Research Article

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DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF SITAGLIPTIN PHOSPHATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM

*Prakash V Diwan, Surendra kumar N, Sanayaima Huidrom, Anusha N, Prasad V V L N
School of Pharmacy, Anurag group of institutions,
Venkatapur (V), Ghatkesar, Rangareddy district, Andhra Pradesh, India - 501301.

Abstract

A simple, precise, selective and accurate isocratic reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Sitagliptin phosphate and Metformin hydrochloride in the tablet dosage form. A chromatographic separation was achieved on reverse phase phenomenex Luna C18 column (250 ×4.6 mm, 5µ). The mobile phase consists of mixture of 0.1% v/v triethylamine and acetonitrile (70:30 v/v). The pH of 0.1% v/v triethylamine was adjusted to pH 3.5 using 0.1%v/v orthophosphoric acid. The flow rate was 1ml/min and the effluents were monitored at the detection wavelength of 254nm.The retention times of Metformin and Sitagliptin were found to be 2.13 and 4.08 min respectively. The method was validated for the linearity, accuracy, precision, robustness, system suitability as per ICH guidelines. Sitagliptin phosphate and Metformin hydrochloride were found to be linear in the range of 10-100 and 5-50µg/ml with the recoveries of 99.21% and 101.69%. The method was also applied for the determination of Metformin and Sitagliptin in the presence of their degradation products formed under variety of stress conditions.

Key words: Degradation, Metformin hydrochloride, Reverse phase HPLC, Simultaneous determination, Sitagliptin phosphate.

Introduction

Sitagliptin phosphate monohydrate chemically (3R)-3-amino-1-[3-(trifluoromethyl)-5,6 dihydrol [1,2,4] Triazolo [4,3-a]pyrazin-7-(2,4,5-trifluorophenyl) butane-1-one phosphate (fig:1) is an oral antidiabetic drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class used in the treatment of type 2 Diabetes. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP- and GIP inactivation, they are able to potentiate the secretion of insulin and suppress the release of glucagon. Metformin hydrochloride is chemically NN -dimethyl amido dicarbonimidic diamide hydrochloride which acts as by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity. Metformin activates

Author for Correspondence:
Prakash V Diwan,
School of Pharmacy, Anurag group of institutions,
Venkatapur (V), Ghatkesar, Rangareddy district,
Andhra Pradesh, India - 501301.
Email: diwanpv@gmail.com

AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in Insulin signaling, whole body energy balance and the metabolism of glucose and fats. The combination of Metformin and a dipeptidyl peptidase 4inhibitor has been shown to be safe, effective and well-tolerated treatment for type 2 diabetes. When both these drugs are combinedly administered the mean HbA1C levels are reduced by 0.65-1.1% from a baseline of 7.8-8.4%. The literature survey reveals that there are some of the methods have been reported for the estimation of Sitagliptin by spectrophotometry [1], UV [2-3], HPLC [4-5] and determination of Metformin by UV [6], HPLC [7-11]. None of the reported analytical methods describe a stability indicating RP-HPLC method development for the simultaneous analysis of Metformin hydrochloride and Sitagliptin phosphate in the tablet dosage form. Hence the present work aims to develop a simple, sensitive, precise, economic and validated stability indicating RP-HPLC method for the simultaneous analysis of Sitagliptin phosphate and Metformin hydrochloride in tablet dosage forms.

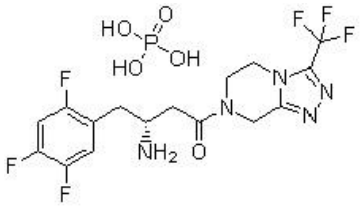

Figure No. 1:
Structure of Sitagliptin phosphate

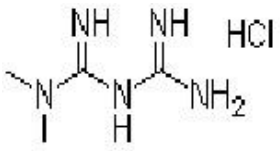

Figure No. 2:
Structure of Metformin hydrochloride

Materials and methods

Acetonitrile and water (HPLC grade) were obtained from Merck India Limited, Mumbai. Sodium hydroxide pellets, Hydrochloric acid, Hydrogen peroxide were obtained from SD Fine chemicals, Mumbai. Triethylamine (GR grade) and orthophosphoric acid 88% (GR grade) were obtained from SD Fine chemicals, Mumbai. Pure drug samples of Sitagliptin phosphate and Metformin Hydrochloride were obtained from Hetero drugs Limited, Hyderabad. JANUMET tablets (Sitagliptin phosphate 50mg and Metformin hydrochloride 500 mg) were purchased from the local pharmacy. Different kinds of equipments like analytical weighing balance, HPLC system(SHIMADZU-SPD 20A), Injector (Rheodyne, 20µl), sonicator, pH meter, vaccum filter pump, Millipore filtration kit, mobile phase reservoir, water bath, sample filtration assembly and glassware's were used throughout the experiment. The HPLC analysis was performed on a reversed-phase high performance liquid chromatographic system with Isocratic elution mode using a mobile phase triethylamine and acetonitrile in the ratio 70:30, v/v (The pH of 0.1% triethylamine was adjusted to pH 3.5 using orthophosphoric acid) with 1ml/min flow rate at 254nm using UV detector.

Preparation of mobile phase and standard solutions

Mobile phase was prepared by mixing buffer (0.1% v/v triethylamine) and acetonitrile in the ratio of (70:30, v/v). The mobile phase is then sonicated using ultra sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram. Standard stock solutions of Sitagliptin and

Metformin were prepared separately by accurately weighing 10mg of each drug and dissolving it in 10ml of mobile phase to get a concentration of 1000 µg/ml each. From these stock solutions Sitagliptin and Metformin were pipetted out in such a way after dilution with mobile phase, they would yield a concentration ranging from 10-100 µg/ml of Sitagliptin and5-50 µg/ml of Metformin. The working solutions of Sitagliptin phosphate and Metformin hydrochloride were thus prepared for the analysis.

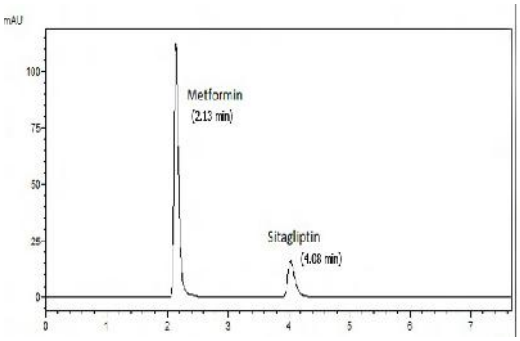

Figure No. 03: Optimized chromatogram of mixture of Sitagliptin and Metformin

Metformin, peak 1 with retention time of 2.13 min and Sitagliptin, peak 2 with retention time of 4.08 min.

Preparation of sample solution:

Twenty tablets (JANUMET) are powdered and average weight was calculated. A quantity equivalent to 10 mg of drug was dissolved in mobile phase and to it 9 mg of Sitagliptin (standard) was added (standard addition method), such that the sample contains each 10 mg of Sitagliptin phosphate and Metformin hydrochloride. The sample stock solution is filtered and finally the working solution of 30mcg/ml each of Sitagliptin phosphate and Metformin hydrochloride was prepared from the filtered solution and subjected for the analysis (table no. 01).

Table No. 01: Analysis of Formulation

Drugs	Amount (mg/tab)		% label claim	%RSD
	labeled	estimated		
Sitagliptin phosphate	50 mg	49.12 mg	98.2 mg	0.31
Metformin hydrochloride	500 mg	501.93 mg	100.386 mg	0.29

Mean of three observations*

Method validation

The proposed method was validated as per International Conference on Harmonization (ICH) guidelines [12-14].

Linearity

A series of solutions of Sitagliptin phosphate and Metformin hydrochloride and were prepared from the standard stock solutions in the concentrations ranging from 10 to 100 µg/ml and 5 to 50 µg/ml and injected into the HPLC system. The linearity of both the drugs was established by plotting their concentration verses their peak area individually and the slope, Y-intercept and the correlation coefficient were calculated and reported as required by ICH guidelines. The correlation coefficient of Sitagliptin and Metformin and were found to be 0.9989 and 0.9987(Fig: 4 and Fig: 5).

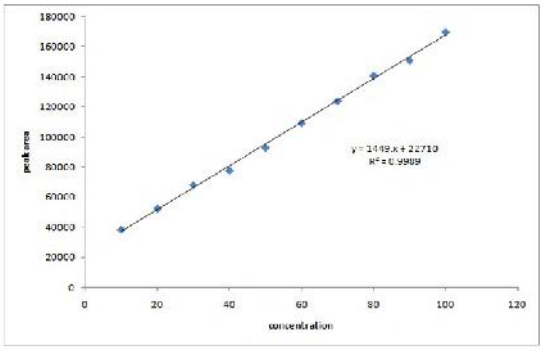

Figure No. 04:
Calibration graph of Sitagliptin Phosphate

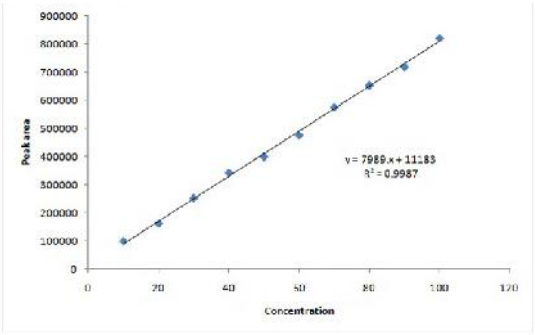

Figure No. 5: Calibration graph of Metformin hydrochloride

Limit of detection (LOD) and Limit of quantitation(LOQ):

The LOD and LOQ of Sitagliptin phosphate and Metformin hydrochloride were calculated by mathematical equation.

LOD= 3.3×standard deviation ÷ slope

LOQ=10×standard deviation ÷slope

The LOD of Sitagliptin and Metformin were found to be 0.4 µg/ml and 0.251µg/ml and the LOQ of Sitagliptin and Metformin were found to be 0.90µg/ml and 0.6 µg/ml.

Accuracy

In order to ensure the suitability and reliability of the proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder (10 mg), 9 mg of standard Sitagliptin was added to it (standard addition method), such that sample contains 10 mg each of Sitagliptin and Metformin. Then a known quantity of standard Sitagliptin and Metformin were added to 50% and 100% level and the contents were reanalyzed by the proposed method. The % recovery and % RSD were calculated (table: 2).

Precision

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done at three levels (intraday, interday and repeatability). Intraday precision was done by analyzing the intermediate concentration of each drugs (Sitagliptin 60 µg/ml and Metformin 30 µg/ml) for three times. Interday precision was measured over three consecutive days for the same drug concentrations. Reproducibility of the method was determined by performing by the same analytical procedure at different laboratories. The %RSD values are calculated for each of them and the low RSD values indicate that the method is precise (table: 2).

System suitability tests (SST):

30 µg/ml of Metformin hydrochloride and 60µg/ml of Sitagliptin phosphate were prepared from the standard stock solutions and analyzed in HPLC. The parameters like number of theoretical plates (efficiency), asymmetry factor, resolution, tailing factor were calculated from the chromatogram (table: 2).

Specificity

The specificity of the method was studied in order to asses' interference from excipients in the pharmaceutical dosage forms prepared as a placebo solution. Specificity was confirmed by obtaining the positive results from the samples

containing analyst, coupled with negative results from the samples which do not contain the analyst. The specificity of the method for the drug was established by checking for interference with drug quantization from degradation products formed during the forced degradation study.

Robustness

The robustness of the analytical procedure is a measure of its capability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during its normal usage. The conditions studied were change in mobile phase composition (68:32 and 72:28v/v of 0.1%v/v triethylamine pH 3.5: acetonitrile) and change in the PH of the buffer (3.3 and 3.7).

Forced degradation studies

Forced degradation studies were performed on Sitagliptin phosphate and Metformin Hydrochloride to prove the stability indicating property of the method. The stress conditions employed for degradation study includes light exposure, acid hydrolysis (0.1N Hcl), base hydrolysis (0.1N NaoH), water hydrolysis, Oxidation (1% Hydrogen peroxide), UV light exposure. The duration of time selected for degradation studies was 6 hours. The photolytic degradation was performed by exposing the solid drugs to sunlight for 6 hours. The solution containing the concentration of 100 mcg/ml of each of Sitagliptin phosphate and Metformin hydrochloride was prepared using respective solvents (NaoH, Hcl, water and Hydrogen peroxide) separately. For each hour solution containing final concentration of 40µg/ml of Sitagliptin and 5µg/ml Metformin was prepared from the above mentioned stock solutions using the mobile phase and analyzed in the HPLC. The % degradation of both the drugs was calculated and the degradation patterns were much more prominent in alkali hydrolysis as shown in the figure: 6.

Table No. 2:
Summary of Validation and SST Parameters

Parameter (units)	Sitagliptin	Metformin
Linearity range(µg/ml)	10-100µg/ml	5-50µg/ml
Correlation coefficient	0.9989	0.9987
Slope	1449	15979
Intercept	22710	11183
LOD(µg/ml)	0.4µg/ml	0.251µg/ml
LOQ(µg/ml)	0.90µg/ml	0.6µg/ml
Recovery (%) 50%	98.7	101.73
100%	99.21	101.69
Precision (%RSD) Intraday (n=3)	0.585	0.356
Interday(n=3)	0.921	0.397
Repeatability	0.377	0.542
Resolution	1.9	2.3
Theoritical plates	5951.547	3568.372
Assymetry factor	1.21	1.30
Tailing factor	1.3	1.02

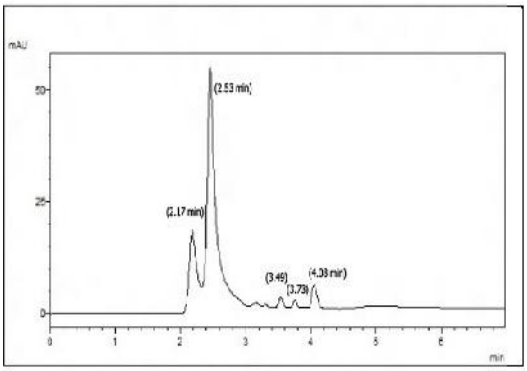

Figure No. 6:
Chromatogram of mixture of Metformin and Sitagliptin degraded with 0.1N NaoH

Metformin, peak 1 with retention time of 2.17 min shows decrease in peak area, but an additional degradation product is observed with retention time of 2.53 min, Sitagliptin peak 5 with retention time of 4.08 min.

Table No. 3: Results of stability indicating studies for Sitagliptin

Conditions	Amount found (µg/ml)							% Degraded
	Duration of time in hours							
	0 hr	1 hr	2hr	3 hr	4hr	5hr	6 hr	
Water (60˚c,6hrs)	99.89	98.91	98.77	98.62	97.67	97.40	97.01	2.99
0.1% Hcl (60˚c,6 hrs)	99.81	99.29	98.71	98.64	98.41	97.81	96.94	3.06
0.1%NaoH (60˚c,6 hrs)	98.97	95.53	92.67	91.10	87.06	84.44	79.14	20.86
1% H2O2 (6 hrs)	99.91	99.74	99.72	99.61	98.99	98.73	98.01	1.99
UV light (6 hrs)	99.91	99.86	99.84	99.67	99.21	99.09	98.94	1.06
Sunlight (6 hrs)	99.95						97.99	2.01

Table No. 4: Results of stability indicating studies for Metformin

Conditions	Amount found (µg/ml)							% Degraded
	Duration of time in hours							
	0 hr	1 hr	2hr	3 hr	4hr	5hr	6 hr	
Water (60˚c,6hrs)	99.89	99.10	98.16	98.14	97.76	97.74	96.97	3.01
0.1% Hcl (60˚c,6 hrs)	99.80	98.64	96.13	95.96	95.911	94.94	94.93	5.07
0.1%NaoH (60˚c,6 hrs)	98.91	97.69	97.19	91.94	87.68	83.27	76.33	23.67
1% H2O2 (6 hrs)	99.91	99.88	99.87	99.19	98.76	98.39	97.91	2.09
UV light (6 hrs)	99.821	99.73	99.423	99.126	98.96	98.94	98.94	1.06
Sunlight (6 hrs)	99.89						98.24	1.76

Results and discussion

The HPLC method selected for the simultaneous analysis of Sitagliptin phosphate and Metformin hydrochloride in tablet dosage form (JANUMET) was validated. Analytical conditions were selected after testing the different conditions affecting the HPLC analysis, for example, mobile phase composition, buffer concentration and other chromatographic conditions. Many preliminary trials were done but peak shapes were not good and finally the mobile phase composition of acetonitrile: 0.1%v/v triethylamine (pH 3.5) in the ratio of 30: 70, v/v showed good peak shape. The pH of the 0.1% triethylamine was adjusted to 3.5 by using 0.1% v/v of orthophosphoric acid. Acetonitrile is used to reduce the retention times and the buffer is used to reduce broadening of peaks, tailing and to improve peak shape.

System suitability was verified by measuring the peak asymmetry, no of theoretical plates, resolution after the analysis of chromatogram of the standard solutions. Sitagliptin phosphate and Metformin hydrochloride showed linearity in the range of 10-100µg/ml and 5-50 µg/ml with correlation coefficients of 0.9989 and 0.9987.

The LOD and LOQ of Sitagliptin and Metformin were calculated according to the mathematical equation. The LOD of Sitagliptin and Metformin were found to be 0.4µg/ml and 0.25µg/ml and the LOQ of Sitagliptin and

Metformin were found to be 0.900µg/ml and 0.6 µg/ml.

The % RSD for precision studies were calculated and the low RSD values obtained indicate that the method is precise (table.2).The recovery studies were determined at 50% and 100% levels .The recoveries of Sitagliptin and Metformin were calculated and tabulated in the (table 2). The forced degradation studies were carried out under various stress conditions and both the drugs Sitagliptin phosphate and Metformin hydrochloride were degraded more in alkaline hydrolysis as shown in the figure:6.

Conclusion

The proposed method is simple, sensitive and reproducible and hence can be used in routine for the simultaneous determination of Metformin and Sitagliptin in bulk as well as in pharmaceutical preparations. Statistical analysis of results has been carried out revealing high accuracy and good precision.

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