

## Evaluation of linagliptin dissolution from tablets using HPLC and UV methods

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Linagliptin (LGT) is used to reduce glucose blood levels in patients with type 2 *Diabetes mellitus*. The proposed conditions for a biowaiver guide can be applied due to high solubility of linagliptin in aqueous media. The aim of the present study was to develop and validate a dissolution test for 5 mg linagliptin coated tablets. After diverse dissolution procedure evaluation, the selected method was achieved using USP apparatus 1 (basket) at 75 rpm and 900 mL of pH 3.5 citrate buffer as dissolution medium. The conditions proposed by biowaiver guide were also applied to this drug, using USP apparatus 2 (paddle) and 900 mL of 0.1 M HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer as dissolution medium. HPLC and UV spectrophotometry were used to LGT quantitation and validated for this purpose. The chromatographic and spectrophotometric methods in the dissolution context proved to be linear in 0.5 – 20.0  $\mu\text{g}\cdot\text{mL}^{-1}$  range, precise with RSD values less than 1.0% and 2.0%, respectively, and accurate (mean recovery 100.29% and 100.59%). The parameters such as specificity, linearity, accuracy, precision and robustness were according to international guidelines for both methods HPLC and UV. Dissolved linagliptin results obtained by the two analytical methods were compared using the Student's t-test and the data found were not significant different ( $p > 0.05$ ). In most dissolution conditions evaluated, LGT presented more than 85% drug dissolved from the tablets in fifteen minutes. The proposed methods can be applied for quality control of this gliptin. According to the results, linagliptin may be a biowaiver candidate.

**Keywords:** Linagliptin; Dissolution test; UV spectrophotometric method; HPLC method; Biowaiver; Quality control.

### Introduction

Diabetes Mellitus (DM) is one of the most common non-transmissible diseases worldwide<sup>1</sup>. Linagliptin (LGT), approved by FDA in May 2011 is a member of the Gliptins class whose act to inhibit the dipeptidyl-peptidase-4 enzyme. This class is used to reduce glucose levels in patients with type 2 DM, preventing the destruction of the incretins, which are hormones that stimulate the pancreas to produce insulin when the glucose level in blood is high.<sup>2,3</sup>

The dissolution test is considered an important tool to characterize a pharmaceutical product. It supplies useful information regarding drug release, both in research and development and in the production and quality control of pharmaceutical forms.<sup>4</sup> The dissolution kinetics is associated with the absorption of the drug by the organism, interfering in the bioavailability and leading to *in vitro-in vivo* correlation (IVIVC) in some cases.<sup>5</sup>

Drugs can be classified according to solubility and permeability, constituting the Biopharmaceutical Classification System (BCS). The FDA has recommendations to carry out dissolution tests for oral solid pharmaceutical forms of immediate release, and the knowledge related to solubility, permeability, dissolution and pharmacokinetics must be considered to define dissolution specifications, aiming to achieve drug registration.<sup>6</sup>

LGT is a drug that was suggested as BCS class III by Boehringer Ingelheim® itself - the laboratory that launched the molecule - as LGT has high solubility and low bioavailability.<sup>3,7</sup> This approach indicates that dissolution is a factor that is most unlikely to limit absorption and the drug could be included in the biowaiver program.<sup>6,8</sup> For these candidate drugs, it is proposed to substitute the studies of relative bioavailability/bioequivalence by *in vitro* assays.<sup>6,9,10</sup> For BCS class III drug products, if the drug substance is highly soluble and the drug product is very rapidly dissolving, the biowaiver guidance could be

applicable. According to the FDA information, LGT is mildly soluble in water (0.9  $\text{mg}\cdot\text{mL}^{-1}$ ), soluble in methanol (60  $\text{mg}\cdot\text{mL}^{-1}$ ), moderately soluble in ethanol (about 10  $\text{mg}\cdot\text{mL}^{-1}$ ), mildly soluble in isopropanol (< 1  $\text{mg}\cdot\text{mL}^{-1}$ ) and mildly soluble in acetone (about 1  $\text{mg}\cdot\text{mL}^{-1}$ ).<sup>3</sup> Wherefore, LGT tablet dose of 5 mg requires less than 10 mL of water for its solubilization.

Otherwise, mild dissolution conditions promoting slower drug dissolution in the beginning of the process usually give better application for define tablet composition.

Therefore, in this work, dissolution studies were conducted using commercial tablets to quantifying the drug, both by UV/VIS spectrophotometry and by HPLC-DAD, and statistical analysis was performed to verify possible interchangeability between the two methods proposed. Biowaiver approach and dissolution conditions for the product developing were focused.

### Material and Methods

#### Material and Reagents

Linagliptin-coated tablets containing 5 mg (Trayenta®), used in the dissolution assay, were acquired at local businesses. Excipients being in the tablets are mannitol, pregelatinized starch, cornstarch, copovidone, and magnesium stearate. In addition, the film coating contains the following inactive ingredients: hypromellose, titanium dioxide, talc, polyethylene glycol, and red ferric oxide. The reference standard of LGT was purchased from Sequoia Research Products (Oxford, UK) and it was properly characterized for this work.<sup>11</sup>

Chromatographic analysis was performed by using an Eclipse Zorbax C8 column (5  $\mu\text{m}$ ; 150 x 4.6 mm - Agilent®), PVDF sample filters (13 mm, 0.45  $\mu\text{m}$  - Merck®) and PVDF filtration membrane (47 mm, 0.45  $\mu\text{m}$  - Merck®).

### **Instrumentation**

All analyses were performed in a Vankel® dissolution apparatus (model VK 7010), composed by an automatic sampler (VK8000) and equipped with eight glass vessels. Quantification by HPLC was conducted by using Shimadzu® 20-A equipment with a CBM-20 A system controller, LC-20AT pump, SIL-20A/C autosampler, CTO-20A/C oven and diode array detector SPD-M20A (Kyoto, Japan). The LC-Solution software was used to control the equipment and to calculate the data and system responses. Quantification by spectrophotometry in UV-Vis region was performed by using Shimadzu UV-1601PC equipment.

### **Dissolution test conditions**

The dissolution media 0.1 M HCl, pH 3.5 50 mM sodium citrate buffer, pH 4.5 50 mM sodium acetate buffer and pH 6.8 50 mM potassium phosphate buffer were evaluated in the dissolution method. Paddle (50 rpm) and basket (75 rpm) apparatus were employed. In order to establish the dissolution profile, aliquots were collected at times 5, 10, 15, 20, 30, 45, 60 and 90 minutes, and the volumes removed were equivalent to 10 mL, without replacement of the medium. The temperature was set in  $37 \pm 0.5$  °C. Filters of 10 µm were assembled in each vessel cannula. During the development phase, drug concentration in the dissolution media was determined by ultraviolet spectrophotometry and for the dissolution method validation high performance liquid chromatography was also used.

In order to verify whether LGT and these tablets could be classified in biowaiver approach, the assays performed in the dissolution media 0.1 M HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer were conducted with  $n=12$  in each condition.<sup>6</sup> The tests were carried out on the same day and the dissolution profiles were obtained by HPLC. The quantities of the LGT aliquoted were always included in the calculation of the dissolved percentage. Since LGT was in the form of coated tablets, the conditions of the dissolution test advocated by the guidelines are medium volume of 900 mL, 50 rpm rotation and apparatus 2 (paddle) with at least three different media. The pH values of the dissolution media were measured before and after the drug tests.

### **Dissolution method validation**

In dissolution test validation, the following mandatory parameters were conducted: specificity, linearity and range, repeatability, intermediate precision and accuracy. To complement the validation, the degassing need of the medium was also evaluated.

After choosing a dissolution condition from diverse ones tested, the accuracy and precision method were validated collecting a single aliquot of 10 mL in a 30 minutes time period, filtered in a Vankel filter (10 µm), submitted to another filtration in a 0.45 µm nylon filter and analyzed by HPLC and UV at 293 nm wavelength. Basket (USP apparatus I) set in 75 rpm and pH 3.5 sodium citrate buffer were used in this validation procedure.

Assays were performed on two consecutive days and the dissolution profiles were obtained by HPLC, with an injection volume of 40 µL from each sample, and by UV spectrophotometry with triplicate analysis for both. The

aliquoted medium was not replaced but the quantities of LGT removed were included in the calculation of the dissolved percentage.

### **Preparation of the standard solution of LGT**

Standard solution was prepared in the selected medium pH 3.5 citrate buffer, using 10 mg of LGT reference standard. The final concentration of stock solution was  $1.0 \text{ mg.mL}^{-1}$ . This solution was diluted in pH 3.5 citrate buffer and filtered through a 0.45 µm membrane before injection into the chromatographic column.

### **Preparation and assay of the sample used to evaluate precision and accuracy**

Twenty LGT tablets were weighed and the mean weight was obtained. They were crushed, forming a pool kept in an amber glass flask that was used for the validation assays. For the crushed powder assay, an equivalent mass to a mean weight of the tablets was transferred to a 50 mL volumetric flask, solubilized in pH 3.5 citrate buffer, shaken mechanically at 200 rpm for 20 minutes and put in an ultrasound bath for 15 minutes. After filtration, a ten fold dilution was performed to obtain a LGT theoretical concentration of  $10 \text{ µg.mL}^{-1}$ .

Samples were analyzed by HPLC, on two consecutive days performing 12 replications. The content obtained in the tablets pool assaying was used to correct the amount of LGT used in the precision and accuracy evaluations throughout the execution of the dissolution validation procedure.

### **Specificity**

Specificity was executed by analyzing formulation placebo, produced from the excipients described in the composition of the coated tablet, submitted to the same conditions as the LGT tablets in the two quantitative methods tested.

### **Linearity**

Standard curves were prepared from the standard solution of LGT at concentrations of 0.5, 2.5, 5.0, 10.0, 15.0 and 20.0  $\text{µg.mL}^{-1}$ , using pH 3.5 citrate buffer as a diluent, evaluated in the HPLC and UV quantification techniques.

### **Accuracy / Precision**

After assaying the crushed tablets, three LGT levels for the recovery evaluation of the dissolution method were established. The levels and the crushed tablets masses that contained the indicated drug quantity according to the content found at assay are shown in Table 1, in order to add strictly the amounts of LGT indicated in the dissolution vessels. The medium aliquots were quantified both by HPLC and by UV spectrophotometry.

**Table 1.** Levels used to determine the concentration of LGT in the accuracy assay

Level	% in relation to the dose	Quantity of drug	Quantity of crushed tablets
Low	80 %	4 mg	147.98 mg
Medium	100 %	5 mg	184.97 mg
High	120 %	6 mg	221.96 mg

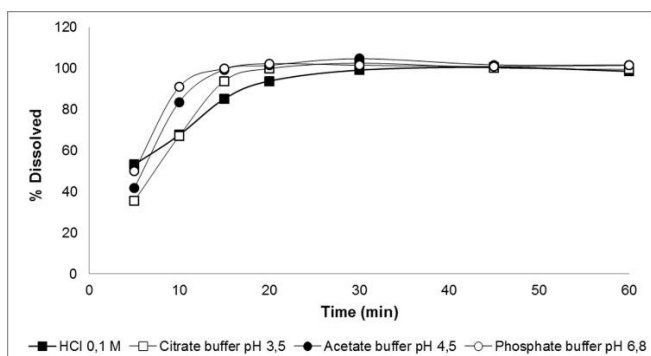
The assays occurred on two consecutive days using three vessels for each level, totalizing six intralevel assays and 18 interlevel assays. Method precision was evaluated through the intralevel relative standard deviation (RSD) at each day (n=3) and on different days (n=6). The interlevel precision was also computed (n=18).

#### Degassing need (robustness)

The interference of the presence of bubbles in the medium was evaluated. The presence of air bubbles in the system may interfere in the results, because acting as a barrier to dissolution, make it easier for particles to adhere both on the vessel surfaces and on the shaking mechanisms.<sup>12</sup> The media were degassed using an ultrasound bath for 20 minutes, as per previous tests.

## Results

According to dissolution guidelines, the three different media with the most significant pH values of the gastrointestinal tract: 0.1 M HCl solution (pH ~1,2), pH 4.5 acetate buffer and pH 6.8 phosphate buffer were tested. Fast LGT dissolution from the tablets were obtained (> 80% in 15 minutes) using the paddle apparatus with a rotation velocity of 50 rpm. A fourth buffer with a perspective of retarding dissolution (pH 3.5 citrate)<sup>8</sup> was also employed (Figure 1) with this apparatus, since the dosage form is a coated tableted of immediate release.



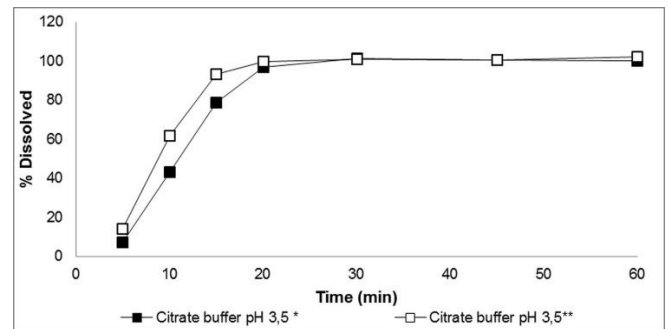
**Figure 1.** LGT dissolution profiles obtained in the media 0.1 M HCl, pH 3.5 citrate buffer, pH 4.5 acetate buffer and pH 6.8 phosphate buffer (n=2) with paddle apparatus (50 rpm) and 900 mL volume.

Additionally, another pH values were tested employing citrate buffer: 5.5 and 6.5 with paddle apparatus at 50 rpm, in order to promote slower drug dissolution in the begging of the process; that aim usually gives better application for define tablet composition. It was observed that as pH

decreased, the dissolution of LGT presented slower kinetics in a 20 minutes period.

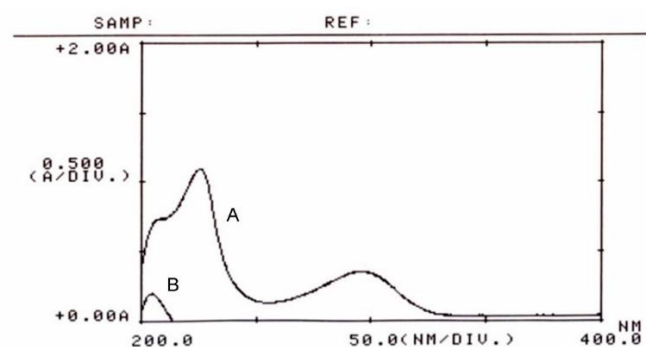
The basket 75 rpm was also used with the same buffers at pH 3.5 and 5.5 and with 500 and 900 mL volumes. There was small difference in dissolution between the volumes tested, 500 and 900 mL, however it was decided to use 900 mL due to the possibility of taking 10 mL aliquots at the times established and to maintain a considerable medium volume above the basket (Figure 2).

Citrate buffer pH 3.5 and basket apparatus at 75 rpm was the chosen condition for subsequent dissolution validation steps aiming to detect possible differences between lots in the quality control routine analysis.

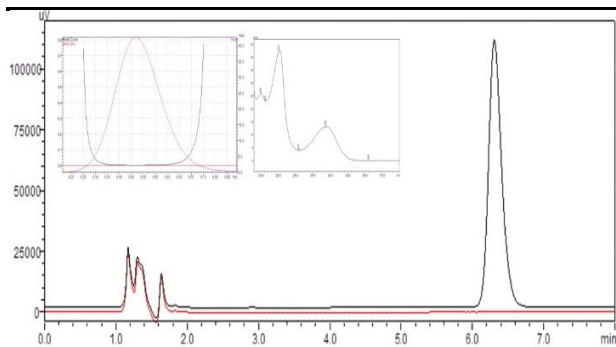


**Figure 2.** Citrate buffer in basket 75 rpm apparatus at pH 3.5 and different volumes (\*500 mL and \*\*900 mL) (n=2)

Specificity evaluation by UV method, both spectra of placebo and sample were traced in the region of 400 to 200 nm using pH 3.5 citrate buffer medium. Figure 3 shows the superposed spectra of LGT sample at the concentration of  $10 \mu\text{g}\cdot\text{mL}^{-1}$  and the placebo at evaluated range. Absorption of the placebo excipients occurred in the range of 200 to 210 nm, but in the two maximum absorptions of the LGT at 225 nm and 293 nm there is no interference from these excipients. At LGT quantitation by UV spectrophotometry, 293 nm wavelength was used. In the HPLC method there was also no interference in the quantification of the drug in employed conditions. Figure 4 shows the superposed chromatograms of LGT tablets and excipients solutions in 293 nm wavelength selected for LGT quantitation.



**Figure 3.** Superposition of UV spectra of a sample of LGT (A) and placebo (B) in the 400 to 200 nm range.



**Figure 4.** Superposition of chromatograms of solutions containing LGT sample and placebo. In minor graphics: LGT peak purity evaluation and UV spectra by DAD.

Three independent standard curves with six concentrations (range of 0.5 – 20  $\mu\text{g}\cdot\text{mL}^{-1}$ ) were built to evaluate method linearity. The linear regression by the least squares method and the analysis of variance (ANOVA) were applied to the data. The linearity was proved to two analytical techniques used. In quantification using UV method, the correlation coefficient ( $r$ ) was equal to 1.0000 and the determination

coefficient ( $R^2$ ) equal to 0.9996. In HPLC method, the correlation coefficient ( $r$ ) was equal to 1.0000 and the determination coefficient ( $R^2$ ) equal to 0.9999. The analysis of variance for linearity of both methods provided a significant result for linear regression ( $p < 0.05$ ) without deviation from linearity ( $p > 0.05$ ). Intercepts was always not statistically significant ( $p > 0.05$ ).

Method accuracy and precision were evaluated by adding the homogenate of crushed tablets to the vessels, in three levels, whose LGT content had been previously determined as 98.76%. The recovery results are expressed in Table 2, as well as the precision evaluated by the intraday and interday relative standard deviation (RSD) of each level. All values of RSD were below 1% and the recovery results between 98.81 and 100.96% demonstrate the accuracy of the analytical methods. Additionally, the two-tailed Student's  $t$ -test was performed with 95% confidence for the spectrophotometric and chromatographic methods used and no significant statistical difference between them was obtained ( $p = 0.068$ ).

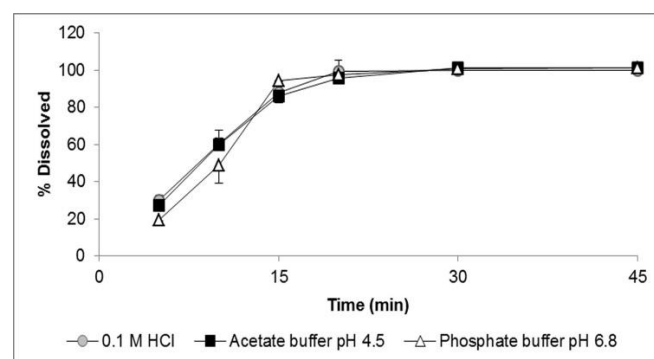
**Table 2.** Accuracy and precision results for LGT dissolved in the two days evaluated by HPLC and UV.

LGT level	Day	Method	Accuracy (Recovery, %)		Precision (RSD, %)	
			Intraday (n=3)	Interday (n=6)	Intraday (n=3)	Interday (n=6)
4 mg (80%)	1	HPLC	100.22	100.58	0.32	0.88
		UV	98.81		0.18	
	2	HPLC	100.94	99.52	0.60	0.89
		UV	100.22		0.67	
5 mg (100%)	1	HPLC	100.58	100.77	0.90	0.68
		UV	99.76		0.85	
	2	HPLC	100.96	100.01	0.14	0.69
		UV	100.25		0.54	
6 mg (120%)	1	HPLC	100.05	100.22	0.74	0.44
		UV	100.39		0.88	
	2	HPLC	100.42	100.66	0.36	0.44
		UV	100.89		0.43	

No differences were observed in the dissolution profiles for the presence of bubbles in the medium and the degassed one whose data (not shown) were obtained using both UV spectrophotometry and HPLC methods.

According to FDA (2000), it is not necessary to calculate the similarity factor ( $f_2$ ) for the different media when the dissolution is greater than or equal to 85% before the 15 minutes of the dissolution test. Besides, the relative standard deviation must be up to 20% in the first points (up to 10 min) and no greater than 10% in the other points evaluated.

Figure 5 shows the mean of the different dissolution media evaluated, with their respective error bars ( $n = 4$ ), employing also paddle apparatus at 100 rpm. The pH values of the different media were measured at the beginning and at the end of the test, and there was a small alteration only in HCl medium, with pH varying from 1.2 to 1.6.



**Figure 5.** LGT dissolution profiles obtained in the media 0.1 M HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer ( $n=4$ ) with paddle apparatus (100 rpm) and 900 mL volume .

The high solubility of members of the gliptin class was pointed out in another dissolution studies. Lange and collaborators developed IVIVC for sitagliptin using the pH 6.8 phosphate medium in the dissolution analysis, velocity 50 rpm and apparatus 2. For vildagliptin, Barden and collaborators carried out a dissolution method in 0.01 M HCl medium using apparatus 2 at 50 rpm.<sup>12,13</sup>

The dissolution profiles found for the different media presented RSDs lower than 20% at the initial points and lower than 10% at the other points, and were within the

range advocated by the FDA.<sup>6</sup> Moreover, there was a dissolution of more than 85% of LGT from the tablets up to 15 minutes of analysis. Thus, the drug attained rapid dissolution requirements of the official guidelines to be a candidate for biowaiver.

These biowaiver candidates are part of the list of essential medications helping to obtain the approval of generic products, especially in developing countries. The LGT is not included in this list but is part of a class of drugs for type 2 Diabetes Mellitus, a metabolic disease considered a pandemic, affecting over 347 million people worldwide.<sup>14</sup>

## Conclusions

According to results obtained, UV analytical method was considered validated for LGT dissolution analysis, respecting all parameters of official validation guidelines.<sup>15,16</sup> The selected dissolution conditions can be used in the quality control routine analysis for LGT tablets. Methods using UV and HPLC proved to be interchangeable for this purpose. To be a highly aqueous soluble drug, as other members of the gliptin class, possibly belonging to BCS Class III, with over 85% dissolution in 15 minutes of analysis in three different media, the LGT could be fit as a biowaiver candidate in accordance with the official guidelines.<sup>3,6</sup>

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