Sitagliptin Phosphate: Development of a Dissolution Method for Coated Tablets Based on In Vivo Data for Improving Medium Sensitivity

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ABSTRACT

Sitagliptin phosphate is a drug used to treat diabetes mellitus type 2, and it belongs to a new therapeutic class called dipeptidyl peptidase IV inhibitors. This hypoglycemic drug is commercially available in coated tablets containing 25, 50, and 100 mg of sitagliptin base. The purpose of this study was to develop and validate the conditions for the dissolution test by investigating a possible in vivo–in vitro correlation. Several parameters were tested to develop the method, and the following conditions were considered satisfactory: pH 6.8 phosphate buffer, 900 mL of dissolution medium, temperature at 37 ± 1 °C, paddle apparatus, and rotation speed at 50 rpm. The dissolved percentage of STG was quantified by high performance liquid chromatography. The sink condition and specificity were determined in all media tested during method development. The parameters evaluated to validate the method were specificity, linearity, precision, and accuracy. The stability of the sample in phosphate buffer solutions for 24 h was also determined. The method is linear in the range of 10.0–70.0 µg/mL, precise, with RSD values less than 2%, and accurate (mean recovery 98.51%). The dissolution method as developed and validated supplied a good IVIVC when employing pH 6.8 phosphate buffer medium, which can be used in quality control of sitagliptin coated tablets since no official method has been described.

KEYWORDS: In vivo–in vitro correlation; dissolution method; sitagliptin phosphate.

INTRODUCTION

Diabetes mellitus (DM) presents as a chronic disease in which the pancreas does not produce enough insulin or occurs when the body cannot effectively use the insulin produced. This pathology reduces the quality of life of people living mainly in low- and middle-income countries (1).

Sitagliptin phosphate (STG) (Figure 1) is used to treat DM type 2 because it improves glycemic control by increasing the levels of active incretin hormones, GLP-1 (peptide-1) and GIP (glucose-dependent insulinotropic peptide). The activation of these incretins in β-pancreatic cells causes increased levels of cyclic adenosine monophosphate (cAMP) and intracellular calcium, with subsequent glucose-dependent insulin secretion (2). This hypoglycemic drug belongs to a new class called dipeptidyl peptidase IV inhibitors (3). STG was approved by the FDA in 2006 (4, 5).

The dissolution test of drugs has been employed as an excellent tool to detect formulation problems that could change drug release in the body (6). The quality of oral solid dosage forms depends on their ability to release the active components in aqueous medium in a consistent
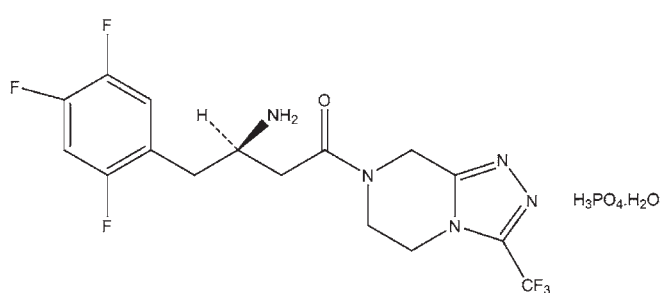

Figure 1. Chemical structure of sitagliptin phosphate.

and reproducible manner, making the active substances available for gastrointestinal absorption (7).

Thus, in vitro dissolution specifications are established to indicate potential problems of bioavailability (6). To characterize a pharmaceutical product, it is necessary to compare the dissolution profiles obtained from different formulations and in diverse conditions. It is thus possible to correlate them with the known aspects of drug bioavailability (8).

The dissolution test is seen as an indispensable tool for the pharmaceutical industry, both in product development and in routine quality control (9–11).

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The solubility and permeability of a drug are considered key parameters for absorption (*12*). The Biopharmaceutics Classification System (BCS) based on these concepts divides drugs into four categories: Class 1, 2, 3, and 4 (*13–15*). The use of the BCS determines the possibility of in vivo–in vitro correlation (*16*).

The FDA has described conditions used for the dissolution test in the STG registration process (dissolution medium: water at 50 rpm) without an indication of the method used for quantification. Nevertheless, no monograph has been described for sitagliptin tablets. Given the above, the purpose of this study was to develop a method based on in vivo data, which were obtained from the literature (*17*).

MATERIAL AND METHOD

Materials and Reagents

Sitagliptin phosphate reference standard, 99.5 % purity, was supplied by Sequoia Research Products (Oxford, UK). The coated tablets (Januvia) used in the dissolution test, which contained 50 mg of sitagliptin base, were obtained commercially. The coated tablets contained the inactive ingredients microcrystalline cellulose, calcium phosphate dibasic anhydrous, croscarmellose sodium, magnesium stearate, sodium stearyl fumarate, polyvinyl alcohol, polyethylene glycol, talc, titanium dioxide, yellow ferric oxide, and red ferric oxide, and they were obtained from several distributors. All other chemical reagents were analytical or HPLC grade.

Instrumentation

The dissolution test was performed using a Vankel VK 7010 multibath (*n* = 8) dissolution testing station attached to a bidirectional peristaltic pump and VK 8000 autosampler.

The quantification of the STG was performed in a high-performance liquid chromatograph (HPLC) Shimadzu 20-A system equipped with a CBM-20A system controller, LC-20AT pump, SIL-20A/C auto sampler, CTO-20A/C column oven, and SPD-M2OA PDA detector (Kyoto, Japan). The LC-Solution manager system software was used to control the equipment and to calculate data and responses from the HPLC system. The experiments were performed on a Waters-XBridge C8 (250 mm × 5 µm) analytical column (Milford, MA). The HPLC system was operated isocratically at 25 ° using a mobile phase composed of a solution of 0.3% triethylamine adjusted to pH 4.0 with phosphoric acid and acetonitrile (75/25, v/v) at a flow rate of 1.0 mL/min, using detection at 207 nm. The injection volume was 20 µL.

Standard Solution Preparation

The standard solution was prepared in pH 6.8 phosphate buffer using an amount of STG equivalent to 10 mg STG base. The concentration of stock solution was 1.0 mg/mL. This solution was diluted with pH 6.8 phosphate buffer and filtered through a 0.45-µm membrane (Millipore) before injection into the analytical column.

Dissolution Test Conditions

Sitagliptin sink conditions were determined in all media used in the study (*9*). The solubility of the drug was tested using an amount of solute (STG) and solvent (dissolution medium) equivalent to three times the formulation dose in 900 mL of medium (*10*). The media 0.1 M HCl, 0.01 M HCl, water, pH 6.8 phosphate buffer, and pH 4.5 and 5.0 acetate buffer were tested. These media were used because they are relevant to physiological pH and are frequently used in dissolution testing (*10*, *14*).

The dissolution tests were conducted using 900 mL of each medium. The media were heated and kept at a temperature of 37 °C. USP Apparatus 2 (paddle) at 50 and 75 rpm was tested, and aliquots of 5 mL were withdrawn at 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min.

In Vivo–In Vitro Correlation (IVIVC)

The correlation between in vitro and in vivo data for STG was checked by plotting the percentage of STG absorbed (fraction absorbed, FA) versus the average percentage of drug dissolved (fraction dissolved, FD). Linear regression analysis was used to evaluate the data obtained (*18*, *19*).

In Vivo Data

Data for plasma concentration of STG versus time (48 h) were obtained from the literature (*17*), and it was possible to verify that the drug complies with the one-compartment pharmacokinetic model. The curve of plasma concentration versus time was fitted using the software Micromath Scientist, v. 1.2, to estimate the plasma concentration of STG at the same time as the dissolution evaluation. FA was calculated using the Wagner–Nelson method.

Method Validation

The dissolution test was validated according to *USP* and ICH monographs for STG phosphate in coated tablets. The parameters evaluated were specificity, linearity, accuracy, and precision. The stability and system suitability were monitored, and parameters such as theoretical plates, retention time, capacity factor, and symmetry were controlled.

Specificity

A simulated mixture of the formulation excipients was prepared and employed in the specificity evaluation of the method. An amount of mixture equivalent to an average weight of STG was transferred to USP Apparatus 2 vessels (*n* = 2) with 900 mL of pH 6.8 phosphate buffer medium at 37 ± 1 °C. After 1 h at 150 rpm, 5-mL aliquots were removed, filtered through a 0.45-µm membrane, and analyzed by HPLC.

Linearity

Method linearity, determined by HPLC, was established by constructing three calibration curves. Aliquots of the

1.0 mg/mL STG standard solution were removed and diluted with pH 6.8 phosphate buffer for concentrations of 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, and 70.0 µg/mL. This range includes the low concentrations found in the initial STG dissolution time points and exceeds the maximum dose that can be dissolved. The levels were prepared in triplicate, and three determinations were performed for each solution.

Accuracy

The accuracy was determined by adding known amounts of a powdered STG tablets pool corresponding to drug levels of 25, 100, and 150% of the labeled amount in an average weight equivalent to a tablet. The dissolution test was run for 30 min using 900 mL of the medium and Apparatus 2 at 50 rpm. This approach was used because of the limited availability of the sitagliptin phosphate reference standard.

Precision

Repeatability and intermediate precision were used to assess method precision. Repeatability was evaluated through the relative standard deviation (RSD) from adding known amounts of the powdered STG tablets pool, corresponding to a drug level of 100% (labeled amount in an average weight equivalent to a tablet). The dissolution test was run for 30 min using 900 mL of medium and Apparatus 2 at 50 rpm, over two days ($n = 3$).

RESULTS AND DISCUSSION

During the dissolution test, several conditions were tested and adjusted to find a condition that would provide a release of STG from the coated tablets that could be correlated with FA obtained from in vivo data.

Although no biopharmaceutics classification of STG was found, it can be inferred that the drug is Class 1, since it presents high solubility and high permeability (bioavailability of 87%). Hence, the absorption process would not be controlled by the solubility and or by permeability. Based on the biopharmaceutics classification, the possibility of in vivo–in vitro correlation is low if the formulation allows rapid drug dissolution, and therefore the rate of absorption is controlled by gastric emptying (13, 20). However, the dissolution rate can be significantly altered when the drug is mixed with excipients during production, and in some cases, this can lead to a reduction in bioavailability and clinical response (21). For example, magnesium stearate, which is present in the formulation of STG and is a hydrophobic lubricant, can slow the rate of dissolution of some drugs by reducing the drug–solvent interfacial area (21). These changes in surface characteristics of the tablets result in a decrease in wettability, prolong the disintegration time, and decreasethe interface between the active and the solvent (21).

Nonetheless, the STG tablets have some other components that may delay drug release from the dosage form.

This justifies attempting a possible correlation between in vitro dissolution and in vivo data.

The release rate of STG from the pharmaceutical formulation is pH dependent. It was observed that the release is faster in acid pH and slower in neutral pH. The agitation speed of the paddle is a very important feature in the development of the dissolution method. The apparatus routinely used for capsules is the basket, while the paddle is frequently used for tablets with a rotational speed ranging from 50 to 100 rpm. The increase from 50 to 75 rpm caused a rapid release of the STG from the pharmaceutical dosage form (22).

The dissolution profiles obtained in different media with paddles at 50 rpm are shown in Figure 2. All tested media reached the sink condition. The pH 6.8 phosphate buffer was the medium that released the drug more slowly, whereas the 0.1 M HCl medium provided a faster drug release profile.

Despite its designation as BCS Class 1, STG did not dissolve rapidly from the tablets in all media evaluated. If the excipients play a role in STG dissolution, as observed in vitro, media employing water and pH 6.8 phosphate buffer could show this slower release. On the other hand, if rapid dissolution of this hypoglycemic agent had been obtained at all pH levels evaluated, the drug could be a candidate for a biowaiver approach in the development of a generic product. As this situation was not achieved, medium that is more sensitive to characterize the formulation can be used more efficaciously in quality control of the tablets and probably in the development of a new product.

The HPLC method had been developed and validated for quantitative analysis of the STG in tablets. However, some changes were necessary for application to the dissolution test, for example, adjustments in the range of linearity and the solvent used for sample dilution. Due to the low concentration of STG in the dissolution medium,
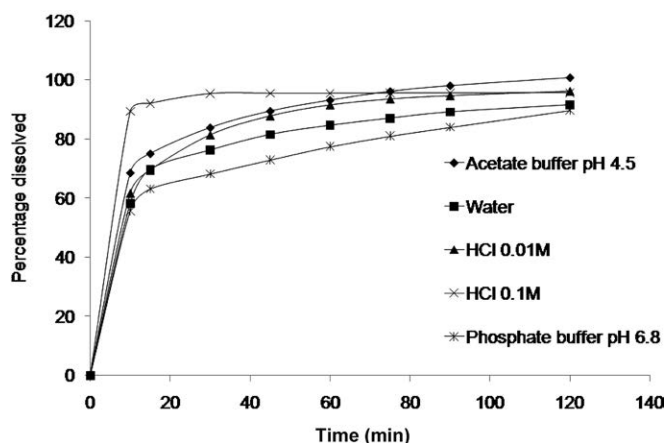

Figure 2. Dissolution profiles of sitagliptin phosphate coated tablets (n = 6) employing pH 4.5 acetate buffer, water, 0.01 M HCl, 0.1 M HCl, and pH 6.8 phosphate buffer at 50 rpm.

it was not possible to perform a second dilution with the mobile phase. The samples were collected, filtered, and analyzed directly.

The linearity was evaluated in the concentration range of 10–70 µg/mL. The representative linear equation for STG was $y = 42031x + 27210$, with a determination coefficient of 0.9995. The data were validated by analysis of variance (ANOVA), which showed significant linear regression and non-significant linearity deviation ($F_{calc} = 0.15 < F_{crit} = 4.53$; $\alpha = 0.05$).

The specificity of the method was evaluated to verify that the formulation excipients and the dissolution medium did not interfere in the analysis of STG. Figure 3 represents the chromatogram obtained for the solutions of the tablets and the formulation excipients. No interference of the formulation excipients was observed in the analysis of STG by HPLC method, as there is no peak at the same retention time as that of STG. The peak purity was determined by Shimadzu LC-Solution software tools.
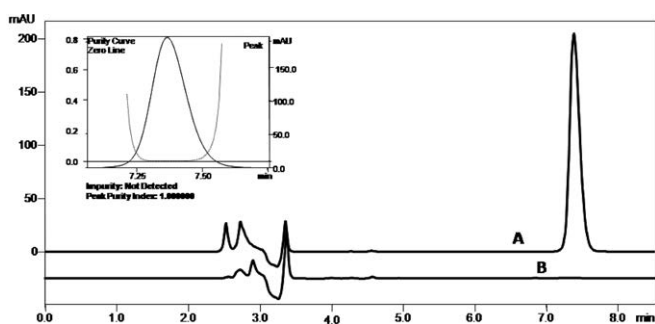

Figure 3. Overlay of chromatograms of (A) STG coated tablets and (B) excipients simulated sample by HPLC.

Table 1. Intraday and Interday Precision Results for STG Dissolved in 900 mL of Medium and Analyzed by LC

Sample	Day 1	Day 2
1	99.74	98.18
2	99.33	98.17
3	98.77	99.36
Average content (%)	99.28	98.57
Intraday RSD (%)	0.49	0.69
Interday RSD (%)	0.66	

Method precision and accuracy were adjusted and redesigned for the execution of validation parameters. Thus, a pool of STG coated tables was prepared, finely triturated, and used for analysis. The pool content was checked every day against a standard solution with known concentration of STG to evaluate precision and accuracy.

The results obtained for the precision of the method are described in Table 1. The values of relative standard deviation (RSD) were less than 2.0% for repeatability and intermediate precision, demonstrating good precision of the dissolution procedure.

The accuracy of the method was demonstrated by the recovery of the known amounts of sitagliptin phosphate added from the tablet pool to the dissolution vessels. Three levels were evaluated, low, medium, and high. The results obtained are described in Table 2. The percentage recoveries ranging from 98% to 102% demonstrate the satisfactory accuracy of the method.

To select the medium (water or pH 6.8 phosphate buffer) that could better correlate with in vivo data, a level A correlation study was attempted.

From the plasma concentration-versus-time data obtained in the literature (17) and knowledge that the drug follows the one-compartment pharmacokinetics model, the Wagner–Nelson approach was applied, and the FA was calculated. To infer the FA from the exact times used in development of the dissolution profile, the Scientist program was used.

The FA for the times 10, 20, 30, 45, 60, 75, 90, 105, and 120 min was determined. Hereafter, the FA was correlated with the FD (data obtained in the dissolution test). As mentioned previously, Class 1 drugs are less likely to achieve a perfect correlation employing the same times for in vivo and in vitro data. The correlation demonstrated in Figure 4 is not ideal; although the correlation coefficient is closer to 1 ($r = 0.9929$), the slope is greater than 1. In other words, the relationship confirms a faster in vitro dissolution as compared with the FA. The adoption of a time-scale factor could improve the slope of the IVIVC, as the x-axis could be stretched by a factor of 3 or 4, for example. However, the introduction of this approach implies, per se, to work with a faster in vitro dissolution profile than in vivo absorption profile.

The correlation between STG FA and FD was tested in all media used in the study (pH 4.5 acetate buffer, water, 0.01 M HCl, 0.1 M HCl, and pH 6.8 phosphate buffer). Water as a dissolution medium showed good results but was not as satisfactory as pH 6.8 buffer phosphate. Although

Table 2. Mean Recovery of STG Added by Means of the Powdered Tablets Pool

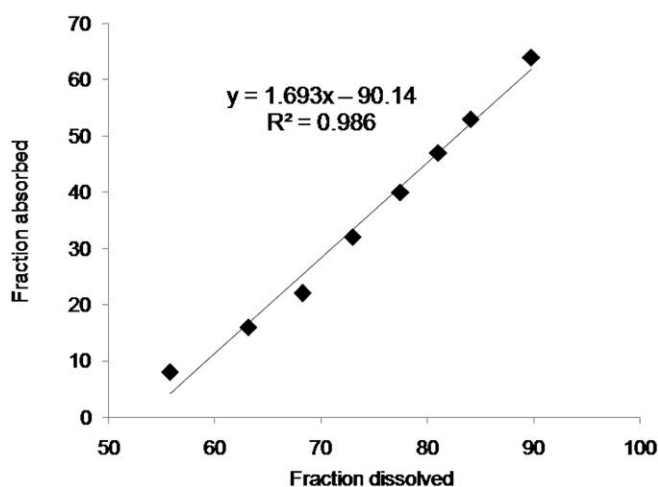| Level (%)	Added STG (mg)	Added tablets "pool" (mg)	Mean recovery ± RSD (%)
25	12.5	51.75	98.99 ± 1.80
100	50.0	207.0	98.73 ± 0.68
150	75.0	310.5	97.81 ± 0.76

Figure 4. Plot of fraction of dose absorbed (FA) in vivo versus fraction of dose dissolved (FD) in vitro for STG phosphate coated tablets.

water is the dissolution medium registered at the FDA for STG, it was not chosen because it presents variable pH values and therefore its use has been discouraged.

CONCLUSIONS

Validation of the dissolution test results shows that the method is specific, linear, precise, and accurate. Both the HPLC analytical method and the in vitro dissolution test were validated and can be used to evaluate the release profile of sitagliptin phosphate from coated tablets. The pH 6.8 phosphate buffer with Apparatus 2 at 50 rpm allowed a slow release of sitagliptin from the pharmaceutical formulation, thus making it possible to correlate the data of the FA with those of the FD. Moreover, a slower dissolution profile usually presents higher discriminative power for use as a control quality tool for formulations and processes.

ACKNOWLEDGMENTS

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