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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING CHROMATOGRAPHIC METHODS FOR ESTIMATION OF HYPOGLYCEMIC DRUGS

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ABSTRACT: A simple, rapid, precise and reliable RP-HPLC method was developed for Simultaneous estimation of Repaglinide and Voglibose in pharmaceutical dosage form. The method is based of precolumn derivatization of Voglibose with Acetonitrile with Water, Acetonitrile with Methanol it cannot directly estimated by UV detector in RP-HPLC method. Precolumn derivatization (PCD) conditions were optimized by evaluating the parameters such as concentration methanol and water, concentration and reaction time of derivatization. The chromatographic separation was achieved on Inertsil-ODS C18 (250mm x 4.6mm, 5µm) column and Methanol: water (50:50v/v) as a mobile phase at a flow rate of 1ml/min. Detection was carried out at 252nm. The Retention time of Repaglinide-4.714min and Voglibose-6.691minute. The method has been validated for linearity, accuracy and precision. Linearity range observed 20-80 ppm. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Repaglinide and Voglibose in its Tablet.

The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and proposed method was successfully applied for the simultaneous estimation of the drugs in commercial combined dosage form.

KEYWORDS: Repaglinide, Voglibose, Derivatization, Analytical Method, Validation, Stability.

INTRODUCTION

Diabetes mellitus is a widespread and persistent health problem. However, the severity and accompanying problems are of little concern to anybody. Both industrialized and developing nations are feeling the effects of diabetes' alarming global rise in incidence. The number of people with diabetes is expected to rise from the current global estimate of 368 million in 2020 to 439 million in 2030, or 7.7 percent of the world's adult

population aged 20-79. According to projections from the International Diabetes Federation's Diabetes Atlas 2009, the number of people with diabetes in India is now about 50.8 million and is predicted to climb to 87 million by the year 2030, making India the diabetes capital of the world. According to the Diabetes Atlas, between 85 and 95 percent of all diagnosed cases of diabetes are due to type 2 diabetes (T2DM). Impaired islet function and insulin resistance contribute to poor glucose tolerance and an abnormal rise in fasting hepatic glucose production, two hallmarks of type 2 diabetes (T2DM). Obesity, lack of exercise, and advanced age all raise the risk of type 2 diabetes. Definition Diabetes is a complex and a multivarious group of disorders that disturbs the metabolism of carbohydrates, protein and fat (KahnCR & Shechter1991, BlissM 2000) characterized by increased fasting (>110mg/dL) and postprandial blood sugar (>140mg/dL) levels. Classification of diabetes Type I (insulin-dependent) and type II (non-insulin-dependent) diabetes mellitus are the two main categories of the disease. Cell-mediated autoimmune destruction of pancreatic -cells causes insulin resistance and type 1 diabetes in children and adolescents (Aikinson MA& McLaren NK 1994, De-Fronzo 2009). However, insulin insufficiency is common in people with non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes (Takeshi K, Shoichi N, 2002). Patients with type I diabetes need an external supply of insulin in order to survive, but those with type II diabetes may be helped by lifestyle modifications, physical activity, and medicine. Bhagwant University Lack of enduring effectiveness in reducing elevated glucose level and failure to address fundamental causes are two of the most major unmet requirements in the therapy of T2DM. Morbidity, mortality, and healthcare costs all rise with type 2 diabetes when glycemic management is inadequate (Turner RC et al., Ohkubo Y et al. 1995.,Koro CE et al., 2000). Although individuals with T2DM may achieve metabolic control with lifestyle adjustment (exercise, food management), this alone is not sufficient to achieve normal blood sugar levels, necessitating the administration of anti-hyperglycemic medicines.

Repaglinide

Repaglinide (S(+)2-ethoxy-4(2((3-methyl-1-(2-(1piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid) is a meglitinide analogue which increases the amount of insulin released by the pancreas. Molecular weight of repaglinide 452.6gm/mol and Molecular formula $C_{27}H_{36}N_2O_4$. Repaglinide is Freely soluble in methanol and in dichloromethane.

Voglibose

Voglibose ((1S,2S,3R,4S,5S)-5-[(1,3-dihydroxypropan-2-yl) amino]- (hydroxymethyl) cyclohexane-1,2,3,4-tetrol.) is an alpha-glucosidase inhibitor which inhibits the intestinal enzymes that cause breakdown of complex sugars into simple sugars such as glucose. Molecular formula of Voglibose is $C_{10}H_{21}NO_7$ and molecular weight is 267.3gm/mol. Voglibose is Soluble in water and slightly soluble in menthol. Voglibose molecule lackes any chromophores capable of giving a reliable signal in the UV region this means that a direct analysis of Voglibose using UV detection is not straightforward. The determination of Voglibose feasible by derivatization with the chromoaphoric agent and subsequent analysis by HPLC. The method is

based on precolumn derivatization of Voglibose with methanol.

A fixed dose combination of Repaglinide(0.5mg) and Voglibose(0.3mg) is used in Type-2 diabetes. Literature review reveals that there are many methods available for single Repaglinide and Voglibose but there are no any RP-HPLC method reported for Repaglinide and Voglibose in combined dosage form so it was thought of interest to develop a simple, accurate, precise and rapid RP-HPLC for analysis of Repaglinide and Voglibose in pharmaceutical dosage form.

MATERIAL AND METHOD

Repaglinide pure sample was kindly provided by Active Pharma Laboratory, Hyderabad, Telangana, India and Voglibose pure sample was kindly provided by Molecule lab., Ahmadabad, India. All chemicals were at least of analytical grade were used. Boric acid, Potassium chloride, Potassium hydroxide, HPLC grade triethyl amine, ortho phosphoric acid, Potassium dihydrogen phosphate (KH_2PO_4) were purchased from Ranbaxy chemicals, New Delhi. HPLC grade Water was purchased from Astron Chemicals, Ahmadabad, India and HPLC grade Methanol was purchased from Finar Limited, Gujarat.

INSTRUMENT

HPLC was performed with Waters model 2690/5 Compact system consisting of Inertsil-C18 ODS Column injector valve with 20.0µL fixed loop. Chromatographic separation was achieved using a Inertsil ODS C18 (250mm × 4.6mm, 5µm particle size) column with Open lab Control panel Software.

METHOD DEVELOPMENT

Preparation of standard stock solution

Fifteen (15) mg of Repaglinide was weight and Transferred to 100ml volumetric flask and volume was mad up to the 100ml with methanol. Ninety (90) mg of Voglibose was weight and Transferred to 100ml volumetric flask and volume was mad up to the 100ml with methanol.

Preparation of derivatization reagents solution:

Borate buffer preparation:

Eight (8) grams of NaOH and 47gm of boric acid weight and transferred to 1000ml beaker. Add 900ml distilled water and dissolved completely. Volume was made up to the mark by adding distilled water..

Derivatization procedure for Voglibose(90µg/ml):

One ml from Voglibose standard stock solution transferred to 10ml volumetric flask. Add 0.5 ml FMOC-Cl solution and mix for 20 second. incubate this solution at 50^0C for 15 min. in water bath for reaction. In order to terminate the reaction 0.1 ml glycine solution was added to the solution mix for 10 second. Volume was made up to by adding borate buffer

Preparation of working standard solution

1 ml from Repaglinide and 1ml from derivatize Voglibose stock solution transferred to 10 ml volumetric flask and volume was made up to the mark by mobile phase.

Phosphate buffer solution

Potassium dihydrogen orthophosphate (6.8gm) were weight and transfer into the 1000ml beaker. 800ml methanol were added and dissolved. volume was made up with HPLC grade water and pH was adjusted by 1% Orthophosphoric acid (pH 3.5) solution.(0.05M potassium dihydrogen ortho phosphate, pH –3.5 buffer).

Pharmaceutical formulation

Twenty tablets were weighed individually and average weight find out. Tablet Powder equivalent to 1.5mg of Repaglinide and 0.9mg of Voglibose was transferred to 100 ml volumetric flask, and add 60 ml of mobile phase and shake for 15 minutes and sonicate for 5 minutes. Made up the volume to the mark with mobile phase. The solution was filtered through Whatman filter paper no-01and first few drops of filtrate were discarded. One ml of this solution was diluted to 10 ml with mobile phase.

STABILITY INDICATING METHOD

Acid degradation

Acid decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solution was added and mixed well and put for 3 hours at RT (25^0C) for Repaglinide and 3 hours at RT (25^0C) for Voglibose(derivatised). This solution was neutralized with 2ml 0.1N NaOH solution then the volume was adjusted with diluent to get 15µg/ml for Repaglinide and 9µg/ml for Voglibose.

Base degradation

Base decomposition studies were performed by transferring 1ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solution was added and mixed well and put for 3 hours at RT (25^0C). This solution was neutralized with 0.1N HCl solution, and then the volume was adjusted with diluent to get 15µg/ml for Repaglinide and 9µg/ml for Voglibose(derivatised).

Oxidative degradation

Oxidation decomposition studies were performed by transferring 1ml of stock solution to 10 ml of volumetric flask. Two ml of 3 % H2O2 solution was added and mixed well and put for 4 hours at RT (25^0C). Then the volume was adjusted with diluent to get 15µg/ml for Repaglinide and 9µg/ml for Voglibose (derivatised).

Thermal degradation

Fifteen (15) mg of Repaglinide was weighed and transferred in a petri dish and put it in the oven at 105^0C for 10 hours, after time period the Repaglinide was transferred in 100 ml volumetric flask and volume was made up to with mobile phase, from this solution take 1ml and transferred to 10 ml volumetric flask to make Repaglinide 15µg/ml. Ninety (90) mg of Voglibose was weighed and transferred it in a Petri dish and Put in the oven at 105^0C for 10 hours, after time period the Voglibose was transferred in 100 ml volumetric

flask and volume was made up to with mobile phase, From this solution take 1ml Voglibose derivatization solution and transferred to 10 ml volumetric flask to make Voglibose 9µg/ml.

Tablet Thermal Degradation:

Tablet powder equivalent to 15 mg of Repaglinide and 90 mg of Voglibose were weighed and transfer in a petri dish and put it in the oven at 105^0C for 10 hours, after time period the Tablet powder was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution take 1ml and transferred to 10ml volumetric flask to make Repaglinide 15µg/ml and Voglibose 9µg/ml.

Photo degradation

Fifteen (15) mg of Repaglinide was weighed and transferred in a petri dish and put in the UV chamber for 48 hours, after time period the Repaglinide was transferred in 100 ml volumetric flask and volume was made up with mobile phase, from this solution take 1 ml and transferred to 10 ml volumetric flask to make Repaglinide 15µg/ml. Ninety (90) mg of Voglibose was weighed and transferred in a petri dish and put in the UV chamber for 48 hours, after time period the Voglibose was transferred in 100 mlvolumetric flask and volume was made up with mobile phase, from this solution take 1ml and transferred to 10 ml volumetric flask to make Voglibose 9µg/ml.

Tablet Photo Degradation:

Tablet powder equivalent to 15 mg of Repaglinide and 9 mg of Voglibose were weighed in a petri dish and put in the UV chamber for 48 hours, after time period the tablet powder was transferred in 100 ml volumetric flask and volume was made up with mobilephase, from this solution take 1 ml and transferred to 10 ml volumetric flask to make Repaglinide 15µg/ml. and Voglibose 9µg/ml.

Selection of wavelength:

Standard solution of 15µg/ml Repaglinide and 9µg/ml of Voglibose in methanol were scanned between 200-400nm using UV- Visible spectrophotometer. Both solutions were scanned between 200-400nm. Wavelength was selected from the overlay spectraof above solution.

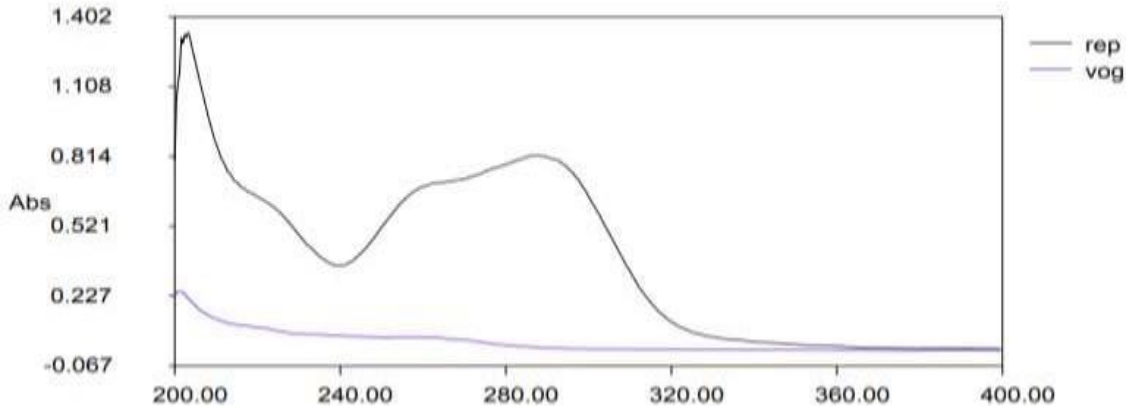


Fig 1: UV Spectra of Repaglinide and Voglibose (257nm Selected)

Mobile Phase selection:

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial

contains various mobile phases which consisted of Methanol, Water, Buffers in different proportions with various pH and different volumes at different flow rate were tried. On the basis of various trials, the mixture of **methanol:water(50:50)**

RESULT AND DISCUSSION

Optimize Chromatographic Condition:

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

Stationary phase (column)	**Inertsil -ODS C18(250 x 4.6 mm, 5 µ)**
Mobile Phase	**Methanol: Water (50:50)**
Flow rate (ml/min)	**1.0 ml/min**
Run time (minutes)	**10 min**
Column temperature (°C)	**Ambient**
Volume of injection loop (µ l)	**20**
Detection wavelength (nm)	**252nm**
Drug RT (min)	**4.714min for Repaglinide and 6.691 for Voglibose.**

METHOD VALIDATION

The method was validated with respect to linearity, limit of detection, limit of quantification, precision, accuracy, recoveryand robustness.

System suitability

It is integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the systemare adequate for the analysis to be performed.

The system suitability of the system was studied by performing the experiment and looking for change in separation, retention times and asymmetry of the peaks. The resolution, areas retention time, theoretical plates values and peak asymmetry were calculated. Result is obtained are given in table 1.

Table:1. System suitability test

Parameters	Repaglinide	Voglibose
Retention Time	4.709	6.684
Theoretical Plates	15237	6420
Asymmetry	1.16334	1.2784

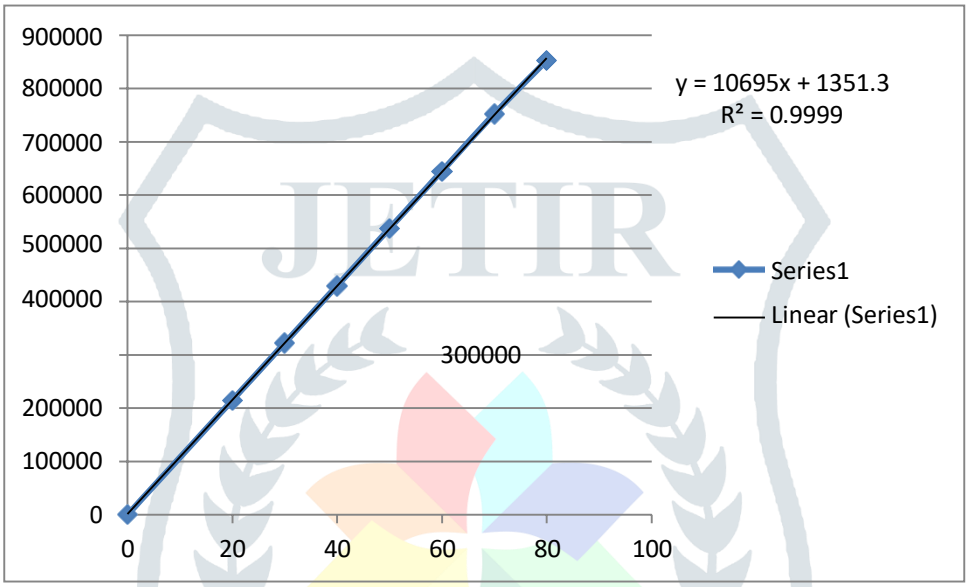
Linearity

The linearity for Repaglinide and Voglibose were assessed by analysis of combined standard solution in range of 20-80 µg/ml respectively. Calibration curve of the area was plotted and found out correlation co-efficient and regression line equation for Repaglinide and Voglibose.Each response was an average of five determinations.

Table 2: Linearity results

Drug	Linearity range	Correlation coefficient
Repaglinide	**20-80 µg/ml**	**0.999**
Voglibose	**20-80 µg/ml**	**0.999**

Fig. 2: Calibration Curve of Repaglinide



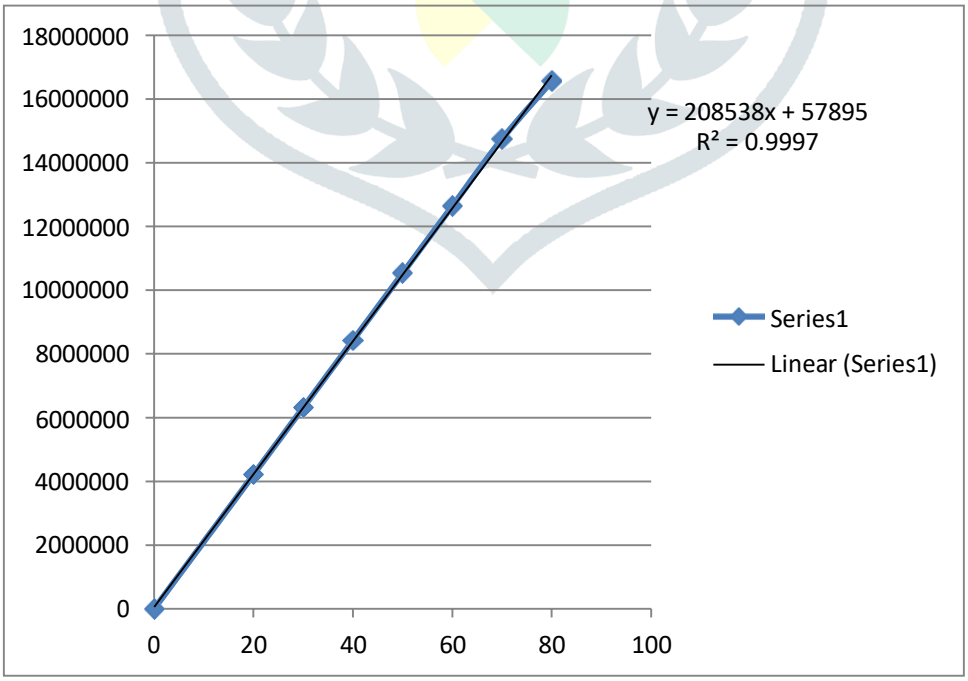


Fig. 3: Calibration Curve of Voglibose

I.**Repeatability**

The data for repeatability of peak area measurement for Repaglinide(15µg/ml) and Voglibose(9µg/ml)based on six measurements of same solution of Repaglinide(15µg/ml) and Voglibose(9µg/ml) and % R.S.D. was calculated.

% RSD of Repaglinide and Voglibose was found to be 0.78541and 0.987541 respectively.

I. Method precision

Standard solution containing Repaglinide (40 ppm) and Voglibose (40 ppm) were analyzed three times on the same day and % R.S.D was calculated.

II. Intermediate precision

Standard solution containing Repaglinide (40 ppm) and Voglibose (40 ppm) were analyzed three times on the different day and % R.S.D was calculated.

Table 3: Precision result

Drug	Method precision (%RSD)	Intermediate precision(% RSD)
Repaglinide	0.875421%	0.68754%
Voglibose	0.587421%	0.987542%

LOD & LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculatedas,

LOD = 3.3 × (SD/Slope)

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves. Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD andLOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration

curve LOQ = 10 * SD/slope of

calibration curve Where, SD = Standard

deviation of intercepts

Table 4: Result for LOD and LOQ

Parameter	Repaglinide	Voglibose
LOD	0.34	0.25
LOQ	1.05	0.77

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are % recovery for Repaglinide was found 99.65-99.93 and Voglibose 98.92-99.96 in range of %.

Table 5: Result of Accuracy

Drug	Concentration %	Amount added(ppm)	Amt. of drug (ppm)	% recovery
Repaglinide	50	20	19.88	99.65
	100	40	39.98	99.89
	150	60	59.91	99.93
Voglibose	50	20	19.96	99.89
	100	40	39.94	98.92
	150	60	59.97	99.96

Robustness

Following parameters were changed one by one and their effect was Observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed 0.8 ml/min,1.0 ml/min and 1.2 ml/min.
2. Ratio of Mobile phase was changed water : Methanol (50:50)

Table 6: Robustness data

Drug	Flow (0.8ml/min) Std area	Tailing factor	Flow (1.0ml/min) Std area	Tailing factor	Flow (1.2ml/min) Std area	Tailing factor
Repaglinide	427089	1.110875	429781.25	1.120887	430847	1.118248
Voglibose	8413218	1.236496	8423027.58	1.247117	8450789	1.24554

Assay

Applicability of the proposed method was tested by analysing the commercially available tablets formulation VOGLI-RAPID 0.5/0.3.The results are shown in table 7.

Table 7: Analysis of marked formulation

Tablet	Label Claim		Assay (% of label claim) Mean ± S.D.	
VOGLI-RAPID 0.5/0.3	**Repaglinide**	**Voglibose**	**%Repaglinide**	**%Voglibose**
	0.5mg	0.3mg	97.80± 0.789	98.26± 0.401

The assay results were comparable to labelled value of each drug in Capsule dosage form. These results indicate that the developedmethod is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 237nm for Repaglinide and 275nm for Voglibose. Common wavelength will be 252nm and the peaks purity was excellent. Injection volumewas selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50 Methanol: Water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

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