International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.2, pp 625-630, April-June 2012

Devlopement and Validation of UV-Visible Spectrophotometric Method for Simultaneous Determination of pioglitazone Hydrochloride, Metformin Hydrochloride and Glipizide in its Bulk and Pharmaceutical Dosage Form (Tablet)

L Adhikari[1]* , S Jagadev[1] , S Sahoo[1], P.N Murthy[2] ,U.S Mishra[2]

[1]Department of Pharmaceutical Analysis and Quality Assurance, School of Pharamaceutical Sciences,Siksha 'O' Anusandhan University ,Kalinga Nagar,Ghatikia,Bhubaneswar,PIN:751003;Orissa,India

[2]Royal College of Pharmacy and Health Sciences,Berhampur ; Orissa,India

*Corres. Author:lopamudraadhikari@gmail.com
Ph:9776354163

Abstract: A simple ,precise, rapid and selective spectroscopic method is used to Quantify three antidiabetics in multicomponent formulations in the present study.Three wave length spectroscopic method and Multiwavelength method was carried out for determination of Metformin Hydrochloride,Glipizide,Pioglitazone Hydrochloride in their bulk and preparations using Acetonitrile: Methanol: Water in the proportion of 5:4:1.The λ_{max} was found at 236.5nm, 226.4nm and 227.3nm respectively. The isobestic point was found to be 254 nm. Method:II is based on multiwavelength spectroscopy. All the three drugs obeys the Beer-Lambert limit within the concentration range of 5-50µg/ml.%COV<2 and S.D<2. Assay results from both the methods with different formulations shows purity around 99.95% to 102.10%.Validation study reveals that the methods are specific, accurate and precise. High recovery and low %COV reveals the reliability and good acceptance of the quantitative study in formulations. The method can be used for routine quantitative analysis of metformin,glipizide and pioglitazone in pure and tablet dosage forms.

Keywords: Metformin Hydrochloride, Glipizide, Pioglitazone Hydrochloride, Three wavelength spectroscopy, Validation.

INTRODUCTION

Metformin Hydrochloride is biguanide group of drugs with chemical name 1, 1-dimethylbiguanides., [1, 2].They do not cause insulin release, but presence of some insulin is essential for their action. It suppresses hepatic gluconeogenesis and glucose output from liver. And it also inhibits intestinal absorption of glucose, other hexodes, amino acid and vit.B_{12} [2]The main use of Metformin is in the use of Diabetes Mellitus 2.The second drug glipizide is a second generation sulfonylurea group of drugs with chemical name 1-cyclohexyl-3-[[4[2-[[5-methylepyrazine-2-yl) carbonyl] amino] ethyl] sulphonyl] urea. [1, 2]. The primary hypoglycemic action of this drug is caused due to the fact that it up regulates the insulin receptor in the periphery and it does not exert a direct effect on glucagons secretion[3]. The initial event in Glipizide action on beta cells appears to be binding to a specific plasma membrane receptor. It is employed for the lower blood glucose label of type II diabetes. [4] The

third drug Pioglitazone hydrochloride is a Thiazolidinedione group of drugs with chemical name 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1, 3Thiazolidine2, 4-dione. Pioglitazone HCL alter the transcription of genes influencing carbohydrate and lipid metabolism, resulting in changed amounts of protein synthesis and, therefore, metabolic changes. Pharmacologically studies indicate that Pioglitazone HCL improves sensitivity to insulin in muscle, adipose tissue and glycemic control while reducing circulating insulin level. It also inhibits hepatic gluconeogenesis [5]. It is used for the decrease insulin resistance and manages the type II diabetes .Literature survey reveals that various spectrophotometric and HPLC method have been developed for the drugs individually[6-10] but records are not available for the simultaneous determination of Metformin Hydrochloride, Glipizide,Pioglitazone Hydrochloride in pharmaceutical dosage forms[11-12]. In this communication we propose a fast, very simple and accurate derivative spectrophotometric method for estimation Metformin Hydrochloride, Glipizide, Pioglitazone Hydrochloride in tablet dosage forms.

EXPERIMENTAL

MTERIALS AND REAGENTS

A double beam UV-VIS spectrophotometer JascoV-630 model with 1cm matched quartz cell .Metformin Hydrochloride, Glipizide and Pioglitazone Hydrochloride pure drugs used for the development of analytical method, were gifted by Ranbaxy pharma labs, Madhya Pradesh; India, Nicholas Piramal India ltd, Madhya Pradesh, India and Dr Reddy's laboratories, Hyderabad; India, respectively. Acetonitrile, Methanol and Water were used as solvents of AR grade (Merck), India, for the analytical purpose.

PREPARATION OF STANDARD AND STOCK SOLUTIONS

Standard stock solution of Metformin and Glipizide was prepared by dissolving 25 mg of drug in 25 ml of Acetonitrile: Methanol: Water (5:4:1) ml in volumetric flask to get a concentration of 1000 µgm/ml. The stock solution was further diluted with the solvent ratio of Acetonitrile: Methanol: Water (5:4:1) ml to get a concentration of 10 µgm/ml and was scanned in the UV region of 400nm to 200nm to obtain the absorption spectra and overlain spectra.

DETERMINATION OF λmax AND ISOABSORPTIVITY POINT

Then the absorbencies were measured at λmax 254nm (Isobestic point) and 226.4 nm (Glipizide) against blank for Metformin HCL and Glipizide. Then the absorbance was measured at λmax 254 nm and 227.3nm against blank for Pioglitazone HCL.

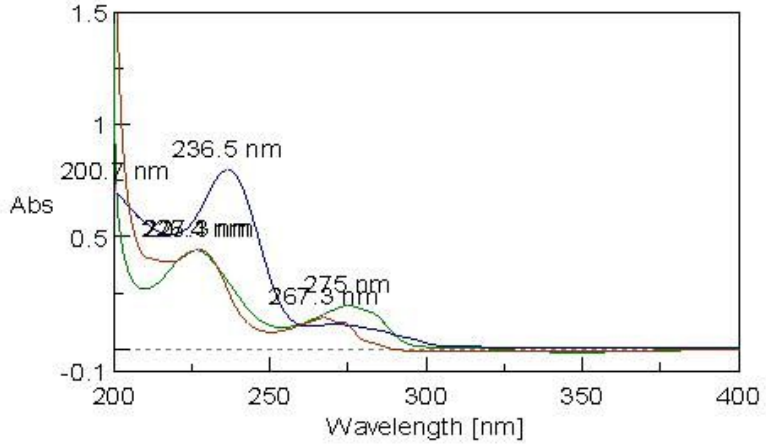

Figure:IOverlain spectrum of Metformin hydrochloride, Glipizide and Pioglitazone hydrochloride

Method 1:Three Wavelength method
Qualitative estimation of Metformin HCL, Glipizide and Pioglitazone HCL shows absorbance maximums at 236.5 nm, 226.4 nm and 227.3 nm, and an isobestic point.Metformin,glipizide was observed at 254 nm, Pioglitazone HCL doesn't absorb UV radiation above 267.3nm so the concentration of Metformin HCL and Glipizide was calculated by an absorbance ratio (Q-ratio) method arrived at by solving the following equation

$$C_{met} = Q_m - Q_y / Q_x - Q_y \times A_1 / ax_1 \quad \text{----------}$$

$$C_{Gli} = A_1 / ax_1 - C_{met} \quad \text{----------}$$

Where C_{met} = concentration of Metformin HCL
C_{Gli} = concentration of Glipizide.
A_1 =Absorbance of sample solution at 254 nm and
ax_1= Absorbtivity of Metformin HCL at 254 nm
Q_y = Absorbtivity value of Glipizide at 226.4nm / Absorbtivity value of
Glipizide at 254 nm
Q_x = Absorbtivity value of Metformin HCL at 226.4nm / Absorbtivity
Value of Metformin HCL 254 nm

Then the concentration of Metformin hydrochloride and Glipizide have been estimated earlier, the estimation of Pioglitazone hydrochloride was carried out at 227.3nm in the concentration ranges from 5 µg / ml to 30 µg / ml. A reference solution containing exactly the same concentration of Metformin hydrochloride and Glipizide as determined from the absorbance measurement at 226.4nm was prepared. The absorbance of the sample solution containing Metformin hydrochloride, Glipizide and Pioglitazone hydrochloride was measured at 226.4 nm against the reference solution containing Metformin hydrochloride and Glipizide. Then concentration of Pioglitazone hydrochloride was then obtained from the calibration curve plotted at 227.3nm.

Estimation of Pioglitazone,Metformin and Glipizide in tablet dosage form
Marketed formulation (Tablet) of Metformin hydrochloride, Glipizide (DIAGLIP-M) was taken and powdered it with the help of mortar and pestle. The powder equivalent to 10 mg Metformin hydrochloride, Glipizide,was taken and dissolved in 10 ml of Acetonitrile: Methanol: Water (5: 4: 1)ml then sonicated and filtered it. Suitable aliquots from the filtrate were taken, further diluted with the same solvent ratio to get the concentrations within the linearity range of standard curve. The absorbencies of the prepared solutions were measured at 254 nm against a blank. The drug content was in each tablet was estimated using the regression line equation as shown in Table:I.

Estimation of Pioglitazone Hydrochloride
Concentration of Metformin Hydrochloride and Glipizide were estimated and taking an exact concentration as contained in the stock solution was prepared as reference solution.The absorbance of the stock solution was measured at 227.3nm against reference solution in the spectrum mode of the instrument and the concentration of Pioglitazone HCl was obtained from the calibration curve of Pioglitazone HCl.

Table 1: ASSAY DATA OF TABLET FORMULATION

Parameter	Metformin HCL		Glipizide		Pioglitazone HCL	
	Method I	Method II	Method I	Method II	Method I	Method II
Label claimed	500	500 mg	5	5 mg	15	15 mg
Amount found ,mean	498.93	499.87	5.020	5.145	14.998	14.994
Label Claim(%)	99.78	99.97%	1.004	102.9%	99.98	99.96%
± SD	0.6524	0.423	0.4217	0.0541	0.4321	0.864
%COV	0.6532	0.436	0.4221	0.0551	0.4523	0.872

Method II:Multiwavelength spectroscopy
The overlain spectrum of Metformin HCl,Glipizide and Pioglitazone HCl is shown in fig: 2.The use of five mix standards and four sampling wavelength 236.5 nm, 226.4 nm , 227.3 nm and 254nm were found to serve the purpose of the experiment.Five mix standard solutions containing Metformin HCl,Glipizide and Pioglitazone HCl in the concentration ratio of 5:0.575:0.15,10:1.15:0.30, 15:1.725:0.46,20:2.3:0.610,25:2.875:0.770(mg/ml) were prepared in given solvent system.All the mixed standard solutions were scanned over the range of 400nm to 200nm in the multicomponent mode using the four smapling wavelengths previously mentioned.Recording the absorbance of the mixed standard solutions was processed by means of matrix equations and was then corrected to determine the concentrations of all the drugs in the tablet sample solutions.A tablet sample solution was prepared as described under method:1.The spectrometric analysis of the resulting solution was carried out using the multicomponent mode of the instrument.

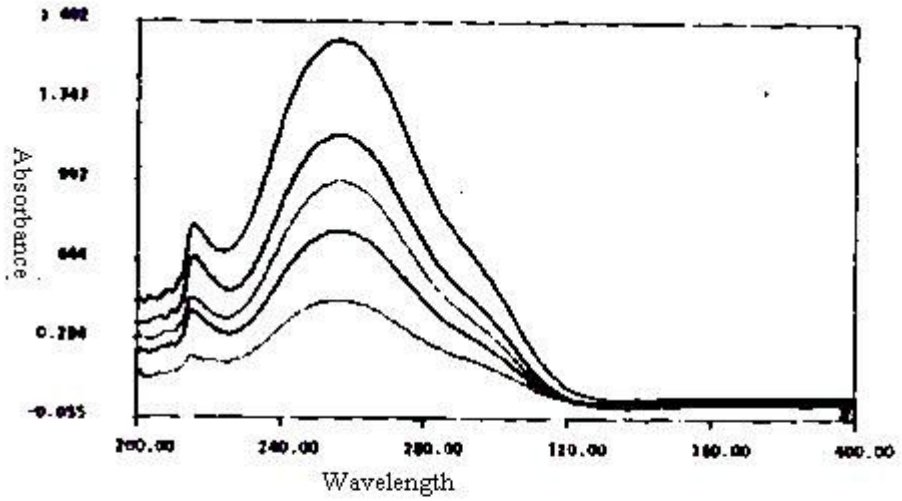

Figure:IIOverlain spectra of mixed standard of Metformin HCl,Glipizide and Pioglitazone HCl

VALIDATION[13]
PRECISION
To determine the precision 7 days measurement (intra-days and interday) of sample solution of Metformin hydrochloride, Glipizide, Pioglitazone hydrochloride were computed with coefficient of variation (C.V. %) for replicate samples (n = 5) using concentration 10µg/ml,15 µg /ml,20 µg /ml Both the intra-day and interday samples were calibrated with standard curve concurrently prepared in the same day of analysis.The observations are given in Table no.2.

ACCURACY
The recovery studies was carried out by adding different amount of 80%, 100%, and 120% of bulk sample of Metformin hydrochloride, Glipizide and Pioglitazone Hydrochloride added to preanalyzed formulation 10µgm/ml the RSD was computed given in the Table no.3.

LINEARITY
For each drug appropriate dilution standard stock solutions were assayed as per developed methods obeys the linearity between concentration ranges of 5 µg/ml to 50µg/ml for Metformin hydrochloride and Glipizide and the linearity ranges of 5 µg/ml to 30 µg/ml for pioglitazone hydrochloride by taking 5 replicates of the analyte
.

SPECIFICITY AND SELECTIVITY

The specificity and selectivity of this proposed method was evaluated with regard to interference due to presence of any other impurities.

LOD AND LOQ

The lowest concentration of the analyte was detected and the result was found out to be 0.6549,0.0374,0.1826 µg/ml for Metformin hydrochloride, Glipizide and Pioglitazone hydrochloride and the lowest quantity of analyte was detected and the result was found out to be 1.636,0.1481,0.5234µg/ml for Metformin HCL, Glipizide and Pioglitazone HCL.

ROBUSTNESS

The optimum UV spectroscopy condition set for this method have been slightly modified for sample Metformin HCL, Glipizide and Pioglitazone HCL respectively and dissolved in the drug matrix as a means to evaluate the method ruggedness.

Lastly the requirements for the system suitability are usually developed after method development and validation.

RESULTS AND DISCUSSION

The proposed Three wavelength spectrophotometry method was carried out by using a solvent ratio of Acetonitrile: Methanol: Water in the proportion of 5:4:1 ml for estimation of Metformin hydrochloride, Glipizide, Pioglitazone hydrochloride and the λmax was found in UV spectroscopy was at 236.5 nm, 226.4 nm and 227.3 nm respectively, and from the overlain spectrum and the isobestic point was found out to be at 254 nm.

The Beer's –Lambert concentration range is 5-50 µg/ml for Metformin hydrochloride, Glipizide, Pioglitazone hydrochloride at 236.5 nm, 226.4 nm and 227.3 nm respectively with coefficient correlation 0.998,0.999,0.999 respectively. Percentage estimation in the tablet dosage form is 99.78, 1.004, 99.98 by method:1 and 99.97%,102.9%,99.96% by method:2 for Metformin HCL, Glipizide and Pioglitazone HCL respectively with standard deviation<2.

In method :II five mixed standards and three sampling wavelengths are selected through rational experimentation keeping in view that the amount of drugs in the formulations and molar absorptivity coefficients(Fig:2). The method requires no manual calculations,produces comparable results to the first method and is more suitable as compared to method:I.

Table 2:PRECISION OF THE PROPOSED METHOD

Concentration of Metformin HCL, Glipizide, Pioglitazone HCL (µg/ml)	Observed concentration of Drug name (µg/ml)			
	Intra day		Inter-day	
	Mean (n=5)	Coefficient of Variation (%)	Mean (n=5)	Coefficient of Variation (%)
10	1.7236	0.765	1.838	0.521
15	1.8919	0.812	1.9257	0.64
20	1.8417	0.513	2.0574	0.689

Table 3:**RECOVERY STUDY OF METFORMIN HCL, GLIPIZIDE, PIOGLITAZONE HCL**

METHOD	Amount Added	PERCENT RECOVERY±SD		
		METFORMIN HCL	GLIPIZIDE	PIOGLITAZONE HCL
I	80%	98.99±0.0456	100.20±0.1045	101.20±0.3558
	100%	99.85±0.0345	101.20±0.7567	98.90±0.4567
	120%	99.50±0.2321	100.10±0.6578	99.80±0.3452
II	80%	100.40±0.2341	99.60±0.567	100.50±0.876
	100%	101.10±0.1671	99.60±0.6784	98.30±0.7850
	120%	99.50±0.2451	100.3±0.3452	99.50±0.8704

The standard deviation ,coefficient of variance and standard error are calculated for Metformin HCL, Glipizide and Pioglitazone HCL(Table:2)). The study reveals %COV not more than 1.0 indicating good repeatability. The LOD values are 0.6549,0.0374,0.1826µg/ml and LOQ values are 1.636,0.1481,0.5234µg/ml for Metformin HCL, Glipizide and Pioglitazone HCL which indicates good sensitivity of the proposed methods.

Assay results from both the methods with different formulations shows purity around 99.95% to 102.10% as given in table 1.

Drug shows good regression values at their respective wavelengths and the recovery study reveals that any small changes in the drug concentration in the solution could be accurately determined by the proposed method and are in good agreement with their respective claims, which suggests non-interference of formulation additives in estimation(Table:3).

CONCLUSION:

Thus the proposed method for the ThreeWave Length estimation of for Metformin HCL, Glipizide and Pioglitazone HCL in the tablet dosage forms was found to be rapid, sensitive, simple, accurate, and economical. High percentage of recovery shows that the method is free from the interference of excipient (s) used in formulation. Therefore the method can be useful in routine quality control of these drugs.

REFERENCES

1. British Pharmacopoeia-2007, Volume-III, 1347-49.
2. Kar A, Medicinal Chemistry; 3rd ed.; New Delhi,591-92.
3. Herman GA, Stein PP, Thornberry NA, Wagner JA. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes. Clin Pharmacol Ther. 2007;81(5):761-7..
4. Tripathi K.D, Essentials of Medicinal pharmacology, 5th Ed, Jaypee brother's medical publisher, New Delhi, 245-53.
5. The merck index, an encyclopedia of chemicals, drugs and biological. Fourteenth ed. USA; 2006.
6. Chaturvedi P K and R., Acta Chromatographica, 2008; 20; 451-61.
7. Patil S, Bonde S C G, Int. J. of Chem Tech. Research, 2009, 1(4), 905-09.
8. Venkatesh P, Harissudhan T, Choudhury H, Mullangi R and Srinavas N R, Biomedical Chromatogr., 2003, 20, 1043-1048.
9. Chaturvedi P K and Sharma R, Anal Lett.,2008, 41, 2133-42.
10. Lakshmi K S, Rajesh T, Sharma S, I.J.P.P.S., 2009, 1(2), 162-66.
11. Patel J K, Suhagia B.N and Patel B.H, Indian J Pharm Sci., 2007, 69 (6) , 844.
12. Patankar S V, Vaidya V.V, Joshi S.S, Gomes N.A,Indian Drugs 2007, 44,(11), 819-25.
13. Deshmukh H,S.Chanda and Prabhu J;E J. Chem.,2011,8(4),1620-25

*****