WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

A New Validated Stability Indicating RP-HPLC Method for the Assay of Belumosudil in Tablet

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ABSTRACT

A simple, accurate, and precise technique for measuring Belumosudil was developed using RP-HPLC technology. The column temperature was 30°C, the detection wave length was 280 nm, and the flow rate was 1 ml/min. The stationary phase was BDS 150 x 4.8 mm, 5 m, and the mobile phase was acetonitrile: 0.01NNa2HPo4 in a 60:40 ratio, with mobile phase serving as the diluent. The conditions determined the appropriate course of action. Belumosudil has a retention time of 2.286 seconds. Its percentage RSD was determined to be 0.6 and 1.1, respectively. % Belumosudil had a recovery rate of 99.73 percent. The Belumosudil regression models produced LOD and LOQ values of 0.24 and 0.71, respectively. The regression equation is: y = 3417.5x + 676.4 from.

Key words: HPLC Belumosudil, ICH Guidelines

INTRODUCTION

That is a widely used analytical method for efficiently extracting and quantifying certain components from complicated mixtures. Disintegrated chemicals can originate from a variety of sources, including food, drugs, pharmaceuticals, plants, animals, the environment, and crop waste..

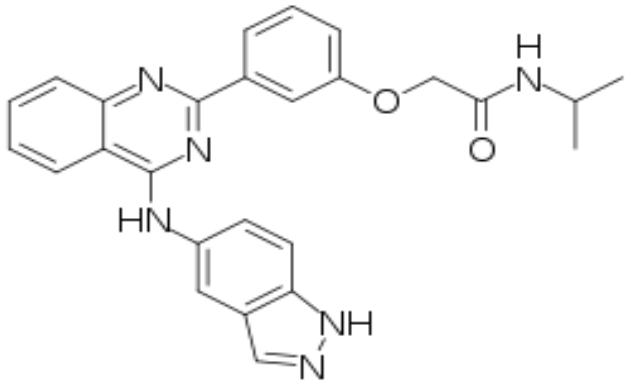


Fig.1 Belumosudil Chemical Structure

MATERIALS AND METHODS

Materials:

- Belumosudil Tablets (brand: Rezurock) are a pure medication (API). Distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen orthophosphate buffer, and orthophosphoric acid. All of the chemicals and solvents listed above are from Rankem.

Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India

- Ultrasonicator-BVK enterprises
- The Waters HPLC 2695 System includes quaternary pumps, a Photo Diode Array detector, and an auto sampler, all integrated with Empower 2 software.

0.1% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water and sonicate to degas.

Buffer:0.01N Potassium dihydrogen Ortho phosphate

Accurately weigh 1.36gm of potassium dihydrogen orthophosphate in a 1000ml volumetric flask, add around 900ml of milli-Q water, degas to sonicate, and lastly make up the volume with water, then add 1ml of triethylamine, and adjust the pH to 4.0 with dil. orthophosphoric acid solution.

Buffer:0.01N Sodium dihydrogen phosphate

Accurately weigh 1.42gm of potassium dihydrogen orthophosphate in a 1000ml volumetric flask. Add around 900ml of milli-Q water and degas to sonicate. Finally, make up the volume with water. Adjust the pH to 3.5 with dil. orthophosphoric acid solution.

Preparation of Standard stock solutions:

Transfer 25mg of Belumosudil to a 50ml volumetric flask after accurately weighing it. 3/4 of the diluents were added to the flask and sonicated for 10 minutes. The flask was filled with diluents and labeled as standard stock solution. (500 µg/mL of Belumosudil)

Preparation of Standard working solution (100% solution):

1ml of each stock solution was pipetted into a 10ml volumetric flask and mixed with diluent. (50 µg/ml Belumosudil).

Preparation of Sample stock solutions:

Ten tablets were weighed, ground, and then A weight comparable to one tablet was put to a 100mL volumetric flask, 50mL of diluent was added, and the mixture was sonicated for 25 minutes before being filtered. (2000 µg/mL of Belumosudil)

Preparation of Sample working solution:

From the filtered solution, 0.25 ml was pipetted into a 10 ml volumetric flask and diluted to 10ml with diluent. (50 µg/ml Belumosudil).

Optimization of chromatographic conditions:

The first trial utilized a Kromosil 250mm column with a mobile phase of OPA and water in a 50:50 ratio at a flow rate of 1.0 ml/min. The column was heated to 30 degrees Celsius, and the photodiode array (PDA) was detected at 280 nm.

A second experiment was done because, while peaks were eluted in the first trial, they were found to be eluted in the void volume. There were insufficient tailing, area, resolution, and USP plate counts.

The third trial was carried out at 30 °C using a Kromosil 250mm x 4.66 column and a mobile phase consisting of Acetonitrile: Na2HPo4 (20:70) at a flow rate of 1 ml per minute. The photodiode array (PDA) was detected at 280 nm.

Observation: Peak where eluted but retention was more so further trial was carried out.

The fourth trial was carried out at 30 °C with a BDS 150x4.88 column and an OPA: Acetonitrile mobile phase flowing at 1 ml/min. The photo-diode array (PDA) was detected 280 nm.

Observation: Peak where eluted but retention was more so further trial was carried out

Optimized Chromatogram

Efficient state **:** BdS 150 mm x 4.8 mm, 5µ.

MP **:** ACN : OPA (40:60)

Start rate **:** 1.0 ml / min

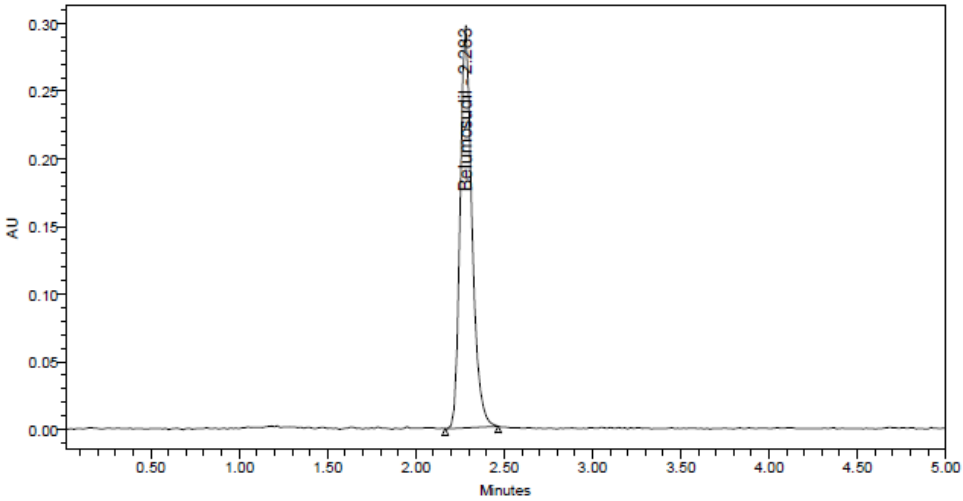
Detector **:** PDA 280nm

Temp **:** 30 ^{0}C

INJ Volume **:** 10 μL

Pump mode **:** Isocratic

Fig..2. OPTIMIZED CHROMATOGRAM



Observation: Belumosudil eluted with good peak shape and retention time and tailing was passed.

Method Validation:

The RP-HPLC method was validated using the ICH recommendations. Method validation characteristics include precision, linearity, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System suitability parameters:

The system appropriateness parameters were established by producing a standard Belumosudil (40ppm) solution, injecting it six times, and determining metrics such as peak tailing, resolution, and USP plate count.

The % RSD for the area of six standard injections results should not be more than 2%.

Table 1: System Suitability Parameters and their recommended limits.

Parameter	**Recommendation**
Capacity Factor (K')	The peak should be well-resolved from other peaks and the
Repeatability	RSD ≤ 1%
Relative Retention	Not required as the resolution is stated
Resolution(Rs)	Rs of > 2 between the peak of interest and the closest eluting
Tailing Factor(T)	T ≤ 2
Theoretical Plates(N)	In general should be > 2000

Discussion: A Standard solution of Belumosudil working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas

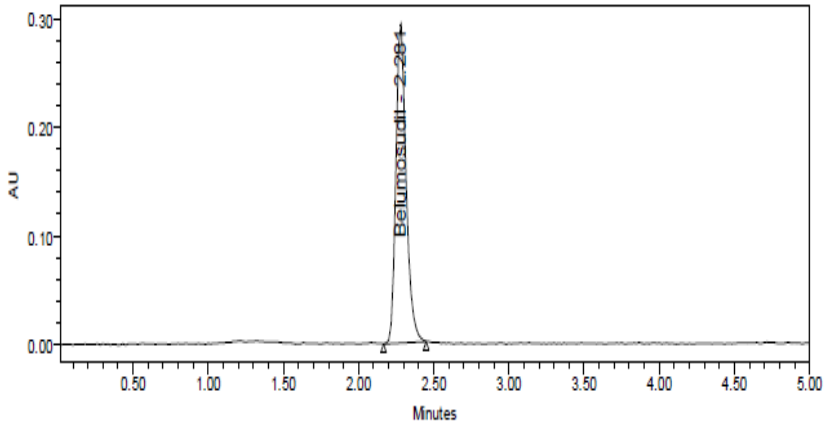


Fig .3.System suitability Chromatogram

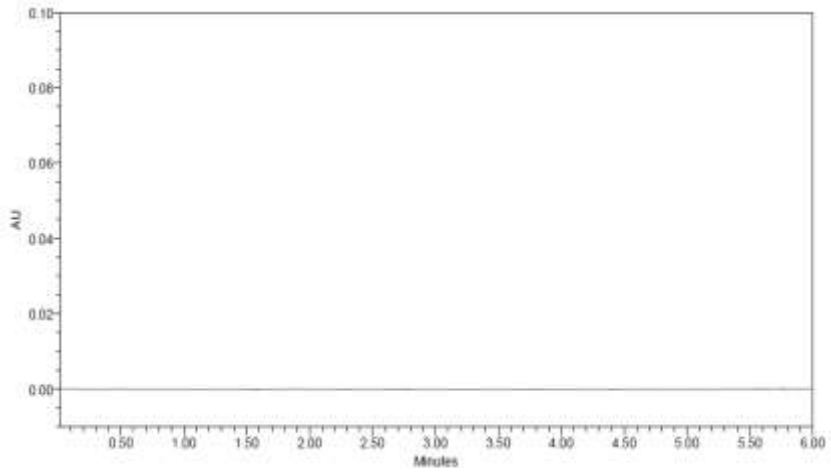


Fig:.4. blank Chromatogram

SPECIFICITY:

Specificity should be investigated throughout test validation, impurity identification, and assay. The approach utilized to explain specificity will be determined by the analytical procedure's intended purpose.

Precision:

The precision of this approach expresses the closeness of agreement (degree of scatter) between the series of measurements acquired from successive samplings of the same homogenous sample under the defined conditions.

Intermediate precision:

On the following day of sample preparation, five working sample solutions of 40ppm were injected, and the%Amount discovered was computed, and the%RSD was determined to be 0.7, and the chromatogram was displayed in fig 6.3.

Table .2. Intermediate precision data

S.No	Peak Area
1	136823
2	136998
3	134597
4	136325
5	137154
6	137367
AVG	136544
STDEV	1017.1
%RSD	0.7

Linearity: The capacity (within a specified range) to get test findings that are proportionate to the analyte concentration (amount) in the sample.

To show the assay method's linearity, inject five standard solutions containing Belumosudil at concentrations ranging from 10ppm to 60ppm. Plot a graph of concentration versus peak area. The slope obtained was 3417.5 xs + 676.4, and the correlation coefficient was 0.999. A linearity plot is presented in Fig 6.15.

Table .3. Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	10	34198
50	20	68448
75	30	102886
100	40	141039
125	50	170750
150	60	204410

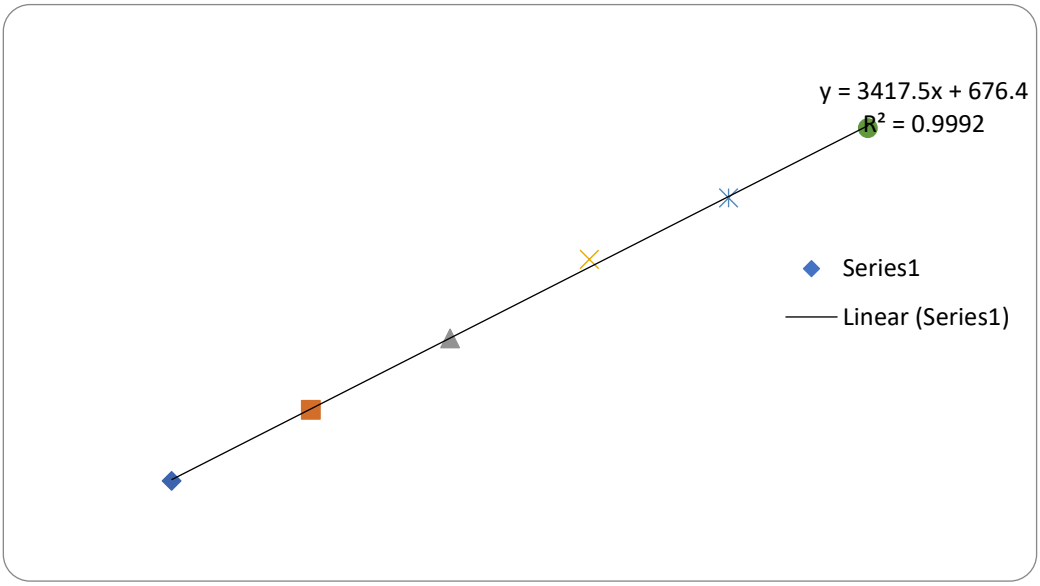


Fig.5.Linearity plot

Accuracy:

The accuracy of an analytical technique expresses the degree of agreement between a value regarded as a conventional true value or an approved reference value and the value found. That is sometimes referred to as trueness. Accuracy should be established within the analytical procedure's stated range.

Table .4. Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.92	99.60	99.73%
	20	20.16	100.81	
	20	19.90	99.51	
100%	40	39.64	99.09	
	40	40.03	100.07	
	40	39.83	99.58	
150%	60	59.51	99.18	
	60	59.72	99.54	
	60	60.10	100.16	

Discussion: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.78%

Robustness:

Small deliberate changes are made to the procedure, such as flow minus, flow plus, mobile phase minus, mobile phase plus, temperature negative, and temperature plus. The %RSD for the aforementioned scenarios is calculated.

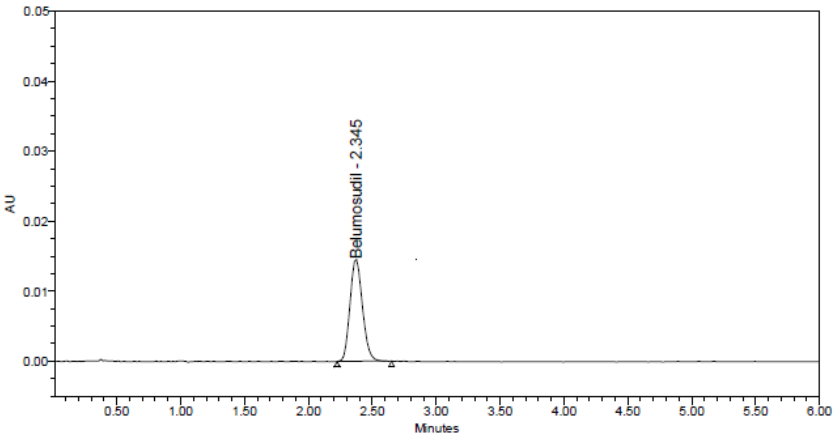
Table .5. Robustness Data

Parameter	%RSD
Flow Minus	0.6
Flow Plus	0.4
Mobile phase Minus	0.6
Mobile phase Plus	0.5
Temperature minus	1.0
Temperature plus	0.7

Detection Limit:

The detection limit of each analytical process is the smallest amount of analyte in a sample that can be detected but not quantified under the specified experimental conditions.

Fig 6. LOD: Detection limit of the Belumosudil in this method was found to be 0.24µg/ml



Quantification limit:

The quantification limit of an individual analytical process is the smallest amount of analyte in a sample that can be quantified with sufficient precision and accuracy.

LOQ: Quantification limit of the Belumosudil in this method was found to be 0.71/ml.

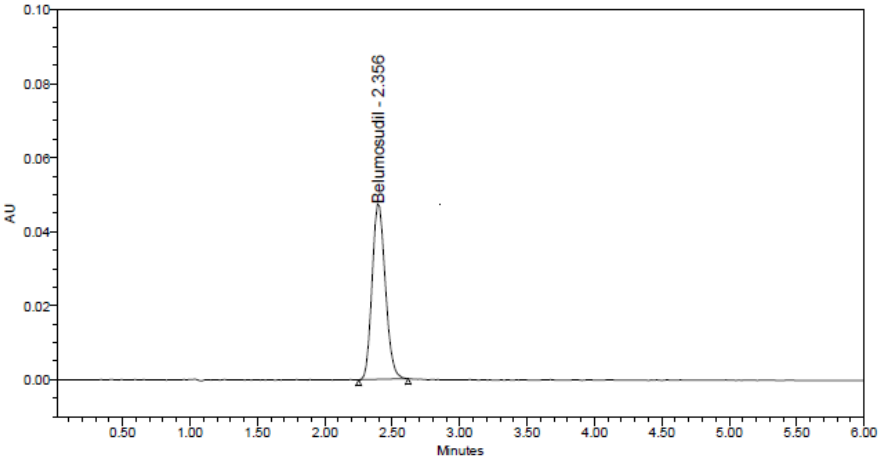


Fig 7. LOQ Chromatogram of Belumosudil

SUMMARY AND CONCLUSION

The chromatographic conditions were as follows: stationary phase BDS (150mm*4.6mm), mobile phase Acetonitrile: 0.01N Na2HPo4 in a 60:40 ratio, flow rate of 1ml/min, detection wave length of 280nm, column temperature of 30oC, and mobile phase diluent. Conditions were finalized using the optimal approach. The system suitability parameters were investigated by injecting the standard six times, and the results were substantially below the acceptance criteria. A linearity analysis was conducted between 25% and 150% levels, and the R2 value was found to be 0.999.Precision was determined to be 1.1 for repeatability and 0.6 for intermediate precision. The limit of detection and limit of quantification

are 0.24µg/ml and 0.71µg/ml, respectively. The above procedure was used to conduct an assay of a commercial product, and 100.31% was present. Belumosudil degradation investigations were conducted, and the purity threshold in all conditions was more than the purity angle and within acceptable limits. The full length procedure was not conducted; if it is, it can be utilized for routine analysis of Belumosudil.

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