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RP-HPLC ANALYSIS OF METFORMIN HYDROCHLORIDE AND VOGLIBOSE AND STUDY OF ITS DIFFERENT ANALYTICAL PARAMETER

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ABSTRACT: A simple, specific, precise, and efficient method for the Simultaneous estimation of Metformin HCl and Voglibose tablet by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase were in a combination of acetonitrile: buffer pH- 6.5 in the ratio of 62:38. Optimized column is a stainless steel column packed with base octa decylsilyl silica gel of 250X4.6mm and at 254 nm wavelength for metformin and Voglibose detection by Spectrofluorimeter and excitation wavelength at 350nm and emission wavelength at 430nm. In our study, the validation of analytical method for determination of Metformin and voglibose tablet formulation was performed in accordance the parameters including-system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines.

INTRODUCTION: Metformin HCl (**Fig. 1**) 1, 1-Dimethylbiguanide hydrochloride[1] is an oral anti-diabetic in the class and has an empirical formula $C_4H_{12}N_5Cl$. It is the drug of choice for the treatment of. It improves hyperglycemia primarily through its suppression of hepatic glucose production(hepatic gluconeogenesis) and activates AMP- activated protein kinase (AMPK)which is required for the inhibitory effect for the production of glucose by liver cells [2-4].

Voglibose (**Fig. 2**) has an empirical formula $[C_{10}H_{21}NO_7]$ 3, 4-Dideoxy-4-[2-hydroxy-1-(hydroxyl methyl) ethyl] amino-2-c-(hydroxymethyl).

As an alpha-glucosidase inhibitor, the compound exerts its activity within the gastrointestinal tract of humans. The drug delays glucose absorption and thus, reduces the post-prandial blood glucose level. Its combination has attracted considerable interests due to its wide range of therapeutic and pharmacological properties, including its excellent inhibitory activity and its action against hyperglycemia [5-7].
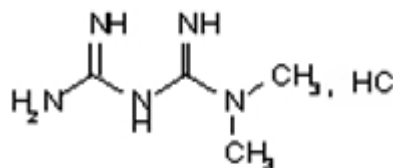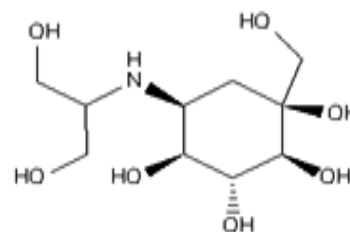
FIGURE 1: METFORMIN HCl

FIGURE 2: VOGLIBOSE

In the present work, an attempt was made to provide a newer, simple, accurate and low cost post column derivatization of spectrophotometric and there derivative method and one HPLC method for the effective quantitative determination of Metformin HCl and Voglibose as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations.

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-2010 variable wavelength programmable UV/Visible detector -2550 and Fluorescence Detector LC-20AT. 20µl of injection volume, stainless steel column of C18:150X4.6mm was used for separation. Mobile phase consisting of a mixture of potassium dihydrogen phosphate and Sodium di hydrogen orthophosphate buffer pH -6.5: acetonitrile (38:62% v/v) was delivered at a flow rate of 1ml/min.

The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 5min. Analysis was performed at ambient temperature. Since voglibose only absorbs UV in the low wavelength region, it cannot be directly detected with high sensitivity. Taurine and sodium periodate were used for the derivatization of voglibose [8-10]. It is validated as per ICH guidelines [11].

MATERIALS AND METHODS: Pure sample of Metformin HCl and Voglibose got as a gift sample by the Micro labs, Hosur. Acetonitrile and water used were of HPLC grade. All other reagents used in this study were of AR grade.

Blank solution: Purified Water is used as diluent.

Standard solution:

- **Solution-A**: 600µg/ml of voglibose was prepared in buffer. This solution 5ml was further diluted with buffer to get a solution of concentration 60 µg/ml.

- **Solution-B**: 1mg/ml of Metformin HCl was prepared along with the 5ml of the solution A and sonicate for 5min.

Preparation of Sample solution: 20 tablets were weighed, average weight was calculated and quantity equivalent to 5 mg of voglibose and metformin were

taken and transfer in to 500 ml V.F. Add 350 ml of buffer and sonicate for 30 min & make up with diluent. And filter through 0.45µ membrane filter paper.

Optimized chromatographic conditions are listed in **Table 1**.

TABLE 1: CHROMATOGRAPHIC CONDITION

Parameters	Description
Diluent	Water
Column	C_{18} :250X4.6mm, 5µ,amino SS Column
Mobile Phase	Acetonitrile: buffer (620:380)
Flow rate	1.0ml/min
Flow rate fluorescence reagent	1.0ml/min
Detection for Metformin HCl	UV-254nm
Detection for Voglibose	Spectrofluorimeter
Excitation wavelength	350nm
Emission wavelength	430nm
Temperature	25°C
Injection volume	20µl
Run time	20min

Method Validation: Once the HPLC method development was over, the method was validated in terms of parameters like specificity, precision, accuracy, linearity, ruggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values were calculated.

The proposed HPLC method was validated as per ICH guidelines.

1. **System precision:**

   a. **Standard solution:** Standard solution was prepared as the same manner as above and injected six replicated injections into the HPLC system and calculates the RSD from six replicate injections.

2. **System suitability:** Standard solution and test solution was prepared as the same manner as above. The results are tabulated in **Table 2**.

3. **Specificity:** Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes.

4. **Precision:** Precision was evaluated in six independent sample preparations in which repeatability and intermediate precision was carried out. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. The results are tabulated in **Table 3**.

5. **Linearity**: Accurately weighed 60mg of Voglibose working standard transfer into 100ml volumetric flask and dissolved with diluent and make up the volume (solution A), weighed 500mg of Metformin HCl working standard and transferred to 500ml volumetric flask and dissolved it in 300ml of diluent. Pipette out 5ml of above solution A and transferred into a 500ml of Metformin HCl volumetric flask and make up the volume with diluent. Further dilution was done and there exists a linear relationship in the concentration range of 500 to 1500ppm for Metformin HCl (**Figure 3**). There exists a linear relationship in the concentration range of 0.3to 0.9ppm for Voglibose (**Figure 4**). The results are tabulated in **Table 4**.

6. **Accuracy / Recovery:** To study the reliability, suitability and accuracy of the method recovery experiments were carried out.

Standard stock solution-A: Accurately weighed 60mg of Voglibose working standard and transferred in to a 100ml volumetric flask and dissolved with diluent and make up the volume with diluent. A placebo of 145mg was added at the level of 50mg to 150mg of metformin and standard stock solution-A 5 to 15ml dissolved in water sonicate about 30min to dissolve the content and made up to 100 ml.

Filter the solution through 0.45μ membrane filter. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 3 times and %RSD was calculated by using the following formula. The contents of metformin and voglibose are the lower values of %RSD of assay indicate the method is accurate. The mean recoveries were in range of 99.8-101.20 % which shows that there is no interference from excipients (**Table 5**). The Recovery curve for metformin and voglibose are shown in figure.5&6.

Stability of analytical solution: To establish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 24hours. Both standard and sample solution was prepared by the same manner. It is shown in **Table 6**.

Robustness: The robustness of the method was determined by carrying out deliberate variations in procedural parameters to remain unaffected and provides an indication of its suitability during normal usage. Deliberately modify the actual chromategraphic conditions specified under the method like flow rate, mobile phase composition, column temperature on lower and higher side of the actual value. Evaluate system suitability and determine the assay of Metformin HCl and Voglibose under these parameters. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria. It is shown in **Table 7**.

RESULTS AND DISCUSSION:

TABLE 2: SYSTEM SUITABILITY PARAMETERS FOR METFORMIN HCl AND VOGLIBOSE

System suitability parameter	Met. HCl	Vogli.	Met. HCl	Vogli.	Met. HCl	Vogli.	Met. HCl	Vogli.
	Retention Time		AUC		Theoretical Plates		Tailing factor	
Solution-1	4.27	15.20	13441854	119290	5978	5978	1.17	1.17
Solution-2	4.25	15.10	13569231	120153	5895	5895	1.15	1.15
Solution-3	4.50	15.10	13442183	119909	5965	5965	1.16	1.16
Solution-4	4.31	15.00	13395011	120661	5988	5988	1.18	1.18
Solution-5	4.55	15.12	13454363	120353	5875	5875	1.05	1.05
Solution-6	4.51	15.02	13460528	120095	5896	5896	1.06	1.06
Mean			13460528.3	120076.8				
S.D.			58020.83	462.8				
R.S.D.			0.431	0.39				

TABLE 3: REPEATABILITY FOR VOGLIBOSE

	Repeatability				Intermediate Precision			
	Metformin HCl		Voglibose		Metformin HCl		Voglibose	
Inj	AUC	%	AUC	%	AUC	%	AUC	%
1	13431738	99.7	120983	100.3	13486738	99.5	121125	100.59
2	13422222	99.8	120974	100.4	13555222	100	120065	100.58
3	13370026	99.3	120641	100.3	13480030	99.5	121104	100.66
4	13418095	99.7	122999	102.4	13489595	99.7	123303	102.8
5	13434540	99.8	122562	102.0	13514540	99.8	119562	101.5
6	13430087	99.6	122700	102.0	13575591	100.1	123254	102.3
Mean		99.65		101.23		99.77		101.41
S.D.		0.19		1.00		0.25		0.96
R.S.D.		0.19		0.99		0.25		0.95

The relative Standard deviation for the assay of six sample preparations of Metformin HCl and Voglibose was found 0.19% and 0.99%.

CALCULATION:

Amount of Metformin HCl present in mg:

$$\frac{A \times Ws \times 500 \times P \times Aw \times 1000}{B \times 500 \times Wt\ taken \times 100}$$

A = peak area of Metformin HCl for sample preparation

B = peak area of Metformin HCl for standard preparation

Ws = weight of Metformin HCl in mg

P = potency of Metformin HCl

Aw = average weight of the tablets

Amount of Metformin HCl in mg:

$$= \frac{13431738 \times 500 \times 500 \times 100 \times 0.7620 \times 1000}{13460528 \times 500 \times 0.7625 \times 100}$$

$= 498.93\,mg$

Assay in % $= \dfrac{498.93 \times 100}{500}$

$= 99.7\ \%$

TABLE 4: LINEARITY FOR METFORMIN HCL & VOGLIBOSE

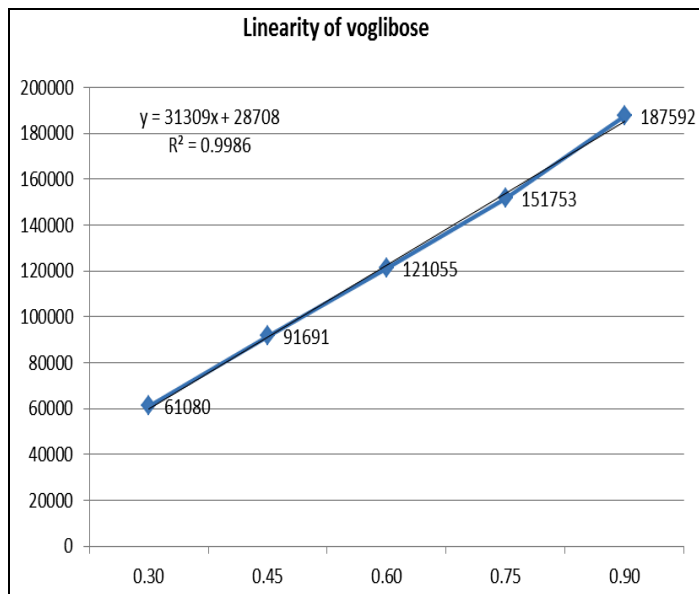|		Metformin HCl				Voglibose			
S. No.	Test con in%	Conc. (ppm)	Repli. inj	Area	Avg. Area	Conc. (ppm)	Repli. inj	Area	Avg. Area
1	50	500	1	6825366	6807014	0.30	1	61259	61080
			2	6795821			2	60958	
			3	6799856			3	61024	
2	75	750	1	10052869	1016942	0.45	1	91256	91691
			2	10256892			2	92563	
			3	10198527			3	91254	
3	100	1000	1	13592568	1353250	0.60	1	121356	121055
			2	13499256			2	120598	
			3	13505695			3	121210	
4	125	1250	1	16892546	1694164	0.75	1	150569	151419
			2	17012036			2	151236	
			3	16920365			3	152453	
5	150	1500	1	20165036	2054057	0.90	1	186025	187925
			2	20935242			2	187536	
			3	20521439			3	190215	

FIGURE 3: LINEARITY OF VOGLIBOSE

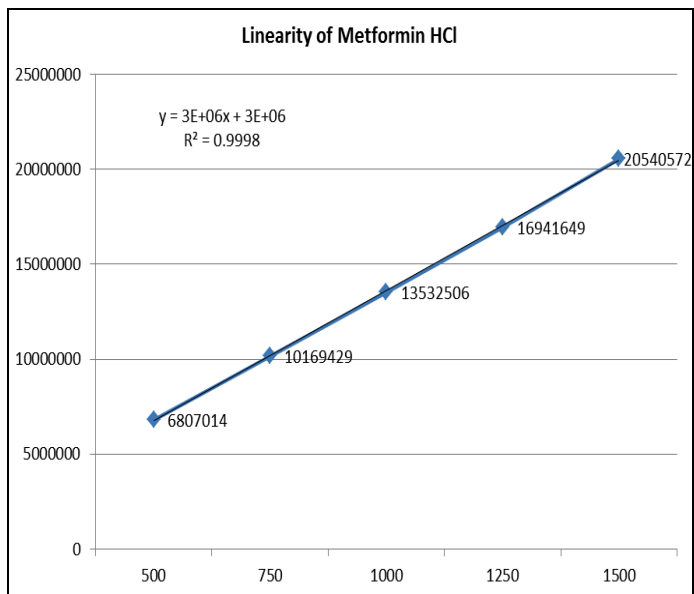**FIGURE 4: LINEARITY OF METFORMIN HCl**

TABLE 5: RECOVERY

| S. No.	Conc. in %	Amount of Placebo added in (mg)	Metformin HCl		Voglibose	
			AUC	Percentage	AUC	Percentage
1	50%	145	6807014	100.6	61080	102.2
2	50%	145	6799013	100.5	61349	102.7
3	50%	145	6805555	100.6	60945	102.0
1	75%	145	10120057	99.7	91691	102.3
2	75%	145	10169429	100.2	91221	101.8
3	75%	145	10233095	100.8	90976	101.5
1	100%	145	13411089	99.1	121055	101.3
2	100%	145	13449139	99.4	122890	102.9
3	100%	145	13532506	100.0	121495	101.7
1	125%	145	16878822	99.8	151419	101.4
2	125%	145	16941649	100.2	151315	101.3
3	125%	145	16836091	99.5	152894	102.4
1	150%	145	20540572	101.2	186259	103.9
2	150%	145	20739237	102.2	182972	102.1
3	150%	145	20280016	99.9	183696	102.5
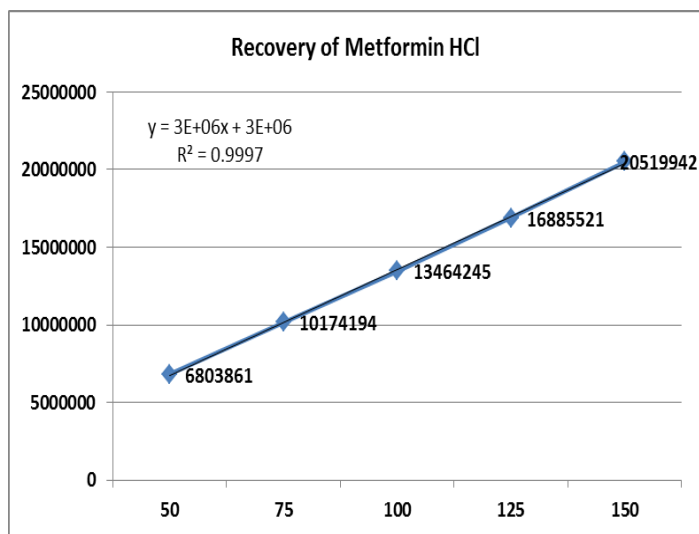
FIG. 5: RECOVERY CURVE FOR METFORMIN HCl
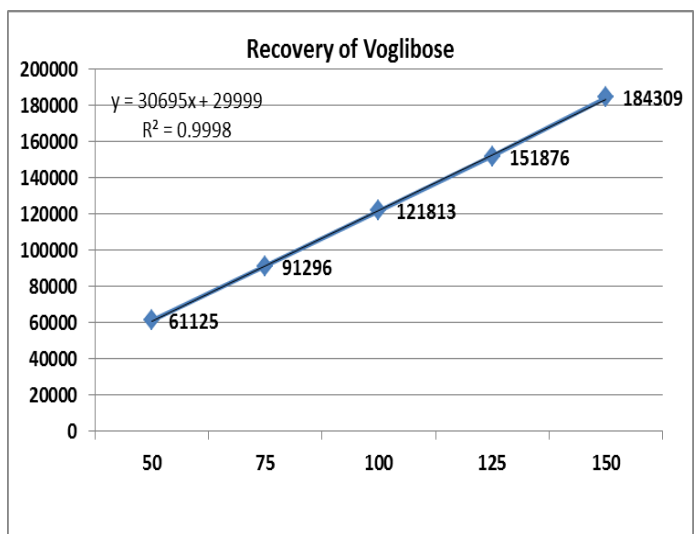

FIG. 6: RECOVERY CURVE FOR VOGLIBOSE

Result: The percentage of recovery of Metformin   HCl and Voglibose was found 97.0% to 103.0.

TABLE 6: STABILITY OF ANALYTICAL SOLUTIONS

Time interval	Peak response for Metformin HCl	Peak response for Voglibose
0th hour	13399963	121125
2th hour	13427856	121883
4th hour	13409897	120452
8th hour	13419360	122453
12th hour	13416331	121563
18th hour	13421622	122325
24th hour	13254286	121350
Mean	**13392759.29**	**121593**
S.D	**61709.67638**	**700.0449986**
R.S.D	**0.46076895**	**0.575728042**

Result: The stability of analytical solution %RSD of Metformin HCl and Voglibose was found 0.46 and 0.57 respectively.

TABLE 7: ROBUSTNESS PARAMETERS

S. No.	Chromatographic parameter	Low	High
1	Flow Rate (1.0ml/min)	0.8ml	1.2ml
2	Column Temperature (25°C)	23°C	27°C
3	Mobile phase composition (Buffer: Acetonitrile 380:620)	400:600	360:640
4	Buffer pH (6.5)	6.3	6.7

CONCLUSION: The HPLC method is simple, specific, precise, linear, sensitive, and also system suitability. The results obtained on the validation parameter met the respective acceptance criteria. The method was found to have suitable application in routine laboratory analysis and with high degree of accuracy and precision. It is also used in routine quality control of raw materials as well as formulations containing the compound.

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