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RESEARCH ARTICLE

Simultaneous Estimation of Metformin HCl and Gliclazide by Q- Analysis Method

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ABSTRACT

Q- Analysis method for simultaneous estimation of Metformin HCl and Gliclazide in tablet dosage form have been developed. The method was simple, precise, accurate, reproducible and economical. Linearity was observed in the concentration range of 2-12 µg/ml for GLZ and 2-12 µg/ml for MET. The result of analysis have been validated as per ICH Guidelines.

KEYWORDS

Gliclazide, Metformin hydrochloride, λmax, Q- Analysis method.

INTRODUCTION

Gliclazide(GLZ), a sulphonyl urea derivative is used as an oral hypoglycemic agent. Chemically it is 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-pmethylpheylsulphonylurea.

It is official in British Pharmacopoeia 2007. Metformin Hydrochloride (MET) is 3-(diaminomethylidene)-1, 1-dimethylguanidine. It is an oral anti-diabetic drug which is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese people and those with normal kidney function. It is an oral anti-diabetic drug from the biguanide class. It is the first-line drug for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidence suggests it may be the best choice for people with heart failure.

The combination of GLZ and MET is used in non insulin dependent diabetes mellitus. Non aqueous method have been reported for analysis of gliclazide in B.P. and metformin hydrochloride in I.P.

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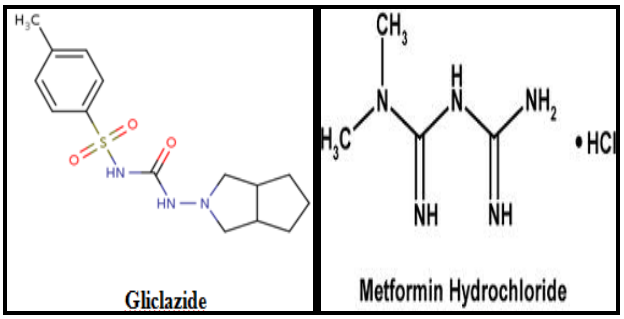
Several HPLC methods have been developed for gliclazide and metformin hydrochloride in plasma. Literature survey revealed that Q-Analysis method is not developed for estimation of gliclazide and metformin hydrochloride in combine dosage forms Simultaneous UV spectrophotometric methods have been reported for estimation of gliclazide and metformin hydrochloride in combine dosage forms. Hence, an attempt has been made to develop new Q-Analysis UV method for its estimation in pharmaceutical dosage formulations with good accuracy, simplicity and sensitivity.



MATERIALS AND METHODS

Instrument

Shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1 cm matches quartz cell.

Materials

Gift samples of MET and GLZ were procured from Emcure Pharmaceutical LTD, Pune. Tablets containing both drugs i.e. Metformin Hydrochloride and Gliclazide were purchased from local pharmacy of commercial brand Glizid-M (Panacea biotech)

Absorbance ratio (Q Analysis) U.V. spectrophotometric method

Apparatus

A Shimadzu UV-1700 UV/VIS spectrophotometer with UV Winlab Software was used. Sartorius ADD-BL-02 balance was used. Calibrated glasswares were used for the study. Trans-o-sonic ultrasonicator, 0.45 µ filter papers (PVDF/Nylon filters) also used.

Selection of Solvent

Water was selected as the solvent for dissolving Metformin Hydrochloride and Gliclazide.

Preparation of Standard Solutions

In order to prepare stock solution, 10 mg Metformin hydrochloride and 10 mg Gliclazide were accurately weighed in two separate volumetric flasks, dissolved in water and diluted to 100 ml with the water. Standard solution was prepared by further diluting 1 ml stock solution with 10 ml water to obtain 10 µg/ml concentration of Metformin hydrochloride and 10 µg/ml concentration of Gliclazide.

Determination of λmax

Both the standard solutions were scanned separately between 400 nm to 200nm. The overlain spectrum of both drugs was recorded, Fig. 1; from the overlain spectrum, two wavelengths 233 nm (λmax of Metformin hydrochloride) and 229 nm (Isoabsorptive point) were selected for estimation of drugs using absorbance ratio method.

Study of Beer-Lambert's Law

Aliquots of standard stock solution of Metformin Hydrochloride and Gliclazide were diluted in a series of 100 ml flasks with water to get concentration in range of 2-12 µg/ml for Metformin hydrochloride and 2-12 µg/ml for Gliclazide. Similarly aliquots of mixed standard stock solution were diluted in a series of 100 ml flasks with water to get concentration in range of 2-12 µg/ml for Metformin hydrochloride and 2-12 µg/ml for Gliclazide. Absorbances of each of the resulting solutions were measured at 233 nm and 229 nm in 1.0 cm cell using solvent blank.

Figure 1: Overlein spectrum of Metformin HCl and Gliclazide

Determination of Absorptivity Value at Selected Wavelength

For Metformin hydrochloride

An accurately weighed quantity of Metformin hydrochloride (10mg) was transferred in 100 ml volumetric flask, dissolved in water, then dilute upto mark with water (concentration of stock solution: 100 µg/ml). 1 ml of above stock solution further diluted to 10 ml with water to form final standard solution 10 µg/ml)

For Gliclazide

An accurately weighed quantity of Gliclazide(10mg) was transferred in 100 ml volumetric flask, dissolved in water, then dilute upto mark with water (concentration of stock solution: 100 µg/ml). 1 ml of above stock solution further diluted to 10 ml with water to form final standard solution 10 µg/ml). The absorbance of each of the above solutions were measured in triplicate in 1.0 cm cell against solvent blank at 233 nm, 229 nm and A (1%,

1cm) values were calculated using formula as given below.

A (1%, 1cm) = Absorbance / Concentration (g/100ml)

The results are shown in Table. 1a, 1b.

Table 1a: A (1%, 1cm) values of Metformin HCl at 229 and 233 nm

Sr. No.	Mean A (1%, 1 cm) for Metformin HCl	
	229 nm	233 nm
1	1017	953
2	1013	954
3	1013	953
4	1014	953
5	1016	954
Mean ± S. D.	1014.6 ± 1.816	953.4 ± 0.547

Table 1b: A (1%, 1cm) values of Gliclazide at 229 and 233 nm

Sr. No.	Mean A (1%, 1 cm) for Gliclazide	
	229 nm	233 nm
1	388	219
2	387	218
3	387	218
4	386	217
5	387	218
Mean ± S. D.	387 ± 0.707	218.0 ± 0.707

Application of Proposed Method for Estimation in Standard Laboratory Mixture

An accurately weighed quantity of Metformin hydrochloride (10 mg) and Gliclazide(10 mg) were transferred to a 100 ml volumetric flask, sufficient quantity of water was added, shaken and dilute upto mark with water. Further 1 ml portion of above stock solution was diluted to 10 ml with water. The absorbance of resulting solution was measured at 233 nm and 229 nm in 1.0 cm cell using water as blank. The contents of Metformin Hydrochloride and Gliclazide were calculated by substituting values in the formulae given below:

For estimation of MET For estimation of FNB

$$Cx = \frac{Qm-Qy}{Qx-Qy} \times \frac{A}{ax} \qquad Cy = \frac{Qm-Qx}{Qy-Qx} \times \frac{A}{ay}$$

Where,

Cx = Concentration of Metformin hydrochloride in g/100ml

Cy = Concentration of Gliclazidein in g/100ml

Qm =Ratio of absorbance of laboratory mixture at 233 nm and 229 nm

Qx =Ratio of absorptivity of Metformin hydrochloride at 233 nm and 229 nm

ax = Absorptivity of Metformin hydrochloride

ay = Absorptivity of Gliclazide

A = Absorbance of mixture at Isoabsorptive point.

Amount of drug estimated = C x D x V

Where,

C = Cx or Cy = Concentration of MET or GLI (mg/ml)

D = Dilution factor = 10

V = Volume of stock solution = 100 ml

From the amount estimated by above method, percentage estimation was calculated by using formula.

$$\% \text{ Estimation} = \frac{\text{Amount of drug estimated}}{\text{Weight of drug taken}} \times 100$$

The results of laboratory mixture study are shown in Table 2.

Table 2: Analysis of Metformin HCl and Gliclazide in standard laboratory mixture

Analysis of Standard Laboratory Mixture				
Sr no.	Metformin estimated	Gliclazide estimated	% Estimation Metformin	% Estimation Gliclazide
1	10.12	10.04	101.2	100.4
2	10.05	9.99	100.5	99.9
3	10.15	10.01	101.5	100.1
4	10.18	10.05	101.8	100.5
5	10.16	10.03	101.6	100.3
Mean ± SD			101.32 ± 0.506	100.24 ± 0.240
% RSD			0.50034709	0.240255

Application of Proposed Method for Estimation of MET and GLI in Tablet Dosage Forms

Sample preparation: 20 tablets were weighed and powdered, quantity of sample powder containing equivalent to 500 mg of MET and 80 mg of GLI was transferred to 200 ml volumetric flask, sufficient quantity of water was added, and sonicated for 15 minutes and diluted upto mark with water. Filtered through 0.45µ membrane filter paper. A 2 ml of filtrate was further diluted to 100 ml with water to get final concentration of about 50 µg/ml MET and 8 µg/ml GLI. The absorbance of resulting solutions was measured at 233 nm and 229 nm in 1.0 cm cell using solvent blank. The results were calculated by using the same formula as described under estimation of standard laboratory mixture. Further % labeled claim was determined using the formula.

$$\% \text{ labeled claim} = \frac{\text{Amt estimated} \times \text{Avg weight}}{\text{Weight taken} \times \text{Label claim}} \times 100$$

The results of estimation in marketed formulation are shown in Table 3.

Accuracy Study

It was done by recovery study. Sample solutions were prepared by spiking at about 80 %, 100 % and 120 % of specification limit to Placebo and analyzed by the proposed method. % Recovery was determined using the formula.

$$\text{Percentage Recovery} = \frac{\text{Amount found}}{\text{Actual amount added}} \times 100$$

$$\text{Actual amt. added (mg)} = \frac{\text{Amount added} \times \text{potency}}{100}$$

Results are shown in Table 4.

Method Validation

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies. Results are shown in Table 4.

Precision

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of the method was studied as intra-day and inter-day variations. 20 tablets were weighed and powdered, quantity of sample powder containing equivalent to 500 mg of MET (and 80 mg of GLI) was transferred to 200 ml volumetric flask, sufficient quantity of water was added, and sonicated for 15 minutes and diluted upto mark with water.

Table 3: Analysis of Metformin HCl and Gliclazide in commercial formulation

Sr. No.	Sample taken in mg	Metformin HCl mg/tab	Gliclazide mg/tab	% Estimation of Metformin HCl	% Estimation of Gliclazide
1	650.81	500.21	80.12	100.04	100.15
2	650.89	499.94	79.89	99.988	99.86
3	651.21	499.98	80.21	99.996	100.26
4	651.12	500.12	79.98	100.024	99.97
5	650.88	499.12	79.96	99.824	99.95
Mean ± S.D.				99.9744± 0.087	100.038 ± 0.163
% RSD				0.0866755	0.162634

Table 4: Standard Addition Technique for determination of Metformin HCl and Gliclazide using Absorption ratio method (n=3)

Compound (mg)	Amount added (%)	Total amount (mg)	Amount found (mg)	Recovery (%)	Average (%)
Metformin (500)	80	900	896.5 ± 0.508	99.61± 0.05	99.39
	100	1000	995.9 ± 0.611	99.59 ± 0.10	
	120	1100	1091.4 ± 0.519	99.21 ± 0.28	
Gliclazide (80)	80	144	145.12 ± 0.810	100.77 ± 0.03	99.68
	100	160	159.96 ± 0.558	99.98 ± 0.04	
	120	176	175.98 ± 0.673	99.99± 0.08	

Table 5: Precision studies of proposed absorption ratio method

Drug	Conc. µg/ml	Intraday Found Conc. ± SD	RSD (%)	Interday Found Conc. ± SD	RSD (%)
Metformin HCl	50	39.87 ± 0.082	0.438	39.89 ± 0.098	0.299
		59.86 ± 0.016	0.027	59.82 ± 0.024	0.190
		79.91 ± 0.029	0.036	80.01 ± 0.028	0.082
Gliclazide	08	39.89 ± 0.043	0.108	39.99 ± 0.045	0.245
		60.07 ± 0.045	0.075	60.04 ± 0.049	0.082
		80.07 ± 0.025	0.031	80.03 ± 0.029	0.036

Filtered through 0.45µ membrane filter paper. A 2 ml of filtrate was further diluted to 100 ml with water to get final concentration of about 50 µg/ml MET and 8 µg/ml GLI. The absorbance of final the solution was measured after 0 hr, 3hr and 6hr in 1.0 cm cell at 229 nm and 233 nm. Similarly the absorbance of the same solution was measured on day 1st, 3rd and 5th and the percent assay was calculated using formulae as described under marketed formulation.

The results are shown in Table.5.

Linearity and Range

Accurately weighed quantities of tablet content equivalent to 80% to 120% of label claim of MET and GLI were taken and dilutions were made as described under marketed formulation. The absorbance of resulting solution was measured at 233 nm and 229 nm in 1.0 cm cell using solvent blank. The graphs was constructed as % label claim versus absorbance plot and found to be linear as depicted in Fig.2, 3.

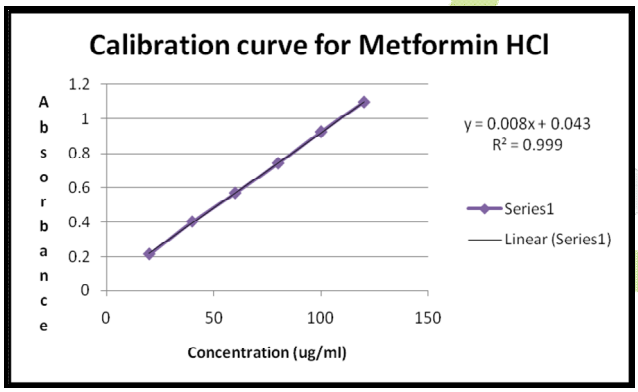


Figure 2: Calibration curve for Metformin Hydrochloride

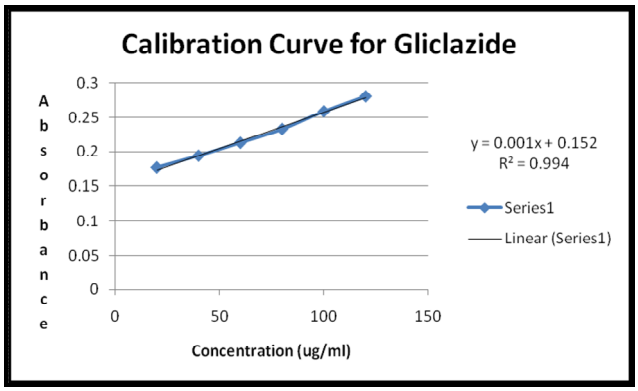


Figure 3: Calibration curve for Gliclazide

Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogeneous slot by two analyst using same operational and environmental conditions; the results are shown in Table.6.

Table 6: Ruggedness study

Analyst 1	**Metformin HCl**	**Gliclazide**
Mean(%Assay)±SD	99.98 ± 0.087	100.040 ± 0.163
% R.S.D	0.090	0.16
Study by ANALYST 2		
Analyst 2	**Metformin HCl**	**Gliclazide**
Mean (%Assay) ± SD	99.98 ± 0.087	100.040 ± 0.163
% R.S.D	0.090	0.16

RESULT AND DISCUSSION

Metformin Hydrochloride and Gliclazide, indicated for the treatment of non-insulin dependent type II diabetes mellitus. Literature scan revealed no absorbance ratio UV spectrophotometric method was developed for the determination of Metformin Hydrochloride and Gliclazide. Fig 1 shows typical overlain spectrum of Metformin Hydrochloride and Gliclazide. The linearity of method was statistically confirmed. The correlation coefficients (r) for calibration curves were not less than 0.99. The relative standard deviation values of the slope were not more than 2%. The analytical recovery at three different concentrations of Metformin Hydrochloride and Gliclazide was determined. In absorbance ratio method, 233 nm which was λ max of Metformin Hydrochloride and 229 nm which was isoabsorptive point, were selected for analysis.

Absorbance ratio UV spectrophotometric method was validated. Therefore proposed validated method was successfully applied to determine Metformin Hydrochloride and Gliclazide in tablet dosage form.

CONCLUSION

Absorption Ratio method was developed and validated as per ICH guidelines for estimation of Metformin HCl and Gliclazide. This method was applied for estimation of these compounds in the marketed formulation. The method has been evaluated for the linearity, accuracy, precision and Robustness in order to ascertain the suitability of the method. It has been proved that the developed method was linear in the concentration range of 2-12 ug/ml.

High percentage recovery showed that method was free from interference of excipients used in the formulations. The results of the study indicates that the proposed absorbance ratio UV spectrophotometric method of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Metformin Hydrochloride and Gliclazide.

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