IJPSR (2024), Volume 15, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 July 2024; received in revised form, 30 August 2024; accepted, 31 August 2024; published 01 September 2024

METHOD DEVELOPMENT AND VALIDATION OF DISSOLUTION METHOD FOR SITAGLIPTIN TABLETS BY UPLC

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Keywords:

Sitagliptin, Dissolution, USP type 1 Dissolution apparatus, Ultra performance liquid chromatography

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ABSTRACT: A fast, efficient and defined method was developed for the real-time assessment of Sitagliptin in tablet dosage form. Dissolution method was developed and carried out to predict *in-vivo* drug release profiles of Sitagliptin tablets. Rate and extent of dissolution of Sitagliptin tablets was studied in different dissolution medias i.e. 0.1 N HCL, pH 4.0 Acetate buffer and pH 6.8 Phosphate buffer using USP Type 1 dissolution apparatus with a RPM of 100. Ultra performance liquid chromatography was performed using BEH C18 Column (2.1x 50mm, 1.7µm). Moving phase containing Phosphate Buffer of pH 7.2, Acetonitrile and Methanol in ratio 60:20:20v/v/v was pumped through the column at 1 mL/min flow rate. Run time is 3 minutes. Injection volume is 1.7µL and column is maintained at a temperature of 25°C. Selected wavelength is 210 nm. The Dissolution method was developed and validated and was found to be Precise, Accurate, Linear in a Concentration Range of 25 % to 175 % of Test concentration. Sitagliptin retention time was found to be 1.592 minutes. As the Retention time and Runtime are less, the method is modest and efficient and can be used in industries for routine quality control tests.

INTRODUCTION: Chromatography is a separation technique where a mixture is distributed between a stationary phase (which has a large surface area) and a mobile phase (which moves through the stationary phase). The separation of components occurs due to their differential affinities for the stationary phase and the mobile phase. Ultra Performance Liquid Chromatography (UPLC) is an advanced form of High-Performance Liquid Chromatography (HPLC). It operates on the same basic principles as HPLC but uses columns with much smaller particle sizes (around 2 µm) and operates at higher pressures (up to about 6000 psi). These factors contribute to increased separation efficiency and faster analysis times compared to traditional HPLC. Dissolution is a *in-vitro* method to study rate and extent of drug release with respect to time. In the pharmaceutical industry, drug dissolution is routinely used to provide critical *in-vitro* drug release information to predict *in-vivo* drug release profiles. USP has listed 7 types of dissolution apparatus. Selection of dissolution apparatus is based on the characteristics of the dosage form being tested and the requirements for the dosage form.

The USP type 1 dissolution apparatuses i.e. basket is chosen for this study based the type of formulation and nature of drug. Sitagliptin is an antidiabetic drug used to treat type 2 to diabetes. It's often prescribed alongside diet and exercise to help control blood sugar in adults with type 2 diabetes. Sitagliptin can be used alone or in combination with other diabetes medications. Common brand names for Sitagliptin include Januvia and Janumet (which combines Sitagliptin with metformin). By inhibiting the enzyme

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DOI: 10.13040/IJPSR.0975-8232.15(9).2868-74

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(9).2868-74

dipeptidyl peptidase 4 (DPP-4), Sitagliptin prevents the breakdown of incretin hormones like GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory polypeptide). These hormones play a crucial role in regulating blood glucose levels by enhancing insulin secretion from beta cells in the pancreas and reducing glucagon release from alpha cells. As a result, Sitagliptin helps lower blood glucose levels in people with type 2 diabetes.

A literature survey revealed that a dissolution method for Sitagliptin tablets using Ultra Performance Liquid Chromatography (UPLC) was not available. Therefore, a dissolution method was developed specifically for Sitagliptin tablets using UPLC. The developed method was thoroughly validated in accordance with International Council for Harmonisation (ICH) guidelines.

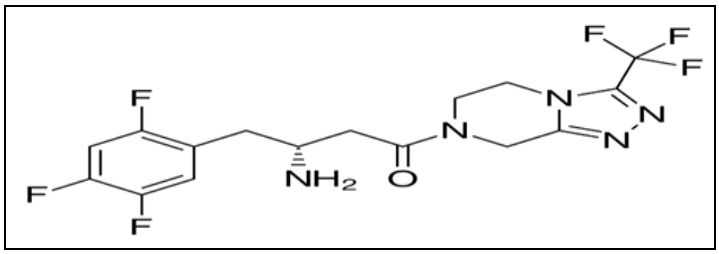

FIG. 1: STRUCTURE OF SITAGLIPTIN

MATERIALS AND METHODS:

Materials: Sitagliptin API, Marketed formulation Januvia 100mg tablets containing 100mg of Sitagliptin, HPLC grade Acetonitrile and Water are procured from Qualigens and Millipore respectively. Potassium dihydrogenphosphate, Dipotassium hydrogen phosphate, Orthophosphoric acid, Methanol and Hydrochloric acid were purchased from Merck.

Instruments: UPLC systems (Make: Waters, Model: 2695 Series, Software: Empower, Injector: Auto Sampler, Detector: PDA Detector), Dissolution Apparatus USP Type-1 (Make: Lab India, Model: DS8000), Weighing Balance (Make: Sartorius, Model: CPA 224S and CPA 225D), Sonicator (Make: Dhaikhan Labtech Power, Model: Sonic 420), pH-meter (Make: Thermoscientific, Model: Orion versastar)

Chromatographic Conditions: Column: Waters BEH C18 (2.1 x 50mm, 1.7µm), Wavelength: 210 nm, Isocratic Flow: 60:20:20 v/v/v (Buffer: Acetonitrile: methanol), Flow rate: 1 mL/min, Injection volume: 1.7µL, Column temperature: 25°C, Sample temperature: ambient and the Run time is 3.0 minutes.

Dissolution Parameters: Dissolution Apparatus USP Type-1 (Basket), Media: 0.1 N HCl, pH 4.0 Acetate buffer, pH 6.8 Phosphate buffer, Media Volume: 900 mL, RPM: 100, Bath Temperature: 37.0±0.5ºC, Dissolution Run Time: 60 minutes.

Solution Preparation:

Preparation of Dilute Ortho Phosphoricacid: Dilute 5 mL of Orthophosphoric acid to 25 mL with water.

Preparation of Buffer: 3.48g of Dipotassium hydrogen phosphate was added to 1000 mL of Milli Q-water then pH was adjusted to 7.2 with dilute OPA after that Filtered through 0.22µm filter and degassed in sonicator for 10 min.

Preparation of Diluent/ Blank: Water: Acetonitrile (50:50 v/v)

Preparation of Moving Phase: Phosphate Buffer of pH 7.2, Acetonitrile and Methanol in ratio 60:20:20 v/v/v.

Preparation of Standard Solution (Concentration 111 ppm): Transfer 46.3 mg of Sitagliptin API into 25 mL volumetric flask, dissolve and dilute to volume with diluent. Transfer 3 mL of above solution into 50 mL volumetric flask. Dilute up to volume with diluent.

Sample preparation (Concentration 111 ppm): 6 tablets were transferred individually into dissolution vessel containing 900 mL dissolution media maintained at a temperature of 37°C±0.5ºC. Dissolution was carried out with USP Type 1 Basket apparatus at a 100 RPM and the samples were withdrawn at regular time intervals (5, 10, 15, 30, 60 minutes) and are filtered through PVDF 0.45 µm filter.

Placebo Preparation: Taken placebo weight equivalent to 100 mg of Sitagliptin tablet weight, transferred into dissolution vessel containing 900 mL dissolution media maintained at a temperature of 37°C±0.5°C. Dissolution was carried out with USP Type 1 Basket apparatus at a 100 RPM and the samples were withdrawn at regular time intervals (5, 10, 15, 30, 60 minutes) and are filtered through PVDF 0.45 µmfilter.

Preparation of Dissolution Media:

pH 6.8 Buffer: 68.021 gm of Potassium dihydrogen phosphate and 9.023 gm of NaOH were dissolved in 10 liters of water and pH was made to 6.8 with diluted OPA or NaOH

pH 4.0 Buffer: 29.013 gm of Sodium acetate trihydrate was dissolved in 10 liters of water and adjusted pH to 4.0 with glacial acetic acid.

0.1 N HCl: 85 mL of Concentrated HCl was measured accurately and dissolved in 10 liters of water.

Needle Wash: Prepared needle wash by mixing methanol: water in the ratio of 80:20 % v/v and sonicated to degas for 5 minutes.

Seal Wash: Prepared seal wash by mixing methanol: water in the ratio of 20:80 % v/v and sonicated to degas for 5 minutes.

Selection of Dissolution Media and Dissolution Time Point: Slower dissolution profile was observed in pH 6.8 Phosphate buffer than 0.1 N HCl and pH 4.0 Acetate buffer. 100 % drug release was achieved at 30 minutes time point in pH 6.8 Phosphate buffer. Slower dissolution profile usually presents higher discriminative power, hence dissolution samples of pH 6.8 Phosphate buffer at 30 minutes time point are considered for method validation.

Method Validation:

System Suitability: Standard solution was prepared and injected into UPLC and % RSD of peak areas, tailing factor, Retention time of five standard injections was checked.

Specificity:

Blank Interference: Dissolution media i.e. pH 6.8 Phosphate buffer was injected into UPLC and checked for blank interference.

Placebo Interference: Placebo weight equivalent to1 tablet placebo weight was transferred into 900 mL of dissolution media. Dissolution was carried out, sample was collected at 30 minutes time point, filtered through 0.45 µm PVDF filter and analyzed to check placebo interference.

Accuracy:

Preparation of 25% Concentration Sample: Placebo weight equivalent to 1 tablet placebo weight and 25 mg of Sitagliptin API was weighed and transferred into 900 mL of dissolution media. Dissolution was carried out, sample was collected at 30 minutes time point, filtered and analyzed to check recovery.

Preparation of 100 % Concentration Sample: Placebo weight equivalent to 1 tablet placebo weight and 100 mg of Sitagliptin API was weighed and transferred into 900 mL of dissolution media. Dissolution was carried out, sample was collected at 30 minutes time point, filtered and analyzed to check recovery.

Preparation of 175 % Concentration Sample: Placebo weight equivalent to 1 tablet placebo weight and 175 mg of Sitagliptin API was weighed and transferred into 900 mL of dissolution media. Dissolution was carried out, sample was collected at 30 minutes time point, filtered and analyzed to check recovery.

Linearity:

Preparation of 25 % Concentration Sample: Transferred 11.57 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 50 % Concentration Sample: Transferred 23.15 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media.

Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 75 % Concentration Sample: Transferred34.71 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above

solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 100 % Concentration Sample: Transferred 46.3 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 125 % Concentration Sample: Transferred 57.85 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 150 % Concentration Sample: Transferred 69.45 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Preparation of 175 % Concentration Sample: Transferred 81 mg of Sitagliptin API into 25 mL volumetric flask, dissolved and diluted to volume with dissolution media. Transferred 3 mL of above solution into 50 mL volumetric flask and made up with dissolution media.

Precision: 6 tablets were transferred individually into dissolution vessel containing 900 mL dissolution media. Dissolution was carried out and samples were withdrawn at 30 minutes time point and are filtered through 0.45 µm PVDF filter and injected into UPLC and Relative standard deviation of six samples was measured.

Robustness: Dissolution was carried out by slightly varying dissolution parameters i.e. ±10 RPM, ±0.2 pH of Dissolution media, ±10 mL Volume of Dissolution media, ±0.5℃ Column oven temperature, ±0.1 mL Flow rate.

RESULTS AND DISCUSSION:

System Suitability: The % RSD for peak areas of five replicate standard injections was found to be 0.5, Tailing factor was 1.0, Theoretical plate count was 12333 **Table 1**.

Specificity: No Blank and Placebo peak interference was observed at the retention time of Sitagliptin peak **Table 2.**

Accuracy: Individual % Recovery, Mean of % Recovery, % RSD were passed at each level **Table 3.**

Linearity: The Square of correlation coefficient was found to be 0.999 and % Y-intercept at 100% response was found to be 0.9 **Table 4.**

Precision: The % Assay for each individual preparation and Mean of six preparations should be between 90.0 % w/w to 110.0 % w/w of labeled amount of Sitagliptin and the results were found to be within the Limits. The % RSD for % Assay of six sample preparations was found to be 0.6 **Table 5.**

Robustness: The System suitability parameters and Release of drug were not affected by the small changes in method parameters. Thus, the developed dissolution method was Robust.

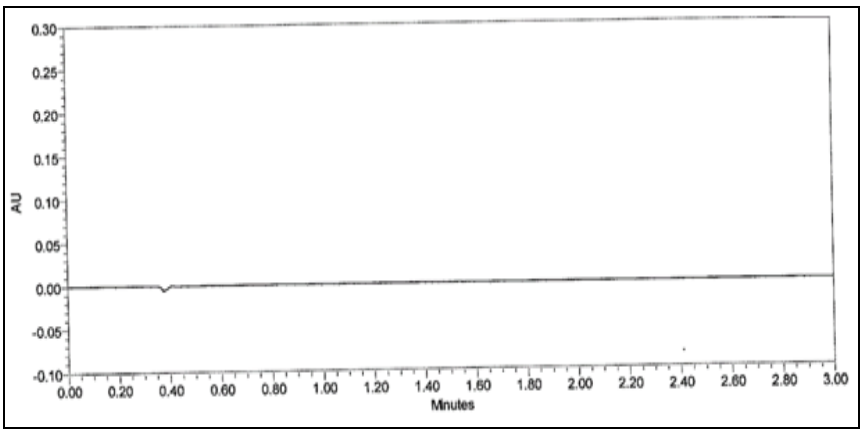

FIG. 2: BLANK CHROMATOGRAM

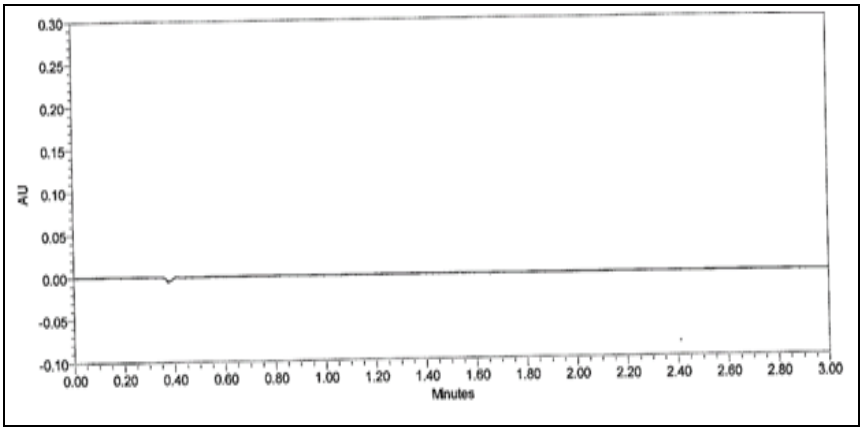

FIG. 3: PLACEBO CHROMATOGRAM

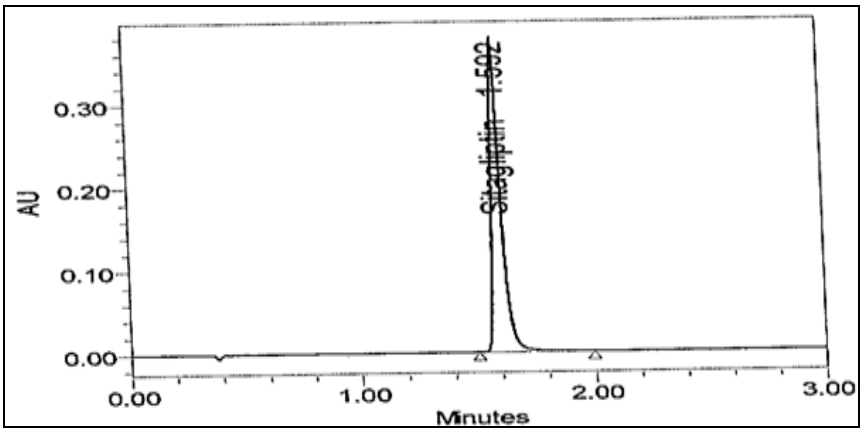

FIG. 4: STANDARD CHROMATOGRAM

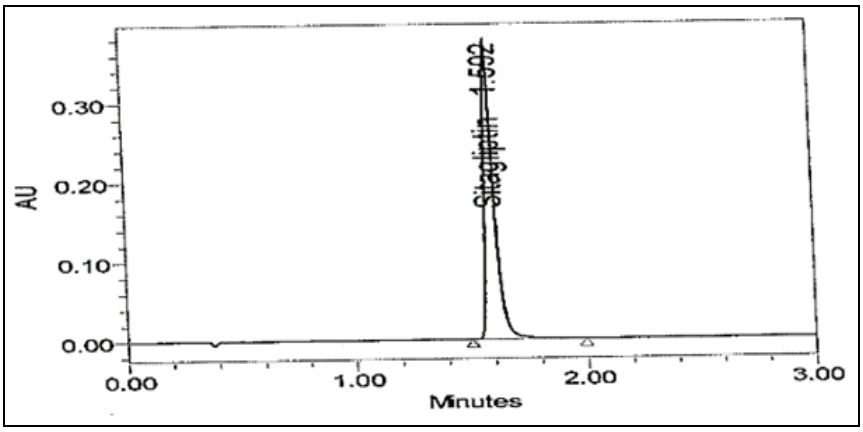

FIG. 5: SAMPLE CHROMATOGRAM

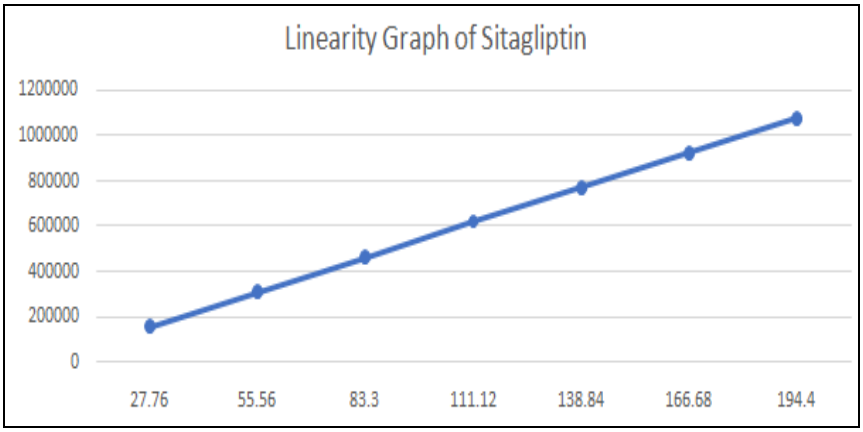

FIG. 6: LINEARITY GRAPH OF SITAGLIPTIN

TABLE 1: RESULTS OF SYSTEM SUITABILITY

Parameter	Observed Value	Acceptance Criteria
%RSD (peak area)	0.5	NMT 2.0
Tailing factor	1.0	NMT 2.0
Plate count	12333	NLT 2000

TABLE 2: RESULTS OF BLANK AND PLACEBO INTERFERENCE

Acceptance Criteria	Results
Blank interference should not be there at the retention time of Sitagliptin peak.	Blank did not interfere with the retention time of Sitagliptin peak.
Placebo interference should not be there at the retention time of Sitagliptin peak.	Placebo did not interfere with the retention time of Sitagliptin peak.

TABLE 3: RESULTS OF ACCURACY

% Level	Added Amount (mg)	Found Amount (mg)	% of Recovery (Limit:98.0 to 102.0)	Mean % of Recovery (Limit:98.0 to 102.0)	% of RSD (NMT 2.0)
25	25	24.5	98.0	99.2	1.1
	24.90	24.8	99.6		
	25	25	100.0		
100	100	99.6	99.6	99.3	0.5
	101	99.7	98.7		
	100.5	100.2	99.7		
175	175.5	175	99.7	99.9	0.2
	175	175.2	100.1		
	174.9	174.6	99.8		

TABLE 4: RESULTS OF LINEARITY

Concentration (µg/mL)	Peak Area
27.76	154568
55.56	309130
83.30	462700
111.12 (100 % Level)	620603
138.84	770900
166.68	926379
194.40	1081022
Square of correlation coefficient(R^2) (Limit: should not be less than 0.999)	0.999
% Y-intercept at 100% response (Limit: should be within ±2.0)	0.9

TABLE 5: RESULTS OF METHOD PRECISION

Sample Number	% Assay (Limit: 90.0to 110.0% w/w)
1	100.5
2	100.6
3	101.7
4	101.4
5	100.2
6	101.5
Mean	101
% RSD (NMT 2.0)	0.6

CONCLUSION: A well-defined UPLC Method was developed and validated as per ICH Q2 guidelines for Dissolution testing of Sitagliptin in Tablet dosage form. Retention time of Sitagliptin was found to be 1.592 minutes. Square of correlation coefficient (R^2) was 0.999. Run time and Retention time areless so developed method was quick and economical and can be adopted in quality control laboratories in industries for dissolution testing of Sitagliptin Tablets to obtain critical *in-vitro* drug release information to predict *in-vivo* drug release profiles.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Author declare there are no conflicts of interest.

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How to cite this article:
Shriya N: Method development and validation of dissolution method for sitagliptin tablets by UPLC. Int J Pharm Sci & Res 2024; 15(9): 2868-74. doi: 10.13040/IJPSR.0975-8232.15(9).2868-74.