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Various Analytical Methods for Analysis of Sitagliptin – A Review

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ABSTRACT: Sitagliptin is a dipeptidyl- peptidase inhibitor used to treat high blood sugar levels caused by type2 diabetes. Absorption of Sitagliptin is 87% orally bioavailable and taking it with or without food does not affect its pharmacokinetics. Sitagliptin reaches maximum plasma concentration in 2 hours. Now in this present analytical research world quality by design or design by expert technique is used to get improved method for method validation. This concise review work can guide an analyst to choose most appropriate method for a best analytical method development and validation. This assessment encompasses various analytical methods such as UV Spectrophotometry, High performance liquid chromatography [HPLC], Liquid chromatography – Mass spectrometry (LC-MS), High performance thin layer chromatography (HPTLC) and Ultra performance liquid chromatrography (UPLC) for the estimation of sitagliptin in single and/or in combination.

KEY WORDS: Sitagliptin, UV - Spectrophotometry, HPLC, HPTLC, LC–MS.

INTRODUCTION

The chemical name for sitagliptin is (3R)3-[3-(trifluoromethyl) amino-1-] [1,2,4] -6,8-dihydro-5H-triazolo [4,3-a][pyrazin-7-yl]-4-(trifluorophenol, 2,4,5-)butan-1-one has a molecular weight of 407.31 and an empirical formula of C16H15 F6N5O. Dipeptidyl peptidase 4 (DPP-4) is competitively inhibited by sitagliptin. The digestive hormones known as incretins, or GLP-1 and GIP, are released after a meal and are broken down by this enzyme. They are able to limit the release of glucagon by the pancreatic alpha cells and enhance the production of insulin by blocking GLP-1 and GIP inactivation. Blood glucose levels move closer to normal as a result. The amounts of insulin produced and glucagon inhibited decrease as blood glucose levels get closer to normal, helping to avoid the "overshoot" and consequent low blood sugar (hypoglycemia) that certain other oral hypoglycemic medications cause. The composition of the chemical was shown in figure 1[(1)].

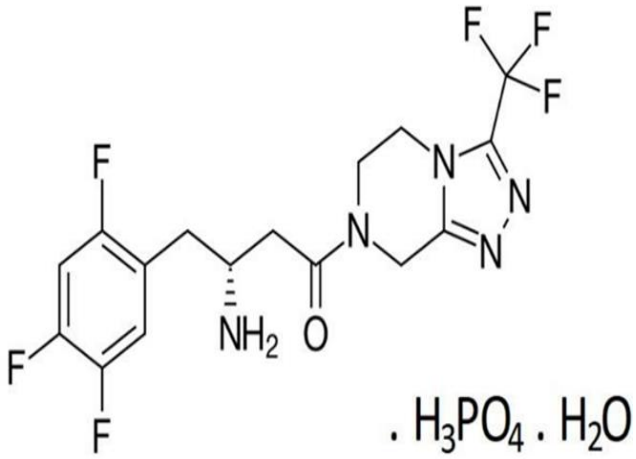

Fig. 1: **Chemical structure of Sitagliptin**

Melting point: 214.92^{O}C
Molecular weight:.407.31 g/mol
Trade Name: Januvia
Dose: 25 mg, 50 mg, 100 mg

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Solubility: The biopharmaceutical classification system (BCS) places the Sitagliptin in class-I, which denotes high permeability and solubility. A saturated water solution containing Sitagliptin has a pH of 4.4. pKa is 7.7 and the partition coefficient is 1.8. The drug's solubility was examined in solvents that are often employed in analytical techniques**.**

Side Effects:

Diarrhea
Stomach pain
Headache
Upper respiratory infection.

Previous studies to estimate sitagliptin:

In addition to various environmental models, other techniques are employed to estimate sitagliptin in pharmaceutical preparations and human serum samples. Numerous analytical techniques, including UV, LC-MS, ,HPLC, HPTLC and UPLC have been documented. An attempt has been made to assemble all of the analytical techniques that have been applied to the sitagliptin analysis in this study.

UV SPECTROPHOTOMETRIC METHOD:

K. Bhavya Sri et al created and approved a UV Spectrophotometric technique for estimating metformin and sitagliptin together in tablet form. Using distilled water as a diluent, sitagliptin demonstrates maximum absorbance at 267 nm whereas metformin exhibits maximum absorbance at 237 nm. For both sitagliptin and metformin, the calibration curve was linear between 10 and 300 µg/ml and 4 and 14 µg/ml, respectively. Less than 2% was the upper limit for the percentage RSD. For sitagliptin, the suggested method's recovery percentage was determined to be 97.12 - 99.46%, while for metformin, it was 98.15 - 99.85%. For sitagliptin, the LOD of the suggested approach was 0.397 µg/ml, and for metformin, it was 0.8952 µg/ml. Sitagliptin's LOQ was 1.2951 µg/ml, while metformin's was 2.7159 µg/ml[(2)].

Parag Pathade et al studied on a simple, sensitive, reproducible and cost effective stability indicating UV Spectrophotometric method that has been developed for quantitative determination of Sitagliptin Phosphate in bulk and pharmaceutical formulations. The UV spectrum was scanned between 200 to 400 nm and 267 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 10-100 µg/ml. Good accuracy (99.87-100.45%), precision (%RSD 1.3147-1.2957) were found, the method was successfully applied to the pharmaceutical dosage form containing the above-mentioned drug without any interference by the excipients. The limit of detection and limit of quantification was found to be 0.16 µg/ml & 0.45 µg/ml respectively [(3)].

Namratha sunkara kandala et al developed a simple UV Spectrophotometric method for the determination of Sitagliptin in bulk and its pharmaceutical formulations. Sitagliptin exhibited maximum absorption at 267 nm in Aqueous solvent as water and obeyed linearity in the concentration range of 2 to 30 µg /ml. The proposed method was statistically validated. From the results obtained for Precision, it was found that % RSD is less than 2%. It indicates that the proposed method has good reproducibility. 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 [(1)].

Gunuputi Sushma et al established five new spectrophotometric methods for the determination of Sitagliptin tablets. SHIMADZU Model No. UV – 1800 double beam spectrophotometer with quartz cells was used for the proposed study. Sitagliptin has shown absorption maxima at 267 nm in reagents such as phosphate buffers (pH 5.0 and 8.0), acetate buffer (pH 4.7), 0.1N NaOH and borate buffer (pH 9.0) Sitagliptin obeys Beer-Lambert's law over the concentration range 5 - 100 µg/ml for all the above mentioned methods and all the methods were validated as per ICH guidelines. The methods are simple, precise, accurate and economical and can be used for the quantification of Sitagliptin tablets[(4)].

P. Ravisankar et al developed UV Spectrophotometric method for the estimation of Sitagliptin phosphate in tablet dosage form. The drug shows maximum absorption at 267 nm in water and obeys Beer's law in the concentration range of 2-10 µg /mL with good correlation coefficient (R^2 =0.9995). The results of analysis were validated by recovery studies. The recovery was found to be 99.53-

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100.41. The relative standard deviation was found to be < 2.0 % in all cases. The proposed spectrophotometric method was validated as 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[5].

G Jeyabalan et al developed a method for the determination of Sitagliptin in pure and tablet dosage form. The proposed method is based on the principle that Sitagliptin exhibiting an absorption spectra of wavelength maxima 267 nm. This method has successfully used for the analysis of drug in marketed preparations in the range of 20-60 µg/ml with correlation coefficient of 0.991. The percentage recovery was found to be 99.62 - 100.48%. LOD and LOQ were found to be 6.03 and 18.28 µg/ml respectively. This method has been validated for linearity, accuracy and precision and found to be rapid, precise, accurate and economical and can be applied for routine estimation of Sitagliptin in solid dosage form. The validation of method was carried out utilizing ICH-guidelines[6].

Safaa M Riad et al developed and validated spectrophotometric method for the determination of Sitagliptin phosphate monohydrate (STA) and Metformin hydrochloride (MTF). The first method was based on measuring the absorbance of STA at 268 nm in the range of 25-500 µg mL-1. The second method was the isosbestic point method. The total mixture concentration was calculated by measuring the absorbance at 257 nm. 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[7].

Shinde G S et al developed a simple, sensitive, accurate, precise and rapid UV spectrophotometric method for the determination of Metformin in human urine. Extraction of Metformin from urine samples was done by dilute and shoot method in which urine was diluted upto 1:10 ratio to avoid interference of matrix. For estimation of Sitagliptin the solvent system employed was water. The Sitagliptin was scanned in range of 200 nm to 400 nm and wavelength of detection (λ_{max}) was found to be 234 nm. The calibration curve was linear in the range of 1-17 µg/ml. The recovery and assay studies of Sitagliptin were within 93-94.85% indicating that the proposed method can be used for the estimation of Sitagliptin[8].

HPLC METHOD

R. Lavanya et al developed and subsequently validated a HPLC 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_2PO_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 C18 (150×4.6 mm, 5µ) with flow rate of 0.7 ml/min using PDA detection at 267 nm. The described method was found to be linear over the range of 5- 30 µg/ml and correlation coefficient was found to be 0.999. The assay of Sitagliptin was found to be 99.89 %. 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[9].

Sai Lakshmi E et al established a HPLC 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 C18 column, (250 x 4.6 particle size of 5µ) at room temperature and UV detection at 248 nm. 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[10].

Ola Ahmed Saleh et al developed and validated a RP-HPLC method for sitagliptin phosphate. The liquid chromatographic determination was achieved isocratically on Poroshell 120 EC-C18 (100 × 4.6 mm, i.d.; particle size, 2.7 µm), Pursuit 5PFP (150 × 4.6 mm, i.d.; particle size, 5 µm) and Chromolith performance RP-18e (100 × 4.6 mm, i.d.; macropore diameter, 2 µm) columns using a mobile phase consisting of methanol : water : triethylamine : acetic acid (60 : 40 : 0.1 : 0.1; v : v : v : v), at a flow rate 0.5 mL/min and UV detection at 268 nm. The method was linear over the concentration range of 100-1000 µg/mL (r = 0.9998) with a limit of detection and quantitation of 10 and 30 µg/mL, respectively. All the validation parameters and stability indicating study were studied on Poroshell 120 EC-C18 column, which achieved the best separation. The proposed method has been found to have the required accuracy, selectivity, sensitivity, and precision to assay sitagliptin phosphate in bulk form and in a pharmaceutical

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dosage form. Degradation products resulting from the stress studies did not interfere with the detection of Sitagliptin phosphate that indicates that the assay are stability-indicating assay[11].

P. Ramalingam et al developed and validated a new stability-indicating high-performance liquid chromatographic method for simultaneous analysis of sitagliptin and simvastatin in pharmaceutical dosage form. The mobile phase consisted of methanol and water (70:30, *v/v*) with 0.2 % of n-heptane sulfonic acid adjusted to pH 3.0 with *ortho* phosphoric acid was used. Retentions of sitagliptin and simvastatin were 4.3 min and 30.4 min, respectively with a flow rate of 1 ml/min on C_8 (Qualisil BDS, 250×4.6 mm, 5 µ). Eluents were detected at 253 nm using photodiode diode array detector. The linear regression analysis data for the linearity plot showed correlation coefficient values of 0.9998 and 0.9993 for sitagliptin and simvastatin, with respective concentration ranges of 20-150 µg/ml and 8-60 µg/ml. The relative standard deviation for inter-day precision was lower than 2.0%[12].

HPTLC METHOD

A. S. Tapkir et al developed and validated a HPTLC method for the estimation of Sitagliptin phosphate (SITA-P). The method was developed using TLC aluminium plates precoated with silica gel 60F254 as the stationary phase using ethyl acetate: methanol: formic acid (8.5:1:0.5 v/v/v) as mobile phase. Densitometric analysis of SITA-P was carried out in the absorbance mode at 265 nm. The retention factor for SITA-P was found to be 0.50 ± 0.04. Linearity was found to be 500 – 2500 ng/band for SITA-P. The method was found to be accurate, precise, and robust according to acceptance criteria. The limit of detection (LOD) and (LOQ) was found to be 124.36 ng/band and 376.87 ng/band for SITA-P respectively. This HPTLC method can be used for the determination for the stability indicating assay methods for bulk drug and its formulations [13].

Darshana K et al studied on high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of two antidiabetic drugs, metformin hydrochloride and sitagliptin phosphate, in tablet dosage form that has been developed and validated. Chromatography was performed on silica gel 60 F254 plates with butanol : water : glacial acetic acid (6 : 2 : 2, v/v/v) as mobile phase. System gave a good resolution for Metformin hydrochloride (R_f value of 0.35 ± 0.01) and sitagliptin phosphate (R_f value of 0.75 ± 0.01). Detection and quantification were carried out at 227 nm. Linear regression data for the calibration plot showed a good relationship with r = 0.9995 and 0.9991 for Metformin hydrochloride and Sitagliptin phosphate, respectively. Method was validated for precision and recovery. Limits of detection and quantification were 13.05 and 39.56 ng/µ µ L for Metformin hydrochloride and 2.65 and 8.03 ng/µ µ L for Sitagliptin phosphate, respectively[14].

T. Raja et al studied on a new simple high performance thin layer chromatographic method for simultaneous determination of antidiabetic drugs, metformin hydrochloride and sitagliptin phosphate in bulk and tablet dosage form. Chromatographic separation of the drugs were performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of acetone: methanol: toluene: formic acid (4:3:2:1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 220 nm and the method was validated. The R_f values and drug content of metformin hydrochloride and sitagliptin phosphate were 0.36 ± 0.02, 0.63 ± 0.02 and 100.1%, 99.84% respectively. The calibration curves of peak area versus concentration, which were linear from 2000-5000 ng per band for Metformin hydrochloride, 200-500 ng per band for Sitagliptin phosphate and regression coefficient (r^2) was greater than 0.99. LOD for Metformin hydrochloride and Sitagliptin phosphate was 45 and 27 ng per band respectively, while LOQ was 150 and 87 ng per band respectively. The method was validated for linearity, accuracy, robustness and application for assay as per ICH guidelines[15].

LC-MS METHOD

Suleman S. Khoja et al studied on Ertugliflozin and Sitagliptin is combination of Antidiabetic drug, a member Antidiabetic drug, is a recent drug developed by Merck Sharp and Dohme Company for the treatment of Type 2 diabetes. Ertugliflozin and Sitagliptin can be used alone or in combination therapy. Chromatographic separation was carried out on Phenomenex Gemini, C18, (150 × 4.6 mm, 5 µm) column. Isocratic method was based on 0.1% formic acid: acetonitrile (10:90), v/v as mobile phase, column temperature at 40°C and flow rate at 0.6 mL/minutes were utilized. The mass spectrometer was operated under multiple reactions monitoring (MRM) mode using electrospray ionization by monitoring the transition pair (precursor to product ion) of m/z 437.10-328.95 in the positive mode for Ertugliflozin and transition pair (precursor to product ion) of m/z 408.10-234.95 in the positive mode for

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Sitagliptin. The method was found linear in the concentration range of 15 to 450 ng/mL and 100–3000 ng/mL for Ertugliflozin and Sitagliptin respectively[16].

Srinivasa Reddy et al studied on simple, sensitive, precise and accurate method for simultaneous estimation of metformin and sitagliptin from human plasma which was developed and validated. Samples extracted from plasma using acetonitrile were separated on an SCX column and estimated using API 4000 Mass Spectrometer in the positive atmospheric pressure ionization mode (Turboionspray) by following multiple reaction monitoring transitions for both parent and daughter ions. A linear calibration plot was achieved for both the analytes in the concentration ranges of 10–2,206 ng/Ml (for Metformin) and 3 –800.5 ng/mL (for Sitagliptin) prepared in K2EDTA pooled plasma. Mean recovery for Metformin was 92% and for Sitagliptin was 104.5%[17].

UPLC METHOD

Sharifa Sultana et al developed UPLC method for the estimation of Sitagliptin in pharmaceutical dosage form. Separation was done by a X-bridge C18 column (4.6 i.d.× 150 mm, 5 µm particle size) 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 linear regression analysis data for the linearity plot showed correlation coefficient values of 0.999 with LOD value of 0.06 µg/ml and LOQ of 0.225 µg/ml. The relative standard deviation (% RSD) for inter-day and intra- day precision was not more than 2.0%. The method was found to be accurate with percentages recovery of 98.50 ± 0.03 to 99.70 ± 0.05 and the % RSD was less than 2[18].

Ramya kuber B et al studied on the separation of Sitagliptin and Ertugliflozin which was done at a retention time of 0.859 min and 1.570 min, respectively. The present method was validated according to the ICH guidelines Q2 R1, and stability-indicating studies were carried out as per ICH guidelines Q1A R2. Intra-day and inter-day precision were found to be within acceptable limits. The linearity of the proposed method was in the concentration range of 25–125 µg/ml and 3.75– 22.5 µg/ml for Sitagliptin and Ertugliflozin, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.5 µg/ml and 1.53 µg/ml for Sitagliptin and 0.13 µg/ml and 0.38 µg/ml for Ertugliflozin, respectively. The recovery of the method was found in between 99.7% and 100.7%[19].

CAPILLARY ELECTROPHORESIS

Mohamed Salim et al developed and validated a method for the simultaneous determination of sitagliptin (SG) and metformin (MF) in pharmaceutical preparations. Separation was carried out in fused silica capillary (50.0 cm total length and 43.0 cm effective length, 49 µm i.d.) by applying a potential of 15 KV (positive polarity) and a running buffer containing 60 mM phosphate buffer at pH 4.0 with UV detection at 203 nm. The samples were injected hydrodynamically for 3 s at 0.5 psi and the temperature of the capillary cartridge was kept at 25 °C. Phenformin was used as internal standard (IS). The method was suitably validated with respect to specificity, linearity, limit of detection and quantitation, accuracy, precision, and robustness. The method showed good linearity in the ranges of 10–100 µg/mL and 50–500 µg/mL with limits of detection of 0.49, 2.11 µg/mL and limits of quantification of 1.48, 6.39 µg/mL for SG and MF, respectively. The estimated amounts of SG/MF were almost identical with the certified values, and their percentage relative standard deviation values (% R.S.D.) were found to be 1.50% (n = 3)[20].

CONCLUSION

Presented systematic review covers the current analytical methods for the determination of sitagliptin and it's combinations in pharmaceutical and biological samples like serum and plasma. UV methods were found to be most widely used for Sitagliptin. The other analytical methods like HPLC, HPTLC, UPLC, LC/MS, capillary electrophoresis are also used for the determination of sitagliptin in blood, serum & pharmaceutical dosage forms. The presented information is useful for the future study for researchers involved in formulation development and quality control of Sitagliptin.

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