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# Development and Validation of Spectrophotometric Method for the Determination of Tofisopam in Bulk and Pharmaceutical Formulation

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## Abstract

A rapid, specific UV spectrophotometric method has been developed using a solvent methanol to determine Tofisopam content in bulk and pharmaceutical formulations. At a pre-determined wavelength at 310 nm, it was proved linear in the range of 4-24 µg/ml and exhibited good correlation coefficient (R2=0.9996) and excellent mean recovery (98-102%). The method was validated statistically and parameters like linearity, precision, accuracy, specificity, and assay were studied according to International Conference on Harmonization guidelines. The obtained results proved that the method can be employed for the routine analysis of Tofisopam in bulk as well as in the commercial formulations.

**Keywords:** Tofisopam; Methanol; UV spectrophotometric method; Validation; Methanol

## Introduction

Tofisopam is chemically 1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5H-2,3-Benzodiazepine. The molecular formula is C22H26N2O4 and the molecular weight is 382.5 Daltons. Tofisopam does not bind to benzodiazepine site of GABA receptors. Tofisopam has anxiolytic action but it is devoid of anticonvulsant, sedative and muscle relaxant activities. It enhances the effect of barbiturates and ethanol only in higher doses. Tofisopam has no sedative effect and no effect on attention; it could prove to be an ideal drug of choice for the treatment of anxiolytic use.

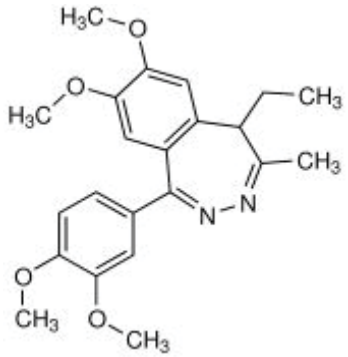


**Figure 1:** Structure of Tofisopam

It is a white pale yellowish white crystalline powder, practically insoluble in water, freely soluble in methanol and acetonitrile, sparingly soluble in ethanol. Its melting point is 155-159°C. Tofisopam is used for the short-term treatment of anxiety disorders (Figure 1) [1].

Several methods have been reported for the determination of Tofisopam. Literature review reveals that there are few methods described for the estimation of Tofisopam in biological fluid and even impurity profiling of Tofisopam and its tablet formulation are reported with respect to HPLC methods which includes normal-phase HPLC and reverse phase-HPLC, gas-liquid chromatography. Similarly literature search for spectrophotometric methods of Tofisopam reports the need of chromophoric reagent and fluorescence agent for its estimation. Thus, there is a need to develop simple, less time consuming and spectrophotometric method for the assay of Tofisopam in bulk and its formulation [2-7].

So, the attempt was made to develop a simple, accurate, precise, specific spectrophotometric method for the direct quantitative estimation of Tofisopam in bulk and formulation. The developed method was validated as per the guidelines of International Conference on Harmonization (ICH Q2-R1) and demonstrated excellent specificity, linearity, precision and accuracy for Tofisopam [8].

## Materials and Methods

### Instrumentation

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study.

### Reagents and chemicals

All chemicals and reagents were of analytical grade. Tofisopam was gifted from Ajanta Pharmaceutical, Mumbai India. The commercial dose formulation containing 50 mg Tofisopam, NEXTRIL Tablet was procured from local market. Analytical grade methanol (Research Labs) was used as solvent.

## Method development

**Preparation of stock solution and working standard solutions:** Accurately weighed 100 mg of Tofisopam were transferred to 100 ml of volumetric flask. The volume was made up to the mark with methanol to obtain stock solution of Tofisopam having concentration 1000 µg/ml of solution.

From the above stock solution, aliquot of 1 ml were pipetted out and placed into 10 ml volumetric flask. The volume was made up to mark with methanol to give solution containing 100 µg/ml of Tofisopam solution.

**Preparation of working standard solution:** Working solution containing 100 µg/ml of Tofisopam was prepared by transferring 1 ml of stock solution to 10 ml of volumetric flask and the volume was made up to mark with methanol, and it was scanned in range of 400-200 nm, and the wavelength corresponding to maximum absorbance was noted at 310 nm (Figure 2).

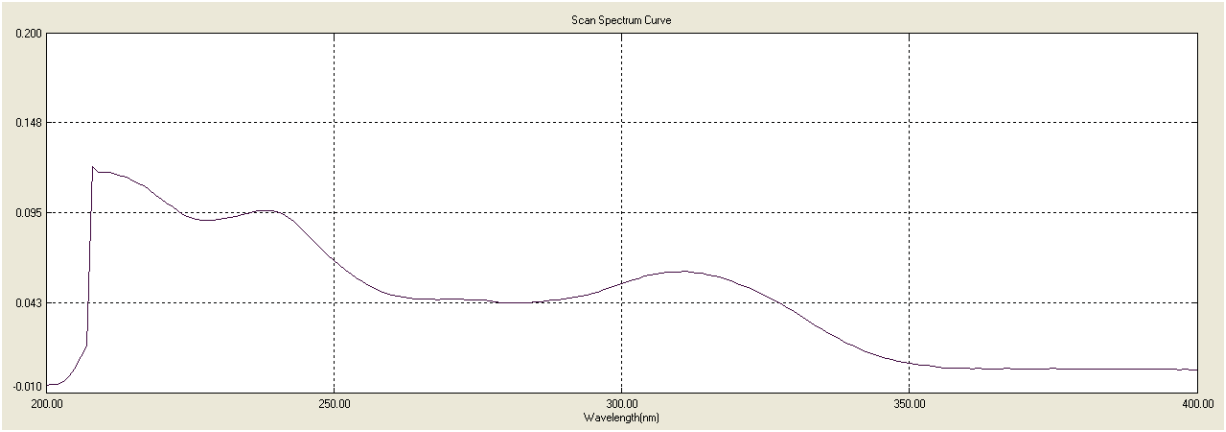


**Figure 2:** UV spectrum of Tofisopam

**Preparation of calibration curve:** For the preparation of standard calibration curve, concentration of 4-24 µg/ml were prepared by pipetting out 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml from the 100 µg/ml solution in to a 10 ml volumetric flask and made up the volume with methanol. The absorbance of each solution was measured at 310 nm against methanol as blank. Calibration curve of the Tofisopam was plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis. The calibration curve is shown in Figure 3.

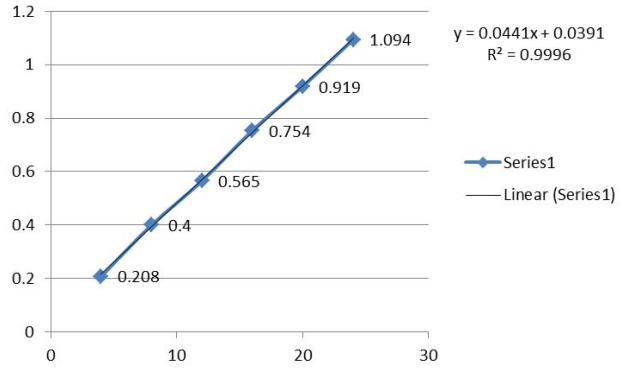


**Figure 3:** Calibration curve of Tofisopam at 310 nm.

## Validation of developed methods

The proposed method has been validated in terms of as linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantitation (LOQ), Assay, and specificity as per to ICH Q2 (R1) guidelines.

**Linearity:** For linearity study, from the stock solution (100 µg/ml) six solutions at different concentrations (4, 8, 12, 16, 20 and 24 mg/mL) were prepared using six point calibration method. The samples were scanned in UV-vis Spectrophotometer against methanol as blank. The selected drug shows linearity between the ranges of 4-24 µg/ml for Tofisopam, with a correlation coefficient ($R^2$) greater than 0.9996.

**Precision:** Variation of results within the same day (Intraday) and between days (interday) was analyzed. The Intraday and Interday precision was determined by analyzing three different concentrations of Tofisopam (8, 12 and 16 µg/ml), obtained by dilution from stock solutions for three times in a day (Intraday) and for three consecutive days (Interday).

**Accuracy (Recovery):** The accuracy of the developed method was determined by calculating percent recovery at three different levels (80, 100 and 120%) in pre analyzed samples using standard addition method.

**Specificity:** Specificity of the developed method was performed by scanning the UV-visible spectra of diluent, standard sample solutions of Tofisopam from 200 to 400 nm. Also spectral homogeneity of Tofisopam samples found to be similar with those obtained for the standard solutions. The specificity of the developed method was established to prove the absence of interference from diluent absorbance, which is a part of required pharmaceutical drug substance preparation. The specificity of Tofisopam is shown in Figure 4.

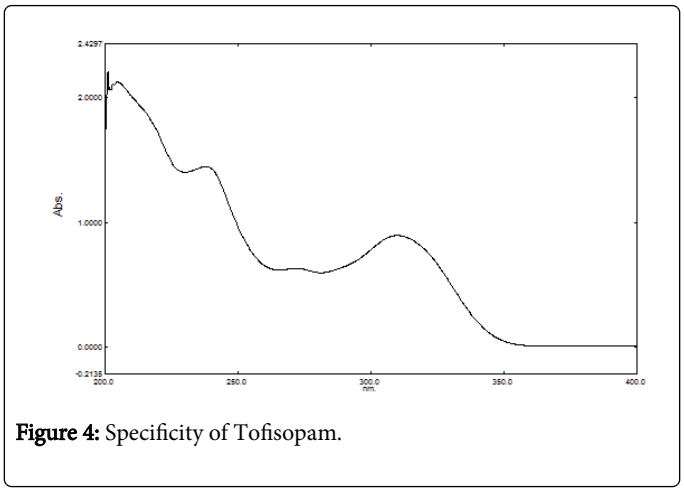


**Figure 4:** Specificity of Tofisopam.

**LOD and LOQ:** Limit of Detection (LOD) and Limit of Quantification (LOQ) of Tofisopam was calculated by using equation given in the ICH guidelines.

LOD=3.3* σ/S and LOQ=10* σ/S

Where, σ=Standard deviation of the response;

S=Slope of the calibration curve.

**Assay (Analysis of marketed formulation):** Twenty tablets were weighed accurately and powdered. A quantity of powder equivalent to 50 mg of Tofisopam was weighed and transferred to a 50 ml volumetric flask. The volume was made by using methanol. Solution was sonicated for 15 mints and resulting solution was filtered. The filtered solution (1 ml) was transferred to 10 ml volumetric flask and diluted with methanol. From this 1 ml was taken and diluted to 10 ml with methanol to get a solution containing 10 µg/ml solution and the absorbance of the solution was measured at 310 nm.

## Results and Discussion

A simple, rapid, accurate, precise spectroscopic method for the estimation of Tofisopam in bulk and pharmaceutical tablet dosage form has been developed and validated. Standard calibration curve for Tofisopam was found to be linear with Correlation Coefficient ($R^2$) value in range of 0.9996 respectively (Figure 3) and statistical data is shown in Table 1.

| S. No. | Parameters | Results |
|---|---|---|
| 1 | Analytical wavelength | 310 nm |
| 2 | Linearity µg/ml | 4-24 µg/ml |
| 3 | Linear regression Equation | y=0.0441x + 0.0391 |
| 4 | Correlation coefficient ($R^2$ ) | 0.9996 |
| 5 | Interday Precision(Mean RSD, n=3) | 0.592032 |
| 6 | Intraday Precision (Mean RSD, n=3) | 0.477814 |
| 8 | Assay in percentage n=6 | 99.45 ± 0.64 |
| 9 | Accuracy | 99.68-100.51% |
| 10 | LOD µg/ml | 0.475 |
| 11 | LOQ µg/ml | 1.36 |

**Table 1:** Summary of validation parameters.

The developed method was found to be precise as the % RSD values for the precision studies were <2% (Tables 2 and 3).

| Concentration (µg/ml) | Absorbance (nm) | | | Mean | Standard Deviation | % Relative Standard Deviation | Average of % RSD |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | | |
| 8 | 0.389 | 0.386 | 0.387 | 0.387 | 0.001528 | 0.39437 | 0.477 |
| 12 | 0.557 | 0.552 | 0.556 | 0.555 | 0.002646 | 0.476712 | |
| 16 | 0.745 | 0.737 | 0.739 | 0.74 | 0.004163 | 0.562359 | |

**Table 2:** Intraday precision.

| Concentration (µg/ml) | Absorbance(nm) | | | Mean | Standard Deviation | % Relative Standard Deviation | Average of % RSD |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | | | |
| 8 | 0.391 | 0.392 | 0.398 | 0.394 | 0.003786 | 0.961712 | 0.592 |
| 12 | 0.557 | 0.561 | 0.556 | 0.558 | 0.002646 | 0.474149 | |
| 16 | 0.737 | 0.742 | 0.74 | 0.74 | 0.002517 | 0.340236 | |

**Table 3:** Interday precision.

Accuracy of the proposed method was determined by recovery studies and the results were found in the range of 99.68-100.51%. Values of standard deviation and coefficient of variance was satisfactorily low, indicating the accuracy of the method (Table 4).

| No of Preparation | Concentration (µg/ml) | | Percent Recovery | Mean % Recovery ± SD | % RSD | Mean RSD |
|---|---|---|---|---|---|---|
| | Formulation | Pure Drug | | | | |
| S1: 80% | 10 | 8 | 100.25 | 100.29 ± 0.036 | 0.19 | 0.24 |
| S1: 80% | 10 | 8 | 100.12 | | | |
| S1: 80% | 10 | 8 | 100.5 | | | |
| S2: 100% | 10 | 10 | 100.65 | 100.5167 ± 0.339 | 0.33 | |
| S2: 100% | 10 | 10 | 100 | | | |
| S2: 100% | 10 | 10 | 100.9 | | | |
| S3: 120% | 10 | 12 | 99.89 | 99.68667 ± 0.205 | 0.2 | |
| S3: 120% | 10 | 12 | 99.69 | | | |
| S3: 120% | 10 | 12 | 99.48 | | | |

**Table 4:** Accuracy of Tofisopam.

The influence of excipients was studied by mixing drug with excipient as per the ratio (Figure 4). LOD and LOQ were found to be 0.475 and 1.36 µg/ml respectively (Table 5). The assay results of tablet formulation are shown in Table 6, which shows good agreement with the labeled claim. So it proved that no interference was observed form the presence of excipients in the amounts, which are commonly present in tablet formulation. Table 1 shows the Summary of all validation parameters.

| S. No. | Parameters | S.D* | b** | Formula | Calculation |
|---|---|---|---|---|---|
| 1 | LOD | 0.043021 | 0.026 | 3.3(./) | 0.4725 |
| 2 | LOQ | 0.043021 | 0.026 | 10(./) | 1.36 |

**Table 5:** LOD and LOQ of UV-vis spectrophotometric method for Tofisopam.

| Tablet Formulation | Label Claim | Sample solution concentration µg/ml | Amount found | % RSD |
|---|---|---|---|---|
| Tofisopam | 50 mg | 10 mg | 99.4% ± 0.0621 | 0.62 |

**Table 6:** Assay, average (n=6).

## Conclusion

The results and the statistical parameters demonstrate that the proposed spectrophotometric method is simple, precise, rapid, specific, and accurate. Therefore, this method can be used for routine analysis of Tofisopam in bulk and pharmaceutical dosage formulation.

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