International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-2, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Simultaneous Estimation of Voglibose and Metformin Hydrochloride High Performance Thin Layer Chromatographic Method by in Tablet Dosage Form

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Manuscript No: IJPRS/V4/I2/00056, Received On: 11/04/2015, Accepted On: 15/04/2015

ABSTRACT

A simple, novel, rapid, precise, accurate, specific and cost effective High performance thin layer chromatographic method has been developed and validated for simultaneous determination of voglibose and Metformin Hydrochloride in combined tablet dosage form. The stationary phase used was precoated silica gel 60F254 plate. The mobile phase used was the mixture of ethyl acetate: methanol (6.5:3.5v/v/v). The detection of spots was carried out densitometrically using a UV detector at 230nm in absorbance mode. Developed method was validated according to the International Conference on Harmonization (ICH) guidelines. Calibration curve was found to be linear between 2000 to 12000 ng/spot for both drug with correlation coefficient 0.995 and 0.9993 for voglibose and Metformin Hydrochloride respectively. Accuracy in terms of % recovery was found to be 98.95-99.50 and 98.41-99.54 for voglibose and Metformin Hydrochloride respectively. Limit of detection for voglibose and Metformin Hydrochloride was found to be 40.60 ng/spot and 755.99ng/spot respectively. Limit of quantitation for voglibose and Metformin Hydrochloride was found to be 100.01 ng/spot and 1269.99ng/spot respectively. Thus the proposed method can be successfully applied for simultaneous determination of voglibose and Metformin Hydrochloride in combined tablet dosage form.

KEYWORDS

Voglibose, Metformin Hydrochloride, High performance thin layer chromatography, Validation

INTRODUCTION

Voglibose (VGB) is chemically 5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol[1]. It is Antideabitic drug.[1] Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates: specifically alpha-glucosidase enzymes in the brush border of the small intestines. Acarbose also blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases.

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Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Individual determination of Voglibose is carried out by UV[7], HPLC[8], HPTLC and LC-MS. A representative structure of Voglibose was shown in Figure-1[1-3].

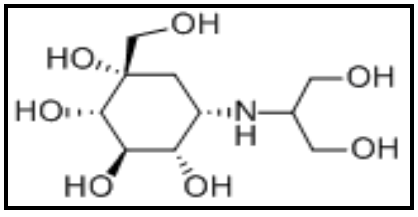

Figure 1: Structure of Voglibose

Metformin HCL **(**MET) is chemically 1-carbamimidamido-N,N-dimethylmethanimid-amide hydrochloride.[1] Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects (except in special circumstances) and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease[12]. Individual determination of MET is carried out by UV[13] and HPLC[14]. A representative structure of MET was shown in Figure-2[4,5].

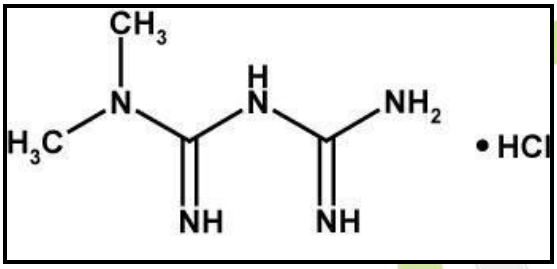

Figure 2: Structure of Metformin hydrochloride

Determination of VGB and MET by RP-HPLC, LC-MS, and UV method.[7,8,9] Till date there have been no published HPTLC methods for simultaneous determination of MET and VOG in bulk or in combined dosage forms. The objective of the work is to develop and validate the simple, economical, precise and accurate UV Spectrophotometric methods for the estimation of Voglibose and Metformin Hydrochloride pharmaceutical formulation.

MATERIAL AND METHODS

Instrument and Apparatus

HPTLC System (Switzerland)

- Linomat v semi-automatic sample applicator (Cemag linomat V, Muttenz, Switzerland)
- TLC Scanner IV (Camag)
- Flat bottom, Twin through developing chamber (10×10 cm^2)(Camag)
- UV cabinet with dual wavelength (254/366)UV lamp (Camag)
- Software : Win-CATS, version 1.4.6
- Digital camera
- Microliter syringe 100µ (Hamiton)
- Stationary phase : Pre coated silica gel aluminum plate 60 F254,(10×10 cm^2 with 250 µm thickness; E. Merk)

Electronic Analytical Balance (Shimadzu)

Hot Air Oven

Ultra Sonic Cleaner (Toshniwal Process Instruments Pvt. Ltd.)

Calibrated Glass Wares

Reagents and Materials

- Voglibose and Metformin HCl were received as gift sample from Dolffin Pharmaceuticals, Surat, Gujarat.
- Methanol and Ethyl acetate from Astron chemicals, Ahmedabad, Gujarat.
- Tablet Formulation (Brand Name) Obimet*V
  - Contains: Voglibose: 0.2mg +Metformin HCl 500mg.

Preparation of Standard Stock Solution

10 mg of voglibose and 10 mg of Metformin Hydrochloride was weighed accurately and transferred to a volumetric flask of 10ml and dissolved in and diluted to mark with methanol to obtain a standard stock solution of 1000µg/ml. It was derivatized using 1N Sulphuric acid.

Prewashing of Plates

HPTLC was performed on 10×10cm precoated silica gel 60F254 precoated plates from E. Merck. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with

methanol, dried, and activated for 30minutes at 110 degree centigrade with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

Sample Application

The standard and formulation samples of voglibose and Metformin hydrochloride were spotted on precoated TLC plates in the form of narrow bands of lengths 6mm, with 10mm from the bottom and left margin and with 9mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150nLs-

Mobile Phase and Migration

Various solvents were used as mobile phase for tried to separate and resolve spot of voglibose and Metformin hydrochloride from its impurities and other excipients of formulation. The mixture of ethyl acetate: methanol (6.5:3.5% v/v/v) could resolve VGB and MET with better peak shape. The drug was satisfactorily resolved with Rf value 0.23±0.03 for VGB and 0.63±0.03 for MET. Pre-saturation of TLC chamber with mobile phase for 30 minutes assured better reproducibility in migration of voglibose and Metformin hydrochloride give better resolution.

Selection of Wavelength

The sensitivity of HPTLC method that is used UV detector depends upon the wavelength of detection. The ideal wavelength is the one that gives measurable response for drugs. For this purpose, standard solution of VGB (12-72ng/spot) and MET (20000-120000ng/spot) were prepared in Methanol. This solutions were than scanned under UV region of 400- 200nm and spectra were overlain. At 254nm both drugs have considerable absorbance.

Method validation[6]

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines for Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Repeatability, and Specificity & Robustness.

Linearity and Calibration Curve

Linearity of the method was evaluated by constructing calibration curves at eight concentration levels. Aliquots of standard working solution of voglibose were applied to the plate to obtain concentration in the range of 12 to 72ng/spot for VGB and 20000 to 120000 ng/spot for MET. The calibration curves were developed by plotting peak areas Vs Concentrations with the help of Win-CATS software. Chromatogram was developed in a twin trough glass chamber; using 20minutes chamber saturation time. The length of chromatogram run was 80mm. The developed plates were air-dried. Scanning was performed in UV mode at 254nm. The slit dimension was kept at 6×0.45mm at scanning speed of 100nm/s. After completion of scanning peak areas were noted. Peak areas were plotted against corresponding concentrations and least square regression analysis was performed to generate the calibration equation.

Precision

To evaluate intra-day precision, three samples at three different concentrations and were analyzed on the same day. The inter-day precision was studied by comparing assays performed on three different days. The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions.

Repeatability

Repeatability of measurement of peak area was determined by spotting 24µl of VGB and 40000µl of MET standard drug solution on TLC plate. After developing the plate separated spot of VGB and MET was scanned six times without changing the position of the plate. Repeatability of sample application was assessed by spotting 24µl and 40000 µl of MET standard drug solution six times on a TLC plate by automatic spotter, followed by development of plate and recording to the peak areas for six spots.

Accuracy

Recovery studies of the drugs were carried out for determining accuracy parameter. It was done

by mixing known quantity of standard drug with the analyzed sample formulation and the contents were reanalyzed by the proposed method. Recovery studies carried out at 80, 100 and 120% levels. The percentage recovery and its %RSD were calculated.

Limit of Detection and Limit of Quantification

In accordance with ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD and LOQ values were estimated as [(standard deviation of repeatability)/(Slope of the regression equation)] by multiplying with 3.3 and 10 respectively.

LOD= 3.3× (SD/Slope)

LOQ= 10× (SD/Slope)

Where,

SD = Standard deviation of Y intercepts of the 5 calibration curve. Slope = Mean slope of 5 calibration curve

Specificity

To confirm the specificity of the proposed method, voglibose was spotted on TLC plate, developed and scanned as described earlier. The UV spectrum of standard voglibose was also compared with spectrum of voglibose extracted from tablet. The peak purity of Voglibose was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot.

Robustness

The parameters selected for the robustness study were mobile phase composition, chamber saturation time and solvent migration distance. By introducing small changes in these parameters the effects on the results were examined.

Stability Studies

When the developed chromatographic plate is exposed to atmosphere, the analytes are likely to decompose. Hence it is necessary to conduct stability studies. Stability of the analyte on the plate was studied at different time intervals and peak areas were compared with the peak area of freshly scanned plate.

Assay Preparations

Twenty tablets were weighed and average weight was calculated. An accurately weighed finely powdered quantity of tablets equivalent to about 500 mg of MET (VOG 0.2 mg) was transferred to 100.0ml volumetric flask. The volume was adjusted up to the mark with methanol. (Stock solution) The stock solution was then filtered through Whatman filter paper and accurately measured; the first few ml was discarded 4µl of solution was spotted on the HPTLC plate for analysis. The amount of VGB and MET per tablet was determine with the help of calibration curve.

RESULTS AND DISCUSSION

Selection of wavelength

The sensitivity of HPTLC method that is used UV detector depends upon the wavelength of detection. The ideal wavelength is the one that gives measurable response for drugs. For this purpose, standard solution of VGB (12-72ng/spot) and MET (20000-120000ng/spot) were prepared in Methanol. This solutions were than scanned under UV region of 400-200nm and spectra were overlain. (Figure 3). At 254nm both drugs have considerable absorbance.

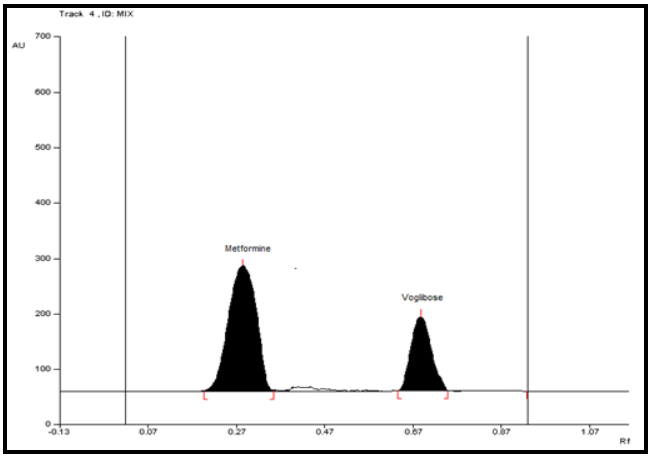

Figure 3: HPTLC chromatogram of VGB (48ng/spot, Rf value =0.29) and MET (80000ng/spot. Rf value = 0.64) in std. mixture using mobile phase ethyl acetate: methanol (6.5:3.5% v/v/v)

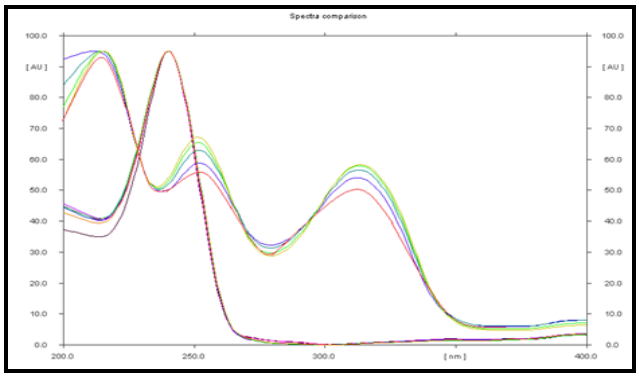

Figure 4: Overlain UV absorption spectra of VGB (12-72ng/spot) and MET (20000-120000ng/spot)

Method Validation

Linearity

A representative calibration curve obtained by plotting peak area of compound against the concentration over the range of 12 to 72ng/spot for VGB and 20000 to 120000 ng/spot for MET. The slope, intercept and correlation co-efficient values were found to be and 0.997 and 0.9993 for VGB and MET respectively. It showed that good correlation between regression coefficient and concentration of the drug.

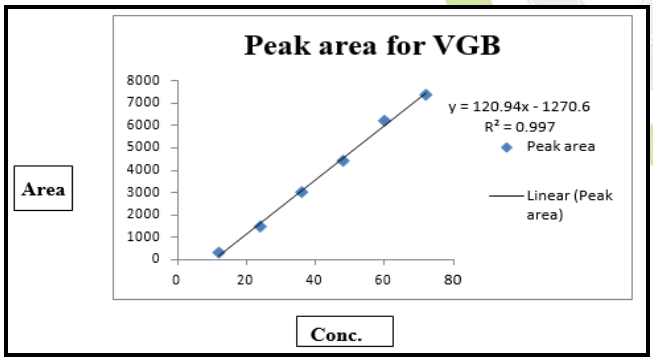

Figure 5: Calibration curve of VGB

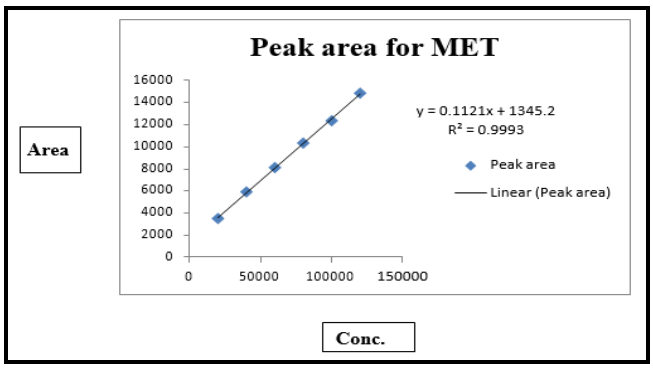

Figure 6: Calibration curve of MET

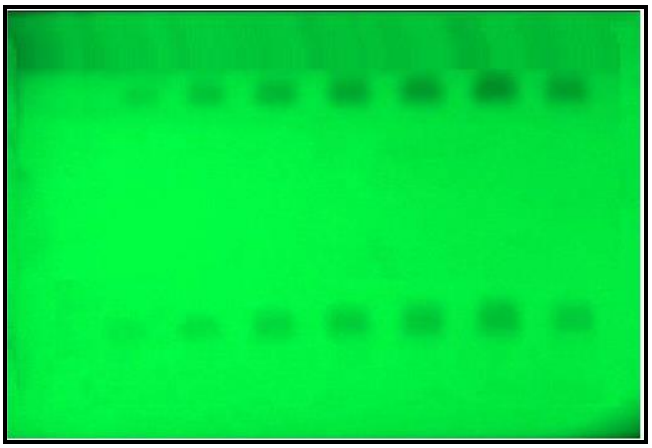

Figure 7: Developed HPTLC Plate of calibration curve of VGB and MET and assay of marketed formulation

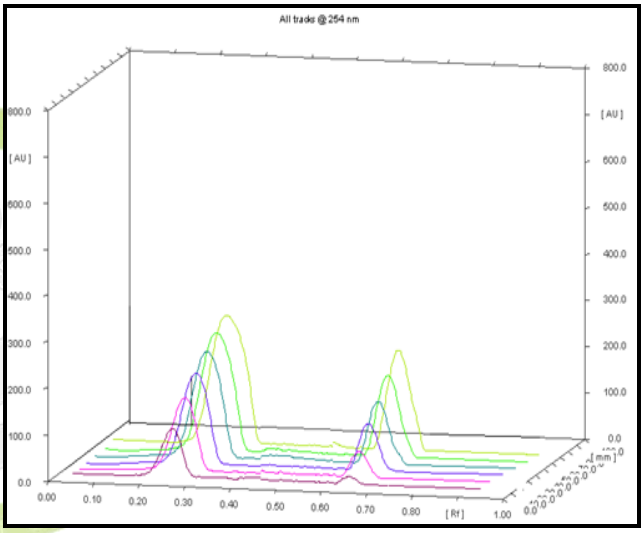

Figure 8: HPTLC chromatogram (3D view) for VGB (Rf value 0.66) and MET (Rf value 0.03) and for Assay preparation

Precision

The intra-day and inter-day relative standard deviations found in the range. The smaller values of intra-day and inter-day variation in the analysis indicate that the method is precise

Repeatability

In repeatability of sample application the %RSD for the peak area values were calculated and found to be 0.37% for VGB and 0.88% for MET. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (i.e. 1%); ensuring proper functioning of HPTLC system.

Intraday Precision

Table 1: Intraday precision of VGB and MET

Conc. taken (ng/spot) VGB	Conc. taken (ng/spot) MET	%Estimated VGB	%Estimated MET	%RSD	
				VGB	MET
24	40000	98.12	98.28		
24	40000	98.4	98.12		
24	40000	100.12	98.17		
	Mean ± SD	99.11±0.64	99.11±1.71	0.64	0.75
36	60000	98.28	98.18		
36	60000	98.39	98.37		
36	60000	100.2	101.3		
	Mean ± SD	98.25±0.66	99.2±0.87	0.66	0.77
48	80000	100.3	98.17		
48	80000	98.13	100.2		
48	80000	98.27	98.43		
	Mean ± SD	98.25±0.62	99.71±1.49	0.65	0.73

Interday Precision

Table 2: Interday precision of VGB and MET

Conc. taken (ng/spot) VGB	Conc. taken (ng/spot) MET	%Estimated VGB	%Estimated MET	%RSD	
				VGB	MET
24	40000	98.12	98.28		
24	40000	98.4	98.12		
24	40000	100.12	98.17		
	Mean ± SD	98.87±0.97	99.66±0.74	0.93	0.75
36	60000	98.28	98.18		
36	60000	98.39	98.37		
36	60000	100.2	101.3		
	Mean ± SD	98.27±0.92	98.89±0.77	0.92	0.77
48	80000	100.3	98.17		
48	80000	98.13	100.2		
48	80000	98.27	98.43		
	Mean ± SD	98.9±0.91	99.59±0.72	0.92	0.73

Table 3: Repeatability of sample application

Conc. taken (ng/spot) VGB	Conc. taken (ng/spot) MET	Area under VGB	Area under MET
24	40000	1488.1	5927.1
24	40000	1458.8	5917.1
24	40000	1465.6	5263.3
24	40000	1464.7	5471.2
24	40000	1444.4	5243.5
24	40000	1484.3	5889.2
	Mean ± SD	1446.4±0.25	5698.5±0.87
	%RSD	0.37	0.88

Table 4: Repeatability of measurement

Conc. taken (ng/spot) VGB	Conc. taken (ng/spot) MET	Area under VGB	Area under MET
24	40000	1488.1	5927.1
24	40000	1458.8	5917.1
24	40000	1465.6	5263.3
24	40000	1464.7	5471.2
24	40000	1444.4	5243.5
24	40000	1484.3	5889.2
	Mean ± SD	1446.4±0.25	5698.5±0.87
	%RSD	0.37	0.88

Accuracy

The % recovery of voglibose was found to be in the range between 98-101% (at 80,100 & 120% levels respectively), which is satisfactory. The results of recovery study indicate that the proposed method is accurate for estimation of drug in tablet dosage.

LOD & LOQ

The limit of detection was found to be ng/spot, while the limit of quantification was found to be ng/spot.

Table 5: LOD and LOQ of VGB and MET

Method	**Ratio derivative Method**	
Drug	**VGB**	**MET**
LOD (ng/spot)	40.60	755.99
LOQ (ng/spot)	100.07	1269.99

Specificity

The good correlation among spectra acquired at start (s), apex (m) and end (e) of the peaks indicates the peak purity of VGB and MET. Hence, it can be concluded that no impurities or degradation products migrated with the peaks obtained from standard solutions of the drug. The proposed HPTLC method was found to be specific.

Table 6: Specificity data of VGB and MET

Drug	**Correlation r (s, m)**	**Correlation r (m, e)**	**Peak purity**
VGB	0.99910	0.99920	Pass
MET	0.9997	0.9998	Pass

Table 7: Recovery data of VGB and MET from Tablet dosage form

Assay Level	**Tablet content taken eq. to (mg)**		**Standard added (mg)**		**Total drugs recovered (mg)**		**%Recovery of standard added**	
	VGB	**MET**	**VGB**	**MET**	**VGB**	**MET**	**VGB**	**MET**
Blank	0.6	100	0	0	0.51	99.5	0	0
	0.6	100	0	0	0.46	100	0	0
	0.6	100	0	0	0.9	99.8	0	0
80%	0.6	100	0.08	80	0.47	80.1	99.08	98.25
	0.6	100	0.48	80	0.48	79.9	100	98.15
	0.0	100	0.48	80	0.49	80.2	99.7	102.5
Mean ± S.D.							99.5±0.46	99.6±2.46
100%	0.6	100	0.6	100	0.59	100.1	98.33	101
	0.6	100	0.6	100	0.61	99.8	101.66	98.13
	0.6	100	0.6	100	0.57	99.9	99.64	99.01
Mean ± S.D.							99.8±1.67	99.3±1.46
120%	0.6	100	0.72	120	0.71	120.1	98.61	100.13
	0.6	100	0.72	120	0.70	119.9	98.89	99.16
	0.6	100	0.72	120	0.69	120.2	101.38	101.06
Mean ± S.D.							99±1.73	99.6±1.72

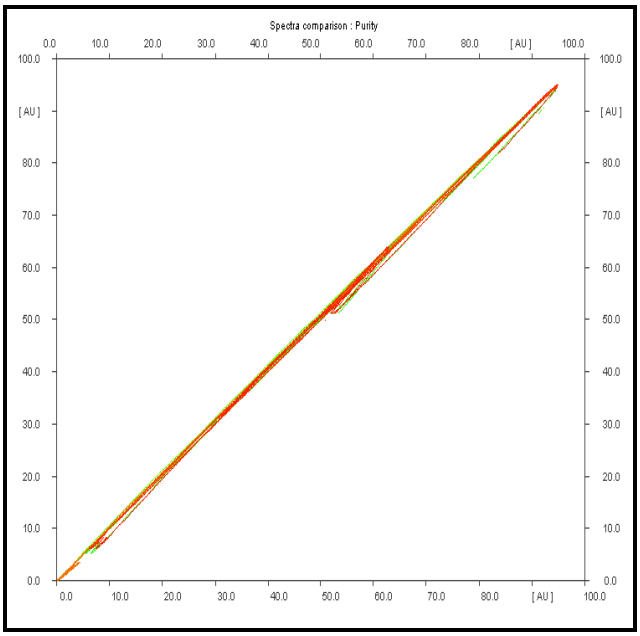

Figure 9: Peak purity of VGB

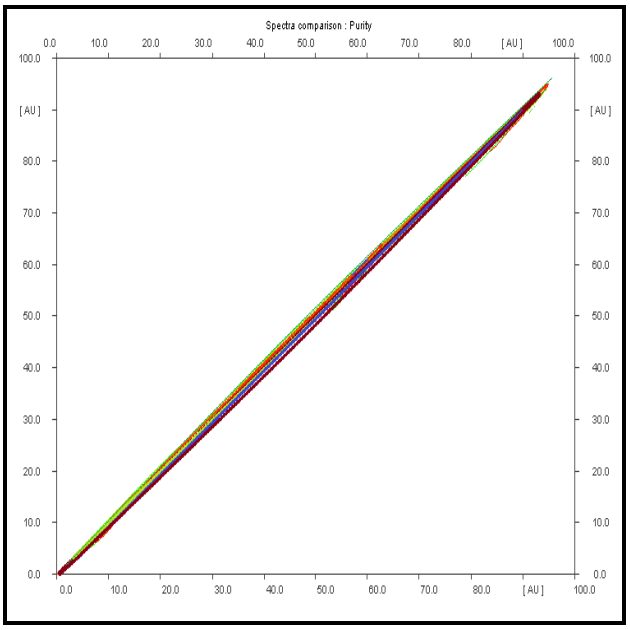

Figure 10: Peak purity of MET

Table 8: Stability studies of VGB and MET

Change Parameters		Peak Area		Mean ± S.D n= 3		%R.S.D	
		ETS	TXA	ETS	TXA	ETS	TXA
Mobile phase composition (Ethy acetate : Methanol)							
1	6:3 v/v/v	3225.63	1511.87	3215.66 ± 9.43	1503.36 ± 8.14	0.29	0.54
2	3:6 v/v/v	3206.88	1495.64				
3	7:3 v/v/v	3214.48	1502.59				
Mobile phase volume							
1	10 ml	3229.43	1516.75	3219.00 ± 9.53	1510.67 ± 6.14	0.30	0.41
2	12 ml	3210.73	1504.47				
3	15 ml	3216.86	1510.79				
Saturation time							
1	15 min	3227.36	1513.28	218.21 ± 8.83	505.61 ± 7.24	0.27	0.48

Table 9: Summary of validation parameters

Sr.No.	Parameters	Result for VGB	Result for MET
1	**Linearity range(µg/ml)**	12-72ng/spot	20000-120000µg/ml
2	**Correlation coefficient**	0.995	0.9993
3	**Precision(%RSD)**	0.37	0.88
	Repeatability(n=6)		
	Intraday	0.65	0.73
	Interday	0.92	0.77
4	**Accuracy (%recovery)**	99.08-101.38	98.25-101.06
5	**LOD (µg/ml)**	0.62	0.86
6	**LOQ (µg/ml)**	1.87	2.60
7	**Specificity**	Ok	Ok
	Correlation r (s, m)	0.99910	0.9997
	Correlation r (m, e)	0.99920	0.9998

Table 10: Analysis of marketed formulation (n=6)

	Mg/tablet		**Assay(% of tablet claim*)**	
Tablet	**VGB**	**MET**	**VGB**	**MET**
Obimet*V	**0.2**	**500**	**98.2±1.24**	**98.46±1.26**

Robustness

Robustness tests examine the effect of the operational parameters on the analysis results. By introducing small changes in mobile phase composition. The deviation obtained by deliberate changes in the mentioned parameters was below 2% RSD which conforms the robustness of the method.

The results indicated that the method was robust.

Application of Method Analysis of Marketed Formulation

Application of proposed method was tested by assay of commercially available tablet formulation Obimet*V 0.2

CONCLUSION

The HPTLC method for the determination of voglibose is simple, precise, specific, accurate, selective, sensitive and reproducible. The amounts found in formulations well agreed with label claim. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of voglibose and metformin Hydrochloride in tablet dosage form.

ACKNOWLEDGEMENTS

Authors are greatly thankful to Bhagwan Mahavir College of Pharmacy, Surat-395017, Gujarat for providing facilities to carry out the work.

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