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An efficient RP-HPLC-based approach for simultaneous determination of sitagliptin and metformin HCl in pharmaceutical drug formulation

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Abstract: This study introduces an innovative, and rapid HPLC method using reverse phase elution for the simultaneous analysis of Sitagliptin and Metformin HCl in pharmaceutical formulations. This combination was explored in bulk and solid dosage forms using Luna Phenomenex C8 column (4.6 x 250 mm, 5 µm) at ambient temperature in isocratic elution. It was found that the mobile phase comprising of 0.1% ortho-phosphoric acid, potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile in ratios 35:35:30, showed a symmetrical peak for Sitagliptin and Metformin HCl. The detection was carried out at 210nm, using a flow rate of 1.0mL/min. The method was linear over the concentration range for Sitagliptin 2.5-7.5 ppm and Metformin HCl 25-75 ppm. The assay recoveries of Sitagliptin and Metformin were found to be 100.36% and 100.20%, respectively. The LOD and LOQ for the Sitagliptin were found to be 0.201 ppm and 0.301 ppm and for Metformin HCl 0.101 ppm and 0.303 ppm, respectively. The proposed methods can be implemented for controlling quality in bulk and solid dosage forms. The analytical methods were validated as per the guideline of ICH Q2 (R2). The developed HPLC methods were effectively employed for the determination of combined dosage forms in pharmaceutical formulations.

Keyword: Sitagliptin, metformin HCl, RP-HPLC, validation, ICH Q2 (R2).

INTRODUCTION

Diabetes is one of the largest non-transmissible diseases all over the world (Abdallah FF *et al.,* 2022). It is among the top five leading death causes worldwide and there are considerable indications that it is becoming epidemic in most of the established states (Aguiree *et al.,* 2013; Maia *et al*, 2022). It is anticipated that the number of individuals with diabetes might be over 370 million worldwide by 2030, in addition to a considerable rise in pre-diabetes, such as unhealthy glucose tolerance (Smyth and Heron, 2006; Lindeman *et al.,* 1998; Gallwitz, 2007) (Alberti and Zimmet, 1998; WHO, 2016).

Diabetes is a metabolic disease characterized by hyperglycemia resulting from a disorder in insulin action or insulin secretion, or both (Popoviciu MS *et al.,* 2023). Diabetes mellitus includes a heterogeneous group of disorders characterized by uncontrolled levels of glucose. The resolution of this heterogeneity has focused on the identification of subgroups of diabetes mellitus with distinct molecular pathology, one example being maturity onset diabetes of the young (MODY) (Reardon *et al.,* 1992; Julier *et al.,* 1991; Vionnet *et al.,* 1992; Bell *et al.,* 1991).

Sitagliptin phosphate monohydrate chemically known as (3R)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one phosphate hydrate (Malleswararao *et al.*, 2012; Jeyabalan and Nyola, 2012), is an oral anti-diabetic drug having dipeptidyl peptidase-4(DPP-4) inhibitor class (Bhende *et al.,* 2012; Jires *et al*, 2024).

Metformin HCl, chemically known as N,N-dimethylimidodicarbonimidic diamide hydrochloride (Vasudevan *et al.,* 2001), is an antihyperglycemic agent and orally administered biguanide, which is commonly used during the treatment of type 2 diabetes (Guo *et al*, 2024; Jain *et al*., 2008; Jeyabalan and Nyola, 2012).

Sitagliptin prevents the decay of GIP and GLP-1 by DPP-4, permitting GIP and GLP-1 to carry out their activities in the metabolism (Nandipati and Reddy, 2012; Baokar *et al.,* 2013). By avoiding GIP and GLP-1 inactivation, they are capable of improving insulin secretion and decreasing

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the production of glucagon from the pancreas. This results in a normal blood glucose level (Santhosha *et al.,* 2012; Yin *et al, 2022*).

Metformin as an antihyperglycemic agent includes less intestinal absorption of glucose, larger uptake of glucose into the tissues from blood, decrease in production of glucose from the liver and reduction in insulin requirements for glucose consumption (Klepser and Kelly, 1997).

In the pharmaceutical industry, the assessment of active pharmaceutical ingredients (APIs), impurities, and degradation products in raw materials, intermediate bulk formulation, and finished goods relies on established pharmacopeias such as USP, EP, and BP. Most pharmacopeias advocate the use of HPLC methods due to their high sensitivity, robustness, accuracy, precision, selectivity, stability-indicating nature, and reproducibility. However, to the best of our knowledge, no pharmacopeia contains the HPLC method for simultaneous determination of metformin and sitagliptin in tablet dosage forms. Whereas, the literature survey reveals a variety of UV, (El-Bagary *et al.,* 2011) HPLC, (Vasudevan *et al.,* 2001) and ion-pair HPLC (Ravanello *et al.,* 2012) methods for estimation of metformin HCl and Sitagliptin alone and with combination in tablet dosage forms (Malayandi *et al.,* 2023).

The literature survey also reveals a few bio-analytical LC–MS and stability-indicating HPLC (Peraman *et al.,* 2013) methods for the determination of sitagliptin alone or with other drugs (Raja and Rao, 2012). Additionally, existing methods were expensive and time consuming, hence the main objective of this research is to develop cost effective LC method for the analysis that has not been developed so far as by the International Council for Harmonisation of Technical Requirements for pharmaceuticals, for human use (ICH) (Validation of Analytical Procedures Q2 (R2)).

MATERIALS AND METHODS

Chemicals and reagents

The working standards of Metformin and Sitagliptin phosphate monohydrate were obtained as a gift from Pharm EVO Pharmaceutical private limited Karachi, Pakistan. Samples of Sitagliptin and Metformin HCl tablet 50/500mg were purchased from a local market Pharmacy, Phosphoric acid (Analytical grade), Potassium di-hydrogen Phosphate (Analytical grade), Purified water (HPLC grade), Acetonitrile (HPLC grade) were purchased from Merck private limited.

HPLC instrumental conditions

The HPLC instrument which was used for analysis have made of Shimadzu equipped with pump with installed degasser, auto-sampler, column oven and UV detector having model no. LC-20AT, SIL-20A, CTO-20A and SPD-20A respectively with operating software (Lab Solution). While another HPLC instrument which was used during intermediate precision have made of water equipped with separation module, column oven and UV detector having model no. Alliance e2695, Alliance C/H/C and 2489 respectively with operating software (Empower).

A chromatographic separation of the two drugs was achieved with a Luna Phenomenex C8 (4.6 x 250 mm, 5 µm) analytical column using ortho-phosphoric acid (0.1%): phosphate buffer (0.05M, pH 3.0): acetonitrile (35:35:30% v/v) in isocratic mode at a flow rate of 1 mL/min, column at ambient temperature and detection of all the drugs were monitored at 210 nm using a PDA detector by injecting 10 µL of solution. The retention time of Sitagliptin and Metformin HCl was about 6.0 and 2.3 minutes, respectively. All the solvents were filtered through 0.45 µm nylon filter and degassed in an ultrasonic bath prior to use.

Analysis of formulation

Preparation of standard solution

In a 10 mL volumetric flask, accurately weighed 12.8 mg of Sitagliptin phosphate monohydrate was added followed by the addition of 8 mL diluent. The sample was dissolved through sonication and the volume was made up to the mark with diluent and mixed thoroughly. A one-tenth dilution of the above solution was also prepared (stock solution). In a 10 mL volumetric flask, accurately weighed 10 mg of metformin HCl was added followed by the addition of 8 mL of diluent. The sample was dissolved through sonication and the volume made up to the mark with diluent and mixed thoroughly (stock solution). A one-twentieth dilution of both stock solutions was made as a working standard solution.

Preparation of sample solution

Weighed 20 tablets of 50+500 mg and crushed to fine powder and the powder was accurately weighed to one fifth of the average weight and transferred into a 100mL volumetric flask. Around 80mL of diluent was added followed by sonication for 10min with intermittent shaking and shaking done for 30min. The volume was then made up to the mark with diluent and mixed thoroughly. The flask was allowed to stand for the separation of solid residue from supernatant. A one-twentieth dilution of the above solution was also prepared.

Analytical method validation

System suitability

System suitability tests were carried out on a freshly prepared standard solution of the sitagliptin and metformin to scrutinize the various optimized parameters. Such as % RSD of areas, plate count, resolution and tailing factor.

Table 1: Analysis of formulation

Drug	Label claim (mg/Tablet)	Mean result (%)	RSD (%)
Sitagliptin	50	100.67	0.79
Metformin	500	100.60	0.61

Table 2: System suitability

System Suitability Parameters	Sitagliptin		Metformin HCl	
	Mean	% RSD	Mean	% RSD
Area	130541.60	0.17	4248411.60	0.04
Number of theoretical plates	6420.60	-	3753.80	-
Tailing factor	1.10	-	1.21	-
Resolution	-	-	13.41	0.16

Table 3: Accuracy/Recovery

Drug	Accuracy level (%)	Actual concentration (ppm)			Concentration found (ppm)			% recovery	% RSD	% Mean recovery
Sitagliptin	50	2.41	2.34	2.34	2.37	2.35	2.34	99.72	1.27	
	80	4.44	4.05	4.05	4.48	4.03	4.05	100.17	0.71	
	100	5.06	4.98	5.14	5.13	5.13	5.16	100.91	0.50	100.36
	120	6.15	5.92	6.15	6.20	5.85	6.20	100.16	1.06	
	150	7.86	7.86	8.10	7.95	7.91	8.14	100.82	0.30	
Metformin	50	24.90	24.80	24.80	24.58	24.52	24.53	98.83	0.11	
	80	40.00	39.10	39.20	39.69	39.48	38.82	99.74	1.07	
	100	50.00	49.00	50.10	49.69	40.68	49.67	99.97	1.23	100.20
	120	62.80	63.00	63.20	63.75	63.69	63.75	101.16	0.32	
	150	78.50	78.80	79.00	79.80	79.81	79.77	101.30	0.34	

Table 4: Precision

	Repeatability		Intermediate precision	
	% results of Sita	% results of Met	% results of Sita	% results of Met
Sample 1	101.21	100.95	98.12	99.08
Sample 2	101.23	101.15	100.77	99.19
Sample 3	100.59	100.37	100.10	99.48
Sample 4	100.99	100.82	101.18	100.02
Sample 5	100.86	100.84	101.00	101.15
Sample 6	99.11	99.46	101.26	99.68
Mean	100.67	100.60	100.40	99.77
%RSD	0.79	0.61	1.19	0.76

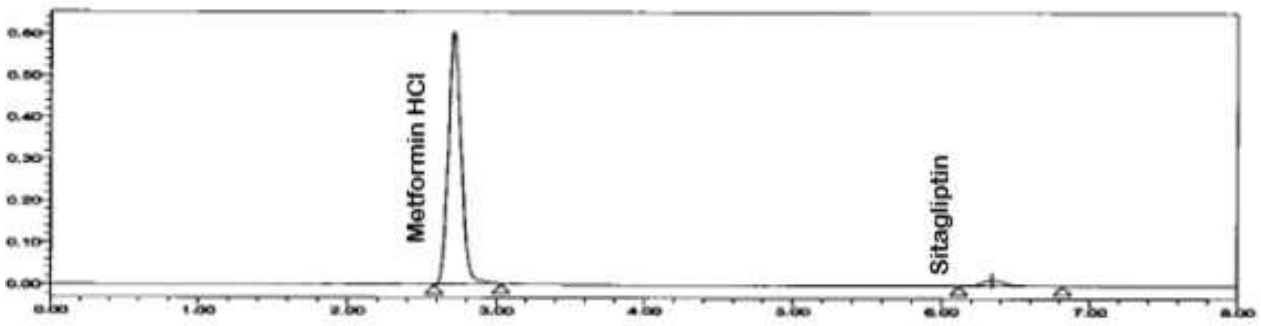

Fig. 1: Chromatogram of standard solution of Sitagliptin and Metformin HCl.

Table 5: Linearity

% level	Conc. of Sita (ppm)	Area of Sita	Conc. of Met (ppm)	Area of Met
50	2.500	40287	25.13	977723
80	4.001	65646	40.20	1616364
100	5.001	83572	50.25	2035719
120	6.001	97319	60.30	2442400
150	7.501	124917	75.38	3057282

Table 6: Robustness

System Suitability Parameters		Sitagliptin		Metformin HCl	
		Mean	% RSD	Mean	% RSD
At –5 nm	Area	117961.400	1.157	2221308.800	0.915
	Number of theoretical plates	6391.000	-	3689.400	-
	Tailing factor	1.116	-	1.147	-
	Resolution	-	-	13.422	0.452
At +5 nm	Area	43364.600	0.129	1894768.600	0.070
	Number of theoretical plates	6421.200	-	3788.800	-
	Tailing factor	1.111	-	1.149	-
	Resolution	-	-	13.430	0.484
At –2 µL	Area	65514.200	0.550	1615173.600	0.168
	Number of theoretical plates	6459.400	-	3828.000	-
	Tailing factor	1.120	-	1.150	-
	Resolution	-	-	13.489	0.493
At +2 µL	Area	97117.600	0.863	2444164.800	0.093
	Number of theoretical plates	6453.200	-	3749.600	-
	Tailing factor	1.137	-	1.133	-
	Resolution	-	-	13.389	0.626

Specificity

To assess the strategy specificity, blank solution/mobile phase, working placebo solution and standard solution having a concentration of 5 and 50 ppm of the sitagliptin and metformin respectively as well as formulations were introduced into the LC system.

Accuracy

Accuracy is represented and determined by recovery experiments. In this process, it was tested at five different levels that were 50, 80, 100, 120 and 150% in 3 replicates and analyzing chromatogram.

Precision

Precision and intermediate precision of the analytical method were established for both system and method by using concentration of 5 and 50 ppm of sitagliptin and metformin by six replicate injections. Method precisions were achieved by individual sample preparations from the same batch of drug.

Limit of detection (LOD)

LOD is based on signal to noise ratio. It is achieved by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration so that analyst can be reliably detected.

Limit of quantification (LOQ)

LOQ is based on signal to noise ratio. It is achieved by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration so that analyst can be reliably quantified.

Linearity

It was demonstrated by preparing and analyzing the standard preparation at 5 different concentrations. The represented methodology shows outstanding linearity over a range of 2.5, 4, 5, 6, 7.5 ppm for sitagliptin, and 25, 40, 50, 60, 75 ppm for metformin.

Robustness

The robustness of the method was studied by negligible variations in the technique such as altering the wavelength of detection at ±5 nm and injection volume at ± 2µL.

RESULTS

Results found from formulation analysis are shown in table 1. The chromatogram of the standard solution of sitagliptin and metformin HCl, is shown in fig. 1. The findings of the system suitability study are summarized in table 2. The outcomes of the recovery study are displayed in table 3. Calibration curves of sitagliptin and metformin

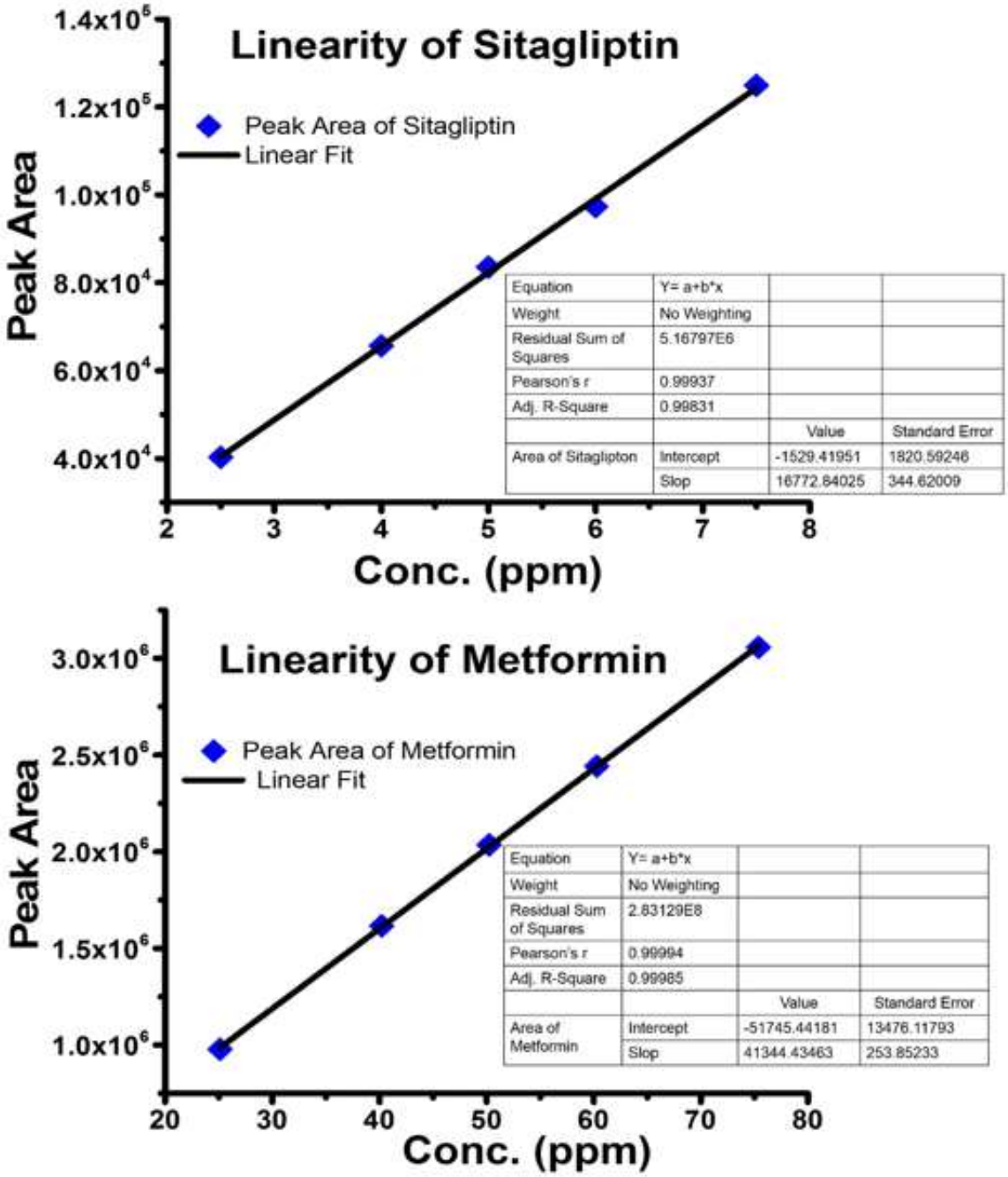

Fig. 2: Calibration curves of sitagliptin and metformin HCl

HCl are shown in fig. 2. The precision of results obtained is shown in table 4. The linearity data and robustness are given in tables 5 and 6 respectively.

DISCUSSION

Method development and optimization of chromatographic techniques

During the method development, several trials were made to achieve the most optimized chromatographic conditions. The λ-max was obtained from UV spectra of drugs and the chromatography was initiated by using stationary phase C18 (150 x 4.6mm, 5µm) and mobile phase of different compositions of few percent of ortho-phosphoric acid, phosphate buffer of acidic pH, methanol, and acetonitrile.

The C18 stationary phase column provides long retention of Sitagliptin peak with an unsymmetrical peak shape. Thus, C8 column was used to minimize the interaction of Sitagliptin with the C18 stationary phase in order to achieve suitable and earlier elution of the Sitagliptin peak.

However, due to this step, Metformin HCl peak was eluted quite earlier with the diluent behavior. To attain resolution and suitable retention of metformin HCl peak in column, 250 x 4.6mm, a 5µm column was used. Different compositions of mobile phase were used such as ortho-phosphoric acid (0.1%): phosphate buffer (0.05M, pH 3.0):ACN=20:20:60, ortho-phosphoric acid (0.05%): phosphate buffer (0.05M, pH 3.0): ACN=30:30:40, ortho-phosphoric acid (0.1%):phosphate buffer (0.05M, pH 4.0):ACN=30:30:40 and ortho-phosphoric acid (0.05%): phosphate buffer (0.05M, pH 4.0):MeOH=30:40:30 and similar. The most optimized resolution, peak shape and elution time were achieved at ortho-phosphoric acid (0.1%), phosphate buffer (0.05M, pH 3.0) and ACN. After further optimization of the mobile phase, the finalized chromatographic conditions were achieved with a Luna phenomenex C8 (250 x 4.6 mm, 5µm) analytical column using ortho-phosphoric acid (0.1%): phosphate buffer (0.05M, pH 3.0): acetonitrile (35:35:30% v/v) in isocratic mode at a flow rate of 1.0 mL/min, column at ambient temperature and detection of both drugs were monitored at 210 nm using a PDA detector by injecting 10 µL of solution.

Analysis of formulations

The drug was analyzed by this developed technique and the percentage of the assay was calculated. Results were found within the limit and shown in table 1. The chromatogram of the standard solution is shown in fig. 1.

Analytical method validation

System Suitability

System suitability was achieved by checking various parameters and found within the ICH limit (table 2) ICH Q2 (R2).

Specificity

It had been found that chromatograms of diluent and placebo solution did not show any interference at the retention time of the sitagliptin and metformin. Consequently, it can be concluded that the main excipients that were present in the formulation do not interfere with the analytical method for the determinations of Sitagliptin and Metformin.

Accuracy

A recovery study was conducted at three different levels and mean recovery results for the sitagliptin and metformin were found to be 100.36 and 100.20% respectively as shown in table 3.

Precision

Precision and intermediate precision of the analytical method were established for both the system and method. The relative standard deviation was found to be less than 2.0% as depicted in table 4.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection of Sitagliptin and Metformin was found 0.201 and 0.101 ppm and the limit of quantification was 0.301 and 0.303 ppm respectively indicating our method was extremely rapid and sensitive.

Linearity

Linearity was performed at five different concentrations for both components of the drug. The described method shows outstanding linearity over a range of 2.5, 4, 5, 6 and 7.5 ppm for Sitagliptin and 25, 40, 50, 60 and 75 ppm for Metformin (figs. 2 and 3). The correlation coefficient, intercept and slope values were also computed and summarized in table 5.

CONCLUSION

It is concluded that the developed analytical method by using RP-HPLC for the estimation of combination of Sitagliptin and Metformin HCl in bulk and pharmaceutical solid dosage form proved to be a sensitive, robust, accurate, simple, precise, selective and rapid approach. This method is quite Adaptable as compared to the other reported methods. The mobile phases were economical and simple in preparation. The results of accuracy were found satisfactory with respect to their label claims and no interference was observed from the excipients of the formulation. The validation of analytical method was carried out by following ICH guidelines and all the acceptance criteria were found satisfactory in all tested parameters. Hence, this method can be effectively used for the determination of combination of Sitagliptin and Metformin HCl in bulk and pharmaceutical solid dosage forms. The method can also be implemented during clinical trials in therapeutic drug monitoring, in bioanalytical labs, in quality control labs of pharmaceutical industries, in research labs, and clinical pharmacokinetics and bio-equivalence studies.

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