Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 834 (2006) 62–67
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of tianeptine in human plasma using high-performance liquid chromatography with fluorescence detection

Sevgi Tatar Ulu *

Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul 34452, Turkey

Received 12 September 2005; accepted 11 February 2006
Available online 7 March 2006

Abstract

A new, selective and sensitive high-performance liquid chromatography (HPLC) method with fluorimetric detection was developed for the determination of tianeptine (TIA) in human plasma using solid phase extraction (SPE) procedures. The method is based on the derivatization of TIA with 4-chloro-7-nitrobenzofurazan (NBD-Cl) in borate buffer of pH 8.5 to yield a yellow, fluorescent product. The HPLC separation was achieved on a Phenomenex C_{18} column (250 mm × 4.6 mm) using a mobile phase of acetonitrile–10 mM orthophosphoric acid (pH 2.5) (77:23, v/v) solvent system at 1 mL/min flow rate. Gabapentin (GA) was used as the internal standard. The fluorometric detector was operated at 458 nm (excitation) and 520 nm (emission). The assay was linear over the concentration range of 5–300 ng/mL. The detection limit (LOD) was found to be 2 ng/mL. The mean recovery was determined to be 88.6%. The proposed method was applied for pharmacokinetic study of 12.5 mg TIA in a healthy volunteer.

Keywords: Tianeptine; 4-Chloro-7-nitrobenzofurazan; High-performance liquid chromatography (HPLC); Pharmacokinetic study

1. Introduction

Tianeptine (TIA); 7-[([3-chloro-6,11]-dihydro-6-methyldibenzo[c,f][1,2]thiazepin-11-yl) amino] heptanoic acid S,S-dioxide (Fig. 1) [1] is a new antidepressant tricyclic effective agent [2–5]. Behavioral tests in rats [6,7] and clinical trials in man [8,9] have clearly demonstrated the antidepressant activity of this drug. Zini et al. [10] were studied the plasma binding of TIA in humans. Ortiz et al. [11] were reported the antidepressant drug TIA on plasma and platelet serotonin concentrations in the rat. The pharmacokinetics and metabolism of in healthy volunteers have been previously studied [12,13]. Onder et al. [14] were studied of fluoxetine, moclobemide, and TIA in the treatment of posttraumatic stress disorder following an earthquake. Ceyhan et al. [15] were investigated of the effects of TIA and fluoxetine on pentylenetetrazole-induced seizures in rats.

The several analytical methods have been developed for the determination in biological fluids. Ion pair and HPLC with UV detection have been reported for the determination of TIA and its metabolites in human plasma, urine and tissues [16]. HPLC method with UV detection has been studied for the determination of TIA in human plasma [17]. The drug has been determined in biological fluids by gas chromatography [18]. Novakova [19] has been reported a thin layer chromatography for the detection in human urine.

In this study, a sensitive HPLC method for the assay of TIA in human plasma by means of the derivative formed with NBD-CI, which is a specific reagent in the analysis of primary and secondary aliphatic amines. A new fluorimetric HPLC method, which has high reproducibility and sensitivity has been developed for the determination of TIA in plasma. In literature research, TIA, for the first time has been derivatized by a reagent and has been determined by using a fluorescent detector. The proposed method was successfully applied to the pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

TIA was a gift sample from Servier (Istanbul, Turkey). GA (internal standard, IS) was purchased from Pfizer (Istanbul,

* Fax: +90 2124400252.
E-mail address: sevgitatar@yahoo.com.

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.02.027

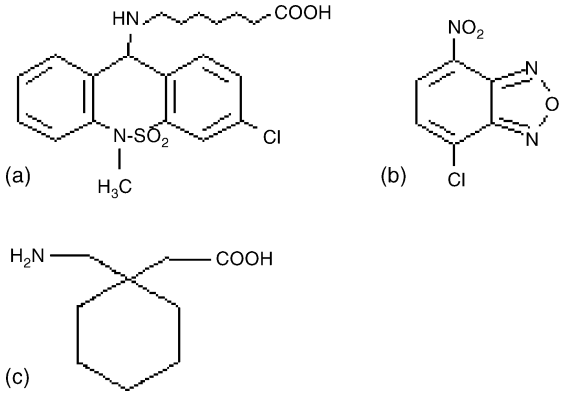

Fig. 1. Chemical structures of tianeptine (a), 4-chloro-7-nitrobenzofurazan (b) and gabapentin (IS) (c).

Turkey). NBD-CI was purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile was purchased from Merck (Germany). All the other chemicals used were of analytical grade. An aquaMAX™ water system (Young Instrument, Korea) produced ultra pure analytical grade water.

Silica cartridges (100 mg, 1 mL) were purchased from Alltech (USA). Blood plasma samples were collected into ethylenediaminetetraacetic acid and centrifuged at 4500 × *g* for 20 min, resultant plasma was separated and stored at −20 °C until assayed.

2.2. Solutions

Stock solutions (0.1 mg/mL) of TIA were prepared in water and 1 μg/mL solutions were made by dilution in water. Then, 5, 10, 20, 40, 50, 100, 150, 200, 250 and 300 ng/mL working standards were prepared in plasma from the 1 μg/mL solution and stored at +4 °C.

The internal standard (GA) working solution was prepared at 0.01 mg/mL in water. The NBD-CI solution was freshly prepared at 4 mg/mL in methanol. Borate buffer (0.1 M) was prepared from boric acid. The pH was adjusted to 8.5 with 0.1 M NaOH.

2.3. Chromatography

A Thermo Separation Products Liquid Chromatograph (TX, USA) consists of a Model P 4000 solvent delivery system, a Rheodyne injection valve with a 20-μL loop, a FU 3000 detector, set an excitation wavelength of 458 nm and emission wavelength of 520 nm and SN 4000 automation system software. Separations were carried out on a Phenomenex C_{18} column (250 mm × 4.6 mm ID, 5 μm; Thermo Separation, TX, USA), with a guard column (4 mm × 3 mm ID, Phenomenex) packed with the same material. For the mobile phase, 10 mM orthophosphoric acid (pH 2.5) solution was prepared and then mixed with acetonitrile in ratio of 77:23 (v/v). Finally, the mobile phase was filtered through a 0.25-μm membrane filter and degassed. The injections volumes for samples and standards were 20 μL and eluted at a flow rate of 1 mL/min at room temperature.

2.4. Sample preparation and derivatization

All of the frozen plasma samples were thawed just before the experiment. To 0.5 mL of plasma, 50 μL the sample and 5 μL the internal standard working solution and 2 mL methanol were added. After shaking for 1 min, the samples were centrifuged at 4500 × *g* for 20 min. An aliquot of 1 mL of the protein-free supernatant was evaporated under nitrogen of 45 °C, and then adding 100 μL of borate buffer and 100 μL of NBD-CI solutions the sample was kept at 80 °C for 20 min. After cooling, 250 μL of 0.1N HCI was added and the contents were extracted 2 × 2.5 mL of ethyl acetate. A 4.5-mL aliquot of the ethyl acetate phase was evaporated to dryness, then passed through a solid-phase extraction column (100 mg, 1 mL) and eluted with ethyl acetate to obtain a finale volume of 4.5 mL. After evaporation the residue was dissolved in 200 μL of the mobile phase and 20 μL of this solution was injected into the HPLC system.

2.5. Method validation

2.5.1. Linearity

Calibration curves were prepared by adding various amounts (2.5, 5, 10, 20, 25, 50, 75, 100, 125 and 150 ng) of TIA to aliquots (0.5 mL) of drug-free human plasma, and a fixed amount (50 ng) of the IS. The samples were analyzed as described in the extraction procedure (Section 2.4) above. Calibration curves were constructed by plotting peak area ratio of TIA to the IS against the known amounts TIA**.** Linear regression analysis of the calibration data was performed using the equation $A = aC + b$ where A is the peak area ratio, C the concentration of TIA; unknown concentrations were computed from the linear regression equation of the peak area ratio against concentration for the calibration curve.

2.5.2. Detection and quantification limits (sensitivity)

The limit of quantification (LOQ) was estimated by analyzing TIA at low concentrations of the calibration curves. The detection limit (LOD) was defined as the lowest concentration level resulting in a peak area of three times the baseline noise (S/N = 3).

2.5.3. Assay precision and accuracy

The intra-day accuracy and precision evaluations were performed by repeated analysis of TIA in human plasma. The run consisted of a calibration curve plus five replicates of each low (5 ng/mL), medium (150 ng/mL) and high (300 ng/mL) plasma samples for TIA on the same day ($n = 7$). The inter-day accuracy and precision were assessed by analysis of samples consisting of a calibration curve and five replicates of low (5 ng/mL), medium (150 ng/mL) and high (300 ng/mL) plasma samples for TIA on five different days ($n = 9$).

2.5.4. Analytical recovery

The absolute recovery was assessed by spiking plasma samples with TIA at 5, 20, 50,150, 200 and 300 ng/mL concentrations. After the derivatization, solid-phase extraction and chromatography processes, the peak areas were compared to the peak

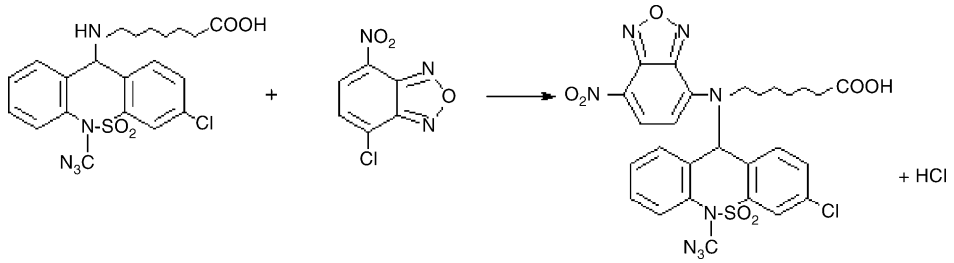

Fig. 2. The reaction between TIA and NBD-CI.

areas obtained from the aqueous solutions of TIA at the same concentration.

2.5.5. Selectivity

Control human plasma, obtained from three healthy volunteers, was assessed by the procedure as described above and compared with respective plasma samples to evaluate selectivity of the method. Drug-free plasma was spiked with therapeutic concentrations of such drugs which included antidepressants (Fluoxetine, Sertraline, Desipramine, Nortriptyline, Tranylcypromine), muscle relaxant (Baclofen), hemostatic (Tranexamic acid), sweetener (Aspartam), some amine and amino acids (hydroxyphenylalkylamine, phenylpropanolamine, prolin, hydroxyprolin, methylamine hydrochloride, ethylamine hydrochloride, propylamine, butylamine, pentylamine, tyramine, phenylethylamine, *N*-nitrosoamines, aspartic acid, glutamic acid, glycine, histidine, lysine, phenylalanine, serine, valine) followed by extraction and analysis as described (Section 2.4) above. The retention times for these drugs under the chromatographic conditions for the TIA assay were determined. No interfering peaks were observed at the retention time for the TIA and IS peaks.

2.5.6. Stability

The short-term stability was examined by keeping replicates of the low and high plasma samples at room temperature for 24 h. Freeze–thaw stability of the samples was obtained over three freeze–thaw cycles, by thawing at room temperature for 2–8 h, refrozen for 12–24 h. Stability of TIA in human plasma was tested after storage at −20 °C for 2–4 months. Long-term stability at −20 °C was determined after 2–4 months. The stability of standard solutions was also tested at room temperature for 2, 24 h and upon refrigeration (4 °C) for 2–4 months. The concentration of TIA after each storage period was related to the initial concentration as determined for the samples that were freshly prepared.

2.6. Pharmacokinetic applications

After oral administration of a single 12.5 mg TIA to a 35-year-old woman volunteer, venous blood samples were drawn into ethylenediaminetetraacetic acid tubes at 15, 30, 45, 60, 90, 120, 180, 270 320 and 480 min. The blood was processed in plasma as described above and samples were stored −20 °C until analysis.

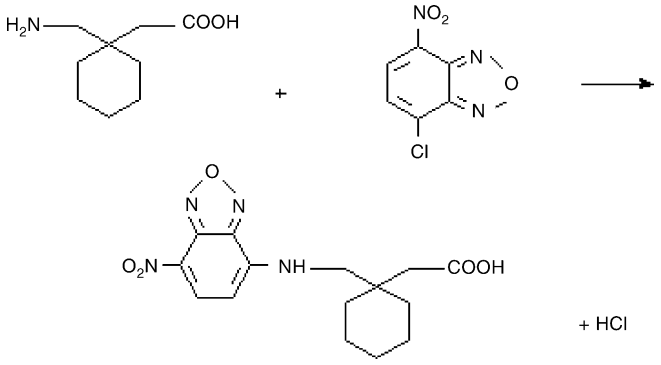

Fig. 3. The reaction between TIA and internal standard (GA).

3. Results and discussion

3.1. Optimization of the reaction conditions and separation

The reaction between TIA and GA (IS) with NBD-Cl (Figs. 2 and 3) in borate buffer of pH 8.5 produces a yellow colored product with maximum fluorescence at 520 nm.

TIA-NBD produced a highly fluorescent derivative with excitation and emission maxima of 458 and 520 nm, respectively. The different experimental parameters affecting the intensity of the color derivatized were studied and optimized to obtain maximum color intensity. The pH was varied over the pH range of 7–10 using borate buffer where the maximum fluorescence was obtained at pH 8.5 as shown in Fig. 4, NBD-Cl is hydrolyzed in alkaline medium. Therefore, it was necessary to acidify the reaction mixture to pH 2 (by adding 250 μL 0.1N HCl) before the measurement was carried out.

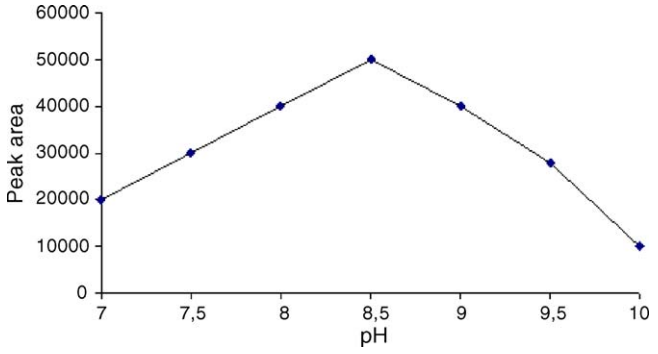

Fig. 4. Effect of pH on the reaction of tianeptine with NBD-CI.

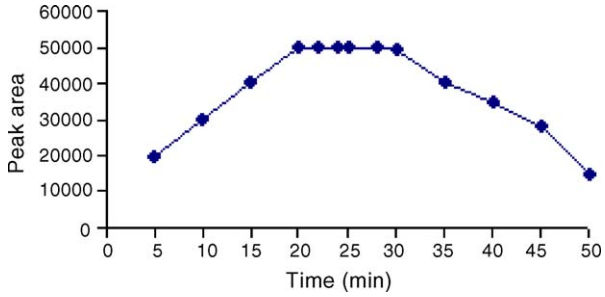

Fig. 5. Effect of time on the reaction of tianeptine with NBD-CI (80 °C).

The influence of different heating temperatures and times was studied using a water bath. Effect of heating time at five different temperatures 50–90 °C for NBD derivatives.

The best results were obtained at 80 °C within 20 min (Fig. 5). The reactions do not go in room temperature and are not completed even at 80 min at 50 °C. However, at 90 °C, although the time of the reaction gets quite less, the stability of the derivative goes down. These optimum conditions have been used for the spectrofluorimetric method and the precolumn derivatization for the HPLC method.

3.2. HPLC method development

A selective and sensitive HPLC method was developed for the determination of TIA in single dosage forms and in spiked human plasma, pharmacokinetic studies.

The separation and resolution of the peaks could be achieved upon using a mixture of 10 mM orthophosphoric acid (pH 2.5) and acetonitrile (23:77, v/v). The effect of pH on the retention of the solutes was investigated by changing the pH (2–6) and concentration of orthophosphoric acid (5–20 mM),and the acetonitrile percentage of elution 40, 50, 60, 70 and 80%, respectively. The optimum separation was accomplished upon using solution of pH 2.5.

3.3. Solid-phase extraction (SPE)

Plasma samples spiked with TIA were put into reaction with NBD-CI under the most suitable conditions after the saturation of the proteins and evaporation of the extraction solvent. Following the extraction with ethyl acetate in acid pH in the reaction environment, solid-phase extraction was carried out. In this process, TIA-NBD derivative was separated from the amine and amino acids in the plasma that react to the reagent. This was found to be the most optimal condition for sample preparation as it resulted in a clean chromatogram.

3.4. Characteristics of the chromatographic peak

According to the conditions described, the retention times were 4.7 and 5.4 min for derivatized TIA and IS (GA), respectively. The total run time for each sample analysis was 8 min. Typical chromatograms obtained from blank human plasma, plasma spiked with TIA (100 ng/mL) and IS (100 ng/mL) are shown in Fig. 6A and B, respectively. Fig. 6C represent the chromatogram of plasma obtained at 4.5 h after a single oral dose of TIA from a healthy volunteer. The chromatogram of blank plasma showed no interfering peaks having the same retention times as TIA or IS derivatives.

3.5. Method validation

3.5.1. Linearity

Calibration curve was obtained by plotting the concentration against the peak area ratios (TIA/GA). The linearity was observed at concentration ranges between 5–300 ng/mL TIA in plasma samples. The regression was $A = 0.022C - 2 \times 10^{-3}$ ($r = 0.9989$), where A = peak-area ratio (TIA/ internal standard) and C = TIA concentration (ng/mL).

3.5.2. Limits of detection and quantification

Under the experimental conditions used, the lower limit of detection (LOD) was 2 ng/mL at (signal to noise ratio of 3) and the lower limit of quantification (LOQ) was found to be 5 ng/mL.

3.5.3. Assay precision and accuracy

Intra-and inter-day precision and accuracy data are shown in Table 1. The intra-day R.S.D. at 5, 150 and 300 ng/mL of TIA were 2.39, 0.48 and 0.22% ($n = 7$), respectively. The inter-day R.S.D. at the above concentrations were 4.55, 0.70 and 0.31% ($n = 9$), respectively (Table 1).

3.5.4. Analytical recovery

The mean relative recoveries for TIA at 5, 20, 50, 150 and 300 ng/mL were 84.00 ± 3.76, 90.50 ± 0.88, 90.76 ± 0.38, 90.87 ± 0.14, 91.00 ± 0.11 and 90.80 ± 0.08% ($n = 6$), respectively. The mean relative recovery for GA (IS) at 100 ng/mL was 94.3 ± 0.13% ($n = 6$) (Table 2).

3.5.5. Selectivity

The assay was found to be selective for TIA, and no interfering peaks were observed in the extracts of the different blank or healthy volunteer plasma samples. Potential interferences by common drugs which are administered concurrently with TIA, such as several drugs (antidepressants, muscle relaxant, hemostatic, sweetener, some amine and amino acids) were tested and found not to interfere with TIA and IS retention time. The complete list of these drugs is reported in Table 3.

Table 1
Intra-day ($n = 7$)and inter-day ($n = 9$) precision and accuracy of TIA in plasma

Concentration (ng/mL)		R.S.D. (%)	R.M.E. (%)
Added	Found (mean ± S.D.)		
Intra-day			
5.00	4.60 ± 0.11	2.39	−8.00
150.00	142.50 ± 0.68	0.48	−5.00
300.00	279.00 ± 0.61	0.22	−0.07
Inter-day			
5.00	4.40 ± 0.20	4.55	−12.00
150.00	139.50 ± 0.98	0.70	−7.00
300.00	274.2 ± 0.87	0.31	−8.60

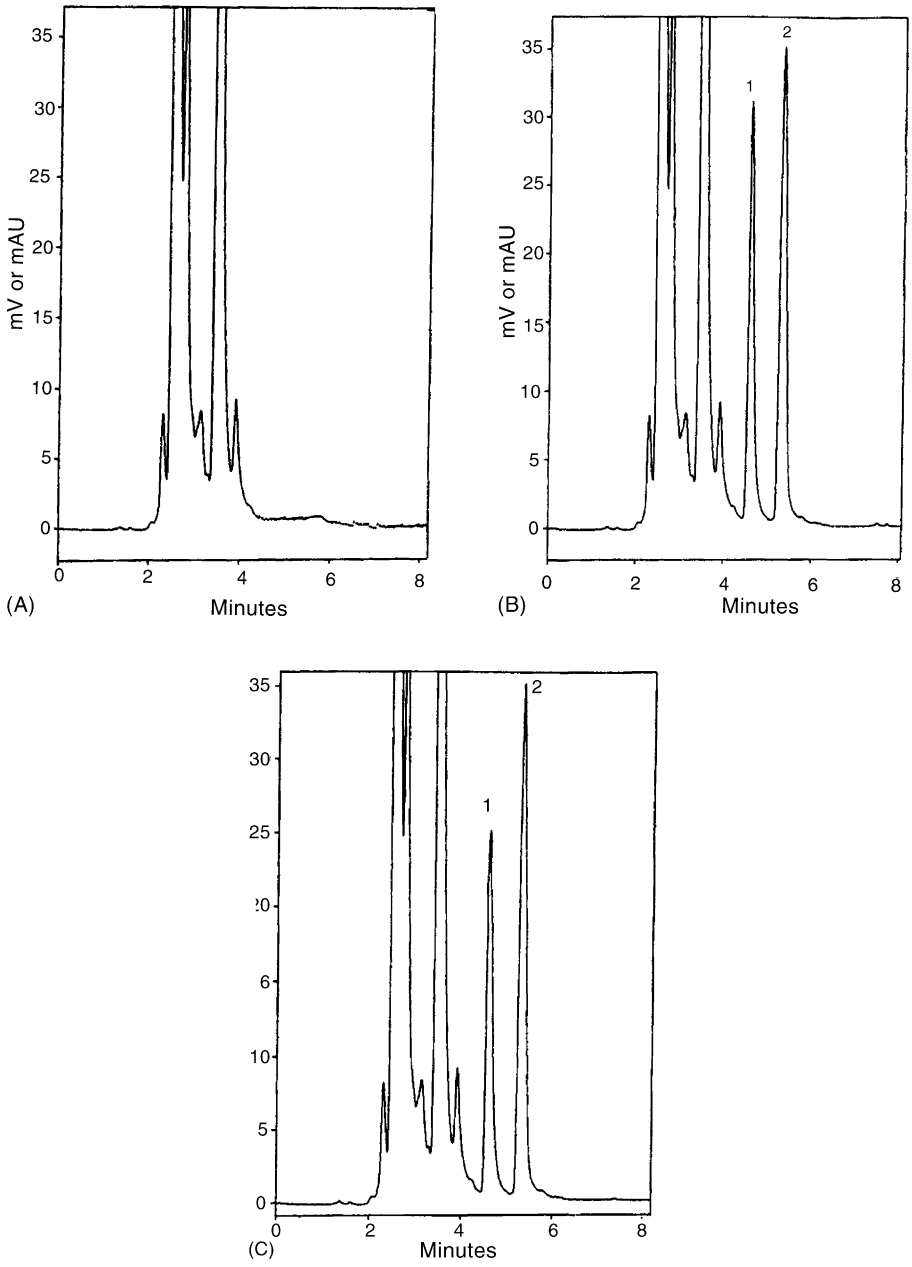

Fig. 6. Chromatogram of (A) blank human plasma, (B) plasma spiked with 100 ng/mL tianeptine100 ng/mL IS, (C) plasma sample obtained at 4.5 h after oral administration of 12.5 mg of tianeptine from a healthy volunteer with 100 ng/mL of the IS.

Table 2
Absolute recovery of TIA from plasma samples ($n = 6$)

Concentration (ng/mL)		Recovery (%)	R.S.D. (%)
Added	Found (mean ± S.D.)		
5.00	4.20 ± 0.158	84.00	3.76
20.00	18.10 ± 0.160	90.50	0.88
50.00	45.38 ± 0.172	90.76	0.38
150.00	136.30 ± 0.187	90.87	0.14
200.00	182.00 ± 0.200	91.00	0.11
300.00	272.40 ± 0.216	90.80	0.08

3.5.6. Stability

The stock solutions were stable for at least 2 months when stored at 4 °C. The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior to the analysis. Long-term stability studies showed no significant degration of TIA in plasma samples stored at −20 °C and at 2–4 months. Mean TIA concentrations ranged from 97.8–101.6% for plasma. Freeze–thawed stability of these plasma was assessed over three cycles: samples were frozen, thawed and analyzed three times; average stability results ranged from 98.1 to 100.9% for plasma.

The TIA-NBD derivative was stable in the ethyl acetate for at least 1 month at 4 °C in the dark. GA-NBD derivative was stable in the ethyl acetate for at least 25 days at 4 °C in the dark.

Table 3
Drugs tested for interference

Drugs	Retention time (min)
Fluoxetine	n.d.
Sertraline	n.d.
Desipramine	n.d.
Nortriptyline	n.d.
Tranylcypromine	n.d.
Baclofen	n.d.
Tranexamic acid	n.d.
Aspartam	n.d.
Amine and amino acids	n.d.

n.d., not detected within a 10-min chromarographic run.

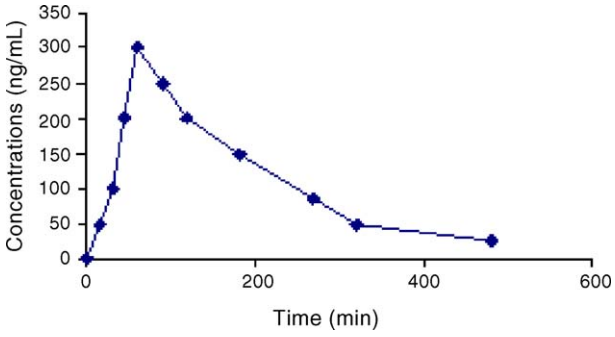

Fig. 7. Plasma concentration–time profile of tianeptine in a healthy volunteer after a single oral administration of 12.5 mg.

3.6. Pharmacokinetic study

The proposed HPLC method was also applied to a pharmacokinetic study. In this study, the drug concentrations were calculated from the regression equations of the calibration curves prepared from plasma samples. After a single oral dose administration of 12.5 mg of TIA to a healthy volunteer, a maximum plasma concentration of 297 ng/mL (C_{max}) was reached at 1 h (t_{max}). The elimination half-life of the drug ($t_{1/2}$) and area under the curve (AUC) were found to be 2 h and 871 ng/mL, respectively (Fig. 7). Pharmacokinetic results are in good agreement with that found previously [2].

4. Conclusion

As a conclusion, a new fluorimetric HPLC method which has high reproducibility and sensitivity for the determination of TIA was developed in this study. According to the literature research, TIA has been derivatized with a reagent and determined using a fluorescent detector for the first time. The analysis has a quantification and detection limits of 5 and 2 ng/mL, respectively, which is as good or superior than those obtained by Nicot et al. using HPLC-UV [16]. The proposed method requires as little as 0.5 mL of plasma whereas the sample volume has varied between 1 and 2 mL in previous methods [16,17], which can be advantageous in clinical pharmacokinetic studies. The absolute recovery is better (84.00–91.00%) than those of the studies reported by Nicot et al. [16] and Gaulier et al. [17], in which the recoveries are 64–69 and 71.5–71.8%, respectively. In this study, the recovery percentage of TIA is high; derivatization and extraction processes do not take much time; additionally, short retention time is an advantage. The method developed can well be proposed for both routine pharmaceutical analysis and the observation of the plasma concentrations of TIA and the bioavailability studies.

References

[1] Tianeptine, The Merck Index, 2001, 1679.
[2] M.I. Wilde, P. Benfield, Drugs 49 (1995) 411.
[3] M.I. Wilde, P. Benfield, R. Defrance, C. Marey, A. Kamoun, Clin. Neuropharmacol. 11 (1988) S74.
[4] E. Mocaer, M.C. Rettori, Clin. Neuropharmacol. 11 (1988) S32.
[5] G. Curzon, K.P. Datla, Eur. Psychiatry 8 (1993) 6S.
[6] C. Labrid, J. Moleyre, J.C. Poignant, C. Malen, E. Mocaer, A. Kamoun, Clin. Neuropharmacol. 11 (1988) S21.
[7] E. Mocaer, M.C. Rettori, A. Kamoun, Clin. Neuropharmacol. 11 (1988) S32.
[8] R. Defrance, C. Marey, A. Kamoun, Clin. Neuropharmacol. 11 (1988) S74.
[9] J.P. Olie, J.D. Guelfi, R. Malka, C. Dulcire, A. Kamoun, H. Loo, L'encephale 14 (1988) 379.
[10] R. Zini, D. Morin, C. Salvadori, J.P. Tillement, Br. J. Clin Pharmacol. 29 (1990) 9.
[11] J.E. Ortiz, E. Mocaer, F. Artigas, Eur. J. Pharmacol. 3 (1991) 335.
[12] C. Salvadori, C. Ward, R. Defrance, et al., Fundam. Clin. Pharmacol. 4 (1990) 115.
[13] R.J. Royer, H. Albin, D. Barrucand, et al., Clin. Neuropharmacol. 11 (1988) S90.
[14] E. Onder, U. Tural, T. Aker, Eur. Psychiatry, in press.
[15] M. Ceyhan, H. Kayir, I.T. Uzbay, J. Psychiatric Res. 39 (2005) 191.
[16] G. Nicot, G. Lachatre, C. Gonnet, J. Mallon, E. Mocaer, J. Chromatogr. 381 (1986) 115.
[17] J.M. Gaulier, P. Marquet, E. Lacassie, R. Desroches, G. Lachatre, J. Chromatogr. B 748 (2000) 407.
[18] M.P. Balem, N. Bromet, S. Courte, J. Guillaudeux, C. Voisin, C. Perris, G. Struwe, B. Jannson, Biol. Psychiatry (1981) 589.
[19] E. Novakova, Soud Lek. 49 (2004) 2.