SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **SPECHROM Solutions 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 SPECHROM Solutions."

TERMS AND CONDITIONS

This Contract shall commence on **11th January 2021** 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 SPECHROM Solutions

For Swarrnim Startup and Innovation University

Swarrnim Startup & Innovation University ★ Gandhinagar

Date: 11.5.2021

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

It is our pleasure to inform you that the project for consultancy which has been under discussion for quite sometimes is granted. The details are as follows:

Project Title:

"Hplc Method Development and Validation for the Estimation of Vildaligliptin in Combinations of Pharmaceutical Dosage Forms'"

Project Timeline:

The project is expected to be completed within the next 3 to 4 months.

Payment:

A total amount payable after successful completion of Project will be Rs. 4,00,000 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 SPECHROM Solutions

SPECHROM SOLUTIONS * AHMEDABAD *

SWARRNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE.
INDIA'S FIRST UNIVERSITY FOR STARTUP

Ref.No.swarrnim/RO/SCR/2021/38 Date: 19.08.2021

To,

SPECHROM SOLUTION.

SF/209, Trade Square, Sabarmati,

Ahmadabad, 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: "**Hplc Method Development and Validation for the Estimation of Vildaligliptin in Combinations of Pharmaceutical Dosage Forms.**"

Date of assigning problem: 11.05.2021

Date of completion: 16.08.2021

Name of person: Dr. Amita Mishra

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 Dr. Amita Mishra.

Thanking you.

Patel

Registrar

Swarrnim Startup & Innovation University, Bhoyan Rathod, Gandhinagar

HPLC Method Development and Validation for the Estimation of Vildagliptin in Pharmaceutical Dosage Form

Research Project Report Submission to

SPECHROM SOLUTION

SWARRNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE.

Submitted by:

Principal Investigator: Dr. Amita Mishra, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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

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- **Declaration**

I, Dr. Amita Mishra, Principal Investigator of the project titled " **HPLC Method Development and Validation for the Estimation of Vildaligliptin in Pharmaceutical Dosage** ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

Name:

Principal Investigator: Dr. Amita Mishra

Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

I extend my sincere gratitude to the Spechrom solutions for funding this project. I also thank my institution, colleagues, and students who supported and contributed to the successful completion of this project. Special thanks to [mention key contributors].

- **Summary**

This study focuses on the development and validation of a High-Performance Liquid Chromatography (HPLC) method for the estimation of Vildagliptin in pharmaceutical dosage forms. The method is optimized for accuracy, sensitivity, and reproducibility. Validation ensures compliance with international standards, evaluating parameters like specificity, linearity, precision, and stability. This validated HPLC method is essential for quality control, ensuring consistent product quality and safety for Vildagliptin-containing medications.

- **Detailed Report**

1. Introduction

The Vildagliptin (Fig. 1) is an oral anti-diabetic drug, potent dipeptidyl peptidase IV (DPP-IV) inhibitor for the treatment of diabetes 1,2. Chemically, it is (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type-2 diabetes3. DPP-IV inhibitors improve fasting and postprandial glycaemic control without hypoglycaemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas4,5.

Vildagliptin is an oral agent which is a member of a new class of hypoglycemic drugs, dipeptidylpeptidase-4 (DPP-4) inhibitors. This review presents the physicochemical properties of vildagliptin and assesses analysis methods for its estimation in substances, medicinal formulations, and biological media. These are chromatographic, spectrophotometric, electrochemical and other analysis methods. The material presented may be useful for developing new methods for analysis of medicinal formulations containing vildagliptin. The most widely used method for assay of vildagliptin is HPLC.

Literature survey revealed that few analytical methods such as spectrophotometric6-8, HPLC9-17 and LC-MS18,19 methods have been reported for the estimation of Vildagliptin in alone or in combination with other drugs. Hence a new sensitive and accurate HPLC method was developed and validated as per ICH guidelines20 for the estimation of Vildagliptin in bulk sample and in pharmaceutical dosage form.

Precision

The intra-day precision study of Vildagliptin was carried out by estimating the correspondence responses six times on the same day with 100 µg/ml concentration and inter-day precision study of Vildagliptin was carried out by estimating the correspondence responses six times next day with 100 µg/ml concentration.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analysed over a period of 8 hours at room temperature.

Robustness

Robustness of the method was studied by changing the composition of organic phase by ±4% and the pH by ± 0.1, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

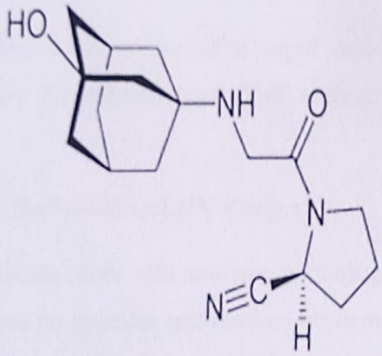


Fig:1 Structure of Vildagliptin

2. Literature Review

Methods for Analysis of Vildagliptin

The Russian literature contains a number of publications on vildagliptin, though all are mainly on pharmacological and medical themes. In the present article we are guided by information in non-Russian scientific journals on methods for analysis of vildagliptin, both in pure form and in mixtures with other antidiabetic components. Vildagliptin is not yet included in any of the world's pharmacopeias. Below we present possible methods for analysis of vildagliptin.

Titrimetric Methods:

Report [17] presented data from studies of the potential use of conductometric titration of vildagliptin and its analogues. This method is based on the ability of vildagliptin and its analogues to form complexes with copper (II) ions in the conductometer cell, which leads to a change in the conductivity of the solution. This method is both simple, rapid, and economical.

IR Spectroscopy:

The IR spectrum of vildagliptin obtained in potassium bromide tablets has characteristic absorption bands at 3294 cm-1 (n, stretch vibrations of -O-H and -N-H), 2992,

2915, 2849 cm-1 (stretch vibrations of -CH in the aliphatic chain), 2238 cm-1 (stretch vibrations of the nitrile group -CN), 1658 cm-1 (stretch vibrations -C=O), 1405, 1354 cm-1 (δ, deformational vibrations (-CH in the aliphatic chain), 1254 cm-1 (, C-N), 1120, 1103 cm-1 (i C-O(H)), 1054, and 1035 cm-1 (, C-O(H)) (cycloalkane in the 3-hydroxyadamantane fragment) [9].

The literature contains descriptions of a rapid and simple method of near-infrared reflection spectroscopy for quality control of vildagliptin and other antidiabetic drugs [17, 18].

Spectrophotometry in the Visual and UV Ranges:

The vildagliptin molecule does not contain a conjugated double-bond system, so its ultraviolet spectrum has no specific and marked absorption peaks. Figure Figure88 shows that the spectrum has a peak at around 200 nm. Despite this, work reported in [10] proposed a validated spectrophotometric method for assay of vildagliptin at an analytical wavelength of 266 nm, with a test solution concentration of 200 μg/ml and water as solvent. Report [19] proposed an analytical wavelength of 244 nm. A wavelength of 202.5 nm in 0.5 M HCl at a vildagliptin test solution concentration of 25 μg/ml has been reported [20]. A spectrophotometric method for assay of vildagliptin with metformin hydrochloride using first derivative spectra has been described [8], as has assay of vildagliptin using the second derivative spectrum [21].

High-Performance Liquid Chromatography with UV Detection:

Studies [29 – 35] used analytical wavelengths of 239, 260, and 290 nm. We obtained UV spectra in buffer solutions with different pH at concentrations of 10 and 200 μg/ml (Fig. (Fig.8).8). As shown in Figs. Figs.88 and and9,9, the expected second absorption bands at around 239, 260, or 290 nm proposed as analytical wavelengths, were not seen.

Vildagliptin is basic in nature (it ionizes in acidic media) [36]. Selection of mobile phase buffer solution pH usually depends on the pKa of the substance being assayed. Vildagliptin has to be in the ionized state. Publications therefore generally use a mobile

phase pH of 7.0 [9, 16, 21, 30, 32, 34]. As shown in Table Table2,2, some authors use buffer solutions with other pH values, as guided by the task being addressed and the properties of substances accompanying vildagliptin.

High-Performance Liquid Chromatography with Mass Detection:

The authors of [16] used mass spectra to propose the structure of a vildagliptin contaminant formed during synthesis. The main degradation products were confirmed and clarified in [44]. The mechanisms of formation of degradation products were described and their structures were determined in [13]. Alkaline hydrolysis produced peaks with products with relative retention times (RRT) of 1.2, 0.6, and 0.4, while acid hydrolysis generated a product with RRT 1.3; m/z values were 337.2, 321.1, and 322.6. A further three decomposition products were also observed on oxidative oxidation of vildagliptin, one with RRT 0.38 and m/z 241.1, the second identical to the product formed on alkaline hydrolysis with RRT 0.6, and a third with RRT 0.8 and m/z 183.1 [13].

Furthermore, there are data on vildagliptin assay in plasma by HPLC-MS in bioanalytical studies [50] and in mixtures with metformin hydrochloride in plasma using an Atlantis HILIC Silica 150 × 2.1 mm, 3µ column [52].

Capillary Electrophoresis:

The literature contains a description of vildagliptin assay by capillary electrophoresis. The assay used fused quartz capillaries with a potential of 25 kV (positive polarity). The supporting electrolyte was 25 mM potassium phosphate pH 8.0 with detection at 207 nm. Electrophoretic separation was run in 6 min and was linear over the concentration range 50 – 200 µg/ml [53]. The authors of [54] assayed vildagliptin in rat plasma, after precipitation of plasma protein with acetonitrile, using sitagliptin as internal standard. Vildagliptin was separated from plasma components using flamed quartz capillaries with supporting electrolyte consisting of 0.25 mM ammonium formate. Detection was by mass spectrometry.

Gas-Liquid Chromatography:

A method for vildagliptin assay by gas chromatography with mass detection was described in [46]. The stationary phase was a capillary column with 5% methylphenyl polysiloxane (30 m × 0.25 mm, 0.25 µ, Agilent Technologies, USA) and the

derivatization agent was N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and catalysts were β-mercaptoethanol and ammonium iodide (NH4I). The trimethylsilyl derivative was attached at the hydroxyl group of vildagliptin. The internal standard was nandrolone.

GLC with flame ionization detection was used to determine the initial product of 3-aminoadamantol as contaminant of vildagliptin [7, 9]

3. Objectives

1. To synthesize high-quality Graphene Oxide (GO) for experimental purposes.
2. To evaluate its adsorption efficiency for removing heavy metals and organic pollutants.
3. To propose an optimized treatment process for real-world applications.

4. Methodology

Instrumentation:

The liquid chromatographic system consisted of Waters HPLC system equipped with a reverse phase Altima C18 column (150 mm x 4.6mm; 5 µm), a 2695 binary pump, a 10 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower 2 software. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials:

The working standard of Vildagliptin was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation GALVUS tablets (Vildagliptin 50 mg) were procured from local market. Acetonitrile of HPLC grade was purchased from E. Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purification through milli-Q water purification system. Orthophosphoric acid of analytical grade was procured from Qualigens, Mumbai, India.

Preparation of standard stock solution

10 mg of Vildagliptin was accurately weighed, transferred to 10 ml volumetric flask and is dissolved in 7 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 ml with diluent to get a concentration of 1 mg/ml stock solution. Further pipetted 0.4 ml of the

above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to obtain required concentrations.

Preparation of sample Solution

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Vildagliptin was transferred to 10 ml volumetric flask and is dissolved in 7 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 ml with diluent. Further pipetted 0.4 ml of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to obtain required concentration.

Chromatographic conditions

HPLC was connected with Altima C18 column (150 mm x 4.6 mm, 5 µm) as stationary phase. A mixture of dilute orthophosphoric acid solution pH 2.6±0.5 as buffer and acetonitrile in the ratio of 72:28 v/v was prepared and used as mobile phase. The orthophosphoric acid buffer solution was prepared by transferring about 1 ml of concentrated orthophosphoric acid into 1000 ml standard flask, add 400 ml of milli-Q water, mix and dilute to volume with illi-Q water, sonicate for five minutes and cool to room temperature, measure the pH of above solution and finally adjusted the pH to 2.6 with orthophosphoric acid solution and filtered through 0.45 µ nylon filter. The 100% water was used as diluent. Injection volume was 10 µL and flow rate was 1.0 mL/min and run time was 7.0 min. The column was maintained at ambient temperature and the eluent was monitored at 266 nm.

Calibration curve

Appropriate aliquots of standard Vildagliptin stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 25, 50, 75, 100, 125 and 150 µg/ml of Vildagliptin. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Vildagliptin was constructed by plotting peak area ratio versus applied concentration of Vildagliptin and regression equation was computed. Similarly, the sample solution was

chromatographed and concentration of Vildagliptin in tablet sample was found out using regression equation.

Method validation

The method was validated for linearity, specificity, limit of detection, limit of quantification, accuracy, precision, stability and robustness by following procedures.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 25-150 µg/ml for Vildagliptin. Evaluation of the drug was performed with UV detector at 266 nm, peak area was recorded for all the peaks. The correlation coefficient value of Vildagliptin was 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Specificity

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Vildagliptin was found to be 0.06 µg/ml and the LOQ for Vildagliptin was found to be 0.21 µg/ml.

Accuracy

The accuracy of the method was determined by calculating recovery of Vildagliptin by the method of standard addition. Known amount of Vildagliptin was added to a pre-quantified sample solution and the amount of Vildagliptin was estimated by measuring the peak area ratios and by fitting these values to the straight-line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range of 50%, 100% and 150% levels. The amount of Vildagliptin was estimated by measuring the peak area ratios by fitting these values to the straight-line equation of calibration curve. From the

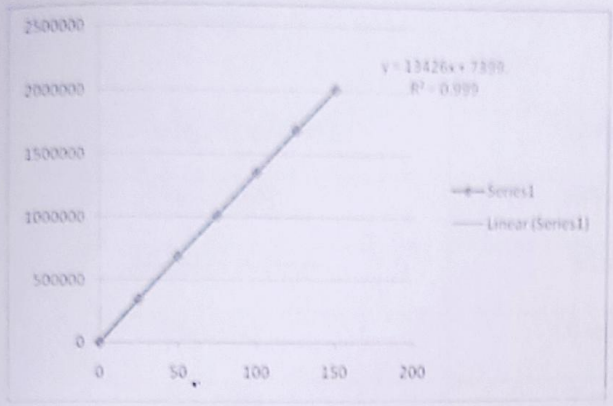
above determination, percentage recovery and standard deviation of percentage recovery were calculated.

5. Results and Discussion

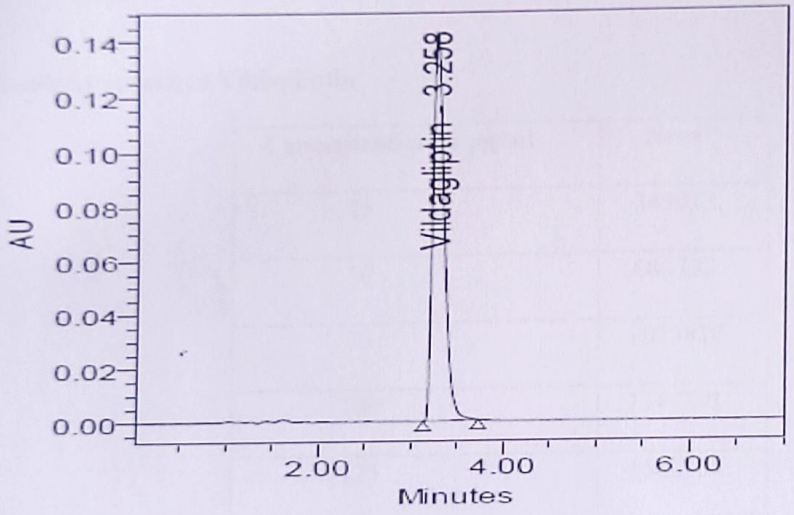
The procedure was optimized with a view to develop an accurate and precise HPLC method in tablet dosage form using Altima C18 column (150 mm x 4.6 mm, 5 µm) in isocratic mode with mobile phase composition of dilute orthophosphoric acid solution pH 2.6±0.5 as buffer and acetonitrile (72:28 v/v) and pH adjusted to 2.6 with orthophosphoric acid. The use of dilute orthophosphoric acid and acetonitrile in the ratio of 72:28 v/v resulted in peak with good shape and resolution. The flow rate was 1.0 ml/min and the drug component was measured with UV detector at 266 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 25 to 150 µg/mL for Vildagliptin with correlation coefficient of 0.999.

The regression equation of Vildagliptin, concentration over its peak area ratio was found to be Y=13426X+7399, where X is the concentration of Vildagliptin and Y is the respective peak area. The linearity results were shown in Table 2 and the linearity curve was shown in Fig. 2. The % recovery was found to be 99.73% for Vildagliptin, which indicate the method is accurate. The results of recovery studies were shown in Table 3. The %RSD for intra-day precision and inter-day precision for Vildagliptin were found to be 0.30 and 0.62, the values were less than 2% which indicate the method is precise. The results of precision studies were shown in Table 4.

The retention time of Vildagliptin was 3.258 min. The number of theoretical plates was 3529 and tailing factor was 1.28 for Vildagliptin, which indicates efficient performance of the column. The limit of detection and limit of quantification for Vildagliptin were found to be 0.06 µg/ml and 0.21 µg/ml, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters. Validated method was applied for the determination of Vildagliptin in commercial formulations. The %assay was found to be 99.88% for Vildagliptin and the assay results were shown in Table 6. Typical chromatogram of drug Vildagliptin was shown in Fig. 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method.



Linearity Plot of Vidalogliptin



Typical HPLC Chromatogram of Vildagliptin

Optimized chromatographic conditions of Vildagliptin

Parameter	Condition
Mobile phase	Dilute orthophosphoric acid: acetonitrile (72:28 v/v)
pH	2.6
Diluent	Water

Column	Altima C18 column (150 mm x 4.6 mm, 5 µm)
Column temperature	Ambient
Wave length	266 nm
Injection volume	10 µl
Flow rate	1.0 ml/min
Run time	7 min
Retention time	3.258 min

Linearity results of Vildagliptin

Concentration in µg/ml	Area
25	343913
50	685732
75	1013810
100	1347391
125	1682700
150	2038528

Recovery results of Vildagliptin

Level	Standard concentration (µg/ml)	Concentration added (µg/ml)	Concentration found (µg/ml)	% Recovery	Mean recovery
50%	100	50	49.70	99.4	99.67%
100%	100	100	99.93	99.7	
150%	100	150	149.78	99.8	

System suitability and validation parameters of Vildagliptin

Parameter	Results
Linearity range (µg/ml)	25-150
Slope	13426
Intercept	7399
Correlation coefficient	0.999
Theoretical plates (N)	3529
Tailing factor	1.28
LOD (µg/ml)	0.06
LOQ (µg/ml)	0.21

Assay results of Vildagliptin

Formulation	Label claim	Amount found	%Assay
GALVUS	50 mg	49.94 mg	99.88 %

6. Financial Statement

Certified that a grant of ₹ 4,00,000 was received from the Spechrom Solution for the project titled " HPLC Method Development and Validation for the Estimation of Vildagliptin in Pharmaceutical Dosage Form ". The amount has been utilized as per the approved budget and guidelines

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹ 75,000
2	Chemicals	₹ 1,20,000
3	Travel	₹ 75,000
4	Contingency	₹ 1,30,000
Total		**₹ 4,00,000**

7. Conclusion

Proposed study describes new HPLC method for the estimation of Vildagliptin in tablet formulation. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of Vildagliptin in its tablet formulation

8. Reference

1.Thornberry NA and Gallwitz B. Mechanism of action of inhibitors of dipeptidyl-peptidase-4 (DPP-4). Best Practice and Research Clinical Endocrinology and Metabolism. 2009;23(4):479-486.

2.McIntosh CHS, Demuth HU, Pospisilik JA and Pederson R. Dipeptidyl peptidase IV inhibitors: how do they work as new antidiabetic agents? Regulatory Peptides. 2005;128(2):159-165.

3.Lankas GR, Leiting B, Sinha Roy R and Eiermann GJ. Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes potential importance of selectivity over dipeptidyl peptidases 8 and 9. Diabetes. 2005;54(10):2988-2994.

4.Weber AE. Dipeptidyl peptidase IV inhibitors for the treatment of diabetes. Journal of Medicinal Chemistry. 2004; 47:4135-4141.

5.Mentlein R. Dipeptidyl-peptidase IV (CD26) role in the inactivation of regulatory peptides. Regulatory Peptides. 1999;85(1):9-24.

6. Moneeb MS. Spectrophotometric and spectrofluorimetric methods for the determination of Saxagliptin and Vildagliptin in bulk and pharmaceutical preparations. Bulletin of Faculty of Pharmacy. Cairo University. 2013;51(2):139-150.

7. Shrikrishna B, Mulgund SV and Ranpise NS. Simultaneous spectrophotometric estimation of Vildagliptin and Metformin in bulk and tablet dosage form. Der Pharma Chemica. 2013;5(1):24-27.

8.El-Bagary RI, Elkady EF and Ayoub BM. Spectrophotometric methods for the determination of Sitagliptin and Vildagliptin in bulk and dosage forms. International Journal of Biomedical Science. 2011;7(1):55-61.

9. Sultana R, Bachar SC and Rahman F., Development and validation of stability indicating assay method of Vildagliptin in bulk and dosage form by RP-HPLC. International Journal of Pharmacy and Life Sciences. 2013;4(4):2530-2534.

10. Aparajita M, Bishwajit B and Dilruba N. Development and validation of RP-HPLC method for estimation of Vildagliptin from tablet dosage form. International Journal of Pharmaceutical and Life Sciences. 2012;1(1):1-6.

11.Pharne AB, Santhakumari B, Ghemud AS, Jain HK and Kulkarni MJ. Bioanalytical method development and validation of Vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP- HPLC method. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(3):119-123.

12.Mohammad MAA, Elkadyb EF and Fouadb MA. Development and validation of a reversed-phase column liquid chromatographic method for simultaneous determination of two novel gliptins in their binary mixtures with Metformin. European Journal of Chemistry. 2012;3(2):152-155.

13.Varma PSRChNP, Lakshmana Rao A and Dinda SC. Simultaneous determination of Metformin and Vildagliptin in solid dosage form by stability indicating RP-HPLC method. International Research Journal of Pharmacy. 2013;4(1):122-128.

14.Pushpalatha K and Ramachandran D. Method development and validation for the simultaneous estimation of Vildagliptin and Metformin in tablet dosage form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(1):459-463.

15.Santhosha B, Ravindranath A and Sundari CH. Validated method for the simultaneous estimation of Metformin hydrochloride and Vildagliptin by RP-HPLC in bulk and the pharmaceutical dosage form. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(3):22-28.

16. Subhakar N, Krishna Reddy V and Ravindranath Reddy T. Development and validation of RP-HPLC method for simultaneous determination of Vildagliptin and Metformin in bulk and formulation dosage. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(3):44-50.

17. Ishaq BM, Prakash KV and Mohan GK. RP- HPLC method for simultaneous estimation of Metformin and Vildagliptin in Bulk and its Tablet Formulation. Journal of Global Trends in Pharmaceutical Sciences. 2012;3(3):747-754.

18. Amanda TB, Barbara S, Schapoval EES and Steppe Stability-indicating RP-LC method for the determination of Vildagliptin and mass spectrometry detection for a main degradation product. Journal of Chromatographic Science.2012;50(5):426-432.

19.Martín J, Buchberger W, Santos JL, Alonso E and Aparicio I. High-performance liquid chromatography quadrupole time-of-flight mass spectrometry method for the analysis of antidiabetic drugs in aqueous environmental samples. Journal of Chromatography B. 2012;895-896:94-101.

20. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, Q2(R1), USA, 2005:1-13.

9.Certified by:

- Principal Investigator: Dr. Amita Mishra
- Head of Institution: Dr. Hemant Chaube

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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

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KARTIK VIKANI

For **Aum Research Labs Pvt Ltd.**

For Swarrnim Startup and Innovation University

Aum Research Labs

FAST • RELIABLE • ACCURATE

Date: 1.5.2021

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:

"Stability Indicating Rp-Hplc Method Development and Validation of Aminocaproic Acid in Pharmaceutical Dosage Form"

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
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Date: 02.10.2021

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: **"Stability Indicating Rp-Hplc Method Development and Validation of Aminocaproic Acid in Pharmaceutical Dosage Form."**

Date of assigning problem: 01.05.2021

Date of completion: 30.09.2021

Name of person: Mr. Mitesh Prajapati

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. Mitesh Prajapati.

Thanking you.

Wpatel

Registrar

Startup & Innovation University
Bhoyan Rathod
Gandhinagar

Stability Indicating Rp-Hplc Method Development and Validation of Aminocaproic Acid in Pharmaceutical Dosage Form

Research Project Report Submission to

Aum Research Lab

Submitted by:

Principal Investigator: Mitesh Prajapati, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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- **Summary**

The quality, safety, and efficacy of pharmaceutical dosage forms depend significantly on the stability of active pharmaceutical ingredients (APIs). Aminocaproic acid, a widely used antifibrinolytic agent, is prone to degradation under various stress conditions. Stability-indicating analytical methods are crucial for monitoring these changes and ensuring regulatory compliance.

This project aims to develop and validate a robust RP-HPLC method for aminocaproic acid that is capable of detecting and quantifying the drug and its degradation products, thereby ensuring reliable quality control and stability assessment.

Detailed Report

. Introduction

.1 Introduction to the Antifibrinolytics:

Fibrinolysis is a physiologic component of haemostasis that functions to limit clot formation. However, after tissue injury associated with trauma or surgery, ischemia and reperfusion, blood contact with large non-endothelial surfaces such as cardiopulmonary bypass (CPB) circuits, or as a contributing factor in other haemostatic disorders, excessive fibrinolysis may contribute to coagulopathy, bleeding, and inflammatory responses. Therefore, antifibrinolytic agents are increasingly used to reduce bleeding, allogeneic blood administration, and adverse clinical outcomes.

The two mainstay antifibrinolytic agents are the synthetic lysine analogues tranexamic acid and aminocaproic acid that use as antifibrinolytic. Antifibrinolytic agents are effective even when bleeding is not associated with laboratory sings of excessive fibrinolysis.

1.1.1 Antifibrinolytic, mechanisms:

Antifibrinolytic agents are bind reversibly to plasminogen interfere with lysin-binding sites on plasminogen. So, Antifibrinolytic block the binding of plasminogen to fibrin and its activation and transformation to plasmin.

1.1.2 Uses of Antifibrinolytic:

- Gastrointestinal Bleeding
- Bleeding in the urinary tract
- Liver transplantation
- Control of Mucosal in High-risk patient

1.1.3 Adverse Effect of Antifibrinolytic:

- Stroke,
- Myocardial infarction
- Coronary-bypass graft occlusion
- Venus and arterial thromboembolism

1.2 High Performance Liquid Chromatography (HPLC): [4-9]

Nowadays High-performance liquid chromatography [HPLC] is the one of the most powerful tools in analytical chemistry. It has the ability to identify, separate and quantify the compounds that are present in

nple that can be dissolved in a liquid. And due to high performance liquid chromatography pounds in trace concentrations as low as parts per trillion [ppt] can be easily be identified.

HPLC can be applied to many samples such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals. In HPLC system commonly used solvents include any miscible combinations of water or various organic liquids and the most common are methanol and acetonitrile. Water may contain buffers or salts to assist in the separation of the analyte components or compounds such as trifluoroacetic acid which acts as an ion pairing agent. HPLC is used either in the liquid-solid adsorption chromatography mode or the liquid-liquid partition chromatography mode, either normal or reversed phase.

1.2.1 Types of HPLC: [4-5]

The principal characteristic defining the identity of each technique is the dominant type of molecular interactions employed. There are three basic types of molecular forces: ionic forces, polar forces and dispersive forces.

There are four main types of HPLC techniques are:

1. Normal Phase HPLC

Normal Phase Chromatography is a powerful complement to the more popular reversed-phase high performance liquid chromatography (RP-HPLC) method for separating non-ionic compounds with moderate molecular weight. In this method, the stationary phase more polar than the mobile phase. Liquid chromatography system includes stationary phases as unmodified silica and other solid adsorbents (adsorption chromatography) and polar bonded phases (normal partition chromatography). All these phases are operated with no aqueous, non-polar or moderately polar mobile phase; Introduction this is an advantage because of the high solute solubility in totally organic solvents.

2. Reverse Phase HPLC

In Reverse Phase chromatography stationary phase is non-polar and mobile phase is polar in nature Reverse Phase Chromatography is the first choice for most regular samples. It is more convenient and rugged than other forms of liquid chromatography and is more likely to result in satisfactory final separation. High Performance Reverse Phase Chromatography columns are efficient, stable detection is obtained easier in the Reverse Phase Chromatography especially by UV detection because of the solvent used, though many organic compounds have limited solubility in aqueous mobile phase that is not a practical limitation because only small amount of sample is injected.

➢ **Separation mechanism of HPLC:**

ıion or liquid-liquid chromatography

.dsorption or liquid-solid chromatography

c) Ion - exchange or ion chromatography

d) Size - exclusion chromatography

e) Affinity chromatography

1.3. Application of HPLC: [8-9]

The High-Performance Liquid Chromatography is most widely used for analysis of various forms of dosage form like capsules, tablets, bulk drug, suspension and other solid and liquid dosage forms. And also having the following application.

- For drug in biological samples
- In biotechnology
- For analysis of surfactant
- For chiral pharmaceutical analysis
- Inorganic chemistry
- For the separation of metabolites of nucleic acid in physiological Fluid
- In food and nutritional analysis
- In physicochemical measurements
- Analysis of organic pollutants in water matrices
- For polymer analysis
- Analysis of ions and inorganic species

1.4 Introduction to Stability Indicating Assay Method: [10-15]

"It is defined as validated quantitative analytical method that can detect changes with time in chemical, physical or microbiological properties of drug substance of drug substance & products & that are specific, so that contents of active ingredient in degradation products & other components of interest, can be measured without interference."

According to FDA guidelines, a stability indicating assay method is defined as

ated analytical procedure that accurately and precisely measure active ingredient drug substance rug product free from potential interference like degradation product, process impurities, pients, or other potential impurities, and the FDA recommends that all assay procedure for stability tudies be stability indicating."

1.4.1 Classification of Stability indicating assay method

- Specific Stability indicating assay method
- Selective Stability indicating assay method

1) Specific Stability indicating assay method:

- A technique that is able to measure drug in presence of all degradation product, excipient and additives expected to be present in the formulation.

2) Selective Stability indicating assay method

- A technique that is able to measure the drug and all degradation products in presence of excipients and additive expected to be present in formulation.

1.4.2 Development of validated Stability Indicating assay method [11-15]

1. Study of drug structure to assess the likely decomposition route
2. Collection of information on physicochemical properties of drug
3. Stress Studies
4. Preliminary separation studies on stressed samples
5. Final method development and optimization
6. Identification and characterization of degradation products, and preparation of standards
7. Validation of stability indicating assay method

1.4.3 Purpose of stability testing: [11]

"Stability testing provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light." The degraded medication item give data into potential contamination or medication that might be created during stability testing. Stability testing permit the foundation of prescribed storage condition, retest period and shelf-lives. Forced degradation studies can likewise be utilized into rapidly asses packaging material similarity or sensitives.

1.5 Introduction to Analytical Method Validation: [16]

on:

dation of an analytical method is documents which provide a high degree of assurance that the ven method will consistently produce a product meeting its predetermined specification and quality attributes."

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 measurements of potential error.
- As per the International Conference on Harmonization (ICH) guidelines, the various parameters are specificity, accuracy, precision, LOD, LOQ, linearity, range and robustness

2.Literature Review

2.2 Aminocaproic acid

2.1.2 Official Method of Aminocaproic acid

Sr.	Pharmacopoeia	Method & Description	Ref.
1	USP 31	**RP-HPLC Method** **Mobile Phase**: Buffer : Methanol (65:35 % v/v) Buffer Preparation: 2 gm of monobasic sodium heptanesulphonate in 1 L water. Add 1 mL triethylamine. Adjust pH 2.2 with orthophosphoric acid. **Stationary Phase**: Legacy L1 (4.6 mm, 150 mm) **Flow Rate**: 0.7 mL/min **Detection Wavelength**: 210 nm **Injection Volume**: 20 µL	17

2	USP 39	**Titrimetric Method** **Mode:** Direct titration **Titrant:** 0.1 N perchloric acid indoxane **Sample solution:** 500 mg Aminocaproic acid + 100 mL Glacial acetic acid **Indicator:** 1 in 500 solution of Crystal violet in chlorbenzene **Detection:** Colourless to blue colour	18

2.1.2 Reported Method for Aminocaproic acid.

Sr.	Title	Method & Description	Ref.
1	Structure elucidation and quantification of impurities formed between 6-aminocaproic acid and the excipients citric acid and sorbitol in an oral solution using high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy	**HPLC-UV Method** **Mobile Phase:** 10 mM ammonium in water (pH=6.8) : Acetonitrile (40:60) % v/v **Stationary Phase:** Thermoscientific Acclaim™ Mixed-Mode WAX-1 column (150×3.0nm, 3µm) **Flow Rate:** 0.5 mL/min **Detection Wavelength:** 210 nm **HPLC-Mass Method** **HPLC: Mobile Phase:** A) 0.1% Formic acid (v/v) B) 0.1% Formic acid in 90% Acetonitrile (v/v) **Stationary Phase:** Phenomenex synergi Hydro-RP column (250×4.6nm, 4µm) **Flow Rate:** 0.5 mL/min **Detection Wavelength:** 210 nm **Mass: Capillary Temp.:** 370^{0}C **Capillary Voltage:** 3.30 kV **Sheath gas:** 25	19

		Auxillary gas: 10 **S-lens RF level:** 50 **Resolution in full scan:** 70,000 **Mass Range:** 100-600 m/z	
2	Assay of Aminocaproic Acid in Dosage Forms by Reversed Phase High Performance Liquid Chromatography with Dansylation	**RP-HPLC** **Mobile Phase:** Methanol : water: acetic acid: triethylamine (60: 38: 1.5 :0.5) %V/V/V **Stationary Phase:** Econsphere C_{18} **Flow Rate:** 1.5 ml/min **Detection Wavelength:** 335 nm	20
3	Development and validation of colorimetric method for the determination of Aminocaproic acid in bulk and pharmaceutical formulation	**Colorimetric Method** **Detection Wavelength:** 540 nm **Linearity Range:** 0.2-1.0 mg/ml **Correlation coefficient:** 0.9986	21
4	Development of Spectrophotometric Method for the Assay of Aminocaproic Acid in Dosage Forms Using Ascorbic Acid	**Colorimetric Method** **Detection Wavelength:** 390 nm, 530 nm **Linearity Range:** 4-20 µg/mL	22
5	Rapid and Sensitive Determination of Aminocaproic Acid Injection by Raman Spectroscopy Combined with	**Raman Microspore Spectrometer** **Spectral resolution:** 2 cm^{-1} **Semiconductor laser:** 780 nm **Semiconductor power:** 24 mW **Aperature slit:** 25 µm	23

	Chemometric Models		

3.Objectives of the work

1. To develop stability indicating RP-HPLC method for estimation of Aminocaproic acid in tablet dosage form
2. To perform forced degradation study in different degradation condition.
3. To perform validation parameter of RP-HPLC method according to ICH Q2 (R1) guidelines

4.Methodology

4.1 EXPERIMENTAL WORK:

Identification of drug

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

Checking melting point:

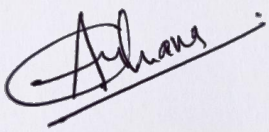
Melting point of Aminocaproic acid was check by using melting point apparatus. Melting point of Aminocaproic acid is show in below table.

Table: 4.1 Melting Point of Aminocaproic acid

Name of drug	Standard melting range	Observed melting range
Aminocaproic acid	207^0-209^0C	206^0-210^0C

IR spectra determination:

- An individual pellet of the drug and KBr (Spectroscopic grade) was prepared using hydraulic pellet press at a pressure of 7-10 tones. FT-IR was scanned from 400-4000 cm^{-1}. Peak observed were shown in IR spectra of each drug.
- IR spectra of Aminocaproic acid(Figure:6.1) was compared with their reference standard I.R. spectra (Figure:5.2) and all spectra were matched with their respective reference standard spectra.



- Principle IR peaks were observed for Aminocaproic acid is shown in Table 5.2 and from this data it was concluded that drug was found to be authentic.

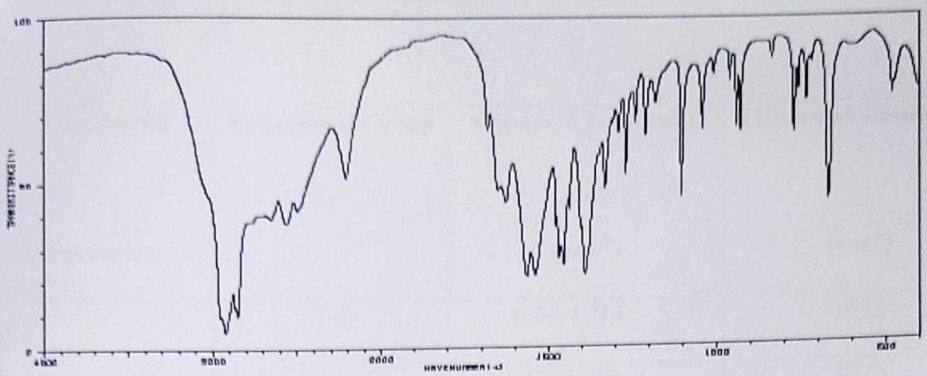


Figure 4.1: FT-IR spectra of reference Aminocaproic aci

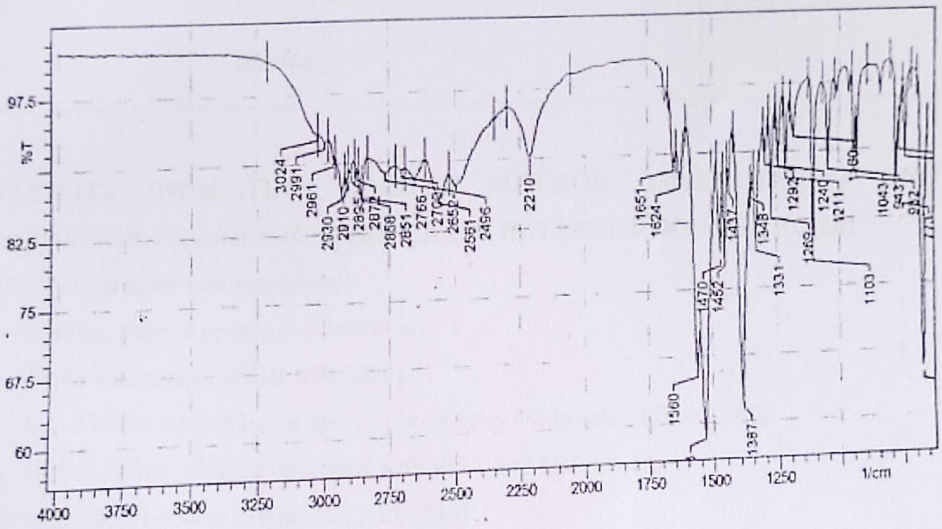


Table 4.2: IR interpretation of Aminocaproic acid data [24]

Compound Parameter	Functional Group	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
IR (cm^{-1})	C=O	1680-1630	1652
	O-H	3400-2400	3020
	CH (Stretching)	2960-2850	2851
	CH_2 (Aliphatic) (Banding)	1465	1470

4.2 STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF AMINOCAPROIC ACID IN TABLET DOSAGE FORM

3.1.2 Instrument and apparatus:

- Melting Point Apparatus (Electro lab)
- FT-IR Instrument (ABB MB 3000)
- UV- Visible double beam spectrophotometer (Shimadzu UV – 1800)
- HPLC (Water alliance equipped with PDA and UV detector)
- Analytical balance (Shimadzu AEU-210)
- pH meter (Metrohm)
- Digital ultra-sonic cleaner (LABMAN – LMCU series)
- Glass ware: Volumetric flask of 10, 20, 100, 250 mL (Borosile), Beaker of 250 and 500 mL (Borosile), Pipette of 1, 2, 5 and 10 mL (Borosile). Measuring cylinder of 10, 50, 100 and 1000 mL (Borosile).

4.3 Materials, Chemicals and Reagents:

Following materials, chemicals and reagents are used for research work.

Table 4.3: Materials

Sr. No.	Material name	Source
1	Aminocaproic acid	Emcure pharmaceutical Limited

Table 4.4: Chemicals and Reagents

Chemicals	Grade	Manufacture
Methanol	HPLC	Fisher scientific
Water	HPLC	Mili Q
Orthophosphoric acid	EMPARTA	Merck
Triethalamine	HPLC	Merck
Monobasic Potassium phosphate	EMPARTA	Merck
Heptane sulphonate monohydrate sodium salt	HPLC	Specroclem
Hydrochloric Acid	AR	Merck
Sodium Hydroxide	EMPARTA	Merck
Hydrogen Peroxide	EMPARTA	Merck

4.3.1 Marketed Formulation:

Table 4.5: Marketed Formulation

Brand Name	Amicar
Company Name	Clover pharmaceuticals Corp.
Label Claim	500 mg

4.3.2 PREPARATION OF SOLUTION:

4.3.2.1 Preparation of stock solution (1000 µg/mL):

curately weighed and transfer 100 mg of Aminocaproic acid in 100 mL clean and dry volumetric lask. 70 mL of diluent (water and Methanol in ratio of 80:20 v/v) added to dissolve the drug. Make up the volume up to mark with diluent and mix well.

4.3.2.2 Preparation of Standard solution of Aminocaproic acid (500 µg/mL):

An aliquot 50 mL of solution was transfer into 100 mL of volumetric flask and volume adjusted up to mark with diluent and mix well.

4.3.2.3 Preparation of sample solution:

Accurately weigh 20 tablets and determine the average weight. Accurately weigh and transfer 10 tablets equivalent to 500 mg in to 250 ml volumetric flask. Add 150 ml of diluent to it, sonicate for 30 minutes with intermittent shaking. Allow to attain room temperature. Dilute up to mark with diluent and mix well. Filter the solution through PVDF 0.45µ syringe filter by discarding initial 5 ml of filtrate and collect remaining filtrate as sample. Transfer

5.0 ml of this solution in to 20 ml volumetric flask. Dilute up to mark with diluent and mix well.

4.3.3 SELECTION OF WAVELENGTH FOR DETECTION:

Standard solution of Aminocaproic acid 500 µg/mL was prepared for selection of wavelength for detection.

Preparation of this solution 5 mL of stock solution was transfer into 10 mL volumetric flask and volume adjusted up to mark with diluent (water and Methanol in ratio of 80:20v/v) and mix well. (as per section 5.2.4.1) for the selection of the wavelength and scanned between 200-400 nm in UV-Visible double beam spectrophotometer at medium scanning speed. Spectra taken which was used for selection of wavelength for detection it was showed responsibly good response at 210 nm. So, 210 nm was selected as wavelength for estimation of Aminocaproic acid. (Figure: 6.1)

4.3.4 SELECTION AND PREPARATION OF MOBILE PHASE:

4.3.4.1 Selection of mobile phase:

For selection of mobile phase the standard solution of Aminocaproic acid injected into HPLC system and run into solvent system. Various composition of mobile phases was shown in Table: 7.1 trial in order to find the best conditions for separation of these drugs. Finally, the optimal composition of mobile phase was determined to be Phosphate buffer: Methanol (65:35 v/v) adjust buffer pH 2.2 with orthophosphoric acid solution.

Preparation of mobile phase:

- Buffer (pH 2.2): Dissolve 2 gm of monobasic potassium phosphate and 1.0 gm of

heptane sulphonic acid sodium salt monohydrate in 1000 mL of water and mix well. Add 1 ml of triethylamine into it. Adjust the pH of this solution to 2.2 with orthophosphoric acid solution. Filter the resulting solution through 0.45 µ nylon membrane filter.

- Preparation of mobile phase: Buffer and Methanol in ratio of 65:35 %v/v.

4.3.5 CHROMATOGRAPHIC CONDITIONS:

- Stationary phase: Inertsil C_8-3 (250 mm × 4.6 mm, 5µm)
- Mobile phase: Buffer: Methanol (65:35 v/v)
- Flow rate: 0.7 mL/minute
- Column oven temperature: 40°C
- Injection volume: 20 µL
- Detection: 210 nm
- Run time: 20 minutes

4.3.6 METHOD VALIDATION:

4.3.6.1 Specificity:

4.3.6.1.1 Interference from blank:

Inject blank solution, standard solution of Aminocaproic acid once, sample preparation once and check the peak purity angle and peak purity threshold of Aminocaproic acid in standard and sample preparation. (Table 6.2)

4.3.6.1.2 Interference from degradation product by stress study:

Standard preparation and sample preparation shall be subjected to acid, base, oxidation, photo, thermal degradation. The stress condition shall be adjusted such that minimum 5-10% degradation achieved in at least two conditions. For each degradation condition

blank solution prepared accordingly. Inject separately blank, standard solution and sample solution for each degradation. Final concentration for each degradation preparation will remain same as per test procedure. Check for separation of degraded product from main peak. Check peak purity angle and peak purity threshold of Aminocaproic acid all the degraded preparation.

4.3.6.1.2.1 Degradation conditions:

2. Acid degradation: Exposed sample in 5 mL 1 N HCl at 80°C in water bath for 2hours.
3. Base degradation: Exposed sample in 5 mL 1 N NaOH at 80°C in water bath for 2 hours.
4. Peroxide degradation: Exposed sample in 5 mL 30 % v/v H_2O_2 at room temperature for 22 hours.
5. Photo degradation: Exposed sample to 1.2 million Lux hours fluorescent light and 200-watt hrs/m^2 in UV chamber for 24 hours.
6. Thermal degradation: Exposed sample to 60° C for 2 hours in hot air oven.

4.3.7 ESTIMATION OF AMINOCAPROIC ACID IN THE MARKETED FORMULATION BY THE PROPOSED RP-HPLC METHOD:

The marketed formulation, Aminocaproic acid was analysed by using the developed method.

Inject diluent as blank, standard preparation and sample preparation and calculate the assay (table 6.25)

For preparation of sample solution: Accurately weigh and transfer as completely as possible, the content of 20 tablets to a suitable container and determine the average net content per tablet. Transfer sample powder equivalent to 500 mg of Aminocaproic acid into 250 mL volumetric flask. Add about 150 mL diluent. Sonicate for 30 minutes with intermittent shaking. Allow the solution to attain room temperature and dilute to volume with diluent and mix well. Filter the solution through PVDF 0.45µ syringe filter by discarding initial 5 ml of filtrate and collect remaining filtrate as sample. Transfer 5.0 ml of this solution in to 20 ml volumetric flask. Dilute up to mark with diluent and mix well Chromatogram was recorded using chromatographic condition mention above. Chromatogram of sample showed peaks at Rt 6.8 mins for Aminocaproic acid. The area of Aminocaproic acid was measured, and amount of drug was calculated using respective regression equation.

sults and Discussion

tability indicating RP-HPLC method development and validation of aminocaproic acid in tablet dosage form

5.1 Selection of wavelength for detection:

Preparation of solution for detection of wavelength as per 5.2.5

It was found that Aminocaproic acid shown good response at 210 nm. So 210 nm was selected as wavelength of estimation.

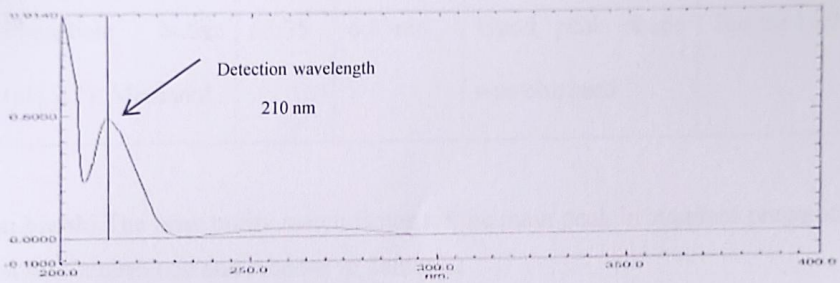


Figure 5.1: Spectra of Aminocaproic acid (500µg/mL) for selection of wavelength for detection (200-400 nm)

5.2 Selection of stationary phase:

Inertsil C_8-3, 250 mm × 4.6 mm column of 5µm particles packing was preferred as stationary phase for method development and this configuration provide a large number of theoretical plates with good peak shape.

5.3 Selection of mobile phase:

Resolution is most important criteria for method, it is imperative to achieve good resolution among the compounds. The standard solution Aminocaproic acid was injected into HPLC system and run into solvent system. Various composition of mobile phases shown in Table 7.1 were tried in order to find best condition for evaluation drug. Trial was done based on literature review. Finally, the optimum composition of mobile phase was determined to be Phosphate buffer: Methanol (65:35 v/v) adjust buffer pH 2.2 with orthophosphoric acid solution. Flow rate was adjusted to 0.7 mL/min. For chromatographic condition see section 5.2.7.

Table 5.1: Observation and Remarks of trials taken for selection of mobile phase

Sr No.	Mobile phase	Ratio	Retention time	Observation	Remarks
1	Monobasic sodium heptane sulphonate methanol (pH 2.2)	65:35	6.7 min	Sharp peak was not observed	No satisfactory
2	Phosphate buffer (pH 2.2): Methanol	65:35	6.8 min	Good peak shape was obtained	Tailing 1.29

Interference from blank: The peak purity match factor for the main peak in standard preparation and sample preparation was determined and recoded in Table 6.2

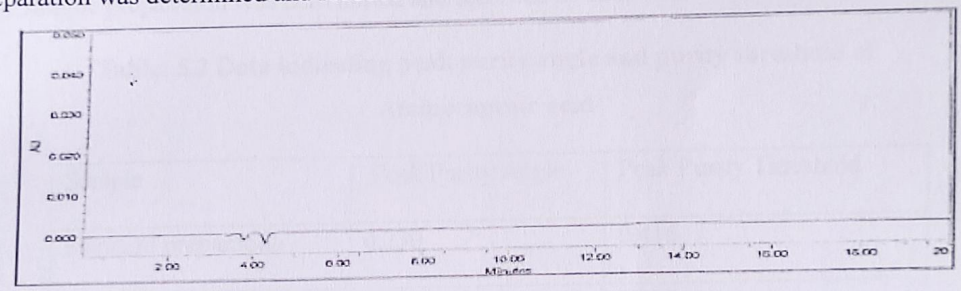


Figure 5.2: Chromatogram of blank preparation at 210 nm.

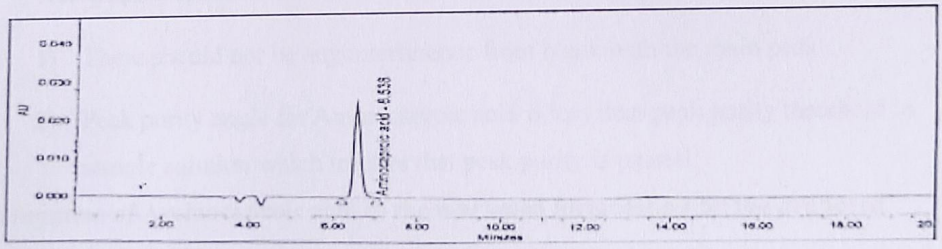


Figure 5.3: Chromatogram of Aminocaproic acid standard (500 µg/mL) at 210 nm.

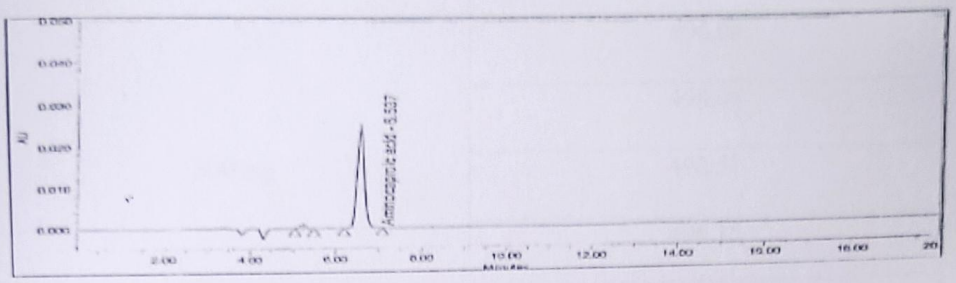


Figure 5.4: Chromatogram of sample preparation at 210 nm.

Result: The peak purity match factor for main peak in standard preparation and in sample preparation was determined and recorded in Table 6.2

Table: 5.2 Data indicating peak purity angle and purity threshold of Aminocaproic acid

Sample	Peak Purity Angle	Peak Purity Threshold
Standard preparation	0.230	0.416
Sample preparation	0.199	0.396

Acceptance criteria:

1) There should not be any interference from blank with the main peak.
2) Peak purity angle for Aminocaproic acid is less than peak purity threshold in sample solution which implies that peak purity is passed.

5.4 Estimation of Aminocaproic acid in the marketed formulation by the proposed RP-HPLC method:

- Applicability of the proposed method was tested by analysing the commercially available formulation **AMICAR ® Tablet**. The assay data are shown in the Table 6.25.
- The assay results were comparable to labelled value of each drug in tablet formulation. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of formulation industries.

Table 5.4: Analysis of Marketed Formulation AMICAR ® Tablet (n=5)

Amount present in one tablet	Amount found in assay
500 mg	496.08
	498.66
	498.51
	496.15
	498.51
Mean amount found ± SD	497.58 ± 1.343795
% RSD	0.27
% Assay	99.51
Standard Limit	97.0%-103.0%

5.4.1 **Validation summary:** All the validation parameters are shown in Table 6.26.

Table 7.26: Summary of validation parameters

Validation parameter	Aminocaproic acid
Linearity	250-750 µg/mL
Accuracy (% Recovery) n=3	99.79-99.92 %
Precision (% RSD)	
Repeatability (n=6)	0.09
Intraday (n=3)	0.28 - 0.60
Interday (n=3)	0.70 – 0.84
LOD (µg/mL)	5.83
LOQ (µg/mL)	17.66
Robustness (% RSD)	

0.5 mL/min	0.04
0.9 mL/min	0.14
35 °C	0.04
45 °C	0.03
2.0 pH	0.06
2.4 pH	0.03
208 nm	0.38
212 nm	0.94

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6.Financial Statement

Certified that a grant of ₹5,00,000) was received from the Aum Research Lab for the project titled " Stability Indicating Rp-Hplc Method Development and Validation of Aminocaproic Acid in Pharmaceutical Dosage Form ". The amount has been utilized as per the approved budget and guidelines

Sr. No.	Particulars	Expenditure Incurred
1	Equipment	₹2,51,747
2	Chemicals	₹1,49,000
3	Travel	₹51,000
4	Contingency	₹48,253
Total		**₹5,00,000**

8. Reference

1. Tripathi KD. In Essential of Medical Pharmacology; 8th Edn; Jaypee Brothers Medical Publisher LTD, New Delhi, 2019, pp 676.
2. Carruthers G, Hoffman B, Melmon K, Nierenberg D. In Clinical Pharmacology; 4th Edn; McGraw-Hill Medical Publication Division, New Delhi, 2000, pp 764.
3. Hardman J, Limbird L. In The Pharmacological Basis of Therapeutics; 10th Edn; Medical Publication Division, New York,2001; pp 1533-1534.
4. Snyder LR., Kirkland JL., and Glajch JL. Practical HPLC Method Development; 2nd Edn; Wiley, New York, 1997, pp 234-295
5. Ahuja S., and Michael WD. Hand book of pharmaceutical analysis by HPLC; 1st Edn; Elsevier Academic Press, 2005, pp 47-61.
6. Braithwaite A., and Smith FJ. Chromatographic Methods; 5th Edn; Kluwer Academic Publisher, Boston, 1996, pp 267-270.
7. Skoog DA., Holler FJ., and Crouch SR. Fundamentals of Analytical Chemistry; 8th Edn; Thomson Books, New York, 2010, pp 970-998.

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8. Engelhardt H., and Aitzetmuller K. Practice of High-Performance Liquid Chromatography; 1st Edn; Springer, New York, 1986, pp 71-75.

9. ICH Harmonization Triptie Guideline, "Stability Indicating Testing of New Drug Substance and New Drug Products Q1A (R2)". International Conference On Harmonization, IFPMA, Geneva, Switzerland, 2003.

10. Bakshi M and Singh S, "Development of validated stability indicating assay methods-critical review." *J. Pharm. Biomed. Anal.* 2002, 28, 1011-1040.

11. Hovorka SW and Schoneich C, "Oxidative degradation of pharmaceuticals." *J. Pharm. Sci.* 2001, 90(3), 253-269.

12. Carstensen JT., and Rhodes CT. Drug Stability: Principle and Practices; 3rd Edn; Marcel Dekker, New York, 2000, pp 339

13. FDA, Guidance for industry "stability Testing of Drug Substance and Drug Product (Draft guidance)". Food and Drug Administration, Rockville, MD, 1998.

14. Baertschi SW., Alsante KM., and Reed RA. Pharmaceutical Stress Testing: Predicting Drug Degradation; 2nd Edn; Informa healthcare, London, 2007, pp 143.

15. ICH, Q2(R1) "Validation of Analytical Procedure" Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.

16. United States Pharmacopeia, NF 26, The United State Pharmacopeial Convention, pp. 1386.

17. United States Pharmacopeia, NF 39, The United State pharmacopeial Convention, pp. 2472

18. Schou A, Cornett C, Nyberg N, Ostergaard J, Hansn S. "Structure elucidation and quantification of impurities formed between 6-aminocaproic acid and the excipients citric acid and sorbitol in an oral solution using high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy." *J. Pharm. Biomed. Anal,* 2015, 107, 333- 340.

19. Lau-Cam C, Roos R. "Assay of Aminocaproic Acid in Dosage Forms by Reversed Phase High Performance Liquid Chromatography with Dansylation." *J. Liq. Chromatogram,* 1993, 16(2), 403-419

20. Shantier S, Adam M, Mohamed R, Gadkariem E. "Development and validation of colorimetric method for the determination of aminocaproic acid in bulk and pharmaceutical formulation." *J. Innova. Pharm. Bio. Sci,* 2017, 4(3), 67-70.

21. Adam M, Shantier S, Alfangari S, and Gadkariem E. "Development of Spectrophotometric Method for the Assay of Aminocaproic Acid in Dosage Forms Using Ascorbic Acid." *Chem. Sci. Trans.* 2015, 4(2), 478-482

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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

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Email: tech.support@gccbiotech.co.in/info@gccbiotech.co.in
Website: www.gccbiotech.co.in

Date: 1.6.2021

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:

"Development and Validation of Analytical Method for Estimation of Bilastinein Bulk and in Tablet dosage Form."

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 beRs. 7,50,000 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/2021/42 Date: 07.10.2021

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: **"Development and Validation of Analytical Method for Estimation of Bilastine in Bulk and in Tablet dosage Form."**

Date of assigning problem: 01.06.2021

Date of completion: 04.10.2021

Name of person: Ms. Anjali Prajapati

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. Anjali Prajapati.

Thanking you.

Registrar

Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Development and Validation of Analytical Method for Estimation of Bilastine in Bulk and in Tablet dosage Form."

Research Project Report Submission to

GCC Biotech Pvt Ltd

Submitted by:

Principal Investigator: Ms. Anjali Prajapati, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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- **Declaration**

I Ms. Anjali Prajapati, Principal Investigator of the project titled " Development and Validation of Analytical Method for Estimation of Bilastine in Bulk and in Tablet dosage Form ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

Name:

Principal Investigator: Ms. Anjali Prajapati

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**

The priority of this study is to develop a reliable and accurate analytical method for the estimation of Bilastine, a second-generation antihistamine, in both bulk and tablet dosage forms, ensuring quality control in pharmaceutical formulations. Method development involves selecting appropriate analytical techniques such as UV-Visible Spectrophotometry, HPLC, or HPTLC. Validation focuses on assessing the accuracy, precision, specificity, sensitivity, linearity, and robustness of the developed method to comply with pharmacopeial standards.

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- **Detailed Report**

1.Introduction:

Bilastine is a second-generation antihistamine primarily used for the treatment of allergic rhinitis and urticaria (hives). It works by blocking histamine receptors in the body, which helps in reducing allergic symptoms without causing significant sedation, a common side effect of first-generation antihistamines. As with all pharmaceutical products, it is essential to ensure that Bilastine in bulk and in tablet formulations is accurately measured to maintain the efficacy and safety of the drug.

The development of a reliable and precise analytical method for the estimation of Bilastine is crucial for its quality control, especially in the pharmaceutical industry. The need arises from the necessity to quantify the active pharmaceutical ingredient (API) in both bulk drug substances and in tablet formulations, ensuring consistent therapeutic effectiveness. Analytical methods must also be validated to ensure they meet regulatory requirements, such as those set by the U.S. Food and Drug Administration (FDA) and the International Council for Harmonization (ICH), which stipulate strict guidelines for method accuracy, precision, sensitivity, and robustness.

Common analytical techniques used for such purposes include High-Performance Liquid Chromatography (HPLC), UV-Visible Spectrophotometry, and High-Performance Thin-Layer Chromatography (HPTLC). These methods offer advantages in terms of accuracy, sensitivity, and reliability, and they are validated to ensure that the methods meet specific criteria such as linearity, precision, and reproducibility.

Developing and validating such methods not only ensures the proper dosage of Bilastine in tablet forms but also guarantees that the drug meets the required pharmacological standards for safe and effective patient use.

2.Literature Review

Bilastine is a second-generation H_1-antihistamine used in the treatment of allergic rhino conjunctivitis and urticaria. It exhibits high selectivity for peripheral H_1-receptors, minimal penetration into the central nervous system, and a favourable pharmacokinetic profile. Due to its increasing use in pharmaceutical formulations, a reliable, accurate, and validated analytical method is essential for quality control and regulatory compliance.

Several studies have been conducted to establish methods for estimating Bilastine in both bulk and pharmaceutical formulations. High-Performance Liquid Chromatography (HPLC) remains the most widely used analytical technique for its quantification due to its se

reproducibility, and specificity.

For instance, *Mehta and Patel (2017)* developed and validated a UV spectrophotometric method for the estimation of Bilastine in bulk drug and tablet dosage form. However, spectrophotometric techniques often lack the specificity required in complex formulations where excipients may interfere.

Rao et al. (2019) reported an RP-HPLC method for the determination of Bilastine in pharmaceutical dosage form using a C18 column with UV detection. The method showed good linearity and precision but had limitations in detecting impurities and degradation products.

Only a few stability-indicating methods have been developed for Bilastine, emphasizing the need for comprehensive methods that can simultaneously quantify the drug and identify its degradation behaviour under various stress conditions. Stability-indicating methods are especially important to ensure the efficacy and safety of the drug throughout its shelf life.

According to ICH guidelines (Q2(R1)), any analytical method used in pharmaceutical quality control must be validated for parameters such as specificity, linearity, accuracy, precision, robustness, and limit of detection and quantification. Therefore, developing and validating a simple, precise, and robust RP-HPLC method is essential for the routine analysis of Bilastine in bulk and tablet dosage forms.

3.Objectives

The primary objective of this study is:

1. To develop and validate a reliable, specific, and accurate analytical method for the quantitative estimation of Bilastine in bulk drug and tablet dosage form using RP-HPLC in accordance with ICH Q2(R1) guidelines.
2. To select a suitable mobile phase, stationary phase, and detection wavelength for optimum separation and quantification of Bilastine.
3. To develop a robust reverse-phase HPLC method for the determination of Bilastine in its pure form and in tablet formulations.
4. To validate the developed method for parameters
5. To perform forced degradation studies to evaluate the stability-indicating nature of the method.
6. To apply the validated method for the routine quality control of Bilastine in commercial pharmaceutical products

4.Methodology:

1. Selection of Analytical Method

The first step is to select an appropriate analytical technique based on the properties of Bilastine, such as solubility, stability, and chemical structure. The most commonly used methods for Bilastine estimation include:

- **UV-Visible Spectrophotometry**: This method relies on measuring the absorbance of Bilastine at a specific wavelength.
- **High-Performance Liquid Chromatography (HPLC)**: HPLC is a widely used method due to its high precision and ability to separate Bilastine from other components in the sample.
- **High-Performance Thin-Layer Chromatography (HPTLC)**: This method is suitable for the separation and quantification of Bilastine in bulk and tablets.

For this study, the most suitable technique, such as HPLC or UV-Visible Spectrophotometry, will be selected based on the drug's properties and the required sensitivity.

2. Preparation of Standard Solutions

- **For UV-Visible Spectrophotometry**: A stock solution of Bilastine (usually 1000 µg/mL) will be prepared using a suitable solvent (e.g., methanol or water) and then diluted to required concentrations for the calibration curve.
- **For HPLC**: A stock solution of Bilastine will be prepared in a mobile phase, ensuring it is properly dissolved and stable.
- **For Tablet Formulation**: The powdered tablet sample will be prepared by accurately weighing the equivalent of a single tablet, dissolving it in the appropriate solvent, and filtering the solution.

3. Method Development

- **UV-Visible Spectrophotometry**: The wavelength of maximum absorption (λ_max) will be determined by scanning the solution of Bilastine across a range of wavelengths (e.g., 200–400 nm) to find the absorbance peak. The linearity of the method will be studied by preparing standard solutions at different concentrations and measuring the absorbance.
- **HPLC**: A suitable stationary phase (e.g., C18 column) and mobile phase (e.g., a mixture of acetonitrile and water or phosphate buffer) will be optimized. The flow rate, [illegible]

wavelength, and column temperature will be adjusted to achieve optimal separation and quantification.

- **HPTLC**: The mobile phase will be selected based on Bilastine's polarity and the stationary phase will be optimized for effective separation.

4. Method Validation

The developed analytical method will be validated according to standard guidelines (e.g., ICH Q2(R1)) by evaluating the following parameters:

- **Specificity**: Ensuring that the method accurately measures Bilastine without interference from excipients or impurities in the formulation.
- **Linearity**: The method's ability to produce results proportional to the concentration of Bilastine, determined by constructing a calibration curve over a specific concentration range.
- **Accuracy**: The closeness of the test results to the true value, which is typically evaluated by recovery studies (spiking known amounts of Bilastine into the sample matrix and measuring the recovery).
- **Precision**: The degree of agreement between replicate measurements, which will be evaluated by performing intra-day and inter-day precision studies.
- **Sensitivity**: The limit of detection (LOD) and limit of quantification (LOQ) of Bilastine, determined by analyzing low concentrations and assessing the signal-to-noise ratio.
- **Robustness**: The method's ability to remain unaffected by small variations in experimental conditions, such as changes in mobile phase composition or temperature.
- **System Suitability**: Ensuring that all system parameters (e.g., column efficiency, resolution, tailing factor) meet predefined specifications during HPLC analysis.

5. Analysis of Bilastine in Tablet Dosage Forms

Once the method is developed and validated, it will be applied to the estimation of Bilastine in commercial tablet formulations. The tablets will be powdered, and a known quantity of the powder will be dissolved in an appropriate solvent. The solution will be filtered, and the concentration of Bilastine will be determined using the developed method (UV, HPLC, or HPTLC).

6. Statistical Analysis

Data from method validation, including calibration curves, precision, and accuracy studies, will be analyzed statistically to ensure the reliability of the developed method. Methods such as linear regression analysis, standard deviation, relative standard deviation (RSD), and recovery percentage will be used to interpret the results.

5.Result and Discussion:

Selection of wavelength for measurement:

To determine Wavelength for measurement, standard spectra of Bilastine was scanned between 200-400nm. It is evident that Bilastine show an absorbance at 215nm respectively. The schematogram for standard and test Bilastine were shown in figure 2 and 3.

- Optimized Chromatographic Condition: HPLC System: LC 2010 CHT (Shimadzu)
- Column: C18 Waters (150mm x 4.5mm, 5µm)
- Mobile Phase: ACN: Ammonium acetate (pH 5.0 adjusted with glacial acetic acid) (85:15 %v/v)
- Flow rate: 1.0ml/min Detection Wavelength: 215nm Injection volume: 20µL
- Column oven temperature: Room temperature
- Run time: 10 min
- Diluents: Methanol

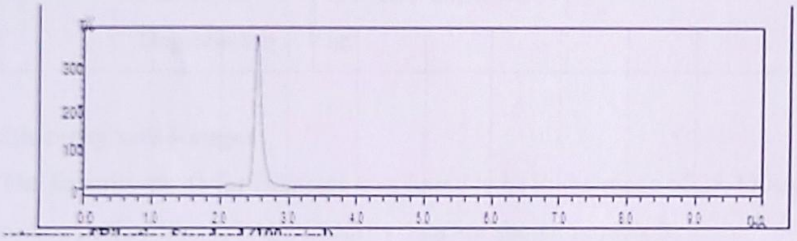

Figure: 2 Chromatogram of Bilastine Standard (100µg/ml)

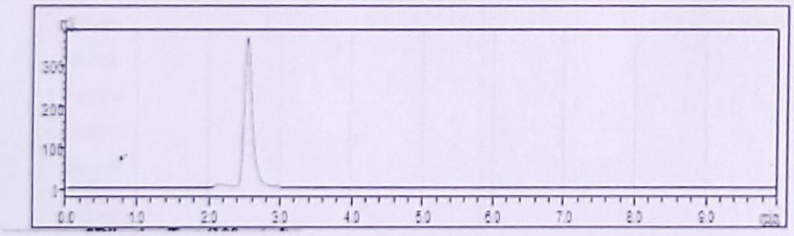

Figure: 3 Chromatogram of Bilastine Standard (100µg/ml)

Results of forced degradation study:

From degradation study it was found that Bilastine was marginally degraded in acid and oxidative conditions and stable in alkali, neutral, thermal, and photolytic conditions. Results of forced degradation study was shown in table 1.

Table: 1 Result of Forced Degradation Study of Bilastine

Sr.No.	Stress type	Condition	No. of peaks	% Degradation
1	Acid Hydrolysis	2 N HCl at 80°C for 30 min.	1	7.25
2	Alkali Hydrolysis	2 N NaOH at 80°C for 2 hr.	-	-
3	Neutral Hydrolysis	H2O at 80°Cfor 2 hr.	-	-
4	Oxidative Degradation	10% H2O2 at 80°C for 30 min.	1	5.24
5	Thermal Degradation	At 70°C for 8 hr.	-	-
6	Photolytic Degradation	UV 254 nm for 24 hr.	-	-

Linearity and Range:

The linearity range for Bilastine was found to be in the range of 25-150µg/ml. Linearity shown in the "Fig. 4." And linearity data are shown in table 3.

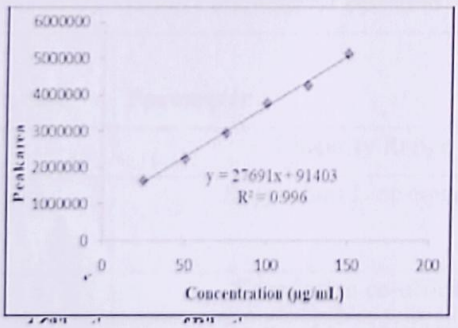

Figure: 4 Calibration curve of Bilastine

Precision:

The %RSD of Intra-day and Inter-day for bilastine was found to be 0.64 to 0.81 and 0.14 to 0.32 respectively.

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation of bilastine at three level (80% - 120%) of standard addition. Percentage recovery for Bilastine was 99.56 - 101.06%, shown in table 2.

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Table: 2 Result of Accuracy study for Bilastine

Level	Test conc. (µg/ml)	Std. conc. (µg/ml)	Amt. of found ± S.D.	% Recovery
80%	50	40	89.69±0.87	99.66
100%	50	50	101.06±0.76	101.06
120%	50	60	109.52±1.10	99.56

Limit of Detection and Limit of Quantitation:

LOD and LOQ was found to be 0.45µg/ml and 1.20 µg/ml respectively.

Robustness:

The typical variations studied under these parameters are flow rate, mobile phase composition, column temperature, change in pH. According to data comparison develop method was robust.

Analysis of marketed formulation:

Applicability of the proposed method was tested by analysising the available tablet formulation BYLOZA.

All method validation parameter of bilastine results were shown in table 3.

Table 3: Validation Parameter of Bilastine

Sr. No.	Parameter	Bilastine
1.	Linearity Range	25-150 µg/ml
2.	Regression Line equation	Y=27691x+ 914034
3.	Correlation co-efficient	0.996
4.	Precision (%RSD)	
	Intra-day Precision	0.637-0.807
	Inter-day Precision	0.140-0.325
5.	Accuracy (%Recovery)	99.78-100.53
6.	Limit of Detection(µg/ml)	0.41
7.	Limit of Quantification(µg/ml)	1.25
8.	% Assay	101.1

9.	Robustness (% RSD of Assay)	0.85

- **Expected Outcomes:**

Reliable Analytical Method: A validated and robust analytical method (e.g., UV-Visible Spectrophotometry, HPLC, or HPTLC) for the accurate estimation of Bilastine in both bulk and tablet dosage forms will be developed. This method will provide reliable results in routine quality control analysis.

Optimal Analytical Conditions: Identification of optimal experimental conditions for the chosen analytical technique, such as the correct solvent, wavelength (for UV spectrophotometry), mobile phase composition (for HPLC), or stationary phase (for HPTLC), will be determined, ensuring maximum accuracy and reproducibility.

Validated Method: The developed method will be validated according to ICH or other regulatory guidelines, ensuring that it meets criteria for:

- Specificity: The method will accurately measure Bilastine without interference from excipients or impurities.
- Linearity: The method will exhibit a linear response over a defined concentration range, allowing precise quantification of Bilastine.
- Accuracy: The method will provide recovery rates within acceptable limits when spiked into formulations, confirming its ability to accurately estimate the drug content.
- Precision: Intra- and inter-day precision tests will show low variability in the results, demonstrating the repeatability of the method.
- Sensitivity: The method will have sufficient sensitivity with determined limits of detection (LOD) and quantification (LOQ) to measure low concentrations of Bilastine.
- Robustness: The method will prove to be robust, with small changes in parameters (e.g., temperature or pH) not significantly affecting the results.
- Accurate Estimation of Bilastine in Tablets: The developed method will be successfully applied to the quantification of Bilastine in commercial tablet formulations, yielding results that are consistent with the label claim, ensuring quality control and product consistency.
- Method Applicability for Routine QC: The validated method will be suitable for routine quality control analysis of Bilastine in both bulk drug substances and tablet dosage forms in pharmaceutical settings.
- Compliance with Regulatory Standards: The developed and validated method will [illegible]

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international regulatory standards (such as those set by the FDA or ICH), ensuring it is acceptable for use in pharmaceutical analysis and can be applied for regulatory submissions or manufacturing processes.

- Data Integrity and Statistical Validation: Statistical analysis will confirm the method's reliability and robustness, with all validation parameters (e.g., linearity, accuracy, precision, and recovery) meeting the required acceptance criteria, ensuring data integrity and consistency.

- **Impact:**

Improved Quality Control in Pharmaceutical Industry: The development and validation of a reliable analytical method for estimating Bilastine in both bulk and tablet dosage forms will significantly enhance the quality control processes within pharmaceutical manufacturing. This ensures that the final product consistently meets the required potency, safety, and quality standards, thereby reducing the risk of underdosing or overdosing patients. Regulatory Compliance: The validated method will meet the regulatory requirements set by authorities such as the U.S. FDA, EMA, and ICH. This is crucial for pharmaceutical companies to ensure their products are in compliance with international standards, enabling smoother regulatory approval and market access for Bilastine formulations. Enhanced Therapeutic Efficacy: Accurate estimation of Bilastine in tablet dosage forms ensures that the correct dosage reaches patients, maintaining the drug's therapeutic efficacy. This contributes to the effective treatment of allergic conditions such as rhinitis and urticaria, improving patient outcomes and overall public health. Cost-Effectiveness for Manufacturers: With a validated, reliable analytical method in place, pharmaceutical manufacturers can reduce the need for more expensive and time-consuming testing methods. The consistency and accuracy of the developed method can streamline production processes and reduce batch rejection rates, leading to cost savings in manufacturing and quality control. Increased Consumer Confidence: Ensuring that Bilastine formulations are accurately tested for dosage and purity strengthens consumer trust in the product. Patients and healthcare providers can be confident that the tablets contain the correct amount of active pharmaceutical ingredient (API), which contributes to overall patient safety and satisfaction. Facilitation of Product Development: The validated analytical method can serve as a foundational tool for the development of new Bilastine formulations or combinations. It provides a standardized approach for assessing Bilastine content in different tablet formulations, allowing for efficient R&D and formulation testing. Environmental and Health Safety: By accurately measuring the concentration of Bilastine in bulk and tablet forms, the method helps to avoid any potential environmental or health hazards that could arise

incorrect dosing. Properly regulated pharmaceutical products reduce the chances of adverse side effects caused by impurities or incorrect dosage amounts. Contribution to Scientific Knowledge: The development of a new, validated method for estimating Bilastine adds to the body of scientific knowledge in analytical chemistry and pharmaceutical analysis. It can be used as a reference for future studies, potentially applying similar techniques to other pharmaceutical compounds. Global Accessibility and Market Expansion: A reliable and validated method enables pharmaceutical companies to meet global standards, allowing them to distribute Bilastine formulations internationally. This can facilitate the expansion of market access and the availability of the drug in various regions, improving global healthcare.

National and International status of work:

The development and validation of analytical methods for active pharmaceutical ingredients (APIs), such as Bilastine, is an essential area of pharmaceutical research. Globally, and within India, various research studies and guidelines have been developed in the realm of pharmaceutical analysis. These works aim to ensure the accuracy, reliability, and regulatory compliance of drug formulations. Below is a summary of the relevant national and international work that directly or indirectly influences the development of analytical methods for estimating Bilastine.

National Work (India)

1. **Regulatory Guidelines by CDSCO (Central Drugs Standard Control Organization)**

The CDSCO (the national regulatory authority for drug standards in India) provides guidelines for analytical method validation for APIs and pharmaceutical dosage forms. These guidelines align with ICH Q2(R1), ensuring that methods for estimating Bilastine in bulk and tablet forms meet requirements for specificity, accuracy, precision, and robustness.

India follows the Pharmacopoeia of India (IP), which sets standards for drug quality, including analytical methods for the estimation of APIs like Bilastine.

2. **Work by Indian Research Institutions**

Various Indian universities and research centers have worked on developing validated methods for the estimation of other antihistamines, which can be adapted for Bilastine. HPLC and UV Spectrophotometry are the most commonly used techniques in these studies.

For example, the Institute of Pharmaceutical Sciences, University of Punjab, and National Institute of Pharmaceutical Education and Research (NIPER) have conducted extensive research on developing analytical methods for antihistamines and other drugs in bulk and tablet

forms. These methods could be adapted and applied to Bilastine.

3. **Studies on Analytical Methods for Antihistamines**

In India, several studies have focused on the development and validation of methods for estimating antihistamines (e.g., Cetirizine, Loratadine, Desloratadine) using methods like HPLC, HPTLC, and UV-Visible Spectrophotometry. These techniques and methodologies have set the groundwork for the development of similar methods for Bilastine.

4. **Indian Pharmacopeia (IP)**

The Indian Pharmacopeia (IP) provides quality standards for pharmaceutical products in India. While Bilastine may not be explicitly listed in the IP, the standards for similar antihistamines (e.g., Cetirizine) can be adapted to create analytical methods for Bilastine.

5. **Work on Green Analytical Chemistry in India**

Some Indian research groups have explored **green chemistry** techniques for the estimation of APIs, focusing on reducing environmental impact, such as minimizing the use of hazardous solvents. This aligns with the growing demand for sustainable analytical methods in drug testing.

International Work

1. **International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)**

The ICH guidelines, specifically ICH Q2(R1), provide internationally accepted standards for the validation of analytical methods in pharmaceuticals. The guidelines emphasize parameters such as accuracy, precision, sensitivity, linearity, and specificity. These principles are directly applicable to the development and validation of methods for Bilastine estimation in bulk and tablet dosage forms.

2. **Pharmacopoeia Standards: USP, EP, and JP**

The United States Pharmacopeia (USP), European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP) provide comprehensive guidelines for the development of analytical methods for various APIs. While Bilastine may not be explicitly listed, methods outlined for similar antihistamines (e.g., Loratadine, Fexofenadine) are relevant and can be adapted for Bilastine estimation in tablet formulations.

The USP provides specific validation protocols for HPLC and UV-Visible Spectrophotometry, which are critical for ensuring that the analytical methods developed for Bilastine meet the standards for pharmaceutical quality control.

3. **Global Research on Analytical Methods for Antihistamines**

Several international studies have focused on the analytical methods for estimating antihistamines, including Bilastine. Research published in journals such as the Journal of Pharmaceutical and Biomedical Analysis has demonstrated the development and validation of methods for estimating antihistamines like Loratadine, Desloratadine, and Cetirizine using techniques like HPLC and UV spectrophotometry.

These studies provide valuable methodologies that can be directly adapted to Bilastine, helping optimize method development for bulk drug and tablet formulations.

4. **International Research on Validation Techniques**

International studies focus on advanced analytical validation techniques for ensuring the accuracy, reliability, and regulatory compliance of drug formulations. For example, research on method validation using HPLC for drugs like Bilastine has been conducted at leading universities and pharmaceutical companies in Europe and the United States.

These studies often follow guidelines outlined by FDA and EMA for analytical testing, ensuring that the methods are compliant with stringent international regulatory standards.

5. **FDA and EMA Guidelines**

The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have set detailed guidelines for the validation of analytical methods used in pharmaceutical testing. Both regulatory bodies ensure that the methods used for estimating drugs, including antihistamines like Bilastine, are reliable, reproducible, and compliant with industry standards. These guidelines are essential for pharmaceutical companies seeking approval for Bilastine tablets and other dosage forms, as they establish the benchmark for quality control testing.

6. **Green Chemistry and Sustainable Analytical Methods**

International research is increasingly focused on green analytical chemistry. Studies on the reduction of solvent usage, development of eco-friendly solvents, and the optimization of analytical procedures to be more sustainable are gaining traction globally. Such research could potentially improve the environmental sustainability of the proposed method for Bilastine estimation.

7. **World Health Organization (WHO) Guidelines**

The World Health Organization (WHO) sets global standards for drug quality control and pharmaceutical testing. Its guidelines on the development and validation of analytical methods for APIs, including antihistamines, influence global practices in drug analysis. WHO's focus on Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) makes its guidelines crucial for ensuring the global acceptance of the developed method for Bilastine.

6.Financial Statement

Certified that a grant of 7,50,000 was received from the GCC Biotech for the project titled " Development and Validation of Analytical Method for Estimation of Bilastine in Bulk and in Tablet dosage Form". The amount has been utilized as per the approved budget and guidelines.

Budget Utilization

Category	Expense
Manpower	₹3,50,000
Consumables	₹2,50,000
Travel	₹85,000
Contingencies	₹65,000
Total (₹)	₹7,50,000

7.Conclusion

The developed method was found to be simple, sensitive and selective for analysis of Bilastine. Bilastine was marginally degraded in acidic and oxidative conditions and was found to be stable in all other conditions. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drugs under non degradation condition. In the proposed study, stability-indicating RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Bilastine.

8.References

1. A new stability indicating RP-HPLC method for determination of bilastine in bulk and pharmaceutical formulation Res. J. Pharm. Technol. (2020)

2. A. Tassinari et al. UV Spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness. Drug Anal. Res. (2017) P. Sharma et al.

3. Method development and validation of bilastine by HPLC.Int. J. Adv. Sci. Technol. (2020)

4. Anuja Uddhav Deokar, Dr Suhas Siddheshwar, Sudarshan. B. Kakad. Analytical Method Development and Validation of Rivaroxaban- A Review. Research Journal of Science & Technology. 2020; 12(1): 36-46.

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Kalol, Gandhinagar

5. Synder LR, Kirkland JL and Glajch JL. Practical HPLC Method Development; 2nd Edn; Wiley Interscience, New York, 1977, pp 1-26.

6. Gandhi J, Godse K, and Godse G. Bilastine: A novel antihistamine. Indian Journal of drugs in dosage form. 2010; 4(1): 3-6.

7. Nishant Sarode, G. S. Chhabra, Shailesh Luhar, Anil Jadhav. Development and Validation of RP-HPLC Method for the Estimation of Montelukast Sodium in Bulk and In Tablet Dosage Form. Research Journal of Science and Technology. 2011; 3(5): 257-260.

8. Ramesh J, Jayalakshmi B, Vijayamirtharaj R, Arul Prakasam K.C. Simultaneous Estimation of Montelukast Sodium and Levocetirizine Hydrochloride by RP-HPLC Method. Asian Journal of Research in Chemistry. 2010; 3(4): 1069-1072.

9. Wikipedia, Bilastine, https://pubchem.ncbi.nlm.nih.gov/compound/Bilastine.

10. Jelena T, Igor P, Ana S, Anja T, Biljana J, Application of Analytical Quality by Design Concept for Bilastine and its Degradation Impurities Determination by Hydrophilic Interaction Liquid Chromatographic Method, Journal of Pharmaceutical and Biomedical Analysis, 125: 385-93.

11. Bilastine detection method. China Patent CN 104730194 A, 2016.

12. Andressa TDS, Gabriela RB, Isadora DM, Lisiane B, Marcelo DM, Clesio SP. UV Spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness. Drug Analytical Research, 2017; 1(2): 38-43.

13. Chowdary V, Kota A, Syed M. Method development and validation of new RP-HPLC method for the estimation of Bilastine in pharmaceutical dosage form. World Journal of Pharmaceutical and Pharmacy Science. 2017; 6(8): 2297-2315.

14. Radio O, Guermouche S. Degradation kinetics of Bilastine Determined by RP-HPLC method and identification of its degradation product in oxidative condition. Spinger Nature. 2019.

15. Sharmin Reza Chowdhury, Mahfuza Maleque, Mahbubul Hoque Shihan. Development and Validation of a Simple RP-HPLC Method for Determination of Caffeine in Pharmaceutical Dosage Forms. Asian Journal of Pharmaceutical Analysis. 2012; 2(1): 01- 04.

16. Akhilesh Gupta, Swati Rawat, Arun Pandey. Method Development and Photolytic Degradation Study of Doxofylline by RP-HPLC and LC-MS/MS. Asian Journal

Pharmaceutical Analysis. 2011; 1 (2) 29-33.

17. Sushil D. Patil, Prajkta Varpe, Sayali Chaure, Siddharth B. Bhalerao, Sanjay Kshirsagar. Development and Validation of Stability Indicating RP-HPLC Method for Piracetam. Asian Journal of Research in Pharmaceutical Sciences. 2017; 7(4): 215- 221.

18. Hemant Shah, Khushbu K. Patel, Pruthviraj Chaudhary, Dr. C. N. Patel. Stability indicating Analytical Method Development and Validation for Estimation of Zonisamide in Pharmaceutical dosage form. World Journal of Pharmaceutical Research. 2020; 9(5): 1204-1215.

19. Zhou H, Fung C, Liu Q, Method for determining related substance of Bilastine intermediate by using high-performance liquid chromatography. China Patent CN 103760260 A, 2014.

20. Method for separating and measuring Bilastine raw material and preparation thereof utilizing liquid chromatography. China Patent CN 103760260 A, 2019.

21. Rupali Tambe, Someshwar Mankar, Santosh Dighe. Analytical Method Development and Validation of Paliperidone: A Review. Research Journal of Science and Technology. 2020; 12(1): 23-35.

22. ICH, Q1A, Stability Testing of New Drug Substances and Products, in: Proceedings of the International Conference on Harmonization, Geneva, October, 1993.

23. Khilesh Gupta, Jaydeep Singh Yadav, Swati Rawat, Mayuri Gandhi. Method Development and Hydrolytic Degradation Study of Doxofylline by RP-HPLC and LC-MS/MS. Asian J. Pharm. Ana. 2011; 1(1): 14-18.

24. Md. Ahsanul Haque, Mohammad Shahriar, Most. Nazma Parvin, S. M. Ashraful Islam. Validated RP-HPLC Method for Estimation of Ranitidine Hydrochloride, Domperidone and Naproxen in Solid Dosage Form. Asian J. Pharm. Ana. 2011; 1(3): 59-63.

25. Vaibhavi.N. Akhani, Mrs. Khushbu K Patel, K. S. Patel, L. M. Prajapati, Dr. C. N. Patel. Development and validation od HPLC method for estimation of prucalopride succinate in pharmaceutical dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2020; 9(6): 1112-1122.

26. L. Satyanarayana, S.V. Naidu, M. Narasimha Rao, Reddy Suma Latha. The Estimation of Nilotinib in Capsule dosage form by RP- HPLC. Asian J. Pharm. Ana. 1(4): Oct. - Dec. 201[illegible] Page 100- 102.**09**; 4: pp 537-546.

SERVICE CONTRACT

This Service Contract ("Contract") is entered into by and between **SPECHROM Solutions 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 SPECHROM Solutions."

TERMS AND CONDITIONS

This Contract shall commence on **11th January 2021** 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 SPECHROM Solutions

For Swarrnim Startup and Innovation University

Swarrnim Startup & Innovation University ★ Gandhinagar

Date: 11.6.2021

To,

The Principal,

Swarrnim Science College

Swarrnim Startup and Innovation University

Gandhinagar Gujrat

Subject: Approval for Consultancy Project

Dear Sir/Madam

It is our pleasure to inform you that the project for consultancy which has been under discussion for quite sometimes is granted. The details are as follows:

Project Title:

"Rp-Hplc Method Development and Validation for the Quantification of Metformin Hydrochloride in Tablet Dosage Form in Various Combinations for reduced cost of Analysis"

Project Timeline:

The project is expected to be completed within the next 3 to 4 months.

Payment:

A total amount payable after successful completion of Project will be Rs. 6,00,000 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 SPECHROM Solutions

SPECHROM SOLUTIONS AHMEDABAD

Ref.No.swarrnim/RO/SCR/2021/44

Date: 24.09.2021

To,

SPECHROM SOLUTION.

SF/209, Trade Square, Sabarmati,

Ahmadabad, 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: **" Rp-Hplc Method Development and Validation for the Quantification of Metformin Hydrochloride in Tablet Dosage Form in Various Combinations for reduced cost of Analysis."**

Date of assigning problem: 11.06.2021

Date of completion: 20.09.2021

Name of person: Ms. Nikita Shah

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. Nikita Shah.

Thanking you.

Patel

Registrar

Swarrnim Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Rp-Hplc Method Development and Validation for the Quantification of Metformin Hydrochloride in Tablet Dosage Form in Various Combinations for reduced cost of Analysis

Research Project Report Submission to

Spechrom Solutions

SWARRNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE.

Submitted by:

Principal Investigator: Ms. Nikita Shah, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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

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- **Declaration**

I, Ms. Nikita Shah, Principal Investigator of the project titled " Rp-Hplc Method Development and Validation for the Quantification of Metformin Hydrochloride in Tablet Dosage Form in Various Combinations for reduced cost of Analysis ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

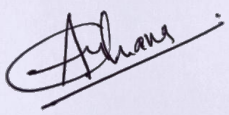
Signature:

Principal Investigator: Ms. Nikita Shah

Head of Institution: Dr. Hemant Chaube

- **Acknowledgment**

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



- **Summary**

Diabetes mellitus, particularly Type 2 diabetes, has become a global health concern due to its increasing prevalence and associated complications. The management of Type 2 diabetes involves the use of oral hypoglycaemic agents, with metformin hydrochloride being one of the most commonly prescribed drugs. Metformin is a first-line treatment for Type 2 diabetes, known for its ability to lower blood glucose levels by improving insulin sensitivity and reducing hepatic glucose production. It is widely used due to its efficacy, safety profile, and cost-effectiveness. Metformin is available in various dosage forms, with tablet formulations being the most prevalent.

To ensure the therapeutic efficacy and safety of pharmaceutical products, it is crucial to develop and validate robust analytical methods for the quantification of active pharmaceutical ingredients (APIs) in dosage forms. High-performance liquid chromatography (HPLC), particularly reverse-phase HPLC (RP-HPLC), is one of the most widely employed techniques due to its precision, sensitivity, and ability to separate compounds in complex matrices. RP-HPLC has proven to be an effective tool for the analysis of metformin in bulk and pharmaceutical formulations.

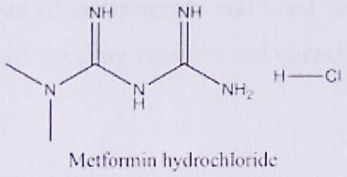
However, the development of a reliable RP-HPLC method for the quantification of metformin hydrochloride requires careful optimization of various chromatographic parameters, such as column type, mobile phase composition, flow rate, and detection wavelength, to achieve accurate and reproducible results. Furthermore, the method must undergo rigorous validation to ensure its reliability, robustness, and compliance with regulatory guidelines such as those from the International Conference on Harmonisation (ICH) and the U.S. Food and Drug Administration (FDA).

This study aims to develop a novel RP-HPLC method for the quantification of metformin hydrochloride in tablet dosage forms. The method will be validated for key analytical parameters such as accuracy, precision, specificity, linearity, and limit of detection (LOD), in line with ICH guidelines. The successful development of this method will offer a reliable tool for quality control, ensuring the consistent production and quality of metformin tablet formulations.

- **Detailed Report**

1. Introduction

Metformin Hydrochloride an antidiabetic drug with a chemical name, 1- carbamimidamido-N,N-dimethylmethanimidamide hydrochloride falls under biguanide class of antihyperglycemic agent. Works by reducing production of hepatic glucose and also increases glucose uptake, it also helps to prevent complications related to cardiovascular system. Its also used for PCOS problems and also decreases levels of triglycerides and low-density lipoprotein cholesterol [4].



Metformin hydrochloride

Figure 1: Metformin Hydrochloride

Drug profile

Chemical name:1-carbamimidamido-N,N-dimethyl- methanimidamide hydrochloride

Molecular Formula: C4H12ClN5

Molecular Weight: 165.62

Appearance: White or almost white crystalline powder

State: solid

Melting point: 223-226 °C

pKa: 12.4

Category: Biguanide class of anti-hyperglycaemic agent

Analytical chemistry helps for the determination of quality, purity, safety of the chemicals and drugs by separating, quantifying and identifying the sample using certain methods and instruments. It helps in both quantitative as well as qualitative analysis of the sample, in qualitative analysis, it determines the purity and quality of the sample whereas, in quantitative analysis the concentration of the sample i.e., the amount of the expected content present within the sample can be found [1].

HPLC is High-Performance Liquid Chromatography is mainly used for the separation, identification and quantification of the components present in a mixture. Till 1960 liquid chromatography in which only glass columns were used and it was used to work in low pressure was developed to HPLC later with metal columns and high pressure. The basic working principle of HPLC is that it separates the constituent of the mixture based on relative affinities of the constituents for the stationary phase and mobile phase, which are used for the separation. Mainly there are two types of HPLC they are; reverse phase uses polar mobile phase and the non-polar stationary phase and the normal phase uses the non-polar mobile phase and polar stationary phase. Major applications of HPLC are analysing drugs, pollutants and synthetic polymers, isolation of components and used in industries in quality control departments to check the purity of the drug samples and chemicals [2].

2. Literature Review

The drug Metformin Hydrochloride is CDSCO approved for symptomatic treatment of allergic rhino-conjunctivitis Diabetes. On literature survey, it was found that few U.V. spectroscopy, HPTLC and RP-HPLC methods, Degradation study are available for the determination of Metformin Hydrochloride in tablet dosage form.[10-18] A literature survey for Metformin Hydrochloride was done. Here are some research papers describing determination of Metformin Hydrochloride by stability indicating RP-HPLC method for in bulk and pharmaceutical formulation.[2] And by UV Spectrophotometric method using experimental design for robustness.[3] Method Development and Validation of Metformin Hydrochloride by HPLC. [4] And new validated Ultra Performance Liquid Chromatography (HPLC) method [5]. Methanol: Acetonitrile: Phosphoric Acid Buffer pH 2.1 in proportion of (21:33:46) was used as mobile phase with flow rate 1.0 ml/min. The column used for the method development is 250× 4.6 mm × 5 µm dimension. Selective RP-HPLC method has been developed [6]

3. Objectives

1. To develop a simple and robust RP-HPLC method for the quantification of Metformin Hydrochloride in tablet dosage forms.
2. To optimize the chromatographic conditions to achieve effective separation and peak resolution for Metformin Hydrochloride in various fixed-dose combinations.
3. To validate the developed method as per ICH guidelines for parameters such as accuracy, precision, specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ).

4. To ensure the developed method is cost-effective and suitable for routine quality control analysis in pharmaceutical industries.
5. To compare the developed method with existing methods to demonstrate its advantages in terms of simplicity, cost, and analytical performance.
6. To apply the validated method for the analysis of marketed tablet formulations containing Metformin Hydrochloride.

4. Methodology

Chemical and reagents:

Metformin Hydrochloride sample was obtained as a gift from Emcure pharmaceuticals Ahmedabad HPLC-grade methanol was purchase from Merck Life Science Private Limited, Mumbai, India and HPLC-grade water were purchased from Merck Life Science Private Limited, Mumbai, India. Metformin Hydrochloride tablet (BYLOZA 20 mg) was purchased for the analytical purpose. All the other chemicals and reagents used were of AR grade and purchased from Avantor Performance Material India Limited, Thane, India. HPLC-grade methanol was purchase from Merck Life Science Private Limited, Mumbai, India and HPLC-grade water were purchased from Merck Life Science Private Limited, Mumbai, India. Metformin Hydrochloride tablet (BYLOZA 20 mg) was purchased for the analytical purpose. All the other chemicals and reagents used were of AR grade and purchased from Avantor Performance Material India Limited, Thane, India.The Double Distilled water HPLC grade was prepared in the laboratory.

Chromatographic conditions and Equipment: Analysis was carried out on a Shimadzu LC_2010 CHT HPLC with UV detector. The output signal was monitored and processed using LC solution software. The chromatographic column used was C18 (150mm × 4.6mm, 5µ). Gradient elution process was adopted throughout the analysis. Mobile phase used was 85:15% v/v (ACN: Ammonium acetate (pH 5.0 adjusted with glacial acetic acid).

Instrumental parameters:

The gradient flow of mobile phase was maintained at 1.0 ml/min. The injection volume was 20µL. Eluted sample was monitored at 215nm and run time was 10 min. The retention time of the sample was about 2.519 min.

Preparation of standard and test solutions: Preparation of stock solution:

Stock solution for method validation were prepared by accurately weighing 100mg of Metformin Hydrochloride and dissolving it in 100ml of methanol by gentle stirring to yield final concentration of 1000µg/ml.

Preparation of working standard solutions:

Working standard solution was prepared by accurately transferring the (0.25, 0.50, 0.75, 1.0, 1.25, 1.5ml) aliquot of the standard stock solution in a series of 10 volumetric flask. The volume was made up to mark mobile phase to obtain concentration of 25-150µg/ml.

Preparation of sample solutions:

Twenty tablets of marketed formulation, BYLOZA (20 mg) were taken and weight of average content was determined. Weight equivalent to 20mg Metformin Hydrochloride was transferred to 100ml volumetric flask and dissolve in methanol. Solution was sonicated and filtered through Whitman filter paper. Average weight of tablet was calculated 113.2mg.

Forced degradation study:

Forced degradation studies of the drug were carried out under conditions of acid hydrolysis, alkali, neutral, oxidative, thermal, photolytic degradation.

Acid Hydrolysis:

Forced degradation in acidic media was performed by taking 2ml stock solution of Metformin Hydrochloride to 10ml volumetric flask. Add 2ml of 2 N HCL in volumetric flask and kept 80° C for 30 min. Then neutralized it with 2 N NaOH and diluted up to the mark with mobile phase. Solution strength of 100µg/ml.

Alkali Hydrolysis:

Forced degradation in basic media was performed by taking 2ml stock solution of Metformin Hydrochloride to 10ml volumetric flask. Add 2ml of 2 N NaOH in volumetric flask and kept at room temp. 80°C for 2 hrs. Then neutralized it with 2 N HCL and diluted up to the mark with mobile phase. Solution strength of 100µg/ml.

Neutral Hydrolysis:

Forced degradation in neutral degradation, was performed by taking 2ml stock solution of Metformin Hydrochloride to 10ml volumetric flask. Add 2ml of HPLC grade w

volumetric flask and kept at 80°C for 2 hrs. Diluted up to the mark with mobile phase. Solution strength of 100 µg/ml.

Oxidative Degradation:

Forced degradation in oxidative condition was performed by taking 2ml stock solution of Metformin Hydrochloride to 10ml volumetric flask. Add 2ml of 10% H2O2 in volumetric flask and kept at 80°C for 30 min. Diluted up to the mark with mobile phase. Solution strength of 100µg/ml.

Thermal Degradation:

Forced degradation in thermal degradation, 10mg accurately weighed amount of Metformin Hydrochloride was exposed to 70°C for 8 hrs. After this exposure, the drug powder was mixed and transferred in to 10ml volumetric flask, dissolved in methanol and diluted up to mark with diluent. Final dilution was done with sample diluent to make final concentration of 100µg/ml.

Photolytic Degradation:

Forced degradation in photolytic degradation, 10mg accurately weighed amount of Metformin Hydrochloride was exposed to 254nm for 24 hrs. After this exposure, the drug powder was mixed and transferred in to 10ml volumetric flask, dissolved in methanol and diluted up to mark with diluent. Final dilution was done with sample diluent to make final concentration of 100µg/ml.

Then 20µl solution of above solutions were injected into HPLC system and analyzed under the chromatographic condition described earlier

Method validation:

Linearity and Range:

Aliquots of working standard solution (0.25, 0.50, 0.75, 1.0, 1.25, and 1.5ml) were transferred into series of 10 ml volumetric flask and diluted up to mark with methanol to obtain final concentration of 25-150µg/ml and mixed properly. 20µl aliquots of each solution were chromatographed three times and analysis was performed by optimized method. The regression equation was derived from the plot of average area of Metformin Hydrochloride peak against the concentration of Metformin Hydrochloride.

Precision:

Intra-day Precision:

Intraday precision was determined by analysing of Metformin Hydrochloride standard solutions in the range 50, 75 and 100 µg/ml of it triplicate in a day. Percentage RSD for Metformin Hydrochloride was calculated.

Inter-day Precision:

Inter-day precision was determined by analysing of SAR standard solutions in the range 50, 75 and 100µg/ml of it in three different days. Percentage RSD for Metformin Hydrochloride was calculated.

Accuracy

Accuracy was determined by calculating recovery of Metformin Hydrochloride by the standard addition method. The known amounts (4.0, 5.0 and 6.0ml) of working standard solutions of Metformin Hydrochloride (100µg/ml) were added to 2ml sample solution of Metformin Hydrochloride (100µg/ml) in 10ml of volumetric flask and diluted up to mark with methanol. Each solution was injected triplicate and recovery was calculated from regression equation of calibration curve by measuring peak areas.

Limit of Detection and Limit of Quantification:

LOD and LOQ of the drug were calculated using following equations according to ICH guidelines.

LOD = 3.3 σ/s and LOQ = 10 σ/s were found. Where, σ is the SD of the response

S is the slope of the calibration curve.

Robustness:

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing four small changes like Flow rate (1.0 ± 0.2 ml/min), Organic phase (70± 5ml), Injection Volume (20

±5µL), pH (5.0±0.5). After each sample solution was injected and area, HETP, tailing factor and retention time were checked.

An aliquot of 20µL from sample solution was injected under a chromatographic condition a peak area was measured and % assay was calculated from regression equation. Respons was an average of six determinations.

5.Result and Discussion

Selection of wavelength for measurement:

To determine Wavelength for measurement, standard spectra of Metformin Hydrochloride was scanned between 200-400nm. It is evident that Metformin Hydrochloride show an absorbance at 215nm respectively. The schematogram for standard and test Metformin Hydrochloride were shown in figure 2 and 3.

- Optimized Chromatographic Condition: HPLC System: LC 2010 CHT (Shimadzu)
- Column: C18 Waters (150mm x 4.5mm, 5µm)
- Mobile Phase: ACN: Ammonium acetate (pH 5.0 adjusted with glacial acetic acid) (85:15 %v/v)
- Flow rate: 1.0ml/min Detection Wavelength: 215nm Injection volume: 20µL
- Column oven temperature: Room temperature
- Run time: 10 min
- Diluents: Methanol

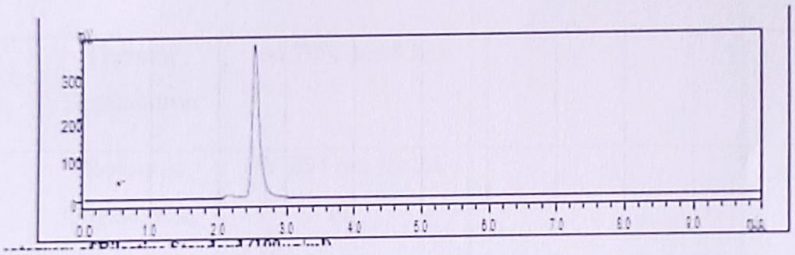


Figure: 2 Chromatogram of Metformin Hydrochloride Standard (100µg/ml)

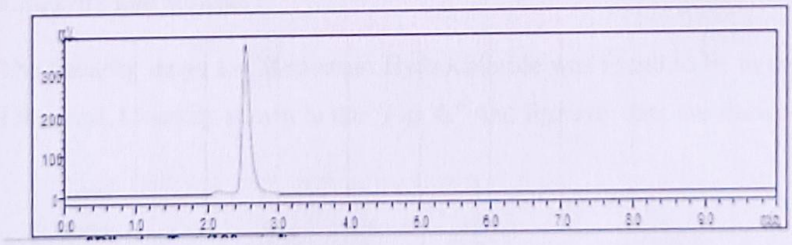


Figure: 3 Chromatogram of Metformin Hydrochloride Standard (100µg/ml)

Results of forced degradation study:

From degradation study it was found that Metformin Hydrochloride was marginally degraded in acid and oxidative conditions and stable in alkali, neutral, thermal, and photolytic conditions. Results of forced degradation study was shown in table 1.

Table: 1 Result of Forced Degradation Study of Metformin Hydrochloride

Sr. No.	Stress type	Condition	No. of peaks	%Degradation
1	Acid Hydrolysis	2 N HCl at 80°C for 30 min.	1	7.25
2	Alkali Hydrolysis	2 N NaOH at 80°C for 2 hr.	-	-
3	Neutral Hydrolysis	H2O at 80°Cfor 2 hr.	-	-
4	Oxidative Degradation	10% H2O2 at 80°C for 30 min.	1	5.24
5	Thermal Degradation	At 70°C for 8 hr.	-	-
6	Photolytic Degradation	UV 254 nm for 24 hr.	-	-

Linearity and Range:

The linearity range for Metformin Hydrochloride was found to be in the range of 25-150µg/ml. Linearity shown in the "Fig. 4." And linearity data are shown in table 3.

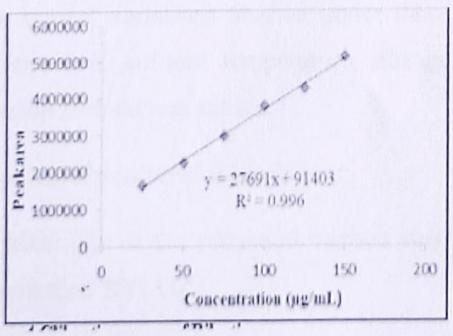


Figure: 4 Calibration curve of Metformin Hydrochloride

Precision:

The %RSD of Intra-day and Inter-day for Metformin Hydrochloride was found to be 0.64 to 0.81 and 0.14 to 0.32 respectively.

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation of Metformin Hydrochloride at three level (80% - 120%) of standard addition. Percentage recovery for Metformin Hydrochloride was 99.56 - 101.06%, shown in table 2.

Table: 2 Result of Accuracy study for Metformin Hydrochloride

Level	Test conc. (µg/ml)	Std. conc. (µg/ml)	Amt. of found ± S.D.	% Recovery
80%	50	40	89.69±0.87	99.66
100%	50	50	101.06±0.76	101.06
120%	50	60	109.52±1.10	99.56

Limit of Detection and Limit of Quantitation:

LOD and LOQ was found to be 0.45µg/ml and 1.20 µg/ml respectively.

Robustness:

The typical variations studied under these parameters are flow rate, mobile phase composition, column temperature, change in pH. According to data comparison develop method was robust.

Analysis of marketed formulation:

Applicability of the proposed method was tested by analysising the available tab formulation BYLOZA.

All method validation parameter of Metformin Hydrochloride results were shown in table 3.

Table 3: Validation Parameter of Metformin Hydrochloride

Sr. No.	Parameter	Metformin Hydrochloride
1.	Linearity Range	25-150 µg/ml
2.	Regression Line equation	Y=27691x+ 914034
3.	Correlation co-efficient	0.996
4.	Precision (%RSD)	
	Intra-day Precision	0.637-0.807
	Inter-day Precision	0.140-0.325
5.	Accuracy (%Recovery)	99.78-100.53
6.	Limit of Detection(µg/ml)	0.41
7.	Limit of Quantification(µg/ml)	1.25
8.	% Assay	101.1
9.	Robustness (% RSD of Assay)	0.85

Expected Outcomes

Development of a Reliable RP-HPLC Method: The project will result in the development of a novel reverse-phase HPLC (RP-HPLC) method that is optimized for the quantification of metformin hydrochloride in tablet dosage forms. This method will be efficient, reproducible, and precise, ensuring accurate measurement of the drug content in pharmaceutical formulations.

Validation of Analytical Parameters: The developed method will be validated according to regulatory guidelines (ICH/FDA) for key analytical parameters, including:

Accuracy: The method will demonstrate its ability to provide results close to the true value of metformin content in the tablets.

Precision: The method will show low variability in repeated measurements (both inter- and intra-day precision).

Linearity: The method will exhibit a direct proportional relationship between metformin concentration and the detector response within a defined range.

Specificity: The method will accurately measure metformin content without interference from excipients or other tablet components.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The method will have low LOD and LOQ, enabling detection and quantification of metformin at trace levels.

Application for Quality Control: The validated RP-HPLC method will be useful for routine quality control in pharmaceutical industries. It will allow for precise quantification of metformin hydrochloride in tablet formulations, ensuring batch-to-batch consistency, product quality, and compliance with pharmacopeial standards.

Regulatory Compliance: The method will comply with international regulatory standards, providing a reliable tool for pharmaceutical companies to meet the quality assurance requirements set by authorities like the FDA, EMA, and ICH.

Publication and Scientific Contribution: The successful development and validation of this method will contribute to the scientific community's knowledge on analytical techniques for anti-diabetic drugs, particularly metformin, and may lead to publications in scientific journals related to pharmaceutical analysis.

Potential for Broader Applications: Although the focus is on metformin hydrochloride, the method developed could serve as a foundation for similar studies involving other anti-diabetic drugs, offering a framework for quantifying drugs in complex pharmaceutical formulations using RP-HPLC.

6.Financial Statement

Certified that a grant of ₹6,00,000 was received from the Spechrom Solutions for the project titled " A Novel RP-HPLC Method Development and Validation for the Quantification of a Potential Anti-Diabetic Drug Metformin Hydrochloride in Tablet Dosage Form". The amount has been utilized as per the approved budget and guidelines.

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BHOYAN RATHOD
Kalol, Gandhinagar

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹2,47,000
2	Chemicals	₹1,53,000
3	Travel	₹99,000
4	Contingency	₹1,01,000
Total		**₹6,00,000**

7.Conclusion and Recommendations

The developed method was found to be simple, sensitive and selective for analysis of Metformin Hydrochloride. Metformin Hydrochloride was marginally degraded in acidic and oxidative conditions and was found to be stable in all other conditions. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drugs under non degradation condition. In the proposed study, stability-indicating RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Metformin Hydrochloride.

8.References

1. Stuart J. Practical HPLC method development by Lloyd R. Snyder (L. C. Resources, Inc.), Joseph J. Kirkland (Rockland Technologies, Inc.), and Joseph L. Glajch (DuPont Merck Pharmaceutical Co.)J Am Chem Soc. 1998;120(14):3540.

2. Metzger S, Kolbesen BO. Application of HPLC for the analysis of organic additives in cleaning chemicals and cleaning mixtures. Solid State Phenom. 2005;103-104:221-6. doi: 10.4028/www.scientific.net/SSP.103-104.221.

3. Rathinavel G, Uma Nath U, Valarmathy J, Samueljoshua L, Thanuja C, Ganesh M. RP-HPLC method for the simultaneous estimation of rosiglitazone and gliclazide in tablets. E-Journal of Chemistry. 2009;6(4):1188-92.

4. Neelima K, Prasad YR. Analytical method development and validation of metformin, voglibose, glimepiride in bulk and combined tablet dosage form by gradient RP-HPLC. Pharm. 2014;5(1):27-33. doi: 10.5530/phm.2014.1.5.

5. Bhole RP, Shinde SS, Chitlange SS, Wankhede SB. A high- performance thin layer chromatography (HPTLC) method for simultaneous determination of diphenhydramine hydrochloride and naproxen sodium in tablets. Anal Chem Insights. 2015;10:47-51.

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SSIU
BHOYAN RATHOD
Kalol, Gandhinagar

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 * VIKANI

For **Aum Research Labs Pvt Ltd.**

For Swarrnim Startup and Innovation University

Date: 11.11.2021

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:

"Hplc Method Development and Validation for Determination of Anti-diabetic Drug Alogliptin Benzoate in Bulk and in 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.**

Ref.No.swarrnim/RO/SCR/2022/11

Date: 14.03.2022

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: **"Hplc Method Development and Validation for Determination of Anti-diabetic Drug Alogliptin Benzoate in Bulk and in Tablets"**

Date of assigning problem: 11.11.21

Date of completion: 11.03.2022

Name of person: Mr. Karnav Patel

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. Karnav Patel

Thanking you.

Patel

Registrar

Startup & Innovation University
Bhoyan Rathod, Gandhinagar

HPLC Method Development and Validation for Determination of Antidiabetic Drug Alogliptin Benzoate in Bulk and Tablets

Research Project Report Submission to

Aum Research Lab

SWARRNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE.

Submitted by:

Principal Investigator: Mr. Karnav Patel, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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

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- **Summary**

This project focuses on the development and validation of an HPLC method for the assay of Alogliptin in injection formulations. The study aims to establish a reliable, reproducible, and efficient analytical method for quality control testing of Alogliptin injections. Through method validation following ICH (International Council for Harmonisation) guidelines, the developed method ensures accuracy, precision, specificity, and robustness. The validation parameters include linearity, accuracy, precision, and limit of detection, providing confidence in the quality assurance of pharmaceutical products.

- **Detailed Report**

1. Introduction

Since the first evidence about a known case of diabetes mellitus nearly 3000 years ago and despite the great deal of research that has been done recently, diabetes mellitus is still a wide spread serious disease that affect the life quality of millions of people worldwide. It is estimated that the number of patients with diabetes mellitus will rise to about 592 million by the year 2035 [1, 2]. It was until the year 1936 that diabetes mellitus was distinguished to Type 1 and Type 2 [1]. Two main features of Type 2 diabetes mellitus is the increased cell resistance to insulin and the dysfunction of the insulin-producing cell in the pancreas (β-cells) [2, 3]. The first line of therapy for the treatment of Type 2 diabetes is metformin, but as the disease progresses, a drug combination may be a must [4]. Incretin hormones are secreted in response to eating food from the gastrointestinal tract to the blood stream and can stimulate insulin secretion and help control glucose levels; that is, they prepare the body against increase in blood glucose. These hormones include glucagon-like peptide-1 and glucose-dependent insulin tropic polypeptide [5, 6]. Dipeptidyl peptidase-4 is an enzyme found in the human body that helps inactivate the incretin hormones, thus terminating their hypoglycaemic effect [2]. Alogliptin a member of dipeptidyl peptidase-4 inhibitors is a recent drug developed in 2010 by Takeda Pharmaceutical Company [2, 7], which is used for the treatment of Type 2 diabetes, and it potentiates the effect of incretin hormones through inhibition of their degradation by the dipeptidyl peptidase-4 enzyme [2, 4]. Alogliptin can be used alone or in combination therapy, and it is now approved in the USA and Europe also [5]. Alogliptin is 2- ({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin 1yl}methyl)benzonitrile (C18H21N5O2), and its structure is shown in Figure 1 [8].

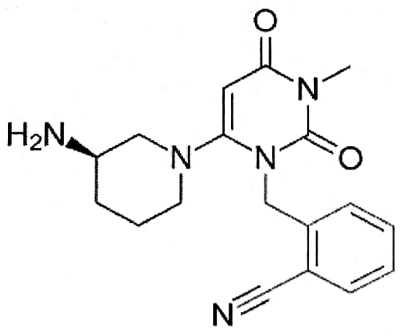

Fig. 1: Chemical structure of Alogliptin

2. Literature Review

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [9–12].

Literature survey revealed a few methods reported for determination of alogliptin benzoate in bulk drug as well as pharmaceutical preparation [2, 5, 13–16].

In this research, a new sensitive and rapid HPLC method was developed for the determination of alogliptin benzoate in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

3. Objectives

1. To develop a simple and efficient HPLC method for the quantitative determination of Alogliptin Benzoate in bulk and tablet dosage forms.
2. To optimize chromatographic conditions for accurate and reproducible results.
3. To validate the developed method according to ICH guidelines.
4. To evaluate key validation parameters such as: Specificity, Linearity, Accuracy, Precision (repeatability and intermediate precision), Limit of Detection (LOD), Limit of Quantification (LOQ), Robustness
5. To ensure the developed method is suitable for routine quality control analysis of Alogliptin Benzoate in pharmaceutical formulations.

4. Methodology

Drugs and Chemicals

Instrumentation. Agilent 1200 HPLC system was used for liquid chromatography method development and validation (Santa Clara, USA), equipped with a pump (model G1312A), an auto sampler (ALS) (model G1329A), and a Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 μm column (Paisley, UK), and the detector consisted of UV/VIS operated at 277 nm. Chemstation Software (Version Rev B.04.03 (16)) was used for data processing and evaluation.

Chemicals and Reagents. A pharmaceutical grade sample of alogliptin benzoate (assigned purity 99.4%) was obtained as gift from Jordan Hikma Pharmaceuticals (Amman, Jor- dan). NESINA tablets containing 8.5 mg alogliptin benzoate were purchased from the local market. Acetonitrile HPLC grade and ammonium carbonate were purchased from Merck (Merck Serono Amman, Jordan). The double distilled water was obtained from a local pharmaceutical company.

Chromatographic Conditions. The mobile phase was prepared by dissolving 1.0 gm ammonium carbonate in 1000 ml water. From the previous solution, 450 ml was mixed with 550 ml of acetonitrile. Prior to use the mobile phase was filtered through 0.45 μm membrane filters and degassed by sonication for 10 min. The analysis was carried out on an Agilent 1200 series HPLC system. The analytes were conducted on an analytical column C18, 5 μm, 250 4.6 mm with a detection wavelength of 277 nm. The operating temperature of the column was set at 30 $^{\circ}$C. The injection volume was 10 μL, and the flow rate was maintained at 1.0 mL/min. The run time was 6 minutes.

Preparation of Standard Solution. A standard solution of alogliptin benzoate was prepared by dissolving an accurately weighed amount of alogliptin benzoate (42.5 mg, which is equivalent to 31.25 mg alogliptin) in 50 ml of the mobile phase, and then 5 mL of the resulting solution was diluted to 25 mL by the same solvent to obtain a standard solution of alogliptin benzoate (170 μg/ml).

Preparation of Sample Solution. Twenty alogliptin tablets were weighed, triturated in porcelain mortar, and mixed, and the average weight of tablet was calculated. Accurately weighed amount of powder equivalent to 25 mg of alogliptin (34 mg alogliptin benzoate) was transferred completely to a 200 mL volumetric flask, and 150 mL of the mobile phase was added and sonicated for 30 minutes. The volume was completed to mark by the same solvent to obtain a solution of alogliptin benzoate with a concentration of 170 μg/ml. The prepared solution was filtered through 0.45 μm membrane filters.

Method Validation. The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD), and robustness [9, 17].

Specificity. Specificity is one of the significant features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture [18]. Specificity of the method was evaluated by injecting 10 μL solutions of standard, sample, blank, and placebo separately.

Linearity. To evaluate the linearity and range of the method, different standard solutions were prepared by di- luting the standard stock solution with the mobile phase in deferent concentrations of alogliptin benzoate: 85, 136, 170, 204, 255, and 306 μg/mL, which cover 50%, 80%, 100%, 120%, 150%, and 180% of the target concentration, respectively. Three injections from each concentration were analysed under the same conditions. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

Sensitivity. Limit of detection (LOD)/limit of quantitation (LOQ) of alogliptin benzoate were determined by analysing different solutions of alogliptin benzoate and measuring the signal-to-noise ratio. The limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3: 1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10: 1 with %RSD (n =3) of less than 10%.

Accuracy. The accuracy of the assay method was determined by recovery studies at three concentration levels (50%, 100%, and 150%), i.e., 85, 170, and 255 μg/ml, and three samples from each concentration were injected. The percentage recovery of added alogliptin benzoate and RSD were calculated for each of the replicate samples.

Precision. The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively [19–22]. System precision was established by ten measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day [23]. The RSD of obtained results was calculated to evaluate repeatability results.

Robustness. Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:

(v) Column temperature: ±5 °C

(vi) Flow rate: ±0.2 mL/min

(vii) Wavelength: ±3 nm

(viii) Mobile phase composition, organic composition ±5%

Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

Stability of Analytical Solutions. The stability of analytical solutions was determined by analysing the standard and sample preparations at 0 h and after one day in refrigerator and at ambient room temperature 30 °C. Three injections from each solution were analysed, and the average of the peak and the RSD were calculated.

5. Results and Discussion

Method Development and Optimization. Several physical and chemical properties of alogliptin benzoate were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC-UV chromatographic conditions, including detection wave- length, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of acetonitrile and ammonium carbonate buffer and optimizing the chromatographic conditions on the Hypersil Gold Thermo Scientific C18 (250 cm 4.6 mm) 5 μm column. The results of method optimization are summarized in Table 1.

The mobile phase consisting of acetonitrile and ammonium carbonate buffer in the ratio 55 : 45 v/v with a flow rate of 1 mL/min, injection volume 10 μl, run time 6 min, and column temperature 30 °C at wavelength (λ) 277 was optimized as the best chromatographic conditions for the entire study where alogliptin benzoate was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 4 min (Figure 2).

Analytical parameters validation

Specificity. Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (alogliptin 170 µg/ml). For this purpose, 10 μl from solutions mobile phase blank, standard solution, and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in Figures 2–5. It can be observed that there no coeluting peaks at the retention time

of alogliptin benzoate interference. This result indicates that the peak of the analyte was pure and this confirmed the specificity of the method.

Linearity and Range. Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figure 6) gave linear relationship over the concentration range of 85–306 μg/ml for alogliptin benzoate. From the regression analysis, a linear equation was obtained: y 17412x + 1.1377, and the goodness-of-fit (r^2) was found to be 1.00, indicating a linear relationship between the concentration of analyte and area under the peak.

Limit of Detection and Limit of Quantification (LOD and LOQ). The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision [24]. The results showed an LOD and LOQ for alogliptin of 0.03 and 0.09 μg, respectively.

Accuracy. The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of 99.4–101.9%, and RDS values were in the range of 0.06–0.43% as shown in Table 2. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the applicability of the method for routine drug analysis.

Precision. The precision of the method is defined as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation [25]. The results of both system and method precision showed that the method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results are within limits. Acceptable precision was not more than 2.0% for the RSD and the tailing factor and not less than 1000 for number of plates, as shown in Tables 3 and 4.

Robustness. The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method, as mentioned in Section 2.6.6. The results of robustness testing showed that a minor change of

method conditions, such as the composition of the mobile phase, temperature, flow rate, and wavelength, is robust within the acceptable limits. The results are summarized in Table 5. In all modifications, good separation of alogliptin benzoate was achieved, and it was observed that the percent of recovery was within acceptable limits and the %RSD is within limit of not more than 2.0%. The tailing factors and number of theoretical plates were found within acceptable limits as well.

Solution Stability. The percent of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard solutions for 24 hr at both conditions. The percent of recovery was within acceptable limits, and the %RSD is within the limit of not more than 2.0%. The tailing factors and number of theoretical plates were found within acceptable limits as well. The results are shown in Table 6.

TABLE 1: Results of method optimization.

Column used	Mobile phase	Flow rate	Wavelength	Observation	Result
Restek C18, 125 × 4.0 mm i.d., 5 µm	(Buffer : methanol) (45 : 55) v/v	1.0 ml/min	216 nm	Poor resolution 1.4	Method rejected
Thermo Scientific C18, 250 × 4.6 mm i.d., 5 µm	(Buffer : acetonitrile) (25 : 75) v/v	1.0 ml/min	277 nm	Poor resolution 1.6	Method rejected
Thermo Scientific C18, 250 × 4.6 mm i.d., 5 µm	(Buffer : acetonitrile) (45 : 55) v/v	1.0 ml/min	277 nm	Good resolution 2.4	Method accepted

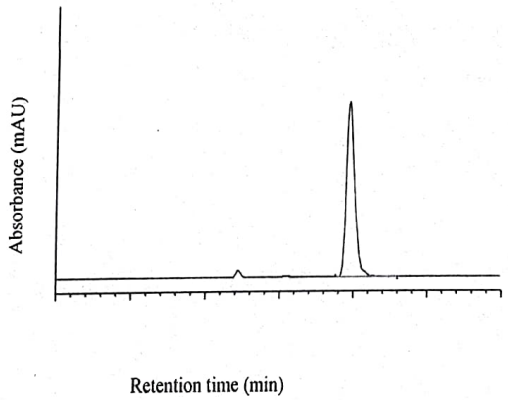

FIGURE 2: Chromatogram of alogliptin standard solution.

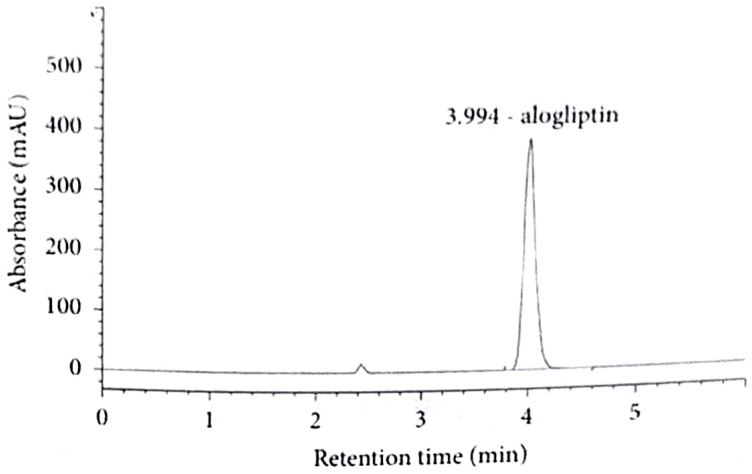

FIGURE 3: Chromatogram of alogliptin sample solution.

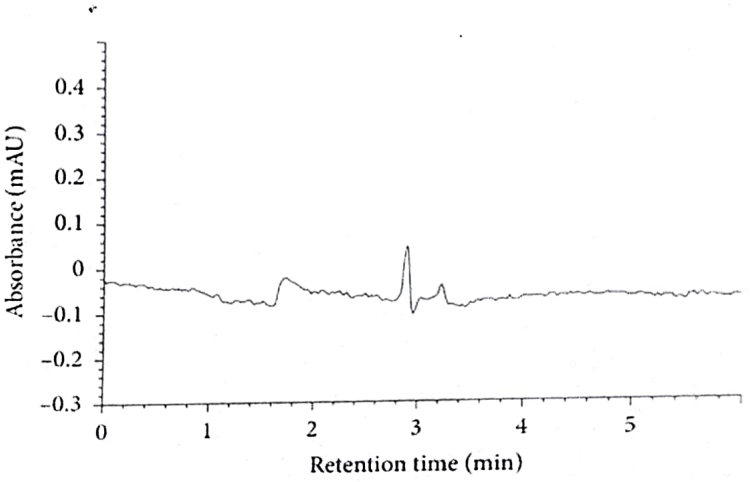

FIGURE 4: Chromatogram of blank solution.

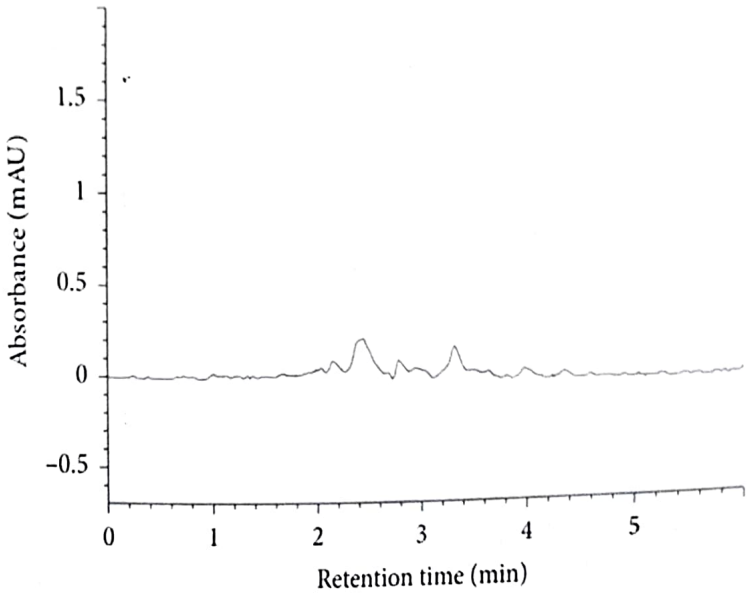

FIGURE 5: Chromatogram of placebo solution.

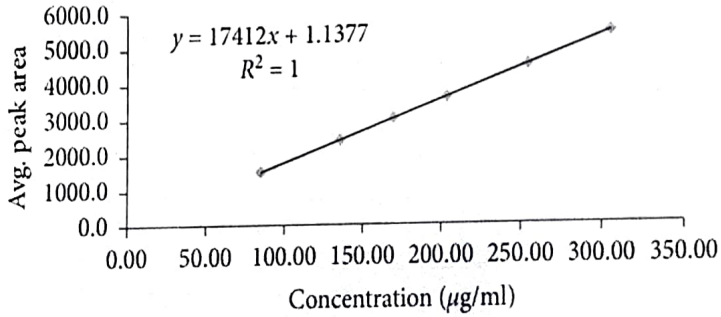

FIGURE 6: Standard calibration curve of alogliptin benzoate.

Table 2: Recovery data of the proposed HPLC method.

% spiked level	Replicate number	Peak area	% recovery	Mean %RSD
50	1	1508.4	101.9	101.5
	2	1495.5	101.0	0.43
	3	1503.5	101.6	
100	1	2950.7	99.4	99.4
	2	2950.8	99.4	0.06
	3	2953.8	99.5	
150	1	4443.5	100.2	100.1
	2	4435.4	100.0	0.13
	3	4431.9	99.9	
Mean (% of recovery)	98.0–102.0		100.318	
%RSD	Max 2.00		0.964149	

Table 3: System precision data from the standard solution of the proposed HPLC method.

Replicate number	RT	Peak area	Number of theoretical plates	Tailing factor
1	3.954	2952	1.32	6274
2	3.956	2951	1.36	6388
3	3.961	2951	1.35	6363
4	3.959	2960	1.33	6364
5	3.961	2953	1.36	6386
6	3.965	2946	1.36	6441
7	3.962	2949	1.38	6479
8	3.965	2950	1.35	6486
9	3.965	2954	1.35	6464
10	3.969	2958	1.33	6471
Average	3.962	2952	1.3	6412
%RSD	—	0.10	—	—

Table 4: Method precision data from the sample solution of the proposed HPLC method.

Alogliptin 6.25 mg tablet					
Replicate number	RT	Peak area	Tailing	Plates	% assay
1	4.025	3009	1.54	8086	99.2
2	4.024	3012	1.52	8049	99.2
3	4.027	3009	1.48	8101	99.2
4	4.027	3009	1.49	8105	98.6
5	4.028	3015	1.50	8039	99.3
6	4.027	3012	1.50	8107	99.5
Average	4.026	3011.0	1.5	8081	99.2
%RSD	—	0.1			0.31

Table 5: Robustness data of the proposed HPLC method.

Parameter		%RSD of standard peak area	%RSD of assay
Column temperature	25°C	0.07	0.15
	30°C (normal)	0.03	0.19
	35°C	0.04	0.2
Wavelength	274 nm	0.06	0.07
	277 nm (normal)	0.03	0.19
	280 nm	0.06	0.17
Mobile phase composition	−5% acetonitrile	0.05	0.20
	Normal	0.03	0.19
	+5% acetonitrile	0.02	0.14
Flow rate	0.8 ml/min	0.04	0.11
	1 ml/min (normal)	0.03	0.19
	1.2 ml/min	0.08	0.23

Table 6: Solutions stability data of the proposed HPLC method.

Parameter		RT	Avg. peak area	RSD peak area (%)	Tailing factor	Recovered (%)	Number of theoretical plates
Standard solution	0 h	4.034	3022.7	0.07	1.5	–	8058
	After 24 h at 30°C	4.035	3021.7	0.2	1.6	100.0	8143
	After 24 h at refrigerator	4.049	2983.7	0.08	1.5	98.7	8137
Sample solution	0 h	4.034	2995.7	0.07	1.5	–	8142
	After 24 h at 30°C	4.035	3001.3	0.3	1.5	100.2	8179
	After 24 h at refrigerator	4.036	3000.0	0.2	1.6	100.1	8188

6. Financial Statement

Certified that a grant of ₹5,00,000 was received from the Aum Research Lab for the project titled " HPLC Method Development and Validation for Determination of Antidiabetic Drug Alogliptin Benzoate in Bulk and Tablets ". The amount has been utilized as per the approved budget and guidelines.

Sr.No.	Particulars	Expenditure Incurred
1	Equipment	₹2,45,000
2	Chemicals	₹1,55,000
3	Travel	₹48,000
4	Contingency	₹52,000
Total		₹5,00,000

Total Project Cost:

This budget focuses on the most critical components of the project, ensuring that key activities, such as translation and basic coordination, can be completed within the financial constraints. While the budget is minimal, it is designed to achieve the project's primary goals effectively.

6.0 Justifications:

Justifications for Project Staff:

The project staff, specifically the Project Assistant (part-time), is essential for supporting the successful execution of the project. The primary responsibilities of the project assistant include assisting in laboratory work, data collection, preparing and analyzing samples, documenting results, maintaining the lab, and ensuring the smooth running of day-to-day activities. Given that this project requires careful monitoring of experimental processes, method validation, and testing, the project assistant's role is crucial to maintaining timelines and meeting the project objectives efficiently. The allocation for the project assistant's salary ensures that the project progresses without delays and maintains a high standard of data integrity and process management.

Justification for Equipment:

The equipment budget is necessary to facilitate the HPLC analysis of alogliptin benzoate in bulk and pharmaceutical dosage forms. This includes essential consumables like HPLC columns, syringes, filters, and other laboratory materials that are necessary to maintain the HPLC system's functionality and accuracy. The HPLC system and consumables such as columns are vital for ensuring precise and accurate measurements in the method development and validation process. Additionally, software for data analysis will be required for efficient processing and interpretation of results, as per the validation and compliance with ICH and FDA guidelines. Without appropriate equipment and consumables, the objectives of the project cannot be met within the set timeframe.

Justification for Other Budget Heads and Amount for the Project:

1. **Consumables**: The consumables budget covers the essential reagents, solvents, and chemicals required for the HPLC method development and validation process. This includes reagents for calibration, sample preparation, mobile phase solvents, and other materials required to maintain a continuous and error-free workflow. These consumables are necessary to conduct multiple test runs, method optimizations, and final validations of the analytical method for alogliptin benzoate.

2. **Testing and Validation**: Testing costs cover the system suitability testing, method

validation (accuracy, precision, specificity, etc.), and analytical tests to ensure that the method adheres to ICH and FDA guidelines. This budget is crucial to ensure the method is precise, accurate, and reproducible. The validation process is an essential part of the project, ensuring the robustness of the method for commercial and regulatory use.

3. **Travel**: Travel expenses are included for any necessary conferences, seminars, or collaborations related to the project. Travel might also be required for field visits or consultations with experts in method development and validation. This amount ensures that the project remains aligned with international best practices, networking with experts, and participating in events that enhance the research outcomes.

4. **Contingencies**: The contingency fund is allocated to cover unexpected expenses that may arise during the course of the project. This could include unforeseen costs for additional reagents, equipment repair, or shipping charges for lab materials. A contingency amount ensures that the project can adapt to unexpected needs without disruption to the work plan.

Each of these categories is essential for the smooth and timely completion of the project, ensuring that the HPLC method for alogliptin benzoate is developed and validated to meet regulatory and scientific standards. The budget allocation reflects the needs for personnel, equipment, consumables, and other resources critical to the success of the project.

7. Conclusion

In the present research, a fast, simple, accurate, precise, and linear stability-indicating HPLC method has been developed and validated for alogliptin benzoate, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for alogliptin benzoate. In addition, the main features of the developed method are short run time and retention time around 4 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

8. Reference

1. A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: a review of current trends," *Oman Medical Journal*, vol. 27, no. 4, pp. 269–273, 2012.
2. K. Zhang, P. Ma, W. Jing, and X. Zhang, "A developed HPLC method for the determination of alogliptin benzoate and its potential impurities in bulk drug and tablets," *Asian Journal of Pharmaceutical Sciences*, vol. 10, no. 2, pp. 152–158, 2015.
3. C. Chen, C. M. Cohrs, J. Stertmann, R. Bozsak, and S. Speier, "Human beta cell mass

and function in diabetes: recent advances in knowledge and technologies to understand disease pathogenesis," *Molecular Metabolism*, vol. 6, no. 9, pp. 943–957, 2017.

4. M. Rendell, A. Drincic, and R. Andukuri, "Alogliptin benzoate for the treatment of type 2 diabetes," *Expert Opinion on Pharmacotherapy*, vol. 13, no. 4, pp. 553–563, 2012.
5. K. Sharma and A. Parle, "Development and validation of HPTLC method for estimation of alogliptin benzoate in bulk drugs and tablet dosage forms," *International Bulletin of Drug Research*, vol. 5, no. 8, pp. 81–89, 2015.
6. W. Kim and J. M. Egan, "The role of incretins in glucose homeostasis and diabetes treatment," *Pharmacological Re- views*, vol. 60, no. 4, pp. 470–512, 2008.
7. K. Y. Kavitha, G. Geetha, R. Hariprasad, M. Kaviarasu, and R. Venkat Narayanan, "Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form," *Journal of Chemical and Pharmaceutical Re- search*, vol. 5, no. 1, pp. 230–235, 2013.
8. P. Supriya, N. L. Madhavi, K. Rohith, G. Ramana, U. Harini, and A. Pawar, "Development and validation of UV spectrophotometric and reversed Phase-high performance liquid chromatography-PDA methods for the estimation of alogliptin benzoate," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 9, no. 1, pp. 282–287, 2016.
9. ICH Guideline, "Validation of analytical procedures: text and methodology," in *Proceedings of International Conference on Harmonization, Topic Q2 (R1)*, Geneva, Switzerland, November 2005.
10. S. Chandran and R. S. P. Singh, "Comparison of various international guidelines for analytical method validation," *Pharmazie*, vol. 62, no. 1, pp. 4–14, 2007.
11. T. M. Kalyankar, P. D. Kulkarni, S. J. Wadher, and S. S. Pekamwar, "Applications of micellar liquid chromatography in bioanalysis: a review," *Journal of Applied Pharmaceutical Science*, vol. 4, no. 1, pp. 128–134, 2014.
12. R. N. El-Shaheny, M. H. El-Maghrabey, and F. F. Belal, "Micellar liquid chromatography from green analysis perspective," *Open Chemistry*, vol. 13, no. 1, pp. 877–892, 2015.
13. M. M. Mabrouk, S. F. Hammad, F. R. Mansour, and M. M. Amer, "Development and

validation of a reversed phase HPLC method for simultaneous determination of antidiabetic drugs alogliptin benzoate and pioglitazone HCl," *Der Pharmacia Sinica*, vol. 7, no. 2, pp. 32–40, 2016.

14. K. S. Potdar, M. S. Kalshetti, and R. Y. Patil, "Development and validation of a novel RP-HPLC method for simultaneous estimation of alogliptin benzoate and pioglitazone HCL in pharmaceutical dosage form," *International Journal of Chemical and Pharmaceutical Analysis*, vol. 4, no. 3, 2017.
15. P. B. Deshpande and S. R. Butle, "Stability indicating high performance thin layer chromatographic determination of alogliptin benzoate as bulk drug and in tablet dosage form," *Eurasian Journal of Analytical Chemistry*, vol. 12, no. 4, pp. 325–335, 2017.
16. N. T. Lamie and M. A. Mahrouse, "Smart spectrophotometric methods based on normalized spectra for simultaneous de- termination of alogliptin and metformin in their combined tablets," *Spectrochimica Acta Part A: Molecular and Bio- molecular Spectroscopy*, vol. 204, pp. 743–747, 2018.
17. FDA, CDER, Beers, and Donald, *Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry*, FDA, Silver Spring, MD, USA, 2015.
18. N. Batrawi, H. Naseef, and F. Al-Rimawi, "Development and validation of a stability-indicating HPLC method for the simultaneous determination of florfenicol and flunixin meglumine combination in an injectable solution," *Journal of Analytical Methods in Chemistry*, vol. 2017, Article ID 1529280, 7 pages, 2017.
19. C. M. Riley, T. W. Rosanske, and S. R. R. Riley, *Specification of Drug Substances and Products: Development and Validation of Analytical Methods*, Elsevier, New York, NY, USA, 2014.
20. A. Agarwal, S. Tiwari, and K. Nagariya, "Method development and its validation for quantitative simultaneous de- termination of latanoprost, timolol and benzalkonium chloride in ophthalmic solution by RP-HPLC," *Journal of Drug Delivery and Therapeutics*, vol. 3, no. 2, 2013.
21. N. R. Poola, D. Bhuiyan, S. Ortiz et al., "A novel HPLC assay for pentamidine: comparative effects of creatinine and inulin on GFR estimation and pentamidine renal excretion in the isolated perfused rat kidney," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 5, no. 2, pp. 135–145, 2002.
22. S. Chandran, R. S. P. Singh, and A. Sajeev Chandran, "Comparison of various international guidelines for analytical method validation," *Pharmazie*, vol. 62, pp. 4–

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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

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Email: tech.support@gccbiotech.co.in/info@gccbiotech.co.in
Website: www.gccbiotech.co.in

Date: 27.5.2021

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:

"Analytical Hplc Method Validation for the Determination of Assay of Levetiracetam in Levetiracetam Injection Formulation"

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. 8,50,000 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

SWARRNIM
STARTUP & INNOVATION
UNIVERSITY
WHERE IDEAS COME ALIVE.
INDIA'S FIRST UNIVERSITY FOR STARTUP

Ref.No.swarrnim/RO/SCR/2021/40

Date: 18.09.2021

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: **"Analytical Hplc Method Validation for the Determination of Assay of Levetiracetam in Levetiracetam Injection Formulation."**

Date of assigning problem: 27.05.2021

Date of completion: 15.09.2021

Name of person: Ms. Dhara Soni

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. Dhara Soni.

Thanking you.

Patel

Registrar

Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Analytical HPLC Method Validation for the Development of Assay of Levetiracetam Injection Formulation

Research Project Report Submission to

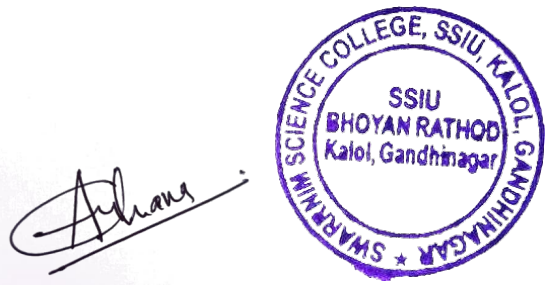
GCC Biotech Pvt Ltd

Submitted by:

Principal Investigator: Ms. Dhara Soni, Swarrnim Science College, Swarrnim Startup & Innovation University, Gandhinagar, Gujarat

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

INDEX



- **Declaration**

I, Ms. Dhara Soni, Principal Investigator of the project titled " Analytical HPLC Method Validation for the Development of Assay of Levetiracetam Injection Formulation ", certify that the project work has been carried out as per the terms and conditions of the University Grants Commission.

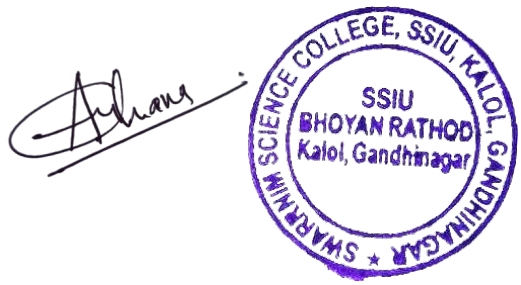
Name:

Principal Investigator: Ms. Dhara Soni

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.



- **Summary**

This project focuses on the development and validation of an HPLC method for the assay of Levetiracetam in injection formulations. The study aims to establish a reliable, reproducible, and efficient analytical method for quality control testing of Levetiracetam injections. Through method validation following ICH (International Council for Harmonisation) guidelines, the developed method ensures accuracy, precision, specificity, and robustness. The validation parameters include linearity, accuracy, precision, and limit of detection, providing confidence in the quality assurance of pharmaceutical products.

- **Detailed Report**

1. Introduction

Pharmaceutical analysis is a vital branch of pharmaceutical sciences that deals with the identification, determination, quantification, and purification of substances. It plays an indispensable role in ensuring the safety, efficacy, and quality of pharmaceutical products. The analytical process helps in monitoring the quality of raw materials, intermediates, and final formulations. As regulations become increasingly stringent, analytical methods must be robust, precise, accurate, and reproducible. With the advancement of analytical techniques, High-Performance Liquid Chromatography (HPLC) has emerged as a cornerstone for the quantification and analysis of pharmaceutical compounds. Method validation is a critical process to confirm that an analytical procedure employed for a specific test is suitable for its intended purpose. Regulatory agencies like the International Council for Harmonisation (ICH), United States Pharmacopeia (USP), and Food and Drug Administration (FDA) emphasize the necessity of validating analytical methods during the development and production of pharmaceuticals. The validated methods ensure reliability, reproducibility, and accuracy in quantification, and they are crucial for establishing the identity, purity, content uniformity, and stability of drug substances and drug products.

A validated analytical method supports drug approval submissions, routine quality control, stability testing, and in-process monitoring. The parameters commonly evaluated during method validation include specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability. High-Performance Liquid Chromatography (HPLC) is a highly accurate and reliable analytical technique used extensively in pharmaceutical industries. It offers several advantages including high resolution, sensitivity, and the ability to separate complex mixtures. HPLC can be applied to determine the content and purity of active pharmaceutical ingredients (APIs), impurities, excipients, degradation products, and dosage forms.

HPLC operates by injecting a liquid sample into a column packed with a solid adsorbent material under high pressure. The analytes in the sample interact differently with the stationary phase, leading to separation based on polarity, molecular weight, or hydrophobicity. The separated components are detected using UV, fluorescence, or other detectors.

The significance of HPLC becomes even more pronounced in the development of injectable formulations, where stringent quality requirements mandate precise quantification of the drug content and the presence of potential impurities.

Injectable drug formulations are parenteral preparations designed to be administered through the skin or mucous membranes using a syringe or needle. They are typically used when the drug cannot be effectively delivered orally due to poor bioavailability, instability in the gastrointestinal tract, or the need for rapid onset of action.

Injection formulations demand exceptional standards of sterility, stability, and accurate dosing. Therefore, the development and validation of robust analytical methods for injectable drugs are crucial. The determination of active drug content in injection formulations must be performed with high precision to comply with pharmacopeial and regulatory guidelines.

Levetiracetam is a second-generation antiepileptic drug (AED) that is chemically designated as (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide. It is primarily used in the treatment of epilepsy and seizure disorders, including partial-onset seizures, myoclonic seizures, and generalized tonic-clonic seizures. It is often used as an adjunctive therapy in patients who do not respond adequately to other medications.

Levetiracetam exhibits unique pharmacokinetic properties such as nearly complete oral bioavailability, rapid absorption, minimal protein binding, and lack of hepatic metabolism, making it suitable for both oral and intravenous administration. The intravenous (IV) injection form is particularly beneficial for patients who are unable to take oral medications or require immediate seizure control. The assay of Levetiracetam injection is critical to ensure that the drug concentration in each unit meets predefined quality specifications. An accurate assay method helps in determining whether the product conforms to the content uniformity and potency requirements prescribed by pharmacopeial standards such as the USP and BP (British Pharmacopeia).

Due to the hydrophilic nature and low protein binding of Levetiracetam, it is challenging to develop a method that can quantify it precisely in an aqueous injection matrix. Hence, the HPLC method must be carefully developed and validated to address factors such as matrix interference, solubility, stability, and sensitivity.

2. Literature Review

Few methods are proposed to quantify levetiracetam in tablet dosage form. They are colorimetry, [10,11] UV spectrophotometry [11, 12] and HPLC. [13-16] Several methodologies for quantifying levetiracetam concentration in human serum or plasma have been developed. They are HPLC, [17-19] UPLC,[20] LC-MS, [21-24] UPLC-MS [25] and GC-MS. [26] Few analytical procedures for determining levetiracetam concentration in saliva was developed using LC-MS,[24] UPLC-MS [25] and GC-MS. [26] To the best of our information through online survey, there is no documented method to evaluate levetiracetam in injectable dosage forms utilizing stability indicating RP-HPLC. Hence, this investigation is aimed at developing and validating a stability indicating RP-HPLC method to assess levetiracetam content in injectable dosage forms.

3.Objectives

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

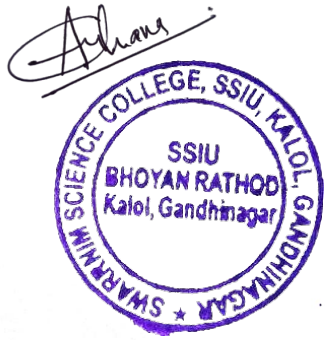
2. To validate the developed method in accordance with ICH guidelines for linearity, accuracy, precision, specificity, and limit of detection (LOD).

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

4. Methodology

1. Development of HPLC Method:
    - Column: C18 (250 mm × 4.6 mm, 5 µm).
    - Mobile Phase: A mixture of phosphate buffer (pH 7.0) and acetonitrile (60:40 v/v).
    - Detection: UV detection at 210 nm.
    - Flow Rate: 1.0 mL/min.
    - Injection Volume: 20 µL.

2.Validation Parameters:



- Linearity: Constructing a calibration curve over a concentration range of 10-100 µg/mL of Levetiracetam.
- Accuracy: Conducting recovery studies to ensure the method's ability to recover the target compound from spiked formulations.
- Precision: Intra-day and inter-day precision testing to ensure consistency in results.
- Specificity: Testing the ability of the method to distinguish Levetiracetam from excipients and other formulation components.
- Limit of Detection (LOD) and Limit of Quantification (LOQ): Determining the lowest detectable and quantifiable amounts of Levetiracetam in the sample.

3.Experimental Setup:

- Sample Preparation: Levetiracetam injection formulations are diluted, filtered, and injected into the HPLC system.
- Data Analysis: Results are analyzed using HPLC software to calculate peak areas and compare with standard solutions.

5. Results and Discussion

1. Calibration Curve: A linear relationship between concentration and peak area with a correlation coefficient (r^2) of ≥ 0.999.

2. Accuracy: Recovery studies showed that the method had an accuracy of 98-102%, which is within the acceptable range for pharmaceutical analysis.

3. Precision: Intra-day and inter-day precisions were found to be less than 2% relative standard deviation (RSD), indicating good repeatability.

4. Specificity: No significant interference from excipients, proving the specificity of the method for Levetiracetam analysis.

5. LOD and LOQ: The method demonstrated LOD and LOQ values of 0.5 µg/mL and 1.5 µg/mL, respectively, indicating high sensitivity.

Validation of the Suggested Method

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

Validation parameters (system suitability, linearity, sensitivity, precision, accuracy, robustness, ruggedness, specificity and selectivity) were validated following criteria of International Conference on Harmonization.[27]

System Suitability

<table>
<tr><td>INJ NO.</td><td>PEAK AREA</td><td>STATISTICAL ASSESSMENT</td><td>CRITERIA FOR ACCEPTANCE</td></tr>
<tr><td>1</td><td>2228299</td><td>Average</td><td rowspan="6">RSD percent for five replicates of standard solution - <2.0%</td></tr>
<tr><td>2</td><td>22304665</td><td rowspan="2">RSD percent</td></tr>
<tr><td>3</td><td>2230925</td></tr>
<tr><td>4</td><td>2230925</td><td rowspan="2">0.05</td></tr>
<tr><td>5</td><td>2230091</td></tr>
<tr><td>Plate count</td><td colspan="2"></td></tr>
</table>

TABLE 1: Levetiracetam system suitability details

Inj. – injection; No. – number; RSD – relative standard deviation

Linearity and Range

Standard linearity was conducted to evaluate to see if a single point calibration could provide adequate accuracy over the method's intended operating range. For standard linearity, five levels of concentration were assessed over a range of 50% level (0.0519 mg/ml) to 150% level (0.1557 mg/ml) of the test concentration (0.1 mg/ml of levetiracetam). Linearity results for standard levetiracetam are shown in Table 2. The results confirmed that the procedure met the criteria (regression coefficient was >0.999) for linearity in the range of 0.0519 mg/ml to 0.1557 mg/ml.

Table 2: Levetiracetam linearity details

Level (with relating to test concentration – 0.1 mg/ml)	Concentration (mg/ml)	Area response
50	0.0519	1173413
75	0.0779	1748966
100	01038	2294224
125	0.1298	2846245
150	0.1557	3362764
Regression equation	y = 21123169.41219 c + 94730	
Regression coefficient	0.9996	

y = area response; c = concentration (mg/ml)

Table 3: Levetiracetam system precision details

Inj. No.	Area response	Statistical assessment	Retention time	Statistical assessment
Inj. 1	2231098		10.338	
		Average:		Average:
Inj. 2	2229853		10.335	
		2230799		10.318
Inj. 3	2230206		10.329	
Inj. 4	2230915		10.314	
		RSD percent:		RSD percent:
Inj. 5	2230599		10.305	
		0.04		0.20
Inj. 6	2232123		10.287	

Inj. – injection; No. – number; RSD – relative standard deviation

Precision

System Precision

Injected the standard solution (0.1 mg/ml levetiracetam) 6 times and determined the percent RSD of area and retention time of levetiracetam peak. The findings are shown in Table 3. The results confirmed that the procedure met the criteria (percent RSD was <2.0%) for system precision

Method Precision

Six injection formulation sample solutions were prepared at concentration level of 100% (0.1 mg/ml). For every sample, the assay of levetiracetam according to the developed method was determined. The percent RSD for assay results were assessed. The findings are shown in Table 4. The results confirmed that the procedure met the criteria (percent RSD was <2.0%) for method precision.

Accuracy

Samples for accuracy study were made by adding levetiracetam reference drug to excipient solution at concentrations of 50% (0.0507 mg/ml), 100 % (0.1014 mg/ml) and 150% (0.1521 mg/ml) relating to test concentration (0.1mg/ml of levetiracetam). For every sample, the assay of levetiracetam according to the developed method was assessed. For individual preparations, the percent recovery was measured at every concentration level and an average of the percent recovery was estimated. For everyconcentration level the percent RSD for percent recovery was also estimated. The findings are shown in Table 6. The results confirmed that the procedure met the criteria (percent recovery was 97.0 – 103.0% and percent RSD was <2.0%) for accuracy

Specificity

To prove that the excipients and diluent do not interfere with the assessment of levetiracetam, the pure substance (levetiracetam - 0.1 mg/ml), diluent, injection formulation (levetiracetam - 0.1 mg/ml) and excipient solution was analyzed individually by the suggested method. The levetiracetam (0.1 mg/ml) spiked in the excipient solution was also analyzed by the suggested method. The retention times in all the cases were compared to establish specificity [Figures 1a-1e]. The results confirmed that the procedure met the criteria for specificity because no peaks due to the excipients/diluent were noted to be interfering with the

assessment of levetiracetam.

Table 3: Levetiracetam system precision details

Inj. No.	Area response	Statistical assessment	Retention time	Statistical assessment
Inj. 1	2231098	Average: 2230799	10.338	Average: 10.318
Inj. 2	2229853		10.335	
Inj. 3	2230206		10.329	
Inj. 4	2230915	RSD percent: 0.04	10.314	RSD percent: 0.20
Inj. 5	2230599		10.305	
Inj. 6	2232123		10.287	

Inj. – injection; No. – number; RSD – relative standard deviation

Table 4: Levetiracetam system precision details

Injection formulation with label claim 5 mg/ml			Injection formulation with label claim 15 mg/ml		
Concentration (mg/ml)	Assay (%)	Statistical assessment	Concentration (mg/ml)	Assay (%)	Statistical assessment
0.1	100.0	Average: 100.1	0.1	101.8	Average: 101.6
0.1	100.0		0.1	101.7	
0.1	100.0		0.1	101.5	
0.1	100.30	RSD percent: 0.10	0.1	101.4	RSD percent: 0.10
0.1	100.10		0.1	101.7	
0.1	100.0		0.1	101.5	

RSD – relative standard deviation

Table 5: Levetiracetam intermediate precision/ruggedness details

Laboratory	Injection formulation with label claim 5 mg/ml			Injection formulation with label claim 15 mg/ml		
	Concentration (mg/ml)	Assay (%)	Statistical assessment	Concentration (mg/ml)	Assay (%)	Statistical assessment
Analytical research and development laboratory	0.1	100.0	Average: 100.3	0.1	101.8	Average: 100.9
	0.1	100.0		0.1	101.7	
	0.1	100.0		0.1	101.5	
	0.1	100.3		0.1	101.4	
	0.1	100.1		0.1	101.7	
	0.1	100.0		0.1	101.5	
Quality control laboratory	0.1	100.4	RSD percent: 0.30	0.1	100.0	RSD percent: 0.8
	0.1	100.6		0.1	100.3	
	0.1	100.6		0.1	100.0	
	0.1	100.5		0.1	100.4	
	0.1	100.6		0.1	99.9	
	0.1	100.8		0.1	100.2	

RSD – relative standard deviation

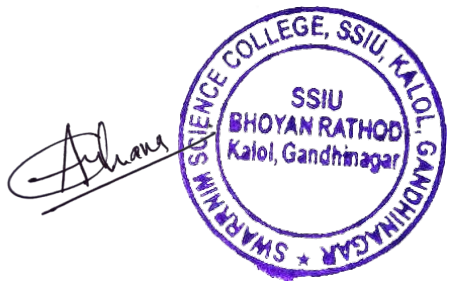


Table 6: Levetiracetam accuracy and recovery details

Level (with relating to test concentration 0.1 mg/ml)	Added amount (mg/ml)	Found amount (mg/ml)	Recovered percent (%)	Statistical assessment
50	0.507	0.0519	102.4	**Mean recovery:** 102.4 **RSD percent:** 0.20
		0.0519	102.4	
		0.0519	102.4	
		0.0521	102.8	
		0.0519	102.4	
		0.0519	102.4	
100	0.1014	0.1024	101.0	**Mean recovery:** 100.9 **RSD percent:** 0.10
		0.1023	100.9	
		0.1023	100.9	
		0.1023	100.8	
		0.1023	100.9	
		0.1026	101.2	
150	0.1521	0.1554	102.1	**Mean recovery:** 100.9 **RSD percent:** 0.90
		0.1553	102.1	
		0.1525	100.3	
		0.1524	100.2	
		0.1525	100.3	
		0.1528	100.4	

Table 8: Levetiracetam robustness details

Condition applied	Assay (%)	Difference (%)
Variation in column lot		
YMC PACK AQ Column ID: LCF 103/12	100.0	0.4
YMC PACK AQ Column ID: LCF 104/12	100.4	
Variation in column oven temperature		
20 °C (optimized)	102.0	1.3
25 °C	100.7	
Variation in flow rate		
0.8 ml/min	100.7	1.3
0.9 ml/min (optimized)	102.0	-
1.0 ml/min	100.6	1.4
Variation in mobile phase buffer pH		
pH 5.3	102.0	0.0
pH 5.5 (optimized)	102.0	-
pH 5.7	101.7	0.3

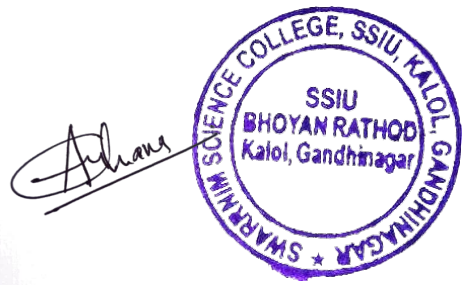


Figure 1a: Typical diluent chromatogram

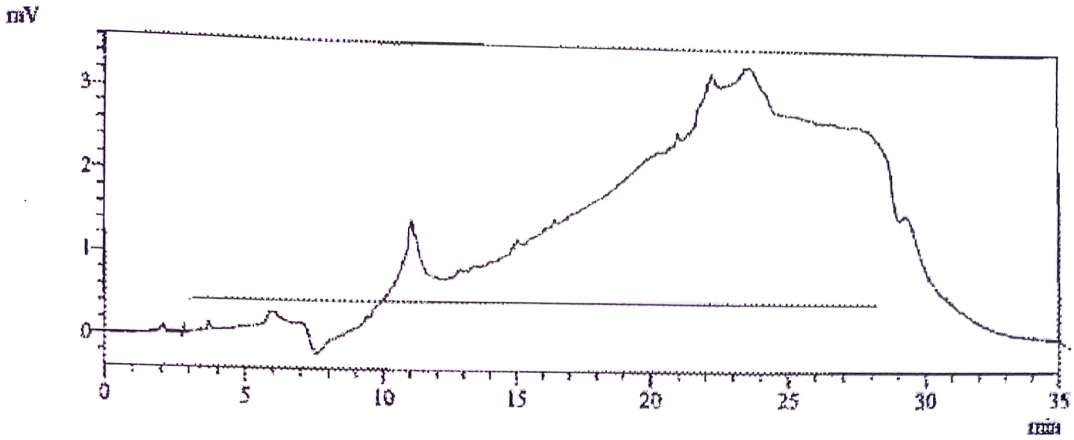


Figure 1b: Typical standard (levetiracetam - 0.1 mg/ml) chromatogram

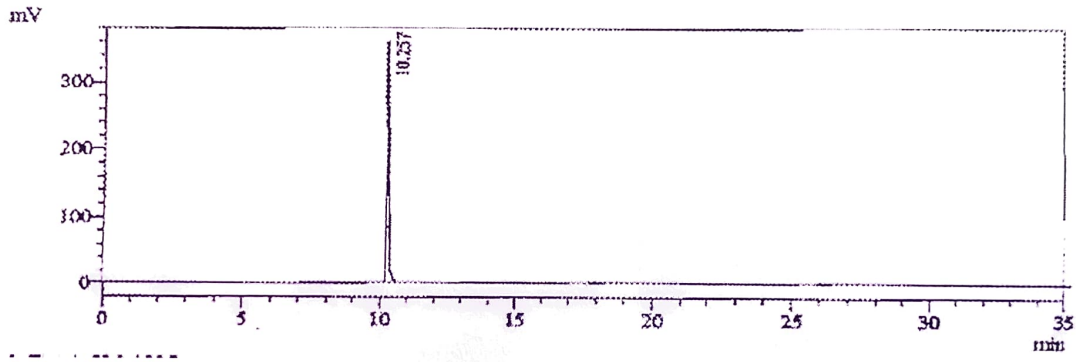


Figure 1c: Typical injection formulation (levetiracetam - 0.1 mg/ml) chromatogram

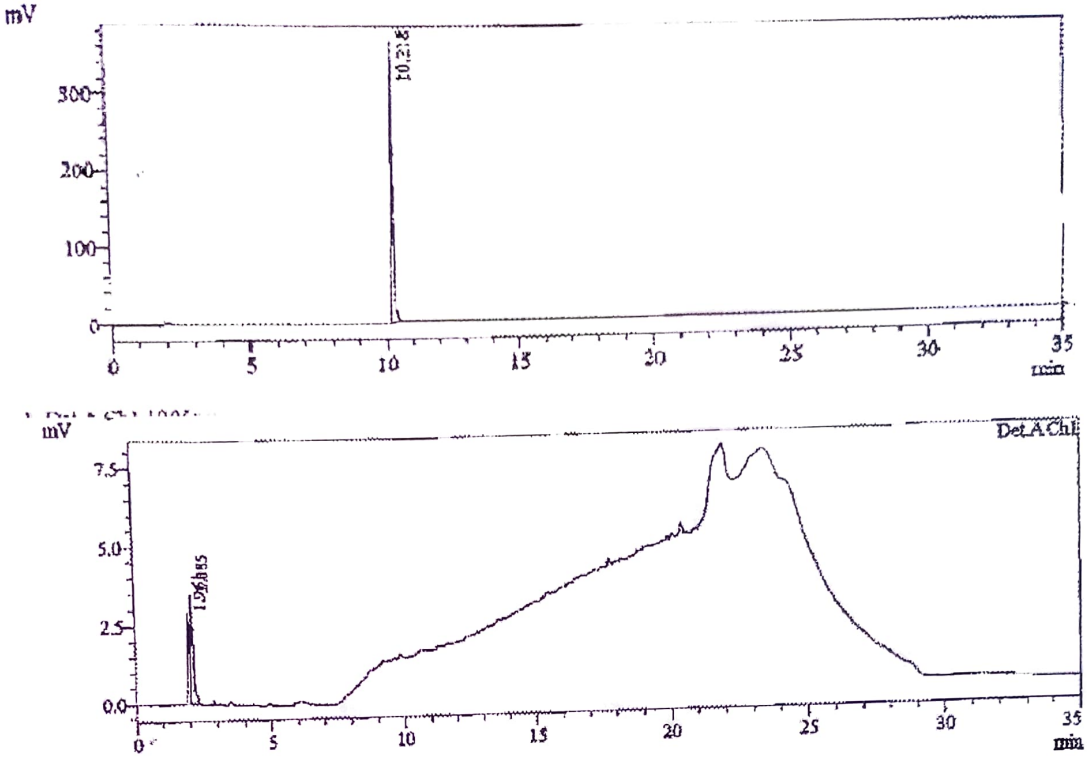
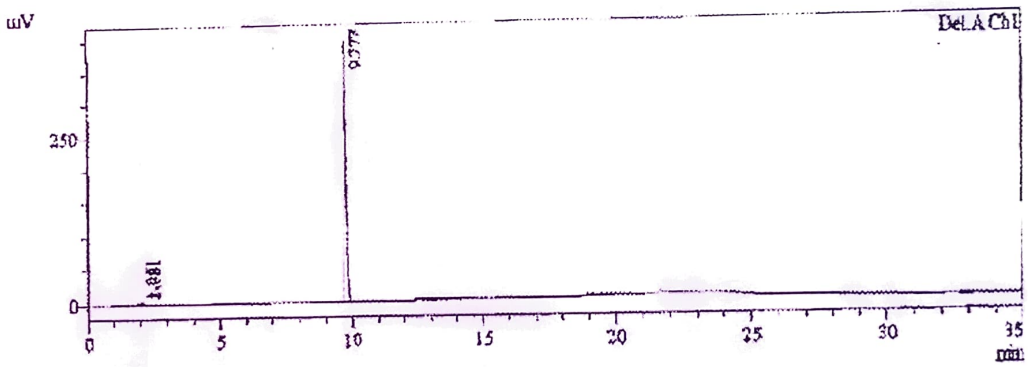


Figure 1d: Typical excipient solution chromatogram

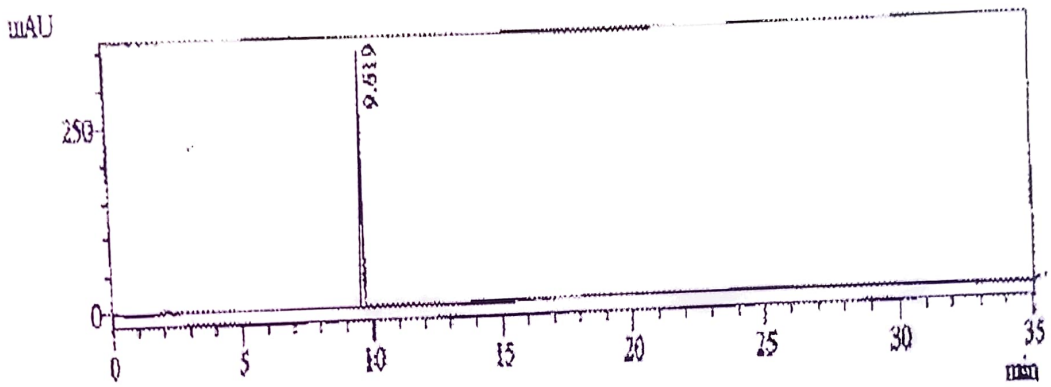


Stability Indicating Characteristic Feature

Developed method's stability indicating characteristics was demonstrated by its ability to resolve levetiracetam from its degradation products. For this, control (undegraded injection sample solution), dry heat and light exposed injection formulation samples prepared were assessed as per the method developed. Excipient placebo solutions spiked with levetiracetam were exposed to acid, base and peroxide stress conditions were also assessed as per the method developed. The chromatograms of all the degradation studies were shown in Figure 2a – 2f. The percent

assay, percent degradation, peak purity and spectral match were determined in all conditions of stress [Table 7]. The peak purity index and similarity index obtained for the degraded stress samples was >0.990 indicating pure peaks devoid of any co-elution and spectrally matched peaks, respectively.The results confirmed that the procedure met the criteria for stability indicating feature because no peaks due to the levetiracetam degradation products were co-eluting with peak of levetiracetam.

Figure 2a: Typical control (undegraded) chromatogram



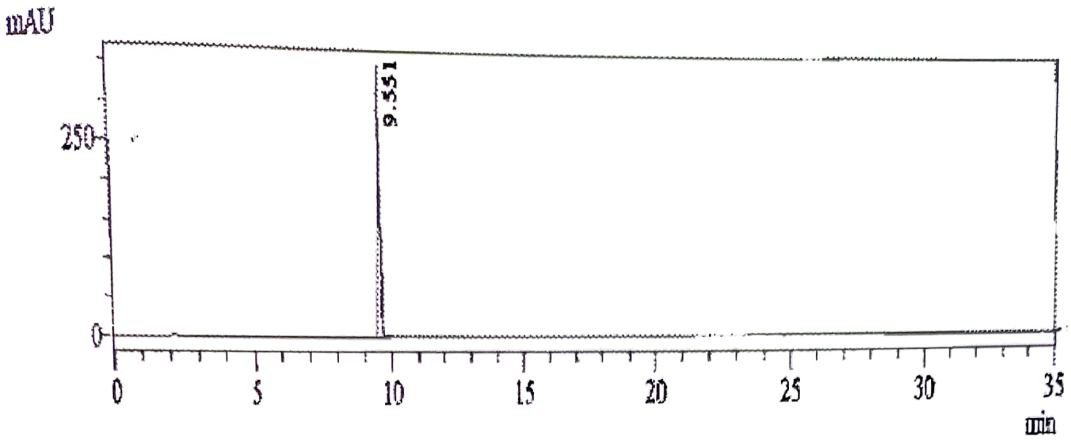
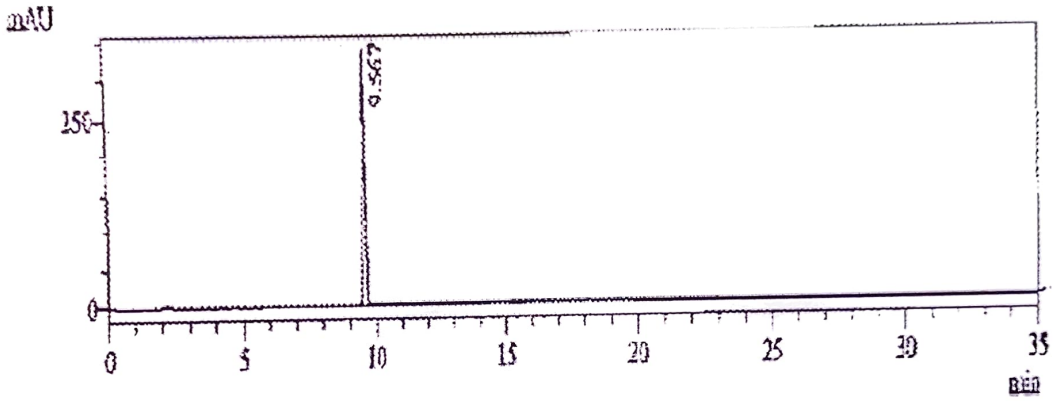


Figure 2b: Typical light exposed sample chromatogram

Figure 2c: Typical dry heat exposed sample chromatogram



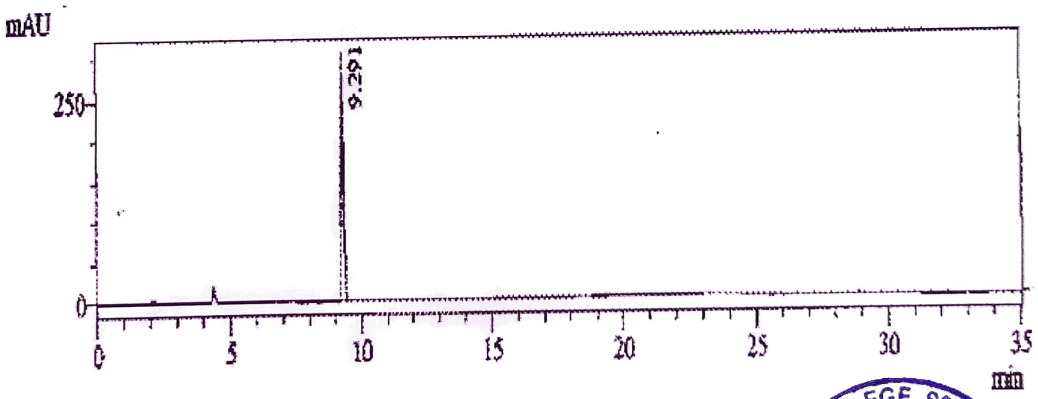


Figure 2e: Typical base exposed sample chromatogram

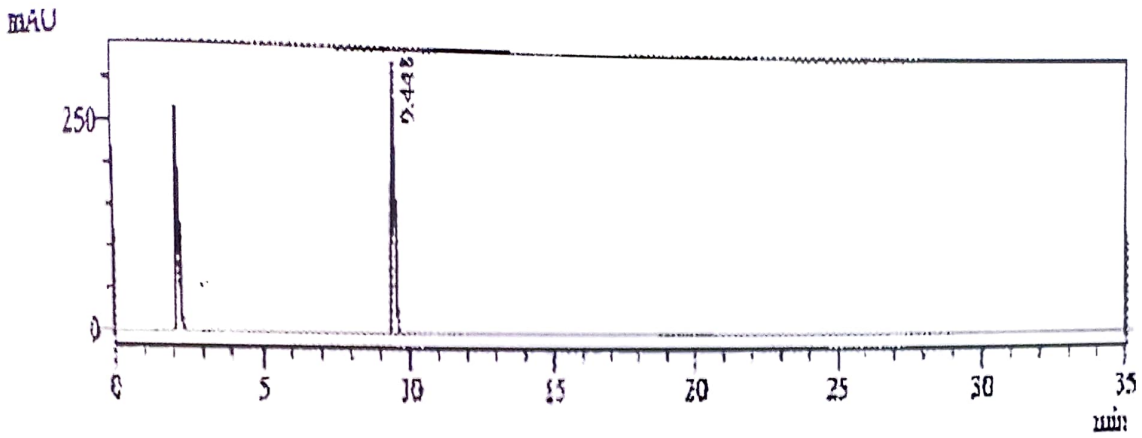


Figure 2f: Typical base exposed sample chromatogram

6.Financial Statement

Certified that a grant of ₹8,50,000 was received from the GCC BIOTECH for the project titled "Analytical HPLC Method Validation for the Development of Assay of Levetiracetam Injection Formulation". The amount has been utilized as per the approved budget and guidelines.

Sr.No	Particulars	Expenditure Incurred
1	Equipment	₹3,25,000
2	Chemicals	₹2,25,000
3	Travel	₹1,20,000
4	Contingency	₹1,80,000
Total		**₹8,50,000**

7. Conclusion and Recommendations

The developed HPLC method is validated and suitable for the routine analysis of Levetiracetam in injection formulations, meeting ICH guidelines. Recommendations for further optimization include exploring the use of other columns or mobile phase conditions for improved separation. The method is reliable for quality control and can be adopted by pharmaceutical companies for the routine testing of Levetiracetam injectable products. The new stability indicating RP-HPLC method developed for the assay of levetiracetam in injection formulation was found to be accurate and precise. The procedure was noticed to be linear for levetiracetam assay over the range of 0.0519 mg/ml to 0.1557 mg/ml. The procedure is repeatable, rugged, specific and stability indicating for levetiracetam. The method is also robust for variations in column lot, flow rate, column oven temperature and buffer pH.

8.Reference

1. Ali, S. M., Bharath, P., Ramakrishna, V. S., & Ramachandran, D. (2022). Quantification of Impurity-G Content in Levetiracetam Injection (100 mg/ml) by Using RP-HPLC Technique. *Journal of Pharmaceutical Research International*, 34(28B), 53–61.

2. Sankar, A. S., & SrinivasaRao. (2022). Analytical method validation for the determination of assay of levetiracetam in levetiracetam injection formulation by HPLC. *Journal of Positive School Psychology*, 6(2).

3. Sonawane, S. S., Chhajed, S. S., Jadhav, D. R., Thombre, N. A., & Kshirsagar, S. J. (2020). Development and Validation of Stability-Indicating HPLC Method for the Quantification of Levetiracetam in Bulk and Oral Solution: Application to Chemical Kinetics. *Pharmaceutical Chemistry Journal*, 54, 870–876.

4. Abd El-Hay, S. S., & Mohram, M. S. (2016). Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation. *Chromatography Research International*, 2016, Article ID 4909547.

5. Parameswaran, S., & Saraswathi, V. B. (2023). Simultaneous Estimation and Validation of Four Antiepileptic Drugs from Bulk and Formulations Using Reverse Phase HPLC. *Brazilian Journal of Pharmaceutical Sciences*, 59.

6. Raju, G. V. H., Ganapathy, S., Sankar, D. G., & Naidu, P. Y. (2010). Development and Validation of an HPLC Method for Analysis of Levetiracetam in Human Plasma. *Asian Journal of Research in Chemistry*, 3(3), 776–780.

7. Ravisankar, P., Niharika, A., & Srinivasa, B. (2015). An Improved RP-HPLC Method for the Quantitative Determination and Validation of Levetiracetam in Bulk and Pharmaceutical Formulation. *International Research Journal of Pharmacy*, 6(8), 537–543.

8. Sonawane, S. S., Chhajed, S. S., Jadhav, D. R., Thombre, N. A., & Kshirsagar, S. J. (2020). Development and Validation of Stability-Indicating HPLC Method for the Quantification of Levetiracetam in Bulk and Oral Solution: Application to Chemical Kinetics. *Pharmaceutical Chemistry Journal*, 54, 870–876.

9. Abd El-Hay, S. S., & Mohram, M. S. (2016). Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in

Pure Form and Pharmaceutical Formulation. *Chromatography Research International*, 2016, Article ID 4909547.

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12. Ravisankar, P., Niharika, A., & Srinivasa, B. (2015). An Improved RP-HPLC Method for the Quantitative Determination and Validation of Levetiracetam in Bulk and Pharmaceutical Formulation. *International Research Journal of Pharmacy*, 6(8), 537–543.

13. Sonawane, S. S., Chhajed, S. S., Jadhav, D. R., Thombre, N. A., & Kshirsagar, S. J. (2020). Development and Validation of Stability-Indicating HPLC Method for the Quantification of Levetiracetam in Bulk and Oral Solution: Application to Chemical Kinetics. *Pharmaceutical Chemistry Journal*, 54, 870–876.

14. Abd El-Hay, S. S., & Mohram, M. S. (2016). Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation. *Chromatography Research International*, 2016, Article ID 4909547.

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16. Raju, G. V. H., Ganapathy, S., Sankar, D. G., & Naidu, P. Y. (2010). Development and Validation of an HPLC Method for Analysis of Levetiracetam in Human Plasma. *Asian Journal of Research in Chemistry*, 3(3), 776–780.

17. Ravisankar, P., Niharika, A., & Srinivasa, B. (2015). An Improved RP-HPLC Method for the Quantitative Determination and Validation of Levetiracetam in Bulk and Pharmaceutical Formulation. *International Research Journal of Pharmacy*, 6(8), 537–543.

18. Sonawane, S. S., Chhajed, S. S., Jadhav, D. R., Thombre, N. A., & Kshirsagar, S. J. (2020). Development and Validation of Stability-Indicating HPLC Method for the Quantification of

Levetiracetam in Bulk and Oral Solution: Application to Chemical Kinetics. *Pharmaceutical Chemistry Journal*, 54, 870–876.

19. Abd El-Hay, S. S., & Mohram, M. S. (2016). Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation. *Chromatography Research International*, 2016, Article ID 4909547.

20. Parameswaran, S., & Saraswathi, V. B. (2023). Simultaneous Estimation and Validation of Four Antiepileptic Drugs from Bulk and Formulations Using Reverse Phase HPLC. *Brazilian Journal of Pharmaceutical Sciences*, 59.

21. Raju, G. V. H., Ganapathy, S., Sankar, D. G., & Naidu, P. Y. (2010). Development and Validation of an HPLC Method for Analysis of Levetiracetam in Human Plasma. *Asian Journal of Research in Chemistry*, 3(3), 776–780.

23. Ravisankar, P., Niharika, A., & Srinivasa, B. (2015). An Improved RP-HPLC Method for the Quantitative Determination and Validation of Levetiracetam in Bulk and Pharmaceutical Formulation. *International Research Journal of Pharmacy*, 6

GUJARAT POLLUTION CONTROL BOARD
Paryavaran Bhavan
Sector - 10 A, Gandhinagar - 382 010.
Environment Audit Cell

R.P.A.D

23 APR 2021

No. GPCB/EA-290/ 588268

To,
Swarrnim Institute of Technology
At Bhoyan Rathod,
Opp. IFFCO (ONGC WSS),
Adalaj-Kalol Highway,
Gandhinagar-382420

Sub:- Amendment to Recognition as Schedule-I Environmental Auditor with team member change.
Ref:- GPCB/EA-290/551732, Dated:20/01/2020

Sir,

This refers to your application for amendment in the recognition as Environmental Auditor with regards to one team member change and subsequent interview by Environment Audit Committee members. It is recommended by the Environment Audit Committee members, to amend your recognition as Schedule-I Environmental Auditor for carrying out the Environmental Audit under Environment Audit Scheme with following conditions.

1) Recognition is valid upto **31/12/2021.**
2) You shall have maximum **One** team for the Environment Audit.
3) You shall carry out maximum **15** nos. of Environment Audit in a year.
4) Team members shall be as under :

Sr. No.	Name	Designation
1	Ms. Saini Kalpana D.	Environment Engineer
2	Ms. Solanki Sejalben J.	Chemical Engineer
3	Mr. Parmar Rakeshkumar J.	Chemist
4	Ms. Patel Roshni K	Microbiologist

5) You shall prepare and submit the Environment Audit report and to comply the conditions for Environment Auditors as per the Hon'ble High Court order dated 20/12/1996, 13/03/1997, 16/09/1999, and also the Guidelines published by

Swarnim 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'

Clean Gujarat Green Gujarat
An ISO 9001: 2008 & ISO 14001: 2004 Certified Organization

Kalpana

1014 6/5/21
CPT 10:30

Collector and District Magistrate,
District Collector Office Sector-11 Gandhinagar

Phone No. 232 56720, 232 56639 Fax No:23245878

No.DI/ERC/Corona/Vashi.842/2021

RG. SERIAL No. 20/E/24
DATE. 08/10/2024

MANOJKUMAR B. MEHTA
NOTARY
GOVT. OF INDIA
08 OCT 2024

Date.08/04/2021

NOTARY * MANOJKUMAR B. MEHTA * TA. KALOL DIST. GANDHINAGAR REGD. NO. 22284/20 EXP. DT. 13/07/2025 GOVERNMENT OF INDIA

Immediate/Deadline:

Taken in reading

(1) Epidemic Diseases Act-1897

(2) Disaster Management Act-2005

(3) Government of India Ministry of Health and Family Welfare, Director General of Health Services, EMR Division Guidelines dated 07.04.2020

(4) Commissioner, Health, Medical Services and Medical Education, Gandhinagar letter No.S. 1/MOU/DI Hospital/sanction/21 dated 03.04.2021

(5) Commissioner, Health, Medical Services and Medical Education Gandhinagar letter No.S.1/MOU/Private/DCHC/DCCC/Grant/21 dated 04.04.2021

Subject: Designating hospital for treatment of Covid-19.

At present, in view of the continuous increase in the number of corona patients in the entire country and in the state, permission has been given from reference letter-4 to recognize the hospitals as dedicated Covid health centers subject to the provisions of the Epidemic Diseases Act-1897 and in accordance with the following conditions. Hence the following Hospital is recognized as a Dedicated Covid Health Center to treat the patients of Covid-19 as per the conditions mentioned in the mentioned letter-4 by charging Rs.2000/- (maximum charge per day) excluding the cost of Inj.Ramdesivir medicine. Along with this, Government of India is involved in the guidelines of Ministry of Health and Family Welfare, Director General of Health Services, EMR Division dated 07.04.2020. To take into account the implementation will be order.

Swarnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

S.NO	Hospital Name	Number of beds
1.	Aarihant Ayurvedic Medical College and Research Institute, Adalaj	125

The treatment cost of the patients cannot be recovered more than the rate mentioned above.

Condition:

1. Hospitals shall voluntarily collect treatment and incidental expenses from patients attending them. And treatment of such patients will be private

2. The State Government/District Administration will not provide any financial or other assistance for this.

3. These hospitals must have all the facilities as per the time-to-time guidelines of Government of India and Government of Gujarat for covid-19. And these facilities have to be provided to the patients.

4. If there are any financial, financial or legal issues with the patient admitted to the hospital, both the patient and the hospital will be responsible for it.

5. As per the time to time checklist of the Government, the details regarding the treatment and the patient should be provided to the concerned officials from time to time.

6. In the private hospitals impaneled under the 'Ma' and 'Ma Vataslya' scheme of Gandhinagar district, if a beneficiary of the 'Ma and Ma Vataslya' scheme receives treatment as a patient, he will not be charged the rates prescribed by this resolution but at the 'Ma' and 'Ma Vataslya' rates. The rates prescribed by the scheme will be applicable.

7. I.D given to all private hospitals as per COVID NHP PORTAL. And patient status, infrastructure and status of N-95 masks, PPE kits and medicines will have to be updated daily on the [illegible] application after logging in with the password.

Swarnim Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

Digitally signed by Ragin Ravindrabhai Shah
DN: c=IN, o=Personal, title=4505, pseudonym=02aef98b11054a5bb4fd53ee0d1fcce, 2.5.4.20=f2d48744d5a03b76d89e3bee3f63b9e9f5408942dc4f9537c58aeb40d7991483, postalCode=380015, st=Gujarat, serialNumber=0f2600a2fa773090e82dc5aebe105fcf7163c41741552ca365cb674075b6b44d6, cn=Ragin Ravindrabhai Shah
Date: 2024.10.10 10:45:46 +05'30'

8. G.M.C. The given dashboard entry on the website is the given I.D. And the number of occupied beds has to be updated three times a day in the morning and evening through the password.

9. On the 6th (sixth) of every month, mock drill and training must be given to all staff under fire safety.

(Dr. Kuldeep Arya)
Collector and District Magistrate,
Gandhinagar.

To, Administrator Sir:

✓ Arihant Ayurvedic Medical College and Research Institute, Adalaj

Copy Courtesy Mail-

✓ Additional Chief Secretary, Energy and Petrochemical Department, Government of Gujarat, Gandhinagar

✓ Senior Secretary, Health and Family Welfare Department, Government of Gujarat, Gandhinagar

✓ Commissioner, Health and Family Welfare Department, Secretariat, Gandhinagar.

Copy Dispatch:

✓ Intimation to Municipal Commissioner, Gandhinagar Mahanagarpalika.

✓ Intimation to District Development Officer, District Panchayat, Gandhinagar.

✓ Necessary action towards Chief District Health Officer, Zilla Panchayat Gandhinagar.

✓ Intimation to Medical Superintendent, Civil Hospital, Gandhinagar.

MY COMMISSION EXPIRES ON 13/07/2025

NOTARIAL NOTARIAL NOTARIAL NOTARIAL NOTARIAL NOTARIAL

This is true and Correct Translation of the Original documents from Gujarati/ [illegible] in to English which produced before me.

Swarnim Startup & Innovation University Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

भारत सरकार
Government of India

भारतीय विशिष्ट पहचान प्राधिकरण
Unique Identification Authority of India

Enrollment No.: 0000/00781/71536

To
Navin Sureshrao Banarase
703 Sapath Hight 1, Near Gayatri Mandir,
VTC: Kalol,
PO: Kalol,
District: Gandhinagar,
State: Gujarat,
PIN Code: 382721,
Mobile: 9930766442

1928319451

MG928319451FI

आपका आधार क्रमांक / Your Aadhaar No.:

3704 8640 0532

मेरा आधार, मेरी पहचान

भारत सरकार
Government of India

Navin Sureshrao Banarase
DOB : 01/12/1977
Male

Issue Date : 28/02/2012

Swarnim Startup & Innovation University
Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

3704 8640 0532

मेरा आधार, मेरी पहचान

કલેક્ટર અને જિલ્લા મેજીસ્ટ્રેટની કચેરી,
જિલ્લા સેવા સદન
સેક્ટર-૧૧ ગાંધીનગર

ફોનનં. ૨૩૨ ૫૬૭૨૦, ૨૩૨ ૫૬૬૩૯ ફેક્સનં.- ૨૩૨ ૪૫૮૭૮

નં.ડિઝા/ઈઆરસી/કોરોના/ વશી.(જરૂ)/૨૦૨૧ તા.૦૮/૦૪/૨૦૨૧

તાત્કાલિક/સમયમર્યાદા:

વંચાણમાં લીધું

(૧) એપેડેમિક ડિસીઝ એક્ટ-૧૮૯૭

(૨) ડિઝાસ્ટર મેનેજમેન્ટ એક્ટ-૨૦૦૫

(૩) ભારત સરકાર મીનીસ્ટ્રી ઓફ હેલ્થ એન્ડ ફેમીલી વેલફેર, ડિરેકટર જનરલ ઓફ હેલ્થ સર્વિસીસ, ઈએમઆર ડીવીઝનની તા.૦૭.૦૪.૨૦૨૦ની માર્ગદર્શિકા

(૪) કમિશનરશ્રી, આરોગ્ય, તબીબી સેવાઓ અને તબીબી શિક્ષણ ગાંધીનગરનો પત્ર નં.એસ.૧/એમઓયુ/ખા.ડેઝી હોસ્પિ/મંજુરી/૨૧ તા.૦૩.૦૪.૨૦૨૧

(૫) કમિશનરશ્રી, આરોગ્ય, તબીબી સેવાઓ અને તબીબી શિક્ષણ ગાંધીનગરનો પત્ર નં.એસ.૧/એમઓયુ/ખાનગી/DCHC/DCCC/મંજુરી/૨૧ તા.૦૪.૦૪.૨૦૨૧

વિષય: હોસ્પિટલને કોવિડ-૧૯ની સારવાર માટે ડેજીગ્નેટ કરવા બાબત.

હાલ સમગ્ર દેશમાં અને રાજયમાં કોરોનાના દર્દીઓની સંખ્યામાં સતત વધારો જોતાં રાજ્યમાં કોવિડ-૧૯નાં દર્દીઓને સારવાર આપી શકે તેવી હોસ્પિટલને એપેડેમિક ડિસીઝ એક્ટ-૧૮૯૭ની જોગવાઈઓને આધીન તથા નીચેની શરતો અનુસાર હોસ્પિટલોને ડેડિકેટેડ કોવિડ હેલ્થ સેન્ટર તરીકે માન્યતા આપવા સંદર્ભદર્શિત પત્ર – ૪ થી અનુમતિ આપેલ છે. આથી નીચે જણાવેલ હોસ્પિટલને સંદર્ભદર્શિત પત્ર-૪માં સુચવેલ રૂ|.૨૦૦૦/- (મહત્તમ ચાર્જ પ્રતિ દિન) Inj.Ramdesivir દવાના ખર્ચ સિવાયના દરે ચાર્જ લઈ જણાવેલ શરતો અનુસાર કોવિડ-૧૯ના દર્દીઓને સારવાર આપવા ડેડિકેટેડ કોવિડ હેલ્થ સેન્ટર તરીકે માન્યતા આપવામાં આવે છે. આ સાથે ભારત સરકાર મીનીસ્ટ્રી ઓફ હેલ્થ એન્ડ ફેમીલી વેલફેર, ડિરેકટર જનરલ ઓફ હેલ્થ સર્વિસીસ, ઈએમઆર ડીવીઝનની તા.૦૭.૦૪.૨૦૨૦ની માર્ગદર્શિકા બિડાણમાં સામેલ છે. જે ધ્યાને લઈ અમલવારી કરવાની રહેશે.

ક્રમ	હોસ્પિટલનું નામ	પથારીની સંખ્યા
૧.	અરિહંત આયુર્વેદિક મેડિકલ કોલેજ અને રીસર્ચ ઈન્સ્ટીટયુટ, અડાલજ	૧૨૫

દર્દીઓનો સારવારનો ખર્ચ ઉપર જણાવેલ દર કરતાં વધારે વસુલ કરી શકાશે નહી.

શરતો

1. સદરહું હોસ્પિટલોમાં સ્વેચ્છાએ તેઓના ત્યાં આવતા દર્દી પાસેથી સારવાર [illegible] પાસેથી મેળવવાનો રહેશે. અને આવા દર્દીઓની સારવાર ખાનગી ગણાશે.

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2. રાજ્ય સરકાર/જીલ્લા વહીવટી તંત્ર આ માટે કોઇ નાણાકીય કે અન્ય સહાય આપશે નહી.
3. આ હોસ્પિટલોમાં કોવિડ-૧૯ માટેની ભારત સરકાર તથા ગુજરાત સરકારની વખતોવખતની ગાઇડલાઇન મુજબની તમામ સુવિધાઓ હોવી ફરજીયાત છે. અને આ સુવિધાઓ દર્દીઓને પુરી પાડવાની રહેશે.
4. હોસ્પિટલ ખાતે દાખલ થનાર દર્દીની સાથે આર્થિક કે નાણાકીય કે કાયદાકીય રીતે જો કોઇ બાબતો ઉપસ્થિત થાય તો તે અંગેની જવાબદારી દર્દી અને હોસ્પિટલ બંનેની રહેશે.
5. સરકારશ્રીની વખતોવખત ચેકલીસ્ટ મુજબ તેની સારવાર તથા દર્દી અંગેની વિગતો સમયાંતરે સંબંધિત અધિકારીશ્રી ને અચુક પુરી પાડવાની રહેશે.
6. ગાંધીનગર જિલ્લાની 'મા' અને 'મા વાત્સલ્ય' યોજના અંતર્ગત જે ખાનગી હોસ્પિટલ એમ્પેનલ્ડ કરાયેલ છે તેવી હોસ્પિટલોમાં 'મા અને મા વાત્સલ્ય' યોજનાના લાભાર્થી દર્દી તરીકે સારવાર મેળવશે તો તેને આ ઠરાવથી નિયત થયેલ દરો નહિ પરંતુ 'મા' અને 'મા વાત્સલ્ય' યોજનાથી નિયત થયેલ દરો લાગુ પડશે.
7. COVID NHP PORTAL અનુસાર તમામ ખાનગી હોસ્પિટલને આપેલ આઇ.ડી. અને પાસવર્ડથી લોગીન થઇ દરરોજ દર્દીની સ્થિતિ, ઇન્ફાસ્ટ્રક્ચર અને એન-૯૫ માસ્ક, પી.પી.ઇ.કીટ અને દવાઓની સ્થિતિ મોબાઇલ એપ્લિકેશન પર દરરોજ ફરજિયાત અપડેટ કરવાની રહેશે.
8. G.M.C. વેબસાઇટ પર આપેલ ડેશબોર્ડની એન્ટ્રી આપેલ આઇ.ડી. અને પાસવર્ડ દ્વારા દિવસમાં ત્રણ વાર ઓક્યુપાઇડ બેડની સંખ્યા સવારે અને સાંજે અપડેટ કરવાની રહેશે.
9. દર મહિનાની ૬(છઠી) તારીખે ફાયર સેફટી અંતર્ગત મોકડ્રીલ અને તમામ સ્ટાફને ટ્રેનીંગ ફરજિયાત આપવાની રહેશે.

(ડો.કુલદીપ આર્ય)
કલેક્ટર અને જિલ્લા મેજીસ્ટ્રેટ,
ગાંધીનગર.

પ્રતિ, સંચાલકશ્રી:
- ✓ અરિહંત આયુર્વેદિક મેડિકલ કોલેજ અને રીસર્ચ ઈન્સ્ટીટયુટ, અડાલજ

નકલ સવિનય રવાનાઃ
- ✓ અધિક મુખ્ય સચિવશ્રી, ઉર્જા અને પેટ્રોકેમિકલ વિભાગ, ગુજરાત સરકાર, ગાંધીનગર
- ✓ અગ્ર સચિવશ્રી, આરોગ્ય અને પરિવાર કલ્યાણ વિભાગ, ગુજરાત સરકાર, ગાંધીનગર
- ✓ કમિશનરશ્રી, આરોગ્ય અને પરિવાર કલ્યાણ વિભાગ, સચિવાલય, ગાંધીનગર.

નકલ રવાનાઃ
- ✓ મ્યુનિસિપલ કમિશનર, ગાંધીનગર મહાનગરપાલિકા તરફ જાણ સારૂ.
- ✓ જિલ્લા વિકાસ અધિકારી, જિલ્લા પંચાયત, ગાંધીનગર તરફ જાણ સારૂ.
- ✓ મુખ્ય જિલ્લા આરોગ્ય અધિકારી,જિલ્લા પંચાયત ગાંધીનગર તરફ જરૂરી કાર્યવાહી
- ✓ મેડિકલ સુપ્રિન્ટેન્ડેટ, સિવિલ હોસ્પિટલ, ગાંધીનગર તરફ જાણ સારૂ.

Bhoyan Rathod, Gandhinagar

Ragin Ravindrabhai Shah

AARIHANT AYURVEDIC MEDICAL COLLEGE AND RESEARCH INSTITUTE		
DEDICATED COVID HEALTH CENTER		
Sr. No	Date	NAME OF PATIENT ADMITTED
1	16-Apr-21	JITENDRA M DAVE
2	18-Apr-21	PATEL VIJAY CHAMANBHAI
3	20-Apr-21	SUDHA VERMA
4	22-Apr-21	PRAJAPATI KOKILABEN VISHNUBHAI
5	22-Apr-21	SUSHIL C. JAIN
6	22-Apr-21	MANOJ MITTAL
7	23-Apr-21	AMARITABEN DASHRATHJI THAKOR
8	23-Apr-21	PATEL BALDEVBHAI SHANKARBHAI
9	23-Apr-21	PATEL KAILASHBEN DASHRATHBHAI
10	23-Apr-21	UPENDRA GADHVI
11	23-Apr-21	PATEL KOKILABEN CHATURBHAI
12	23-Apr-21	YASHVANT MEENA
13	24-Apr-21	BHAILALBHAI SOMABHAI PATEL
14	24-Apr-21	PATEL VIKRAM AMBALAL
15	24-Apr-21	BAHRAT CHAUDHARY
16	24-Apr-21	RAVAL AJAYBHAI BALDEVBHAI
17	24-Apr-21	SHANTABEN C BAROT
18	26-Apr-21	DABHI CHIRAG VIKRAMSINH
19	26-Apr-21	MEERABEN RAMLESH BAROT
20	26-Apr-21	CHANDUBHAI POPATBHAI PATEL
21	26-Apr-21	AASHABEN JAYANTILAL BAROT
22.	26-Apr-21	BAROT JAYNTILAL
23	26-Apr-21	VAJESHING SOLANKI

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24	27-Apr-21	PATEL PRAVINBHAI MAGANBHAI
25	27-Apr-21	BHARVI M PATEL
26	27-Apr-21	MANEKLALPRABHUDAS PRAJAPATI
27	28-Apr-21	JOSHI RAKESH HASMUKHLAL
28	28-Apr-21	GEETABEN M DARJI
29	29-Apr-21	PATEL GOVINDBHAI KACHRADAS
30	29-Apr-21	PATEL GAURIBEN KALIDAS
31	29-Apr-21	YAGNESH N JOSHI
32	29-Apr-21	DAVE MILAN KANTIBHAI
33	30-Apr-21	JASHIBEN CHANNALAL PATEL
34	01-May-21	CHANDUBHAI POPATBHAI PATEL
35	01-May-21	VIRENDRA M SHAH
36	01-May-21	DHARMENDRA RAVAL
37	01-May-21	RAHUL JAWAHAR
38	01-May-21	LALITBHAI J SHAH
39	01-May-21	BAROT SURESH BHARATBHAI
40	01-May-21	ASHVINBHAI NATVARBHAI PATEL
41	01-May-21	RATHOD GEETA AMARSINH
42	01-May-21	VIASHNAV JAGDISHBHAI
43	01-May-21	RANJANBEN PATEL
44	02-May-21	SMITABEN B PATEL
45	02-May-21	ASHABEN N PATEL
46	02-May-21	NAMAN ASHVINKUMAR DESAI

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Ragini Ravindrabhai Shah

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47	02-May-21	BHARATBHAI G PATEL
48	02-May-21	PATEL KALPESH NATHABHAI
49	02-May-21	PRAJAPATI BHARATBHAI KANJIBHAI
50	03-May-21	JOSHI DINTABEN ARVINDKUMAR
51	03-May-21	PATEL PRAVIN DWARKABHAI
52	03-May-21	RAVAL BALDEVBHAI VITHHALBHAI
53	03-May-21	PRAJAPATI HARDIK BAKOLBHAI
54	04-May-21	CHETANKUMAR H PATEL
55	04-May-21	PATEL MANIBEN AMBALAL
56	04-May-21	BHAGWATIBEN M BAROT
57	04-May-21	MANGALBHAI SHANKARDAS PATEL
58	04-May-21	DAXABEN DILIPBHAI PATEL
59	04-May-21	ACHLA RAM DAHYAJI
60	04-May-21	MAHENDRA RAVAL
61	05-May-21	ANILKUMAR RAI
62	05-May-21	LILABEN AMARATBHAI PATEL
63	08-May-21	RAJUBHAI JIVANBHAI PATEL
64	10-May-21	MANISHABEN V JAIN
65	10-May-21	SAROJBEN GHANSHAYMBHAI PATEL
66	10-May-21	PATEL MITTALBEN
67	10-May-21	DHIREN CHANARIYA
68	10-May-21	CHANDULAL SHIVRAMDAS PATEL
69	11-May-21	PATEL VIMALKUMAR

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70	12-May-21	PATEL MAHENDRAKUMAR PRABHUDAS
71	12-May-21	SOLANKI PARSOTTAM
72	15-May-21	KAUSHIKBHAI L JOSHI
73	16-May-21	PATEL GANGABEN NATHALAL
74	17-May-21	MEHTA RUPALBEN KETANBHAI
75	17-May-21	SHASHIKALABEN K PATEL
76	18-May-21	PATEL NARAYAN Z.
77	19-May-21	KATARA BACHUBEN K
78	20-May-21	SHRAVAN KAMLE
79	21-May-21	JASHIBEN J THAKOR
80	21-May-21	JASHIBEN J THAKOR
81	21-May-21	PATEL JAGDISHBHAI P

Principal
Aarihant Ayurvedic Medical College and Research Institute

Bhoyan Rathod, Gandhinagar

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