ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1137-1145

ORIGINAL RESEARCH ARTICLE

Simultaneous UV-Spectrophotometric Estimation of Glipizide and Metformin in Bulk and Its Dosage Form

Rashmi Ranjan Sarangi*, Satya Narayana Panda, Susanta Kumar Panda, Kanhu Charana Sahu.

Department of Pharmaceutical Analysis and Quality Assurance,Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur-760002, Odisha, India

Received 13 May 2011; Revised 28 Jul 2011; Accepted 03 Aug 2011

ABSTRACT:

A simple, accurate, validated and reproducible UV-Spectrophotometric method has been developed for the simultaneous estimation of Glipizide Hydrochloride and Metformin in both bulk and tablet formulation. Glipizide and Metformin in combined tablet formulation were estimated by using the multi component mode at 276 nm for Glipizide and 237 nm for Metformin in their solution in methanol. The Beer's law obeyed the concentration range of 2-20µg/ml for both Glipizide and Metformin. Mean recovery of 99.90% for Glipizide and 99.99% for Metformin respectively signifies the accuracy of the method. This method can be used for the routine simultaneous estimation of Glipizide and Metformin in industries and other analytical laboratories.

Key Words: Glipizide, Metformin, Multi component mode, Simultaneous estimation, UV Spectroscopy

INTRODUCTION

Glipizide is an oral anti-hyperglycaemic agent. Chemically it is *N*-[2[4[[[(Cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-5-methylpyrazinecarboxamide][1]. Glipizide mainly acted by stimulation of insulin release from the β cells of the pancreas by blocking the ATP-sensitive K^+ Channels, resulting in depolarization and Ca^{2+} reduction in hepatic glucose production. Metformin is also another class oral ant hyperglycaemic agent [2]. Chemically it is N, N-Dimethyl imidodicarbonimidic diamide. Metformin mainly acted by decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Extensive literature review reveals that several liquid chromatography tandem mass spectrometry method for simultaneous determination of anti diabetic drugs Metformin and glyburide in human plasma[3], stripping voltammetric quantification of the anti-diabetic drug Glipizide in bulk form and pharmaceutical formulation[4], method of ION-pair liquid chromatography technique[5] for the estimation of Metformin in its multi component dosage forms, two new UV-Spectrophotometric methods[6] have been developed for simultaneous estimation of Pioglitazone hydrochloride and Metformin hydrochloride in tablets, simple, stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination of Glipizide in guinea pig plasma[7-8]. Therefore, in this communication we report a new simultaneous UV-Spectrophotometric estimation of both the drugs which indicating accuracy, precision and sensitivity of the method. Using methanol as a solvent. All the chemicals used were of analytical grade. Spectral and absorbance measurement were made on Shimadzu Double beam UV- spectrophotometer 1700.

MATERIALS AND METHODS:

Apparatus:

Digital balance, Ultrasonicator, a double-beam UV-Visible spectrophotometer, 1700 pharmaspec with spectral band width of 2nm, wavelength accuracy ± 0.5nm and a pair of 1-cm matched quartz cells were used to measure absorbance of the resulting solutions.

Solvent used: Methanol was used as solvent.

Preparation of standard drug solution:

Ten mg of bulk Glipizide was weighed and transferred to a calibrated, clean and dried 100ml

***Corresponding Author:** Rashmi Ranjan Sarangi**, Email:** sarangi_ranjan@yahoo.co.in, **Phone No:** +91-9438338628

volumetric flask containing some amount of solvent. Then the drug was diluted and finally volume was made up to 100ml with Methanol. Similarly 10mg of Metformin was weighed and prepared in same procedure. The final conc. of solutions of both Glipizide and Metformin was 100µgm/ml. The std. drug solutions were scanned between 400-200nm in spectrum mode. Glipizide and Metformin show the absorption maxima at 276nm and 237nm respectively. The overlay spectrum is shown in **Fig 1 & Fig 2**. The calibration curves were plotted and recorded in the quantitative mode of instrument. The calibration curves for Glipizide and Metformin are shown in **Fig 3 & Fig 4**. The linearity of calibration curve was calculated by least square method which showed co-efficient of correlation 0.999 for Glipizide and Metformin respectively.

Selection of wavelength:

The absorption maxima of Glipizide and Metformin at 276 nm and 237 nm respectively given satisfactory results.

Preparation of mixed standard:

The standard drug solution of Glipizide and Metformin were used for preparation of mixed standard solutions. Four mixed standards of Glipizide and Metformin were prepared from the standard drug solution. The mixed standards were prepared by taking the ratio of Glipizide and Metformin (1:25) in the formulations in to consideration.

Analysis of Lab sample by multicomponent method:

The method was validated by analyzing the physical admixture prepared in the laboratory. Three mixed standards of Glipizide and Metformin were prepared (**Table 17**) .The lab solution were prepared by taking the ratio of each component in the formulation in consideration and random samples were prepared. The mixed standards were then scanned in the Multi component mode in the range of 400-200nm using 276nm, 237nm & 218nm as sampling wavelength. The prepared lab samples were scanned and conc. was displayed through the inbuilt microprocessor of the instrument. The statistical data of the results were calculated by least square method and tabulated below.

Analysis of formulation by multi component method:

Individual weights of five tablets were taken & average weight was calculated. Then the tablets were triturated and average weight of the triturated tablet was taken and dissolved in the solvent i.e. methanol. The sample solutions were prepared as per (**Table 20**). The prepared samples were scanned in the Multicomponent mode and conc. was recorded from the display through the inbuilt microprocessor of the instrument. The stastical data of the results were calculated by least square method and tabulated (**Table 21 & 22**).

Validation of Glipizide and Metformin in bulk and its pharmaceutical dosage forms by UV-VIS Spectrophotometric method:

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples within the linearity range and added to the pre-analyzed formulation of Concentration 50 µg/ml and from that percentage recovery values were calculated.

Precision:

The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance by the proposed method. From the absorbance, Mean, Standard deviation and %RSD were calculated. Different parameters included for precision study were repeatability, intraday and interday precision.

Ruggedness:

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions. These conditions included different analysts and different instruments etc. In this method different analysts was considered. The data was subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Robustness:

Robustness of the method was studied by deliberate variations of the analytical parameter such as solvent composition. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

RESULTS:

Fig 1: UV-Vis-Overlay Spectra of Glipizide at 276nm.

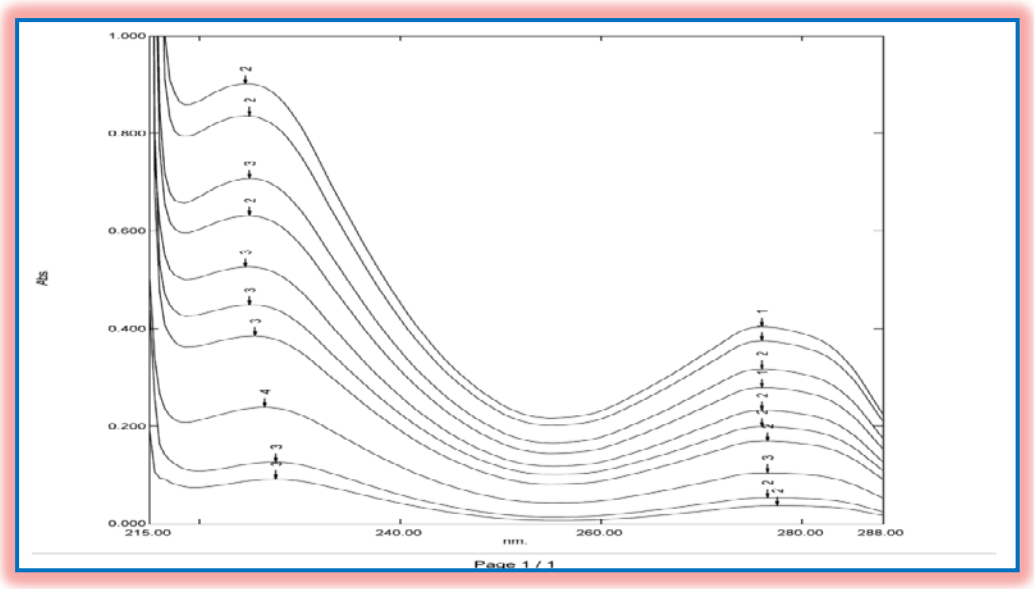

Table 1: Calibration Table of the UV-Vis Spectrophotometric Method for Glipizide

Concentration (µg/ml)	Mean Absorbance (n=6)	Statistical analysis
2	0.030	
4	0.080	
6	0.108	Mean=0.410
8	0.152	SD=0.568
10	0.190	%RSD =1.38
12	0.235	Slope =0.20
14	0.272	Intercept =0.009
16	0.318	R^2 =0.999
18	0.356	
20	0.398	

Fig 2: Calibration Curve of Glipizide at 276nm

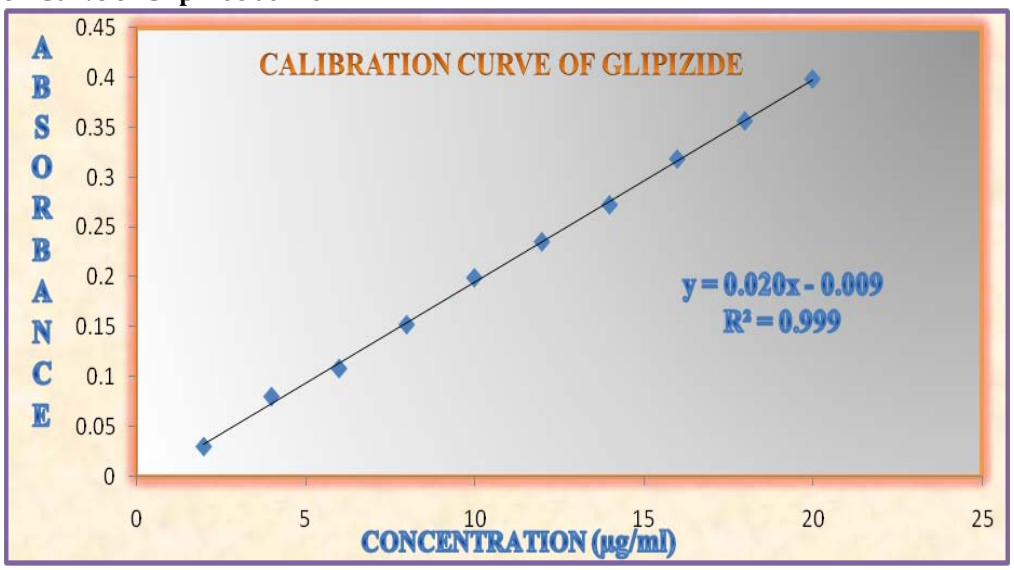

Table 2: Optical Characteristics of Glipizide

Beer's Law limit (µg/ml)	2-20 mcg/ml
Correlation coefficient	0.999
Regression equation (Y)	Y=0.020X-0.009
Slope (a)	0.020
Intercept (b)	0.009

Table 3: Accuracy Data of the UV-Vis Spectrophotometric Method for Glipizide

No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis		
	Amount present in Formulation	Amount of drug added		Mean	SD	%RSD
S_1 : 80 %	10	8	98.08	99.023	0.935	0.009
S_2 : 80 %	10	8	99.04			
S_3 : 80 %	10	8	99.95			
S_4 : 100 %	10	10	99.00	98.96	0.915	0.009
S_5 : 100 %	10	10	99.86			
S_6 : 100 %	10	10	98.03			
S_7 : 120 %	10	12	99.69	99.903	0.378	0.003
S_8 : 120 %	10	12	100.34			
S_9 : 120 %	10	12	99.68			

Table 4: Precision Data Showing Repeatability of the UV-Vis Spectrophotometric Method for Glipizide

Concentrations (mc g/ml)	Absorbance	Calculated Amount (mcg/ml)	Statistical Analysis
10	0.192	9.05	Mean =9.034 SD =0.064 %RSD =0.007
10	0.189	9.00	
10	0.190	9.02	
10	0.191	9.03	
10	0.188	8.96	
10	0.192	9.15	

Table 5: Intraday Precision Data of the UV-Vis Spectrophotometric Method for Glipizide

Conc.(mcg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Statistical Analysis
10	0.192	0.191	0.190	Mean =9.04 SD =0.011 %RSD =0.01
10	0.191	0.187	0.187	
10	0.188	0.192	0.188	
10	0.190	0.191	0.192	
10	0.191	0.190	0.191	
10	0.193	0.189	0.190	
Mean	0.190	0.190	0.189	
Calcd.Amt (mcg/ml)	9.05	9.05	9.03	

Table 6: Interday Precision Data of the UV-Vis Spectrophotometric Method for Glipizide

S.No	Concentration (mcg/ml)	Day 1	Day 2	Day 3	Statistical Analysis
1	10	0.191	0.188	0.190	Mean =9.04 SD =0.011 %RSD =0.01
2	10	0.190	0.189	0.189	
3	10	0.187	0.191	0.188	
4	10	0.188	0.189	0.190	
5	10	0.191	0.188	0.188	
6	10	0.192	0.193	0.191	
	Mean	0.189	0.189	0.189	
	Calc. Amt.(mcg/ml)	9.04	9.04	9.04	

Table 7: Ruggedness Data of the UV-Vis Spectrophotometric Method by Different Analysts for Glipizide

Analyst-1				Analyst-2			
Conc. (mcg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (mcg/ml)	Abs.	Calc. Amt.	Statistical Analysis
10	0.190	9.15	Mean=9.16 S.D=0.024 %RSD=0.003	10	0.190	9.15	Mean=9.16 SD=0.016 %RSD=0.001
10	0.191	9.17		10	0.193	9.19	
10	0.193	9.19		10	0.190	9.15	
10	0.189	9.13		10	0.191	9.17	
10	0.190	9.15		10	0.191	9.17	
10	0.192	9.19		10	0.190	9.15	

Table 8: Robustness Data of the UV-Vis Spectrophotometric Method by Different Solvent Composition for Glipizide

Solvent (92:08)				Solvent (88:12)			
Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis
10	0.192	9.15		10	0.185	8.80	
10	0.193	9.16	Mean=9.13 SD=0.021 %RSD=0.002	10	0.186	8.85	Mean=8.85 SD=0.071 %RSD=0.008
10	0.190	9.12		10	0.185	8.80	
10	0.191	9.13		10	0.185	8.80	
10	0.189	9.10		10	0.189	8.98	
10	0.190	9.12		10	0.187	8.88	

Fig 2: UV-Vis Overlay Spectra of Metformin at 237nm

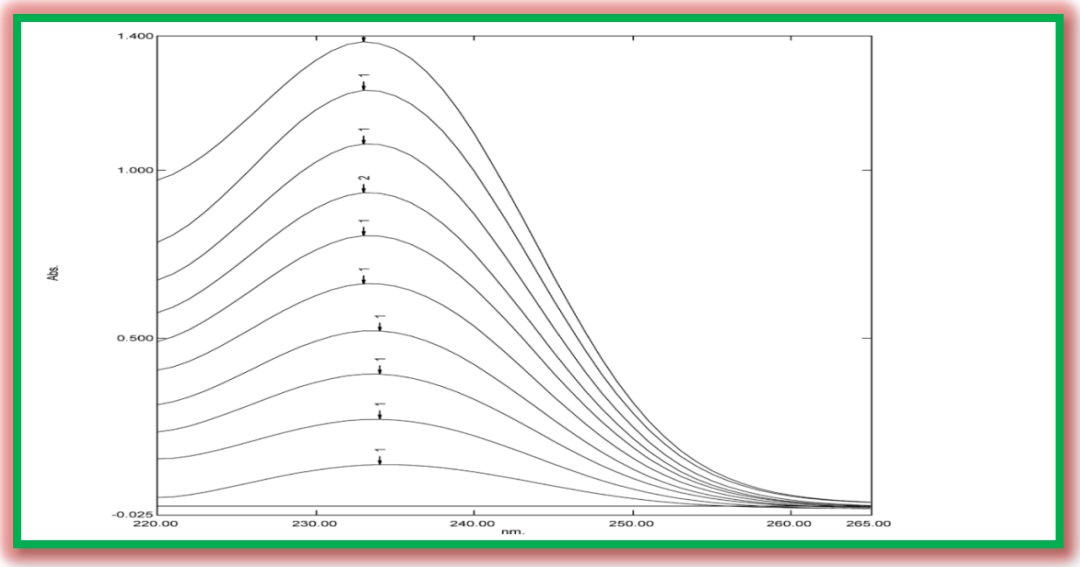

Table 9: Calibration Table of the UV-Vis Spectrophotometric Method for Metformin

Concentration (µg/ml)	Mean Absorbance (n=6)	Statistical analysis
2	0.160	
4	0.289	
6	0.432	
8	0.590	Mean=0.815
10	0.749	SD=0.446
12	0.915	%RSD =0.5473
14	1.047	Slope =0.073
16	1.173	Intercept =0.005
18	1.320	R^2 =0.999
20	1.476	

Fig 4: Calibration Curve of Metformin at 237nm

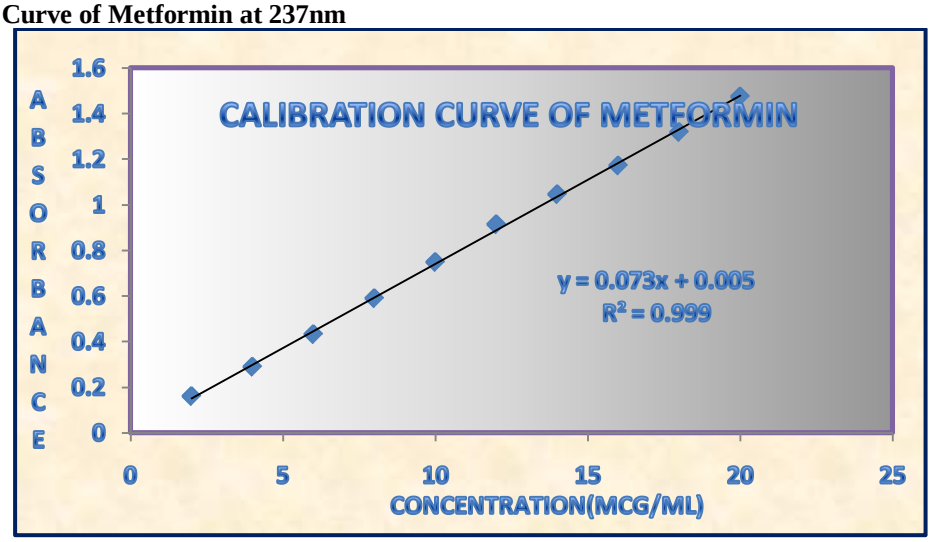

Table 10: Optical characteristics of Metformin

Beer's Law limit (µg/ml)	2-20mcg/ml
Correlation coefficient	0.999
Regression equation (Y*)	Y=0.073X+0.005
Slope (a)	0.07
Intercept (b)	0.005

Table 11: Accuracy Data of the UV-Vis Spectrophotometric Method for Metformin

No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis		
	Amount present in Formulation	Amount of drug added		Mean	SD	%RSD
S_1 : 80 %	10	8	98.08	99.023	0.939	0.009
S_2 : 80 %	10	8	99.04			
S_3 : 80 %	10	8	99.95			
S_4 : 100 %	10	10	99.00	98.99	0.915	0.009
S_5 : 100 %	10	10	99.86			
S_6 : 100 %	10	10	98.03			
S_7 : 120 %	10	12	99.69	99.993	0.379	0.003
S_8 : 120 %	10	12	100.34			
S_9 : 120 %	10	12	99.68			

Table 12: Precision Data of the UV-Vis Spectrophotometric Method for Metformin

Concentrations (mcg/ml)	Absorbance	Calculated Amount (mcg/ml)	Statistical Analysis
10	0.748	9.05	Mean =9.09 SD=0.068 %RSD =0.007
10	0.750	9.15	
10	0.749	9.02	
10	0.748	9.06	
10	0.748	9.08	
10	0.749	9.20	

Table 13: Intraday Precision Data of the UV-Vis Spectrophotometric Method for Metformin

Conc.(mcg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Statistical Analysis
10	0.748	0.749	0.747	Mean =10.13 SD =0.011 %RSD =0.002
10	0.749	0.748	0.750	
10	0.749	0.747	0.748	
10	0.750	0.750	0.749	
10	0.749	0.749	0.749	
10	0.751	0.751	0.749	
Mean	0.749	0.749	0.748	
Calc.Amt. (mcg/ml)	10.12	10.14	10.14	

Table 14: Interday Precision Data of the UV-Vis Spectrophotometric Method for Metformin

Sl.No.	Concentration	Day 1	Day 2	Day 3	Statistical Analysis
1	10	0.748	0.749	0.749	Mean =10.11 SD =0.02 %RSD =0.001
2	10	0.749	0.749	0.749	
3	10	0.748	0.751	0.751	
4	10	0.749	0.749	0.750	
5	10	0.749	0.747	0.748	
6	10	0.748	0.748	0.748	
	Mean	0.748	0.748	0.749	
	Calc. Amt.(mcg/ml)	10.09	10.10	10.14	

Table 15: Ruggedness Data of the UV-Vis Spectrophotometric Method by Different Analysts for Metformin

Analyst-1				Analyst-2			
Conc. (µg/ml)	**Abs.**	**Calc. Amt.**	**Statistical Analysis**	**Conc. (µg/ml)**	**Abs.**	**Calc. Amt.**	**Statistical Analysis**
10	0.748	10.12	Mean=10.11 SD=0.02 %RSD=0.002	10	0.749	10.14	Mean=10.12 SD=0.01 %RSD=0.001
10	0.749	10.14		10	0.750	10.12	
10	0.750	10.09		10	0.749	10.14	
10	0.749	10.10		10	0.748	10.09	
10	0.749	10.14		10	0.748	10.14	
10	0.749	10.12		10	0.748	10.12	

Table 16: Robustness Data of the UV-Vis Spectrophotometric Method by Different Solvent Ratio for Metformin

(92:08)				(88:12)			
Conc. (mcg/ml)	**Abs.**	**Calc. Amt.**	**Statistical Analysis**	**Conc. (mcg/ml)**	**Abs.**	**Calc. Amt.**	**Statistical Analysis**
10	0.749	10.12	Mean=10.11 S.D=0.02 %RSD=0.001	10	0.749	10.12	Mean=10.11 S.D=0.02 %RSD=0.001
10	0.748	10.14		10	0.748	10.14	
10	0.749	10.08		10	0.750	10.12	
10	0.750	10.10		10	0.749	10.08	
10	0.751	10.08		10	0.748	10.10	
10	0.749	10.14		10	0.750	10.14	

Table 17: Concentrations of Glipizide & Metformin in Mixed Standards (1:25)

S.No	Glipizide Conc.(mcg/ml)	Metformin Conc.(mcg/ml)
Mix-1	0.2	0.5
Mix-2	0.4	01
Mix-3	0.6	1.5

Table 18: Analysis of Result of Lab. Solution of Glipizide

S.No.	Conc. Of drug (mcg/ml)	Conc. Of drug after Multicomponent (mcg/ml)	Percentage of result obtained	Statistical analysis
SAMPLE-1	0.2	0.202	101.45	Mean=100.4 SD=1.64 %RSD=0.025
SAMPLE-2	0.4	0.405	101.25	
SAMPLE-3	0.6	0.591	98.50	

Table 19: Analysis of Result of Lab. Solution of Metformin

S.No.	Conc. Of drug in (mcg/ml)	Conc. Of drug after Multicomponent (mcg/ml)	Percentage of result obtained	Statistical Analysis
SAMPLE-1	0.5	0.49	98	Mean=99.6 S.D. = 1.42 %RSD=0.014
SAMPLE-2	1	1.002	100.2	
SAMPLE-3	1.5	1.49	100.67	

Table 20: Concentrations of Glipizide & Metformin in the Sample of Formulation

S.No.	Glipizide Conc.(µg/ml)	Metformin Conc.(µg/ml)
Mix-1	0.2	5
Mix-2	0.4	10
Mix-3	0.6	15
Mix-4	0.8	20
Mix-5	1	25

Fig 5: Absorbance Curve of Glipizide and Metformin Formulation in (1:25) Ratio

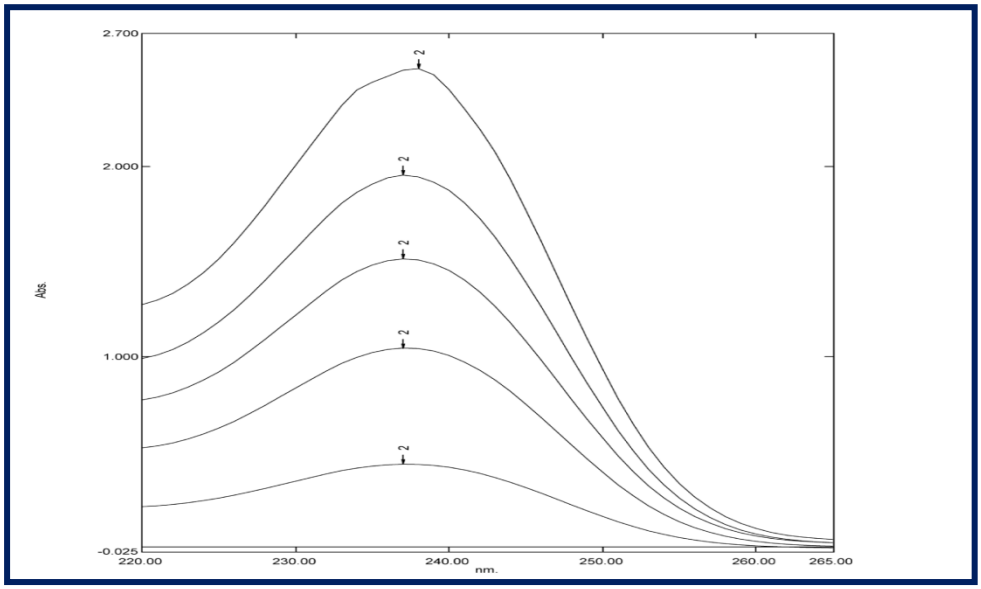

Table 21: Analysis of Result of Glipizide in Formulation

S.No.	Conc. Of drug in (µg/ml)	Conc. Of drug after Multicomponent (µg/ml)	Percentage of result obtained	Statistical analysis
SAMPLE-1	0.5	0.502	100.4	%Mean=99.54 SD=0.925
SAMPLE-2	0.7	0.690	98.57	
SAMPLE-3	0.9	0.897	99.66	

Table 22: Analysis of Result of Metformin in Formulation

S. No.	Conc. Of drug in (µg/ml)	Conc. Of drug after Multicomponent (µg/ml)	Percentage of result obtained	Statistical analysis
SAMPLE-1	1.25	1.29	100.03	%Mean=99.61 S.D.=0.704
SAMPLE-2	1.75	1.73	98.8	
SAMPLE-3	2.25	2.28	100.01	

DISCUSSION

Glipizide obeys the Beer's limit in the range of 2-20 µg/ml with the regression coefficient of 0.999. The results of recovery studies reveals that the method is accurate with the mean % recovery values between 98.96 – 99.903, S.D. values between 0.378– 0.935 and the % R.S.D. values between 0.003– 0.009. While performing the repeatability of the method it was found that the method is precise with the S.D. value of 0.064 and % R.S.D. value of 0.007. The % R.S.D values of the intraday and Interday precision studies also supports this preciseness of the method. Further, the method was found rugged by showing the S.D. value between 0.024– 0.016 and % R.S.D value ranging from 0.001 – 0.003. The method was robust with an average S.D. and % R.S.D of 0.045 and 0.005 respectively and Metformin obeys the Beer's limit in the range of 2-20 µg/ml with the regression coefficient of 0.999. The results of recovery studies reveals that the method is accurate with the mean % recovery values between 98.99– 99.993, S.D. values between 0.379–0.939 and the % R.S.D. values between 0.003– 0.009. While performing the repeatability of the method it was found that the method is precise with the S.D. value of 0.068 and % R.S.D. value of 0.007. The % R.S.D values of the intraday and Interday precision studies also supports this preciseness of the method. Further, the method was found rugged by showing the S.D. value between 0.01– 0.02 and % R.S.D value ranging from 0.001– 0.002. The method was robust with an average S.D. and % R.S.D of 0.02 and 0.001 respectively.

CONCLUSION

By the multi component method the laboratory samples, as well as the marketed formulations were analyzed. The results were validated statistically and the results obtained are found well within the prescribed limits. From these results it can be concluded that, the developed multi component method is simple, accurate, precise, rapid and economical. Hence, this method can be used routinely for the simultaneous estimation of

Glipizide and Metformin in industries and different analytical laboratories.

ACKNOWLEDGEMENTS

The authors thanks to Prof. P.N. Murthy Director cum Principal and Prof. Dr. Uma Shankar Mishra HOD of P.A. & Q.A., Royal College of Pharmacy & Health Sciences, Berhampur, Odisha, for providing required facilities to carry out this research work.

REFERENCES

1. Sweetman SC, Martindale-The complete drug reference, 34th Edn, Pharmaceutical press: 5083 (2005).
2. Beckett A.H., Stenlake J.B, Practical Pharmaceutical Chemistry, 4th Edn, CBS Publishers and Distributers: 221 (2005).
3. Dhabale, Seervi CR, Simple accurate precise reproducible and economical procedures for simultaneous estimation of Glipizide and Metformin hydrochloride in tablet dosage form, International Journal of ChemTech Research, Vol.2.2: 813-817 (2009).
4. Radjenovic J, Perez S, Petrovic M and Barcelo D, To develope and validated a RP- HPLC method For simultaneous Analysis of a three-Component Tablet Formulation Containing Metformin Hydrochloride Pioglitazone Hydrochloride and Glibenclamide, Journal of Chromatography A,Vol. 2.2: 142-153 (2008).
5. Ming Wang, Miksa Irina R, To develope the spectral data analyses and Structure elucidation of Hypoglycemic Drug Glipizide Journal of Chromatography, Vol. 5, 318-327 (2007).
6. Mura P, Cirri M, M Gines-Dorado J M, Bettinetti G. P, To develope the method of ION-pair liquid chromatography technique for the estimation of Metformin in its multicomponent dosage forms, Journal of Pharmaceutical and Biomedical Analysis. Vol. 30-2: 227-237(2002).
7. Sujana K, to develop two new UV-Spectrophotometric methods have been developed for simultaneous estimation of Pioglitazone hydrochloride and Metformin hydrochloride in tablets, Biomed J. and Res, Vol 2-2: 102-107 (2010).
8. Yu H Nola, Ho NM Emmie, Tang P. W Francis, Wan S.M. Terence, To developed the simple, stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination of Glipizide in guinea pig plasma, Journal of Chromatography A, Vol.1, Issues 1-2: 426-434 (2008).