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

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Abstract **Original Research Article**

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 × 250mm, [5µ particle size]) analytical column. The optimised 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 1ml/min. The simultaneous estimation was carried out at detection wavelength of 206nm using variable wavelength detector. Both drugs metformin HCl and sitagliptin phosphate were resolved and retained at 10mins. 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 [1].

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. 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 [2]. 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. The pumps convey the desired flow and composition of the mobile phase through the column. 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 [3].

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. The 1200 series is a pace forward from the innovative 1100 series, building on the modular design and configuration capabilities of the original. The outcome is a superior combination of speed, resolution, and sensitivity that has fostered widespread implementation in many labs [4].

Metformin HCl is glucose lowering agent that is global used for controlling for type2 diabetes [5]. Sitagliptin phosphate is an oral anti hypoglycaemic drug which is highly selective dipeptidyl peptidase-4 (DDP-4) inhibitors that enhance the action of incretins, hormones that stimulate postprandial insulin secretion via direct action on pancreatic β- cells and suppress glucagon secretion by the α- cells [6]. These drugs are generally co-administered to diabetic patients. They are decided in combined tablet dosage form [7]. Hence, the initiate experimental work was aimed to develop and validate RP-HPLC Method for simultaneous estimation of Metformin and Sitagliptin.

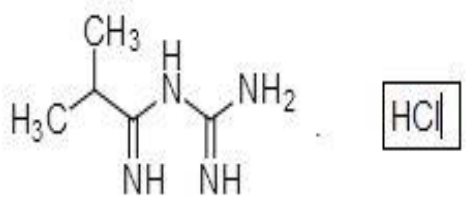

Fig-1: Metformin HCl

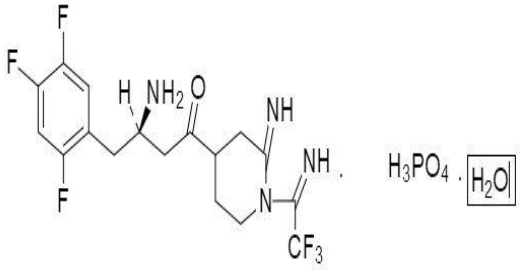

Fig-2: Sitagliptin Phosphate

MATERIALS AND METHODS

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

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

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.

Column temperature selected – The column temperature was set at 20 ^{0} C to get high plates numbers.

Injection volume – 20 µl was selected as the sample injection volume, because sample size 25µl to avoid excessive band broadening.

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

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)
  - Flow rate – 0.8ml/min
    - Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 20mins.
- 2nd trial
  - Isocratic flow
  - Mobile phase used – a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (pH2.5)
  - Flow rate – 1ml/min
    - Problem in the chromatogram - metformin peak was observed but sitagliptin peak was not observed in the run time of 10mins
- 3rdtrial
  - Isocratic flow
  - Mobile phase used – a mixture of 200ml of acetonitrile and 800ml of phosphate buffer(pH 2)
  - Flow rate – 1ml/min
    - Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 10 mins

- 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 – 1ml/min
        - Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 10mins
- 5th trial
    - Isocratic flow
    - Mobile phase used – a mixture of 150 volume of acetonitrile and 850 volume of phosphate buffer (pH2.5)
    - Flow rate – 1ml/min
        - Problem in the chromatogram – metformin peak was observed but sitagliptin peak was not observed in the run time of 20mins
- 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 – 1ml/min
        - Problem phase used – metformin peak was observed but sitagliptin peak was not observed in the run time of 20 mins

The method was optimized after all the trials

HPLC Instrument settings

- Isocratic flow mode
- Flow rate = 1 ml/min
- Temperature = 20 ^{0}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 = 5µm

Materials and Reagents

Fixed dose combination tablets (Brand: Janumet) containing 500mg of metformin and 50mg 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 100mg Metformin HCl and 10mg Sitagliptin Phosphate working standard into 100ml & 10ml clean dry volumetric flask respectively. Volume was made up to 100ml & 10ml with diluents to prepare 1000ppm 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 7.2mg was weighed and transferred to 10ml volumetric flask. Volume was made up to 10ml with diluent to prepare 500ppm 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 1.36gm of KH_2PO_4 in sufficient volume of Milli Q water & the volume was made to 1 litre. 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: 223nm, 206nmand 253nm (However, it was selected at 206nm). It was found that at low concentration the signal to noise ratio at 253 and 223nm was not clear. Hence, 206nm was selected as the working wavelength so that the method can be used in the lower concentration range.

Optimisation of Chromatographic Conditions

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

Analytical Method Validation

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 was reported.

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 has to be accurate, precise and linear over the selected range.

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)

Accuracy

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

Robustness

Robustness of the current method was studied by analyzing a sample of metformin hydrochloride and sitagliptin phosphate. Robustness 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)

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).

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 100ppm solution of each Metformin, Sitagliptin individually and their mixture were taken and 200-400nm was selected as a detection wavelength for simultaneous chromatographic determination of Metformin and Sitagliptin.

Optimization of Chromatographic Conditions

Assay

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

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%]

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 table 1, 2, 3,4,5,6.

Metformin HCl

200PPM

Table-1: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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

400PPM

Table-2: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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		26860812
SD		167562.9
RSD		0.006238

600PPM

Table-3: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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		47242208
SD		124279.7
RSD		0.002631

Sitagliptin Phosphate

20 PPM

Table-4: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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		869579
SD		9538.436
RSD		0.010969

40PPM

Table-5: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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

60PPM

Table-6: Results obtained for samples [JANUMET] analysis by the proposed HPLC method

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

Chromatograms

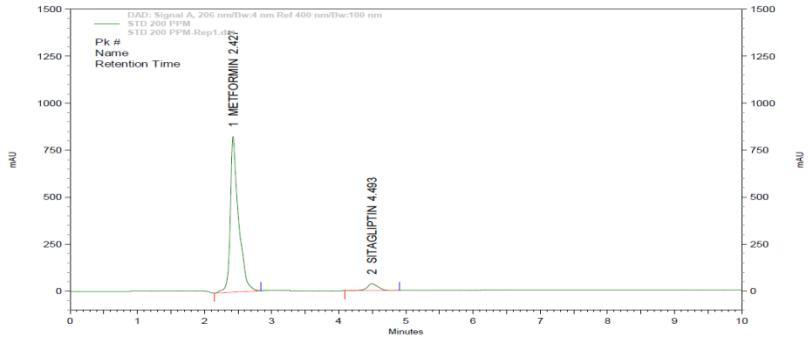

Fig-3: (200ppm of JANUMET sample) [Chromatogram of concentration of 200ppm after System Suitability procedure]

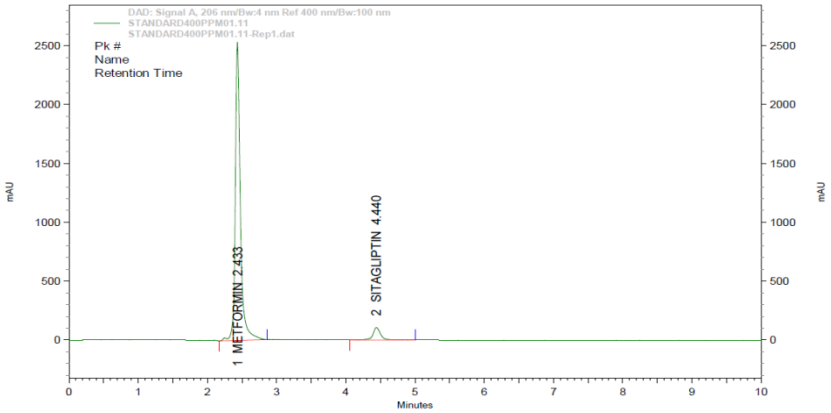

Fig-4: (400ppm of JANUMET sample) [Chromatogram of concentration of 400ppm after System Suitability procedure]

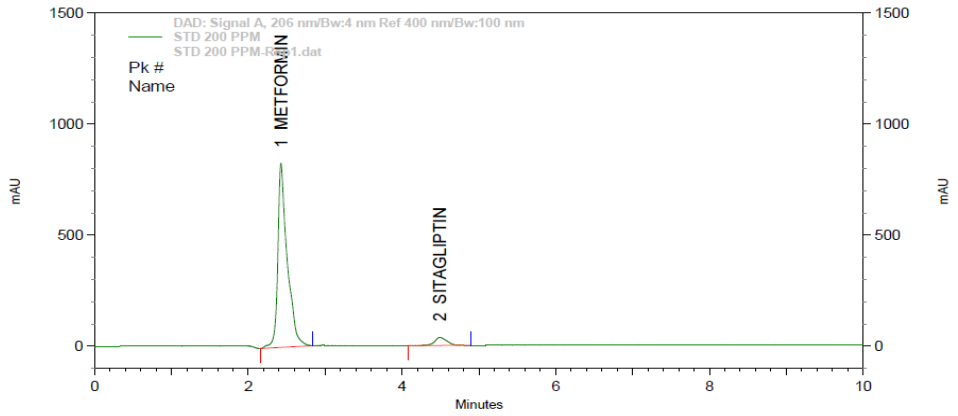

Fig-5: (600ppm of JANUMET sample) [Chromatogram of concentration of 600ppm after System Suitability procedure]

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 plotting peak area versus concentration of metformin and sitagliptin respectively, and the standard plot and regression equation was determined. Linearity chromatograms at different concentrations % level are shown in diagram 1 &2.

Metformin HCl

Table-7: Analysis characteristics of samples [METFORMIN HCl] by the proposed HPLC method

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

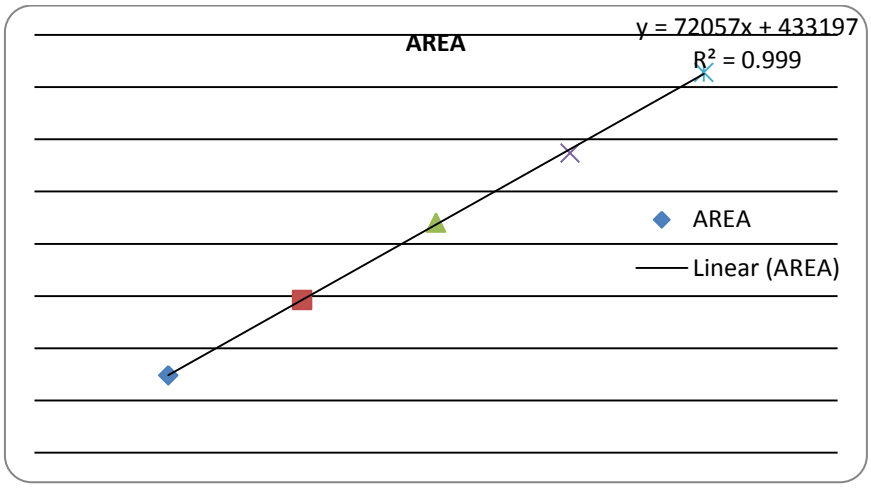

Fig-6: (Linearity data of Metformin HCl of 5 different concentrations)

Sitagliptin Phosphate

Table-8: Analysis characteristics of samples [SITAGLIPTIN PHOSPHATE] by the proposed HPLC method

CONC ppm	AREA(REP 1)	AREA(REP 2)	AVG. AREA
20	854167	865291	859729
40	1663338	1657120	1657120
60	2615279	2619334	2617307
80	3405079	3479118	3442099
100	4403221	4321198	4362210

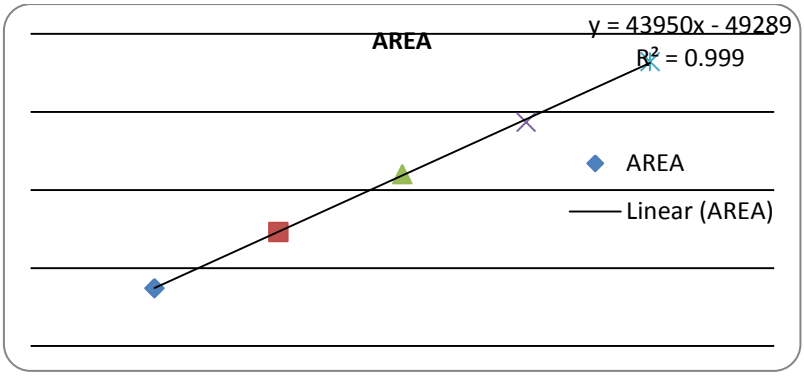

Fig-7: (Linearity data of Sitagliptin Phosphate of 5 different concentrations)

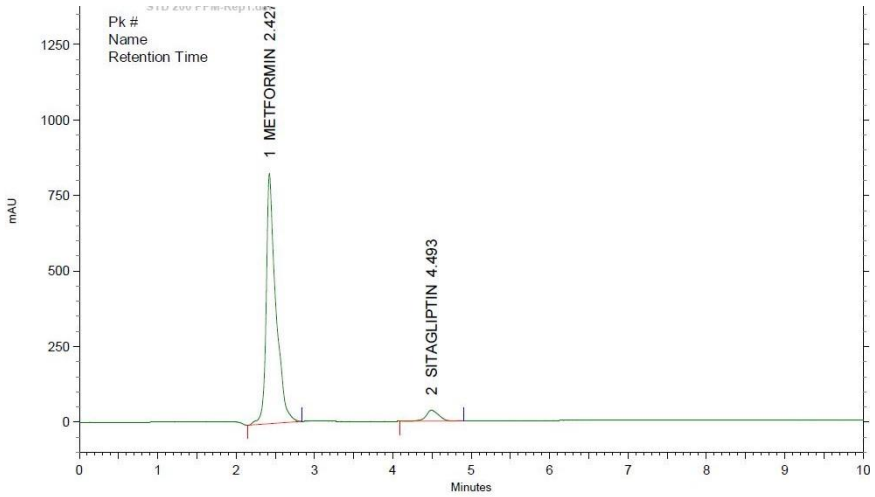

Fig-8: (800ppm) [Chromatogram of concentration of 800ppm after linearity procedure]

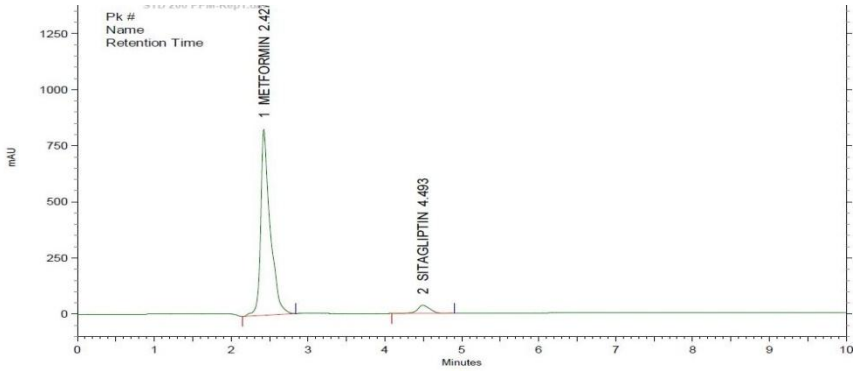

Fig-9: (1000ppm) [Chromatogram of concentration of 1000ppm after linearity procedure]

Precision

Precision of the method was estimated with respect to both repeatability (intra-day) and intermediate precision (inter-day).

Interday Precision - (METFORMIN)

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

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

(Interday Precision-(SITAGLIPTIN)

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

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

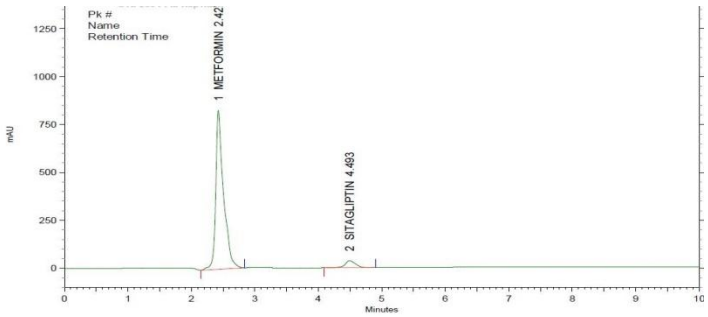

Fig-10: (1000ppm of JANUMET sample) [Chromatogram of concentration of 200ppm after Precision procedure]

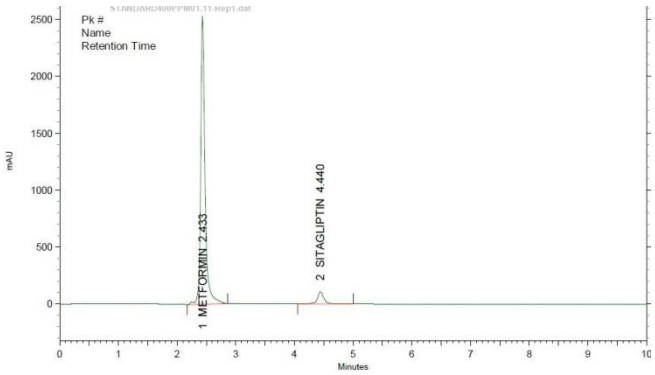

Fig-11: (400ppm of JANUMET sample) [Chromatogram of concentration of 4ppm after Precision procedure]

(Intraday Precision- (METFORMIN)

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

Conc (ppm)	Rt (Rep 1)	Rt(Rep 2)	Avg Rt
200	2.48	2.47	2.43
400	2.43	2.42	2.41
600	2.39	2.40	2.39
STD DEV.			0.04
AVG.			2.431
% RSD			0.01

Where AVG: Average, STD DEV: Standard deviation, RSD: Relative Standard Deviation

Intraday Precision- (SITAGLIPTIN)

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

Conc. (ppm)	Rt (Rep 1)	Rt (Rep 2)	Avg. Rt
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

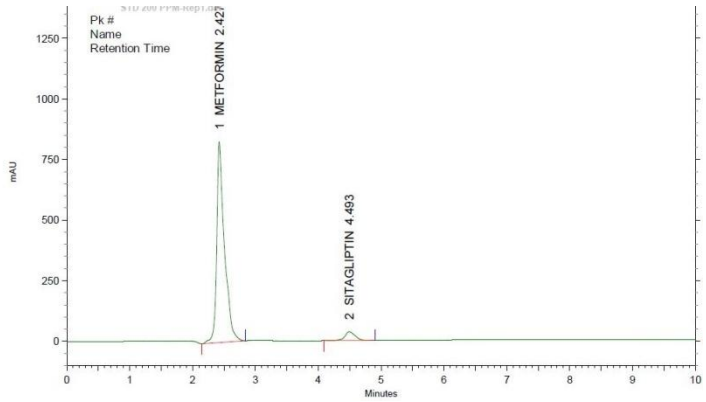

Fig-12: (600ppm of JANUMET sample) [Chromatogram of concentration of 600ppm after Precision procedure]

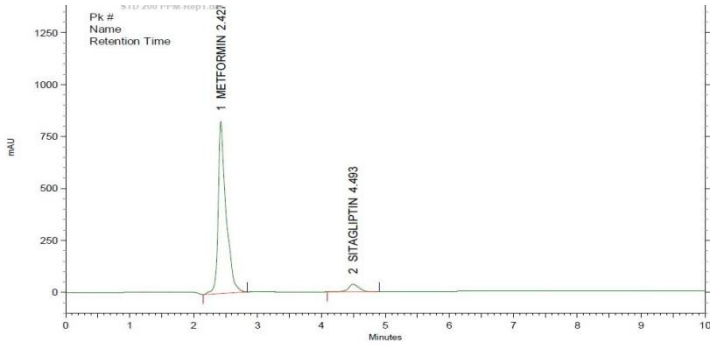

Fig-13: (200ppm of JANUMET) [Chromatogram of concentration of 200ppm after Precision procedure]

Accuracy

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

(Accuracy-% Recovery)

Table-13: Accuracy data of samples [JANUMET] by the proposed HPLC method

Drug name	Conc.% of Spiked level	% Recovery	Mean % recovery	Standard deviation	%RSD
METFORMIN	200	98.34	98.89	0.61118	0.60161
	400	98.72	98.86	1.18034	1.1920
	600	99.71	99.51	1.55794	1.56493
SITAGLIPTIN	20	98.32	98.29	0.02564	0.03284
	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

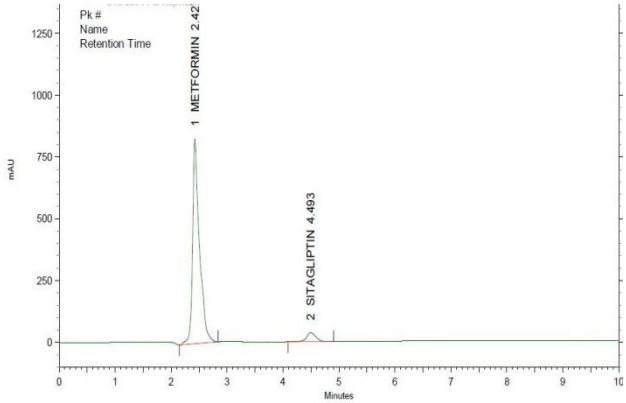

Fig-14: (200ppm of JANUMET sample) [Chromatogram of concentration of 200ppm after accuracy procedure]

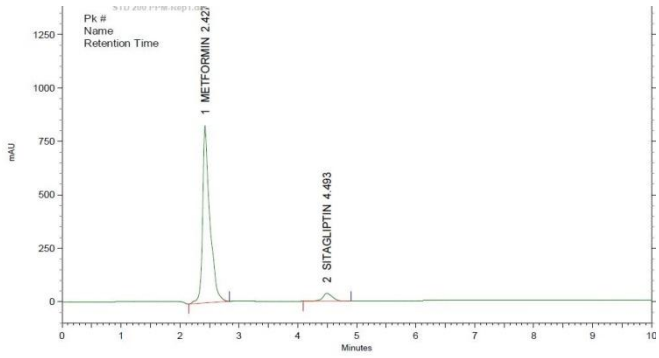

Fig-15: (400ppm of JANUMET sample) [Chromatogram of concentration of 400ppm after accuracy procedure]

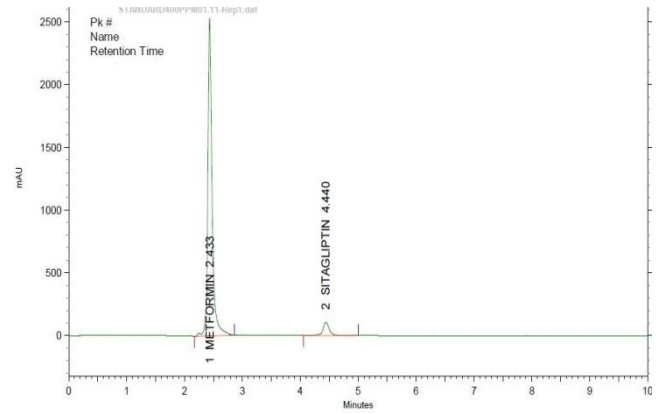

Fig-16: (600ppm of JANUMET sample) [Chromatogram of concentration of 600ppm after accuracy procedure]

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).

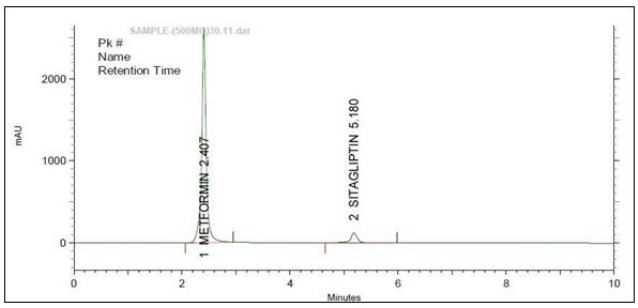

Fig-17: (200ppm of JANUMET sample) [Chromatogram of concentration of 200ppm after effect of small variation on pH i.e. pH 3.06]

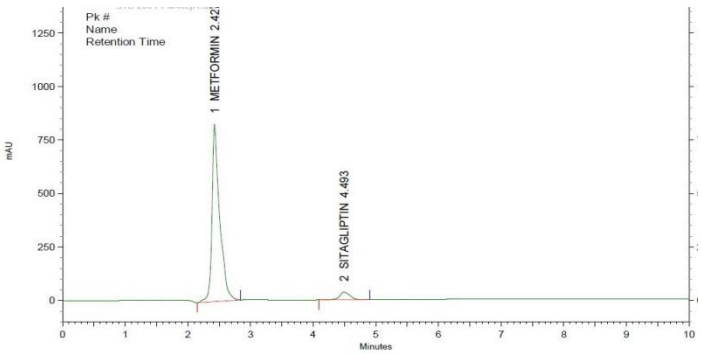

Fig-18: (200ppm of JANUMET sample) [Chromatogram of concentration of 200ppm after effect of small variation on pH i.e. pH 3.15]

Ruggedness

Ruggedness is measured by the relative standard deviation of measurement caused by the different analyst, at different day as shown in table 1 & 2.

Ruggedness by analyst 1st on day 1

Table-14: Ruggedness data of samples [JANUMET] by the proposed HPLC method

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

AVG: Average, SD: Standard Deviation, RSD: Relative Standard Deviation

Ruggedness by analyst 2nd on day 2

Table-15: Ruggedness data of samples [JANUMET] by the proposed HPLC method

Conc (ppm)	AREA (Rep 1)	AREA (Rep 2)	Avg. AREA
200	15134763	15176579	15155671
400	28759233	28685095	28722164
600	49837114	49924018	49880566
STD DEV.			17500219
AVG.			31252800
% RSD			0.559

AVG: Average, SD: Standard Deviation, RSD: Relative Standard Deviation

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

Table-16: Ruggedness data of samples [JANUMET] by the proposed HPLC method

Conc (ppm)	AREA (Rep 1)	AREA (Rep 2)	Avg. AREA
200	15689979	15155671	15422825
400	30857939	28722164	29790052
600	46689008	49880566	48284787
STD DEV.			16474126
AVG.			31165888
% RSD			0.5285

AVG: Average, SD: Standard Deviation, RSD: Relative Standard Deviation

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).

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 0.017µg/ml.

LOQ was found to be 0.056µg/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 0.017µg/ml and 0.056µg/ml respectively.

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Rational use of drugs combination
(Metformin HCl with Sitagliptin Phosphate)

Till now these drugs combination has not been officially developed and validated in any pharmacopoeias. Therefore, I decided to work on this drug.

No studies have been performed specifically examining the safety and efficacy of JANUMET in patients previously treated with other oral antihyperglycemic agents and switched to JANUMET. Any change in therapy of type 2 diabetes should be undertaken with care and appropriate monitoring as changes in glycemic control can occur.

Rational for development of estimation of metformin and sitagliptin simultaneously. In the literature review, simultaneous estimation of metformin and sitagliptin, there is no official method for this combination. Therefore, this research work was undertaking.

REFERENCES

1. Pulla RP, Sastry BS, Prasad YR, Raju NA. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. Research Journal of Pharmacy and Technology. 2011;4(4):646-9.
2. Pathade P, Imran M, Bairagi V, Ahire Y. Development and validation of stability indicating UV spectrophotometric method for the estimation of sitagliptin phosphate in bulk and tablet dosage form. Journal of pharmacy research. 2011 Mar;4(3):871-3.
3. Kolte BL, Raut BB, Deo AA, Bagool MA, Shinde DB. Simultaneous determination of metformin in combination with rosiglitazone by reversed-phase liquid chromatography. Journal of chromatographic science. 2004 Feb 1;42(2):70-3.
4. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. Journal of chromatography A. 2003 Feb 14;987(1-2):57-66.
5. DeCensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, Gandini S. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer prevention research. 2010 Nov 1;3(11):1451-61.
6. Herman GA, Bergman A, Liu F, Stevens C, Wang AQ, Zeng W, Chen L, Snyder K, Hilliard D, Tanen M, Tanaka W. Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. The Journal of Clinical Pharmacology. 2006 Aug;46(8):876-86.
7. Sekaran CB, Rani AP. Development and validation of spectrophotometric method for the determination of DPP-4 inhibitor, sitagliptin, in its pharmaceutical preparations. Eclética Química. 2010 Sep;35(3):45-53.