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Stability Indicating RP-HPLC Method for the Assay of Sitagliptin Phosaphate Monohydrate in Pharmaceutical Dosage Form

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ABSTRACT

Presently a stability indicating RP-HPLC method was developed for the estimation of Sitagliptin Phosphate monohydrate in pharmaceutical dosage forms . A 0.1 M KH_2 PO_4 solution was used as the buffer solution .Buffer and acetonitrile were mixed in the ratio 50:50 ,the resulting solution was sonicated to dissolve and degassed by vacuum filtration through 0.4 µ membrane filter and the resulting solution was used as mobile phase and diluent. Chromatograms were recorded using Inertsil ODS, C_{18} ,150 mm x 4.6 mm , 5 µ column at a temperature of 30^0 C with isocratic elusion .The detection wave length was 268 nm at a flow rate of 1.0 mL per minute .Each injection volume was 20 µL. The retention times for Sitagliptin Phosphate standard and sample were 3.613 and 3.618 min respectively. Linear calibration curve was obtained over the concentration range of 2.5 -15.0 µg /mL with a slope, intercept, Standard deviation and correlation coefficient of respectively 35792,10262,669649.4 and 0.999 respecively. The present method was validated as per ICH guidelines for Specificity, Linearity, Accuracy ,Precision, Ruggedness and found suitable for the determination of Sitagliptin Phosphate .The stability indicating studies under different stress conditions revealed that the drug was stable and the % of degradation of the drug was in the range 4.75 -11.57 % .The limit of detection was 61.74 µg-mL and limit of quantification was187.09 µg – mL . The present method was rapid and sensitive for the determination of Sitagliptin Phosphate monohydrate.

Introduction

Sitagliptin phosphate monohydrate was described chemically as 7-[(3R)-3-amino-1-oxo-4-(2,4,5 trifluorophenyl) butyl]-5, 6, 7, 8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate. The empirical formula $C_{16}H_{15}F_6N_5O{\bullet}H_3PO_4{\bullet}H_2O$ and the molecular weight 523.32.

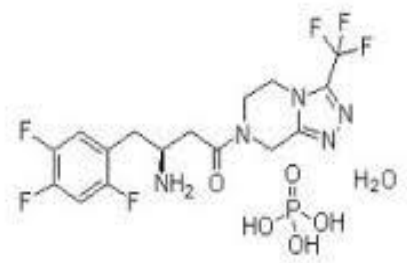

Figure 1. Chemical Structure of Sitagliptin Phosphate monohydrate

It is a well known oral hyperglycemic drug of dipeptidylpeptidase 4 inhibitar class.Sitagliptin increased incretin levels which inhibit glucogon release ,inturn decrease blood glucose,but more significantly increases insulin secretion.In the market, it is available in the form of Januvia tablets of dosage 25 mg,50 mg and 100 mg.As per the available literature, Sitagliptin Phosphate was detrmined in bulk and pharmaceutical formulations mostly by chromatographic methods either individually[1-12] or in combinations with other drugs[13-25]. However, no stability indicating RP-HPLC method is available for the estimation of Sitagliptin Phosphate monohydrate alone in its bulk or in formulations and hence we report a stability indicating RP-HPLC for the routine ,rapid and sensitive analysis of the drug.

Chemicals, Reagents and Method

Sitagliptin Phosphate Monohydrate standard was donated by Hetero drugs, Hyderabad and the formulation Januvia was procured from the local market. All the chemicals used in the method development and validation were of Merck grade and Ultra pure water was used in this method.

Buffer Solution

0.1M Potassium Dihydrogenorthophosphate solution was prepared and used as buffer.

Mobile Phase(Diluent)

Buffer and Acetonitrile were mixed in the ratio 50:50 ,Sonicated to dissolve and degassed by vacuum filtration through 0.4µ membrane filter. It was used as mobile phase and diluents .

Preparation of Stock Solution

Accurately 100mg of Sitagliptin Phosphate Monohydrate was transferred into a 100ml volumetric flask and 50mL diluent was added, sonicated to dissolve and made upto the mark by the diluent.

Preparation of Standard solution

1 mL of the Stock solution was taken in a 100mL volumetric flask and made upto the mark with the diluent.

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Preparation of Sample Solution

20 Januvia tablets were crushed into powder in mortar and pestile and accurately 100mg of Sitagliptin Phosphate

Monohydrate sample was taken in 100mL volumetric flask,50mL of diluent was added, sonicated to dissolve and made upto the volume by the diluent and filtered.1 mL of the filtrate was diluted to 100mL by the diluent.

Method

In this method Waters HPLC2 2695 series containing 4 pump auto sampler with 5 racks, each has 24 vials holding capacity with temperature control was used. Each time 20 µL of solution was injected on to the Inertsil ODS, C_{18}, 150 mm x 4.6 mm,5 µ column at a temperature of 30^0C was used at a flow rate of 1.0 mL / minute.0.1M Potassium Dihydrogenorthophosphate was used as buffer and a 50 :50 ratio mixture of buffer and acetonitrile was used as mobile phase (Diluent). Waters(Alliance), HPLC system was equipped with empower software-2 software. The UV detection wavelength was 268 nm.

Results and Discussion

In the presently developed method, Sitagliptin Phosphate Monohydrate was detected at a UV wavelength of 268 nm using Inertsil ODS ,C_{18},150 mm x 4.6 mm , 5µ column at a flow rate of 1.0 mL / minute and at an ambient temperature of 30^o C. 0.1 M Potassium Dihydrogenorthophosphate solution was used as buffer and a mixture of buffer and acetonitrile in the ratio of 50:50 was used as mobile phase .In the concentration range of 2.5 µg / mL-15µg / mL, linear calibration curve was obtained with slope ,Intercept, Standard deviation and Correlation Coefficient of 35792,103262,669649.4 and 0.999 respectively for Sitagliptin Phosphate Monohydrate. The drug was stable and in degradation studies the % of degradation of the drug in different conditions was found to be in the range 4.75-11.57.The % recovery was 99.96.

Validation of the method

1)Specificity: The specificity of the present method was determined by injecting 20 µL of blank, Standard and sample solutions onto the column and Chromatograms were recorded. In the Chromatograms, no additional peaks were observed except that of the Drug .The chromatograms were shown in Figures 2-4.The retention times of standard and sample were 3.613 min and 3.618 min respectively and the peak areas of standard and sample were 1453411,1455644.The results of specificity were shown in Table 1.

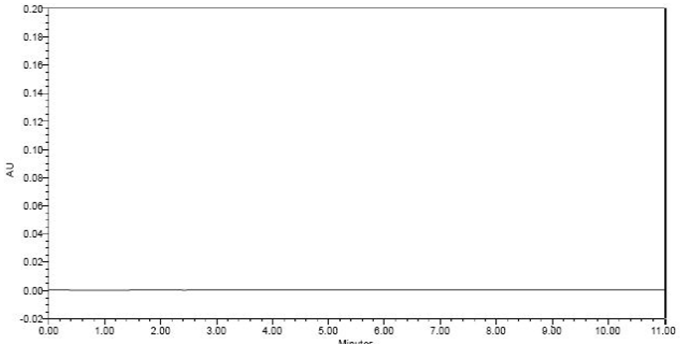

Figure 2. Chromatogram of the Blank

2)Linearity The linearity of the present method was tested from the chromatograms of the solution of concentrations of 25% to 150% of the target concentration of the Sitagliptin Phosphate monohydrate and plotting Concentration vs Peak area Graphs. The regression equation of the Calibration curve was found to be y=35792x + 10262.The slope, Intercept, Standard deviation and Correlation Coefficient were 35792, 103262, 669649.4 and 0.999 respectively for Sitagliptin Phosphate Monohydrate**.**

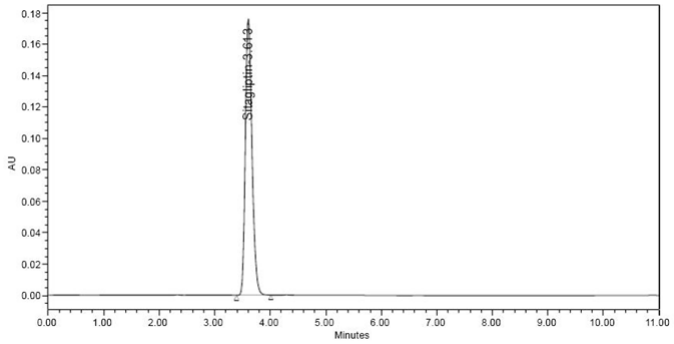

Figure 3. Chromatogram of sitagliptin phosphate monohydrate standard

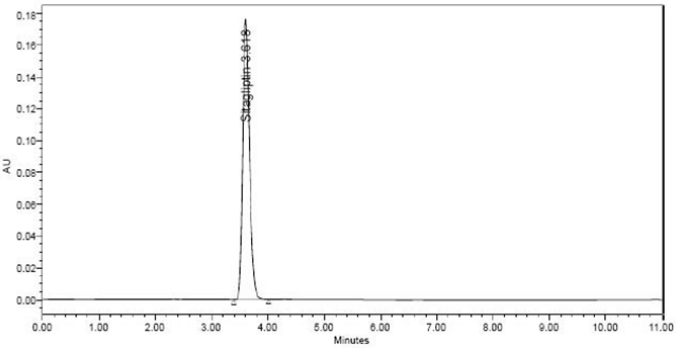

Figure 4. Chromatogram of Sitagliptin Phosphate Monohydrate Sample

The calibration curve was shown in Figure 5.

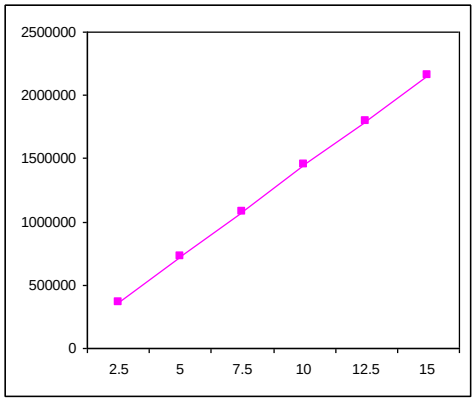

Figure 5. Linearity curve of Sitagliptin Phosphate Monohydrate

3 a) System Precision: It was evaluated by making six identical solutions of 10µg/mL concentration, 20 mL of each was injected into the HPLC system six times separately and Chromatograms are recorded under identical conditions. By using statistical methods mean, standard deviation, %RSD were calculated and were shown inTable 3

3b)Method Precision: By transferring 1mL of stock solution into 100mL volumetric flask and diluted upto the mark.6 solutions of 10µg/mL concentration were prepared.20µL of each solution was injected into the HPLC system under identical conditions and chromatograms were recorded. The results were shown in Table 3.

The %RSD in RT and Peak Area for system precision and method precision were less than 2 and hence the present method was precise.

4)Accuracy : By studying % recovery, the accuracy of the present method was tested. Solutions of 80%,100% and 120 %with respect to the precision concentration were prepared and each solution was injected three times into the HPLC system and chromatograms were recorded. The results were shown in Table

4.The percentage recovery was with in the limit 100 ± 2, hence the method is considered to be accurate.

5) Ruggedness: Under the identical experimental conditions the analysis was carried out with different instruments / on different days / with different columns and the ruggedness was determined. The results were shown in Table 5.

The % RSD for retention time and peak area were less than 2 hence the method is rugged.

6) Robustness: The robustness of the method was studied by making intentional changes in the experimental conditions in the present method, the flow rate and the temperature, were altered and chromatograms were recorded..The results were shown in Table 6.RT and area show some difference from the standard RT and area.

7) Degradation Studies: The degradation of the drug under different conditions was studied and % of drug degraded was calculated from the concerned chromatograms.

7a) Acid Hydrolysis:100 mg of Sitagliptin Phosphate was taken in a 250mL RB flask and 100mL of 0.1 N HCl solution was added and kept it for 10 hours, filtered and the solution was neutralized with NaOH solution .1mL of the filtrate was diluted to 100mL with the diluent. The result of Acid Hydrolysis was shown in Table 7 and the chromatogram in Figure 6.

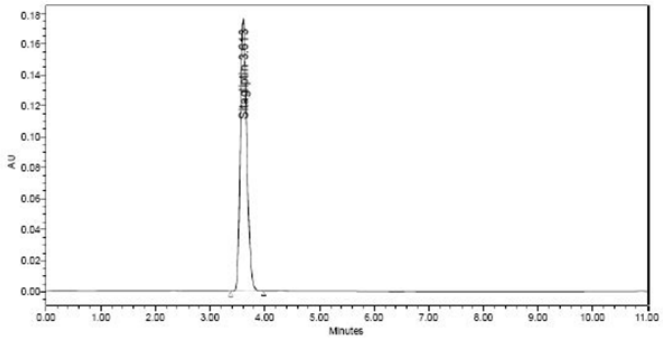

Figure 6. Typical chromatogram of Acid Hydrolysis

7b) Base Hydrolysis :100 mg of Sitagliptin Phosphate was taken in a 250mL RB flask and 100mL of 0.1 N NaOH solution was added and kept it for 10 hours, filtered and the solution was neutralized with HCl solution .1mL of the filtrate was diluted to 100mL with the diluent.The result of Base Hydrolysis was shown in Table 7. And the chromatogram in Figure 7.

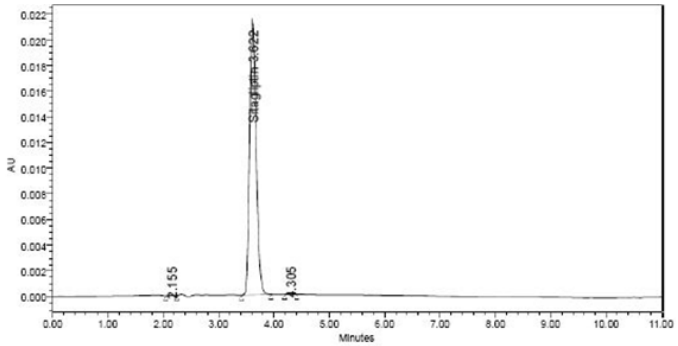

Figure 7. Typical Chromatogram of Base Hydrolysis

7c) Oxidative Degradation: 100 mg of Sitagliptin Phosphate was taken in a 250mL RB flask and 100mL of 1% H_2O_2 solution was added and kept it for 10 hours and filtered. 1mL of the filtrate was diluted to 100mL with the diluent. The results of Oxidative Degradation were shown in Table 7 and the chromatogram in Figure 8. **7d) Thermal Degradation:** On a clean dry petridish 100mg of Sitagliptin Phosphate was taken and spread it through out the plate. The petridish was kept in a oven at a Temperature of 100^0 C for 12 hours.

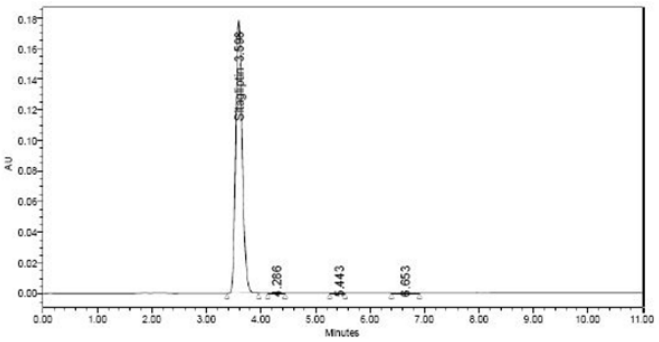

Figure 8. Typical Chromatogram of Oxidative Degradation

After 12 hours the contents were transferred to a 100mL volumetric flask sonicated with 50mL diluent to dissolve and made upto the mark and filtered the solution.1mL of the filtrate was diluted to 100mL with the diluent. The results were shown in Table 7 and chromatogram in Figure 9.

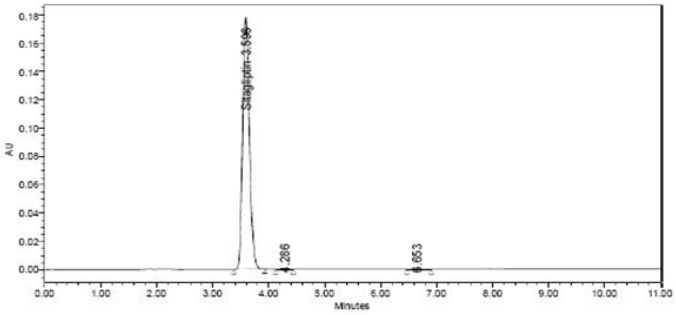

Figure 9. Typical Chromatogram of Thermal Degradation

7e) UV Degradation:- 100 mg of Sitagliptin phosphate was taken on a clean dry petridish and it was kept in UV chamber for 10 hours. After exposure the contents were transferred into a 100mL volumetric flask, sonicated by 50mL of diluent to dissolve and filtered.1mL of the filtrate was diluted to 100mL by the diluent and chromatogram was recorded. The results of UV degradation were shown in Table 7 and chromatogram in Figure 10.

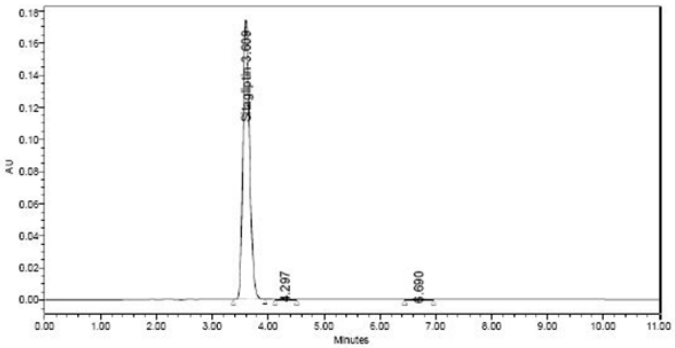

Figure 10. Typical Chromatogram of UV degradation.

As per the degradation studies, the drug was least degraded in Base Hydrolysis and most degraded in Oxidation with Hydrogen Peroxide.

Determination of Assay of Sitagliptin Phosphate Sample

Nearly 20 Januvia tablets were crushed into powder and powder equivalent to 100mg of Sitagliptin was transferred into a 100mL volumetric flask,50mL of diluen was added ,sonicated to dissolve degassed and filtered.

Table 1. Results of specificity

S.no	Name	RT in min	Peak area
1	Blank	----	-----
2	Sitagliptin Standard	3.613	1453411
3	Sitagliptin Sample	3.618	1455644

Table 2. Results of Linearity Studies

S.no	Level of the solution	Volume of the stock solution in mL	Final Volume in mL	Concentration of solution µg/mL	Peak Area
1	25%	0.25	100	2.5	362394
2	50 %	0.5	100	5.0	731125
3	75 %	0.75	100	7.5	1080074
4	100 %	1.0	100	10.0	1453666
5	125%	1.25	100	12.5	1797233
6	150%	1.5	100	15.0	2153477

Table 3. Results of system precision and method precision

Property	System Precision		Method Precision	
	RT in min	Peak Area	RT in min	Peak Area
Average	3.613	1450316	3.612	1453006
Std.Dev	0.0042	3782.0113	0.0023	4084.8168
RSD	0.117	0.261	0.062	0.281

Table 4. Results of Recovery studies of Sitagliptin Phosphate

S.no	Level of the solution	Amount added(µg)	Amount recovered(µg)	Percentage recovered
1	80%	8	7.93	99.23
2	100%	10	9.982	99.82
3	120%	12	11.976	99.8

Table 5. Results of Ruggedness

S.no	Intraday Precision		Interday Precision	
	RT	Peak Area	RT	Peak Area
1	3.613	1453422	3.615	1454665
2	3.608	1444213	3.612	1455222
3	3.614	1450452	3.608	1448875
4	3.615	1454265	3.613	1453162
5	3.618	1451825	3.612	1456102
6	3.607	1447721	3.615	1453345
Avg	3.613	1450316	3.613	1453562
Std.Dev	0.0042	3782.0113	0.0026	2553.3847
RSD	0.117	0.261	0.072	0.176

Table 6. Results of Robustness Studies

S.no	Parameter	Retention Time Min	Peak area
1	Standard	3.612	1450316
2	Robustness Flow 1	3.173	1249667
3	Robustness Flow 2	4.201	1666822
4	Robustness Oven Temp-1	3.611	1432445
5	Robustness Oven Temp-2	3.508	1419081

Table 7. Results of Degradation Studies

Acid Hydrolysis

	Peak Area	Found Assay (µg)	Percentage Assay	Percentage Degradation
Degraded API	1365175			
Standard	1454285	93.87	99.96	6.09

Base Hydrolysis

	Peak Area	Found Assay (µg)	Percentage Assay	Percentage Degradation
Degraded API	1388766			
Standard	1454285	95.21	99.96	4.75

Oxidative Degradation

	Peak Area	Found Assay (µg)	Percentage Assay	Percentage Degradation
Degraded API	1285425			
Standard	1454285	88.39	99.96	11.57

Thermal Degradation

	Peak Area	Found Assay (µg)	Percentage Assay	Percentage Degradation
Degraded API	1345112			
Standard	1454285	92.4	99.96	7.56

UV Degradation

	Peak Area	Found Assay (µg)	Percentage Assay	Percentage Degradation
Degraded API	1337057			
Standard	1454285	92.3	99.96	7.93

Table 8. Results of Assay of Januvia tablets

S.no	Formulation	Dosage(mg)	Found Assay (mg)	Percentage Assay
1	Januvia	25	24.995	99.98
2		50	49.865	99.73
3		100	100.23	100.23

The solution was made upto the mark by the diluent. 1 mL of the above solution was transferred in to a 100Ml volumetric flask and made upto the mark by the diluent.20 µL of the solution was injected into the HPLC system and chromatogram was recorded. The results were shown in Table 8.

Conclusion

A simple , rapid and sensitive stability indicating RP-HPLC method was developed for the determination of Sitagliptin Phosphate in pharmaceutical dosage form . The presently developed method was found to be simple, rapid , sensitive , accurate , precise and rugged. Hence it can be used for the analysis of Sitagliptin Phosphate in quality control.

Acknowledgements

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