www.wcprj.com 2026 Page: 01-09 Review Article

World Current Pharmaceutical Research Journal ISSN (O): Applied Volume: 02 Issue: 03

COMPARATIVE ANALYTICAL STUDY OF TOPIROXOSTAT USING UV SPECTROSCOPY AND HPLC METHODS

C. A. Shaik Fayaaz Ahamed*[1], P. Parveen[2], V. Prabavathi[2], S. Nithish[2], R. Pradeep[2], J. Pradeep Raj[2]

*[1]Associate Professor, Department of Pharmaceutical Analysis,

[2]B. Pharm Final Year Students,

Aadhi Bhagawan College of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu.

Article Received: 21 January 2026

Article Revised: 02 February 2026

Published on: 22 February 2026

***Corresponding Author: C. A. Shaik Fayaaz Ahamed**

Associate Professor, Department of Pharmaceutical Analysis, Aadhi Bhagawan College of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu. **DOI:** https://doi.org/10.5281/zenodo.18827575

ABSTRACT

Topiroxostat is a selective, non-purine xanthine oxidoreductase (XOR) inhibitor indicated for the treatment of hyperuricemia and gout. It reduces serum uric acid levels by inhibiting the enzymatic conversion of hypoxanthine and xanthine into uric acid during purine metabolism. Chemically known as 4-[5-(4-Pyridinyl)-1H-1,2,4-triazole-3-yl]-2-pyridinecarbonitrile, Topiroxostat has a molecular formula of $C_{13}H_8N_6$ and a molecular weight of 248.24 g/mol. The drug exhibits good solubility in organic solvents, limited aqueous solubility, and a half-life of approximately 5 hours. Pharmacokinetically, Topiroxostat is rapidly absorbed with peak plasma concentrations reached within 0.67 hours, exhibits high plasma protein binding (>97.5%), undergoes hepatic metabolism primarily via glucuronidation, and is excreted through feces and urine. In addition to gout management, it shows potential benefits in chronic kidney disease, heart failure, and diabetic nephropathy. Several analytical techniques including UV spectrophotometry, HPLC, and RP-HPLC have been developed and validated for its quantitative estimation, demonstrating excellent linearity, precision, accuracy, and sensitivity. This review summarizes the physicochemical properties, mechanism of action, pharmacokinetics, therapeutic uses, adverse effects, and analytical methods of Topiroxostat.

KEYWORDS: Topiroxostat, Xanthine oxidoreductase inhibitor, Hyperuricemia, Gout, HPLC, RP-HPLC, UV spectroscopy, Pharmacokinetics.

1. INTRODUCTION

Hyperuricemia is a metabolic disorder characterized by elevated serum uric acid levels, often leading to gout, renal dysfunction, and cardiovascular complications. Uric acid is the final product of purine metabolism and is formed by the catalytic action of the enzyme xanthine oxidoreductase (XOR). Inhibition of this enzyme plays a crucial role in reducing uric acid production. Topiroxostat is a non-purine selective XOR inhibitor developed for the management of hyperuricemia. Unlike purine analog inhibitors, it binds tightly to both oxidized and reduced forms of the enzyme, thereby effectively suppressing uric acid synthesis. This selective inhibition reduces urate crystal deposition and prevents gout flare-ups and renal complications.

The drug is available under various trade names and is administered orally. It demonstrates rapid absorption, extensive tissue distribution, and high protein binding. Hepatic metabolism via glucuronidation and dual excretion pathways contribute to its therapeutic profile. Due to its growing clinical importance, accurate and reliable analytical methods are essential for its quantitative estimation in bulk and pharmaceutical dosage forms. Several validated methods such as UV spectrophotometry, HPLC, and RP-HPLC have been reported, ensuring precision, sensitivity, and regulatory compliance in pharmaceutical analysis.

1.1 ANALYTICAL CHEMISTRY

Analytical chemistry is a branch of chemistry concerned with the identification, separation, and quantification of chemical substances. It plays a fundamental role in pharmaceutical sciences, ensuring the quality, safety, and efficacy of drug substances and dosage forms. Analytical chemistry involves the development and application of techniques to determine the chemical composition of materials and to measure the concentration of analytes accurately and precisely.

Analytical methods are broadly classified into classical methods (such as titrimetric and gravimetric analysis) and instrumental methods, including UV–Visible spectroscopy, High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Liquid Chromatography–Mass Spectrometry (LC-MS/MS). Instrumental techniques offer higher sensitivity, selectivity, and reproducibility, making them indispensable in modern pharmaceutical analysis.

In drug analysis, analytical chemistry is applied for identification of active pharmaceutical ingredients (API), assay of formulations, impurity profiling, stability studies, dissolution testing, and bioanalytical investigations. Proper analytical method development ensures reliable results that comply with regulatory standards.

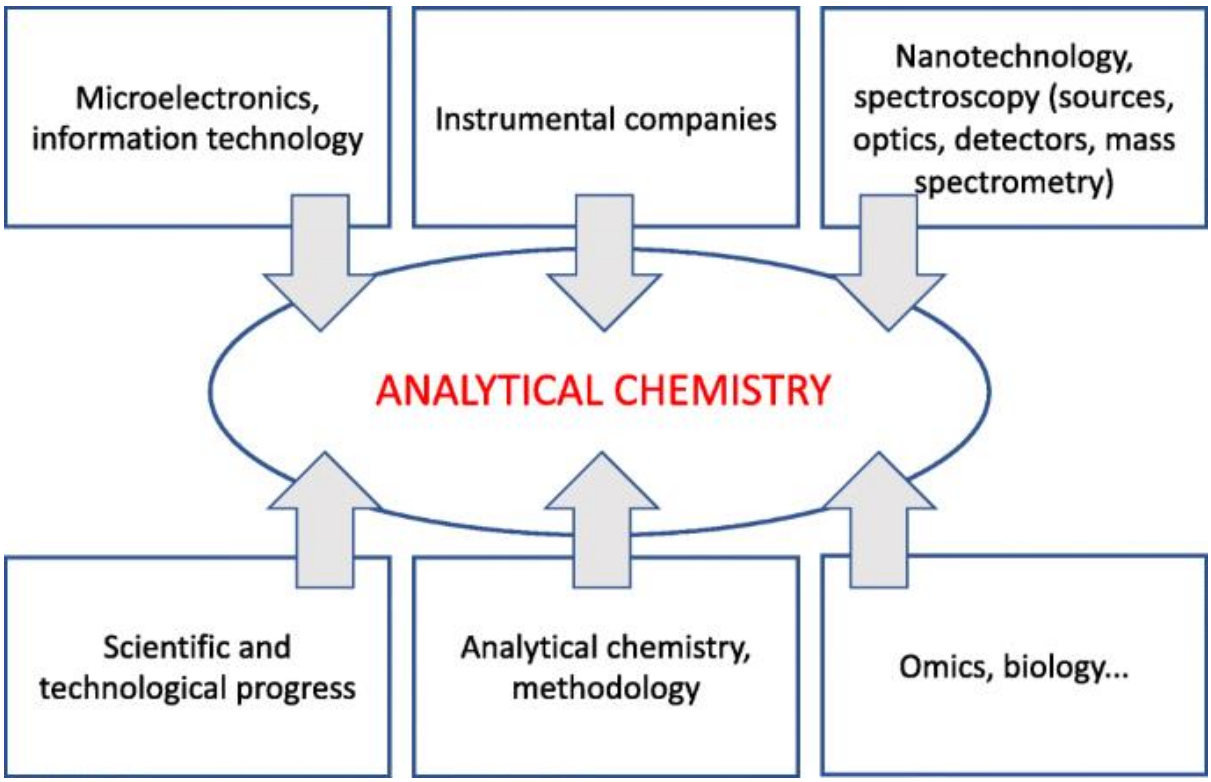

Fig. 1: Analytical Chemistry.

1.2 ANALYTICAL METHOD VALIDATION

Analytical method validation is the process of establishing documented evidence that an analytical procedure is suitable for its intended purpose. It confirms that the method consistently produces accurate, precise, and reliable results. Validation is essential in pharmaceutical industries to meet regulatory requirements and ensure product quality.

According to International Council for Harmonisation (ICH) guidelines (ICH Q2(R1)), analytical method validation includes the evaluation of several parameters such as:

- **Specificity** – Ability to measure the analyte accurately in the presence of impurities, excipients, or degradation products.
- **Linearity** – Ability to obtain test results directly proportional to concentration within a given range.
- **Accuracy** – Closeness of measured values to the true value.

- **Precision** – Degree of agreement among individual test results (repeatability, intermediate precision).
- **Limit of Detection (LOD)** – Lowest amount of analyte detectable but not necessarily quantifiable.
- **Limit of Quantification (LOQ)** – Lowest amount of analyte quantifiable with acceptable precision and accuracy.
- **Robustness** – Ability of the method to remain unaffected by small deliberate variations in method parameters.
- **Range** – Interval between upper and lower concentration levels with acceptable precision and accuracy.

Validation ensures reliability, reproducibility, and compliance with regulatory standards. Properly validated analytical methods are essential for drug approval, routine quality control, stability studies, and regulatory submissions.

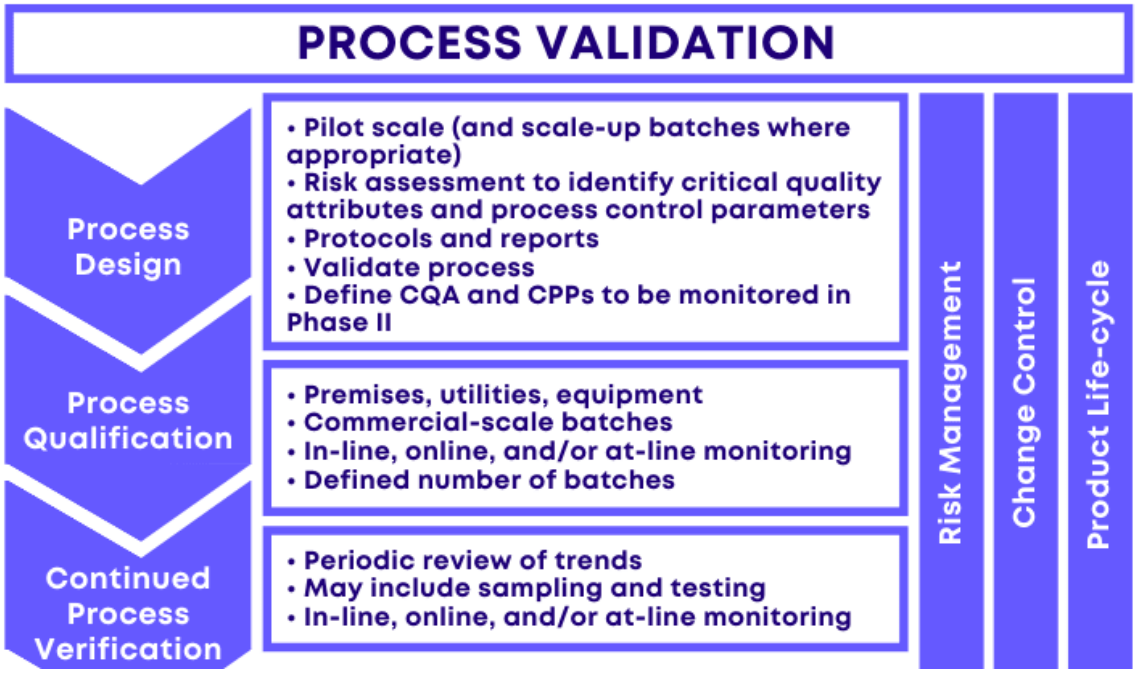

Fig. 2: Validation Process.

2. DRUGS PROFILE FOR TOPIROXOSTAT

2.1 Physio-Chemical Properties

- **Trade name**: Topiroxo-20 (Alkem Laboratories Ltd), Toxur-20 (Blisson Medi plus Pvt. Ltd) and Topimac-20 (Macleods Pharmaceuticals Ltd).
- **Drug category**: Hyperuricemia

- **Class**: Non-purine xanthine oxidase inhibitor.
- **Description**: Topiroxostat is an orally administered, non- purine, selective xanthine oxidase (XO) inhibitor developed for the treatment of hyperuricemia specifically for patients used in gout.
- **Chemical name**: 4-[5-(4-Pyridinyl)-1H-1,2,4-triazole-3-yl]-2-pyridinecarbonitrile
- **Storage**: Stored at room temperature
- **Molecular weight**: 248.24 g
- **Molecular formula**: $C_{13}H_8N_6$
- **Melting point**: 293°C
- **Solubility**: soluble in organic solvents. Sparingly soluble in aqueous buffers.
- **Half-life**: Approximately ~5 hours.

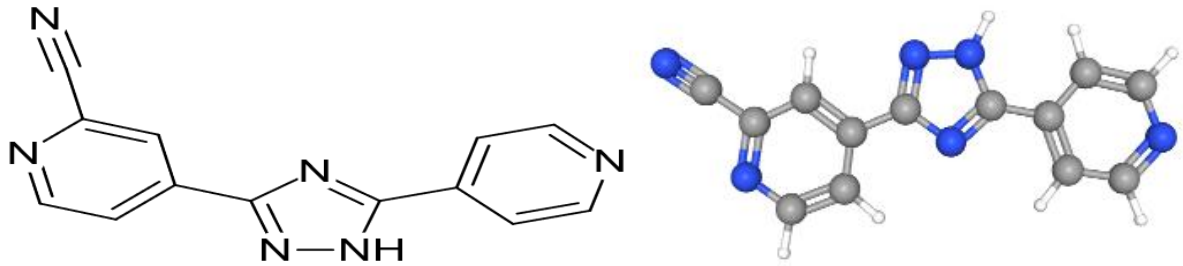

Fig. 3: Structure of Topiroxostat.

2.2 Mechanism of Action

Topiroxostat is a selective xanthine oxidoreductase (XOR) inhibitor used in the management of hyperuricemia and gout. It acts by inhibiting the enzyme xanthine oxidoreductase, which is responsible for the conversion of hypoxanthine to xanthine and xanthine to uric acid during purine metabolism. By blocking this enzyme, Topiroxostat reduces the formation of uric acid in the body, thereby lowering serum uric acid levels. It is a non-purine type inhibitor that forms a tight-binding interaction with the enzyme and inhibits both the oxidized and reduced forms of XOR. Through this mechanism, it helps prevent uric acid crystal deposition, gout attacks, and renal complications associated with elevated uric acid levels.

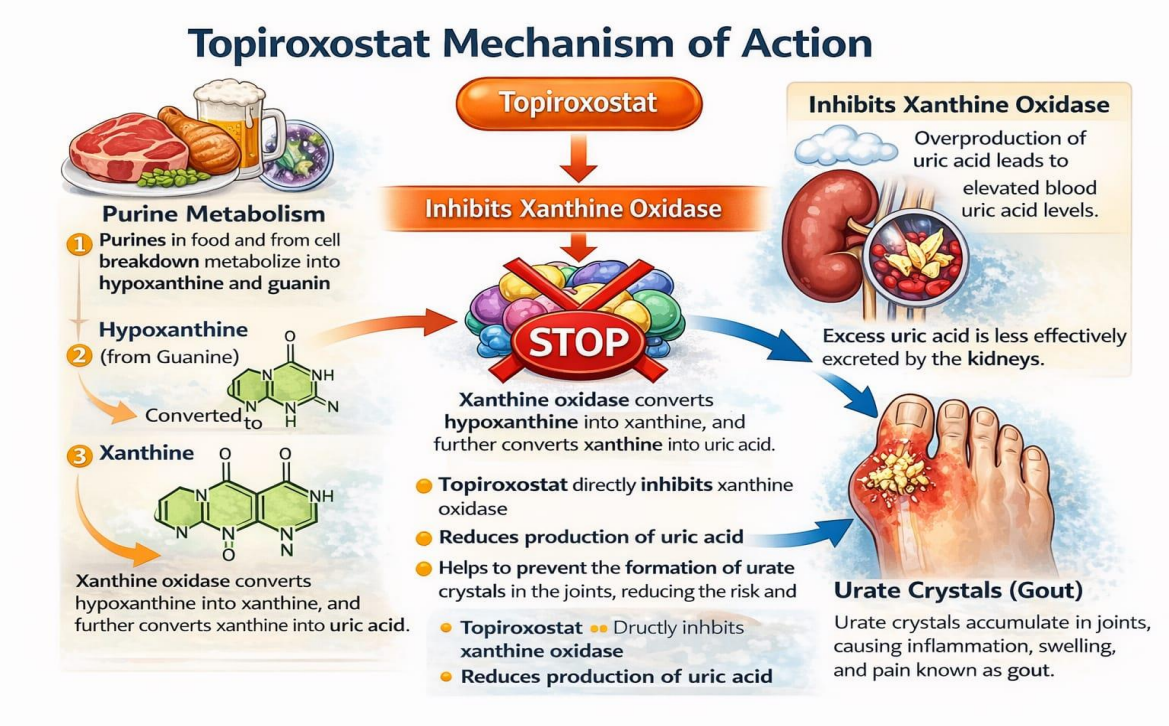

Fig. 4: Mechanism Of Topiroxostat.

2.3 Uses

- Hyperuricemia management – lowers uric acid levels in conditions like gout and tumor lysis syndrome..
- Chronic kidney disease (CKD) – reduces uric acid to slow CKD progression.
- Heart failure – may improve cardiac function by reducing oxidative stress.
- Diabetic nephropathy– renal protective effects.

2.4 Adverse Effects:

- Gastrointestinal discomfort – nausea, vomiting, diarrhea, abdominal pain.
- Liver enzyme elevation
- Hypersensitivity reactions
- Musculoskeletal pain – joint pain, muscle aches.
- Dizziness & headache
- Kidney function impact – reduced urine output, swelling, fatigue; renal function tests needed.
- Cardiovascular signals – chest pain, palpitations, shortness of breath (potential cardiovascular risk).

2.5 Pharmacokinetics

1. **Absorption**: Taken orally; peak plasma levels ~0.67 hours after ingestion.
2. **Distribution**: extensive tissue distribution. It shows high plasma protein binding (>97.5%) and reaches peak plasma concentration (229.9 ng/mL) in 0.67 hours.
3. **Metabolism**: Primarily hepatic (glucuronidation).
4. **Excretion**: ~40% via feces and ~30% in urine.

3. REVIEW OF LITERATURE

Table 1: High Performance Liquid Chromatography (HPLC).

Stationary Phase	Mobile Phase	Flow Rate Method of Detection Retention Time	Results	Reference
Column Agilent Zorbax Bonus RP C18 (250 x 4.6 mm,5 µm).	50mM/L Dihydrogen Potassium Phosphate (pH 3.3 set with ortho-phosphoric acid): Acetonitrile (20:80%v/v)	**Flow rate**= 1.0mL/min **Retention time (Rt)** = 6.99 min.	**Linearity range** 0.01–120 µg/mL **LOD**= 0.075 µg/mL **LOQ**= 0.229 µg/mL **%Accuracy (n = 3)** 100.01–100.90 **Intermediate precision** = 0.18	**Vaibhavi V Kunjir (2026)**

Table 2: Reverse phase High Performance Liquid Chromatography (RP-HPLC).

Stationary Phase	Mobile Phase	Flow Rate, Method of Detection, Retention Time	Results	Reference
Phenomenex Luna C18 column (250 mm × 4.6 mm × 5 µm particle size)	Potassium dihydrogen orthophosphate pH 2.5 adjusted with orthophosphoric acid and acetonitrile (60:40 % v/v)	**Flow rate**=1mL/min. **method of detection UV at a** 215 nm. **Retention Time(Rt)** = 3.81 minutes	**Assayrecovery percentage** = 100.08%, **Linearity** =18–42µg/mL **Slope** = 417,484 **Theoretical plates (N)** = 9926	***Medidi Srinivas (2024)***
Zodiac-100 C8 (5µm; 150 x 4.6 mm ID.)	Mixed phosphate buffer: Acetonitrile (45:55)	**Flow rate**=1ml/min **method of detection UV at a** 240 **Retention Time (Rt)** = 3.52 minutes	**LOQ** = 8. 08µg.ml^{-1} **LOD** = 2.42 µg.ml^{-1} **Linearty** = 3.92 -0.5µg/ml^{-1} **Repeatability (%RSD)** = 1.67 **Theoretical plates (N)** = 3160	**M. Kuranjekar & Dr. Anup Barsagade (2024)**

Table 3: UV Spectrophotometric Method.

Stationary Phase/ Instrumentation	Mobile Phase/ Solvent	Flow Rate, Method Of Detection, Retention Time	Result	Reference
Stationary Phase Not applicable (NA) **Instrumentation**	methanol and acetonitrile (50:50)	**FlowRate**: Not applicable (NA), **Method Of Detection**|: UV absorbance at 320nm,	**λmax (nm)** =320.05 **Beer's range(µg/ml)**=10-50 **Correlation coefficient (r2)**=0.09995 **Intercept (C)**=0.0085	Pallavi Suthar (2023)

UV-VIS 2080N double-beam spectrophotometer		**Retention Time (Rt) =** Not applicable	**Slope (m)**=0.0205 **LOD** =3.0µg/ml **LOQ**=9.23µg/ml **Precision (%RSD)** =0.49	

4. CONCLUSION

Topiroxostat is an effective and selective non-purine xanthine oxidoreductase inhibitor used in the treatment of hyperuricemia and gout. Its mechanism of action involves inhibition of uric acid synthesis, thereby preventing complications associated with elevated uric acid levels. The drug exhibits favorable pharmacokinetic properties, including rapid absorption, high protein binding, hepatic metabolism, and dual elimination pathways.

Validated analytical methods such as UV spectroscopy and chromatographic techniques provide accurate and reliable quantification of Topiroxostat in pharmaceutical formulations. Overall, Topiroxostat represents an important therapeutic option in the management of hyperuricemia with well-established analytical and pharmacological profiles.

5. REFERENCES

1. Sharma BK, Instrumental Methods of Chemical Analysis; 23rd Edn, Goel Publication House, New Delhi, 2002; 1-16.
2. Michael A and Swartz, Ultra Performance Liquid Chromatography (UPLC): An Introduction; 3rd Edn, Nirali Publication, Pune, 2005; 120.
3. Jeffery GH, Bassett J, Mehdham J and Denney RC, Text of quantitative Chemical Analysis; 5th Edn, 1989; 3-4.
4. Swarbrick J, and Boylan JC, Encyclopedia of pharmaceutical technology; 4th Edn, Marcel Dekker Inc, 2002; 363-416.
5. Sharma PP, How to Practice GMPs (Good Manufacturing Practices); 3rd Edn, Vandana Publication Pvt Ltd, New Delhi, 2001; 214.
6. Richette P, Perez-Ruiz F, Doherty M, Jansen TL, Nuki G, Pascual E, *et al.* Improving cardiovascular and renal outcomes in gout: What should we target? Nat Rev Rheumatol., 2014; 10: 654-61.
7. Okafor ON, Farrington K, Gorog DA. Allopurinol as a therapeutic option in cardiovascular disease. Pharmacol Ther, 2017; 172: 139-50.
8. Grimaldi-Bensouda L, Alpérovitch A, Aubrun E, Danchin N, Rossignol M, Abenhaim L, *et al.* Impact of allopurinol on risk of myocardial infarction. Ann Rheum Dis., 2015; 74: 836-42.

9. MacIsaac RL, Salatzki J, Higgins P, Walters MR, Padmanabhan S, Dominiczak AF, *et al.* Allopurinol and cardiovascular outcomes in adults with hypertension. Hypertension, 2016; 67: 535-40.
10. Struthers AD, Donnan PT, Lindsay P, McNaughton D, Broomhall J, MacDonald TM. Effect of allopurinol on mortality and hospitalisations in chronic heart failure: A retrospective cohort study. Heart., 2002; 87: 229-34.
11. Chen C, Lu JM, Yao Q. Hyperuricemia-related diseases and xanthine oxidoreductase (XOR) inhibitors: an overview. *Medicinal Scientific Monitoring,* 2016; 22: 2501–2512.
12. Nakamura T, Murase T, et al, Effects of topiroxostat and febuxostat on urinary albumin excretion and plasma xanthine oxidoreductase activity in db/db mice. *European Journal of Pharmacology*, 2016; 780: 224–231.
13. Kumar SD, Kumar DH. Importance of RP-HPLC in analytical method development: a review. *International journal of pharmaceutical sciences and research*, 2012; 3(12): 4626.
14. Lindholm J. Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 2004; 13-14.
15. Kaushal C, Srivastava B, A Process of Method Development: A Chromatographic Approach. Journal of Chemical and Pharmaceutical Research, 2010; 2(2): 519-545.
16. Marinho ES, Marinho MM. A DFT study ofsynthetic drug topiroxostat: MEP, HOMO, LUMO. Int. J. Sci. Eng. Res., 2016 1 Jul; 7(8): 1264.
17. Kamal AH, El-Malla SF, Hammad SF. A review on UV spectrophotometric methods for simultaneous multicomponent analysis. Eur J Pharm Med Res., 2016 Jan 27; 3(2): 348-60.
18. Kalra K. Method development andvalidation of analytical procedures. QualityControl of Herbal Medicines and Related Areas, 2011 Nov 4; 4: 3-16.
19. Suthar P, Ram M. UV spectrophotometricmethod development and validation for theestimation of Topiroxostat in bulk and pharmaceutical dosage form.
20. Francis R, Burt T. Optical characterization of thin films using a new Universal Measurement Accessory for the AgilentCary UV-Vis-NIR spectrophotometers. Agilent Application Note 5991-1356EN; 2013.