World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

RP HPLC METHOD FOR DETERMINATION OF DASIGLUCAGON IN PHARMACEUTICAL DOSAGE FORM

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Received: 05-09-2023 / Revised Accepted: 13-09-2023 / Published: 20-09-2023

ABSTRACT

A simple, precise, accurate sensitive and specific RP-HPLC method for the determination of Dasiglucagon in pharmaceutical dosage form. Chromatogram was run through Kromosil 150 Acetonitrile: Orthophosphoric acid taken in the ratio 70:30 was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 270.0nm. Retention time of Dasiglucagon was found to 2.096min. %RSD of the Dasiglucagon were and found to be 0.3%. %RSD of Method precision of Dasiglucagon was found to be 0.6%. %Recovery was obtained as 99.59% for Dasiglucagon. LOD, LOQ values obtained from regression equation of Dasiglucagon were 0.04, 0.11, Regression equation of Dasiglucagon is y = 135481x + 6696.7 Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: HPLC, Dasiglucagon, Method development. ICH Guidelines.

INTRODUCTION

Hypoglycemia is a condition in which your blood sugar (glucose) level is lower than the standard range. Glucose is your body's main energy source. Hypoglycemia is often related to diabetes treatment. But other drugs and a variety of conditions many rare can cause low blood sugar in people who don't have diabetes. Hypoglycemia needs immediate treatment. For many people, a fasting blood sugar of 70 milligrams per deciliter (mg/dL), or 3.9 millimoles per liter (mmol/L), or below should serve as an alert for hypoglycemia. But your numbers might be different.[1] Dasiglucagon is a glucagon analog used to treat severe hypoglycemia in pediatric and adult patients with diabetes. Dasiglucagon is a glucagon analog that acts to increase blood sugar levels.[2] It consists of 29 amino acids similar to endogenous glucagon;

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How to Cite this Article: Dr. K. Swathi Priya, V.Purna, Dr. K. Atchuta Kumar. Rp Hplc Method For Determination Of Dasiglucagon In Pharmaceutical Dosage Form. World J Pharm Sci 2023; 11(01): 35-47; https://doi.org/10.54037/WJPS.2022.100905

however, it contains seven substituted amino acids for improved physical and chemical stability in its drug formulation.[3] In March 2021, the FDA approved dasiglucagon to treat severe hypoglycemia in patients six years and older with diabetes. It is available as a subcutaneous injection marketed as ZEGALOGUE.[2]

Severe hypoglycemia is an acute, life-threatening medical condition resulting from a profound drop in blood glucose levels. It is characterized by neurological impairment, with manifestations like loss of consciousness and seizure. Hypoglycemia is a common side effect of antidiabetic treatments, most notably insulin and sulfonylureas. Although it tends to be more common in type 1 diabetes mellitus, occurring in about 22% to 46% of patients annually, about 7% to 25% of patients with type 2 diabetes mellitus treated with insulin experience severe hypoglycemia a year.[3] Even with close monitoring of blood glucose levels, it is not always possible to prevent severe hypoglycemic events in patients with diabetes, and children are particularly at risk for experiencing severe hypoglycemia.[4] Treatments for severe hypoglycemia have mostly been limited to intravenous dextrose and different glucagon formulations.[3] The approval of dasiglucagon marks the first glucagon analog approved for severe hypoglycemia treatment that does not require administration by a healthcare professional.[3]

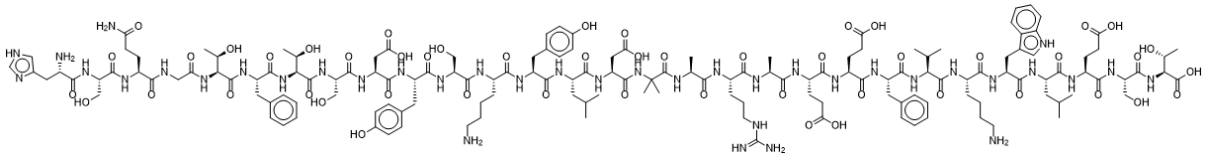

Figure 1. Structure of Dasiglucagon

Preclinical studies and noncontrolled trials suggest that intravenous administration of supraphysiologic concentrations of glucagon can increase heart rate, blood pressure, and heart contractility.[5,6,7] These positive chronotropic and inotropic effects form the basis for clinical use of glucagon to treat overdoses induced by cardioinhibitory drugs such as beta blockers and calcium channel blockers.[7] Elevated levels of glucagon may also be associated with chronic tachycardia, which, in addition to recurrent hypoglycemia, is a risk factor for cardiovascular morbidity.[8,9,10] Therefore, it is important to understand any potential chronotropic effects of novel pharmacologic agents that regulate glucose metabolism and blood glucose levels.

Generic Name Dasiglucagon, sold under the brand name Zegalogue.

A study of the literature says that there are different ways to measure these medicines at the same time, as well as ways to measure them separately or in combination with other medicines. Using RP-HPLC and UV-Spectrophotometry A review of the literature shows that there isn't a standard way to measure Dasiglucagon RP-HPLC simultaneously while showing stability in pharmacy dosage form. The main goal of this work is to come up with an RP-HPLC method that is quick, easy, and accurate for figuring out the amount and type of Dasiglucagon medicines. As suggested by the ICH, a tried-and-true method was also used to guess how much Trilaciclib was present. [11-16]

MATERIALS AND REAGENTS

Spectrum Pharma Research Solutions in Hyderabad sent us pure Dasiglucagon, drugs. Dasiglucagon (Zegalogue), 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:

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of Standard stock solutions: Accurately weighed 3mg of Dasiglucagon 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. (60µg/ml of Dasiglucagon)

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. (6µg/ml of Dasiglucagon).

Preparation of Sample stock solutions: Pipette out 0.6ml of Dasiglucagon injection sample from autosampller vial into a 10 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. (60µg/ml of Dasiglucagon)

Preparation of Sample working solutions (100% solution): of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (6µg/ml of Dasiglucagon)

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation Dasiglucagon 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 Dasiglucagon 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 Sample stock solutions: Pipette out 0.6ml of Dasiglucagon injection sample from auto sampler vial into a 10 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. (60µg/ml of Dasiglucagon)

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 (0.9ml/min), Flow plus (1.1ml/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 each of Dasiglucagon, 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 0.9asml each of Dasiglucagon, 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 solution of Dasiglucagon (6ppm) and the solution 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 Dasiglucagon, 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 6µg/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 Dasiglucagon, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. For HPLC study, the resultant solution was diluted to obtain 6µg/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 Dasiglucagon, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. For HPLC study, the resultant solution was diluted to obtain 6µg/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 6µg/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 120µg/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 6µg/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 6µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No.	Dasiglucagon		
Injection	**RT (min)**	**USP Plate Count**	**Tailing**
1	2.049	6211	1.33
2	2.051	6433	1.40
3	2.051	6495	1.39
4	2.052	6489	1.39
5	2.056	6173	1.38
6	2.059	5713	1.39

Table 2. Specificity data

Sample name	retention time(Mins)	Area
Dasiglucagon	2.096	816212

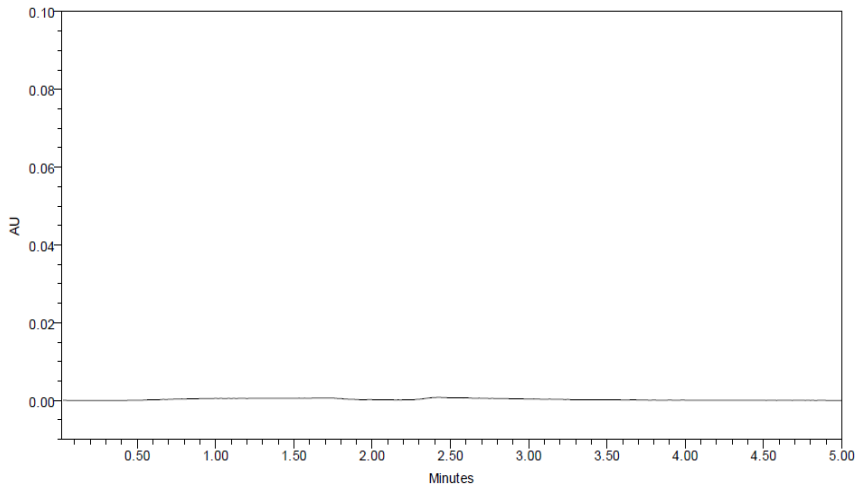

Figure 2.Blank Chromatogram

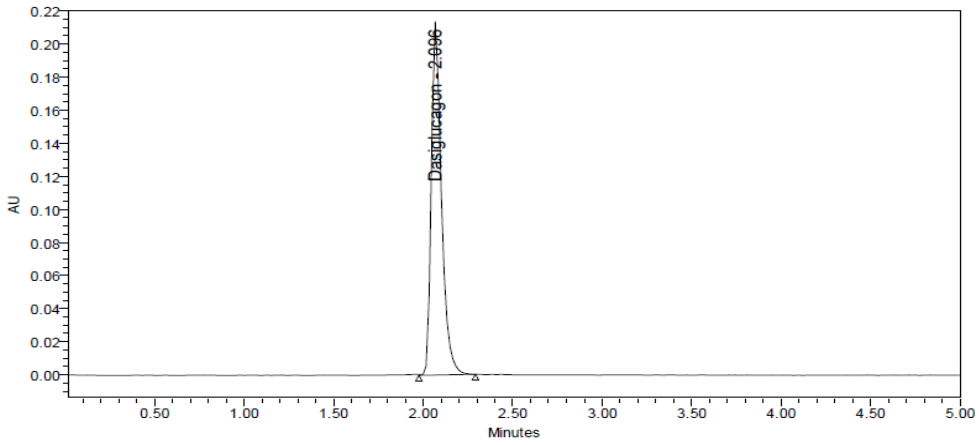

Figure 3. Specificity Chromatograms of Dasiglucagon

Linearity:

Table 3. Linearity table for Dasiglucagon:

Dasiglucagon		
Linearity Level (%)	**Concentration (ppm)**	**Area**
0	0	0
25	1.5	209565
50	3	404297
75	4.5	628758
100	6	840993
125	7.5	1020092
150	9	1210812

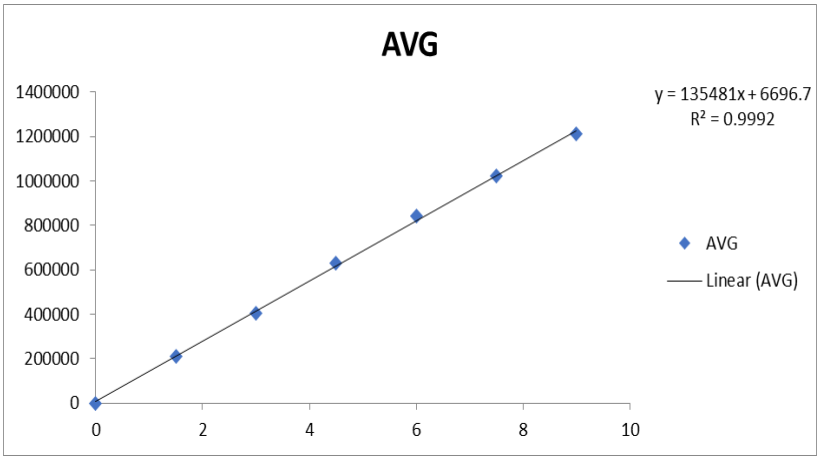

Figure 4. Dasiglucagon calibration Curve

Accuracy:

Table 4. Accuracy table of Dasiglucagon

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	3	3.002	100.07	99.59%
	3	2.975	99.17	
	3	2.985	99.49	
100%	6	5.966	99.43	
	6	6.035	100.58	
	6	5.965	99.41	
150%	9	8.920	99.11	
	9	8.912	99.03	
	9	9.006	100.07	

System Precision: With regard to the working strength of Dasiglucagon 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 Dasiglucagon
1.	835436
2.	836478
3.	837148
4.	840029
5.	838939
6.	843192
Mean	**838537**
S.D	**2822.8**
%RSD	**0.3**

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

Method precision: Analyzing a sample of Dasiglucagon 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	Dasiglucagon
1	845939
2	837480
3	836759
4	833120
5	836338
6	832067
Avg	**836951**
Std dev	**4899.1**
%RSD	**0.6**

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

Table 7: Robustness

S.No.	Condition	%RSD of Dasiglucagon.
1	Flow rate (-) 0.9ml/min	0.3
2	Flow rate (+) 1.1ml/min	0.7
3	Mobile phase (-) 60B:40A	0.4
4	Mobile phase (+) 70B:30A	0.9
5	Temperature (-) 25°C	0.9
6	Temperature (+) 35°C	0.4

Table 8: Forced degradation for Dasiglucagon

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

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 Dasiglucagon

Type of degradation	Dasiglucagon	
	% RECOVERED	% DEGRADED
Acid	93.45	6.55
Base	98.99	1.01
Peroxide	93.36	6.64
Thermal	98.42	1.58
Uv	98.58	1.42
Water	99.78	0.22

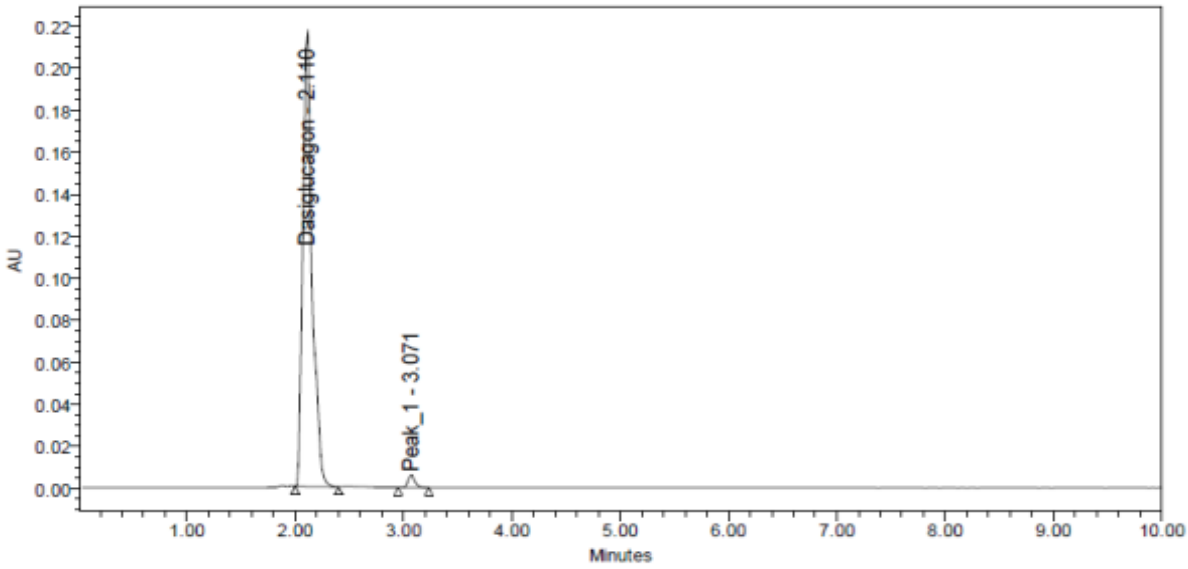

Figure 5. Acid chromatogram of Dasiglucagon

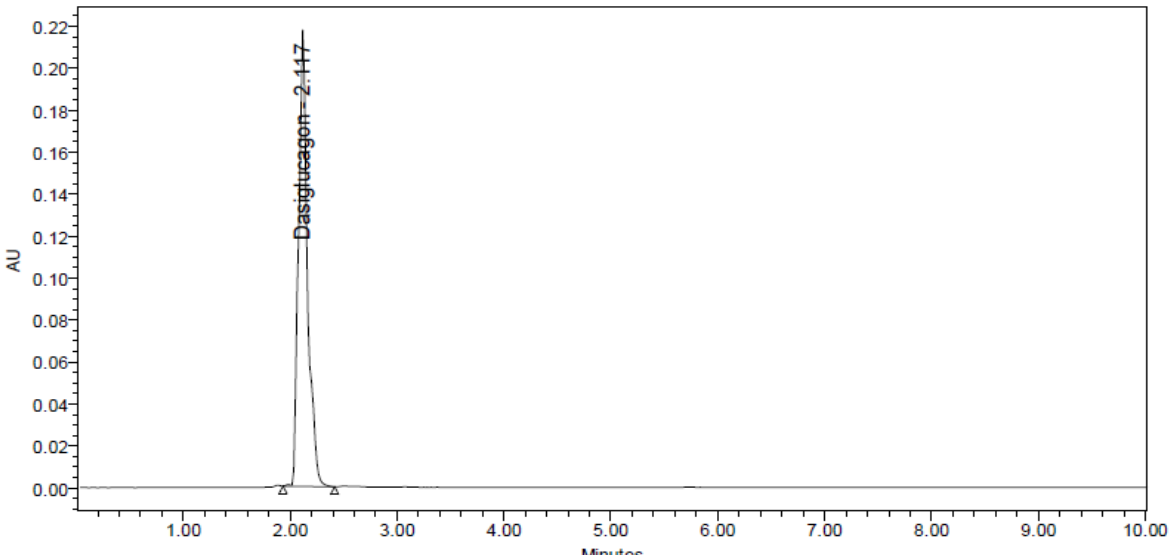

Figure 6. Base chromatogram of Dasiglucagon

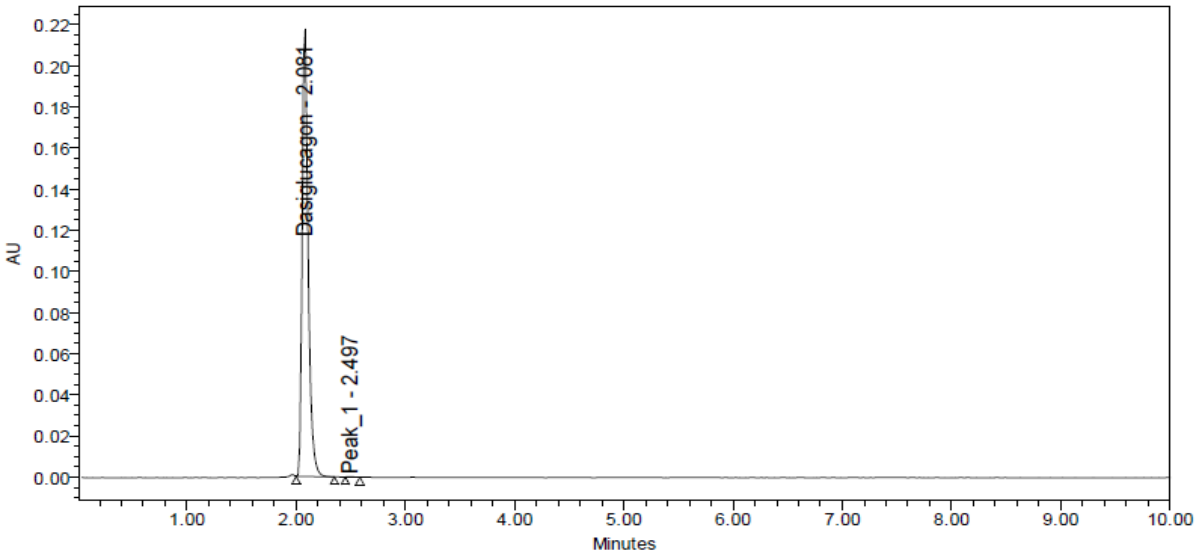

Figure 7. Peroxide chromatogram of Dasiglucagon

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: (Zegalogue**)** bearing label claim, Dasiglucagon 0.6 mg , assay was carried out by injecting sample into HPLC System.

Table 10: Assay data of Dasiglucagon

S.No.	Standard Area	Sample area	% Assay
1	835436	845939	100.6
2	836478	837480	99.6
3	837148	836759	99.5
4	840029	833120	99.1
5	838939	836338	99.4
6	843192	832067	98.9
Avg	**838537**	**836951**	**99.51**
Stdev	**2822.8**	**4899.1**	**0.58**
% RSD	**0.3**	**0.6**	**0.6**

Table 11: Assay outcome for Dasiglucagon

Drug Name	Label claim dose	%Assay	Brand Name
Dasiglucagon	0.6 mg	99.51%	Zegalogue

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Dasiglucagon in tablet dosage form. Chromatographic conditions used are stationary phase Ascentis C18 (150mm*4.6mm2.8m), Mobile phase 0.01N Kh2Po4: Methanol in the ratio of 55:45 and flow rate was maintained at 1.0ml/min, detection wave length was 253nm, column temperature was set to 30oC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R2 value was found to be as 0. 999.Precision was found to be 0.9 for repeatability and 0.7 for intermediate precision.LOD and LOQ are 0.14µg/ml and 0.41µg/ml respectively. By using above method assay of marketed formulation was carried out 100.31% was present. Degradation studies of Dasiglucagon were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Dasiglucagon.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmaceutical Analysis and Quality Assurance, Srinivasa Rao College of Pharmacy, P.M Palem, Affiliated to Andhra University, Visakhapatnam, Andhra Pradesh, India and Spectrum Pharma Research Solution, Hyderabad, Telangana, India, for providing Dsiglucagon as gift samples.

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