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Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Metformin Hydrochloride, Ramipril, Atorvastatin and Glimepiride in Bulk Drugs and Combined Tablet Dosage Form

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Abstract: A simple, rapid, and reliable reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of metformin hydrochloride, ramipril, atorvastatin, and glimepiride in bulk drugs and combined tablet dosage form. Chromatographic separation was achieved using a GRACE ODS column (150 × 4.6 mm, 5 µm; L1 packing) maintained at ambient temperature. The optimized mobile phase consisted of methanol and 0.03 M potassium dihydrogen phosphate (KH_2PO_4) buffer in a ratio of 74:26 (v/v), delivered at a flow rate of 1.0 mL/min. The analytes were monitored at 210 nm. Under the optimized chromatographic conditions, the retention times of metformin hydrochloride, ramipril, atorvastatin, and glimepiride were approximately 1.473, 2.7, 4.7, and 6.2 min, respectively, demonstrating satisfactory resolution without interference from adjacent peaks. The method exhibited a linear response over the investigated concentration range of 0.1–140 µg/mL. The limits of quantification for metformin hydrochloride, ramipril, atorvastatin, and glimepiride were 100, 0.5, 2.0, and 0.2 µg/mL, respectively, while the corresponding limits of detection were 10 µg/mL, 20 ng/mL, 50 ng/mL, and 200 ng/mL. The proposed method was found to be rapid, sensitive, and suitable for the simultaneous determination of all four drugs. Therefore, it can be applied to the routine quality-control analysis of metformin hydrochloride, ramipril, atorvastatin, and glimepiride in bulk materials and combined pharmaceutical formulations.

Keywords: *Metformin Hydrochloride; Ramipril; Atorvastatin; Glimepiride; Simultaneous Estimation; RP-HPLC; Method Validation.*

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I. INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disorder frequently accompanied by hypertension, dyslipidaemia, and an increased risk of cardiovascular complications. Consequently, patients may require multiple medicines to achieve adequate glycaemic control and simultaneously manage associated cardiovascular risk factors. Metformin hydrochloride and glimepiride provide complementary glucose-lowering effects, whereas ramipril and atorvastatin are used to control hypertension and dyslipidaemia, respectively. Formulations containing these

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pharmacologically distinct agents may therefore offer therapeutic convenience and reduce the complexity of multidrug regimens. However, the presence of four active pharmaceutical ingredients with markedly different physicochemical properties and dose strengths creates considerable challenges for their simultaneous analysis and quality control [1–4].

Metformin hydrochloride is a biguanide antihyperglycaemic agent widely employed in the management of type 2 diabetes mellitus. It reduces hepatic glucose production, decreases intestinal glucose absorption, and improves insulin sensitivity by enhancing peripheral glucose uptake and utilization [1]. Chemically, metformin is highly polar and exhibits relatively weak retention on conventional reversed-phase stationary phases. Its high therapeutic concentration compared with the other components of the proposed formulation can also complicate simultaneous chromatographic determination.

Glimepiride is a third-generation sulfonylurea that lowers blood glucose primarily by stimulating insulin secretion from functional pancreatic β-cells through interaction with sulfonylurea receptors and closure of ATP-sensitive potassium channels [2]. In contrast to metformin, glimepiride is comparatively lipophilic and is generally present at a much lower concentration in combined formulations. These differences in polarity, solubility, chromatographic retention, and dosage level require careful optimization of the stationary phase, mobile-phase composition, pH, detection wavelength, and sample-preparation procedure.

Ramipril is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and for reducing cardiovascular risk in appropriate patients. Its active metabolite, ramiprilat, inhibits the conversion of angiotensin I into angiotensin II, thereby decreasing vasoconstrictor activity and aldosterone secretion [3]. Ramipril is susceptible to degradation and may form related substances through hydrolytic, oxidative, and thermal pathways. Therefore, an analytical method intended for its determination in a multicomponent formulation must be sufficiently selective to distinguish the intact drug from its potential degradation products.

Atorvastatin is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme involved in cholesterol biosynthesis. It is extensively used to reduce low-density lipoprotein cholesterol and prevent cardiovascular events [4]. Atorvastatin can undergo degradation under acidic, alkaline, oxidative, thermal, and photolytic conditions. Its simultaneous determination with metformin, ramipril, and glimepiride is analytically demanding because the four compounds differ substantially in chemical structure, ionization behaviour, polarity, solubility, concentration, and ultraviolet absorption characteristics.

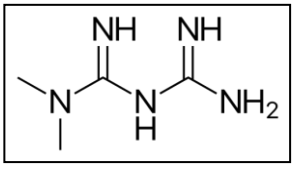
Reversed-phase high-performance liquid chromatography is one of the most widely employed techniques for pharmaceutical quality-control analysis because of its selectivity, reproducibility, sensitivity, and suitability for multicomponent formulations. Several chromatographic procedures have been reported for the determination of these drugs individually or in combinations with other pharmaceutical agents. Stability-indicating RP-HPLC methods have also been described for the simultaneous determination of metformin, atorvastatin, and glimepiride [5]. In addition, an RP-HPLC method for the simultaneous assay of metformin hydrochloride, ramipril, atorvastatin, and glimepiride in bulk materials and tablets has previously been reported [6]. Nevertheless, a routine assay method cannot automatically be considered stability-indicating unless its specificity is demonstrated by resolving all active ingredients from their degradation products, impurities, and formulation excipients.

A stability-indicating analytical procedure is essential for monitoring drug quality throughout formulation development, manufacturing, storage, and shelf-life evaluation. According to ICH Q1A(R2), stability studies should establish how the quality of a drug substance or product changes under the influence of environmental factors such as temperature, humidity, and light [7]. Forced-degradation experiments performed under acidic, alkaline, oxidative, thermal, and photolytic conditions are particularly important for establishing degradation behaviour and demonstrating the selectivity of an analytical method. Furthermore, ICH Q2(R2) requires analytical procedures to be validated for characteristics relevant to their intended purpose, including specificity, response, range, accuracy, precision, detection limit, quantification limit, and robustness, as applicable [8].

Considering the therapeutic importance of these drugs and the analytical complexity of their combined determination, there is a need for a rapid, accurate, precise, robust, and genuinely stability-indicating chromatographic procedure. Therefore, the present study aims to develop and validate an RP-HPLC method for the simultaneous estimation of metformin hydrochloride, ramipril, atorvastatin, and glimepiride in bulk drugs and combined tablet dosage form. The stability-indicating capability of the proposed method will be established through systematic forced-degradation studies and evaluation of peak purity and chromatographic resolution. The developed procedure is expected to provide an efficient tool for routine assay, stability testing, and quality-control evaluation of the four-component pharmaceutical formulation.

➢ *Drug Profile:*

- *Metformin HCL*



IUPAC name : 1,1-Dimethylbiguanide hydrochloride

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Molecularformula : $C_4H_{12}ClN_5$

Molecular weight : 165.625 g/mol

Solubility: soluble in water, methanol & acetonitrile.

Category: anti diabetic agent

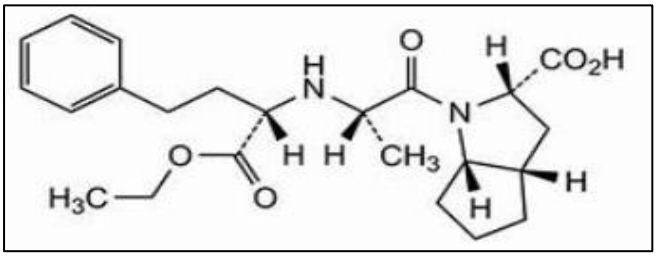
P^KA value: 2.8 and 11.5.

UV maxima: 236 nm

Melting point: 222 deg C. to 226 deg C

Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. Metformin administration also increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. The rare side effect, lactic acidosis, is thought to be caused by decreased liver uptake of serum lactate, one of the substrates of gluconeogenesis. In those with healthy renal function, the slight excess is simply cleared. However, those with severe renal impairment may accumulate clinically significant serum lactic acid levels. Other conditions that may precipitate lactic acidosis include severe hepatic disease and acute/decompensated heart failure.

- *Ramipril*



IUPACname:(2S,3as,6as)-1-[(2S)-2-{[(2S)-1-Ethoxy-1-oxo-4-phenyl-2-butanyl]amino}-propanoyl]octahydrocyclopenta[b]pyrrol-2-carbonsäure

Molecular formula : $C_{23}H_{32}N_2O_5$

Molecular weight : 416.511 g/mol,

Solubility: methanol, acetonitrile, acetic acid and chloroform ,hydrochloric acid ,sodium hydroxide, ethanol& pyridine.

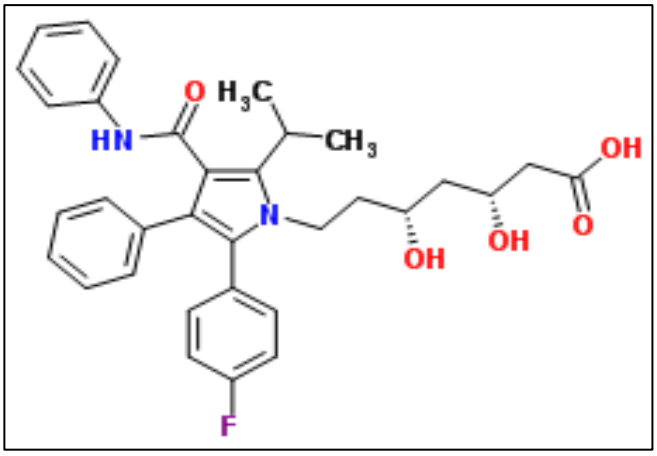
Category: angiotensin-converting enzyme (ACE) inhibitor

Melting point: 109 °

ACE inhibitors inhibit the actions of angiotensin converting enzyme(ACE), thereby lowering the production of angiotensin II and decreasing the breakdown of bradykinin. The decrease in angiotensin II results in relaxation of arteriole smooth muscle leading to a decrease in total peripheral resistance, reducing blood pressure as the blood is pumped through widened vessels. Its effect on bradykinin is responsible for the dry cough side effect.

Ramipril, a prodrug or precursor drug, is converted to the active metabolite ramiprilat by carboxylesterase1 .

- *Atorvastatin*



IUPAC Name : (3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid

Molecular formula : $C_{33}H_{35}FN_2O_5$.

Molecular weight : 558.640 g/mol.

Solubility : methanol, acetonitrile, acetic acid and chloroform, ethanol and pyridine.

Category: lipid-lowering agent

Melting point: 159-161°C

Atorvastatin is a competitive inhibitor of HMG-CoA reductase. Unlike most others, however, it is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases *de novo* cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

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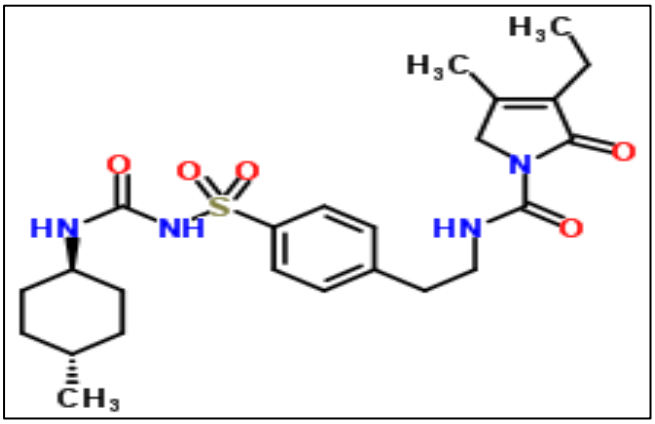
- *Glimepiride*



IUPAC Name : 1-{[4-(2-{[(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1H-pyrrol-1-yl)carbonyl]amino}ethyl)phenyl]sulfonyl}-3-(trans-4-methylcyclohexyl)urea

Molecular formula : $C_{24}H_{34}N_4O_5S$

Molecular weight : 490.617 g/mol

Solubility: methanol, acetonitrile, acetic acid and chloroform.

Category: anti diabetic agent

Melting point: 207 °C

Glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin.

➢ *Experimental Procedure*

- *Experimental Requirements*

- *Equipment and Apparatus*

The chromatographic analysis was performed using a Shimadzu LC-10AD HPLC system equipped with an LC-10ADVP reciprocating pump and a UV–Visible detector. Data acquisition and chromatographic processing were carried out using N2000 chromatography software. Separation was achieved on an Inertsil C18 column (250 × 4.6 mm i.d., 5 µm particle size).

A Shimadzu UV-1800 UV–Visible spectrophotometer was used for wavelength selection and spectral analysis. Other instruments used during the study included an electronic analytical balance, digital pH meter, ultrasonic bath sonicator, and vacuum filtration apparatus. Sonication was performed using a Citizen digital ultrasonic cleaner.

- *Materials and Chemicals*

Reference standards of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride of suitable pharmaceutical purity were used for method development and validation. HPLC-grade methanol was obtained from Merck Pvt. Ltd. Double-distilled/Grade-2 water was used throughout the chromatographic analysis. Orthophosphoric acid of analytical-reagent grade was used for pH adjustment wherever required.

All solvents and solutions used for chromatographic analysis were filtered through a suitable membrane filter and degassed before use.

II. METHOD DEVELOPMENT

➢ *Selection of Detection Wavelength*

Selection of an appropriate detection wavelength was carried out to obtain adequate sensitivity for the simultaneous determination of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride. Individual working standard solutions of each drug, having an approximate concentration of 10 µg/mL, were prepared using a suitable diluent. The solutions were scanned individually over the UV region of approximately 200–400 nm using a Shimadzu UV-1800 UV–Visible spectrophotometer. The absorption spectra of all four analytes were recorded and their respective absorption maxima were examined. Based on the overlay spectra and comparative absorbance of the four drugs, 210 nm provided satisfactory absorbance and adequate detector response for all the analytes. Therefore, 210 nm was selected as the common detection wavelength for subsequent RP-HPLC method development and quantitative analysis.

➢ *Preparation of Mobile Phase*

Different proportions of organic solvent and aqueous buffer were investigated during preliminary chromatographic trials to obtain satisfactory separation, symmetrical peak shapes, acceptable resolution, and a reasonable analysis time.

The optimized mobile phase consisted of methanol and 0.03 M potassium dihydrogen phosphate (KH_2PO_4) buffer in the ratio of 74:26, v/v.

The required quantities of methanol and phosphate buffer were measured separately and mixed thoroughly. The prepared mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication before chromatographic use.

➢ *Preparation of 0.03 M Potassium Dihydrogen Phosphate Buffer*

The molecular weight of potassium dihydrogen phosphate (KH_2PO_4) is approximately 136.09 g/mol. Therefore, approximately 4.08 g of KH_2PO_4 was accurately weighed and dissolved in about 800 mL of double-distilled water. The solution was mixed thoroughly and the volume was made up to 1000 mL with double-distilled water.

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Where necessary, the pH of the buffer was adjusted using dilute orthophosphoric acid. The prepared buffer was filtered through a suitable membrane filter before mixing with the organic phase.

➢ *Preparation of Standard Stock Solutions*

Accurately weighed quantities of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride reference standards were transferred separately into suitable volumetric flasks.

A portion of the diluent was added to each flask, and the solutions were sonicated until complete dissolution of the respective drug was achieved. The solutions were subsequently diluted to volume with the diluent to obtain individual primary stock solutions of known concentration.

Appropriate aliquots from the individual stock solutions were further diluted with the mobile phase or selected diluent to prepare a mixed working standard solution containing all four analytes at their required analytical concentrations.

➢ *Chromatographic Conditions*

The optimized chromatographic conditions employed for simultaneous analysis were as follows:

Table 1 Chromatographic Conditions

Chromatographic parameter	Optimized condition
HPLC system	Shimadzu LC-10AD
Column	Inertsil C18
Column dimensions	250 × 4.6 mm i.d.
Particle size	5 µm
Mobile phase	Methanol:0.03 M KH_2PO_4 buffer
Mobile-phase ratio	74:26, v/v
Elution mode	Isocratic
Flow rate	1.0 mL/min
Detection wavelength	210 nm
Detector	UV–Visible detector
Column temperature	Ambient temperature
Injection volume	Approximately 20 µL*
Data-processing software	N2000

*The Injection Volume should be Replaced with the Actual Experimentally used value if it Differed from 20 µL.

➢ *Chromatographic Procedure*

Before starting the analysis, the HPLC system was equilibrated with the optimized mobile phase until a stable baseline was obtained. The column was conditioned by passing the mobile phase at a flow rate of 1.0 mL/min.

The blank solution was initially injected to confirm the absence of interfering peaks. Subsequently, the mixed standard solution containing Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride was injected into the chromatographic system.

The chromatogram was recorded at 210 nm, and the retention time, peak area, peak symmetry, theoretical plate count, and resolution of the individual analytes were evaluated. Several preliminary chromatographic trials were performed by varying the mobile-phase composition, flow rate, and other chromatographic parameters until satisfactory separation of all four compounds was achieved.

The optimized chromatographic system using methanol:0.03 M KH_2PO_4 buffer (74:26, v/v) at a flow rate of 1.0 mL/min provided satisfactory separation and was therefore selected for further analytical method validation.

➢ *System Suitability Testing*

Before quantitative analysis, system suitability was established by injecting the mixed standard solution repeatedly under the optimized chromatographic conditions. Parameters such as retention time, peak area, theoretical plate number, tailing factor, and resolution between adjacent peaks were evaluated.

The system was considered suitable for analysis when the chromatographic parameters complied with the predefined acceptance criteria, including adequate theoretical plate count, acceptable peak symmetry, and sufficient resolution between the four analytes.

➢ *Preparation of Sample Solution*

A suitable number of combined tablets containing Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride were accurately weighed and finely powdered. A quantity of tablet powder equivalent to the required amount of each active pharmaceutical ingredient was transferred into an appropriate volumetric flask.

Approximately 60–70% of the final volume of diluent was added, and the mixture was sonicated to facilitate complete extraction of the drugs from the tablet matrix. The solution was allowed to cool, diluted to volume with the diluent, and mixed thoroughly.

The resulting solution was filtered through a suitable membrane filter, discarding the initial portion of the filtrate. An appropriate aliquot was subsequently diluted with the mobile phase to obtain concentrations within the established calibration range.

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The prepared sample solution was injected into the HPLC system under the optimized chromatographic conditions, and the concentrations of the four drugs were calculated by comparing their respective peak areas with those obtained from the standard solutions.

➢ *Proposed Method Validation*

The developed RP-HPLC method should be validated in accordance with current ICH Q2(R2) principles for analytical procedure validation. The validation study should include evaluation of specificity/selectivity, linearity and range, accuracy, precision, repeatability, intermediate precision, limit of detection, limit of quantification, robustness, system suitability, and solution stability, as applicable to the intended quantitative analytical procedure.

This experimental approach provides the basis for establishing a simple, reproducible, and efficient RP-HPLC procedure for the simultaneous estimation of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride in combined pharmaceutical dosage forms.

- *Over Lay Spectra's of Metformin HCL, Ramipril, Atorvastatin and Glimepiride*

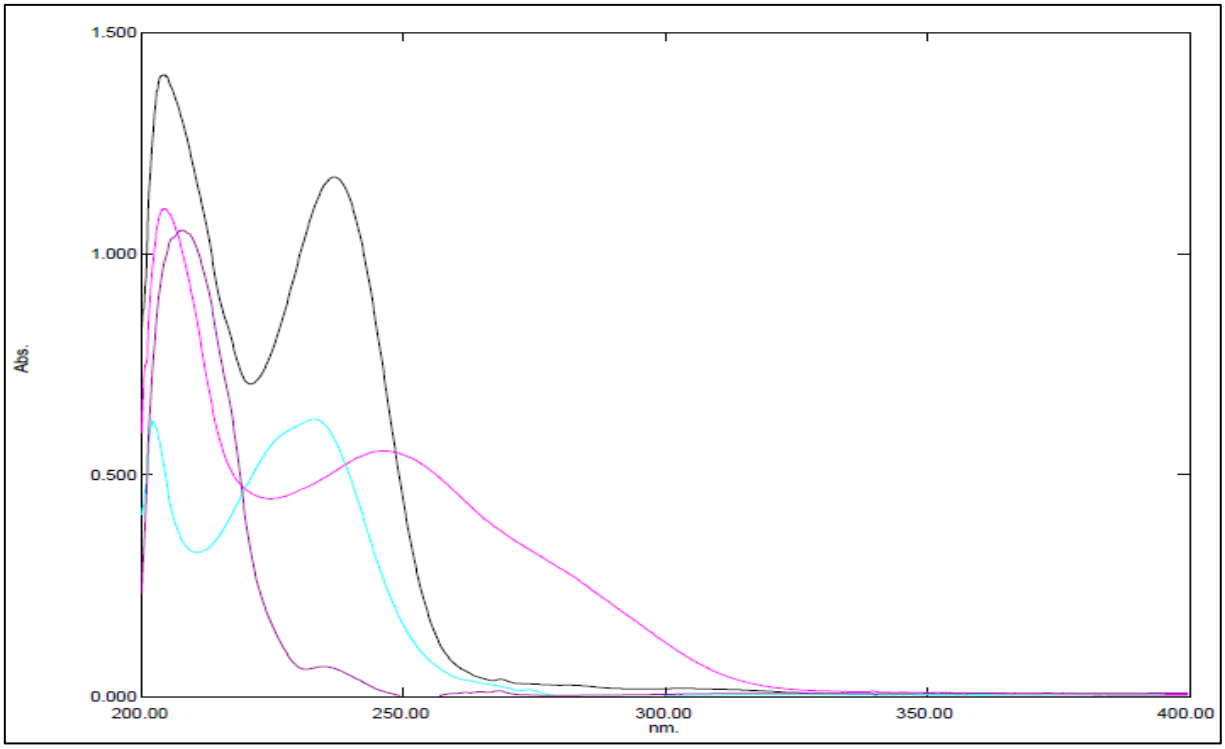

Fig 1 Over Lay Spectra's of Metformin HCL, Ramipril, Atorvastatin and Glimepiride

✓ Blue Line - glimepiride
✓ Pink Line -Aorvastatin
✓ Purple Line :Ramipril
✓ Brown Line - Metformin HCL

The developed RP-HPLC method provided satisfactory simultaneous separation and quantification of Metformin hydrochloride, Ramipril, Atorvastatin, and Glimepiride in the combined formulation. After establishing the optimized chromatographic conditions, the HPLC system was allowed to equilibrate until a stable baseline was obtained. Equal injection volumes of 20 µL of blank, standard, and test preparations were introduced separately into the chromatographic system, and the corresponding chromatograms were recorded. The absence of interfering peaks at the retention times of the analytes in the blank chromatogram demonstrated adequate method specificity.

Under the optimized chromatographic conditions, Metformin HCl, Ramipril, Atorvastatin, and Glimepiride were eluted at retention times of approximately 1.5, 2.7, 4.9, and 6.2 min, respectively. All four components were separated within a relatively short chromatographic run, demonstrating the rapid nature of the proposed method.

➢ *System Suitability Results*

The system suitability parameters confirmed the adequacy of the chromatographic system. The theoretical plate counts ranged from 2,248 to 2,498, indicating acceptable column efficiency for all four analytes. Atorvastatin showed the highest plate count of 2,498, whereas Glimepiride exhibited a plate count of 2,248.

The tailing factors were 1.8, 1.4, 1.3, and 1.2 for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride, respectively. These values indicate satisfactory peak symmetry without excessive peak tailing. The slightly higher tailing observed for Metformin HCl may be attributed to its highly polar and basic nature and its comparatively weaker retention on the reversed-phase stationary phase; nevertheless, its chromatographic performance remained suitable for quantitative analysis.

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A critical feature of a simultaneous multicomponent method is adequate resolution between neighboring peaks. The observed resolution values ranged from 2.1 to 2.9, demonstrating clear baseline separation of the analytes. Since all resolution values were above the commonly accepted minimum of approximately 1.5, interference between adjacent peaks was effectively avoided. The highest resolution was observed for Glimepiride, with a value of 2.9, confirming excellent separation from the preceding Atorvastatin peak.

➢ *System Suitability Parameters of the Optimized RP-HPLC Method*

Table 2 System Suitability Parameters of the Optimized RP-HPLC Method

S. No.	Analyte	Retention Time (min)	Peak Area	USP Plate Count	USP Tailing Factor	Resolution (Rs)
1	Metformin HCl	1.5	515,294	2,421	1.8	2.1
2	Ramipril	2.7	365,245	2,356	1.4	2.3
3	Atorvastatin	4.9	674,339	2,498	1.3	2.5
4	Glimepiride	6.2	391,819	2,248		

The elution order reflected differences in physicochemical properties and interactions of the analytes with the reversed-phase stationary phase. Metformin HCl, being highly hydrophilic, showed the shortest retention time of 1.5 min. Ramipril was retained moderately and eluted at 2.7 min, followed by Atorvastatin at 4.9 min. Glimepiride exhibited the longest retention time of 6.2 min, consistent with its greater hydrophobic interaction with the stationary phase. This orderly separation demonstrates that the optimized mobile-phase composition provided an appropriate balance between retention and selectivity for compounds having substantially different chemical characteristics.

➢ *Standard And Test Chromatograms*

The optimized standard chromatogram showed four well-defined, sharp, and adequately separated peaks corresponding to the four drugs. The test chromatogram exhibited peaks at retention times comparable with those obtained from the standard preparation. The close agreement between the retention times of standard and sample solutions supports reliable identification of each drug in the combined dosage form.

No significant additional peaks were observed at the retention positions of the analytes, indicating that formulation excipients did not interfere with the determination. Consequently, the method demonstrated satisfactory selectivity for routine simultaneous analysis of the four active pharmaceutical ingredients.

➢ *Assay Results*

Table 3 Assay Results

Component	Assay (%)
Metformin HCl	98.90
Ramipril	98.00
Atorvastatin	98.15
Glimepiride	98.30

The assay values obtained for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride were 98.90%, 98.00%, 98.15%, and 98.30%, respectively. The results were close to the nominal drug contents and demonstrated satisfactory quantitative recovery from the combined tablet formulation. The narrow range of assay values, from 98.00% to 98.90%, suggests that the analytical procedure was capable of accurately estimating compounds present together despite their substantially different physicochemical properties.

Metformin HCl showed the highest assay value of 98.90%, followed by Glimepiride at 98.30%, Atorvastatin at 98.15%, and Ramipril at 98.00%. No substantial positive or negative bias was evident from the assay results. Thus, the developed chromatographic procedure appears suitable for determination of the labeled drug content in pharmaceutical quality-control applications.

➢ *Overall Discussion*

A major analytical challenge in this combination is the simultaneous chromatographic determination of four drugs exhibiting markedly different polarity and retention characteristics. The optimized RP-HPLC conditions successfully addressed this challenge by producing distinct peaks with retention times distributed from 1.5 to 6.2 min. Importantly, adequate resolution was achieved without an unnecessarily long run time.

The combination of acceptable theoretical plate counts, controlled peak tailing, resolution values greater than 2.0, reproducible retention behavior, and assay values close to 100% collectively demonstrates satisfactory chromatographic performance. The method therefore provides a rapid, selective, efficient, and practical approach for simultaneous estimation of Metformin hydrochloride, Ramipril, Atorvastatin, and Glimepiride in combined pharmaceutical dosage forms.

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➢ *Method Validation:*

- *Linearity Graphs:*

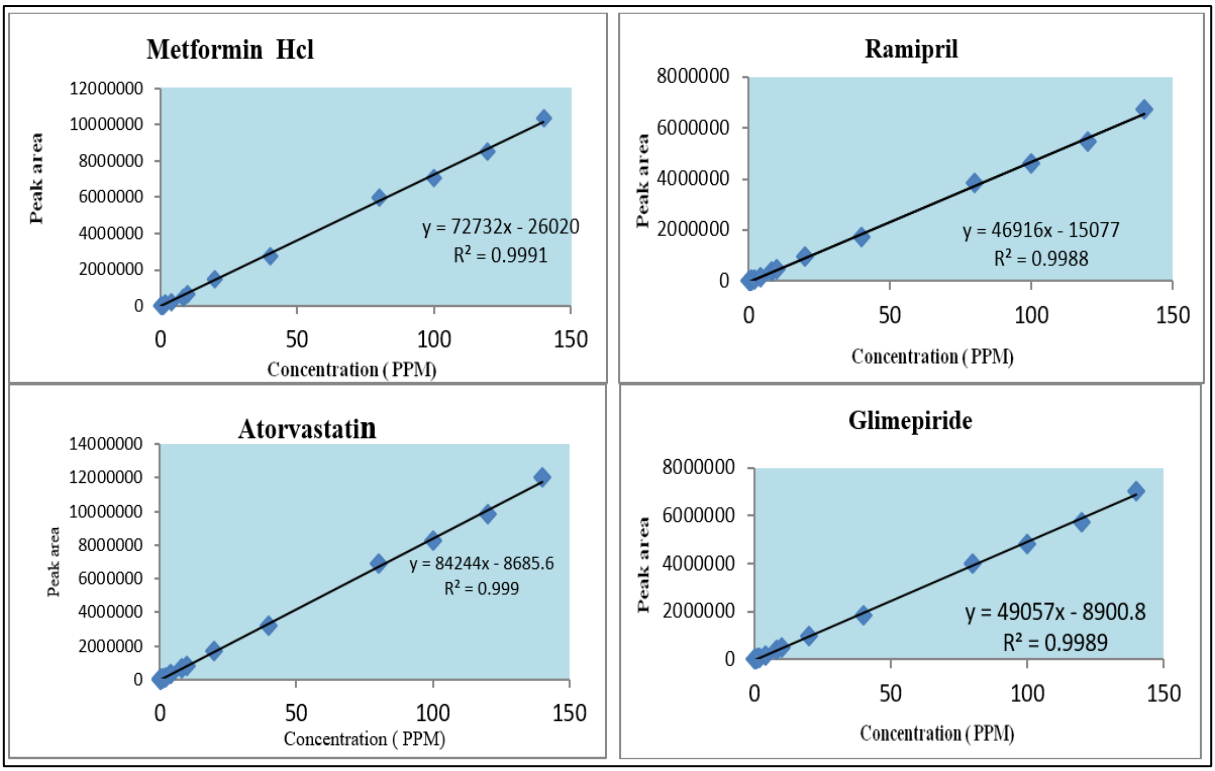

Fig 2 Linearity Graphs

- *Overlay of Linearity Chromatograms*

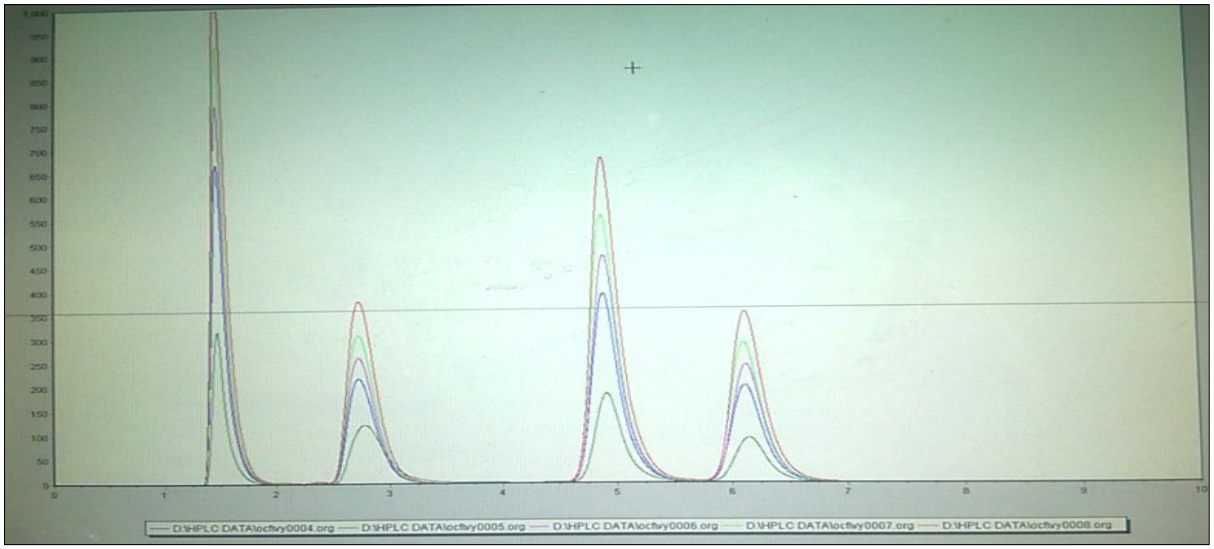

Fig 3 Overlay of Linearity Chromatograms

- *Assay:*

Table 4 Assay

Component	Assay
Metformin Hcl	98.9%
Ramipril	98%
Atorvastatin	98.15%
Glimepiride	98.3%

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III. RESULTS AND DISCUSSION — PRECISION (REPEATABILITY)

The repeatability of the proposed RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride was evaluated by analyzing six replicate preparations under identical chromatographic conditions. Precision was assessed from the peak-area responses and expressed as the percentage relative standard deviation (%RSD).

➢ *Precission : Repeatability:*

Table 5 Repeatability/Method Precision Results

S. No	Metformin Hcl	Ramipril	Atorvastatin	Glimepiride
1	6985007	47658	204330	32589
2	6915793	47549	207745	32320
3	3846438	48186	204078	32823
4	6849093	48362	206850	32638
5	6822128	47460	204848	33260
6	6822128	48460	204841	33262
Average :	6873431	47945.83	205448.7	32815.33
S D:	64542.54	440.8176	1489.771	380.8746
% RSD	0.939	0.919	0.725	0.160

➢ *Discussion*

The precision results demonstrate that the developed RP-HPLC method provides highly reproducible chromatographic responses for the simultaneous determination of all four analytes. The reported %RSD values were 0.939% for Metformin HCl, 0.919% for Ramipril, 0.725% for Atorvastatin, and 0.160% for Glimepiride. All values are below the commonly applied analytical-method precision acceptance criterion of 2.0%, indicating minimal variation among replicate measurements.

Among the four analytes, Glimepiride exhibited the lowest %RSD (0.160%), indicating particularly consistent detector response, while Metformin HCl showed the highest reported %RSD (0.939%), which nevertheless remains well within the acceptance limit. Overall, the low variability in peak-area measurements supports the repeatability, reliability, and precision of the proposed RP-HPLC method for routine simultaneous quantitative analysis.

➢ *Conclusion of Precision Study*

The repeatability study indicates that the proposed method satisfies the precision requirement, with the reported %RSD values for all four drugs being <2.0%. Therefore, the method can be considered precise and suitable for simultaneous estimation of Metformin HCl, Ramipril, Atorvastatin, and Glimepiride in the intended pharmaceutical analysis.

Important data check: The Metformin HCl value for injection/replicate No. 3 (3,846,438) is inconsistent with the reported mean of 6,873,431 and %RSD of 0.939%. It is likely a transcription error (possibly 6,846,438). This value should be verified from the original chromatogram or instrument report before manuscript submission; otherwise, the reported Metformin precision statistics will not mathematically agree with the raw data.

➢ *Reproducibility:*

The reproducibility of the developed RP-HPLC method was evaluated by Analyst 1 using six replicate determinations of the standard/sample preparation containing Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride under the optimized chromatographic conditions. Reproducibility was assessed based on the consistency of chromatographic peak-area responses, and the results were expressed as mean, standard deviation (SD), and percentage relative standard deviation (%RSD).

➢ *Analyst 1:*

Table 6 Analyst 1

S. NO	Metformin HCL	Ramipril	Atorvastatin	Glimepiride
1	6985007	47658	204330	32589
2	6915793	47549	207745	32320
3	6846438	48186	204078	32823
4	6849093	48362	206850	32638
5	6822128	47460	204848	33260
6	6822128	48460	204841	33262
Average :	6873431	47945.83	205448.7	32815.33
S D:	64542.54	440.8176	1489.771	380.8746
% RSD	0.939	0.919	0.725	0.160

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➢ *Results and Discussion*

The reproducibility study demonstrated satisfactory consistency in the chromatographic responses obtained by Analyst 1. The reported mean peak areas were 6,873,431 for Metformin HCl, 47,945.83 for Ramipril, 205,448.7 for Atorvastatin, and 32,815.33 for Glimepiride. The corresponding reported %RSD values were 0.939%, 0.919%, 0.725%, and 0.160%, respectively.

The low %RSD values indicate limited variability among the six replicate determinations and demonstrate satisfactory precision of the analytical procedure. Metformin HCl and Ramipril showed %RSD values below 1%, while Atorvastatin also exhibited excellent reproducibility with a %RSD of 0.725%. Glimepiride showed the lowest reported variation, further suggesting consistent chromatographic response.

Overall, the results indicate that the proposed RP-HPLC method provides consistent and reproducible analytical performance for the simultaneous determination of the four drugs. The observed precision supports the suitability of the method for routine quality-control analysis of combined pharmaceutical formulations.

Based on the reported results, the %RSD values for all four analytes were below 2.0%, indicating satisfactory reproducibility for Analyst 1. The developed method can therefore be considered precise, reliable, and reproducible for the simultaneous quantitative determination of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride.

➢ *Analyst 2*

Intermediate precision of the developed RP-HPLC method was evaluated by performing replicate analyses on two different days to determine the influence of normal within-laboratory variations on the simultaneous estimation of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride. Six replicate measurements were performed under the specified chromatographic conditions, and precision was evaluated in terms of mean peak area, standard deviation (SD), and percentage relative standard deviation (%RSD).

On Day 1, the mean peak-area responses obtained for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride were 2,745,291, 1,753,850, 3,172,969, and 1,834,431, respectively. The corresponding %RSD values were 1.780%, 0.846%, 0.855%, and 0.968%, respectively. The low variability among replicate measurements demonstrates satisfactory precision of the analytical procedure under the Day 1 experimental conditions.

For the second-day analysis, the mean peak areas were 2,790,816 for Metformin HCl, 1,762,297 for Ramipril, 3,192,532 for Atorvastatin, and 1,839,672 for Glimepiride. The respective %RSD values were 1.984%, 1.622%, 1.677%, and 1.537%. Although slightly greater variability was observed on the second day compared with Day 1, all reported %RSD values remained below 2.0%, demonstrating satisfactory day-to-day precision.

The comparison between the two analytical days showed that the chromatographic responses remained reasonably consistent despite routine variations associated with different days of analysis. Metformin HCl exhibited the highest reported variability, with %RSD values of 1.780% and 1.984%, whereas Ramipril, Atorvastatin, and Glimepiride also remained within the predefined precision criterion. These findings indicate that minor variations in routine laboratory conditions did not substantially affect the performance of the analytical method.

Therefore, the intermediate-precision results support the reliability, reproducibility, and robustness of the developed RP-HPLC procedure for simultaneous quantitative determination of the four analytes. The method may consequently be considered suitable for routine quality-control analysis of combined pharmaceutical dosage forms, subject to completion of the other validation characteristics.

- *Intermediate Precission:*

Table 7 Day 1

S. No	Metformin HCL	Ramipril	Atorvastatin	Glimepiride
1	2723096	1730577	3139015	18104228
2	2758354	1759039	3180177	1834867
3	2687848	1744492	3139012	1815776
4	2816267	1751636	3185150	1843352
5	2704745	1768077	3195850	1847861
6	2781435	1769277	3198608	1854304
Average: SD: % RSD	2745291 48889.5 1.780	1753850 14842.05 0.846	3172969 2715578 0.855	1834431 17772.05 0.968

Table 8 Data 2

S.no	Metformin HCL	Ramipril	Atorvastatin	Glimepiride
1	2711289	1704716	3091450	1790121
2	2745940	1778248	3202057	1841138
3	2807918	1775902	3199069	1839936
4	2795728	1779231	3217912	1866545

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5	2815117	1767841	3194365	1832784
6	2868906	1815338	3250336	1867510
Average : S D: % RSD	2790816 55396.01 1.984	1762297 28647.28 1.622	3192532 53553.4 1.677	1839672 28291.61 1.537

- *Accuracy (% Recovery):*

The accuracy of the developed RP-HPLC method for the simultaneous determination of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride was evaluated by recovery studies using the standard-addition approach at 50%, 100%, and 150% spiking levels. The percentage recovery obtained at each concentration level was used to assess the closeness of the experimentally determined amount to the amount added to the sample matrix.

For Metformin HCl, recoveries of 98.66%, 98.00%, and 99.40% were obtained at the 50%, 100%, and 150% levels, respectively. The overall mean recovery was 98.68%, with an SD of 0.700 and %RSD of 0.709%. The low variability across the three concentration levels indicates satisfactory recovery of Metformin HCl from the formulation matrix.

Ramipril showed recoveries of 98.38%, 100.00%, and 100.97% at the respective accuracy levels. The reported mean recovery was 99.92%, with an SD of 1.087 and %RSD of 1.087%. These results demonstrate that the method can accurately quantify Ramipril despite its comparatively low concentration in the combined formulation.

For Atorvastatin, percentage recoveries were 99.60%, 99.50%, and 100.35% at 50%, 100%, and 150% levels, respectively. The mean recovery was 99.81%, while the SD and %RSD were 0.464 and 0.465%, respectively. The close agreement of the recovery values with 100%, together with the very low %RSD, demonstrates excellent accuracy and consistency for Atorvastatin determination.

Similarly, Glimepiride exhibited recoveries of 99.00%, 98.70%, and 100.31%, with a reported overall mean recovery of 99.33%. The SD and %RSD were 0.856 and 0.851%, respectively. These results indicate satisfactory extraction and quantification of Glimepiride without substantial interference from the other active ingredients or formulation excipients.

Overall, the recovery values for all four analytes were approximately 98–101%, while the reported %RSD values were below 2%. The consistency of recovery across the 50–150% concentration range demonstrates that the analytical response is not substantially affected by changes in analyte concentration or by the formulation matrix. The results therefore support the accuracy and reliability of the proposed RP-HPLC method for simultaneous quantitative analysis of Metformin HCl, Ramipril, Atorvastatin, and Glimepiride in the studied combined dosage form.

Table 9 Accuracy (% Recovery)

Component	**At 50% level**	**at 100%**	**At 150 % level**	**%Recovary**	
Metformin Hcl	98.66%	98%	99.4%	Average: SDV: % RSD:	98.68% 0.700 0.709
Ramipril	98.38%	100%	100.97%	Average: SDV: % RSD:	99.92% 1.087 1.087
Atorvastatin	99.6%	99.5%	100.35%	Average: SDV: % RSD:	99.81% 0.464 0.465
Glimepiride	99%	98.7%	100.31%	Average: SDV: % RSD:	99.33% 0.856 0.851

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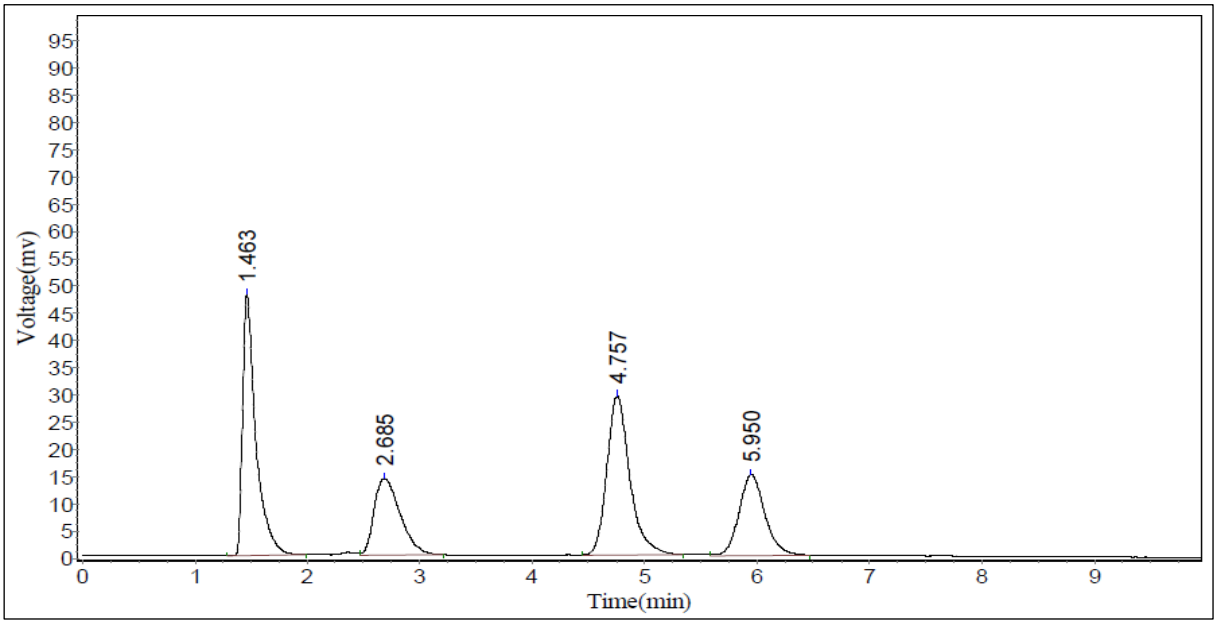


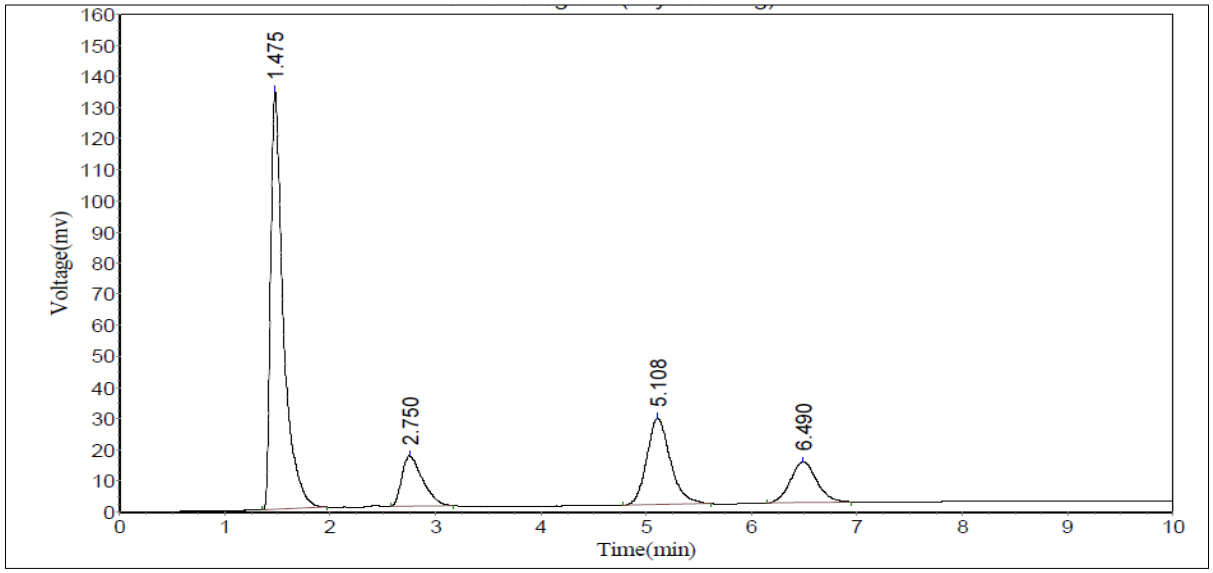
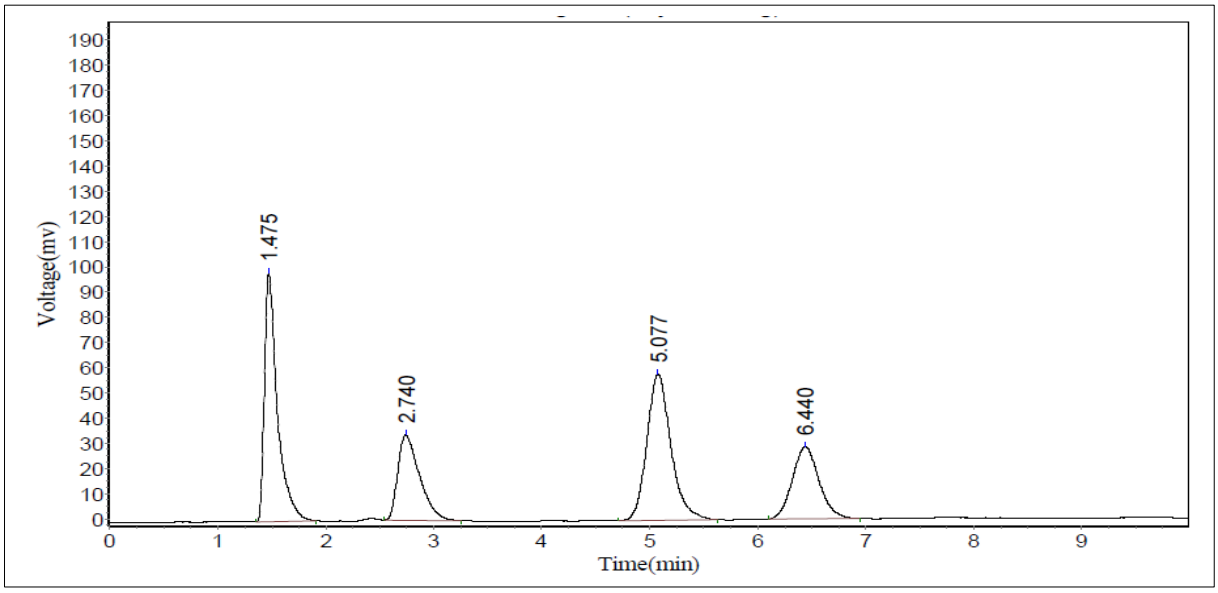


Fig 4 Standard Chromatogram (5 $\mu g/ml$) Spicked Chromatogra 100% Level



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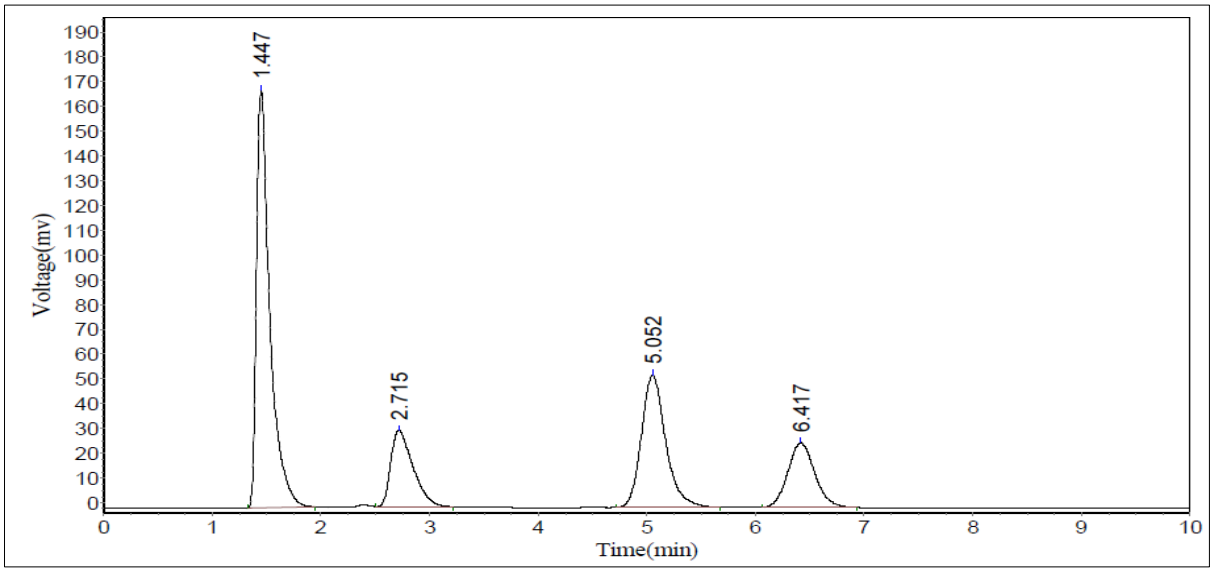
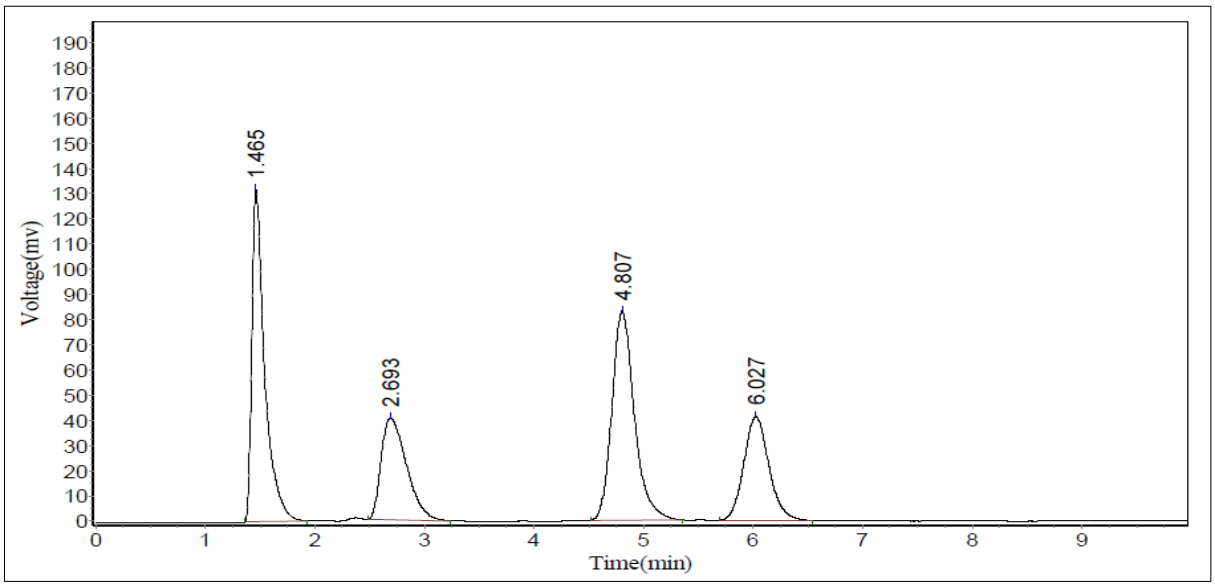


Fig 5 Standard Chromatogram (1 0 $\mu g/ml$) Spiked Chromatogram 150% Level



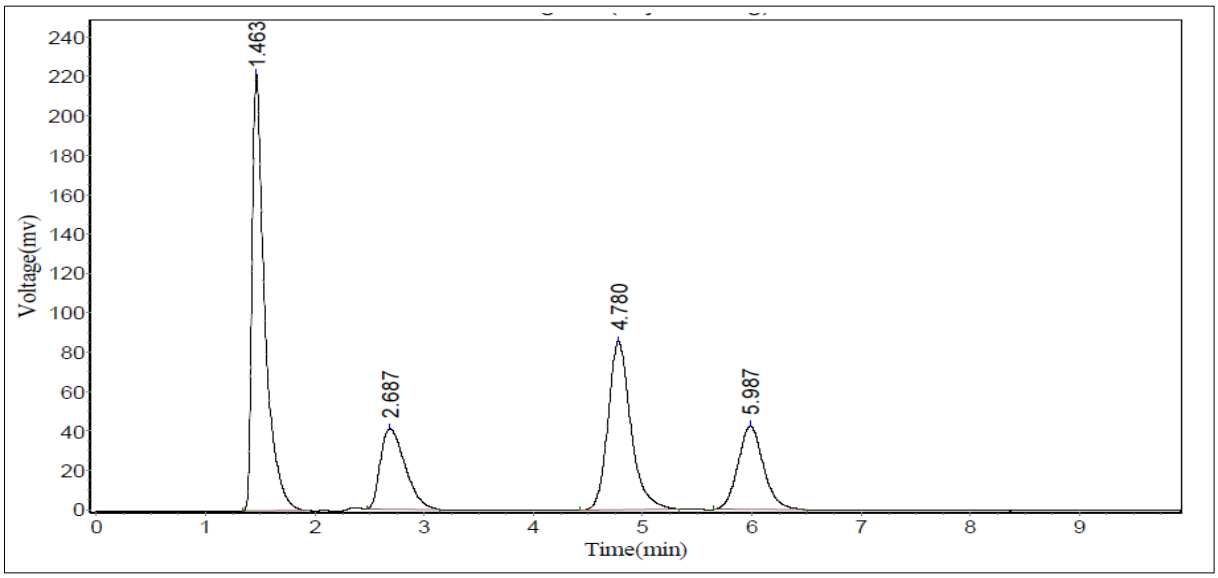


Fig 6 Standard Chromatogram (15 $\mu g/ml$) Spiked Chromatogram

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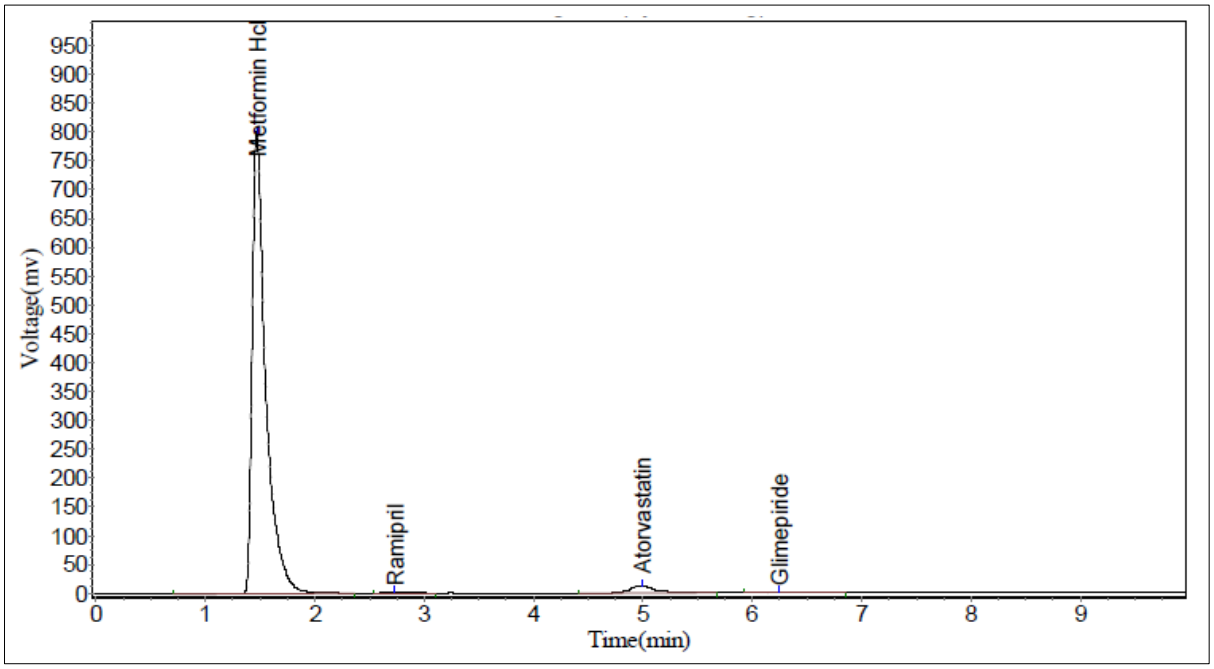

Fig 7 Test Chermomatpgram

➢ *Robustness:*

The robustness of the developed RP-HPLC method for the simultaneous estimation of Metformin HCl, Ramipril, Atorvastatin, and Glimepiride was evaluated by introducing small deliberate variations in critical chromatographic parameters, including flow rate, detection wavelength, and mobile-phase composition. The effect of these changes on chromatographic response was assessed primarily from the peak-area variability and percentage relative standard deviation (%RSD).

- *Effect of Flow-Rate Variation*

The influence of flow rate was investigated at 0.8, 1.0, and 1.2 mL/min, with 1.0 mL/min representing the nominal chromatographic condition. At 0.8 mL/min, the %RSD values for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride were 0.330%, 0.581%, 1.246%, and 0.504%, respectively. At the nominal flow rate of 1.0 mL/min, the corresponding reported %RSD values were 0.800%, 0.408%, and 1.653% for Metformin HCl, Ramipril, and Atorvastatin, respectively. For Glimepiride, the supplied mean and SD values were 968,316.2 and 22,502.75; these correspond to a calculated %RSD of approximately 2.32%, so this entry should be verified against the original data before publication.

At 1.2 mL/min, %RSD values of 0.616%, 0.462%, 1.616%, and 1.353% were obtained for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride, respectively. Overall, the data indicate that modest changes in flow rate did not produce excessive variability for most analytes. The observed changes in absolute peak areas with flow rate are expected because alteration of mobile-phase velocity can influence analyte residence time and detector response.

- *Effect of Detection Wavelength*

The robustness of the detection system was also examined by making small changes around the optimized wavelength of 210 nm. At 210 nm, the %RSD values were 0.853%, 1.202%, 0.385%, and 0.835% for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride, respectively. Under the altered wavelength condition reported as “2012 nm”, the corresponding %RSD values were 1.241%, 0.904%, 0.899%, and 1.073%.

These relatively low %RSD values indicate that small changes in detection wavelength did not substantially compromise the repeatability of detector response. This finding supports the robustness of the method with respect to minor wavelength fluctuations.

Important correction: “2012 nm” appears to be a typographical error and is most likely intended to be 212 nm. In addition, although 208 nm is listed in the table, no corresponding experimental results were supplied. These points should be corrected/completed before manuscript submission.

- *Effect of Mobile-Phase Composition*

Robustness was further assessed by deliberately modifying the mobile-phase composition. Under the altered composition reported as 77:26, the %RSD values for Metformin HCl, Ramipril, Atorvastatin, and Glimepiride were 1.106%, 1.39%, 0.625%, and 1.654%, respectively. The relatively low variability indicates that a minor alteration in mobile-phase composition did not markedly affect the reproducibility of the chromatographic response.

However, 77:26 totals 103%, so this mobile-phase ratio is internally inconsistent. If your optimized mobile phase is methanol:0.03 M KH_2PO_4 buffer (74:26, v/v), the robustness levels should normally represent deliberate changes around this composition and each ratio should total 100%. The original laboratory records should therefore be checked before reporting the final ratio.

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- *Overall Discussion*

The robustness study demonstrates that the developed RP-HPLC procedure generally maintained consistent analytical responses following deliberate variations in flow rate, detection wavelength, and mobile-phase composition. Most reported %RSD values were low, indicating satisfactory repeatability under slightly modified experimental conditions. These findings suggest that the simultaneous determination of Metformin HCl, Ramipril, Atorvastatin, and Glimepiride is not highly sensitive to minor variations in the selected chromatographic parameters.

Consequently, the method shows promising robustness and suitability for routine quality-control analysis. Nevertheless, the Glimepiride result at 1.0 mL/min, the "2012 nm" wavelength, the missing 208-nm results, and the 77:26 mobile-phase ratio should be verified and corrected before the robustness data are used in a final manuscript or validation report.

Table 10 Overall Discussion

S.no			Metformin HCL	Ramipril	Atorvastatin	Glimepiride
			Peak area			
Flow rate (ml / min)	0.8 ml/min.	Average: SDV: %RSD:	2049515 6767 0.330	1238963 7205.85 0.581	2224445 22728.56 1.246	1268794 6394.192 0.503958
	1.0 ml/ min.	Average: SDV: %RSD:	1535951 12300.51 0.800	9857258 40250.2 0.408	1740454 28785.23 1.653	968316.2 22502.75
	1.2ml / min.	Average: SDV: %RSD:	1307560 8060.132 0.616	814690.8 3764.819 0.462	1453031 23488.22 1.616	851363 11522.36 1.353
	Peak area					
Wavelength (nm)	208nm	Average: SDV: %RSD:				
	210nm	Average: SDV: %RSD:	7118581 60763.23 0.853	4274978 51407.31 1.202	8077624 31129.03 0.385	4743436 39626.65 0.835
	2012nm	Average: SDV: %RSD:	7365789 91743.04 1.241	4253486 38465.49 0.904	7216117 64897.61 0.899	4577831 48833.28 1.073

Moble phase composition (77:26)		Metformin Hcl	Ramipril	Atorvastatin	Glimepiride
	Average: SDV: %RSD:	913263.7 10102..4 1.106	549950.2 7673.303 1.39	1019727 6373.49 0.625	568846.8 9408.885 1.654

- *Robustness Chromatograms :*
- *Flow Rate Change*

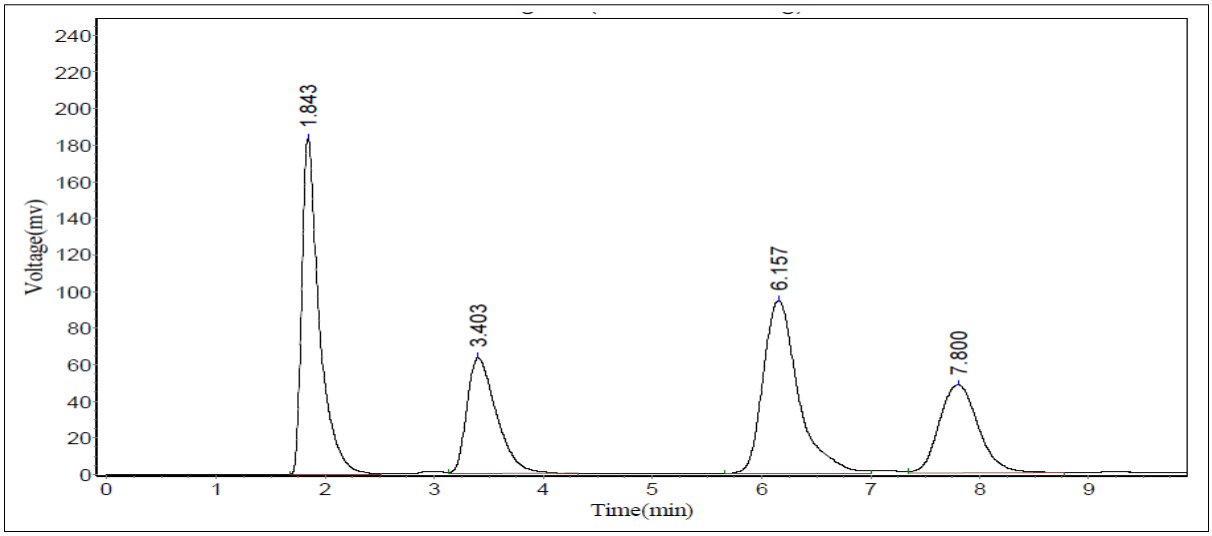

Fig 8 Flow Rate 0.8 ml/minute

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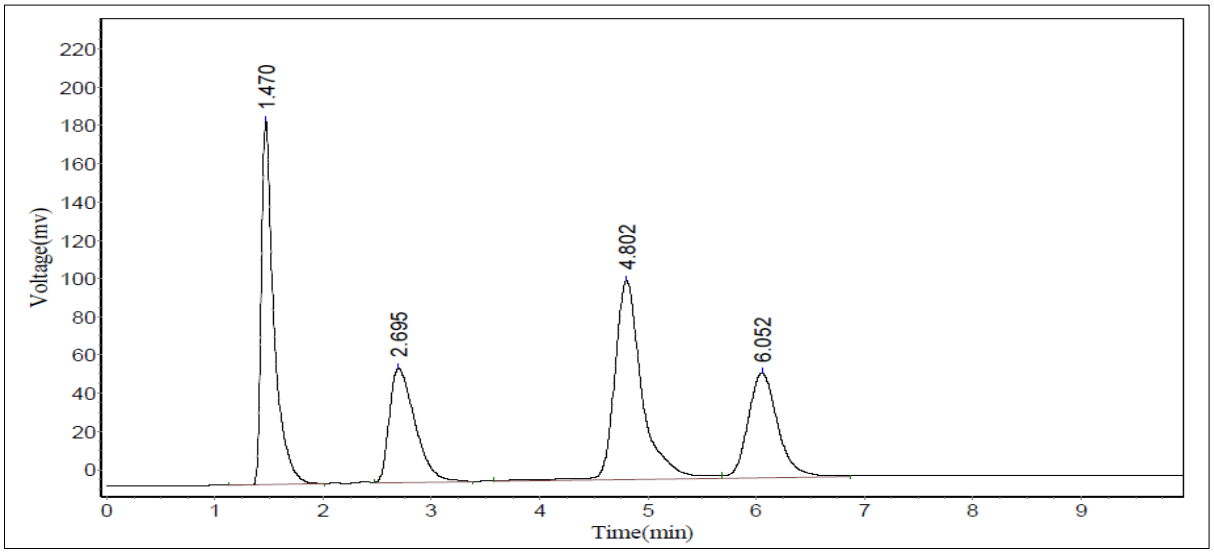
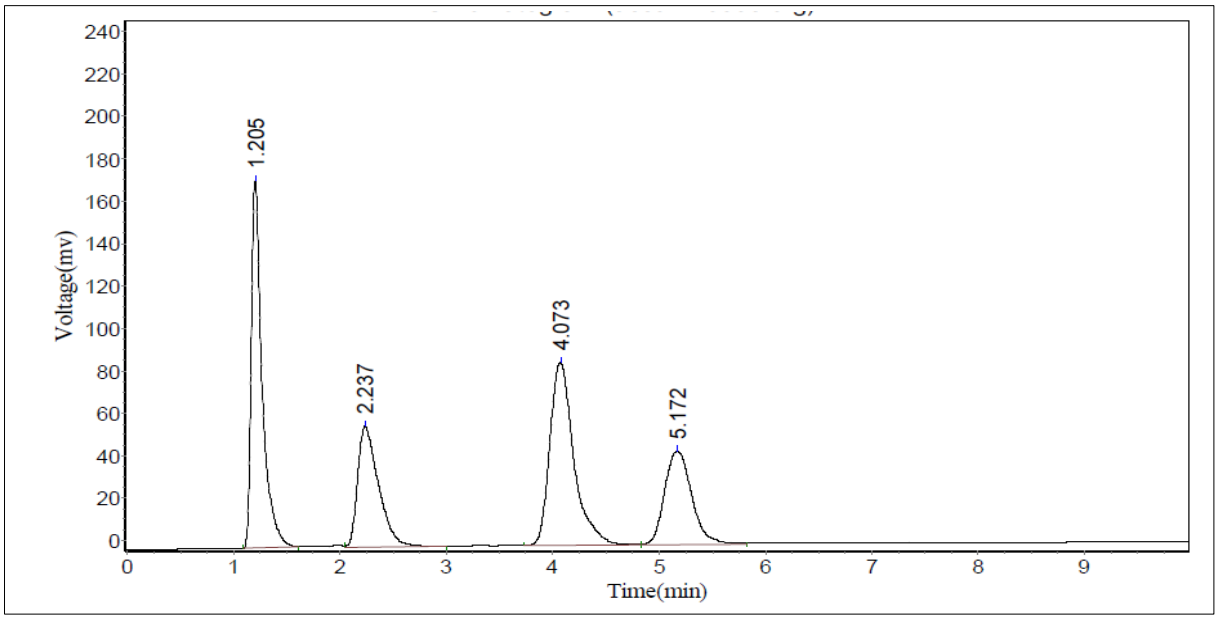


Fig 9 Flow Rate 1.0 ml/minute



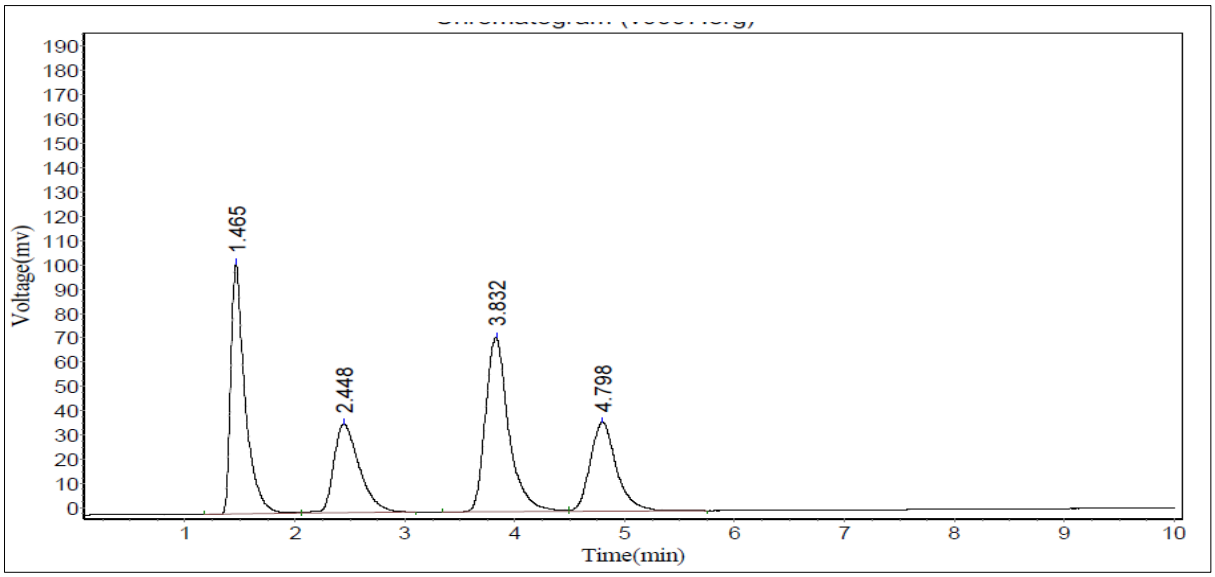


Fig 10 Flow Rate 1.2 ml/minute

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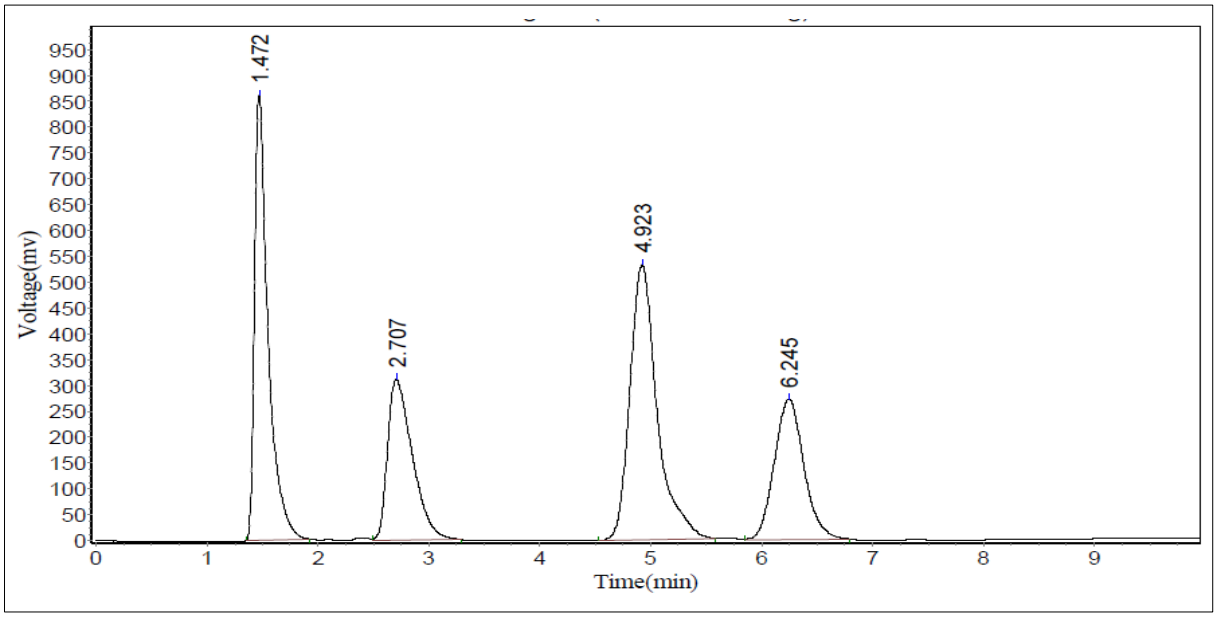

Fig 11 Chromatogram at 210 nm

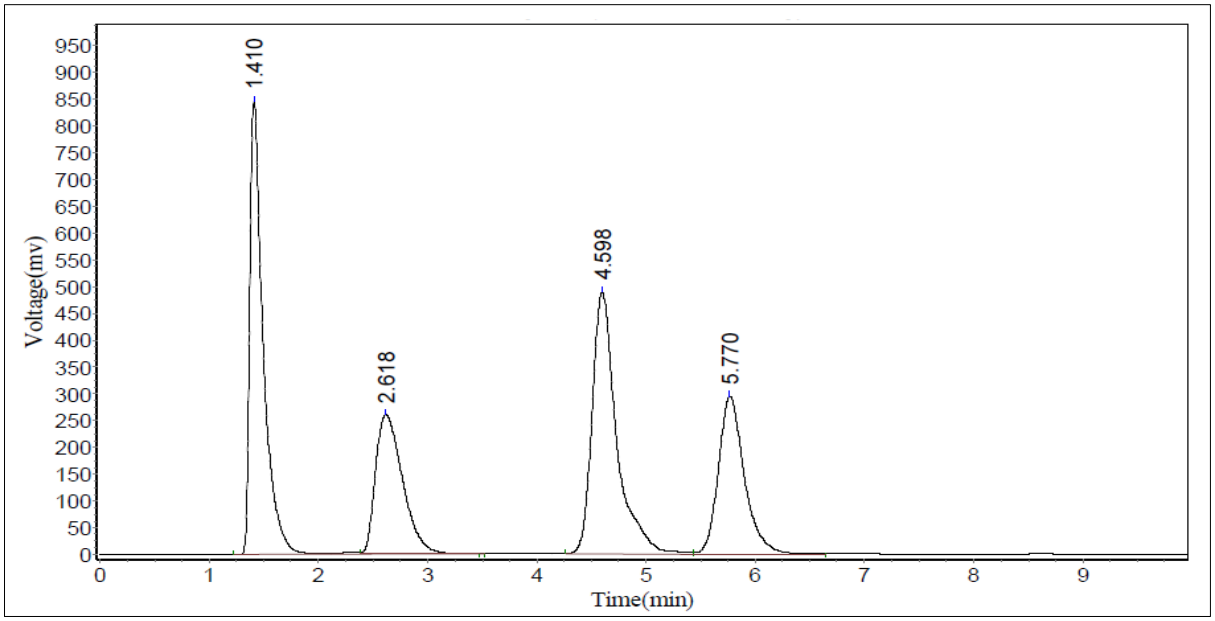

Fig 12 Chromatogram at 208 nm

IV. CONCLUSION

The present study successfully developed a simple, rapid, precise, accurate, and stability-indicating RP-HPLC method for the simultaneous determination of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride in bulk drugs and combined tablet dosage forms. Effective chromatographic separation of all four analytes was achieved using a GRACE ODS column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of methanol and 0.03 M KH_2PO_4 buffer (74:26, v/v) at a flow rate of 1.0 mL/min, with detection at 210 nm.

The developed method demonstrated satisfactory system suitability, linearity, accuracy, precision, specificity, robustness, and sensitivity, supporting its suitability for simultaneous quantitative analysis of this multicomponent formulation. The low percentage relative standard deviation (%RSD) values observed during validation indicate good repeatability and reproducibility of the analytical procedure.

Importantly, the method was capable of distinguishing the four active pharmaceutical ingredients from degradation products generated under the applied forced-degradation conditions, demonstrating its stability-indicating capability. This feature makes the method particularly valuable for evaluating drug stability and monitoring product quality during formulation development, manufacturing, and storage.

The simultaneous determination of four therapeutically distinct drugs within a single chromatographic run reduces analytical time, solvent consumption, sample handling, and overall laboratory workload compared with separate analytical procedures. Therefore, the validated RP-HPLC

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method represents a practical, economical, and reliable analytical approach that can be routinely applied for quality-control testing, assay determination, and stability studies of Metformin Hydrochloride, Ramipril, Atorvastatin, and Glimepiride in combined pharmaceutical dosage forms.

REFERENCES

[1]. Ramesh D, Habibuddin M. Stability indicating RP-HPLC method for the simultaneous determination of atorvastatin calcium, metformin hydrochloride, and glimepiride in bulk and combined tablet dosage form. Int Sch Res Notices. 2014;2014:754695. doi:10.1155/2014/754695.

[2]. Polagani SR, Pilli NR, Gajula R, Gandu V. Simultaneous determination of atorvastatin, metformin and glimepiride in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. J Pharm Anal. 2013;3(1):9–19. doi:10.1016/j.jpha.2012.09.002.

[3]. Joseph L, George M, Rao BVR. Simultaneous estimation of atorvastatin and ramipril by RP-HPLC and spectroscopy. Pak J Pharm Sci. 2008;21(3):282–284.

[4]. Panchal HJ, Suhagia BN. Simultaneous determination of atorvastatin calcium and ramipril in capsule dosage forms by high-performance liquid chromatography and high-performance thin layer chromatography. J AOAC Int. 2010;93(5):1450–1457.

[5]. Elzanfaly ES, Abdel-Gawad SA. Simultaneous quantification of ramipril, glimepiride and metformin in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry. J Appl Pharm Sci. 2017;7(7):62–69. doi:10.7324/JAPS.2017.70711.

[6]. Mubeen G, Noor K. Spectrophotometric method for analysis of metformin hydrochloride. Indian J Pharm Sci. 2009;71(1):100–102.

[7]. AbuRuz S, Millership J, McElnay J. Determination of metformin in plasma using a new ion pair solid phase extraction technique and ion pair liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2003;798(2):203–209.

[8]. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Rapid determination of metformin in human plasma using ion-pair HPLC. J Pharm Biomed Anal. 2003;31(1):197–200.

[9]. Lakshmi KS, Rajesh T. Development and validation of RP-HPLC method for simultaneous determination of metformin and pioglitazone in pharmaceutical dosage form. Int J Pharm Pharm Sci. 2009;1(2):162–166.

[10]. Sahoo PK, Sharma R, Chaturvedi SC. Simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by RP-HPLC method from combined tablet dosage form. Indian J Pharm Sci. 2008;70(3):383–386.

[11]. Bonfilio R, Giannini C, Okamoto RT, Araújo MB. Development and validation of a stability-indicating liquid chromatographic method for the determination of glimepiride in tablets. J AOAC Int. 2011;94(3):862–869.

[12]. Khan IU, Aslam F, Ashfaq M, Asghar MN. Determination of glimepiride in pharmaceutical formulations using HPLC and first-derivative spectrophotometric methods. J Anal Chem. 2009;64:171–175.

[13]. Musmade P, Subramanian G, Srinivasan KK. High-performance liquid chromatography and pharmacokinetic study of glimepiride in rats. Indian J Pharm Sci. 2008;70(2):253–258.

[14]. Yunoos M, Sankar DG, Kumar BP, Hameed S. HPLC method for simultaneous estimation of glimepiride and metformin in pharmaceutical dosage forms. Asian J Chem. 2009;21:1377–1382.

[15]. Lad NR, Bhoir SI, Bhoir IC, Sundaresan M. Concurrent assay of metformin and glimepiride in tablets using RP-HPLC with wavelength programming. Indian J Pharm Sci. 2003;65:650–653.

[16]. Jain N, Raghuwanshi R, Jain D. Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and glimepiride in tablet dosage form. Indian J Pharm Sci. 2008;70(2):263–265.

[17]. Patil SS, Bonde CG. Development and validation of analytical method for simultaneous estimation of glibenclamide and metformin HCl in bulk and tablets using UV-visible spectroscopy. Int J ChemTech Res. 2009;1(4):905–909.

[18]. Stanisz B. The stability of quinapril hydrochloride—a mixture of amorphous and crystalline forms—in solid phase. Acta Pol Pharm. 2003;60(6):443–450.

[19]. Kurbanoglu S, Esim O, Ozkan SA. Stability profiling and analytical approaches for cardiovascular pharmaceutical compounds. Crit Rev Anal Chem. 2018;48:1–18.

[20]. Alhasani KF, Mohsin K, Shakeel F, Alanazi FK. Development and validation of stability-indicating ultra high-performance liquid chromatography for ramipril analysis in pharmaceutical dosage forms and its application in lipid-based formulations. Orient J Chem. 2018;34(1). doi:10.13005/ojc/340107.

[21]. Pawar PY, Joshi RS, Sandhan VS, Wagh S. Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of losartan potassium and ramipril in tablets. Indian J Pharm Sci. 2012;74(4):378–383. doi:10.4103/0250-474X.106061.

[22]. Manju Latha YB, Gowri Sankar D. Stability indicating RP-HPLC method for determination of ramipril in pure and pharmaceutical formulation. Asian J Pharm Clin Res. 2013.

[23]. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Guideline Q2(R2): Validation of Analytical Procedures. Geneva: ICH; 2023. This should be your principal reference for validation characteristics.

[24]. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Guideline Q14: Analytical Procedure Development. Geneva: ICH; 2023. The guideline covers science- and risk-based analytical

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug1079

procedure development, robustness, parameter evaluation and analytical procedure control strategy.
[25]. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
[26]. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline Q1B: Photostability Testing of New Drug Substances and Products. Geneva: ICH; 1996.
[27]. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—a review. J Pharm Anal. 2014;4(3):159–165. doi:10.1016/j.jpha.2013.09.003.
[28]. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. J Pharm Biomed Anal. 2002;28(6):1011–1040. doi:10.1016/S0731-7085(02)00047-X.
[29]. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken, NJ: John Wiley & Sons; 2010.
[30]. Kazakevich Y, Lobrutto R, editors. HPLC for Pharmaceutical Scientists. Hoboken, NJ: John Wiley & Sons; 2007.