Research Article

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SIMPLE, RAPID RP-HPLC METHOD FOR ESTIMATION OF SITAGLIPTIN FROM URINE AND ITS APPLICATION IN PHARMACOKINETICS

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Abstract: RP-HPLC method was developed and validated for the estimation of Sitagliptin from urine. The chromatographic separation was carried out on a reverse phase Enable C18 column (250 × 4.6mm, 5µ). A mixture of Potassium dihydrogen phosphate solution (10mM, pH 3) and acetonitrile (70:30 v/v) was used as mobile phase, pumped at 1.0 ml/min and the detection was carried out at 267 nm. Sitagliptin eluted at 6.3 min and analysis can be completed within 8minutes. The method was validated according to USFDA guidelines and found to be linear in the range of 1-150µg/ml. The obtained results indicate that method is linear, precise and accurate. The developed method was used for estimation of sitagliptin in real biological samples and pharmacokinetic studies were done.

Keywords: Sitagliptin, RP-HPLC, Pharmacokinetics, Urine.

INTRODUCTION

A bioanalytical method is used for quantitative measurement of analyte in the different biological fluids such as blood, plasma, serum, saliva and urine. It is also used for determination of pharmacokinetic parameters like bioavailability, bioequivalence and drug interaction studies. Among all biological fluids plasma is preferred for drug estimation in the body because it is direct approach for assessing the pharmacokinetics in the body. Estimation of drug in plasma has some disadvantages such as it is invasive method hence less patient compliance and more matrix interference. Therefore good extraction procedure is required for sample preparation. Measurement of drug in urine is an indirect method to ascertain the bioavailability of a drug but it is non-invasive and involves simple matrix. The rate and extent of drug excreted in the urine reflects the rate and extent of systemic drug absorption. The determination of drug excretion in urine can thus be used to establish various pharmacokinetic parameters.

Sitagliptin phosphate (2*R*)-4-oxo-4- [3-(trifluoromethyl) -5, 6-dihydro [1,2,4] triazolo [4,3*a*] pyrazin -7 (8*H*)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine [Fig.1] is an oral anti- hyperglycemic of the dipeptidyl peptidase-4 (DPP-4) inhibitor class.

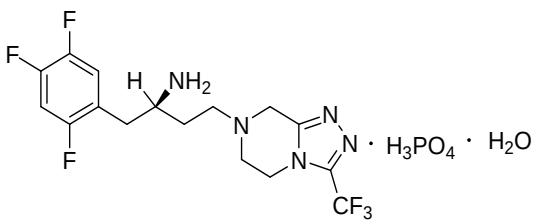

Fig. 1: Chemical structure of sitagliptin phosphate

The sitagliptin is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or simvastatin) for treatment of type 2 diabetes mellitus. Sitagliptin competitively inhibit the enzyme DPP-4. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, it leads to increased secretion of insulin and suppression of glucagon secretion by the pancreas which results in decrease in the blood glucose levels towards normal [1-4].

For the determination of sitagliptin UV [5,6,7], RP-HPLC[8,9] methods are reported for its analysis alone or in combination with other drugs. Few bio analytical methods are reported for estimation of sitagliptin in human and rat plasma but these are cumbersome and are costly [10, 11]. In the present study a simple, sensitive, accurate and reproducible bio analytical method for estimation of sitagliptin in diabetic rat urine was developed and validated.

MATERIALS AND METHODS

Materials:

Pure sample of sitagliptin phosphate was obtained as a gift sample from Watson Pharma Pvt. Ltd. Ambernath, Maharashtra, India. Methanol, acetonitrile (HPLC grade), potassium dihydrogen phosphate and ortho phosphoric acid were obtained from Thomas Baker (Chemicals) Pvt. Ltd. The urine was collected from diabetes induced Wistar rats housed in metabolic cages.

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Instrumentation:

The analysis was carried on JASCO HPLC 2000 Series having PU-2080 HPLC isocratic pump, a JASCO UV- 2075 variable wavelength detector and Rheodyne injector (20µl). Borwin software version 1.5 was used for data analysis. Separation was made on the Enable C18 column (250x4.6mm, 5µm) at ambient temperature.

Preparation of Sitagliptin Standard Solution:

Stock solution was prepared by taking 10 mg Sitagliptin phosphate in 10 ml of volumetric flask and making the volume up to 10ml with mobile phase to get concentration of 1000 µg/ml.

Preparation of mobile phase:

10mM Phosphate buffer solution was prepared by dissolving 0.68gm of potassium dihydrogen phosphate in 500ml water. The pH of Phosphate solution was adjusted to 3.0 with o-phosphoric acid. Phosphate buffer and HPLC grade Acetonitrile were mixed in 70:30 v/v. Mobile phase was filtered through 0.22µ membrane filters and sonicated before use.

Preparation of spiked urine samples:

The diabetic rat urine was diluted with double distilled water (1 to 10 ml) and filtered. The spiked urine samples were prepared by taking 1, 25, 50, 75, 100, 125 and 150µl of sitagliptin standard stock solution (1000 µg/ml) and 999, 975, 950, 925, 900, 875 and 850µl of diluted urine to obtain concentrations 1-125µg/ml.

Development of bioanalytical method:

Sitagliptin is a weakly basic, polar compound having pKa 7.7 and it is soluble in water but slightly soluble in methanol. Potassium dihydrogen phosphate and acetonitrile was used in various proportions at different pH to have optimum system suitability parameters.

Validation:

Method was validated according to USFDA guidelines [12]. System suitability parameters [13] were calculated from the chromatogram. Selectivity of the method for sitagliptin was checked by interference of drug peak with urine matrix. The standard curve consisting of seven points ranging from 1 to 150µg/ml was developed. Spiked Sitagliptin samples at LQC (25 µg /ml), MQC (75µg /ml) and HQC (125µg/ml) were used to determine the Precision (intra as well as inter-day) and accuracy. Stability was evaluated at LQC (25 µg /ml) and HQC (125µg/ml) levels. For this spiked samples were exposed to freeze thaw conditions (3cycles), short term storage at room temperature (up to 4hrs) and long term storage at room temperature (7 and 21 days). Stock solution and processed samples were tested up to 6hrs for stock and post preparative stability respectively.

Estimation of sitagliptin from diabetic rat urine:

All the experimental procedures and protocols used in this study were reviewed and approved by Institutional Animal Ethics Committee [SIOP/IAEC/2012/52]. Six rats of weight (200-250gm) were selected for study. Rats were made diabetic by a single Intra peritoneal injection of Alloxan monohydrate (60mg/kg) [14]. Blood glucose levels were measured daily for 3 days prior and 7days after Alloxan administration. Rats with blood glucose level higher than 150mg/dl were chosen for study and sitagliptin was injected to them intravenously (5 mg/kg) [10]. Treated rats were kept in metabolic cages. Urine samples were collected at 30 min intervals after drug administration. Urine concentration of sitagliptin was analyzed by developed bio analytical method.

RESULTS AND DISCUSSION

Development of Bioanalytical method:

After several trials, 10mM potassium dihydrogen phosphate (pH 3): Acetonitrile (70:30 v/v) was chosen as the mobile phase, which gave good resolution from urine matrix and acceptable peak parameters. Cationic form of sitagliptin was found to have good retention on said mobile phase.

Validation:

Selectivity: For selectivity, the samples of the urine were obtained from six different diabetic rats. Each blank sample was tested for interference of matrix at retention time of sitagliptin. The urine matrix and sitagliptin peak were well resolved. Results are shown in fig. 2 and fig. 3.

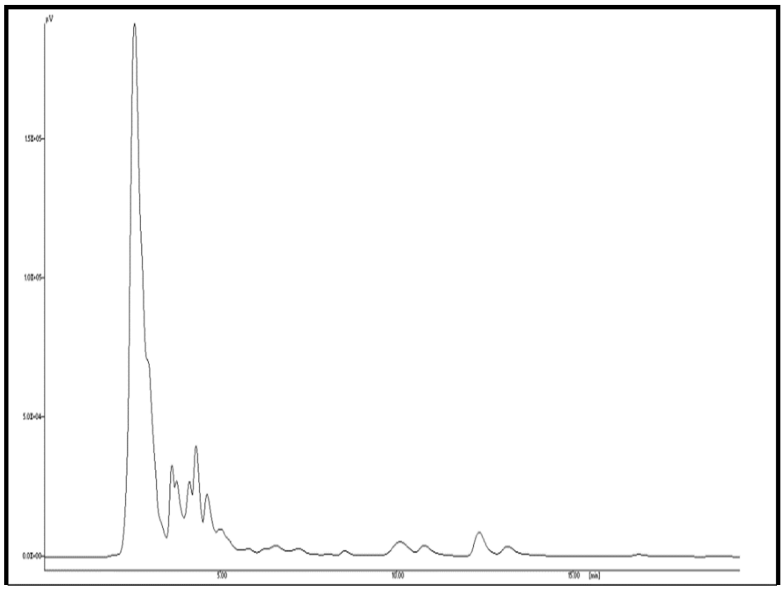

Fig.2A: typical chromatogram of blank rat urine

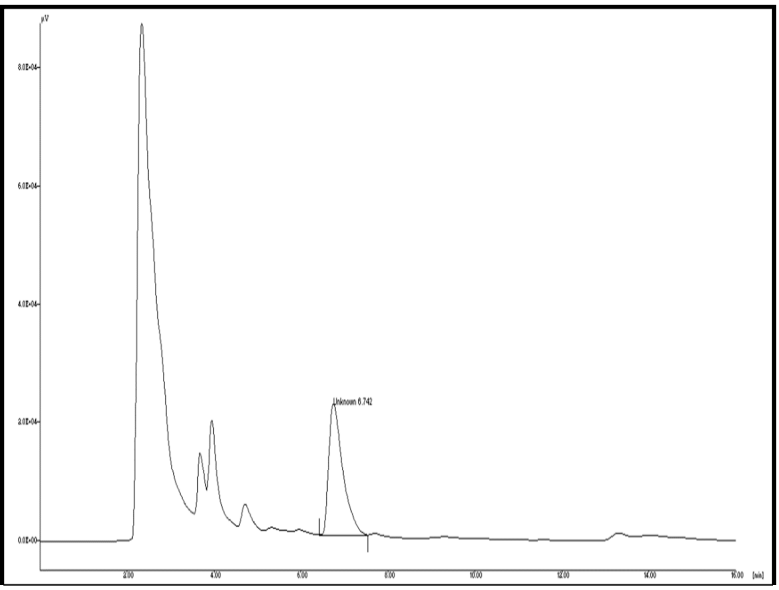

Fig.3A: typical chromatogram of Sitagliptin spiked in urine

Linearity and Range: Linearity range of sitagliptin was observed at 1, 25, 50, 75, 100, 125 and 150µg/ml. This range was selected because pharmacokinetic data of sitagliptin in rat (oral dose 80mg/kg) shows 87% absorption and 79-85% excretion in urine. For all curves the correlation coefficient (r^2) is more than 0.997.The result obtained is shown in Table 1 and Fig. 4.

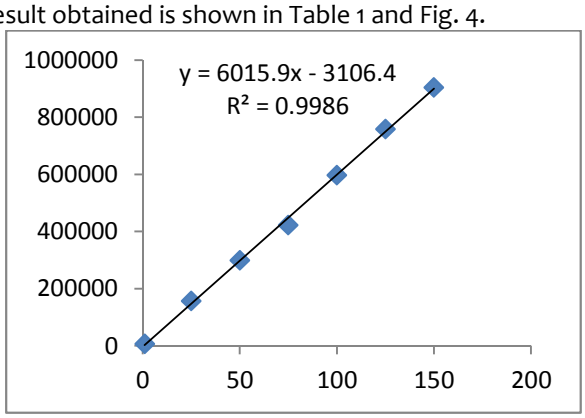

Fig.4: Calibration of Sitagliptin spiked in urine

Table.1: Linearity of Sitagliptin spiked in urine

Sr. No.	concentration (µg/ml)	Mean Area
1	**1**	**6272**
2	25	156846
3	50	299007
4	75	422013
5	100	596523
6	125	757915
7	150	904032

Accuracy and Precision: %CV of Intra and inter-day precision was 2.51, 1.72, 0.72 and 0.75, 0.44, 0.32 for the spiked concentration at 25.0, 75.0 and 125.0µg/ml respectively. The % accuracy of sitagliptin was 93, 94 and 98 for the spiked concentration at 25, 75 and 125 µg /ml respectively. The results are shown in table 2. Hence method is accurate and reproducible.

Table 2: Results of precision for Sitagliptin

variables	Precision						Accuracy		
	Intra-day(µg/ml)			Inter-day(µg/ml)					
Concentrations	25	75	125	25	75	125	25	75	125
Found conc.	23	70	123	23	72	122	24	66	116
Precision (%CV)	2.51	1.74	0.72	0.75	0.44	0.32	3.18	1.36	0.73

Recovery: The percent recovery of sitagliptin from rat urine was found to be 81.47%, 88.18% and 90.60% for LQC, MQC and HQC level. The recovery of sitagliptin was consistent and reproducible. Results are shown in Table 3.

Table.3: Recovery result of Sitagliptin

Conc.	Avg .peak area standard (n=3)	Avg. peak area after spiking (n=3)	% Recovery	% CV
25	153841	125345	81.47	1.68
75	395346	348638	88.18	1.01
125	741175	671571	90.60	3.06

Stability: The stability studies were conducted for sitagliptin spiked in urine. Sitagliptin did not show significant alteration in its concentration even after three cycles of freeze and thaw. The % mean stability for short-term temperature, stock solution and post-preparative stability study was within the 90-100% and sitagliptin samples were stable in urine matrix for time period under the indicated storage condition. It is evident from long-term stability study that sitagliptin samples are stable up to 7 days but concentration decreased up to 30% within 21 days. Results are shown in table 4.

Table.4: Stability study of Sitagliptin

	Conc.µg/ml	Area of standard	Avg. area of stability samples	%Mean stability
Freeze and Thaw	25	147712	135653	91.83
stability (3 cycles)	125	736224	706362	95.94
Short term	25	135667	125377	92.41
Stability (4hrs)	125	730855	677908	92.75
Long term	25	154095	140600	91.24
Stability (7days)	125	704331	694172	98.55
Stock solution	25	156161	151957	97.30
stability (6hrs)	125	686011	677809	98.80
Post prepara-	25	152558	151044	99.0
tive stability (6hrs)	125	732237	727403	99.33

Estimation of sitagliptin from diabetic rat urine:

Urine drug concentration-time curve shows initial rapid increase in drug concentration, reaching maximum within 2-2.5 hrs followed by progressive decrease. Drug gets completely eliminated by 12 hrs. The peak plasma concentration (C_{max}) and time needed to reach the peak plasma concentration (T_{max}) was noted directly from the generated data. The area under the plasma level-time curves ($AUC_{0\text{-}84h}$) was calculated using Trapezoidal rule. The elimination rate constant (K_{el}) was calculated from the semi-log plot of the data

using slope of the terminal elimination phase. Results are shown in fig. 5 and table 5.

Table.5: Excretion pattern of sitagliptin in rats (5mg/kg i.v.)

Sample	Time of urine collection 't' (hrs)	Average (µg/ml)	Concentration
1	0		0
2	0-1.0		0.7
3	1.0-1.5		1.5
4	1.5-2.0		2.27
5	2.0-2.5		3.33
6	2.5-3.0		2.27
7	3.0-3.5		2.11
8	3.5-4.0		1.77
9	4.0-6.0		1.41
10	6.0-8.0		0.89
11	8.0-12.0		0.51
12	12.0-24.0		-
13	24.0-48.0		-

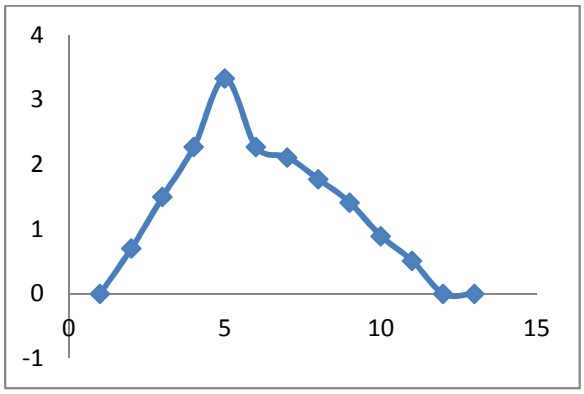

Fig.5: Urine drug concentration-time curve

Table.6: Pharmacokinetic parameters of sitagliptin Excretion

Pharmacokinetic Parameter	Result
C_{max} (µg/ml)	3.33
T_{max} (hrs)	2.5
AUC_{0-48h}(µg hrs/ml)	19.56
K_{el} (hrs^{-1})	0.06

CONCLUSION

Developed bioanalytical method is selective, reliable, accurate, precise and reproducible for quantification of sitagliptin from urine. The method was found to be suitable for pharmacokinetic studies in rat urine. Excretion pattern of sitagliptin in the wistar rats was studied after I.V. administration of dose 5mg/kg. It was observed that sitagliptin starts excreting in about 1 hr reaching to its maximum concentration in 2-2.5 hrs followed by continuous decreasing over the time period of 10 hrs and it is completely eliminated in 12 hrs. Hence developed method is suitable for estimation of sitagliptin from real biological samples.

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