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Development of stability indicating RP- HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate monohydrate in bulk as well as in pharmaceutical formulation

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ABSTRACT

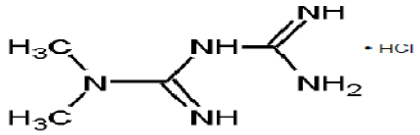
A new simple, accurate, precise, reproducible stability indicating Reverse Phase-High Performance Liquid Chromatography method was developed for the simultaneous estimation of Metformin and Sitagliptin in bulk as well as in pharmaceutical dosage form by using Symmetry C18 column (4.6 x 150mm, 3.5µm, Make: XTerra) in isocratic mode. The mobile phase was prepared by using Potassium Dihydrogen Phosphate and Acetonitrile in different ratio at different pH. Several trials were performed and it was found that the ratio 0f 65:35 of Potassium Dihydrogen Phosphate (with pH 5.8 which was adjusted by using Sodium Hydroxide) and Acetonitrile respectively shown a good peak. The detection was carried out at 254 nm. The method was linear over the concentration range for Metformin 100-300ppm and Sitagliptin 10-30ppm. The % recoveries of Metformin and Sitagliptin were found to be 98.8 to 100.7% and 99.1 to 100.6% respectively. The LOD for the drug Metformin was found to be 0.06µg/ml, LOQ for the Drug Metformin was found to be 0.2µg/mL & the LOD for the drug Sitagliptin was found to be 0.1µg/mL, LOQ for the drug Sitagliptin was found to be 0.4µg/mL. The drug content formulations were quantified by using the proposed analytical method. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms. The method was also applied for the determination of Metformin & Sitagliptin in the presence of their degradation products formed under variety of stress conditions. The method was applied for the determination of Metformin & Sitagliptin in the presence of their degradation products formed under the variety of stress conditions. The validation of method was carried out utilizing International Conference on Harmonization (ICH) guidelines. The described High Performance Liquid Chromatography method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Key words: Metformin, Sitagliptin, Simultaneous Estimation, Reverse Phase –High Performance Liquid Chromatography, Validation, ICH- Guideline, Degradation.

INTRODUCTION

As the number of individuals affected by diabetes is continuing to increase worldwide, the need for effective management assumes ever greater urgency. Newer classes of medications, particularly those which work via the incretin pathway, achieve glucose lowering and minimizing risks associated with more traditional therapies. Ideally, combination therapies should be well tolerated, convenient to take, have few contraindications, have a low risk of hypoglycemia and weight gain, and be reasonably effective over both the short and long term such as the

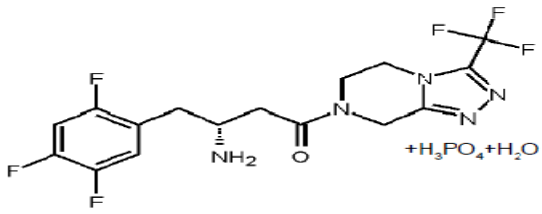
combination of Metformin (MF) and the dipeptidyl peptidase-4 (DPP-4) inhibitor Sitagliptin (SG). The chemical structure of the drugs was represented in Fig. no.1 & 2 respectively.



N,*N*-dimethylimidodicarbonimidic diamide

Metformin

Fig. no.1Structure of Metformin



(3*R*)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a] pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

Sitagliptin

Fig. no.2 Structure of Sitagliptin

Sitagliptin phosphate monohydrate (SPM) chemically, (3*R*)-3-amino-1-[3-(trifluoromethyl)-5, 6-Dihydro [1,2,4]triazolo[4,3-*a*] pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate hydrate(Fig. 2) is oral hypoglycemic drug of the dipeptidyl peptidase-4(DPP-4) inhibitor class. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide- 1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control [1-3]. Several analytical methods based on UV [4-6], Spectroflourimetry [6], RP-HPLC [7-8], LC-MS/MS [9-11] was reported for the determination of Sitagliptin phosphate monohydrate in plasma and urine of humans, rats and dogs. Metformin hydrochloride (MTF) ($C_4H_{11}N_5$.HCl) is 1: 1 dimethylbiguanidine monohydrochloride is an anti-diabetic drug from the biguanide class of oral Hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus[12] .Major action of Metformin HCl in increasing glucose transport across the cell membrane in skeletal muscle[13-14]. Several analytical methods based on UV [15-18], Spectroflourimetry [15], Reverse Phase-HPLC [19-27], HPTLC [28] and LC-MS/MS [29] was reported for the determination of Metformin. Although literature survey reveals that various methods were reported for Metformin (MTF) and Sitagliptin (SPM) both for single estimation and in combination with others drugs, but no method was reported for the analysis of these drugs in combination.

MATERIALS AND METHODS

Chemical and Reagent Used: The following chemicals were procured for the process Water [HPLC Grade], Metformin & Sitagliptin **[**Working Standards], Methanol [HPLC Grade] & Sodium Hydroxide all the chemicals were procured from STANDARD SOLUTIONS, HCL procured from FINAR CHEMICAL LIMITED, NaOH procured from S D FINE- CHEM LIMITED & H_2O_2 procured from ALPHA PHARMA LIMITED. Metformin & Sitagliptin Tablets 500mg&50mg were collected from the Local market and the manufacturer was MSD, Brand name Janumet[30].

Apparatus and Chromatographic Conditions:

Equipment Used	High Performance Liquid Chromatography Equipped with Auto Sampler & DAD or UV Detector
Column Used	Symmetry C18 (4.6 X 150nm, 3.5 µm, Make: XTerra) or Equivalent
Flow Rate Maintained	0.9 mL per min.
Wavelength Selected	254 nm
Injection Volume	20µL
Column Oven Maintained	Ambient
Run Time	7 min.
Detector	Photo diode array
Soft ware	Empower 2
MFD By	WATERS

Preparation of Phosphate buffer [31-32]: The Buffer Solution was prepared by weighing 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml of water [HPLC grade]. Then the pH was adjusted to 5.8with Sodium hydroxide.

Preparation of mobile phase: The Mobile Phase was prepared by mixing a mixture of above buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%) and degas in ultrasonic water bath for 5 minutes. Then it was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The same Mobile Phase was used as Diluent.

Preparation of the Metformin & Sitagliptin Standard & Sample Solution:
Standard Solution Preparation: The Standard Stock Solution of the drug was prepared by weighing accurately and transferred 10 mg Metformin and 10mg Sitagliptin working standard into a 10ml & 100ml clean dry volumetric flask respectively. About 7ml&70ml of Diluent were added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation: The Sample Stock Solution was prepared by weighing accurately and transferred 936.9 mg of Metformin and Sitagliptin Tablet powder into a 100ml clean dry volumetric flask. 70ml of the diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution 0.4ml solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

Standard & Sample Solution Injected inside the Column [33]: About 20µL of Standard and Sample Solutions were injected into the chromatographic system and the peak area were measured for the Metformin and Sitagliptin respectively. Then the %Assay was calculated by using the suitable formulae.

System Suitability [34]: The Tailing factor of the peaks due to Metformin & Sitagliptin in Standard solution should not be more than 1.5. The Theoretical plates for the Metformin& Sitagliptin peaks in Standard solution should not be less than 2000.

System Suitability Results (Metformin):
1) The Tailing factor obtained from the standard injection was **1.5**
2) The Theoretical Plates obtained from the standard injection was **4817.5**

Assay of Metformin:

$$\textbf{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT= Weight of Sample taken in mg.

DS = Dilution of Standard solution.
DT = Dilution of sample solution.
P = Percentage purity of working standard.
LC = Label Claim of Metformin mg/ml.

Assay % = $\frac{2015521}{2020755} \times \frac{10}{10} \times \frac{2}{10} \times \frac{100}{936.9} \times \frac{10}{0.4} \times \frac{99.9}{100} \times \frac{936.9}{500} \times 100 = 99.6\%$

System Suitability Results:
1). The Tailing factor obtained from the standard injection was **1.2**
2). The Theoretical Plates obtained from the standard injection was **4267.5**

Assay Calculation for Sitagliptin:

Assay % = $\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
WT= Weight of Sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard
LC = Label Claim of Sitagliptin mg/ml**.**

Assay % = $\frac{130152}{130835} \times \frac{10}{100} \times \frac{2}{10} \times \frac{100}{936.9} \times \frac{10}{0.4} \times \frac{99.8}{100} \times \frac{936.9}{50} \times 100 = 99.2\%$

VALIDATION METHOD [35-40]

1. Precision: The precision of an analytical procedure expresses the closeness of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The standard solution was injected for five times and measured the area for all five injections. The %RSD for the area of five replicate injections was found to be within the specified limits and the results were summarized in Table no 1 & 2.

Table no.1The Precision results of Metformin

Injection	Area
Injection-1	1988914
Injection-2	2025739
Injection-3	2019189
Injection-4	2018510
Injection-5	2033936
Average	2017258
Standard Deviation	17020.5
%RSD	0.84

Table no.2The Precision results of Sitagliptin

Injection	Area
Injection-1	128478
Injection-2	130962
Injection-3	130097
Injection-4	130484
Injection-5	130460
Average	130096
Standard Deviation	955.3
%RSD	0.73

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%.

2. Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits and the results were summarized in Table 3 & 4.

Table no.3The Ruggedness results of Metformin

Injection	Area
Injection-1	1960848
Injection-2	1940400
Injection-3	1942932
Injection-4	1947900
Injection-5	1952215
Average	1948859
Standard Deviation	8102.3
%RSD	0.42

Table no.4The Ruggedness results of Sitagliptin

Injection	Area
Injection-1	122532
Injection-2	126721
Injection-3	125998
Injection-4	126435
Injection-5	126663
Average	125670
Standard Deviation	1777.0
%RSD	1.41

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. Standard solutions with Accuracy -50%, 100% and 150% were injected into chromatographic column and calculated the Amount found and Amount added for Metformin & Sitagliptin. Same time the Individual recovery and Mean recovery values were also calculated (Table no. 5 & 6).

Table no.5 The Accuracy results of Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1009442	5.0	4.94	98.8%	99.9%
100%	2047373	10.0	10.0	100.2%	
150%	3085210	15.0	15.1	100.7%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table no. 6The accuracy results for Sitagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65699.3	5.0	4.95	99.1%	100.1%
100%	133312	10.0	10.0	100.5%	
150%	200131	15.0	15.0	100.6%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

3. Linearity: The linearity of the analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample. Different levels were prepared & injected into the chromatographic system and measured the peak areas. A graph was plotted between

peak area versus concentration and correlation coefficient value was calculated (Table no 7 & 8).

Table no.7The Linearity results of Metformin

Sl. No.	Linearity Level	Concentration	Area
1	I	100ppm	1322402
2	II	150ppm	1669399
3	III	200ppm	2032985
4	IV	250ppm	2365299
5	V	300ppm	2688465
Correlation Coefficient			0.999

Acceptance Criteria: The Correlation coefficient should be not less than 0.999

Table no.8 The Linearity results of Sitagliptin

Sl. No.	Linearity Level	Concentration	Area
1	I	10ppm	85152
2	II	15ppm	108768
3	III	20ppm	130477
4	IV	25ppm	152589
5	V	30ppm	177212
Correlation Coefficient			0.999

Acceptance Criteria: The Correlation coefficient should be not less than 0.999.

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non instrumental or instrumental.

a. Limit of Detection for Metformin:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV

Signal obtained from LOD solution (0.3% of target assay concentration) : 131 µV

S/N =131/44 = **2.97**

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

b. Limit of Detection for Sitagliptin:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOD solution (0.5% of target assay concentration) : 129 µV

S/N = 129/44 = **2.93**

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

Limit of Quantification: The Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/ or degradation products. Several approaches for determining the Quantification limit are possible, depending on whether the procedure is a non- instrumental or instrumental.

a. Limit of Quantification of Metformin:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOQ solution (1.0% of target assay concentration) : 437µV

S/N = 437/44 = **9.93**

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

b. Limit of Quantification of Sitagliptin:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOQ solution (2.0% of target assay concentration) : 435µV

S/N = 435/44=**9.88**

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) Variation at flow rate (0.8 ml/min to 1.0 ml/min): The Standard solution of Metformin (200ppm) and Sitagliptin (20ppm) was prepared and analysed using various flow rates along with actual flow rate. On evaluation of the above results, it was concluded that the variation in flow rate did not affect the method significantly. Hence it indicated that the method was robust even by change in the flow rate ±10% (Table no 9 & 10).

Table no. 9The results for System suitability for Metformin

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3421.6	1.4
2	0.9	4817.5	1.5
3	1.0	2398.9	1.4

Table no. 10The results for System suitability for Sitagliptin

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3023.0	1.2
2	0.9	4267.5	1.2
3	1.0	2264.6	1.3

b) Variation in organic composition of the Mobile phase from 25% to 15%. The Standard solution of Metformin (200 µg/ml) and Sitagliptin (20 µg/ml) was prepared and analysed using the various Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase did not affected the method significantly. Hence it indicated that the method was robust even by change in the Mobile phase ±1. (Table no 11 & 12).

Table no. 11The results for System suitability for Metformin.

Sl. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3815.9	1.4
2	Actual	4817.5	1.5
3	10% more	2891.5	1.4

Table no. 12The results for System suitability for Sitagliptin.

Sl. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3128.9	1.2
2	Actual	4267.5	1.2
3	10% more	2759.6	1.3

Forced degradation Studies* [41-42]*: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Metformin and Sitagliptin using the proposed method. Drug product and placebo were subjected to forced degradation at various stressed conditions like Hydrolytic degradation under acidic condition, Hydrolytic degradation under alkaline condition, Thermal induced degradation, Oxidative degradation & Photolytic degradation. All the samples were analyzed for purity peak of Metformin and Sitagliptin. In all the samples, Peak purity meets the acceptance limits. (Purity angle should be less than purity threshold. peak should not have any flag in purity results table (For Waters Empower-2 software). The results were summarized in Table 13 & 14.

a. Hydrolytic degradation under acidic condition: 2ml stock solution of Metformin and 0.2ml of Sitagliptin solution was prepared and taken in a 10 ml of volumetric flask; 3 ml of 0.1N HCl was added. Then the volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N NaOH and the volume was made upto the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

b. **Hydrolytic degradation under alkaline condition:** 2ml Metformin stock solution and 0.2ml of Sitagliptin solution was prepared and taken in a 10ml volumetric flask; 3 ml of 0.1N NaOH was added. Then the volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N HCL and the volume was made upto the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

c. Thermal induced degradation: 2ml Metformin stock solution and 0.2ml of Sitagliptin solution was prepared and taken in a 10ml of volumetric flask; 3 ml of the diluent was added. Then the volumetric flask was kept at reflex condition for 60 minutes and further the volume was made upto the mark with the diluent. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

d. **Oxidative degradation:** 2ml Metformin stock solution and 0.2ml of Sitagliptin solution was prepared and taken in a 10ml volumetric flask; 1 ml of 3 % w/v of hydrogen peroxide solution was added and the volume was made up to the mark with diluent . Further the volumetric flask was kept at room temperature for 15 min. The resultant solution was filtered with 0.45 microns syringe filters and placed in the vials.

e. Photolytic degradation: 2ml Metformin stock solution and 0.2ml of Sitagliptin solution was prepared and exposed to near ultra violet lamp in photostablity chamber providing illumination for 1hr, 5hr. Then 10 mg of sample was dissolved in water and the volume was made up to mark [10 ml]. From the above prepared solution dilutions were carried out to achieve the appropriate concentration (30µg/ml) and then the solution was taken in the vials.

Table no. 13Forced Degradation Data for Metformin

Sl. No.	Degradation Studies	Retention Time	Area	Height	USP Plate Count	USP Tailing Factor	Purity Angle	Purity Threshold
1	Hydrolytic degradation under acidic condition	2.581	1866941	313702	4265.8	1.4	0.19	0.22
2	Hydrolytic degradation under alkaline condition	2.586	1826791	306956	4159.8	1.5	0.23	0.29
3	Thermal induced degradation	2.588	1766567	296836	4203.5	1.5	0.31	0.35
4	Oxidative degradation	2.587	1726418	290090	4365.8	1.5	0.35	0.38
5	Photolytic degradation	2.584	1686269	283344	4285.6	1.5	0.33	0.35

Table no. 14Forced Degradation Data for Sitagliptin

Sl. No.	Degradation Studies	Retention Time	Area	Height	USP Plate Count	USP Tailing Factor	Purity Angle	Purity Threshold
1	Hydrolytic degradation under acidic condition	4.292	121203	11860	4217.8	1.3	0.16	0.19
2	Hydrolytic degradation under alkaline condition	4.291	118597	11605	4169.8	1.3	0.38	0.43
3	Thermal induced degradation	4.293	113384	11095	4186.9	1.3	0.22	0.29
4	Oxidative degradation	4.296	109474	10712	4316.9	1.4	0.45	0.49
5	Photolytic degradation	4.295	113384	10967	4215.8	1.3	0.34	0.41

RESULTS AND DISCUSSION

Present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of Metformin & Sitagliptin in Bulk as well as in pharmaceutical dosage forms. In order to method development under isocratic conditions, mixtures of Phosphate Buffer with the pH 5.8 and Acetonitrile [HPLC grade] in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 3.5µm, Make: XTerra) column. A binary mixture of Phosphate Buffer pH 5.8 and Acetonitrile in 65:35 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for Metformin & Sitagliptin were around 2.592 & 4.307 min respectively. A model chromatogram was shown in Fig. no.3.

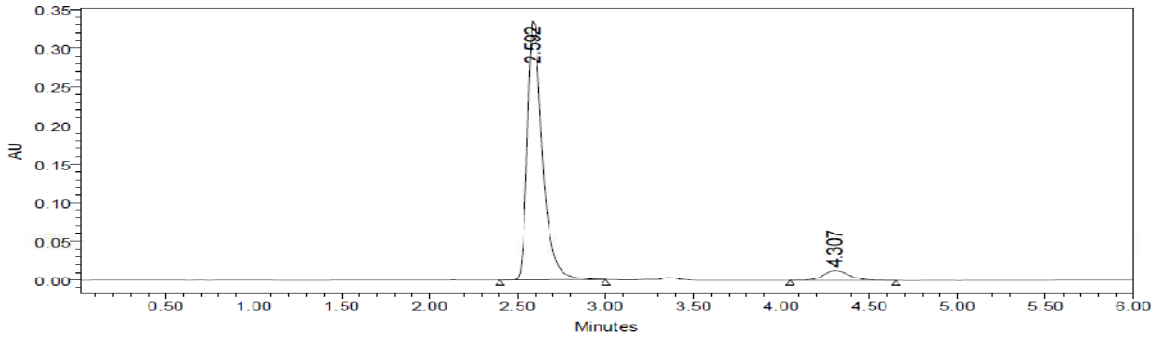


Fig. no.3A model Chromatograph showing the separation of the Drug

The Precision data was represented by Table no. 1 & 2. When Metformin & Sitagliptin were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation results, a low coefficient of variation was observed (Table no. 3 & 4). Above data showed the preciseness of present HPLC method and it was represented by Fig no. 4.

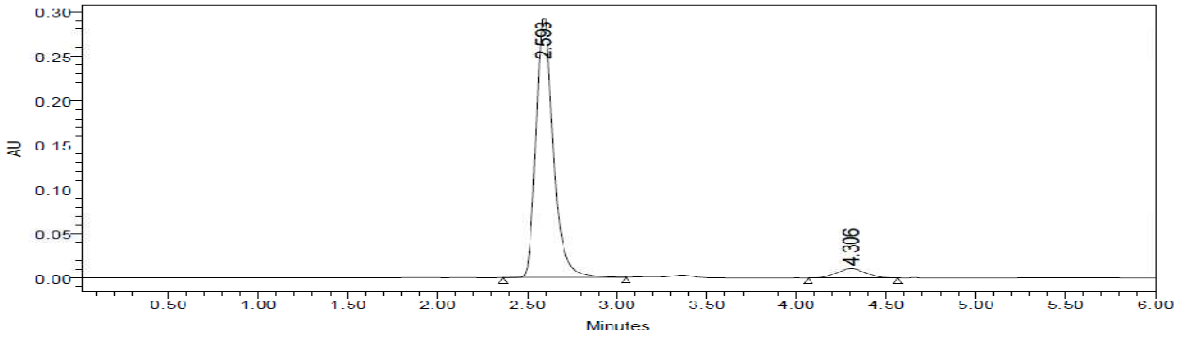


Fig.no.4The Ruggedness Chromatograph for the Drug Metformin & Sitagliptin

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found (Table no. 5 & 6). In order to test the linearity of the method, five dilutions of the working standard solutions of the drug in the range of 100ppm to 300ppm for the drug Metformin and 10ppm to 30ppm for the drug Sitagliptin were prepared respectively (Table no.

7 & 8). Each of the dilutions was injected into the column and the graph for the Linearity Curve was represented in Fig no. 5 & 6.

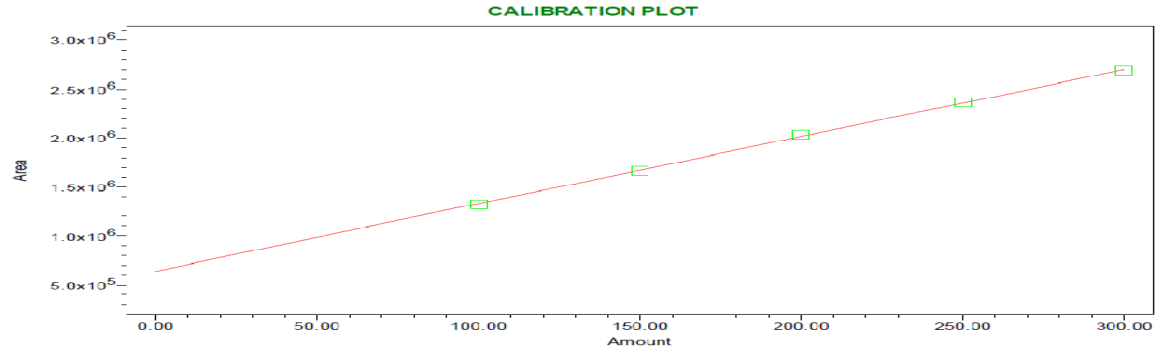


Fig. no.5The Linearity curve of Metformin

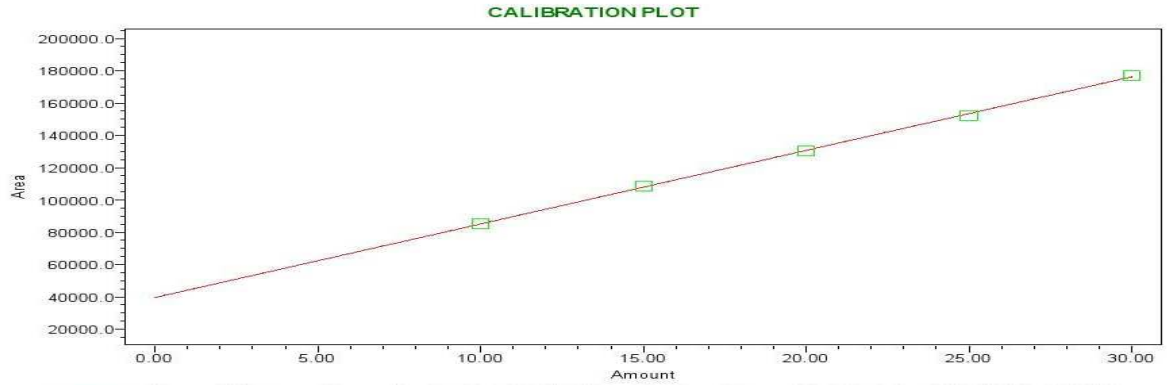


Fig.no.6 The Linearity curve for Sitagliptin

The method was duly validated by evaluation of the required parameters. Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The Fig.no.7, 8, 9 & 10 was represented the Robust nature of the chromatograph.

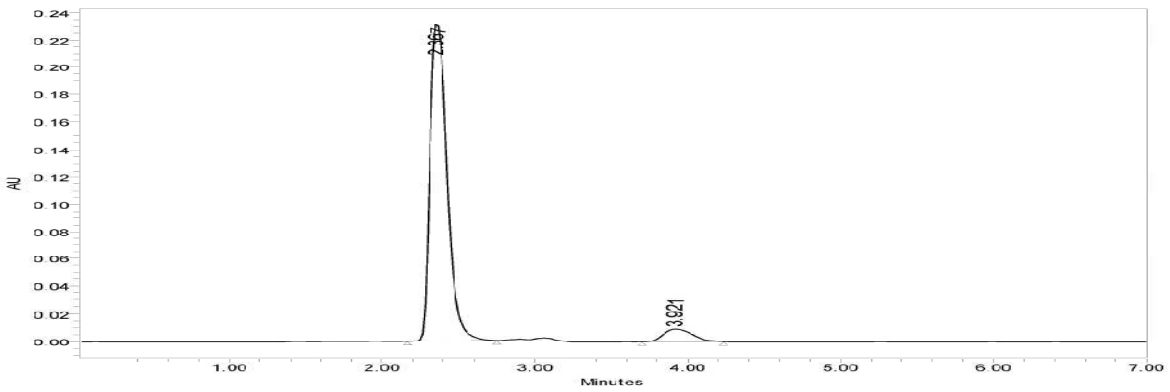


Fig. no 7 Robustness Chromatograph with increase composition of the Mobile Phase

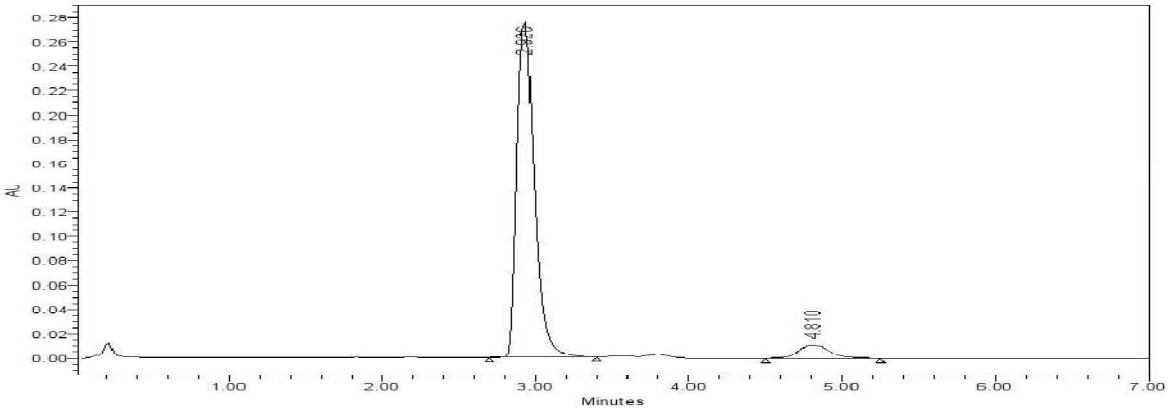


Fig. no 8 Robustness Chromatograph with decrease composition of the Mobile Phase

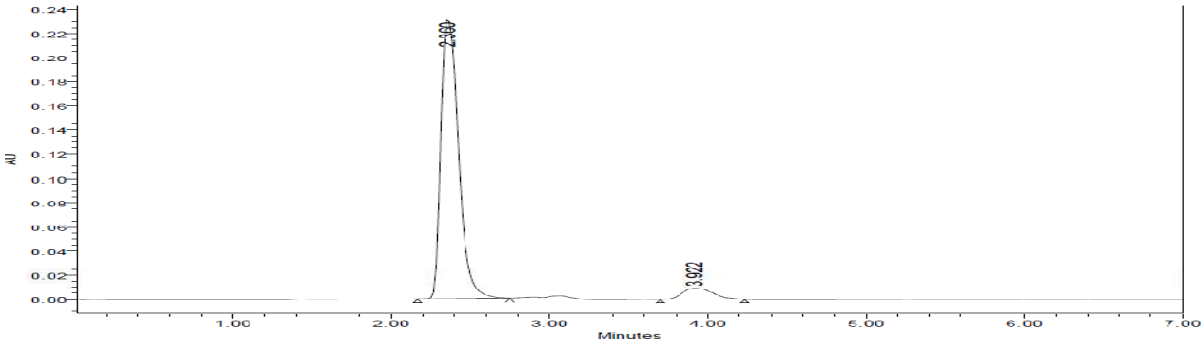


Fig. no. 9 Robustness Chromatograph with increase in the Flow Rate

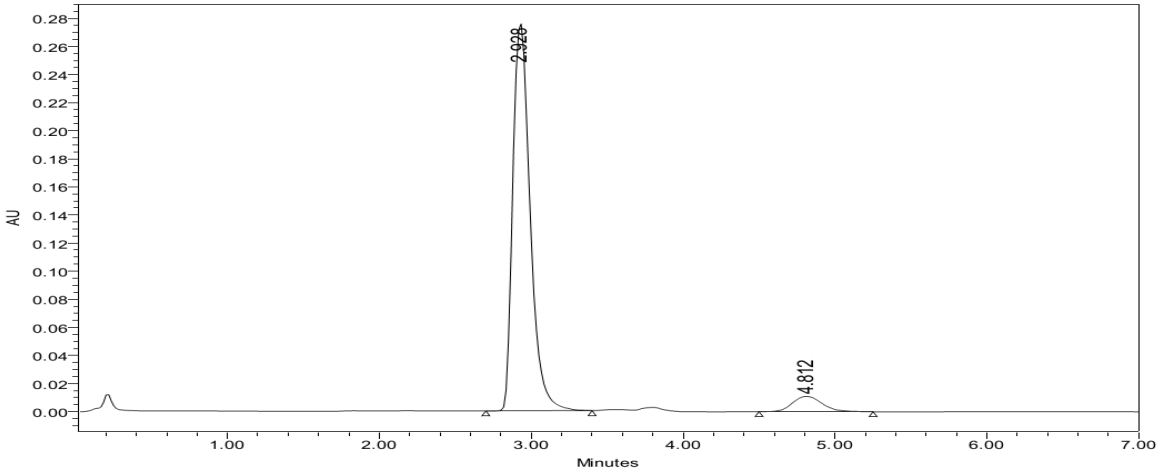


Fig. no. 10The Robustness Chromatograph with decrease in the Flow Rate

The system suitability parameters were within the limits as shown in Table 9, 10, 11 and 12. Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drug Metformin was found to be 0.06µg/ml, LOQ for the Drug Metformin was found to be 0.2µg/mL & the LOD for the drug Sitagliptin was found to be 0.1µg/mL, LOQ for the drug Sitagliptin was found to be 0.4µg/mL. The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. In order to evaluate the stability of Metformin & Sitagliptin and ability of the method to separate Metformin & Sitagliptin from its degradation products, Metformin & Sitagliptin was subjected to various stress conditions such as Hydrolytic degradation under

acidic condition (using 0.1N HCl & 0.1 N NaOH), Hydrolytic degradation under alkaline condition (using0.1N NaOH & 0.1N HCL), Thermal induced degradation (Reflex Condition for 60 mins), Oxidative degradation (by using 3 % w/v of hydrogen peroxide), Photolytic degradation (exposed to near ultra violet lamp in photostablity chamber providing illumination for 1hr, 5hr). The following chromatograph represents the degradation studies for the drug [Metformin & Sitagliptin] which were represented in fig. no. 11, 12, 13, 14 & 15.

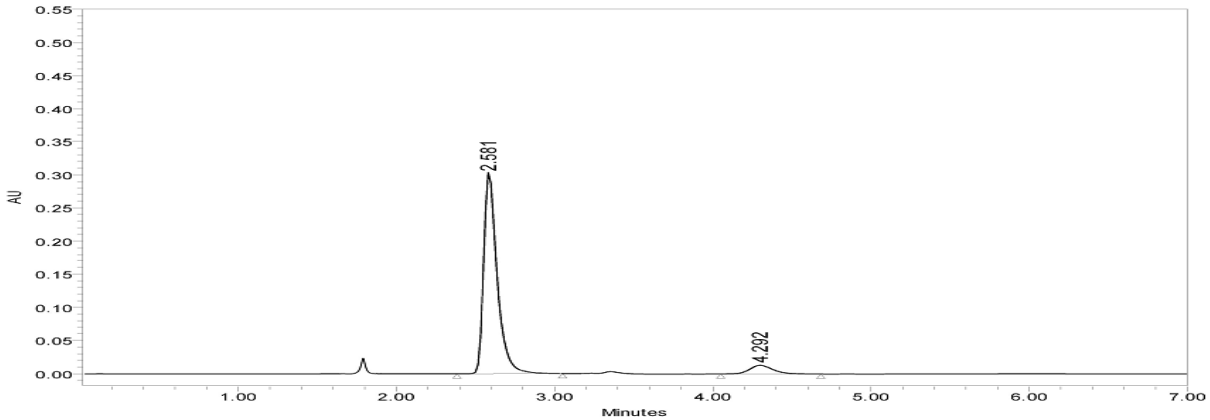


Fig. no. 11.The chromatograph represents the Hydrolytic degradation under acidic condition

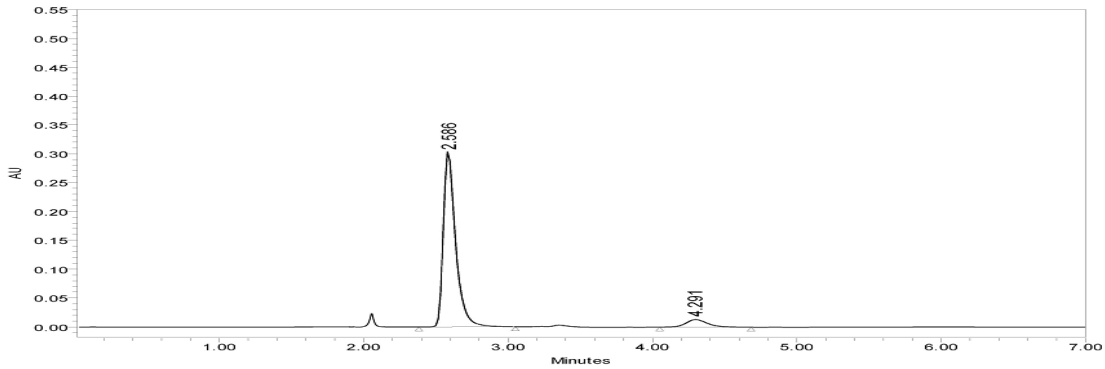


Fig. no. 12.The chromatograph represents the Hydrolytic degradation under alkaline condition

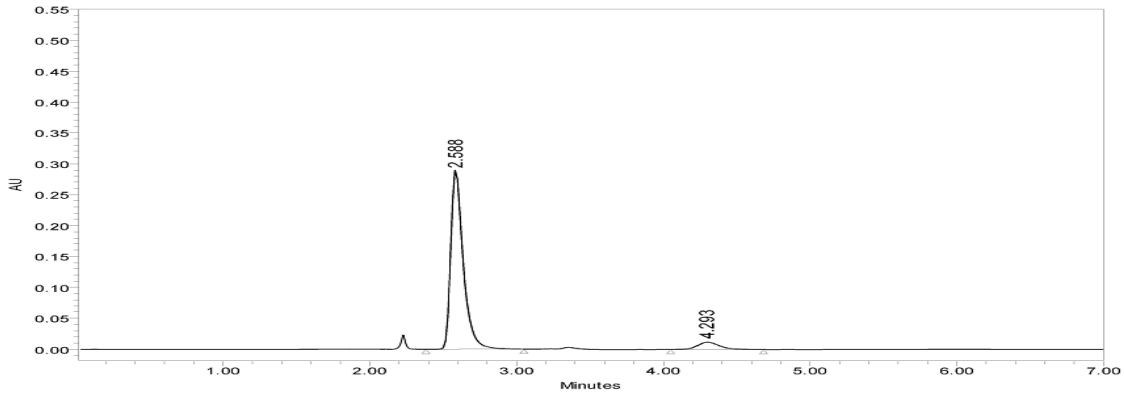


Fig. no. 13.The chromatograph represents the Thermal induced degradation

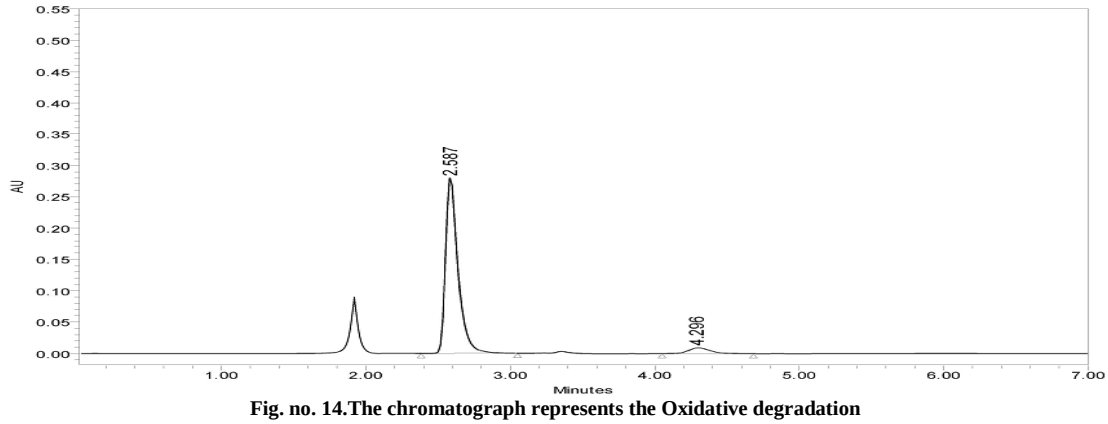


Fig. no. 14.The chromatograph represents the Oxidative degradation

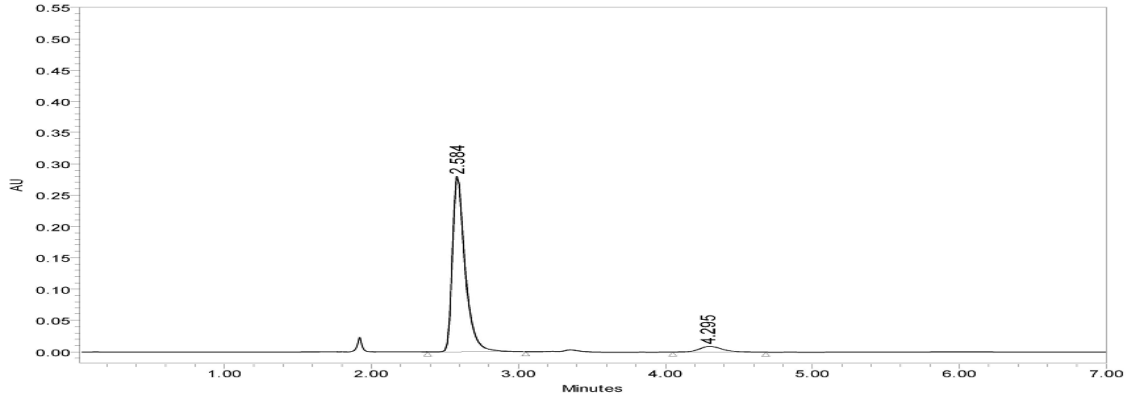


Fig. no. 15.The chromatograph represents the Photolytic degradation

The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. It was concluded that the proposed RP-HPLC method was sufficiently sensitive and reproducible for the analysis of Metformin & Sitagliptin in the Tablet formulation dosage forms within a short analysis time.

CONCLUSION

It was concluded that the proposed RP-HPLC method developed for the quantitative determination of Metformin & Sitagliptin in bulk and in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Metformin & Sitagliptin from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of Metformin & Sitagliptin in bulk and pharmaceutical dosage forms. Hence this method can easily be adopted as an alternative method to reported ones for the routine determination of Metformin & Sitagliptin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method will also find use in clinical, biological and pharmacokinetic studies of Metformin & Sitagliptin at future.

REFERENCES

[1] Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W et al. *J Clin Pharmacol;* **2006**; 46 (8): 876–886.
[2] Dubal A, Khatwal R, Kosaraju J, Meda V, Samanta M. *Int J Pharm Pharm Sci*; **2012**; 4, (2): 691-694.
[3] Balasekaran C, Rani P A. *Int J Pharm Pharm Sci;* **2010**; 2(4): 138-142.

[4] Parag P, Imran M, Bairagi V, Ahire Y. *J Pharm Res*; **2011**; 4(3): 871-873.
[5] Khan, Agrawal Y P, Sabarwal N, Jain A, Gupta A K. *Asian J Biochem Pharma Res*; **2011**; 1(2): 352-358.
[6] Ramzia El -Bagary, Ehab E F, Bassam A M. *Int J Biomed Sci*; **2011**; 7(1): 62-69.
[7] Ravi P P, Sastry B S, Rajendra P Y, Appala R N. *Res J Pharm Tech*; **2011**; 4(4): 646-649.
[8] Shyamala M, Mohideen S, Satyanarayana T, Narasimha R, Suresh K, Swetha K. *American J Pharm Tech Res;* **2011**; 1(2): 93-101.
[9] Zeng W, Xu Y, Constanzer M, Woolf E J. *J Chromatogr B;* **2010**; 878(21):1817-1823.
[10] Zeng W, Musson D G, Fisher A L, Chen L, Schwartz M S, Woolf E J et al. *J Pharm Biomed Anal;* **2008**; 46(3): 534-542.
[11] Nirogi R, Kandikere V, Mudigonda K, Komarneni P, Aleti R, Boggavarapu R. *Biomed Chromatogr*; **2008**; 22(2): 214–222.
[12] Campbell DB, Lavielle R, Nathan C. *Diab Res Clin Prac;* **1991**; 14:S21-S36.
[13] Moses R., *Metab Syndr*; **2009**; 2:101-9.
[14] Tripathi K D. Essential of Medical Pharmacology, 5th Edn, Jaypee Brothers Medical, New Delhi. **2006**, pp: 515-516.
[15] Hassasaad S.M., Mahmoud W H., Elmosallamy M A, Othman A H. *Anal Chimic.* **1999**; 378(1-3): 299-311.
[16] Patil S.S., Bonde C. G. *Int J Chem Tech Res;* **2009**; 1(4): 905-909.
[17] Lakshmi KS, Rajesh T, Sharma S, Lakshmi S. *Der Pharma Chemica*; **2009**; 1 (1): 238-246.
[18] Freddy H Havaldar, Dharmendra L Vairal. *Int J App Bio Pharm Tec*; **2010**; 1(3):1000-1005.
[19] Abu Ruz S, Millership J, Mc Elnay J. *J Chromatogr B*; **2005**; 817(2): 277-286.
[20] Jain D, Jain S, Jain D, Maulik A. *J Chromatogr Sci;* **2008**; 46:501-504.
[21] Havele S, Dhaneshwar S. *Webmed central pharmaceutical sciences;* **2010**; 1(10):wmc001078
[22] Al Rimawi F. *Talanta*; **2009**; 79(5):1368-71.
[23] Havele S, Dhaneshwar S. *J Nanomedic Nanotechnolo*; **2010**; 1: 102.doi:10.4172/2157-7439.1000102
[24] Georgita C, Albu F, David V, Medvedovici A. *J Chromatogra B;* **2007**; 854(1-2):211-218.
[25] ICH Harmonized Tripartite Guideline. Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva; **1994**; 1-5.
[26] Lakshmi K S, Rajesh T, Sharma S. *Int J Pharm Pharm Sci;* **2009**; 1(2): 162-166.
[27] Alexandar S, Diwedi R, Chandrasekar M. *Res J Pharm Bio Chem Sci;* **2010**; 1(4): 858-866.
[28] Florentin T, Monica A. *Revue Roumainede Chimie* **2007**; 52(6):603–609.
[29] Pawar S, Meshram G, Jadhav R, Bansal Y. *Der Pharma Chemica;* **2010**; 2(4): 157-168.
[30] Indian Pharmacopeia **2007**, Volume I, The Indian Pharmacopoeia Commission, 477-478.
[31] S. Ashutosh Kumar, Manidipa Debnath, J.V.L.N.Seshagiri Rao. *Am. J. Pharm Tech Res.* **2013**; 3(3); 556-575.
[32]Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists 1st ed, Wiley-VCH; **2007**; 369-382.
[33]Stavros Kromidas. HPLC Made to Measure, A Practical Book for Optimization. Published by Wiley-VCH; **2006**; 62-66.
[34] Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists.1st ed, Wiley-VCH; **2007**; 389-391.
[35]Sharma Ajay, Sharma Rohit: Validation of analytical procedures: a comparison of ICH VS PHARMACOPOIEA (USP) and FDA. *Inter National Research Journal of Pharmacy* **2012**; 3(6) 39-42.
[36] Validation of analytical procedure: Methodology Q2B, ICH Harmonized Tripartite Guidelines, **1996**; 1-8.
[37] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonized tripartite guideline Validation of analytical procedures: Text and Methodology Q2 (R1) 6 November **1996**.
[38] Ravichandran V, Shalini S, Sundram K. M and Harish Rajak: *International Journal of Pharmacy and Pharmaceutical Sciences* **2010**; vol 2, suppl 3.
[39]Tangri Pranshu, Rawat Prakash Singh, Jakhmola Vikash: *Journal of Drug Delivery & Therapeutics* **2012**; 2(3): 34-40.
[40]ICH, Validation of Analytical Procedure, Text and Methodology Q2 (R1), International conference on Harmonization, IFPMA, Geneve, Switzerland, **2005**.
[41] ICH harmonized tripartite guideline. Impurities in New Drug products Q3B (R2) current step 4 versions dated 2 June **2006**.
[42]International Conference on Harmonization, ICH Q1 A(R2); Stability Testing of New Drug Substances and Products **2003**.
[43] Ram VR, Kher GJ, Dubal KL, Pandya GP, Joshi HS. *Der Chemica Sinica*, **2011**, 2 (2), 58-65.

[44] Nagrajan G, Kavimani S. *Der Pharmacia Sinica*, **2010**, 1 (3), 109-116.
[45] Syeda Humaira, Akalanka Dey, S. Appala Raju, Syed Sanaullah. *Der Pharmacia Lettre*, **2010**, 2(4): 315-325.
[46] Leenata P. Mandpe and Varsha B. Pokharkar, *Der Chemica Sinica*, **2011**, 2 (2): 230-239.
[47] Joshi Pryanka and Kumar Mukesh, *Der Pharmacia Sinica*, **2011**, 2 (3): 211-219.