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Review Article

Simultaneous estimation of pioglitazone, glimepiride & metformin hydrochloride in bulk & tablet dosage form by UV, RP-HPLC method

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ABSTRACT

A stability-indicating UV spectroscopic and high-performance liquid chromatography (RP-HPLC) method is developed for the quantification of Pioglitazone, Glimepiride & Metformin Hydrochloride drug substances. UV spectroscopic method was developed and validated, the wavelength selected for simultaneous estimation were 226nm for pioglitazone, 229nm for glimepiride and 232nm for metformin hydrochloride. The isosbestic point found for the analysis was 229nm. Selected mobile phase was a combination of methanol and water with a ratio of 70% Methanol and 30 % HPLC water with the flow rate of 0.85ml/min. The analyte was analysed on the C18 HPLC column having the pore size of 5 microns at room temperature. The method is validated according to ICH guidelines, the retention time of about 4.0min for metformin, 5.5min for Pioglitazone and 6.8min for Glimepiride was observed. The linearity range with regression co-efficient for Pioglitazone, Glimepiride & Metformin Hydrochloride is 3-15 µg/mL, 0.4-1.2 µg/mL and 100-500 µg/mL and 0.9998, 0.9991, 0.9991 respectively.

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1. Introduction

Metformin Hydrochloride is a anti-diabetic drug which belongs to Biguanides drug class. The chemical formula for Metformin hydrochloride is $C_4H_{12}ClN_5$. Metformin is prescribed with other medication to control high blood sugar with instruction to follow proper diet and exercise program. It is used to treat patients with type 2 diabetes. Metformin exerts its anti-diabetic action through suppression of generation of glucose in the liver.¹⁻³ Till date, two important molecular targets of metformin have been identified, both of which are present in mitochondria.²⁻⁴ Metformin acts on mitochondrial respiratory complex I by inhibiting its function, which results in increasing in the cellular ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) as a result of a reduction in the efficiency of ATP production. This increase in

the ratio of AMP: ATP triggers the activation of AMP-activated protein kinase (AMPK), which has a lot of effects on energy metabolism, also down regulation of the expression of gluconeogenic genes is controlled by it.² The activity of adenylate cyclase is also thought to be inhibited by increase in AMP concentration⁵, it is an important mediator of glucagon action, therefore results in the inhibition of gluconeogenesis. The second target of metformin in mitochondria is mitochondrial glycerol-3-phosphate dehydrogenase,⁴ which plays a key role in the glycerophosphate shuttle. This oxidation-reduction shuttle between the cytosol and mitochondria is necessary for production of the oxidized form of coenzymes required for biochemical reactions, such as nicotinamide adenine dinucleotide in gluconeogenic reactions. The activity of mitochondrial glycerol-3-phosphate dehydrogenase is also inhibited by metformin, which suppresses gluconeogenic reactions including the conversion of lactate to pyruvate.⁴

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Fig. 1: Structure of metformin hydrochloride

Fig. 2: Structure of pioglitazone

Pioglitazone hydrochloride is another anti-diabetic drug with chemical formula CHNOS . Pioglitazone hydrochloride belongs to drug class thiazolidinedione that depends on the presence of insulin for its mechanism of action. Pioglitazone hydrochloride decreases insulin resistance in the periphery and in the liver which results in increasing the insulin-dependent glucose disposal and decreasing hepatic glucose output. Pioglitazone is secretagogue.⁶ Pioglitazone has agonist action for peroxisome proliferator-activated receptor-gamma (PPARgamma). PPAR receptors are found in tissues such as adipose tissue, skeletal muscle, and liver which are important for insulin action.^{7–16} PPARgamma metabolism.

Glimepiride is a potent anti-diabetic drug which belongs to Sulfonylurea drug class. Its chemical formula is $\text{C}_{24}\text{H}_{34}\text{N}_4\text{O}_5\text{S}$. It is used to treat patients with type 2 diabetes. It is prescribed with other medication with proper diet and regular exercise routine. Route of administration is oral it is available in tablet form and the elimination half life of glimepiride is 5-8 hours. Glimepiride exerts its action by increasing insulin release from the pancreas and by improving glucose tolerance. It acts on the "sulfonylurea receptors" on pancreatic β -cell membrane and reduces

conductance of ATP sensitive K^+ channels and thus causes depolarization. Due to this the Ca^{2+} influx and degranulation is enhanced which in turn increases insulin secretion rate at any glucose concentration.¹⁷ It primarily increases 2nd phase insulin secretion and has little effect on 1st phase. The hepatic degradation of insulin and minor action of reducing glucagon release and increasing somatostatin release is also slowed down. It sensitizes the target tissues such as liver to insulin action and exerts strong extrapancreatic action. Number of insulin receptors are increased and by a post receptor action translation of receptor activation is enhanced.

Fig. 3: Structure of glimepiride

2. Materials and Methods

All the three drugs i.e Pioglitazone, Glimepiride & Metformin Hydrochloride were obtained from RAP Analytical research and training centre and all the chemicals used were of HPLC grade were purchased from Thermofisher pvt ltd. The balance used for weighing is a high precision balance from Wensar with model no. PGB 100. The mobile phase was degassed using Wensar Ultra sonicator and was filtered using Ultra filtration with a membrane filter size of 0.45 microns. For UV spectroscopy, double beam instrument manufactured by Analytical Technologies Ltd. with Model no- UV-2012 was used. The software used for analysis was UV-VIS Analyst.^{18–25} The instrument used for the High Performance Liquid Chromatography consisted of binary gradient system. The software used of the HPLC instruments for the method development and the validation purpose was HPLC workstation. The maker of the HPLC system is analytical technologies Pvt. Ltd. with model number HPLC 3000 series. Reciprocating type of pump was used for the analysis of model no. P-3000M. The detector used was UV-VIS detector of single wavelength with the model no. of UV3000M. The C18 type of column was used for the separation which was from cosmosil make having the dimension of 250mm X 4.6mm (ID), particle size of

5micron.

2.1. Sample preparation

Accurately 10mg of Pioglitazone, glimepiride, metformin hydrochloride drug sample was weighed and transferred to 10ml volumetric flask individually, then it was dissolved using 5ml solvent(mobile phase) and then it was diluted up to the mark which makes stock solution of 1000 µg/ml. Working solution was made as per the requirement.

2.2. Method development

In order to obtain resolution of the three drugs that is to develop sharp and well resolved peaks various trials were carried out. By changing the ratio of mobile phase methanol and water various trial were taken and it was observed that all the three drugs were well resolved at the 70% methanol and 30% water at 229 nm. The flow rate of the mobile phase was taken at 0.8 ml/min. Optimised results was shown below of all three drugs

Fig. 4: Chromatogram of optimised trail

Rank	Time	Area	Resolut.	T.Plake	Num asymmetry
1	4.033	1618046	6.25	6955	1.03
2	5.523	327268	4.64	7446	1.00
3	6.951	148683	0.00	7768	1.16

3. Result

All the System Suitability parameters i.e resolution, number of theoretical plates, asymmetry of peaks were found to be within the range which is indicating the performance of the system.

3.1. Linearity

Statistically the response of the analyte and the concentration were calculated in order to check the linearity of all the three drugs.

Concentration range from 100-500 ug/ml were taken for metformin, 3-15 ug/ml for pioglitazone, 0.4-1.2 ug/ml for glimepiride and the calibration curve was plotted as shown below

Fig. 5: Calibrationcurve for metformin

Fig. 6: Calibration curve for pioglitazone

Fig. 7: Calibration curve for glimepiride Regression value for all the three drugs were found within the limit.

3.2. Accuracy

Triplicates of all the three drugs were taken for the recording the data of accuracy and the %RSD for all the drugs were calculated whose data is as follows

From the above results it is observed that for all the three drugs the % RSD is less than 2% which is within the acceptance limit.

3.3. % Recovery

Three concentration range of percentages were injected in order to analyse the recovery of the analyte in the formulation. Following are the results for the same.

3.4. Assay

Assay is the analysis of the marketed formulation which is checked against the standard solution. The results obtained for three drugs are:

It is observed that the assay of the formulation were found within the criteria limit.

3.5. Precision

Variations in the results were observed by performing interday and intraday of the drugs. Following are the observed results for the same.

As the % RSD in the above all three results were within the range ensures the repeatability of the results.

3.6. Robustness

It is performed to check the variations in the experiment i.e. in the chromatographic conditions. The results obtained were as follows.

As the RSD is less than 2% showing that the results are unaffected with the experimental changes.

3.7. Ruggedness

Wide range of concentration were injected for all the three drugs and their calibration curve was plotted as the response of analyte with the range if concentration.

Fig. 8: Calibration curve of ruggedness for metformin

Fig. 9: Calibration curve of ruggedness for pioglitazone

Fig. 10: Calibration curve of ruggedness for glimepiride

Results are showing that the developed method is stable as regression coefficient of all are within the limit hence change doesn't affects the results.

Limit of detection (LOD) and limit of quantification (LOQ)

It is calculated by slope and the standard deviation. Following are the formulas used to estimate the LOD and LOQ:

$$3.7.1. LOD = 3.3\sigma/S$$

1. Where, σ = the standard deviation of the y-intercept
2. S = slope of calibration curve of analyte

$$3.7.2. LOQ = 10\sigma/S$$

1. Where, σ = the standard deviation of the y-intercept
2. S = slope of calibration curve of analyte
3. The result of LOD and LOQ of respective drugs are as follows:

3.7.3. Metformin

1. LOD = 1.3608,
2. LOQ = 4.1239

3.7.4. Pioglitazone

1. LOD = 0.04710,
2. LOQ = 0.14274

3.7.5. Glimepiride

1. LOD = 0.00468,
2. LOQ = 0.01421

Table 1: Analytical data of accuracy of metformin

Conc.	Conc. ($\mu\text{g/ml}$)	Area	Standard Deviation		Accuracy %SD	Precision %RSD
			Mean	SD		
1	100	2046657 2056223 2047347	2050075.667	5334.913807	0.2602301	0.113609507
3	300	3890897 3884716 3893092	3889568.333	4343.197017	0.1116627	
5	500	5822073 5819626 5817773	5819824	2156.827068	0.03706	

Table 2: Analytical data of accuracy of pioglitazone

Comp.	Conc. ($\mu\text{g/ml}$)	Area	Standard Deviation		Accuracy %SD	Precision %RSD
			Mean	SD		
1	3	150344 149471 149774	149863	443.2527496	0.295772	0.133370602
3	9	295283 295312 295112	295235.6667	108.0755908	0.0366065	
5	15	440904 440692 441630	441075.3333	491.9119162	0.1115256	

Table 3: Analytical data of accuracy of glimepirideS

Comp	Conc. ($\mu\text{g/ml}$)	Area	Standard Deviation		Accuracy %SD	Precision %RSD
			Mean	SD		
1	0.4	16831 16731 16778	16780	50.02999101	0.2981525	0.135143733
3	1.2	39633 39540 39575	39582.66667	46.97162264	0.1186672	
5	2	61560 61519 61537	61538.66667	20.55075018	0.0333949	

Table 4: Statistical data of % recovery of metformin

Sr.no.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	3890897	3881879	99.76822825
2	100% Recovery	4914355	4917149	100.0568538
3	150% Recovery	5822073	5816256	99.90008713

Table 5: SStatistical data of % recovery of pioglitazone

Sr.no.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	295283	295340	100.0193035
2	100% Recovery	371066	371107	100.0110492
3	150% Recovery	440904	440600	99.93105075

Table 6: SStatistical data of % recovery of glimepiride Method was found to be accurate by observing the recovery of the analyte.

Sr.no.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	39633	39530	99.74011556
2	100% Recovery	49640	49603	99.92546334
3	150% Recovery	61560	61593	100.0536062

Table 7: tatistical data of assay of metformin

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	3890897	3896759	100.151

Table 8: Statistical data of assay of pioglitazone

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	295283	295185	99.9668

Table 9: Statistical data of assay of glimepiride

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	39633	39730	100.245

Table 10: Data for intraday precision of metformin

Morning	Evening	Mean	%RSD
3890897	3890160		
3884716	3883158	3888863	0.10%
3893092	3891157		

Table 11: Data for Interday precision of metformin

Day 1	Day 2	Mean	%RSD
3890897	3893449		
3884716	3886600	3892884	0.10%
3893092	3892884		

Table 12: Data for intraday precision of pioglitazone

Morning	Evening	Mean	%RSD
295283	294750		
295312	295249	295087.2	0.08%
295112	294817		

Table 13: Data for interday precision of pioglitazone

Day 1	Day 2	Mean	%RSD
295283	294668		
295312	294876	295017	0.08%
295112	295017		

Table 14: Data for intraday precision of glimepiride

Morning	Evening	Mean	%RSD
39633	39610		
39540	39576	39586.33	0.08%
39575	39584		

Table 15: Data for Interday precision of Glimepiride

Day 1	Day 2	Mean	%RSD
39633	39594		
39540	39560	39516	0.08%
39575	39516		

Table 16: Data of robustness of metformin (Change in flow rate)

Conc. ($\mu\text{g/ml}$)	Parameter	Condition	Area	Mean	SD	%SD
200	Change in flow rate	0.65	2905068			
		0.85	2899046	2899733	5026.37	0.17333906
		1.05	2895086			

Table 17: Data of robustness of metformin (Change in wavelength)

Conc. ($\mu\text{g/ml}$)	Parameter	Area	Mean	SD	%SD
200	Change in Wavelength	2897199	2895475	4677.67	0.16155113
		2899046			
		2890180			

Table 18: Data of robustness of pioglitazone (Change in flow rate)

Conc. ($\mu\text{g/ml}$)	Parameter	Area	Mean	SD	%SD
6	Change in flow rate	221047	220905	494.122	0.22368101
		221312			
		220355			

Table 19: Data of robustness of pioglitazone (Change in wavelength)

Conc. ($\mu\text{g/ml}$)	Parameter	Area	Mean	SD	%SD
6	Change in Wavelength	221868	221490	327.237	0.14774343
		221312			
		221291			

Table 20: Data of robustness of glimepiride (Change in flow rate)

Conc. ($\mu\text{g/ml}$)	Parameter	Area	Mean	SD	%SD
0.8	Change in flow rate	28725	28761	38.7427	0.13470582
		28802			
		28756			

Table 21: Data of Robustness of glimepiride (Change in wavelength)

Conc. ($\mu\text{g/ml}$)	Parameter	Area	Mean	SD	%SD
0.8	Change in Wavelength	28733	28760.7	36.4737	0.12681811
		28802			
		28747			

Table 22: Statistical data of ruggedness of metformin

Conc.	Area
100	2043536
200	2896093
300	3889571
400	4907803
500	5822486

Table 23: Statistical data of ruggedness of pioglitazone

Conc.	Area
3	150608
6	221964
9	295029
12	370883
15	441183

Table 24: Statistical data of ruggedness of glimepiride

Conc.	Area
0.4	16812
0.6	28766
0.8	39607
1.0	49589
1.2	61511

Table 25: Statistical data of degradation studies of metformin

Sr. no.	Degradation	Area of Standard	Area of degraded Sample	Degraded up to %	Actual % degradation
1	Acid Degradation	5822073	5566418	95.60886646	4.391133536
2	Basic Degradation	5822073	5445290	93.52837039	6.471629607
3	H2O2 Degradation	5822073	5818895	99.94541463	0.054585368
4	Thermal	5822073	5819926	99.9631231	0.036876899
5	Photolytic	5822073	5821547	99.99096542	0.009034583

Table 26: Statistical data of degradation studies of pioglitazone

Sr. NO.	Degradation	Area of Standard	Area of degraded Sample	Degraded up to %	Actual % degradation
1	Acid Degradation	440904	426845	96.81132401	3.188675993
2	Basic Degradation	440904	413594	93.80590786	6.194092138
3	H2O2 Degradation	440904	440145	99.82785368	0.172146318
4	Thermal	440904	440233	99.84781268	0.152187324
5	Photolytic	440904	440374	99.8797924	0.120207574

Table 27: Statistical data of degradation studies of glimepiride

Sr. NO.	Degradation	Area of Standard	Area of degraded Sample	Degraded up to %	Actual % degradation
1	Acid Degradation	61560	59446	96.56595192	3.434048083
2	Basic Degradation	61560	58265	94.64749838	5.352501624
3	H2O2 Degradation	61560	59546	96.72839506	3.271604938
4	Thermal	61560	61541	99.9691358	0.030864198
5	Photolytic	61560A	60563	98.38044185	1.619558155

3.8. Degradation studies

Five degradation parameters (acidic, basic, thermal, photolytic and peroxide) were performed to analyse the stability of all three drugs. Drugs are forcefully degraded under specified conditions and results were calculated to ensure the % degradation.

It was observed that all the three drugs are stable under the degradation parameters also. All the three drugs were carried out under five stressed conditions and the results obtained showing that the drugs are not affected that much i.e. all the results are under the limit criteria of degradation only.

4. Discussion

For simultaneous estimation of metformin, pioglitazone and glimepiride, an analytical method was developed and validated by UV spectroscopy and reverse phase chromatography. The proposed method was found to be specific, accurate and precise. The method was found to be linear which was performed by linearity study for all the three drugs which was studied as per their respective concentration. Around more than 0.99 regression coefficient value was observed which was within the limit. The retention time for metformin was observed at around 4.0 min, for pioglitazone it was observed 5.5 min and for glimepiride it was observed around 6.8 min. The

recovery for the method was also accurate within the limit as per the guidelines only which was observed within 98% to 102%. The percent relative standard was also observed below 2 value which falls under the guidelines. No degradation interference was observed while developing and validating the analytical method. It was also observed that the results are unaffected for the small variations in the method showing the repeatability of the developed analytical method. The method was found to be simple, rapid and robust for the analysis of metformin, pioglitazone and glimepiride.

5. Conclusion

For the simultaneous estimation of metformin, pioglitazone and glimepiride of stability indicating analytical method development and validation in the formulation by RP-HPLC technique, the method was found to be accurate, sensitive, reproducible, linear and precise. The results which was observed was found to be relevant that are carried out as per International Council on Harmonisation guidelines. Hence, for the quality control analysis the proposed method was carried out for the routine analysis in the tablet dosage form.

6. Source of Funding

None.

7. Conflict of Interest

None.

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