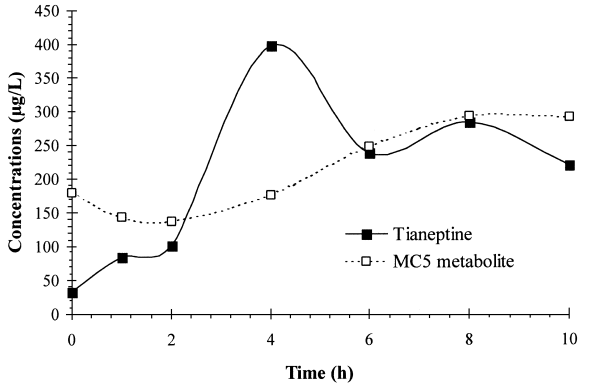


Fig. 4. Pharmacokinetic of tianeptine and its MC_5 metabolite in an elderly patient following long-term administration of a 50-mg prolonged release form of tianeptine.

In summary, the present procedure is suitable for routine analyses of tianeptine and its MC_5-metabolite in human plasma, as well as for pharmacokinetic studies owing to the reasonable sample volume required.

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