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New knowledge for omarigliptin tablets: dissolution assay -behavior in different media and biopharmaceutical classification

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Omarigliptin (OMG) is an active substance for the treatment of type 2 diabetes and it is representing of the dipeptidyl peptidase IV inhibitors class, an important enzyme in the regulation of blood glucose. It was launched on the Japanese market in 2015 as a weekly dose innovation. This work aimed to report the results obtained of the OMG tablets solubilization in different media, evaluate the dissolution profiles in the chosen media, propose the biopharmaceutical classification, in addition to validate the dissolution method. After solubility test, the dissolution assay was conducted and a rapid process of tablets disintegration and dissolution was observed in three media having different pH values, they were acid hydrochloric 0.01M at pH 3.1, sodium acetate buffer at pH 4.5 and potassium phosphate buffer at pH 6.8. The potassium phosphate buffer medium was chosen to proceed with the dissolution method validation. With the profiles obtained in the three tested media it was possible to conclude that the dissolution is very fast, with 100% of the active substance dissolved in less than 10 min for all tested media. In addition, with the evaluation of the dissolution profile obtained in this work and data available in the literature, it was possible to suggest that OMG belongs to class I of the biopharmaceutical classification system. The proposed method was validated and contained all the necessary parameters recommended by the official guides.

Keywords: Omarigliptin, Dissolution assay, Solubility, Biopharmaceutical classification, Validation.

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Introduction

Omarigliptin is an active substance used to treat type 2 diabetes mellitus (T2DM) and it represents of the dipeptidyl-peptidase 4 (DPP-4) inhibitors class. This enzyme is capable of cleaving endogenous peptides important in regulating glucose metabolism, named incretins (Glucagon-like peptide-1 and the gastric inhibitory polypeptide), which are secreted in response to food intake (1, 2). DPP-4 inhibitors increase native incretins, which effectively stimulates insulin secretion, suppresses glucagon release, and improves glucose control in patients with T2DM (3). Almost all of the currently marketed DPP-4 inhibitors are prescribed as once or twice-daily oral medications; however, OMG, approved in Japan for the treatment of T2DM, was discovery once-weekly oral dosing in September 2015, which favors patients' adherence to treatment (4). It is marketed only in Japan, with the commercial name Marizev. Among the various tests for the control and monitoring of medicines, the tablet dissolution test stands out. This assay associated with the safety and efficacy of the treatment, verifies the drug release and its solubilization under physiological conditions, which are factors that can modify the absorption of the drug after administration of oral dosage forms. Thus, in vitro dissolution specifications are established to indicate potential bioavailability problems (5). The dissolution test is seen as an indispensable tool for the pharmaceutical industry, both in product development and in the quality control routine for batch release and stability monitoring (6). The development typically involves selection of dissolution apparatus, optimizing dissolution media and, others conditions (e.g., stirring) to provide sufficient reproducibility and sensitivity or discriminatory power, and defining appropriate dissolution specifications for the products (7). The dissolution assay for OMG tablets is not described in any pharmacopoeia, in addition, its biopharmaceutical classification has not been reported either. Only one published work briefly reports the profile of the tablets' dissolution in only one medium (0.1N HCl) by spectrofluorimetric detection and the dissolution method used has not been validated by the researchers (8). Thus, the present study aimed to develop an OMG tablet dissolution assay using HPLC method for sample detection. After evaluating the dissolution profile of the OMG in different media covering the physiological pH range, the biopharmaceutical classification of the OMG was proposed and the method was in accordance with official guides (9).

Experimental

Chemicals

The OMG standard (purity of 98%) was purchased by Acchemblock (USA). Marizev® tablets 25mg for oral administration were purchased in the commercial pharmacy from Japan. All chemical reagents used in the

preparation of mobile phase were HPLC grades. Methanol, trimethylamine and acid hydrochloric (HCl) were purchased from Merck, the monobasic potassium phosphate and sodium acetate from Sigma Aldrich.

Instrumentation

The high performance liquid chromatography (HPLC) method was carried out in an Agilent 1200 series (auto-injector, quaternary pump, vacuum degasser and diode array and multiple wavelength) connected to a computer loaded with Agilent ChemStation Software (Agilent Technologies, Santa Clara, CA, USA) that was used to control the equipment and to calculate data and responses from HPLC system. The experiments were performed on an analytical column Agilent – ZORBAX Eclipse C8 (250 mm x 4.6 mm x 5 µm) (USA). The system was operated isocratically at 25ºC in the column oven, using a mobile-phase composed by 10µM monobasic potassium phosphate buffer and methanol (45:55 v/v), adjusted to pH 7.0 with triethylamine at a flow-rate of 0.8 $mL.min^{-1}$, using detection at 230 nm. The injection volume was 10 µL. The quantification method used was previously validated by Emanuelli & Schapoval (10). The dissolution test was performed using a Vankel® VK 7010 multi-bath (n=8), auto sampling consisting of a bidirectional peristaltic pump and to the VK 8000 auto-sampler. The test was conducted at 37 °C ± 0.5, using paddles at 50 rpm.

Preparation of reference and sample solution for solubility test

The stock solution of OMG standard ($1mg.mL^{-1}$) was prepared in a 10 mL volumetric flask by dissolving an accurately weighed amount of OMG in methanol. Working dilutions were prepared from this in different media to establish the standard curve. The sample solutions were prepared by weighing the amount of powder from the previously crushed tablet to obtain the final concentration intended.

Solubility test and stability

The media used for solubility test were water, 0.01M HCl, 0.1M HCl, 0.05M sodium acetate buffer (SAB) and 0.05M potassium phosphate buffer (PPB). The amount of OMG used to perform the test was three times the highest commercialized dose (25mg) to guarantee the sink condition of the experiment. From crushed tablets, powder amounts equivalent to 3mg of OMG were weighed and placed in contact with 20 mL of each medium. This proportion was chosen to simulate OMG amount three times in 500 mL of dissolution medium. The preparations were kept under stirring with magnetic bar at 37 ºC ± 0.5 ºC and collections were performed at 0.5, 5 and 9h to ensure that in the analyzed HPLC time range the area would be maintained without the presence of degradation products. In addition, the pH of these preparations was measured at the beginning and at the end of 9h.

Dissolution test

Omarigliptin 25mg tablets were dissolved in 500 mL of 0.01M HCl, 0.05M PPB and 0.05M SAB, thermostatized at 37ºC ± 0.5 ºC using paddles and rotation of 50rpm. The collected samples were filtered through 0.45 µm membrane filter. For HPLC analysis, aliquots of 1 mL were withdrawn from the dissolution medium in pre-established times (5, 10, 20, 30, and 60 min).

Method Validation

To validate the dissolution method, the parameters evaluated were specificity, linearity, precision, and accuracy. The dissolution medium used was PPB (pH 6.8) maintained at 37 °C ± 0.5 using paddles, at 50rmp.

Specificity

Simulated mixture of the formulation placebo were prepared and employed in the specificity evaluation of the method. The solution containing excipients was prepared by mixing mannitol, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, hypromellose, hydroxypropylcellulose, carnauba wax and titanium dioxide. An amount of excipients equivalent to a tablet average weight of OMG was transferred to different vessels (n=3) containing 500mL of potassium phosphate buffer medium, at 37 ºC ± 0.5. After 1 hour of shaking at 150 rpm, 1 mL aliquot was removed and filtered through 0.45 µm filter and analyzed by HPLC. The chromatogram obtained from the placebo was compared to the chromatogram obtained for the analysis of OMG tablet under the same conditions. A mobile-phase composed by 10µM monobasic potassium phosphate buffer and methanol (45:55 v/v), adjusted to pH 7.0 with triethylamine was used to the OMG quantification.

Linearity

The linearity was established through the construction of the three calibration curves. Aliquots of OMG standard solution in methanol ($1\ mg.mL^{-1}$) were removed and diluted with potassium phosphate buffer pH 6.8 to obtain final concentration range of 20-140 $\mu g.mL^{-1}$. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method and analysis of variance (ANOVA).

Precision

Repeatability and intermediate precision were executed to assess method precision. Repeatability was evaluated through relative standard deviation (RSD) from the recovery data corresponding to 100% drug level. The intermediate precision was evaluated by comparing the assays on two days and different analysts. The dissolution test was done for 30 min using vessels containing 500 mL of the dissolution medium, at 50 rpm (n=3). Aliquots of 1

mL were filtered through 0.45 µm filter and analyzed by HPLC in triplicate.

Accuracy

The accuracy was determined by adding known amounts of a powered OMG tablets pool, corresponding to three drug levels 75, 100 and 125% of the labeled amount in an average weight equivalent to a tablet. The dissolution test was done for 30 min using vessels containing 500 mL of dissolution medium, at 50 rpm (n=3), in triplicate.

Results and discussion

Solubility test and stability

The solubility test of OMG tablets was evaluated by testing traditional media and covering the physiological pH range. The calibration curve in the different media was constructed in the range of 20 to 160 µg mL^{-1} in order to contemplate the theoretical OMG concentration obtained in the solubility test (150 µg.mL^{-1}). For all media, the determination coefficient found was greater than 0.9980. Table 1 (end of the text) contains the values of the concentrations found for each medium analyzed and the initial and final pH value of the experiment. As can be seen in Table 1, the pH values of the media remained stable during the analysis time (p> 0.05). The theoretical concentration of the OMG dilution (3 mg) in 20 mL in different media was always close to 150 µg.mL^{-1} and OMG was soluble respecting the sink condition for all dissolution media tested (p> 0.05). When 0.1M HCl media was employed in OMG tablets dissolution, an additional indicative peak degradation product was observed at the chromatograms, although the decrease in OMG area was not statistically significant (p> 0.05) and in the water media no peak purity was observed for OMG. Therefore, the dissolution media chosen for the dissolution test were 0.01 M HCl, PPB, and SAB, covering three different pH ranges capable of simulating different gastrointestinal regions to observe the release behavior of OMG.

Table 1. Different dissolution media used, concentrations found in the different times and the initial and final pH value obtained in the evaluation of sink conditions for OMG tablets (n=3, p=0.05)

Medium	Concentrations (µg.mL^{-1}) 0.5h	5h	9h	pH (a)	pH (b)
Water	149.5 ± 0.30	148.5 ± 0.70	149.1 ± 0.59	6.37 ± 0.08	6.45 ± 0.09
HCl 0.01M	148.5 ± 0.75	147.5 ± 1.35	147.9 ± 1.16	3.16 ± 0.11	3.25 ± 0.11
HCl 0.1M	149.5 ± 0.15	148.7 ± 0.18	148.9 ± 0.40	1.17 ± 0.10	1.21 ±0.09
SAB	150.1 ± 0.12	149.5 ± 0.06	149.1 ± 0.58	4.56 ± 0.11	4.61 ±0.07
PPB	148.5 ± 1.23	148.2 ± 1.56	147.9 ± 0.98	6.75 ±0.06	6.79 ± 0.08

a: initial pH; b: final pH

Dissolution test

The dissolution profile is an important physical-chemical test to demonstrate in vitro the performance of products that require dissolution for its absorption and consequent therapeutic effect (11). The graph of the OMG profiles obtained in the different media is showed below:

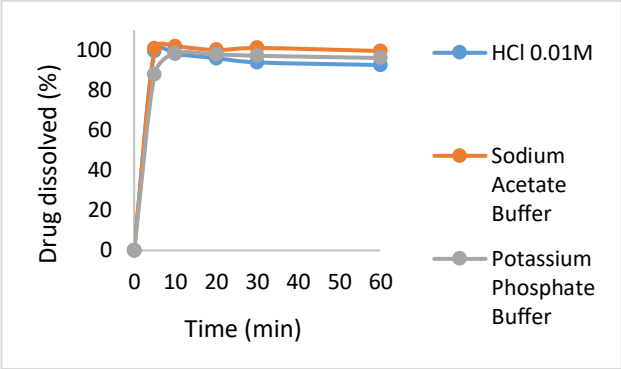

Figure 1. OMG release profile obtained in different dissolution media tested (HCl 0.01M, pH 3.1; Sodium acetate buffer, pH 4.5; Potassium phosphate buffer, pH 6.8)

As can be seen in Fig. 1, the OMG release profile of the tablets was very similar to the three media tested. Practically, 100% of the drug was dissolved within five minutes in 0.01 M HCl and SAB dissolution media, and within 10 min in PPB. According to ANVISA (11), substances with a dissolution profile of at least 85% of the active substance available in less than 15 min fit in very fast dissolutions and this is an important data for comparing dissolution profiles in bioequivalence studies. The disintegration pattern observed was the same in the three tested media, with instantaneous and rapid disintegration after contact of the tablet with the different media. The pharmacokinetic data found in the literature demonstrate maximum plasma concentration values between 0.5 and 2h after the administration of 25 mg of OMG in healthy patients, being, therefore, a compound considered highly permeable (12) with a bioavailability value greater than 87% (13). Considering the obtainment of pharmacokinetic data in the literature and the analysis of the dissolution profile obtained in this work, we can suggest that OMG belongs to class I of the biopharmaceutical classification system created by Amidon et al. (14), therefore presenting high solubility and high permeability. Substances with these characteristics are considered to be well absorbed by the gastrointestinal tract and the limiting factor for absorption is dissolution and/or gastric emptying. If there is a very rapid release of the active substance in the medium, as seen in the dissolution profile of OMG tablets, only the gastric emptying is responsible for the absorption time and no correlation with the dissolution rate is expected (15). In an in vitro release study of OMG developed by Ayoub et al. (8) using 0.1M HCl as a media it was demonstrated that 90% was released within 15 minutes. In this work, we performed the dissolution of OMG in other pH ranges not

yet studied and we verified the rapid disintegration and dissolution of the tablets in different media that contemplate the gastrointestinal physiological pH range.

Method validation

Due to the OMG presenting a similar dissolution profile in the three tested media, we chose to perform the dissolution validation with the medium containing potassium phosphate buffer (pH 6.8), simulating a pH value of intestinal absorption. The specificity was evaluated to verify that the formulation excipients in the dissolution medium did not interfere in the OMG analysis. Figure 2 represent the chromatogram obtained for the tablets' solutions and the excipients formulation. No interference of the excipients' formulation was observed in the OMG analysis by HPLC method, as there is no peak observed, confirming that the method is specific for OMG analysis.

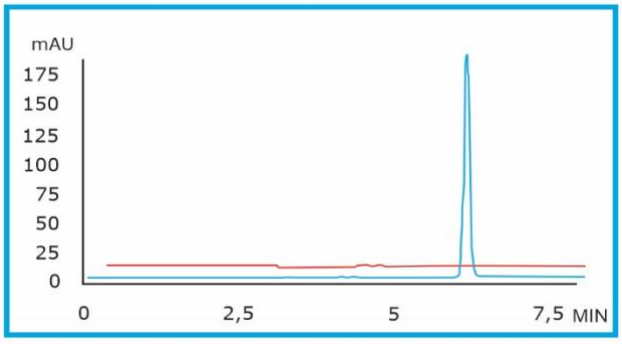

Figure 2. Chromatogram obtained for the solutions of the tablets (blue line) and the excipients formulation (red line) in potassium phosphate buffer medium (pH 6.8)

To assess linearity, three OMG calibration curves were constructed, plotting concentration versus area. The determination coefficient was 0,9998 in the concentration range studied (20-140 $\mu g.mL^{-1}$). The representative linear equation was y= 12931x - 13529. The data were validated by means of the analysis of variance (ANOVA), which demonstrated significant linear regression (F calculated = 15.746 > F critical= 4.60; p=0.05) and there is no significant linearity deviation (F calculated = 0.1 < F critical = 2.96; p=0.05). The statistical p-value obtained for the intercept (0.1118) shows that there is no difference from zero point in the calibration range worked at the 95% confidence level. The chosen concentration range was adequate for detecting the rapid concentration (50 $\mu g.mL^{-1}$) obtained in a few minutes of experiment. The results obtained for the precision and accuracy of the method are described in Table 2 and 3, respectively.

Table 1. Intra-day and inter-day precision results (n=3) for OMG dissolved in 500 mL of the medium and analyzed by HPLC

Sample	Repeatability		Intermediate precision
	Day 1	Day 2*	
1	100.31	99.08	
2	98.03	99.02	
3	98.76	100.15	
Mean (%)	99.03	99.42	99.18
RSD (%)	1.16	0.64	0.87

* Different analyst

Table 2. Mean of recovery (%) and relative standard deviation (%) for OMG added (n=3)

Levels (%)	Mean of recovery (%)	RSD (%)
75	98.26	0.30
100	101.32	0.44
125	101.94	0.51

For the method precision, a pool of OMG tables was prepared, triturated and used for analysis. 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 method accuracy was demonstrated by the recovery of the known amounts of OMG added from the tablet pool to the dissolution vessels. Three levels were evaluated, low, medium, and high. The percentage recoveries ranging from 98% to 102% demonstrate the satisfactory method accuracy.

Conclusion

It was possible to conduct an OMG tablet dissolution assay in media that meet the recommended pH range for this type of study. A rapid dissolution profile was obtained in the three media analyzed and is consistent with the rapid absorption reported in the literature. It was possible to suggest the biopharmaceutical classification of OMG and to conduct the validation of the proposed dissolution method. This article is unprecedented in demonstrating the dissolution profile of OMG in different media and suggest biopharmaceutical classification.

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Conflict of interest

The authors declare no conflicts of interest.

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Data availability

Part of the processed data required to reproduce these findings are available to download from [https://doi.org/10.1016/j.microc.2020.105084].

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