



Simple and Accurate Simultaneous HPLC Method Development and Validation for an Anti-Diabetic Multi-Component Dosage Form

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Abstract

The complexity of the multicomponent dosage forms is that multiple entities and excipients poses a considerable challenge to the analytical chemist during the development of an assay procedure. One of such formulation is of an anti-diabetic medicine comprising metformin hydrochloride (MET), gliclazide (GLZ) and pioglitazone (PIO). Many methods have been reported in the literature for the estimation of MET, GLZ and PIO individually and with other components by chromatographic methods. However, there is no simple reverse phase high performance liquid chromatography (RP-HPLC) method has been reported for the simultaneous estimation of MET, GLZ and PIO. Thus, we have attempted to develop and validate a simple HPLC method for MET, GLZ and PIO. The RP-HPLC method was successfully developed and validated according to ICH guidelines. The proposed method is simple, accurate, precise, and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of MET, GLZ and PIO in tablet dosage form without any interference from the excipients.

Keywords

Gliclazide, Metformin hydrochloride, Pioglitazone, RP-HPLC.

INTRODUCTION

The complexity of the multicomponent dosage forms is that multiple entities and excipients poses a considerable challenge to the analytical chemist during the development of an assay procedure. [1] Earlier, colorimetric and spectrophotometric methods were employed for drug analysis as they were economical and easily available. However, the use of these methods have reduced extensively due

to the lack of specificity, sensitivity and accuracy. For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method. [2]

One of the most multi-component dosage form is for diabetic disease. Metformin hydrochloride (MET), an insulin-sensitizing biguanide used to treat type-2 diabetes, has been found to be as effective as insulin

or sulfonylureas when used as monotherapy.[3-7] For many patients with type 2 diabetes, monotherapy with an oral anti-diabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control.[8] In such cases a combination of metformin hydrochloride and one of the sulfonylureas are used.[9] The fixed dose combination of gliclazide (80 mg) and metformin hydrochloride (500 mg) once or twice daily with meals to a maximum of 4 tablets per day (depending upon the glycemic control) showed significant efficacy in improving the glycemic control in type 2 diabetics.[10]

Gliclazide (GLZ) is a second generation sulphonylurea which that is widely used in the treatment of patients with type 2 diabetes because it has efficacy similar to other sulphonylureas but a lower risk of hypoglycemia. [11,12]

Pioglitazone hydrochloride (PIO) is an oral anti-hyperglycemic agent which acts primarily by decreasing insulin resistance. It is used in the treatment of type-II diabetes mellitus. [13]

Many methods have been reported in the literature for the estimation of MET, GLZ and PIO individually and with other components. However, there is no simple method has been reported for the simultaneous estimation of metformin hydrochloride, pioglitazone with gliclazide. Thus, we have attempted to develop and validate a simple HPLC method for MET, GLZ and PIO. [14-23]

MATERIAL AND METHODS

Chemicals

MET, GLZ and PIO API procured as gift sample from Ipca Laboratories Limited Mumbai. The marketed formulation (Glycinorm Total) with fixed dose combination tablets of the three drugs, (MET, GLZ, PIO) were purchased from retail pharmacy in Mumbai (Maharashtra, India). Other chemicals and reagents of analytical grade were purchased from Merck (India).

Instrumentation

The study was carried out on Jasco Autosampler HPLC (AS-5040, PU- 2080, UV-2075). The column used was Hypersil ODS C18 (25 * 4.6 mm, 5 μ m).

Development of HPLC method

Selection of mobile phase

Various solvent systems were tried for the development of suitable HPLC methods for the analysis of MET, GLZ and PIO using API and extracted formulation sample. The suitability of the solvent system was decided on the basis of the sensitivity of the assay, retention time, tailing factor and selection of flow rate.

Selection of flow rate

To determine the effect of flow rate, the programmed controller was set at different flow rates 0.5ml/min, 0.7 ml/min, 1.0 ml/min, 1.1 ml/min, 1.2 ml/min and 1.5 ml/min operate were performed at each flow rate.

Selection of analytical wavelength

An appropriate dilution of standard stock solution with mobile phase, various concentrations of MET, GLZ and PIO were accurately prepared. The solutions were scanned between the wavelength range 400-200 nm using the UV spectrophotometer.

Preparation of stock solution of API

MET, GLZ and PIO were weighed in ratio 33:4:1 and transferred in single vial. The stock solution of was prepared by dissolving the Api in methanol. Further dilutions were made to obtain 66, 8 and 2 μ g/ml solutions of MET, GLZ and PIO respectively.

Preparation of sample solution

Twenty tablets were weighed and crushed. The powdered drug equivalent to 500 mg of MET, GLZ of 60mg, PIO of 15mg and transferred in a single vial. 1ml of methanol was used to dissolve the powder completely.

Method validation: [24, 25]

Linearity and range

Under the optimized conditions, a calibration curve was prepared for MET, GLZ and PIO. Standard mixtures of different concentration were prepared for determining the working range. The corrected peaks were used to construct the calibration graph. Linearity range, regression equation, correlation coefficient, were evaluated.

Accuracy

In order to examine the accuracy of the method standard addition method was carried out. In this method, three different concentration of MET, GLZ and PIO were added to a constant known concentration of the composite solution. Each solution was injected and the amounts determined were compared to theoretical.

Precision

The precision of the method was tested by performing intra-day and inter-day studies. For intra-day studies, a triplicate of prepared samples was analyzed on the same day. For inter-day validation, concentrations were determined on a separate day. The % RSD values obtained from peak area for MET, GLZ and PIO were observed.

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

The LOD and LOQ were separately determined based on the signal to noise ratio. For LOD the S/N ratio is 3:1 and for LOQ the ratio is 10:1.

System suitability parameter

The system suitability test was carried out on freshly prepared standard stock solution of MET, GLZ and PIO. Parameters such as resolution, peak tailing HETP were studied to evaluate the suitability of the system.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and temperature. Effects of variation in the flow rate and wavelength were studied at two different concentrations.

Estimation of content of drugs in tablet formulation

The content of all three drugs was estimated in tablet formulation (Glycinorm Total) by the developed and validated HPLC method.

RESULTS AND DISCUSSION

Method development

To achieve the desired separation (resolution: $R_s \geq 1.5$) and retention time value (Rt: 0.2 - 0.8) different mobile phases of various combinations were used. The mobile phase containing methanol: water (70:30 v/v) with the flow rate of 1 ml/min was selected as it gave well resolved peaks of standard MET, GLZ and PIO. The optimum wavelength 229 nm selected for detection and quantitation. The Rt for standard MET, GLZ and PIO was found to be 10.025, 3.133 and 5.817 respectively. (Figure- 1).

Method validation

Linearity

The calibration curves were found to be linear for the concentration range of 66-330 $\mu\text{g/ml}$ for MET, 8-40 $\mu\text{g/ml}$ for GLZ and 2-10 $\mu\text{g/ml}$ for PIO. The standard working curve equation for MET was found to be $y = 89838x - 101064$ with correlation coefficient value $r^2 = 0.9953$, for GLZ standard working curve equation was found to be $y = 5320.7x + 1E+06$ with correlation coefficient value $r^2 = 0.9975$ and for PIO it was $y = 23093x + 128750$ and $r^2 = 0.9987$. The results of linearity are given in Table-1 and Figure- 2, 3, and 4.

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for MET, GLZ and PIO are given in Table-2.1, 2.2, 2.3. It was found that the method was accurate as the

percent recovery was in the range of 99 % for MET, 98% for GLZ and 100% for PIO.

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision was found to be 0.97 and 1.56, 1.76 and 0.42, 0.05 and 1.29 for MET, GLZ and PIO respectively and % RSD of intermediate (inter-day) precision was found to be 0.66 and 1.17, 0.81 and 1.28, 1.93 and 0.98 for MET, GLZ and PIO respectively. The results of precision studies are shown in Table-3.1, 3.2, 3.3 and 4.1, 4.2, 4.3.

Limit of detection and Limit of quantitation

It was calculated by standard deviation of the response and the slope of calibration curve. LOD and LOQ of the method were calculated and found to be 0.15 $\mu\text{g/ml}$ and 0.45 $\mu\text{g/ml}$ of MET, 0.12 $\mu\text{g/ml}$ and 0.33 $\mu\text{g/ml}$ of GLZ and 0.06 $\mu\text{g/ml}$ and 0.16 $\mu\text{g/ml}$ of PIO.

System Suitability

The method developed for the simultaneous estimation of MET, GLZ and PIO was found to be suitable as the resolution factor and symmetry were within the acceptable range. The resolution (R_s) was 6.135, 1.785 and symmetry was 0.809, 0.967 and 1.023 for GLZ, PIO and MET respectively. The tailing factor was also in limits, the data is shown in Table-5.

Robustness

It was measured by multiple injections of a homogenous sample containing MET, GLZ and PIO in concentration of 132 and 264 $\mu\text{g/ml}$ of MET, 16 and 32 $\mu\text{g/ml}$ of GLZ and 16 and 32 $\mu\text{g/ml}$ of PIO that indicates the performance of the HPLC instrument under chromatographic conditions by changing flow rate 0.8ml/min and 1.2 ml/min and by changing Wavelength i.e 226nm and 232nm nm. The method was found to be robust in the range of deliberate changes made. (Table-6.1, 6.2, 6.3, 6.4, 6.5, 6.6)

Estimation of content of drugs in tablet

The development and validation of HPLC method estimated that the content of MET, GLZ and PIO in tablet formulation was 99.68%, 100.30 % and 98.00 % respectively. The detailed data is mentioned in Table-7.

Table-1: Linearity data of MET, GLZ and PIO

Linearity MET		Linearity GLZ		Linearity PIO	
Concentration (µg/ml)	Area Average	Concentration (µg/ml)	Area Average	Concentration (µg/ml)	Area Average
66	6563341	8	1255545	2	176823
132	11024426	16	1304830	4	221546
198	17547338	24	1348685	6	263589
264	23154003	32	1385478	8	312088
330	30145042	40	1428048	10	362477

Table-2.1: Recovery data of MET

Level (%)	Drug Conc (mg)	Amt added (mg)	Total Amt (mg)	Amt recovered (mg)	% Recovery
80%	500	400	900	898.3	99.7
100%	500	500	1000	999.6	99.96
120%	500	600	1100	1098	99.81

a) Conc= Concentration, Amt= Amount

Table- 2.2: Recovery data of GLZ

Level (%)	Drug Conc (mg)	Amt added (mg)	Total Amt (mg)	Amt recovered (mg)	% Recovery
80%	60	48	108	106.0	98.14
100%	60	60	120	119.2	99.33
120%	60	72	132	130.0	98.48

a) Conc= Concentration, Amt= Amount

Table- 2.3: Recovery data of PIO

Level (%)	Drug Conc (mg)	Amt added (mg)	Total Amt (mg)	Amt recovered (mg)	% Recovery
80%	15	12	27	27.65	102.00
100%	15	15	30	30.12	100.40
120%	15	18	33	33.04	100.12

a) Conc= Concentration, Amt= Amount

Table- 3.1: Precision study (intra- day) of MET

Conc µg/ml	Area	AVG	SD	%RSD
132	11806552			
132	11984775	11937935	115332	0.97
132	12022478			
264	24554023			
264	25302254	24989620	388976	1.56
264	25112584			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table- 3.2: Precision study (intra- day) of GLZ

Conc µg/ml	Area	AVG	SD	%RSD
16	1246881			
16	1250244	1236006	21814	1.76
16	1210892			
32	1361128			
32	1352411	1354663	5684	0.42
32	1350451			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-3.3: Precision study (intra- day) of PIO

Conc µg/ml	Area	AVG	SD	%RSD
4	210153			
4	209963	210090.7	111	0.05
4	210156			
8	297684			
8	305410	301747.0	3879	1.29
8	302147			

- a) Conc= Concentration
b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-4.1: Precision study (inter-day) of MET

Conc µg/ml	Area	AVG	SD	%RSD
132	11901552			
132	11884775	11938068	78228.5	0.66
132	12027878			
264	24714023			
264	24162254	24396287	285248.8	1.17
264	24312584			

- a) Conc= Concentration
b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-4.2: Precision study (inter-day) of GLZ

Conc µg/ml	Area	AVG	SD	%RSD
16	1256881			
16	1288524	1282099	22698	1.77
16	1300892			
32	1360128			
32	1362411	1357663	6350	0.47
32	1350451			

- a) Conc= Concentration
b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-4.3: Precision study (inter-day) of PIO

Conc µg/ml	Area	AVG	SD	%RSD
4	220153			
4	219963	221091	1791	0.81
4	223156			
8	302684			
8	295410	299747	3834	1.28
8	301147			

- a) Conc= Concentration
b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-5: System suitability study

Std	Tailing	HETP	Resolution
MET	0.806	2343	6.195
GLZ	1.073	2891	Na
PIO	0.960	3208	1.785

a) Std= Standard

b) HETP= Height Equivalent to the Theoretical Plate

Table-6.1: Robustness study with change in flow rate of MET

Conc µg/ml	Area	AVG	SD	%RSD
132	11756552			
132	12004775	11954602	178337	1.49
132	12102478			
264	24558023			
264	24851546	24841297	278291	1.12
264	25114321			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table- 6.2: Robustness study with change in flow rate of GLZ

Conc µg/ml	Area	AVG	SD	%RSD
16	1246881			
16	1248524	1235432	21268	1.72
16	1210892			
32	1360128			
32	1352411	1354330	5116	0.38
32	1350451			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-6.3: Robustness study with change in flow rate of PIO

Conc µg/ml	Area	AVG	SD	%RSD
4	211863			
4	216663	216227	4164	1.93
4	220156			
8	292684			
8	296710	293513	2873	0.98
8	291147			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-6.4: Robustness study with change in wavelength of MET

Conc µg/ml	Area	AVG	SD	%RSD
132	11806552			
132	12084775	11971268	146010	1.22
132	12022478			
264	24554023			
264	24862254	24842954	279780	1.13
264	25112584			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-6.5: Robustness study with change in wavelength of GLZ

Conc µg/ml	Area	AVG	SD	%RSD
16	1282881			
16	1298524	1278099	23189	1.81
16	1252892			
32	1360127			
32	1372411	1361663	10068	0.74
32	1352451			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-6.6: Robustness study with change in wavelength of PIO

Conc (µg/ml)	Area	AVG	SD	%RSD
4	210153			
4	209963	211091	1791	0.85
4	213156			
8	292684			
8	295410	296414	4320	1.46
8	301147			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-7: Assay Results of Tablet Dosage Form

Parameter	MET	PIO	GLZ
Label claim amount (mg)	500	15	60
Amount found (mg)	498.4	14.7	60.2
% Purity	99.68	98.00	100.30

a) GL= GLZ, PI=PIO, M= MET

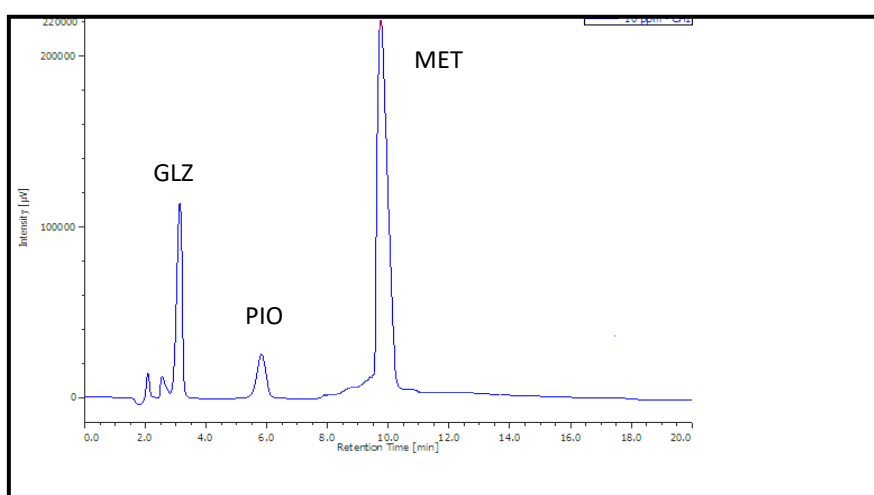

Figure-1: HPLC Chromatogram with three resolved peaks of GLZ, PIO and MET

Figure-2: Linearity curve of standard MET

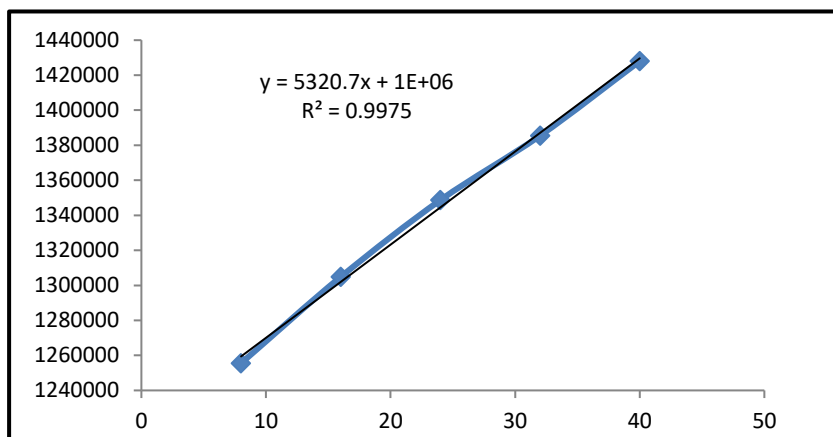


Figure-3: Linearity curve of standard GLZ

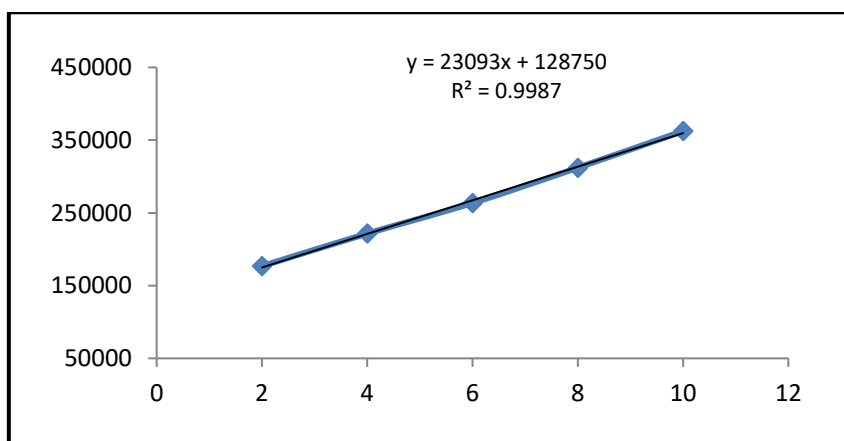
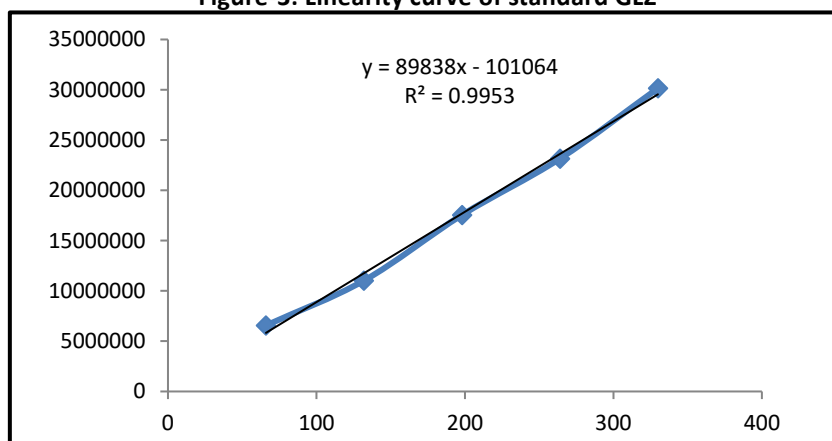


Figure-4: Linearity curve of standard PIO

CONCLUSION

The RP-HPLC method was successfully developed and validated. The proposed method is simple, accurate, precise, and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of MET, GLZ and PIO in

tablet dosage form without any interference from the excipients. From this study it was concluded that, the method can be employed for routine Quality Control analysis.

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