RP-HPLC Method for Simultaneous Estimation of Metformin HCL, Ramipril and Glimepiride in Bulk and Their Combination Tablet Dosage Form.

M. Vishnu Priya[1], Dr.P.Madhavan[2,] Dr.Pramod Kumar[3,] Dr.Raj Kumar[4]

[1, 4]Usha Biotech, Sundari Reddy Bhavan,Vengal Rao Nagar, Hyderabad,India
[2]CPS, Dr.Reddys Laboratories Ltd, Miyapur, Hyderabad India
[3]Retired Professor, CCST, IST JNTU Hyderabad. India

Abstract: *A simple, accurate, precise assay and rapid stability-indicating reversed phase high performance liquid chromatography (RP-HPLC) method has been developed with an effective resolution and subsequently validated for the simultaneous estimation of Metformin HCl, Ramipril and Glimiperide from their combination drug product. The method was developed using Hypersil BDS C18, 250 x 4.6 mm, 5μm , L1 packing, maintained at an ambient temperature.The optimum mobile phase used in this method was a mixture of Methanol and 0.02M KH_2PO_4 buffer in the ratio of 850:150 v/v ,flowrate of mobile phase was set 0.8mL min $^{-1}$. At 210 nm compounds was eluted and monitored. Metformin HCl, Ramipril and Glimepiride are subjected to stress conditions of acid, base, oxidative, thermal and photolytic degradation. The degradation products were well separated from the main peak proving the stability-indicating ability of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines.[ICHQ2(R 1)]*

Keywords: *Metformin HCl, Ramipril, Glimepiride Validation, Degradation, RP HPLC.*

I. Introduction

Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin hydrochloride (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

Ramipril is chemically (2*S*, 3*aS*, 6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid. It is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. It is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes.

Chemically, glimepiride is identified as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea ($C_{24}H_{34}N_4O_5S$) with a molecular weight of 490.62. Glimepiride is a sulfonylurea antidiabetic medicine. It works by causing the pancreas to release insulin, which helps to lower blood sugar.

There is no reported method for these three combinations of drugs and there are very few analytical methods reported for the estimation of Metformin HCl, Ramipril and Glimepiride which includes RP-HPLC. But these methods are sophisticated, expensive and time consuming when compared to the simple HPLC method. There is a need for an interest to develop simple, accurate, specific, sensitive, precise and reproducible simultaneous RP HPLC method for the estimation of Metformin HCl, Ramipril and Glimepiride in bulk and its formulation

II. Experimental

2.1 Materials and reagents

The reference samples of Metformin HCl, Ramipril & Glimepiride were donated from Sohan healthcare private Limited, Pune & Dr.Reddys Laboratories Limited, IDA Bollaram Hyderabad. HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (Diakit-3, 1.0/2.5/500mg dosage) were purchased from the local pharmacy.

2.2 Instrument and Chromatographic Conditions

The HPLC system used for the method development and validation consisted of gradient pumps from Agilent 1260 Technologies, Ultraviolet detector from Agilent Technologies. USA, with auto sampler and auto

injector. The HPLC system was equipped with data acquision and processing software "EZ Chrome software" Agilent Technologies. USA.

The column used for separation of analytes is Hypersil BDS C18, 250 x 4.6 mm, 5μm, with L1 Packing. Mobile phase consisting of 0.02M KH_2PO_4 buffer, and methanol in the ratio of 150:850 at a flow rate of 0.8 ml/min. It was filtered through 0.45μm nylon filter and sonicated for 15 min in ultrasonic bath. Sample analyzed at 210 nm at an injection volume of 20 μL.

2.3 Preparation of 0.02M KH_2PO_4 Buffer

Accurately weighed 2.72g of KH_2PO_4 and adjusted the P^H to 3.00 with dilute Ortho-phosphoric acid solution in a 1000mL of Milli-Q water and sonicated for 15 minutes to degas.

2.4 Preparation of Mobile phase

Taken above 150mL of buffer solution and added 850mL of methanol and degassed.

2.5 Diluent: Mobile phase

2.6 Preparation of Solutions:

2.6.1 Metformin HCl. Ramipril and Glimepiride stock preparation (100μg/mL): Accurately weighed and transferred each 10 mg of Metformin HCl. Ramipril and Glimepiride in to 100ml of the clean dry volumetric flask, add 50mL of diluent (Mobile phase), then sonicated for 10min and made up the volume with diluent.
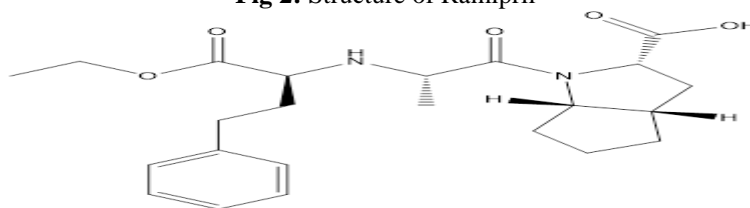
Fig 1: Structure of Metformin HCl
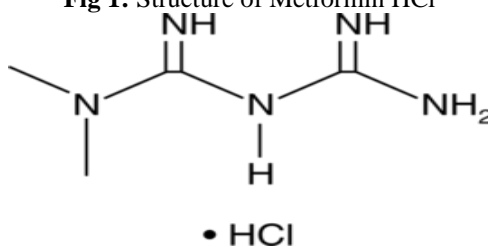
Chemical Name: 1, 1-Dimethylbiguanide mono hydrochloride
Molecular formula: $C_4H_{11}N_5 \bullet HCl$
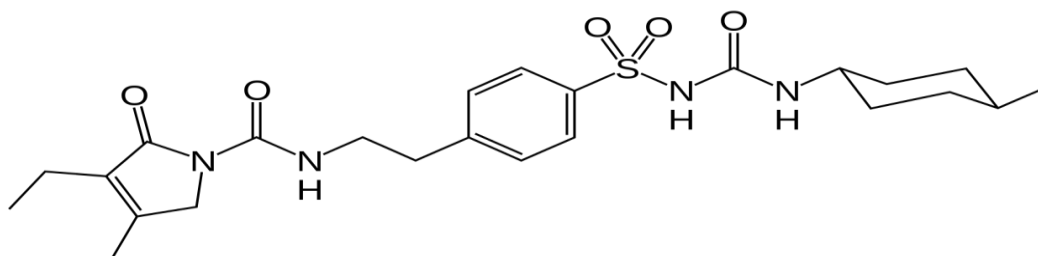Molecular weight: 165.62

Fig 2: Structure of Ramipril

Chemical Name: (2S,3aS,6aS)-1-(((S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl) -L-alanyl) octahydrocyclopenta[b]pyrrole-2-carboxylic acid.
Molecular formula: $C_{23}H_{32}N_2O_5$
Molecular weight: 416.52

Fig 3: Structure of Glimepiride

Chemical Name: 3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4- methylcyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide

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

Molecular weight: 490.617

2.7 METHOD VALIDATION [ICHQ2(R1)]

The validation of the method was carried out as per ICH Guidelines (ICH Q2 R1)**.** The parameters assessed were specificity, linearity, precision, accuracy, stability, Robustness, LOD and LOQ.

2.7.1 Specificity

Specificity is the ability of the analytical method to   measure the analyte response in the presence of Interferences including degradation products. The specificity of the developed HPLC method for the combination of Metformin HCl, Ramipril & Glimepiride was carried out in the presence of its degraded impurities. Stress studies were performed for Metformin HCl, Ramipril & Glimepiride bulk drugs provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), heat (80°C), acid (0.5N HCl), base (0.1N NaOH), and Oxidation (3.0 % H_2O_2) to evaluate the ability of the proposed method to separate Metformin HCl, Ramipril & Glimepiride from its degradation impurities. For all degradation studies, period was 24 hours. Assay and degradation studies were carried out for stress samples against qualified Metformin HCl, Ramipril & Glimepiride reference standard.

2.7.2 Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels 50%,100% and 150 % of test concentration (0.1 mg/mL). The percentages of recoveries for Assay were calculated from the slope and Y- Intercept of the calibration curve.

2.7.3 Precision

The precision of the assay method was evaluated by carrying out six independent assays of Metformin HCl, Ramipril & Glimepiride test samples against a qualified reference standard and calculate the % RSD of assay. The intermediate precision of the Assay method was also evaluated using different analyst and different instrument in the same laboratory.

2.7.4 Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

2.7.5 Robustness

Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

2.7.6 Limit of Detection & Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, LOD=3.3×SD/S and LOQ=10×SD/s, where SD = standard deviation, S= slope of the calibration curve.

### 2.7.7 Solution stability and Mobile phase stability:

The solution stability of Metformin HCl, Ramipril & Glimepiride in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 24 hrs. The same sample solutions were assayed for 6 hrs. interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solution against freshly prepared reference standard solution for 6 hrs interval up to 48 hrs. Mobile phase prepared was kept constant during the study period. The % RSD for the assay of Metformin HCl, Ramipril & Glimepiride was calculated during mobile phase and solution stability experiment.

The solution stability of Metformin HCl, Ramipril & Glimepiride in the assay method was carried out by leaving spiked sample solution in the tightly capped volumetric flasks at room temperature for 24 hrs. Content of Metformin HCl, Ramipril & Glimepiride were checked in the test solutions.
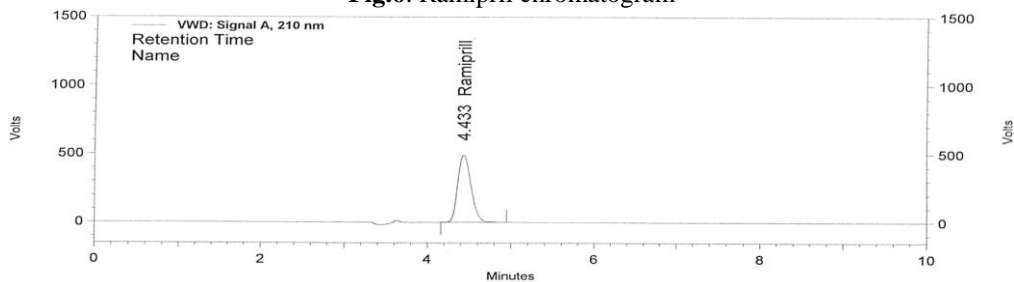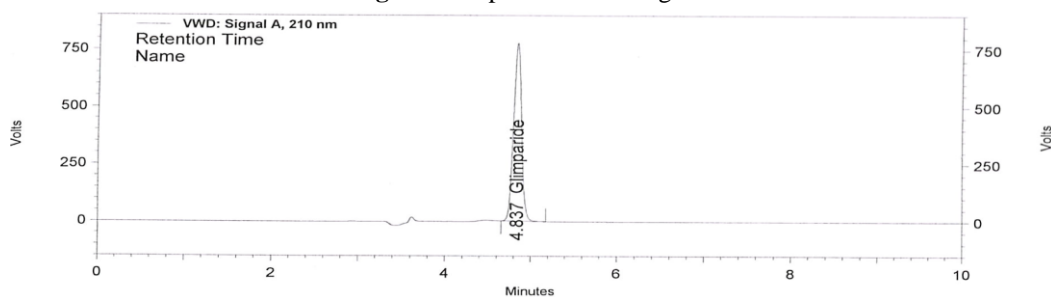
Fig.4. Blank (H_2O_2) chromatogram
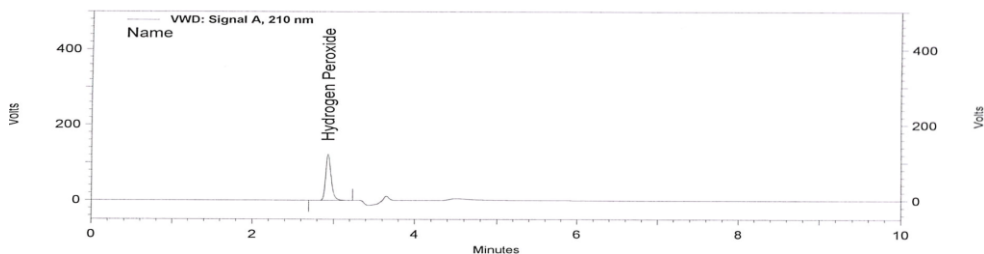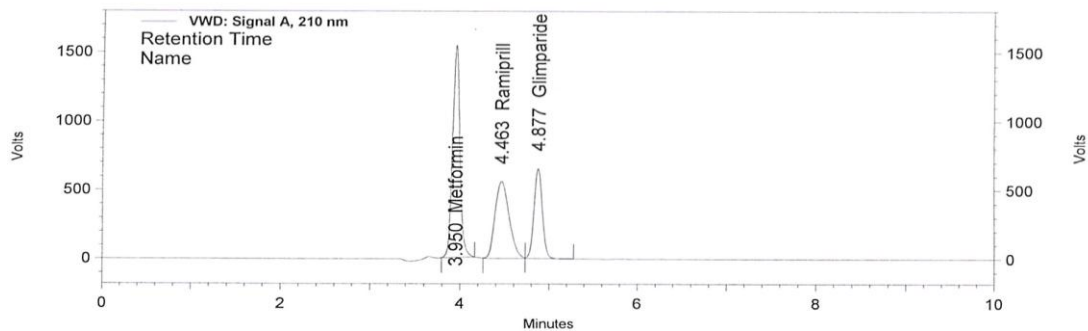

Fig.5. Metformin HCl chromatogram
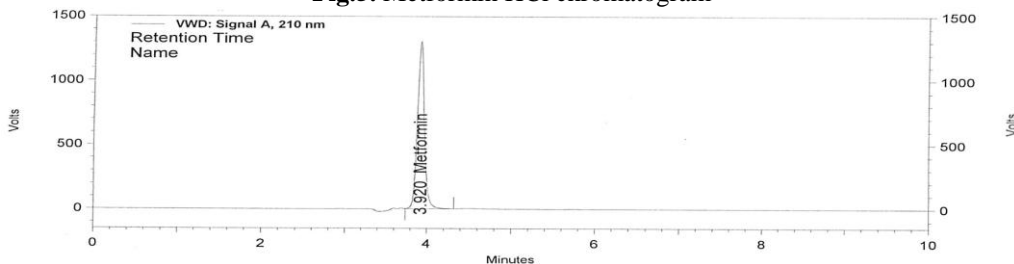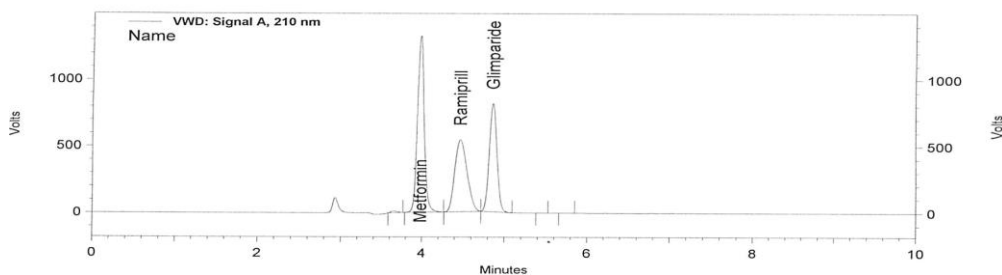

Fig.6. Ramipril chromatogram

Fig.7. Glimepiride chromatogram

Fig.8. Metformin HCl, Ramipril & Glimepiride spiked chromatogram

Fig.9. Peroxide degradation (Metformin HCl, Ramipril & Glimeprie) chromatogram

Fig.10. Thermal degradation (Metformin HCl, Ramipril & Glimeprie) chromatogram

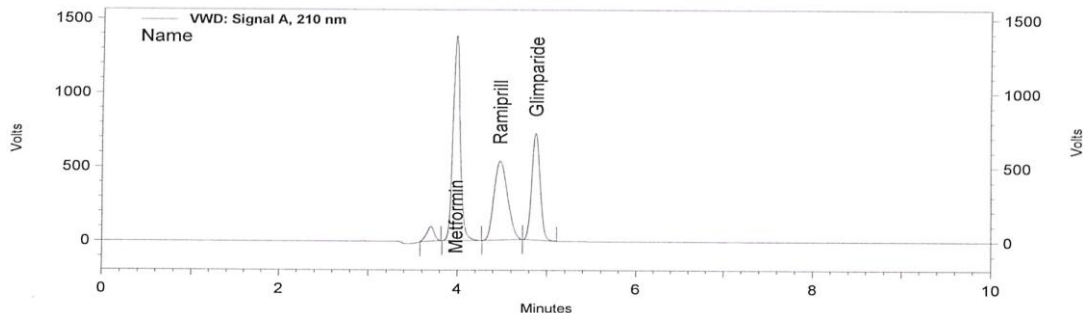**Fig.11**. Photo degradation (Metformin HCl, Ramipril & Glimeprie) chromatogram
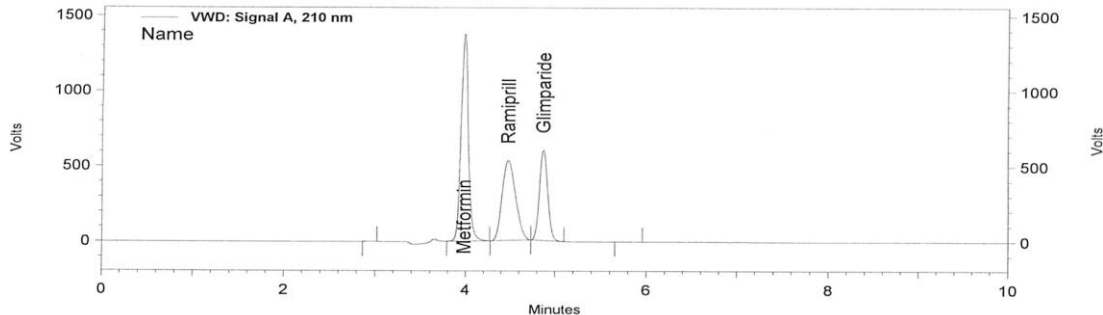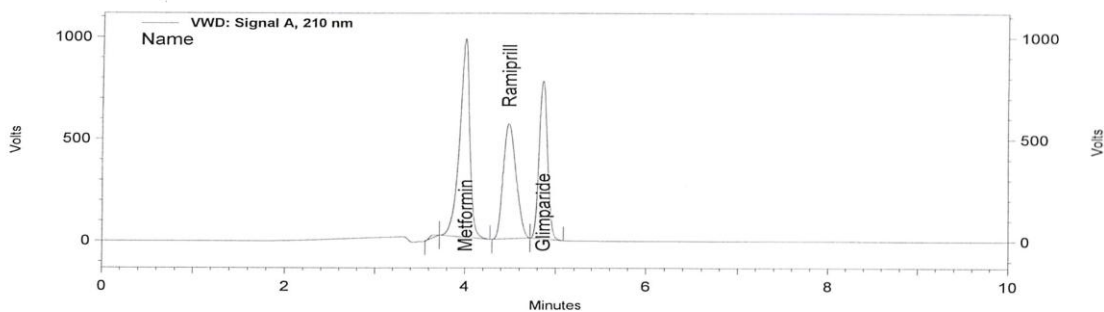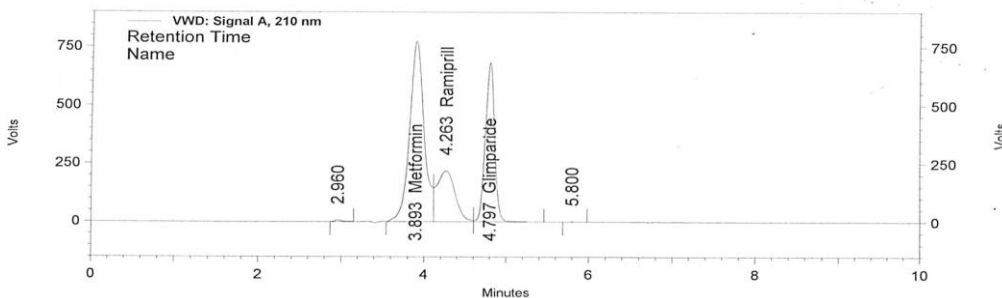

Fig.12. Acid degradation (Metformin HCl, Ramipril & Glimeprie) chromatogram

Fig.13. Base degradation (Metformin HCl, Ramipril & Glimeprie) chromatogram

Table 1: Method development and optimized chromatographic conditions

Parameter	Condition
Mobile phase	0.02M KH_2PO_4 Buffer: Methanol (150:850)
Column	Hypersil *BDS C18, 250 x 4.6 mm, 5μm , L1 Packing*
Wavelength	210nm
Flow Rate	0.8mL/min
Injection volume	20 μL
Run time	10 min
Diluent	Mobile phase

Table 2: Forced degradation studies

S.No	Stress conditions	Time (hours)	Assay of Active substance (%)	Total impurities (%)	Mass balance Assay impurities (%)
1	Normal	24	100	-	100
2	Acid hydrolysis	24	99.67	0.33	100
3	Base hydrolysis	24	99.76	0.24	100
4	Oxidation (3%H_2O_2)	24	99.60	0.40	100
5	Thermal at 80^0C	24	96.88	3.12	100
6	UV light	24	99.97	0.03	100

Table3- Limit of Detection (LOD) and Limit of Quantification (LOQ)

Name	Metformin HCl	Colchisine	Glimepiride
LOD	2.88	2.88	2.88
LOQ	8.72	8.73	8.72

Table4- Linearity data

Name	Metformin HCl	Ramipril	Glimepiride
Linearity (n=3)			
Intercept	309130.71	-5622605.07	140267.76
Slope	114997080.14	101447173.52	86475985.73
R	1.00	1.00	1.00

Table5- Accuracy data

Name	Metformin HCl	Ramipril	Glimepiride
Accuracy % Recovery	99.9- 100.5	99.8-100.8	99.5-100.6

III. Results and Discussion

To establish and validate an efficient method for analysis of these drugs in bulk and pharmaceutical formulations, preliminary tests were performed. Different chromatographic conditions were employed for the analysis of the Metformin HCl, Ramipril & Glimepiride in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using 0.02M KH_2PO_4 Buffer: Methanol in the ratio of 150:850 % v/v at a flow rate 0.8 mL/min. Samples were analyzed at 210nm at an injection volume of 20 μL and separation was carried by using Hypersil *BDS C18, 250 x 4.6 mm, 5μm* with L1 packing. The proposed method was optimized to give very sharp peak with greater resolution (Fig 5. The optimized conditions were given in table 1.

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out at 24 hours under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis and the drug substances were observed high degradation in thermal, comparative remaining in all conditions. Thermal degradation conditions were performed by the drug sample at 80^oC and degraded product peak were observed at retention time 3.70, 3.98, 4.47 & 4.87 min Metformin HCl, Ramipril & Glimepiride with degraded products respectively.

Acid and base hydrolysis was performed by exposing the drug substances with 0.5N HCl & 0.1N NaOH at 25^oC for 24 hours and it was showed slight degradation of Metformin HCl, Ramipril & Glimepiride. And base hydrolysis also performed by exposing the drug substance with 0.5N NaOH at 25^oC for 24 hours and it was showed complete degradation of Metformin HCl, Ramipril & Glimepiride. And there was no degradation occurs under UV light and slight degradation occur under oxidation (3% H_2O_2) conditions. The results of forced degradation studies were given in table.2

Precision was evaluated by a known concentration of Metformin HCl, Ramipril & Glimepiride was injected six times and corresponding peaks were recorded and % RSD was calculated and found within the limits. The low % RSD value was indicated that the method was precise and reproducible.

Accuracy of the method was proved by performing recovery studies on the commercial bulk and formulation for Assay at 25%,50%, 100% 150%,200 and 250% level. Recoveries of Metformin HCl, Ramipril & Glimepiride ranges from 99.5% to 100.8% in proposed method and the results were shown in the (Table 5).

Linearity was established by analyzing different concentrations (for assay (25%, 50%, 100% 150%,200% and 250% level of Metformin HCl, Ramipril & Glimepiride respectively. The calibration curve was plotted with the area obtained versus concentration Metformin HCl, Ramipril & Glimepiride. In the present study six concentrations were chosen ranging between 25-250 μg/mL. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance and the results were shown in the (Table 4)

Robustness of the method is the ability of the method to remain unaffected by small deliberate changes in parameters like flow rate, mobile phase composition and column temperature. To study the effect of flow rate of the mobile phase it was changed to 0.1 units from 0.8 mL to 0.7 mL and 0.9 mL. The effect of column temperature also checked by changing temperature to $\pm 5^{o}$C. This deliberate change in the above parameters has no significant effect on chromatographic behavior of the samples. LOD and LOQ of Metformin HCl, Ramipril & Glimepiride were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 0.05 μg/mL and 0.15 μg/mL of Metformin HCl, Ramipril & Glimepiride respectively. The quantitation limit were found to be 0.10μg/mL for Metformin HCl, Ramipril & Glimepiride. The results were given in the (Table 3).

IV.   Conclusion

A new stability- indicating RP-HPLC method has been developed for simultaneous estimation of Metformin HCl, Ramipril & Glimepiride in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of Metformin HCl, Ramipril & Glimepiride in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out and the results revealed suitability of the method to study stability of Metformin HCl, Ramipril & Glimepiride under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally it was concluded that the method is simple, sensitive and has the ability to separate combination of three drugs from degradation products found in the pharmaceutical dosage form.

Acknowledgements

References

[1].   ICH Q2 (R1), Validation of Analytical procedures, Text and Methodology, *International Conference on Harmonization. Geneva. 2005. p. 1-17.*

[2].   M.Suchitra, D.Sunitha, C.Prathiban, B.Siddartha and C.Madhavi, Method development and validation of Metformin, Glimepiride and Pioglitazone in tablet dosage form by RP HPLC, International research journal of pharmacy *Analytical and Bioanalytical Chemistry. 2013, 4 (08), 250-254.*

[3].   A. Praveen Kumar, G. Aruna, K. Rajasekar3, P. Jayachandra Reddy, Analytical method development and validation of Alogliptin and Metformin hydrochloride tablet dosage form by Rp-Hplc method *International Bulletin of Drug Research.,2013, 3(5): 58-68.*

[4].   Kishore Kumar Hotha, Narasimha Reddy Yarramu, Thriveni Kandibedala, Vijaya Bharathi Dasari*, Venkateswarlu Vobalaboina**, Simultaneous Determination of Atorvastatin and Glimepiride by LC-MS/MS in Human Plasma and Its Application to a Pharmacokinetic Study, *American Journal of Analytical Chemistry, 2012, 3, 559-569.*

[5].   Srinivasa Rao Polagania, Nageswara Rao Pillib, Ramakrishna Gajulab, Venkateswarlu., Simultaneous determination of atorvastatin, metformin and glimepiride in human plasma by LC–MS/MS and its application to a human pharmacokinetic study, Journal of Pharmaceutical Analysis 2013,3(1), 9–19

[6].   Gadapa Nirupa,1 and Upendra M. Tripathi, RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms, Hindawi Publishing Corporation Journal of Chemistry Volume 2013, Article ID 726235, 8 pages

[7].   Mihirkumar G. Patel, Pravin O. Patil*, Sanjay B. Bari, Validated Rp- Hplc method for simultaneous estimation of Metformin hydrochloride and Benfotiamine in bulk drug and in pharmaceutical dosage form, *International Journal of Analytical and Bioanalytical Chemistry 2012; 2(3): 196-200*

[8].   R. Pravin Cumar, M.Vasudevan and Deecarama, A Validated Rp – Hplc method for simultaneous estimation of Metformin and Saxagliptin in tablets, *Rasayan journal of chemistry, 2012, Vol. 5 (2) 137-141*

[9].   Ramanjaneyulu Juvvigunta*, G. Nagarjuna Reddy, D. Dhanalakshmi and B. Ramesh, A New Analytical Method Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Tablet Dosage form by RP-HPLC, *International Journal of Pharma Sciences Vol. 3, No. 5 (2013): 360-364*

[10].   Sattar Navaneetha and Medidi Srinivas, Rp-Hplc method for the simultaneous estimation of Metformin hydrochloride and Telmisartan in bulk and in a synthetic mixture, *International Journal of ChemTech Research, 2014, 6 (11), 4737-4745*

[11].   P. Raja, J. C. Thejaswini, B. M. Gurupadayya, K. Sowjanya, Determination and Validation of Metformin, Glimepiride, Pioglitazone Using Atorvastatin as an Internal Standard in Bulk Drug and Pharmaceutical Dosage Form, *Journal of Applied Chemical Research, 2011,18, 61-68.*

[12].   Rajesh Sharma, Sunil khanna, and Ganesh P. Mishra, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ramipril, Aspirin and Atorvastatin in Pharmaceutical Preparations, *European -Journal of Chemistry http://www.ejchem.net 2012, 9(4), 2177-2184*

[13]. G. Nagarajan, B. Govardhan1, B. V. Ramana, K. Sujatha, S. Rubina, T. Arundathi and R. Soumya, Development and validation of a RP- HPLC method for simultaneous estimation of enalapril maleate and Ramipril in bulk and tablet dosage form, *Der Pharmacia Lettre, 2013, 5 (1):69-76.*

[14]. Validation of analytical procedures, Text and methodology, ICH Q2 (R1) Harmonized tripartite guideline, 2006, 15-64.

[15]. Validation of compendial methods The United States pharmacopeia, USP-37, Page no.1157-1162.

[16]. FDA Guidance for Industry, Analytical Procedures and Methods Validation (draft guidance), August 2000.

[17]. D. Adukondalu, P. S. Malathy, J. V. Rao, and Y. M. Rao,"Development and validation of HPLC method for detection of pioglitazone hydrochloride in dosage forms," *International Journal of Pharmacy and Biological Sciences*, 2011, 1(4), 474–478.

[18]. Lad NR, Bhoir SI, Bhoir IC, Sundaresan M. Concurrent assay of metformin and glimepiride in tablet using RP-HPLC with wavelength Programming. *Indian Journal Pharmaceutical Sciences. 2003, 65, 650–653*