

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND VOGLIBOSE IN ITS TABLET DOSAGE FORM.

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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 9- Fluorenyl methyl oxycarbonyl chloride (FMOC - chloride) due to lack of chromophoric group it cannot directly estimated by UV detector in RP-HPLC method. Precolumn derivatization (PCD) conditions were optimized by evaluating the parameters such as concentration of borate buffer, concentration of FMOC-Cl and reaction time of derivatization. The chromatographic separation was achieved on Hypersil BDS C₁₈ (250mm x 4.6mm, 5µm) column and Buffer (0.5M potassium dihydrogen ortho phosphate, pH was adjusted with 1% orthophosphoric acid, Buffer, (pH3.5): methanol (30:70v/v) as a mobile phase at a flow rate of 1ml/min. detection was carried out at 240nm. The Retention time of Repaglinide- 3.670 and Voglibose- 5.333minute. The method has been validated for linearity, accuracy and precision. Linearity observed for Repaglinide 7.5-22.5 µg/ml and Voglibose 4.5-13.5 µg/ml. 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, FMCO-cl, Analytical Method, Validation, Stability.

INTRODUCTION

Repaglinide

Repaglinide (S(+)-2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) 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 C₂₇H₃₆N₂O₄. 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 C₁₀H₂₁NO₇ and molecular weight is 267.3gm/mol. Voglibose is Soluble in water and slightly soluble in menthol. Voglibose molecule lacks 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 9 – Fluorenyl methyl oxy carbonyl chloride (FMCO-chloride). The reaction of FMOC- chloride reacts with secondary amines to form a polar UV – absorbing which can be detected by UV absorbance.

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 Molecule Laboratory, Ahmedabad, Gujrat, India and Voglibose pure sample was kindly provided by Molecule lab., Ahmadabad, India. 9-flourenylmethylchloroformate (FMOC-Cl) and Glycine were purchased from Sigma-Aldrich, Ahmadabad. 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 Agilent Technologies, 1220 infinity using LC -20 AT pump and Column injector valve with 20.0 μL fixed loop. Chromatographic separation was achieved using a Hypersil BDS C18 (250mm \times 4.6mm, 5 μm particle size) column with Open lab Control panel Software. Detection was carried out by Systronics 119 UV spectrophotometer.

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.

FMOC-Cl and glycine stock solution preparation:

Five hundred (500) mg FMOC-Cl weight and transferred to 100ml flask. Volume was made up to the mark by adding acetonitrile. Hundred (100) mg glycine weight and transferred to 100ml flask and volume was made up to the mark by adding water.

Derivatization procedure for Voglibose(90 $\mu\text{g}/\text{ml}$):

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 $^\circ\text{C}$ 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-01 and 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°C) for Repaglinide and 3 hours at RT (25°C) 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°C). 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°C). 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°C 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°C 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°C 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 ml volumetric 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 mobile phase, 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 spectra of above solution.

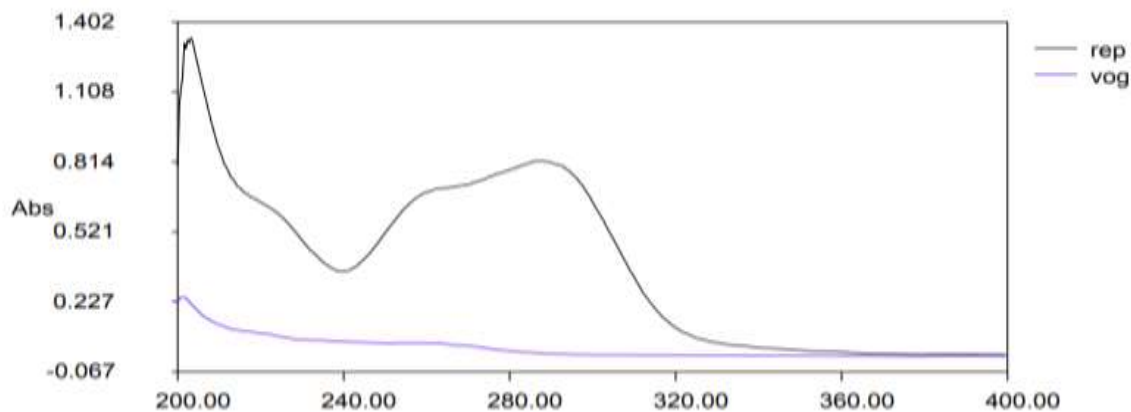


Fig 1: UV Spectra of Repaglinide and Voglibose (240nm Selected)

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 **KH₂PO₄ Buffer, pH 3.5: Methanol (30:70%v/v)**

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.

- ✓ **Mode of Elusion:** Isocratic
- ✓ **Column:** C18 (25 cm × 0.46 cm) Hypersil BDS
- ✓ **Mobile Phase:** Buffer (pH 3.5): Methanol (30:70V/V)
- ✓ **Flow Rate:** 1.0 ml/min
- ✓ **Detection Wavelength:** 240 nm
- ✓ **Run time:** 20 min
- ✓ **Injection volume:** 20.0 μ l
- ✓

METHOD VALIDATION

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

System suitability

It is integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are 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	3.670	5.333
Theoretical Plates	7985	8437
Asymmetry	1.261	1.313
Resolution	-	8.389

Linearity

The linearity for Repaglinide and Voglibose were assessed by analysis of combined standard solution in range of 7.5-22.5 µg/ml, and 4.5-13.5 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	7.5-22.5 µg/ml	0.999
Voglibose	4.5-13.5 µg/ml	0.999

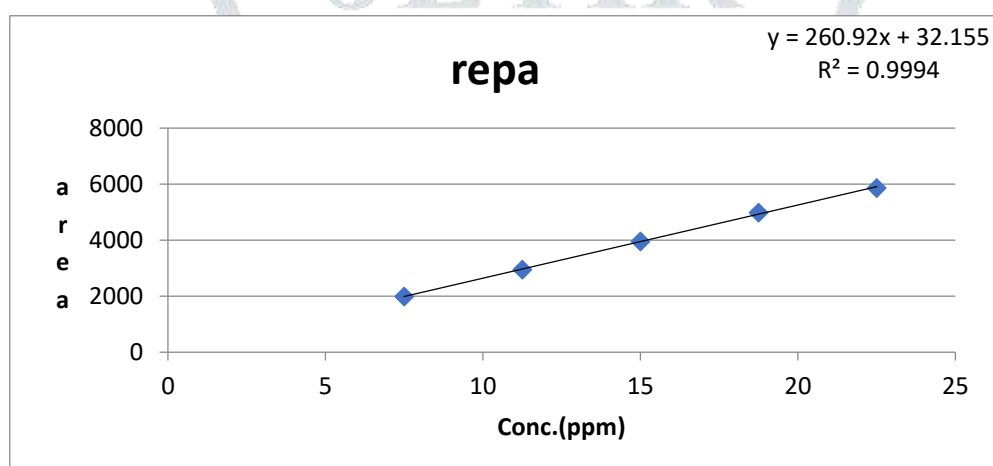


Fig. 2: Calibration Curve of Repaglinide

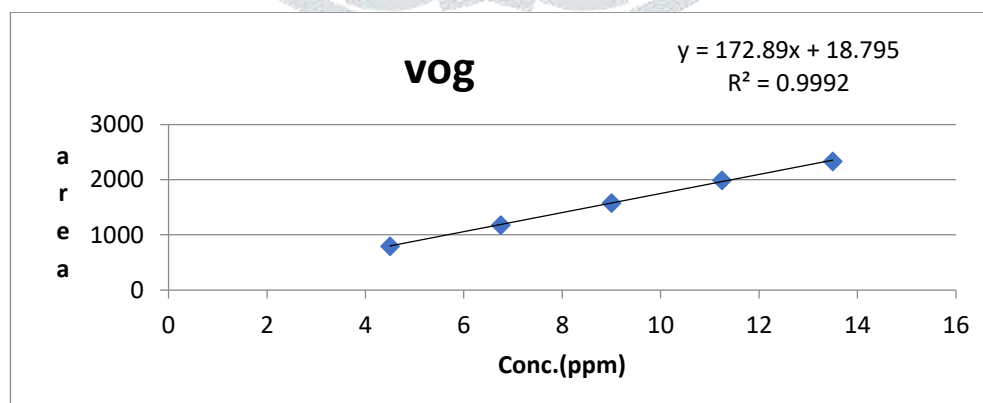


Fig. 3: Calibration Curve of Voglibose

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

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 1.452 and 1.321 respectively.

II. Intraday precision

Standard solution containing Repaglinide (7.5,15,22.5µg/ml) and Voglibose (4.5,9,13.5µg/ml) were analyzed three times on the same day and % R.S.D was calculated.

III. Interday precision

Standard solution containing Repaglinide (7.5,15,22.5µg/ml) and Voglibose (4.5,9,13.5µg/ml) were analyzed three times on the different day and % R.S.D was calculated.

Table 3: Precision result

Drug	Intraday precision (%RSD)	Inter day precision (% RSD)
Repaglinide	0.804-0.958%	1.117- 1.460%
Voglibose	0.631- 0.653%	1.308- 1.313%

LOD & LOQ

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

$$\text{LOD} = 3.3 \times (\text{SD}/\text{Slope})$$

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,

$$\text{LOQ} = 10 \times (\text{SD}/\text{Slope})$$

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 and LOQ were calculated as follows:

$$\text{LOD} = 3.3 * \text{SD}/\text{slope of calibration curve}$$

$$\text{LOQ} = 10 * \text{SD}/\text{slope of calibration curve}$$

Where, SD = Standard deviation of intercepts

Table 4: Result for LOD and LOQ

Parameter	Repaglinide	Voglibose
LOD	0.541µg/ml	0.386µg/ml
LOQ	1.639µg/ml	1.171µg/ml

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 100.02-101.43 and Voglibose 99.32-100.83 in range of %.

Table 5: Result of Accuracy

Drug	Amt. of drug (U=µg/ml)	Amount of drug added (µ/ml)	Amt. recovered Mean (µ/ml)	Mean % recovery ± S.D. (n=3)	Mean % RSD
Repaglinide	7.5	6	6.00	100.025±1.22	1.22
	7.5	7.5	7.60	101.43±1.11	1.09

	7.5	9	9.05	100.61±0.99	0.98
Voglibose	4.5	3.6	3.57	99.325±1.25	1.26
	4.5	4.5	4.53	100.80± 1.89	1.87
	4.5	5.4	5.44	100.83± 1.38	1.37

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 (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 3.3 and 3.7
3. Ratio of Mobile phase was changed (± 2) Buffer: Methanol (28:72) and Buffer: Methanol (32:68)

Table 6: Robustness data

Drug	Area at Flow Rate (+0.2 ml/min)	Area at Flow Rate (-0.2 ml/min)	Area at Mobile phase (+2)	Area at Mobile phase (-2)	Area at pH (+0.2)	Area at pH (-0.2)
Repaglinide	3851.567	4032.572	3795.889	4024.366	3954.017	3851.725
Voglibose	1539.024	1603.228	1519.001	1611.095	1578.209	1527.075

Assay

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

Table 7: Analysis of marked formulation

Tablet	Label Claim		Assay (% of label claim)	
	Repaglinide	Voglibose	Mean \pm S.D.	
EUREP A-V 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 developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of Repaglinide and Voglibose in its tablet. The method is based of precolumn derivatization of Voglibose with 9- Fluorenyl methyl oxycarbonyl chloride (FMOC - chloride) due to lack of chromophoric group it cannot directly estimated by UV detector in RP-HPLC method. The fine separation with better resolution achieved by C₁₈ (250mm x 4.6mm, 5 μ m) column and Buffer (0.5M potassium dihydrogen ortho phosphate, pH was adjusted with 1% orthophosphoric acid, Buffer(pH3.5): methanol (30:70v/v) as a mobile phase at a flow

rate of 1ml/min. Detection was carried out at 240nm. The Retention time of Repaglinide- 3.670 and Voglibose- 5.333minute. The method has been validated for its all parameters like – linearity, precision, accuracy, and robustness. Linearity observed for Repaglinide 7.5-22.5 µg/ml and Voglibose 4.5-13.5 µg/ml. The developed method found precise and accurate for simultaneous estimation of Repaglinide and Voglibose.

The degradation behaviour of Repaglinide and Voglibose was investigated under different stress degradation (Hydrolytic, oxidative, photolytic and thermal) conditions recommended by International Conference on Harmonization (ICH) using HPLC. A stability indicating RP-HPLC method was developed that could separate drug from degradation products formed under various stressed conditions.

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