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Stability-Indicating RP-HPLC Method for the Determination of Belumosudil in Bulk and Pharmaceutical Dosage Form

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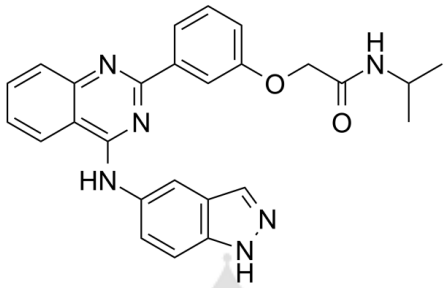
ABSTRACT

A straightforward, sensitive, focused, and accurate RP-HPLC technique is provided for the measurement of belumosudil in pharmaceutical dosage form and API. BDS C18 150 x 4.6 mm, 5m was used to conduct the chromatogram. At a flow rate of 1.0 ml/min, the mobile phase comprising Opa: Acetonitrile in the ratio 45:55 was passed through the column. Orthophosphoric acid buffer was employed in this procedure. 30°C was kept as the temperature. The chosen optimized wavelength was 225.0 nm. Belumosudil's retention time was discovered to be 2.439 minutes. Belumosudil's %RSD was determined to be 0.5. Its %RSD for method accuracy was determined to be 0.8. Its recovery was determined to be 99.97%. Belumosudil's regression equation yielded LOD and LOQ values of 0.38 and 1.16. Belumosudil's regression equation is y = = 27756x. + 7235. As a result of shorter retention durations and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

INTRODUCTION:

An oral active rho-associated coiled-coil protein kinase (ROCK) inhibitor called belumosudil (REZUROCKTM) was created by Kadmon Pharmaceuticals for the treatment of systemic sclerosis and chronic graft-versus-host disease (cGVHD). The US FDA approved belumosudil as an orphan medicine through its Real-Time Oncology Review pilot programme.[1].Belumosudil should be taken in the prescribed dosage of 200 mg once daily (with food) until cGVHD progresses to the point where new systemic therapy is necessary. In patients taking concurrent strong CYP3A4 inducers or proton pump inhibitors, the dosage of belumosudil needs to be increased to 200 mg twice daily.[2,3]



Structure of Belumosudil

Pharmacodynamics:

Belumosudil inhibits signal transducer and activator of transcription 3 (STAT3) phosphorylation, increases STAT5 phosphorylation, and shifts the balance of T helper 17 (Th17)/T regulatory (Treg) cells toward the Treg phenotype to suppress proinflammatory responses.[4,5].Belumosudil significantly reduced the severity of cGVHD in several mouse models.[4]

Pharmacokinetics:

Over a dosage range of 200-400 mg once a day, there are approximately dose proportionate increases in the maximum plasma concentration of belumosudil (Cmax) and the area under the plasma concentration-time curve (AUC). Lumosudil has an accumulation ratio of 1.4. The median time to Cmax at a steady state was 1.26-2.53 h after belumosudil 200 mg was given once or twice daily to patients with cGVHD. Belumosudil Cmax and AUC dropped 2.2-fold and 2-fold, respectively, when a single dose was given together with a high-fat, high-calorie meal, while the median time to Cmax was prolonged by 0.5 h. Belumosudil had a mean

bioavailability of 64% after a single dose. Following a single dose, the geometric mean volume of distribution of belumosudil is 184 L. 99.9% of the medication is bound. Over a dosage range of 200-400 mg once a day, there are approximately dose proportionate increases in the maximum plasma concentration of belumosudil (Cmax) and the area under the plasma concentration-time curve (AUC). Lumosudil has an accumulation ratio of 1.4. The median time to Cmax at a steady state was 1.26-2.53 h after belumosudil 200 mg was given once or twice daily to patients with cGVHD. BelumosudilCmax and AUC dropped 2.2-fold and 2-fold, respectively, when a single dose was given together with a high-fat, high-calorie meal, while the median time to Cmax was prolonged by 0.5 h. Belumosudil had a mean bioavailability of 64% after a single dose. Following a single dose, the geometric mean volume of distribution of belumosudil is 184 L. 99.9% of the medication is bound.[2]

Adverse Events:

Phase II trials using belumosudil to treat cGVHD showed that it was well tolerated.[6,7]The most frequent adverse events (AEs) associated with belumosudil 200 mg once daily (incidence 20%; n = 83) were infection (53%); asthenia (46%); nausea (42%); diarrhoea (35%); dyspnea (33%); cough (30%); oedema (27%); haemorrhage (23%); musculoskeletal pain (22%); abdominal pain (22%); headache (21%) and hypertension (21%).[2]

Allogeneic hematopoietic cell transplantation (HCT), a potential cure for a variety of hematological malignancies, is a therapy option for belumosudil. The prevalence of chronic graft-versus-host disease (cGVHD), a frequent and clinically relevant complication that affects up to 50% of HCT patients, can reduce the efficacy of allogenic HCT[8]. According to United States (US) claims analysis, approximately 14,000 individuals have cGVHD at this time, and 5,000 new cases are identified every year.[9]

Treatment :

There are several methods for treating cGVHD, but many of them have limitations that reduce their overall efficacy. A considerable portion of patients fail systemic therapy or develop steroid resistance even though systemic corticosteroids are the cornerstone of first-line treatment for cGVHD. Within two years of receiving initial systemic treatment, 50–70% of patients with cGVHD require additional treatment.[10]. even though there are numerous second-line standard-

of-care options (such as mTOR inhibitors, calcineurin inhibitors, extracorporeal photopheresis, mycophenolate mofetil, steroid combination therapy, ibrutinib, and ruxolitinib) available, these therapies are connected to increasing immunosuppression and side-effects, and there is no agreement on which secondary cGVHD therapy is the most beneficial.[9,11]. Due to a higher risk of infections, cytopenias, and second primary cancers, current treatment options could also put patients' chances of recovering in danger. Due to these challenges, individuals with cGVHD use a significant amount of resources in the US healthcare system, and their inability to return to labor costs the country's economy an estimated $27 billion annually.[12]

CLINICAL TRIALS[13,14,15]:

The terms "belumosudil" and "chronic graft-versus-host disease" were used in a literature search. NCT03640481; KD025-213) ROCKstar: Efficacy and safety of belumosudil were examined in phase 2 US-based, multicenter, randomized, open-label trial in patients with cGVHD who needed further treatment after receiving 2 lines of systemic medication. Patients with platelets less than 50 109/L, absolute neutrophil count (ANC) less than 1.5 109/L, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 ULN, total bilirubin greater than 1.5 ULN, corrected QT interval using Fridericia's formula (QTcF) greater than 480 ms, eGFR less than 30 mL/min/1. Patients who met the criteria were randomly assigned to receive either belumosudil 200 mg once daily or a placebo.

MATERIALS AND METHODS

Chemicals and reagents

Belumosudil pure drug (API), Belumosudil tablets (Rezurock), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises

- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector, and Autosampler integrated with Empower 2 Software.

- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring the absorbance of Belumosudil solution.

Preparation of Standard stock solution:

Accurately weighed 20mg of Belumosudil is transferred to a 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up of diluents and labeled as a Standard stock solution. (400µg/ml of Belumosudil).

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Belumosudil).

Preparation of Sample stock solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents were added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400µg/ml of Belumosudil).

Preparation of Sample working solution: 0.2ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent. (40µg/ml of Belumosudil).

Chromatographic conditions:

Mobile phase	**:**	45**%** OPA: 55% Acetonitrile
Flow rate	**:**	1.0ml/min
Column	**:**	BDS (4.6 x 150mm, 5µm).
Detector wavelength:	294nm	
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	5 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50

Results: Belumosudil drug peak has good resolution, tailing Factor, theoretical plate count, and resolution.

Degradation: To conduct the forced degradation experiment, standard stock solutions of Belumosudilwas exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL of 2N NaOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60^oC. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 50µg/ml of Belumosudil for degradation studies. To examine sample stability, 10µl samples were fed into the system, and chromatograms were obtained.

Method Validation: The method was validated following ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions of Belumosudil (50µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All of the system's appropriate parameters were passed and remained within the limitations. Table 1 shows the results.

Specificity: In the optimized method, the interference is checked. Belumosudil had a retention time of 2.346 minutes. We did not find any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be particular. Figures 2, 3, and 4 show the chromatograms for specificity.

Linearity: Six linear concentrations of Belumosudil(12.5-75µg/ml) was injected in a triplicate manner. The correlation coefficient obtained was 0.999 for all three drugs. The results were shown in table 2 and fig 5.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml Belumosudil) were created. Each injection was given from each working sample solution, and the results are shown in Table 3.

The average area, standard deviation, and % RSD for the medication were computed and found to be 0.5% for Lamivudine. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml of Belumosudil) were prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.7% for Belumosudil. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Belumosudilwas found to be 99.99 %. Tables 5 show the outcomes. Because satisfactory recovery values were achieved, the accuracy of this approach was passed.

Robustness: Robustness conditions such as flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35 v/v), mobile phase plus (55:45 v/v), temperature minus (25°C), and temperature plus (35°C) were maintained, and samples (50µg/ml Belumosudil) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay: Aciherpen tablets had a label claim of Belumosudil 400mg per unit formulation. The aforementioned formulation was used for the assay. The average % assay achieved for Belumosudilwas 100.08%.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using the proposed method. Assay % of Belumosudilin the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7. The purity plots obtained in degradation studies are shown in figs. 6 and 7.

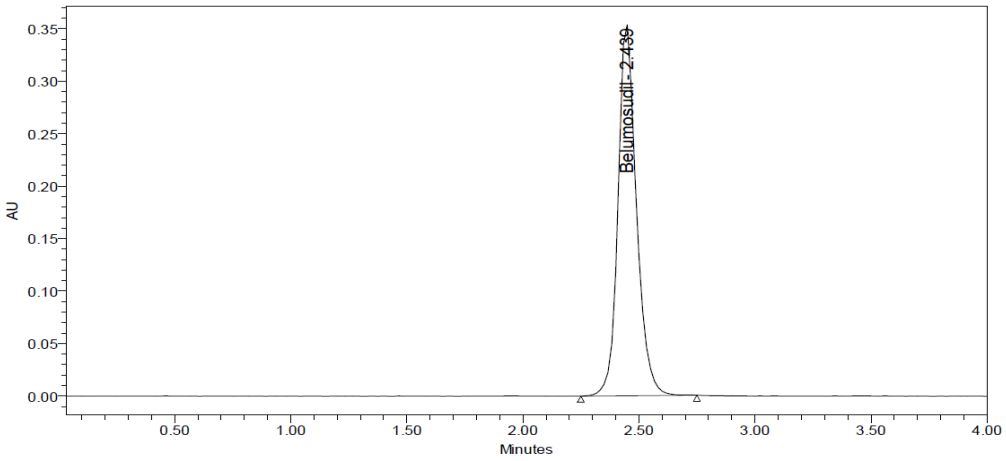


Fig No.1: Optimised Chromatogram

Table No.1: System suitability parameters

S no	**Belumosudil**		
Inj	RT(min)	USP Plate Count	Tailing
1	2.434	4848	1.16
2	2.434	4708	1.15
3	2.435	4789	1.15
4	2.435	4657	1.16
5	2.435	4578	1.14
6	2.437	4609	1.17

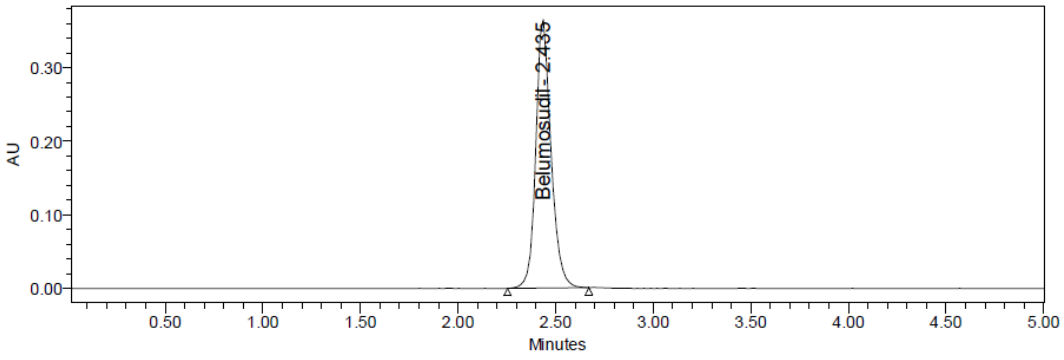


Fig No.2: Standard solution chromatogram

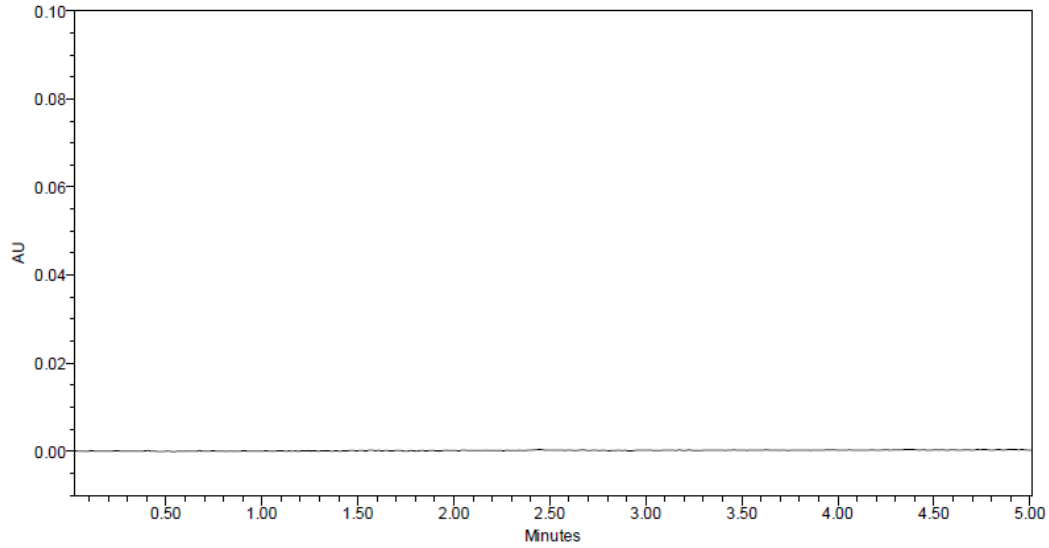


Figure No.3: Blank chromatogram

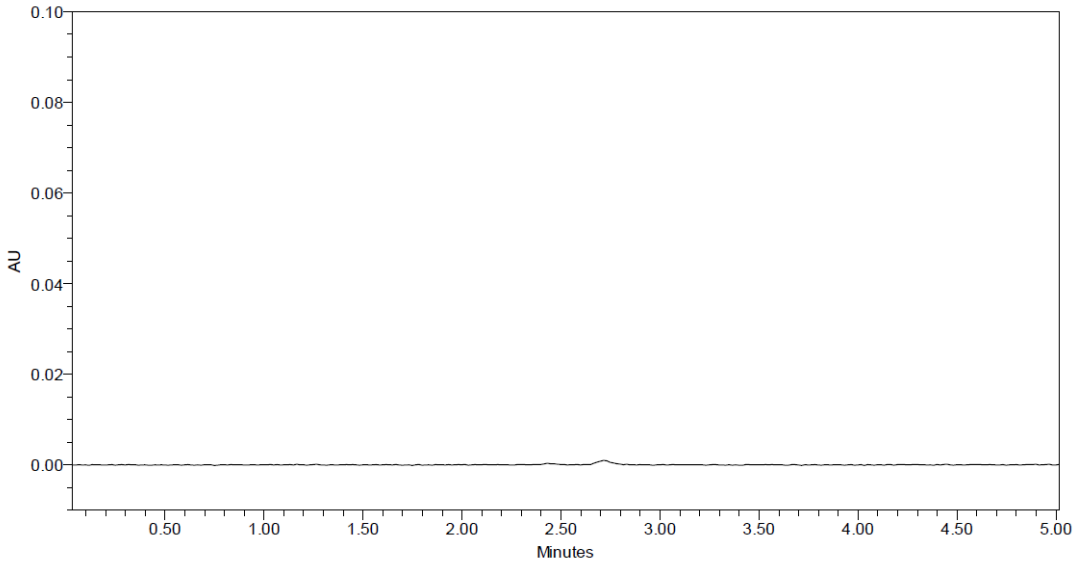


Fig No.4: Placebo chromatogram

Table No.2: Linearity table for Belumosudil,

Belumosudil	
Conc (µg/mL)	**Peak area**
0	0
10	303519
20	562411
30	821646
40	1133848
50	1366777
60	1691273

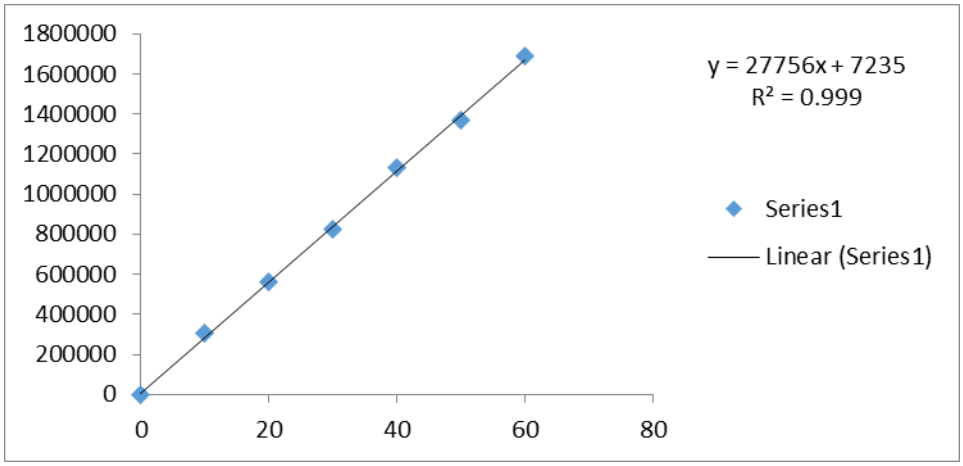


Fig No 5: Calibration curve of Belumosudil

Table No.3: Repeatability for Belumosudil

S. No	Area of Belumosudil
1.	1129853
2.	1142738
3.	1133946
4.	1124501
5.	1142684
6.	1122964
Mean	1132781
S.D	8626.4
%RSD	0.8

Table No.4: Intermediate Precision for Belumosudil

S. No	Area of Belumosudil
1.	1111893
2.	1108797
3.	1126677
4.	1134395
5.	1123308
6.	1131298
Mean	1122728
S.D	10363.1
%RSD	0.9

Table No.5: Accuracy for Belumosudil

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	40.68	101.69	99.97%
	20	40.02	100.04	
	20	40.42	101.05	
100%	40	39.39	98.49	
	40	40.21	100.51	
	40	39.77	99.42	
150%	60	39.89	99.74	
	60	39.74	99.34	
	60	39.80	99.49	

Table No.6: Robustness Data

S.no	Condition	%RSD of Belumosudil
1	Flow rate (-) 0.9ml/min	0.4
2	Flow rate (+) 1.1ml/min	0.9
3	Mobile phase (-) 75B:25A	0.8
4	Mobile phase (+) 65B:35A	0.7
5	Temperature (-) 27°C	0.4
6	Temperature (+) 33°C	0.9

Table No.7: Degradation Data

S.NO	Degradation Condition	% Drug Undegraded	% Drug Degraded
1	Acid	93.31	6.69
2	Alkali	95.33	4.67
3	Oxidation	94.63	5.37
4	Thermal	97.89	2.11
5	UV	98.19	1.81
6	Water	99.04	0.96

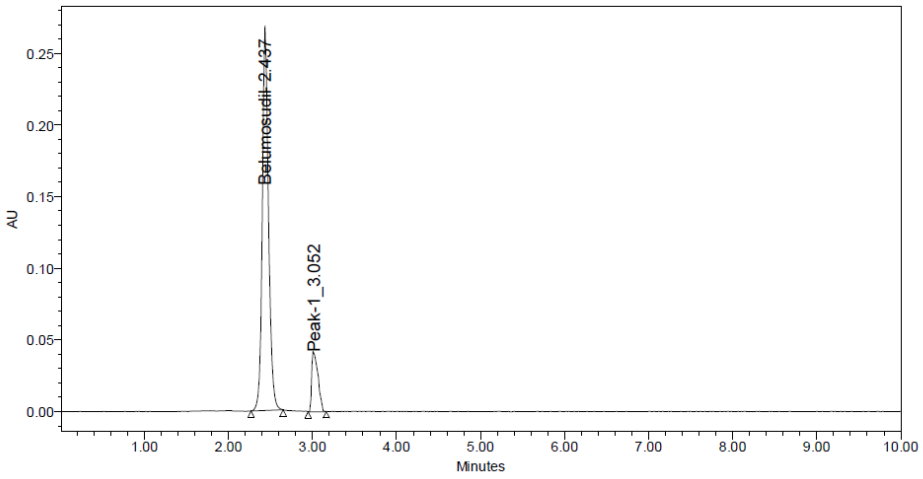


Fig No 6: Degradation Chromotogram

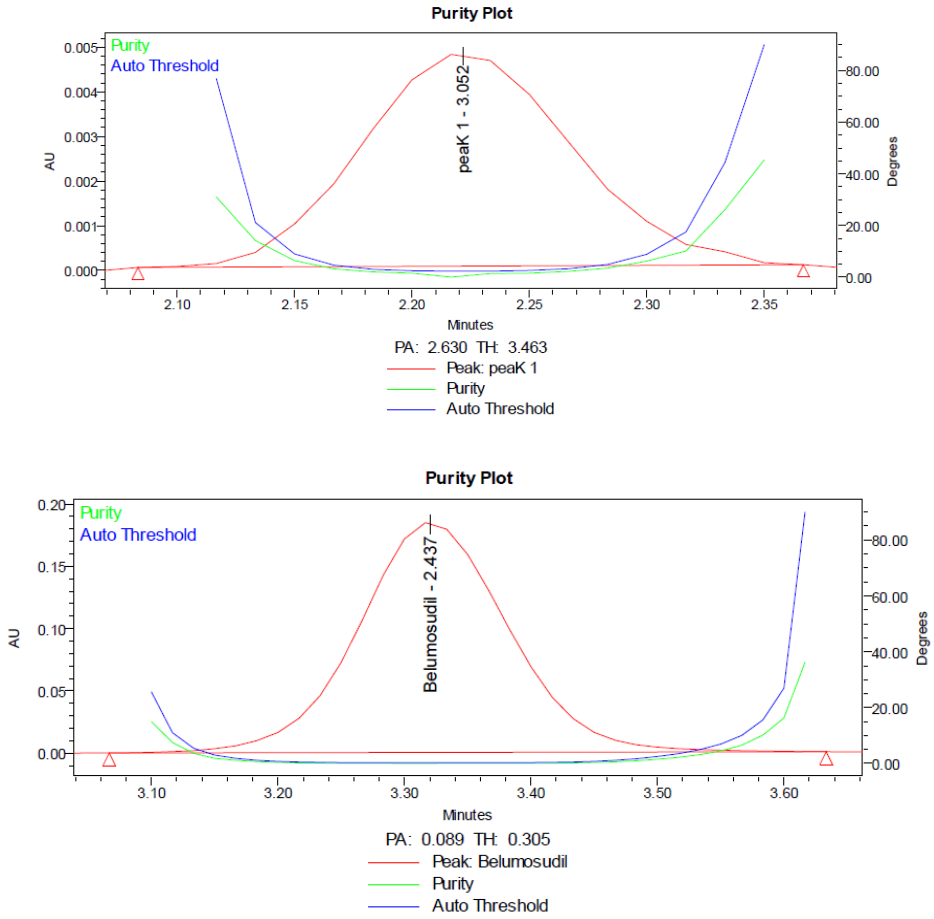


Fig No 7: Purity Plots of Degradation(Acid)

CONCLUSION:

This work shows how to determine Belumosudil in the presence of substances using a simple and established stability-indicating RP-HPLC approach. The devised approach was exact, sensitive, particular, quick, and durable. The approach is capable of distinguishing active medicinal components from degradation products generated during forced degradation tests. The suggested approach may be utilized for routine quantitative Belumosudil analysis in the quality-control department.

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