A New Spectrophotometric Method for the Determination of Tianeptine in Tablets Using Ion-Pair Reagents

Sevgi Tatar ULU* and Zeynep AYDOGMUS

Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University; Istanbul 34452, Turkey.
Received January 18, 2008; accepted August 10, 2008; published online October 1, 2008

A new rapid and sensitive procedure assay is proposed for the spectrophotometric determination of tianeptine. The developed method involves formation of colored chloroform extractable ion-pair complexes of tianeptine with bromophenol blue (BPB), bromocresol green (BCG), bromothymol blue (BTB) and methyl orange (MO) in acidic medium. Beer's law is obeyed in the concentration ranges 3.0—12.0, 4.0—16.0, 4.0—14.0 and 2.0—10.0 μg ml^{-1} with BPB, BCG, BTB and MO, respectively. The detection limit of tianeptine was found to be 1.8 μg ml^{-1} for BPB, 2.0 for BCG, 2.0 μg ml^{-1} for BTB and 1.0 μg ml^{-1} for MO. Validation of the method was performed in terms of linearity, limit of detection (LOD), quantification (LOQ), accuracy and precision. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed method. The proposed method has been applied to determination of the examined drugs in pharmaceutical formulations and the results demonstrated that the method is equally accurate, precise, and reproducible as the official method. The *t*-test showed no significant difference at 95% confidence level.

Key words tianeptine; spectrophotometry; ion pair; pharmaceutical preparation; validation

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 an antidepressant agent with a novel neurochemical profile. It increases serotonin (5-hydroxytryptamine; 5-HT) uptake in the brain (in contrast to most antidepressant agents) and reduces stress-induced atrophy of neuronal dendrites. Like the selective serotonin reuptake inhibitors (SSRIs) and in contrast with most tricyclic antidepressant agents, tianeptine does not appear to be associated with adverse cognitive, psychomotor, sleep, cardiovascular or bodyweight effects and has a low propensity for abuse.[2]

Various methods have been reported in the literature for the analysis of Tia including spectrofluorimetry[3,4] and voltametry.[5]

Reverse-phase high performance liquid chromatography/fluorescence detection,[6] UV detection[7,8] and gas chromatography[9] are also reported for determination of Tia from human biological fluids.

According to the literature research,[3—9] Tia has been ion-pair associated with bromophenol blue (BPB), bromocresol green (BCG), bromothymol blue (BTB), methyl orange (MO) and determined by a spectrophotometric method for the first time. The proposed method for tianeptine determination has many advantages over other analytical methods due to its rapidity, lower cost and environmental safety. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and is not costly. All statistical values (percentage recoveries, RSD, confidence limits of the slope and intercept, LOD and LOQ) were within the acceptable limits. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. The proposed method reports a new for the determination of Tia in pharmaceuticals.

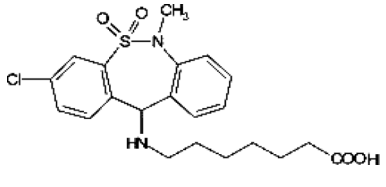

Fig. 1. Molecular Structure of Tianeptine

The present paper thus describes fast, simple, sensitive and validated spectrophotometric method for the determination of Tia.

Experimental

Materials Tia and 12.5 mg tablets (Stablon®) were gifts from Servier (Istanbul, Turkey). BPB, BCG, BTB, and MO were purchased from Merck (Darmstadt, Germany). All chemicals and solvents used were of analytical and pharmaceutical grades. Water was always distilled.

Solutions A stock solution of 1.0 mg ml^{-1} was prepared by dissolving 100 mg of Tia in 100 ml of water. Working standard solutions of Tia of 100 μg ml^{-1} were prepared by appropriate dilution of the stock solution with water and were stored 4 °C. A 0.02% (w/v) of BPB, BCG, BTB and MO solutions was prepared by dissolving the accurately weighed amount of 20 mg in 100 ml of distilled water.

Phthalate buffer was prepared by dissolving 2.042 g of potassium hydrogen phthalate in 50 ml of water. The pH was adjusted to 3.0 with 0.2 M HCl solution and the volume was made up to 200 ml with water.

Apparatus A Shimadzu UV-160A UV–VIS spectrophotometer with 1 cm quartz cells was used. UV–Visible spectra were automatically obtained by Shimadzu UV-160A system software.

Methods. General Procedure Into a series of 15 ml glass tubes 1 ml buffer solution of pH 3.0 and 1 ml of BPB (BCG, BTB or MO) was added to 1 ml of Tia (100 μg ml^{-1}) solution and mixed well. After 3 min vortexing the tubes were allowed to separate the two layers. The Tia–BPB, Tia–BCG, Tia–BTB and Tia–MO complexes were extracted three times with 3 ml of chloroform. After the phases had been separated by centrifugation, the combined extracts were adjusted to 10 ml with the same solvent. Then the blank experiment was made in which only buffer, reagent and chloroform without Tia solution was used. The absorbance of the organic phase was measured at 411, 414, 415 and 424 nm for Tia–BPB, Tia–BCG, Tia–BTB and Tia–MO complexes, respectively.

Assay Procedure for Tablets Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of Tia was weighed into a 100 ml volumetric flask, 30 ml water was then added and shaken thoroughly for about 15 min. The volume was diluted to the mark with water, mixed well and filtered. An aliquot of 10 ml of this solution was transferred to a 100 ml volumetric flask and water added to make up the volume to produce a final concentration of 100 μg ml^{-1}. Thereafter, the general procedure was followed.

Stoichiometric Relationship Job's method of continuous variation[10] of equimolar solutions was employed: a 2.0×10^{-5} M standard solution of drug

* To whom correspondence should be addressed. e-mail: sevgitatar@yahoo.com

base and 2.0×10^{-5} M solution of BPB, BCG, BTB and MO, respectively, were used.

Method Validation The analytical method was validated according the International Conference for Harmonization (ICH) guidelines[11,12] under the optimized experimental conditions: linearity, accuracy, precision, specificity and stability of ion pair complex.

Linearity The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method.

The Limit of Detection (LOD) and Quantification (LOQ) The limit of detection was calculated by LOD$=3.3\sigma/S$, where σ is the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines and S is the slope of the calibration curve. The limit of quantification was calculated by LOQ$=10\sigma/S$ under the ICH guidelines.[11,12]

Precision and Accuracy In order to assess the intra- and inter-day precision and accuracy of the assay, ion pair complexes were prepared as described above. The inter-day precision and accuracy were determined by analyzing the three level samples on 3 different days. Precision was expressed as the relative standard deviation (RSD %). Accuracy was expressed as the mean relative error (RME %).

Recovery The recovery method was performed by adding known amounts of the studied compounds to a known concentration of the commercial pharmaceutical tablets (standard addition method).

Specificity A study of some potential interfering substances in the spectrophotometric determination of Tia was performed by selecting them as the excipients (lactose, glucose, saccarose, magnesium stearate) often used in table formulations.

Results and Discussion

Absorption Spectra The absorption spectra of the reaction product between Tia (containing amino group) and BPB, BCG, BTB and MO reagents in acidic medium is shown in Fig. 2. The absorption spectra of the ion-pairs extracted in chloroform show a maximum at 411, 414, 415 and 424 nm for Tia–BPB, Tia–BCG, Tia–BTB and Tia–MO complexes, respectively.

Effect of pH The effect of pH on the drug–reagent complex was investigated over the pH range 2.5—4.5 using different types of buffer solutions (acetate, borate, phosphate and phthalate). Phthalate buffer (pH=3) solution is the best for complex formation to obtain the highest absorbance value in addition to the stability of the colour without affecting the absorbance (Fig. 3).

Effect of Solvents The solvents studied were water, methanol, ethanol, acetonitrile, acetone, chloroform and dichloromethane. Solvents like *n*-butanol and benzene cannot be used for extraction of the ion-pairs formed, while 1,2-dichloroethane, methylene chloride and chloroform can extract those ion pairs quantitatively. Experimental results indicated that chloroform gave the maximum and stable absorbance for studied drugs.

Effect of Reagent Concentration When the general procedure was followed with varied amounts of 0.02% (w/v) reagent concentration, maximum and constant absorbance was obtained with 1.0 ml for Tia-complex with BTB, BCG, BPB and MO.

Effect of Reaction Time and Temperature The optimum reaction time was investigated by following the colour development at ambient temperature (25±2 °C). Complete colour intensity was attained after 3 min of mixing for all complexes.

Stoichiometric Relationship The molar ratio of the drug to reagent in the complex formed was investigated by Job's method of continuous variations which was found to be 1 : 1 (Fig. 4).[10]

Method Validation Calibration graphs were constructed by measuring the absorbance at five concentration levels which showed linear response of absorbance in relation to concentration of Tia over the range of 3.0—12.0, 4.0—16.0,

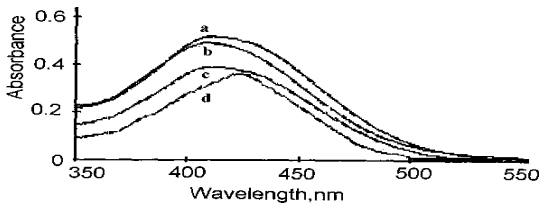

Fig. 2. Absorption Spectra of: (a) Tia–BPB Complex (8.0 μg ml^{-1}); (c) Tia–BCG Complex (6.0 μg ml^{-1}); (b) Tia–BTB Complex (10.0 μg ml^{-1}); (d) Tia–MO Complex (4.0 μg ml^{-1})

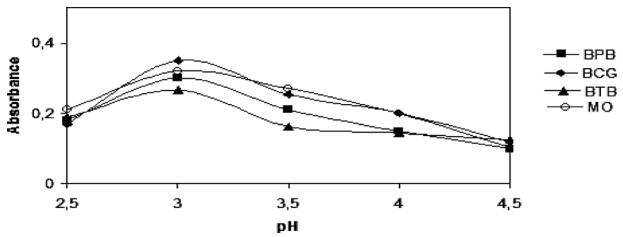

Fig. 3. Effect of pH on the Absorbance of Tianeptine
—■— BPB; —◇— BCG; —▲— BTB; —○— MO.

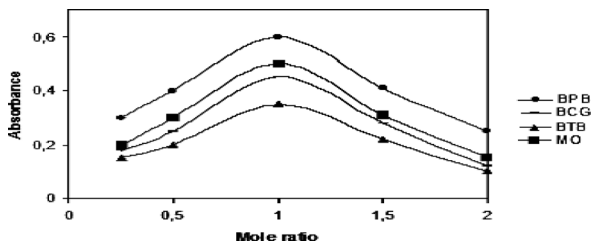

Fig. 4. Job's Method of Continuous Variation of Tianeptine–Dyes
—●— Tia–BPB; —◆— Tia–BCG; —▲— Tia–BTB and —■— Tia–MO.

Table 1. Analytical Parameters for the Determination of Tia Using the Proposed Method[a]

Parameter	BPB	BCG	BTB	MO
λ_{max} (nm)	411	414	415	424
Concentration range (μg ml^{-1})	3.0—12.0	4.0—16.0	4.0—14.0	2.0—10.0
Linear regression equation $A=mC+b$				
Regression equation				
Intercept (b)	1.27×10^{-3}	0.029	0.016	2.87×10^{-3}
Slope (m)	0.066	0.053	0.052	0.029
Correlation coefficient (r)	0.9999	0.9989	0.9999	0.9989

a) $n=4$.

4.0—14.0 and 2.0—10.0 $\mu g\,ml^{-1}$ for BPB, BCG, BTB and MO methods, respectively; this is good to that reported in another paper.[5)]

For the BPB method, the regression equation was $A=0.066C+1.27\times10^{-3}$ with $r=0.9999$. The limit of quantification (LOQ) was found to be 3.0 $\mu g\,ml^{-1}$. The limit of detection (LOD) was 1.8 $\mu g\,ml^{-1}$. For the BCG method, the regression equation was $A=0.053C+0.029$ with $r=0.9989$. The LOQ was found to be 4.0 $\mu g\,ml^{-1}$. The LOD was 2.0 $\mu g\,ml^{-1}$. For the BTB method, the regression equation was $A=0.052C+0.016$ with $r=0.9999$. The LOQ was found to be 4.0 $\mu g\,ml^{-1}$. The LOD was 2.0 $\mu g\,ml^{-1}$. For the MO method, the regression equation was $A=0.029C+2.87\times10^{-3}$ with $r=0.9999$. The LOQ was 2.0 $\mu g\,ml^{-1}$. The LOD was 1.0 $\mu g\,ml^{-1}$ (Table 1).

Table 2. Intra-day and Inter-day Precision and Accuracy of Tia Proposed Methods[a)]

Method	Added concentration ($\mu g\,ml^{-1}$)	Found concentration ($\mu g\,ml^{-1}$)	RME (%)	RSD (%)
Intra day				
BPB	3.0	3.04	1.33	0.48
	6.0	6.05	0.83	0.35
	9.0	9.03	0.33	0.28
	12.0	12.02	0.17	0.22
BCG	4.0	4.03	0.75	0.56
	6.0	6.05	0.83	0.42
	8.0	8.05	0.63	0.38
	16.0	16.05	0.31	0.25
BTB	4.0	4.10	2.50	0.65
	6.0	6.08	1.33	0.54
	8.0	8.07	0.88	0.45
	14.0	14.05	0.36	0.34
MO	2.0	2.20	10.0	0.58
	4.0	4.12	3.00	0.50
	6.0	6.07	1.17	0.46
	10.0	10.06	0.60	0.39
Inter day				
BPB	3.0	3.10	3.33	0.65
	6.0	6.09	1.50	0.46
	9.0	9.12	1.33	0.42
	12.0	12.08	0.67	0.35
BCG	4.0	4.09	2.25	0.68
	6.0	6.10	1.67	0.55
	8.0	8.12	1.50	0.48
	16.0	16.09	0.56	0.32
BTB	4.0	4.23	5.75	0.72
	6.0	6.14	2.33	0.66
	8.0	8.12	1.50	0.52
	14.0	14.09	0.64	0.45
MO	2.0	2.28	14.0	0.67
	4.0	4.34	8.50	0.53
	6.0	6.13	2.17	0.58
	10.0	10.11	1.10	0.46

a) $n=6$.

Accuracy and precision was determined from Tia samples at three different concentrations in the calibration range in six replicates. The relative standard deviation (RSD) on the absorbance from three replicate solutions was found to be between 0.22 and 0.72%. The data is summarized in Table 2.

The recoveries of the standard addition method (Table 3) suggested that high accuracy of the proposed methods. Recoveries were found to be between % 98.67 and 100.23, better than those reported in other paper.[5)]

A stability study of the colored ion-pair complex was developed and showed that yellow color was stable for 8 h at room temperature.

A study of some potential interfering substances in the spectrophotometric determination of Tia was performed by selecting some excipients often used in table formulations. There was no interference from most of the common ingredients such as lactose, glucose, saccarose, and magnesium stearate It was shown that these compounds do not interfere with the proposed method.

Analysis of Pharmaceutical Formulations The developed method was applied for the determination of Tia in tablets. The results were compared statistically with those obtained by the official method[13)] using *t*- and *F*-tests. There was no significant difference between the two methods in the respect of mean values and standard deviations at 95 % con-

Table 3. Results of Recovery Studies by Standard Addition Method[a)]

Added concentration ($\mu g\,ml^{-1}$)	Recovery±RSD %
BPB	
3.00	98.87±033
6.00	98.82±0.27
9.00	99.11±0.21
12.00	99.42±0.39
BCG	
4.00	98.67±0.24
6.00	98.88±0.19
6.00	98.92±0.14
16.00	99.12±0.11
BTB	
4.00	100.02±0.65
6.00	100.13±0.57
8.00	100.17±0.48
14.00	100.23±0.32
MO	
3.00	100.1±0.71
4.00	99.43±0.68
6.00	99.24±0.56
10.00	99.48±0.51

a) $n=6$.

Table 4. Determination of Tianeptine in Pharmaceutical Preparations (Stablon 12.5 mg)

Statistical value	Proposed methods				Official method[13)]
	BPB	BCG	BTB	MO	
Mean	12.43	12.37	12.41	12.45	12.44
Recovery (%)	99.44	99.12	99.28	99.60	99.52
RSD (%)	0.43	0.39	0.42	0.53	0.34
t-test of significance[a)]	1.66	1.32	1.47	1.21	
F-test of significance[a)]	1.60	1.31	1.54	2.40	

a) $n=6$, $p=0.05$, $t=2.23$, $F=5.05$

fidence level.

Conclusion

The proposed method for Tia determination has many advantages over other analytical methods due to its rapidity, lower cost, instrumental simplicity, environmental safety and better sensitivity.

The method can be successfully employed for Tia quantification in all types of pharmaceutical preparations and liquid samples, such as urine, serum, plasma.[6)]

Acknowledgment This work was supported by the Research Fund of the University of Istanbul (project number 595/15122006).

References

1) O'neil M. J., Smith A., Heckelman P. E., Obenchain J. R., Gallipeau J. A. R., D'Arecca M. A., "Merck Index," 13th ed., Merck Co., Inc., 2001, p. 1679.
2) Wagstaff A. J., Ormrod D., Spencer C. M., *CNS Drugs*, **15**, 231—259 (2001).
3) Bulaceanu-Mac-Nair M., Aaron J. J., Prognon P., Mahuzier G., *Analyst*, **123**, 2267—2270 (1998).
4) Dikici E., Deo S. K., Daunert S., *Anal. Chem. Acta*, **500**, 237—245 (2003).
5) Gazy A. A., Mahgoub H., Khamis E. F., Youssef R. M., El-Sayed M. A., *J. Pharm. Biomed. Anal.*, **41**, 1157—1163 (2006).
6) Ulu S. T., *J. Chromatogr. B*, **834**, 62—67 (2006).
7) Gaulier J. M., Marquet P., Lacassie E., Desroches R., Lachatre G., *J. Chromatogr. B*, **748**, 407—414 (2000).
8) Nicot G., Lachatre G., Gonnet C., Mallon J., Mocaer E., *J. Chromatogr. Biomed. Appl.*, **54**, 115—126 (1986).
9) Nicot G., Lachatre G., Gonnet C., Valette J. P., Merle L., Nouaille Y., Bromet N., *J. Chromatogr. Biomed. Appl.*, **31**, 279—290 (1984).
10) Job P., *Ann. Chim.*, **9**, 113—203 (1928).
11) Validation of Analytical Procedures, Methodology ICH Harmonised Tripartite Guideline, Having Reached Step 4 of the ICH Process at the ICH Steering Committee meeting on November 6, 1996.
12) Shabir G. A., *J. Chromatogr. A*, **987**, 57—66 (2003).
13) European Directorate for the Quality of Medicines (*EDQM*) of the Council of Europe, "European Pharmacopeia," 5th ed., Strasbourg, 2005, p. 2575.