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Research Article

ESTIMATION OF BEXAGLIFLOZIN BY USING RP-HPLC METHOD

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ABSTRACT
A simple, Accurate, precise method was developed for the simultaneous estimation of the An easy, sensitive, specific, and precise RP-HPLC method for determining API and pharmaceutical bexagliflozin. Discovery C18 150 x 4.6 mm, 5m chromatogram analysis. Column was pumped with 0.01N Na2Hpo4: Methanol 55:45 mobile phase at 0.9ml/min. This buffer was 30°C. Selecting 220.0nm optimized wavelength. Bexagliflozin retained 2.854 Min. Method precision was 0.4 and Bexagliflozin's % RSD was 0.9. Bexelifluzin recovered 99.63%. In regression equation, Bexagliflozin LOD and LOQ were 0.440 and 1.33. Bexagliflozin regression equation: y = 2121x + 4049.8 Reduced retention and run times made the method simple and cost-effective for regular quality control tests in industries.

Keywords: HPLC Bexagliflozin, Method development. ICH Guidelines.

INTRODUCTION
Bexagliflozin (Brenzavvy), an oral sodium-glucose co-transporter 2 (SGLT2) inhibitor, has gained recognition for enhancing glycemic control in adults with type 2 diabetes mellitus. Developed by TheracosBio, this SGLT-2 inhibitor received FDA approval in January 2023, following comprehensive evaluation in 23 clinical trials involving more than 5000 adult participants. Beyond its primary role in diabetes treatment, SGLT-2 inhibitors have demonstrated versatility in various health aspects, offering benefits beyond glucose reduction. This drug class has exhibited unique kidney protective actions, improved cardiovascular (CV) outcomes, and other physical benefits[1]
bexagliflozin can improve glycemic control in adults with type 2 diabetes mellitus. It can be used alone or in combination with other diabetes medications. It is essential to note that bexagliflozin is not indicated for patients with type 1 diabetes because it may elevate the risk of diabetic ketoacidosis (DKA) in these patients.

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Bexagliflozin reduces hemoglobin ~0.5% with similar reductions in systolic blood pressure and body weight to other SGLT2 inhibitors. No cardiovascular outcomes trial is published, nor ongoing at this time. Adverse effects are similar to other SGLT2 inhibitors (genital mycotic and urinary tract infections, increased urination) including a warning for lower extremity amputation similar to Bexagliflozin.

Bexagliflozin is a highly specific and potent sodium-glucose co-transporter 2 (SGLT2) inhibitor.[3,4] Similar to other SGLT2 inhibitors, bexagliflozin contains three basic moieties: glucose, two benzene rings and a methylene bridge.[2] SGLT2 is responsible for 60% to 90% of renal glucose re-uptake, and unlike other isoforms such as SGLT1, SGLT2 is mainly expressed in the kidney. By inhibiting SGLT2, bexagliflozin reduces renal reabsorption of filtered glucose and increases urinary glucose excretion, which reduces blood glucose levels independently of insulin sensitivity.[5,6] In January 2023, bexagliflozin was approved by the FDA for the treatment of adults with type 2 diabetes. Its use is not recommended in patients with type 1 diabetes since it may increase their risk of diabetic ketoacidosis.[6]

The following current practice guidelines for pharmacotherapy for type 2 diabetes mellitus (T2DM) in the United States recommend the initial use of metformin in uncomplicated diabetes, with agents that reduce cardiorenal risk, such as glucagon-like peptide 1 receptor agonists and sodium-glucose cotransporter 2 (SGLT2) inhibitors recommended for patients with, or at high risk for, atherosclerotic cardiovascular disease, heart failure, and/or chronic kidney disease (CKD).

In Bexagliflozin the treatment of recent years, SGLT2 inhibitors have emerged as broadly useful treatment options for adults with T2DM. They typically promote weight loss because of caloric wasting and have been shown to provide reductions in cardiovascular risks and preservation of renal function[7] in addition to improved glycaemic control. This work evaluated the effectiveness and safety of bexagliflozin for the management of T2DM in adults inadequately controlled by metformin alone

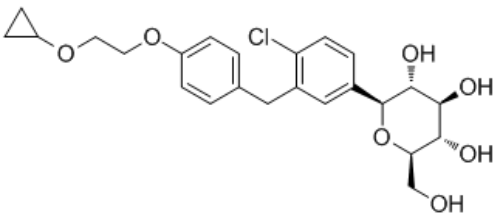

Figure 1: Structure of Bexagliflozin

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs. Utilizing UV-Spectrophotometry RP-HPLC. There is no established technique for the stability-indicating simultaneous measurement of Bilastine and Montelukast by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Bilastine and Montelukast in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Bilastine and Montelukast. [15-23]

MATERIALS AND REAGENTS

Spectrum Pharma Research Solutions in Hyderabad sent us pure Bexagliflozin drugs. Bexagliflozin (Brenzavvy), a mixture drug, was bought at a nearby pharmacy. All of the

materials and buffers used in this method came from Rankem in India. These included acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid, distilled water, and phosphate buffer.

Instrumentation and Chromatographic Conditions For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50.

Preparation of buffer: 0.01N Ammonium Formate Buffer 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 then PH adjusted to 4.8 with dil.

Preparation of Standard stock solutions: Accurately weighed 20 mg of Bexagliflozin, transferred to 50ml volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (400µg/ml of Bexagliflozin)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Bexagliflozin)

Preparation of Sample stock solutions: 10 Injection vial were taken was transferred into a 100 ml 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 (400 µg/ml of Bexagliflozin).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of Bexagliflozin)

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation Bexagliflozin drug material in accordance with the ICH criteria.

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.

Linearity: stock solutions of Bexagliflozin is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: That is sometimes term of trueness. The Accuracy should be established across the specified range of the analytical procedure.

Preparation of Standard stock solutions: Accurately weighed 20mg of Bexagliflozin is transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Bexagliflozin)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.

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 Guide lines. Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.3ml of Bexagliflozin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml of Bexagliflozin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Bexagliflozin (40ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Degradation studies:

Oxidation: To 1 ml of stock solution of Bexagliflozin 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 (40ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of s tock s solution Bexagliflozin 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c .The resultant solution was diluted to obtain (40ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.7

Alkali Degradation Studies: To 1 ml of stock solution Bexagliflozin 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain (40ppm) solution and 10 µl 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 1050c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (40ppm) solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the (400ppm) 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 (40ppm) solutions and 10 µl 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 60ºc. For HPLC study, the resultant solution was diluted to (40ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS:

Table 1: System suitability table

S no	Bexagliflozin		
Injection	**RT(min)**	**USP plate count**	**Tailing**
1	2.840	7663	1.40
2	2.841	7233	1.39
3	2.842	7310	1.42
4	2.842	7713	1.41
5	2.851	8799	1.34
6	2.853	7731	1.35

Table 2: Specificity data table

Sample name	Retention time(Mins)	Area
Bexagliflozin	2.854	662867

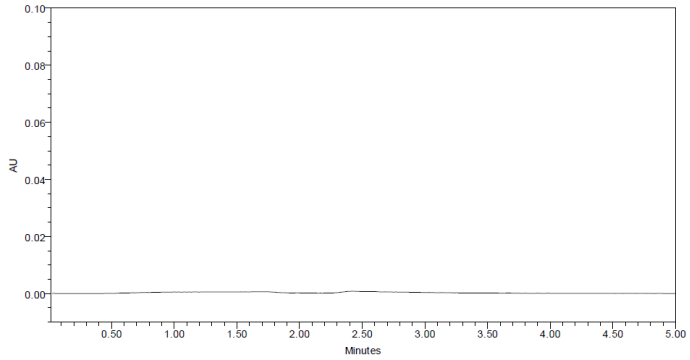

Figure 2. Blank Chromatogram

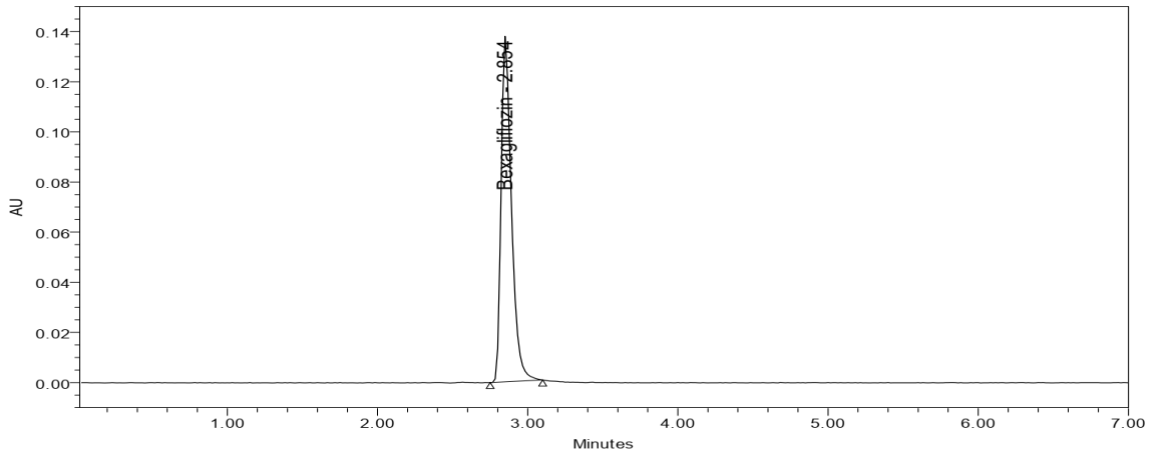

Figure 3. Specificity Chromatograms of Bexagliflozin

Linearity

Table 3. Linearity table for Bexagliflozin:

Bexagliflozin		
Linearity level (%)	**Conc (µg/mL)**	**Peak area**
0	0	0
25	10	213193
50	20	434153
75	30	653274
100	40	851303
125	50	1072654
150	60	1275170

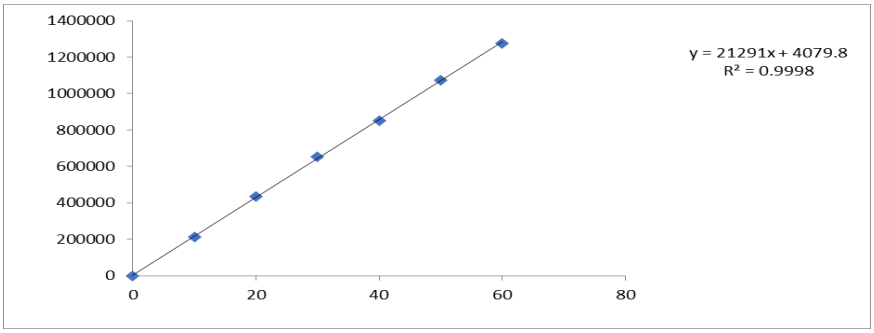

Figure 4. Bexagliflozin calibration Curve

Accuracy:

Table 4. Accuracy table of Bexagliflozin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.68	98.40	
	20	19.85	99.23	
	20	20.13	100.63	
100%	40	40.12	100.30	
	40	39.92	99.79	99.63%
	40	40.02	100.05	
150%	60	59.53	99.22	
	60	59.74	99.57	
	60	59.67	99.45	

System Precision: With regard to the working strength of Bexagliflozin, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 5: System precision

S. No	Area of Bexagliflozin
1.	582538
2.	580044
3.	584275
4.	579185
5.	571435
6.	574019
Mean	**578583**
S.D	**4948.9**
%RSD	**0.9**

The % RSD for the peak areas of Bexagliflozin obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Bexagliflozin allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 6. Method precision

S.no	Bexagliflozin
1	574649
2	578184
3	574593
4	573752
5	579876
6	575228
Avg	**576047**
Std dev	**2418.0**
%RSD	**0.4**

Results shows, the % RSD of Repeatability study was within the range for **Bexagliflozin** is (<2%).

Table 7: Robustness

S.No.	Condition	%RSD of Bexagliflozin.
1	Flow rate (-) 0.9ml/min	0.5
2	Flow rate (+) 1.1ml/min	0.7
3	Mobile phase (-) 60B:40A	0.6
4	Mobile phase (+) 70B:30A	1.0.
5	Temperature (-) 25°C	0.7
6	Temperature (+) 35°C	0.7

Table 8. Forced degradation for Bexagliflozin

Stress condition	Solvent	Temp (0C)	Exposed time
Acid	2N HCL	600c	30 mins
Base	2N NAOH	600c	30 mins
Oxidation	20% H2O2	600c	30 mins
Thermal	Diluent	1050c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	600c	

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 9. Degradation results of Bexagliflozin

Type of degradation	Bexagliflozin	
	% RECOVERED	% DEGRADED
Acid	95.57	4.43
Base	94.12	5.88
Peroxide	92.09	7.91
Thermal	96.30	3.70
Uv	97.38	2.62
Water	97.44	2.56

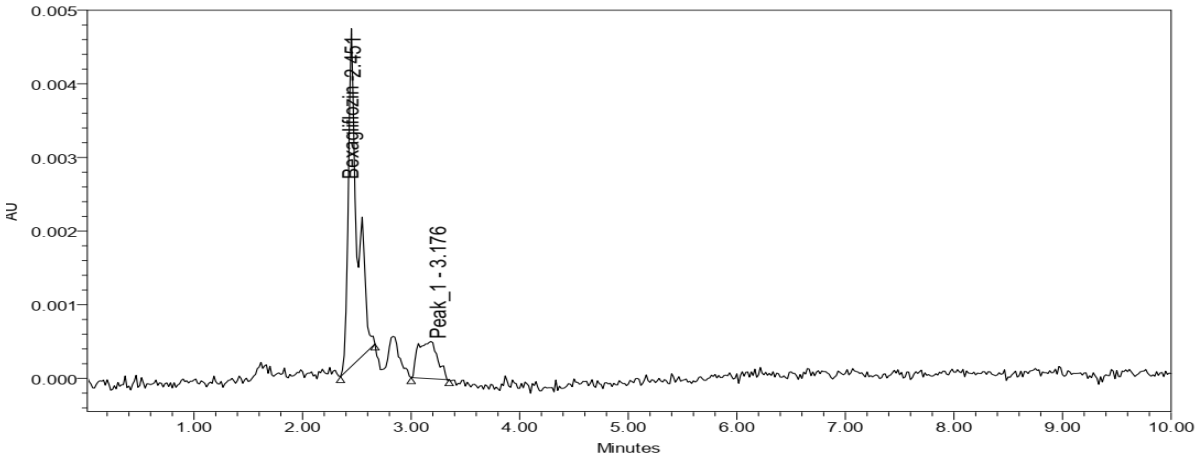

Figure 5. Acid chromatogram of Bexagliflozin

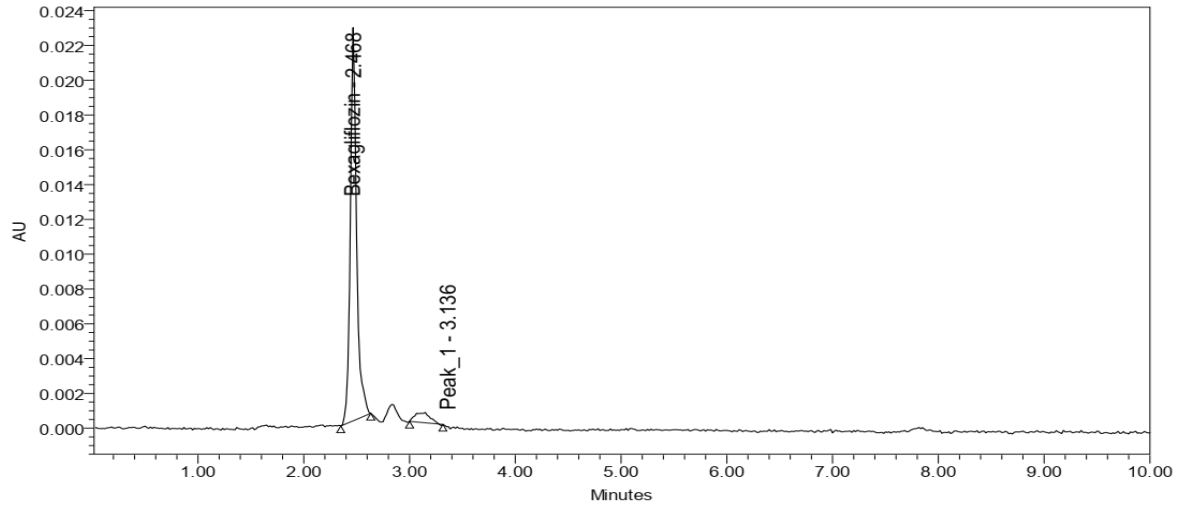

Figure 6. Base chromatogram of Bexagliflozin

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: Bexagliflozin bearing label claim **Brenzavvy**, 20mg , assay was carried out by injecting sample into HPLC System.

Table 10. Assay data of Bexagliflozin

S.No.	Standard Area	Sample area	% Assay
1	582538	574649	99.22
2	580044	578184	99.83
3	584275	574593	99.21
4	579185	573752	99.07
5	571435	579876	100.12
6	574019	575228	99.32
Avg	**578583**	**576047**	**99.46**
Stdev	**4948.9**	**2418.0**	**0.4175**
% RSD	**0.9**	**0.4**	**0.42**

Table 11: Assay outcome for Bexagliflozin

Drug Name	Label claim dose	%Assay	Brand Name
Bexagliflozin	20mg	99.46	Brenzavvy

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Bexagliflozin in tablet dosage form. Easy, sensitive, specific, and precise RP-HPLC method for Bexagliflozin API and pharmaceutical dosage form determination. Bexagliflozin retained 2.854 min. Bexagliflozin had 0.9 %RSD and 0.4 %method precision. Recovery for Bexagliflozin was 99.63%. From Bexagliflozin regression equation, LOD and LOQ were 0.440, 1.33. Bexagliflozin regression equation: y = 21291x +4079.8. The method developed was simple and economical for regular quality control tests in industries because retention and run times were reduced.

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