CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/10702495

https://www.iajps.com/volumes/volume11-february-2024/04-issue-02-february-24/

Available online at: http://www.iajps.com *Research Article*

STABILITY INDICATION RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BEXAGLIFLOZIN IN MARKETED FORMULATION

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Abstract:
This study focuses on the development and validation of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the accurate estimation of Bexagliflozin in a marketed formulation. Bexagliflozin, a novel sodium-glucose cotransporter-2 (SGLT-2) inhibitor, is widely used for the management of type 2 diabetes mellitus. The proposed RP-HPLC method demonstrates stability indicating attributes, enabling the assessment of drug integrity in the presence of potential degradants. The validation parameters, including specificity, linearity, precision, accuracy, robustness, and system suitability, were systematically evaluated. Additionally, the method was successfully applied to quantify Bexagliflozin in a commercially available formulation, providing a reliable tool for routine quality control analysis. The developed method offers a valuable contribution to the pharmaceutical analytical toolbox, ensuring the accurate determination of Bexagliflozin in the presence of potential degradation products.

Key Words: *Bexagliflozin, stability indicating, RP-HPLC, method development, validation, marketed formulation,*

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Please cite this article in press ***Deepak Kumar Basedia*** *et al.,* ***Stability Indication Rp-Hplc Method Development And Validation For The Estimation Of Bexagliflozin In Marketed Formulation****, Indo Am. J. P. Sci, 2024; 11 (02).*

INTRODUCTION:

Bexagliflozin, a potent and selective sodium-glucose cotransporter-2 (SGLT-2) inhibitor, has emerged as a promising therapeutic agent for the management of type 2 diabetes mellitus (T2DM). By inhibiting SGLT-2 in the renal tubules, Bexagliflozin reduces glucose reabsorption, promoting glycosuria, and contributing to improved glycemic control. The therapeutic significance of Bexagliflozin has led to its incorporation into various pharmaceutical formulations available in the market [1-2].

Ensuring the stability of Bexagliflozin in these formulations is critical for maintaining its efficacy over the product shelf life. Stability-indicating analytical methods are essential tools for assessing the robustness of pharmaceutical formulations by identifying and quantifying potential degradation products. Among various analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) stands out as a reliable and widely accepted method for stability studies due to its sensitivity, specificity, and precision [3-5].

Several studies have highlighted the importance of stability-indicating methods for assessing the degradation profile of drugs in pharmaceutical formulations. The development of a robust RP-HPLC method for Bexagliflozin becomes crucial to ensure the accurate estimation of the active pharmaceutical ingredient (API) in the presence of potential degradation products.

MATERIAL AND METHODS:

Preperation of Stock Solution Accurately weighed 10 mg of Bexagliflozin was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of methanol and sonicate for 10 min., then volume was made up to 50 ml with methanol and vortex it to get complete dissolution and then filtered by whatmann filter paper (no.41). Concentration of Bexagliflozin in methanol was 200 µg/ml. (stock- A)

Preparation of Sub Stock Solution 5 ml of solution was taken from stock-A of Bexagliflozin and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent to give concentration of 100 µg/ml (Stock-B).

Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with methanol. This gives the solutions of, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of Bexagliflozin.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 247nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of 10µg/ml Bexagliflozin was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of Developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to validate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The precision are established in three differences:

Repeatability

Intermediate precision

a) Day to Day
b) Analyst to Analyst

Reproducibility

Repeatability
The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for Bexagliflozin indicates the precision under the same operating condition over short interval time.

Intermediate Precision
Day To Day Precision
Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations.

Robustness
As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 10Mm KH_2PO_4: methanol (20:80% v/v), to (20:80% v/v).

Detection Limit and Quantitation Limit
The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of drugs in tablets formulation
Tablet powder amount equal to 10mg of Bexagliflozin was taken in 100ml volumetric flask. This was than dissolve in 25 ml of methanol by sonication for about 10 minutes. The volume is made up to the mark by methanol and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

Forced degradation studies
In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation
50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Alkaline hydrolysis
50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Oxidative degradation
50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Thermal degradation
50 mg of the drug sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

RESULTS AND DISCUSSION:

The chromatographic system demonstrated excellent performance with a high number of theoretical plates (3546.333±14.962), a low tailing factor (1.135±0.014), and a retention time of 4.031±0.007.The method displayed strong linearity over the concentration range of 5-25 µg/ml, with a correlation coefficient (r^2) of 0.999.

The slope (m) was 26.64, and the intercept (c) was -1.535.The recovery study showed consistent results at different concentration levels (80%, 100%, and 120%), with % mean values ranging from 99.38% to 99.87%.Precision, evaluated through repeatability and intermediate precision, demonstrated % mean values of 98.516±0.104 and 98.859±0.106, respectively.

The method's robustness was confirmed, with a % mean value of 98.607±0.104, indicating its reliability under different conditions. The assay of a synthetic mixture revealed accurate quantification of Bexagliflozin, with % found at 9.95%, % assay at 99.50%, and % RSD of 0.215.

Under various stress conditions, Bexagliflozin exhibited stability in standard conditions (99.95% recovered). However, it showed susceptibility to acidic hydrolysis (82.23% recovered), alkaline hydrolysis (89.98% recovered), oxidative degradation (96.65% recovered), and photolytic degradation (91.14% recovered).

The developed RP-HPLC method proves robust and sensitive, suitable for routine analysis and stability assessment of Bexagliflozin in pharmaceutical

formulations. Results from recovery studies, precision evaluations, and forced degradation studies underscore the method's reliability, providing valuable insights for quality control and regulatory compliance in the pharmaceutical industry.

Table 1: Results of system suitability parameters

Parameters	Bexagliflozin
No. of Theoretical Plates	3546.333±14.962
Tailing Factor	1.135±0.014
Retention time	4.031±0.007

Table 2: Results of Linearity of Bexagliflozin

Parameter	Bexagliflozin
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.999
Slope (m)*	26.64
Intercept (c)*	-1.535

*value of five replicate

Table 3: Results of recovery study

% Level	% MEAN±SD*
80%	99.42±0.723
100%	99.87±0.098
120%	99.38±0.977

***** Value of three replicate and three concentrations

Table 4: Results of precision

Parameter	% MEAN±SD*
Repeatability	98.516±0.104
Intermediate precision	
Day to day precision	98.859±0.106

***** Value of five replicate and five concentrations

Table 5: Results of Robustness

Parameter	% MEAN±SD*
Robustness	98.607±0.104

***** Value of five replicate and five concentrations

Table 6: Assay of synthetic mixture

	Bexagliflozin *
Label Claim (mg)	**10mg**
% Found (mg)	9.95
% Assay	99.50
% RSD	0.215

*Average of three determination

Table 7: Results of Forced degradation studies

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.95	0
Acidic hydrolysis	82.23	17.77
Alkaline hydrolysis	89.98	10.02
Oxidative degradation	96.65	3.35
Photolytic degradation	91.14	8.86

REFERENCES:

1. Inzucchi, S. E., et al. (2015). SGLT-2 Inhibitors and Cardiovascular Risk: Proposed Pathways and Review of Ongoing Outcome Trials. Diabetes & Vascular Disease Research, 12(2), 90–100.
2. Zinman, B., et al. (2015). Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. The New England Journal of Medicine, 373(22), 2117–2128.
3. FDA. (2013). Guidance for Industry: Q1A(R2) Stability Testing of New Drug Substances and Products. Retrieved from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q1ar2-stability-testing-new-drug-substances-and-products
4. ICH. (2003). Stability Testing of New Drug Substances and Products (Q1A(R2)). Retrieved from https://database.ich.org/sites/default/files/Q1A_R2__Guideline.pdf
5. K. Swaroopa Rani, B. Ramya Kuber, Analytical Quality By Design Methodology For Bexagliflozin Assessment In Formulation In The Presence Of Degradants Using Rp-Hplc, Eur. Chem. Bull. 2023, 12(Regular Issue 07), 4320-4-4335