ABSTRACT
Analytical method development and Validation are the continuous and inter-dependent task associated with the research & development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. Validations determine that the analytical procedure is suitable for its intended purpose. Literature survey reveals that the analytical methods based on UV spectrometry, RP-HPLC and HPTLC for the determination of Sitagliptin personally and in combination with different drugs. The parameters were validated according to ICH guideline in terms of accuracy, precision, robustness, and other components of analytical validation. The developed methods are simple, sensitive and reproducible and can be used for the analysis of Sitagliptin in bulk and Tablet dosage form.

KEYWORDS: Sitagliptin, UV, HPLC, Validation, ICH Guidelines.

INTRODUCTION
Sitagliptin is an anti-diabetic medication used to treat type 2 diabetes. Sitagliptin is a dipeptidyl peptidase-4 inhibitor which is used in the combination with diet and exercise, either alone or in the combination with other oral hypoglycemic agents.
Sitagliptin is chemically known as 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetra -[3-(trifluoromethyl)-1,2,4,-triazolo[4,3-a]pyrazine phosphate (1:1)Monohydrate. with a molecular formula of C_{16}H_{15}F_{6}N_{5}O and a molecular weight of 407.314 g/mol. Sitagliptin substance is white crystalline powder and Soluble in water and N,N-dimethyl formamide, slightly soluble in methanol, soluble in ethanol, acetone and acetonitrile.

Review of literature

1. Safaa M. Raid\textsuperscript{[1]} et al., have developed two simple, accurate and precise spectrophotometric methods for the determination of sitagliptin phosphate monohydrate. The first method was based on measuring the absorbance of Sitagliptin phosphate monohydrate at 268nm in the range of 25-500µg/ml. The second method was the isosbestic point method. The total mixture concentration was calculated by measuring the absorbance at 257nm. The proposed methods used to determine each drug in binary mixture. The results were statistically compared using one-way analysis of variance (ANOVA). The developed methods were satisfactory applied to the analysis of the pharmaceutical formulation and proved to be selective and accurate for the quality control of the cited drugs in their pharmaceutical formulation.

2. P. Ravisankar\textsuperscript{[2]} et al., have developed a reliable UV spectrophotometric method for the estimation of Sitagliptin phosphate in tablet dosage form. The drug shows maximum absorption at 267nm in water and obeys Beer’s law in the concentration range of 2-10µg/ml with good correlation coefficient (R\textsuperscript{2} =0.9995). The results of analysis were validated by recovery studies. The Proposed spectrophotometric method was validated as
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per the ICH Q2 (R1) guidelines. The proposed method can be used for the reliable quantification of Sitagliptin in bulk form and routine analysis of pharmaceutical formulations.

3. C. Bala Sekaran[3] et al., have developed an improved spectrophotometric method for the determination of sitagliptin phosphate in bulk and in pharmaceutical formulations. The proposed method is based on condensation of the primary amino group of sitagliptin phosphate with acetyl acetone and formaldehyde producing a yellow colored product, which is measured spectrophotometrically at 430nm. The color was stable for about 1 hour. Beer’s law is obeyed over a concentration range of 5-25μg/ml. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the method was tested by analyzing sitagliptin phosphate in its pharmaceutical preparations.

4. Namratha Sunkara[4] et al., have been developed a simple UV Spectrophotometric method for the determination of Sitagliptin in bulk and its pharmaceutical formulations. Sitagliptin exhibited maximum absorption at 267nm in Aqueous solvent as water and obeyed linearity in the concentration range of 2 to 30μg/ml. The proposed method was statistically validated. From the results obtained for Accuracy, it was found that Percentage Recovery values of pure drug from the analyzed formulation was 99.75 which indicates that the method is accurate and commonly used excipients and additives present in the formulation was not interfering in the proposed method.

5. Madhuri Ajay Hinge[5] et al., has been developed a simple, accurate and precise spectroscopic method for simultaneous estimation of Metformin and Sitagliptin in marketed formulation using Q-Absorbance Ratio Method. In this spectroscopic method, 237nm and 253nm (iso absorptive point for both drugs) were selected for measurement of absorptivity. Both the drugs show linearity in a concentration range of 5-25μg/ml for Metformin and 0.5-2.5μg/ml for Sitagliptin at 237nm and 253.26nm respectively. Accuracy, precision and recovery studies were done by QC samples covering lower, medium and high concentrations of the linearity range. The relative standard deviation for accuracy, precision studies were found to be within the acceptance range.

6. Jain Pritam[6] et al., have developed a new simple, rapid, accurate and economical First order UV-derivative spectrophotometric method for estimation of sitagliptin from bulk
and pharmaceutical formulation. The $\lambda_{\text{max}}$ of sitagliptin in methanol and water was found to be 267nm. The same spectrum was derivatised in to first order derivative showed maximum amplitude of the trough at 275nm. The drug follows linearity in the concentration range 10-60µg/ml with correlation coefficient value 0.998. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D value less than 2 indicate that the method is precise. The above method was a rapid and cost effective quality-control tool for routine analysis of sitagliptin in bulk and in pharmaceutical dosage form.

7. **Amruta B. Loni**\(^{[7]} \) et al., have been developed two simple, precise and economical UV methods for the simultaneous estimation of Sitagliptin phosphate and Metformin hydrochloride in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength of 266nm and 232nm for Sitagliptin phosphate and Metformin hydrochloride respectively. Method B is area under curve in the wavelength range of 244-279nm for Sitagliptin phosphate and 222-240nm for Metformin hydrochloride. The developed method was validated with respect to linearity, accuracy, precision and specificity.

8. **Dr. Sanjeev Kumar Subudha**\(^{[8]} \) et al., have developed a simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method for the determination of Sitagliptin in bulk and pharmaceutical dosage form. The chromatographic method was standardized using develosil ODS HG-5 RP C\(_{18}\), 5µm, 15cm x 4.6mm i.d. column with UV detection at 255nm and (0.05M) Phosphate Buffer : Acetonitrile with 30:70 (pH-2.8) ratio at a flow rate of 1.0ml/min. The proposed method was successfully applied to the determination of Sitagliptin in bulk and pharmaceutical dosage form. The method was linear over the range of 30-70µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization guidelines.

9. **D. China Babu**\(^{[9]} \) et al., have developed a selective, sensitive RP-HPLC method for the simultaneous estimation of the Ertugliflozin and Sitagliptin in bulk and its dosage form. The separation and determination was carried on water’s C\(_{18}\) column, retention times of Ertugliflozin and Sitagliptin were found to be 2.39 and 4.60min. respectively. The wavelength was fixed at 215nm with PDA detection. The mobile phase was consisted mixture of 0.5M potassium dihydrogen ortho phosphate buffer: Methanol in the ratio of
55:45 v/v, pH 5.3 was adjusted with HCl and flow of mobile phase was maintained 1ml/min. The quantization limit and detection limit of the method were found 0.1 & 0.3µg/ml and 0.4 and 1µg/ml for Ertugliflozin and Sitagliptin.

10. A. S. K. Sankar[10] et al., have developed a simple, accurate, specific and reliable RP-HPLC method for the simultaneous estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Pharmaceutical dosage form. In the present method, SHIMADZU HPLC with UV detector LC 10 AT VP with analytical column PHENOMENEX Luna (C18) A 100 RP Column, 250 mm x 4.6 mm x 5µm, an injection volume of 20µl was injected and eluted with mobile phase 0.02M Potassium dihydrogen phosphate pH(4.0) Acetonitrile (60:40) pumped at a flow rate of 1.0ml/min. Sitagliptin Phosphate and Metformin Hydrochloride were eluted at 2.718 and 1.925min. The detection was carried out at a wavelength 252nm.

11. El-Zaher[11] et al., has been developed a new Liquid Chromatographic method for precise, efficient, and selective determination of sitagliptin phosphate, simvastatin were simultaneously determined with a simple reversed phase liquid chromatography method in which a sitagliptin phosphate, simvastatin binary mixture, present in a dosage form brand was considered central for its development. Chromatographic separation was achieved with a mobile phase of acetonitrile and 0.02M potassium dihydrogen phosphate (pH 5.2) (77 + 23, v/v) flowing through a C18 column at 1.2ml/min at ambient temperature. UV detection was programmed to be carried out at 210nm for Sitagliptin. The developed method is simple, rapid, accurate and suitable for the routine QC analysis of the cited drugs in pharmaceutical products by conventional HPLC.

12. Sai Datri Arige[12] et al., have developed a simple, precise and accurate RP-HPLC method for simultaneous estimation of Sitagliptin phosphate and Simvastatin. In RP-HPLC method, mixture of pH 4.0 sodium phosphate buffer and acetonitrile in the ratio of 20:80 v/v was selected as a mobile phase and equal proportions of water and acetonitrile with one drop of phosphoric acid was selected as solvent which gives good resolution and good peak shapes for Sitagliptin and Simvastatin. The linearity range was established over the range of 25-150µg/ml and 10-60µg/ml concentration range Sitagliptin and Simvastatin. The correlation coefficient of Sitagliptin and Simvastatin was found to be 1. The method validation data showed excellent results for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness.
13. Vasanth P. M. et al., have developed a simple, accurate and precise method for estimation of reversed phase HPLC method for the simultaneous determination of sitagliptin and Metformin by using hypersil BDS C\textsubscript{18} (100 x 4.6mm, 5µm particle size) column and mobile phase of at 215nm. A mobile phase has a composition of potassium dihydrogen orthophosphate and methanol(50:50v/v) adjusted the pH8.5 with o-phosphoric acid was used and flow rate 1.0ml/min. Retention times of Sitagliptin and Metformin were 2.3min and 4.6min respectively. The method developed validated as per ICH guidelines.

14. Muhammad Ashraf et al., has been developed a new HPLC method for the quantification of sitagliptin and its application in spiked plasma and tablet dosage form. The method was developed by using the C\textsubscript{18} ODS Hypersil column of 150 x 4.6mm id with 5µm particle size, mobile phase of acetonitrile and 0.01N potassium dihydrogen phosphate at a flow rate of 1.0ml/min. Eluate was detected at 269nm with the retention time of 5.6min. This developed method is more sensitive than the already reported methods and reproducible with all validation parameters with FDA guidelines.

15. R. Lavanya et al., have developed a new simple and precise reverse phase high performance liquid chromatographic method for the estimation of Sitagliptin phosphate monohydrate in bulk and its pharmaceutical dosage form. The chromatographic separation was performed by using mobile phase consisting of 0.01M KH\textsubscript{2}PO\textsubscript{4}, methanol in the ratio of 50:50%v/v and the pH 2.5 adjusted with 0.2% orthophosphoric acid. The column used was zorbax eclipse XDB C\textsubscript{18} (150x4.6 mm, 5µ) with flow rate of 0.7ml/min using PDA detection at 267nm. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, reliable, accurate and economical which is useful for the routine determination of Sitagliptin phosphate in bulk and its pharmaceutical dosage form.

16. Sai Lakshmi. E. et al., have developed a new simple, rapid, specific, accurate, precise and novel Reverse Phase High Performance Liquid Chromatography method for the estimation of Sitagliptin Phosphate in the pharmaceutical dosage form. The chromatographic separation for Sitagliptin was achieved with mobile phase containing methanol. Thermoscientific C\textsubscript{18} column at room temperature and UV detection at 248nm. The compounds were eluted in the isocratic mode at a flow rate of 1ml/min. The retention
time of Sitagliptin was 1.91 min. The above method was validated in terms of linearity, accuracy, precision, LOD and LOQ in accordance with ICH guidelines.

17. Vinit Chavhan\[17\] et al., have developed a simple and new Reverse Phase High Performance Liquid Chromatographic for the simultaneous estimation of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage form. column at ambient temperature in isocratic mode with mobile phase containing acetonitrile, methanol and 10 ml phosphate buffer (65:25:10% v/v/v) pH 4 adjusted with orthophosphoric acid, pumped at flow rate of 1.2 ml/min and eluent was monitored at 250 nm. The selected chromatographic conditions were found to be effectively separate Sitagliptin phosphate and Simvastatin with retention time of 2.2 and 6.8 min respectively. The results of validation parameters indicates that the proposed method was also found to be accurate, precise, robust and sensitive.

18. Sharifa Sultana\[18\] et al., have developed a novel reversed phase ultra-high performance liquid chromatographic for the estimation of sitagliptin in pharmaceutical dosage form. Separation was done by a X-bridge C\(_18\) column with a flow rate of 1 ml/min using phosphate buffer (pH 6) and acetonitrile (70:30 v/v) as mobile phase at 268 nm using photodiode array plus (PDA+) detector. The retention time was found at 4.607 min. The developed method was validated as per the requirements of ICH-Q2B guidelines for specificity, system suitability, linearity, precision, accuracy, sensitivity and robustness. The results showed that the proposed method is highly convenient for routine analysis of sitagliptin.

19. Chellu S. N. Malleswararao\[19\] et al., has been develop a ultra performance liquid chromatographic method for the simultaneous determination of Sitagliptin phosphate monohydrate and Metformin hydrochloride in pharmaceutical dosage forms. The chromatographic separation was achieved on aquity ultra performance liquid chromatographic, C\(_8\) 100 x 2.1 mm, 1.7 \(\mu\)m, column using a buffer consisting of 10 M potassium dihydrogen phosphate and 2 M hexane-1-sulfonic acid sodium salt (pH adjusted to 5.50 with diluted phosphoric acid) and acetonitrile as organic solvent in a gradient program. The flow rate was 0.2 ml/min and the detection wavelength was 210 nm. This method was validated with respect to linearity, accuracy, precision, specificity and robustness.
20. K. R. Patil\textsuperscript{[20]} \textit{et al.}, have developed a new simple, accurate, precise and stability indicating HPTLC method for the determination of sitagliptin in tablet dosage form. The chromatographic separation was achieved by using Toluene:Ethyl acetate: Methanol (3:6:1v/v/v) as mobile phase and UV detection at 238nm. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of condition of acid hydrolysis, alkali hydrolysis, photolysis, thermal degradation. Results found to be linear in concentration range of 100-500ng/band. The developed method can be used for the quantification of bulk drug as well in formulation.

CONCLUSION

Literature survey suggested that various UV\textsuperscript{1-7}, HPLC\textsuperscript{8-19}, HPTLC\textsuperscript{20} and few simultaneous methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis proved that the published methods were reproducible and selective. Thus, it can be concluded that the reported and published methods can be successfully applied for the estimation of the Sitagliptin in pure and pharmaceutical dosage form.

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