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Research Article

Analytical Method Development And Validation For Metformin Hcl In Transdermal Patch

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ABSTRACT
The aim of the study was to develop and validate an analytical method for the estimation of the drug using High-Performance Liquid Chromatography (HPLC), and to assess its stability-indicating properties through forced degradation studies, as per the guidelines of the International Council for Harmonisation (ICH) for analytical method development and validation. Extraction was performed using phosphate buffer (pH 7.4), and analysis was carried out with a mobile phase of 1% orthophosphoric acid and acetonitrile (90:10 v/v) using a YMC C18 column. The retention time was found to be 3.4 minutes. The method was validated per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, and forced degradation studies. The method exhibited excellent linearity over 50–150 µg/mL with an $R^2 > 0.999$ Precision was high, with %RSD values below 2%. Accuracy, determined through recovery studies, showed results between 99% and 102.5%, aligning with the acceptable 98%–102% range. A forced degradation study was conducted under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic environments. Significant degradation was observed under acidic and basic hydrolysis, and the degradation products were well-resolved from the main drug peak, demonstrating the stability-indicating capability of the method proved suitable for routine quality control analysis of Metformin in transdermal formulations.

Keywords: Metformin HCl, Transdermal Patch, RP-HPLC, Method Validation, ICH Guidelines, Force degradation study

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among the various forms of diabetes, type 2 diabetes mellitus (T2DM) is the most prevalent, often associated with obesity, dyslipidemia, and

hypertension, contributing significantly to global morbidity and mortality. [1, 2]

Metformin hydrochloride (HCl), a biguanide class oral antihyperglycemic agent, is widely recognized as the first-line pharmacological treatment for T2DM. Approved by the U.S. Food and Drug Administration(FDA) in 1995, metformin has gained prominence due to its low cost, high efficacy, favorable safety profile, and its ability to improve cardiovascular outcomes.[3,4] Its primary mechanism of action involves inhibition of hepatic gluconeogenesis, thereby lowering fasting plasma glucose levels. Additionally, it offers ancillary benefits such as reduction in macrovascular complications and improvement in lipid profiles. [5, 6]

However, despite its advantages, the oral administration of metformin is often associated with gastrointestinal side effects like nausea, diarrhea, and abdominal discomfort, particularly at higher doses. Furthermore, due to its relatively short half-life, multiple daily dosing is required, which can compromise patient compliance. [7] These limitations have led to growing interest in alternative drug delivery systems.

The transdermal drug delivery system (TDDS) offers a promising approach for delivering metformin. TDDS bypasses the gastrointestinal tract and hepatic first-pass metabolism, thereby minimizing systemic side effects and potentially enhancing bioavailability and patient adherence. [7] By employing the skin as a route of administration, TDDS provides sustained drug release and stable plasma concentrations over extended periods.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. [8]

As per International Council for Harmonisation (ICH) guidelines Q2(R1), a validated method must demonstrate specificity, linearity, accuracy, precision, detection and quantitation limits, robustness, and system suitability. [9] Moreover, forced degradation studies are vital for establishing the stability-indicating nature of the method by identifying potential degradation products under stress conditions. [10]

A comprehensive literature survey reveals that no validated analytical method has been reported specifically for the estimation of metformin HCl in transdermal patch formulations. This underscores the need for a novel, validated, and stability-indicating method. [11, 12, 13, 14, 15]

This research focuses on the formulation of a metformin hydrochloride transdermal patch using chitosan and PVP as the polymeric base and the development and validation of a robust, stability-indicating RP-HPLC method for its quantitative estimation. Given the therapeutic importance of metformin and the increasing interest in transdermal drug delivery systems (TDDS), the study aims to support both quality control and stability assessment of the formulation. Additionally, forced degradation studies were conducted to ensure the method's specificity and its ability to indicate stability by detecting degradation products. The overall goal is to establish a simple, cost-effective, and validated analytical method suitable for routine evaluation of metformin in transdermal patches.

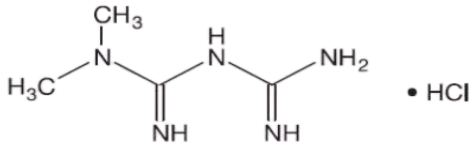


Figure No.1 Structure of Metformin HCL

EXPERIMENTAL

Chemicals and Reagents

The Active Pharmaceutical Ingredient (API), Metformin Hydrochloride (Metformin HCl), along with excipients such as Polyvinylpyrrolidone (PVP), Acetic Acid, and Glycerin used in the formulation of the transdermal patch, were generously provided by Acetopharma (India) pvt Ltd . Chitosan was procured from Longshore Technologies, India. All HPLC-grade solvents and reagents, including Water, Acetonitrile, Potassium Dihydrogen Phosphate, and Orthophosphoric Acid, were also supplied by Theta Beta Acetopharma (India) pvt Ltd

Instruments

The Development and Validation of the method were performed on a Waters 2695 Separation Module equipped with quaternary pumps, an autosampler and a photo diode array detector. Empower 3 software was applied for data collection and processing.

Formulation of transdermal patch of metformin

Metformin transdermal patches were formulated using Chitosan (1.45 g) as the polymer, Metformin HCl (290 mg) as the active drug, PVP (0.5–0.7 g) as the plasticizer, and 1% Acetic acid (60 mL) as the solvent. Chitosan was dissolved in 50 mL of 1% acetic acid and stirred until homogenous.

Separately, Metformin and PVP were dissolved in the remaining 10 mL of the acetic acid. Both solutions were combined, stirred, and poured into glycerin-greased petri dishes. The patches were dried in a hot air oven at 40 °C (4–5 hours). Final patches were

peeled, cooled, and stored in a desiccator for further evaluation.

Figure No.2: Metformin transdermal patch at room temperature

Figure No.3: Metformin dried patch

Extraction of Metformin HCL (API) from Transdermal Patch

To extract Metformin HCl from the developed transdermal patch for HPLC analysis and validation, a physiologically relevant medium—phosphate buffer (pH 7.4)—was employed to simulate skin conditions and ensure effective in vitro drug release. A patch weighing 84 mg, equivalent to approximately 10 mg of Metformin HCl std (for a concentration of 100 µg/mL), was accurately weighed based on the label claim of 290 mg. The patch was finely cut using sterilized scissors to increase the surface area for efficient extraction and transferred into a 100 mL volumetric flask. In that phosphate buffer was added making up the solution of 100µg/ml.

A 0.1 M phosphate buffer of pH 7.4 was prepared by accurately weighing 1.421 g of disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and 0.619 g of monosodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), which were dissolved in approximately 80 mL of distilled water in a 100 mL volumetric flask. The pH of the solution was measured using a calibrated pH meter and adjusted to 7.4 using either dilute sodium hydroxide (NaOH) or orthophosphoric acid (H_3PO_4), if necessary. After pH adjustment, the volume was made up to 100 mL with distilled water. The patch- buffer mixture was then subjected to ultrasonication in a bath sonication for 4 hours, while maintaining the temperature below 40°C to avoid any potential thermal degradation of the drug. The sonication process assisted in breaking the polymeric chitosan-PVP matrix and enhancing drug diffusion into the extraction medium. Following ultrasonication, the flask was allowed to cool to room temperature. undissolved polymeric residues or excipients, yielding a clear filtrate.

This clear solution was collected as the test sample for HPLC injection and further method validation studies. The extraction method was found to be efficient, reproducible, and free from interfering substances, confirming its suitability for routine drug content estimation and analytical validation of Metformin HCl in transdermal patches.

Preparation of Mobile phase

Mobile phase was prepared by mixing the 1% Orthophosphoric acid and acetonitrile in the ratio of 90:10.

Preparation of Standard Solution

Accurately weighed 100 mg of Metformin HCl standard was transferred into a 100 ml volumetric flask. A sufficient quantity of diluent was added, and the solution was sonicated to ensure complete dissolution. The volume was then made up to the mark with the same diluent to obtain a stock solution of 1000 µg/ml. From this stock solution, 10 ml was pipetted into a 100 ml volumetric flask and diluted to volume with the diluent to obtain a final working standard solution of 100 µg/ml.

Preparation of Sample Solution

Accurately weighed one transdermal patch of Metformin HCL containing 84 mg of Metformin HCl (equivalent to 10 mg for a 100 µg/ml solution), was transferred into a 100 ml volumetric flask. A sufficient quantity of phosphate buffer pH 7.4 was added to dissolve the sample with the aid of sonication. The volume was then made up to the mark with the same buffer to obtain a final concentration of 100µg/ml. The sample solution was filtered through a 0.45µ syringe filter. HPLC vial was filled after discarding an initial 4-5 ml of filtrate. 10 µl injected into the HPLC system. The Chromatograms were recorded and the % Assay was calculated.

METHOD VALIDATION

Validation was done according to ICH guidelines for the determination of the developed RP-HPLC method for Metformin HCL in transdermal dosage form. Validation was carried out for the

Specificity

To evaluate for excipient interference, diluent, placebo, and sample solution were injected to determine specificity.

Linearity

Accurately weighed 100 mg of Metformin HCL and transferred to 100ml volumetric flask. Diluted up to the mark with solvent to get concentration of 1000 µg/ml. From the above solution 10 ml was pipette out and transfer to 100 ml volumetric flask and diluted up to the mark with solvent to get concentration of 100 µg/ml. From the above solution suitable solutions were made to obtain concentration in the range of 50-150 µg/ml. Linearity response was assessed over the range of 50-150 µg/ml. Linear relationship should be evaluated should be evaluated across concentration and peak area. Each linearity preparation was injected six times. Solvent was used as blank. Calibration curve was obtained by plotting peak area Vs concentration.

Accuracy

Accuracy of the method was determined by spiking known amount of standard in sample solution at 3 different levels (i.e. 50%, 100%, 150%) in duplicates as per the method of analysis and % recovery was calculated.

Precision

System Precision

Six different standard preparations 100µg/ml Metformin HCL injection samples were prepared the same as specified earlier procedure. The % RSD was calculated.

Method precision

Six different sample preparations 100µg/ml Metformin HCL injection samples were prepared the same as specified earlier procedure. The % Assay was calculated for each preparation.

Robustness

Robustness of an analytical procedure is it's ability to remain unaffected by small, but deliberate variations. Parameters like flow rate, temperature, wavelength were changed one by one in triplicate and %RSD calculated.

FORCE DEGRADATION STUDIES

To evaluate the stability indicating nature of the developed RP-HPLC method, the samples were subjected to various stress conditions including acidic oxidative thermal and photolytic degradation as per ICH guidelines. Following exposure to each stress condition the samples were analyzed using the developed RP-HPLC method. The percentage degradation of Metformin was calculated for each condition to assess the method's capability to separate the drug from its degradation products thereby confirming the specificity and stability indicating property of the method.

Acid Degradation

Pipette out 2 mL of the sample solution and add 2 mL of 0.5 N HCl. Transfer the mixture into a 20 mL volumetric flask and allow it to stand for 24 hours at room temperature. After 24 hours, add 2 mL of 0.5 N NaOH to neutralize the solution. The final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45 µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Alkaline Degradation

Pipette out 2ml from sample solution and transfer in 20 ml of volumetric flask and add 2ml of 0.5N NaOH. Transfer the mixture into a 20 mL volumetric flask and allow it to stand for 24 hours at room temperature. After 24 hours, add 2 mL of 0.5 N HCL to neutralize the solution. The final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45 µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Oxidation degradation

Pipette out 2ml from sample solution and transfer in 20 ml of volumetric flask and add 2ml of 3% H2O2. Above solution was kept for 24hrs. After 24hrs, the final volume was then made up to with diluent. The solution was mixed thoroughly and filtered through a 0.45µm syringe filter. The resulting solution was then analyzed by HPLC. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

Thermal degradation

A patch weighing 84 mg, equivalent to 10 mg of standard drug, was placed in a 100 mL volumetric flask. It was exposed to thermal stress by keeping it in a hot air oven at 60 °C for 24 hours. After 24 hours, the sample was allowed to cool to room temperature. The

volume was then made up to 100 mL using the diluent. The solution was filtered through a 0.45 µm syringe filter and analyzed using a validated HPLC method. The chromatogram was recorded, and the percentage degradation was calculated based on the peak area.

Photolytic degradation

The patch of 84mg equivalent to 10mg of standard drug was placed in 100ml of a volumetric flask and subjected to photolytic stress by keeping it in UV chamber (254nm) for 24 hours. After 24 hrs, the final volume was then made up to with diluent. The solution was then filtered through a 0.45 µm syringe filter and analyzed by a Validated HPLC method. The chromatogram of the sample was recorded, and the percentage degradation was calculated based on the peak area.

RESULTS AND DISCUSSION

New method was developed for the assay and stability indicating through force degradation studies of Metformin HCL transdermal patch (290 mg label claim): the following are conditions optimized.

Table No.1: Optimized Chromatographic Conditions

Sr. No.	Specification	Description
1	Column	YMC C_{18} (250 x 4.6 mm, ID 5µm)
2	Detector	Photo Diode Array Detector
3	Diluent	Phosphate buffer 7.4
4	Mobile Phase	90:10 1% Orthophosphoric acid and Acetonitrile
5	Flow Rate	0.8 ml/min
6	Wavelength	230 nm
7	Injection Volume	10 µl
8	Column Temperature	25℃
9	Elution Mode	Isocratic
10	Run Time	10 minutes
11	Retention time	3.4 minutes

ASSAY METHOD

Six replicates of Standard and Sample solutions were prepared and % Assay was calculated.

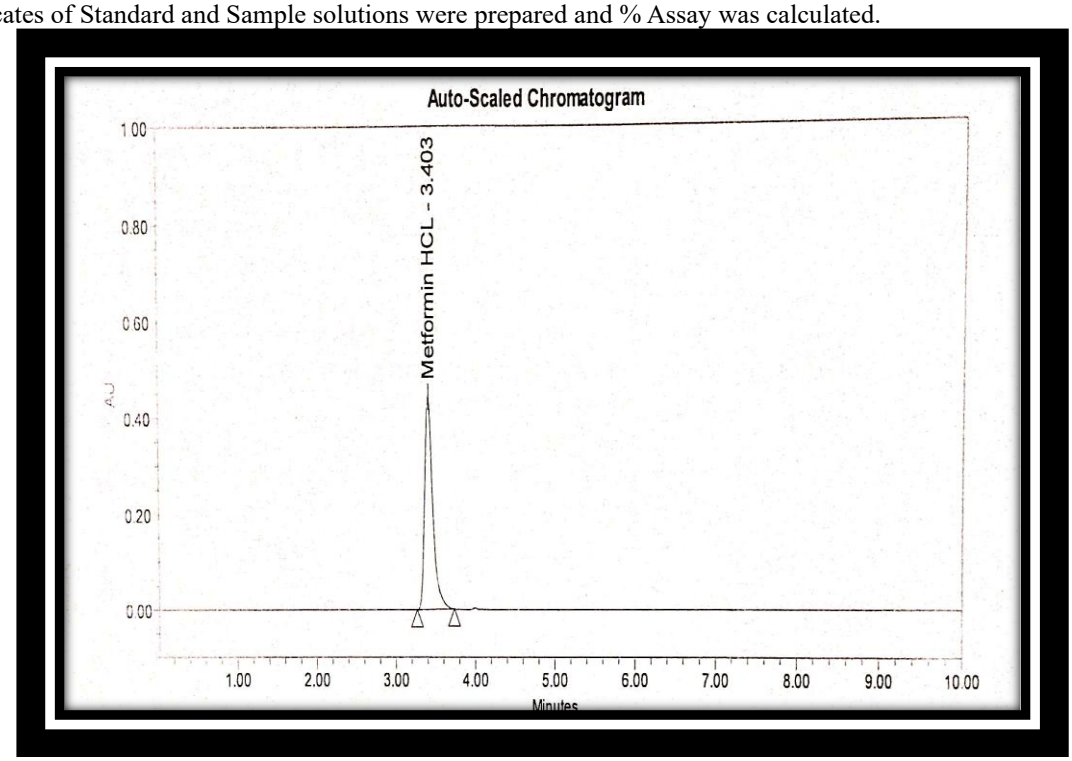


Figure No. 4: Chromatogram of Metformin HCL Standard for Assay

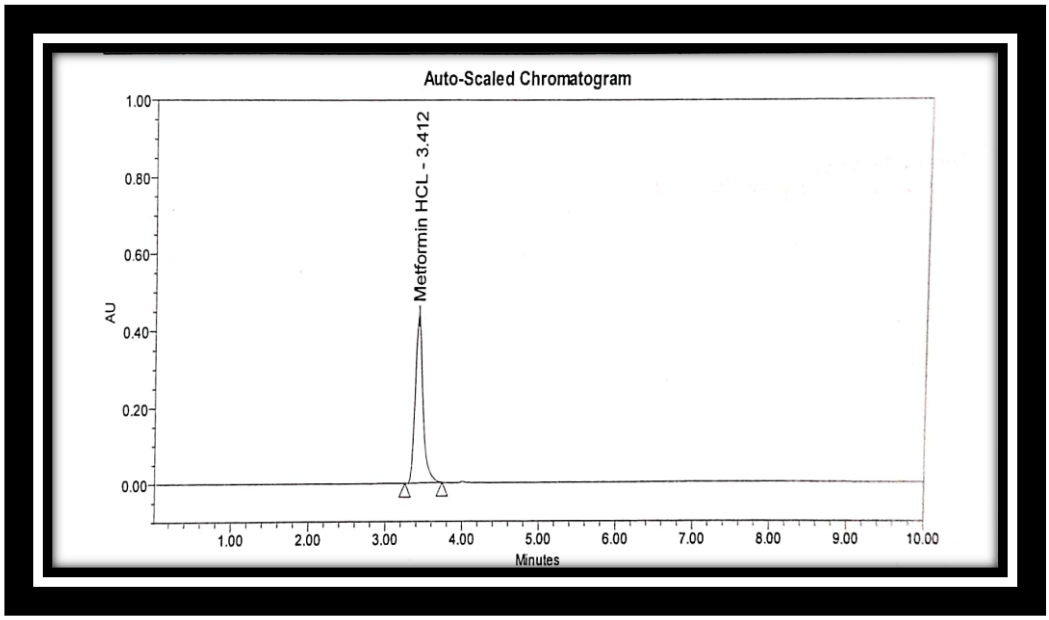


Figure No.5: Chromatogram of Metformin HCL Sample for Assay
Table No.2: Assay Data for HPLC

Table No.5.1: Assay Data for HPLC

Sr. No.	Area of sample at 230nm	Assay
1	3068625	98.22
2	3046866	97.40
3	3083429	98.22
4	3041507	97.35
5	3071781	99.03
6	3073072	99.55
Average	3064216	98.29
SD	16376.55	0.87
%RSD (Limit NMT 2%)	0.53	0.89

Observation: The Metformin HCL present in analyze dosage form was found to be 98.29 Acceptance criteria: Analyzed dosage form should be 98 % and 102 % pure respectively.

HPLC Validation Parameters
Specificity

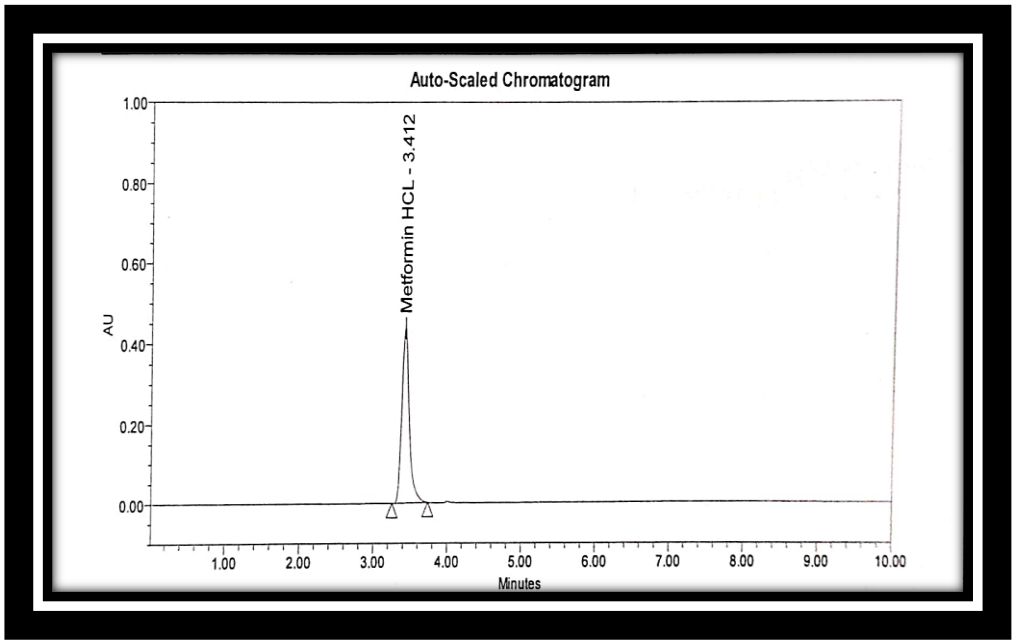


Figure No.6: Chromatogram for Specificity of Metformin HCL

Observation: From the above chromatogram it can be observe that there is no major interference of mobile phase, solvent and impurities in the drug analysis.

Linearity

Five different concentrations of solution were injected into the chromatographic system and Peak area was measured. Graph was plotted of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and correlation coefficient was calculated.

Table No.3: Linearity data of Metformin HCL

Metformin HCL (µg/mL)	Area
50	1562522.33
75	2359216.00
100	3092882.00
125	3934553.33
150	4735202.33

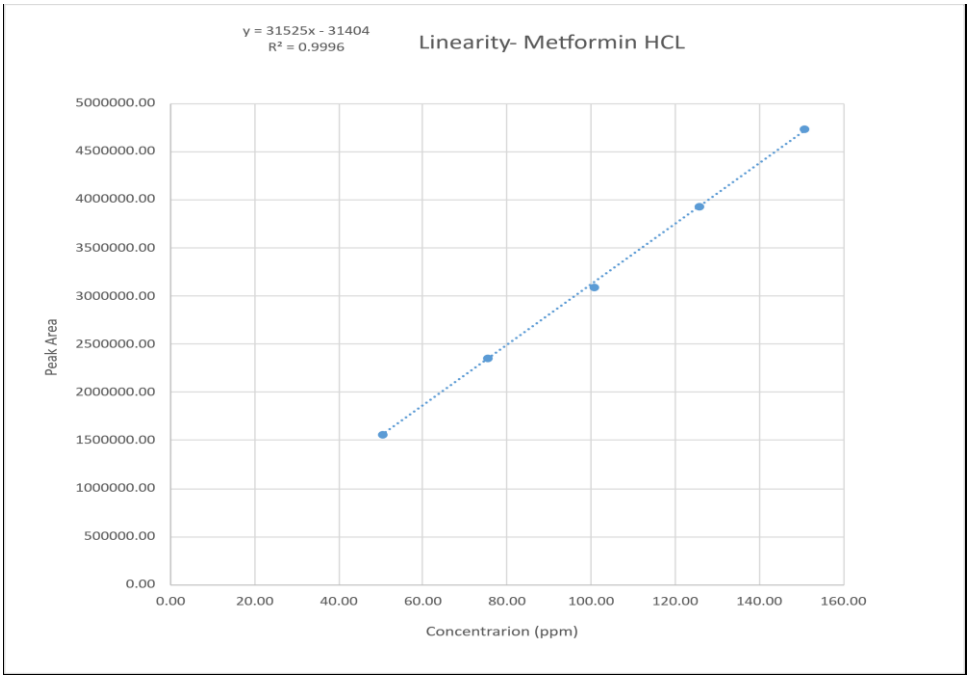


Figure No.6: Linearity of Metformin HCL

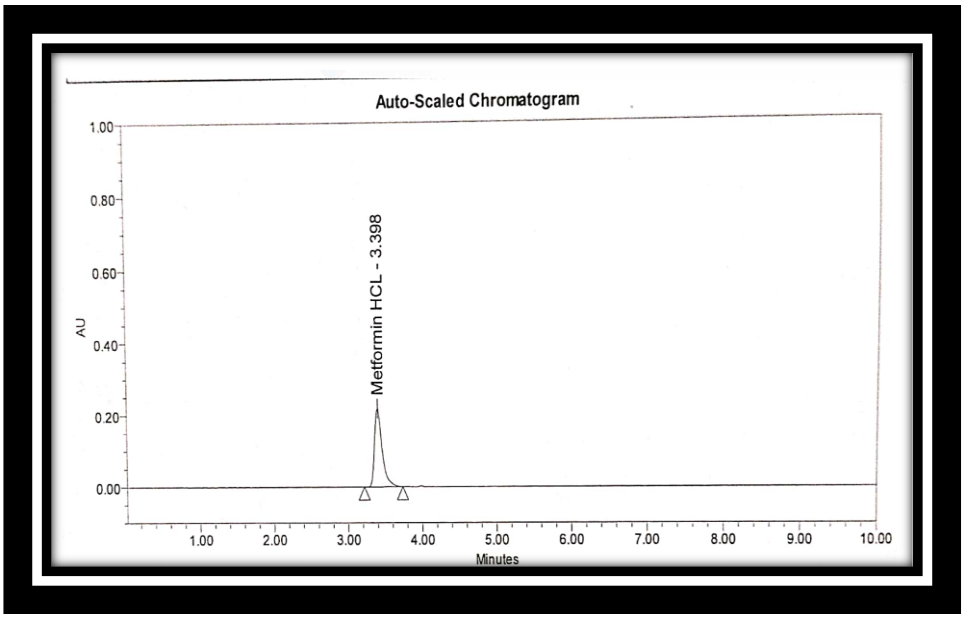


Fig No.7: Chromatogram of Linearity at 50µg/ml for Metformin HCL

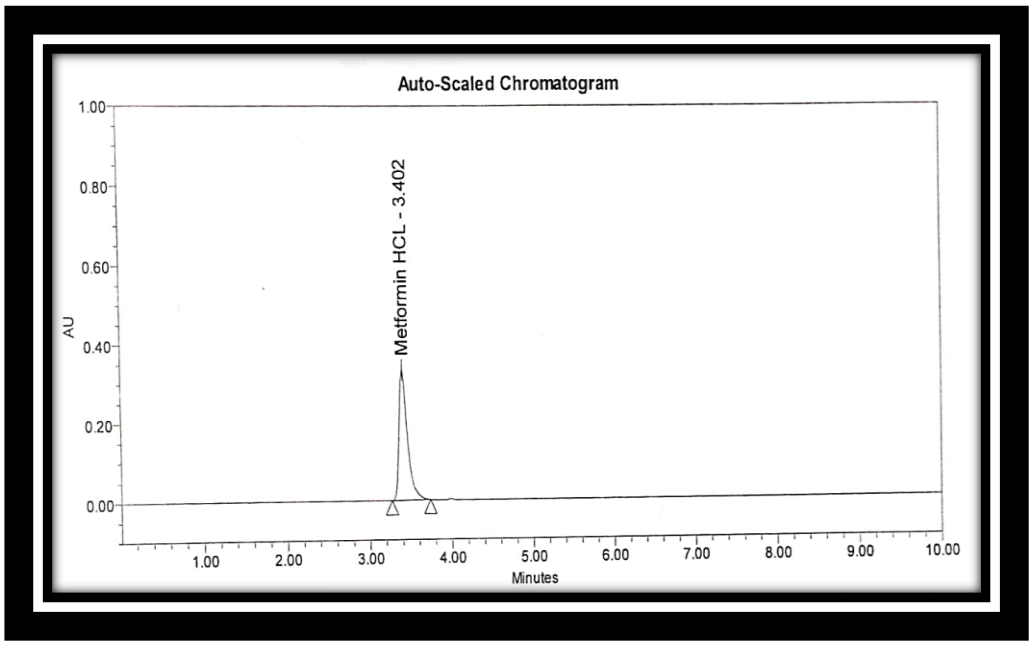


Fig No.8: Chromatogram of Linearity at 75µg/ml for Metformin HCL

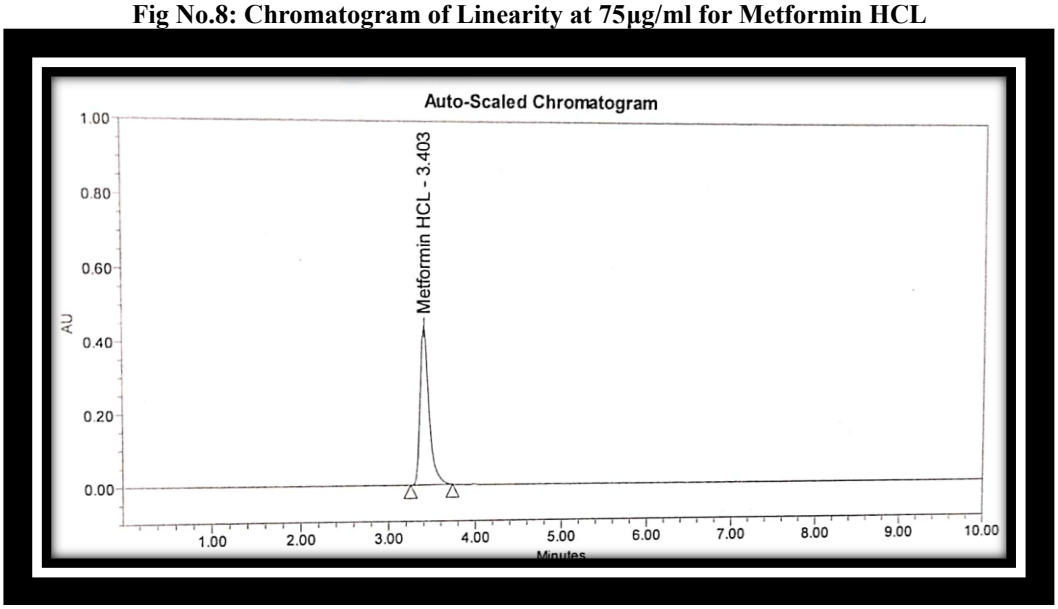


Fig No.9: Chromatogram of Linearity at 100µg/ml for Metformin HCL

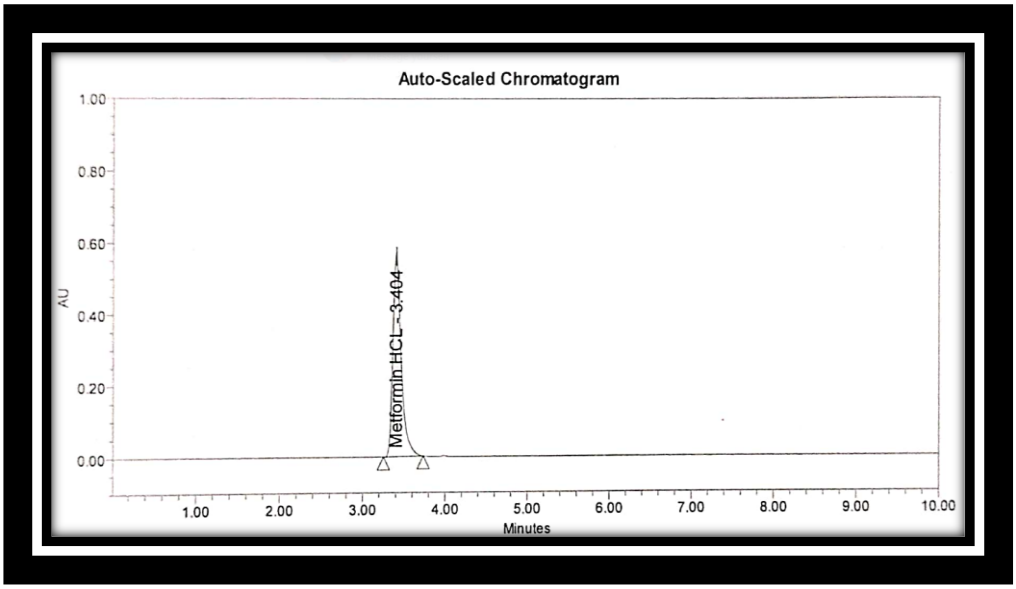


Fig No.10: Chromatogram of Linearity at 125µg/ml for Metformin HCL

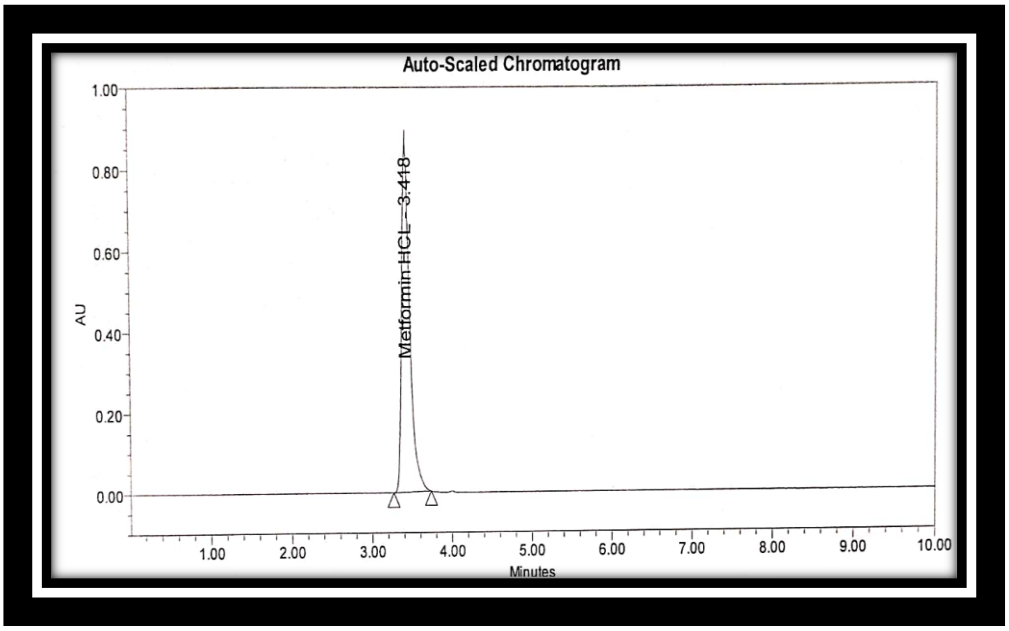


Fig No.11: Chromatogram of Linearity at 150µg/ml for Metformin HCL

Observation: The correlation coefficient for the linear curve obtained between concentrations vs. area for standard preparations of Metformin HCL was found to be 0.9996. The relationship between the concentrations of both drugs was linear in the range examined and the correlation coefficient was within the limit.

Acceptance Criteria: The relationship between the concentration and area of the drug should be linear in the specified range and the correlation coefficient should not be less than 0.999.

Precision

System Precision

Six replicates of standard solution were prepared and %RSD was calculated.

Method Precision

Six replicates of sample solution were prepared and % RSD was calculated.

Table No.4: System Precision data

System Precision of Metformin HCL	
Injection No.	Area at 230 nm
1	3140840
2	3154447
3	3156212
4	3172127
5	3169559
6	3160185
Average	3160185
SD	11944.28
%RSD (Limit NMT 2%)	0.38

Table No.5: Method Precision data

Method Precision of Metformin HCL	
Sr no.	Assay
1	98.22
2	97.40
3	98.22
4	97.35
5	99.03
6	100.22
Average	99.55
SD	0.87
%RSD (Limit NMT 2%)	0.89

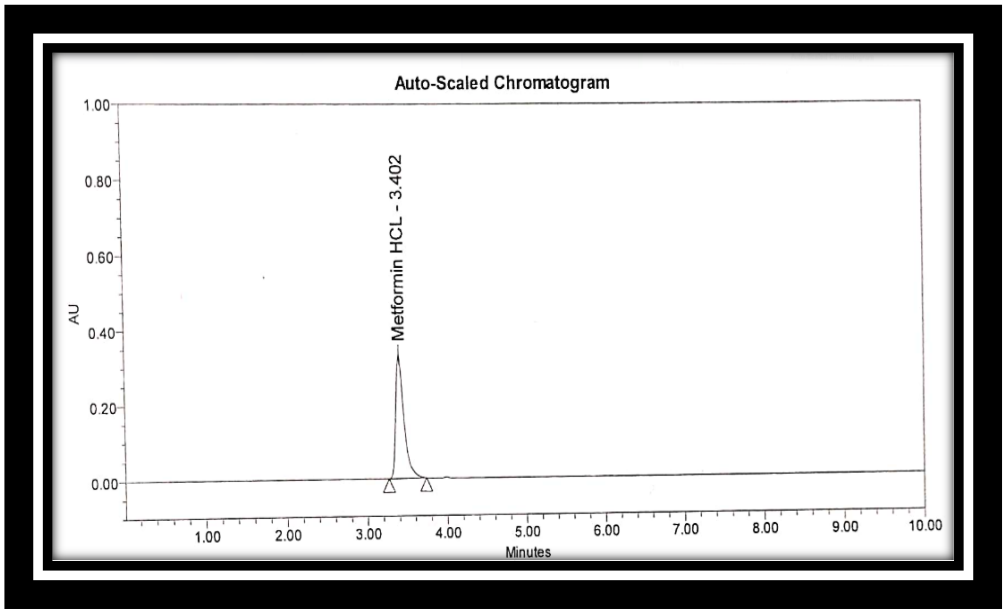


Figure No.12: Chromatogram for System Precision

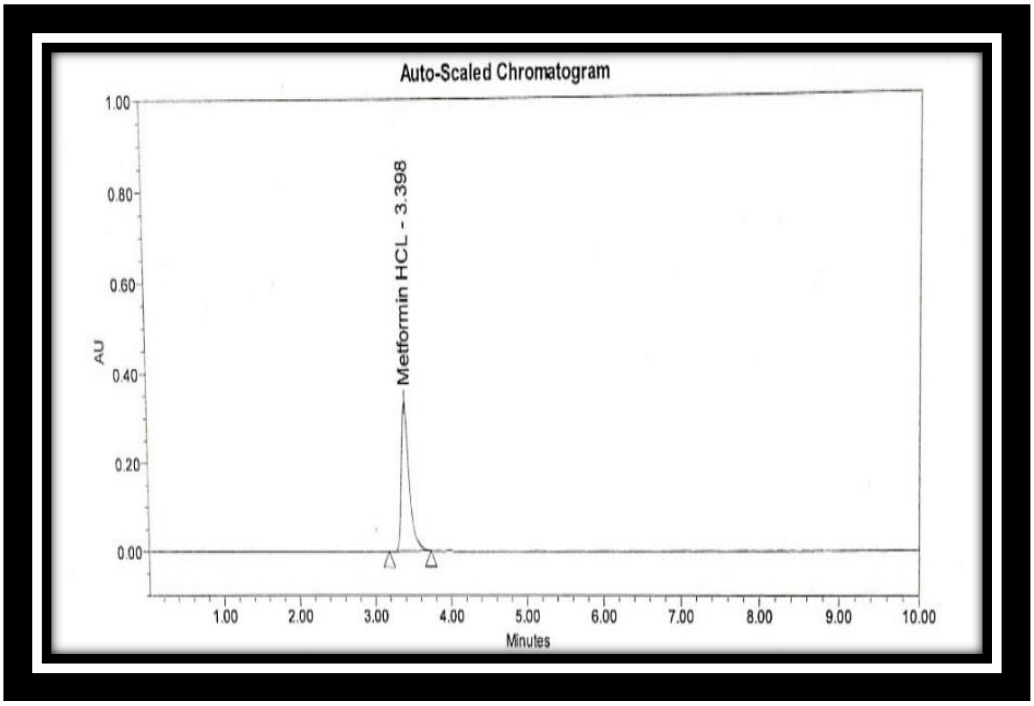


Figure No.13: Chromatogram for Method Precision

Observation: The % RSD for system and method precision was found to be 0.53% and 0.89% respectively for Metformin HCL. Acceptance Criteria: The % RSD should not be more than 2.

Accuracy:

The recovery studies were carried out three times, chromatogram was recorded and %Recovery and Mean % Recovery was calculated.

Table No.6: Accuracy Study data

% LEVEL	Amount Spiked (µg/ml)	Amount Found (µg/ml)	%Recovery	Mean % Recovery	SD	%RSD
50	50	51.04	102.1	102.0	0.1005	0.099
50	50	50.93	101.9			
100	100	101.00	100.0	100.5	0.707	0.70
100	100	102.01	101.0			
150	150	149.62	99.7	99.0	0.99	1.00
150	150	147.41	98.3			

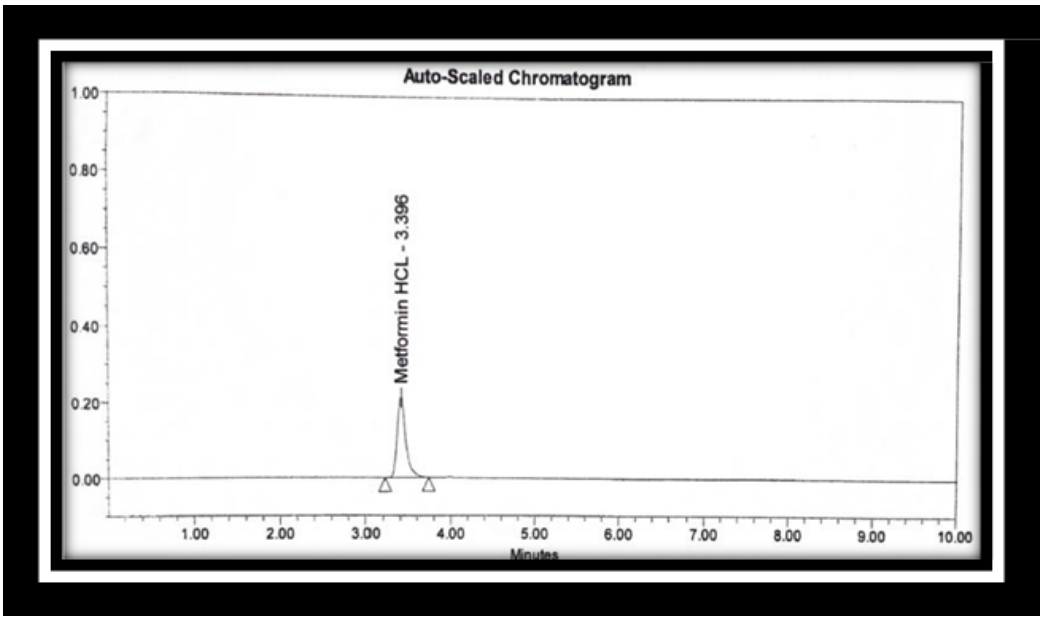


Figure No.14: Chromatogram of Accuracy at 50% for Metformin HCL

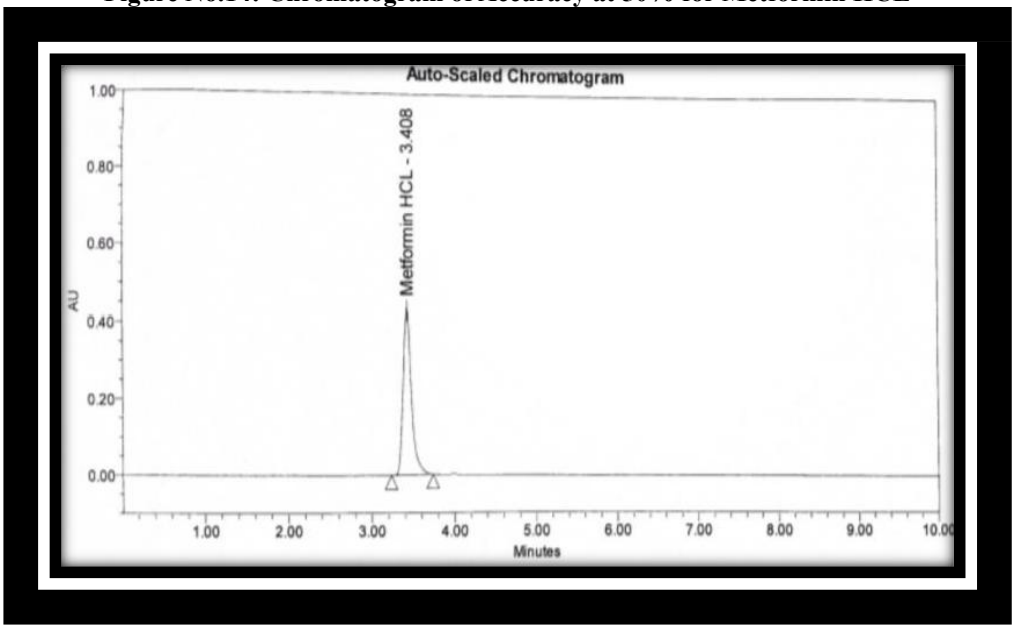


Figure No.15: Chromatogram of Accuracy at 100% for Metformin HCL

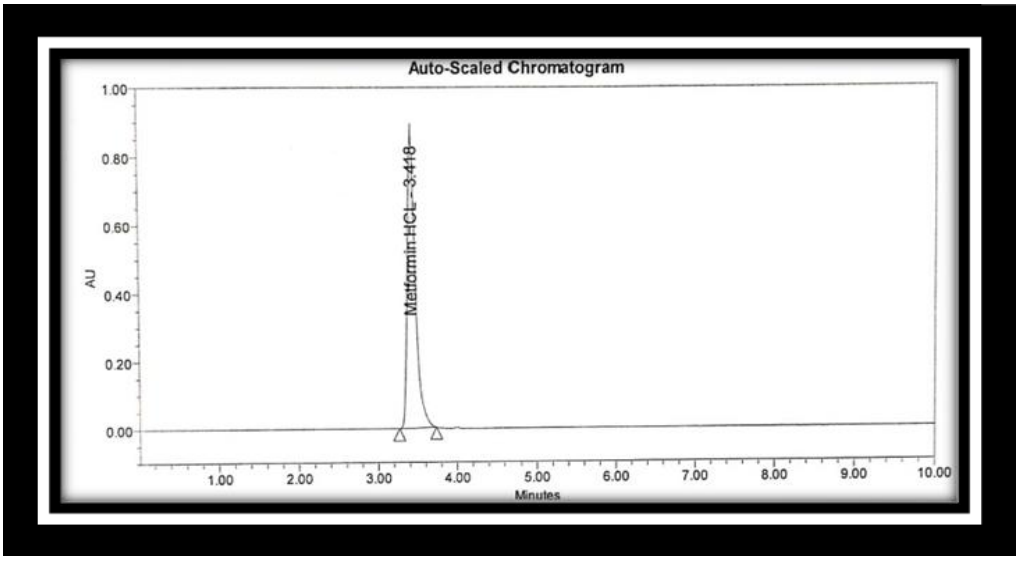


Figure No.16: Chromatogram of Accuracy at 150% for Metformin HCL

Observation: Mean % Recovery for Metformin HCL was found to be 102.0% (50%), 100.5% (100%), and 99.0% (150%).
Acceptance Criteria: Mean % Recovery should be between 98-102.

Robustness

The robustness research conducted revealed that the devised technique is robust, with the RSD falling within the allowed range (less than 2%) at various wavelengths, flow rates, and column oven temperatures.

Table No.7: Robustness Study data

Parameters	Changes in conditions	Metformin HCL	% RSD
Flow rate	1 min/ml	AREA	0.23
		RT	0.21
		NTP	1.9
	0.6 min/ml	AREA	0.4
		RT	0.03
		NTP	1.16
Wavelength	225 nm	AREA	0.44
		RT	0.02
		NTP	1.55
	235 nm	AREA	0.42
		RT	0
		NTP	1.23
Temperature	30 0C	AREA	0.18
		RT	0.08
		NTP	1.27
	20 0C	AREA	0.48
		RT	0.06
		NTP	1.55

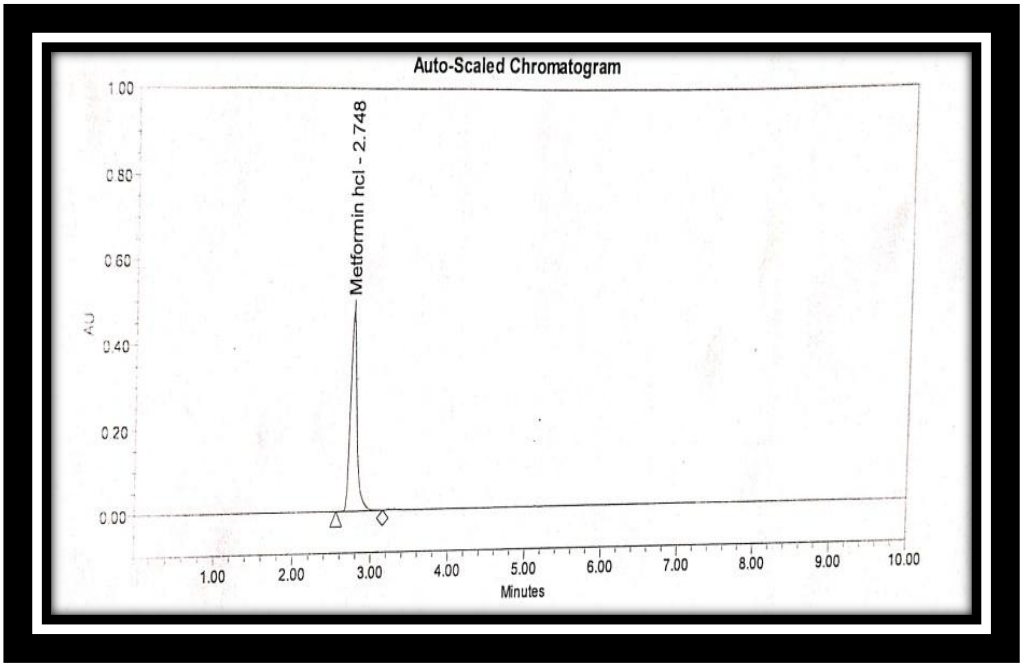


Figure No.17: Chromatogram of robustness at 1 min/ml flow rate

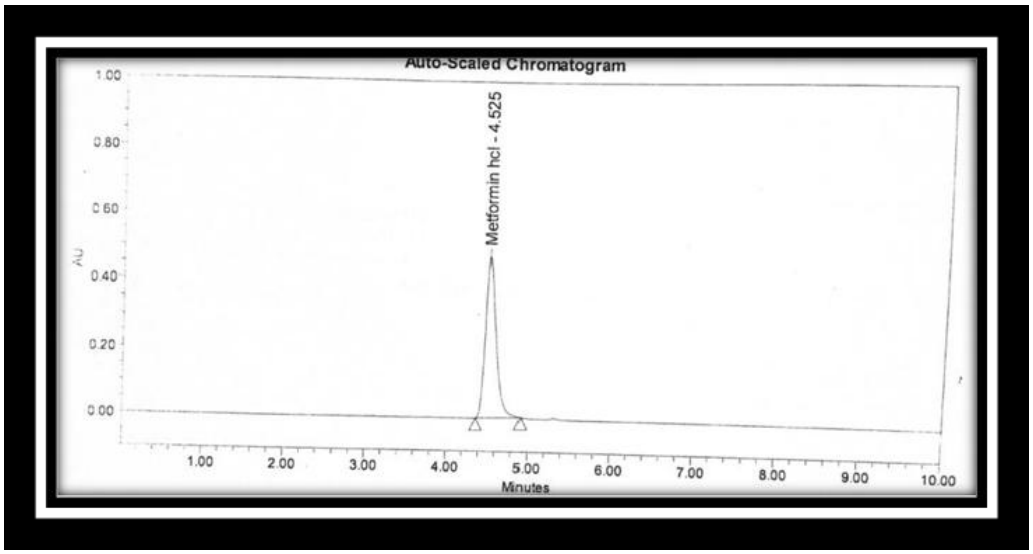


Figure No.18: Chromatogram of robustness at 0.6 min/ml flow rate

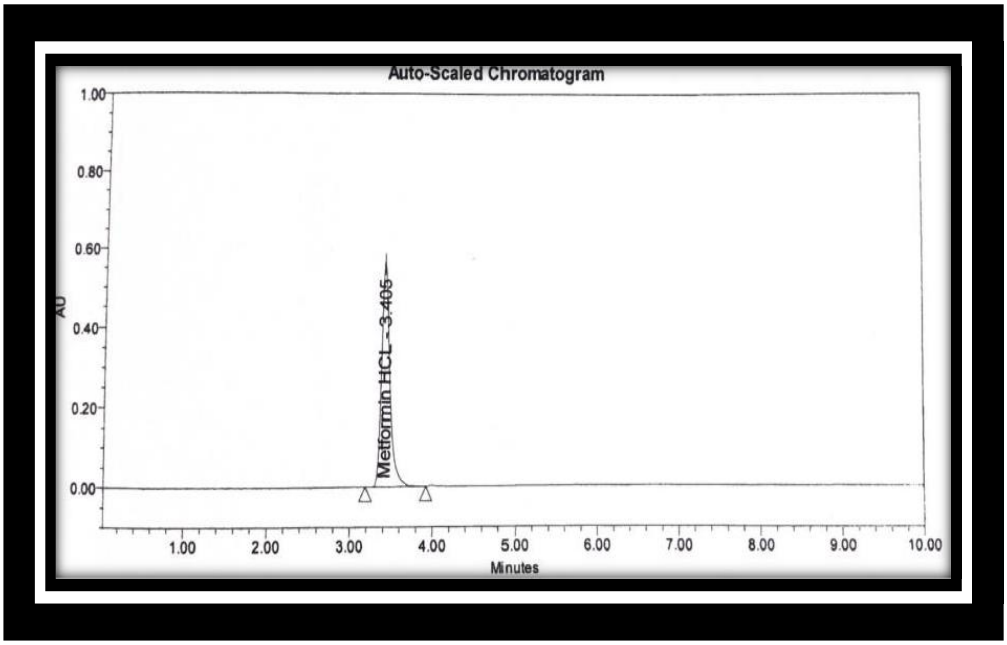


Figure No.19: Chromatogram of robustness at 225nm

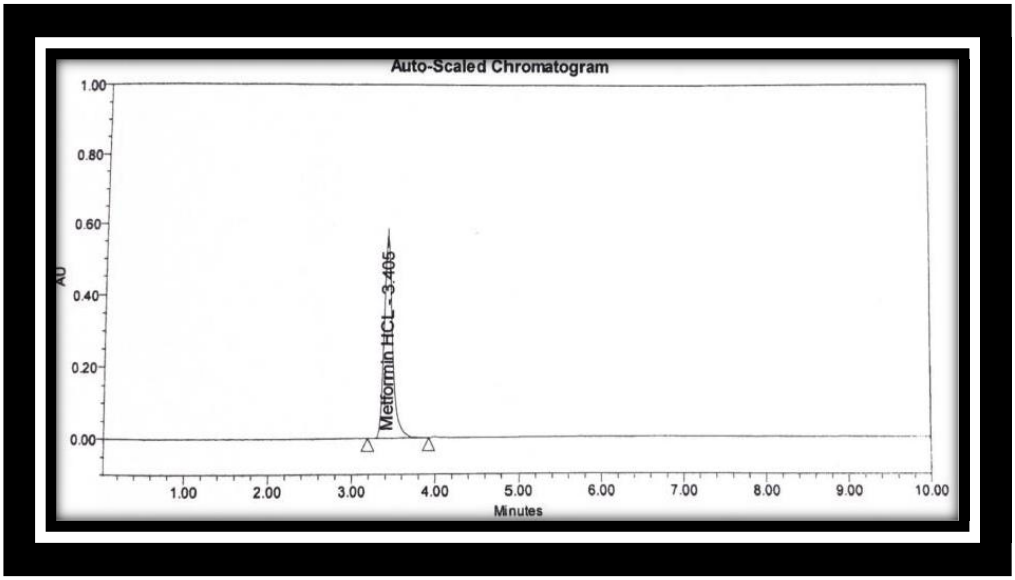


Figure No.20: Chromatogram of robustness at 235nm

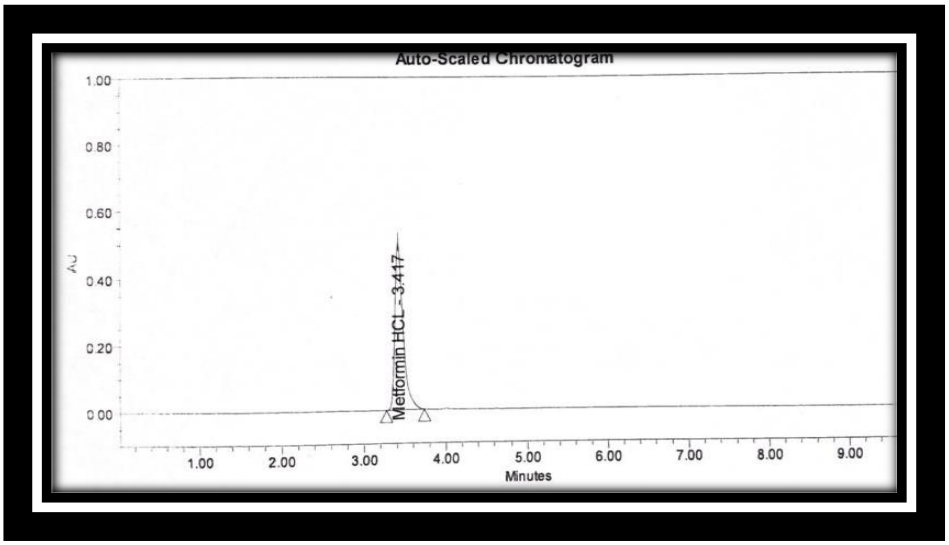


Figure No.21: Chromatogram of robustness at 30°C

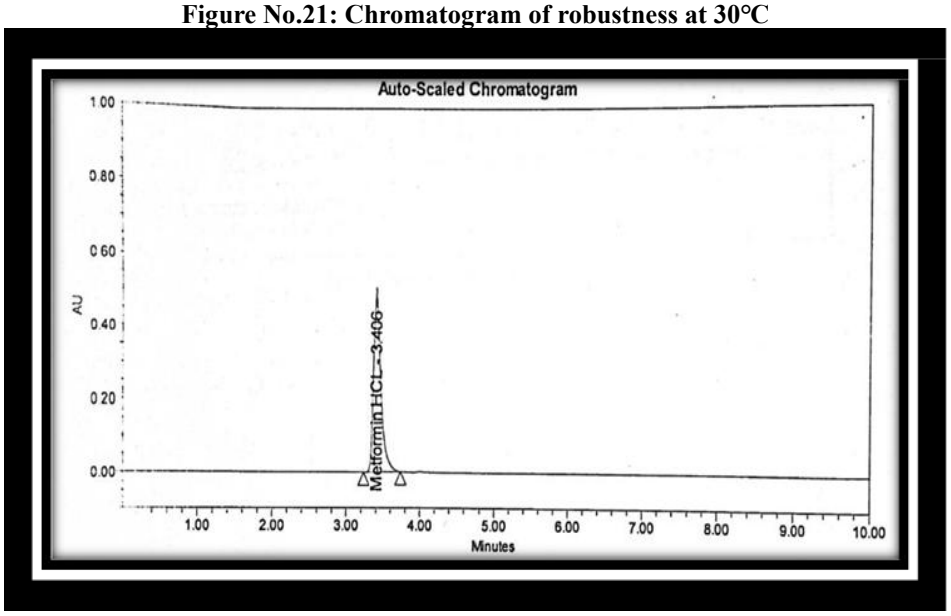


Figure No.22: Chromatogram of robustness at 20°C

Force Degradation Studies by RP- HPLC Method

To evaluated the stability condition of the development RP – HPLC method stress in condition as acid base, oxidation, thermal and photolytic degradation. In all studies % degradation was calculated.

Acid degradation

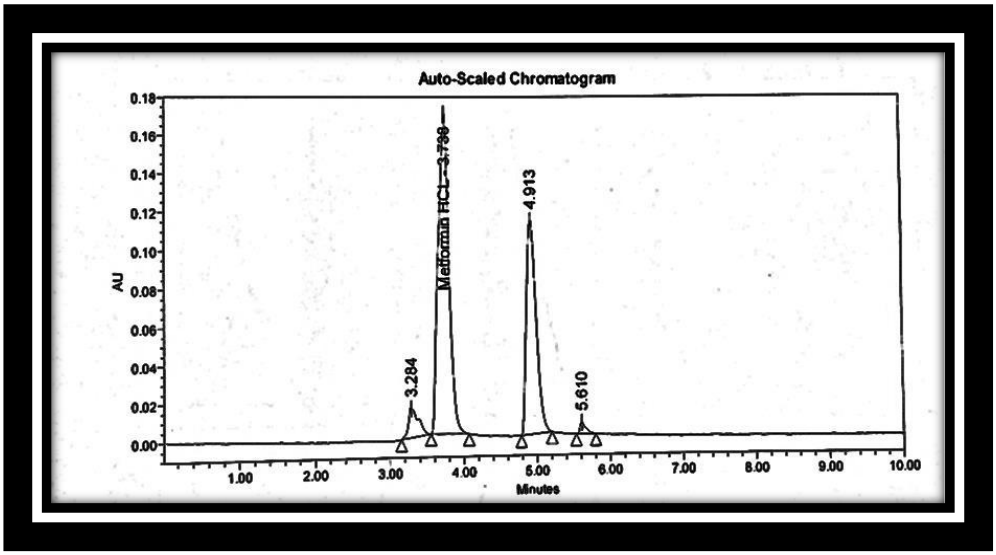


Fig No.23: Chromatogram of Metformin HCL by Acid Degradation

Base degradation

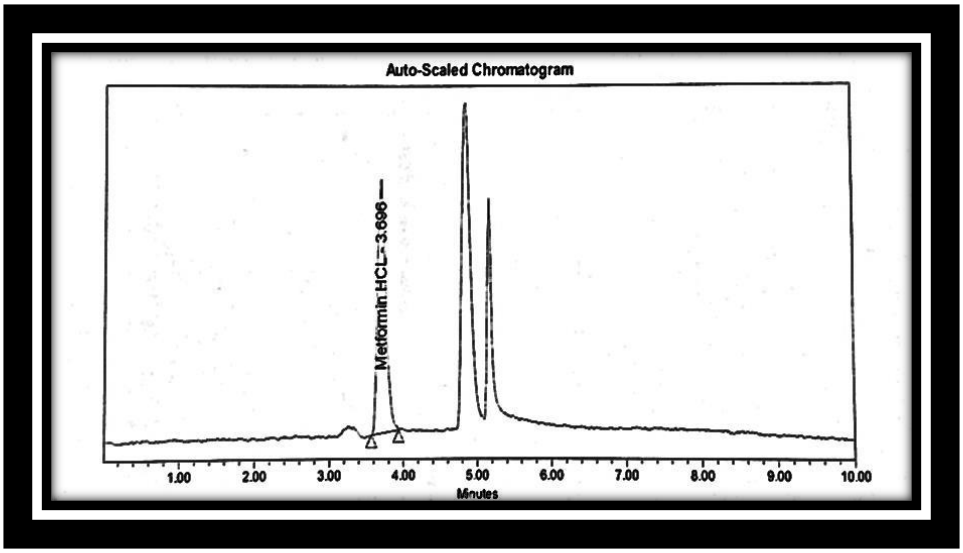


Fig No.24: Chromatogram of Metformin HCL by Base Degradation

Oxidation degradation

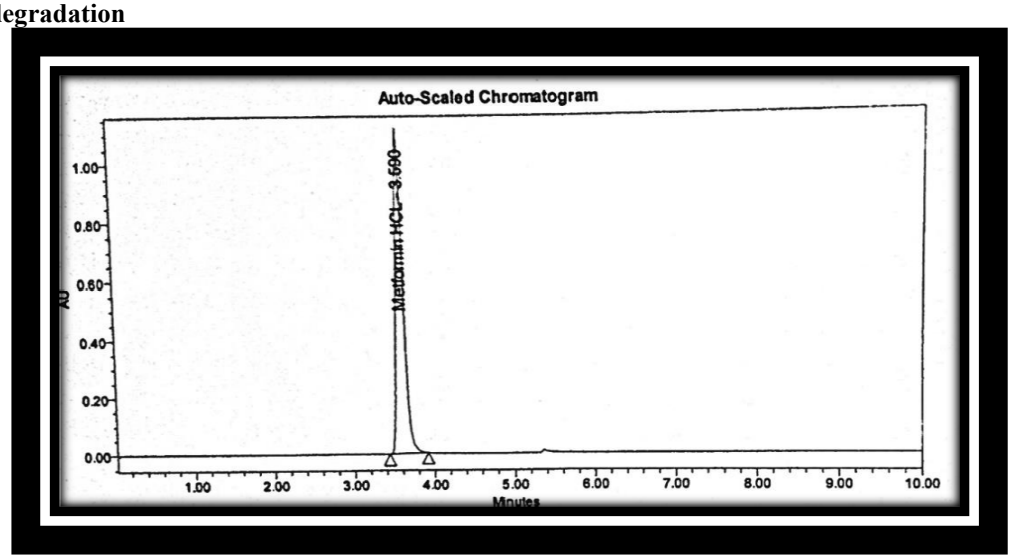
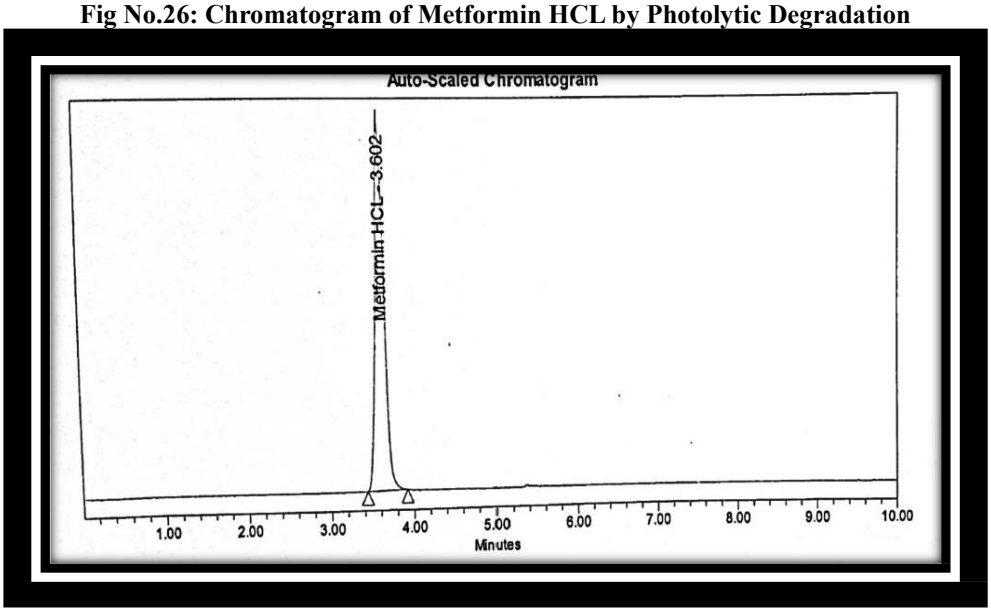


Fig No.25: Chromatogram of Metformin HCL by Oxidation Degradation

Photolytic degradation

Fig No.26: Chromatogram of Metformin HCL by Photolytic Degradation



Thermal degradation

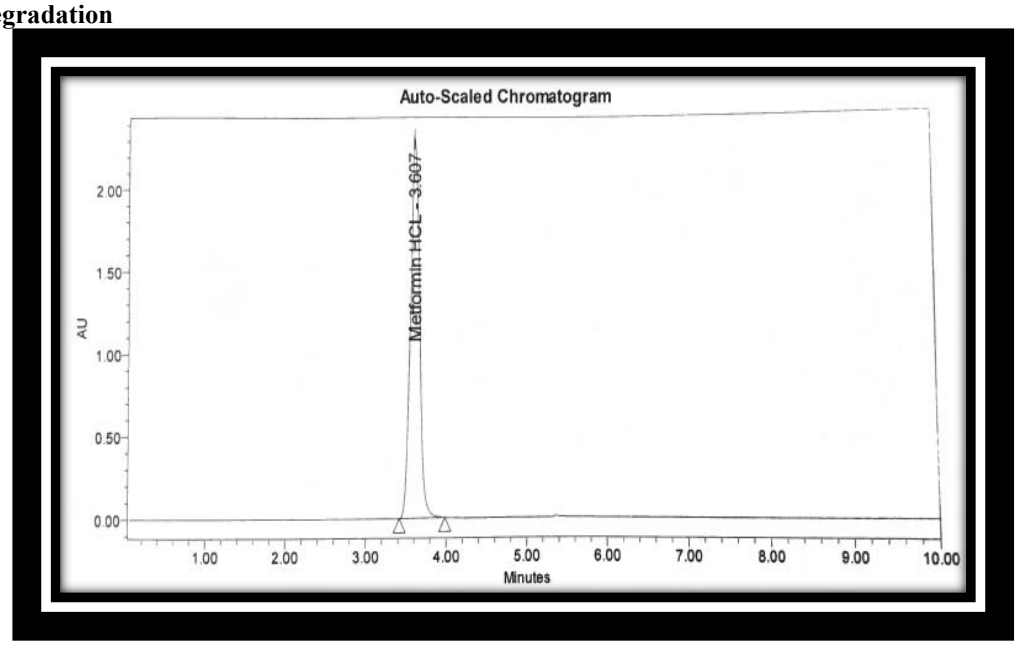


Fig No.27: Chromatogram of Metformin HCL by Thermal Degradation

Table No.8: Force degradation Study

Metformin HCL			
Sr. No	Degradation condition	Time	% Degradation
1	Acid	24hrs	7.8
2	Base	24hrs	7.0
3	Oxidation	24hrs	2.0
4	Thermal	24hrs	0.08
5	Photolytic	24hrs	0.26

CONCLUSION

A stability-indicating RP-HPLC method was successfully developed and validated for the quantitative estimation of Metformin Hydrochloride in its pharmaceutical dosage form—specifically, a transdermal patch. The method demonstrated accuracy, precision, robustness, linearity, and specificity in accordance with ICH Q2(R1) guidelines. Importantly, no interference was observed from excipients or degradation products, confirming the method's selectivity. Force degradation studies conducted under acidic, alkaline, oxidative, photolytic, and thermal conditions Results indicates that Metformin HCl underwent the most substantial degradation in an acidic environment and basic environment, thereby confirming the method's effectiveness as a stability-indicating assay. The validated method was effectively applied to the analysis of Metformin HCl in transdermal patch formulations. Due to its simplicity, reliability, and sensitivity, the proposed method is suitable for routine quality control, quantitative analysis, and stability testing of Metformin HCl in transdermal drug delivery systems.

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