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NOVEL RPHPLC METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF TOPIROXOSTAT AND DOTINURAD

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ABSTRACT

A novel reverse-phase high-performance liquid chromatography (RP-HPLC) method was designed and developed for the simultaneous estimation of Topiroxostat and Dotinurad in synthetic mixtures. The method exhibited high accuracy, precision, and robustness, making it ideal for routine analytical applications. Assay results demonstrated 99.93% for Topiroxostat and 99.95% for Dotinurad, indicating minimal error and reliable quantification of active pharmaceutical ingredients. Linearity assessments revealed a correlation coefficient of 0.999 for both compounds, confirming excellent linearity and sensitivity across a wide concentration range. The method showed remarkable precision with relative standard deviation (RSD) values of 0.6% for Topiroxostat and 0.8% for Dotinurad, well within the acceptance criteria of RSD not more than 2.0%. Intermediate precision, with RSD values of 0.6% for both drugs, demonstrated the method's reliability across different days and analysts. Accuracy was validated through recovery studies, yielding 99.60% for Topiroxostat and 100.15% for Dotinurad, within the acceptable range of 97.0% to 103.0%, indicating reliable recovery of analytes. Limits of detection (LOD) were 3.07 µg/mL for Topiroxostat and 2.95 µg/mL for Dotinurad, while limits of quantification (LOQ) were 10.09 µg/mL and 9.93 µg/mL, respectively, showcasing the method's capability for trace analysis. Robustness testing confirmed consistent performance under varied mobile phase composition and flow rate conditions. The method's robustness and system suitability, coupled with high precision and accuracy, underscore its potential for routine quality control, dissolution studies, and bioequivalence assessments of formulations containing Topiroxostat and Dotinurad.

Keywords: Topiroxostat, Dotinurad, High Performance Liquid Chromatography, Reverse Phase Chromatography, Validation Parameters.

INTRODUCTION

Chromatography was originally developed by the Russian botanist Michael Tswett in1903 for the separation of colored plant pigments by percolating a petroleum ether extract through a glass column packed with powdered calcium carbonate [1]. It is now, in general, the most widely used separation technique in analytical chemistry having developed into a number of related but quite different forms that enable the components of complex mixtures of organic or inorganic components to be separated and quantified [2]. In RP-HPLC the stationary phase is non-polar often a hydrocarbon and the mobile phase is relatively polar such as water, methanol or Acetonitrile. In RPC the solutes are eluted in the order of their decreasing polarities [3]. Retention by interaction of the stationary phase non-polar hydrocarbon chain with non-polar parts and sample molecules [3].

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Topiroxostat is a selective xanthine oxidase inhibitor developed for treatment and management of hyperuricemia and gout [3].

Topiroxostat and its metabolites are shown to be unaffected by renal complications, thus may be effective in patients with chronic kidney diseases. Approved for therapeutic use in Japan since 2013, topiroxostat is marketed under the name Topiloric and Uriadec and is orally administered twice daily. Topiroxostat is shown to inhibit ATP-binding cassette transporter G2 (ABCG2) in vitro, which is a membrane protein responsible for recovering uric acid in the kidneys and secreting uric acid from the intestines. Dotinurad is under investigation in clinical trial NCT03372200 (Febuxostat-controlled, Double-blind, Comparative Study of FYU-981 in Hyperuricemia with or without Gout) [4,5]. Gout suppressants that act directly on the renal tubule to increase the excretion of uric acid, thus reducing its concentrations in plasma [6]. Validation may be defined as a process involving confirmation or establishing by laboratory studies that a method/ system/ analyst gives accurate and reproducible result for intended analytical application in a proven and established range. Linearity is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. Precision of the method when repeated by the same analysts [7], same test method and under same set of laboratory conditions (reagent, equipments), within a short interval of time, the only difference being the sample. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. LOQ defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions [8]. Design and development of novel simultaneous RP-HPLC method for the estimation of Topiroxostat and Dotinurad in synthetic mixture and validation of the developed method.

METHODOLOGY

Wave length selection

UV spectrum of 10 µg/ml Topiroxostat and 10 µg/ml Dotinurad in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 255 nm. At this wavelength both the drugs show good absorbance.

HPLC method development

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer pH 3.0: Methanol in proportion 70: 30 v/v respectively [9].

Optimization of Column

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (150 x 4.6, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.5 ml/min flow.

Preparation of phosphate buffer

Take 6.8gm of potassium di hydrogen orthophosthate in 1000ml volumetric flask and make up with HPLC water and adjust the pH with OPA solution up to 3.5, and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above Buffer and 300 ml (30%) of Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Assay

Preparation of Synthetic Mixture of Standard Solution

Accurately weigh and transfer 300 mg of Topiroxostat and 200 mg of Dotinurad working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) [10]. Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the SM solution into the chromatographic system and measure the areas for Topiroxostat and Dotinurad peaks and calculate the %Assay by using the formulae.

$$\%\,Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where,
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

METHOD VALIDATION

Linearity

Preparation of stock solution Accurately weigh and transfer 300 mg of Topiroxostat and 200 mg of Dotinurad working standard into a 100 ml clean dry

volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Various level of solution preparation

0.25ml, 0.5ml, 0.75ml, 1ml 1.25ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration and calculate the correlation coefficient

Precision

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION/RUGGEDNESS

The standard solution prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC [11]. The %RSD for the area of six replicate injections was found to be within the specified limits.

Specificity

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retiontime of the analytical peaks.

Accuracy

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Topiroxostat & Dotinurad and calculate the individual recovery and mean recovery values.

LOD and LOQ

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method.

Stability Studies

Hydrolytic degradation under acidic condition, alkaline condition, oxidative degradation, Thermal induced degradation were done.

RESULTS AND DISCUSSION

Assay

Sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below

Validation Parameters

Linearity:

The linearity range was found to lie from 75µg/ml to 375µg/ml of Topiroxostat, 50µg/ml to 250µg/ml of Dotinurad and chromatograms are shown below. Correlation coefficient (R2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit.

Precision

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below

Ruggedness (Intermediate Precision)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation. %RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The percentage recovery was found to be within the limit (97-103%).The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of Detection for Topiroxostat And Dotinurad

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Signal to noise ratio shall be 3 for LOD solution. The result obtained is within the limit.

Limit of Quantification for Topiroxostat And Dotinurad

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Signal to noise ratio shall be 10 for LOQ solution

Robustness

The samples of Topiroxostat and Dotinurad were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The Retention time, USP plate count, USP tailing

factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

Table: 1 Results of Assay for Topiroxostat and Dotinurad

	Claim (mg)	**% Assay**
Topiroxostat	300	99.93
Dotinurad	200	99.95

Table: 2 Precision findings

Injection	Area for Topiroxostat	Area for Dotinurad
Injection-1	469199	378542
Injection-2	466480	370422
Injection-3	463505	377395
Injection-4	465113	375692
Injection-5	463129	375700
Injection-6	460972	372893
Average	464733.0	375107.3
Standard Deviation	2876.4	2985.9
%RSD	0.6	0.8

Table: 3 Intermediate Precision findings

Injection	Area for Topiroxostat	Area for Dotinurad
Injection-1	466111	372909
Injection-2	463354	378218
Injection-3	467721	375833
Injection-4	463219	376144
Injection-5	469297	379868
Injection-6	462378	377714
Average	465346.7	376781.0
Standard Deviation	2797.8	2398.4
%RSD	0.6	0.6

Table: 4 Accuracy (recovery) data for Topiroxostat

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	233775.3	150	150.42	100.28	99.60
100%	462242.7	300	297.42	99.14	
150%	695121.3	450	447.25	99.39	

Table: 5 Accuracy (recovery) data for Dotinurad

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	188250.7	100	100.19	100.19	100.15
100%	374491	200	199.32	99.66	
150%	567073.3	300	301.81	100.60	

Table: 6 Results for Stability of Topiroxostat and Dotinurad

Sample Name	Topiroxostat		Dotinurad	
	Area	% Degraded	Area	% Degraded
Standard	465326.7		375025.0	
Acid	446578	4.03	359788	4.06
Base	453567	2.53	362545	3.33
Peroxide	439786	5.49	343876	8.31
Thermal	448788	3.55	349675	6.76

Photo	437675	5.94	351989	6.14

Figure:1 Structure of Dotinurad & Topiroxostat

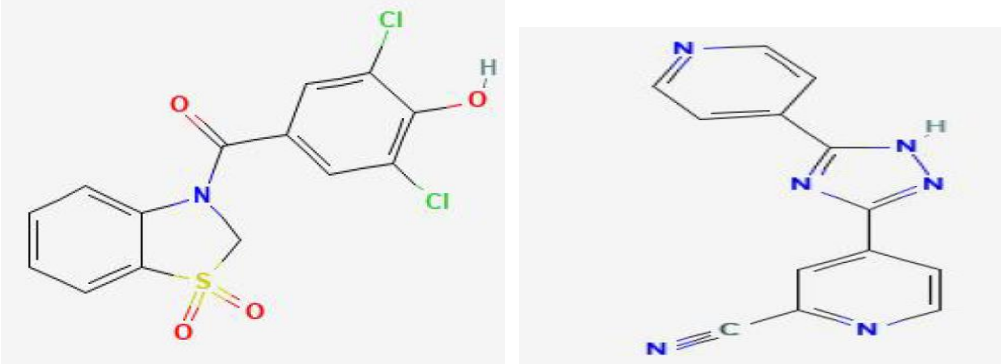


Figure:2 Chromatogram of Method Optimization a. Trial 1; b. Trial 2; c. Trial 3

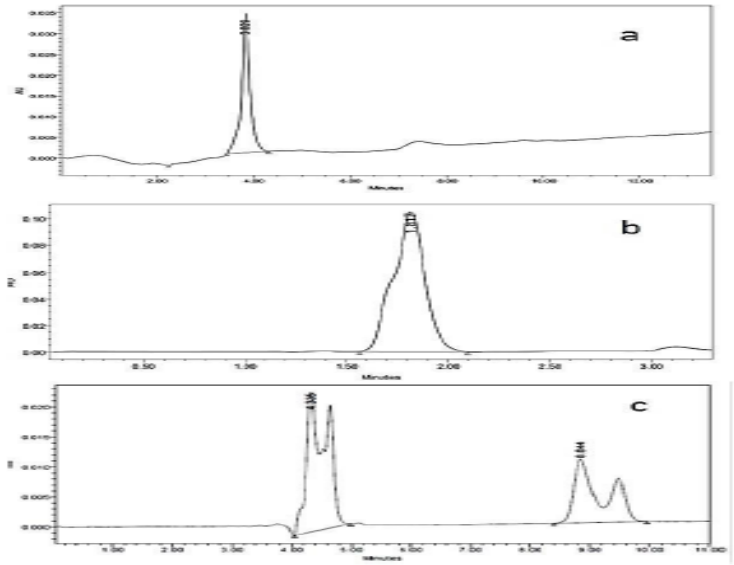


Figure: 3 Chromatogram of Method Optimization a. Trial 4; b. Trial 5; c. Trial 6

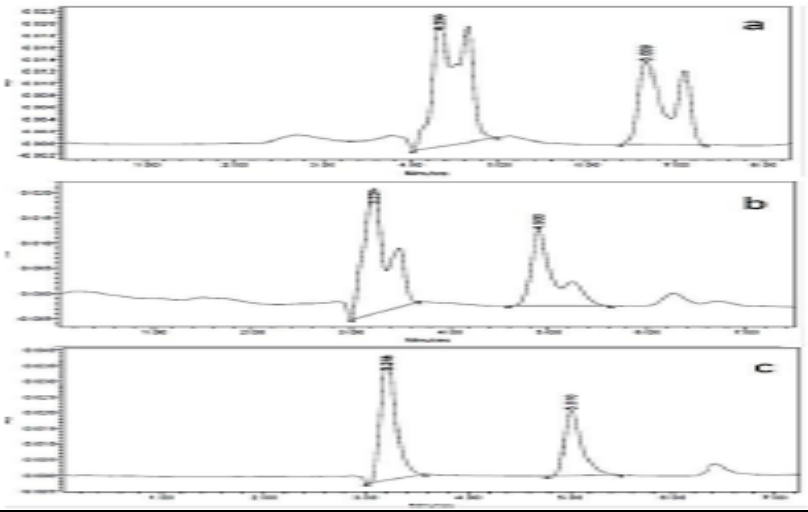


Figure: 4 Chromatogram for Assay of Synthetic Mixture

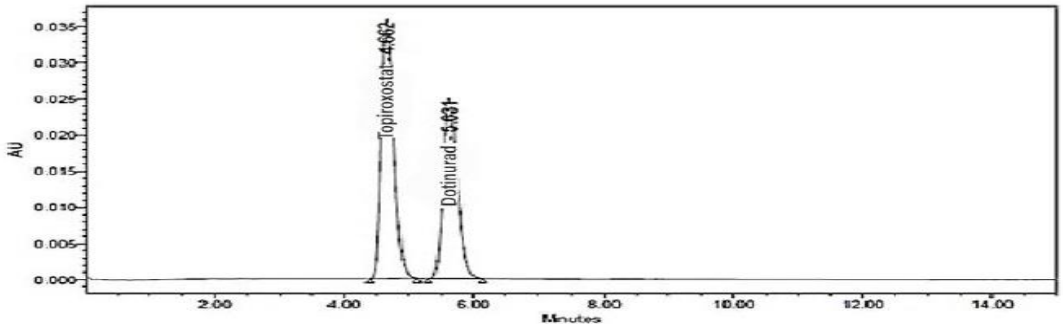


Figure: 5 Chromatogram for Linearity

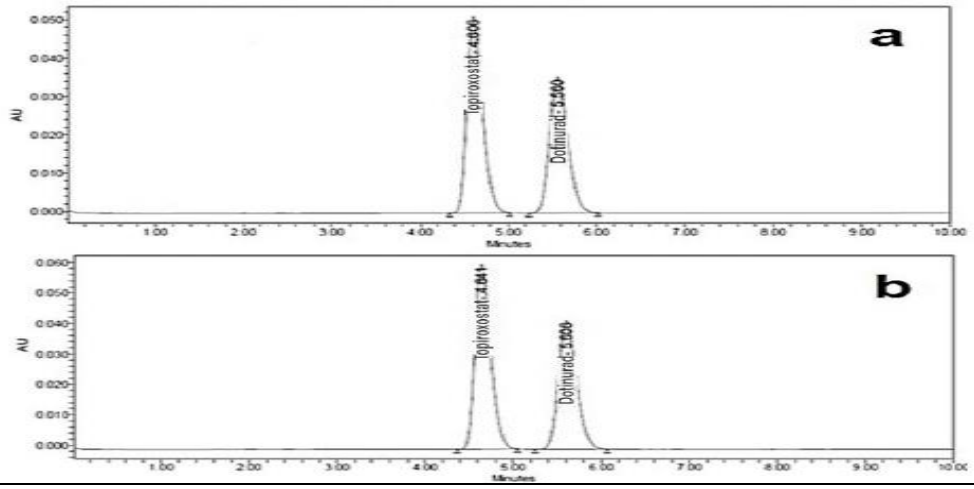


Figure: 6 Calibration graph for Topiroxostat

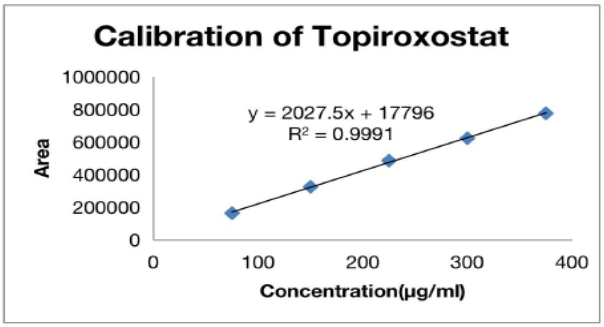


Figure: 7 Calibration graph for Dotinurad

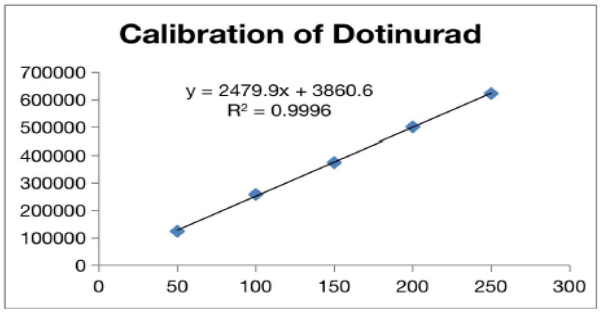


Figure: 8 Chromatogram of Topiroxostat & Dotinurad showing LOD

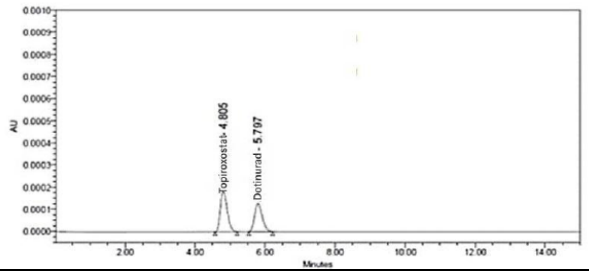
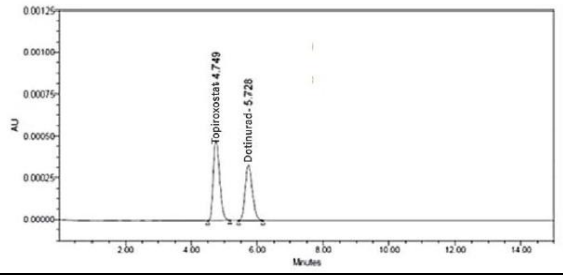


Figure:9 Chromatogram of Topiroxostat & Dotinurad showing LOQ



DISCUSSION

The RP-HPLC method developed for the estimation of Topiroxostat and Dotinurad in a synthetic mixture demonstrated high accuracy, precision, and robustness, making it suitable for routine analysis. The assay results of 99.93% for Topiroxostat and 99.95% for Dotinurad indicate that the method is highly effective in quantifying these compounds with minimal error. The high assay values underscore the method's reliability in ensuring the accurate measurement of the active pharmaceutical ingredients in the synthetic mixture. The linearity assessment revealed a correlation coefficient of 0.999 for both Topiroxostat and Dotinurad, signifying excellent linearity over the tested concentration range. This high degree of linearity confirms the method's sensitivity and its capability to produce consistent and proportional responses across varying concentrations of the analytes. Precision is a critical parameter in analytical method validation, and the developed method showed remarkable precision with RSD values of 0.6% for Topiroxostat and 0.8% for Dotinurad. These values are well within the acceptance criteria of RSD not more than 2.0%, indicating the method's repeatability and reliability under consistent conditions. Furthermore, intermediate precision, with RSD values of 0.6% for both compounds, suggests that the method maintains its precision across different days and potentially different analysts, enhancing its applicability for routine quality control. Accuracy, measured through recovery studies, yielded results of 99.60% for Topiroxostat and 100.15% for Dotinurad, which are within the acceptable range of 97.0% to 103.0%. These recovery rates confirm that the method can accurately recover known amounts of the analytes from the matrix, proving its reliability in practical applications. The limits of detection (LOD) and quantification (LOQ) are crucial for determining the method's sensitivity. The LOD values of 3.07 µg/mL for Topiroxostat and 2.95 µg/mL for Dotinurad, along with LOQ values of 10.09 µg/mL and 9.93 µg/mL respectively, demonstrate that the method is sensitive enough to detect and quantify low concentrations of these drugs, ensuring its suitability for trace analysis. Robustness testing, involving variations in the mobile phase composition and flow rate, showed that the method's performance remained within acceptable limits. The consistent degradation results further emphasize the method's robustness and system suitability under varied conditions, ensuring reliable performance during routine analytical procedures.

CONCLUSION

The proposed HPLC method is precise, specific, accurate, rapid, and economical for the simultaneous estimation of Topiroxostat and Dotinurad in synthetic mixtures. Sample recoveries were consistent with expected quantities, indicating the method's suitability for routine analysis. It is applicable for quality control of raw materials, formulations, dissolution studies, and bioequivalence studies in laboratories.

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