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Research Article

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Development of Reverse Phase HPLC Method and Validation for the Estimation of Metformin Hydrochloride and Glipizide in Combined Dosage Form

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of Metformin hydrochloride and Glipizide in tablet dosage form. A Symmetry C18 (4.6 × 150 mm, 5µm, Make: Thermosil), Ambient, with mobile phase containing methanol: phosphate buffer pH 4.5 (65:35), pH 4.5 adjusted with Ortho Phosphoric acid were used. The flow rate was 0.8 mL per min. The retention time of Metformin hydrochloride and Glipizide were 2.572 and 3.833 min respectively. The proposed method is accurate, precise, specific and rapid for estimation of Glipizide and Metformin hydrochloride in tablet dosage form.

Keywords: Reverse phase high performance liquid chromatography; Metformin hydrochloride; Glipizide; Symmetry C18

INTRODUCTION

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention, or treatment of diseases in human beings or animals or for alternating any structure or function of the body of human being or animals. Analytical chemistry deals with methods for determining the chemical composition of the samples of matter. A qualitative method may yields information about the identity of atomic or molecular species, or the functional groups in the sample; a quantitative method in contrast, provides numerical information as to the relative amount of one or more of these components. It is necessary to distinguish between analytical methods and analytical techniques. A technique is a fundamental scientific phenomenon that has proved useful for providing information about the composition of substances. A method is a specific application of technique to solve an analytical problem. Two other terms associated with chemical analysis is procedure and protocol. A procedure is a written instruction for carrying out a method. A procedure assumes that the user has some prior knowledge of analytical methodology, thus it does not provide great detail, only a general outline of the steps to be followed. In contrast, the most specific description of a method is a protocol. The detailed direction must be followed. Analytical methods are classified as being classical or instrumental. In classical methods, the analyses are carried out by separating the components of interest (the analyte) in a sample by precipitation, extraction or distillation. In contrast, in instrumental methods the physical properties of analyte such as conductivity, electrode potential, light absorption or emission, mass to charge ratio and fluorescence are used for qualitative and quantitative analysis for a variety of inorganic, organic and bio-chemical analytes. Now highly efficient chromatographic and electrophoretic techniques are available. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Principle types of chemical instrumentation are:

UV-Visible Spectrometry, Potentiometry, HPLC, Thermal Analysis, and GC-MS, an instrument for chemical analysis converts information stored in the physical or chemical characteristics of the analyte to information that may be manipulated or interpreted by humans. In this project work attempt would be made to develop and validate an analytical method for the simultaneous estimation of Metformine hydrochloride and Glipizide in bulk and pharmaceutical dosage form by RP HPLC [1-7].

EXPERIMENTAL SECTION

Preparation of Mobile Phase

Preparation of phosphate buffer for mobile phase:

Accurately Weighed and transferred 7.0 grams of potassium di hydrogen ortho phosphate into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. pH was adjusted to 4.5 with Orthophosporic acid.

Preparation of mobile phase l:

Mix a mixture of above buffer 350 mL (35%) and 650 mL of Methanol HPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of Standard Stock Solution Solution of Metformin and Glipizide

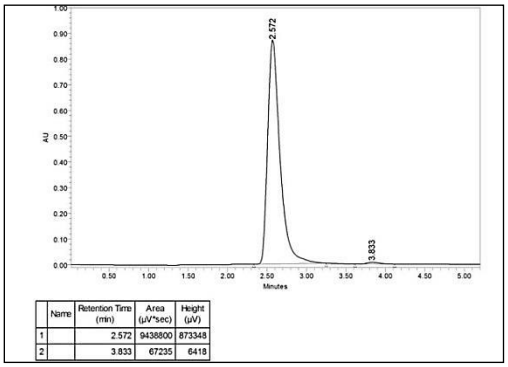
Accurately weighed and transferred 10 mg of Metformin HCL and Glipizide working standard into two different 10 mL clean dry volumetric flasks containing about 7 mL of diluent and sonicated for 3 mins to dissolve it completely. Then the volume was made up to the mark with the same diluent. Further pipette 3 ml and 0.03 ml of Metformin and Glipizide respectively and transferred into a 10 ml volumetric flask and dilute up to the mark with diluents to obtain concentration of the drugs i.e., 300 µg/ml (Metfomine HCl) and 3 µg/ml (Glipizide).

Preparation of Sample Solution

Accurately weighed and transferred 12.5 mg tablet sample powder (equivalent to 10 mg of Metformin and Glipizide) into a 10 mL clean dry volumetric flask containing about 7 mL of diluent and then it was sonicated for 3minutes to dissolve it completely and made the volume up to the mark with the diluent. Then the sample solution was filtered with the help of Whatman filter paper No 41. Then the 1 ml was further transferred into a 10 ml volumetric flask and then the 20 µl solution was injected into the instrument under optimized chromatographic conditions.

Optimize Chromatographic Parameters (Figure 1)

Equipment : High performance liquid chromatography equipped With Auto Sampler and DAD or UV detector
Column : Symmetry C18 (4.6 × 150 mm, 5 µm, Make: Thermosil)
Flow rate : 0.8 mL per min
Wavelength : 225 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 10 min
Mobile phase : methanol: phosphate buffer pH 4.5 (65:35)



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1		2.572	9438800	873348
2		3.833	67235	6418

Figure 1: Chromatogram of metformin and glipizide under optimized chromatographic conditions

Validation Method

Following parameters were observed under validation *viz.*, linearity, specificity, precession, accuracy, ruggedness, robustness, limit of detection, limit of quantitation.

Linearity (Table 1)

Preparation of Level – I (100 µg/ml of Metformine HCL and 1 µg/ml of Glipizide)
Preparation of Level – II (200 µg/ml of Metformin HCL and 2 µg/ml of Glipizide)
Preparation of Level – III (300 µg/ml of Metformine HCL and 3 µg/ml of Glipizide)
Preparation of Level – IV (400 µg/ml of Metformine HCL and 4µg/ml of Glipizide)
Preparation of Level – V (500 µg/ml of Metformine HCL and 5 µg/ml of Glipizide)

Table 1: Linearity of metformin HCL and glipizide

Sl no	Name	RT	Area	Height
1	Metformine	2.546	5355165	516428
2	Glipizide	3.779	24573	2612
3	Metformine	2.548	6732263	645332
4	Glipizide	3.78	27141	2808
5	Metformine	2.551	8046209	766630
6	Glipizide	3.782	30686	3280
7	Metformine	2.548	9015546	840685
8	Glipizide	3.78	55538	5696
9	Metformine	2.56	10069356	904788
10	Glipizide	3.786	38980	4052

The linear regression equation was found to be Y = 12,891.6650 X + 4,112,208.30 and r 2 = 0.9992 for Metformine HCL. The linear regression equation was found to be Y = 3,274.30 X + 20,798.10, r 2 = 0.9994 for Glipizide.

Specificity

Standard 300 µg/ml Metformine HCL and 3 µg/ml Glipizide combination used for specificity injection study. It was injected for three times and found that there was no additional peak in the chromatogram (Tables 2 and 3).

Table 2: Specificity of glipizide

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Glipizide	3.782	29680	3145	3463.3	1.2
2	Glipizide	3.782	30469	3209	3390.8	1.2
3	Glipizide	3.784	30033	3162	3370.9	1.2
Mean			30061			
Std Dev.			395.1			
%Of RSD			1.31			

Table 3: Specificity of metformin HCL

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin HCL	2.555	7914776	738312	1426.6	1.5
2	Metformin HCL	2.555	7927462	744872	1443.8	1.5
3	Metformin HCL	2.557	7870049	732278	1396.6	1.5
Mean			7904096			
Std Dev.			30160.2			
%Of RSD			0.38			

Precision

300 µg/ml Metformine HCL and 3 µg/ml of Glipizide was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits (Tables 4 and 5).

Table 4: Precision of metformin HCL

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin HCL	2.558	7784847	733940	2431.2	1.5
2	Metformin HCL	2.555	7810031	723735	2396.6	1.5
3	Metformin HCL	2.552	7887781	747176	2459.8	1.5
4	Metformin HCL	2.551	7888213	745996	2443.4	1.5
5	Metformine HCL	2.552	7884380	752924	2466.4	1.5
Mean			7851050			
Std. Dev			49765.6			
% of RSD			0.63			

Table 5: Precision of glipizide

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Glipizide	3.789	30488	3152	3480.8	1.2
2	Glipizide	3.784	30869	3157	3428	1.2
3	Glipizide	3.779	30910	3251	3386.7	1.2
4	Glipizide	3.781	30872	3258	3391.9	1.2
5	Glipizide	3.779	30282	3291	3468.7	1.2
Mean			30684			
Std. Dev			283.1			
% of RSD			0.92			

The % RSD for the area of five standard injections results should not be more than 2%

Accuracy

It was found out by recovery study using standard addition method. Known amounts of standard Metformine HCL and Glipizide were added to pre-analyzed samples (100 µg/ml + 1 µg/ml) at a level from 100% and 150% and then subjected to the proposed RP-HPLC method (Tables 6 and 7).

Table 6: Recovery data of metformin HCL and glipizide

Analyte	% Level of recovery	Formulation (µg/ml)	Amount of standard drug added (µg/ml)	Amount of pure drug found (µg/ml)	C.I. RSD	%	SE	t
Metformin HCL	100	100	100	98.89	100.27 ± 1.757	1.101	0.55	0.5
		100	100	101.1				
		100	100	99.89				
		100	100	101.23				
Glipizide		1	1	1.03	101.5 ± 2.054	1.271	0.64	2.32
		1	1	1.02				
		1	1	1.01				
		1	1	1				
Metformin HCL	150	100	150	151	99.925 ± 0.964	0.6	0.302	0.24
		100	150	149.34				
		100	150	148.98				
		100	150	150.23				
Glipizide		1	1.5	1.51	100.166 ± 3.16	1.987	0.995	0.167
		1	1.5	1.54				
		1	1.5	1.47				
		1	1.5	1.49				

SD: Standard deviation, SE: standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 4). Theoretical' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 3) = 3.18

Table 7: Ruggedness of glipizide

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Glipizide	3.789	29113	3151	3380.8	1.2
2	Glipizide	3.784	29568	3152	3128	1.2
3	Glipizide	3.779	29654	3254	3286.7	1.2
4	Glipizide	3.781	29547	3255	3191.9	1.2
5	Glipizide	3.779	29647	3251	3168.7	1.2
Mean			29505			
Std. Dev			224.5			
% of RSD			0.76			

Ruggedness

It was done by five replicate injections of the standard stock solution (300 µg/ml Metformin HCL and 3 µg/ml Glipizide). The % RSD was found with in the 1 (Table 8).

Table 8: Ruggedness of metformin HCL

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Metformin HCL	2.558	7784847	733140	2431.2	1.5
2	Metformin HCL	2.555	7710031	723715	2196.6	1.5
3	Metformin HCL	2.552	7795841	747276	2059.8	1.5
4	Metformin HCL	2.551	7777486	745946	2343.4	1.5
5	Metformin HCL	2.552	7768954	752914	2066.4	1.5
Mean			7767432			
Std. Dev			35573.2			
% of RSD			0.43			

Robustness

The flow rate was varied at 0.7 ml/min to 0.9 ml/min (Tables 9 and 10).

Table 9: System suitability results for metformin HCL

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.7	2477.9	1.6
2	0.8	2396.6	1.5
3	0.9	2329.4	1.2

Table 10: System suitability results for glipizide

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.7	3811.7	1.2
2	0.8	3370.9	1.2
3	0.9	3541.7	0.9

The Organic composition in the Mobile phase was varied from 60% to 70% Standard solution 300 µg/ml of Metformin HCL 3 µg/ml of Glipizide was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method (65 methanol and 35 phosphate buffer). Less organic (55% methanol and 45% Phosphate buffer pH 4.5) (Tables 11 and 12).

Table 11: System suitability results for metformin HCL

Change in Organic Composition in the Mobile Phase	System Suitability Results	
	USP Plate Count	USP Tailing
10% less	2574.6	1
* Actual	2396.6	1.5
10% more	2422	1.6

Table 12: System suitability results for glipizide

Change in Organic Composition in the Mobile Phase	System Suitability Results	
	USP Plate Count	USP Tailing
10% less	3499.3	0.9
* Actual	3370.9	1.2
10% more	3331.6	1.6

***** Results for actual Mobile phase composition (65:35Methanol: Buffer) have been considered from standard.

Limit of Detection

LOD of Metformin HCL S/N = 154/52 = 2.96
LOD of Glipizide S/N = 153/52 = 2.94

Quantitation Limit

LOQ of Metformin HCL S/N = 519/52 = 9.96
LOQ of Glipizide S/N = 517/52 = 9.94

RESULTS AND DISCUSSION

The method was chosen after several trials with various proportions of buffer and methanol i.e., 40:60, 50:50, 30:70, 25:75, and 35:65 and at different pH values i.e., 2, 2.45, 3, 3.5, 4, 4.5, 2.48. A mobile phase consisting of buffer (pH 4.5) and methanol in the ratios of 35:65 was selected to achieve best chromatographic peaks for Metformin HCL and Glipizide RT 2.55 and 3.78 minutes respectively and sensitive. The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 99 to 101. The results of analysis of average recoveries obtained in each instance were compared with the theoretical value of 100 percent by means of Student's 't' test.

CONCLUSION

As the calculated't' values are less than theoretical' values, it is concluded that the results of recoveries obtained in agreement with 100 percent for each analyte. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The lower limit of detection and the limit of quantitation were found to be within the acceptable range. The % RSD was less than 2 in all the parameters of robustness study. So, it indicates that the method is robust. This demonstrates that the developed RP=HPLC method is new, simple, linear, accurate, robust, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms.

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