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Research Article

DEVELOPMENT AND VALIDATION OF UV DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF GLIMEPIRIDE, METFORMINE HCL AND PIOGLITAZONE HCL IN BULK AND MARKETED FORMULATION

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ABSTRACT
UV Derivative Spectrophotometric methods for the simultaneous determination of Glimepiride (GLM), Metformin HCL (MFN) and Pioglitazone HCL (PLZ) in tablets were developed in the present work. The various parameters, such as linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to (ICH) International Conference on Harmonization guidelines.The first derivative UV spectrophotometric method was performed at 227nm, 233nm and 265.5nm for GLM, MET and PIO respectively in 0.1N NaOH solution and distilled water (50:50). The proposed methods are highly sensitive, precise and accurate and therefore can be used for its intended purpose.
KEYWORDS: Anti-diabetic drugs; Glimepiride, Metformin HCL and Pioglitazone HCL, Validation.

INTRODUCTION

UV Spectrophotometry is applicable for colorless compounds which is having double or triple bonds in structure. Absorption of sample increases with the increase in sample concentration. By preparing different concentrations in beer's law range and constructing the calibration curve sample can be estimated quantitatively.

Diabetes is one of the costliest health problems in the world. Globally, diabetes is likely to be the fourth leading cause of death[1]. Approximately 90% of people with diabetes have type 2 diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises; the pancreas gradually loses its ability to produce insulin. Type II diabetes is associated with older age, obesity, family history of gestational diabetes, impaired glucose metabolism, physical inactivity and race/ ethnicity[2]. If the glycemic target level is not achieved with one oral agent alone, combination oral and/or insulin therapy is recommended[3, 4]. Combination oral therapy becomes an obvious choice when glycemic control is not achieved with conventional monotherapy[5]. The advantages of oral dose combinations as compared to their components which are taken alone are lower cost and better patient compliance[6].

Combination therapy has been shown to achieve greater blood glucose lowering than monotherapybecause different classes have different and complimentary mechanisms of action. Therefore, it is more logical to add another drug than replace the existing drug. The rapid introduction of combination therapy with two or three complementary oral anti diabetics help in targeting the dual effect and also reduced adverse effects[8].

Chemically, metformin is 1,1-dimethyl biguanide hydrochloride, pioglitazone is (±)-5-[p- [2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione whereas glimepiride is 1-(4-(2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamido)ethyl)phenylsulfonyl)-3-(4-meth ylcyclohexyl)urea[10] (structures shown in figure 1a, 1b and 1c). Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis, pioglitazone has been shown to effect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues whereas glimepiride is a sulfonylurea group oral anti-diabetic drug with prolonged effect and more over it maintains a more physiological regulation of insulin secretion than glibenclamide during physical exercise, suggesting that there may be less risk of hypoglycaemia with glimepiride, and act by increasing the secretion of insulin by the functioning β-cells of the pancreas[11]. Fig 1 shows the structure of [A] Metformine, [B] Pioglitazone and [C] Glimepiride

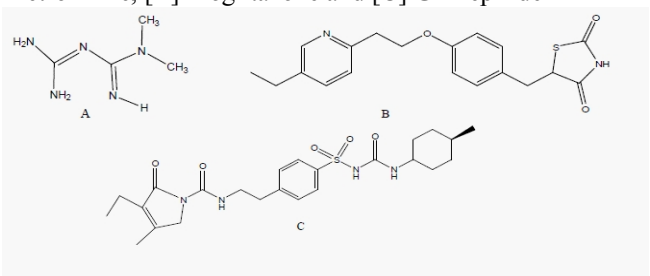


Fig 1: [A] Metformin, [B] Pioglitazone and [C] Glimepiride

This combination can be achieved by taking each of the drugs separately or alternatively fixed formulations have been developed. A combination tablet formulation is beneficial in terms of its convenience and patient compliance. The review of literature reveals that there were analytical methods of all the three drugs individually in pharmaceutical dosage forms and even in biological samples[12-20] and a few methods reported for combination of either of the two drugs. [20-22]

MATERIAL AND METHODS

Apparatus

A double beam UV/Visible spectrophotometer, Shimadzu UV- 1700 Pharmaspec, was employed with a pair of 1 cm quartz cells for all analytical work.

Reagents and chemicals

Glimepiride was obtained from Zim Lab. Nagpur, Metformin and Pioglitazone were obtained from Gen Pharmaceuticals Ltd. Pune, Maharashtra, India as gift sample and were used as working standards. Sodium hydroxide of analytical grade and double distilled water were used throughout the analysis.

Commercial formulation

A commercial pharmaceutical preparation, Gluconorm PG-2tablet (2 mg Glimepiride, 15 mg Pioglitazone and 500mg Metformin) was procured from the local market.

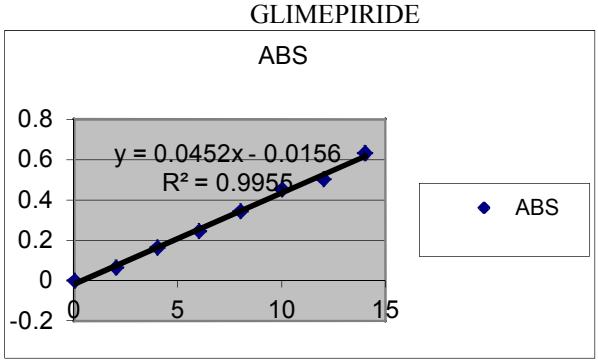
Preparation of standard solution

Standard stock solution of GLM, MFN and PLZ was prepared by dissolving 10 mg of each drug separately in 100mL volumetric flask using 0.1N sodium hydroxide as solvent up to 50 ml and volume make up with distilled water and both sample sonicate upto 20 min. Stock solutions of 100 µg/mL were obtained in this manner. From these stock solutions, working standard solutions of concentration were prepared by appropriate dilutions. Working standard solutions were scanned in the entire UV range to determine the λmax. The λmax of GLM, MFN and PLZ were found to be 227 nm,233nm and 265.5 nm respectively.

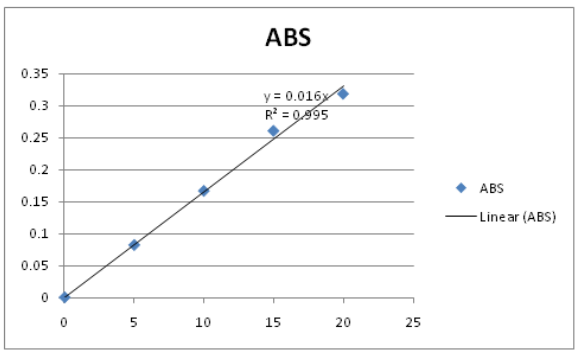
Calibration curve

Standard dilutions of each drug were prepared separately having concentrations of 2-20 µg/mL for GLM and MFN and concentration of 5-50µg/mL for PLZ.The absorbances of these standard solutions were measured at 227nm,233nm and 265.5nm and calibration curve was plotted. The absorptivity coefficients of the three drugs were determined using calibration curve graph shown below;

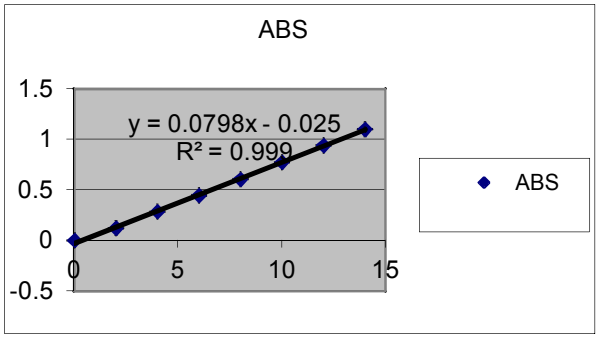
GLIMEPIRIDE



PIOGLITAZONE HCL



METFORMINE HCL



Preparation of sample solutions

Sample solution containing both the drugs was prepared by dissolving 10 mg of each drug in 100mL volumetric flask using 50 ml of 0.1N Sodium hydroxide to give stock solutions then both sonicate for 20 min and further with distilled water and made 100 µg/mL stock solution, working standard solution of 10 µg/mL concentration was prepared by appropriate dilution. Seven standard dilutions of concentrations of 5, 10, 15,20,25,30,35,40,45 and 50µg/mL was prepared from working standard solution. The absorbance of this sample solution was measured at 227nm, 233nm and 265.5nm and their concentrations were determined using proposed analyticalmethods.

Simultaneous equations method and preparation of solutions

Method was based on simultaneous equation method of Vierodt. The method is applicable in the case of sample containing two drugs, each of which absorbs at the λ max of the other (Beckett et al, 1997). Three equations were developed using absorptivity coefficient values as an X componant. The content in the mixture was determined by using the following three component equations/ Cramer's rule:

X COMPONANT =A1 (β2γ3 - β3γ2) -A2 (β1γ3- β3γ1) + A3 (β1γ2- β2γ1)/α1 (β2γ3 - β3γ2) - α 2 (β1γ3- β3γ1) + α 3 (β1γ2-β2γ1)

Similarly, y and z component can be estimated.

Triple combination equations were constructed based upon the fact that the absorbance of the mixture of GLM, MFN and PLZ at 227nm, 233nm and 265.5nm is the sum of the absorbances at respective wavelengths and the spectra shown in fig no 1. From the absorbance value obtained of all the three λ max, absorptivity were calculated and shown in table 1.

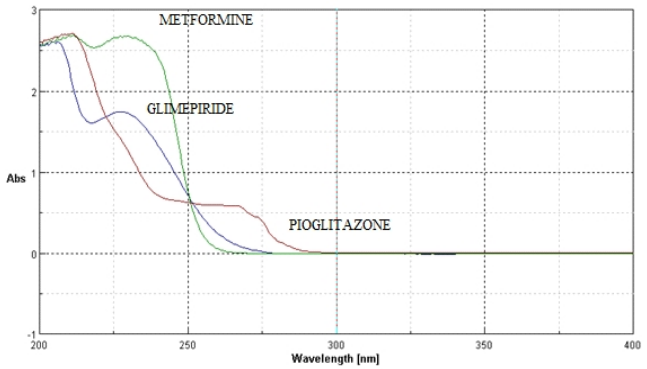


Fig: 1Spectra of Mixture i.e; GLM, MFN and PLZ in 25: 50: 50 ratios

Quantitative equations method

Method was based on Quantitative equation method. Primary stock solution was prepared by using 0.1N NaOH. From this different dilutions were prepared to determine λmax and beer's law range. Calibration curve was prepared by using different concentrations of standard solution. GLM, MFN and PLZ in dosage form were estimated by calibration curve[25, 26]. Developed method was validated as per ICH[27, 28] guidelines with the help of several parameters like accuracy, precision, LOD, LOQ, and stability.[29, 30]

Estimation in the marked formulation

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10mg of GLM, MFN and PLZ was transferred to a 10mL volumetric flask, dissolved in 5mL 0.1N NaOH, shaken for 10 min and the volume was made up to the mark with 0.1N NaOH. The solution was then filtered through Whatman filter paper no. 41. The solution was further diluted to get different concentrations in the range of 5-50µg/mL of both the drugs. The analysis procedure was repeated three times with the formulation. The result of analysis of the formulation is shown in Table 1.

***M*ethod validation**

The method validation parameters like linearity, precision, accuracy, repeatability, limit of detection and limit of quantitation were checked as per ICH guidelines.

***Linearity* and range**

The linearity for GLM, MFN and PLZ were determined at some concentration levels for GLM and MFN from 2-20 µ/mL and for PLZ ranging from 5-50µ/mL using working standards.

Precision and Accuracy

Journal of Applied Pharmaceutical Science 01 (01); 2011: 46-49 the precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of standard and sample were analyses thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in table 1-6.

The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table 1-3.

Limit of detection and limit of quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that give the measurable response. LOD was calculated using the following formula and shown in Table 4-7.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 4-7.

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULTS AND DISCUSSION

In the present work, new method, namely, simultaneous equation method (Vierordt's method) was used for the simultaneous spectroscopic estimation of GLM, MFN and PLZ in commercially available tablet dosage form. The concentrations in the range of 2-20 µg/mL of mixed working standard and three sampling wavelengths of 227nm (λ max of GLM), 233nm (λ max of MFN) and 265.5nm (λ max of PLZ) gave optimum accuracy, precision, time, economy and sensitivity for this method. The proposed procedure was successfully applied to the determination of GLM, MFN and PLZ in the commercially available tablets dosage form.

The recovery studies were carried out at different concentrations by spiking a known concentration of standard drug to the reanalyzed sample and contents were reanalyzed by proposed methods. The results of marketed formulation analysis and Recovery studies are depicted in Table2. The method was validated statistically for range, linearity, precision, accuracy, repeatability, LOD, and LOQ Table 3-5. Accuracy was ascertained on the basis of Recovery studies. Precision was calculated as inter and intraday Variation for both the drugs table 6-8. The percentage recoveries for of GLM, MFN and PLZ were found to be 99.77%±1.5409, 100.29%±1.7891 and 99.99±0.7662 for this method respectively. The relative standard deviation was found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

CONCLUSION

The proposed method based on the UV is suitable for determination of GLM, MFN and PLZ in the commercial tablets. The methods are simple, reliable, fast and reproducible. The spectrophotometric method requires only wavelength scan and automatic calculation of the first derivative value. Furthermore, the proposed methods are inexpensive and low polluting, because small volumes are required for preparation of samples.

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Table 1: The absorptivity values of GLM, MFN and PLZ in the proposed method

Absorptivity value	227nm	233nm	265.5 nm
Ax1	0.0639	-	-
Ax2	-	0.0599	-
Ax3	-	-	0.0233
Ay1	0.0526	-	-
Ay2	-	0.0523	-
Ay3	-	-	0.000484
Az1	0.03834	-	-
Az2	-	0.0286	-
Az3	-	-	0.01547

Whereas; Ax1, Ax2 and Ax3 = are the absorptivity value of GLM at the respective wavelength.
Ay1, Ay2 and Ay3 = are the absorptivity value of MFN at the respective wavelength.
Az1, Az2 and Az3 = are the absorptivity value of PLZ at the respective wavelength.

1mg / ml solution was used as primary stock solution. The working solution of 0.1 mg / ml prepared by transferring 5ml from respective stock solution to a 50 ml volumetric flask and completing to volume with the distilled water. The drug proporation for Q-method was 25µg/ml GLM, 50 µg/ml MFN and 50 µg/ml PLZ (means the proportion is 25:50:50)

Table 2: Determination of Accuracy by percentage recovery method for GLM, MFN and PLZ

Ingredients	Tablet amount (µg/ml)	Amount added (µg/ml)	Level of addition	Amount recovered (µg/ml)	Percentage recovery	Average % recovery
GLM	25(µg/ml) 25(µg/ml) 25(µg/ml)	2(µg/ml) 2.5(µg/ml) 3(µg/ml)	80% 100% 120%	4.5(µg/ml) 5 (µg/ml) 5.5(µg/ml)	99.97% 99.08% 100.25%	99.77%±1.5409
MFN	50(µg/ml) 50(µg/ml) 50(µg/ml)	2(µg/ml) 2.5(µg/ml) 3(µg/ml)	80% 100% 120%	4.5(µg/ml) 5(µg/ml) 5.5(µg/ml)	99.98% 99.99% 100.90%	100.29%±1.7891
PLZ	50(µg/ml) 50(µg/ml) 50(µg/ml)	2(µg/ml) 2.5(µg/ml) 3(µg/ml)	80% 100% 120%	4.5(µg/ml) 5(µg/ml) 5.5(µg/ml)	99.77% 99.98% 100.24%	99.99±0.7662

Table 3: Validation parameters for GLM

Sr. No	Parameters	Results
1	Absorption (nm)	227nm
2	Linearity range (µg/ml)	2-20 µg/ml
3	Standard regression equation	y=0.045x- 0.015
4	Correlation coefficient (r^2)	r^2=0.995
5	A (1%, 1cm)	61636
6	Accuracy (% recovery± SD)	99.77%±0.1518
7	Precision (% CV)	100.6 %, 101.3%
8	Specificity	A 25 µg/ml solution of candidate drug in solvent (0.1 N NaOH and distilled water mixture in the ratio of 50:50 respectively) at UV detection ʎ of 227 nm will show an absorbance value of 1.5409
9	LOD	0.02261
10	LOQ	0.07565

Table 4: Validation parameters for MFN

Sr.No	Parameters	Results
1	Absorption (nm)	233nm
2	Linearity range (µg/ml)	2-20 µg/ml
3	Standard regression	Y = 0.079
4	equation	r^2 = 0.999
5	Correlation coefficient (r^2)	35781
6	A (1%, 1cm)	100.29%±0.2567
7	Accuracy (% recovery±	100.7 %,101.3%
8	SD) Precision (% CV) Specificity LOD LOQ	A 25 µg/ml solution of candidate drug in solvent (0.1 N NaOH and distilled water mixture in the ratio of 50:50 respectively) at UV detection ƛ of 233 nm will show an absorbance value of 1.7891
9		0.067
10		0.2055

Table 5: Validation parameters for PLZ

Sr.No	Parameters	Results
1	Absorption (nm)	265.5nm
2	Linearity range (µg/ml)	5-25 µg/ml
3	Standard regression	y=0.016x
4	equation	r^2=0.995
5	Correlation coefficient (r^2)	15324
6	A (1%, 1cm)	99.99±0.1315
7	Accuracy (% recovery± SD)	101.5 %,100.9%
8	Precision (% CV) Specificity	A 50 µg/ml solution of candidate drug in solvent (0.1 N NaOH and distilled water mixture in the ratio of 50:50 respectively) at UV detection ƛ of 265.5 nm will show an absorbance value of 0.7662
9	LOD	0.0077
10	LOQ	0.0235

Table 6: Precision data for the developed method Assays of GLM as % of labeled amount

Sample number	Analyst –I (Intra-day precision)	Analyst –II (Inter-day precision)
1	101.0	101.2
2	100.6	101.6
3	99.9	101.9
4	100.3	101.5
5	100.1	101.4
6	100.8	101.1
Average S.D.	100.6 0.423	101.3 0.327

Table 7: Precision data for the developed method Assays of MFN as % of labeled amount

Sample number	Analyst –I (Intra- day precision)	Analyst –II (Inter- day precision)
1	100.4	101.2
2	100.6	101.5
3	100.8	101.1
4	100.8	101.5
5	100.4	101.0
6	100.7	101.1
Average S.D.	100.7 0.444	101.3 0.343

Table 8: Precision data for the developed method Assays of PLZ as % of labelled amount

Sample number	Analyst –I (Intra- day precision)	Analyst –II (Inter- day precision)
1	101.6	101.2
2	101.9	100.1
3	101.5	100.8
4	101.4	101.0
5	101.1	100.6
6	100.3	99.9
Average S.D.	101.5 0.324	100.9 0.493