SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **M/s Aum Research Labs Pvt Ltd.** and **Swarrnim Startup and Innovation University**, Gandhinagar, Gujarat.
In consideration of the mutual promises and covenants contained herein, the receipt and sufficiency of which are hereby acknowledged, the parties agree to the following terms:

SERVICES

"The Provider agrees to develop and validate low cost and efficient analytical methods for the APIs and formulations for Aum Research Labs Pvt Ltd.."

TERMS AND CONDITIONS

This Contract shall commence on **2nd April 2019** and will remain in effect for five years. The contract may be extended for an additional period as mutually agreed by both parties. Should either party wish to terminate the contract, a written notice must be provided 90 days prior to the intended termination date.

PAYMENT

For each completed service, the Provider will submit an invoice as services are delivered. The Client agrees to make payment upon receipt of the invoice, after deducting any applicable TDS (Tax Deducted at Source).

KARTIK VAKANI

For **Aum Research Labs Pvt Ltd.**

For Swarrnim Startup and Innovation University

Aum Research Labs

FAST • RELIABLE • ACCURATE

Date: 3.5.2019

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

We are delighted to share the news with you that the consultancy project for which we were exchanging ideas in our earlier meetings has been permitted. The project will proceed as follows:

Project Title:

"Development and Validation of Stability- Indicating Rp-Hplc Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets"

Project Timeline:

The project is expected to be completed within the next 4 to 5 months.

Payment:

A total amount payable after successful completion of Project will be Rs. 5,00,000 plus GST will be made after raising invoice of the same after due TDS.

Should you require any further clarification regarding the project, please feel free to reach out to us. We are excited about this collaboration and look forward to a positive working experience with **Swarrnim Startup and Innovation University**.

Best Regards,

KARTIK VIKANI

For Aum Research Labs Pvt. Ltd.

Aum Research Labs Pvt. Ltd.
Plot No. 555/1, 5A, Opp. Fresh & Fresh Bakery, Behind Gulab Oil, Rakanpur-382721, Ta. Kalol, Dist. Gandhinagar, Gujarat, India
+91 97274 68107 | +91 97129 68107 info@aumresearchlabs.com www.aumresearchlabs.com

Ref.No.swarrnim/RO/SCR/2019/46 Date: 13.09.2019

To,

Aum Research Lab Pvt. Ltd.

Kalol, Gandhinagar

Gujarat.

Subject: - Submission of completion report regarding your shared problem.

Dear Sir/Madam

Please find enclosed herewith all data related to the problem shared by your prestigious company the details are as follows:

Project title: **"Development And Validation of Stability- Indicating Rp-Hplc Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets."**

Date of assigning problem: 03.05.2019

Date of completion: 10.09.2019

Name of person: Ms. Richa Raval

We are thankful for providing the opportunity to support you and the profession. We will always ready to solve such problems with our best effort.

For any technical support please contact person who has completed the project, the name is Ms. Richa Raval.

Thanking you.

Wpatel

Registrar

Swarrnim Startup & Innovation University Bhoyan Rathod Gandhinagar

Development And Validation of Stability- Indicating Rp-Hplc Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets.

Research Project Report Submission to

AUM Research Labs

Submitted by:

- **Principal Investigator:** Ms. Richa Raval, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

SWARRNIM SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar

INDEX

Sr. No	Content
1	Introduction
2.	Literature Review
3.	Objectives
4.	Methodology
5.	Result and Discussion
6.	Financial statement
7.	Conclusion
8.	References

- **Declaration**

I, Ms. Richa Raval, Principal Investigator of the project titled " Development And Validation of Stability- Indicating Rp-Hplc Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets". Certified that the project work has been carried out as per the terms and conditions of the University Grants Commission.

Name:

Principal Investigator: Ms. Richa Raval

Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

I extend my sincere gratitude to the AUM for funding this project. I also thank my institution, colleagues, and students who supported and contributed to the successful completion of this project.

- **Executive Summary**

The article "Development and Validation of Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets" describes the creation and validation of a novel analytical method for the simultaneous quantification of two widely used antidiabetic drugs—Ertugliflozin Pidolate (an SGLT2 inhibitor) and Metformin Hydrochloride (a biguanide)—using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). The primary goal of this study was to develop a stability-indicating method capable of assessing the stability of these drugs under various stress conditions (e.g., heat, light, oxidation) and ensuring accurate measurement of both drugs in their bulk and tablet formulations.

SSIU
BHOYAN RATHOD
Kalol, Gandhinagar
SWARNIM SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR

- **Detailed Report**

1. Introduction:

The development and validation of a stability-indicating RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method for the simultaneous determination of Ertugliflozin Pidolate and Metformin Hydrochloride in bulk and tablet formulations is crucial in pharmaceutical analysis. This project aims to establish a reliable, efficient, and precise analytical method for the determination of these two drugs, ensuring their quality, safety, and efficacy during their shelf life and in various conditions such as storage and handling.

Ertugliflozin Pidolate: Ertugliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor, primarily used in the management of Type 2 Diabetes Mellitus (T2DM). It works by inhibiting the SGLT2 protein in the kidneys, preventing glucose reabsorption into the bloodstream and promoting glucose excretion through urine. Ertugliflozin is commonly administered as the pidolate salt form in combination therapies to help control blood glucose levels in diabetic patients. However, as with any pharmaceutical compound, its stability in various conditions needs to be monitored.

Metformin Hydrochloride: Metformin is one of the first-line oral medications for the treatment of Type 2 Diabetes. It works by decreasing glucose production in the liver and increasing insulin sensitivity. Due to its widespread use and the necessity to ensure proper dosages, it is important to monitor the stability and concentration of Metformin in both bulk drugs and formulations.

Importance of Stability-Indicating Methods: Stability-indicating methods are essential in the pharmaceutical industry for ensuring the quality and potency of drugs during their shelf life. The stability of a drug can be affected by various factors, such as light, temperature, humidity, and oxidation. Therefore, it is crucial to evaluate and validate a method that can accurately quantify the active pharmaceutical ingredients (API) in the presence of degradation products.

Role of RP-HPLC: Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely used for the separation and quantification of complex mixtures, making it an ideal method for determining drugs in a pharmaceutical matrix. In RP-HPLC, the stationary phase is non-polar, while the mobile phase is polar. The separation of compounds occurs due to the differential interaction between the sample and the stationary phase, allowing for precise quantification even in the presence of impurities or degradation products.

Stability-indicating RP-HPLC methods are particularly valuable in pharmaceutical quality control to detect any degradation or impurities that may arise due to storage conditions or manufacturing processes.

This method can be adapted for the simultaneous determination of both Ertugliflozin and Metformin, ensuring that the final pharmaceutical product maintains its integrity.

Validation of Analytical Methods: Validation of the RP-HPLC method ensures that the developed technique is accurate, precise, robust, specific, and linear across a suitable range of concentrations. Validation parameters like precision, accuracy, specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ) must be assessed. The method must also demonstrate the ability to distinguish the active ingredients from any degradation products or other impurities.

Need for Simultaneous Determination: The combination of Ertugliflozin and Metformin in a single tablet formulation is a common treatment option for Type 2 diabetes, offering patients a more convenient treatment regimen. The simultaneous determination of both drugs in a single analytical run improves efficiency and reduces the time and cost associated with individual analyses.

Challenges in Stability Testing:

- Degradation studies are necessary to assess how each drug behaves under stress conditions such as heat, humidity, light, and oxidation.
- The ability to detect and separate degradation products without interference from the active ingredients is a challenge that must be addressed when developing the RP-HPLC method.

2. Literature Review

Month 1:

- **Literature Review**: Conduct a detailed review of existing analytical methods, focusing on RP-HPLC techniques used for the determination of Ertugliflozin Pidolate, Metformin Hydrochloride, and other anti-diabetic drugs.
- **Requisition of Materials**: Procure Ertugliflozin Pidolate, Metformin Hydrochloride,

solvents, buffers, and HPLC columns (e.g., C18).

- **Lab Setup**: Ensure the availability of the HPLC system and related laboratory equipment, such as pH meters, balances, and centrifuges.
- **Study Regulatory Guidelines**: Familiarize with ICH, USP, and other relevant regulatory guidelines for analytical method development and validation.
- **Prepare Project Plan**: Set milestones and define timelines for each stage of the project.

Month 2: Method Development

- **Preparation of Stock Solutions**: Prepare standard solutions of Ertugliflozin Pidolate and Metformin Hydrochloride in appropriate solvents.
- **Optimization of Chromatographic Conditions**:
  - Test different **mobile phases** (combinations of organic solvents and buffers) for optimal separation.
  - Adjust **flow rate**, **column type**, and **temperature** to obtain the best resolution and peak symmetry.
  - Identify the **detection wavelength** based on the absorbance maxima of the two drugs.
- **Initial Testing**: Run test samples and adjust chromatographic parameters to achieve well-separated peaks for both drugs.

Month 3: Forced Degradation and Stability Testing

- **Forced Degradation Studies**: Perform forced degradation studies under various conditions, including:
  - Acidic, basic, oxidative, and thermal stress to generate degradation products.
  - Analyze the stability of Ertugliflozin and Metformin and ensure no overlap between the degradation products and the main peaks.
- **Optimization of Degradation Conditions**: Refine the chromatographic method based on the results of degradation studies, ensuring the method is stability-indicating.
- **Preliminary Data Analysis**: Assess the integrity and purity of the drug peaks by comparing them with degradation products.

Month 4: Method Validation – Linearity, Accuracy, and Precision

- **Validation Testing**:
  - **Linearity**: Prepare a series of standard solutions at different concentrations for both drugs and construct calibration curves to assess linearity.
  - **Accuracy**: Perform recovery studies by spiking known amounts of drugs in tablet formulations or excipient matrices and calculate recovery percentage.
  - **Precision**: Determine intraday and interday precision by conducting multiple

measurements (at least 6) on the same day and across multiple days.

- **Documentation**: Record results and verify that the method meets the criteria for linearity, accuracy, and precision according to regulatory standards.

Month 5: Method Validation – Sensitivity, Robustness, and System Suitability

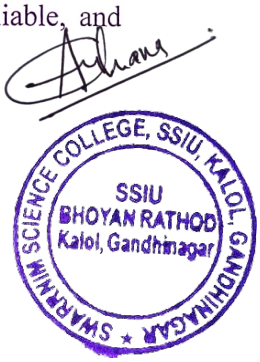
- **Sensitivity Testing**: Determine **Limit of Detection (LOD)** and **Limit of Quantification (LOQ)** for both drugs.
- **Robustness**: Evaluate the method's robustness by making slight variations in chromatographic conditions (e.g., flow rate, temperature, mobile phase composition) and assessing the impact on the results.
- **System Suitability Tests**: Perform tests such as resolution, peak tailing factor, and theoretical plates to ensure the system meets required standards.
- **Final Validation**: Complete all method validation parameters, ensuring the method is accurate, precise, stable, and robust under different conditions.

Month 6: Application to Tablet Formulations and Final Reporting

- **Tablet Sample Analysis**: Analyze the content of Ertugliflozin Pidolate and Metformin Hydrochloride in commercial tablet formulations using the validated method.
- **Data Interpretation**: Calculate the drug content in the tablets and compare results with the labeled amounts to ensure accuracy and consistency.
- **Final Report Compilation**: Summarize the methodology, validation results, forced degradation studies, and tablet analysis data. Ensure all results comply with regulatory guidelines for submission.
- **Presentation**: Prepare a final presentation summarizing the key findings, methodology, and outcomes of the project for stakeholders or regulatory bodies.

3.Objective:

The primary objective of this project is to develop a stability-indicating RP-HPLC method for the simultaneous determination of Ertugliflozin Pidolate and Metformin Hydrochloride in bulk drug substances and in tablet formulations. This method will then be validated following established guidelines (such as ICH guidelines) to ensure that it is accurate, reliable, and suitable for routine quality control testing in pharmaceutical manufacturing



4. Methodology:

Project Setup Activities:

- **Conduct an in-depth review of:**

- Existing HPLC methods for the analysis of Ertugliflozin Pidolate and Metformin Hydrochloride.
- Stability-indicating methods and their applications in pharmaceutical analysis.
- Various chromatographic techniques used for simultaneous determination of multiple drugs in tablet formulations.
- **Identify Equipment and Reagents:**
  - Make sure you have access to an HPLC system, appropriate columns, and solvents.
  - Order necessary reagents such as Ertugliflozin Pidolate, Metformin Hydrochloride, buffers, and mobile phase solvents.
- **Set Up Lab Environment:**
  - Arrange for lab facilities including the HPLC system, balance, pH meter, and other required instruments.
  - Prepare a project plan detailing all the stages, milestones, and deliverables.
- **Familiarization with HPLC:** Understand the operation of the HPLC system and necessary software for chromatogram analysis.

Month 2: Method Development - Optimization of Chromatographic Conditions

Activities:

- **Preparation of Standard Solutions:**
  - Prepare stock solutions for Ertugliflozin Pidolate and Metformin Hydrochloride.
  - Prepare working solutions in various concentrations for method development.
- **Selection of Mobile Phase:**
  - Experiment with different mobile phases (e.g., water, acetonitrile, phosphoric acid, methanol) to optimize the separation of both drugs.
  - Test various pH levels and buffer strengths for optimal peak resolution.
- **Column Selection and Flow Rate Optimization:**
  - Test different columns (e.g., C18, C8) for separation efficiency.
  - Adjust flow rates (e.g., 1.0 mL/min) and column temperatures (e.g., ambient temperature) to achieve optimal resolution.

- **Initial Chromatographic Analysis:**
  - Inject standard solutions of Ertugliflozin and Metformin separately to obtain retention times and identify the optimal conditions for separation.

- Monitor the UV response (typically at 240 nm) and adjust parameters to minimize peak overlap.

Month 3: Method Development - Forced Degradation Studies & Optimization

Activities:

- **Forced Degradation Studies:**

- Perform forced degradation studies under conditions like acid, base, oxidation, heat, and light to evaluate the stability of both drugs.
- Expose the drug solutions to different stress conditions and analyze the samples using the optimized HPLC conditions.
- Identify any degradation products and assess if the method can separate these from the active ingredients.

- **Optimization of Chromatographic Conditions:**

- Fine-tune the mobile phase, pH, and flow rate based on the degradation results to ensure that the method can efficiently separate degradation products from the API peaks.
- Ensure the method is stability-indicating by confirming that degradation products do not interfere with the analysis.

- Data Analysis:

- Evaluate chromatograms to ensure proper separation and resolution of Ertugliflozin and Metformin from degradation products.
- Make adjustments to the method as needed to improve specificity and selectivity.

Month 4: Method Validation - Accuracy, Precision, and Linearity

Activities:

- **Validation Studies:**

- Linearity: Prepare calibration curves over a concentration range (e.g., 10–200 µg/mL) for both drugs. Ensure that the calibration plots are linear (correlation coefficient ≥ 0.999).
- Accuracy: Conduct recovery studies by spiking known amounts of Ertugliflozin and Metformin in tablet matrix samples. Calculate the percentage recovery to assess accuracy.
- Precision:
  - Repeatability: Perform the same analysis six times on the same sample to calculate the relative standard deviation (RSD).
  - Intermediate Precision: Conduct the same tests on different days and by different analysts to assess intra-laboratory variation.

- Specificity: Ensure the method is capable of quantifying Ertugliflozin and Metformin without interference from excipients or degradation products.

- **Documentation:**

- Prepare reports detailing the results of the linearity, accuracy, and precision tests, ensuring compliance with regulatory standards.

Month 5: Method Validation - Sensitivity, Robustness, and Stability

Activities:

- **Sensitivity:**

- Limit of Detection (LOD) and Limit of Quantification (LOQ): Determine the LOD and LOQ using the signal-to-noise ratio (e.g., S/N ≥ 3 for LOD and S/N ≥ 10 for LOQ).

- **Robustness:**

- Assess the robustness of the method by making small deliberate variations in key parameters such as flow rate, pH of the mobile phase, and column temperature. Observe the effect on retention time, peak area, and resolution.

- **System Suitability Testing:**

- Perform system suitability tests like resolution, tailing factor, and theoretical plates to ensure the system is working within the specified parameters.

- **Stability Studies:**

- Analyze the stability of the stock and working solutions of Ertugliflozin and Metformin to ensure no significant degradation occurs over time under storage conditions.

- **Data Analysis:**

- Evaluate the results of sensitivity and robustness tests, ensuring the method is stable and reliable for routine use in pharmaceutical quality control.

Month 6: Application to Tablet Formulations & Final Report Writing

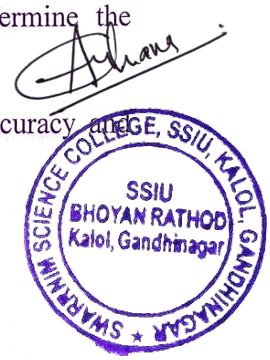
Activities:

- **Application to Tablet Formulation:**

- Prepare tablet sample solutions by accurately weighing and dissolving powdered tablets in suitable solvents.

- Analyze the tablet samples using the validated RP-HPLC method to determine the concentrations of Ertugliflozin Pidolate and Metformin Hydrochloride.

- Compare the tablet results with the theoretical values to evaluate method accuracy and precision in a real-world sample matrix.



- **Final Method Validation Report:**

o Compile all data and results obtained throughout the project into a comprehensive final report.

o The report should include:

- Method development and optimization process.
- Forced degradation study findings.
- Validation parameters (specificity, accuracy, precision, linearity, LOD, LOQ, robustness, etc.).
- Application to tablet formulations.
- Conclusions and recommendations for future work or improvements.

- **Presentation and Submission:**

o Prepare a presentation summarizing key findings from the project (development, validation, and application of the method).

o Submit the final report for review.

Key Deliverables:

- Month 1: Literature review, project setup, and equipment preparation.
- Month 2: Development of chromatographic conditions and optimization of separation.
- Month 3: Forced degradation studies and final optimization of method.
- Month 4: Validation of accuracy, precision, and linearity.
- Month 5: Validation of sensitivity, robustness, and stability, followed by system suitability.
- Month 6: Application to tablet samples and submission of the final report.

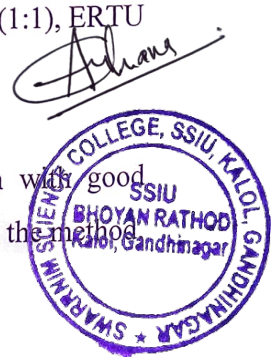
5. Result and Discussion

Result:

Based on the solubility studies, the diluent selected was HPLC grade water: ACN (1:1), ERTU being sparingly soluble in water and MET freely soluble in water.

Method optimization

Method was optimized by trial and error method to obtain a chromatogram with good resolution, acceptable number of theoretical plates, and tailing factor. To optimize the method,



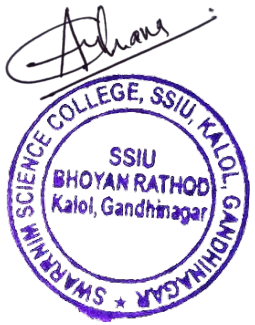
preliminary trial runs were performed by changing mobile phase and column type (Table 1). Buffer selected for the present analysis was 0.1% ortho-phosphoric acid, as ERTU (566 Da) and MET (165.62 Da) being regular samples with basic nature requiring the control of pH by the addition of buffer in the mobile phase. The pH of the buffer should be ± 1.5 U of the pKa value to retain the drug in single state which avoids peak splitting [16]. pH of the buffer selected was 2.7 as the pKa of ERTU was 11.98 and MET was 12.33 to retain both the selected drugs in completely ionized state in order to avoid peak splitting. PDA detector has an advantage of simultaneously collecting the chromatograms in the entire UV range during a single run [16]. When the drug samples were scanned between 200 and 400 nm, the ideal λ max was found to be 224 nm. UV spectrum is shown in Fig. 3. C18 columns are rugged, highly retentive, and widely available [16]. Optimal separation and peak shapes were obtained on Kromasil C18 column with dimensions of 150mm× 4.6 mm, 5 µm indicating that it was suitable for the simultaneous estimation of ERTU and MET. Theoretical plates are an important characteristic of the column which indicates the ability of the column to produce sharp, narrow peaks for achieving good resolution. Under optimized test conditions, a column with a length of 150mm and a particle diameter of 5 µm produces

theoretical plates of 10,000–12,000 [16]. In the optimized trial, the observed system suitability parameters were theoretical plates (11,025 (MET), 11,261 (ERTU)), tailing factor (1.2 (MET), 1.1 (ERTU)) and resolution of 7.7 for both the drugs reflecting that the selected column was ideal for the estimation of the drugs. In the trial runs, for the ideal separation of the selected drugs, mixtures of solvents like methanol and acetonitrile with or without buffers (0.1% ortho-phosphoric acid and KH2PO4) in different proportions were tried on C18 column. In the optimized method, the mobile phase selected was 0.1% ortho-phosphoric acid (pH 2.7):acetonitrile (65:35% v/v), as the resolution and peak shape of ERTU and MET were good with optimum system suitability parameters. The flow rate was optimized based on the peak resolution and minimal consumption of the mobile phase, and the flow rate selected was 1.0 ml/min. MET and ERTU were eluted at 2.170 min and 2.929 min confirming that MET (log P − 0.92) was more polar over ERTU (log P 2.21). Optimized chromatogram is shown in Fig. 4.

Table 1: Preliminary trial runs

Trial	Column	Mobile phase	Observation

1	BDS C18 (250 mm × 4.6 mm, 5 µm)	Methanol: 0.1 % OPA (40:60)	Only MET was eluted with less theoretical plates
2	BDS C18 (250 mm × 4.6 mm, 5 µm)	ACN: KH_2PO_4 (40:60)	Only MET was eluted
3	Agilent C18 (250 mm × 4.6 mm, 5 µm)	ACN: 0.1 % OPA (60:40)	Peak symmetry of both the peaks was good but with increased retention time
4	Kromasil C18 (150 mm × 4.6 mm, 5 µm)	ACN: 0.1 % OPA (50:50)	Peak symmetry was good but MET eluted at 1.6 min
5	Kromasil C18 (150 mm × 4.6 mm, 5 µm)	ACN: 0.1 % OPA (65:35)	Peak symmetry was good with system suitability parameters in limits



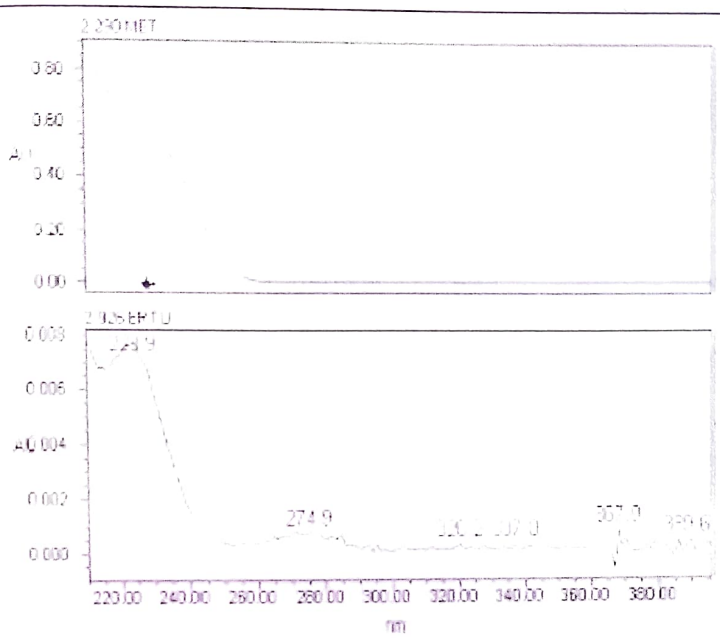


Figure 3: UV spectrum of ERTU and MET

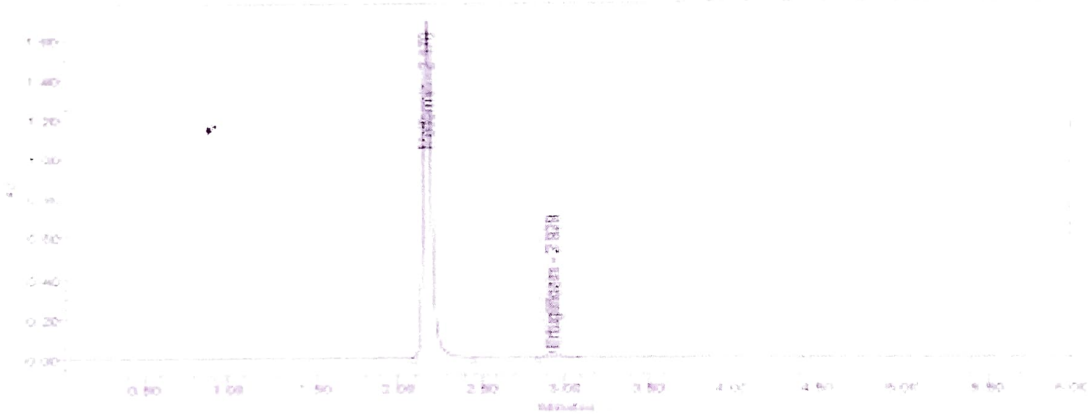


Figure 4: Optimized chromatogram of ERTU and MET

The developed method was validated as per ICH guidelines. Good association was observed between the retention times of the standard and sample peaks confirming the drugs in the tablet were ERTU and MET. Additional peaks were absent in the chromatograms indicating no interference of excipients with the drugs at the retention times, and the developed method was specific for the simultaneous estimation of the drugs in tablet formulation.

The important part of validation of a stability indicating method is to assess the presence of impurities under the main analyte peak. The analyte peak should be checked for its purity/homogeneity, which is usually evaluated by determining the purity angle and purity threshold. According to the ICH Q2 (R1) guidelines, in forced degradation studies, purity threshold should be greater than purity angle and % degradation should be less than 20 to consider the method as stable [12]. The developed method was specific and stable for the

simultaneous estimation of the drugs. The % degradation of ERTU and MET were 1.02–7.55 and 0.90–7.71, respectively.

The co-elution of degradants with the drugs was absent. The purity threshold for both the drugs (1.966– 2.341 (ERTU), 1.752–2.927 (MET)) was found to be greater than the purity angle (1.493–1.936 (ERTU), 1.348–2.753 (MET)) in the presence of 2N HCl, 2N NaOH, 20 % v/v H2O2 at 60 ± 2 °C for 30 min, in the oven at 105 ± 2 °C for 6 h, in the UV chamber for 7 days and in the presence of HPLC grade water at 60 ± 2 °C for 30 min.

In forced degradations studies, purity threshold was found to be greater than purity angle for both the drugs inferring the absence of co-elution of degradants with the drugs, and the analyte peaks were pure. Percent degradation of less than 10 for both the drugs demonstrates that the developed method was specific and stable (Table 2).

Table 2: Forced Degradation of ERTU and MET

Stressor	ERTU				MET			
	Purity angle	Purity threshold	% Assay	% Degraded	Purity angle	Purity threshold	% Assay	% Degraded
Acid	1.493	1.966	92.45	7.55	1.448	1.801	92.29	7.71
Alkali	1.766	2.146	94.56	5.44	1.402	1.752	93.07	6.93
Oxidative	1.693	2.130	95.42	4.58	1.348	2.270	94.47	5.53
Thermal	1.936	2.341	96.86	3.14	2.753	2.927	97.07	2.93
Photolytic	1.659	2.009	98.30	1.70	2.489	2.786	98.21	1.79
Neutral	1.780	2.134	98.98	1.02	2.358	2.915	99.10	0.90

Accuracy is presented in terms of % recovery and should be 98–102 [12]. Accuracy was demonstrated at 80, 100, and 120% of the target concentration, and the percent recovery for ERTU was 99.27–100.60, and MET was 99.11–101.13 (Table 3) reflecting that the developed method was accurate.

Table 3 Accuracy (% recovery) of ERTU and MET

ERTU					MET			
% Spiked level	Fixed sample concentration (µg/ml)	Amount Spiked (µg/ml)	Statistical Analysis Mean % Recovery ± SD % RSD		Fixed sample concentration (µg/ml)	Amount Spiked (µg/ml)	Statistical Analysis Mean % Recovery ± RSD SD	
80	3.75	3.0	100.04 ± 0.306	0.306	250	200	99.7 ± 0.54	0.54
100	3.75	3.75	99.99 ± 0.135	0.135	250	250	99.91 ± 0.57	0.57
120	3.75	4.50	99.87 ± 0.64	0.604	250	300	100.65 ± 0.47	0.47

The % RSD is used to express the precision of the method, and ICH-stated limits are that % RSD should be not more than 2.0 for intra-day precision and inter-day precision [12]. The % RSD of intraday precision was 0.25 and 0.42 (Table 7) for ERTU and MET. % RSD of inter-day precision was 0.70 and 0.56 (Table 7) for ERTU and MET implying that the deviation was less among repeated results and the developed method was more precise.

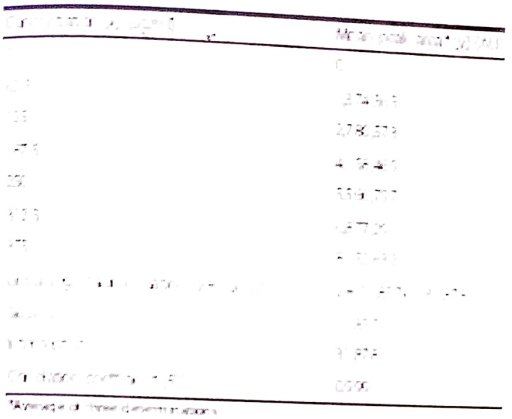
The linearity is expressed in terms of correlation coefficient (R2) and should be not less than (NLT) 0.999 [12]. The developed method was found to be linear in concentration range of 0.9375–5.625 µg/ml for ERTU (Table 4) and 62.5–375 µg/ml for MET (Table 5)with a correlation coefficient of 0.999 for both the drugs indicating the linear relationship between the peak area and concentration of the drugs (Figs. 5 and 6).

Table 4 Linearity of ERTU

Concentration (x) (µg/ml)	Mean peak area* (y) (AU)
0	0
0.9375	24,802
1.875	49,541
2.8125	74,457
3.75	99,190
4.6875	126,714
5.625	145,645
Linear regression equation (y = mx + c)	y = 26,223x + 49,009
Slope (m)	26,223
Intercept (c)	49,009
Correlation coefficient (R^2)	0.999

*Average of three determinations

Table 5 Linearity of MET



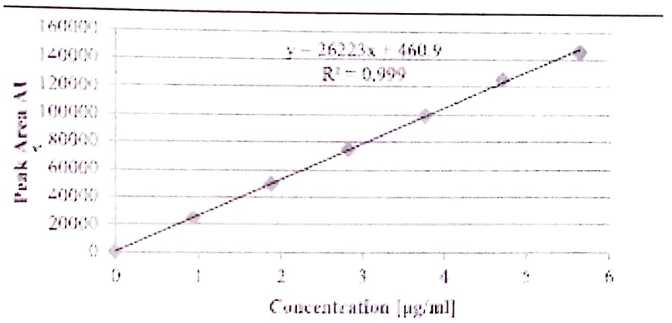


Figure 5: Calibration curve of ERTU

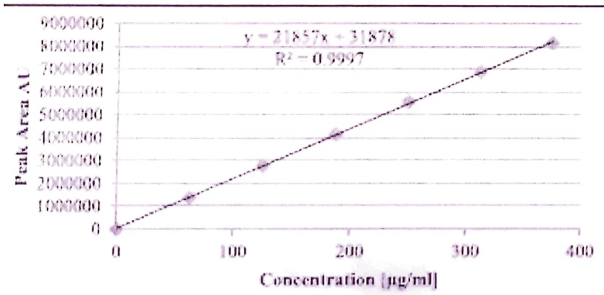


Figure 6: Calibration curve of MET

LOD for ERTU and MET were 0.025 µg/ml and 0.87 µg/ml (Table 7) respectively indicating that the developed method was suitable for the estimation of 0.1 µg of the drugs. LOQ for ERTU and MET were 0.076 µg/ml and 2.63 µg/ml (Table 7). The stability of standard solution is expressed in terms of % assay difference, and it should be no more than (NMT) 2.0 [12]. In the present study, % assay difference was 0.76 for 12 h and 1.74 for 24 h for ERTU and was 0.83 and 1.51 for MET (Table 7). The % assay difference of ERTU and MET reflect that the solutionwas stable for 24 h at 30 ± 2 °C.

System suitability parameters are used for expressing the robustness of the method. To consider the method is robust, % RSD should be less than 2.0, theoretical plates should be more than 2000, tailing factor should be less than 2.0, and resolution should be more than 2.0 [12]. The system suitability parameters observed were % RSD 0.531–1.377 (ERTU), 0.382–1.893

(MET), theoretical plates 11,564–13,396 (ERTU), 9463–11,751 (MET), tailing factor 1.14–1.26 (ERTU), 1.14–1.33 (MET), and resolution of 6.78–9.2 for ERTU and MET reflecting that the developed method was robust for the simultaneous determination of ERTU and MET (Table 6).

Table 6: Robustness of ERTU and MET

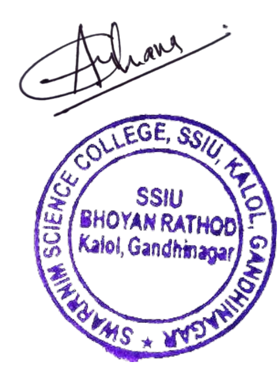
Parameter	Modified condition	% RSD of peak area (NMT 2.0 %)		Theoretical plates (N > 2000)		Tailing factor (< 2.0)		Rs*
		ERTU	MET	ERTU	MET	ERTU	MET	
Flow rate (ml/min)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Temperature (°C)	25	[illegible]	1.278	13,306	[illegible]	[illegible]	[illegible]	[illegible]
	30	0.531	0.382	11,679	9947	1.23	[illegible]	[illegible]
	35	0.574	0.908	13,306	[illegible]	[illegible]	[illegible]	[illegible]

* All the values were expressed as mean of six determinations

In the present study, the % RSDs 0.531 (ERTU) and 0.382 (MET), theoretical plates 11,679 (ERTU) and 9947 (MET), tailing factors 1.23 (ERTU) and 1.25 (MET), and resolution of 7.21 for ERTU and MET indicate that the system was suitable for the simultaneous analysis of ERTU and MET (Table 7). On the application of the developed method to tablets, the mean amount of ERTU and MET present in the tablets were found to be 7.45 ± 0.16 mg and 497.4 ± 0.97 mg against the labeled claim of 7.5 mg (ERTU) and 500 mg (MET), reflecting that the method was suitable for the simultaneous estimation of ERTU and MET in tablet formulation (Table 7).

Table 7 Summary of validation parameters of ERTU and MET

Parameter	Results		ICH limits
	MET	ERTU	
Retention time (min)	2.170	3.929	
System suitability parameter			
% RSD	0.382	0.531	NMT 2.0
Theoretical plates	9947	11,679	NLT 2000
Tailing factor	1.25	1.23	NMT 2.0
Range (µg/ml)	62.5–375	0.9375–5.625	
Linearity (R^2)	0.999	0.999	NLT 0.999
% Recovery	99.11–101.13	99.27–100.60	98–102
% RSD			
Intra-day precision	0.42	0.25	NMT 1.0
Inter-day precision	0.56	0.70	NMT 2.0
LOD (µg/ml)	0.87	0.025	
LOQ (µg/ml)	2.63	0.076	
% Assay difference at 24 h	1.51	1.74	NMT 2.0
% Assay	99.48	99.31	



e. Discussion

The stability-indicating RP-HPLC method is an analytical procedure that is capable of discriminating between the major active pharmaceutical ingredients from any degradation/decomposition products formed under defined storage conditions. Stability-indicating assay method development studies the effect of stressors on a drug which helps in

understanding the stability of the drug during storage conditions and analysis. Few methods were reported for the simultaneous estimation of ERTU and MET by RP-HPLC. In the present method, MET and ERTU were eluted at 2.170 and
2.929 min with a resolution of 7.21. The present method was developed using 0.1% ortho-phosphoric acid (pH 2.7): ACN as the mobile phase. The developed the method was found to be sensitive and cost-effective with the reduced ratio of organic solvent in
the mobile phase.

6. Financial statement

Utilization Certificate

Certified that a grant of 5,00,000 was received from the Aum Research Lab for the project titled " Development And Validation of Stability- Indicating Rp-Hplc Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets ". The amount has been utilized as per the approved budget and guidelines.

Category	Utilized
Manpower	₹2,20,000
Consumables	₹1,70,000
Travel	₹75,000
Contingencies	₹35,000
Total	₹5,00,000

Budget Breakdown and Justifications:

The total budget for the proposed project on the "Development and Validation of Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Ertugliflozin Pidolate and Metformin Hydrochloride in Bulk and Tablets" is ₹500,000. Below is a detailed breakdown of each category and the justification for the proposed expenses.

SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar
SWARNIM

1. Manpower (₹2,20,000)

Justification:

- Project Staff Compensation:
  - The project requires a multidisciplinary team comprising researchers, lab technicians,

and a project manager to handle the various tasks associated with method development, validation, and analysis.

- A budget of ₹40,000/month for six months is allocated to ensure the continuous engagement of the project staff, particularly in critical phases such as method development, forced degradation studies, and validation.

- Key Roles:
- Researcher/Scientist (₹20,000/month): Responsible for developing and optimizing the HPLC method, running the chromatographic analyses, and interpreting data.
- Lab Technicians (₹10,000/month): Handle sample preparation, standard solution preparation, and general laboratory maintenance.
- Project Manager (₹10,000/month): Oversees the overall progress, ensures adherence to timelines, manages resources, and prepares the final report.

2. Consumables (₹170,000)

Justification:

- Chemicals and Reagents:
- Ertugliflozin Pidolate and Metformin Hydrochloride are the primary drugs being studied, along with other essential reagents such as solvents (methanol, acetonitrile), buffers, and pH modifiers.
- The consumables category also includes the procurement of HPLC columns (such as C18), which are crucial for method development and validation.
- In addition, standard solutions, excipients, vials, filters, syringes, and other laboratory supplies are required for sample preparation and analysis.
- Consumables are expected to be more heavily utilized in the later months for stability studies and tablet analysis, which is reflected in the incremental cost in months 3 to 6.

Breakdown:

- Initial Consumables (Month 1): ₹20,000 (reagents, solvents, basic chemicals)
- Subsequent Consumables (Month 2-6): ₹30,000 per month to cover consumables for method development, forced degradation studies, stability testing, and tablet analysis.

SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar
SWARNIM

3. Travel (₹75,000)

Justification:

- Project-related travel:
- This allocation covers the costs associated with potential travel for the project team,

including attending scientific meetings, conferences, or visits to collaborating labs (if necessary).

- ₹5,000 per month is estimated for travel-related expenses such as transport, accommodation, and registration fees. While travel may not be a major focus of the project, this budget ensures that any travel necessary for knowledge exchange or collaboration is accounted for.

- Travel Purpose:
- Participation in conferences or training related to HPLC analysis, stability testing, and regulatory compliance may help enhance the project's outcomes.
- Site visits to pharmaceutical companies or industry experts for discussions on method application and validation are also considered.

4. Contingencies (₹35,000)

Justification:

- Unforeseen Expenses:
- The contingencies fund is meant to cover any unexpected costs that may arise during the project, such as unplanned equipment repairs, additional consumables, or replacements of critical items.
- Given the nature of the project, there could be unforeseen costs for equipment maintenance, purchase of additional reagents, or extra consumables if certain materials run out during validation or testing.
- A ₹5,000/month allocation for contingencies ensures that the project remains on track without financial delays.
- Equipment-Related Costs: For example, if an HPLC system experiences technical issues or if additional calibration or maintenance is required.

Summary of Justifications:

1. Manpower: Covers salaries for key project personnel to ensure proper execution, monitoring, and timely completion of the project tasks.
2. Consumables: Includes essential chemicals, reagents, equipment, and HPLC consumables for both method development and validation processes, ensuring the ability to perform all planned tests.
3. Travel: Budgeted to support any necessary travel for training, conferences, or project-related collaboration.

SHARDABEN SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar

4. Contingencies: Provides a buffer to manage unexpected costs without disrupting the project flow.

The total project cost of ₹500,000 is strategically allocated to balance the needs for skilled personnel, necessary laboratory materials, potential travel, and unforeseen expenses, ensuring the successful completion of the project within the given timeframe.

7. Conclusion:

This project aligns with both national and international research efforts in the field of pharmaceutical analysis. While there are existing methods for the analysis of individual drugs like Metformin and Ertugliflozin, the simultaneous analysis of these drugs, especially with an emphasis on stability testing, is an area that needs further exploration. The project will not only contribute to advancing analytical methodologies but also help bridge gaps in regulatory standards for combination drug products, benefiting the pharmaceutical industry both in India and globally.

8. Reference

1. Bansal, G., & DeStefano, A. (2007). Key elements of a successful stability indicating HPLC method. *Journal of Liquid Chromatography & Related Technologies, 30*(5), 709–738. https://doi.org/10.1080/10826070701273775

2. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs—A review. *Journal of Pharmaceutical Analysis, 4*(3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003

3. ICH Q2(R1). (2005). *Validation of Analytical Procedures: Text and Methodology*. International Council for Harmonisation.

4. International Conference on Harmonization (ICH). (2003). *ICH Q1A(R2) Stability Testing of New Drug Substances and Products*.

5. Chunduri, B. R., Dannana, G. S., & Pilli, N. R. (2017). Development and validation of stability-indicating UPLC method for simultaneous determination of ertugliflozin and sitagliptin in bulk and tablet dosage form. *Future Journal of Pharmaceutical Sciences, 3*(1), 84–89.

6. Bhavsar, D., & Patel, D. (2017). RP-HPLC method for simultaneous estimation of metformin and dapagliflozin in tablet dosage form. *International Journal of Pharmaceutical Sciences, 9*(3), 167–170.

7. Maheshwari, D. G., & Pujara, P. D. (2019). Development and validation of RP-HPLC method for simultaneous estimation of ertugliflozin and metformin hydrochloride. *World Journal of Pharmacy and Pharmaceutical Sciences, 8*(4), 1026–1038.

8. Narayana, A., & Reddy, M. P. (2020). A validated RP-HPLC method for the estimation of ertugliflozin and metformin in bulk and tablet dosage forms. *Research Journal of Pharmacy and Technology, 13*(5), 2195–2199.

9. Bhaskaran, S., & Sabarinathan, T. (2019). A new stability indicating RP-HPLC method for simultaneous estimation of ertugliflozin and metformin in pharmaceutical dosage forms. *Asian Journal of Research in Chemistry, 12*(6), 345–350.

10. Pindwal, A., & Sharma, P. (2020). A review on analytical methods for determination of metformin hydrochloride. *International Journal of Pharmaceutical Sciences Review and Research, 60*(1), 65–71.

11. Rao, B. M., & Reddy, S. (2019). RP-HPLC method development and validation for the simultaneous estimation of ertugliflozin and metformin in tablet dosage form. *Asian Journal of Pharmaceutical Analysis, 9*(4), 201–205.

12. Joshi, P. R., & Shah, D. A. (2018). A review on recent analytical methods for estimation of SGLT2 inhibitors in pharmaceutical formulations. *PharmaTutor, 6*(6), 35–42.

13. Gadge, N. B., & Sawant, R. L. (2021). Method development and validation of stability indicating RP-HPLC method for ertugliflozin and metformin. *Asian Journal of Pharmaceutical and Clinical Research, 14*(3), 145–149.

14. Patel, H. R., & Gandhi, T. R. (2018). Analytical method development and validation for simultaneous estimation of ertugliflozin and metformin in bulk and tablet dosage form. *Journal of Drug Delivery and Therapeutics, 8*(6), 253–259.

15. Chavan, S. D., & Vaidya, R. R. (2020). RP-HPLC method development and validation of ertugliflozin and metformin in bulk and tablet dosage form. *International Journal of Pharmaceutical Sciences and Research, 11*(2), 987–993.

16. Bhatt, K. K., & Mehta, R. S. (2020). Development and validation of RP-HPLC method for simultaneous determination of antidiabetic drugs. *International Journal of Chemical and Pharmaceutical Sciences, 8*(1), 35–40.

17. Sharma, S., & Jain, A. (2016). HPLC method development and validation for the estimation of metformin hydrochloride. *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, 4*(3), 132–136.

18. Sahoo, P. K., & Bera, A. K. (2021). RP-HPLC method for simultaneous determination of metformin and gliflozin drugs in combination therapy. *International Journal of Pharmacy and*

Pharmaceutical Sciences, 13(1), 100–105.

19. Patel, R. B., & Patel, M. R. (2015). Recent trends in method development and validation of stability indicating RP-HPLC. *Journal of Analytical Chemistry, 70*(4), 456–464.

20. Sharma, D., & Mahajan, R. (2019). Analytical method validation for pharmaceutical analysis: A review. *Journal of Drug Delivery and Therapeutics, 9*(3-s), 430–435.

21. Prajapati, H., & Patel, A. (2021). Simultaneous estimation of metformin hydrochloride and canagliflozin by RP-HPLC. *Research Journal of Pharmacy and Technology, 14*(10), 5361–5364.

22. Nair, A. B., & Al-Dhubiab, B. E. (2020). Stability indicating methods for pharmaceutical drugs. *Saudi Pharmaceutical Journal, 28*(1), 1–15.

23. Dave, D. J., & Patel, H. P. (2021). Validated HPLC method for determination of metformin and its degradation products. *Analytical Chemistry Letters, 11*(1), 65–74.

24. Kaur, R., & Gill, H. (2021). RP-HPLC method development and validation: Review. *International Journal of Research in Pharmaceutical Sciences, 12*(2), 1321–1328.

25. Roy, S., & Dey, R. S. (2017). Development and validation of RP-HPLC method for simultaneous estimation of metformin and other antidiabetic drugs. *Journal of Chromatographic Science, 55*(9), 907–913.

26. Reddy, D. R., & Sireesha, G. (2018). RP-HPLC method for simultaneous estimation of metformin and empagliflozin in bulk and tablet dosage form. *International Journal of Pharmaceutical Sciences and Research, 9*(3), 1001–1006.

27. Shah, D. A., & Patel, B. A. (2016). Stability indicating RP-HPLC method for simultaneous estimation of gliptins and gliflozins with metformin. *Pharma Science Monitor, 7*(1), 153–159.

28. Singh, R., & Sharma, P. (2020). Forced degradation studies and stability indicating HPLC method development. *International Journal of Pharmaceutical Investigation, 10*(2), 70–76.

29. Dubey, R. N., & Dwivedi, S. (2018). Review on RP-HPLC analytical method development and validation. *World Journal of Pharmaceutical Research, 7*(15), 534–553.

30. Aithal, K. S., & Udupa, N. (2017). Analytical method development and validation for the estimation of drugs in pharmaceutical dosage forms: A review. *Journal of Applied Pharmaceutical Science, 7*(6), 135–143.

GCC BIOTECH®

GCC BIOTECH (INDIA) PVT. LTD.
An ISO 9001:2015 Certified Co.
Off.& Lab.: Joychandipur, Bakrahat, 24-Pgs (South)
PIN-743377 (W.B.), INDIA
Ph.: 91-033-24951044 / 0004 Fax No.:91-33-40608482
Regd. Off.: 351, BMK, Giri Nagar, Kalkaji, New Delhi-110019-INDIA
Ph.:91-11-26440301 Fax No.: 91-11-26418606
Email: tech.support@gccbiotech.co.in/info@gccbiotech.co.in
Website: www.gccbiotech.co.in

SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **M/s GCC Biotech** and **Swarrnim Startup and Innovation University**, Gandhinagar, Gujarat.

In consideration of the mutual promises and covenants contained herein, the receipt and sufficiency of which are hereby acknowledged, the parties agree to the following terms:

SERVICES

The Provider agrees to develop and validate low cost and efficient analytical methods for the APIs and formulations for GCC Biotech Pvt Ltd.

TERMS AND CONDITIONS

This Contract shall commence on **11th March 2019** and will remain in effect for five years. The contract may be extended for an additional period as mutually agreed by both parties. Should either party wish to terminate the contract, a written notice must be provided 90 days prior to the intended termination date.

PAYMENT

For each completed service, the Provider will submit an invoice as services are delivered. The Client agrees to make payment upon receipt of the invoice, after deducting any applicable TDS (Tax Deducted at Source).

For GCC Biotech Pvt Ltd

GCC LABORATORY GCCL

ForSwarrnim Startup and Innovation University

Swarrnim Startup & Innovation University Gandhinagar

GCC BIOTECH®

GCC BIOTECH (INDIA) PVT. LTD.
An ISO 9001:2015 Certified Co.
Off.& Lab.: Joychandipur, Bakrahat, 24-Pgs (South)
PIN-743377 (W.B.), INDIA
Ph.: 91-033-24951044 / 0004 Fax No.:91-33-40608482
Regd. Off.: 351, BMK, Giri Nagar, Kalkaji, New Delhi-110019-INDIA
Ph.:91-11-26440301 Fax No.: 91-11-26418606
Email: tech.support@gccbiotech.co.in/info@gccbiotech.co.in
Website: www.gccbiotech.co.in

Date: 8.7.2019

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

We are pleased to inform you that the consultancy project for which we were in discussion in our earlier meetings has been granted. The project will proceed as follows:

Project Title:

"Method Development And Validation Of Carglumic Acid And Its Related Substances In Tablet Dosage Form By Rp-Hplc."

Project Timeline:

The project is expected to be completed within the next 5 to 6months.

Payment:

A total amount payable after successful completion of Project will be Rs. 5,00,000 plus GST will be made after raising invoice of the same after due TDS.

Should you require any further clarification regarding the project, please feel free to reach out to us. We are excited about this collaboration and look forward to a positive working experience with **Swarrnim Startup and Innovation University**.

Best Regards,

For GCC Biotech

GCC LABORATORY GCCL MODASA

Ref.No.swarrnim/RO/SCR/2019/44 Date: 09.12.2019

To,

GCC BIOTECH (INDIA) PVT. LTD.

Joychandipur, Bakrahat, 24- pgs (south)

West Bengal, India.

Subject: - Submission of completion report regarding your shared problem.

Dear Sir/Madam

Please find enclosed herewith all data related to the problem shared by your prestigious company the details are as follows:

Project title: **"Method Development and Validation of Carglumic Acid and Its Related Substances in Tablet Dosage Form by Rp-Hplc."**

Date of assigning problem: 08.07.2019

Date of completion: 06.12.2019

Name of person: Mr. Milan Pansuriya

We are thankful for providing the opportunity to support you and the profession. We will always ready to solve such problems with our best effort.

For any technical support please contact person who has completed the project, the name is Mr. Milan Pansuriya.

Thanking you.

Registrar

Startup & Innovation University
Bhoyan Rathod
Gandhinagar

Method Development and Validation of Carglumic Acid and Its Related Substances in Tablet Dosage Form by Rp-Hplc

Research Project Report Submission to

GCC Biotech

STARTUP & INNOVATION UNIVERSITY

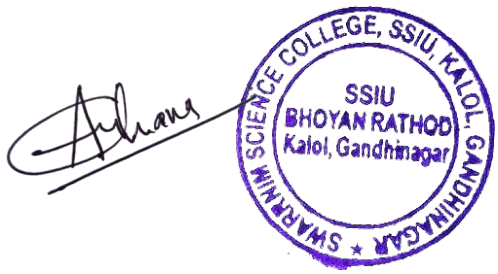
Submitted by:

Principal Investigator: Mr. Milan Pansuriya, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

SWARRNIM SCIENCE COLLEGE, SSIU, KALOL, GANDHINAGAR
SSIU
BHOYAN RATHOD
Kalol, Gandhinagar

INDEX

Sr. No	Content
1	Introduction
2.	Literature Review
3.	Objectives
4.	Methodology
5.	Result and Discussion
6.	Financial Statement
7.	Conclusion
8.	References



- **Declaration**

I, Mr. Milan Pansuriya, Principal Investigator of the project titled " Method Development and Validation of Carglumic Acid and Its Related Substances in Tablet Dosage Form by Rp-Hplc ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

Name:

Principal Investigator: Mr. Milan Pansuriya

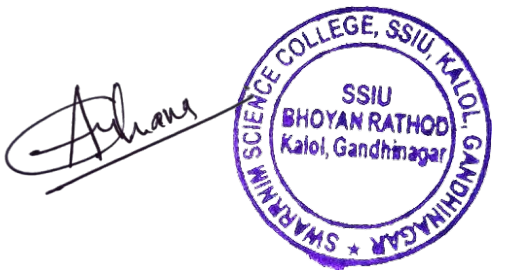
Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

I extend my sincere gratitude to the GCC biotech for funding this project. I also thank my institution, colleagues, and students who supported and contributed to the successful completion of this project.

- **Executive Summary**

Carglumic acid is an important medication used primarily for the treatment of hyperammonemia associated with organic acidurias, a condition where the body is unable to process certain amino acids. Due to its therapeutic significance, accurate and reliable methods to quantify carglumic acid and detect its related substances are crucial for ensuring drug safety, efficacy, and quality control in pharmaceutical formulations. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely used analytical technique that offers high precision, sensitivity, and selectivity for the quantification of active pharmaceutical ingredients and their impurities in tablet formulations. This project aims to develop and validate a robust RP-HPLC method for the determination of carglumic acid and its related substances in tablet dosage forms, which is essential for regulatory compliance and quality assurance in the pharmaceutical industry.



- **Detailed Report**

1.Introduction

Carglumic acid is used to treat for hyperammonemia.

1.6 Introduction of Hyperammonemia [1-2]

Hyperammonemia is a metabolic disturbance characterized by an excess of ammonia in the blood. It is a dangerous condition that may lead to brain injury and death.

❖ Types of Hyperammonemia Primary hyperammonemia

Primary hyperammonemia is caused by several inborn errors of metabolism that are characterized by reduced activity of any of the enzymes in the urea cycle.

The most common example is ornithine transcarboxylase deficiency which is inherited in an X- linked fashion.

Secondary hyperammonemia

Secondary hyperammonemia is caused by inborn errors of intermediary metabolism, which are characterized by reduced activity of enzymes that are not part of the urea cycle or dysfunction of cells that makes major contributions to metabolism.

Examples of the former are propionic acidemia and methylmalonic acidemia, examples of the latter are acute liver failure and hepatic cirrhosis with liver failure.

Acquired hyperammonemia

Acquired hyperammonemia is usually caused by diseases that result in either acute liver failure, such as overwhelming hepatitis B or exposure to hepatotoxins, or cirrhosis of the liver with chronic liver failure. Chronic hepatitis B, chronic hepatitis C, and excessive alcohol consumption are common causes of cirrhosis. The physiologic consequences of cirrhosis include shunting of blood from the liver to the inferior vena cava resulting in decreased filtration of blood and removal of nitrogen- containing toxins by the liver and then hyperammonemia. This type of hyperammonemia can be treated with antibiotics to kill the bacteria that initially produce the ammonia, though this doesn't work as well as removal of protein from the colon prior to its digestion to ammonia, achieved by lactulose administration for frequent (3-4 per day) bowel movements.

Medication induced hyperammonemia can occur with valproic acid overdose and is due to a deficiency in carnitine. Its treatment is carnitine replacement.

Severe dehydration and small intestinal bacterial overgrowth can also lead to acquired hyperammonemia. Glycine toxicity causes hyperammonemia, which manifests as CNS symptoms and nausea. Transient blindness can also occur.

Congenital hyperammonemia

Congenital hyperammonemia is usually due to genetic defects in one of the enzymes of the urea cycle, such as ornithine transcarboxylase deficiency, which leads to lower production of urea from ammonia.

❖ Treatment of Hyperammonemia

Following are the types of hyperammonemia

3. Neonatal hyperammonemia
4. Intercurrent hyperammonemia

Treatment of both the types of hyperammonemia is described as below

Treatment of neonatal hyperammonemia coma

Protein intake should be stopped.

Calories should be supplied by giving hypertonic 10% glucose.

Hemodialysis should be started promptly in all comatose neonates with plasma ammonium levels greater than 10 times reference range. Plasma ammonium levels are reduced quickly and the total dialysis time is shorter with hemodialysis than with peritoneal dialysis. Continuous arteriovenous or Veno venous hemofiltration may be used as an alternative method.

Intravenous sodium benzoate and phenylacetate should be started once the plasma ammonium level falls to 3-4 times the upper limit of the reference range. Intravenous arginine should be provided.

Corticosteroids are not indicated for the management of increased intracranial pressure in hyperammonemia because they induce negative nitrogen balance. Mannitol is not effective in treating cerebral edema induced by hyperammonemia.

Valproic acid should not be used to treat seizures as it decreases urea cycle function and increases serum ammonia levels.

Treatment of intercurrent hyperammonemia

Patients with urea cycle defects may present with episodes of hyperammonemia secondary to increased protein intake, increased catabolism, or noncompliance with therapy. This should be recognized early and treated as an emergency.

Treatment should be started if the plasma ammonium level is 3 times the reference level. All nitrogen intake should be stopped.

High parenteral intake of calories from 10-15% glucose and intralipids should be provided. Intravenous infusion of sodium benzoate and phenylacetate should be started.

Plasma ammonium levels should be checked at the end of the infusion and at every 8 hours. Once the ammonia level is near normal, oral medication should be started.

If the level does not decrease in 8 hours, hemodialysis should be started.

Osmotic demyelination syndrome has been reported as a potential serious complication of standard therapy for hyperammonemia in patients with ornithine transcarbamylase deficiency.

1.7 HIGH PERFORMANCE CHROMATOGRAPHY: [3-18]

- HPLC is one of the most powerful tool in analytical chemistry. It has the ability to separate, identify and quantify the compounds that are present in any sample that can dissolved in liquid. Now a days, compounds in trace concentrations as low as parts per trillion (ppt) may be easily identified.
- High-pressure liquid chromatography is frequently called high- performance liquid chromatography (both are abbreviated HPLC or simply LC) because it offers improved performance over classical liquid chromatography.
- HPLC is the premier analytical technique in pharmaceutical analysis, which is predominantly used in pharmaceutical industry for a large variety of samples. It is the method of choice for checking the purity of new drug candidates, monitoring changes or scale-ups of synthetic procedures, evaluating new formulations and scrutinizing quality control/ assurance of final drug products.
- Most of the drugs in multi-component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method.
- HPLC method eliminates tedious extraction and isolation procedures.

Table: 1.1 Characteristics of primary HPLC methods [6]

Method/column	When the method is preferred?
RP-HPLC Uses water-organic mobile phase Columns: C18(ODS), C8, phenyl, Trimethylsilyl (TMS), Cyno	First choice for most samples, especially neutral, or non- ionized compounds that dissolves in water-organic mixtures.
NP-HPLC Uses mixtures of organic solvents as mobile phase Columns: Cyno, Diol, Amino, Silica	Good second choice when reverse phase or ion pair is ineffective, first choice for lipophilic samples that do not dissolve well in water-organic mixtures; first choice for mixtures isomers and for preparative scale HPL (silica best)

Ion-pair HPLC Uses water-organic mobile phase, a buffer to control pH and a ion-pair reagent Columns: C18, C8, Cyno	Acceptable choice for ionic or ionizable compounds, especially bases or cations.

Also, HPLC are classified by mechanism of separation or by the type of stationary phase includes:

Partition or liquid-liquid chromatography

Adsorption or liquid-solid chromatography

Ion exchange or ion chromatography

Size exclusion chromatography

Affinity chromatography

Chiral chromatography

1.8 METHOD DEVELOPMENT: [14-18]

For routine analysis of various mixtures with HPLC method is performed with the ideal combination of suitable column, mobile phase and detection wavelength that assures faster delivery of desire result of validated method of separation.

Mobile phase

In reverse-phase chromatography, the mobile phase is more polar than the stationary phase. Mobile phase in these systems is usually mixtures of two or more individual solvents with or without additives or organic solvent modifiers. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solutes.

Detector

UV-visible detectors are the most popular as they can detect a broad range of compounds and have a fair degree of selectivity for some analytes. Table 1.1 summarizes some of the available options.

Column length

Method development can be streamlined by starting with shorter columns; 150, 100 or even 50mm long. This is simply because they have proportionally shorter run times.

Stationary phase

For RP-HPLC method, various columns are available but our main aim is to resolve drugs in the presence of excipients. So, the C-18 column was selected which provides high peak symmetry, good

retention to drugs and facilitates the separation of the drugs from each other without the interference of excipients within short run time.

Gradient programming

The fastest and easiest way to develop a method is to use a gradient mobile phase. Always start with a weak solvent strength and move to a higher solvent strength. To begin, use a very fast gradient (e.g.,10 minutes) and then modify the starting and finishing mobile phases to achieve a suitable separation. Of course, the choice of solvents and buffers may need to be modified during method development. (Different HPLC instruments will give different results for the same gradient, so if a method is to be validated for use by several different laboratories, isocratic methods are recommended).

Retention

Analytes may be too strongly retained (producing long run times). If this occurs, the solvent strength should be increased. In reverse phase analysis this means a higher % of organic solvent in the mobile phase.

Poor separation

Analytes often co-elute with each other or impurities. To overcome this, the analysis should be run at both higher and lower solvent strengths so the best separation conditions may be determined. Varying solvents may help - try methanol instead of acetonitrile for reversed phase analysis. Using buffers and modifying the pH (within the column's recommended pH range) can also assist the separation.

Peak shape

This is often a problem, especially for basic compounds analyzed by reversed phase HPLC. To minimize any potential problems always use a high purity silica phase such as Wakosil II. These modern phases are very highly deactivated so secondary interactions with the support are minimal. Buffers can be used effectively to give sharp peaks. If peak shape remains a problem, use an organic modifier such as triethylamine, although this should not be necessary with modern phases like Wakosil. One point often forgotten is the effect of temperature changes on a separation. To maximize the reproducibility of a method, it is best to use a column heater to control the temperature of the separation. A temperature of 35 – 40°C is recommended.

Buffer selection

In RP-HPLC, the retention of analytes is related to their hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and, therefore, it retention decreases. When separating mixtures containing acid and/or bases by reversed phase HPLC, it is necessary to control the pH of mobile phase using appropriate buffer in order to achieve reproducible results.

Selection of pH

- The pH range most often used for RP-HPLC is 2- 8 and can be divided into low pH (2 - 4) and intermediate pH (4 - 8) ranges. Each range has a number of advantages. Low pH has the advantage of creating an environment in which peak tailing is minimized and method ruggedness is maximized. For this reason, operating at low pH is recommended. At a mobile phase pH greater than 7, dissolution of silica can severely shorten the lifetime of columns packed with silica- based stationary phases.

1.9 Introduction to Related Substances: [19-21]

➢ Definition:

Related substances are structurally related to drug substance. These substances may be identified or unidentified degradation products or impurities arising from manufacturing process or during storage of material.

➢ Classification of Impurities

4. Organic impurities, (process and drug related)
5. Inorganic impurities,
6. Residual solvents

- Organic impurities can arise during manufacturing process and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile. These include:

➢ Starting materials,
➢ By-products,
➢ Intermediates,
➢ Degradation products,
➢ Reagents, ligands and catalysis

- Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

➢ Reagents, ligands and catalysts
➢ Heavy metals or other residual metals
➢ Inorganic salts
➢ Other materials (e.g., filter aids, charcoal)

- Solvents are inorganic or organic liquids, used as vehicles for preparation of solutions or suspensions in synthesis of new drug substance. Since these are generally of known toxicity, the selection of appropriate controls is easily accomplished.

❖ Qualification of Impurities

- Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity, or a given impurity profile at the levels specified. The applicant should provide a rationale for establishing impurity acceptance criteria that includes safety considerations.
- The level of any impurity that present in a new drug substance that has been adequately tested in safety and/or clinical studies would be considered qualified. Impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified.
- A level of a qualified impurity higher than that present in new drug substance can also be justified based on an analysis of the actual amount of impurity administered in previous relevant safety studies.
- If data are unavailable to qualify the proposed acceptance criterion of an impurity, studies to obtain such data can be appropriate when the usual qualification thresholds given as below are exceeded. Higher or lower thresholds for qualification of impurities can be appropriate for some individual drugs based on scientific rationale and level of concern, including drug class effects and clinical experience.

1.10 INTRODUCTION TO VALIDATION [22]

Definition:

"Validation is process of establishing documented evidence, which provides high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermine specification and quality characteristics."

Purpose of validation:

- To recognize technique is appropriate for its intended purpose to get predictable, dependable and exact information.
- Satisfy FDA requirements.
- Establish evidence that strategy can be used for decision making.
- Identification of sources and measurement of potential error.

Types of analytical method validated

It is directed to the most common types of analytical procedure:

- Identification tests, Quantitative tests for impurities content,
- Limit test for impurities,
- Quantitative tests for active ingredient in sample of drug substances or drug product or other selected component(s) in drug product.

➢ As per the International Conference on Harmonization (ICH) Q2 (R1) guidelines, the method validation parameters to be checked are specificity, accuracy precision, LOD, LOQ, linearity, range and robustness.

2. Literature Review

2.2 Carglumic acid is not official in IP, USP, BP

Table: 2.1 Literature review of Carglumic acid

Sr.No	Title	Method & Description	
1	Pharmaceutical parenteral formulation containing carglumic acid.	HPLC METHOD: Stationary phase: column Develosil 5mm, RPAQUEOUS-AR C30, 250x4.6mm Mobile phase A: KH2PO4 50 mM pH 2.0 per H3PO4 85% Mobile phase B: Methanol Wavelength: 215 nm Flow rate: 1.0 mL/min Retention time :6.6 min	23
2	Preparation of stable pharmaceutical dosage forms.	HPLC METHOD: Stationary phase: T3 column (250 x 4.6 mm, 5.0 mm) Mobile phase A: KH2PO4 pH 2.5 Mobile phase B: Methanol Wavelength: 205 nm Flow rate: 1.0mL/min Retention time: 70 min	4

3	Carglumic acid in human plasma by LC-MS/MS	LC -MS/MS METHOD: Stationary phase: ACE 5 CN column (150 x 4.6 mm) Mobile phase: MeCN: MeOH: 0.1% acetic acid pH 3.2 (40:40:20) %v/v Flow rate: 1.0mL/min	5

3.Objectives

1. To develop a robust HPLC method for the assay of Levetiracetam in injection formulations.

2. To validate the developed method in accordance with ICH guidelines for linearity, accuracy, precision, specificity, and limit of detection (LOD).

3. To apply the validated method to assess the Levetiracetam content in various injection formulations.

4.Methodology

4.5 Experimental work:

4.5.1 List of Instruments, Chemicals, Materials & Reagents

To develop analytical method & to validate Carglumic acid & its related substances, following instruments, chemicals, materials and reagents were used.

The detailed of each of them were listed in Table 5.1 & 5.2.

Table: 4.1 List of Instruments and Apparatus used

Sr No.	Instrument	Manufacturer
1	Visual Melting Range Apparatus	LABINDIA
2	FT-IR	Perkin Elmer
3	Analytical Balance	Mettler Toledo

4	HPLC (UV & PDA Detector) Empower3 Software	Waters Alliance 2393
5	pH Meter	Metrohm
6	Ultra Sonicator	Leelasonic
7	Hot Air Oven	Patel scientific instrument
8	Micropipette	Enderson

Table: 4.2 List of Chemicals, Materials & Reagents used

Chemical and Reagent	Grade	Source/Supplier
Potassium dihydrogen phosphate	AR	Merck
Sodium hydroxide	AR	Merck
Orthophosphoric acid	AR	Spectrochem
Methanol	HPLC	RANKEM
Water	Milli-Q	Milli-Q
Carglumic acid	Emcure pharmaceuticals Ltd. Gandhinagar	
Placebo		
Impurities		

4.3 Identification of Drug and its Related Substances

- Identification of standard Carglumic acid was carried out by melting point study and infrared spectroscopic study.

Determination of melting point:

- Melting point of Carglumic acid was checked by using melting point apparatus. Melting point of Carglumic acid is shown in table 5.3.

Table: 4.3 Melting point of Carglumic acid

Name of drug	Standard melting range	Observed melting range
Carglumic acid	155-165 °C	158-162°C

4.5.2 IR spectra analysis

The standard sample of Carglumic Acid and related substances was scanned from 400-4000 cm^{-1}, using an FT-IR instrument.

Interpretation of Carglumic acid and related substances are shown in table (5.4, 5.5 & 5.6).

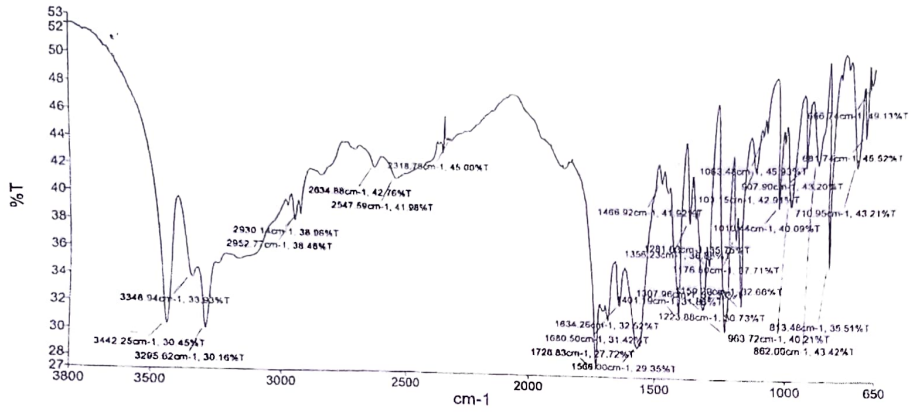
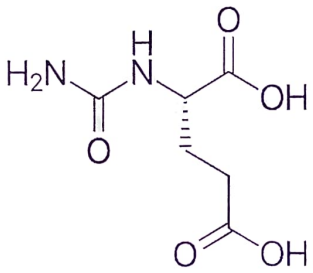


Fig. 4.1: FT-IR Spectra of Standard sample of Carglumic acid



Structure of Carglumic acid

Table: 4.4 IR interpretation of Carglumic Acid

Sr No.	Functional Group	Standard frequency (cm-1)	Observed frequency (cm-1)
1	O-H (Stretching)	3440-2400	3442.25
2	N-H (Stretching)	3500-3100	3295.62
3	C-H (Aliphatic Stretching)	3000-2850	2952.77
4	C=O (Stretching) Carboxylic acid	1725-1700	1728.83
5	C=O (Stretching) Amide	1680-1630	1680.5

6	C-H (Aliphatic Bending)	1465	1466.92

4.6 Preparation of solution

4.6.1 Preparation of Diluent

Weigh and dissolve about 2.72 gm of potassium dihydrogen phosphate in 1000 mL of water. Adjust the pH to 2.5 □ 0.05 with dilute orthophosphoric acid. Filter the solution through Millipore PVDF 0.45□ membrane filter.

4.6.2 Preparation of blank solution

Use diluent as a blank solution.

4.6.3 Preparation of standard stock solution (40g/mL)

Accurately weigh and transfer about 20 mg of carglumic acid working/reference standard into a 50 mL clear and dry volumetric flask. Add about 30 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well. Transfer 5 mL of this solution into a 50 mL clean and dry volumetric flask, dilute to volume with diluent and mix well.

4.6.4 Preparation of Impurity stock solution (100g/mL)

0.1mg/mL of HPA impurity standard in diluent.

4.6.5 Preparation of standard solution (4g/mL)

Transfer 5 mL of standard stock solution and 2 mL of Impurity stock solution into a 50 mL clean and dry volumetric flask, dilute to volume with diluent and mix well.

4.6.6 Preparation of sensitivity solution (0.2g/mL)

Transfer 1 mL of standard stock solution into a 200 mL clean and dry volumetric flask, dilute to volume with diluent and mix well.

4.3.7 Preparation of excipient blend (placebo) solution

Accurately weigh and transfer excipient blend equivalent to 800 mg of carglumic acid into a 100 mL clean and dry volumetric flask. Add about 70 mL of diluent and sonicate (at about 20□C) for 30 minutes with intermittent shaking. Allow solution to attain room temperature. Dilute to volume with diluent and mix well. Filter solution through 0.45□ Nylon filter (make: Millipore Millex HN) by discarding initial first 5 mL of filtrate.

4.6.7 Preparation of sample solution (8000g/mL)

Accurately weigh 20 intact tablets and determine the average weight. Transfer 4 intact tablets into a 100 mL clean and dry volumetric flask. Add about 70 mL of diluent and sonicate (at about 20□C) for 30 minutes with intermittent shaking. Allow solution to attain room temperature. Dilute to volume with diluent and mix well. Filter solution through 0.45□ Nylon filter (make: Millipore Millex HN) by discarding initial first 5 mL of filtrate.

4.7 Development of the chromatographic method

4.7.1 Selection of Wavelength for Detection:

The standard solution of Carglumic acid was prepared for the selection of wavelength and scanned between 200-400 nm with the help of PDA detector. 205nm was selected as wave-length for estimation of related substances of Carglumic acid as shown in Fig.7.4.

4.7.2 Selection and Preparation of Mobile Phase

4.7.2.1 Selection of mobile phase

The standard and sample solution of Carglumic acid was injected into the HPLC system and run into solvent system. Various composition of the mobile phases showed in table 6.7 were tried in order to find the satisfactory conditions for separation of related substances of Carglumic acid. The optimum composition of the mobile phase selected is describe as below.

4.7.2.2 Preparation of the optimized mobile phase

E. Preparation of buffer solution:

Weigh and dissolve about 2.72 gm of potassium dihydrogen phosphate in 1000 mL of water. Adjust the pH to 2.5 □ 0.05 with dilute orthophosphoric acid. Filter the solution through Millipore PVDF 0.45□ membrane filter.

F. Preparation of mobile phase-A:

Use buffer solution as a mobile phase-A.

G. Preparation of mobile phase-B:

Prepare a mixture of methanol and buffer solution in the ratio of 60:40%v/v and sonicate to degas.

H. Preparation of diluent:

Use mobile phase-A as a diluent.

4.7.3 Chromatographic Conditions

Column: X-select HSS T3, 250 mm x 4.6 mm, 5.0 □m (Make: Waters)

Wavelength: 210nm Flow rate: 1.0mL/minute Injection volume: 50µL

Column oven temperature: 30°C Sample cooler temperature: 5°C Run time: 60minutes

Retention time: ~ 6.8minutes

Mode: Gradient

Table: 4.7 Gradient program

Time (minutes)	Mobile phase-A (%v/v)	Mobile phase-B (%v/v)
0.0	100	0
23.0	99	1
35.0	70	30
43.0	55	45
46.0	100	0
60.0	100	0

4.8 Method Validation

Method validation parameters studied as per ICH guidelines and follows acceptance criteria as per In-house method of Emcure Pharmaceuticals Ltd.

4.8.1 Specificity

4.8.1.1 Interference from blank &placebo

Specificity is shown that the procedure is unaffected by the presence of impurities or excipients. Specificity of an analytical method indicates that the analytical method is able to measure accurately and specifically the analyte of interest without any interference of blank. It was performed by injected blank solution, placebo solution, sample solution, standard solution, HBA Impurity solution, Diaza Impurity solution, spiked sample solution. In this parameter, the peak purity angle,

peak purity threshold, peak purity were checked for interference of blank and placebo in standard solution, standard, sample solution and spiked sample solution as shown in Table 7.6.

4.8.2 Linearity and Range (n=5)

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. Linearity has carried out by preparing a series of solutions of carglumic acid (conc. range-0.206 to 12.384g/mL) & related substances (conc. range-0.083 to 12.384□g/mL) over the range of LOQ to 150% of limit level as given in Table 7.7, 7.8 & 7.9

4.8.3 Limit of Detection (LOD)

According to the ICH recommendation, the approach based on the standard deviation of the response and slope was used for the determination of LOD.

LOD=3.3 σ/s Where; σ = Standard deviation of the response

S = Slope of the calibration curve

4.8.4 Limit of Quantitation (LOQ)

According to the ICH recommendation, the approach based on signal to noise ratio was used for the determination of LOQ value of Carglumic acid & its related substances Table 7.10 to 7.12.

4.8.5 Accuracy

It was carried out to determine the stability and reliability of the proposed method. It should be assessed on samples spiked with known amounts of impurities. Accuracy was determined by calculating the % recovery of recovered impurities in the sample by the method in which known amounts of related substances was spiked at LOQ, 50 %, 100% ,150 % levels to the pre-analyzed samples. The recovered amounts of impurities were calculated at each level and % recovery was reported as discussed in 6.3 summary section in chapter 6.

For performing accuracy study known amounts of related substances in sample solution at LOQ, 50%, 100% and 150% level of target concentration was added to a pre-quantified sample solution of 8000µg/mL of Carglumic acid. Each solution was injected in triplicate and the % recovery was calculated by measuring the peak areas as discussed in 6.3 summary section in chapter 6.

4.8.5.1 Preparation of HPA impurity stock solution (200g/mL)

Accurately weigh and transfer about 10 mg of HPA impurity standard in to a 50 mL clean and dry volumetric flask, add about 10 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

4.8.5.2 Preparation of HPA impurity solution (8g/mL)

Transfer 1 mL of HPA impurity stock solution into a 25 mL clean and dry volumetric flask. Dilute to volume with diluent and mix well.

4.8.5.3 Preparation of Diaza impurity stock solution (200g/mL)

Accurately weigh and transfer about 10 mg of Diaza impurity standard in to a 50 mL clean and dry volumetric flask, add about 10 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

4.8.5.4 Preparation of Diaza impurity solution (8g/mL)

Transfer 1 mL of Diaza impurity stock solution into a 25 mL clean and dry volumetric flask. Dilute to volume with diluent and mix well.

4.8.5.5 Preparation of recovery solution at LOQ Level (Spiked in Excipient blend): Accurately weigh and transfer excipient blend equivalent to 800 mg of carglumic acid into a 100 mL clean and dry volumetric flask. Add about 70 mL of diluent, to this add 1 mL of HPA impurity solution and 1 mL of Diaza impurity solution and sonicate (at 20□C) for 30 minutes with intermittent shaking. Allow solution to attain room temperature. Dilute to volume with diluent and mix well. Filter solution through 0.45□ Nylon filter (make: Millipore Millex HN) by discarding initial first 5 mL of filtrate.

4.8.5.6 Preparation of Recovery solution at 100% Level (Spiked in sample):

Transfer 4 intact tablets into a 100 mL clean and dry volumetric flask. Add about 70 mL of diluent, to this add 4 mL of HPA impurity stock solution and 4 mL of Diaza impurity stock solution and sonicate (at 20□C) for 30 minutes with intermittent shaking. Allow solution to attain room temperature. Dilute to volume with diluent and mix well. Filter solution through 0.45□ Nylon filter (make: Millipore Millex HN) by discarding initial first 5 mL of filtrate.

4.8.6 Precision

It provides an indication of random error in results and was expressed as % related standard deviation (%RSD). It is performed in terms of repeatability and intermediate precision.

4.8.6.1 Repeatability (Method Precision)

Method precision was carried out by analyzing six set of spiked 100% level sample solution which was performed as discussed in 6.3 summary section in chapter 6.

4.8.6.2 Intermediate Precision

Intermediate precision was performed by analyzing six set of spiked 100% level sample solution which was performed as discussed in 6.3 summary section in chapter 6. By different analyst using different instruments by using a column of same make but different lot.

4.8.7 Robustness

Robustness was carried out by analyzing standard solution and spiked 100% level sample solution as discussed in 6.3 summary section in chapter 6. By changing the following parameters one by one and observed their effects on system suitability.

Change in flow rate by ± 0.1mL/min (i.e. 0.9mL/min and 1.1mL/min)

Change in pH of buffer solution ± 0.2pH (i.e. pH2.3 and pH2.7)

Change in wavelength ± 2nm (i.e. 208nm and 212nm)

Change in column oven temperature ± 5°C (i.e. 25°C and 35°C)

Change in organic ratio of mobile phase B ±5% (i.e. 45:55%v/v and 35:65%v/v)

5. Results and Discussion

5.1 Method Development and Validation of Carglumic acid and Its Related Substances in Tablet Dosage form by RP-HPLC.

5.1 Selection of Wavelength for Detection:

The standard solution of Carglumic acid was prepared for the selection of wavelength and scanned between 200-400 nm with the help of PDA detector. 205nm was selected as wave-length for estimation of related substances of Carglumic acid.

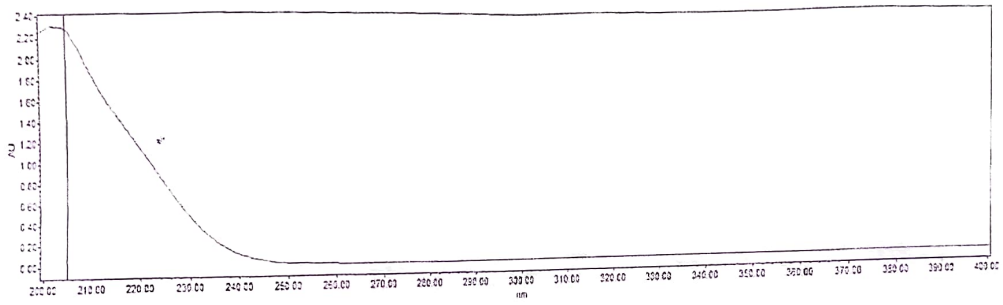


Fig. 5.1 PDA Spectra of Carglumic Acid for selection of wavelength (200-400 nm)

5.1 Selection of mobile phase:

Various compositions of the mobile phases were tried as shown in table 6.1. They were taken in order to find the best conditions for estimation of related substances of Carglumic acid.

Time (min)	M.P A	M.P B
0	100	0
30	95	5
40	50	50
63	50	50

64	100	0
70	100	0

Time (min)	M.P A	M.P B
0	100	0
30	95	5
40	50	50
63	50	50
64	100	0
70	100	0

Time (min)	M.P A	M.P B
0	100	0
30	95	5
40	50	50
63	50	50
64	100	0
70	100	0

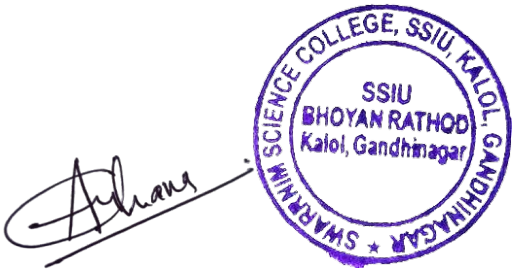
5.1.1. Method Development

Table: 5.1: Trials for selection of chromatographic condition

Trial	Diluent / Solvent Composition	Retention Time (min) - Carglumic Acid	Retention Time (min) - HPA Impurity	Retention Time (min) - Diaza Impurity
1	Phosphate buffer pH 2.5	6.843	12.772	17.79

Trial	Diluent / Solvent Composition	Retention Time (min) - Carglumic Acid	Retention Time (min) - HPA Impurity	Retention Time (min) - Diaza Impurity
2	Phosphate buffer pH 2.5: Methanol (70:30)	6.999	12.772	17.765
3	Phosphate buffer pH 2.5: Methanol (90:10)	6.358	13.303	17.700
4	Water	6.727	12.541	17.492
5	Water	6.727	12.541	17.492

Parameter	Value
Buffer Preparation	Phosphate buffer pH 2.5
M.P.A	Buffer solution
M.P.B	Buffer: Methanol (5:95% v/v)
Column	X-select HSS T3, 250 mm × 4.6 mm, 5 µm
Wavelength	205 nm
Flow Rate	1.0 mL/min
Injection Volume	50 µL
Column Oven Temp	30°C
Sample Cooler Temp	5°C
Run Time	70 min
Mode of Operation	Gradient



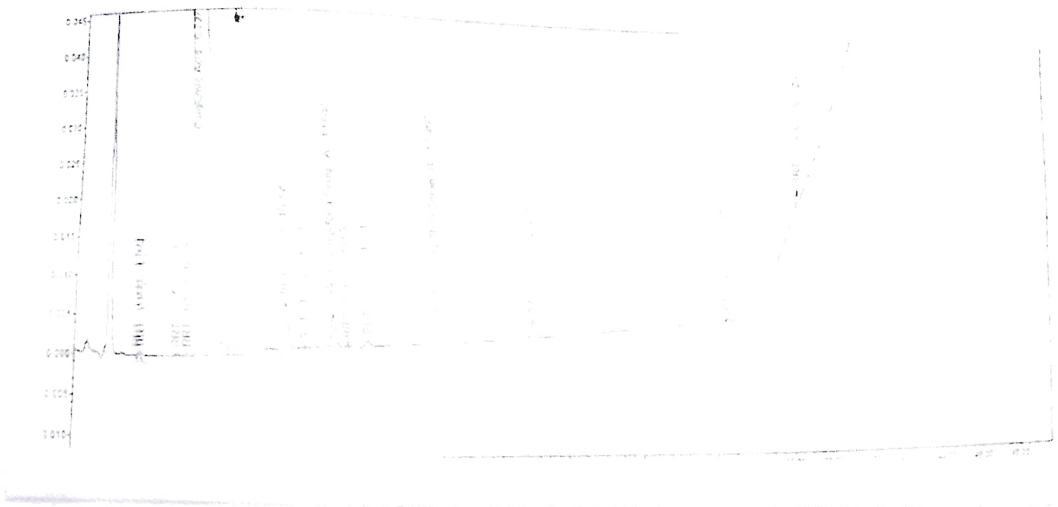


Fig 5.2: Chromatogram of sample at 205 nm Observation: Placebo interfere the main peak

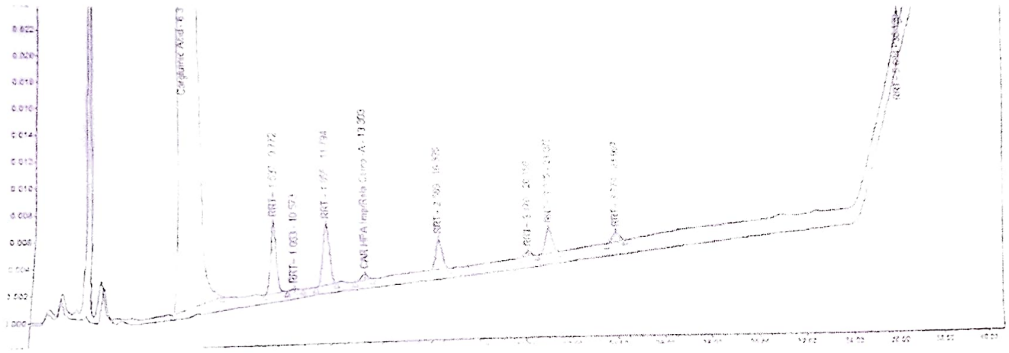


Fig 6.5: Chromatogram of sample at 205 nm Observation: Placebo interfere the main peak.

5.1Assay of marketed formulation

Applicability of the proposed method was tested by analysing the commercially available formulation Carbaglu tablet. The data are shown in the Table 6.2.

The % impurity were comparable to labelled value of drug in tablet formulation.

These results indicate that the development method is accurate and precise. It can be used in the routine quality control of formulation in industries.

Table 6.2 Data of Marketed formulation

Amount present in one tablet	Name of Related Substances	%Results
200 mg	HPA impurity	0.1
	Diaza impurity	0.1
	Unknown impurities	0.1
	Total impurities of Carglumic acid	0.75

5. Financial Statement

Certified that a grant of 5,00,000 *was received from the GCC BIOTECH for the project titled "* Method Development and Validation of Carglumic Acid and Its Related Substances in Tablet Dosage Form by Rp-Hplc*". The amount has been utilized as per the approved budget and guidelines.*

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹2,60,000
2	Chemicals	₹1,50,000
3	Travel	₹40,000
4	Contingency	₹50,000
	Total	₹5,00,000

7.Conclusion

The method has shown adequate separation of main peaks from their associated degradation products. Separation was achieved on X-select HSS T3 (250 mm x 4.6 mm, 5µm) column at 30°C temperature by using a mobile phase A: B as mobile phase A: Buffer(pH:2.5) & Mobile phase B:

Buffer: Methanol (40:60%v/v) with gradient program at a flow rate of 1.0 ml/min and detection was at 210 nm. Specificity of the method was established by determining the peak purity of the drug in samples using PDA detector. Data suggests that peak purity angle was found to be less than peak purity threshold, so there is no co-elution of any degradation products with main peaks and the results obtained were found within the acceptance criteria.

8.Reference

1. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs—A review. *Journal of Pharmaceutical Analysis, 4*(3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003
2. Bansal, G., & DeStefano, A. (2007). Key elements of a successful stability indicating HPLC method. *Journal of Liquid Chromatography & Related Technologies, 30*(5), 709–738.
3. International Council for Harmonisation (ICH). (2005). *ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology.*
4. International Council for Harmonisation (ICH). (2006). *ICH Q3A(R2): Impurities in New Drug Substances.*
5. International Council for Harmonisation (ICH). (2006). *ICH Q3B(R2): Impurities in New Drug Products.*
6. Ali, I., & Aboul-Enein, H. Y. (2006). Validated stability-indicating HPLC methods for drug stability testing and determination. *Journal of Liquid Chromatography & Related Technologies, 29*(5), 703–754.
7. ICH Q1A(R2). (2003). *Stability Testing of New Drug Substances and Products.*
8. Sahoo, U., & Sahoo, S. (2015). Development and validation of RP-HPLC method for determination of related substances of an active pharmaceutical ingredient. *International Journal of Pharmaceutical Sciences and Research, 6*(4), 1724–1730.
9. Barot, B. S., Parejiya, P. B., Mehta, D. M., & Shelat, P. K. (2012). Stability indicating RP-HPLC method for determination of drug and its impurities. *Journal of Liquid Chromatography & Related Technologies, 35*(1), 106–120.
10. Vaishnav, P., & Dholakia, R. (2021). Stability indicating RP-HPLC method development and validation of carglumic acid in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology, 14*(4), 2123–2128.
11. Hote, B. S., & Bhinge, S. D. (2015). Stability indicating RP-HPLC method for estimation of pharmaceutical impurities. *Journal of Applied Pharmaceutical Science, 5*(7), 98–105.
12. Pagar, S. V., & Saudagar, R. B. (2020). Analytical method development and validation: A concise review. *World Journal of Pharmaceutical and Medical Research, 6*(9), 422–426.

13. Patil, P. M., & Jadhav, S. B. (2021). Development and validation of analytical method for determination of pharmaceutical impurities. *Asian Journal of Pharmaceutical Analysis, 11*(1), 33–38.

14. Puranik, M. P., & Rathod, M. K. (2019). Analytical method development and validation for identification and quantification of related substances. *International Journal of Pharmaceutical Sciences Review and Research, 56*(2), 43–48.

15. Malode, S. J., & Dhabale, P. N. (2020). RP-HPLC method for simultaneous estimation of drugs and related substances. *International Journal of Current Pharmaceutical Research, 12*(1), 40–45.

16. Vaidya, A., & Vyas, M. (2020). Stability indicating HPLC methods for pharmaceutical dosage forms: A review. *Pharma Science Monitor, 11*(2), 120–135.

17. Pawar, P. A., & Narkhede, S. B. (2019). Analytical techniques for determination of related substances and impurities in pharmaceuticals. *Asian Journal of Pharmaceutical Analysis, 9*(1), 15–19.

18. Trivedi, R. K., & Patel, M. C. (2020). RP-HPLC method development and validation for quantitative estimation of carglumic acid and its impurities. *World Journal of Pharmacy and Pharmaceutical Sciences, 9*(12), 125–136.

19. Gandhi, S. V., & Patel, A. K. (2018). A validated RP-HPLC method for analysis of amino acid-based drugs. *Journal of Chromatographic Science, 56*(3), 205–212.

20. Raval, P., & Patel, A. (2017). Analytical method development and validation for identification and quantification of pharmaceutical drug impurities. *Asian Journal of Research in Chemistry, 10*(3), 352–358.

21. Maheshwari, D. G., & Bhatt, K. K. (2016). Analytical method development for determination of drug substance and its impurities. *International Journal of Pharmaceutical Sciences and Research, 7*(12), 5026–5031.

22. Goud, K. V., & Rao, V. R. (2015). A review on stability indicating analytical method development and validation. *International Journal of Pharmacy and Biological Sciences, 5*(2), 73–86.

23. Kumar, R., & Singh, A. (2018). Development of RP-HPLC method for quantification of active pharmaceutical ingredients. *International Journal of ChemTech Research, 11*(4), 85–91.

24. Wankhede, S. B., & Deshmukh, S. N. (2020). HPLC methods for the analysis of drug impurities: An overview. *Asian Journal of Pharmaceutical and Clinical Research, 13*(2), 45–50.

25. Patel, H. R., & Chaudhary, A. B. (2019). Forced degradation and stability studies of carglumic acid by RP-HPLC. *PharmaTutor, 7*(6), 45–51.

To, **Gpcb Id**: 10653
Industry Name: Jay Chemical Industries Ltd (Unit-3)

Plot No.: 172, Near Ajanta Industrial Estate, Vasana-Iyava, Sanand 382170

To, **Aud Id**: 1349
Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1349) shall carry out Environment audit work of M /s **Jay Chemical Industries Ltd (Unit-3)** (**10653**) situated at Plot No. 109/220, Phase 2, GIDC Vatva, Ahmedabad 382445 for the financial ye

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653
Industry Name: Jay Chemical Industries Ltd (Unit-3)

Plot No.: 172, Near Ajanta Industrial Estate, Vasana-Iyava, Sanand 382170

To, **Aud Id**: 1228
Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1228) shall carry out Environment audit work of M /s **Jay Chemical Industries Ltd (Unit-3)** (**10653**) situated at Plot No. 109/220, Phase 2, GIDC Vatva, Ahmedabad 382445 for the financial ye

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653
Industry Name: Matangi Industries LLP

Plot No.: Plot No. 28, Phase 2, GIDC Vatva, Ahmedabad 382445

To, **Aud Id**: 1056
Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1349) shall carry out Environment audit work of M /s **Matangi Industries LLP** (**1056**) situated at Plot No. 28, Phase 2, GIDC Vatva, Ahmedabad 382445 for the financial year 2019-20

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653
Industry Name: Odhav Enviro Project Ltd.

Plot No.: 25, 12-B, GIDC Odhav

Dist:Ahmedabad - 382415

To, **Aud Id**: 1011
Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1011) shall carry out Environment audit work of M /s **Odhav Enviro Project Ltd.** (**10653**) situated at Plot No. 25, 12-B, GIDC Odhav, Ahmedabad 382415 for the financial year **2019-20** with the following terms and conditions:

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653

Industry Name: Rhythm Biocare(Common Biomedical Waste Treatment Facility)

Plot No.: Survey No.331, Chadotar gadh Road, Vedancha-385511, Ta:- Palanpur, Dist:- Banaskantha

To, **Aud Id**:1540

Auditor Name: Swarnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarnim Startup and Innovation University** (1540) shall carry out Environment audit work of M /s **Rhythm Biocare(Common Biomedical Waste Treatment Facility)** (1435) situated at Survey No.331, Chadotar gadh Road, Vedancha- 385511, Ta:- Palanpur, Dist:- Banaskantha. for the financial year 2019-20

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653
Industry Name: Sanand Eco Project Ltd

Plot No.: 172, Near Ajanta Industrial Estate, Vasana-Iyava, Sanand 382170

To, **Aud Id**: 1124
Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1114) shall carry out Environment audit work of M /s **Sanand Eco Project Ltd.** (**10653**) situated at Plot No. 172, Near Ajanta Industrial Estate, Vasana-Iyava, Sanand 382170 for the financial year **2019-20** with the following terms and conditions:

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

To, **Gpcb Id**: 10653

Industry Name: The Bavla Eco Project.
Jal Shuddhikaran sahakari Mandli Ltd.(CETP)

Plot No.: Survey No. 1440/1,
Opp.Vijyalaxmi Rice Mill, Bavla,
Ahmedabad-382220.

To, **Aud Id**:1435

Auditor Name: Swarrnim Startup and Innovation University

At Post-Bhoyan Rathod, Opp. IFFCO (ONGC WSS), Adalaj-Kalol Highway, Gandhinagar-382420

Phone : 9016931688

Subject: Allotment Of Auditor Vice versa Industry under Environment Audit Scheme for the year **2019-20**

The Hon'ble High Court of Gujarat introduced the Environment Audit Scheme in its order dt 20 / 12 / 1996 in (Misc. Civil Application No. 1863 of 1995 with Misc. Civil Application No. 1967 of 1995 with Misc. Civil Application No. 93 of 1995 and in its order dated 13/3/1997) Special Civil Application No. 770 of 1995.

The Environment Audit Scheme modified vide Office Order No. GPCB / EAS - C- 28 / 301928 Dated: 23-01-2015.

Without prejudice to the power of this Board under the Environment Acts, this is to inform you that **Swarrnim Startup and Innovation University** (1435) shall carry out Environment audit work of M /s **The Bavla Eco Project. Jal Shuddhikaran sahakari Mandli Ltd.(CETP)** (1435) situated at Survey No. 1440/1, Opp.Vijyalaxmi Rice Mill, Bavla, Ahmedabad-382220. for the financial year 2019-20

1. The Industry shall allow environment auditor to carry out environment audit work and shall provide relevant data and documents required for environment audit work as per Environment Audit Scheme.(Which Is Available on www.gpcb,gov.in)

2. The Industry shall pay environment audit fee to the assigned environment auditor as per the provision of Office Order No. GPCB / EAS -C-28 / 301928 dated: 23-01-2015.

3. Any non - payment towards environment audit work to the auditor by the industry , shall be liable for legal actions under prevailing rules and regulations.

4. In case of excess charges to the industry by the auditor, under the Environment Audit Scheme, the auditor shall be liable for legal actions under prevailing rules and regulations , which may lead to de-recognition as Environment Auditor.

5.The environment auditor shall Upload Report in XGN as per formate of audit report prescribed in environment audit Scheme.within 15 day from date of monitoring carried out in industry.Non compliance in this regards will attract legal actions under prevailing rule and regulation,which may lead to de-recognition as Environment Auditor.

6.The auditor shall prepare environment audit report on the environment management system as per Environment audit scheme

7. The Environment audit report shall be submitted by 30th June, 2024.

For and on behalf of
Gujarat Pollution Control Board

Place: Gandhinagar

D. M. Thaker.

(Member Secretary)

Swarrnim Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

Digitally signed by Ragin Ravindrabhai Shah
DN: c=IN, o=Personal, title=4505, pseudonym=02aef9bb114542e5bb44d53ee0d1fcce, 2.5.4.20=f2d4874d45a02b76d89e3beeaf63b5e9f5408942de4f9537c58eeb40d7951483, postalCode=380015, st=Gujarat, serialNumber=0f260a2fa77309ae82dc5aebe10f5cf7163c41741552ca365cb67407666b44d6, cn=Ragin Ravindrabhai Shah
Date: 2024.10.10 10:45:46 +05'30'