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RP - HPTLC METHOD DETERMINATION OF TIANEPTINE IN SOLID DOSAGE FORM

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ABSTRACT

Tianeptine is used as an antidepressant agent. A new rapid, simple, Economical and environmental friendly High-Performance Thin-Layer Chromatography (HPTLC) has been developed and validated for quantitative determination of Tianeptine in marketed formulation. HPTLC separation was performed on aluminium plates precoated with silica gel 60 RP-18F-$_{254}$S as the stationary phase using Chloroform: Methanol (90:10% v/v) as mobile phase. Quantification was achieved by densitometric analysis at 210nm over the concentration range of 200–1200 ng/band. The method was found to give compact and well resolved band for Tianeptine at Retention factor (R_f) 0.70± 0.74. The linear regression analysis data for calibration graph showed good linear relationship with r^2 = 0.999. The method was validated for precision, recovery, robustness, ruggedness and sensitivity as per International conference on Harmonization (ICH) guidelines. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 5.23 ng and 15.85 ng, respectively. The proposed developed RP-HPTLC method can be applied for identification and quantitative determination of Tianeptine in marketed formulation.

Keywords: Tianeptine, RP- HPTLC, ICH, LOD, LOQ.

1.0 Introduction

Depression is a chronic and recurrent psychiatric disorder that affects mental and physical health, and has a significant impact on healthcare resources and costs.1 It is a heterogeneous mental disorder with psychological, behavioural, and also physiological symptom. Tianeptine,4-Chloro-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,belongs to that the class antidepressants and chemically related to the amineptine.[3][4] It is a serotonin reuptake stimulator and works opposite to the action of selective serotonin reuptake inhibitor (SSRIs) and in contrast with most tricyclic antidepressant agents.[5-

7] A sensitive and selective high performance thin layer chromatography method has been developed for the determination of Tianeptine tablet. The method on the basis of derivatisation of Tianeptine.[8-10]

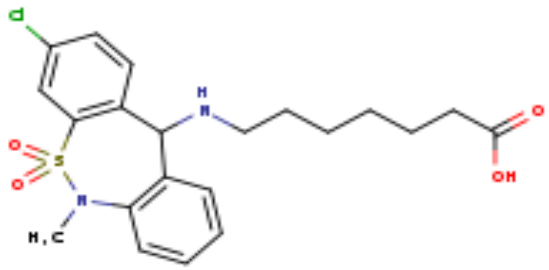

Fig.1 Structure of Tianeptine

Some methods are reported for determination of Tianeptine in pharmaceutical preparations such as Liquid chromatography using prominence fluorescence detector and mobile phase of acetonitrile: orthophosphoric acid(35:65 % v/v) (Patel et al. 2009), HPLC method for determination of Tianeptine using UV–visible detector and mobile phase of buffer : acetonitrile (50:50 % v/v) (Bharati et al., 2010), Stability indicating HPLC method Polarography using a 0.01 M KH2PO4 water solution (pH 4.5) as supporting electrolyte (Gianfranco et al. 2009), Comparison of Microbiological and UV-Spectrophotometric Assays for Determination of Tianeptine in Tablets and HPTLC determination in marketed formulation.

So far no HPTLC method for the analysis of Tianeptine was been reported. Therefore, in the present research paper a simple, accurate, sensitive and precise RP-HPTLC method has been developed for determination of Tianeptine in solid dosage form.

2.0 EXPERIMENTATION

2.1 MATERIALS& METHODOLOGY

Tianeptine was supplied as a gift sample from Glenmark Pharmaceutical LTD, Sinnar, and Nasik, India. All chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

2.2 CHROMATOGRAPHIC CONDITION

The plates were prewashed with methanol. Then the plates were activated at 100^{o}C for 10 min prior to chromatography. The drug standard and samples were spotted in the form of bands of 6 mm width. The bands spotted with a Camag microlitre syringe on precoated silica gel aluminium plates 60 RP-18 F254

S(20 · 10 cm with 200 mm thickness) using a CamagLinomat 5 applicator. The slit dimension was kept at 6.00 · 0.45 mm (micro). The scanning speed 20mm/s was employed. The mobile phase consisted of Chloroform: Methanol (9:1 v/v), and 10 mL of mobile phase was used. Linear ascending development was carried out in a 20 · 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (35 C ± 2). The length of the chromatogram run was approximately 80 mm. After development; the HPTLC plates were dried in a current of air with thehelp of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by winCATSsoftware(Version 1.3.0).

2.3 PREPARATION OF STANDARD SOLUTION AND LINEARITY STUDY

Stock standard solution was prepared by dissolving 12.5 mg of Tianeptine in 10 mL methanol to obtain concentration 1.25mg/mL. The aliquots of standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of Tianeptine were transferred into six separate 10 mL volumetric flasks. And then volumes were made up to the mark using same solvent. An appropriate volume 10 lL was applied with the help of microlitre syringe, using Linomat 5 applicator on RP-HPTLC plate to obtain concentrations of 200,400, 600, 800, 1000 and 1200 ng/band. The standard curves were assessed for within day and day-to-day reproducibility. Each experiment was repeated for six times.

2.4 METHOD VALIDATION

2.4.1 PRECISION

Precision can be performed at two different levels i.e. repeatability and intermediate precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same band (600 ng/band of Tianeptine). The intermediate precision results from the variations such as different days, analysts and equipments. The intra-day variation experiments were studied using three different concentrations over the linearity range within same day. The inter-day variations in the methods were assessed by studying three different concentrations for three different days over a period of week. The intra and inter-day variation for the determination of Tianeptine was done at three different concentration levels of 800, 1000, and 1200 ng/band.

2.4.2 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

In order to determine limit of detection and limit of quantification, Tianeptine concentrations in the lower part of the linear range of the calibration curve were used. Tianeptine solutions of 200, 400, 600, 800, 1000 and 1200 ng/band were prepared and applied on RP-HPTLC plate. The LOD and LOQ = 5.230 N/B and LOQ = 15.85032 N/B, where, ‘N’ is standard deviation of the peak areas of the drugs (n =917.0756), taken as a measure of noise, and ‘B’ is the slope of the corresponding calibration curve.

2.4.3 SPECIFICITY

The specificity of the method was determined by examining Tianeptine standard and Tianeptine extracted from the solid dosage form. The spot for Tianeptine in sample was confirmed by comparing the Rf values and spectra. The peak-purity of Tianeptine was assessed by comparing the spectra at three different levels, i.e., peak- start (S), peak- apex (M) and peak- end (E) positions of the band was assessed by comparing the spectra at three different levels, i.e., peak- start (S), peak- apex (M) and peak- end (E) positions of the band..

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2.4.4 ACCURACY

The pre- analysed samples were spotted with extra 80 %, 100% and 120% of the Tianeptine standard and the mixtures were re-analysed by the proposed method. This was performed to check the recovery of the drug at different levels in the formulations.

2.4.5 ROBUSTNESS

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate various in method parameters. By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions of Chloroform: Methanol (9:1 v/v), Chloroform: Methanol (9.5:0.5 v/v) tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity varied in the range of ±5%. The plates were prewashed with methanol and activated at 100 ± 5 C for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning varied from 20, 30 and 40 min.

3.0 Results and discussion

3.1 Development of optimum mobile phase

Different compositions of the mobile phase for RP-HPTLC analysis were experimented with an objective to obtain high resolution and reproducible peaks. The required objective was achieved using Chloroform: Methanol (9:1% v/v) as the mobile phase. The wavelength of 257 nm was found to be optimal for the highest sensitivity. Sharp and well defined peaks for the Tianeptine were obtained at Rf 0.48 ± 0.02 when the chamber was saturated with mobile phase for 25 min at room temperature. The chromatogram of Tianeptine standard is shown in Fig. 2.

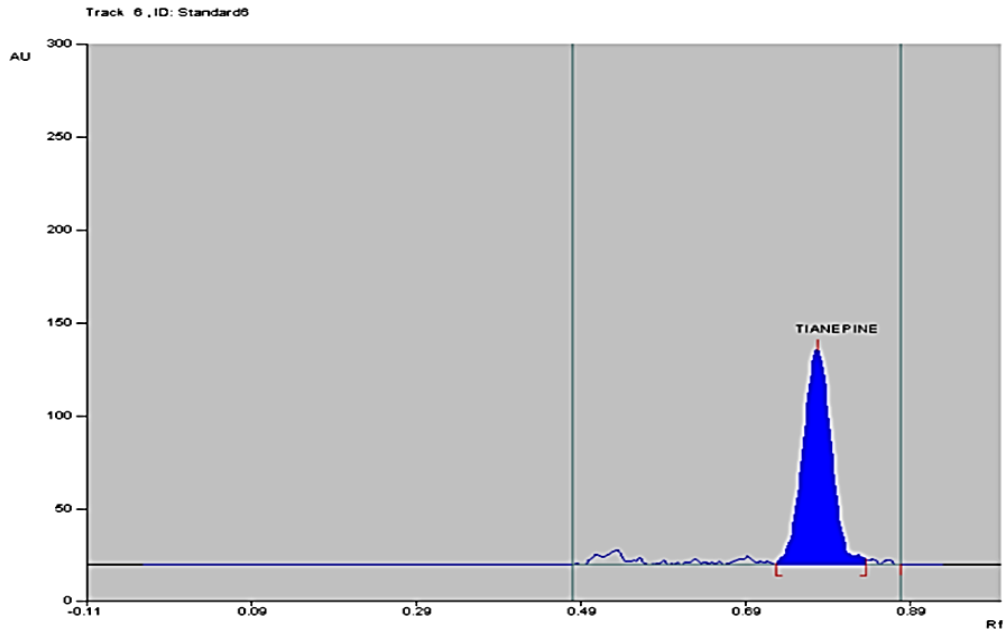

Fig.2 Chromatogram Showing Tianeptine

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Table 1: Intra-day and Inter-day precision of RP-HPTLC method.

Drug	Conc. ng/band	Intra-day		Inter-day	
		%Amount found[a]	% R.S.D.	% Amount found[a]	% R.S.D
Tianeptine	800	101.81	0.64	96.22	0.79
	1000	100.08	0.65	98.83	0.94
	1200	100.39	0.78	97.67	1.00

Mean of three estimations.

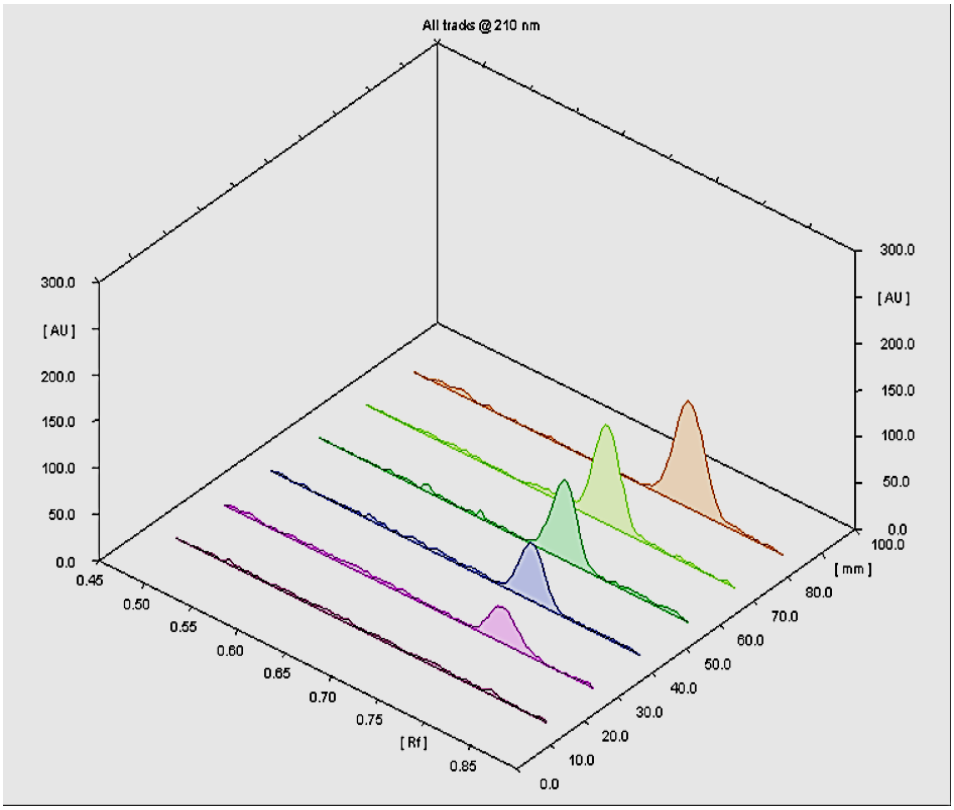

Fig.3 HPTLC 3D Densitogram of Tianeptine

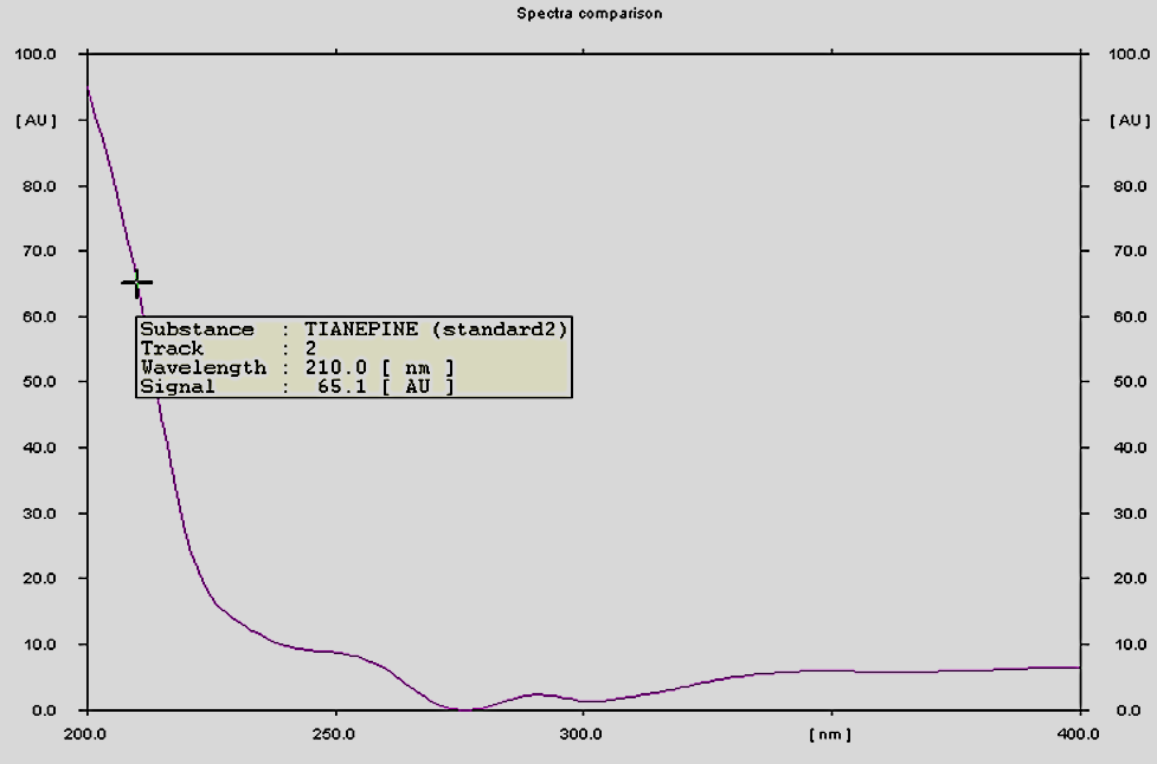

Fig.4 Spectra Comparison of Tianapine

Table 2: Recovery study

Drug/Label claim	Initial amount of drug (ng/band)	Amount of standard drug added (%)	% Recovery[a]	%RSD
Tianeptine 1%(w/w)	500	80	98.47	1.06
		100	101.10	0.85
		120	100.31	0.61

A

Mean of three estimations at each level.

4.0 VALIDATION OF METHOD

4.1 PRECISION

The precision of the developed RP-HPTLC method was expressed in terms of % relative standard deviation (%RSD). The % RSD value for repeatability of sample application and amount of Tianeptine was estimated and was found to be less than 2. The results depicted revealed high precision of the method and are presented in Table **4.2.2.**0 ng respectively. This indicates that the sensitivity of the method is adequate.

4.2 RUDGENESS

The method was executed out by two different analysts under the same experimental and environmental conditions; the results were calculated in terms of % RSD of amount found. The % RSD was found to be less than 2 which indicate that the method is rugged.

4.3 RECOVERY STUDY

The accuracy of the method is studied to measure that other components in the pharmaceutical formulation do not interfere with analytical method.

The proposed method when used for extraction and subsequent quantification of Tianeptine from the marketed formulation after over spotting with 80%, 100% and 120% of additional drug, gives excellent recovery of Tianeptine. The amounts of drug added were determined and the % recovery is shown in Table 2. The results obtained indicate that other components do not interfereDetection limit and quantification limit were calculated by the method as described above. The LOD and LOQ were found to be 19.99 ng and 60.6 with the analytical method.

4.4 ROBUSTNESS OF THE METHOD

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low value of % RSD, Table 3 indicates the reliability of analytical method during normal usage.

5.0 CONCLUSION

The present RP-HPTLC method is precise, specific, sensitive and accurate. Statistical analysis proved the method is reproducible and selective for analysis of Tianeptine in the marketed formulations. The method can be used to determine the purity of the commercially available drug. The additives usually present in the marketed formulations of the assayed samples did not interfere with determination of Tianeptine.

6.0 ACKNOWLEDGEMENTS

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