INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.3 | Issue 4 | Oct-Dec-2014
Journal Home page: www.ijpar.com

Research article Open Access

Development and validation of RP-HPLC method for simultaneous estimation of gliclazide and metformin in pure and tablet dosage form

Nirupama.D*, P.Venkateswara Rao, B.Thangabalan, S.Manohar Babu.

Department of Pharmaceutical Analysis, Sims College of Pharmacy, Vijayawada road, Guntur, India.

*** Corresponding author: Nirupama.D**

E-mail id: dnreddy.niru@gmail.com

ABSTRACT

A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Gliclazide(GLZ) and Metformin(MET) in pure and tablet dosage form. The method was carried on Phenomex (kromosil-250 mm × 4.6 mm, 5 µm) column with mobile phase comprising of phosphate buffer and methanol in the ratio 60:40 v/v. Flow rate was adjusted to 1.0ml/min and effluents were monitored at 230 nm. The retention time obtained for Gliclazide and Metformin was 5.0 and 3.2 mint respectively. The calibration curves were linear in the concentration range of 10-100µg/ml for Gliclazide and Metformin 62.5-625µg/ml for. The developed method was validated in accordance to ICH guidelines.

Keywords: Gliclazide, Metformin, RP-HPLC and Simultaneous estimation.

INTRODUCTION

Gliclazideis chemically 3-[(3aR, 6aS)-octahydrocyclopenta[c]pyrrol-2-yl]-1-(4-methyl-benzenesulfonyl) urea. It is an oral hypoglycemic agent used in treatment of diabetes mellitus type II. analytical methods including radioimmunoassay, gas chromatography, HPLC, Evaporative Light Scattering Detection, Charged Aerosol Detection and simultaneous estimation of gliclazide and metformin hydrochloride in combined dosage forms have been reported for determination of gliclazide.

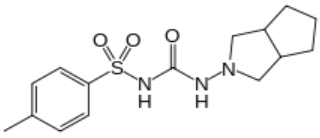

Figure 1: chemical structure of gliclazide.

Metformin[2] Hydrochlorideis chemically N, N-d imethylimido di carbonimidicdiamide hydrochloride. Metformin activates 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. AMPK probably also plays a role, as metformin administration increases AMPK activity in skeletal muscle.

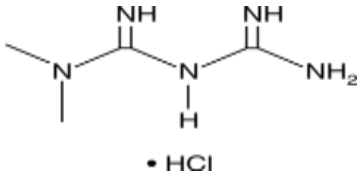

Figure 2: chemical structure of metformin

The objective of this study was to develop reverse phase high performance liquid chromatography method for the simultaneous estimation of gliclazide[3] and metformin in pure and pharmaceutical dosage form without any derivatization[4] and having the short retention time. The method was found to be linear, The analytical separations were carried out on a waters 2487 HPLC system with Photo Diode Array detector. The output of signal was monitored and integrated using LC-solutions 2000 software. The analytical column was phenomex (kromosil-250mm× 4.6mm,5µm).Mobile phase consisted phosphate buffer(pH-6.5) and methanol in the ratio of 60:40. Mobile phase was mixed, filtered through 0.45µ membrane filter and degassed under ultrasonication. The mobile phase was used as diluent. The flow rate was 1.0ml/min and runtime was 7minute. The column was maintained at ambient temperature. UV detection was measured at 230nm and the volume of sample injected was 10µl.

Preparation of mobile phase

Accurately weighed portion of 2.722g of potassium dihydrogen orthophosphate was dissolved in 200 ml of HPLC water. Separately 700mg of di-sodium hydrogen orthophosphate was weighed and dissolved in 20ml of HPLC water, the pH adjusted to 6.5 using disodium hydrogen ortho phosphate, and then the solution was filtered through a 0.22µm filter membrane and stored in closed container.

Preparation of standard stock solution

Accurately weighed 62.5mg of Metformin and 10mg of gliclazide was dissolved in diuent, in 100 ml volumetric flask, that gave 625 µg/ml of Metformin and 100 µg/ml of Gliclazide[1]. From this into a series of six 10ml volumetric flasks 1, 2, 4, 6, 8, 10ml were transferred and diluted to 10ml with diluents, that gave 62.5, 125, 250, 375, 500 and 625 µg/ml of Metformin and 10, 20, 40, 60, 80, and 100 µg/ml of Gliclazide.

Preparation of sample solution

20 tablets of combined formulation of Metformin and Gliclazide were weighed, average weight was calculated and triturated in a mortar with pestle from that, powder equivalent to 62.5 mg of Metformin and 10 mg of Gliclazide was weighed and dissolved in diluent and test concentration was prepared by further dilution with same.

RESULTS AND DISCUSSION

HPLC method development and optimization

To optimize the chromatographic conditions, different columns, mobile phases, flow rates etc., were tested. Buffer and methanol in the ratio of 60:40 was preffered as mobile phase. Because it resulted in a greater response to gliclazide, metformin after several preliminary investigatory runs compared with the different mobile phase combinations. The effect of the flow rate was studied in the range 0.9 to 1.1ml/min and 1.0ml/min was preffered to be effective. Under these conditions, the analyte peak obtained was well-defined and free from tailing. The retention time(RT) was found to be 5.0 and 3.2min. The optimized chromatographic parameters[5] were listed in table 1.

precise, accurate, sensitive, specific, and robust, and therefore suitable for routine analysis.

MATERIALS AND METHOD

HPLC Instrumentation and chromatographic conditions

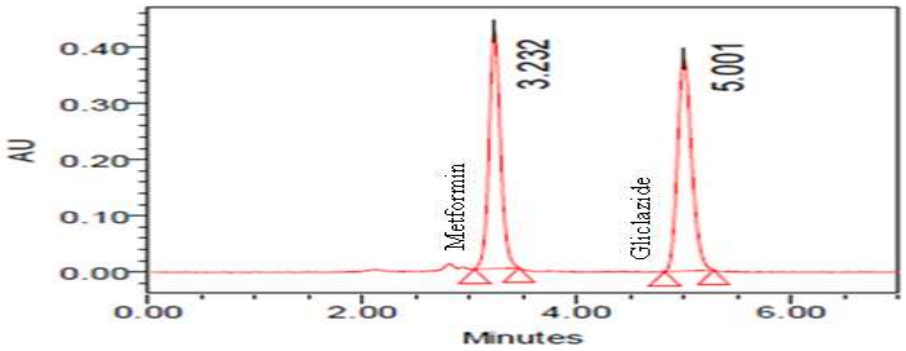

Fig 1: optimized chromatogram

Table 1: Optimized chromatographic parameters

S.NO	Optimized	Chromatographic parameters
1	Mobile Phase	Buffer:Methanol(60:40 v/v)
2	Pump Mode	Isocratic
4	Diluents	Mobile phase
5	Column	Phenomex C18 (150mm×4.6mm×5µ)
6	Column Temperature	Ambient
7	Wavelength	230 nm
8	Injection Volume	10 µl
9	Flow Rate	1 ml/min
10	Run Time	7min
11	Retention Time	5.0 min & 3.2 min

VALIDATION OF THE METHOD

When method development and optimizations are complete, it is necessary to accomplish method validation[6]. the validation studies include linear range (Correlation coefficient), method precision (RSD,%), method accuracy (%Recovery and RSD%), sensitivity studies (LOD & LOQ), and robustness.

System suitability studies

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), tailing factor(T), and peak asymmetry (AS), resolution (RS) were evaluated. The system suitability test was performed before analysis of sample. The system suitability method acceptance criteria set in each validation run were: capacity factor>2.0; tailing factor <2.0 and theoretical plates>2000. In all cases, the relative standard deviation(R.S.D) for the analytic peak area for two consecutive injections was <2.0%. system suitability parameters were shown in table 2.

Table 2: system suitability parameters

parameters	gliclazide	metformin
Retention time	5.0	3.2

Linearity

The linearity of the method was evaluated by preparing six series of standard solutions of gliclazide and metformin in the range of 10-100 µg/ml, 62.5-625µg/ml in mobile phase and injecting the solution into the HPLC system. Excellent correlation between gliclazide, metformin peak area and concentration was observed with (R^2)=0.999, (Figure.3). the regression equation was found to be Y=4617.x + 11446 and Y=793.3x + 13107 respectively. The statistical data are presented in table 3. And the calibration curve was shown in figure 3.

Table 3: linearity results for gliclazide and metformin

	Gliclazide		Metformin	
S.No	**Conc(µg/ml)**	**Peak Area**	**Conc(µg/ml)**	**Peak Area**
1	10	154403	62.5	175033
2	20	206452	125	232162
3	40	305646	250	330190
4	60	397427	375	437433
5	80	482011	500	525305
6	100	572203	625	623402

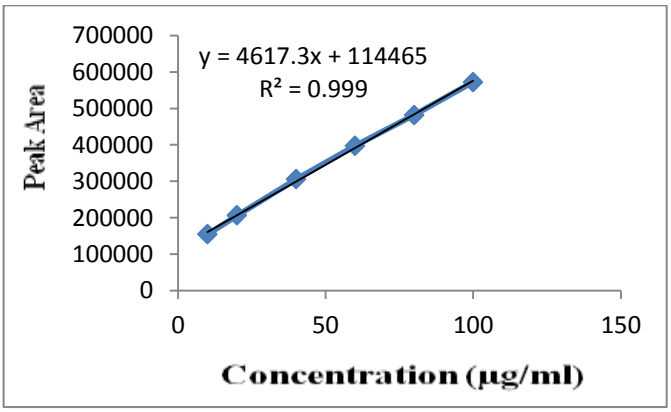

Fig 2. Calibration curve for gliclazide

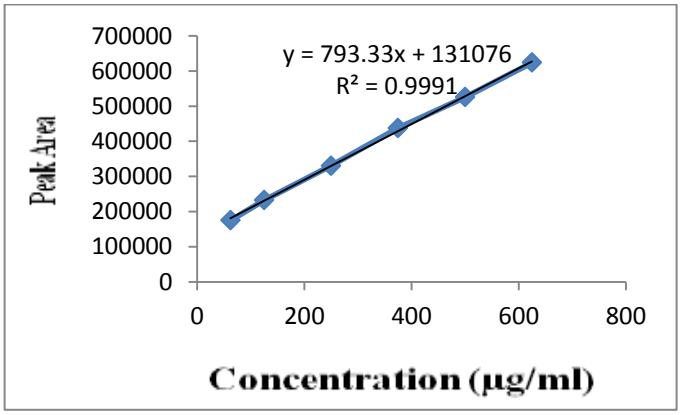

Fig 3. Calibration curve for metformin

Precision

System precision(Repeatability)

To study precision, five replicate standard solutions of gliclazide (40µg/ml), metformin(250µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation[7] (%RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4: system precision results for gliclazide and metformin

S.No	Gliclazide		Metformin	
	Rt(min)	Peak Area	Rt(min)	Peak Area
1	4.975	350236	3.234	315642
2	4.962	350654	3.233	315371
3	4.943	350693	3.231	315598
4	4.965	350398	3.239	315877
5	4.978	350451	3.221	315668
Mean	4.9646	350486.4	3.2316	315631.2
St.deviation	0.013795	188.7731	0.006618	180.7587
%RSD	0.277866	0.05386	0.204795	0.057269

Method

The intraday and inter-day precision of the proposed method was determined by analyzing the correspomding responses 6 times of the same day and on different days for concentration of 10µg/ml of gliclazide and 100µg/ml of metformin. The results was reported in terms of relative standard deviation (%RSD), results of method precision studies were shown in table 5.

Precision (Reproducibility)

Table 5: Method precision results for gliclazide and metformin

S.No	Gliclazide		Metformin	
	Rt(min)	Peak Area	Rt(min)	Peak Area
1	5.005	3503557	3.234	3152466
2	5.007	3505877	3.233	3151745
3	5.006	3503969	3.231	3158954
4	5.006	3508933	3.229	3158663
5	5.004	3501546	3.230	3155433
Mean	5.0056	3504776	3.2314	3155452
St.deviation	0.00114	2786.708	0.002074	3362.772
%RSD	0.022778	0.079512	0.064172	0.10657

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts. (Analyst 1 and Analyst 2) for concentration of sample solutions of gliclazide (40µg/ml), metformin (250µg/ml). The percent relative standard deviation (%RSD) for peak responses was calculated. The results for intermediate were shown in table 6.

Table 6: results of intermediate precision for gliclazide, metformin.

Repeatability (% RSD) (n=6)	Intermediate precision (% RSD) (n=6)			
	Day 1		Day 2	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
GLZ	0.0815	0.0398	0.0632	0.0808
MET	0.0777	0.0128	0.0521	0.0636

Accuracy

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at different concentrations are accordance with ICH guidelines, by replicate analisis (n=3). The closeness of obtained value to the true value indicates that the proposed method is accurate. Recovery studies were shown in table 7.

Table 7: Results of recovery studies for gliclazide, metformin

Sample	Spiked Amount (mg)	Recovered Amount (mg)	% Recovered	% Average Recovery
GLZ	20	19.76	99.54	99.90
	40	40.03	100.03	
	60	60.01	100.006	
MET	125	125.02	100.7	100.06
	250	250.01	100.27	
	375	375.01	101.07	

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition, the robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength.

- Change the dection wavelength ± 2nm(228nm and 232nm)
- Change in flow rate by ± 0.2ml/minute (1.1ml/min and 0.9ml/min)

After each change, sample solution was injected and %assay with system suitability parameters were checked. Robustness values were given in table 8.

Table 8: results of robustness for gliclazide and metformin

Drug	Parameters count	Changes	RT(min)	USP Tailing	USP Plate
GLZ	Flow rate (ml/min)	0.9	6.1	1.2	7487
		1.2	4.1	1.1	5954
MET	Flow rate (ml/min)	0.9	3.9	1.2	5203
		1.2	2.6	1.1	7487

Limit of Detection and Quantitation

Detection and quantitation limit were calculated by the method based on the standard deviation and slope of the calibration plot, using the formula.

Limit of Detection = $3.3\times\sigma/S$, Limit of Quantitation=$10\times\sigma/S$. Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Table 9: Results if LOD, LOQ for gliclazide, metformin

Name of drug	LOD (µg/ml)	LOQ (µg/ml)
GLZ	0.0750µg/ml	0.2273µg/ml
MET	0.6557µg/ml	1.9872µg/ml

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions were compared in order to provide an indication of specificity of the method.

Assay

The proposed validated method was successfully applied to determine gliclazide, metformin in their pharmaceutical dosage form and the % assay results were shown in table 10.

Table 10: Results of % assay by using RP-HPLC method

Sample	Spiked Amount (mg)	Recovered Amount (mg)	% Recovered	% Assay
GLZ	20	19.76	99.54	99.90
	40	40.03	100.03	
	60	60.01	100.006	
MET	125	125.02	100.7	100.06
	250	250.01	100.27	
	375	375.01	101.07	

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method for the analysis of gliclazide and metformin in pure and in pharmaceutical dosage forms had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed is cost-effective due to short retention time which enabled analysis of gliclazide and metformin samples with a small amount of mobile phase. From the%RSD values of precision and recovery studies the method was found to be precise and accurate. The low detection and quantification limits achieved indicate the method is very sensitie. The robustness data gathered during method validation showed that the method is not susceptible to small changes in chromatographic conditions, the proposed RP-HPLC method developed by the author is suitable for routine analysis ana quality assessment of gliclazide, metformin in pharmaceutical products.

Table 11: summary of validated parameters for proposed method

Parameters	GLZ	MET
Linearity (µg/ml)	10-100	62.5-625
Regression equation	4617.x + 11446	793.3x + 13107
Slope (m)	4617	793.3
Intercept (C)	11446	13107
Correlation coefficient (r^2)	0.999	0.999
Method precision (%RSD, n=5)	0.06	0.10
LOD (µg/ml)	0.0750µg/ml	0.2273µg/ml
LOQ (µg/ml)	0.6557 µg/ml	1.9872µg/ml
% Assay	99.89%	99.98%

REFERENCES

[1] Bhagyashri V. Vadnere, Aarti M. Jain and Priyanka S. Jadhav, Prof. S. P. Patil, Dr. S. D. Barhate have developed and validation for the simultaneous estimation of gliclazide metformin and in tablet dosage form by RP-HPLC.Journal of Pharmacy Research 2012, 5(10), 5036-5038.

[2] http://en.wikipedia.org/wiki/Metformin.

[3] http://en.wikipedia.org/wiki/Gliclazide.

[4] Edigasasikirangoud, V.Krishna Reddy, Chandra K Sekharhave developed andvalidation for the simultaneous estimation of metformin and gliclazide in tabletdosage form by RP-HPLC. International Journal of Pharmacy and Biological Sciences,Volume 2, Issue 4, OCT-DEC-2012

[5] A.anusha, N.Prajwala. M.Sandhya, Dr.UmaMaheswara Rao have developed and validation for the simultaneous estimation of metformin and gliclazide in tablet dosage form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences, Volume- 5, Suppl 4, 2013

[6] KanijFatema, Md.zakirRahman, TasnuvaHaque, Mohammad abulkalamazad, and Md.selimreza, Development and validation of a simple method for simultaneous estimation of Metformin hydrochloride and gliclazide in tablets by using RP-HPLC, Dhaka UnivJ.PharmSci , 2010, 9(2) : 83-89

[7] B.V.V.Ravikumar, A.K. Patnaik, Saroj Kumar Raul, NagireddyNeelakanta Rao Development and Validation of a simple method for the Estimation of Gliclazide in bulk and Pharmaceutical Dosage Forms by RP-HPLC. Journal of Applied Pharmaceutical Science Vol. 3 (04), pp. 059-062, April, 2013.