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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TIANEPTINE IN API AND TABLET DOSAGE FORM

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Tianeptine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C_{18} (4.6 x 250mm, 5µm) column using a mixture of Water and Methanol (85:15% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 218nm. The retention time of the Tianeptine was 5.430±0.02min respectively. The method produce linear responses in the concentration range of 10-50 µg/ml of Tianeptine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Tianeptine, RP-HPLC, Method development, Validation, Tablet Dosage form.

INTRODUCTION

Tianeptine (TIA) is a tricyclic antidepressant compound of dibenzothiazepine type and neuro protective, anxiolytic and mood-brightening serotonin reuptake enhancer. TIA, designated (RS)-7-[(3-chloro-6, 11-dihydro-6-methyl dibenzo [c, f] [1, 2] thiazepin-11-yl) amino] heptanoic acid S,S-dioxide mono sodium salt1 and has the molecular formula of C21H24ClN2O4SNa. Its molecular weight is 458.93. The drug exists as two isomers, of which the leavo isomer seems to be the therapeutically active form and shows serotonergic activity by enhancing the presynaptic reuptake of serotonin. The drug is official in European Pharmacopoeia 2 and suggests potentiometrictitration method for the determination of TIA in bulk and tablet formulations. The drug is mainly metabolized by the external route; oxidation of its heptanoic side chain is the major metabolic pathway and the pentanoic (MC5) and propionic (MC3) acid side chain derivatives are the major metabolites in urine and plasma, inhibits the mitochondrial oxidation of medium and short chain fatty acids in mice, further displaying therapeutic activity.

Literature survey revealed Spectrophotometric RP-HPLC methods were developed for Tianeptine determination in combination with other drugs. Stability indicating and bioanalytical chromatographic methods have also been done. Detailed survey of literature for Tianeptine revealed few methods have been developed on RP-HPLC for its determination in pharmaceutical. Hence the aim of this paper is to develop a simple, sensitive, accurate and selective method through RP-HPLC for Tianeptine in both bulk and pharmaceutical formulations. The proposed method describes a simple, sensitive accurate and precise RP-HPLC method for the Tianeptine in bulk and in marketed dosage forms.

MATERIALS AND METHODS

Chemical and reagents

Tianeptine (TIA) was procured as a gift sample from Sura labs. Tablet was purchased from the local market (stablon containing Tianeptine250 mg, marketed by Serida Pharmaceuticals .Acetonitrile, methanol, were of HPLC grade and were procured from Merck.A High

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Performance Liquid Chromatographic System (Alliance 2695,with Empower2 software) with Analytical Column Zorbax C_{18} (4.6×250mm) 5µ was used for the analysis. The mobile phase constituted Water: Methanol (85:15% v/v) and the flow rate 1.0ml/min. Detection was performed at 218 nm.

Method development and optimization

Selection of chromatographic method

Proper selection of the method depends upon the nature of sample (ionic/ ionizable/ neutral molecule); its molecular weight and solubility. The reversed phased HPLC was selected because of its simplicity, speed and suitability. The sensitivity of the HPLC method depends upon the proper selection of wavelength. The detection wavelength of 218.0 nm was selected as the drug showed optimal absorbance at that wavelength.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Tianeptine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3 ml of the above Tianeptine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Chromatographic conditions

Chromatographic separation was performed a tambient temperature on a reverse phase Zorbax C_{18} (4.6×250mm) 5µ .The mobile phase used in this analysis consists of a mixture of Water: Methanol (85:15% v/v). The mobile phase was filtered, degassed before use. The flow rate was adjusted to 1ml/min. the detector wavelength was set at 218 nm. The injector volume of the standard and sample was 10 µl. The Optimized chromatographic is shown (Fig No:2)& Table No:4.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofTianeptine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3 ml of Tianeptine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

ASSAY

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalentweight into HPLC system. Calculate the percentage purity of Tianeptine present in tablet.

Analytical method validation

System Suitability

System suitability testing is an integral part of analytical procedures. The purpose of system suitability test is to ensure that the complete testing system is suitable for the intended purpose.

Six injections standard preparation (30 µg/mL for Tianeptine) were injected and checked for the system suitability parameters like resolution, tailing factor, peak purity, theoretical plates. % RSD of six injections was calculated. results are given in Table No:5.

Accuracy

Accuracy is the measure of the closeness of agreement between the values, which is accepted either as a conventional, true value or an accepted reference value and the value found. To document accuracy the ICH guideline on methodology recommends collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range (for example : three concentrations, three replicates each) (Results are reported from Table No:6.

Precision

Precision is measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples

PRECISION

REPEATABILITY

Preparation of Tianeptine Product Solution For Precision

Accurately weigh and transfer 10 mg of Tianeptine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Tianeptine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.(Table No:7.)

Linearity

Linearity is the ability of the method to elicit test results that are directly proportional to the analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be

determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results obtained by the method. The ICH guidelines specify a minimum of five concentration levels, along with certain minimum specified ranges. For assay, the minimum specified range is from 10-50% of the target concentration. results are given in Table No: 8.

Preparation of Calibration Standard Solutions

Accurately weigh and transfer 10 mg of Tianeptine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

From the above solution 0.1, 0.2 0.3, 0.4, 0.5 mL was pipetted in to 10 mL volumetric flasks and make upto volume with diluent such that the final resulted concentrations are 10, 20, 30, 40, 50 µg/mL respectively. System suitability was checked with six replicate injections of standard solution. All standard solutions were injected in duplicate. Correlation coefficient was calculated to check the linearity of analyte in the above concentration range. results are given in graph Fig No:1.

Limit of detection

The limit of detection (LOD) is defined, as the lowest concentration of an analyte in a sample that can be detected, not quantified.results are given in Table No:9.

Limit of quantification

The Limit of Quantification (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. results are given in Table No:9.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic, pH, flow rate, temperature etc., and determining the effect (if any) on the results of the method.

For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions such as

Influence of variations in mobile phase flow rate (±10%)

Effect of Variation of mobile phase organic composition

The results are given in section: shown results from Table No: 10.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Acceptance Criteria

The percentage recovery was found to be within the limit (98-102%).

REPEATABILITY

Obtained Five(5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Linearity

Validation criteria: The response linearity is verified if the Correlation Coefficien

is 0.99 or greater.

Correlation Coefficient (r) is 0.99, and the intercept is 4318. These values meet the validation criteria.

LOD and LOQ

Robustness

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Assay

Optimized chromatogram of Tianeptine

Table 1. List of Drug used

S.no	Drug Name	Formulation	Manufacturer	Puarchased
1	stablon	tablet	Serida Pharmaceuticals	Local pharmacy
2	Tianeptine	-	-	Sura labs

Table 2. List of Instrument used

Sl.No	Instruments	Software	Model	Company
1	HPLC	Empower2	Alliance 2695 separation, 996 PDA detector	Waters
2	pH meter	N/A	AD102U	ADWA
3	Weighing machine	N/A	XEX 200	LabIndia
4	Volumetric flasks	N/A		Borosil
5	Pipettes and Burettes	N/A		Borosil
6	Beakers	N/A		Borosil
7	Digital ultra sonicator	N/A	SE60US	Enertech

Table 3. Chemicals used

S.No	Chemical	Grade	Company
1	Tianeptine	Sura labs	Sura labs
2	Water and Methanol for HPLC	HPLC Grade	Lichrosolv (Merck)
3	Acetonitrile for HPLC	AR	Merck

Table 4. Optimized Chromatogram (Standard)

S.No	Name	R_t	Area	Height	USPTailing	USPPlate Count
1	Tianeptine	5.430	530023	56127	1.03	9118

System Suitability

Table 5. System Performance for Tianeptine

Drug substances	Retention time	USPTailing factor	USPPlateCount
Tianeptine	5.474	1.1	8931.7

Table 6. The accuracy results for Tianeptine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	80848	15	14.74	98.2%	99.3%
100%	146118	30	29.86	99.5%	
150%	212196.3	45	45.16	100.3%	

Table 7. Results of repeatability for Tianeptine

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tianeptine	5.419	507837	54219	8931.7	1.1
2	Tianeptine	5.405	510468	08	8957.7	1.1
3	Tianeptine	5.478	514561	55259	8764.6	1.1
4	Tianeptine	5.466	515381	55552	9037.7	1.1
5	Tianeptine	5.466	516416	55653	8972.4	1.1
Mean			512932.6			
Std.dev			3633.862			
%RSD			0.7			

Table 8. Chromatographic Data For Linearity Study

S.No	Concentrationµg/ml	AveragePeak Area
1	10	182423
2	20	356108
3	30	511715
4	40	678851
5	50	873452

Table 9. LOD and LOQ of Cefuroxime Axetil

Limit of Detection concentration in µg/mL	2.25
Limit of Quantitation concentration in µg/mL	6.8

Table 10. Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0 mL/min	534995	5.453	9124	1.01
Less Flow rate of 0.9 mL/min	566441	5.599	9364	1.02
More Flow rate of 1.1 mL/min	459187	4.576	7559	0.98

More Organic phase	93382	3.827	6274	1.07
Less organic phase	24366	7.415	12009	1.00

Table 11. Assay Resultls ofTianeptine

Formulation	Label Claim (mg)	% of Assay
stablon	12.5	99.0 %

Fig 1. Linearity Graph

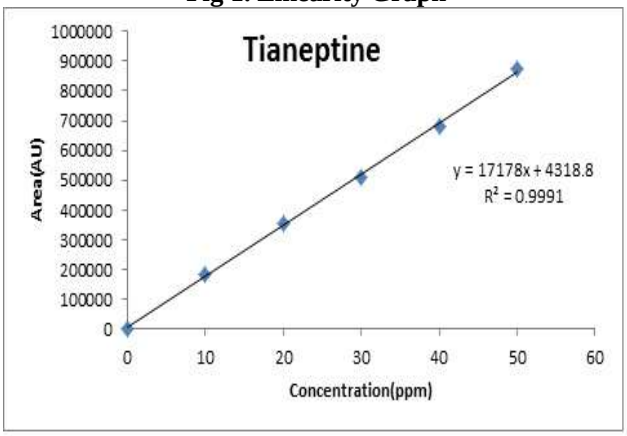
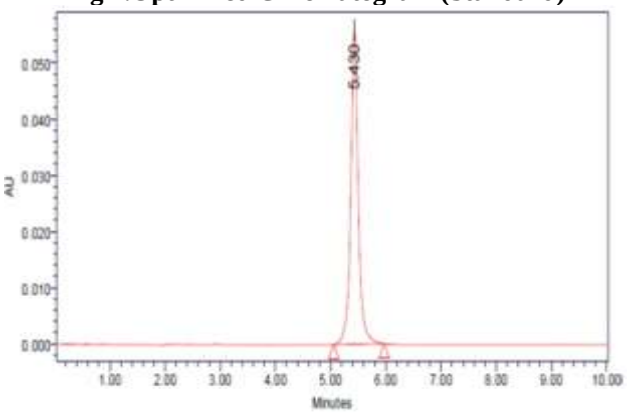


Fig 2.Optimized Chromatogram (Standard)



CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Tianeptine in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective label claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories. Tianeptine was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Methanol(85:15% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

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