Journal of Pharmaceutical and Biomedical Analysis Letters

Journal Home Page: www.pharmaresearchlibrary.com/jpbmal

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Metformin and Gemigliptin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

Y. Krishna Reddy*, Kedhareshwari, Y. Harikha, V. Harikrishna, Muzahid, K. Gopi

Bomma Institute of Pharmacy, Khammam, Telangana, India

A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Metformin and Gemigliptin by using RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of metformin and gemigliptin were found to be from 100-500 µg/ml of metformin and 1-5µg/ml of gemigliptin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of metformin and gemigliptin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Metformin, Gemigliptin, HPLC, LOD and LOQ

A R T I C L E I N F O

CONTENTS

Article History: Received 19 May 2016, **Accepted** 21 June 2016, **Available Online** 18 July 2016

***Corresponding Author**
Y. Krishna Reddy
Bomma Institute of Pharmacy,
Khammam, Telangana, India
Manuscript ID: JPBMAL3050

PAPER-QR CODE

Citation: Y. Krishna Reddy, *et al.* Analytical Method Development and Validation for the Simultaneous Estimation of Metformin and Gemigliptin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form. *J. Pharm, Biomed. A. Lett*., 2016, 4(2): 93-99.

1. Introduction

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [5, 6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

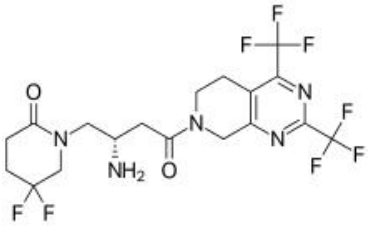

Figure 1: Metformin

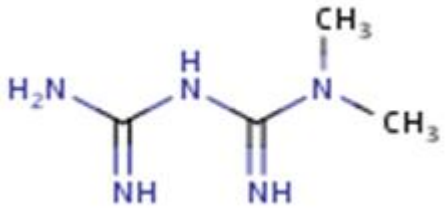

Figure 2: Gemigliptin

2. Materials and Methods

Apparatus: The instrument used for the study was WATERS, software: Empower, 2695 separation module, PDA detector.

Reagents & Materials: The solvents used were Potassium dihydrogen orthophosphate, Methanol, Acetonitril, Orthophosporic acid and Water [10].

Selection of detection wavelength:

UV spectrum of 10 µg / ml Metformin and Gemigliptinin diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260 [11]. At this wavelength both the drugs show good absorbance.

Selection of mobile phase

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively [12].

Optimization Chromatographic trials for Simultaneous Estimation of Metformin and Gemigliptin by RP-HPLC [Optimization chromatographic conditions]

Parameters Description

Column : Inertsil ODS (4.6 x 150mm, 5µm)
Mobile phase ratio : 30% buffer 70% Methanol
Detection wavelength: 260 nm
Flow rate: 1ml/min Injection volume: 10µl Column temperature: Ambient Auto sampler temperature: Ambient

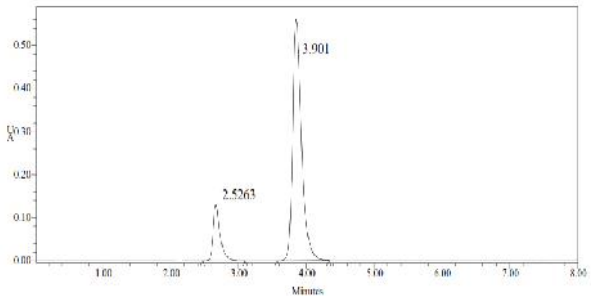

Figure 3: Optimization Chromatogram

Observation:

The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method [13].

Procedure

Preparation of Buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid [14].

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [15].

Standard Preparation:

Accurately weigh and transfer 10 mg of Metformin and Gemigliptin10mg of working standard into a 10Ml & 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution), Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents[16].

Assay

Preparation of samples for Assay

Standard preparation:

Weigh accurately 10mg Amlodipine Working Reference Standard and 15mg of Perindopril Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark [17]. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution), Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents [18].

Sample preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Metformin and Gemigliptin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [19]. (Stock solution)Further pipette 3 ml of Metformine and Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [20].

Assay procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Metformin and Gemigliptin peaks and calculate the %Assay by using the formulae [21].

3. Results and Discussion

Method Validation Parameters

1. Specificity

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Serial dilutions of 100µg/ml to 500µg/ml of Metformin, 5µg/ml to 25µg/ml 0f Gemigliptin were injected into the column and detected at a wavelength set at 256 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range: The linearity study was performed for the concentration of 100ppm to 500ppm and 5ppm to 25ppm level of Metformin and Gemigliptin. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Percentage method:

For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria:

The mean % recovery of the Metformin and Gemigliptin at each level should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

Method Precision:

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions [21]. The % RSD of peak areas of six samples was calculated. The method precision was performed on Metformin and Gemigliptin formulation. The % RSD of the assay value for six determinations should not be more than 2.0%.

Intermediate Precision:

Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated.

Method Validation Parameters

1. Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Method validation

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 1.0–20.0 µg/mL was linear with a correlation coefficient (R^2) greater than 0.998. The LOD and LOQ were calculated as 0.9160 µg/mL and 3.053 µg/mL respectively. Figure 4 shows the linearity curve of Tenofovir.

Intra-day and inter-day precision

The intra-day and inter-day precision study of the developed method confirmed adequate sample stability and

method reliability where all the RSDs were <2%. Results of precision were shown in table 1.

Accuracy/recovery

Results within the range of 99.84–99.91% ensure an accurate method as well as indicate non-interference with the excipients of formulation. Table 2 shows the accuracy results.

Specificity

The specificity of the analytical method was proved by comparing the spectra of placebo, Active pharmaceutical ingredient with tablet sample solution. The tablet spectra show no interference with excipients. Figure 2 & 3 shows the results of specificity.

Assay: Tenofovir content of the marketed product determined by the proposed method and was in good agreement with the label claims and was in the range of 98.38–100.53% with the RSD value was 0.841.

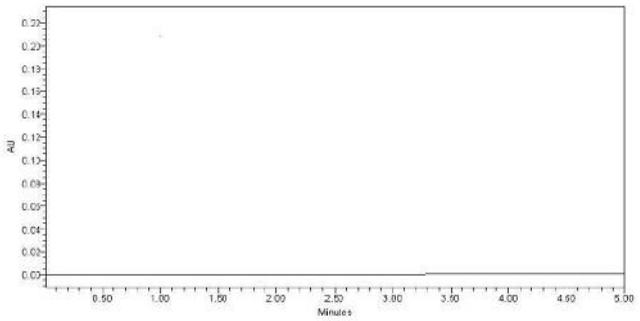

Figure 4: Chromatogram of Blank

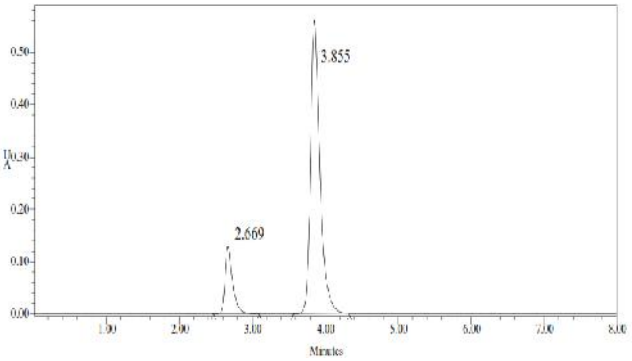

Figure 5: Chromatogram of Sample

Linearity: The linearity study was performed for the concentration of 100 ppm to 500ppm for Metformin and 5ppm to 25ppm for Gemigliptin and chromatograms are shown below.

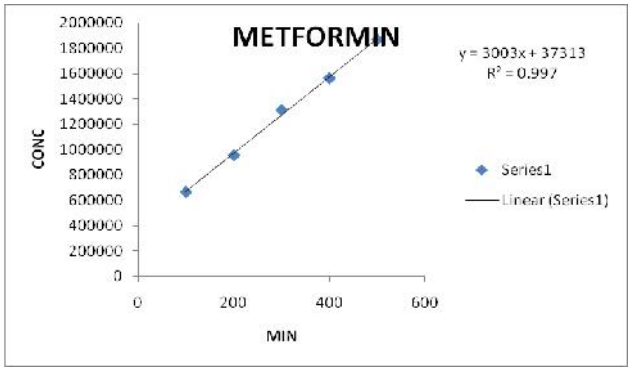

Figure 6: Calibration graph of Metformin

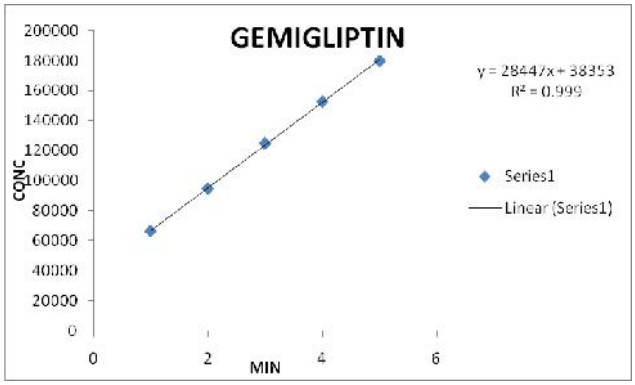

Figure 7: Calibration graph of Gemigliptin

Recovery studies

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Detection limit

The LOD was performed for Metformin and Gemigliptin was found to be 2.9and 3 respectively.

Quantitation limit

The LOQ was performed for Metformin and Gemigliptin was found to be 10.03 and 10.1 respectively.

Table 1: Area of different concentration of Metformin

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 2: Area of different concentration of Gemigliptin

S.No.	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731

5	V	5ppm	179732
Correlation Coefficient			0.999

Table 3: Accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 4: Accuracy (recovery) data for Gemigliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 5: Flow Rate (ml/min) data for Metformin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 6: Flow rate (ml/min) data for Gemigliptin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 7: Results of method precession for Metformin

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 8: Results of method precession for Gemigliptin

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 9: Results of Intermediate precision for Metformin

Injection	Area
Injection-1	1300148
Injection-2	1304520

Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 10: Results of Intermediate precision for Gemigliptin

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 11: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Metformin	52	152	2.9
Gemigliptin	52	156	3

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metformin	52	522	10.03
Gemigliptin	52	524	10.1

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of metformin and gemigliptin was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of metformin and gemigliptin were found to be from 100-500 µg/ml of metformin and 1-5µg/ml of gemigliptin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of metformin and gemigliptin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] Dnyaneshwar Thakare, Development and Validation of spectrophotometric method for the determination of DPP-4 Inhibitor, Metformin and Linagliptin, in its pharmaceutical preparations, World journal of pharmacy & pharmaceutical sciences volume 2,issue3,1332-1341.

[2] Kavitha. K.Y, Development and Validation of spectrophotometric method for the determination of DPP-4 Inhibitor Metformin and Linagliptin, in its pharmaceutical preparations. Journal of Chemical and Pharmaceutical Research, 2013, 5(1):230-235).

[3] Geetha, G **et al,** Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Metformin and Linagliptin in Bulk and Tablet Dosage Form. Journal of Chemical & Pharmaceutical Research;2013, Vol. 5 Issue 1, p230)

[4] Ghazala Khan, Dinesh SahuAgrawal Y P, NeetuSabarwal, Avnish Jain, Gupta A K. Simultaneous Estimation of Metformin and Sitagliptin In Tablet Dosage Form. Asian J. Biochem. Pharma. Res. 2011; 1(2): 352-358.

[5] Rutvik H Pandya. Spectroflourometric and Spectrophotometric Methods for the Determination of Linagliptin in Binary Mixture with Metformin and Ternary Mixture with Metformin and Linagliptin Alkaline Degradation Product. Pharmacophore. 2014, 5(2), 202-218)

[6] Ravi PratapPulla, Sastry B S, Rajendra Prasad Y, AppalaRaju N. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. Res. J. Pharm. Tech. 2011; 4(4): 646-649.

[7] Janardhan Swamy et al, Validated RP-HPLC for simultaneous estimation of Linagliptin and Metformin hydrochloride in tablet dosage form. Int J Pharm. 2013; 3(3): 594-600)

[8] Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptinin human plasma using protein precipitation and tandem mass spectrometry. J. Chromatogr. B. 2010; 878(21): 1817-1823.

[9] Wei Zeng, Donald Musson G, Alison Fisher L, Li Chen, Michael Schwartz S, Eric Woolf J, Amy Qiu Wang. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J. Pharm. Biomed.Anal.2008; 46(3): 534-542.

[10] Ramakrishna Nirogi, Vishwottam Kandikere, Koteshwara Mudigonda, Prashanth Komarneni, Raghupathi Al eti, Rajeshkumar Boggavarapu. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid–liquid extraction. Biomed. Chromatogr. 2008; 22(2): 214–222.

[11] Campbell DB, Lavielle R, Nathan C. The mode of action and clinical pharmacology of gliclazide: a review. Diab Res Clin Prac.1991; 14:S21-S36.

[12] Robert Moses, Fixed combination of repaglinide and metformin in the management of type 2 diabetes, Diabetes, Metabolic syndrome and obesity: Targets and Therapy Dove press, open access to scientific and medical research.2009; 2:101-9.

[13] Tripathi K.D. Essential of Medical Pharmacology, 5th Edn, Jaypee Brothers Medical publisher New Delhi. pp: 515-516.

[14] Hassa Saad S.M., Mahmoud Wagiha H., Elmosallamy Mohamed A.F, Othman Abdel Hammeed M.Determination of metformin in pharmaceutical preparations using potentiometry, spectrofluorimetry and UV–visible spectrophotometry. Anal. Chimica Acta .1999; 378(1-3): 299-311.

[15] Patil S.S., Bonde C. G .Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV visible spectroscopy, Int. J. Chem. Tec. Res. 2009;1(4):905-9

[16] Lakshmi KS, Rajesh T, Sharma S, Lakshmi S. Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Metformin, Pioglitazone and Glimepiride in Pharmaceutical Formulations. Der Pharma Chemica. 2009; 1 (1): 238-246.

[17] Freddy H. Havaldar, Dharmendra L.Vairal. Simultaneous estimation of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride in the tablets dosage form. Int. J.Appl .Bio. Pharm.Tec.2010;1(3):1000-1005.

[18] AbuRuz, S., Millership J,McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. J Chromatogr. B, 2005; 817(2): 277-286.

[19] Jain D, Jain S,Jain D ,Maulik A. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation Journal of Chromatogr. Sci.2008; 46:501-504.

[20] Lakshmi KS, Rajesh T,Sharma S. Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int.J Pharm. Pharm. Sci. 2009; 1(2): 162-166.