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Research Article

STABILITY INDICATING REVERSE PHASE-HPLC METHOD DEVELOPMENT AND VALIDATION FOR BELZUTIFAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:
A Simple, sensitive, specific and precise RP-HPLC method for the pharmaceutical dose estimation of Belzutifan. Chromatogram was run through Agilent C18 250 x 4.6 mm, 5m. Mobile phase containing 0.1% OPA: Acetonitrile taken in the ratio 50:50 v/vwas pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 251nm. Retention time of Belzutifan was found to be 2.351 min. standard %RSD of the Belzutifan were and found to be 0.8. %RSD of Method precision of Belzutifan was found to be 0.2. %Recovery was obtained as 99.64% for Belzutifan. LOD, LOQ values obtained from regression equation of Belzutifan were 0.03, 0.08. Regression equation of Belzutifan is y = 120739x + 6111.9.

Key Words: Belzutifan, RP-HPLC, validation, Method Development.

Introduction:

Antineoplastic antibiotics function similarly to quinolones and are also referred to as anticancer or antitumor antibiotics. Antibiotics and antineoplastic antibiotics differ primarily in that the former target bacterial cells, whereas the latter target malignant or tumorous cells within the human body. Antineoplastic antibiotics interfere with DNA synthesis and replication by either inserting themselves into DNA strands or generating superoxide, which breaks DNA strands and stops tumorous or malignant cells from proliferating.[1] Belzutifan is an inhibitor of hypoxia-inducible factor 2α (HIF-2α) used in the treatment of von Hippel-Lindau (VHL) disease-associated cancers[2] is used to treat some malignancies that include renal cell carcinoma (RCC) linked with Von Hippel-Lindau (VHL) disease.[3] Approved by the U.S. Food and Drug Administration (FDA) in 2021, Belzutifan represents the first-in-class HIF-2α inhibitor indicated for adult patients with von Hippel–Lindau disease–associated renal cell carcinoma, central nervous system hemangioblastomas, and pancreatic neuroendocrine tumors that do not require immediate surgery. Ongoing studies are evaluating its efficacy in sporadic clear cell renal cell carcinoma and other solid tumors, highlighting its potential as a key therapeutic agent targeting hypoxia-driven malignancies.[4-8] Belzutifan Chemically known as 3-{[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methanesulfonyl-2,3-dihydro-1H-inden-4-yl]oxy}-5-fluorobenzonitrile.[9]

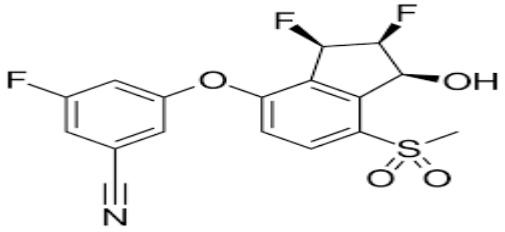

Figure 1: Structure of Belzutifan

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Belzutifan (MK-6482), a novel drug used in the treatment of cancers associated with von Hippel-Lindau (VHL) disease. In the review of literature, more economical methods were observed[10-13],

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hence a simple, cost-effective stability-indicating simultaneous estimation of Belzutifan by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

Materials and Methods:

Belzutifan (API), Belzutifan tablets (Welireg), Acetonitrile, Methanol, Ortho Phosphoric Acid, Distilled water. All of the solvents and chemicals were of HPLC quality and obtained from Rankem Chemicals Pvt Ltd.

Instrumentation:

The Method Development and Validation was performed by Waters HPLC Model 2695 equipped with PDA Detector and Empower 3 Software. Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven.

Chromatographic Condition:

An Isocratic Elution carried out by using Acetonitrile and 0.1% OPA 50:50 v/v as the Mobile Phase, Diluent used was Combination of Acetonitrile and Water in 1:1 ratio. Agilent C18 (4.6 x 250mm, 5µm) column was used to determine the Method at a flow rate of 1ml/min, by maintaining the column Temperature at 30 0C. In addition, with an injection volume of 10µL and the wavelength detected at 251nm.

API Formulation

Preparation of Standard stock solutions and Working Solution: Accurately weighed 10mg of Belzutifan is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labelled as Standard stock solution to make 200µg/ml of Belzutifan solution. Moreover, from this 1ml from stock solution was pipetted out, taken into a 10ml volumetric flask, and made up with diluent to make 20µg/ml of Belzutifan

Sample Formulation

Preparation of Sample stock solutions and Working 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, 80ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters to make 400µg/ml of Belzutifan solution. From this 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent to make 20µg/ml of Belzutifan.

Method Validation

As per ICH guidelines, the developed method is validated for validating the analytical procedures. The validation parameters include system suitability, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity and Degradation Studies were performed.

System suitability parameters:

The working standard solution was injected six times into HPLC system as and the chromatographic study was performed as per the developed and optimized conditions. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, theoretical plates and peak areas from six replicate injections.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

The method and system precision were demonstrated by preparing the standard solution at a known concentration and six repeatable injections were given to ensure the repeatability of the proposed method. The intermediate precision was also performed by preparing six working sample solutions by injecting each solution six times. The area was noted, mean, standard deviation, and percentage of RSD were calculated. The results were satisfactory and below 2% as per the limit.

Linearity:

The linearity of the drug was studied by preparing serial dilutions in the range of 5–30 µg/ml. The correlation between peak area response and drug concentration was shown on a graph. It was observed to be linear for the specified drug concentration.

Accuracy:

Accuracy was performed in triplicate for various concentrations of Belzutifan equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure.

Sensitivity:

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines.

Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

Assay:

The assay and % purity were calculated for brand Weligra with label claim 40g. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form

Degradation Studies

These studies are performed at various stress conditions to describe the stability of the pure drug substance and are helpful in determining a suitable storage conditions. These studies include base, peroxide, acid, neutral hydrolysis, photo, and thermal degradation.

Oxidation:

To 1 ml of stock solution of Belzutifan, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Belzutifan, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Belzutifan, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 300µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULT AND DISCUSSION

Table.1 Optimized Condition

Parameter	***Condition***
Mobile phase	*Acetonitrile: OPA (50:50 v/v)*
Flow rate	*1 ml/min*
Column	*Agilent C18 (4.6 x 250mm, 5µm)*
Detector wave length	*251nm*
Column temperature	*30°C*
Injection volume	*10µL*
Diluent	*Water and Acetonitrile in the ratio 50:50*

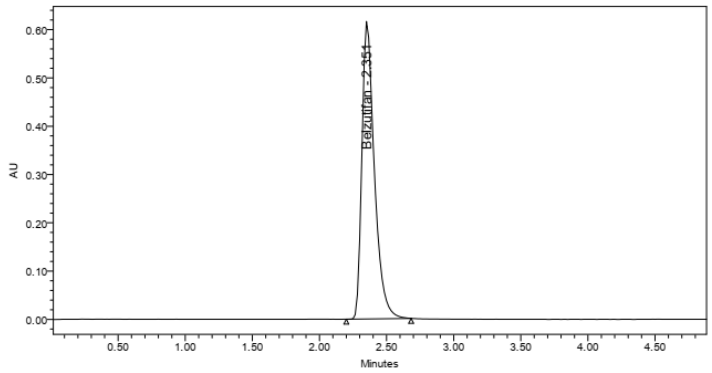

Figure 2: Optimized Chromatogram

System Suitability:

Table 2: System Suitability

S no			*Belzutifan*	
Injection	*RT*	*area*	*Plate Count*	*Tailing*
Injection-1	*2.185*	*2417963*	*2913*	*1.43*
Injection-2	*2.265*	*2397388*	*3452*	*1.42*
Injection-3	*2.288*	*2436020*	*3075*	*1.43*
Injection-4	*2.310*	*2457902*	*3313*	*1.43*
Injection-5	*2.334*	*2432183*	*3413*	*1.43*
Injection-6	*2.343*	*2434182*	*2999*	*1.42*
Mean		*2429273*		
Std ev		*20210.3*		
RSD		*0.8*		

Standard solution was injected six times, and their corresponding chromatograms were obtained. The theoretical plate count exceeded 2,000, the USP tailing was under 2, and the percent RSD was under 2%, according to observations. All of the requirements for system suitability were met and fall within acceptable ranges.

Linearity:

Six concentrations ranging from 5 to 30 µg/ml were prepared and linearity was estimated in a duplicate manner. The linearity equation for belzutifan was y = 120739x + 6111.9. For the alibration curve over the concentration range, the data have shown a good correlation.

Table 3: Linearity Data

Concentration (ppm)	****Peak area***
0	*0*
5	*606877*
10	*1211958*
15	*1823212*
20	*2430736*
25	*3044340*
30	*3603248*
y :	*120739x + 6111.9*
R^2	*0.9999*
Slope	*120739*
Intercept	*6111.9*

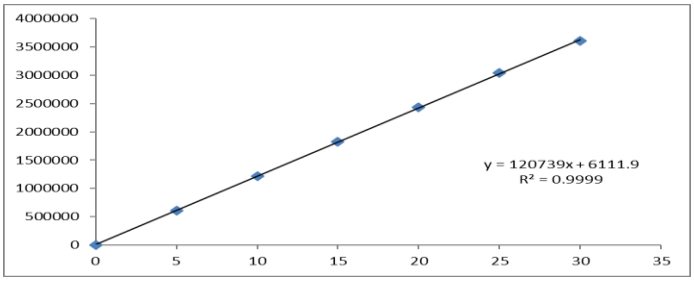

Figure 3: Calibration Curve of Belzutifan

Accuracy:

Three doses were given at each level, and the mean % recovery was calculated. Belzutifan's recovery rate was observed to be between 99% and 100.%, which is within the acceptable ranges

Table 4: Accuracy Data

% Level	*Amount Spiked (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery*	*Avg %*	*Mean %Recovery*
		7.47	*99.56*		
50%	***7.5***	*7.49*	*99.91*	*99.69*	
		7.47	*99.60*		
		14.85	*99.02*		
100%	***15***	*14.86*	*99.10*	*99.11*	***99.41%***
		14.88	*99.22*		
		22.35	*99.35*		
150%	***22.5***	*22.38*	*99.47*	*99.41*	
		22.37	*99.42*		

Precision:

Table 5: Precision Data

S. No	*Method Precision*	*Intermediate Precision*
Injection-1	*2419264*	*2409793*
Injection-2	*2418414*	*2412783*
Injection-3	*2414410*	*2406647*
Injection-4	*2416248*	*2411701*
Injection-5	*2414681*	*2409533*
Injection-6	*2408311*	*2415693*
Mean	*2415221*	*2411025*
S.D	*3906.2*	*3106.4*
%RSD	*0.2*	*0.1*

Sensitivity:

Table 6: LOD and LOQ Data

Molecule	*LOD*	*LOQ*
Belzutifan	*0.03µg/ml*	*0.08 µg/ml*

Robustness:

Table 7: Robustness data

Parameter	*Optimized condition and %RSD*		*Used condition*	*Obtained %RSD*
Flow rate (±0.1ml/min)	*1ml/min*		*0.9ml/min*	*0.7*
			1.1 ml/min	*0.7*
MP Composition (5%v/v)	*50:50*	*0.8%*	*55:45*	*1.0*
			45:55	*0.6*
Column Temp (±3^{0}c)	*30^{0}c*		*27 ^{0}C*	*0.6*
			33 ^{0}C	*0.5*

Assay

Table 8: % Assay Purity Data

Formulation	*Label claim(mg)*	*% Assay**
Weligra	*Belzutifan 40 mg*	*99.32 %w/w*

Degradation studies:

Table 9: Force Degradation Studies

S.No	*% Degradation*	*Peak area*	*Stress Conditions*	*Peak Purity*
1	*6.92*	*2263468*	*Acid*	*Passes*
2	*6.82*	*2265804*	*Base*	*Passes*
3	*6.14*	*2282397*	*Oxidation*	*Passes*
4	*3.19*	*2354088*	*Thermal*	*Passes*
5	*2.03*	*2382390*	*Photolytic*	*Passes*
6	*2.03*	*2414116*	*Hydrolytic*	*Passes*

CONCLUSION:

In conclusion, the Belzutifan HPLC analysis findings demonstrate that this technique can accurately evaluate the drug's concentration and purity. This method's consistent repeatability, sharp peak resolutions, and reliable retention lengths make it ideal for routine quality control and pharmacokinetic research. HPLC is a vital tool that ensures the efficacy and safety of Belzutifan for usage in clinical settings when assessing its analytical qualities.

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References:

1. *Sufia Hena, Hussein Znad, in Comprehensive Analytical Chemistry, 2018.*
2. *FDA Approved Drug Products: Welireg (belzutifan) tablets for oral use*
3. *Sravya D, Kuber BR. A stability-indicating reverse phaseHPLC method development and validation for newly approved drug, Belzutifan in bulk and pharmaceutical dosage forms. J Appl Pharm Sci, 2023; 13(07):233–240.*
4. *Chen W, Hill H, Christie A, et al. Targeting renal cell carcinoma with a HIF-2 antagonist. Nature. 2016; 539(7627):112–117.*

5. *Xu R, Lu Y, Dong L, et al. Mechanism and clinical significance of HIF-2α inhibition in cancer therapy. Front Oncol. 2022; 12: 837541.*
6. *Choueiri TK, Kaelin WG Jr. Targeting the HIF2–VHL pathway in renal cell carcinoma. Nat Med. 2020; 26 (10): 1519 –1530.*
7. *Merck & Co., Inc. WELIREG™ (belzutifan) prescribing information. 2021.*
8. *Gossage L, Eisen T. *Alterations in VHL as potential biomarkers in renal-cell carcinoma. Nat Rev Clin Oncol. 2010;7(5):277–288.*
9. *https://go.drugbank.com/drugs/DB15463*
10. *Priyanka P Shyamala et al.., Development And Validation Of Rp-Hplc Method For Determination Of New Anticancer Agent Belzutifan In Bulk And Its Pharmaceutical Formulation, European Journal of Biomedical and Pharmaceutical Sciences 2019, 6(4); 465-470.*
11. *Anusha A, Pushpa Latha E et al.., Stability indicating RP-HPLC method development and validation for the determination of Belzutifan in bulk drug and capsule dosage form, International Journal of Bio-Pharma Research 2019, 8(8); 2758-2762.*
12. *Gujju. Hima Bindu, Mukthinuthalapati Mathrusri Annapurna. A sensitive stability indicating RP-HPLC method for the determination of Ibrutinib - An anti-cancer drug, Research Journal of Pharmacy and Technology 2018, 11(10); 4587-4591.*
13. *Acerta Pharma BV. Belzutifan (ACP-196): A Covalent Bruton Tyrosine Kinase Inhibitor with a Differentiated Selectivity and In Vivo Potency Profile, The Journal Of Pharmacology And Experimental Therapeutics 2017.*