Development, Validation and Stability Indicating of RP-HPLC method for estimation of Metformin and Sitagliptin in Pharmaceutical dosage form

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ABSTRACT

The main objective of the present work was to develop a simple, precise, specific & stability indicating RP-HPLC method for simultaneous estimation of Metformin and Sitagliptin in bulk tablet dosage form. Chromatographic separation of Metformin and Sitagliptin was achieved on Inertsil C8, 150mm x 4.6mm, 5µm and the mobile phase containing P^H 2.5 buffer, acetonitrile and methanol in the ratio of 65:25:10 v/v as mobile phase. The flow rate was 1.0 ml/min, detection was carried out by absorption at 229 nm u sing a photodiode array detector. The RT of Metformin hydrochloride and Sitagliptin is found to be 2.8 and 5.2 min respectively. . The linearity of the method was excellent over the range 50- 740µg/ml and 0.5-7.5 µg/ml for Metformin and Sitagliptin respectively. The forced degradation studies were performed as per the guidelines of ICH under acidic, alkaline, oxidative, thermal, photo stability & neutral conditions and chromatograms from the stressed samples, obtained by use of the photodiode-array detector. The correlation coefficient was found to be 0.999. The proposed method was validated according to ICH guidelines. And it was found to be suitable accurate and sensible method for quantitative analysis of drug from Dosage form and study of its stability.

INTRODUCTION

Sitagliptin (rINN) is (1S,3S,5S)-2-((2S)-Amino (3-hydroxytricyclo (3.3.1.13,7) dec-1- yl) acetyl) - 2-azabicyclo (3.1.0) hexane-3- carbonitrile an orally active hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4) affecting the action of natural hormones in the body called incretins they are able to potentiate the secretion of insulin and suppress the release the glucagon by the pancreas. This drives blood glucose levels to normal[1].

Metformin hydrochloride is N,Ndimethyl imidocar boni-midic diamide is used in the treatment of type 2 diabetes. Metformin hydrochloride activates AMP- activated proteinkinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for Metformin hydrochloride inhibitory effect on the production of glucose by liver cells[2].

Literature survey reveals good number of analytical methods for the estimation of Metformin and Sitagliptin individually or in combination with other drugs using UV spectrophotometry [3], HPLC9-[4], HPTLC[5] and LC-MS/MS[6]. Moreover, few methods were reported for the estimation of the selected drugs in their combinations using UV spectrophotometry[7-8], HPLC25[9-11]. As per the data available, few stability indicating RP-HPLC method was reported so far for the estimation of the Metformin and Sitagliptin. Hence, present research work revealed to develop a simple, accurate stability indicating HPLC method for the estimation of selected drugs. The developed method has been validated as per the guidelines of ICH[12]. To establish stability indicating nature of the method forced degradation studies were planned for the proposed method under acidic, alkaline, oxidative, thermal, photo stability and neutral conditions[13-14]

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure Metformin hydrochloride and Sitagliptin were obtained as gift samples Goa and Hyderabad, India. Tablet (Istamet) was purchased from the local market. Buffers, Acetonitrile and all other chemicals were analytical grade.

Instruments

All analytical works performed on Shimazdu model LC-20-AD dual pump, a Shimadzu model DGU-20A degasser, Shimadzu model SPD-M20A photo diode array (PDA) detector and a Shimadzu model SPD 20 HT auto injector, Empower2 solution version software, phenominex Gemini C18 (250×4.6mm×5µ) as stationary phase, a calibrated electronic single pan balance Ohaus (AUX-220), a pH meter of Elico (LI-120) and Sonicator were also used during the research.

Methodology

Preparation of buffer pH 4.5

Prepare about 0.02M dipotassium hydrogen phosphate in a suitable conical flask and adjust the pH to 4.5 with orthophosphoric acid.(0.02M of di-potassium hydrogen phosphate is prepared by taking 1.3602mg of dipotassium hydrogen phosphate in a volumetric flask , and make up to 1L with water).

Preparation of mobile phase:

Prepare a mixture of buffer 4.5 pH, and acetonitrile in the ratio 70:30 filter through 0.45µ membrane filter and degas it.

Mobile phase: Prepare a mixture of Buffer and Acetonitrile in the ratio of (70:30). Filter and degas.

Chromatographic condition Use suitable High Performance Liquid Chromatography equipped with UV-visible detector.

Column : Phenominex Gemini C18 (250×4.6mm×5µ).

Wavelength : 229 nm Injection Volume : 10µL

Column Temperature: Ambient

Flow rate : 1.0 mL/min.

Retention time of Metformin hydrochloride is about 3.0-4.0 min and Sitagliptin is about 5.0-6.0min.

Preparation of Diluent: Used mobile phase as diluents.

Preparation of standard solution of Metformin:

Weigh accurately about 50mg of Metformin, working standard to a 100ml volumetric flask. Dissolve it

completely and sonicate it. Make up to 100ml mobile phase. Take 5ml from the above flask and make up to 50ml with mobile phase.

Preparation of standard solution of Sitagliptin:

Weigh accurately about 10mg ofSitagliptin working standard are taken into 200ml volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5mL to 50mL with the diluent.

Sample preparation:

Weigh accurately 20 tablets equivalent to 92.4mg to a 100ml volumetric flask. Mobile phase to dissolve it completely and sonicate for 10min with intermediate shaking Make up to 100ml with mobile phase and filter through 0.45µ GHP filter. Further dilute 3ml with 50ml mobile phase.

Method Validation

Method validation was done as per the guidelines of ICH

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The other component may include excipients, impurities, degradation product etc.

Peak purity test may be useful to show that the analyte chromatographic peak is not contributed by more than one component (e.g. .diode array, mass, spectroscopy).

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range.

Linearity is generally reported as the variance of the slope of the regression line. Linearity should be evaluated by visual inspection of a plot of signal as a function of analyte concentration. The correlation coefficient, y-intercept, slope of the regression line and the residual sum of squares should be calculated.

System precision

The system precision was evaluated by measuring the peak response of Metformin HCL and Sitagliptin, WS solution prepared as per the proposed method and chromatograms were recorded.

Accuracy

To document accuracy, the ICH guideline on methodology recommends collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters.

The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength or temperature and determining the effect on the results of the method. Robustness tests were generally introduced to avoid problems in linear laboratory studies and to identify the potentially responsible factors.

Ruggedness

To determine the degree of reproducibility of the results by this method involved the studies of the analyst to analyst and day to day; that is to carry out precision study in six replicate of an assay of a single batch sample by two different analysts on two different days.

LOD and LOQ

ICH has recommended some method for determining the limit of detection. The method may be either instrumental or non-instrumental. . It is calculated using formula

LOD = 3.3 σ/S; where, σ = S.D; S =Slope

Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of calibration curve.

LOD = 3.3s / S

Where,

s = Standard deviation of the response

S = Slope of calibration curve

Force Degradation Studies

Metformin and Sitagliptin standard samples were subjected to degradation under different stress conditions like acidic, alkali, oxidative, thermal, photo stability and neutral conditions. For acidic & alkali degradation samples were refluxed with 2N HCl & 2N NaOH at 60°C for 30 min. For oxidative degradation 20% v/v, H_2O_2 was used and the same was refluxed at 60°C for 30 min. For thermal degradation, sample was placed in oven at 105°C for 6 hr; and for photo stability degradation, drug was exposed to UV light by keeping the sample in UV chamber for 7 days or 200 watt hours/m2 in photo stability chamber; for neutral degradation, the drugs were refluxed in water for 6 hours at a temperature of 60°C. All the samples were diluted to obtain a final concentration of 20 µg/ml of Sitagliptin & 500 µg/ml of Metformine. Ten micro liters of the samples were injected into the system and the chromatograms were recorded to assess the stability of the sample. The stability of the drug solution was determined for the short term stability, auto sampler stability. Short term stability was carried out by keeping the samples, at room temp (25°C) for 24 hrs. Auto sampler stability was determined by storing the samples for 24 hrs in the auto sampler. Each sample was injected 6 times into HPLC & the results obtained were compared with nominal values of QC samples.

RESULT AND DISCUSSION

A reversed-phase column procedure was selected as a suitable method for the determination of and Metformin hydrochloride and Sitagliptin in combined dosage forms. The chromatographic conditions were

optimized by changing the mobile phase composition, p^H, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of P^{H}2.5 buffer, Acetonitrile and methanol in the ratio of 65:25:10 v/v was used. After injection of sample in the specified concentration the chromatograms was recorded and measure the peak responses for Metformin hydrochloride & Sitagliptin. The System suitability parameters should be met as per specifications. The content of Metformin hydrochloride & Sitagliptin in the sample was calculated from the peak responses.
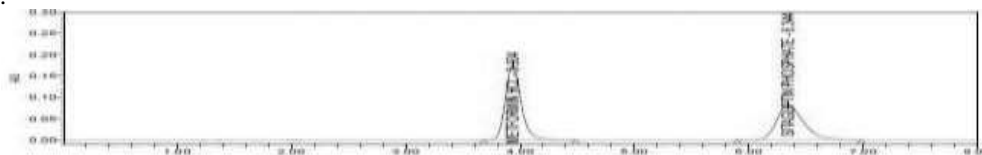

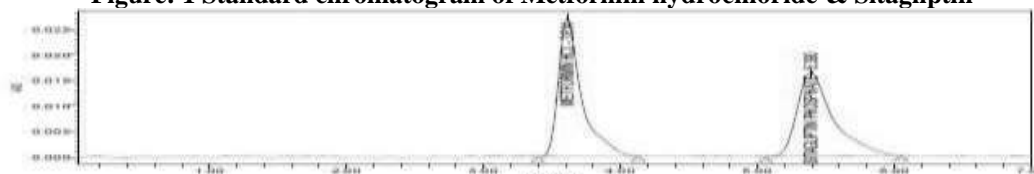**Figure: 1 Standard chromatogram of Metformin hydrochloride & Sitagliptin**

Figure: 2 Sample chromatogram of Metformin hydrochloride & Sitagliptin

Table 1. Percent purity of Metformin hydrochloride and Sitagliptin in combined dosage form.

Drug	Label claim	% Drug found ± SD	% RSD
Metformin hydrochloride	500 mg	99.9	0.17
Sitagliptin	5 mg	99.7	0.25

Evaluation of system suitability

A typical chromatogram obtained by using the above mentioned mobile phase from 10µl of the assay 3.614 and 5.390 min, respectively. The linearity of the method was tested from 50-750µg/ml for Metformin hydrochloride and 0.5-7.5 µg/ml for Sitagliptin. Correlation coefficients for the regression line were found to be 0.9996 and 0,9987 for Metformin hydrochloride and Sitagliptin respectively.

Relative Standard Deviation of sample injected of Standard preparation for Metformin hydrochloride & Sitagliptin peaks should not be more than 2.0%.

The tailing factor for Metformin hydrochloride & Sitagliptin peaks should be more than 2.0 and plate count will be not less than 3000.

Table: 2 System suitability parameters.

Parameter	Metformin hydrochloride	Sitagliptin
USP tailing factor	1.29	1.08
Theoretical plates	7488	6583
%RSD for Areas	0.48	0.355
%RSD for RT	0.11	0.14

Method validation

This method described above had been validated as per the ICH guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness were determined and mentioned in the results.

System suitability

The system suitability was assessed using five replicate analyses of drugs at concentration of 500µg/mL for MET and 5µg/mL for TEL by increasing the injection volumes 10-50µL.

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the plots with blank and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by purity index data.

Linearity

The linearity responses in the concentration range of 80-730 µg/mL for Metformin hydrochloride and 8-70µg/mL for Sitagliptin was determined and the co-relation coefficient was NLT 0.99.

Table 5: Linearity of Metformin hydrochloride.

Concentration (µg/ml)	Area
50	122322
100	257538
200	495755
261	648447
300	760152
400	972342
500	1240791
600	1512821
740	1835059

Table 6: Linearity of Sitagliptin.

Concentration (µg/ml)	Area

0.5	110950
1	260050
2	477785
2.5	621862
3	728070

4	933052
5	1196880
6	1463882
7.5	1772580

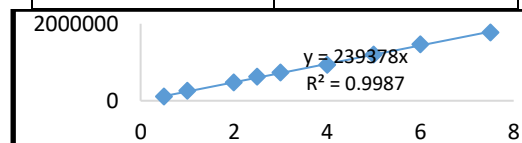**Fig: 11 Linearity graph of Metformin Hcl.**

Fig:12 Linearity graph of Sitagliptin.

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at a concentration of 500µg/mL for Metformin hydrochloride and 5µg/mL for Sitagliptin. And the RSD calculated from replicates of assay values NMT 2.0%.

Table 7: Precision data of the proposed method.

Drug	Concentration added, µg mL	Intra-day precision		Inter-day precision	
		Mean amount found, µg/mL	% RSD (n = 6)	Mean amount found, µg/mL	% RSD (n = 6)
Metformin hydrochloride	500	499.53±0.61	0.42	499.71±0.31	0.51
Sitagliptin	5	4.95.2 ±0.52	0.34	4.88±0.54	0.37

Accuracy

Accuracy (Recovery) of the method was determined by spiking 50, 100 and 150% of working standard at a concentration of 500µg/mL for Metformin hydrochloride and 5µg/mL for Sitagliptin. Samples were injected in triplicate across its range according to the assay procedure. The RSD calculated from replicates of assay values NMT 2.0% and the percentage recovery was in between 99% to 102%.

Recovery

S.NO	Level	% Recovery for Metformin hydrochloride	%Recovery for Sitagliptin
1	50%	99.6	99.6
2	100%	100.10	98.8
3	150%	99.7	99.8

Detection and quantitation limits

The LOD and LOQ values were determined by the formulae LOD = 3.3 s/ m and LOQ =10 s/m (Where, s is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate (1± 0.1 mL/min), wavelength (±1nm), organic phase (± 10%) and ph(±0.2)

Chromatograms for forced Degradation Studies

Forced degradation studies of both drugs were carried out under various stress conditions as follows:

Effect of Acid, Alkaline and Neutral Hydrolysis: Metformin and Sitagliptin were found to undergo 8.35% & 10.36% decomposition under acidic stress condition with a degradation product at retention time of about 2.88 min and 8.30 min respectively and minute decomposition about 7.32 % & 8.72% under basic stress condition with a degradation product at retention time of about 2.55 min. and 8.80 min respectively. Under neutral degradation condition, no degradation was observed.

Effect of Oxidation: In oxidation stress condition, almost 12.49% & 9.65% of Metformin and Sitagliptin were degraded and degradation peak appeared in chromatogram.

Effect of Heat: Under dry thermal stress condition, Metformin and Sitagliptin were degraded about 1.06% & 1.35% with degradation product.

Effect of light: When Metformin and Sitagliptin in solution state were exposed to sun light; and Metformin and Sitagliptin in powder state were exposed to UV light, no degradation was observed, respectively.

The samples exposed to acidic, alkaline, neutral, oxidative, thermal and photolytic conditions were colorless. In Photolytic stability, Metformin and Sitagliptin were found to be stable showing no degradation. All degradates were resolved from Metformin and Sitagliptin peaks and the percentage degradation for each condition indicated that there were no interference from degradates in determination of the Metformin and Sitagliptin in tablet dosage form. Thus, the proposed, method was found to be "Stability Indicating".

ACKNOWLEDGEMENTS

Fig: 5 Acid Degradation Chromatogram for Metformin hydrochloride and Sitagliptin
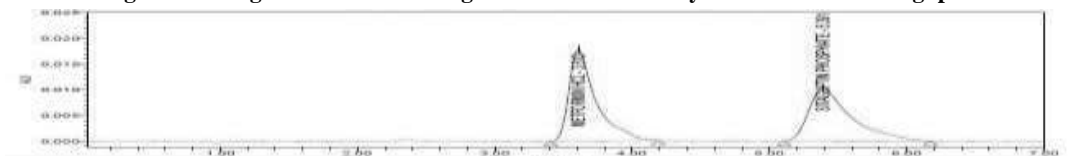

Fig:6 Alkali Degradation Chromatogram for Metformin hydrochloride and Sitagliptin
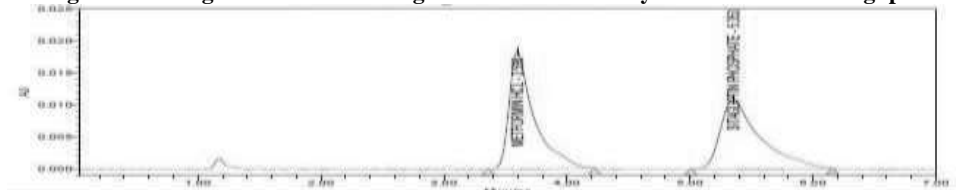

Fig:7 Peroxide Degradation Chromatogram for Metformin hydrochloride and Sitagliptin
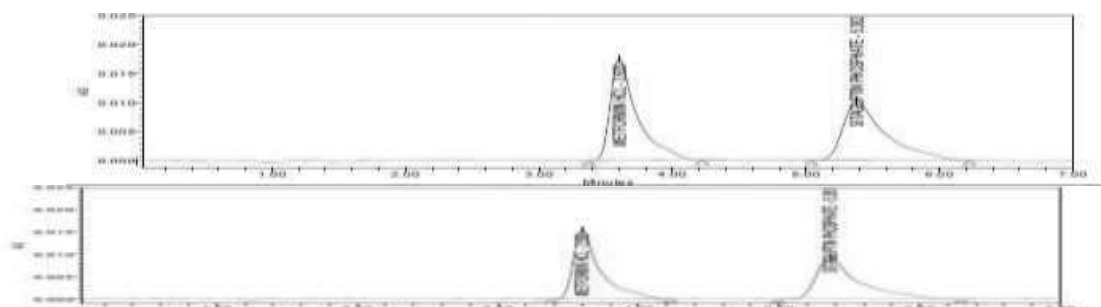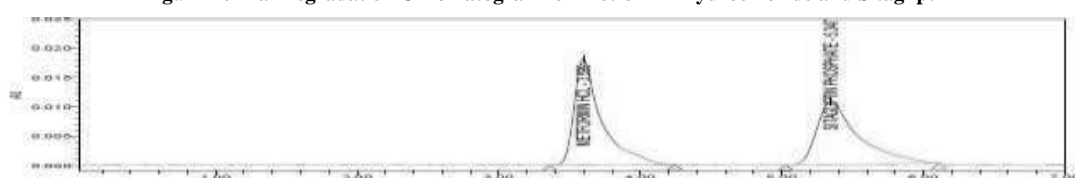

Fig:8 Reduction Degradation Chromatogram for Metformin hydrochloride and Sitagliptin

Fig:9 Thermal Degradation Chromatogram for Metformin hydrochloride and Sitagliptin

CONCLUSION:

A simple specific stability-indicating HPLC method has been developed for the quantification of Metformin hydrochloride and Sitagliptin. This method has been validated and found to be specific, precise, accurate, linear, robust, and linear for the detection and quantification of Metformin hydrochloride and Sitagliptin. This method exhibited an excellent performance in terms of sensitivity and speed. The major advantage of this technique is that it is less time consuming and also eco-friendly because of its low consumption of organic solvents as compared to other analytical techniques. It helps in estimation of Metformin hydrochloride and Sitagliptin in pharmaceuticals dosage forms. This method is suitable for routine analysis and quality control of pharmaceuticals.

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