DRUG FORMULATIONS AND CLINICAL METHODS

# Determination of Tianeptine in Tablets by High-Performance Liquid Chromatography with Fluorescence Detection

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**A sensitive and selective high-performance liquid chromatographic method has been developed for the determination of tianeptine (Tia) in tablets. The method is based on derivatization of Tia with 4-chloro-7-nitrobenzofurazan (NBD-Cl). A mobile phase consisting of acetonitrile–10 mM orthophosphoric acid (pH 2.5; 77 + 23) was used at a flow rate of 1 mL/min on a $C_{18}$ column. The Tia-NBD derivative was monitored using a fluorescence detector, with emission set at 520 nm and excitation at 458 nm. Gabapentin was selected as an internal standard. Linear calibration graphs were obtained in the concentration range of 45–300 ng/mL. The lower limit of detection (LOD) was 10 ng/mL at a signal-to-noise ratio of 4. The lower limit of quantitation (LOQ) was 45 ng/mL. The relative standard values for intra- and interday precision were <0.46 and <0.57%, respectively. The recovery of the drug samples ranged between 98.89 and 99.85%. No chromatographic interference from the tablet excipients was found. The proposed method was validated in terms of precision, robustness, recovery, LOD, and LOQ. All the validation parameters were within the acceptance range. The proposed method was applied for the determination of Tia in commercially available tablets. The results were compared with those obtained by an ultraviolet spectrophotometric method using *t*- and *F*-tests.**

Tianeptine (Tia;7-[(3-chloro-6,11]-dihydro-6-methyl-dibenzo[c,f][1,2]thiazepin-11-yl)amino]heptanoic acid *S,S*-dioxide; Figure 1; 1) is a novel and tricyclic effective antidepressant agent (2–5) that belongs to the class of antidepressants and is chemically related to amineptine. It is a serotonin reuptake accelerator and works opposite to the action of the selective serotonin reuptake inhibitors (SSRIs) and in contrast with most tricyclic antidepressant agents. Tia does not appear to be associated with adverse cognitive, psychomotor, sleep, cardiovascular, or bodyweight effects and has a low propensity for abuse. The antidepressant and anxiolytic properties of Tia and its action on somatic complaints make this medicine particularly suitable for the treatment of the entire range of depressive symptomatology (6).

Articles on the determination of Tia in tablets were not found in the literature. Tia has been determined in biological fluids by high-performance liquid chromatography (LC; 7, 8) and gas chromatography (9) methods. Novakova (10) reported a thin-layer chromatography method for detection in human urine, and Ulu (11) studied fluorescence detection for determination in human plasma. This report describes a sensitive, reproducible, accurate, robust, and specific LC procedure with fluorescence detection for determining Tia in tablets by means of the derivative formed with 4-chloro-7-nitrobenzofurazan (NBD-Cl).

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## Experimental

### *Chemicals*

Tia and gabapentin [Ga; internal standard (IS)] were gifts from Servier (Istanbul, Turkey) and Pfizer (Istanbul, Turkey), respectively. Tia 12.5 mg tablets (Stablon®) were obtained from Servier. NBD-Cl and other chemicals were purchased from Merck (Darmstadt, Germany). All reagents used were analytical grade except acetonitrile, which was LC grade. Water was purified for LC with an aquaMAX™ Ultra purification system (Young Lin Instrument Co., Anyang, Republic of South Korea).

### *Instrumentation*

A Thermo Separation Products liquid chromatograph, consisting of a Model P solvent delivery system, and Rheodyne injection system with a loop of 20 µL was used. A 3000 fluorescence detector (Thermo Separation)was set in an excitation wavelength of 458 nm and an emission wavelength of 520 nm. Chromatograms were recorded by means of a computer. Data acquisition was performed using SN 4000 chromatography software from Thermo Separation.

Separation was performed on a Phenomenex (Torrance, CA) Luna $C_{18}$ column (250 mm × 4.6 mm id, 5 µm particle size) with a guard column (4 mm × 3 mm id) packed with the same material. A mobile phase consisting of acetonitrile–10 mM orthophosphoric acid (pH 2.5; 77 + 23) was used. The mobile phase was delivered isocratically at a flow rate of 1 mL/min, and the injection volume was 20 µL. The total analysis time was 11 min.

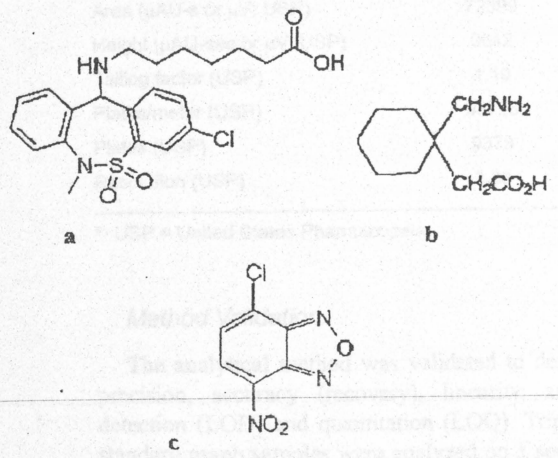


**Figure 1. Chemical structures of tianeptine (a), gabapentin (internal standard; b), and 4-chloro-7-nitrobenzofurazan (c).**

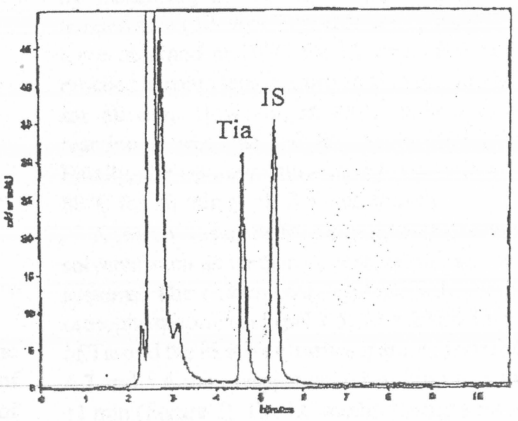


**Figure 2. Liquid chromatogram of 150 ng/mL Tia derivative (retention time 4.7 min) and 75 ng/mL internal standard derivative (retention time 5.4 min).**

### *Standard Solutions*

(a) *LC method.*—Stock solutions of Tia were prepared by dissolving the compound in water at a concentration of 0.1 mg/mL. The standard solution (1 μg/mL) was prepared by dilution of the stock solution with water.

(b) *Ultraviolet (UV) spectrophotometry method.*—100 mg Tia was accurately weighed and dissolved in 100 mL water. A 10 mL volume of the solution was adjusted to 100 mL with water to prepare the stock solution. Standard solutions were obtained by diluting the stock solution for the preparation of calibration curves in the concentration range of 10–60 μg/mL.

### *Sample Solutions*

(a) *LC method.*—Ten tablets were accurately weighed and finely powdered. Powder equivalent to about 10 mg Tia was accurately weighed and transferred into a 100 mL volumetric flask, and 50 mL water was added and the flask was shaken mechanically for 30 min. The volume was made up with water, mixed, and filtered. The filtrate was diluted to obtain appropriate concentrations (similar to standard working solutions, 1 μg/mL).

(b) *UV spectrophotometry method.*—Tablet powder equivalent to ca 100 mg Tia was accurately weighed and transferred into a 100 mL volumetric flask; 50 mL water was added and the flask was shaken mechanically for 30 min. The volume was diluted with water, mixed, and filtered. A 10 mL volume of the filtrate was adjusted to 100 mL with water in a volumetric flask. For UV spectrophotometric method, a 3 mL aliquot of the resulting solution was transferred to a 10 mL volumetric flask and the volume was adjusted with water. The absorbance of Tia was measured at 274.3 nm wavelength.

### *Internal Standard*

The IS solution was prepared at a final concentration of 1 μg/mL. The IS working solution was prepared at 0.01 mg/mL.

### *Reagent Solution*

NBD-Cl was freshly prepared at $2.7 \times 10^{-2}$ M in methanol.

### *Buffer Solution*

A borate buffer (0.1 M) was prepared by dissolving 0.620 g boric acid and 0.750 g potassium chloride in 100 mL water. The pH was adjusted to 8.5 with 0.1 M NaOH solution.

### *Assay Procedure*

To a set of 12 mL volumetric flasks, increasing volumes from the standard solution of the Tia were quantitatively transferred so as to contain the drug within the concentration range 45–300 ng/mL. Next, 75 μL IS and 100 μL buffer and NBD-Cl solutions were added, and the reaction mixture was kept at 80°C for 20 min in a water bath. The mixture was cooled and acidified with 250 μL of 0.1 N HCl. The derivative was extracted with 2 × 2.5 mL ethyl acetate, and the organic layer was transferred to a tube. The organic phases were dried on anhydrous sodium sulfate. A 4.5 mL aliquot of the extract was evaporated under nitrogen at 45°C. The residue was then dissolved in 200 μL of the mobile phase. Typically, 20 μL aliquots of this solution are used for determination by LC.

Peak area ratios ($A_{Tia}/A_{IS}$) were plotted against corresponding concentrations in the 45–300 ng/mL concentration range. The amount of Tia in tablets was calculated from this regression equation. The Tia-NBD derivative is stable in the dark at 4°C in ethyl acetate for at least 1 month.

**Table 1. System suitability in Tia determination ($n$ = 6)**

| Parameter | Data | RSD, % |
|---|---|---|
| Retention time, min | 4.71 | 0.95 |
| Capacity factor (USP)[a] | 8.42 | 0.91 |
| Area (μAU-s or μV; USP) | 72399 | |
| Height (μAU-sec or μV; USP) | 9842 | |
| Tailing factor (USP) | 1.19 | 0.94 |
| Plates/meter (USP) | 93728 | |
| Plates (USP) | 9373 | |
| Resolution (USP) | 3.33 | 0.97 |

[a] USP = United States Pharmacopeia.

### *Method Validation*

The analytical method was validated to demonstrate the precision, accuracy (recovery), linearity, and limits of detection (LOD) and quantitation (LOQ). Triplicate sets of standard graph samples were analyzed on 3 separate days to determine the inter- and intraday validation. The recovery was determined by comparing the content of Tia for 3 replicates of the high-, medium-, and low-level samples. The study of robustness was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions for the determination of Tia in tablets. The factors chosen for this study were the wavelength (nm), flow (mL/min), and mobile phase composition.

## Results and Discussion

### *Method Development*

As the derivatization reaction is a typical nucleophilic reaction, the pH of derivatization is an important factor affecting the derivatization efficiency. For Tia, which is a secondary amine, pH 7–10 is the most suitable condition. The results of the pH study indicated that maximum fluorescence was obtained at pH 8.5. The influence of different heating temperatures and times was studied using a water bath. The effect of the reaction time on the reaction course was studied by measuring the corresponding fluorescence at different temperature (50–90°C) for different periods. The best results were obtained at 80°C for 20 min. The reaction does not proceed at room temperature and is not complete even at 50°C for 80 min. However, at 90°C, although the time of the reaction is less, the stability of the derivative goes down. Finally, the optimum fluorescent derivatization condition of 80°C for 20 min at pH 8.5 was chosen.

A search was made for a simple mobile phase using several solvents such as methanol, acetonitrile, and water and buffer systems. The chosen mobile phase was acetonitrile–10 mM orthophosphoric acid (pH 2.5; 77 + 23), with which the peaks of Tia and the IS were clearly separated with retention times of 4.7 and 5.4 min, respectively, resulting in a total run time of 11 min (Figure 2). The LC method using a reversed-phase $C_{18}$ column with this mobile phase at a flow rate of 1 mL/min and ambient temperature yielded the best results.

### *Method Validation*

(a) *System suitability.*—To ascertain the resolution and reproducibility of the LC method, system suitability tests were performed using the working standard solution of Tia. Resolution (Rs), relative standard deviation (RSD), N (theoretical plate number), $k'$ (capacity factor), and T (tailing factor) were measured as the criteria for system suitability testing. The RSD of triplicate injections was 0.95%, mean N was 9373, mean $k'$ was 8.41, mean T was 1.18, and Rs was 3.33. These results are satisfactory compared to the minimum values necessary for an acceptable method: $1 < k' < 10$), Rs >

**Table 2. Intra- and interday precision and accuracy of Tia derivative ($n$ = 6)**

| Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|
| Added | Found | RSD, % | RME, % | Added | Found | RSD, % | RME, % |
| LC method[a] | | | | | | | |
| 45 | 43.5 | 0.46 | –3.33 | 45 | 42.5 | 0.57 | –5.56 |
| 150 | 149.6 | 0.33 | –0.27 | 150 | 149.1 | 0.36 | –0.60 |
| 300 | 297.78 | 0.22 | –0.74 | 300 | 293.3 | 0.25 | –2.23 |
| UV spectrophotometric method[b] | | | | | | | |
| 10 | 9.57 | 0.58 | –4.30 | 10 | 9.48 | 0.62 | –5.20 |
| 35 | 33.60 | 0.46 | –4.00 | 35 | 33.55 | 0.51 | –4.14 |
| 60 | 59.35 | 0.29 | –1.08 | 60 | 59.20 | 0.35 | –1.33 |

[a] Concentration added and found in ng/mL.

[b] Concentration added and found in μg/mL.

**Table 3. Recovery of Tia derivative ($n$ = 6)**

| Added | Found | Recovery, % | RSD, % |
|---|---|---|---|
| | LC method[a] | | |
| 45 | 44.5 | 98.89 | 1.23 |
| 150 | 149.78 | 99.85 | 0.21 |
| 300 | 298.22 | 99.41 | 0.11 |
| | UV spectrophotometric method[b] | | |
| 10 | 9.91 | 99.10 | 1.23 |
| 35 | 34.72 | 99.20 | 0.21 |
| 60 | 59.64 | 99.40 | 0.11 |

[a] Concentration added and found in ng/mL.
[b] Concentration added and found in μg/mL.

2, T < 2, and N > 2000 (Table 1; 12). Also, the system reproducibility was demonstrated: from an individual standard solution of the analytes, 6 replicates were injected onto the column on 5 different days, and the RSD was calculated.

(b) *Quantitation.*—A calibration graph was constructed, and the proposed method was evaluated by its correlation coefficient (r = 0.999). Characteristic parameters for the regression equation $A = aC + b$, where $A$ is the peak area ratio ($A_{Tia}/A_{IS}$) and $C$ the concentration of Tia, obtained by least-squares treatment of the results, confirmed the good linearity of the method developed.

For the UV spectrophotometric method, linear correlation was obtained between the absorbance and concentration of Tia at the range of 10–60 μg/mL. Calibration graphs were obtained with 5 concentrations of the standard solutions ($n$ = 6). Linearity was evaluated by linear regression analysis, which was calculated by the least-squares regression method.

(c) *Accuracy.*—Accuracy was determined at 3 concentration levels within the range of calibration: near the LOQ at 45 ng/mL, in the middle of the calibration range at 150 ng/mL, and at 300 ng/mL for the LC method, and at 10, 35, and 60 μg/mL for the UV spectrophotometric method. The accuracy of the method expressed as relative mean error (RME) was below 0.27%.

(d) *Precision.*—Intraday precision was expressed as a coefficient of variation of 6 repeated measurements of samples at the same 3 concentration levels as in accuracy determination. Interday precision was determined by analyzing the same set of samples on 3 different days. For the determination of interday precision, Tia was analyzed on 4 different days. The intra- and interday RSDs were 0.22–0.46 and 0.25–0.57% for HPLC method respectively, and 0.29–0.58 and 0.35–0.62%, respectively for the UV spectrophotometric method, indicating good precision. The results are presented in Table 2.

(e) *Recovery.*—Recover of Tia wrer found to be 98.89%, 99.85%, and 99.41% for the HPLC method and 99.10, 99.20, and 99.40% for the UV-spectrophotometric method Table 3.

(f) *Linearity.*—The ratio of the peak area of Tia to that of the IS was used for the determination of Tia in tablets. The calibration graphs were linear in the concentration range of 45–300 ng/mL. The regression equation was:

$$A_{Tia}/A_{IS} = 0.017C - 0.001 \text{ with } r = 0.9997$$

where $C$ = concentration of Tia.

For the UV spectrophotometric method, linear correlation was obtained between the absorbance and concentration of Tia at the range of 10–60 μg/mL. The regression equation was:

$$A = 0.018C + 0.0212 \; r = 0.9999$$

More sensitive values of the range of linearity were obtained by these methods than for literature methods (6).

(g) *LOD and LOQ.*—For the HPLC method, the LOD was calculated by the equation:

$$LOD = 3SDa/b$$

where $SDa$ is the standard deviation (SD) of the intercept and $b$ is the slope of the regression line. LOQ was calculated by the equation:

$$LOQ = 10SDa/b$$

where $SDa$ is the SD of the intercept and $b$ is the slope of the regression line (12). LOD was 10 ng/mL at a signal-to-noise ratio of 4, and LOQ was 45 ng/mL. For the UV spectrophotometric method, LOD and LOQ were found to be 2 and 10 μg/mL, respectively.

(h) *Robustness.*—The study of robustness was performed to evaluate the influence of small but deliberate variations in the chromatographic conditions for the determination of Tia in

**Table 4. Robustness data for the proposed method (Tia derivative 100 ng/mL)**

| Conditions | Mean ± SD | RSD, % |
|---|---|---|
| Optimum | 98.70 ± 0.37 | 0.37 |
| Mobile phase | | |
| 78 + 22, v/v | 98.40 ± 0.41 | 0.42 |
| 76 + 24, v/v | 98.20 ± 0.43 | 0.44 |
| Flow rate | | |
| 0.9 mL/min | 98.60 ± 0.42 | 0.43 |
| 0.8 mL/min | 98.50 ± 0.43 | 0.44 |
| Excitation and emission wavelengths | | |
| λex = 459 nm, λem = 521 nm | 98.40 ± 0.38 | 0.39 |
| λex = 457 nm, λem = 519 nm | 98.60 ± 0.39 | 0.40 |

**Table 5. Assay results of tablets containing 12.5 mg Tia**

| Statistical value | LC method | Comparison method |
|---|---|---|
| Mean[a] | 12.45 | 12.39 |
| RSD, % | 0.97 | 0.85 |
| $t^b$ | 0.78 | |
| $F^c$ | 1.30 | |

[a] $n = 6$.
[b] $P = 0.05$.
[c] $F = 5.05$.

tablets. The factors chosen for this study were the wavelength (nm), flow (mL/min), and mobile phase composition. No significant differences could be observed in the results obtained (Table 4).

(i) *Effect of interfering substances.*—A study of some potential interfering substances in the LC determination of Tia was performed by selecting some excipients often used in tablet formulations. Samples containing a fixed amount of the Tia (150 ng/mL) and variable concentrations of excipients were measured. There was no interference from most of the common ingredients, such as mannitol, magnesium stearate, ethyl cellulose, glycerol oleate, sodium carboxymethyl cellulose, silica, talc, titanium dioxide, bicarbonate, or sucrose.

(j) *Pharmaceutical applications.*—The developed method was applied for the determination of Tia in tablets. The results were compared statistically with those obtained by the UV spectrophotometric method using *t*- and *F*-tests. There was no significant difference between the 2 methods in terms of the mean values and SD at the 95% confidence level (Table 5).

## Conclusions

Tia has been determined for the first time in tablets by first derivatizing it with NBD-Cl and then monitoring it with a fluorescence detector. The method showed no interference from the formulation excipients and good resolution between drugs. The proposed method was sensitive, accurate, precise, and robust and can be successfully applied for the quantitative analysis of Tia in pharmaceutical preparations and biological fluids (11). It is a simple and inexpensive method that is suitable for routine quality control of Tia in pharmaceutical tablets.

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