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## DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF GLICLAZIDE AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY SIMULTANEOUS EQUATION METHOD

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### ABSTRACT

A simple, rapid, accurate, precise, specific and economical spectrophotometric method for simultaneous estimation of Gliclazide (GLC) and Metformin hydrochloride (MET) in combined tablet dosage form has been developed. It employs formation and solving of simultaneous equation using two wavelengths 227.0 nm and 237.5 nm. This method obeys Beer's law in the employed concentration ranges of 5-25 µg/ml and 2.5-12.5 µg/ml for Gliclazide and Metformin hydrochloride, respectively. Results of analysis were validated statistically and by recovery studies.

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**INTRODUCTION:** Metformin hydrochloride (*N, N*-dimethylimidodicarbonimidic diamide hydrochloride or 1, 1-dimethyl biguanide hydrochloride) is oral antihyperglycemic drugs used in the management of type 2 diabetes<sup>1,2</sup>. It is an antihyperglycemic agent, not hypoglycemic which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. It does not cause insulin release from the pancreas and does not cause hypoglycemia, even in large doses. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin<sup>3,4</sup>.

Gliclazide (1- (3- azabicyclo [3.3.0] oct- 3- yl) - 3- p-tolylsulfonylurea or 1-(hexahydrocyclopenta [c]pyrrol-2 (1H)- yl)- 3- [(4-methylphenyl) sulfonyl] urea) is hypoglycemic agent used in the treatment of type-II diabetes mellitus<sup>2,5</sup>. It reduces blood glucose levels by correcting both defective insulin secretion and peripheral insulin resistance, increasing the sensitivity of β-cells to glucose, decreasing hepatic glucose production, and increasing glucose clearance. It also has anti-platelet adhesive activity and reduces levels of free radicals, thereby preventing vascular complications. It also has been reported to reduce plasma cholesterol and triglyceride levels after repeated administration<sup>3,6</sup>. Gliclazide and Metformin hydrochloride are now widely used in combination for the effective treatment of type-II diabetes mellitus.

Literature survey revealed that several methods like UV-Visible spectrophotometry, HPLC are available for the drug identification, impurity analysis, metabolite identification and assay of Gliclazide and Metformin hydrochloride as an individual drug and in combination with other drug/s in bulk drug, formulations and biological fluids<sup>7-26</sup>.

The HPLC method has been reported for the simultaneous estimation of Gliclazide and Metformin hydrochloride in tablet dosage form. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible U.V method for simultaneous estimation of Gliclazide and Metformin hydrochloride in tablet dosage form.

## MATERIALS AND METHODS:

**Apparatus:** A double beam UV-visible Spectrophotometer (Elico Ltd, SL 164, India), attached to a computer software UV-VIS Spectrasoft, with a spectral width of 2 nm, wavelength accuracy of  $\pm 0.5$  nm and pair of 1 cm matched quartz cells, Analytical balance (Keroy (Balance) Pvt. Ltd, KEROY, Varanasi, India), Ultrasonicator (Toshniwal Process Instruments Pvt. Ltd., TOSHCON SW 4, Ajmer, India), Corning volumetric flasks and pipettes of borosilicate glass were used for the development of proposed method.

**Reagents and Materials:** Gliclazide (GLC) and Metformin Hydrochloride (MET) were provided by RPG Life Sciences Ltd (Ankleshwar, India) and Troikaa Pharmaceuticals Ltd (Ahmedabad, India) respectively. Methanol, acetonitrile were of analytical reagent grade and purchased from S. D. Fine Chemicals Ltd., India. HPLC grade methanol and acetonitrile were purchased from S. D. Fine Chemicals Ltd., India. The marketed formulations, tablet(s), were procured from the local market which had a label claim of Gliclazide 80 mg and Metformin hydrochloride 500 mg.

**Preparation of Standard Stock Solution:** Stock solutions (100 $\mu$ g/ml) of Gliclazide and Metformin

hydrochloride were prepared by dissolving separately 10 mg of drug in methanol and making up the volume with methanol. The stock solution was suitably diluted to produce solution of concentration 10  $\mu$ g/ml. These working solutions were scanned in the entire UV range (200-400 nm) to determine the  $\lambda_{max}$ .

**Procedure for Calibration Curve:** Standard solutions of Gliclazide in the concentration range of 5  $\mu$ g/ml to 25  $\mu$ g/ml obtained by transferring (0.5, 1, 1.5, 2 and 2.5 ml) of Gliclazide stock solution (100  $\mu$ g/ml) to the series of 10 ml volumetric flasks and standard solutions of Metformin hydrochloride in the concentration range of 2.5  $\mu$ g/ml to 15  $\mu$ g/ml were obtained by transferring (0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml) of Metformin hydrochloride stock solution (100  $\mu$ g/ml) to the series of 10 ml volumetric flasks. Then methanol was added to each volumetric flask up to 10 ml. All dilutions were scanned in wavelength range of 200 nm to 400 nm. The absorbances were plotted against the respective concentrations to obtain the calibration curves.

**Estimation of Gliclazide and Metformin hydrochloride in Pharmaceutical tablet Dosage form:** Twenty tablets were weighed and finely powdered. The powder equivalent to 16 mg GLC and 100 mg MET was accurately weighed and transferred to volumetric flask of 100 ml capacity contains 25 ml of the methanol and sonicated it for 5 min. The flask was shaken and volume was made up to the mark with methanol. This solution was carefully filtered through Whatman filter paper (No. 41). Aliquot (0.06 ml) was pipette out and transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with HPLC grade methanol to give a solution containing 6.0  $\mu$ g/ml MET and 0.96  $\mu$ g/ml GLC. The absorbance of sample solution was measured at 227.0 nm and 237.5.0 nm against blank. The content of Gliclazide and Metformin hydrochloride in tablet was calculated using two framed simultaneous equations and results of analysis are shown in **Table 1**.

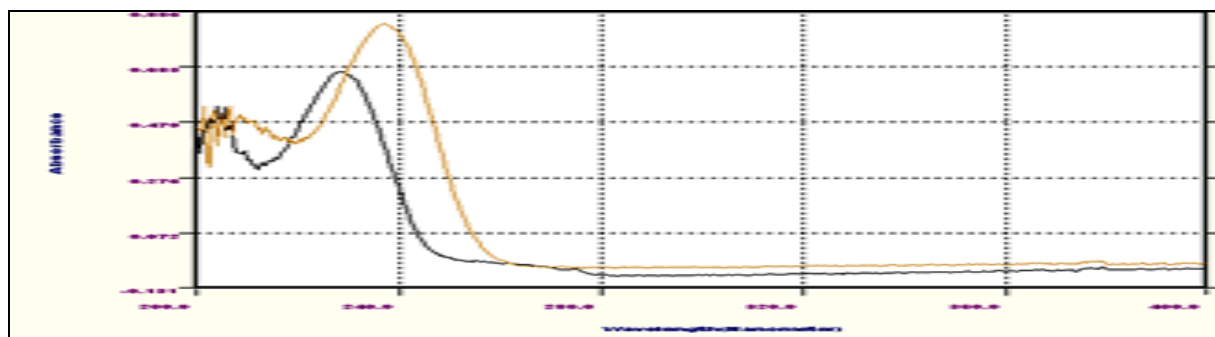
TABLE 1: RESULTS OF ANALYSIS OF TABLET FORMULATIONS

Formulation	Label claim Amount (mg)		Amount Found (mg)		%Recovery $\pm$ SD	
	GLC	MET	GLC	MET	GLC	MET
Formulation A	80	500	79.808	499.55	99.76 $\pm$ 0.0213	99.91 $\pm$ 0.0089
Formulation B	80	500	79.232	500.3	99.04 $\pm$ 0.0385	100.06 $\pm$ 0.0159

**Recovery Studies:** Recovery studies were done so as to check the accuracy of the method. The accuracy of the method was assessed by taking known amounts of Gliclazide and Metformin hydrochloride in standard mixture solution and absorbance were determined at 227.0 nm and 237.5 nm. Concentration of the drugs in the mixture was calculated using the equations. The analysis was done in a set of 3 replicates and results of analysis are shown in **Table 2**.

**TABLE 2: RECOVERY STUDIES**

Amt. of drug added ( $\mu\text{g/ml}$ )		% Recovery $\pm$ S.D.	
GLC	MET	GLC	MET
1	2	99.29 $\pm$ 2.15	100.03 $\pm$ 0.30
2	2	100.38 $\pm$ 1.31	99.90 $\pm$ 0.24
3	1	99.36 $\pm$ 0.25	100.25 $\pm$ 0.13
Mean % recovery $\pm$ S.D.		99.68 $\pm$ 1.24	100.06 $\pm$ 0.22

**FIG. 1: OVERLAIN SPECTRUM OF GLICLAZIDE AND METFORMIN HYDROCHLORIDE**

**Formation of Simultaneous Equation:** A set of two simultaneous equations were framed using the mean absorptivity.

$$C_x = \left( \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \right)$$

$$C_y = \left( \frac{A_2 a_{x1} - A_1 a_{x2}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \right)$$

Where,  $A_1$  and  $A_2$  are the absorbance of sample solutions at 227.0 nm and 237.5 nm respectively.  $C_x$

## RESULTS AND DISCUSSION:

**Absorption Maxima:** Absorption maxima of Gliclazide and Metformin hydrochloride were detected at 227 nm ( $\lambda_1$ ) and 237.5 nm ( $\lambda_2$ ), respectively and overlain spectra was recorded **Fig. 1**.

**Absorptivity Coefficients:** The absorptivity coefficients of the two drugs were determined by using Beer's law:  $A = E (1\%, 1\text{cm}) CL$ . The absorptivity coefficients of Gliclazide at 227.0 nm and 237.5 nm were 29.4689 $\pm$ 1.0844 and 16.7762 $\pm$ 0.7069 and for Metformin hydrochloride were 52.5711 $\pm$ 2.6003 and 83.2329 $\pm$ 1.9087.

and  $C_y$  are concentration of Gliclazide and Metformin hydrochloride in mg/mL in sample solution. By substituting the values of  $A_1$  and  $A_2$  the values of  $C_x$  and  $C_y$  can be calculated by solving the two equations simultaneously. Here,  $a_{x1}$  and  $a_{x2}$  are the absorptivity coefficient of Gliclazide at 227.0 nm and 237.5 nm respectively;  $a_{y1}$  and  $a_{y2}$  are the absorptivity coefficient of Metformin hydrochloride at 227.0 nm and 237.5 nm respectively. The optical parameters & regression characteristics for Gliclazide and Metformin hydrochloride are shown in **Table 3**.

**TABLE 3: OPTICAL AND REGRESSION CHARACTERISTICS**

Characteristics	Gliclazide		Metformin hydrochloride	
	227.0	237.5	227.0	237.5
Beer's Law Limit ( $\mu\text{g/ml}$ )	5-25	5-25	2.5-12.5	2.5-12.5
Molar Absorptivity	9530.24	5425.42	8707.35	13785.87
Sandell's sensitivity ( $\mu\text{g/cm}^2/0.001$ absorbance unit)	0.034	0.059	0.019	0.012
Regression Equation	$y = 0.030x - 0.0063$	$y = 0.017x + 0.0009$	$y = 0.053x - 0.0008$	$y = 0.084x - 0.0049$
Slope	0.0300	0.0166	0.0527	0.0841
Intercept	-0.0063	0.0009	-0.0008	-0.0049
$r^2$	0.9999	0.9998	0.9995	1.0000

**Method Validation:** The linearity range for Gliclazide and Metformin hydrochloride were 5-25 µg/mL and 2.5-12.5 µg/ mL respectively. Recovery studies was carried out by addition of standard drug solution to pre-analyzed tablet sample solution at three different concentration levels taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to be

satisfactory and shown in Table 2. The results obtained from recovery study (accuracy study) indicated that mean percentage recovery were 99.68±1.24 and 100.06±0.22 for Gliclazide and Metformin hydrochloride, respectively. Other validation parameters were found to be satisfactory and are shown in **Table 4**.

**TABLE 4: VALIDATION PARAMETERS**

Parameters	Gliclazide		Metformin hydrochloride	
Wavelength (nm)	227	237.5	227	237.5
Repeatability(%RSD)	0.0374	0.0437	0.0499	0.0265
Precision (C.V.)				
Intra-day	1.0976–1.8373	1.2295–2.4684	1.8373–2.3531	1.4064–2.9557
Inter-day	2.1376–2.8528	1.6059–3.3340	3.6422–3.7655	1.4617–3.9687
Reproducibility* (Mean ±S.D)				
Analyst 1	0.294 ± 0.0151	0.166 ± 0.0047	0.519 ± 0.0270	0.836 ± 0.0135
Analyst 2	0.296 ± 0.0105	0.164 ± 0.0046	0.525 ± 0.0175	0.844 ± 0.0212
LOD (µg/ml)	0.7334	0.3725	0.3320	0.4664
LOQ (µg/ml)	2.2223	1.1287	1.0061	1.4133

\* t- Test was performed for comparison of results

**CONCLUSION:** Based on the results obtained, it was concluded that the proposed method of analysis is accurate, precise, reproducible & economical and can be employed for routine quality control of Gliclazide and Metformin Hydrochloride in tablet formulations.

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## REFERENCES:

1. The Indian Pharmacopoeia, Vol. I, II, Government of India, The Controller of Publication, Delhi, 1996.
2. The British Pharmacopoeia, Vol. I, III, IV, HSMO, London, 2004.
3. Brunton LL, Lazo JS, Parker KL: Goodman and Gillman's The Pharmacological Basis of Therapeutics. Mcgraw-Hill medical publishing division, 11<sup>th</sup> edition 2006, 1634-1639.
4. Rang HP, Dale MM, Ritter JM, Moore PK: Pharmacology. Churchill Livingstone, 5<sup>th</sup> edition 2003, 385-391.
5. Maryadele J. O'Neil: The Merck Index. Merck Research Laboratories, Division of Merck And Co Inc, 13<sup>th</sup> edition 2001, 5963 & 4452.
6. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd, 5<sup>th</sup> edition 2004, 245-253.
7. Amini H, Ahmadiani A and Gazerani P: Determination of metformin in human plasma by high-performance liquid chromatography. Journal of Chromatography B 2005; 824: 319-322.
8. Zhang M, Moore GA, Lever M, Gardiner SJ, Kirkpatrick CMJ, Begg EJ: Rapid and simple high-performance liquid chromatographic assay for the determination of metformin in human plasma and breast milk. Journal of Chromatography B 2001; 766: 175-179.
9. Gandhimathi M, Anandkumar K, Cherian A, Ravi TK: Simultaneous estimation of metformin and gliclazide in tablets using reverse phase high performance liquid chromatography. Indian Journal of Pharmaceutical Sciences, Sept-Oct 2003; 65(05): 530-531.
10. Khanolkar DH, Shinde VM: RP-HPLC method for the estimation of Glibenclamide and Metformin HCL from combined dosage form. Indian Drugs Dec 1999; 36(12): 739-742.
11. Shukla IC, Dubey A: Microgram determination of Glipizide and Metformin hydrochloride in pharmaceutical preparation by HPLC. Indian Drugs Aug 2002; 39(8): 446-448.
12. Lad NR, Bhoir SI, Bhoir IC, Sundaresan M: Concurrent Assay of Metformin and Glimepiride in tablets using RP-HPLC with wavelength programming. Indian Journal of Pharmaceutical Sciences Nov-Dec 2003; 65(06): 650-653.
13. Bhanu R, Kulkarni SK, Kadam AB: Simultaneous estimation of Gliclazide and Metformin in pharmaceutical dosage by reverse phase high performance liquid chromatography. Indian Drugs Jan 2006; 43(1): 16-20.

14. AbuRuz S, Millership J, McElnay J: The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimeperide in plasma. *Journal of Chromatography B* 2005; 817: 277-286.
15. Vasudevan M, Ravi J, Ravisankar S, Suresh B: ION-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. *Journal of Pharmaceutical and Biomedical Analysis* 2001; 25: 77-84.
16. Chen X, Gu Q, Qiu F, Zhong D: Rapid determination of metformin in human plasma by liquid chromatography-tandem mass spectrometry method. *Journal of Chromatography B* 2004; 802: 377-381.
17. Wang Y, Tang Y, Gu J, Fawcett JP, Bai X: Rapid and sensitive liquid chromatography- tandem mass spectrometric method for the quantitation of metformin in human plasma. *Journal of Chromatography B* 2004; 808: 215-219.
18. Puranik M, Wadher SJ, Yeole PG, Thakur S: Simultaneous estimation of Metformin HCl and Rosiglitazone maleate in solid dosage form by Ultra-Violet spectrophotometry. *Indian Drugs* July 2005; 42(7): 428-431.
19. Lalhriatpuii TC, Kawathekar N: Derivative spectrophotometric estimation of Pioglitazone and Metformin HCl. *Indian Drugs* Nov 2005; 42(11): 740-743.
20. Rouini MR, Mohajer A, Tahami MH: A simple and sensitive HPLC method for the determination of gliclazide in human serum. *Journal of Chromatography B* 2003; 785: 383-386.
21. Bari VR, Dhorda UJ, Sundaresan M: Analysis of Gliclazide in pooled human plasma by a sensitive HPLC method with amperometric detection. *Indian Drugs* July 1999; 36(7): 459-462.
22. Gayatri S, Shantha A, Vaidhyalingam V, Aruna A, Niraimathi V: Simultaneous estimation of gliclazide and rosiglitazone from its pharmaceutical dosage form by HPLC method. *Indian Drugs* June 2004; 41(6): 374-375.
23. Park JY, Kim KA, Kim SL, Park PW: Quantification of gliclazide by semi-micro high-performance liquid chromatography: application to a bioequivalence study of two formulations in healthy subjects. *Journal of Pharmaceutical and Biomedical Analysis* 2004; 35: 943-949.
24. Gayatri S, Shantha A, Vaidhyalingam V, Ajithadas A, Niraimathi V: Simultaneous spectrophotometric estimation of gliclazide and rosiglitazone from its pharmaceutical dosage form. *Indian Drugs* Feb 2004; 41(2): 112-113.
25. El-Enany N: Spectrophotometric determination of gliclazide in pharmaceuticals and biological fluids through ternary complex formation with eosin and palladium(II). *IL FARMACO* 2004; 59: 63-69.
26. Gayatri S, Shantha A, Vaidyalingam V: Simultaneous HPTLC determination of Gliclazide and Rosiglitazone in tablets. *Indian Journal of Pharmaceutical Sciences* Nov-Dec 2003; 65(06): 663-665. Guideline.

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