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# UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TOPIROXOSTAT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

A simple, sensitive, rapid, accurate, reproducible and less time consuming UV visible spectroscopic method has been developed for the determination of topiroxostat in bulk and tablet dosage form. The wavelength maxima for topiroxostat was found to be 320.0nm. Beer's law was obeyed in the concentration range of 10-50µg/ml. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 3µg/ml and 9.27µg/ml. The percentage recovery of the drug for the proposed method was 99.63% indicating no interference of tablet excipients. The results demonstrate that proposed method is accurate, precise and reproducible while being simple and rapid too for determination of topiroxostat in tablet dosage form.

**KEYWORDS:** Validation, Range, Concentration, Method development, Percentage recovery.

## INTRODUCTION

Topiroxostat is a selective xanthine oxidase inhibitor developed for the treatment and management of hyperuricemia and gout.[1] Chemically, it is 4-[5-(4-Pyridinyl)-1H-1,2,4-triazol-3-yl]. -2-pyridinecarbonitrile. It is a selective xanthine oxidoreductase (XOR) inhibitor for the management of hyperuricemia in patients with or without gout. Xanthine oxidase, or xanthine oxidoreductase (XOR), regulates purine metabolism, and inhibition of the enzyme results in an efficacious reduction of serum urate levels.[2] Topirastat has a significant inhibitory effect on both oxidised and reduced XOR, so its effect of reducing uric acid is more powerful and long-lasting, so this product can be used to treat chronic hyperuricemia in gout. Furthermore, topiroxostat and febuxostat showed similar renal protective and anti-inflammatory effects after 6 months of treatment in hyperuricemic patients with cardiovascular disease.[3] The molecular formula is C13H8N6 and has a molecular mass of 248.24 g/mol. It is a white powder. It is soluble in DMSO (dimethyl sulfoxide), insoluble in water, and in ethanol.

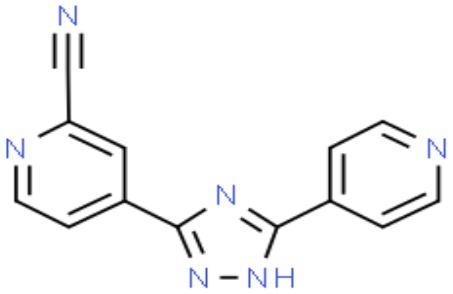


**Figure 1: Chemical structure of topiroxostat.**

From the literature survey, it was found that topiroxostat was estimated by analytical methods such as Spectrophotometry, HPLC and HPTLC in single form or combination with other drugs. The aim of the present study is to develop a simple, precise and accurate spectrophotometric method for the estimation of topiroxostat in pharmaceutical dosage form as per ICH guidelines.[4-10]

## MATERIALS AND METHODS

### Instruments and Reagents

A UV-VIS 2080N double-beam spectrophotometer connected to a computer with UV professional software installed was used for all the spectrophotometric measurements. The samples were weighed on an electronic balance (A120) by an analyst. Topiroxostat tablets were procured from a local manufacturer, Blisson

Biopharma Pvt. Ltd. (Toxur 20). All the reagents were of analytical grade. Glass-distilled water was used throughout the experiment.

**Selection of solvent**

The solubility of Topiroxostat is determined in a variety of solvents as per pharmacopoeia standards. A solubility test was carried out in different solvents like distilled water, methanol, ethanol, and 0.1N sodium hydroxide. From the solubility studies, it was found that Topiroxostat is insoluble in distilled water, ethanol, and 0.1N sodium hydroxide. It is soluble in acetonitrile, methanol, and DMSO. Because of their easy availability and cost effectiveness, a mixture of methanol and acetonitrile was selected as the solvent for the analysis of topiroxostat. In this study, methanol and acetonitrile (50:50) were selected as solvents.

**Preparation of standard stock solution**

An accurately weighed quantity of 100mg was taken in a 100-ml volumetric flask. 40 ml of solvent (methanol:acn) were added to it and sonicated for 15 minutes. Then the volume was adjusted with solvent to get a concentration of 1000 µg /mL. From this, 10 mL were taken into a 100 mL volumetric flask, and 80 mL of solvent were added to it. This was sonicated for 10 minutes, and the volume was diluted up to the mark with solvent to get a concentration of 100 µg /ml.

**Determination of $\lambda_{max}$**

The standard solution of Topiroxostat (100µg /ml) was scanned in the UV region (200–400 nm) and the spectrum was recorded. Solvent was used as a blank. It was seen that at 320.05nm maximum absorbance was found, as shown in figure 2. Therefore, 320.05nm was selected for this study.

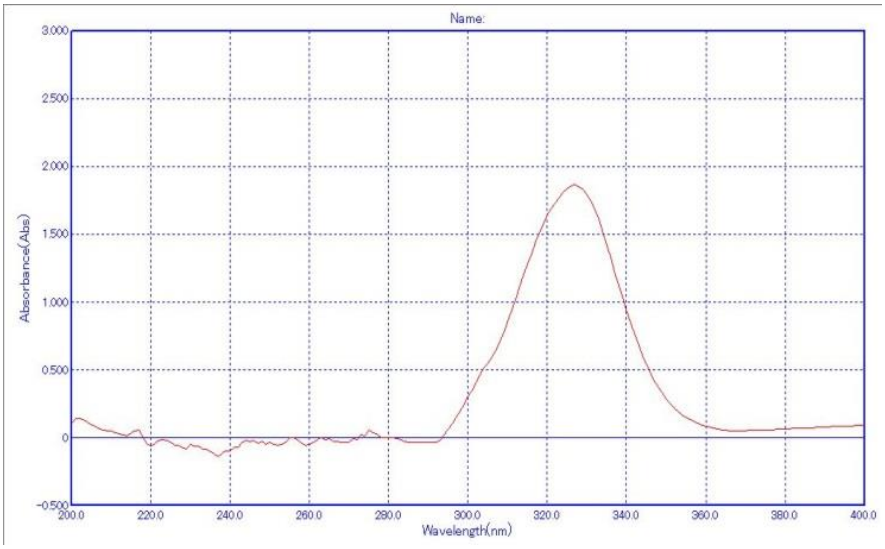


**Figure 2: Absorbance spectra of Topiroxostat.**

**Method Validation**[11-14]

The objective of method validation is to demonstrate that the method is suitable for its intended purpose. The method was validated for linearity, precision, accuracy, robustness, ruggedness, LOD, LOQ, and specificity as per ICH guidelines.

**1. Linearity**

From the standard stock solution, the various dilutions with concentrations of 10 µg /ml, 20 µg /ml, 30 µg /ml, 40 µg /ml, and 50 µg /ml were prepared. The solutions were scanned at 320.05 nm, and the absorbance was recorded, as shown in table 1. From this calibration curve, the absorbance versus concentration of Topiroxostat was plotted, and the linearity graph was represented in Figure 3. The correlation coefficient was found to be 0.9995.

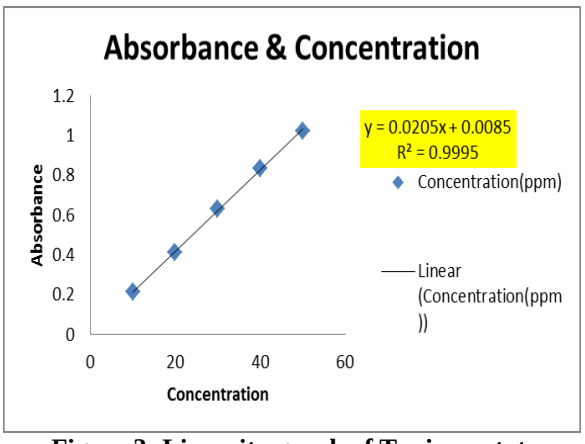


**Figure 3: Linearity graph of Topiroxostat.**

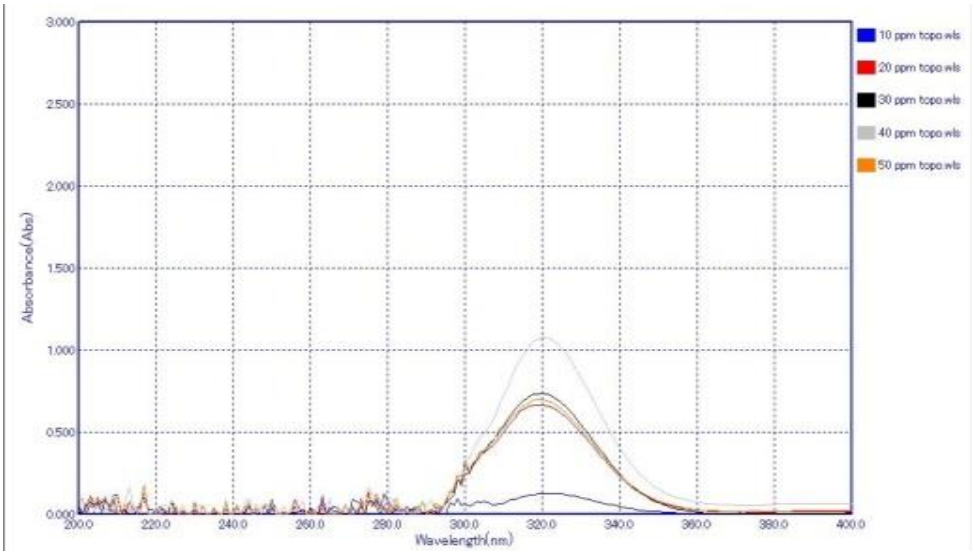


**Figure 4: linear spectra of Topiroxostat.**

**Table 1: Linearity parameter for topiroxostat.**

| Concentration(µg/ml) | Absorbance |
|---|---|
| 10 | 0.212 |
| 20 | 0.412 |
| 30 | 0.63 |
| 40 | 0.835 |
| 50 | 1.024 |

**2. Precision**

The repeatability of the method was checked by scanning 10 µg /ml of solution six times. Intra-day precision was determined by checking the absorbance of 10 µg /mL on the same day. Inter-day precision was determined by checking the absorbance of 10 µg /ml on three different days. The %RSD was found to be 0.49% for intra-day and 0.55% for inter-day, as shown in Table 2.

**Table 2: Precision parameter for topiroxostat.**

| Scans | Intra-day | Inter-day |
|---|---|---|
| **1** | 0.212 | 0.214 |
| **2** | 0.213 | 0.212 |
| **3** | 0.211 | 0.213 |
| **4** | 0.214 | 0.211 |
| **5** | 0.212 | 0.212 |
| **6** | 0.213 | 0.211 |
| **Mean** | 0.2125 | 0.212167 |
| **SD** | 0.001049 | 0.001169 |
| **% RSD** | 0.493557 | 0.551003 |

**3. Accuracy**

Accuracy study was conducted by spiking at three different concentration levels (80%, 100%, 120%). At each level samples were scanned and from the absorbance recovery percentage was determined and presented in table 3.

**Table 3: Accuracy parameter for topiroxostat.**

| Level of recovery | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|
| 80% | 8 | 7.95 | 99.45 | 99.41% |
| 100% | 10 | 9.95 | 99.58 | |
| 120% | 12 | 11.90 | 99.22 | |

**4. Robustness**

To determine robustness of the method two parameters (wavelength and diluent composition) were made slightly different from the selected wavelength and diluent composition. No significant difference was found in the absorbance and hence the proposed method was considered as robust which is shown in table 4.

**Table 4: Robustness parameter for topiroxostat.**

| Method parameters | Adjusted to | Average absorbance | S.D | %RSD |
|---|---|---|---|---|
| **Wave length (320nm)** | 318 | 0.210 | 0.001 | 0.476 |
| **Diluent composition (50:50)** | 45:55 | 0.213 | 0.002 | 0.938 |

**5. Ruggedness**

The ruggedness of the developed method was checked by analysing the samples by different analysts on different days in similar operational conditions. The statistical analysis showed no significant differences were observed between results obtained employing different analysts, as given in Table 5.

**Table 5: Ruggedness parameter for topiroxostat.**

| Analyst | Days | Average absorbance | S.D | % RSD |
|---|---|---|---|---|
| 1 | 1 | 0.212 | 0.001049 | 0.439 |
| 2 | 2 | 0.213 | 0.001169 | 0.551 |
| 3 | 3 | 0.211 | 0.001063 | 0.529 |

**6. Limit of detection and Limit of quantification**

Limit of detection is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. Limit of quantification is the lowest amount of analyte in a sample that can be quantified under stated experimental conditions. The LOD and LOQ for topiroxostat were found to be 3.05µg/ml and 9.27µg/ml.

**7. Specificity**

A solution containing mixture of tablet excipients were prepared using the sample preparation procedure to evaluate the possible interference of the excipients. From the absorbance result no interference was observed from the excipients present in the formulation, indicated that the method is specific.

**Assay of topoxistat tablets**

Two different brands of topoxistat were analysed using the validated method. For the analysis six replicates of each brand were assayed. 20 tablets of topiroxostat were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 50mg of topiroxostat was taken in a 50ml volumetric flask. 10ml of methanol was added to it followed by 20ml of solvent. The solution was sonicated for 15 min and then filtered through Whatmann filter paper (No.41) and volume adjusted with the solvent. From this further dilution was made to get final concentration of 20µg/ml. The results were presented in table 6.

**Table 6: Assay for topiroxostat tablets.**

| Sample | Label claim(mg) | Amount found(mg) (± S.D) | % Amount found |
|---|---|---|---|
| Taxur 20 **(blisson)** | 20 | 19.66±0.35 | 98.31 |

**RESULTS AND DISCUSSION**

The method was validated and developed as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD, LOQ, and specificity. Beer's law is obeyed over the concentration range of 10–50 µg/ml. Using regression analysis, the linear equation Y = 0.0205x + 0.0085 has a correlation coefficient of r2 = 0.09995. The precision results show a % RSD of less than 2 at each level, which indicates clearly that the method is precise enough for the analysis of Topiroxostat. The accuracy of the method was checked by recovery studies. The high recovery values indicated the accuracy of the developed method. The robustness and ruggedness studies reveal that the method is robust and rugged enough. The LOD and LOQ values indicate the sensitivity of the method. There was no interference observed from the excipients present in the formulation, which indicates that the method is specific. The determination of Topiroxostat in the tablet formulation of the brand showed that the content of Topiroxostat was very close to the labelled amount. The %RSD values in all the parameters were within the acceptable limit. The optical characteristics of the method are represented in table 7.

**Table 7: Optical characteristics of the proposed developed method.**

| Parameter | Value |
|---|---|
| $\lambda_{max}$ (nm) | 320.05 |
| Beer's range(µg/ml) | 10-50 |
| Correlation coefficient($r^2$) | 0.09995 |
| Regression equation (Y= mX+C) | Y= 0.0205x+0.0085 |
| Intercept (C) | 0.0085 |
| Slope (m) | 0.0205 |
| LOD (µg/ml) &LOQ (µg/ml) | 3.0 & 9.23 |
| Precision (%RSD) | 0.49 |

**CONCLUSION**

A validated UV spectrophotometer method has been developed for the estimation of Topiroxostat in bulk as well as pharmaceutical dosage forms. The developed method was found to be simple, accurate, precise, specific, reproducible, and linear over the concentration range studied. The proposed method can be used for routine analysis of Topiroxostat in bulk as well as pharmaceutical formulations.

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