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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF GLIBENCLAMIDE AND METFORMIN HCL IN BULK AND TABLETS USING UV – VISIBLE SPECTROSCOPY

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ABSTRACT

Two simple, precise and economical UV methods have been developed and validated for the simultaneous estimation of Glibenclamide and Metformin hydrochloride in bulk and pharmaceutical combined dosage form. The method employed simultaneous equation method for analysis using 0.01N NaOH as a solvent. The two wavelengths 226.60nm and 233 nm were selected for estimation of Glibenclamide and Metformin HCl respectively. Linearity was observed in the concentration range of 2-10µg/ml for both Glibenclamide and Metformin HCl respectively. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents.

KEYWORDS: Glibenclamide, Metformin HCl, Simultaneous estimation.

INTRODUCTION

Glibenclamide

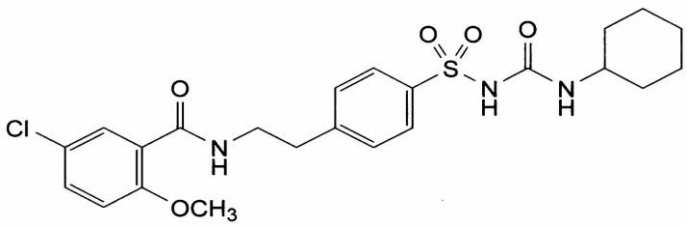

Fig. 1(a): Chemical structure of Glibenclamide.

Glibenclamide is 1-[4-[2-(chloro-2-methoxybenzamido) ethyl]-benzenesulphonyl]-cyclohexylurea, 5-chloro-N-[2[4[[[(cyclohexyl (amino) carbonyl]-amino] sulphonyl] phenyl] ethyl]-2-methoxy benzamide or 1-[[p-[-2-(5-chloro-oanisamido) ethyl] phenyl]-sulphonyl-3-cyclohexylurea. A sulphonyl urea derivative is a second generation oral hypoglycemic agent which is more potent than those of first group1 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.

Metformin Hydrochloride

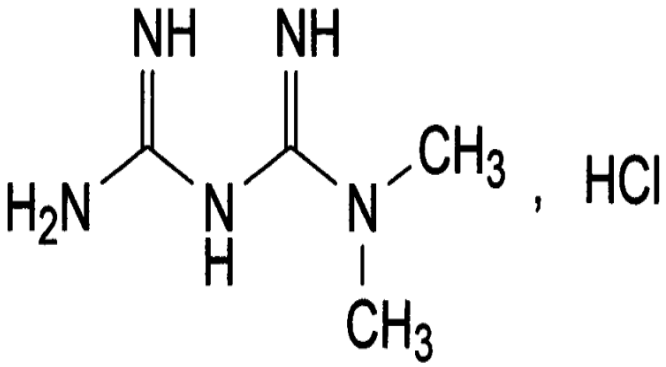

Fig. 1(b): Chemical structure of Metformin HCL.

Metformin chemically N, Ndimethyl imido dicarbonimidic 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. The chemical structure of Glibenclamide and Metformin HCL are shown shown in fig. 1.(a, b) several assay techniques have been described for quantitative determination of glibenclamide in biological fluids; these include procedures based on high performance liquid chromatography (HPLC) fluorometry, radioimmunoassay and gas chromatography. A few reports deal with the analysis of the drug in these dosage forms; such procedures include: micellar electrokinetic capillary chromatography 18, RPHPLC19, fluorometry, TLC-UV spectrophotometry, derivative spectrophotometry, UV spectrophotometry and colorimetry.

Few UV Spectrophotometric methods, HPLC and ion-pair HPLC method have been reported for the estimation of MET.

EXPERIMENTAL

Instrumentation

UV experimentation was performed on **Shimadzu 1800** UV-visible spectrophotometer equipped with Photo Diode Array (PDA) detector, with 1 cm quartz cell. Citizen Digital Ultrasonic Cleaner was used for solubility purpose.

Preliminary Solubility Study

Solubility of both drugs was determined at 27 ±1°C. MET (10mg) was added in 10ml volumetric flask and 10ml 0.01NAOH was added in it. The clear solution of MET was obtained. GLB (10mg) was added in 10ml volumetric flask and 10ML 0.01N NAOH was added in it. Then sonicate it for 10 min and The clear solution of GLB was obtained.

Preparation of stock solution

GLB and MET (10mg) were accurately weighed and transferred to two separate 10 ml volumetric flasks. Each drug was dissolved in 0.01N NaOH, shaken manually for 10 min and volume was made up to the mark with the same solvent to obtain concentration 1000 µg/ml each. Then from that solution pipette out 0.1ml solution & dilute to 10 ml in volumetric flask with same solvent to obtain final concentration 10µg/ml each.

Study of Spectra and Selection of Wavelength

The aliquot portions of standard stock solutions of GLB and MET were diluted appropriately with distilled water to obtain concentration 10 µg/mL of both drugs. The solutions of both drugs were scanned separately in the range of 400 – 200nm. and the two wavelength 226.60nm (λ max of GLB) and 233nm (λ max of MET) were selected for further study .The overlain UV absorbance spectrum of GLB and MET is shown in **Fig. 2 (A,B,C).**

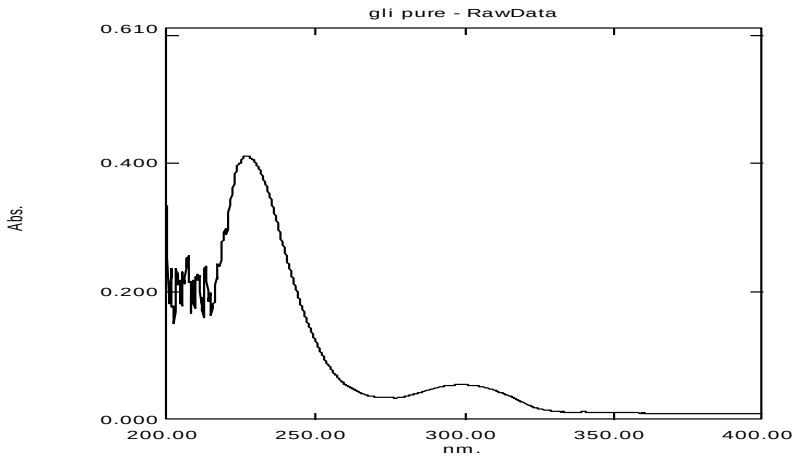

Fig 2 (A)

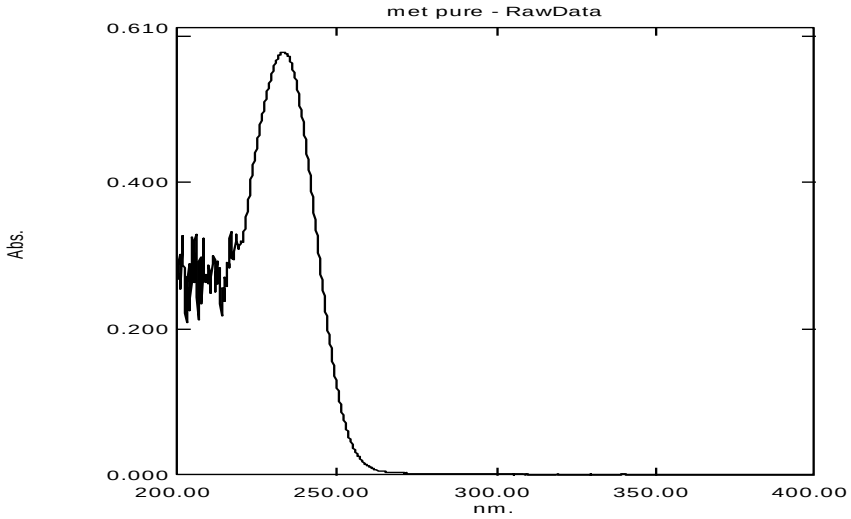

Fig 2 (B).

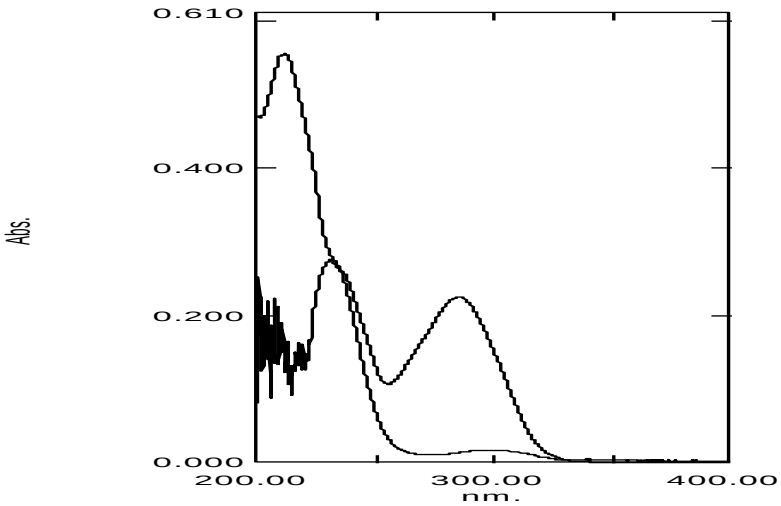

Fig. 2: UV Spectra of GLB (A), MET (B) and overlain spectra (C) of GLB and MET.

From the overlain spectrum the wavelengths selected for estimation of drugs were 226.60nm as λ max of GLB and 233nm as λ max of MET.

Study of linearity curves

The aliquot portions of standard stock solutions of GLB and MET were diluted appropriately with distilled water to get a series of concentration from 2-10µg/ml for both drugs. The absorbance of these drugs was measured at 226 nm and 233 nm respectively and calibration curves were plotted as concentrations versus absorbances.

Simultaneous equation method

Two wavelengths selected for the method are 226.60nm and 233 nm that are absorption maxima of GLB and MET respectively. Absorptivity (A 1%, cm) values at all the

wavelengths were determined from the calibration curve. Absorptivity (A 1%, 1 cm) values for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

Cx = (A2ay1-A1ay2)/ (ax2ay1-ax1ay2).

Cy = (A1ax2-A2ax1)/ (ax2ay1-ax1ay2).

Where, A1 and A2 are absorbances of mixture at 226.60nm and 233nm respectively, ax1 and ax2 are Absorptivity values of AM at λ1 and λ2 respectively and ay1 and ay2 are Absorptivity value of AT at λ1 and λ2 respectively. Cx and Cy are concentrations of GLB and MET respectively.

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 226nm and 233 nm for GLB and MET, respectively and calibration curves were plotted at selected wavelengths; The E (1%, 1cm) of each drug at both wavelengths was determined; results are presented in table 2. The overlain spectra of GLB and MET are shown in fig. 2. Two simultaneous equation (in two variables C1 and C2) were framed by using E (1%, 1cm)

A1 = (60.70) C1 + (84.20) C2 (I).

A2 = (58.20) C1 + (110.20) C2 (II).

Where, C1 and C2 are the concentrations of GLB and MET measured in g /100 ml, in the sample solutions. A1 and A2 are the absorbances of the sample solutions, at selected wavelength i.e. 229.5 nm and 237 nm, respectively. By applying the Cramer's rule (Beckett and Stenlake, 2005) to equations I and II, the concentrations CGLB and CMET can be determined as follows:

CGLB = A2 (58.20) - A1 (110.20)/ - 2045.23 (III).

CMET = A1 (84.80) - A2 (60.70) / - 3019.65 (IV).

Table no. 1: E (1%, 1cm) for GLB and MET.

***E(1%, 1cm) at 226 nm ± SD**		***E(1%, 1cm) at 233 nm ± SD**	
GLB	MET	GLB	MET
ax1= 60.17 ± 0.48	ay1=84.80 ± 0.21	ax2=58.20 ± 0.60	ay2= 110.20 ± 0.81

*mean of ten readings.

Analysis of Marketed Formulation by Proposed Method

20 Tablets were accurately weighed, and reduced to fine powder. A quantity of tablet powder was transferred to 10ml volumetric flask and 10ml 0.01N NAOH was added in it. Sonicate it for 11 min. The solution was filtered through Whatman filter paper no. 41. The filtrate was further diluted with distilled water to get final concentration (1000µg/ml). From this solution 10µg/ml was prepared. The absorbance of sample solution was measured at 226 nm and 233 nm and the results are shown in **Table No. 2.**

Sample	Label Claimed	% Label Claim* ± SD	%RSD
DAONIL-M	Glibenclamide 5mg	101.17± 0.73	0.72
	Metformin HCL500mg	99.96± 0.65	0.67

Validation of Method

Accuracy

Accuracy of each of the proposed method was ascertained on the basis of recovery studies performed by standard addition method as shown in the table no.2.

Table no. 3.

Drug name	Sr. no.	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
GLB	1	80	6	4.8	0.321 ± 0.0004	4.22 ± 0.02	87.91± 0.35
	2	100	6	6	0.521 ± 0.0004	5.75± 0.01	95.88 ± 0.18
	3	120	6	7.2	0.655 ± 0.0004	7.03± 0.01	97.62± 0.15
MET	1	80	6	4.8	0.397 ±0.0004	3.58±0.01	74.67±0.26
	2	100	6	6	0.497 ± 0.0004	4.56± 0.01	75.97± 0.21
	3	120	6	7.2	0.522 ± 0.0008	7.19± 0.03	99.85± 0.39

*mean of each 3 reading.

Precision

Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed method as shown in table no.3.

Table no. 4.

Drug name	Conc. In µg/ml	Inter day			Intra Day		
		Mean*± S.D.	Amt. Found	% Amt. Found	Mean*± S.D.	Amt. Found	%Amt. Found
GLB	4	0.153±0.001	4.08	101.96	0.148±0.001	3.97	97.39
	6	0.222±0.001	6.11	101.80	0.213±0.001	6.03	100.51
	8	0.300±0.001	8.42	105.27	0.296±0.001	7.58	94.75
MET	4	0.198±0.001	4.04	100.91	0.186±0.001	3.78	94.38
	6	0.264±0.001	5.49	91.55	0.281±0.001	5.84	97.34
	8	0.385±0.001	8.28	101.54	0.393±0.001	8.28	103.44

*mean of each 3 reading.

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no. 04.

Table no. 5.

Sr.No.	Conc		Abs		Amt Found		% Amt Found	
	GLB	MET	GLB	MET	GLB	MET	GLB	MET
1	6	6	0.187	0.289	5.06	6.02	84.31	100.36
2	6	6	0.186	0.288	5.03	6.00	83.82	100.00
3	6	6	0.184	0.296	4.97	6.17	82.84	102.90
4	6	6	0.185	0.287	5.00	5.98	83.33	99.64
5	6	6	0.182	0.286	4.91	5.96	81.86	99.28
6	6	6	0.186	0.289	5.03	6.02	83.82	100.36
7	6	6	0.187	0.287	5.06	5.98	84.31	99.64
8	6	6	0.188	0.286	5.09	5.96	84.80	99.28
9	6	6	0.181	0.287	4.88	5.98	81.37	99.64
10	6	6	0.180	0.288	4.85	6.00	80.88	100.00
	MEAN		0.185	0.285	4.99	6.01	83.14	100.11
	SD		0.003	0.003	0.077	0.060	1.28	1.00
	% RSD		1.42	1.42	1.54	1.00	1.54	1.00

Linearity and Range

The suitable aliquots were taken to obtain 2, 4, 6, 8, 10 µg/ml. from GLB stock solution. The suitable aliquots were taken to obtain 2, 4, 6, 8, 10 µg/ml from MET stock solution. The results are shown in table no 05, Figure no. 3 and Figure no. 4.

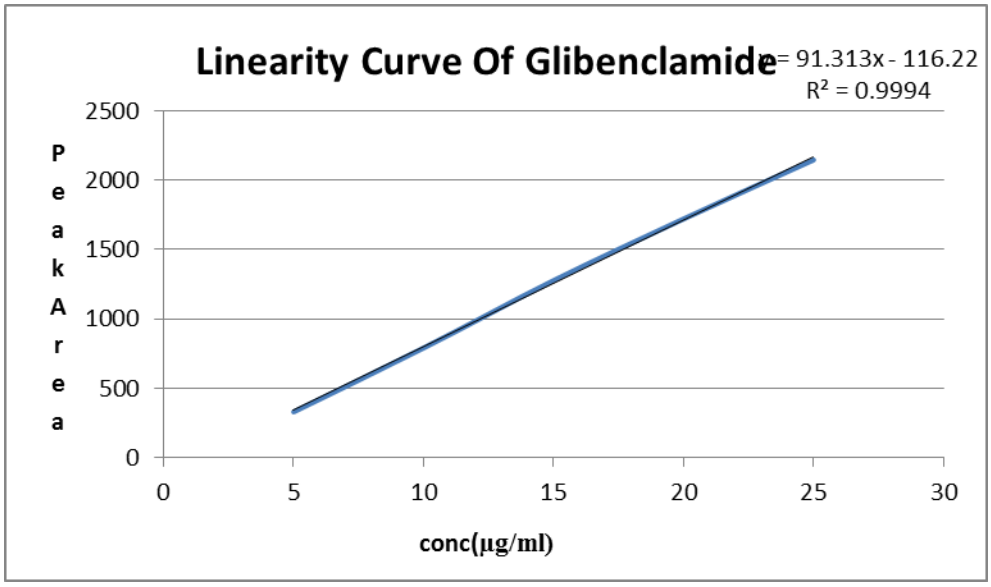

Fig 3 linearity curve of Glibenclamide at 226.60nm.

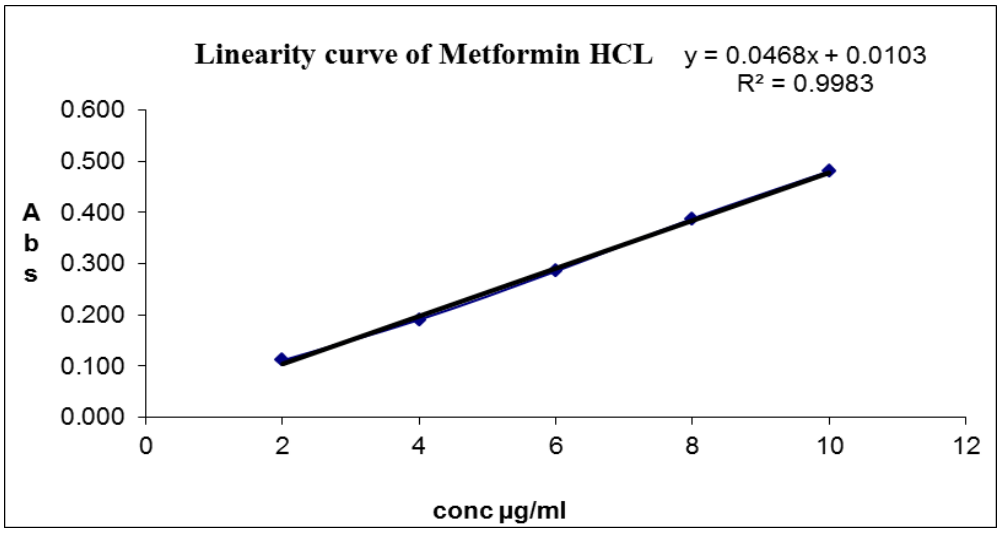

Fig. 4 linearity curve of Meformin HCL at 233nm.

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 no 06.

Table no. 6: Validation Parameter.

Parameters	GLB	MET
Working wavelengths	226.60nm	233nm
Linearity range (µg/mL)	2-10	2-10
Precision [%RSD] Inter-day [n=3] Intra-day[n=3]	0.192-0.376 0.195-0.676	0.150-0.292 0.147-0.311
Repeatability (Mean* ±SD)	83.14±1.28	100.11±1.00
Ruggedness [%RSD] Analyst I[n=3] Analyst II [n=3]	1.01 0.45	1.00 0.99
% Recovery [n=3] %RSD	0.15-0.40	0.28-0.39

RESULTS AND DISCUSSION

In this method precision was studied as repeatability (% RSD < 2) and inter and intra-day variations (%RSD < 2) for both drugs. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80,100 and 120 % level. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The % recovery, repeatability data, ruggedness data were presented in **Table-6.**

CONCLUSION

The developed was found to be accurate, precise, economic, rapid and rugged. Further, the developed method is simple and can usually be used for estimation of both these drugs in their combined dosage form. This method is used for routine analysis of drugs in bulk and pharmaceutical formulation.

REFERENCES

1. 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.
2. Martindale: The Complete Drug Reference, Pharmaceutical Press, 2007; 411.
3. Seema M. Dhole1, Pramod B. Khedekar, Nikhil D. Amnerkar, UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Pioglitazone HCl, Metformin HCl and Glibenclamide in Multicomponent Formulation, Int. J. Analytical & Bioanalytical Chem, 2013; 3(1): 18-22.
4. Subhashini Edla and B. Syama Sundhar, New Analytical Method Development And Validation For The Simultaneous Estimation Of Metformin And Glibenclamide in Bulk And Tablet Dosage Form Using Rp-Hplc, RJC, 2014; 7(1): 55-63.

5. Narendra Nyola and Govinda Samy Jeyabalan, Method Development of Simultaneous Estimation Of Sitagliptin And Metformin Hydrochloride In Pure And Tablet Dosage Form By Uv-Vis Spectroscopy, WJPPS, 2012; 1(4): 1392-1401.
6. Patil Sudarshan S., Bonde C. G., Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy, Int. J. Chem Tech Res., 2009; 1(4): 905-909.
7. Narsimha rao. Doredla, Shanmugasundaram. P, Hemant vaishnav, 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, Int. J. ChemTech Res., 2011; 3(4): 2011-2017.
8. Abida Bilal, Kanwal Rehman, Muhammad Sajid Hamid Akash, Khalid Hussain, Muhammad Ibrahim and Syed Saeedul Hussan, Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of Tablet Dosage form Using UV-Visible Spectroscopy, J Mol Genet Med, 2013; 7(3): 1-6.
9. Shweta s. Havele,sunil r. Dhaneshwar, Determination of Glibenclamide, Metformin Hydrochloride And Rosiglitazone Maleate By Reversed Phase Liquid Chromatographic Technique In Tablet Dosage Form, Chem. Ind. Chem. Eng. Q., 2014; 20(1): 39−47.
10. Amruta B. Loni, Minal R. Ghante, S. D. Sawant, Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form, Der Pharma Chemica, 2012; 4(3): 854-859.
11. Asit Kumar De, Ayan Kumar Dey, Angshuman Biswas, Simultaneous Estimation Of Metformin Hydrochloride And Glibenclamide By Rphplc Method From Combined Tablet Dosage Form, IJSIT, 2012; 1(2): 98-105.