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Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in API and Tablet Dosage by HPLC Method

Pragati Sinha[1]*, Dharam Pal Pathak[2] and Jai Prakash[3]

[1]*Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences & Research, New Delhi, India*

[2]*Director of Delhi Institute of Pharmaceutical Sciences & Research, New Delhi*

[3]*Principal of Pharmacovigilance, Indian Pharmacopoeial Commision, Ghaziabad*

***Corresponding author:** Pragati Sinha, Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences & Research, New Delhi, India, Tel: +91 8709324537; E-mail: p5pragatisinha@gmail.com

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Abstract

A simple, accurate, precise and rapid reversed phase high performance liquid chromatography (RP-HPLC) method had been developed and subsequently validated for the simultaneous estimation of metformin HCl and sitagliptin phosphate in bulk & tablet dosage form. The proposed method was based on the separation of the two drugs in reversed phase mode using C18 (4.6 × 250 mm, [5 µ particle size]) analytical column. The optimized mobile phase consisted of phosphate buffer (pH adjusted to 3.11 using o-phosphoric acid): Acetonitrile in the ratio of 850:150. Flow rate was kept at 1 mL/min. The simultaneous estimation was carried out at detection wavelength of 206 nm using variable wavelength detector. Both drugs metformin HCl and sitagliptin phosphate were resolved and retained at 10 minutes. This method was statistically validated as per ICH guideline for analytical method validation.

Keywords: Janumet; Metformin HCl; RP-HPLC; Sitagliptin phosphate; Validation

Introduction

Chromatography is the laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid, known as the mobile phase, which carries it through a structure holding another material, known as the stationary phase [1]. The different components of the mixture travel at different speeds, causing them to separate. The separation is depending on dissimilar partitioning between the mobile and stationary phases [2].

High-performance liquid chromatography (HPLC; previously referred to as high-pressure liquid chromatography), is an operating procedure in analytical chemistry used to separate, identify, and quantify each component in a mixture [3]. HPLC basically depend on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components [4]. The stately of a HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler guides the sample mixture into the mobile phase stream which carries it into the column [5]. The pumps convey the desired flow and composition of the mobile phase through the column [6]. The detector creates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components [7].

The Agilent 1200 Series HPLC System was instigate in 2010, with a modular design allowing users to define a configuration preferably suited to meet their HPLC and UHPLC applications and requirements [8]. The 1200 series is a pace forward from the innovative 1100 series, building on the modular design and configuration capabilities of the original [9]. The outcome is a superior combination of speed, resolution, and sensitivity that has fostered widespread implementation in many labs [10].

Metformin HCl is glucose lowering agent that is global used for controlling for type2 diabetes [11]. Sitagliptin phosphate is an oral anti hypoglycaemic drug which is highly selective dipeptidyl peptidase-4 β- cells and suppress glucagon secretion by the α- cells [12]. These drugs are generally co-administered to diabetic patients. They are decided in combined tablet dosage form [13]. Hence, the initiate experimental work was aimed to develop and validate RP-HPLC Method for simultane ous estimation of Metformin and Sitagliptin (Figures 1-2).

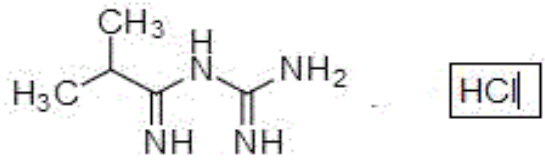

Figure 1: Metformin HCl.

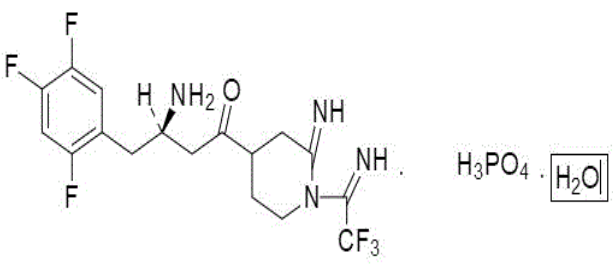

Figure 2: Sitagliptin Phosphate.

Materials and Methods

HPLC Method development for the quantitative estimation of Metformin hydrochloride and Sitagliptin phosphate in marketed tablets.

1. Reverse phase HPLC was selected for the quantitative estimation of Metformin HCl and Sitagliptin phosphate. RP-HPLC has many advantages because of which it was preferred over the other HPLC technique.
   - It is robust
   - It is reproducible
   - It is efficient
2. This size of the packing particle was selected as 5 microns because this size is a good compromise between the column efficiency and back pressure.
3. Column temperature selected – The column temperature was set at 20°C to get high plates numbers.
4. Injection volume – 20 µL was selected as the sample injection volume, because sample size 25 µL to avoid excessive band broadening.
5. Selection of wavelength- PDA scan of the standard drug dilution showed that the drug absorbs in three regions of UV: 223 nm, 206 nm and 253 nm (However, it was selected at 206 nm). It was found that at low concentration the signal to noise ratio at 253 and 223 nm was not clear. Hence, 206 nm was selected as the working wavelength so that the method can be used in the lower concentration range.
6. Optimization of mobile phase-

1st trial

- Isocratic flow
- Mobile phase used- a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (pH 2.5)
- Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 20 minutes.
- Flow rate – 0.8 mL/min

2nd trial

- Isocratic flow
- Mobile phase used – a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (Ph 2.5)
- Flow rate – 1 mL/min
- Problem in the chromatogram - metformin peak was observed but sitagliptin peak was not observed in the run time of 10 minutes.

3rd trial

- Isocratic flow
- Mobile phase used – a mixture of 200 mL of acetonitrile and 800 mL of phosphate buffer (pH 2)
- Flow rate – 1 mL/min
- Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 10 minutes

4th trial

- Isocratic flow
- Mobile phase used – a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (pH 2.2)
- Flow rate – 1 mL/min
- Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 10 minutes

5th trial

- Isocratic flow
- Mobile phase used – a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (pH2.5)
- Flow rate – 1 mL/min
- Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 20 minutes

6th trial

- Isocratic flow
- Mobile phase used – a mixture of 200 volume of acetonitrile and 800 volume of phosphate buffer (pH 2.5)
- Flow rate – 1 mL/min
- Problem phase used – metformin peak was observed but sitagliptin peak was not observed in the run time of 20 minutes.

The method was optimized after all the trials.

HPLC instrument settings:

- Isocratic flow mode
- Flow rate=1 mL/min
- Temperature=20 °C
- Injection volume=20 µL
- Run time=10 mins
- Detection wavelength=206 nm

Column characteristics:

- Column=INERTSIL (ODS)
- Column Length=250 mm
- Internal diameter=4.6 mm
- Particle size=5 µm

Materials and reagents

Fixed dose combination tablets (Brand: Janumet) containing 500 mg of metformin and 50 mg sitagliptin were procured from MSD company, India.

Acetonitrile, Water and Active Pharmaceutical Ingredients (Metformin HCl and Sitagliptin Phosphate) from IPC, Ghaziabad.

Instrument

The HPLC system was Agilent 1200 series equipped with variable wavelength detector. The chromatogram was recorded using EZChrome software.

Experimental Analytical Method Development

Preparation of standard stock

The standard stock solution of the drug was prepared by weighing accurately and transferred to 100 mg Metformin HCl and 10 mg Sitagliptin Phosphate working standard into 100 mL & 10 mL clean dry volumetric flask respectively. Volume was made up to 100 mL & 10 mL with diluents to prepare 1000 ppm solution respectively. Aliquots were drawn from working solution and diluted suitably to prepare the solution to be injected into the HPLC.

Preparation of sample solution for simultaneous estimation from marketed tablet formulation

Tablet sample equivalent to 7.2 mg was weighed and transferred to 10 mL volumetric flask. Volume was made up to 10 mL with diluent to prepare 500 ppm solution. Aliquots were drawn from the working solution and diluted suitably to prepare the sample to be injected into the HPLC.

Preparation of mobile phase

850: 150 v/v ratios of Buffer and acetonitrile respectively. Buffer was 1.36 gm of KH_2PO_4 and final pH was adjusted to 3.11 using orthophosphoric acid. This buffer was prepared by dissolving 1.36 gm of KH_2PO_4 in enough volume of Milli Q water & the volume was made to 1 liter. Final pH was adjusted to 3.11 using orthophosphoric acid. Final buffer was filtered through 0.45-micron membrane filter.

Selection of detection wavelength

PDA scan of the standard drug dilution showed that the drug absorbs in three regions of UV: 223 nm, 206 nm and 253 nm (However, it was selected at 206 nm). It was found that at low concentration the signal to noise ratio at 253 and 223 nm was not clear. Hence, 206 nm was selected as the working wavelength so that the method can be used in the lower concentration range.

Optimization of chromatographic conditions

Many preliminary trails were carried out for selection and optimizations of stationary phase, mobile phase, flow rate, injection volume and column temperature.

Analytical method validation

1. Linearity

Preparation of calibration curve for HPLC method. Reference standard was weighed and transferred to volumetric flask. Aliquots were drawn from the working solution and diluted suitably to prepare different dilutions. Each dilution was prepared in duplicate. Dilution was injected into the HPLC system and peak area was noted and calibration curve for HPLC method was plotted.

- Linearity was determined from the HPLC calibration curve of metformin hydrochloride and sitagliptin phosphate.
- Regression coefficient, correlation coefficient, slopes and intercept were reported.

2. Range

Range was selected based upon the linearity. Following the selection of range the test concentration was selected. Range should at least be 80 to 100% of the test concentration. Later, accuracy and precision studies were carried out within the range itself as the method must be accurate, precise and linear over the selected range.

3. Precision

Concentrations selected for precision studies were (200, 400, 600 600, 1000) ppm.

- Interday Precision: It was determined by analyzing a sample 6 times a day (n=6)
- Intraday precision: It was assessed by analyzing a sample, 3 different concentration (n=6)

4. Accuracy

The accuracy of the method was evaluated in two replicates by analysis at different three concentration levels i.e., (200 ppm, 400 ppm, 600 ppm) of the drug concentration.

5. Robustness

Robustness of the current method was studied by analyzing a sample of metformin hydrochloride and sitagliptin phosphate. Mobile Phase of the method was determined by bringing small changes in the method and the comparing the results with the result of standard method. The small changes applied to the method were:

- pH changes (± 0.5)
- Flow rate (± 0.2 mL/min)

6. Specificity

A solution containing tablet excipients was prepared using sample preparation procedure and injected into the HPLC, to evaluate if excipient interferes in the method. Also, the peak purities of metformin hydrochloride and sitagliptin phosphate peaks, obtained with the tablet sample solution were evaluated. Peak purity was determined using three brands (Janumet, Istamet, and Zitamet).

7. System suitability

Once, the method was developed and validated requirements for system suitability were developed.

Results and Discussion

Analytical method development

Selection of wavelength: UV absorption spectra 100 ppm solution of each Metformin, Sitagliptin individually and their mixture were taken, and 200-400 nm was selected as a detection wavelength for simultaneous chromatographic determination of Metformin and Sitagliptin [8-10].

Optimization of chromatographic conditions assay: The solution of the drug was prepared by weighing accurately and transferred to 100 mg Metformin HCl and 10 mg Sitagliptin Phosphate working standard into 100 mL & 10 mL clean dry volumetric flask respectively. Volume was made up to 100 mL & 10 mL with diluents to prepare 1000 ppm solution respectively. Purity by HPLC is firm by measuring the area of the peak that corresponds to the composite of interest [11-14].

Assay of metformin HCl:

Assay %=AT/AS × WS/DS × DT/WT × Avg Wt/ Label claim × Potency

Where AT=Avg area counts of sample preparation
AS=Avg area counts of standard preparation
WS=Weight of working standard
WT=Weight of sample
DS=Dilution of standard solution
DT=Dilution of sample solution

Assay %=33880482/49837114 × 80.6/100 × 10/7.2 × 700.2/500 × 100
=104.97 % (Limit=99-110%)

Assay of sitagliptin phosphate:

Assay % - AT/AS × WS/DS × DT/WT × Avg Wt/ Label claim × Potency

Where AT=Avg area counts of sample preparation
AS=Avg area counts of standard preparation
WS=Weight of working standard
WT=Weight of sample
DS=Dilution of standard solution
DT=Dilution of sample solution

Assay %=33880482/ 49837114 × 8.3/100 × 10/7.2 × 700.2/50 × 100
=102.01% [Limit=99-110%]

Chromatograms

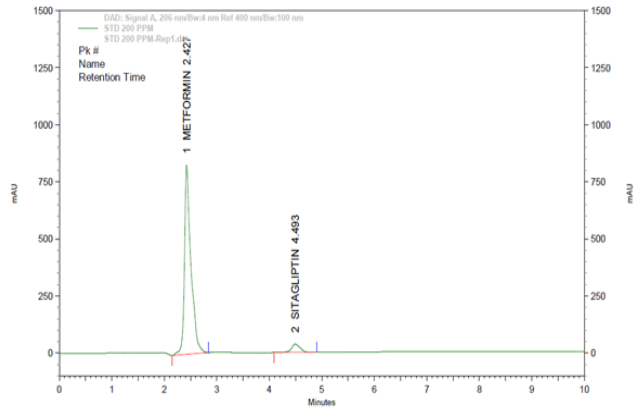

Figure 3: (200ppm of JANUMET sample) (Chromatogram of concentration of 200 ppm after System Suitability procedure).

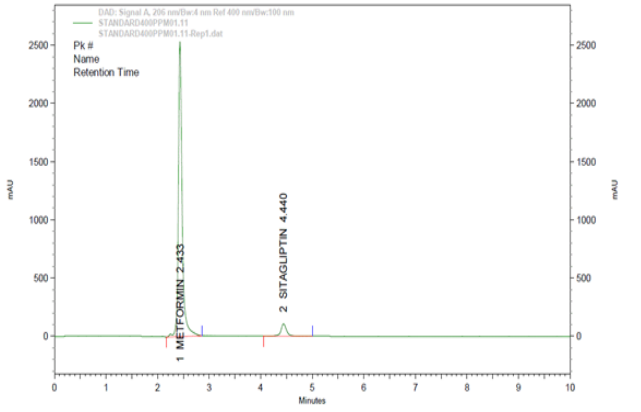

Figure 4: (400ppm of JANUMET sample) (Chromatogram of concentration of 400ppm after System Suitability procedure).

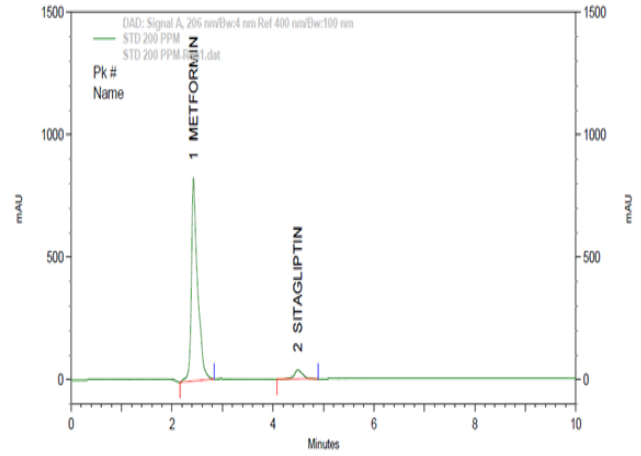

Figure 5: (600ppm of JANUMET sample) (Chromatogram of concentration of 600ppm after System Suitability procedure).

1. System suitability

System suitability parameters were measured so as to validate the system performance. System precision was determined on six replicate injections of standard preparations. All-important characteristics including area were measured as shown in Tables 1-6 and Figures 3-5.

Metformin HCl

A) 200 PPM

S No	Injection	Area
1	Injection 1	14268698
2	Injection 2	14299239
3	Injection 3	14291383
4	Injection 4	14266521
5	Injection 5	14254839
6	Injection 6	14254861
Average		14272590
SD		18680.16
RSD		0.001309

Table 1: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

B) 400 PPM

S No	Injection	Area
1	Injection 1	14268698
2	Injection 2	14299239
3	Injection 3	14291383
4	Injection 4	14266521
5	Injection 5	14254839
6	Injection 6	14254861
Average		26860812
SD		167562.9
RSD		0.006238

Table 2: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

C) 600 PPM

S No	Injection	Area
1	Injection 1	14268698
2	Injection 2	14299239
3	Injection 3	14291383
4	Injection 4	14266521
5	Injection 5	14254839
6	Injection 6	14254861
Average		47242208
SD		124279.7
RSD		0.002631

Table 3: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

A) 420 PPM

S No	Injection	Area
1	Injection 1	27057321
2	Injection 2	26969532
3	Injection 3	26876931
4	Injection 4	26937231
5	Injection 5	26710626
6	Injection 6	26613233
Average		1709364
SD		7447.562
RSD		0.004357

Table 4: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

B) 40 PPM

S No	Injection	Area
1	Injection 1	27057321
2	Injection 2	26969532
3	Injection 3	26876931
4	Injection 4	26937231
5	Injection 5	26710626
6	Injection 6	26613233
Average		1709364
SD		7447.562
RSD		0.004357

Table 5: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

C) 60 PPM

S No	Injection	Area
1	Injection 1	27057321
2	Injection 2	26969532
3	Injection 3	26876931
4	Injection 4	26937231
5	Injection 5	26710626
6	Injection 6	26613233
Average		2612949
SD		21314.05
RSD		0.008157

Table 6: Results obtained for samples (JANUMET) analysis by the proposed HPLC method.

2. Linearity

The linearity of the method was determined at five different concentration level ranging from 200 ppm to 1000 ppm for metformin HCl and sitagliptin. The calibration curve was constructed by peak plotting area versus concentration of metformin and sitagliptin respectively, and the standard plot and regression equation was determined. Linearity chromatograms at different concentrations % level is shown in Figures 6-9 and Tables 7-8.

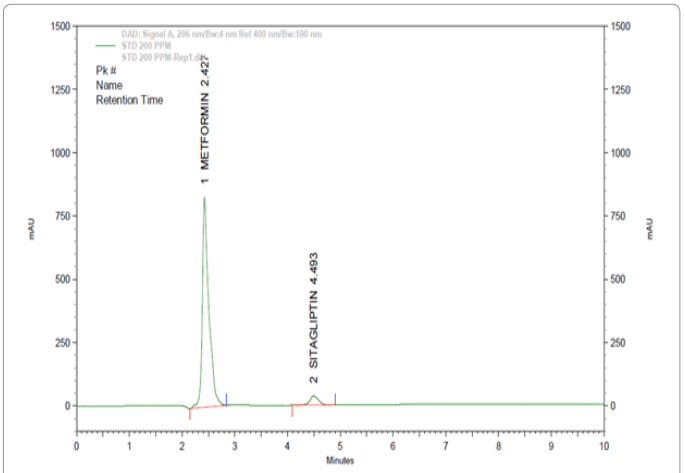

Figure 6: (800ppm) Chromatogram of concentration of 800ppm after linearity procedure.

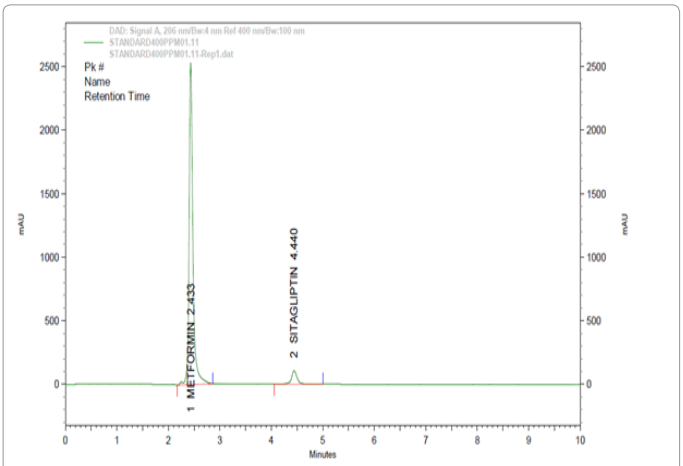

Figure 7: (1000ppm) Chromatogram of concentration of 1000ppm after linearity procedure.

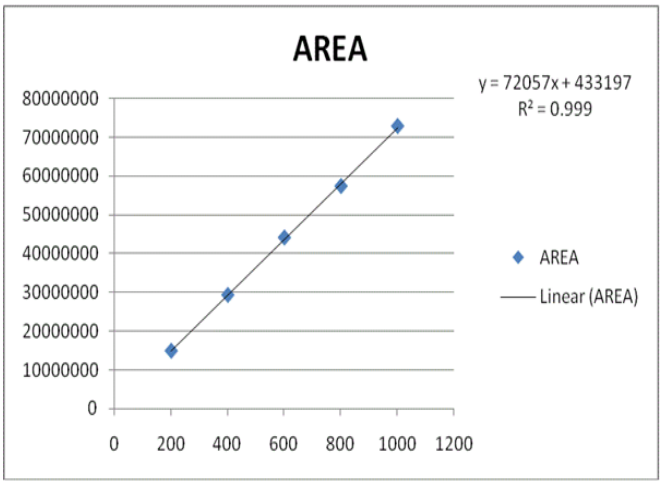

Figure 8: Linearity data of Metformin HCl of 5 different concentrations.

Metformin HCl

Conc ppm	Area (REP 1)	Area (REP 2)	AVG.
200	14807495	14853005	14830250
400	29213600	29188038	29245964
600	43378124	44765279	44071701
800	57323028	57389802	57356415
1000	72995531	72667888	72831709

Table 7: Analysis characteristics of samples (METFORMIN HCl) by the proposed HPLC method.

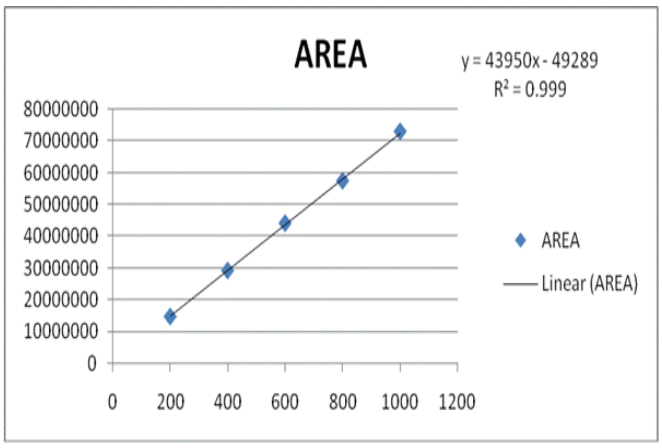

Figure 9: Linearity data of Sitagliptin Phosphate of 5 different concentrations.

Sitagliptin Phosphate

Conc ppm	Area (REP 1)	Area (REP 2)	AVG.
200	854167	865291	859729
400	1663338	1657120	1657120
600	2615279	2619334	2617307
800	3405079	3479118	3442099
1000	4403221	4321198	4362210

Table 8: Analysis characteristics of samples (SITAGLIPTIN PHOSPHATE) by the proposed HPLC method.

3. Precision

Precision of the method was estimated with respect to both repeatability (intra-day) and intermediate precision (inter-day) in Figures 10-13 and Tables 9-12.

Interday Precision - (METFORMIN)

Conc ppm	Rt (Rep 1)	Rt (Rep 2)	AVG.
200	2.43	2.43	2.43
400	2.41	2.41	2.41
600	2.39	2.39	2.39
STD DEV.			0.02
AVG.			2.41
% RSD			0.008
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 9: Precision data of samples by the proposed HPLC method.

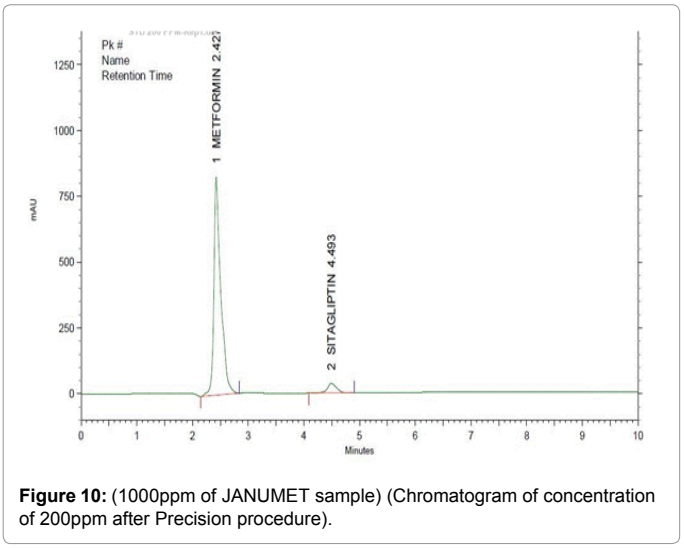

Figure 10: (1000ppm of JANUMET sample) (Chromatogram of concentration of 200ppm after Precision procedure).

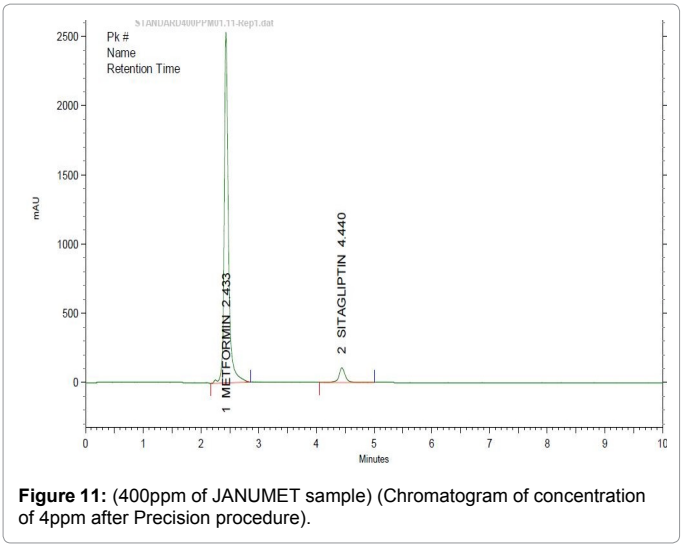

Figure 11: (400ppm of JANUMET sample) (Chromatogram of concentration of 4ppm after Precision procedure).

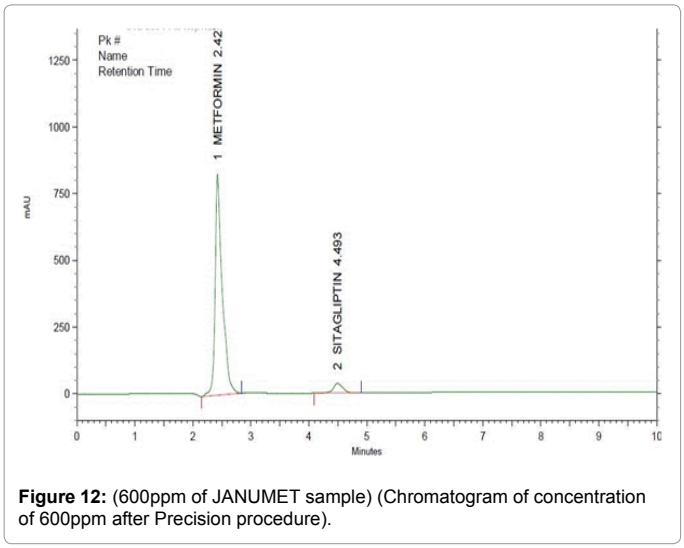

Figure 12: (600ppm of JANUMET sample) (Chromatogram of concentration of 600ppm after Precision procedure).

(Interday Precision -(SITAGLIPTIN)

Conc ppm	Rt (Rep 1)	Rt (Rep 2)	AVG.
20	4.49	4.53	4.51
40	4.53	4.56	4.54
60	4.57	4.59	4.58
STD DEV.			0.035
AVG.			4.545
% RSD			0.0077
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 10: Precision data of samples by the proposed HPLC method.

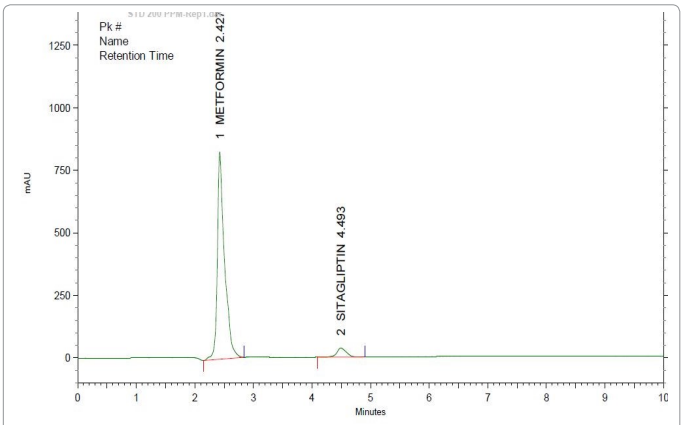

Figure 13: (200ppm of JANUMET) (Chromatogram of concentration of 200ppm after Precision procedure).

(Intraday Precision- (METFORMIN)

Conc ppm	Rt (Rep 1)	Rt (Rep 2)	AVG.
200	2.48	2.47	2.43
400	2.43	2.42	2.41
600	2.39	2.4	2.39
STD DEV.			0.04
AVG.			2.431
% RSD			0.01
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 11: Precision data of samples by the proposed HPLC method.

(Intraday Precision -(SITAGLIPTIN)

Conc ppm	Rt (Rep 1)	Rt (Rep 2)	AVG.
20	4.77	4.77	4.77
40	4.44	4.43	4.435
60	4.66	4.67	4.665
STD DEV.			0.171
AVG.			4.623
% RSD			0.037
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 12: Precision data of samples by the proposed HPLC method.

4. Accuracy

The accuracy of the method was evaluated in two replicates by analysis at different three concentration levels i.e. 200, 400, 600 ppm of the drug concentration in Table 13 and Figures 14-16.

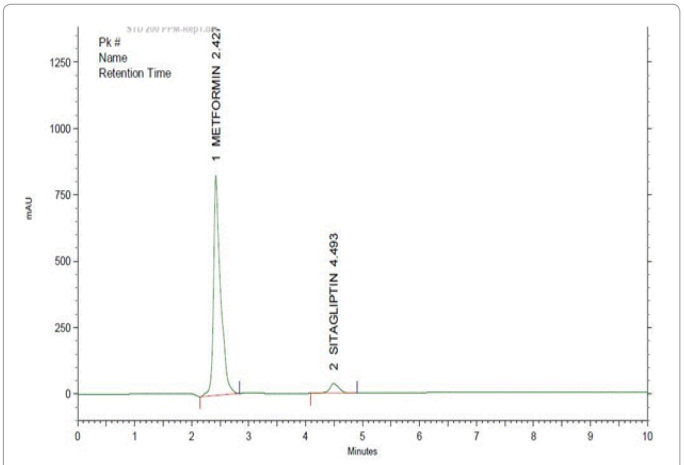

Figure 14: (200ppm of JANUMET sample) (Chromatogram of concentration of 200ppm after accuracy procedure).

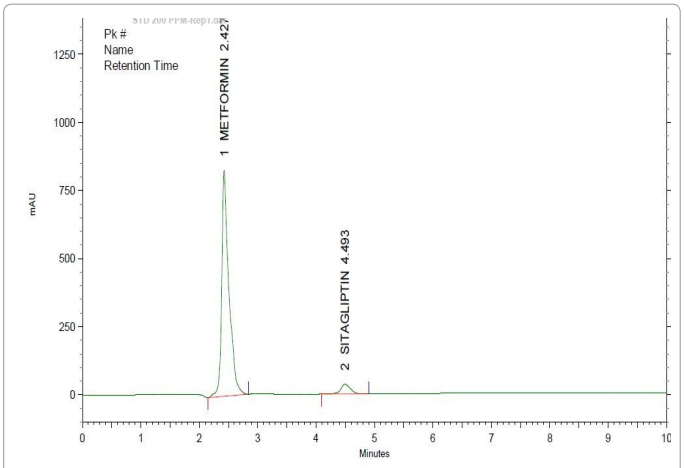

Figure 15: (400ppm of JANUMET sample) (Chromatogram of concentration of 400ppm after accuracy procedure).

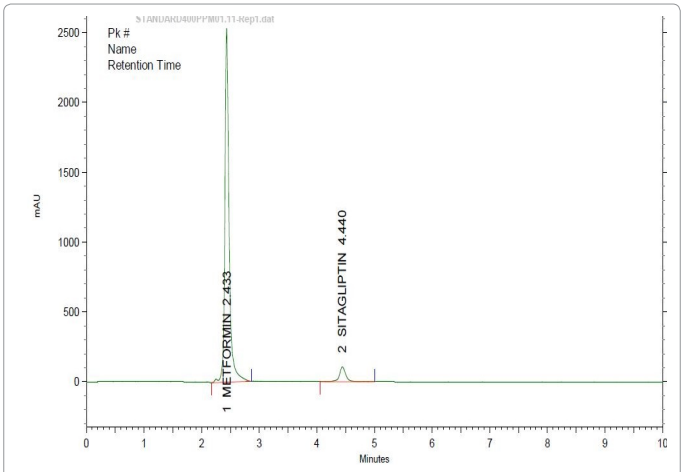

Figure 16: (600ppm of JANUMET sample) (Chromatogram of concentration of 600ppm after accuracy procedure).

(Accuracy-% Recovery)

Drug Name	Conc.% of	% Recovery	Mean %	Standard	%RSD
	Spiked level		recovery	deviation	
	200	98.34	98.89	0.61118	0.60161
METFORMIN	400	98.72	98.86	1.18034	1.192
	600	99.71	99.51	1.55794	1.56493
	20	98.32	98.29	0.02564	0.03284
SITAGLIPTIN	40	99.22	99.61	0.0596	0.0529
	60	98.11	98.25	0.0622	0.0655
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation					

Table 13: Accuracy data of samples (JANUMET) by the proposed HPLC method.

5. Robustness

The concept of robustness of an analytical method procedure has been defined by the ICH guidelines as; a measure of its capacity to remain unaffected by small but deliberate variation in method parameters. This was studied by testing influence of small changes in pH of buffer (pH 3.11) in Figures 17-18.

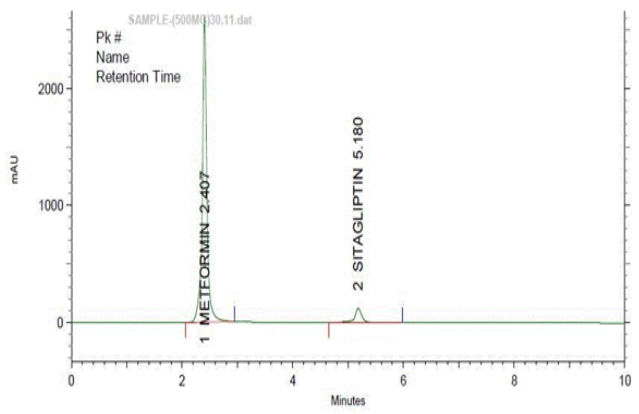

Figure 17: (200ppm of JANUMET sample) (Chromatogram of concentration of 200 ppm after effect of small variation on pH i.e. pH 3.06).

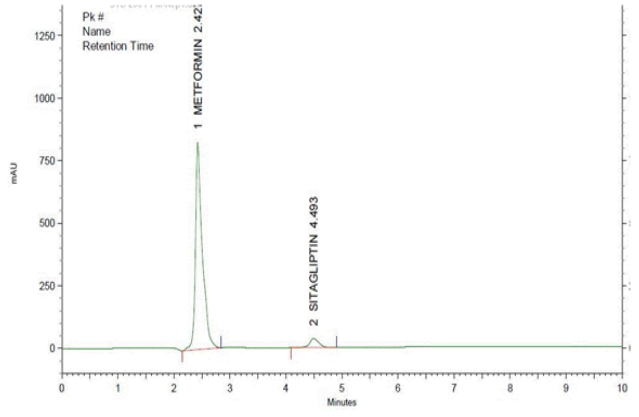

Figure 18: (200ppm of JANUMET sample) (Chromatogram of concentration of 200 ppm after effect of small variation on pH i.e. pH 3.15).

6. Ruggedness

Ruggedness is measured by the relative standard deviation of measurement caused by the different analyst, at different day as shown in Tables 14-16.

Ruggedness by analyst 1st on day 1

Conc ppm	Area (Rep 1)	Area (Rep 2)	Avg. Area
200	15661662	15718296	15689979
400	30876938	30838940	30857939
600	45993403	47384613	46689008
STD DEV.			15500697
AVG.			31078975
% RSD			0.498
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 14: Ruggedness data of samples (JANUMET) by the proposed HPLC method.

Ruggedness by analyst 2nd on day 2

Conc ppm	Area (Rep 1)	Area (Rep 2)	Avg. Area
200	2.48	2.47	2.43
400	2.43	2.42	2.41
600	2.39	2.4	2.39
STD DEV.			0.04
AVG.			2.431
% RSD			0.01
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 15: Ruggedness data of samples (JANUMET) by the proposed HPLC method.

Avg. area of analyst 1 and Avg. area of analyst 2

Conc ppm	Area (Rep 1)	Area (Rep 2)	Avg. Area
200	2.48	2.47	2.43
400	2.43	2.42	2.41
600	2.39	2.4	2.39
STD DEV.			0.04
AVG.			2.431
% RSD			0.01
Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation			

Table 16: Ruggedness data of samples (JANUMET) by the proposed HPLC method.

7. Detection limit

The detection limit of an individual analytical means is the lowest amount of analyte in a sample which can be detect but not necessarily quantities as a precise value.

The detection limit (LOD) may be expressed as:

$$LOD = 3.3\ \sigma/S$$

Where σ=Relative standard deviation of the response. S=the slope of the calibration curve (of the analyte).

8. Quantitation limit

The Quantitation limit of an analytical means is the lowest amount of analyte in a trial, which can be quantitatively determined with suitable precision.

Quantitation Limit (LOQ) may be expressed as:

$$LOQ = 10\ \sigma/S$$

Where σ=Relative standard deviation of the response. S= the slope of the calibration curve (of the analyte)

LOD was found to be 0.017 µg/mL

LOQ was found to be 0.056 µg/mL

Conclusion

The present study describes RP-HPLC method for the estimation of Sitagliptin Phosphate and Metformin HCl in standard and tablet. The % RSD of sitagliptin phosphate and metformin HCl for injection reproducibility and interday precision was less than 2% indicating high degree of precision. The results of the robustness study also indicate that the method is robust and is unaffected by small variations in the chromatographic conditions. The result of LOD & LOQ study was found to be 0.017 µg/mL and 0.056 µg/mL respectively.

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