International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Report

Development and validation of new RP-HPLC method for the estimation of Voglibose in bulk and pharmaceutical dosage form

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Abstract

Background: Voglibose is used to lower blood glucose level in patients with Type 2 Diabetes Mellitus, especially when other medicines are not able to provide the desired results.
Aim and Objective: To develop and validate a simple, rapid, accurate and precise RP-HPLC method validated for the determination of Voglibose in table dosage form.
Materials and Method: Chromatographic analysis of the drug was achieved on a C18 column (250 mm x 4.6 mm, 5 µm) as the stationary phase with a mobile phase consisting of acetonitrile and water in the ratio of 70: 30 v/v.
Results: The method showed a good linear response in the concentration range of 10-60 µg/mL with correlation coefficient of 0.999. The flow rate was maintained at 1.0 mL/min and detection was carried out at 214.5 nm. The retention time was 3.08 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity and sensitivity. The results obtained in the study were within the limits of ICH guidelines.
Conclusion: The method was found to be simple, sensitive and economical used for the determination of Voglibose in tablet formulation.
Keywords: Voglibose; RP-HPLC; Method Development; ICH; Validation; Tablet dosage form.

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***Article History:**
Received: 07/10/2020
Revised: 18/10/2020
Accepted: 19/10/2020
DOI: https://doi.org/10.7439/ijbr.v11i10.5523

QR Code

How to cite: Debnath R, Manjunath S. Y. and Hemant Kumar T. Development and validation of new RP-HPLC method for the estimation of Voglibose in bulk and pharmaceutical dosage form. *International Journal of Biomedical Research* 2020; 11(10): e5523. DOI: 10.7439/ijbr.v11i10.5523 Available from: https://ssjournals.com/index.php/ijbr/article/view/5523

1. Introduction

Voglibose, a potent α-glucosidase inhibitor is used for the treatment of diabetes mellitus [1-2]. It acts as glucosidase inhibitor, remaining active within the gastrointestinal tract of humans by delaying the glucose absorption thereby preventing the sudden surge of glucose in the human body after meals. Most commonly used glucosidase inhibitors include acarbose, mitglitol & voglibose. Voglibose is the safest and most effective of them all [3,4]. It is most commonly available in the form of tablets with the dosages of 0.2 mg to 0.3 mg per tablet. Structure of voglibose (Fig.1) is [5-(1, 3-dihydroxypropane-2-yl -amino)-1-(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol] similar to that of carbohydrate .Voglibose exist as colorless crystalline solid having a molecular formula $C_{10}H_{21}NO_7$ and molecular weight 267.27 g/mol. Literature survey reveals various analytical methods reported for estimation of voglibose in API and pharmaceutical formulations includes UV spectrophotometric[5,6] HPLC[7,8], LC-MS[9] and HPTLC[10]. Out of these analytical methods HPLC[11-13] is most widely used technique in quantitative analysis of drugs. In spite of various HPLC methods available for the estimation of voglibose , still there is a need of an economical and rapid HPLC method for its estimation in dosage forms. The present study, a new RP-HPLC method was developed for estimation of voglibose in pharmaceutical formulation, which showed high reproducibility, sensitivity and economical. The developed method was validated as per ICH guidelines [14-16].

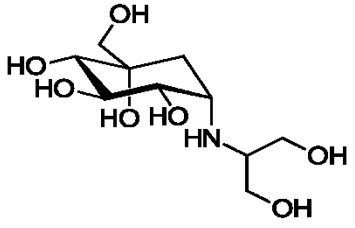

Figure 1: Structure of voglibose

2. Materials and Methods

2.1 Instrumentation

Chromatographic separation was performed on a Cyberlab HPLC system equipped with a Flowrosil C18 column (250 × 4.6 mm, with 5 µm particle), single pumps, degasser, variable wave length detector and Rheodyne injector with 20 µl loop volume. 'LC solution' software was used to collect and process the data. Ultra sonicator (Citizen ultra sonicator) was used for sonicating the drug and sample solution. Digital weighing balance (SHIMADZU AUX 220) is used for weighing.

2.2 Chemicals and Reagents

All reagents and chemicals used were grade of analytical reagent and H_2O (HPLC grade) was used during the study. Voglibose standard gifted from Sun Pharmaceutical Industries Limited Mumbai, Maharashtra. A commercial tablet of VOLIBO (Sun Pharmaceutical Industries Ltd) containing 0.3 mg of voglibose was purchased from local medical store.

2.3 Chromatographic conditions

The chromatographic system used for method development and validation includes the LC-P100 pump, variable wavelength programmable LC-UV100 UV detector and SCL20A system controller at CYBERLAB HPLC. A Rheodyne injector 7725i equipped with a 20 µL loop was used and the data was recorded and evaluated using LC solution software version 5.0. Separation was performed at Flowrosil C18 (250 × 4.6 mm i.d., 5µm) at the ambient temperature. A mixture of water and acetonitrile in a 30: 70 v / v ratio was found to be the ideal mobile phase for the ideal chromatographic analysis of voglibose. The solvent mixture was filtered through a 0.22 µ membrane filter and sonicated before use. It is pumped through the column at a flow rate of 1.0 mL / min. The injection volume is maintained in the column at 20 µL and room temperature. The column was balanced by pumping the mobile phase through the column for at least 20 min before injecting the drug solution. The detection was monitored at 214.5 nm. Run time is set to 10 minutes. .

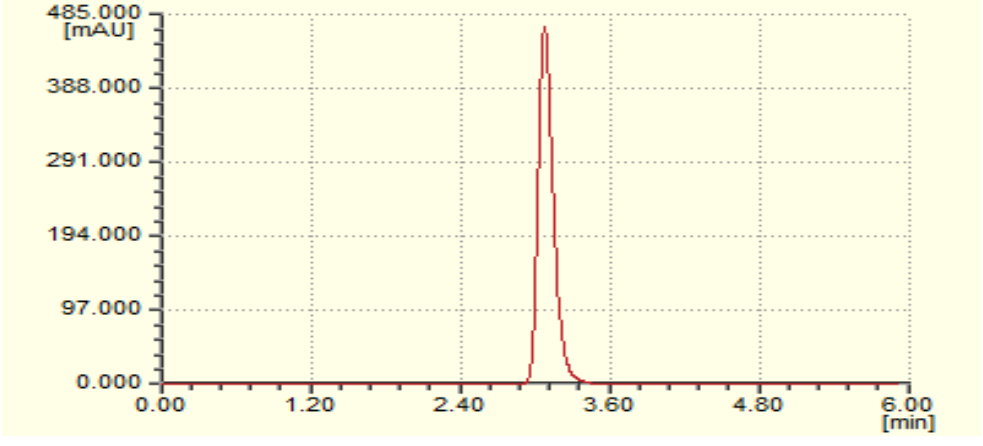

Fig. 2: Chromatogram of standard solution of voglibose

2.4 Preparation of mobile phase

Mobile phase was prepared by mixing 700 mL of HPLC grade acetonitrile with 300 mL water (HPLC grade water). The mobile phase was sonicated for 60 min and filtered through the 0.22 µm membrane filter.

2.5 Preparation of standard stock solutions

The standard stock solutions of 1000 µg/mL of the drug were prepared by dissolving 10 mg of voglibose standard into 10 mL of volumetric flask, Add 10 mL of the mobile phase and volume was made up to the mark. From the above solution transferred 1 mL in to 10 mL of volumetric flask and made upto the mark with mobile phase (100 µg/mL). From this solution pipetted out 1, 2, 3, 4, 5 & 6 mL in to 10 mL volumetric flasks and made upto the mark with mobile phase (10, 20, 30, 40, 50, 60 µg/mL).

2.6 Preparation of sample solution

To determine the content of Voglibose in tablet dosage form (Label claim: 0.3 mg/tablet) five tablets were accurately weighed and triturated to fine powder. The powder weight equivalent to 1.5 mg of voglibose was taken and dissolved in 10 mL of mobile phase. The resulting solution (2 mL) was transferred to a 10 mL volumetric flask and diluted up to the mark with mobile phase. The final solution was filtered through 0.22 µ membrane filter using injection filter. A 20 µL of the filtrate was injected into chromatographic system. The peak area of the voglibose was determined and the concentration was found using a linear regression equation obtained from calibration curve.

2.7 Method Validation

The developed method was validated as per ICH guidelines by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficients of variation and relative errors of less than 2 % were considered acceptable.

2.7.1 System Suitability Test

Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, and also relative standard deviation (RSD) of the peak areas.

2.7.2 Stability

Stability was assessed by analyzing QC standard solutions after keeping them at room temperature for 48 hr. Obtained results were investigated as recovery values and compared to the freshly prepared solutions.

2.7.3 Linearity

A stock solution of voglibose of 1000 µg/mL was prepared with the mobile phase. From it, various working standard solutions were prepared in the range of 10 to 100 µg/mL and injected into HPLC. It was shown that the selected drug had linearity in the range of 10–60 µg/mL. The calibration plot (peak area of voglibose versus voglibose concentration) was generated by replicate analysis (n=6) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.7.4 Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 80 %, 100 % and 120 %, and then comparing the difference between the spiked value (theoretical value) and actual found value.

2.7.5 Precision

The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (30 µg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD).

2.7.6 Robustness

Robustness of the proposed method for voglibose was carried out by the slight variation in flow rate, analytical wavelength and mobile phase ratio. The percentage recovery and RSD were noted for voglibose.

2.7.7 Ruggedness

The test solutions were prepared as per the test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

2.7.8 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:

LOD=3.3SD/slope

LOQ=10SD/slope

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y-intercept of the regression line, and s is the slope of the calibration curve.

2.7.9 Specificity

The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as blank and excipients like starch, lactose, magnesium stearate were used as placebo.

3. Results and Discussion

3.1 Method validation

3.1.1 System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. To determine the parameters, the study was performed with a standard solution of 30 µg/mL concentration and the results were acquired from six injections. System suitability parameters of the method were demonstrated in Table 1. According to the results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

Table 1: Results of system suitability test (n = 6)

Parameter	Criteria	Result
Capacity factor(k')	$k' > 2$	3.824
Tailing factor (T)	T < 2	1.5
Theoretical plates (N)	N> 2000	2543
% RSD (peak area)	% RSD ≤ 2	0.94

3.1.2 Stability

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results was found within acceptable limits (% RSD < 2), which are summarized in Table 2.

Table 2: Stability data of voglibose (standard solutions)

Time (hr)	Assay (%)	% Difference
Initial	100.08	----
After 12 hr	100.02	0.05
After 24 hr	99.87	0.21
After 36 hr	99.16	0.92
After 48 hr	98.32	1.76

3.1.3 Linearity and sensitivity

Linearity study was performed with calibration standards with 10, 20, 30, 40, 50, and 60 µg/mL concentrations. The standards were injected in triplicate. Calibration curves were obtained by plotting the peak areas against the given concentrations. The calibration curve was evaluated by the determination coefficient. The

determination coefficient (R^2) of the calibration curves was 0.999. Therefore, the calibration curve for voglibose was found to be linear within the range of 10–60 µg/mL concentrations as showed in Fig. 3.

The regression equations were calculated from the calibration graphs. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ are given in Table 3. The low values of LOD and LOQ indicate the sensitivity of method.

Table 3: Spectral and statistical data for determination of Istradefyllin from proposed RP-HPLC method

Parameter	Result
Detection wavelength (nm)	214.5
Linearity range (µg/mL)	10-60
Coefficient of determination (r^2)	0.999
Regression equation (Y^a)	Y= 8151x + 4666
Slope (m)	8151
Intercept (c)	29037
Limit of detection, LOD (µg/mL)	0.054
Limit of quantitation, LOQ (µg/mL)	0.16

[a]$Y = mx + c$, where x is the concentration (µg/mL).

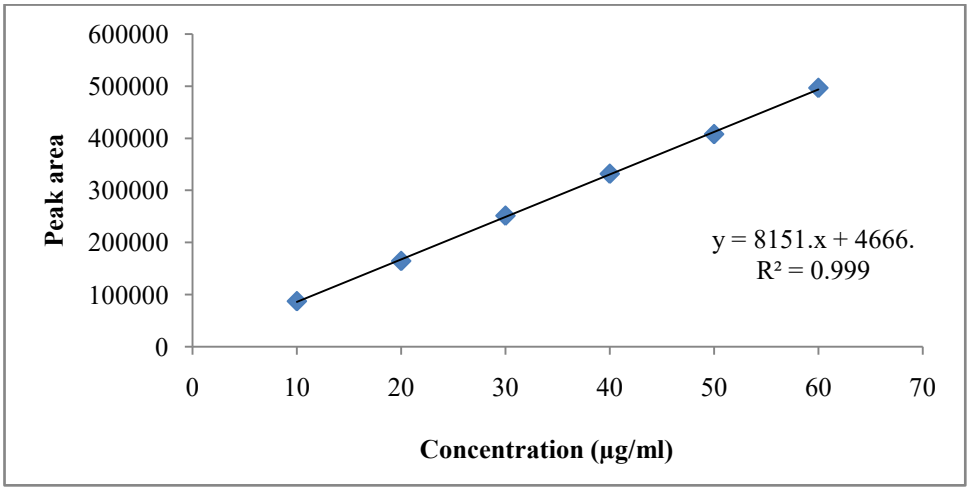

Figure 3: calibration curve of Voglibose

3.1.4 Accuracy

To study the reliability, the suitability, and the accuracy of the method, recovery experiments were carried out. Known quantities of the pure drug were added to the preanalyzed sample to make samples at the levels of 80 %, 100 %, and 120 %, and were assayed by the proposed method. Accuracy was calculated as the percentage of recovery. The recovery and relative standard deviation for each of the analytes are given Table 4. From the recovery studies it is evidence that the method is highly accurate and can give excellent results.

Table 4: Accuracy results

% spike Level	Sample	Amount Added (Std)	Amount Found (µg/mL)	% Recovery	Statistical Parameters
80	30	24	23.76	99.0	Mean = 99.1 SD= 0.458 % RSD=0.462
	30	24	23.90	99.6	
	30	24	23.68	98.7	
100	30	30	29.65	99.5	Mean = 98.9 SD= 0.655 % RSD=0.663
	30	30	29.46	98.2	
	30	30	29.70	99.0	
120	30	36	35.67	99.1	Mean = 98.9 SD= 0.529 % RSD=0.535
	30	36	35.38	98.3	
	30	36	35.74	99.3	

3.1.5 Precision

The precision was demonstrated at three levels: repeatability, intermediate precision, and reproducibility (between laboratories' precision). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 20, 30 and 40 µg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The results of three levels of precision are shown in Table 5. The developed method was found to be precise as the RSD values for repeatability, intermediate precision and reproducibility studies were < 2 %, respectively as recommended by ICH guidelines (ICH Q2 (R1), 2005).

Table 5: Precision results

Precision	Results		
	Concentration (µg/mL)	% RSD of Peak area	% RSD of Retention Time
Repeatability	20	0.89	0.02
	30	1.21	0.08
	40	1.11	0.12
Intermediate precision	20	1.42	0.08
	30	0.75	0.06
	40	0.67	0.06
Reproducibility	20	1.64	0.11
	30	0.78	0.17
	40	0.85	0.09

3.1.6 Robustness and ruggedness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0±0.1 mL/min), mobile phase composition (± 5 % organic phase) and analytical wavelength (±2 nm). The results are given in Tables 6. The result shown that have the negligible effect on retention time, recoveries and peak area of voglibose indicating the developed method is robustness. Ruggedness of the method was carried out by different analysts. The results are displayed in Table 7. There is no variation in peak areas and retention time of voglibose from studies carried out by two analysts as indicated by % RSD < 2 gives the method ruggedness.

Table 6: Robustness studies

Parameter	Variation	Observed value			
		% RSD of area	% RSD of R.T	Tailing factor	Theroteical plates(N)
Flow rate (m L)	0.9	0.35	0.91	1.5	2541
	1.1	0.59	0.73	1.5	2534
Mobile Phase Composition	60% acetonitrile	0.42	0.14	1.4	2562
	80 % acetonitrile	0.54	0.13	1.5	2551
Wavelength (nm)	212 nm	0.55	0.61	1.5	2545
	216 nm	0.62	0.74	1.5	2552

Table 7: Ruggedness studies

Analyst	Observed value			
	% RSD of area	% RSD of R.T	Tailing factor(T)	Theroteical plates(N)
Analyst I	0.45	0.63	1.5	2565
Analyst II	0.52	0.72	1.5	2553

3.1.7 Mobile phase stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4–8 °C for 1 week. The aged mobile phase was compared using a freshly prepared one. The mobile phase was stable up to 1 week at 4–8 °C.

3.1.8 Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purity of voglibose was assessed by comparing the retention times of standard voglibose and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. There is no interference of blank and placebo on drug peaks hence, the method is specific.

3.1.9 Sample Analysis

The developed and validated method was applied for analysis of tablet formulation contains voglibose. The sample was analyzed in triplicate. Analysis results were evaluated using a calibration curve. The amount of voglibose in the samples was calculated from calibration curve equation and recovery and RSD values were determined. The results of analysis are given in Table 8. The recoveries were in good agreement with the label claims. The chromatogram obtained was clear as shown in Fig. 4. It was concluded that the method can be applied successfully for the analysis of voglibose in tablet dosage form.

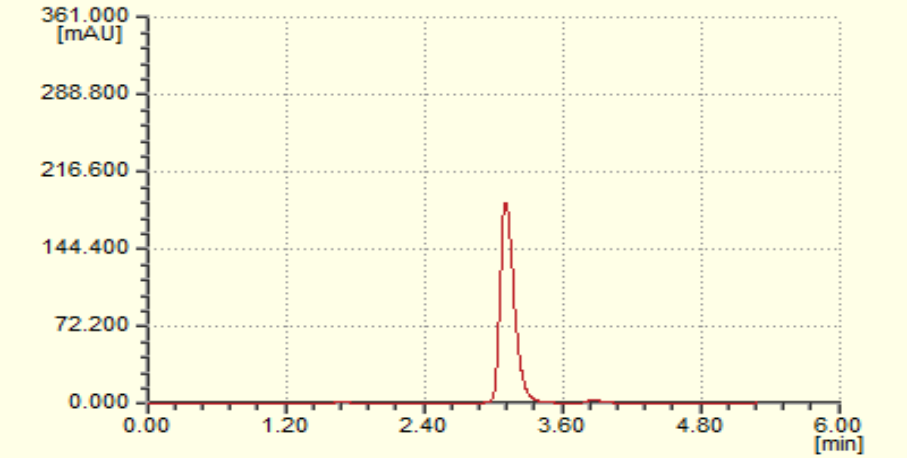

Fig. 4: Chromatogram of sample Voglibose

Table 8: Assay results from commercial formulation

Tablet	Drug	Labeled Claim (mg)	Amount found (mg)	% Mean Recovery* ± % RSD
Volibo (Sun Pharmaceutical Industries Ltd)	Voglibose	0.3 mg	0.29	99.32 ± 0.94

* Average of five determinations

4. Conclusion

The proposed method for the estimation of voglibose was validated as per the ICH guidelines and it is simple, specific and economical. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of voglibose in pharmaceutical formulations without any interference from the excipient.

Acknowledgement

The authors are grateful to Sun Pharma Ltd for supplying the gift samples of pure drug. The authors thankful to the management of Srikrupa Institute of Pharmaceutical Sciences for providing the instrumental and chemical facilities.

References

[1]. Maryadele JO, The Merck Index: An encyclopedia of chemicals drugsand biologicals, Merck Research Laboratories, Division of Merck Co, Inc, Whitehouse Station, Neil (Eds), New Jersey; 2006.

[2]. Maryadele J, Heckelaman PE, Koch CB, The Merck Index: An encyclopedia of chemicals. 14th ed. Drugs and Biologicals. Merk Research Laboratories, USA; 2006.

[3]. Vichayanrat A, Ploybutr S, Tunlakit M and Watanakejorn P. Efficacy and safety of voglibose in comparison with acarbose in type 2 diabetic patients. *Diabetes Res Clin Pract* 2002; 5: 99-103.

[4]. Satoh N, Shimatsu A, Yamada K, Aizawa-Abe M, Suganami T, Kuzuya H and Ogawa Y. An alpha-glucosidase inhibitor, voglibose, reduces oxidative stress markers and soluble intercellular adhesion molecule 1 in obese type 2 diabetic patients". *Metabolism* 2006; 55 (6): 86-93.

[5]. Udhan RR, Jaybhaye S, Pathan IB. Development and validation of a simple UV spectrophotometric method for the determination of desloratadine both in bulk and marketed dosage formulation. *Int J Pharm Res Sci* 2013; 1(1):33-6.

[6]. Bhagyalakshami RM, Ravi TK. Development and validation of UV-spectroscopic method for estimation of voglibose in bulk and tablets. *J Chem Pharm Res* 2010; 2(2):350-6.

[7]. Kishore VS, Kumar JP, Chakravarthy TK, Naik SV, Reddy MH and Babu EN. Development and Validation of RP-HPLC Method for Quantitative Analysis Voglibose in Pure and Pharmaceutical formulations. *Int J of Pharma Chem and Biol Sci* 2013; 3(2): 336-41.

[8]. Mallikarjuna RN. RP-HPLC method development and validation for estimation of Voglibose in bulk and tablet dosage forms. *Int J Res Pharm Sci* 2010; 1(2) : 190-4.

[9]. Rajput M, Dahiyaet M, Kumari P, Kalra K, Aggarwal M and Khandal RK. Development and Validation for Determination of Voglibose in tablet Formulation Using LC-MS/MS. *E Journal of Chemistry* 2011; 8(4), 1770- 83.

[10]. Shinde, C. C., S. Chopade, and S. S. Dhaneshwar. Development and Validation of HPTLC Method For Estimation of Voglibose In Pharmaceutical Dosage Forms. *Int J Pharm Pharm Sci* 2015; 7(5): 288-93.

[11]. Hemant Kumar T, Swathi Sri D, Vara Prasada Rao K and Srinivasa Rao Y. Validated RP- HPLC Method For Determination Of Rosuvastatin Calcium In Bulk And Pharmaceutical Formulation. *Int J Pharm Sci & Res* 2015; 6(7): 1000-05.

[12]. Kumar TH, Jhanavi D, Rao KVP and Rao YS: RP-HPLC method for quantification of orotic acid in capsule formulation. *Int J Pharm Sci & Res* 2019; 10(5): 2343-7.

[13]. T. Hemant Kumar**,** C Kasi Krishna Prajna, K. Varaprasad Rao, Y. Srinivas Rao. RP-HPLC Method For Estimation Of Tramadol Hydrochloride And Paracetamol In Pharmaceutical Formulation. *GSC Biological and Pharmaceutical Sciences* 2019; 8(1):89-97.

[14]. ICH Harmonized Tripartite Guidelines, Validation of analytical procedure: Text and methodology'Q2 (R1); 2005.

[15]. ICH. Q2B Validation of analytical procedure: Methodology. International conference on harmonization,Geneva;1996.

[16]. ICH Harmonized Tripartite Guideline, Validation of analytical procedure: Methodology (Q2B); 1996.