Research Article

Analytical Method Development and Validation of Metformin, Losartan and Glimepiride in Bulk and Combined Tablet Dosage form by Gradient RP-HPLC

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ABSTRACT

Purpose: A simple, sensitive, linear, precise, and accurate method by gradient reversed-phase-high performance liquid chromatography for the simultaneous estimation of metformin (MET), losartan (LOS) and glimepiride (GLI) in bulk and in their combined tablet dosage form was developed and validated.

Methodology: The separation of the three drugs was based on the use of Luna c18 (250 × 4.6 mm, i.e. 5 µm) column in a gradient mode. Mobile phase consisted of Methanol (solvent A) and 0.1% Orthophosphoric acid [OPA] (solvent B) was set with gradient programming for 18 min and was delivered at 1 ml/min flow rate and effluents are achieved with variable wavelength: Photodiode array detector at 284 nm. The retention times of MET, LOS and GLI were found to be 3.11, 7.12 and 13.52mins respectively. The percentage assay of MET, LOS and GLI was found to be 100.5%, 100.5 and 100.4%, respectively. Calibration curves were linear for MET, LOS and GLI at concentration ranges of 30- 450 ng/ml, and 15-225ng/ml and 1-18ng/ml with the regression coefficient of 0.999 for all the three drugs and precise with (% RSD <2). The drug was subjected to various stress conditions of acid and base hydrolysis, oxidation, photolysis, thermal degradation and condition.

Findings: Considerable degradation was found under all stress conditions and the degradation products were well resolved from Metformin (MET), Llosartan (LOS) and Glimepiride (GLI) in the proposed gradient RP-HPLC method.

Conclusion: The method was validated by determining its linearity, accuracy, precision, system suitability and can be employed for routine quality control analysis.

Keywords: RP-HPLC, Validation, Stability studies, ICH guidelines.

INTRODUCTION

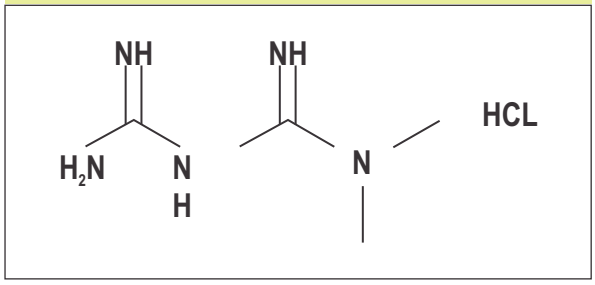
Metformin: The chemical name of Metformin (MET) is 1-Carbamimidamido-N, N-dimethylmethanimidamide.[1] It has a molecular formula of $C_8H_{11}N_5$ and a molecular weight of 129.1636 g/mol. Soluble in water, Freely soluble as HCL salt.

MOA: Metformin's mechanisms of action differ from other classes of oral antihyperglycemic agents. 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. It has the following structural formula shown in fig.1(a)

Losartan: The chemical name of Lorsartan (LOS)is [2-butyl-4-chloro-1-({4-[2-(2H-1,2,3,4-tetrazole-5-yl) phenyl]phenyl} methyl)-1H-imidazole-5-yl]methanol[2]. It has a molecular formula of $C_{22}H_{23}ClN_6O$ and a molecular weight of 422.911 g/mol. Soluble in water.

MOA: Losartan competitively inhibits the binding of angiotensin II to AT1 in many tissues including vascular smooth muscle and the adrenal glands.

Fig. 1(a): Structure of Metformin



Losartan is metabolized to its active metabolite, E-3174, which is 10 to 40 times more potent than losartan and acts as a non-competitive AT1 antagonist. Inhibition of angiotensin II binding to AT1 inhibits its AT1-mediated vasoconstrictive and aldosterone-secreting effects and results in decreased vascular resistance and blood pressure. It has the following structural formula shown in fig.1(b).

Glimepiride: The chemical name of Glimepiride (GLI)is 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl) carbamoyl] amino}sulfonyl)phenyl]ethyl}-2-oxo2,5- dihydro-1H-pyrrole-1-carboxamide[3]. It has a

molecular formula of $C_{24}H_{34}N_4O_5S$ and a molecular weight of 490.616 g/mol. Insoluble in water.

MOA: The mechanism of action of 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. It has the following structural formula shown in fig.1(c).

Fig. 1(b): Structure of HCL Losartan (c) Glimepiride

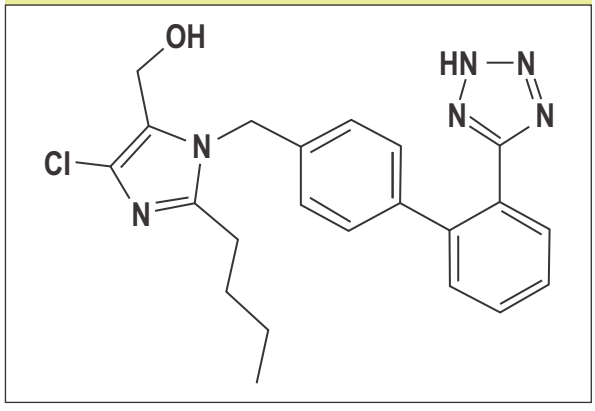
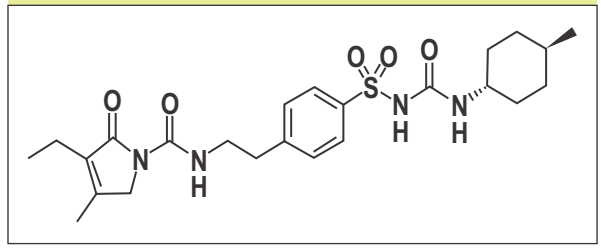


Fig. 1(c): Structure of Glimepiride



The literature survey revealed that there is very few HPLC[4-12], GC[16-18] and spectroscopic[13, 14] methods available for the determination of Metformin HCL, Losartan and Glimepiride in pure and combined dosage forms. The present study was aimed to develop a new HPLC method for simultaneous estimation of Metformin HCL, Losartan and Glimepiride in bulk and their combined pharmaceutical dosage form using more economical chromatographic conditions.

MATERIALS AND METHODS

Chemicals and reagents

Metformin HCL (99.5%), Losartan (99.6%) and Glimepiride (99.4%) are obtained as gift samples from Hetero laboratories, Hyderabad, India. The formulation used was Glucoryl-MV-2 tablets (Label claim: 500 mg of MET, 25 mg of LOS, and 2 mg of GLI) were procured from the local market. Ortho Phosphoric Acid was purchased from Merck (Mumbai, India), HPLC grade Water (Milli Q or equivalent) all chemicals (AR Grade) were used for entire study.

Instrumentation: All HPLC experiments were carried out on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in gradient mode using Auto sampler. Data collection and processing was done using EMPOWER PDA 2 software. The analytical column used for the separation was Luna phenyl hexyl (250mm x 4.6mm, 5µm) Column, Other equipments used were ultra-sonicator (model 3210) Analytical balance (Contech balance).

Preparation of solutions

Diluent: Methanol and HPLC grade water in the ratio of 50:50

Mobile Phase: Methanol and 0.1% Orthophosphoric acid is programmed on gradient flow indicated in Table 1.

Preparation of standard solution

Standard stock solution was prepared by dissolving separately 500 mg of MET, 25 mg of LOS and 2 mg of GLI in 100 ml clean dry volumetric flask. Dissolved and diluted with mobile phase up to the mark and filtered through 0.45µm membrane filter. From the prepared standard stock solution, 5 ml was transferred to 50 ml volumetric fl ask and volume made up with the mobile-phase to obtain concentration of 500 µg/ml for MET, 25 µg/ml for LOS, and 2 µg/ml for GLI respectively.

Chromatographic conditions:

The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. The column used in the development for the determination is Luna Phenyl Hexyl, (250mm x 4.6mm, 5µm). Various combinations of mobile phases were screened and finally, the mobile phase consisting of Methanol (solvent A) and 0.1 % Orthophosphoric acid (solvent B) was set with gradient programming for 18 min was optimized at a flow rate of 1 ml/min, 284 nm wavelength, injection volume of 10 µL and ambient temperature was maintained during the entire process. The corresponding peak and retention times were recorded for each drug. From the chromatogram retention times for Metformin HCL, Losartan and Glimepiride were found to be 3.11, 7.12 and 13.52mins respectively. Typical chromatogram of Metformin HCL, Losartan and Glimepiride was shown in fig.2 and optimized chromatographic conditions as shown in the table.2.

Table 1: Time Programming of Gradient elution

	Time [min]	Flow [ml/min]	Methanol	Buffer
1	0.01	1	20	80
2	5.00	1	80	20
3	6.00	1	20	80
4	16.00	1	20	80

Fig. 2:Typical Chromatogram of Metformin, Losartan and Glimepiride

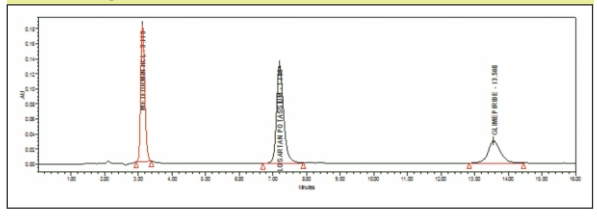


Table. 2: Optimization Chromatographic conditions

Column	Luna C18, 250 x4.6mm, 5µ			
Mobile phase:	**Time**	**Flow**	**Methanol**	**Buffer**
Elution mode: Gradient	1 m	1 ml/m	20	80
	5 m	1 ml/m	80	20
	6 m	1 ml/m	20	80
	16 m	1 ml/m	20	80
Flow rate	1ml/min			
Column temperature	Ambient			
Injection volume	10µl			
Detection Wavelength	284 nm			
Run time	18 mins			
Retention time (Mins)	MET-3.119, LOS-7.196, GLI-13.560			

Method Development:

To saturate the column, the mobile phase was pumped for about 30 minutes thereby to get the base line corrected. The separate standard calibration lines were constructed for each drug. A series of aliquots were prepared from the above stock solutions using diluents to get the concentrations 30-450 ng/ml for Metformin HCL, 15-225 ng/ml for Losartan and 1-18 ng/ml Glimepiride. Each concentration 6 times was injected in to chromatographic system. Each time peak area and retention time were recorded separately for all the drugs. Calibration curves were constructed as by taking average peak area on Y-axis and concentration on X-axis separately for all the drugs. From the calibration curves regression equations were calculated, these regression equations were used to calculate drug content in formulation.

Estimation of LAM, ABA and DOL in tablet dosage forms

The formulation consists of Label claim: 500 mg of MET, 25 mg of LOS and 2 mg of GLI. Twenty tablets of combined dosage form of MET, LOS and GLI were weighed and made to a fine powder. 650.2 mg of powdered tablets equivalent to 500 mg of MET, 25 mg of LOS and 2 mg of GLI were weighed accurately and transferred into a 100 ml clean dry volumetric flask. Dissolved and diluted with mobile phase up to the mark and filtered through 0.45 µm membrane filter. From the prepared standard stock solution, 5 ml was transferred to 50 ml volumetric flask and the volume made up with the mobile phase to obtain concentration of 500 µg/ml, 25 µg/ml and 2 µg/ml for MET, LOS and GLI respectively. The assay procedure was repeated 6 times (n=6) the drug content was estimated using above calculated regression equation; the results of laboratory mixture are shown in the table.3.

METHOD VALIDATION: The analytical method was validated for various parameters as per ICH guidelines

Linearity: The linearity of the method was determined in concentration range of 30-450 ng/ml for Metformin HCL, 15-225 ng/ml for Losartan and 1-18 ng/ml Glimepiride. Each solution was injected six times. The peak area versus concentration data was analyzed with least squares linear regression. The slope and intercept of the calibration curve were reported. The results were shown in Table 4, and obtained graphs were shown in fig. 3 a-c

Fig. 3(a): Linearty plot for Metformin

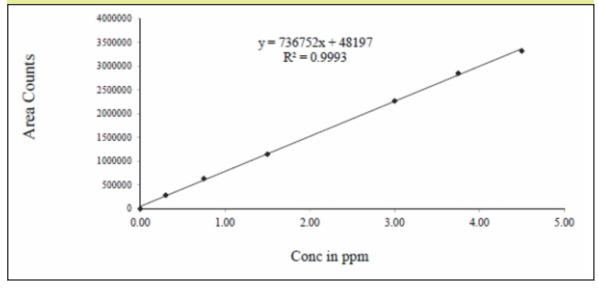


Fig. 3(b): Linearty plot Losartan c)Glimepiride

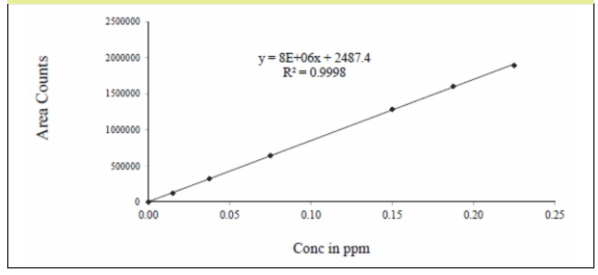
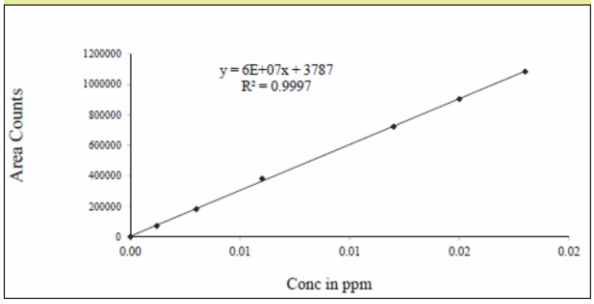


Table 3: Assay of commercial tablet

Drug	Brand name	Label claim (mg)	Test concentration (ng/ml)	Mean amount estimated(ug/ml) (n=6)	% Assay	% RSD
Metformin	Glucoryl-MV-2	500	15	15.5	100.5	0.117
Losartan		25	75	75.5	100.5	0.229
Glimepiride		2	6	6.51	00.4	0.444

Fig. 3(c): Linearty plot Glimepiride



Accuracy: Accuracy was evaluated in triplicate, at three different concentration levels equivalent to 50, 100 and 150% of the target concentration of active ingredient, by adding a known amount of each of the Standard to a pre-analysed concentration of all drugs (MET, LOT and GLI) and calculating the % of recovery. The results were shown in Table. 5.

Precision: The precision at 100% concentration was evaluated by carrying out six independent assays of MET, LOS and GLI with the reference standard of the same drugs as shown in Tables.6 a, 6b.

LOD and LOQ

LOD: It is lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is

usually expressed as the concentration of analyte. The standard deviation and response of the slope.

$$LOD = 3.3*\text{standard deviation } (\sigma)/s$$

LOQ: The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined

Table 4: Optical Characteristics of Metformin, Losartan, and Glimepiride

Parameters	Metformin	Losartan	Glimepiride
Linearity range (µg/ml)	0.30-4.5	0.015-0.225	0.001-0.018
Regression line equation	y=736752x+48197	y=8E+06x+2487.4	y=6E+07x+3787
Correlation coefficient (*r*)	0.999	0.999	0.999
LOD [µg/ml]	0.032	0.028	0.059
LOQ [µg/ml]	0.10	0.097	0.189

Table.5: Results of the Recovery studies

Drugs	% of Recovery levels	Pre-analysed conc (ug/ml)	Amount Added (ug/ml)	Amount Found (ug/ml)	%Recovery	% RSD
Metformin	50	1.5	0.75	2.2610	0.4	
	100	1.5	1.5	3.03	101.0	0.06
	150	1.5	2.25	3.77	100.5	
Losartan	50	0.075	0.0375	0.113	100.4	
	100	0.075	0.075	0.155	100.3	0.11
	150	0.075	0.1125	0.190	100.2	
Glimepiride	50	0.006	0.003	0.0095	100.4	
	100	0.006	0.006	0.0124	100.3	0.200
	150	0.006	0.009	0.0157	100.2	

Table 6a: Results for precision of the Standard

Injection	Metformin		Losartan		Glimepiride	
	Retention Time	Area	Retention Time	Area	Retention Time	Area
1	3.119	2402567	7.196	1208345	13.560	710370
2	3.108	2408840	7.184	1205355	13.479	716845
3	3.099	2407589	7.162	1204613	13.423	715602
4	3.089	2407773	7.166	1207265	13.449	718171
5	3.086	2409890	7.164	1201188	13.442	712676
6	3.086	2406723	7.178	1206939	13.464	710026
Mean		2406723		1205617		713949
% RSD		0.194		0.134		0.480

Table 6b: Results for precision of the Sample

Injection	Metformin		Losartan		Glimepiride	
	Retention Time	Area	Retention Time	Area	Retention Time	Area
1	3.026	2487538	7.172	1272865	13.453	752177
2	3.025	2482341	7.163	1258506	13.434	750160
3	3.029	2476941	7.168	1287259	13.452	755875
4	3.029	2463222	7.172	1299329	13.465	750662
5	3.028	2420318	7.171	1297696	13.463	750121
6	3.021	2448155	7.173	1260959	13.484	753065
Mean		2463086		1279436		752010
Std Dev		25348.1		17960.9		2231.1
% RSD		1.524		0.907		0.297

with suitable precision and accuracy. The standard deviation and response of the slope and the results obtained.

LOQ= 10* standard deviation (σ)/s

The results of LOD & LOQ are shown in the table.4

System suitability parameters: For assessing system suitability, six replicates of working standards samples of MET; LOS and GLI were injected and studied the parameters like plate number (N), tailing factor (K), resolution, relative retention time and peak symmetry of samples. The results were tabulated in Table.7.

Robustness: The robustness of the assay method wasestablished by introducing small changes in the chromatographic condition which included percentage of acetonitrile in mobile phase, flow rate (0.8 and 1.2mL/min) and organic phase, column oven temperature (25°C and 35°C). The results were tabulated in Table.8.

Table 7: System suitability results for Metformin Losartan and Glimepirid

S.no	Parameters	Metformin	Losartan	Glimepiride
1	Theoretical plates	3205	6152	7397
2	Tailing factors	1.3	1.25	1.08
3	Resolution		13.64	12.33
4	Relative retention time [mins]	3.11	7.12	13.52

Fig. 4 (a): Specificity chromatogram with blank

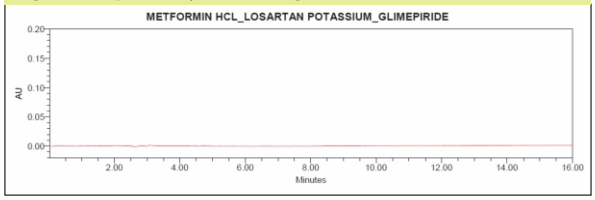
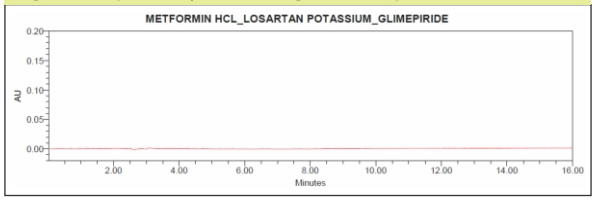


Fig. 4 (b): Specificity chromatogram with placebo



Selectivity: Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard solution of MET, LOS and GLI, commercial product solution and blank solutions were run in the instrument one after another. The results of the tests proved that the components other than the drug did not produce any detectable signal at the retention time of MET, LOS and GLI.There were no interfering peaks at retention time of MET, LOS and GLI. Fig.4 shows the chromatogram of blank and working placebo solution respectively.

FORCE DEGRADATION

Preparation of sample stock solution: 3.9 mg of powdered sample drug combined dosage form of Metformin, Losartan and Glimepiride were accurately weighed and dissolve in 10 ml of diluent that consist methanol and HPLC grade water [50:50] then sonicated for 2 minutes.

1. Acid Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask, to thisadd order of 1ml 5N HCL and 1ml 5N NaOH then make up to 10 ml of diluent.

2. Alkali Degradation: From the sample stock solution 1ml is pipetted out and taken into 10ml volumetric flask, to this add order of 1ml 5N NaOH and 1ml 5N HCL then make up to 10 ml of diluent.

3. Oxidative Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask, to this add 1ml 30% H_2O_2 then make up to 10 ml of diluent.

4. Reduction Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask, to this add 1ml 10% Sodium Bisulphate then make up to 10ml of diluent.

5. Thermal Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask then make up to 10 ml of diluent,then allowed to heat 105^{0}C in incubator for 24 hours.

6. Photolytic Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask then make up to 10ml of diluent, then allowed to kept in sunlight for 12 hours.

7. Hydrolysis Degradation: From the sample stock solution 1ml is pippeted out and taken into 10ml volumetric flask, to this add 5ml HPLC grade water then make up to 10ml of diluent.

Fig. 5 (a): chromatograms of degradation- acid degradation

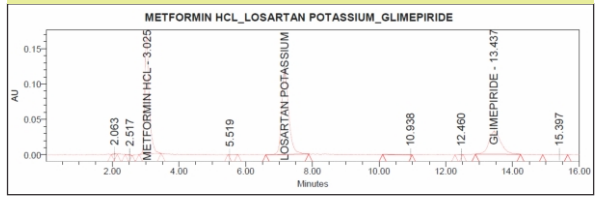


Fig. 5 (b): chromatograms of degradation-Alkali degradation

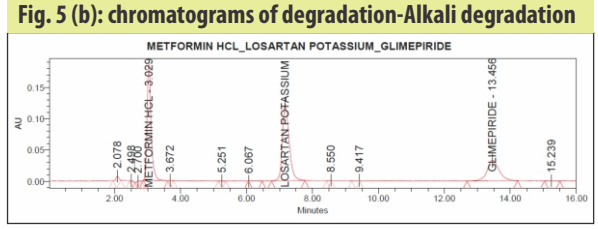


Fig. 5 (c): chromatograms of degradation- of peroxide degradation

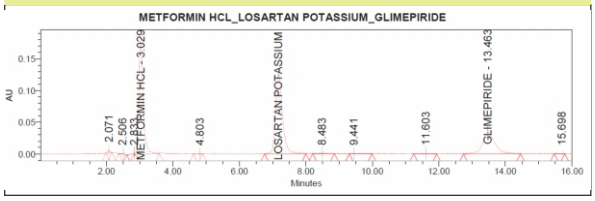


Fig. 5 (d): chromatograms of degradation - Reduction degradation

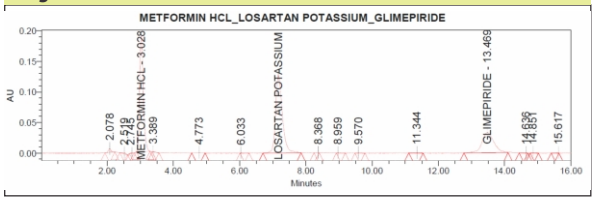


Fig. 5 (f): chromatograms of degradation- thermal degradation

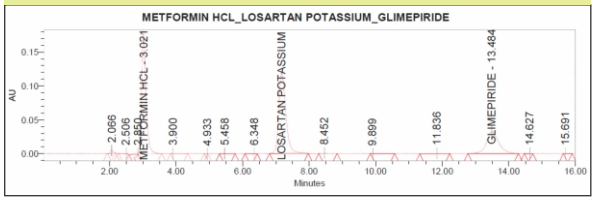


Fig. 5 (g): chromatograms of degradation- Photolytic degradation

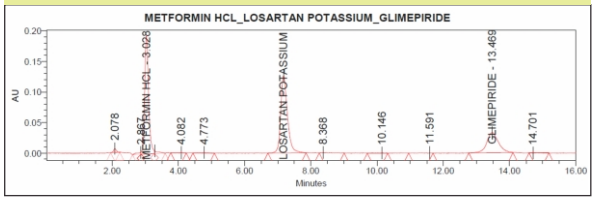
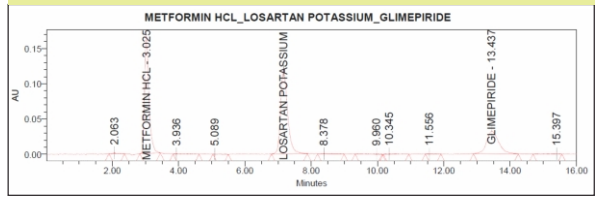


Fig. 5 (e): chromatograms of degradation - Hydrolysis degradation



RESULTS AND DISCUSSION

Optimization of chromatographic conditions

A gradient, rapid and simple RP-HPLC method was developed and validated for the simultaneous estimation of MET, LOS and GLI. Mobile phase consisting of Methanol (solvent A) and 0.1% Orthophosphoric acid (solvent B) was set with gradient programming for 18 min. Chromatographic conditions were optimized for mobile phase using Luna C18 (250 × 4.6 mm, i.d., 5µm) column at a flow rate of 1 ml/min. Effluents were detected at 284 nm by variable wavelength PDA detector. Column compartment temperature was in ambient. Chromatogram of MET, LOS and GLI (Fig.2) and optimized chromatographic condition is shown in Table.2.

Selectivity: Fig.4 shows the chromatogram of blank and working placebo sample solution. There were no interfering peaks at retention time of MET, LOS and GLI.

Linearity and range: The linearity regression co-efficient (R2) values were found to be 0.999 for MET

Table 8: Results of Robustness by variation in flow rate and organic phase

Parameters	Retention time			Peak area			% Recovery		
	MET	LOS	GLI	MET	LOS	GLI	MET	LOS	GLI
Flow Minus(0.8)	3.768	8.975	17.005	2265529	1264804	915510	100.3	100.5	100.2
Flow Plus(1.2)	2.519	5.958	11.122	2474083	1432847	636888	100.7	100.3	100.3
Organic Minus	3.303	8.973	18.027	1576454	1266717	732757	100.5	100.4	100.2
Organic Plus	2.836	6.165	11.117	2413871	1285531	767266	100.1	100.3	100.3

Table 9: Stability studies for Metformin, Losartan and Glimepiride

Stress condition	%Assay			%Degradation			Purity Angle			Purity Threshold		
	MET	LOS	GLI	MET	LOS	GLI	MET	LOS	GLI	MET	LOS	GLI
Control	100.8	100.6	100.4	-0.8-	0.6	-0.4	0.692	0.079	0.104	5.124	5.081	5.102
Acid	80.4	80.2	80.3	20.4	20.4	20.1	0.904	0.088	0.122	5.131	5.088	5.116
Alkali	80.8	78	78.9	20	22.6	21.5	0.883	0.078	0.116	5.131	5.075	5.112
Oxidative	79.9	80.5	78.7	20.9	20.1	21.7	0.803	0.085	0.122	5.132	5.089	5.12
Reduction	80.3	80.1	80	20.5	20.5	20.4	0.334	0.075	0.122	5.099	5.075	5.116
Thermal	79.2	79.9	74	21.6	20.7	26.4	0.459	0.089	0.141	5.155	5.087	5.137
Photolytic	79.2	78.1	72.4	21.6	22.5	28	0.432	0.075	0.122	5.099	5.075	5.116
Hydrolysis	78.6	78.9	72.7	22.2	21.7	27.7	0.999	0.08	0.122	5.127	5.08	5.116

and 0.999 for LOS and 0.999 for GLI. Linearity equation obtained for MET, LOS and GLI were y = 736752x+48197, y = 8E+06x+2487.4 and y = 6E+07x+3787, respectively. Fig. 3 a-c show linearity graphs for MET, LOS and GLI respectively.

Accuracy and Precision: Accuracy as recovery was evaluated by spiking previously analyzed test solution with additional Placebo at three different concentration levels (table-5). Recovery of previously analyzed test solution drug concentration added was found to be 100.5 % for MET, 100.3 % for LOS and 100.3% for GLI with the value of RSD less than 2% indicating that the proposed method is accurate for the simultaneous estimation of all drugs from their combination drug products in presence of their degradation products. The low RSD values indicate the repeatability and reproducibility of the Method table-6.

Linearity, LOD and LOQ: The calibration plot was linear over the concentration range investigated (0.30-4.50 µg/ml; n= 3), (0.015-0.225 µg/ml; n= 3) and

(0.001-0.018 µg/ml; n = 3) for metformin (MET), losartan (LOS) and glimepiride (GLI) respectively. Average correlation coefficient r=0.999 for all the drugs with %RSD values ⩽ 2.0 across the concentration ranges studied was obtained from regression analysis. The limit of detection for MET, LOS and GLI was found to be 0.032 µg/ml, 0.028 µg/ml, 0.059 µg/ml, and limit of quantitation for MET, LOS and GLI was found to be 0.10 µg/ml, 0.097 µg/ml, and 0.189 µg/ml, respectively. The Regression results indicate that method was linear in the concentration range studied and can be used for detection and quantification of metformin (MET), losartan (LOS) and glimepiride (GLI) in a very wide concentration range.

Specificity and Selectivity: Specificity is checked in each analysis by examining blank and placebo samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other excipients.

Robustness: Results of the robustness (table.8). The elution order and resolution for all components were not significantly affected. RSD of peak areas were found to be well within the limit of 2.0%.

System suitability: The system suitability parameters were found to be within acceptance criteria. Good peak with resolution between two drugs is >1.5, asymmetric factor <2 shows that the three drugs were better separated. Parameters calculated for system suitability were a number of theoretical plates, tailing factor, resolution, retention time, and area.Results are shown in table 7..

Assay: The proposed method was applied for the analysis of Anti-diabetic and anti-hypertensive tablets and the results of the assay of MET, LOS and GLI are shown in Table.3

Degradation studies

Acid hydrolysis: Upon performance of acid degradation studies 20.4 % of MET, 20.1 % LOS and 20.4% of GLI was degraded.

Base hydrolysis: Upon performance of base degradation studies 20 % of MET, 22.6 % LOS and 21.5% of GLI was degraded.

Peroxide hydrolysis: Upon performance of peroxide degradation studies 20.9 % of MET, 20.1 % LOS and 21.7% of GLI was degraded.

Reduction degradation: Upon performance of Reduction degradation studies 20.5 % of MET, 20.5 % LOS and 20.4% of GLI was degraded.

Photolytic degradation: Upon performance of Photolytic degradation studies 21.6 % of MET, 22.5 % LOS and 28% of GLI was degraded.

Hydrolysis degradation: Upon performance of Hydrolysis degradation studies 22.2 % of MET, 21.7 % LOS and 27.7 % of GLI was degraded.

Thermal degradation: Upon performance of Thermal degradation studies 21.6 % of MET, 20.7 % LOS and 26.4% of GLI was degraded.

All the stability studies results were shown in table- 9 and figure-5 a-g

CONCLUSION

A simple, rapid, accurate and precise stability-indicating gradient RP-HPLC analytical method has been developed and validated for the quantitative analysis of metformin (MET), losartan (LOS) and glimepiride (GLI) in bulk drugs and combined dosage forms. The newly developed gradient RP-HPLC method for separation of different degradation products along with the pure drugs were found to be capable of giving faster retention times while still maintaining good resolution than that achieved with conventional HPLC. This method exhibited an excellent performance in terms of sensitivity and speed. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability-indicating. The proposed method has the ability to separate these drugs from their degradation products in tablet dosage forms and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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