**IJPSR (2013), Vol. 4, Issue 4** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 December, 2012; received in revised form, 12 February, 2013; accepted, 15 March, 2013

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

K. Sonia* and K. Prasad babu

Department of Pharmaceutical Analysis, K.K College of Pharmacy, Chennai, Tamil Nadu, India

**Keywords:**
RP-HPLC, Metformin HCl and Voglibose, Simultaneous estimation, Method development, Method validation

**Correspondence to Author:**

**K. Sonia**

Lecturer, Department of Pharmaceutical Analysis, K.K College of Pharmacy, 1/161, Sankaralinganar Road, Gerugambakkam, Chennai-600 122, Tamil Nadu, India

E-mail: soniapharm68@yahoo.com

**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 was 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.

QUICK RESPONSE CODE

IJPSR: ICV (2011)- 5.07

Article can be accessed online on: www.ijpsr.com

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].

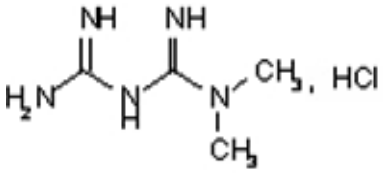


**FIGURE 1: METFORMIN HCl**

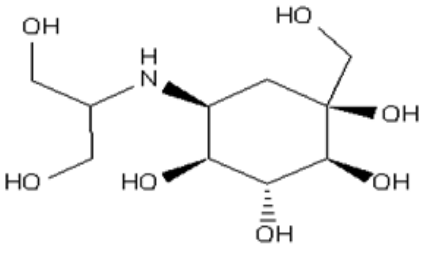


**FIGURE 2: VOGLIBOSE**

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 ambi-ent 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 chro-matographic conditions are listed in **Table 1**.

**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:**

   **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 transfered to 500ml volumetric flask and dissolved it in 300ml of diluent.

Pipette out 5ml of above solution A and transfered 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 transfered 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 chro-matograms.

   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 shown in table 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**.

7. **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 (**Table 6**).

8. **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 chromatographic 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 preci-sion under given set of conditions and were within the acceptance criteria (**Table 7)**.

## RESULTS AND DISCUSSION:

**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$^{o}$C |
| Injection volume | 20µl |
| Run time | 20min |

**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:**

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

= 498.93mg

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

= 99.7 %

**TABLE 4: LINEARITY FOR METFORMIN HCl & VOGLIBOSE**

| | Metformin HCl | | | | | Voglibose | | | |
|---|---|---|---|---|---|---|---|---|---|
| S. No. | Test conc. 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 | 60958 | |
| | | | 2 | 6795821 | | | 3 | 61024 | |
| | | | 3 | 6799856 | | | | | |
| 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 | |

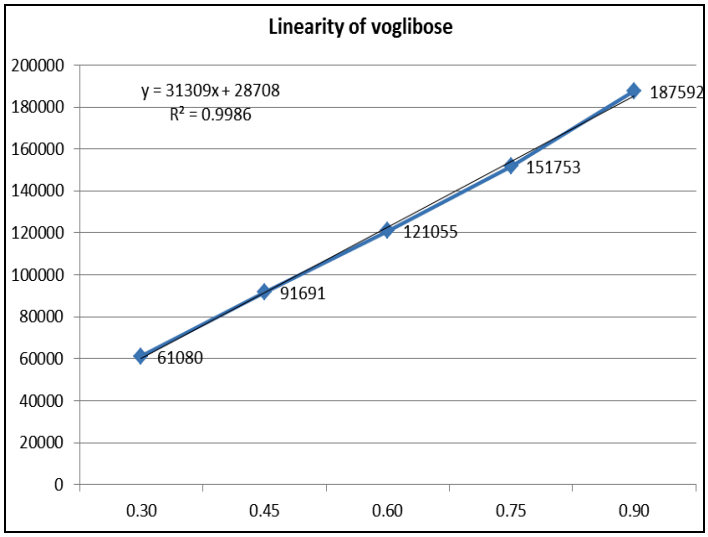


**FIGURE 3:**

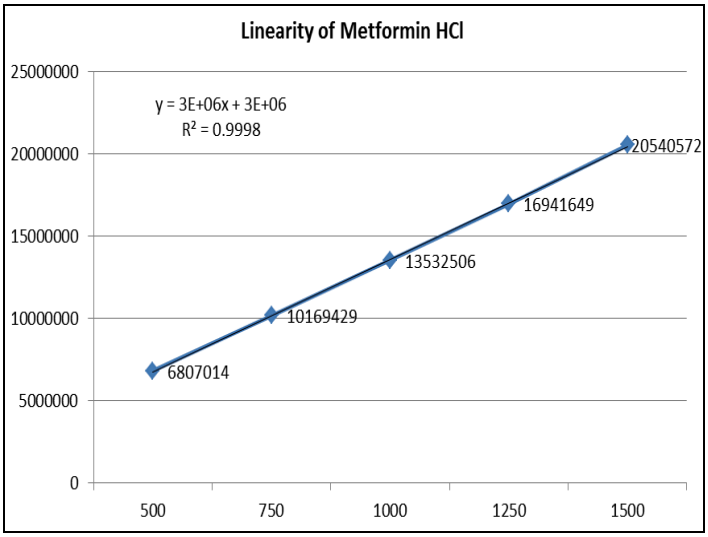


**FIGURE 4**

**TABLE 5: RECOVERY**

| S. NO. | Con 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 |

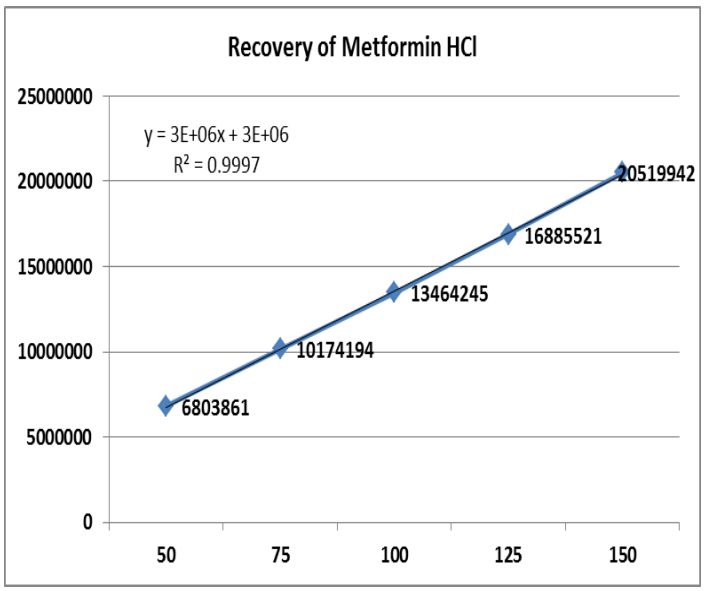


**FIG 5: RECOVERY CURVE FOR METFORMIN HCl**

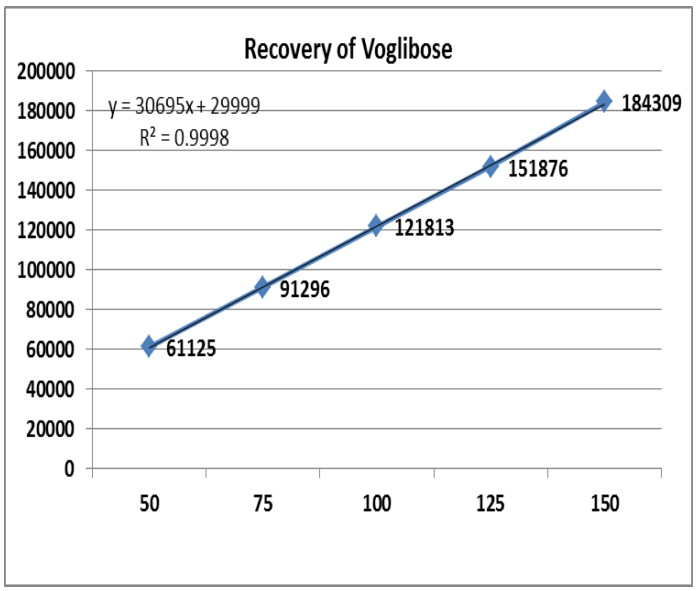


**FIG. 6: RECOVERY CURVE FOR VOGLIBOSE**

**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$^{o}$C) | 23$^{o}$C | 27$^{o}$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.

## REFERENCES:


1. United States Pharmacopoeia, 25th Review, The National Formulary, 19th Review, US Pharmacopoeia Convention, Rockville, MD, 2002.
2. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res. 2007;100(3):328–41
3. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman M, Goodyear L, Moller D: Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108(8):1167–74.
4. Kim YD, Park KG, Lee YS: Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes. 2008; 57(2):306–14.
5. Benalla W. Bellahcen S. Bnouham M: Antidiabetic medicinal plants as a source of alpha glucosidase inhibitors. [Review]". Current Diabetes Reviews. Jul 2010; 6(4):247-54.
6. Ji F. Xiao G. Dong L. Ma Z. Ni J. Zhongguo Zhong Yao Za Zhi/Zhongguo Zhongyao Zazhi: "Development of alpha-glucosidase inhibitor from medicinal herbs. China Journal of Chinese Materia Medica. Jun. 2010; 35(12):1633-40,
7. Samantha J. Venable; Diane S. Aschenbrenner, Drug Therapy In Nursing, Hagerstown, MD: Lippincott Williams & Wilkins, ISBN 0-7817-4839-9
8. Sharma BK: Instrumental methods of chemical analysis, In; Introduction to Analytical chemistry. Goel Publishing House Meerut, 23thedition 2004.
9. Willard HH, Merritt LL, Dean JJA, Frank AS: Instrumental method of analysis. CBS Publishers and Distributors, New Delhi, 7th Edition 1986.
10. Skoog, Holler, Nieman: Principals of Instrumental Analysis. Harcourt Publishers International Company, 5th Edition 2001.
11. Method validation guidelines International Conference on harmonization; GENEVA; 1996.


**How to cite this article**:
Sonia K and Babu KP: RP-HPLC analysis of Metformin hydrochloride and Voglibose and study of its different analytical parameter. *Int J Pharm Sci Res* 2013; 4(4); 1469-1474.