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Development and Validation of a New Analytical Method for the Determination of Belzutifan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A Simple, sensitive, specific and precise RP-HPLC method for the determination of Belzutifan in Pharmaceutical dosage form is developed. Chromatogram was run through Inertsil C18 (150mm x 4.6mm, 5µm), Mobile phase containing 0.01N Disodium hydrogen phosphate: Acetonitrile taken in the ratio 70:30 % v/v was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. The optimized wavelength selected was 228 nm. Retention time of Belzutifan was found to be 2.231 min. % RSD of the Belzutifan was and found to be 0.3. % RSD of Method precision of Belzutifan was found to be 0.5. % Recovery was obtained as 99.88 % for Belzutifan. % Assay was obtained as 99.49% for Belzutifan. LOD, LOQ values obtained from regression equation of Belzutifan were 0.17, 0.51. Regression equation of Belzutifan is y = 78150x + 29893. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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INTRODUCTION:

Belzutifan is an inhibitor of hypoxia-inducible factor 2α (HIF-2α) used in the treatment of von Hippel-Lindau (VHL) disease-associated cancers. The HIF-2α protein was first identified in the 1990s by researchers at UT Southwestern Medical Center as a key player in the growth of certain cancers[1,2]. Belzutifan inhibits the complexation of HIF-2α with another transcription factor, HIF-1β, a necessary step in its activation - by preventing the formation of this complex, belzutifan can slow or stop the growth of VHL-associated tumors. Belzutifan received FDA approval for the treatment of select VHL-associated cancers on August 13, 2021[4].Upon oral administration, belzutifan binds to and blocks the function of HIF-2alpha, thereby preventing HIF-2alpha heterodimerization and its subsequent binding to DNA. This results in decreased transcription and expression of HIF-2alpha downstream target genes, many of which regulate hypoxic signaling. This inhibits cell growth and survival of HIF-2alpha-expressing tumor cells. HIF-2alpha, the alpha subunit for the heterodimeric transcription factor HIF-2, is overexpressed in many cancers and promotes tumorigenesis[4,5]. Belzutifan exerts its therapeutic effects by inhibiting a transcription factor necessary for the growth of solid tumors associated with VHL disease[2]. Hypoxia-inducible factor 2α (HIF-2α) is a transcription factor that aids in oxygen sensing by regulating genes that promote adaptation to hypoxia. In healthy patients, when oxygen levels are normal, HIF-2α is broken down via ubiquitin-proteasomal degradation by von-Hippel Lindau (VHL) proteins. In the presence of hypoxia, HIF-2α translocates into cell nuclei and forms a transcriptional complex with hypoxia-inducible factor 1β (HIF-1β) - this complex then induces the expression of downstream genes associated with cellular proliferation and angiogenesis[2,6]. Structure of Belzuitifanis known as3-{[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methanesulfonyl-2,3-dihydro-1H-inden-4-yl]oxy}-5-fluorobenzonitrile.[5]

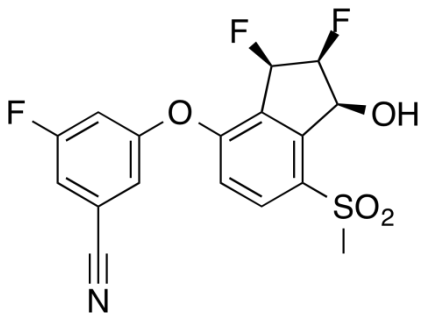

Fig 1: Chemical Structure of Belzuitifan

There are no RP-HPLC methods have been reported in the literature for the determination of Belzutifan in bulk and pharmaceutical dosage form by Rp-hplc. An attempt has been made to develop an RP-HPLC method that is simple, specific, rapid, precise, and economical method for the quantitative determination of Belzutifanin bulk and pharmaceutical dosage form. This method has been validated as per International Conference on Harmonization (ICHQ2 (R1) guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Belzuitifan standard (Purity ≥ 99.6 as is basis), Acetonitrile (HPLC grade), HPLC grade water (Millipore), Disodium hydrogen phosphate, 0.1% Orthophosphoricacid, Sodium hydroxide, Hydrochloric acid and Hydrogen peroxide were purchased from Rankem, India.

Instrumentation: The instrument used in the study was HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 2 software. electronic balance, sonicator, hot air oven, digital pH meter and UV-Visible chamber.

Preparation of Standard stock solution:

Accurately weighed 10 mg of Belzutifan is transferred to 50 ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as a Standard stock solution. (200µg/ml of Belzuitifan).

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (20 µg/ml of Belzutifan).

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, 50 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (200µg/ml of Belzutifan)

Preparation of Sample working solution:0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Belzutifan)

Chromatographic conditions:

Flow rate **:** 1ml/min

Column **:** Inertsil C18 150x 4.6 mm, 5 µm

Wavelength **:** 228.0 nm

Column temperature: 30°C

Injection volume **:** 10.0 µL

Run time **:** 5.0minutes

Diluent **:** 0.01N Disodium hydrogen phosphate:Acetonitrile (50:50)

Observation: Belzuitifan Retention time was at 2.231 mins with good resolution (Fig.2). The plate count and tailing factor were highly excellent, the technique conditions were optimized and the same conditions were used for validation.

Degradation: To conduct the forced degradation experiment, standard stock solutions of Belzuitifan was 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^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 20µg/ml of Belzuitifan for degradation studies. To examine sample stability, 10 µl samples were fed into the system and chromatograms were obtained.

Method Validation: The method was validated by 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 Belzuitifan (20µg/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. Belzuitifan, had a retention time of 2.231 minutes. Method did not found 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 3, 4, and 5 show the chromatograms for specificity.

Linearity: Six linear concentrations of Belzuitifan (5-30µg/ml) was injected in triplicate manner. The correlation coefficients obtained was 0.999 for Belzutifan drug. The results were shown in Table 2 and Fig 6.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (20 µg/ml Belzuitifan) 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 0.3% for Belzuitifan. 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 (20 µg/ml of Belzuitifan) 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 medicationwas computed and found to be 0.4% for Belzuitifan. 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 Belzuitifan was found to be 99.88 %. Tables 5 show the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Robustness: Robustness conditions such as flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (75:25 v/v), mobile phase plus (65:35 v/v), temperature minus (27°C), and temperature plus (33°C) were maintained, and samples (20µg/ml Belzuitifan) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay: Belzuitifan tablets had a label claim of Belzuitifan 40 mg per unit formulation. The aforementioned formulation was used for the assay. The average % assay achieved for Belzuitifan was 99.49%.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using proposed method. Assay % of Belzuitifanin the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in Table 7.

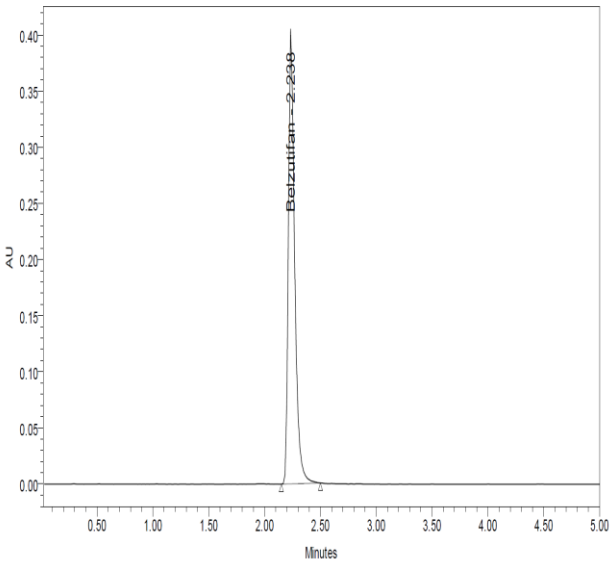

Fig No.2: Optimized Chromatogram

Table No.1: System Suitability Parameters

S no	Belzuitifan		
Inj	RT (min)	USP Plate Count	Tailing
1	2.223	7147	1.38
2	2.223	7211	1.38
3	2.224	7042	1.38
4	2.226	6920	1.39
5	2.226	7092	1.40
6	2.231	7301	1.31

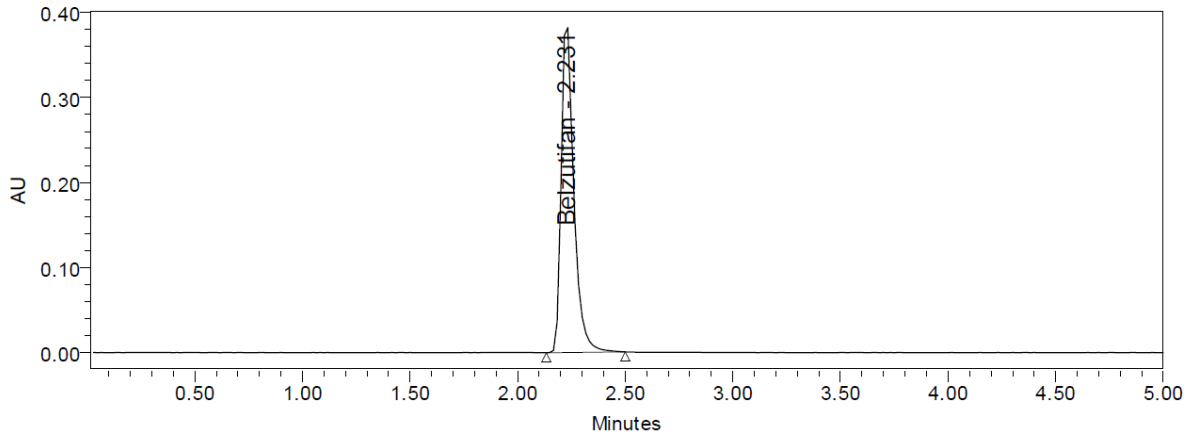

Fig No. 3: Standard solution chromatogram

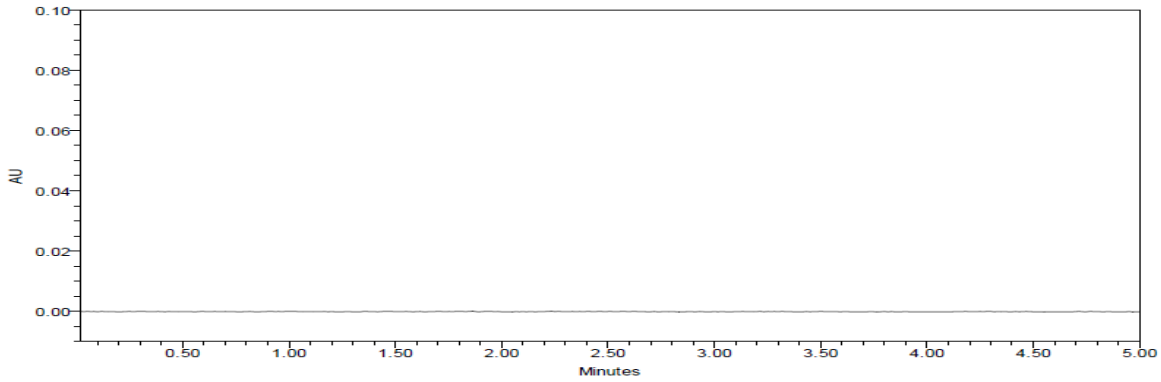

Figure No.4: Blank chromatogram

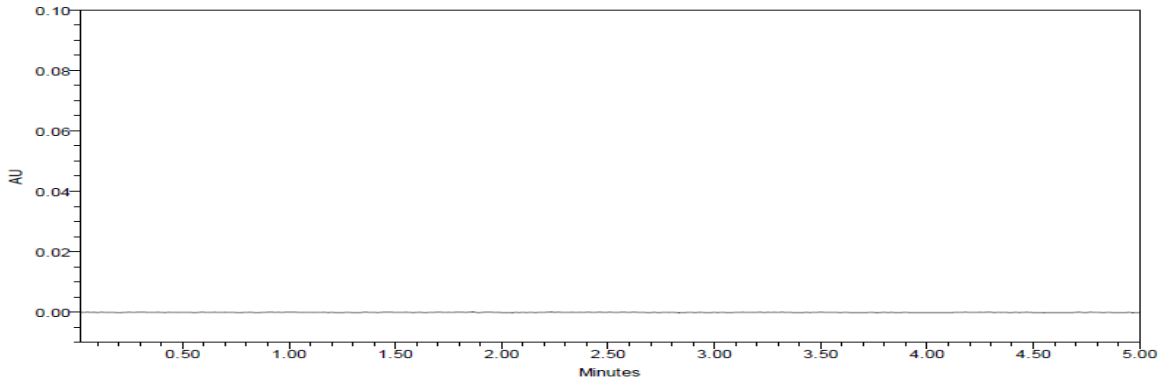

Fig No.5: Placebo chromatogram

Table No.2: Linearity table for Belzuitifan

Belzuitifan	
Conc (µg/mL)	**Peak area**
37.5	434473
75	823227
112.5	1214971
150	1616252
187.5	1961286
225	2364775

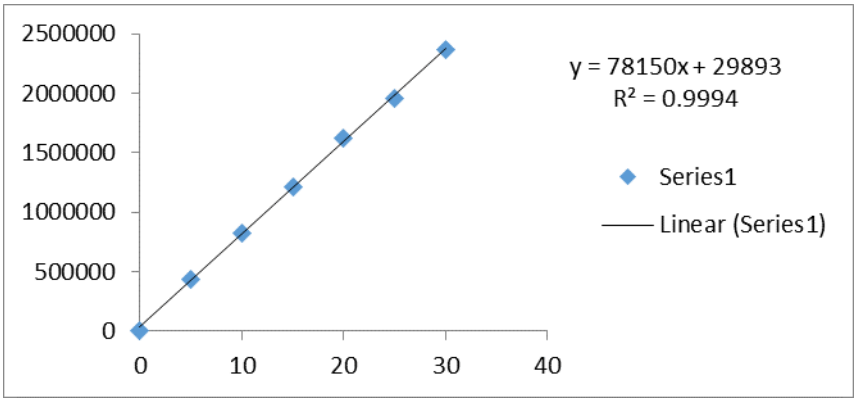

Fig No 6: Calibration curve of Belzuitifan

Table No.3: Repeatability for Belzuitifan

S.no.	Belzuitifan
1	1607426
2	1613076
3	1611183
4	1608542
5	1606885
6	1618268
Mean	1610897
S.D	4309.3
%RSD	0.3

Table No.4: Intermediate Precision for Belzuitifan

S.no.	Belzuitifan
1	1613149
2	1610334
3	1590261
4	1599694
5	1610713
6	1601608
Mean	1604293
S.D	8733.8
%RSD	0.5

Table No.5: Accuracy for Belzuitifan

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	10	9.89	98.89	99.88%
	10	10.09	100.85	
	10	9.89	98.90	
100%	20	20.17	100.83	
	20	19.98	99.92	
	20	20.14	100.72	
150%	30	30.03	100.10	
	30	29.77	99.22	
	30	29.84	99.47	

Table No.6: Robustness Data

S.no	Condition	%RSD of Belzuitifan
1	Flow rate (-) 0.9ml/min	0.4
2	Flow rate (+) 1.1ml/min	0.2
3	Mobile phase (-) 60B:40A	0.1
4	Mobile phase (+) 50B:50A	0.3
5	Temperature (-) 27°C	0.5
6	Temperature (+) 33°C	0.4

Table No.7: Degradation Data

S.No.	Condition	%Degraded	%Obtained
1	**Acid**	5.47	94.53
2	**Base**	4.49	95.51
3	**Oxidation**	5.10	94.90
4	**Dry heat**	2.02	97.98
5	**UV Light**	1.71	98.29

CONCLUSION:

For the identification and quantification of Belzuitifan in pure and pharmaceutical formulations, a simple, quick, validated, and isocratic RP-HPLC technique with UV detection was devised. The approach was validated according to ICH requirements, and statistical results confirmed the suggested method's selectivity, linearity, sensitivity, precision, and accuracy. The provided method can also be used to investigate the stability of analytical solutions. The new method appears to be relevant as a quality control tool for Belzuitifan assay in pharmaceutical businesses due to the necessity of low retention time in regular drug analysis.

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