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RP-HPLC Method Development and Validation for Sitagliptin in Human Plasma

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ABSTRACT

A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Sitagliptin in human plasma was developed and validated as per US-FDA guidelines. The drug was spiked in the plasma and extracted with mobile phase by precipitation method. The extracted analyte was injected into an INTERSIL C18 column (150 mm × 4.6 mm, 5µm), maintained at ambient temperature and effluent was monitored at 267 nm. The mobile phase consisting of acetonitrile: methanol: buffer (2:3:5 v/v). The pH of the mobile phase was adjusted to 4.0 by using *O*-phosphoric acid. The flow rate was maintained at 1.0 mL/min. The developed method shows high specificity for sitagliptin. Calibration curve was plotted with a range from 25-125µg/mL (r^2>0.9994). The lower limit of quantification (LLOQ) was found to be 25µg/mL. The method was validated for parameters like accuracy, precision, recovery, linearity, range, stability and sensitivity. This RP-HPLC method is suitable for determining the concentration of sitagliptin in human plasma and it was applied to routine analysis for determination of the Sitagliptin from dosage form during pharmacokinetic study.

Keywords: Sitagliptin, RP-HPLC, Human plasma, Validation.

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INTRODUCTION

Dipeptidyl-peptidase IV (DPP4) inhibitors are a novel class of antidiabetic drugs, which include Sitagliptin, Vildagliptin[1] and Saxagliptin[2]. They increase insulin release and decrease glucagon levels by preventing the inactivation of incretin hormones, glucagon like peptide-1 and glucose dependent insulin tropic polypeptide, to treat the type 2 diabetes[3, 4]. Sitagliptin, which is the first DPP4 inhibitor approved by the United States Food and Drug Administration (US-FDA) in October 2006 [5], has been proven to be effective in reducing the levels of glycosylated haemoglobin (HbA1c), fasting plasma glucose and two hour postprandial glucose in patients with type 2 diabetes[6]. Sitagliptin chemically, 7-[(3R)-amino-1-oxo-4(2,4,5trifluorophenyl)butyl]5,6,7,8tetrahydro,3-(trifluoromethyl)-1,2,4-triazolo(4,3a) pyrazineophosphate (fig.1), having molecular weight 523.32. Sitagliptin phosphate is not official in IP, BP and USP.

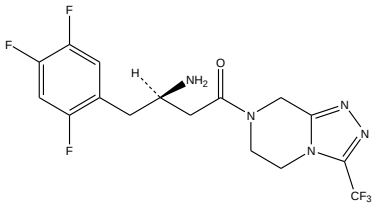

Figure 1: Chemical structure of Sitagliptin

The available HPLC bioanalytical techniques for estimation of sitagliptin phosphate were found to be tiresome. Literature survey state that validated RP-HPLC method for the quantification of sitagliptin in human plasma is not reported[8,9].

MATERIALS AND METHODS

Reagents and Materials

Dihydrogen phosphate (AR Grade), Methanol (HPLC Grade), Acetonitrile (HPLC Grade), Sitagliptin Phosphate, Human plasma (pooled).

Instrumentation

The HPLC system consisted of a HPLC Jasco-2000 series (Borwin software) connected with UV VIS Detector. Analysis was carried out at 267 nm using an INTERSIL C18 column (150x4.6mm, 5µm) at ambient temperature.

Preparation of mobile phase

Prepare 0.05M potassium dihydrogen phosphate buffer and pH adjusted to 4 with O-phosphoric acid. The mobile phase was prepared by using HPLC grade acetonitrile, methanol and buffer in the ratio of 2:3:5 v/v.

Preparation of Sitagliptin standard stock solution

Accurately weighed 10 mg of Sitagliptin phosphate were transfer into 10 mL volumetric flask. The volume was made up by using mobile phase and sonicate for 10 minutes to get a final concentration. Resulting solution was diluted and injected into HPLC system for analysis.

Extraction of Sitagliptin from Human Plasma

Serial dilutions of analyte were prepared in mobile phase and 1ml of each dilution were spiked into 100µL of plasma in a polypropylene centrifuge tubes. Then all the tubes were centrifuge for 20 min at 3000 rpm. Supernatant were collected in another eppendorf tube and 20µL supernatant was injected into the analytical column.

Method validation

The validation of the method was carried out as per US-FDA Guidelines[7]. The parameters were specificity, linearity, precision, accuracy, stability.

RESULTS AND DISCUSSION

Method development[8, 9]

Separately 20µL of sample of Sitagliptin API were injected into the HPLC system and the chromatograms were recorded. The mobile phase was Acetonitrile: Methanol: Buffer in the ratio 2:3:5(v/v), pH-4 was adjusted by using *O*-phosphoric acid. The column used was INTERSIL ODS (150 mm X 4.6 mm i.d. 5µ). The flow rate was maintained at 1mL/min during run and detection was carried out at 267 nm.

Specificity

The method was found to be precise and specific to the analytes. There is no interferences were found in the retention of drug extracted from plasma. (Figure.3)

Linearity

The linearity of the method was carried out in the range 25-125 µg/mL for Sitagliptin. Linearity solutions were injected and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/mL. The calibration curve was found to be linear for the mentioned concentrations and the correlation coefficient (r^2) of the regression line was 0.9994, (Table. 1).

Precision

The precision of the analytical method was determined by analyzing the homogeneous samples of drug concentrations was spiked in plasma with three levels of sitagliptin in six replicates. (Table. 2 and 3)

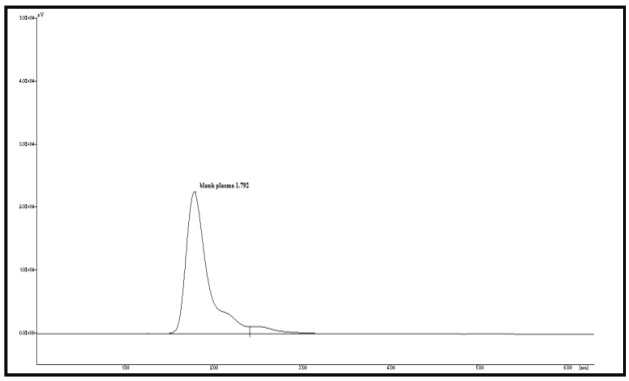

Figure 2: Typical chromatogram of blank plasma

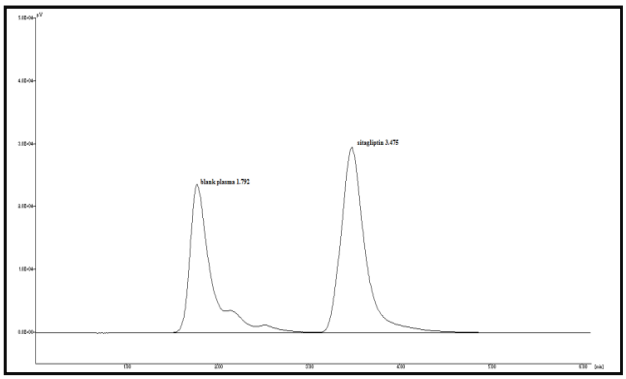

Figure 3: Typical chromatogram of sitagliptin phosphate in plasma

Table 1. Linearity data

Concentration	Peak area	r^2 Value
25 µg/mL	118891.50	
50 µg/mL	211388.57	
75 µg/mL	310670.11	**0.999**
100 µg/mL	417526.94	
125 µg/mL	511298.10	

Table 2. Precision data. Interday precision

Conc. Of Drug (µg/mL)	Sitagliptin Phosphate	
	R_t	Peak area
100	3.31	374276.95
	3.32	371682.59
	3.31	374678.58
	3.34	368977.45
	3.33	372680.67
	3.31	371879.75
Average	3.32	372362.66
SD	0.0126	2063.51
% RSD	0.3809	0.5540

Table 3. Precision data. Intraday precision

Conc. of Drug (µg/mL)	Sitagliptin phosphate	
	R_t	Peak area
100	3.31	370674.95
	3.37	375480.59
	3.33	373878.58
	3.34	371676.45
	3.35	368875.67
	3.34	371379.75
Average	3.34	371994.33
SD	0.02	2351.59
% RSD	0.5988	0.6321

Table 4. Accuracy data (Recovery)

Accuracy level	Conc. in formulation (µg/mL)	Std. Drug added (µg/mL)	Area of sample	Amount recovered (µg/mL)	% Recovery	% Average recovery	SD	% RSD
80%	0.5	0.4	636505.2	357.31	99.25	99.73	0.5061	0.5074
			642980.2	360.95	100.26			
			639340.0	358.91	99.69			
100%	0.5	0.5	714899.5	401.32	100.33	100.25	0.5326	0.5317
			709388.5	398.23	99.55			
			716663.8	402.31	100.57			
120%	0.5	0.6	777110.5	436.25	99.14	99.93	0.7089	0.7094
			787902.0	442.31	100.52			
			784807.2	440.57	100.13			

Table 5. Stability data

A) Freeze and thaw stability

Replicate No.	Concentrations			
	LQC (25 µg/mL)		HQC (125 µg/mL)	
	Standard Sample	Stability Sample	Standard sample	Stability sample
	Peak area			
1	143060.05	146315.68	208661.10	215563.09
2	145836.0	145883.76	205980.65	210030.52
3	147668.25	143019.09	207619.34	209279.45
Mean	145521.79	145071.11	207420.28	211624.35
SD	2320.15	1494.53	1351.23	3431.65
% RSD	1.6	1.2	0.7	1.6
% Mean stability	97.45%		102.02%	

B) Short term stability

Replicate No	Concentrations			
	LQC (25 µg/mL)		HQC (125µg/mL)	
	Comparison sample	Stability Sample	Comparison sample	Stability Sample
	Peak area			
1	150787.46	145104.16	163103.94	171415.41
2	144891.50	148284.39	163858.80	179414.10
3	145942.82	146868.41	161634.17	160216.79
Mean	147207.26	146752.32	162865.63	173779.56
SD	3144.78	1593.29	1131.29	4900.68
% RSD	2.1	1.1	0.7	2.8
% Mean stability	99.69%		104.59%	

C) Long term stability

Replicate No	Concentrations			
	LQC (µg/mL)		HQC (µg/mL)	
	Comparison sample	Stability sample	Comparison sample	Stability sample
	Peak area			
1	154157.04	153660.07	389335.91	403211.62
2	155260.24	157919.63	371137.80	405030.95
3	160713.74	157384.97	382035.85	392530.20
Mean	156710.50	156323.39	384568.21	400257.35
SD	3510.36	2316.81	4131.61	6753.65
% RSD	2.2	1.5	1.1	1.7
% Mean stability	99.75%		105.09%	

Accuracy

The accuracy of the method was studied by recovery studies. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations. Three samples were prepared for each recovery level. The recovery values for sitagliptin ranged from 99.16-100.05%. (Table.4)

Stability

The stability of the drug extracted, was subjected to freeze and thaw stability at -20ºC ± 2ºC, short term stability for period of 24 hours stored at room temperature, long term stability for period of 15 days stored at 4ºC. All the stability parameters compared against the freshly weighed stock solution assessed for stability. (Table.5)

CONCLUSION

A simple bioanalytical method is developed to quantify Sitagliptin in human plasma. The validated method covers the wide range of linearity over 25-125 µg/mL and is therefore suitable for the determination of Sitagliptin in human plasma at different therapeutic dose levels. Samples were prepared by using protein precipitation method for analysis.

The mobile phase used is acetonitrile: methanol: buffer (2:3:5 v/v). The % mean recovery was found to be in the range of 99.73% -100.25%. The developed method was simple, selective, precise and accurate. Sitagliptin has been found to be stable when subjected under different stability conditions. The proposed method can be applied to monitor plasma concentrations of Sitagliptin in pharmacokinetic studies. It can also be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis.

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