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Research **Article**

QUANTITATIVE DETERMINATION OF BEXAGLIFLOZIN IN TABLET FORMULATION AND BULK BY USING RP-HPLC.

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Abstract

A new, accurate and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) as analytical method for the quantitative determination of Bexagliflozin was developed. For HPLC analysis, Reverse phase Chromotography method with photo diode array detector was chosen. The separation of the analyzed compound was conducted by means of a Discovery® C18 (5 µm particle size, L × I.D. 15 cm × 4.6 mm) analytical column, analyzed drugs were determined within 6.0 min using phosphate buffer (4.2ph_dipotassium hydrogen phosphate 0.01N) in water and Methanol in isocratic elution mode as mobile phase at a flow rate of 1.0 mL/min, Temperature was maintained at 30°C. Optimized wavelength selected was 220.0nm. Retention time of Bexagliflozin was found to be 2.167 min. the method was validated to fulfill International Conference on Harmonization (ICH) requirements and this validation included specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. The calibration curve was linear over the concentration range from 5 to 30 µg/ml, The accuracy and precision of the method were within the acceptable limit of ±20% at the lower limit of quantitation and ±15% at other concentrations, all results were acceptable and this confirmed that the method is suitable for its intended use in routine quality control and assay of drugs.

Keywords: RP-HPLC, Bexagliflozin, Method development.

INTRODUCTION

Approximately ninety percent of all occurrences of diabetes are type 2 diabetic mellitus (T2DM). Insulin resistance is the reduced ability to respond to insulin in people with type 2 diabetes. When insulin is inefficient in this situation, the body responds by producing more insulin at first to keep glucose homeostasis stable.[1,2] However, over time, this declines and leads to type 2 diabetes. People over 45 years old are most frequently diagnosed with T2DM. Even Nevertheless, the prevalence of obesity, physical inactivity, and diets high in energy are contributing factors to its increased occurrence in kids, teens, and young adults, Insulin resistance and beta-cell dysfunction relate to type 2 diabetes (T2DM).[3,4] Insulin secretion first increases in response, keeping blood glucose levels within normal bounds. Hyperglycemia is caused when insulin secretion is inadequate to maintain glucose homeostasis due to changes in beta cells brought on by the progression of the disease. The majority of T2DM patients are obese or have greater body fat percentages, which are mostly concentrated in the abdomen. By adipokine dysregulation and increased FFA release, among other inflammatory

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processes, this adipose tissue itself contributes to insulin resistance.[5] The risk of acquiring type 2 diabetes is further increased by inactivity, dyslipidemia, and previous GDM in individuals with hypertension.[6] Adipokine dysregulation, inflammation, aberrant incretin biology with reduced incretins such glucagon-like peptide-1 (GLP-I) or incretin resistance are all suggested to have a role by emerging findings. a novel hypoglycemic agent bexagliflozin was approved by the FDA for the treatment of adults with type 2 diabetes.[7,8]

Bexagliflozin: -

A powerful and extremely selective inhibitor of sodium-glucose co-transporter 2 (SGLT2) is bexagliflozin. They have a minor effect on plasma glucose concentrations in euglycemic people, but they decrease it in patients with hyperglycemia. Moreover, they have been linked to decreased blood pressure, weight loss, and a decreased risk of hypoglycemia in comparison to other frequently used antidiabetic medications like insulin and sulfonylureas. Bexagliflozin has three basic components, like other SGLT2 inhibitors: glucose, two benzene rings, and a methylene bridge.[9,10] A relatively recent class of oral antidiabetic medications called sodium-glucose cotransporter-2 (SGLT-2) inhibitors lowers the renal threshold for glucose absorption in the proximal tubule, which results in glycosuria and natriuresis and inhibits renal sodium and glucose reabsorption.[11] structure elucidation was figured out in Figure No-1.

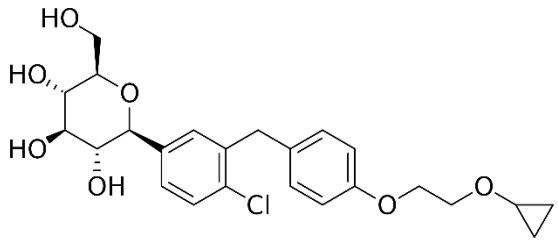


Figure No-1 Structure of Bexagliflozin

High performance liquid chromatography (HPLC) can be used for determination of drug and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product. It has the advantages of being accurate, sensitive, rapid, selective, and reproducible. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of Bexagliflozin in different type of pharmaceutical formulations and environmental water samples Upon literature survey, few methods were reported for determination of Bexagliflozin in pharmaceutical formulations using different analytical techniques; UV–VIS spectroscopy methods, and chromatographic method, Although, few HPLC methods were previously reported but up to our knowledge no stability-indicating HPLC method has been published yet for determination of Bexagliflozin.[14,15] Accordingly, the proposed work aims to develop and validate for the first time a stability-indicating HPLC-PDA for determination of Bexagliflozin in its pharmaceutical dosage form. The chemical stability of Bexagliflozin was evaluated under different stress conditions such as hydrolysis, oxidation, photolytic, and thermal stress conditions.

Chemicals and Equipment's: -

Pure medication of Bexagliflozin were delivered by Piramal's (Gujarat), india with 99.98%. An India Mart pharmacy provided Bexagliflozin tablet (Brenzavvy) from B And B Pharmaceuticals Kolkata, West Bengal. All the chemicals and buffers used in this method were given by Rankem in India, Analysis was performed on waters alliance HPLC system (Waters, USA) equipped with quaternary pump (M00SM4493M), autosampler (M17VSM029N), column oven (B18HLP981G) and photodiode array detector (2998PDA).

Buffer preparation: -

0.01N NA_2HPO_4 Buffer: Accurately weighed 1.41gm of Sodium Hydrogen Phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water (4.0-pH) by adding OPA 0.1% to the phosphate buffer.

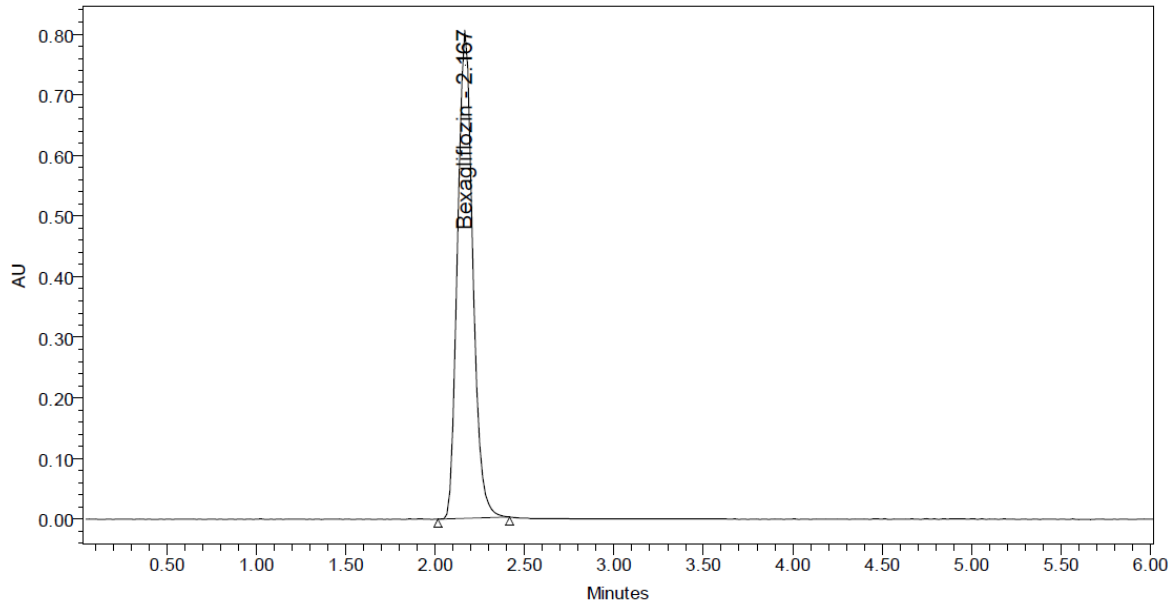
Standard solutions: Accurately weighed 10mg of Bexagliflozin is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Bexagliflozin), from the stock solution, 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Bexagliflozin).

Analysis of Bexagliflozin in its pharmaceutical formulation: -

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 Bexagliflozin), from the stock solution 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Bexagliflozin)

Optimized Conditions: -

Chromotography method was achieved using isocratic elution mode on Discovery C18 150 x 4.6 mm, 5m stationary phase, The mobile phase used was comprised of 70 mm 0.01N Kh2Po4 buffer (pH 4.2 ± 0.05) and Methanol (30 by volume), delivered at flow rate of 1.0 mL/min, The column temperature was kept at 26.5 °C. for each sample, 10 µL was injected in hplc. The photo diode array signal for Bexagliflozin was monitored at 220.0 nm, WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower 2, Figure No-2.



	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Bexagliflozin	2.167	4683045	3024.9	1.2

Figure No-2 Optimized Chromatogram

METHOD VALIDATION

The validation of the HPLC method was carried out in accordance with the ICH recommendations for the simultaneous estimation of Bexagliflozin material to show that the method is suitable for routine analysis.

System suitability:

The system suitability parameters were determined by preparing standard solutions of Bexagliflozin (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined, Results are displayed in Table 1, Figure No 3. The % RSD for the area of six standard injections results should not be more than 2%.

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. Figure No 4 and 5.

Table 1: System suitability results

S no	Bexagliflozin		
Injects	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.162	3035	1.10
2	2.164	3033	1.08
3	2.165	3008	1.10
4	2.166	2896	1.14
5	2.167	3025	1.16
6	2.162	3035	1.10

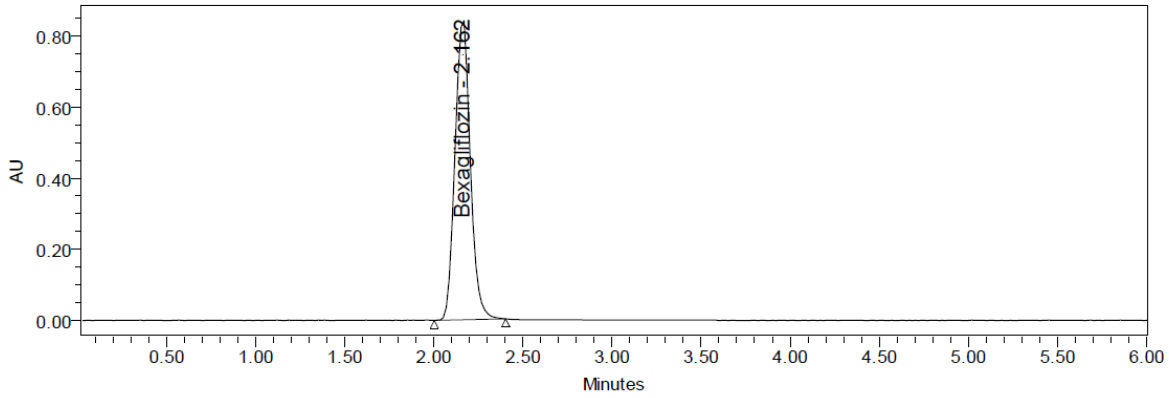


Figure No 3: System suitability Chromatogram of Bexagliflozin

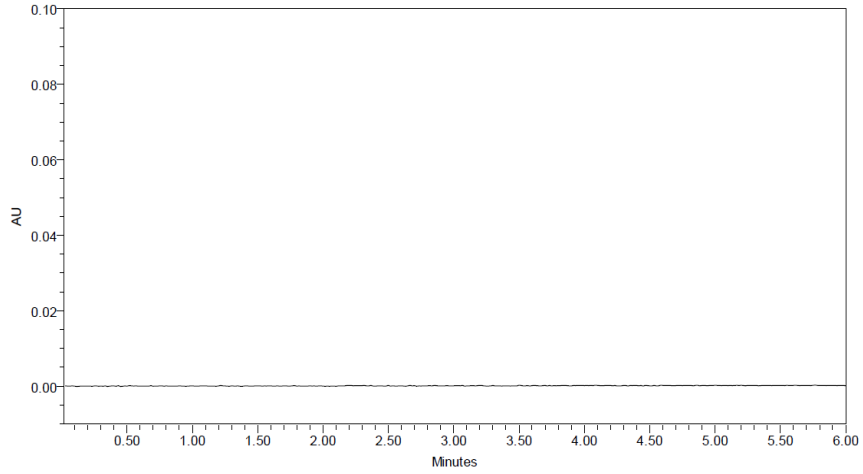


Figure No. 4 Blank

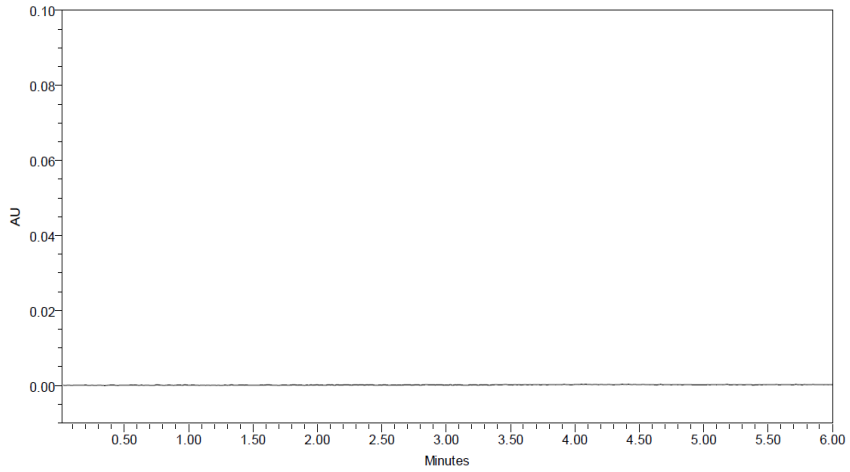


Figure No. 5 Placebo

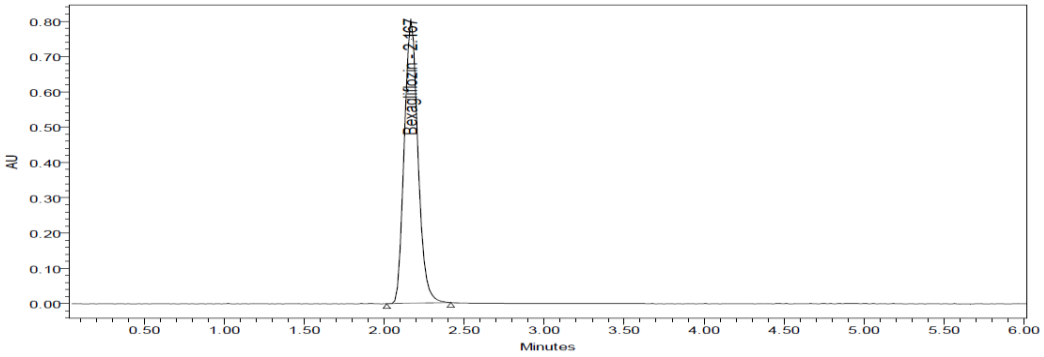


Figure No. 6 Chromatograms of Specificity

Discussion: Bexagliflozin had retention durations of 2.167 minutes. Using this strategy, we were unable to discover any interfering peaks in the blank and placebo at the retention times of these medications. It was said that this approach was specific.

Linearity and construction of calibration curve

Bexagliflozin linearity was examined across a concentration range of 5–30 µg/mL. Using the previously mentioned chromatographic settings, triplicate injections of the prepared solutions were made into the HPLC-PDA system. Peak area values were plotted against the appropriate concentrations of Bexagliflozin to create the calibration graph was plotted in fig no 5, and the regression equation was then calculated. Results are displayed in Table 2.

Table 2: Sparsentan Linearity

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	5	1150502
50	10	2364894
75	15	3450426
100	20	4682529
125	25	5783331
150	30	6949739

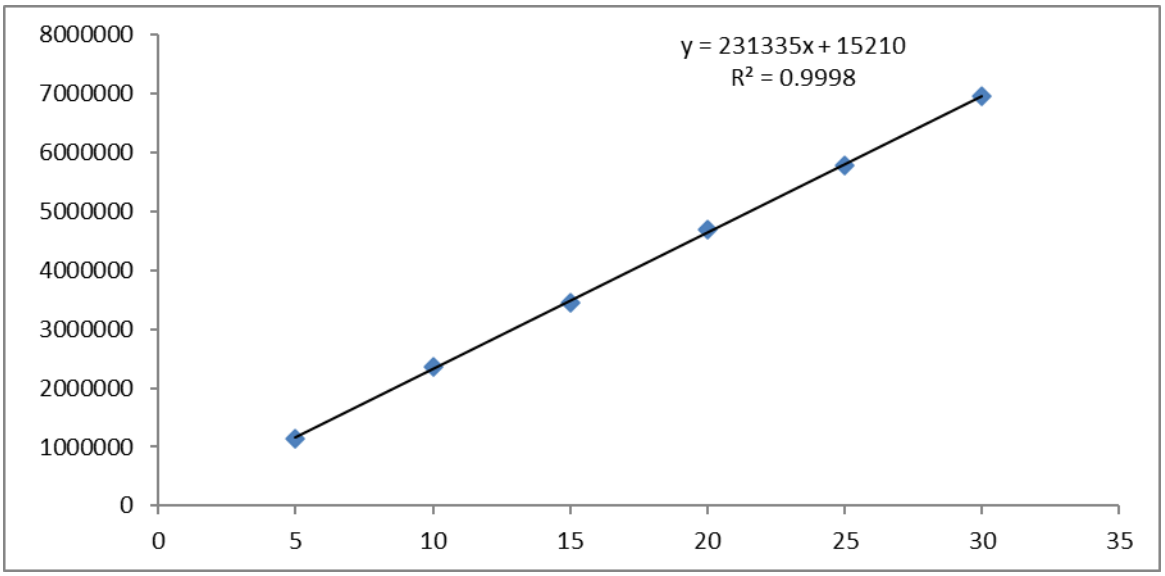


Figure No. 7: Bexagliflozin Calibration curve

Discussion: Accuracy is established across the specified range of the analytical procedure. Three concentration levels, in triplicates, (50.0, 100.0 and 150.0 µg/mL) of pure samples of Bexagliflozin were analyzed by the proposed method.

Table 3: Accuracy (%Recovery data)

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	9.95	99.52	100.31%
	10	10.02	100.16	
	10	10.08	100.80	
100%	20	20.24	101.18	
	20	20.12	100.60	
	20	20.06	100.28	
150%	30	30.05	100.17	
	30	29.97	99.90	
	30	30.07	100.22	

Observation: - The standard addition method was used to prepare three levels of accuracy samples. For every accuracy level, three injections were administered, and the mean percentage of recovery for Bexagliflozin was found to be 100.31%.

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 Bexagliflozin. Results of peak area are summarized in Table 4**.**

Table :4 System precision data

S. No	Area of Bexagliflozin
1.	4636048
2.	4619965
3.	4620226
4.	4613572
5.	4683045
6.	4657818
Mean	**4638446**
S.D	**27064.1**
%RSD	**0.6**

Discussions: The % RSD for the peak areas of Bexagliflozin obtained from six replicate injections of standard solution was within the limit.

Method Precision: Six working sample solutions are injected and the % Amount found was calculated and %RSD was found to be 0.7.

Table 5: **Method precision data**

Injection	Bexagliflozin
1.	4692992
2.	4683237
3.	4674968
4.	4672596
5.	4616938
6.	4612761
Mean	**4658915**
S.D	**34899.7**
%RSD	**0.7**

Discussions: From the above results, the % RSD of method precision study was within the limit for Bexagliflozin.

Robustness parameter

Samples were injected in triplicate under robustness settings that included Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), Mobile Phase minus (65:35A), Mobile Phase plus (75B:25A), Temperature minus (27°C), and Temperature plus (33°C). All the system suitability parameters passed with little to no impact. %RSD was not over the upper bound.

Table 6: Robustness results

Chromatographic condition	Bexagliflozin (SRN)
Flow rate (-) 0.9ml/min	0.4
Flow rate (+) 1.1ml/min	0.7
Mobile phase (-) 45B:55A	0.4
Mobile phase (+) 55B:45A	1.0
Temperature (-) 25°C	0.4
Temperature (+) 35°C	0.4

Stress stability studies

Stress studies were carried out as per ICH guidelines under different acid, base, oxidative, photolytic, and thermal conditions.

Table 7: Forced degradation conditions for Sparsentan.

S.NO	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	**Acid**	98.70	1.30
2	**Alkali**	98.55	1.45
3	**Oxidation**	94.08	5.92
4	**Thermal**	97.87	2.13
5	**UV**	97.03	2.97
6	**Water**	99.70	0.30

Discussion: From the results, no degradation was observed when the samples were exposed to base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

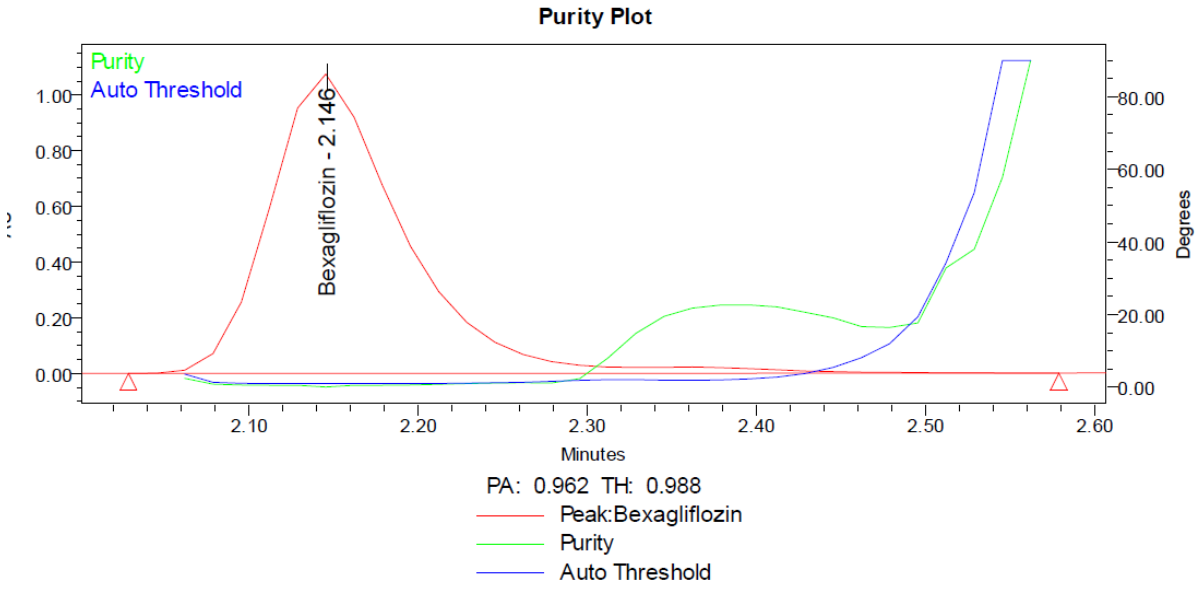


Figure No.8. Degradation purity plots

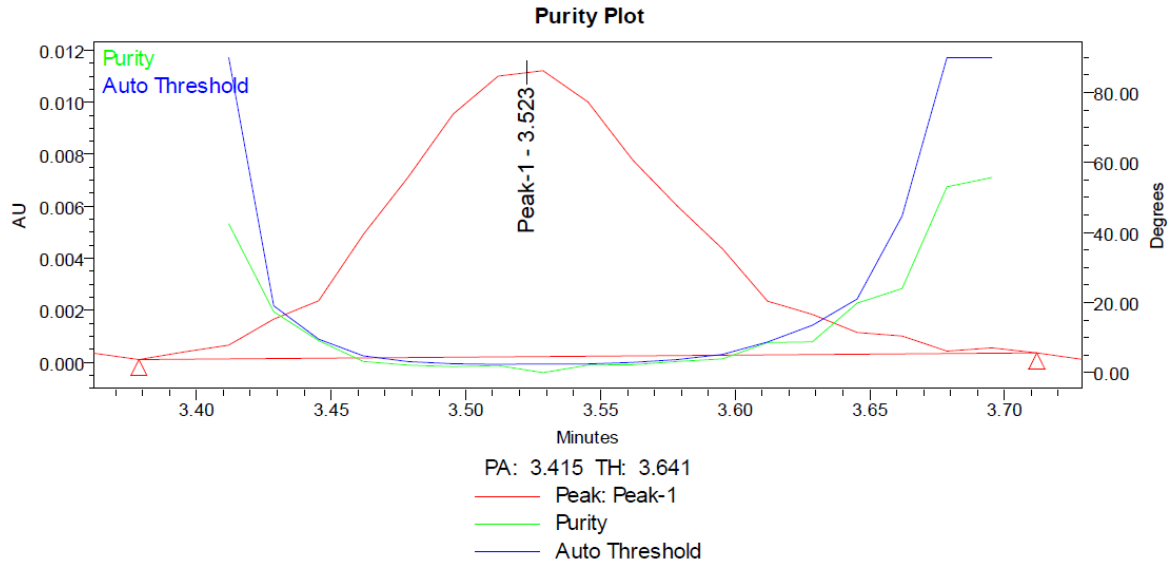


Figure No.9. Degradation purity plots

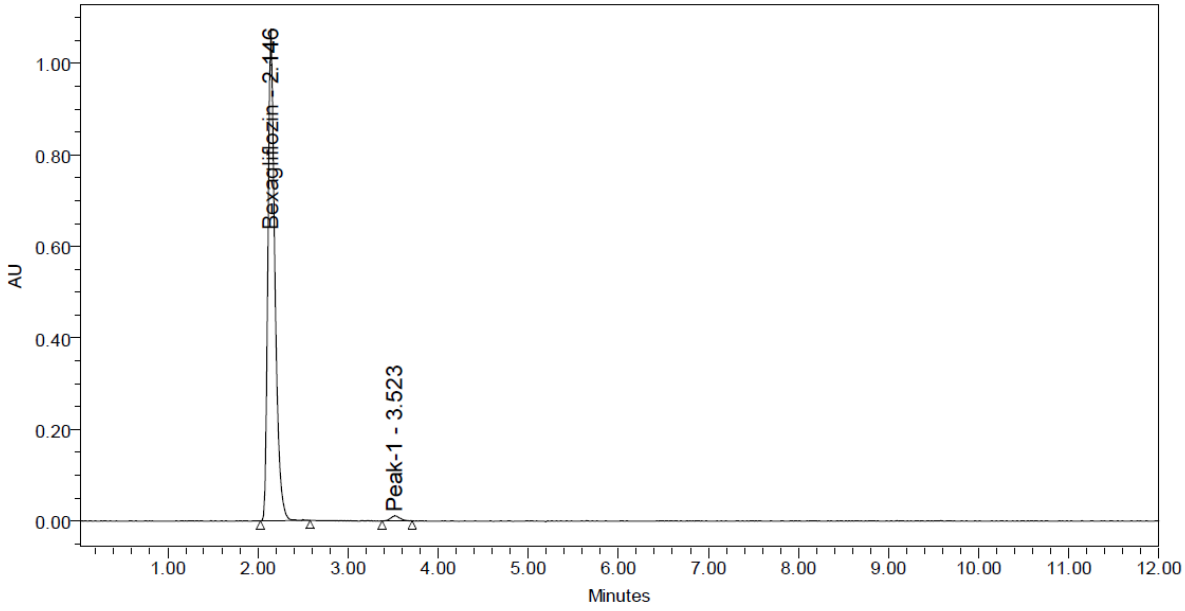


Figure No.10. Acid degradation chromatogram

Analysis of Bexagliflozin in its pharmaceutical formulation.

Brenzavvy bearing the label claim Bexagliflozin 20mg. Assay was performed with the above formulation. Average % Assay for Bexagliflozin obtained was 99.73%.

Table 8: Assay data of Sparsentan and % assay

S.no	Standard Area	Sample area	%Assay
1	4636048	4692992	100.97
2	4619965	4683237	100.76
3	4620226	4674968	100.59
4	4613572	4672596	100.53
5	4683045	4616938	99.34
6	4657818	4612761	99.25
Avg	**4638446**	**4658915**	**100.24**
Stdev	**27064.1**	**34899.7**	**0.75**
%RSD	**0.6**	**0.7**	**0.7**

Conclusion

In this study, accurate, simple, and rapid HPLC method was developed and validated for the determination of Bexagliflozin in pharmaceutical formulations and industrial waste water samples. The method was selective using L1 analytical column and applicable to pharmaceutical preparations. Thus, the developed method was recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in view of its high recovery and precision. PDA is a valuable tool in the recommended HPLC-PDA approach for identifying peak purity and selecting the right wavelength for analysis. The ability to

successfully assess Bexagliflozin in a pharmaceutical formulation that is on the market without interference from excipients found in typical tablets or potential degradation products indicates the feasibility of this technology as a stability-indicating method for regular quality control laboratories.

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