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**Research Article**

# STABILITY INDICATING RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF DASIGLUCAGON IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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## ABSTRACT
Targeted RP-HPLC methodology for the quantification of Dasiglucagon in pharmaceutical formulations. The chromatogram was analysed using an Agilent 150, 4.6 mm, 5µm column. Orthophosphoric acid and acetonitrile were combined in an 80:20 ratio and pushed through the column at a flow rate of 1.0 ml/min. The temperature was sustained at 30°C. The chosen optimised wavelength was 271.0 nm. The retention time of Dasiglucagon was determined to be 2.171 minutes. %The relative standard deviation (RSD) of Dasiglucagon was determined to be 0.2%. The method precision RSD for Dasiglucagon was found to be 0.5%.The recovery rate for Dasiglucagon was 99.72%. The LOD and LOQ values derived from the regression equation for Dasiglucagon are 0.03 and 0.09, respectively. The regression equation is expressed as y = 135330x + 20334. The retention durations and run times were reduced, indicating that the new approach is straightforward and cost-effective, suitable for routine quality control testing in industries.
**Key Words:** Dasiglucagon, Method development, Validation, RP-HPLC

## INTRODUCTION[1-10]
Dasiglucagon is a novel, next-generation glucagon analog developed to rapidly treat severe hypoglycemia in patients with diabetes. Hypoglycemia, particularly in individuals with type 1 diabetes (T1D), poses a serious risk of seizures, unconsciousness, and even death if not treated immediately. Traditional glucagon treatments require reconstitution before administration, which can be time-consuming and prone to error in emergency situations. Dasiglucagon, in contrast, is a ready-to-use formulation, offering a fast, simple, and effective solution for managing acute episodes of hypoglycemia.

Dasiglucagon functions by mimicking the effects of endogenous glucagon, a hormone that raises blood glucose levels by stimulating glycogenolysis, the breakdown of glycogen into glucose in the liver. It is administered via subcutaneous injection and has shown to rapidly elevate blood glucose levels within minutes. Its stability in liquid form and ease of administration provide a significant advantage over traditional glucagon, especially in emergency settings.

The approval of dasiglucagon by the U.S. Food and Drug Administration (FDA) in March 2021 under the brand name Zegalogue marked a significant advancement in hypoglycemia management. The efficacy and safety of dasiglucagon were demonstrated in clinical trials involving individuals with type 1 diabetes, showing rapid recovery from insulin-induced hypoglycemia. Compared to older formulations, dasiglucagon's key advantage lies in its ready-to-use format and fast action, which can be crucial in preventing severe complications from hypoglycemia.

Dasiglucagon is also under investigation for use in other therapeutic areas, such as congenital hyperinsulinism, where its ability to raise blood glucose may help manage persistent hypoglycemia caused by excessive insulin

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production in infants and children. Additionally, dasiglucagon is being studied for its potential use in artificial pancreas systems, offering a more complete approach to glucose management for patients with T1D.

Dasiglucagon works by binding to glucagon receptors in the liver, activating pathways that promote glycogen breakdown into glucose. This glucose is then released into the bloodstream, counteracting the effects of insulin and increasing blood glucose levels. The ready-to-use nature of dasiglucagon, which does not require reconstitution, allows for rapid intervention during episodes of severe hypoglycemia.

The advent of dasiglucagon provides a critical option for individuals with diabetes, especially those prone to severe hypoglycemia. Its ready-to-use formulation, rapid glucose-raising effect, and safety profile make it a significant improvement over traditional glucagon therapies. In an emergency setting, where time is of the essence, dasiglucagon's ease of use ensures prompt and effective treatment, potentially reducing the risk of long-term complications associated with severe hypoglycemia.

**Analytical Background**[11]

Dasiglucagon works to increase blood glucose levels under normal and hypoglycemic conditions. After administration of dasiglucagon in adult patients with type 1 diabetes, the mean glucose increase from baseline at 90 minutes was 168 mg/dL. It is chemically known as L-histidyl-L-seryl-L-glutaminyl-glycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-alpha-aspartyl-L-tyrosyl-L-seryl-L-lysyl-L-tyrosyl-L-leucyl-L-alpha-aspartyl-alpha-methyl-alanyl-L-alanyl-L-arginyl-L-alanyl-L-alpha-glutamyl-L-alpha-glutamyl-L-phenylalanyl-L-valyl-L-lysyl-L-tryptophyl-L-leucyl-L-alpha-glutamyl-L-seryl-L-threonine

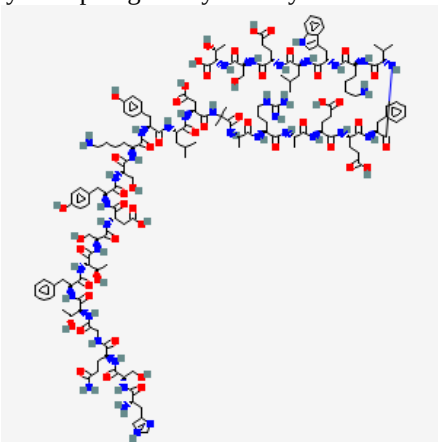

**Figure 1 structure of Dasiglucagon**

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Dasiglucagon, In the review of literature, more economical methods were observed [12-13], hence a simple, cost-effective stability-indicating simultaneous estimation of Dasiglucagon by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

**MATERIALS:**

Dasiglucagon pure drug (API), Dasiglucagon formulation (**Tepmetko**), 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.

**Table 1: Chromatographic Conditions**

| | |
|---|---|
| **Mobile phase** | 0.1% OPA: MeCN (80:20 v/v) |
| **Flow rate** | 1.0 ml/min |
| **Column** | Agilent C18 (4.6 x 150mm, 5µm) |
| **wave length** | 271 nm |
| **Column temperature** | 30°C |
| **Injection volume** | 10µL |
| **Run time** | 10.0 min |

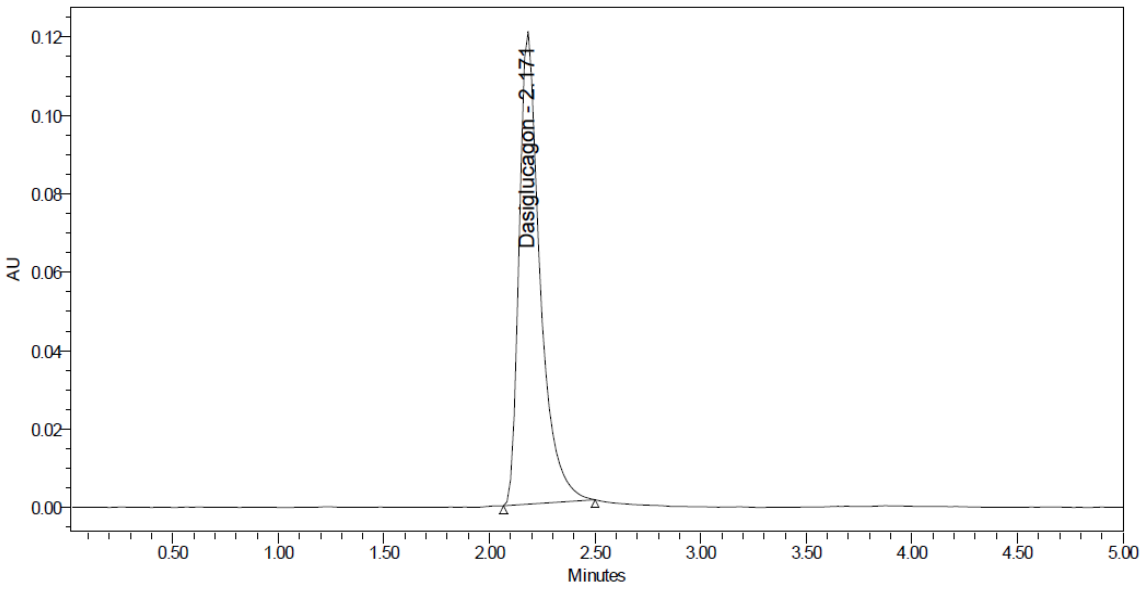


**Figure 2: Optimized Chromatogram**

**Methods:**

**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 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):** 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (6µg/ml of Dasiglucagon)

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solution of Dasiglucagon (6 ppm) 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 (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 3 and experimental data is given in Table 2

**Table: 2 System suitability parameters for Dasiglucagon**

| S no | Dasiglucagon | | |
|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing |
| 1 | 2.179 | 2524 | 1.49 |
| 2 | 2.180 | 2609 | 1.52 |
| 3 | 2.181 | 2617 | 1.50 |
| 4 | 2.186 | 2734 | 1.53 |
| 5 | 2.186 | 2744 | 1.53 |
| 6 | 2.186 | 2724 | 1.51 |

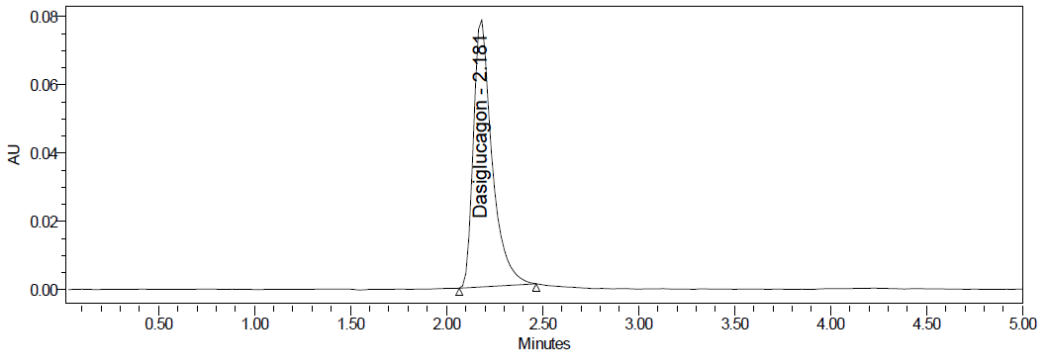


**Figure 3: System Suitability Chromatogram of Dasiglucagon**

**Table 3: Specificity Data**

| Peak name | Rt | Area | USP plate count | Tailing |
|---|---|---|---|---|
| **Dasiglucagon** | 2.171 | 850101 | 2555.6 | 1.3 |

**Specificity:**

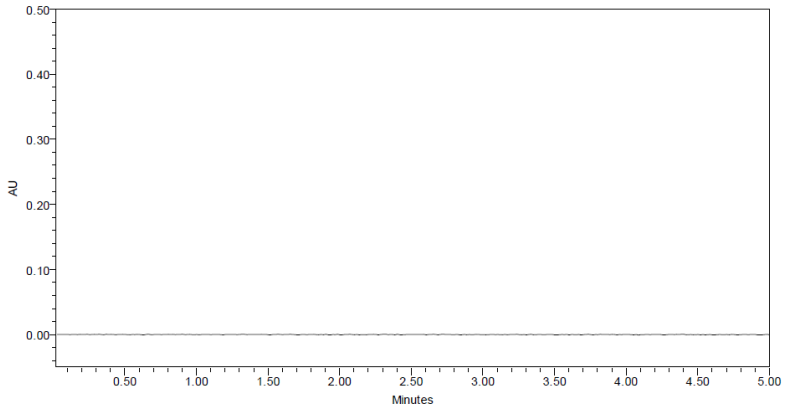


**Figure 4 Chromatogram of blank.**

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

**Table 4: Forced degradation conditions for Dasiglucagon**

| 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 Condition | % Drug Undegraded | % Drug Degraded |
|---|---|---|
| **Acid** | 95.11 | 4.89 |
| **Base** | 94.42 | 5.58 |
| **Oxidation** | 96.08 | 3.92 |
| **Thermal** | 97.78 | 2.22 |
| **Photolytic** | 98.3 | 1.67 |
| **Hydrolytic** | 99.3 | 0.74 |

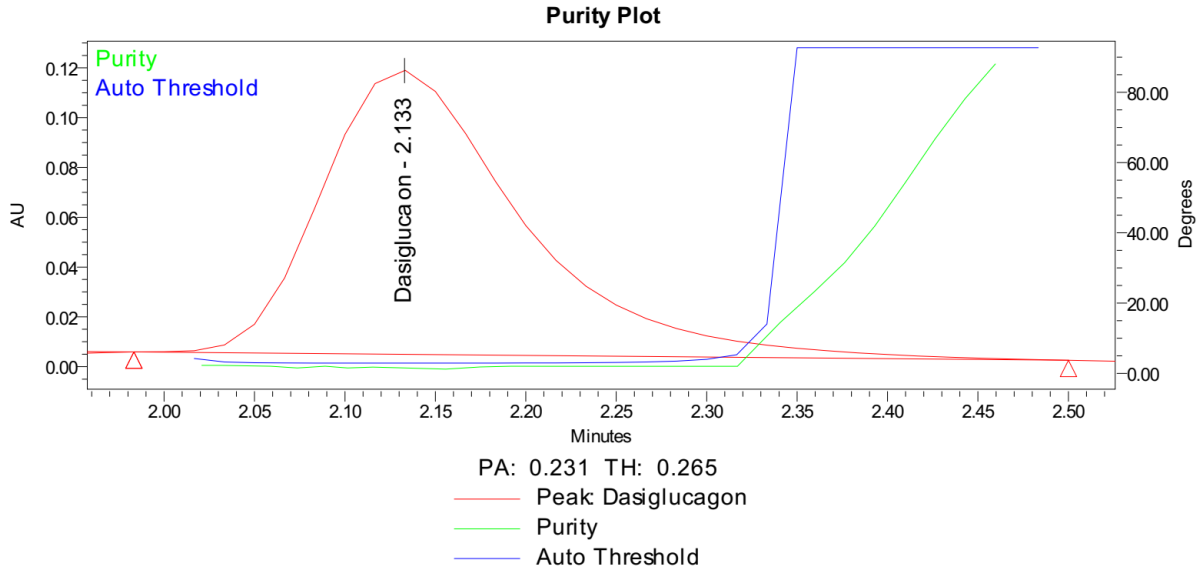


**Figure 5: Purity Plot of Acid**

**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 Dasiglucagon are listed in Table 6.

**Table 6: Summary of limit of detection**

| Sample | Conc (µg/ml) |
|---|---|
| LOD | 0.03 |
| LOQ | 0.09 |

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

**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 Dasiglucagon**

| Dasiglucagon | |
|---|---|
| **Conc (µg/mL)** | **Peak area** |
| 0 | 0 |
| 1.5 | 219105 |
| 3 | 421821 |
| 4.5 | 631501 |
| 6 | 849382 |
| 7.5 | 1033769 |
| 9 | 1229328 |

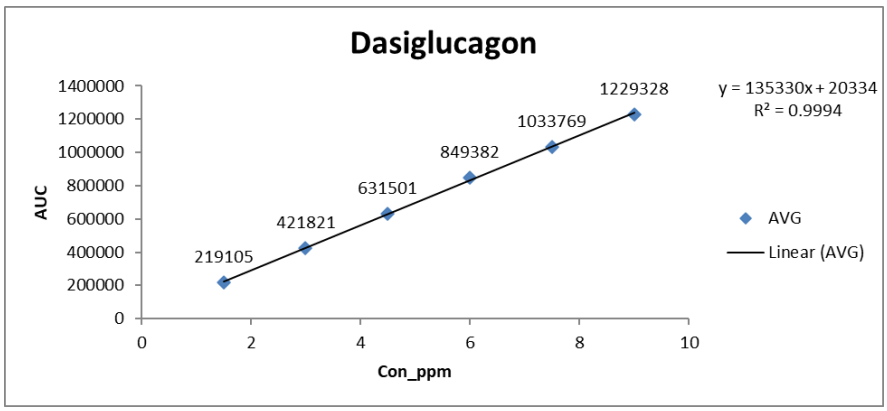


**Figure 6: Calibration curve of Dasiglucagon**

**Table 8: regression data**

| **Parameter** | Dasiglucagon |
|---|---|
| **Conc range (µg/mL)** | 1.5-9 µg/ml |
| **Regression Equation** | y = 135330x + 20334 |
| **Co-relation** | 0.999 |

**Accuracy:** The accuracy of the method was determined by using solutions containing spiked samples of Dasiglucagon 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 Dasiglucagon**

| **% Level** | **Amount Spiked (µg/mL)** | **Amount recovered (µg/mL)** | **% recovery** |
|---|---|---|---|
| **50%** | 3 | 2.964 | 98.81 |
| | 3 | 2.972 | 99.07 |
| | 3 | 2.986 | 99.55 |
| **100%** | 6 | 6.058 | 100.96 |
| | 6 | 5.994 | 99.90 |
| | 6 | 5.987 | 99.78 |
| **150%** | 9 | 8.981 | 99.78 |
| | 9 | 8.981 | 99.79 |
| | 9 | 8.989 | 99.88 |
| **Mean % recovery** | | | **99.72** |

**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 Dasiglucagon. Results of peak area are summarized in Table 10

**Table 10 System precision table of Dasiglucagon**

| **S. No** | **Area of Dasiglucagon** |
|---|---|
| 1. | 847219 |
| 2. | 849289 |
| 3. | 846670 |
| 4. | 845629 |
| 5. | 849366 |
| 6. | 845509 |
| **Mean** | **847280** |
| **S.D** | **1710.3** |
| **%RSD** | **0.2** |

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

**Table 11 Repeatability table of Dasiglucagon**

| S. No | Area of Dasiglucagon |
|---|---|
| 1. | 845935 |
| 2. | 849486 |
| 3. | 850904 |
| 4. | 840699 |
| 5. | 842435 |
| 6. | 845833 |
| **Mean** | **845882** |
| **S.D** | **3923.3** |
| **%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 Dasiglucagon**

| S. No | Area of Dasiglucagon |
|---|---|
| 1. | 844648 |
| 2. | 846929 |
| 3. | 843642 |
| 4. | 845783 |
| 5. | 846012 |
| 6. | 844302 |
| **Mean** | **845219** |
| **S.D** | **1226.8** |
| **%RSD** | **0.1** |

**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 Dasiglucagon**

| Condition | %RSD of Dasiglucagon |
|---|---|
| Flow rate (-) 0.9ml/min | 0.2 |
| Flow rate (+) 1.1ml/min | 0.5 |
| Mobile phase (-) 75B:25A | 0.2 |
| Mobile phase (+) 85B:15A | 0.4 |
| Temperature (-) 27°C | 0.3 |
| Temperature (+) 33°C | 0.5 |

**Assay data: -**

Tepmetko Tablet bearing the label claims Dasiglucagon 300 mg. Assay was performed with the above formulation. Average % Assay for Dasiglucagon obtained was 99.54%. Assay data shown in table no 14.


**Figure 7: Dasiglucagon Marketed 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 | Average Peak area of Carboprost in test solution |
| AS | Mean peak area of Carboprost in standard solution |
| WS | Weight of Carboprost working standard taken in mg |
| P | Assay of Carboprost working standard in % on dried basis |
| L.C | Label Claim |
| FV | Filled volume(1ml of a vail) |

**Table 14: Assay Data of Dasiglucagon**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 847219 | 845935 | 99.5 |
| 2 | 849289 | 849486 | 100.0 |
| 3 | 846670 | 850904 | 100.1 |
| 4 | 845629 | 840699 | 98.9 |
| 5 | 849366 | 842435 | 99.1 |
| 6 | 845509 | 845833 | 99.5 |
| **Avg** | **847280** | **845882** | **99.54** |
| **Stdev** | **1710.3** | **3923.3** | **0.46** |
| **%RSD** | **0.2** | **0.5** | **0.5** |

## CONCLUSION

The outcomes of the Dasiglucagon HPLC investigation indicate that this approach can precisely measure the concentration and purity of the medication. This method is ideal for pharmacokinetic studies and routine quality control due to its capacity for repeated usage with sharp peak resolutions and consistent retention times. Ensuring Dasiglucagon efficacy and safety for medical use, along with verifying its chemical makeup, relies on HPLC analysis.

## ACKNOWLEDGEMENT:

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## REFERENCES

1. Ilag, L. L., Lankisch, M., Yamamura, A., et al. (2021). Dasiglucagon: A novel glucagon analog for the treatment of severe hypoglycemia in diabetes. Diabetes Technology & Therapeutics, 23(5), 304-309. https://doi.org/10.1089/dia.2020.0545
2. Rickels, M. R., Ruedy, K. J., Foster, N. C., et al. (2021). Dasiglucagon for rapid treatment of insulin-induced hypoglycemia in patients with type 1 diabetes: Results from a randomized, double-blind trial. The Lancet Diabetes & Endocrinology, 9(7), 450-457. https://doi.org/10.1016/S2213-8587(21)00127-1
3. de Vries, J. H., Jansen, H. J., et al. (2020). Dasiglucagon: Ready-to-use glucagon for severe hypoglycemia in patients with type 1 diabetes. Diabetes, Obesity and Metabolism, 22(10), 1731-1740. https://doi.org/10.1111/dom.14122
4. Pieber, T. R., Aronson, R., Hövelmann, U., et al. (2021). Dasiglucagon for the treatment of severe hypoglycemia: A new and rapid-acting glucagon analog. Journal of Clinical Endocrinology & Metabolism, 106(9), e3524-e3532. https://doi.org/10.1210/clinem/dgab292
5. Sherr, J. L., Tauschmann, M., Battelino, T., et al. (2021). The use of dasiglucagon in dual-hormone artificial pancreas systems: A review of potential applications. Diabetes Care, 44(Suppl 2), S225-S230. https://doi.org/10.2337/dc21-s004
6. Hövelmann, U., Brøndsted, L., Kristensen, P. L., et al. (2019). Safety and efficacy of dasiglucagon in pediatric patients with congenital hyperinsulinism: A clinical trial. Pediatric Diabetes, 20(8), 1008-1014. https://doi.org/10.1111/pedi.12932
7. Karlstad, Ø., Jørgensen, M. E., & Hovstadius, B. (2022). The role of dasiglucagon in preventing severe hypoglycemia and its use in combination with insulin. Endocrine Practice, 28(4), 398-405. https://doi.org/10.1016/j.eprac.2022.01.021

8. Einarson, T. R., Acs, A., Ludwig, C., et al. (2020). Economic evaluation of dasiglucagon for the treatment of severe hypoglycemia. PharmacoEconomics, 38(10), 1033-1045. https://doi.org/10.1007/s40273-020-00937-5
9. Franklin, B., & Couper, J. (2021). Dasiglucagon in the management of hypoglycemia in diabetes: A review. Expert Review of Endocrinology & Metabolism, 16(3), 183-190. https://doi.org/10.1080/17446651.2021.1925036
10. Grunberger, G., Handelsman, Y., Bloomgarden, Z. T., et al. (2021). Safety and efficacy of dasiglucagon: A perspective on the management of diabetes emergencies. Clinical Diabetes, 39(4), 344-350. https://doi.org/10.2337/cd21-0042
11. https://pubchem.ncbi.nlm.nih.gov/compound/Dasiglucagon
12. Dr. K. Swathi Priya, RP HPLC Method for Determination of Dasiglucagon In Pharmaceutical Dosage Form. World Journal of Pharmaceutical Sciences, 11(01), 2024.
13. Durgam Harika, Ramya Kuber Banoth, Sri Padmavati, Mahila Visvavidyalayam, Development And Validation Of Stability Indicating Rp-Hplc Method For The Determination Of Dasiglucagon In Bulk And Pharmaceutical Formulation, Journal of Xidian University 16(1, 2):692-709.