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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF BELZUTIFAN IN ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The RP-High Performance approach is straightforward, delicate, precise, and consistent to gauge Belzutifan in drug (forms of administration and Extracts. Used materials in chromatographic techniques like stationary phase is Kromosil (4.6mm x 150mm, 5µm) was applied along with the solvent fissure MeCn and Dipotassium buffer. The instrumental conditions setup after method optimization are warmth setup of 30oC and Solvent fissure at 60 and 40 where buffer has been loaded higher than organic, and effluent velocity maintained at 1.0ml/min and last frequency of lamda seen the analyte absorbance was 251.0nm. The Belzutifan maintenance season was observed to last 2.248 minutes. the Eluite % R was evaluated as 0.8, while the %RSD of its methodology precision was evaluated as 0.9. the fraction of trueness percent for Belzutifan was 101.03%. The detective and quantification values obtained from Regression method were 0.31 and 0.94 and R2 is y = 93023x + 81190. Since the duration of retention and the run period were shortened, the procedure was simple and cost-effective, making it suitable for use in periodic inspection and quality tests in organizations.

Key Words: Belzutifan, RP-HPLC.

INTRODUCTION

Antineoplastic antibiotic is also known as anticancer or antitumor antibiotic and it acts quite similar as quinolones. The main difference between antibiotics and antineoplastic antibiotics is the former one act on bacterial cells, while the latter act on tumorous or cancerous cells in human body. Antineoplastic antibiotic affects DNA synthesis and replication by inserting into DNA strands or by producing superoxide that cause breakage in DNA strands and prevent the tumorous or cancerous cells to divide further.[1]

Most anticancer drugs approved by the regulatory agencies are small molecules or monoclonal antibodies that inhibit specific targets that are upregulated in cancer. The main cause of the clinical failure of standard cancer chemotherapy is the limited drug concentration that reaches the tumour site and the high cytotoxicity to both cancer and normal cells of most of anticancer drugs.[2] Antineoplastic agents are extensively utilized in cancer therapy as they can inhibit growth via disrupting cell division and through destroying the actively growing cells.[3]

Chemotherapeutic drugs are generally administered parenterally, orally, or as local therapies. Pentavalent antimonial, already developed in the 1940s, are still used as first-line medication in the treatment of both CL and VL, including the drugs, meglumine antimoniate and sodium stibogluconate.[4] Conventional chemotherapeutic drugs cause severe adverse effects during clinical uses. A compact delivery vehicle triggered to release its payload in the tumour microvasculature without affecting healthy tissues which could be a very likely to improve the therapeutic window of any anticancer drug.[5]

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In recent years, targeted therapies have revolutionized cancer treatment by specifically targeting molecular abnormalities unique to cancer cells. Drugs like imatinib and trastuzumab inhibit specific proteins or receptors essential for cancer cell survival and proliferation, resulting in more precise and often less toxic treatment options compared to traditional chemotherapy.[6]

Analytical Background:

Belzutifan represents a significant advancement in targeted therapy for cancers associated with VHL disease. Belzutifan, also known by its generic name belzutifanib (MK-6482), is a novel anticancer drug belonging to a class of medications known as hypoxia-inducible factor (HIF)-2α inhibitors. Developed by Merck (known as MSD outside the United States and Canada), belzutifan inhibits the activity of HIF-2α, a transcription factor involved in the regulation of genes related to cellular adaptation to low oxygen levels (hypoxia). In cancer, HIF-2α plays a critical role in promoting tumor growth, angiogenesis (formation of new blood vessels), and resistance to therapy.[7] Belzutifan is an inhibitor of hypoxia-inducible factor 2α (HIF-2α) used in the treatment of von Hippel-Lindau (VHL) disease-associated cancers.[8]

On August 13, 2021, the Food and Drug Administration approved belzutifan (Welireg, Merck), 9 it is chemically known as 3-{[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methanesulfonyl-2,3-dihydro-1H-inden-4-yl]oxy}-5-fluorobenzonitrile.[10]

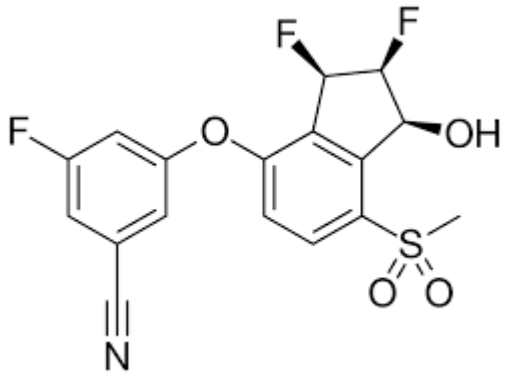

Figure 1: Structure of Belzutifan

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 [10-14], 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:

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

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Chromatographic Conditions

Table 1: Chromatographic Conditions:

Mobile phase	**Methanol: KH_2PO_4(60:40 v/v)**
Flow rate	1 ml/min
Column	Sunfire C18 (4.6 x 250mm, 5µm
wave length	251 nm
Column temperature	26°C
Injection volume	10µL
Run time	5.0 min
Buffer	Na2hpo4, Kh2po4, OPA

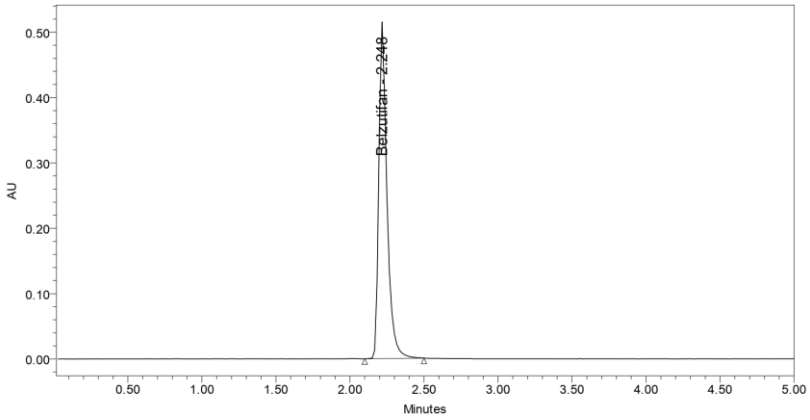

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: 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 labeled as Standard stock solution. (200µg/ml of Belzutifan)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Belzutifan).

Preparation of Sample stock solutions: 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 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 solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Belzutifan)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Belzutifan (50ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

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

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.

System suitability:

The system suitability parameters were determined by preparing standard solution of Belzutifan (50ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Specificity (Selectivity): 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. Representative chromatogram is shown in Figure 4 and experimental data is given in Table 2

Table: 2 System suitability parameters for Belzutifan

S no	Belzutifan		
Inj	RT(min)	USP Plate Count	Tailing
1	2.206	7541	1.41
2	2.206	7841	1.41
3	2.207	7629	1.41
4	2.216	8117	1.42
5	2.220	8260	1.41
6	2.221	8065	1.40

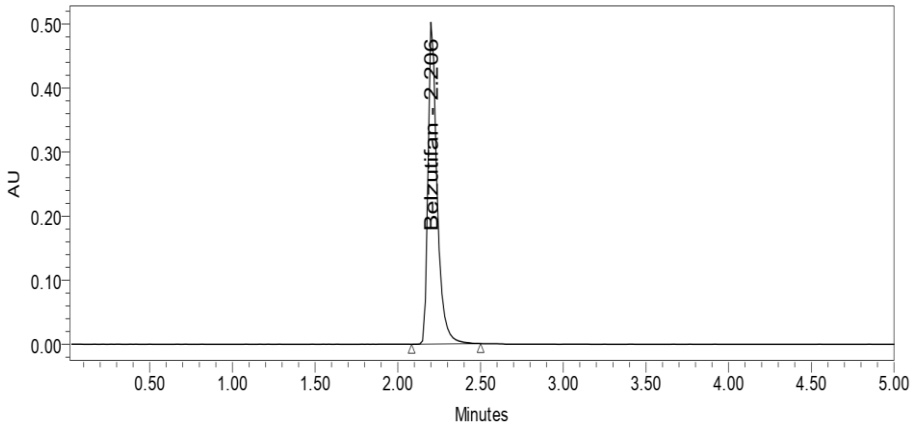

Figure 3: System Suitability Chromatogram of Belzutifan

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Belzutifan	2.248	1967074	7998	1.4

Specificity:

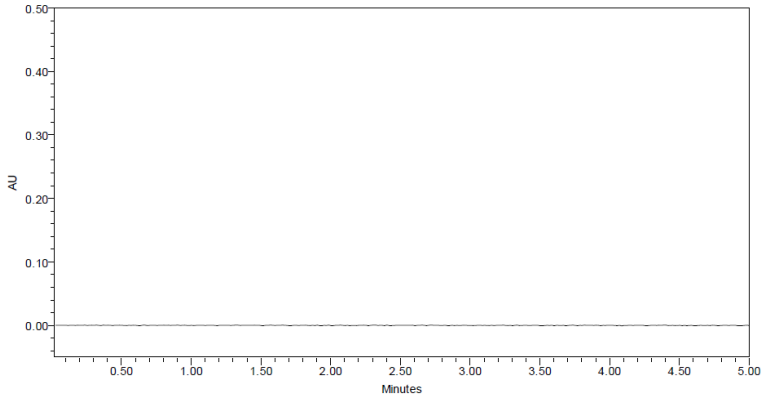

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 3 and the results are mentioned in Table 4

Table 4: Forced degradation conditions for Belzutifan

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Conditon	% Drug UnDegeraded	& Drug Degeraded
Acid	95.66	4.34
Base	95.09	4.91
Oxidation	95.00	5.00
Thermal	97.12	2.88
Photolytic	98.36	1.64
Hydrolytic	99.10	0.90

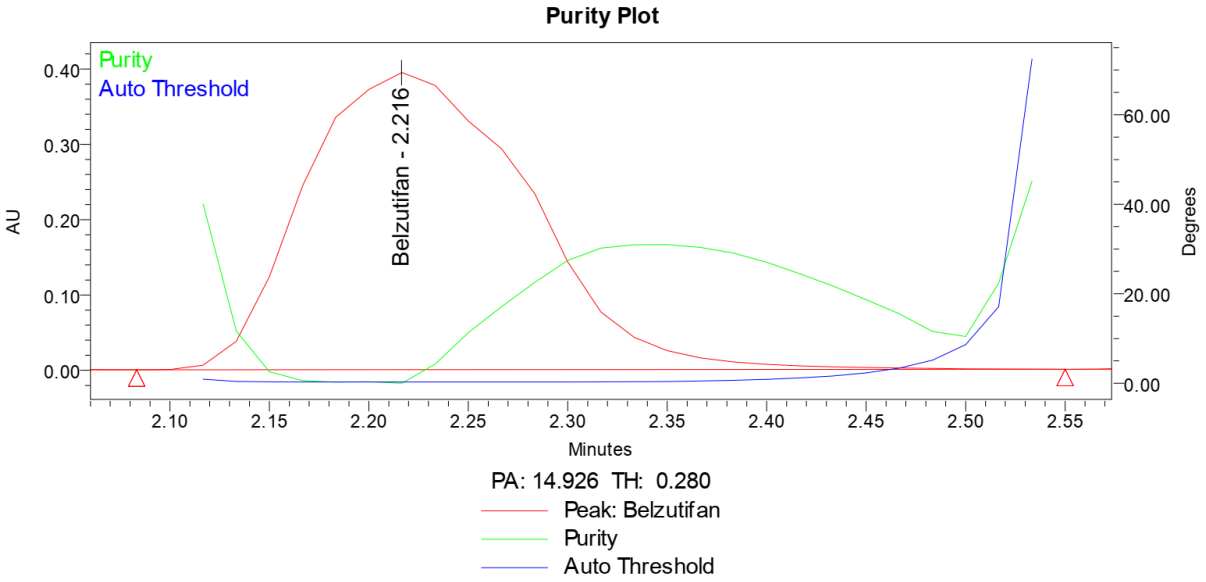

Figure 5: Purity Plots of Belzutifan

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Belzutifan are listed in Table 5.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)	Peak Area
LOD	0.16	36549
LOQ	0.49	55893

Linearity: The linearity of the method was demonstrated for Belzutifan by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Belzutifan was 0.999. This indicates good linearity (Figures 8).

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Belzutifan

Belzutifan	
Conc (µg/mL)	**Peak area**
0	0
5	554825
10	1009657
15	1449425
20	1975037
25	2385820
30	2879819

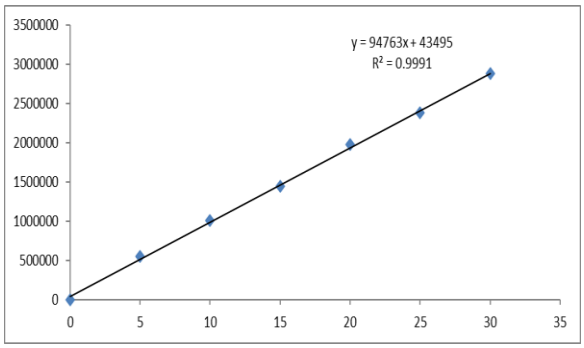

Figure 6: Calibration curve of Belzutifan

Table 8: regression data

Parameter	Belzutifan
Conc range (µg/mL)	5-30µg/ml
Regression Equation	y = 94763x + 43495.
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Belzutifan at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Belzutifan

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	10	9.96	99.63
	10	9.92	99.24
	10	9.95	99.54
100%	20	19.79	98.97
	20	19.91	99.57
	20	19.86	99.31
150%	30	29.79	99.29
	30	29.85	99.51
	30	29.76	99.20
Mean % recovery			**99.36**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Belzutifan. Results of peak area are summarized in Table 10

Table 10 System precision table of Belzutifan

S. No	Area of Belzutifan
1.	1961243
2.	1980375
3.	1957137
4.	1963031
5.	1953291
6.	1943781
Mean	**1959810**
S.D	**12181.3**
%RSD	**0.6**

Method Precision: The precision of the method was determined by analyzing a sample of Belzutifan). Data obtained is summarized in Table 11

Table 11 Repeatability table of Belzutifan

S. No	Area of Belzutifan
1.	1960514
2.	1969654
3.	1967074
4.	1948145
5.	1976337
6.	1964498
Mean	**1964370**
S.D	**9555.9**
%RSD	**0.5**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Belzutifan

S. No	Area of Belzutifan
1.	1958889
2.	1954941
3.	1962928
4.	1938810
5.	1954238
6.	1964377
Mean	**1955697**
S.D	**9227.0**
%RSD	**0.5**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Belzutifan

Condition	%RSD of Belzutifan
Flow rate (-) 0.9ml/min	0.1
Flow rate (+) 1.1ml/min	0.8
Mobile phase (-) 75B:25A	0.6
Mobile phase (+) 65B:35A	0.4
Temperature (-) 27°C	0.7
Temperature (+) 33°C	0.5

Assay data: -

Welireg Tablet bearing the label claims Belzutifan 40 mg. Assay was performed with the above formulation. Average % Assay for Belzutifan obtained was 99.93%. Assay data shown in table no 8.

Figure 7: marketed Belzutifan Drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 8: Assay Data of Belzutifan

S.no	Standard Area	Sample area	% Assay
1	1961243	1960514	99.74
2	1980375	1969654	100.20
3	1957137	1967074	100.07
4	1963031	1948145	99.11
5	1953291	1976337	100.54
6	1943781	1964498	99.94
Avg	**1959810**	**1964370**	**99.93**
Stdev	**12181.3**	**9555.9**	**0.49**
%RSD	**0.6**	**0.5**	**0.5**

Conclusion

Finally, the results of the Belzutifan HPLC analysis show that the drug's concentration and purity can be precisely measured using this method. This approach is well-suited for regular quality control and pharmacokinetic research because to its constant repeatability, crisp peak resolutions, and dependable retention durations. When it comes to evaluating the analytical properties of Belzutifan, HPLC is an indispensable instrument that guarantees its effectiveness and safety for use in clinical settings.

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