Research Article

A Validated HPLC Stability Indicating Method for the Determination of Sitagliptin in Bulk Drug Substance and Tablets

Mohamed Karam Qassas[1], Mohammad Ameral-mardini[2], Heba Ghazal[3]
Faculty of Pharmacy, University of Damascus, Syria.
[1]Dept. of Pharmaceutical Chemistry and Quality control, Faculty of Pharmacy, University of Damascus, Syria.
[2]PhD - Dept. of Pharmaceutical Chemistry and Quality control, Faculty of Pharmacy, University of Damascus, Syria.
[3]Heba Ghazal, School of Pharmacy and Chemistry, Kingston University, London, United Kingdom.
*Corresponding author's E-mail: karamqassas@hotmail.com

ABSTRACT

A simple, rapid and precise reversed-phase High performance liquid chromatographic (RP-HPLC) stability indicating method was developed and validated for the determination of Sitagliptin in the bulk and tablets. The method involves the use of commonly available and inexpensive laboratory reagents. Chromatographic separation of sitagliptin was achieved using a reverse phase C18 column (250 mm × 4.6 mm, 5.0 μm) with isocratic flow using a mobile phase consisting of methanol and phosphate potassium buffer pH 6.8 (60:40, v/v). The flow rate was 1mL/min and the eluents were monitored at 260 nm. The method was successfully validated in compliance to ICH guidelines acceptance criteria in terms of linearity, accuracy, precision, limit of detection, limit of quantification and robustness where all validation parameters were within the acceptance range. The proposed method was found to be suitable and accurate for the quantitative determination of sitagliptin in bulk samples and it was successfully applied for the quantitative determination of sitagliptin in commercially available tablet dosage forms.

Keywords: Sitagliptin, RP-HPLC, Validation, stability-indicating, Tablets.

INTRODUCTION

Sitagliptin phosphate monohydrate (Fig. 1) is an oral hypoglycemic drug that belongs to a relatively new therapeutic class; dipeptidyl peptidase IV (DPP-IV) inhibitors. These agents increase insulin secretion and decrease glucagon levels resulting in a decrease in the fasting glucose concentrations in people with type 2 diabetes. Sitagliptin improves glycemic control by prolonging and potentiating the actions of incretin hormones including glucagon-like peptide-1 (GLP-1) and GIP (glucose-dependent insulinotropic peptide).[1]

Sitagliptin was approved by the FDA in 2006 either alone or in combination with metformin or thiazolidinediones to improve glycemic control.[2] It is commercially available in coated tablets containing 25, 50, and 100 mg of sitagliptin base.

The literature review reveals several methods for the determination of sitagliptin in tablets alone or in combination with other hypoglycaemic agents such as Metformin.[3] Few methods were reported using spectrophotometric method directly or after chemical derivatization[4-6], or using HPLC coupled with UV detection.[7] Other methods used sophisticated instrumental techniques such as and tandem mass[8], UPLC[9] and capillary zone electrophoresis.[10] However, only few methods used simple HPLC methods with commonly available columns and detectors like UV.

The aim of the present study is to develop a rapid, accurate and precise reverse phase HPLC stability indicating method for the determination of sitagliptin in

bulk samples and in tablet dosage forms based on materials commonly available and inexpensive. The proposed method was optimized and validated in accordance with the International Conference on Harmonization (ICH) guidelines.
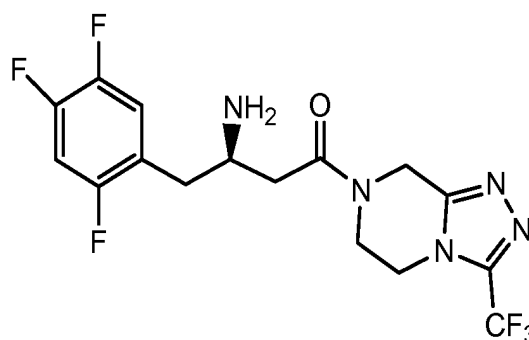
Figure 1: Chemical structure of Sitagliptin

MATERIALS AND METHODS

Chromatographic Conditions

The analysis of the drug was carried out using SHIMADZU HPLC system, UFLC-20A-DAD model, Japan equipped with an autosampler-SIL-20AC, pump-LC-20AD and a dual absorbance DAD, using reverse phase C18 (250x 4.6mm, 5μm particle size). The data were evaluated by LC solution Software.

Chemicals and Solvents

The reference sample of sitagliptin phosphate monohydrate was purchased from Sigma-Aldrich Chemical Co. (Hamburg, Germany). Sitagliptin tablets

(100 mg) were purchased from local pharmacy. HPLC grade methanol was purchased from Merck. Potassium dihydrogen ortho phosphate and orthophosphoric acid were obtained from Merck. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

Preparation of Standard Stock Solution

Accurately weighed 12.8 mg of sitagliptin phosphate monohydrate was transferred to a 100 ml volumetric flask, 50 ml of mobile phase was added and allowed to sonicate for 20 min and finally the volume was made up to the mark by the mobile phase. Standard stock solution of Sitagliptin phosphate monohydrate (100µg/ml) was prepared.

Preparation of Phosphate Buffer (pH 6.8)

27.22 g of monobasic potassium phosphate (KH_2PO_4) was dissolved in water, and diluted with water to 1000 mL. 50 mL of the monobasic potassium phosphate solution 0.2 M placed in a 200-mL volumetric flask, 22.4 ml of sodium hydroxide 0.2 M solution were added, and then water added to volume (USP32-NF27).

Preparation of Mobile Phase

400 ml of the phosphate buffer was mixed with 600 ml Methanol. The solution was degassed in an ultrasonic water bath for 15 minutes and filtered through 0.45µm filter under vacuum.

Optimization of the Chromatographic Conditions

The chromatographic conditions were optimized through several trials to achieve peaks with good resolution and symmetry within reasonable short time run. It was found that a mixture of phosphate buffer and methanol (40:60, v/v) was appropriate.
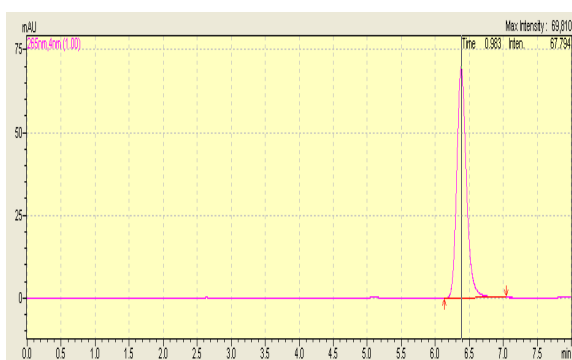
Procedure

Figure 2: Typical chromatogram of Sitagliptin

A mixture of buffer pH6.8and methanol in a ratio of 40:60, v/v was found to be the most suitable mobile phase for ideal separation of Sitagliptin. The solvent mixture was filtered through a 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1ml/min. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the

drug solution. The pump pressure was set at 98 psi. The injection volume was 50 µL. The detection of the drug was monitored at 265nm. The run time was set at 9 min.

Under these optimized chromatographic conditions the retention time obtained for the drug was 6.382 min. A typical chromatogram showing the separation of the drug is given in (Fig. 2).

Validation of the Proposed Method

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines.

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method.

Estimation of Sitagliptin in Tablet Dosage Forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to measure sitagliptin in tablet dosage forms. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to100mg of Sitagliptin was transferred into a 50mlvolumetric flask, dissolved in 25ml water and sonicated for 15 min. Then, the volume was made up to 50ml and shaken continuously to dissolve the drug.

Thereafter, the solution was filtered through a 0.45µ membrane filter and injected into the HPLC column. The peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug.

Forced Degradation Studies

Drug at a concentration of 200 µg/mL was used in all degradation studies. Solutions for stress stability studies were prepared as follows:

- Solution for acidic hydrolysis: (10) ml of stock solution was treated with 0.1 N HCl and incubated for a period of one month. Then the solution was neutralized to pH7 by 0.1N NaOH.
- Solution for basic hydrolysis: (10) ml of stock solution was treated with 0.1N NaOH and incubated for two weeks. Then the solution was neutralized to pH7 by 0.1N HCl.
- Solution for oxidative degradation: (10) ml of stock solution was treated with 33 % H_2O_2 and incubated for 24 hour at room temperature.
- Solution for thermal degradation: (10) ml of stock solution was incubated at a temperature of 80°C for two weeks and then the concentration was modified to 100 µ/ml.
- Solution for UV-degradation: (10) ml of stock solution was exposed to UV light 256 in UV chamber for two week at room temperature.

For HPLC study, the resultant solutions were diluted to obtain 100 µg/ml solutions and 50µl were injected into the HPLC system and the chromatograms were recorded to assess the stability of sample.

RESULTS

Linearity

Linearity is the ability of the method to elicit test results directly proportional to analyte concentration within a given range. Standard plots were constructed with five concentrations containing 80%-120% of the standard concentration (0.217 mg/ml). The samples were injected into HPLC apparatus and the analyses were performed in triplicate to test linearity. The peak area of sitagliptin was plotted against the concentration to obtain the calibration graph (Fig 3).

The results indicated good linearity, the equation of the regression line was y= 3268585.3x – 8194.3 with a correlation factor of (r=0.9994).
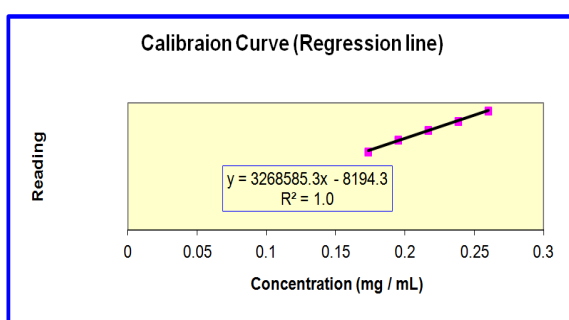

Figure 3: Calibration Curve for Sitagliptin

Accuracy and Recovery

Accuracy was determined by applying the method to samples of placebo, to which known amounts of the drug were added covering the working range 80% to 120% of the standard solution, then the recovery was calculated from the test results as the percentageof sitagliptin recovered by the assay and it was found 99.41 %.

Precision

The precision is the degree of repeatability of an analytical method under normal operational conditions. It was studied with respect to both repeatability (intraday) and intermediate (intraday) precision studies. The intra and inter-day variability or precision data are summarized in Table 1.

Repeatability

Nine samples of sitagliptin covering the working range 80% to 120% of the standard solution were injected into HPLC apparatus in the same day, then the RSD of the results was calculated and it did not exceed 2%.

Intermediate Precision

Nine samples of sitagliptin covering the working range 80% to 120% of the standard solution were injected into HPLC apparatus in the same day by different analysts

then the RSD of the results was calculated and it did not exceed 2%. The results of repeatability and intermediate precision are displayed in Table 1.

Table 1: Results of Repeatability and Intermediate precision of HPLC method

Level %	Number of samples	Repeatability %	Intermediate precision %
80	3	102.86	99.77
100	3	100.63	99.67
120	3	99.34	99.67
	Avg	100.94	99.7
	RSD	1.9	0.93

System Suitability Parameters

To ensure adequate performance of the chromatographic system, the system suitability parameter namely, tailing factor and number of theoretical plates were calculated. The system suitability parameters are given in Table 2.

Table 2: System Suitability Parameters

Correlation Coefficient	1.553
Retention time (min)	6.382
Theoretical plates (N)	10354.780
Tailing factor	1.225
LOD µg/mL	0.0011
LOQ µg/mL	0.0033

Detection Limit (LOD)

The detection limit as defined by the ICH is the lowest amount of the analyte that can be detected but not necessarily quantitated as an exact value by the method. LOD was calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula 3.3 (SD/S) and it was found 0.0011 µg/mL.

Quantitation Limit (LOQ)

The Quantitation Limit is defined as the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOQ was calculated according to the formula 10 (SD/S) and it was found 0.0033 µg/mL.

Robustness

The robustness is to evaluate the method capacity to remain unaffected by small and deliberate variations. In the present study the flow rate was increased from 0.9 t0 1.1 and mobile phase pH from 6.6 to 7. This did not cause important differences on the assay value and the relative retention time.

Tablet Assay

The results of assay show that the amount of the drug in each tablet is within the usual requirements of the tablets (90–110%) of the label claim. No interfering peaks were detected in the formulations' chromatograms within the

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
196

run time suggesting that the excipients did not interfere with the drug analysis.

Table 3: Tablet Assay Studies

Assay studies Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation I	100	99.75	99.75
Formulation II	100	108.56	108.5

Forced Degradation Studies

Forced degradation studies were performed on sitagliptin to prove the stability indicating property of the method. The stress conditions employed for degradation study of sitagliptin phosphate included acidic hydrolysis, basic hydrolysis, oxidation, thermal and uv-degradation.

The results of these studies and the chromatogram of UV-induced degraded samples are displayed in Table 4 and Figures (4).

The results indicate that the method can differentiate between the drug and its degradation products.

Table 4: Result of Forced Degradation

Degradation condition	Time	Result of Assay (%)
HCl 0.1 N	1 month	94.88
NaOH 0.1 N	2 weeks	30.09
H2O2 33%	24 h	92.48
Dry Heat 80Cº	2 weeks	97.75
UV 256	2 weeks	77.045

Figure 4: Chromatogram of sitagliptin subjected to UV-induced degradation

DISCUSSION

In the proposed method, the retention time of sitagliptin was found to be 6.38 min. The number of theoretical plates calculated was 10354.780, which indicates efficient performance of the column (N > 2000) and the tailing factor is 1.225 ($T \leq 2$) which is within than the required limit as per ICH.

The limit of detection and limit of quantification were found to be 0.0011µg/ml and 0.0033 µg/ml, respectively, which indicate the sensitivity of the method.

The high percentage of the recovery indicates that the proposed method is highly accurate. The precision results showed good reproducibility with percent relative standard deviation (RSD %) below 2.0. This indicated that the method is highly precise. Specificity of the method was proved through non-interference of the excipients or the degradation products.

The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability sitagliptin under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations.

CONCLUSION

A simple, accurate robust and precise stability indicating HPLC method has been developed for the determination of sitagliptin in bulk drug and as well in solid dosage forms.

The method uses inexpensive reagents commonly available in any analytical laboratory and so it can be reliably adopted for routine quality control analysis.

REFERENCES

1. Zerilli T, Pyon EY. Sitagliptin Phosphate: A DPP-4 Inhibitor for the treatment of type 2 Diabetes Mellitus. Clinical Therapeutics. 29(12), 2007, 2614-2634.

2. Januvia (Sitagliptin) Tablets [Prescribing Information]. Whitehouse Station NJMC, Inc., 2006, 2007. Accessed 2014, at: http://www.merck.com/product/usa/pi_circulars/j/januvia/januvia_pi.pdf. [cited 2014].

3. Chirag B. Patel MHM, Purvi A. Shah, Kalpana G. Patel, Tejal R. Gandhi. Validated HPTLC Method for the Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Marketed Formulation. International Journal in Marketed Formulation. International Journal of Analytical and Bioanalytical Chemistry. 3, 2013, 47-51.

4. Bala Sekaran. C1* Pra. Development and Validation of Spectrophotometric Method for the Determination of DPP4 Inhibitor, Sitagliptin, in its Pharmaceutical Dosage Forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2, 2010, 138-142.

5. El-Bagary RI, Elkady EF, Ayoub BM. Spectrophotometric Methods for the Determination of Sitagliptin and Vildagliptin in Bulk and Dosage Forms. International Journal of Biomedical Science: IJBS. 7(1), 2011, 55-61.

6. Sekaran CB, Rani AP. Development and Validation of Spectrophotometric Method for the Determination of DPP-4 Inhibitor, Sitagliptin, in its pharmaceutical preparations. Ecletica Quimica. 35, 2010, 45-53.

7. Nallagatla V. A novel RP-HPLC method for the quantification of Sitagliptin in formulations. The Experiment. 5, 2012, 315-323.

8. Nirogi R, Kandikere V, Mudigonda K, Komarneni P, Aleti R, Boggavarapu R. Sensitive Liquid Chromatography Tandem Mass Spectrometry method for the quantification of Sitagliptin. A DPP-4 Inhibitor in Human Plasma using liquid-liquid extraction. Biomedical Chromatography: BMC. 22(2), 2008, 214-222.

9. Malleswara Rao CSN, Suryanarayana MV, Mukkanti K. Simultaneous Determination of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Tablets by a validated UPLC Method. Scientia Pharmaceutica. 80(1), 2012, 139-152.

10. Salim M, El-Enany N, Belal F, Walash M, Patonay G. Simultaneous Determination of Sitagliptin and Metformin in Pharmaceutical Preparations by Capillary zone Electrophoresis and its application to Human Plasma analysis. Analytical Chemistry Insights. 7, 2012, 31-46.

Source of Support: Nil, **Conflict of Interest:** None.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
198