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Research Article

Development and Validation of Stability Indicating Assay Method for Simultaneous Estimation of Glibenclamide and Metformin

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ABSTRACT

A high performance reverse phase liquid chromatographic procedure is developed for simultaneous estimation of Metformin and Glibenclamide in combined tablet dosage form. The method was carried out on Agilent Hypersil ODS (4.6 x 250 mm) column with a mobile phase used consisting of Methanol: Water (0.1 % OPA) OPA= Ortho Phosphoric acid (80:20) and the pH of buffer was adjusted to 3 using 2M Orthophosphoric acid. The detection of the combined dosage form was carried out at 228 nm and a flow rate employed was 1 ml/min and column oven temperature at 300C. The retention times of Metformin & Glibenclamide were 3.4667 & 7.3500 minutes respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification as per ICH norms. The proposed method can be used for the estimation of these combined drugs.

Keywords: Glibenclamide, Metformin, HPLC, ICH.

INTRODUCTION

Metformin is a potent anti-diabetic drug of class biguanide class considered as the first line treatment of diabetes mellitus particularly in overweight and obese people and people with normal kidney function[1,2]. It has been extensively used in the treatment of non-alcoholic fatty liver disease and premature puberty, three other diseases that feature insulin resistance. It is used in the treatment of gestational diabetes and also polycystic ovary syndrome[3,4]. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It also reduces LDL Cholesterol and triglyceride levels and not associated with gaining weight[5,6]. Metformin has also been reported to decrease the blood levels of thyroid-stimulating hormone in people with hypothyroidism[7].

Glibenclamide acts as anti-diabetic drug belongs to the class of sulfonylureas commonly known as sulfa drugs. It is also helpful in improving the out coming results on animal stroke models by preventing brain swelling and enhancing neuroprotection[8]. Recent studies reveled that Glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulin secreting medications, and has potential to lower some of side effects. So the present work is aimed to achieve a new RP-HPLC method determination estimation of Metformin and Glibenclamide API and tablet dosage forms[9].

MATERIALS AND METHODS

Reagents and Chemicals

Methanol used was of HPLC grade of Merck and Milli Q water was used for the preparation of the mobile phase. All other reagents used were of HPLC or AR grade.

Drugs used

Metformin (potency: 99.1%) and Glibenclamide (potency: 100%) were in Shree Reliable Industrial Training Center, Jalgaon. Tablet formulation DIAMET containing Metformin (500 mg) and Glibenclamide (2.5 mg) was purchased from market for analysis.

Determination of Wavelength Maxima using ultra violet visible spectroscopy

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte was analyzed at different wavelengths. As per the response of analyte, 228 nm was selected.

Metformin standard stock solution

An accurately weighed quantity of Metformin 10 mg was transferred to the 10 ml volumetric flask and dissolved in Methanol. The volume was made up to the mark with the same to make (1000 ug/ml). The aliquot portions of stock standard solutions were diluted appropriately with diluents Methanol to obtain concentration 100ug/ml of each drug. The solutions were scanned in the range of 400–200 nm in 1 cm cell against blank.

Glibenclamide standard stock solution

An accurately weighed quantity of Glibenclamide 10 mg was transferred to the 10 ml volumetric flask and dissolved in Methanol. The volume was made up to the mark with the same to make (1000 ug/ml).

The aliquot portions of stock standard solutions were diluted appropriately with Diluents Methanol to obtain

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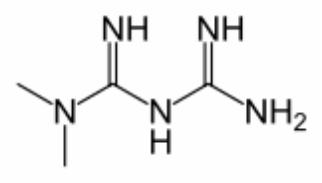
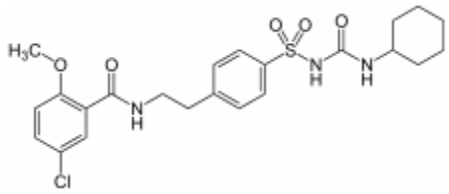


Metformin



Glibenclamide

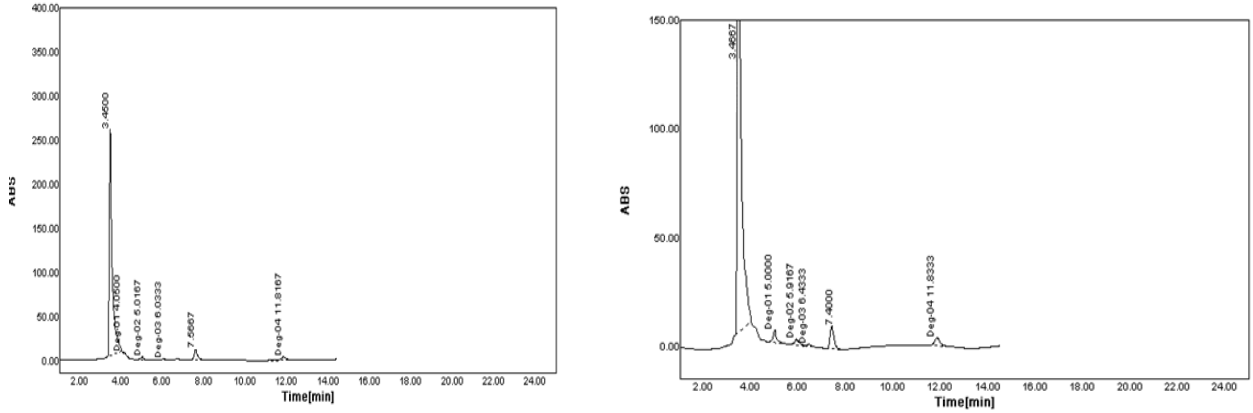


Figure 1: Chromatogram of Metformin and Glibenclamide on Acid hydrolysis (0.1N HCl) after 1 hr & 3 hrs.

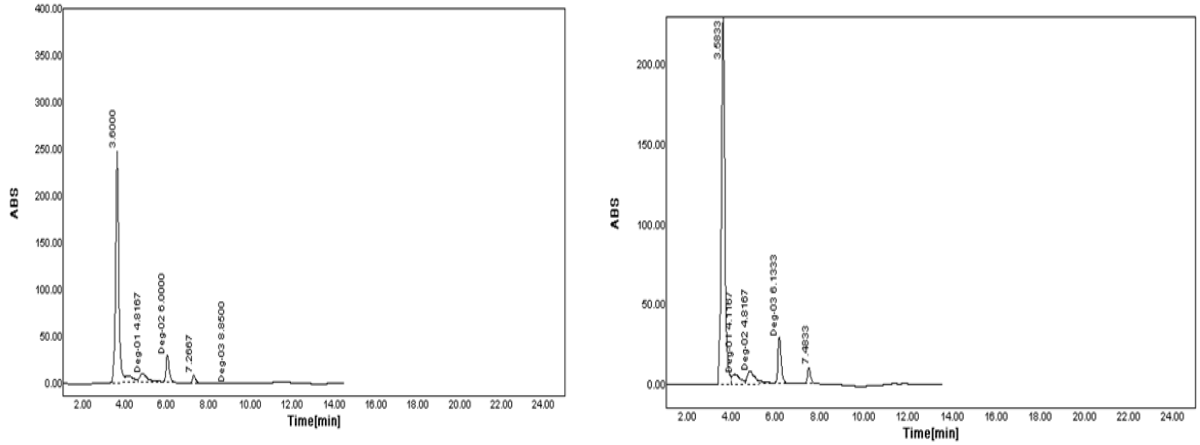


Figure 2: Chromatogram of Metformin and Glibenclamide on Alkali hydrolysis (0.1N NaOH) after 1 hr & 3 hrs

Table 1: Linearity study of Glibenclamide.

Sr. No.	Conc.	AREA-I	AREA-II	Mean	SD	RSD
1	1	85.22	83.7	84.46	1.07	1.27
2	2	166.39	167.89	167.14	1.06	0.63
3	3	251.16	254.17	252.67	2.13	0.84
4	4	335.05	341.791	338.42	4.77	1.41
5	5	424.54	432.8	428.67	5.84	1.36

Table 2: Linearity study of Metformin.

Sr. No.	Conc.	AREA-I	AREA-II	Mean	SD	% RSD
1	100	1159.92	1162.32	1161.12	1.70	0.15
2	200	2211.71	2215.7	2213.71	2.82	0.13
3	300	3122.6	3161.08	3141.84	27.21	0.87
2	400	4270.99	4260.26	4265.63	7.59	0.18
3	500	5279.91	5292.46	5286.19	8.87	0.17

concentration 100ug/ml of each drug. The solutions were scanned in the range of 400–200 nm in 1 cm cell against blank.

Mobile Phase Selection

HPLC grade Methanol and Water of analytical reagent grade in the ratio of 80:20 v/v with 0.1% Ortho Phosphoric

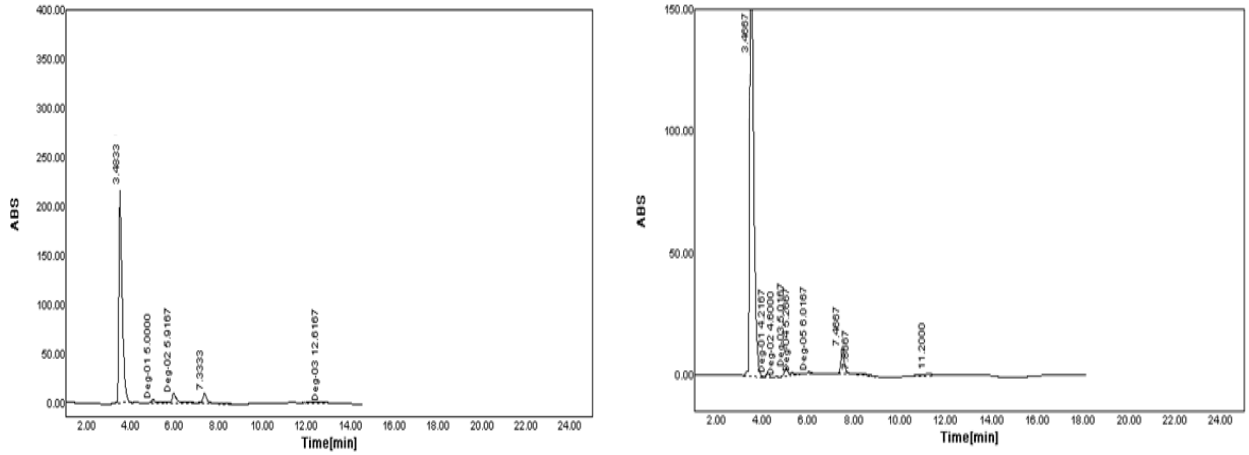


Figure 3: Chromatogram of Metformin and Glibenclamide on neutral degradation after 1 hr & 3hrs.

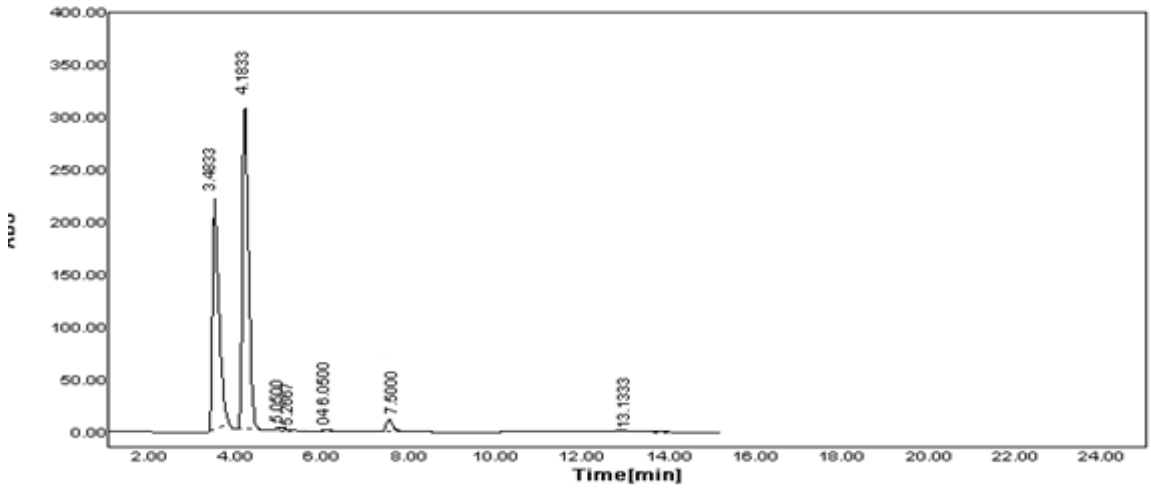


Figure 4: Chromatogram of Metformin and Glibenclamide on Oxidative degradation after 1 hr.

Table 3: Chromatogram of Metformin and Glibenclamide for precision study.

Sr. No.	Conc.	Area	II	Mean	Amount Found	% Amount Found	SD	%RSD
1	200	2202.46	2212.32	2207.39	202.36	101.18	6.97	0.32
2	300	3112.53	3128.68	3138.71	292.78	97.59	11.42	0.36
3	400	4282.8	4278.23	4280.51	403.64	100.91	3.23	0.08

Table 4: Result of Intraday & Interday precision study for Metformin.

Sr. No.	Conc.	Area	II	Mean	Amount Found	% Amount Found	SD	%RSD
				Intraday				
1	200	2202.46	2212.32	2207.39	202.36	101.18	6.97	0.32
2	300	3112.53	3128.68	3138.71	292.78	97.59	11.42	0.36
3	400	4282.8	4278.23	4280.51	403.64	100.91	3.23	0.08
				Interday				
1	200	2202.46	2212.32	2207.39	202.36	101.18	6.97	0.32
2	300	3112.53	3128.68	3138.71	292.78	97.59	11.42	0.36
3	400	4282.8	4278.23	4280.51	403.64	100.91	3.23	0.08

Acid was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of the drug in different solvents.

Forced degradation (stress study) of Metformin and Glibenclamide

Forced degradation carried out by applying various stress conditions to study the effect over wide range of pH, heat, and oxidation and photo degradation using the following

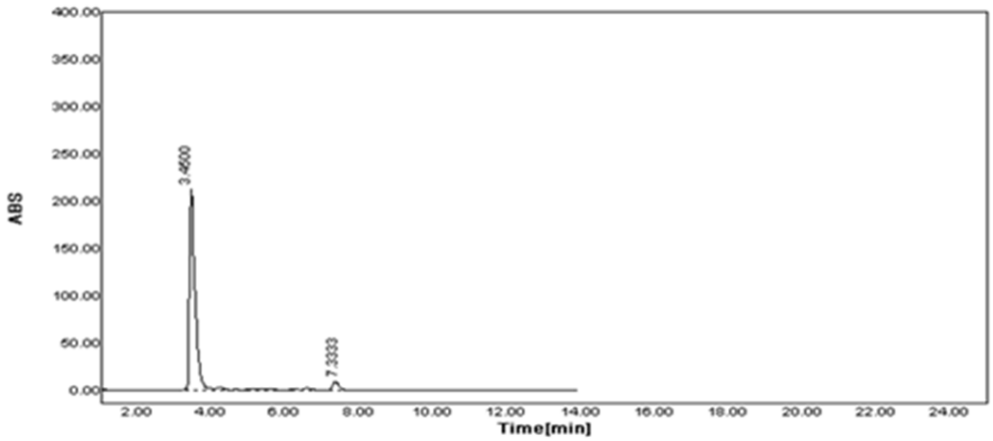


Figure 5: Chromatogram of Metformin and Glibenclamide on Photo Degradation after 34 hrs.

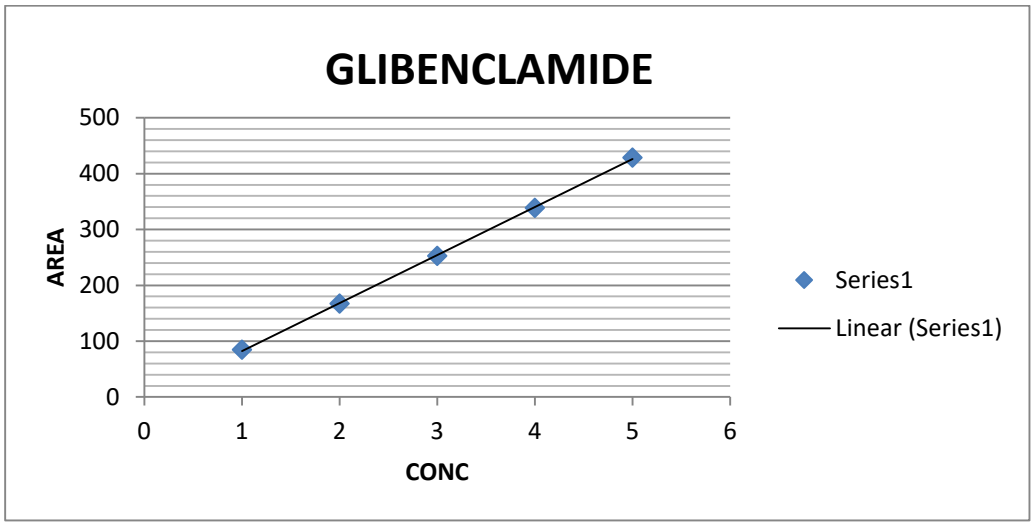


Figure 6: Calibration curve for Glibenclamide.

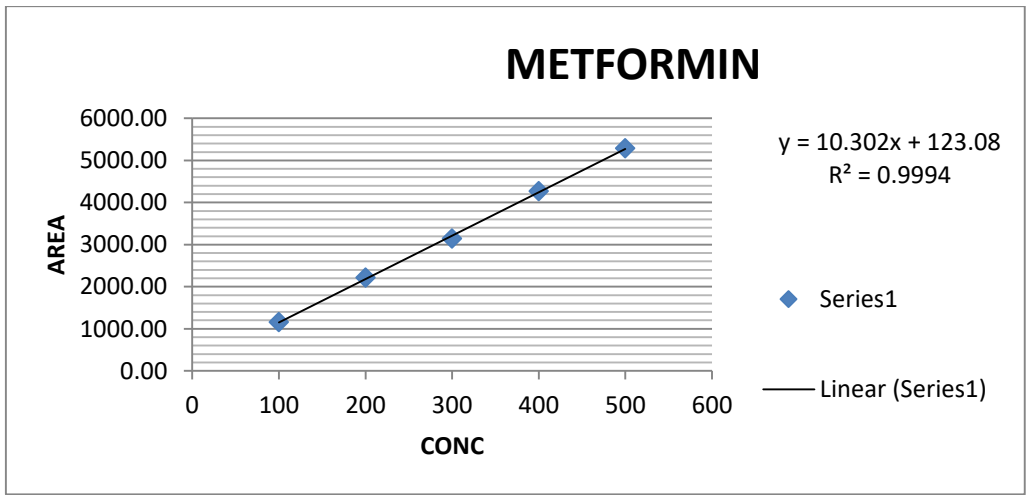


Figure 7: Calibration curve for Metformin.

approach. Stress studies were conducted in aqueous solutions.

Acid Degradation

Accurately weighed tablet equivalent to 300 mg of Metformin & 03 mg of glibenclamide were dissolved in 5.0 ml of aqueous 0.1N hydrochloric acid in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1 hr. and with heat after 3 hr.

Alkali Degradation

Accurately weighed tablet equivalent to 300 mg of Metformin & 03 mg of glibenclamide were dissolved in 5.0 ml of aqueous 0.1N sodium hydroxide in a separate

Table 5: Result of Intraday & Interday precision study for Glibenclamide.

Sr. No.	Conc.	Area	II	Mean	Amount Found	% Amount Found	SD	%RSD
				Intraday				
1	2	163.39	160.68	162.04	1.92	96.00	1.92	1.18
2	3	254.87	260.54	257.70	2.95	98.50	4.01	1.56
3	4	329.65	333.97	331.81	3.90	97.50	3.05	0.92
				Interday				
1	2	163.39	160.68	162.04	1.92	96.00	1.92	1.18
2	3	254.87	260.54	257.70	2.95	98.50	4.01	1.56
3	4	329.65	333.97	331.81	3.90	97.50	3.05	0.92

Table 6: Result for Accuracy study (80%) of Metformin.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount rcvd	% rcvd
1	100	80	1969.28	179.25	79.25	99.06
2	100	80	1986.24	180.89	80.89	101.12
3	100	80	1976.89	179.76	79.85	100.02
			Mean	180.07	80.07	100.09
			SD	1.16	1.16	1.46
			%RSD	0.64	1.45	1.46

Table 7: Result for Accuracy study (100%) of Metformin.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount rcvd	% Rcvd
1	100	120	2395.08	220.59	120.59	100.49
2	100	120	2389.77	220.07	120.07	100.06
3	100	120	2390.78	220.54	120.45	100.23
			Mean	220.33	120.33	100.28
			SD	0.37	0.37	0.30
			%RSD	0.20	0.29	0.16

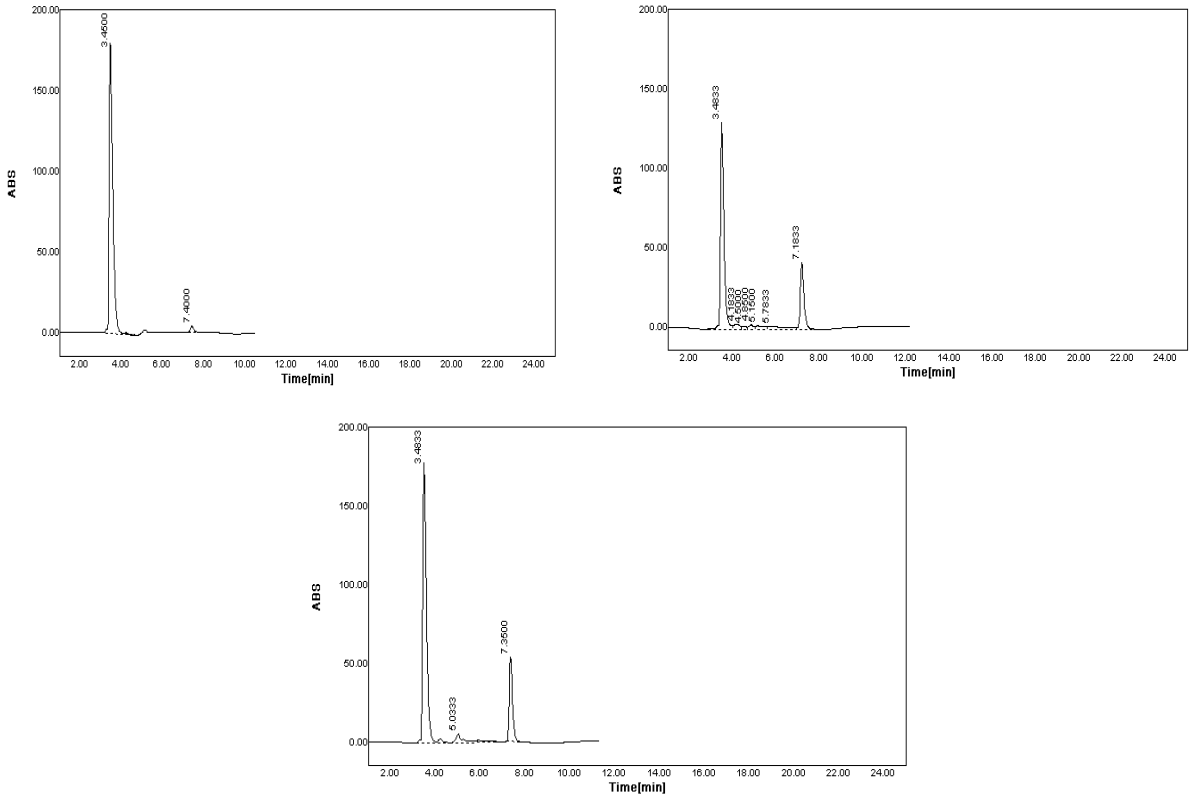


Figure 8: Chromatogram of Metformin and Glibenclamide for Accuracy (80%,100%,120%).

Table 8: Result for Accuracy study (120%) of Metformin.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount rcvd	% Rcvd
1	1	0.8	150.41	1.79	0.79	98.98
2	1	0.8	151.22	1.80	0.80	100.00
3	1	0.8	150.56	1.80	0.79	99.76
			Mean	1.80	0.80	99.49
			SD	0.01	0.01	0.72
			%RSD	0.39	0.89	0.72

Table 9: Result for Accuracy study (80%) of Glibenclamide.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount rcvd	% Rcvd
1	1	1	168.14	1.99	0.99	99.81
2	1	1	169.63	2.01	1.01	101.00
3	1	1	168.98	1.90	0.98	99.90
			Mean	2.00	1.00	100.41
			SD	0.01	0.01	0.84
			%RSD	0.71	1.41	0.84

Table 10: Result for Accuracy study (100%) of Glibenclamide.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount recvd	% Recv
1	100	100	2206.1	202.8	102.8	102.8
2	100	100	2202.1	201.6	101.6	101.6
3	100	100	2204.1	201.7	102.3	101.8
			Mean	202.7	102.7	102.7
			SD	0.30	0.30	0.30
			%RSD	0.15	0.29	0.29

Table 11: Result for Accuracy study (120%) of Glibenclamide.

Sr. no.	Ng/Band	Amount added	Area	Amount found	Amount rcvd	% Rcvd
1	1	1.2	184.51	2.18	1.18	98.33
2	1	1.2	187.36	2.22	1.22	100.41
3	1	1.2	186.79	2.20	1.20	99.98
			Mean	2.20	1.20	99.37
			SD	0.03	0.03	1.47
			%RSD	1.29	.36	1.48

volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr and with heat after 3 hr.

Neutral Degradation

Accurately weighed tablet equivalent to 300 mg of Metformin & 03 mg of glibenclamide were dissolved in 10.0 ml of water in a separate volumetric flask and kept at room temperature for 1hr and with heat after 3 hr.

Oxidative Degradation

Accurately weighed tablet equivalent to 300 mg of Metformin & 03 mg of glibenclamide were dissolved in 10.0 ml of 3% H_2O_2 in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr and without heat after 3 hr.

Photo Degradation

Accurately weighed tablet equivalent to 300 mg of Metformin & 03 mg of glibenclamide were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in sunlight for 3 days.

RESULT AND DISCUSSION

Method validation

This method described above had been validated as per the ICH guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. And the results were summarized below.

Linearity

The linearity responses in the concentration range of 2-10 µg/ml for MET and 6-30 µg/ml glibenclamide for was determined. And the co-relation coefficient was NLT 0.99

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at a concentration of 300µg/ml for MET and 3µg/ml for glibenclamide. And the RSD calculated from replicates of assay values NMT2.0%.

Accuracy

Accuracy (Recovery) of the method was determined by spiking 80, 100 and 120% of working standard at a concentration of 300µg/ml for MET and 3µg/ml for glibenclamide. Samples were injected in triplicate across its range according to the assay procedure. The RSD calculated from replicates of assay values NMT 2.0% and the percentage recovery was in between 99% to 102%.

Table 12: Robustness study for Metformin.

Sr.no.	Parameter		Conc. (ug/ml)	Area	Mean	S.D.	% RSD
			500	4867.09			
			500	4875.86			
		1.1	500	4873.70	4871.48	6.20	0.13
	Flow Rate		500	5609.28			
	Change	0.9	500	5680.85			
1	(ml)		500	5656.45	5645.07	50.61	0.90
		81+19	500	5370.96			
			500	5380.14		6.77	
2	Mobile phase		500	5372.06	5375.75		0.13
	composition	79+21	500	5302.00			
			500	5311.25	5306.63	6.54	0.12
			500	5309.96			
3	Wavelength	227	500	5330.25			
	Change		500	5374.63	5352.44	31.38	0.59
	(nm)		500	5326.64			
		229	500	5351.86			
			500	5328.96	5340.41	16.19	0.30
			500	5335.36			

Table 13: Robustness study for Glibenclamide.

Sr. no.	Parameter		Conc. (ug/ml)	Area	Mean	S.D.	% RSD
			500	479.71			
			500	485.32			
		1.1	500	480.52	482.52	3.97	0.82
			500	427.28			
	Flow Rate Change	0.9	500	432.65			
1	(ml)		500	431.45	429.97	3.80	0.88
		81+19	500	460.38			
			500	475.63		10.78	
2	Mobile phase		500	472.06	468.08		2.30
	composition	79+21	500	447.03			
			500	452.69	449.86	4.00	0.89
			500	450.29			
3	Wavelength Change	227	500	472.28			
	(nm)		500	480.39	476.34	5.73	1.20
			500	475.52			
		229	500	490.42			
			500	481.69	486.06	6.17	1.27
			500	486.36			

Detection quantitation limits

The LOD for Metformin and Glibenclamide was found to be 0.3µg/ml and 0.11µg/ml, respectively. The LOQ was 0.93 µg/ml and 0.34µg/ml for Metformin and Glibenclamide, respectively.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate (1± 0.1 ml/min), wavelength (±1nm),organic phase(± 10%) and ph(±0.2)

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of metformin and glibenclamide the mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. The most striking feature of this method is its simplicity and rapidity also best separation of the analyte against the method available. The recovery studies revealed excellent accuracy and high precision of the method. The HPLC method was found to give better results and can be employed for routine analysis in quality control analysis. The described method gives accurate and precise results for determination of metformin and glibenclamide mixture in tablet dosage form.

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