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Validation of Stability Indicating RP-HPLC Assay Method of Tofisopam in Pharmaceutical Dosage Form

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ABSTRACT

Reversed phase high performance liquid chromatographic method was developed and validated in the present investigation is simple and accurate for Tofisopam determination in its dosage form. This method is suitably equipped with UV-visible detector consisting of Luna Phenyl Hexyl, 250 mm x 4.6 mm, 5 µm columns using a mobile phase mixed acetonitrile and water in the ratio 50+50. The tofisopam retention time is found to be about 3.320 min respectively. For tofisopam the linearity was established and it is in the range 187411 - 2715567 µV*sec. The recovery percentage of tofisopam was measured and it is in the range 99.9 – 100%. This drug was subjected to acid, alkali, oxidation, dry heat and UV degradation. These studies revealed that this method can be successfully employed for routine analysis of tofisopam in pure and formulations.

Keywords: Tofisopam, RP-HPLC, Stability indicating method, Stability, Linearity and Accuracy,

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INTRODUCTION

Tofisopam is a 1, 2-benzodiazepine. It is similar with other anxiolytic benzodiazepines which are generally 1, 4- or 1, 5-substituted. It does not have anticonvulsant, calming [1], skeletal reduce tension. It has motor skill-damaging or amnestic features [2] (Figure-1). Tofisopam has chemical formula $C_{12}H_{22}N_2O_4$. It is chemically known as 1-(3, 4-dimethoxyphenyl)-5-ethyl-7, 8-dimethoxy-4-methyl-5H-2, 3-benzodiazepine. Tofisopam (marketed under brand names Emandaxin and Grandaxin) is a 2, 3-benzodiazepine drug which is a benzodiazepine derivative. Tofisopam a 2, 3-benzodiazepine (2, 3-BDZs). It is a unique drug among CNS-active compounds. It is an anxiolytic without sedative–hypnotic or muscle relaxant effects. It is used for the therapy of worry and alcohol retraction. It is prescribed in a dose of limit 50 -300 mg daily, into three doses. Tofisopam is not reported to cause dependence to the same extent as other benzodiazepines, but is suitably advised to be prescribed for 12 weeks maximum. The efforts encouraged are aimed at pharmacological effectiveness of tofisopam (TOF) and its inherent toxicity for the development of different assay methods are drug. Spectrofluorometry [3], high performance liquid chromatography (HPLC) using reverse-phase chromatography [4-6] or enantiomeric separation [7, 8], gas chromatography (GC) [9] and super critical chromatography [10]. These methods have been cited in the literature for the determination of tofisopam in its dosage form. These methods require much time, derivatization procedure, even though they are sensitive; they are of high price and are not obtainable in most quality control laboratories. Rao et al. have reported their work on different materials in their earlier studies [11-34].

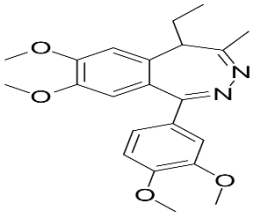

Figure-1: Structure of tofisopam

EXPERIMENTAL

Chemicals and Reagents

Tofisopam was obtained from Hetero Drugs, Hyderabad as gift sample and Emandaxin (Tofisopam) Tablets (10 mg) available in the local pharmacy were used in the present study. Acetonitrile (HPLC) grade and HPLC grade water were used in the preparation of different solutions (Mobile phase, Stock and working) in the present work.

Instrumentation

Waters 2695 HPLC system equipped with quaternary, low-pressure mixing pump and Waters 2996 Photodiode Array Detector (wavelength range of 190-800 nm) was used for quantitative performance of HPLC. Waters Empower software was used to monitor and integrate the output signal. The separation was done by a Hypersil ODS C18 column (250 mm × 4.6 mm, 5 µm). In the present investigation Shimadzu analytical electronic balance was used.

Preparation of Mobile Phase

Mix Acetonitrile and Water in the ratio 50+50 mL in 100 ml volumetric flask. This solution was mixed and filtered which is used for the mobile phase preparation. Filter through 0.45 µ membrane filter paper.

Preparation of Standard Tofisopam Stock Solution

About 10 mg of tofisopam reference standard was exactly weighed and dissolved in a 10 mL volumetric flask. The stock solution was prepared by adding 7 mL of diluent (mobile phase) and further diluted with the mobile phase according to the requirement (concentration within the linearity limits i.e., 5.0 – 25 µg/mL).

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RESULTS AND DISCUSSION

Method Development

In the present investigation selection of appropriate mobile phase was taken care by method development. For this initial trials were exclusively carried-out by the author using inject 10 µL of standard preparation five times and sample preparation in the Chromatograph. Record the chromatograms and measure the peak responses for tofisopam. The system suitability parameters should be met from the peak responses and suitably calculates the content of tofisopam in the sample.

Validation of the Proposed Procedure

The parameters like selectivity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness are systematically studied to validate the proposed RP-HPLC method as per the ICH guidelines for the estimation of tofisopam.

Selectivity

This method was selected for the tofisopam with Retention time about3.320 min. The analysis of the chromatogram of tofilopam revealed the following efficiencies of the column for tofisopam N = 5574 (where N represents theoretical plate number) and asymmetry 1.24 with the retention time of 4.010 min. The typical excipients included in the drug formulation do not interfere with selectivity of the method.

Accuracy

Accuracy was assessed by determination of the recovery of the method at three different concentrations (Figure-2 to Figure-4) (corresponding to 50, 100 and 150 % of test solution concentration) by addition of known amounts of standard (Table-1). For each concentration, three sets were prepared and injected in duplicate.

Table-1: Accuracy measurements of tofisopam

S. No.	Vial	Name	Retension Time (min)	Sample Name	Area (µV*Sec)	USP Tailing	USP Plate Count
1	108	Tofisopam	3.318	Accuracy 50 %-1	843974	1.17	1717
2	109	Tofisopam	3.316	Accuracy 50 %-2	844889	1.17	1656
3	110	Tofisopam	3.328	Accuracy 50 %-3	837384	1.16	1555
Mean					842082		
Std. Dev.					4094.5		
% RSD					0.486		

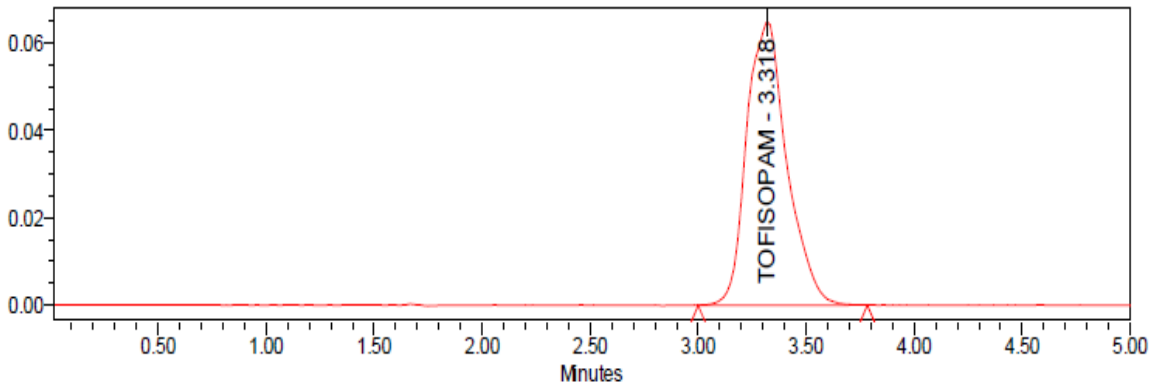

Figure-2: Accuracy measurement of tofisopam peak for 50 % concentration

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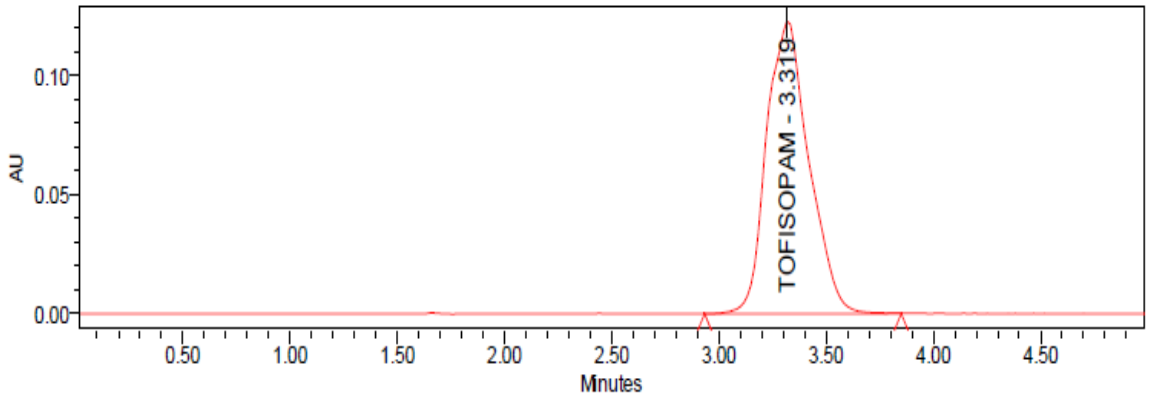

Figure-3: Accuracy measurement of tofisopam peak for 100 % concentration

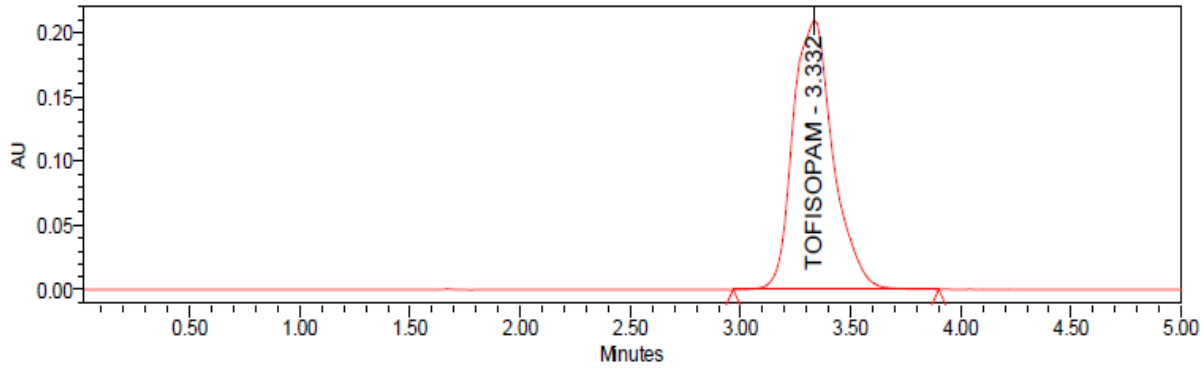

Figure-4: Accuracy measurement of tofisopam peak for 150 % concentration

Linearity

Linearity of the present method was evaluated by diluting the standard tofisopam solutions over the range 5.0 – 25 µg mL^{-1} at five different concentration levels (Table-2). Figure-5 shows the linearity measurement of tofisopam peak for 50 % concentration. The peak areas were recorded by injecting 20 µl of these solutions in triplicate in to HPLC system. A linearity plot was drawn by plotting obtained peak area verses the concentration data was calibrated by least-squares linear regression analysis to obtain the results of calibration data. The linearity range was between 5.0 – 25 µg mL^{-1} presented by the equation 39332 x + 23819 with correlation coefficient (r^2 = 0.9995) closed to unity revealing the good linearity of the present method.

Table-2: Linearity measurements of tofisopam

S, No.	Vial	Name	Retension Time (min)	Sample Name	Area (µV*Sec)	USP Tailing	USP Plate Count
1	102	Tofisopam	3.331	Linearity-1	187411	1.15	2036
2	103	Tofisopam	3.314	Linearity-2	469843	1.16	2026
3	104	Tofisopam	3.312	Linearity-3	875605	1.16	2071
4	105	Tofisopam	3.319	Linearity-4	1728428	1.17	1734
5	106	Tofisopam	3.314	Linearity-5	2215410	1.14	2161
6	107	Tofisopam	3.322	Linearity-6	2715567	1.13	2210
Mean					1365377		
Std. Dev.					1010627.3		
% RSD					74.016		

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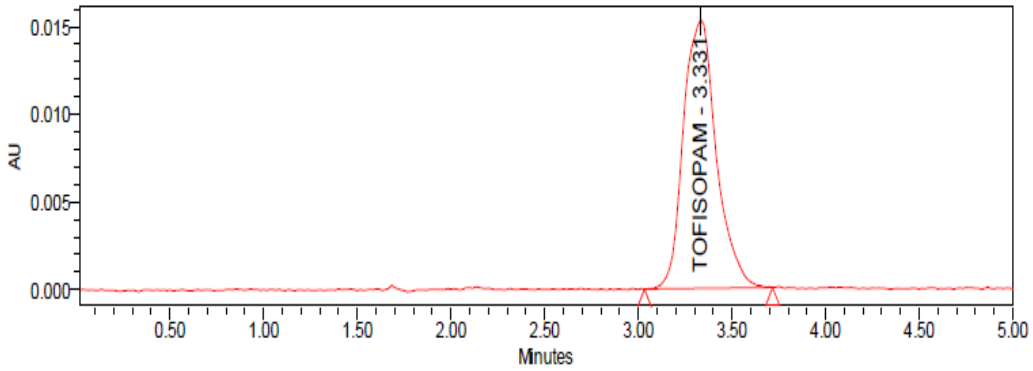

Figure-5: Linearity measurement of tofisopam peak for 50 % concentration

Stability

Stability of solution was evaluated for the standard solution and the test preparation. The solutions were stored at 5° and at ambient temperature without protection of light and tested after 6, 12, 24, 36 and 48 h. The responses for the aged solution were evaluated by comparing with the freshly prepared solutions. Figure-6 and Figure-7 represent the stability measurements of tofisopam for 6 hours and initial stability measurement of tofisopam in the beginning. This data is given in Table-3 and Table-4.

Table-3: Stability measurements of tofisopam for 6 hours

S. No.	Vial	Name	Retension Time (min)	Sample Name	Area (µV*Sec)	USP Tailing	USP Plate Count
1	11	Tofisopam	3.300	Stability 6 hrs	1695955	1.44	4728
Mean		Tofisopam			1695955		
Std. Dev.		Tofisopam					
% RSD							

Table-4: Initial Stability measurements of tofisopam

S. No.	Vial	Name	Retension Time (min)	Sample Name	Area (µV*Sec)	USP Tailing	USP Plate Count
1	11	Tofisopam	3.300	Stability Initial	1696158	1.44	4690
Mean					1696158		
Std. Dev.							
% RSD							

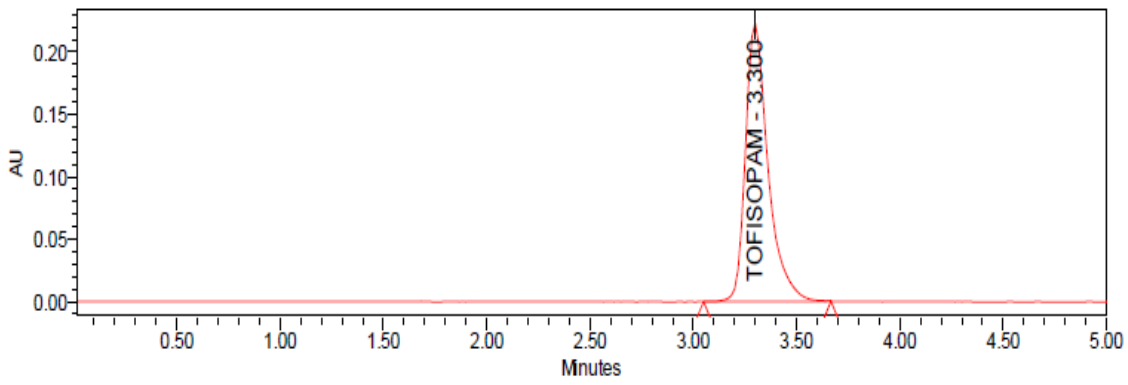

Figure-6: Stability measurements of tofisopam peak for 6 hours

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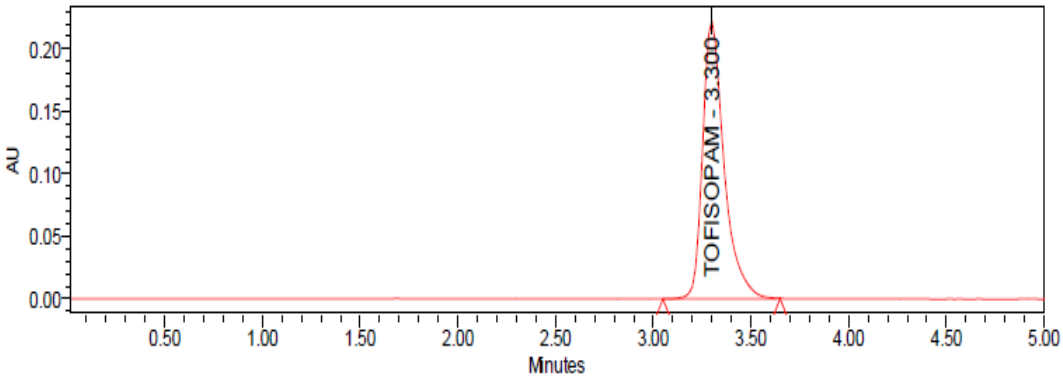

Figure-7: Initial Stability measurements of tofisopam peak

System suitability

The chromatographic system was tested suitably before each stage of validation. Five replicate injections of standard preparation were injected and asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

CONCLUSIONS

High performance liquid chromatographic method was developed for the assay of tofisopam in its dosage form. This method is simple, economical, rapid, accurate, sensitive and should be suitable for routine quality control of in a dosage form. The retention time was measured and it is around 3.320 min for tofisopam. The established Linearity for tofisopam was in the range 187411 - 2715567 µV*sec. The recovery percentage was found to be in the range 99.9 – 100 % for tofisopam. These studies revealed that this method can be employed for stability indicating for routine analysis of tofisopam in pure and formulations successfully

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