RESEARCH PAPER

Development and Validation of a Sustainable Area under Curve Based Spectrophotometric Method for the Determination of Tofisopam

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ABSTRACT

Background: Tofisopam, an anxiolytic and a 1,3-benzodizepines with a unique CNS activity of not binding to the GABA receptor. Tofisopam is prescribed in 50-300mg perday and does not cause dependence as other benzodiazepines.

Objectives: The objective of the present investigation is to develop a simple, sustainable and stability indicating spectrophotometric method and to validate the same for estimation of Tofisopam in bulk, and pharmaceutical formulation.

Method: UV-Spectrophotometric method was developed by using Methanol: Water (25:75%) as a diluent. The Developed method was validated as per ICH guidelines, in terms of specificity/selectivity, linear range, Accuracy, Precision, LOD, LOQ, robustness, ruggedness and system suitability also considering the Area under Curve (AUC) method. The newly developed and validated method was successfully applied for the estimation of Tofisopam in pharmaceutical dosage forms. Stability studies were performed using acidic, basic, thermal, oxidative, and photolytic conditions.

Results: Tofisopam exhibits λmax at 309nm. Beer's law was obeyed in the concentration range of 4-12µg/mL with the correlation coefficient (R^2) of 0.9995. The limit of detection and limit of quantification were found to 0.73µg/mL and 2.22µg/mL respectively. Recovery of Tofisopam was in the range of 92-100%.The percentage relative standard deviation was found to be less than 2% for all the precision, robustness, ruggedness and system suitability studies. The assay of Tofisopam was found to be 91.72%. Upon degradations studies, it was observed that 6-20% of the drug was degraded on exposure to Acidic, basic, thermal, oxidation, and photolytic conditions.

Conclusion: The newly devised UV Spectroscopic method for determining Tofisopam in bulk and Pharmaceutical formulations was found to be simple, specific, reproducible, and sustainable. It has been shown to be the only investigation performed using Area under curve method proving to be reliable and practical method for determining Tofisopam in both pure and commercial formulations. According to ICH guidelines, all validation parameters were confirmed to be within acceptable ranges. As a result, it can be utilised in the laboratory for daily Tofisopam analysis with a high degree of precision and accuracy.

Keywords: Method validation, Stability Indicating, Tofisopam, Toficalm, Area Under Curve (AUC), Diluent {methanol:water (25:75% v/v)}.

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INTRODUCTION

Tofisopam, chemically a 2,3-benzodiazepine and empirical formula C22H26N2O4. Unlike 1,4- and 1,5- substituted benzodiazepines Tofisopam has a unique CNS activity, being a anxiolytic it also enhances the anticonvulsant action of Diazepam and Muscimol. Tofisopam serves as an isoenzyme-selective inhibitor of phosphodiesterases (PDEs)[1-3]. Several spectrophotometric, chromatographic and stability indicating methods were developed and validated for the determination of Tofisopam in bulk as well

as pharmaceutical dosage forms using different organic solvents, and chromophores, also the methods were developed for the determination of Tofisopam in biological fluids and the impurity profiling[6-15] on the other had Area Under Curve methodology was not considred for estimation of Tofisopam. Therefore, the study was undertaken with the view of developing a new, simple, specific, cost-effective and stability indicating method for the assay of Tofisopam in bulk and its pharmaceutical formulation using Area Under Curve methodology.

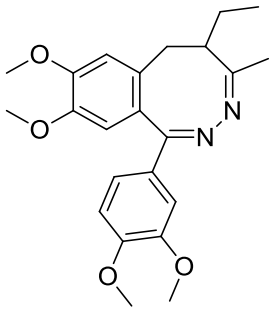

Figure 1: Structure of Tofisopam

METHOD AND MATERIALS:

Instrumentation: Shimadzu UV-1800 with UV Probe software & Shimadzu UV-1900 with Lab Solutions software were used for the determination of Tofisopam. An MJL ultrasonic cleaner was used for sonication, Merk Millipore was used for type III Millipore water, Calibrated weighing balance was used for weighing of the drug.

Reagents and Chemicals: Tofisopam (Standard) was obtained as a gift sample from Symed labs Pvt. LTD. The commercial formulation containing 50mg of Tofisopam, Toficalm tablets were procured from the local market of Belgaum. Analytical grade methanol and Type III millipore water were used as solvents from Merk Millipore.

Method Development

Selection of detection wavelength: Different solvents and solvent ratios were used to assess the solubility of the Tofisopam. Based on solubility pattern of Tofisopam, the diluent containg 25 portions of methanol and 75 portions of water was found to be suitable for analysis. The UV-spectrophotometer was used to scan tofisopam (10 µg/mL) between 400 nm to 200 nm, tofisopam showed highest absorption at 309 nm with the AUC of 2.456 (Figure 2).

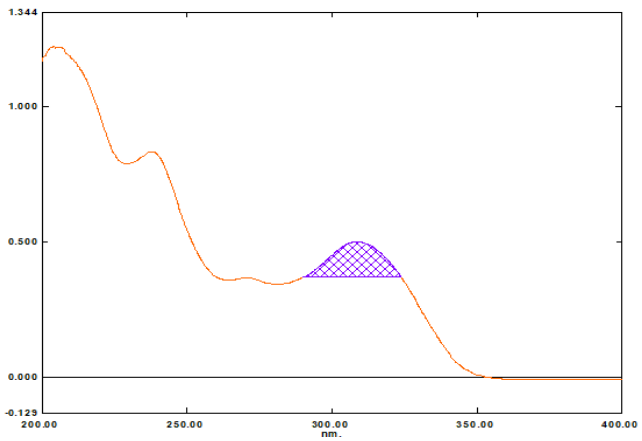

Figure 2: UV Spectrum of Tofisopam

Preparation of standard solutions: The stock solutions of Tofisopam was prepared in diluent containing methanol:water (25:75% v/v) at a concentration of 1000µg/mL by dissolving an accurate amount of Tofisopam equivalent to 50mg in 50ml diluent, followed by serial dilution to reach the concentration range of 4 to 12µg/mL after preliminary checking the validity of Beer Lambert Law.

Preparation of Sample solution: Ten tablets were weighed to obtain the average weight and was finely powdered. A portion of finely powdered tablets equivalent to about 10mg of total label claim was weighed accurately and transferred to 10ml volumetric flask and diluted to mark using diluent, the solution was filtered, the transparent filtrate was serially diluted to obtain an equivalent concentration of about 10µg/mL.

Method Validation

The developed method was validated in terms of linearity,

accuracy, precision, specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), assay, Robustness, Ruggedness and stability as per ICH Q2(R2) guidelines. Specificity and selectivity were evaluated in order to ensure that the selected solvent is specific for the newly developed method and the solvent will not interfere by showing its absorbance in the analysis. Linearity examines the relationship between analyte concentration and the instruments response which was determined at the concentration range of 4-12µg/mL by plotting concentration verses absorbance and concentration versus area curves, corelation coefficient was calculated.

Preparation of calibration curve:
From the IInd stock, serial dilutions containing concentrations of 4, 6, 8, 10 and 12µg/mL were prepared by pipetting out 0.4, 0.6, 0.8, 1.0, and 1.2 ml from IInd stock in 10ml volumetric flask, and the volume was made up using methanol:water (25:75%v/v) solution. The solutions were then scanned in the photometric mode at 309nm and the absorbance for each solution was noted at 309nm against methanol;water (25:75%v/v). The calibration curve was plotted as Concentration on X-axis and Absorbance on Y-axis. The calibration curve is shown in figure 3.

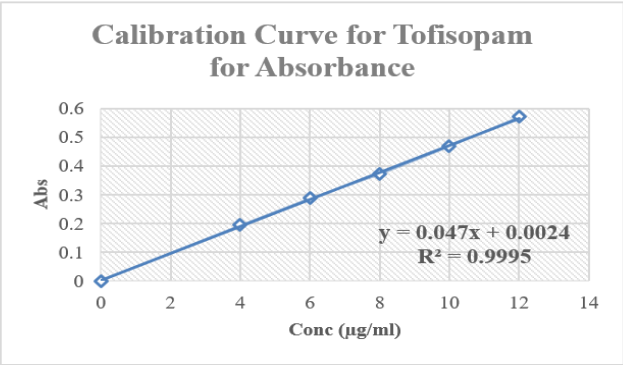

Figure 3: Calibration curve of Tofisopam at 309nm

Specificity and selectivity: For specificity and selectivity studies, the blank solution was scanned in UV-Vis spectrophotometer in the range of 200-400nm, and as a result, a straight line was obtained, similarly the sample was also scanned in the range of 200-400nm and it showed the maximum absorbance at 309nm. Therefore the method was found to selective and specific.

Linearity: For the linearity studies 5 solutions were prepare having concentrations 4µg/mL, 6µg/mL, 8µg/mL, 10µg/mL and 12µg/mL solution were prepared and scanned in UV-Vis spectrophotometer against methanol:water (25:75%v/v) as blank. The drug shows linearity in the range of 4-12µg/mL.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection (LOD) and Limit of Quantification (LOQ) was performed as per ICH guidelines using the following equations,
LOD = 3.3*σ/s
LOQ = 10* σ/s
Where,
σ = Standard deviation
S = Slope of the calibration curve

Precision: The precision was tested for system precision, Intraday (Thrice on the same day) Interday (Three times on the different days) precision. The study aimed at observing the reproducibility of results at different times on the same day and also on different days. The relative standard deviation was calculated from the obtained results and was found to be less than 2%.

Robustness: The robustness study was performed by changing the wavelength to 308nm, 309nm and 310nm, also by changing the solvent ratio to Methanol: Water (23:77% v/v and 27:73% v/v), to observe analyte response on small variations in the applied methodology.

Ruggedness: The ruggedness was performed by the different analyst on UV 1800 and UV 1900 UV-Vis spectrophotometer and the results were compared to identify variations occur due to change of analyst and the instrument.

Accuracy: The accuracy was performed by standard addition method, to the fixed amount of pre-analyzed mixtures, 80%, 100% and 120% of standard solution were added and the % recovery was calculated.

Assay: Ten Tofiscalm tablets (each containing 50mg of Tofisopam) were weighed accurately and powdered. A quantity equivalent to 10mg was weighed accurately and transferred to a 10ml volumetric flask and was made up to the mark using Methanol: water (25:75% v/v). The solution was sonicated for 15 minutes and was filtered. From the filtered solution, 1ml was transferred to a 10ml volumetric flask and was diluted with Methanol:water (25:75%v/v). From this 1ml was pipetted out and diluted using methanol:water(25:75%v/v) to obtained 10µg/mL concentration and the absorbance was noted at 309nm.

Forced Degradation Studies:[12-13]The force degradation study aimed at observing the response of API in the presence of its degradation product. The API was exposed to alkaline, acidic, thermal, oxidative, and photolytic conditions.

Alkaline Degradation: 1ml of the drug solution (pipetted out from Stock II) was transferred to a 10ml volumetric flask, to this solution 1ml of 0.1N NaOH was added and was heated at 80°Cfor 2hrs. The solution was then cooled. The volume was made up to the mark using the solvent to obtain 10µg/mL concentration and the solution was scanned in the photometric mode at 309nm and the results were compared with that of standard.

Acid Degradation: 1ml of the drug solution (pipetted out from Stock II) was transferred to a 10ml volumetric flask, to this solution 1ml of 0.1N HCL was added and was heated at 80°Cfor 2hrs. The solution was cooled. The volume was made up to the mark using the solvent to obtain 10µg/mL concentration and the solution was scanned in the photometric mode at 309nm and the results were compared with that of standard.

Thermal Degradation: 1ml of the drug solution was pipetted out from stock II and was transferred to a 10ml volumetric flask and the volume was made up to the mark using the solvent to obtain 10µg/ml solution. The above solution was heated at 80°Cfor 2hrsand the solution was cooled. The obtained solution was then scanned in the photometric mode at 309nm and the results were compared with that of standard.

Oxidative Degradation: 1ml of the drug solution (pipetted out from Stock II) was transferred to a 10ml volumetric flask, to this solution 1ml of H2O2 was added and was heated at 80°Cfor 2hrs. The solution was cooled and made up to the mark using the solvent to obtain 10µg/mL concentration and the solution was scanned in the photometric mode at 309nm and the results were compared with that of standard.

Photolytic Degradation: An accurately weighed 10mg of the drug sample was exposed directly to UV light for 24hr and it was transfered to a 10ml volumetric flask. serial dilutions were prepared to obtain 10µg/ml solution. The obtained 10µg/ml solution was then scanned in the photometric mode at 309nm and the results were compared with that of standard.

RESULTS AND DISCUSSION

A simple, accurate, precise, and rapid spectrophotometric method for the determination of Tofisopam in bulk and pharmaceutical dosage form was developed and validated.

Specificity and selectivity: The newly developed method was found to selective and specific as it shows 0% solvent interference and maximum absorbance at 309nm respectively. The UV spectrum of the solvent is shown in Figure 4.

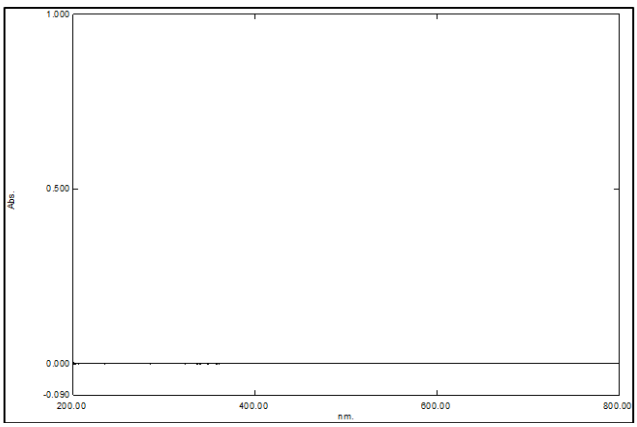

Figure 4: UV spectrum of the solvent

Linearity: The drug shows linearity in the range of 4-12µg/mL with the correlation coefficient (R^2)=0.999 The overlay spectra for the concentration range of 4-12µg/mL is shown in figure 5. The statistical data for linearity has been given in table no. 1.

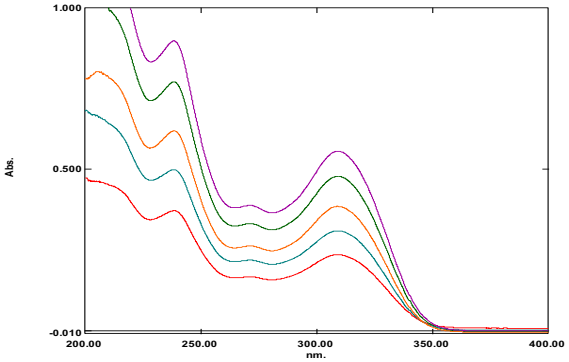

Figure 5: Overlay Spectra of 4-12ug/ml solution of Tofisopam

Table 1: Linearity data of Tofisopam

Sr. No.	Concentration (µg/mL)	Absorbance	Area
1	0	0	0
2	4	0.195	1.191
3	6	0.288	1.609
4	8	0.372	2.075
5	10	0.468	2.546
6	12	0.570	2.929
r^2 =0.9995			
LOD=0.73µg/ml			
LOQ=2.22µg/ml			

System Precision: The system precision was performed on 6 replicates of 3 different concentrations (i.e. 4µg/mL, 8µg/mL, and 12µg/mL) and the Percentage relative standard deviation was found to be less than 2%. The data of system precision has been given in table no. 2

Table 2: System Precision data for Tofisopam

Conc. (µg/ml)	4		8		12	
	Abs	Area	Abs	Area	Abs	Area
Mean	0.228	1.162	0.464	2.372	0.583	3.045
SD	0.004	0.007	0.004	0.012	0.010	0.013
%RSD	1.938	0.631	0.826	0.497	1.689	0.417

x̄ = Mean absorbance of six replicates

Intraday and Interday Precision: The Intraday and interday precision was performed on 6 replicates of 3 different concentrations, the percentage relative standard deviation was found to be less than 2%. The results for intraday precision and interday precision have been given in table no 3 to 8.

Table 3: Intraday precision data of Tofisopam

1st Hour						
Sl. No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.240	1.165	0.377	2.081	0.575	2.898
2	0.238	1.167	0.383	2.083	0.575	2.913
3	0.237	1.164	0.384	2.104	0.583	2.923
4	0.232	1.148	0.390	2.096	0.584	2.195
5	0.233	1.168	0.388	2.112	0.588	2.904
6	0.234	1.162	0.380	2.108	0.587	2.909
Mean	0.236	1.162	0.384	2.097	0.582	2.924
SD	0.003	0.007	0.005	0.013	0.006	0.036
%RSD	**1.333**	**0.631**	**1.263**	**0.621**	**0.984**	**1.230**

x̄ = Mean absorbance of six replicates

Table 4: Intraday precision data of Tofisopam

4th Hour						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.192	1.285	0.424	2.462	0.583	3.305
2	0.189	1.259	0.427	2.469	0.588	3.308
3	0.196	1.286	0.422	2.421	0.594	3.32
4	0.187	1.256	0.422	2.42	0.587	3.307
5	0.194	1.286	0.425	2.46	0.588	3.309
6	0.192	1.288	0.425	2.461	0.591	3.319
Mean	0.192	1.277	0.424	2.449	0.589	3.311
SD	0.003	0.015	0.002	0.022	0.004	0.006
%RSD	**1.704**	**1.168**	**0.458**	**0.906**	**0.634**	**0.195**

$\bar{x}$ = Mean absorbance of six replicates

Table 5: Intraday precision data of Tofisopam

8th Hour						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.252	1.226	0.498	2.453	0.666	3.331
2	0.257	1.218	0.504	2.472	0.669	3.305
3	0.256	1.221	0.501	2.448	0.667	3.329
4	0.255	1.222	0.503	2.445	0.665	3.332
5	0.251	1.215	0.497	2.482	0.661	3.306
6	0.258	1.225	0.499	2.441	0.666	3.328
Mean	0.255	1.221	0.500	2.457	0.666	3.322
SD	0.003	0.004	0.003	0.016	0.003	0.013
%RSD	**1.094**	**0.341**	**0.561**	**0.668**	**0.399**	**0.383**

$\bar{x}$ = Mean absorbance of six replicates

Table 6: Interday Precision data of Tofisopam

Day 1						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.224	1.165	0.377	2.081	0.575	2.898
2	0.228	1.167	0.38	2.108	0.575	2.913
3	0.227	1.164	0.383	2.083	0.583	2.923
4	0.222	1.169	0.384	2.104	0.584	2.915
5	0.224	1.168	0.39	2.096	0.588	2.904
6	0.225	1.166	0.388	2.112	0.582	2.913
Mean	0.225	1.167	0.384	2.097	0.581	2.911
SD	0.002	0.002	0.005	0.013	0.005	0.009
%RSD	**0.974**	**0.160**	**1.263**	**0.621**	**0.894**	**0.302**

$\bar{x}$ = Mean absorbance of six replicates

Table 7: Interday Precision data of Tofisopam

Day 2						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.192	1.285	0.424	2.462	0.583	3.305
2	0.189	1.259	0.427	2.469	0.588	3.308
3	0.192	1.286	0.422	2.421	0.594	3.32
4	0.191	1.25	0.423	2.46	0.591	3.303
5	0.186	1.284	0.42	2.44	0.585	3.305
6	0.188	1.257	0.425	2.441	0.592	3.309
Mean	0.190	1.270	0.424	2.449	0.589	3.308
SD	0.002	0.017	0.002	0.018	0.004	0.006
%RSD	**1.277**	**1.302**	**0.574**	**0.735**	**0.724**	**0.019**

Table 8: Interday Precision data of Tofisopam

Day 3						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.246	1.265	0.401	2.067	0.595	3.048
2	0.246	1.275	0.401	2.07	0.59	3.044
3	0.246	1.278	0.403	2.079	0.594	3.057
4	0.244	1.277	0.402	2.066	0.593	3.045
5	0.248	1.266	0.404	2.078	0.591	3.055
6	0.245	1.279	0.401	2.068	0.592	3.046
Mean	0.246	1.273	0.402	2.071	0.593	3.049
SD	0.001	0.006	0.001	0.006	0.002	0.005
%RSD	**0.541**	**0.488**	**0.315**	**0.276**	**0.316**	**0.180**

Ruggedness: Ruggedness was performed by analyzing the API on different spectrophotometers (i.e. UV1800 and UV1900). The results have been given in table no 6.

Table 9: Ruggedness data for Tofisopam

Analyst 1						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.246	1.265	0.401	2.067	0.595	3.048
2	0.246	1.275	0.401	2.07	0.59	3.044
3	0.246	1.278	0.403	2.079	0.594	3.057
4	0.244	1.277	0.402	2.066	0.593	3.045
5	0.248	1.266	0.404	2.078	0.591	3.055
6	0.245	1.279	0.401	2.068	0.592	3.046
Mean	0.246	1.273	0.402	2.071	0.593	3.049
SD	0.001	0.006	0.001	0.006	0.002	0.005
%RSD	**0.541**	**0.488**	**0.315**	**0.276**	**0.316**	**0.180**

$\bar{x}$ = Mean absorbance of Six replicates

Table 10: ruggedness data of Tofisopam

Analyst 2						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.224	1.165	0.377	2.081	0.575	2.898
2	0.228	1.167	0.38	2.108	0.575	2.913
3	0.227	1.164	0.383	2.083	0.583	2.923
4	0.222	1.169	0.384	2.104	0.584	2.915
5	0.224	1.168	0.39	2.096	0.588	2.904
6	0.225	1.166	0.388	2.112	0.582	2.913
Mean	0.225	1.167	0.384	2.097	0.581	2.911
SD	0.002	0.002	0.005	0.013	0.005	0.009
%RSD	**0.974**	**0.160**	**1.263**	**0.621**	**0.894**	**0.302**

Robustness: Robustness was performed by changing the method conditions like change in the absorption wavelength and change instrument. Results of the change in absorption wavelength have been given in table no. 7a and Results of the change in the solvent ratio have been given in 7b.

Table 11: Robustness (Change in Absorbace wavelength) data for Tofisopam

Wavelength	**308nm**		**309nm**		**310nm**	
Sl. No.	**4ug/ml**	**Area**	**4ug/ml**	**Area**	**4ug/ml**	**Area**
1	0.262	1.168	0.262	1.171	0.261	1.169
2	0.263	1.171	0.264	1.168	0.263	1.173
3	0.265	1.166	0.264	1.169	0.263	1.179

4	0.262	1.169	0.261	1.166	0.265	1.176
5	0.267	1.166	0.266	1.164	0.262	1.181
6	0.269	1.17	0.262	1.173	0.264	1.14
Mean	0.265	1.168	0.263	1.169	0.263	1.170
SD	0.003	0.002	0.002	0.003	0.001	0.015
%RSD	**1.086**	**0.177**	**0.697**	**0.280**	**0.538**	**1.295**

$\bar{x}$ = Mean absorbance of six replicates

Table 12: Robustness (Change in Absorbace wavelength) data for Tofisopam

Wavelength	**308nm**		**309nm**		**310nm**	
Sl. No	**6ug/ml**	**Area**	**6ug/ml**	**Area**	**6ug/ml**	**Area**
1	0.417	2.083	0.417	2.087	0.417	2.079
2	0.42	2.109	0.421	2.101	0.42	2.11
3	0.429	2.086	0.429	2.081	0.427	2.109
4	0.416	2.107	0.425	2.099	0.425	2.1
5	0.425	2.099	0.416	2.098	0.412	2.107
6	0.425	2.111	0.422	2.107	0.422	2.111
Mean	0.422	2.099	0.422	2.096	0.421	2.103
SD	0.005	0.012	0.005	0.010	0.005	0.012
%RSD	**1.218**	**0.577**	**1.159**	**0.459**	**1.300**	**0.582**

Table 13: Robustness (Change in Absorbace wavelength) data for Tofisopam

Wavelength Sl. No.	**308nm**		**309nm**		**310nm**	
	12ug/ml	**Area**	**12ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.605	2.899	0.605	2.893	0.604	2.889
2	0.606	2.91	0.608	2.991	0.607	2.974
3	0.61	2.919	0.61	2.923	0.608	2.969
4	0.605	2.919	0.605	2.925	0.603	2.932
5	0.604	2.899	0.601	2.901	0.606	2.896
6	0.601	2.905	0.609	2.91	0.608	2.893
Mean	0.605	2.909	0.606	2.924	0.606	2.926
SD	0.003	0.009	0.003	0.035	0.002	0.039
%RSD	**0.484**	**0.313**	**0.549**	**1.202**	**0.346**	**1.328**

Table 14: Robustness (Change in instruments) data for Tofisopam

Conventional Instrument						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.224	1.165	0.461	2.372	0.583	3.034
2	0.228	1.167	0.457	2.366	0.586	3.027
3	0.227	1.164	0.461	2.371	0.587	3.044
4	0.232	1.148	0.464	2.361	0.589	3.06
5	0.233	1.168	0.463	2.369	0.591	3.055
6	0.234	1.162	0.464	2.395	0.59	3.051
Mean	0.230	1.162	0.462	2.372	0.588	3.045
SD	0.004	0.007	0.003	0.012	0.003	0.013
%RSD	**1.712**	**0.631**	**0.576**	**0.497**	**0.501**	**0.417**

$\bar{x}$= Mean absorbance of six replicates

Table 15: Robustness (Change in instruments) data for Tofisopam

Other Instrument						
Sl.No.	**4ug/ml**	**Area**	**8ug/ml**	**Area**	**12ug/ml**	**Area**
1	0.246	1.265	0.498	2.453	0.666	3.331
2	0.246	1.275	0.504	2.472	0.669	3.305
3	0.246	1.278	0.501	2.448	0.667	3.329
4	0.244	1.277	0.503	2.445	0.665	3.332
5	0.248	1.266	0.497	2.482	0.661	3.306
6	0.245	1.279	0.499	2.441	0.666	3.328
Mean	0.246	1.273	0.500	2.457	0.666	3.322
SD	0.001	0.006	0.003	0.016	0.003	0.013
%RSD	**0.541**	**0.488**	**0.561**	**0.668**	**0.399**	**0.383**

Accuracy and Recovery: Accuracy was determined by performing recovery studies, at three different levels 80%, 100%, and 120% of the sample solutions. And the percent recovery is found in the range of 97-100%. The results of the same have been given in table no. 8.

Table 16: Accuracy and Recovery data for Tofisopam

%	Standard Conc.	Sample Conc.	Total Conc.	Standard absorbance	Sample absorbance	% recovery
80%	5	4	9	0.241	0.434	100%
100%	5	5	10	0.241	0.475	97%
120%	5	6	11	0.241	0.526	98%

Assay: On the analysis of pharmaceutical formulation, the results were obtained following the label claim. The results of percentage purity of tofisopam tablets were found to be 91.72%.

Limit of detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were found to be 0.73µg/ml and 2.22µg/ml respectively.

Force Degradation studies:
In these studies, the drug was exposed to base, acid, heat, oxidation, and UV light, it was found the10.11% drug was degraded on alkaline degradation,6.43% on acidic and 19.42% was degraded on thermal degradation, 10.07% on oxidative degradation and 14.28% on photolytic degradation.

CONCLUSION:
It can be concluded that the developed method for estimation of Tofisopam in marketed formulationis simple, sensitive, accurate, precise, reproducible.
The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the methods for this formulation.

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