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Method Development and Validated of Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide in Pure and Tablet dosage form by Spectrophotometric Multi component Method

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Abstract: A method was developed for simultaneous estimation of Metformin HCl, pioglitazone HCl and glibenclamide in pure and tablet dosage form by using methanol as a solvent. Metformin HCl, pioglitazone HCl and glibenclamide show absorbance maxima at 237 nm, 270 nm and 230 nm respectively. Shimadzu UV 1700, capable of multicomponent analysis, was used for quantitation. This method is based on a multiwavelength spectroscopic method. Validation study reveals that the methods are specific, accurate, precise, and reproducible. All three drugs obey Beer's law in the concentration ranges used for the methods. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% COV< 2.0 and S.D. < 2.0) for both accuracy and precision study. High recovery and low % COV reveals the reliability of the method for quantitative study of three drugs in tablet formulation. The methods are simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of Metformin HCl, pioglitazone HCl and glibenclamide in pure and tablet dosage form.
Keywords: Multi-wavelength spectroscopy, Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide.

INTRODUCTION

Metformin hydrochloride (MET) chemically N,N dimethylimidodicarbonimidic diamide hydrochloride is used as antidiabitic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin lay in increasing glucose transport across the cell membrane in skeletal muscle [(1)]

Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-(5-ethyl-2- pyridinyl) ethoxy] phenyl] methyl] -2, 4-] thiazolidinedione monohydrochloride. It is a potent agonist for peroxisome proliferatoractivated receptor-gamma (PPARγ), activation of which modulates the transcription of a number of insulin responsive genes involved in the control of glucose

and lipid metabolism. Glibenclamide (GLB) is 1- [4-[2- (chloro-2-methoxybenzamido)ethyl]-benzene sulphonyl]3cyclohexylurea,5-chloro-N-[2-[4[[[(cyclo hexyl(amino)carbonyl]-amino]sulphonyl] phenyl] ethyl] 2-methoxy benzamide or 1-[[p-[-2-(5-chloro-oanisamido) ethyl]phenyl]-sulphonyl-3-cyclohexyl urea,a sulphonyl urea derivative is a second generation oral hypoglycaemic agent which is more potent than those of first group[(2)] and is used to assist in the control of mild to moderately severe type II. diabetes mellitus (adult, maturity-onset) that does not require insulin, but that can be adequately controlled by diet alone. It is drug of choice for initiating treatment in noninsulin-dependent diabetes when diet and weight control fails. It stimulates the secretion and enhances the utilization of insulin by appropriate tissues.[(3)] The chemical structures of Metformin HCl, Pioglitazone HCl and Glibenclamide are shown in fig. 1.

Several assay techniques have been described for quantitative determination of metformin, pioglitazone and glibenclamide in individual and in combination. The UV Spectroscopy determination [(4-5)], UV and HPLC determination [(6)], HPLC determination [(7-15)], HPTLC determination [(16-19)]

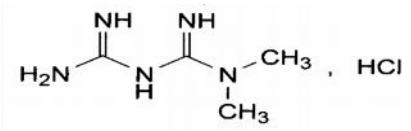

Fig1 (a) chemical structure of Metformin HCl

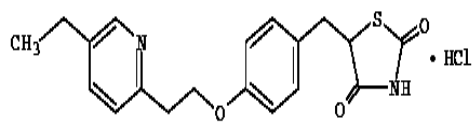

Fig1 (b) chemical structure of Pioglitazone HCl

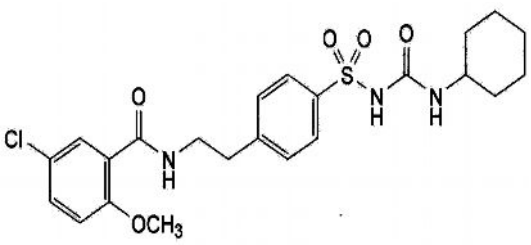

Fig1(c) chemical structure of Glibenclamide

EXPERIMENTAL

Instrument

Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Shimadzu Model 1700) using UV Probe software version 2 was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells.

Chemicals

Metformin HCl (MET), Pioglitazone (PIO) and Glibenclamide (GLB) were supplied by Aribindo pharmaceuticals, India as gift sample and used as such. Methanol used was from Qualigen fine chemicals Ltd, India. Water used was generated by double distillation.

Preparation of standard stock solution:

Standard stock solution of PIO, MET and GLB were prepared by dissolving 10mg of drug in methanol and making up the volume to 10ml in three different 10ml volumetric flasks to get 1mg/ml.

Preliminary solubility studies of drugs

Solubility of three drugs was determined at 28±1 C. A small quantity of standard drugs were dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and pH 4, 7, 9.2 buffer solutions. By the solubility studies we determined that all the three drugs were dissolved in methanol.

Determination of λ max:

From the stock solutions, a working standard was prepared. The absorption spectrum for MET, the absorption spectrum was recorded using 10µg/mL solution and the maximum absorption was found to be 237 nm.For PIO was recorded using the concentration of 10 µg/mL and it was found to show two absorption maxima at 225, 270 nm. For GLB the absorption spectrum was recorded using 10 µg/mL solution and the maximum absorption was found to be 230nm. The UV spectra of the three drugs were shown in fig: 2, 3 and 4

The Calibration curves were prepared for MET, PIO and GLB in the concentration range of 1-10 µg/mL, 10-50 µg/mL and 1-50 µg/mL at selected wave lengths by diluting aliquot portions of stock solution of each drug. The plots of Beer's law limit are reported in table 1.

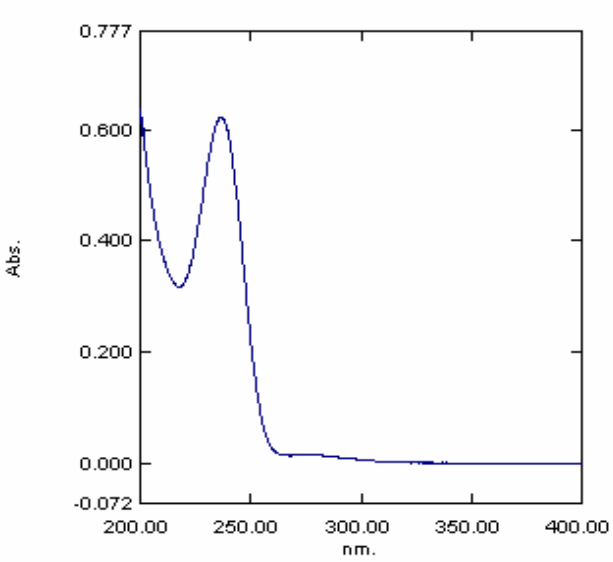

Fig:2 UV Spectra of Metformin standard

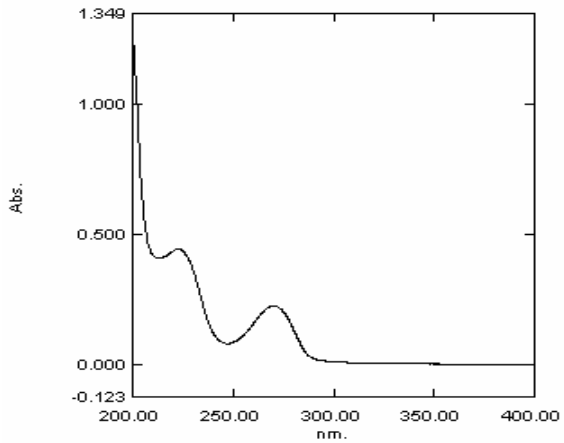

Fig:3 UV Spectra of Pioglitazone standard

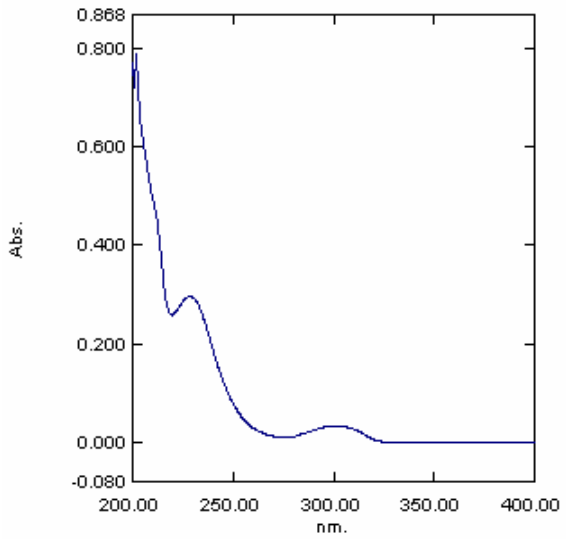

Fig: 4 UV Spectra of Glibenclamide standard

Table 1: Optical characteristic and linearity data

Parameters	Metformin	Pioglitazone	Glibenclamide
λmax (nm)	237	270	230
Beer's law limits in µg/mL	1-10	1-50	1-10
Correlation coefficient	0.99894	0.99964	0.99821
Regression equation Y=mx+c	Y=0.1083x+0.0129	Y=0.01059x+0.0175	Y=0.1787x+0.0882
Intercept(c)	0.0129	0.0175	0.0882
slope	0.1083	0.01059	0.1787
Std error	0.009112	0.005122	0.09982
Molar Abs	18151.39	4848.386	44400.72

Multi-component Method

Different aliquots were taken from the stock solutions and diluted with the same solvent to prepare a series of concentrations. The absorbances of these solutions were measured at 237 nm, 270 nm and 230nm for MET, PIO and GLB respectively and calibration curves were plotted at selected wavelengths, the optical characteristics and linearity data is shown in table.1.In this method four mixed standards of Metformin hydrochloride, Pioglitazone hydrochloride and Glibenclamide in the ratio of 10:3:1having concentrations in g/ml was prepared in methanol by diluting appropriate volumes of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (230 nm 237nm, 270nm, 280nm) were selected on the trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of four mixed standards(Fig 5).

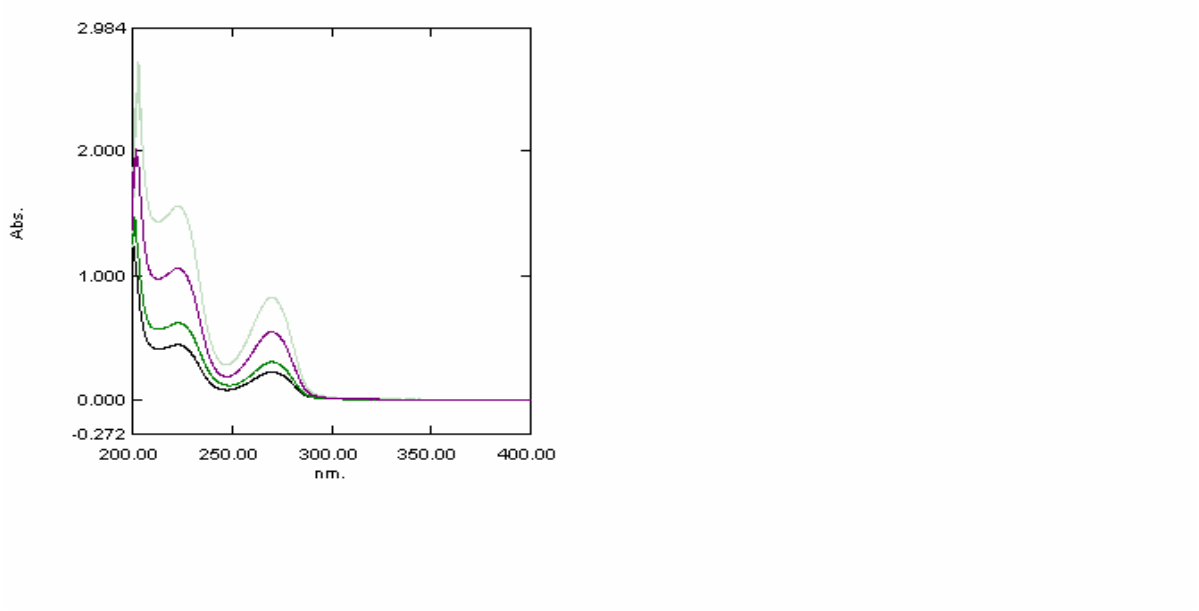

Fig 5: Overlain spectra of mixed standards of MET, PIO and GLB

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 10 mg of was transferred to 100 mL volumetric flask and dissolved in methanol with frequent shaking for 15minutes and final volume was made up with methanol the sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 1mL of solution was taken and diluted to 10ml with methanol to get a solution containing 10 µg/mL of Glibenclamide. This solution contains Metformin100 µg/mL, Pioglitazone 30 µg/mL and Glibenclamide10 µg/mL. The solution contains 10:3:1 ratio of Metformin, Pioglitazone and Glibenclamide. The result of analysis of tablet formulation was reported in Table 2.

Validation of Method (ICH guidelines, 2005)

The method was validated with reference to accuracy, precision, and ruggedness.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of three drugs to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods; the results are shown in table 3.

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for six times. The results are shown in table 4.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. The results are shown in table 4.

Table 2: Analysis data of Tablet formulation

Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.*	% COV	S.E*
MET	500	500.5	100.4	0.057	0.033	0.009
PIO	15	15.4	102.6	0.115	0.066	0.005
GLB	5	4.84	98.8	0.500	0.287	0.099

MET: Metformin, PIO: Pioglitazone, GLB: Glibenclamide, S.D: Standard deviation,
COV: Coefficient of variation, S.E: Standard error,*Average of four estimation of tablet formulation.

Table 3: Accuracy studies

% conc	Metformin		Pioglitazone		Glibenclamide		%Recovery Amount found ×100/Amount Added		
	Amount Found in mg	Amount Added in mg	Amount Found in mg	Amount Added in mg	Amount Found in mg	Amount Added in mg	MET in %	PIO in %	GLB in %
80%	8.33	8.3	7.98	8.0	7.97	8.0	100.4	99.7	99.6
100 %	9 .98	10.0	9.92	10.1	9.89	10.0	99.8	99.2	98.9
120 %	12.10	12.0	11.97	12.0	11.84	12.0	100.9	99.7	98.6
Mean recovery							100.3	99.5	99.0

Average of three determinations

Table 4: Results from precision and ruggedness

Parameters	Metformin 237nm	Pioglitazone 270nm	Glibenclamide 230nm
Precision (%RSD)			
Intra-day (n = 3)	0.560– 0.985	0.120-0.548	0.123 – 0.915
Inter-day (n = 3)	0.562 – 1.73	0.134-0.748	0.170 – 1.81
Repeatability (n=6)	0.35	0.48	0.61
Ruggedness (%RSD)			
Analyst 1 (n = 3)	0.61	0.12	0.12
Analyst 2 (n = 3)	0.70	014	0.16

RESULTS AND DISCUSSION

Three wavelengths 237nm (λmax for MET) 270 nm (λmax for PIO) and 230 nm (λmax for GLB) were selected for analysis of the drugs in methanol. Linearity was observed in the range 1 - 10µg/ml (r2=0.99894) for MET, 1-50 µg/ml (r2 =0.99964) for PIO and 1 - 10µg/ml (r2=0.99821) for GLB the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. Both the methods were found to be precise as indicated by the repeatability, inter-day, intra-day analysis, showing %RSD less than 2. The results did not show any statistical difference between operators suggesting that methods developed were rugged. The results of precision and ruggedness are shown in table 4. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulation containing three drugs.

REFERENCES

1. Martindale: The Complete Drug Reference, Pharmaceutical Press, London, 2007, 411
2. Grodsky, G.M., Epstein, G.H., Fanska, R. And Karam, J.H. Pancreatic action of the sulfonylureas. Fed Proc., 1977, 36(13): 2714-2719.
3. Drell, D.W. and Notkins, A.L. Multiple immunological abnormalities in patients with type 1 (insulin dependent) diabetes mellitus. Diabetologia. 1987, 30(3): 132-143.
4. Patil Sudarshan .S, Bonde C.G **(**2009) Development and Validation of analytical method for Simultaneous estimation of Glibenclamide and metformin HCl in bulk and tablets using UV – visible spectroscopy International Journal of ChemTech Research,1, 4, 905-909.
5. Sujana .K, Swathi.G, Rani, Bhanu Prasad .M, Saheethi.M (2010) Simultaneous estimation of Pioglitazone hydrochloride and metformin hydrochloride using UV Spectroscopic Method**,** Journal of Biomed Science and Research. 2 (2), 102-107.
6. Lakshmi.K.S., Rajesh.T, Sharma.S, Lakshmi.S (2009) Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Metformin, pioglitazone and glimepiride in pharmaceutical formulations.Der Pharma Chemica; 1 (1) 238-246.
7. Mir Imam Ibne Wahed (2007) Simultaneous High-performance Liquid Chromatographic determination of Metformin HCl and rosiglitazone maleate in pharmaceutical dosage form.Research Journal of Medicine and Medical Sciences, 2(2), 115-121.
8. Ali M. Qaisi , Maha F.Tutunji , and Charl A. Sahouri (2006) Determination of Metformin in Human plasma using Normal phase High performance liquid chromatography. Saudi Pharmaceutical Journal, 14, 2, 108-118.
9. Florentin Tache and Monica Albu (2007) Specificity of an Analytical HPLC Assay Method of Metformin Hydrochloride**.** Revue Roumaine de Chimie, 52(6) 603–609.
10. Ceren Yardımc, Nuran O¨ zaltın, Alper G¨urle., k (2007) Simultaneous determination of rosiglitazone and metformin in plasma by gradient liquid chromatography with UV detection. Talanta 72, 1416–1422.
11. AbuRuza. S, Millershipb. J, McElnayb.J (2005) The development and validation of liquid Chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma Journal of Chromatography, 81, 277–286.
12. Ms. Shweta Havele, Dr. Sunil Dhaneshwar (2010) Development and Validation of a HPLC Method for the Determination of Metformin Hydrochloride, Gliclazide and Piogliglitazone Hydrochloride in Multicomponent Formulation. WebmedCentral pharmaceutical sciences, 2046-1690, 1-16.
13. Praveen Kumar reddy.B, Boopathy.D, Bibin Mathew, Prakash.M, and Perumal. P **(**2010**)**Method Development and validation of simultaneous determination of pioglitazone and glimepiride in pharmaceutical dosage form by RP-HPLC. International Journal of ChemTech Research, 2,1, 50-53.
14. Lakshmi. K.S, Rajesh.T, Shrinivas Sharma (2009) Determination of pioglitazone and glimepiride in pharmaceutical formulations and rat plasma by RP-LC. International Journal of PharmTech Research, 1,3, 496-499
15. Lakshmi. K.S, Rajesh.T, Shrinivas Sharma (2009) Simultaneous determination of metformin and pioglitazone by Reversed phase HPLC in pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences.1, 2,162-166.16. Kalyankar.T.M, Badguja.M.R, Mitkare S.S and Kakde.R.B (2010) HPTLC Method for Simultaneous analysis of pioglitazone HCL and glimepiride in pharmaceutical preparations. Journal of Pharmacy Research. 3(12), 3118-3120
17. Ms. Shweta Havele, Dr. Sunil Dhaneshwar (2010) Estimation of Metformin in Bulk Drug and in Formulation by HPTLC. Journal of Nanomedicine & Nanotechnology,1,1,1-3
18. Meeta A Jiladia SS Pandya and Viidyasagar G (2009) A Simple and Sensitive HPTLC Method for Estimation of Pioglitazone In bulk and tablet dosage forms. Asian J. Research Chem. 2(2), 72-76.
19. Uihlein.M and Sistovariw N (1982) Thin-layer chromatographic determination of human serum Glibenclamide at therapeutic levels. Journal of Chromatography, 227, 93-101.

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