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Method Development and Validation for the Estimation of Related Compounds in Tianeptine Sodium Tablets by RP-HPLC

Dasi Shalem Raju*, Karavadi Thejomoorthy and Ch. Saibabu

Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Kanumalla, Singarayakonda-523101

ABSTRACT

The purpose of this work is to develop a sensitive, selective and validated HPLC method for the estimation of Related Compounds in Tianeptine Sodium in its tablet form. Tianeptine with concentration 0.002mg/ml has a maximum absorbance at 220nm. Stock solutions of related compounds were prepared with a concentration of 0.1mg/ml in diluent. Mobile Phase A was prepared by mixing 210ml Methanol, 315ml ACN, 475ml Buffer and mobile phase B was prepared by mixing 200ml Methanol, 200ml Buffer and 600ml ACN. pH of the mobile phase was adjusted to 2.5 with Orhto Phosphoric Acid. Column used was Nucleosil C_{18} , 150X4.6mm , 3µ and flow rate was 1.0ml/min. The effluents were Tia Alcohol, Tia Ketone, Tia Imine, Tianeptine Sodium and Tia Methyl Ester. The effluents were monitored at 220nm using UV detector. The method was validated for System suitability, Specificity, LOD, LOQ, Linearity, Accuracy, Precision, Filter study, Ruggedness, Robustness and Solution stability at room temperature (25±5^{0}C).

Keywords: Tianeptine sodium, related substances, method development, validation RP-HPLC

Introduction

Tianeptine, sold under the brand names Stablon, Tatinol, and Coaxil among others, is an atypical tricyclic antidepressant which is used mainly in the treatment of major depressive disorder, although it may also be used to treat anxiety, asthma, and irritable bowel syndrome.

Tianeptine has antidepressant and anxiolytic effects with a relative lack of sedative, anticholinergic, and cardiovascular side effects.[8][12] It has been found to act as an atypical agonist of the μ-opioid receptor with clinically negligible effects on the δ- and κ-opioid receptors. This may explain part of its antidepressant and anxiolytic effects, however, it is thought that tianeptine also modulates glutamate receptors, and this may also explain Tianeptine's antidepressant/anxiolytic effects. The recommended dosage is 1 tablet (Tianeptine Sodium 12.5mg) three times a day (morning, midday and evening) before the main meals of the days. In chronic alcoholics, whether cirrhotic or not, no alteration of dosage is necessary. In subjects aged over 70 years, and in subjects with renal insufficiency, the dosage should be restricted to 2 tablets per day, or according to Physician's advice. It decreases extracellular levels of *Serotonin and e*nhances the mesolimbic release of *Dopamine* and potentiates CNS D2 & D3 receptors. Side effects are Dry mouth, Constipation, Dizziness. It is White or yellowish powder, very hygroscopic. It is freely soluble in water, in methanol and in methylene chloride.

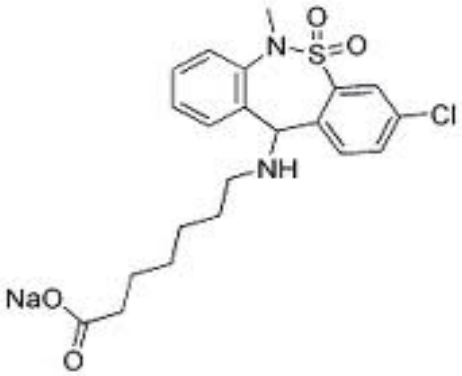

Figure 1: Chemical structure of tianeptine sodium

As per the literature survey RS estimation of tianeptine has been done in tablets, capsules, oral formulation and in human plasma by HPLC, LC-MS-MS. Stability indicating assay and qualitative determination by fluorescent UV techniques has been done to the drug. To propose an accurate and sensitive technique for the quantitative determination of related compounds in tianeptine sodium by using HPLC. Validation of the method was done in accordance with USP and ICH guidelines. The methods were validated for parameters like accuracy, linearity, precision, specificity, ruggedness, robustness, and

system suitability. This proposed method is suitable for the pharmaceutical analysis in analytical laboratories and have been successfully applied to the quantitative analysis in spectrophotometric, chromatographic and electrochemical data.

Materials and methods

Equipment

The Method development and Validation was carried out using Waters Alliance-HPLC system equipped with waters 1525 binary HPLC pump, 2695-separation module connected to 2996-photo diode array detector, and Waters 2707 auto sampler. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents

Tianeptine sodium working standard was kindly given as gift sample by Mylan labs Pvt. Ltd, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Nucleosil C_{18} , 150 X 4.6 mm , 3µ as a column with gradient mode of elution using various compositions of mobile phases A and B. The samples were analyzed using 10 µL injection volume, Flow rate was maintained at 1.0mL/min with runtime of 70 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 220 nm wavelength.

Preparation of Buffer:

2gm of Sodium lauryl sulphate was dissolved in 1000ml of water. Sonicated and mixed well.

Preparation of Mobile Phase A:

A mixture of 210ml Methanol, 315ml ACN and 475ml of Buffer was prepared and pH adjusted to 2.5 with Orthophosphoric acid. The solution was filtered through 0.45µm nylon membrane filter.

Preparation of Mobile Phase B:

A mixture of 200ml Methanol, 200ml Buffer was prepared and pH adjusted to 2.5 with Orthophosphoric acid,600ml ACN was added and the solution was filtered through 0.45µm nylon membrane filter.

Gradient program

Time (min)	Mobile Phase A	Mobile Phase B
0	100	0
35	100	0
45	40	60
60	40	60
70	100	0

Preparation of Tia Alcohol Standard Stock Solution:

Accurately weighed 10mg of Tia Alcohol standard into a 100ml volumetric flask and added 30ml diluent, sonicated and diluted upto the mark with diluent. This solution contained 0.1mg/ml of Tia Alcohol.

Preparation of Tia Ketone Standard Stock Solution:

Accurately weighed 10mg of Tia Ketone standard into a 100ml volumetric flask and added 30ml diluent, sonicated and diluted upto the mark with diluent. This solution contained 0.1mg/ml of Tia Ketone.

Preparation of Tia Imine Standard Stock Solution:

Accurately weighed 10mg of Tia Imine standard into a 100ml volumetric flask and added 30ml diluent, sonicated and diluted upto the mark with diluent. This solution contained 0.1mg/ml of Tia Imine.

Preparation of Tia Methyl Ester Standard Stock Solution:

Accurately weighed 10mg of Tia Methyl Ester standard into a 100ml volumetric flask and added 30ml diluent, sonicated and diluted upto the mark with diluent. This solution contained 0.1mg/ml of Tia Methyl Ester.

Preparation of Standard Stock Solution:

Accurately weighed 50mg Tianeptine Sodium WRS , added 30ml diluent , sonicated and the volume was made upto 5oml with diluent.

Preparation of Diluted Standard Solution:

2ml of the above solution was diluted to 100ml with diluent. Further 5ml of the solution was diluted to 50ml with diluent. This solution contained 0.002mg/ml of Tianeptine Sodium.

Preparation of Placebo Solution :

The placebo powder equivalent to 50mg was weighed, added 30ml diluent and sonicated for 20min with intermittent shaking and diluted to 50ml with diluent. The solution was filtered through 0.45µm nylon membrane filter.

Preparation of Sample Solution:

20 tablets were weighed and crushed. Powder equivalent to 50mg was taken, added 30ml diluent and sonicated for 20min. Diluted to 50ml with diluent and filtered through 0.45µm nylon membrane filter.

Preparation of Spiked Sample Solution:

20 tablets were weighed and crushed. Powder equivalent to 50mg was taken, added 30ml diluent and sonicated for 20min. 2.5ml each of Tia Alcohol, Tia Ketone, Tia Imine and Tia Methyl Ester standard stock solutions were transferred to 50ml volumetric flask and the volume was made to 50ml with diluent.

Table: 1 Chromatographic Conditions

Column	Nucleosil C18, 150X4.6mm, 3µ
Flow	1.0 ml/min
Detector	UV, 220nm
Temperature	30^0C
Injection Volume	10µL
Run Time Program	70 min
Injection Delayed Time	10 min

Table: 2 Gradient Program:

Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	100	0
35	100	0
45	40	60
60	40	60
70	100	0

Results and Discussion

System Suitability:

The % RSD for area of Tianeptine Sodium peak is NMT 10.0 from diluted standard solution. The Theoretical plates of Tianeptine Sodium peak are NLT 2000 from diluted standard solution. The Tailing factor for Tianeptine Sodium peak is NMT 2.0 from diluted standard solution. The result obtained meets the system suitability requirement, which indicates that the system is suitable for analysis.

Table : 3 System Suitability Data

Injections	**Rt**	**Area**	**Theoretical Plates**	**Tailing Factor**
1	25.903	40681	12551	1.0
2	25.830	40625	12788	1.0
3	25.876	40509	12672	1.0
4	25.810	40616	12769	1.0
5	25.850	40770	12750	1.0
6	25.900	40669	12708	1.0
AVG	-	**40645**	-	-

SD	-	**86.29**	-	-
%RSD	-	**0.2**	-	-

Specificity:

From the observation it is clear that all the known impurities of Tianeptine Sodium were adequately resolved from main peak and hence the method is specific for the determination of related compounds of Tianeptine Sodium.

Table : 4 Specificty Data

Name of The Peak	**RT(mins)**	**RRT**	**Peak Purity**
Diluent	-	-	-
Placebo	-	-	-
Tia Alcohol	3.78	0.15	0.9997
Tia Ketone	7.11	0.28	0.9968
Tia Imine I	10.46	0.40	0.9961
Tai Imine II	16.30	0.63	0.9949
Tianeptine Sodium	25.83	1.00	1.0000
Tia Methyl Ester	43.10	1.67	1.0000

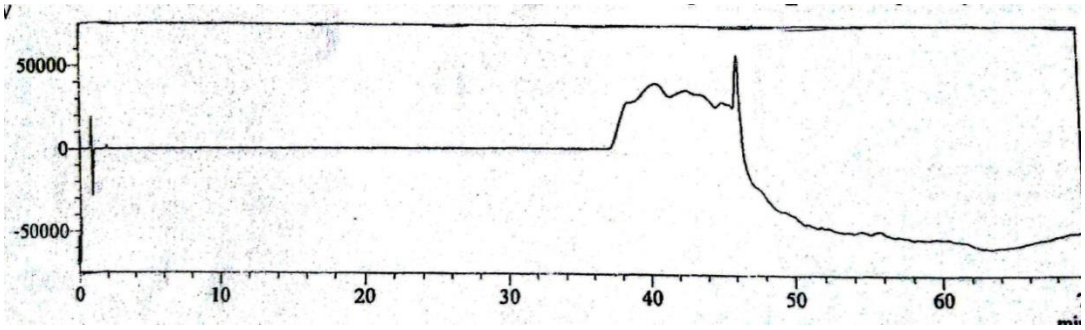

Fig 2 Chromatogram of Blank

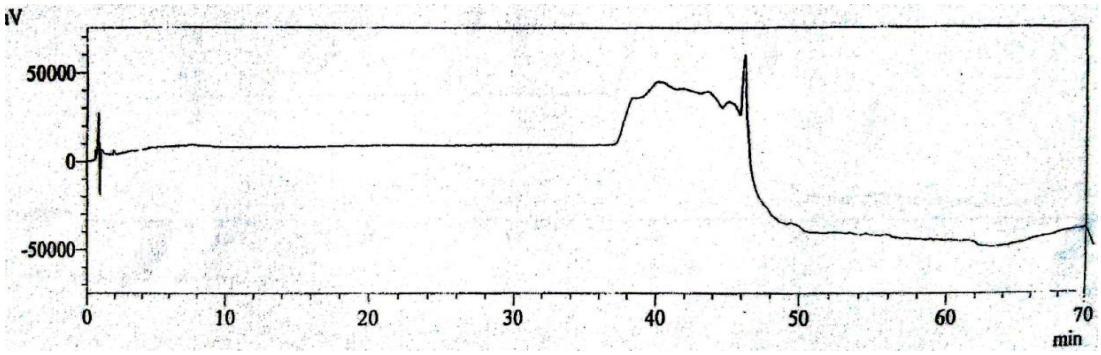

Fig 3 Chromatogram of Blank

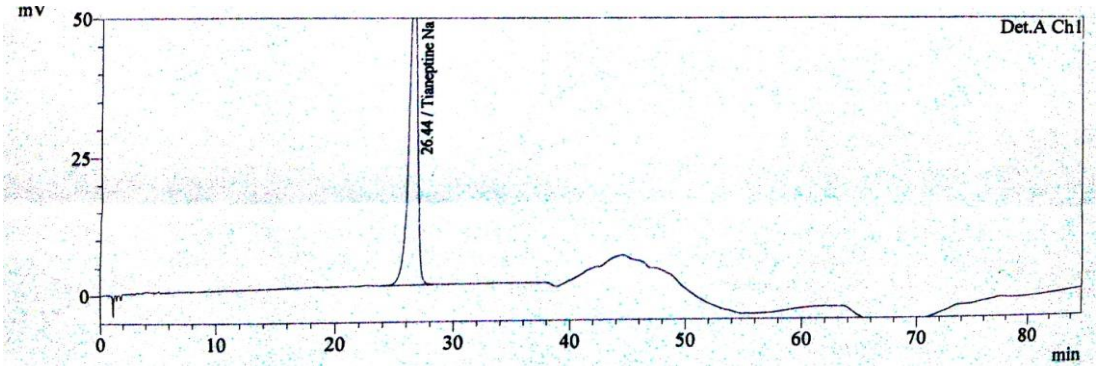

Fig 4 Chromatogram of Standard solution

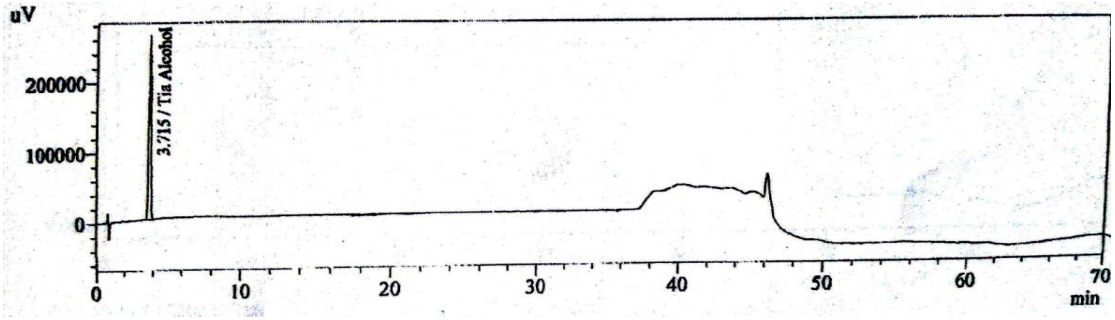

Fig 5 Chromatogram of Tia Alcohol

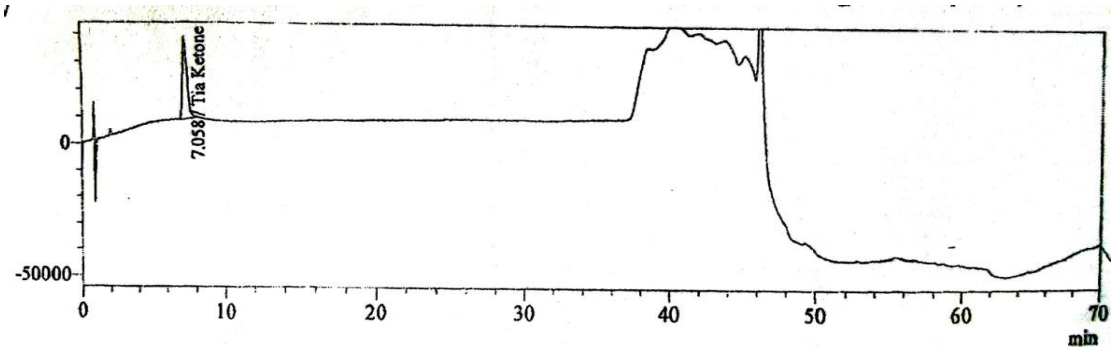

Fig 6 Chromatogram of Tia Ketone

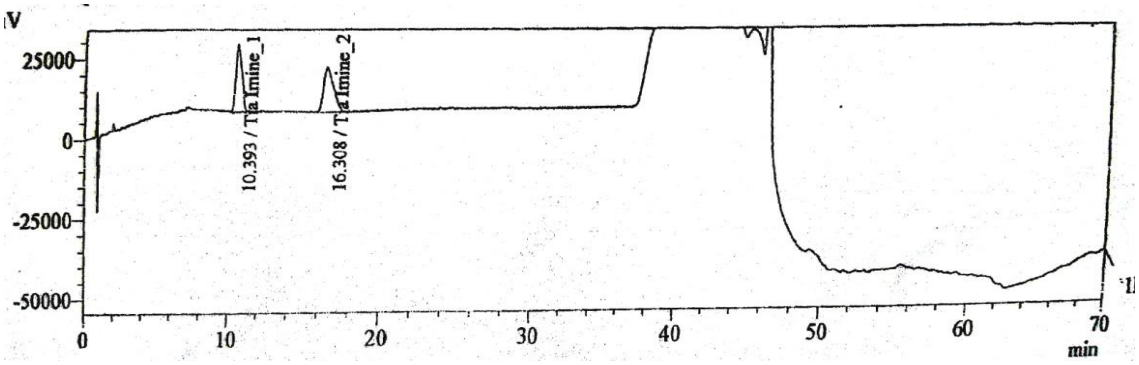

Fig 7 Chromatogram of Tia Imine

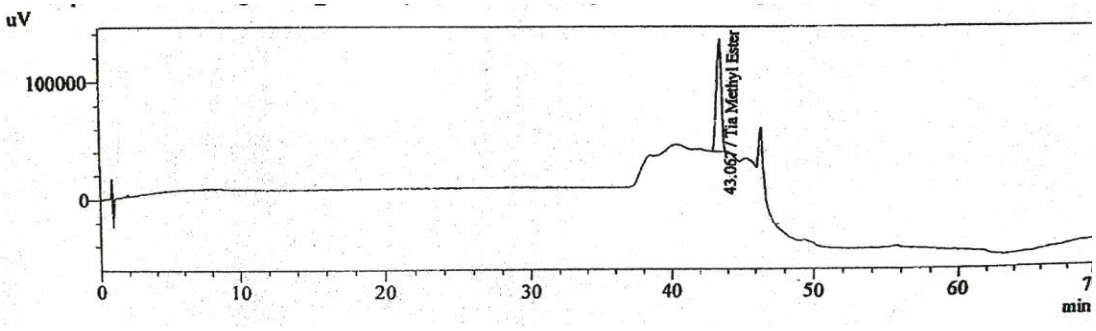

Fig 8 Chromatogram of Tia Methyl Ester

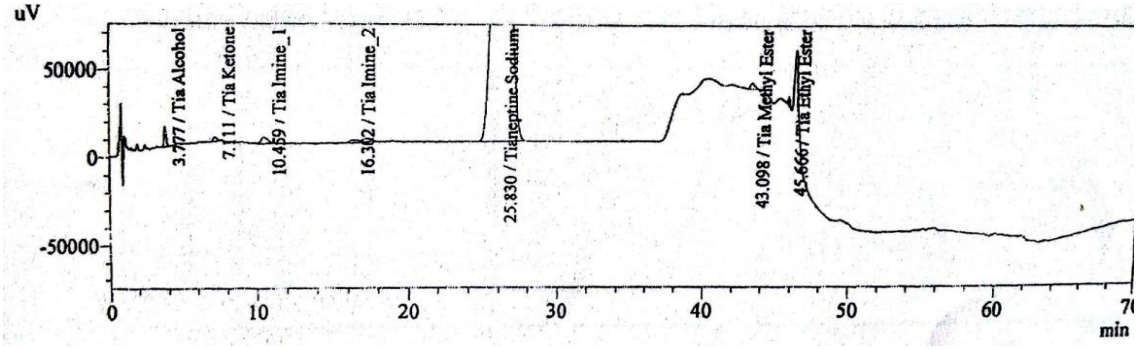

Fig 9 Chromatogram of Spiked Sample

Limit of Detection :

LOD is determined by calculating S/N ratio and by comparing test results from sample with known concentration of analyte with those of blank. Signal to Noise ratio should be about 3:1

Table : 5 LOD Data

Name of The Peak	**%LOD**	**S/N Ratio**
Tia Alcohol	0.1789	2.85
Tia Ketone	0.3709	3.77
Tia Imine	0.9167	4.57
Tianeptine Sodium	3.1251	3.94
Tia Methyl Ester	0.4184	3.74

The LOD values were within the limit for each impurity.

Limit of Quantitation:

LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. Signal to Noise ratio should be about 10:1

Table : 6 LOQ Data

Name of The Peak	**%LOD**	**S/N Ratio**
Tia Alcohol	0.5420	8.95
Tia Ketone	1.1240	8.44
Tia Imine	2.7780	17.29
Tianeptine Sodium	9.4700	10.84
Tia Methyl Ester	1.2680	11.49

The LOQ values obtained for each impurity were within the acceptance criteria.

Linearity and Range

The study proved that the responses for impurity peaks are linear over the range of 50% to 150% of specification limit.

Table: 7 Linearity Data

Tianeptine Sodium		
Level	**Concentration (µg/ml)**	**Peak Area**
50%	1.0076	25690
80%	1.6122	40549
100%	2.0152	47307
120%	2.4183	57415
150%	3.0229	73308
Tia Alcohol		
50%	2.7060	73626
80%	4.3296	113794

100%	5.4120	145503
120%	6.4944	173470
150%	8.1181	214908
Tia ketone		
50%	2.8080	54021
80%	4.4929	81087
100%	5.6161	107310
120%	6.7393	117691
150%	8.4241	143289
Tia Imine		
50%	2.6709	69667
80%	4.2734	125198
100%	5.3418	156236
120%	6.4102	182249
150%	8.0127	234278
Tia Methyl Ester		
50%	2.2644	58950
80%	3.6230	93757
100%	4.5287	115293
120%	5.4344	136186
150%	6.7931	170325

Accuracy

Table: 8 Preparation of accuracy solution

Level	**Sample Wt. equivalent to Tianeptine Sodium(mg)**	**Known Impurity standard stock (ml)**	**Diluted Volume(ml)**
50%	100.0	2.5	100
100%	100.0	5.0	100
150%	100.0	7.5	100

The % recovery values obtained for impurities were in the range of about 83.12-107.62 which are within the specified criteria.

The %RSD of recoveries obtained for impurities were in th range of 0.4%-5.3%

Precision

Table: 9 Method Precision Data

Spiked	**Tia Alcohol**	**Tia Ketone**	**Tia Imine**	**Tia Methyl Ester**
Prep#1	0.52	0.51	0.49	0.48
Prep#2	0.51	0.53	0.49	0.48
Prep#3	0.51	0.53	0.47	0.48
Prep#4	0.51	0.52	0.49	0.47
Prep#5	0.52	0.52	0.49	0.48
Prep#6	0.51	0.52	0.49	0.48
Avg	**0.51**	**0.52**	**0.49**	**0.48**
SD	**0.001**	**0.006**	**0.008**	**0.003**
%RSD	**0.2**	**1.2**	**1.6**	**0.6**

The content of Impurities was found to be in an acceptable RSD of less than 10.0%, which implies that the method is precise.

Robustness

Flow rate changed by ± 0.1ml/min (i.e., 0.9ml/min and 1.1ml/min). Column temperature to be changed to $\pm 5^0$C (i.e., 25^0C and 35^0C). Mobile phase pH changed to ±0.2 (i.e., 2.3 and 2.7).

Table 10: Recovery Values in Robustness

Parameter Condition	**Tia Alcohol**	**Tia Ketone**	**Tia Imine**	**Tia Methyl Ester**
Actual	0.51	0.52	0.49	0.48
Low Flow :0.9ml/min	0.48	0.47	0.49	0.46

High Flow:1.1ml/min	0.48	0.46	0.51	0.47
Low Mobile Phase pH :2.30	0.53	0.50	0.48	0.48
High Mobile Phase pH :2.70	0.52	0.47	0.45	0.49
Low Temperature : 25^0C	0.53	0.53	0.49	0.48
High Temperature : 35^0C	0.56	0.52	0.50	0.48

From the obtained values it is clear that the %RSD was found to be in the range of 0.4% - 2.2% which states the method is acceptable.

Solution Stability

Chromatograph diluted standard solution and spiked sample solution for minimum of 24hrs.**.** Individual impurity stability values should be within ± 0.05% of the original value and total impurities should be within ± 0.10 % from the initial value.

Table: 11 Solution Stability Data

Station	**Tia Alcohol**	**Tia Ketone**	**Tia Imine**	**Tia Methyl Ester**
Initial	0.47	0.42	0.51	0.46
6^{th} Hour	0.48	0.47	0.51	0.47
Difference	0.01	0.04	0.00	0.01
12^{th} Hour	0.48	0.47	0.50	0.42
Difference	0.01	0.04	-0.01	-0.04
18^{th} Hour	0.48	0.51	0.50	0.45
Difference	0.01	0.09	-0.01	-0.01
24^{th} Hour	0.48	0.47	0.50	0.46
Difference	0.01	0.05	-0.01	0.00
30^{th} Hour	0.48	0.47	0.50	0.46
Difference	0.01	0.04	-0.01	0.00

No significant variation in the % of impurities was observed upto 30hrs for spiked sample solution and standard solution. Both the solutions are stable for a period of 30hrs at 25^0C.

Conclusion

The objective of the proposed work was method development for the estimation of related compounds in Tianeptine sodium tablets by RP-HPLC and to validate the developed method according to USP and ICH guidelines and applying the same for use in the quality control samples in pharmaceutical industry. As there is no official method for the estimation of related compounds in tianeptine, so we tried to develop a method by which we can quantify the amount of impurities present in the given sample. The optimum wavelength for detection was 220 nm. The average retention times for Tia Alcohol, Tia Ketone, Tia Imine and Tia Methyl Ester was found to be 3.71 min, 7.05 min, 10.39 min, 43.06min respectively. The correlation coefficient are 0.999, 0.992, 0.998 and 0.999 for Tia Alcohol, Tia Ketone, Tia Imine and Tia Methyl Ester respectively.The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 70-130%. Finally, it can be concluded that the values of formulation were the same as mentioned in the label claim with the RSD of < 10%. The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for the routine analysis of the drugs in tablet dosage forms.

References

1. J.M. Gaulier, P. Marquet, E. Lacassie, R. Desroches, and G. Lachatre. High-performance liquid chromatographic determination of tianeptinein plasma applied to pharmacokinetic studies. J. Chromatogr. B
2. Biomed.Appl. 748: 407–14 (2000). G. Nicot, G. Lachatre, C. Gonnet, J. Mallon, and E. Mocaer. Ion-pair extraction and high-performance liquid chromatographic determination of tianeptine and its metabolites in human plasma, urine and tissues. J. Chromatogr. 381: 115–26 (1986). Impurities in new drug substances.
3. Fed. Regist. 61(3): January 4,1996, p. 372. “European Pharmacopeia”, vol. 2, 5th ed. Strasbourg, Council of Europe, 2005, 2575- 2576.
4. O’ neil M.J., Smith A., Heckelman P.E, Obenchain JR., Gallipeau JAR, D’Arecca MA., “Merck Index” 13 th ed., Merck Co., Inc., 2001, 1679.
5. Hindmarch I. Expanding the horizons of depression: beyond the monoamine hypothesis. Hum Psychopharmacol 2001; 16: 203-218.
6. Oluyomi AO, Datla KP, Curzon G. Effects of the (+) and (-)enantiomers of the antidepressant drug tianeptine on 5-HTPinduced behavior Neuropharmacology 1997; 36: 383-7.

7. R. Defrance, C. Marey, and A. Kamoun. Antidepressant and anxiolytic activities of tianeptine: an overview of clinical trials. Clin. neuropharm 11(suppl 2): S74–82 (1988).

8. M.B. Nair, J.J. Aaron, P. Prognon, and G. Mahuzier. Photochemically induced fluorimetric detection of tianeptine and some of metabolites. Application to pharmaceutical preparation. Analyst. 123: 2267–701998

9. Mantanus, J.& Ziémons, E.& Lebrun, P.& Rozet, E.& Klinkenberg, R.& Streel, B.& Evrard, B.& Hubert, P., "Moisture content determination of pharmaceutical pellets by near infrared spectroscopy: method development and validation", Anal. Chim. Acta, vol. 642, 2009, p.186-192

10. Morilak DA, Frazer A (2004) Antidepressants and brain mono aminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. Int J Neuropsychopharmacol 7:193–218.