Full Paper

Development and Validation Dissolution Analytical Method of Nimesulide β-Cyclodextrin 400 mg Tablet

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Abstract: The nimesulide (*N*-(4-nitro-2-phenoxyphenyl)methanesulfonamide) belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs and category II of the biopharmaceutical classification, The complexation of nimesulide with β-cyclodextrin is a pharmacological strategy to increase the solubility of the drug The objective of this study was to develop and validate an analytical methodology for dissolving the nimesulide beta-cyclodextrin 400 mg tablet and meets the guidelines of ANVISA for drug registration purposes. Once developed, the dissolution methodology was validated according to the RE of parameters no. 899/2003. In the development of the method it was noted that the duration of the dissolution test was 60 minutes, the volume and the most suitable dissolution medium was 900 mL of aqueous solution of sodium lauryl sulfate 1% (w/ v). It was also noted that rotation of 100 rpm and the paddle apparatus was the most appropriate to evaluate the dissolution of the drug. Spectrophotometric methodology was used to quantify the percentage of dissolved drug. The wavelength was 390 nm using the quantification. The validation of the methodology, system suitability parameters, specificity/selectivity, linearity, precision, accuracy and robustness were satisfactory and proved that the developed dissolution methodology was duly executed.

Keywords: analytical methodology; dissolution study; validation; nimesulide beta-cyclodextrin

1. INTRODUCTION

The nimesulide (*N*-(4-nitro-2-phenoxyphenyl)methanesulfonamide) belongs to the class of non-steroidal antiinflammatory drugs (NSAIDs). Nimesulide is presented as a pale yellow crystalline powder, slightly oily to the touch, odorless and non-hygroscopic. This drug has melting range between 143.3-144.5 °C and is characterized by being practically insoluble in water and very soluble in ethanol, methanol and acetone [1].
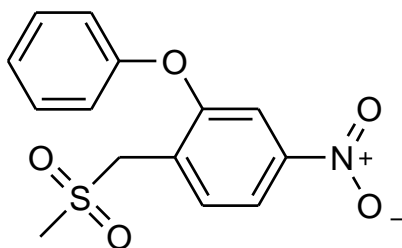
Figure 1. Structural formula of nimesulide flat
Source: ChemDraw (2003).

This drug is prescribed for the treatment of musculoskeletal and osteoarticular inflammations, feverish states, headache, myalgia and postoperative pain due to their analgesic activity, anti-inflammatory and antipyretic properties [2, 3]. Nimesulide has weakly acidic character with an approximate *p*Ka 6.5 and belongs to the category II of the biopharmaceutical classification, or is classified as a drug with low solubility and high permeability. In this category, the dissolution of the drug is a rate-limiting step in oral absorption, so it is possible to obtain a strong correlation to in vitro dissolution and in vivo absorption by the dissolution test. Drugs belonging to biopharmaceutical class II are subject to formulation strategies for improving the dissolution rate and consequently oral bioavailability. The complexation of nimesulide with beta-cyclodextrin is a pharmacological strategy to increase the solubility of the drug [4, 5].

The β-cyclodextrin exhibit good solubility in

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water, therefore the association of drugs with β-cyclodextrin occurs because they possess the ability to encapsulate nonpolar drug molecules. In this case, they form water soluble reversible complexes. As encapsulation of consequences is observed improvement in dissolution rate, bioavailability and stability of pharmaceutical forms [6, 7]. However, there is no dissolution methodology for the association of nimesulide with β-cyclodextrin in official textbooks and literature. Thus, it is essential to the development and validation of methodology dissolution for nimesulide β-cyclodextrin. The resolution directory collegiate number 31 [8], provides that in the absence of analytical method of dissolution described in official compendium (pharmacopoeia), standards or approved specific regulations and approved by ANVISA (National Health Surveillance Agency) the development and validation of dissolution method is necessary.

The development must assess the solubility of the active substance experimentally for each manufacturer in at least three different dissolution media within the physiological pH range (1.2 to 6.8), whereas the temperature of 37 °C ± 1 °C. The choice of means should take into account the chemical nature of the drug, its dissociation constant (pK's), pharmacokinetics and route of administration. Also up should evaluate drug stability in dissolution media, interference of the membrane filters the results of solubility and finally must be held dissolution profiles to determine the most appropriate parameters for the test run. At this stage is that define the apparatus, the rotation, the dissolution medium, the volume of the dissolution medium, the test run time and the minimum amount to be released to allow the drug to be approved in the dissolution test [9].

To ensure and demonstrate confidence in the results provided by the analytical methodology it must demonstrate that it is appropriate for the intended purpose [10]. Because of the large impact that nimesulide is for the public health, the evaluation of the quality of medicines containing the drug is imperative. Considering the absence of dissolution methods to analyze the nimesulide β-cyclodextrin drug, propose a discriminative dissolution methodology and validated to analyze possible quality deviations in medicaments containing the mixture of nimesulide and β-cyclodextrin.

2. MATERIAL AND METHODS

Instrumentation and materials

All the reagents and solvents were purchased from Sigma Aldrich and used as received, unless otherwise indicated. Except nimesulide β-cyclodextrin was courtesy that Brainfarma Chemical and Pharmaceutical Industry S/A. The standard of the nimesulide was purchased from Brazilian Pharmacopeia.

The filter test analyze was performed using a spectrophotometer Agilent Model: 8453 by following the spectrophotometric parameters: wavelength = 390 nm, working concentration = 0.0444 mg/mL nimesulide β-cyclodextrin and blank = NaOH 0.01M.

In liquid chromatography technique High Efficiency equipment Waters model 2695 Separations Module Alliance Waters 2998 Photodiode Array Detector and chromatographic column: LiChrospher C8 RP Select B; 125 x 4 mm; 5 µm; 60 A, mobile phase: ammonium phosphate buffer 6.5mM *p*H: 7.0: acetonitrile in the ratio 65:35, flow rate: 1.3 mL/min and injection volume of 10 µL for analysis the study stability and solubility.

The incubator with orbital shaking platform used to determine the solubility of nimesulide beta-cyclodextrin was Brand New Ethics Model: 430/RDBP.

Dissolution profiles were performed in equipment Hanson model SR8 Plus.

Preparation of standard solution nimesulide

It was weighed 33.3 mg nimesulide standard to 100.0 mL volumetric flask and completed with NaOH 0.01M. After was transferred 2.0 mL this solution to 50.0 mL volumetric flask and completed with NaOH 0.01M. Concentration of nimesulide = 0.0133 mg/mL.

Preparation of sample solutions of nimesulide beta-cyclodextrin 400 mg tablet drug - Study of the dissolution medium

It was weighed tablet nimesulide β-cyclodextrin 400 mg tablet to 900 mL of sodium lauryl sulfate 1.0 % present in a dissolutor. After elapsing 60 minutes of dissolution, collected, about 10.0 mL, filtered through filter paper, pipetted 3.0 mL to 25.0 mL volumetric flask and completed with NaOH 0.01M. Sample concentration of nimesulide = 0.0133 mg/mL equivalent to 0.0532 mg/mL nimesulide β-cyclodextrin.

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Preparation of the placebo solution nimesulide β-cyclodextrin 400 mg tablet

It was weighed 277.5 mg of the placebo nimesulide β-cyclodextrin 400 mg tablet to 900 mL of sodium lauryl sulfate 1.0% present in a dissolutor. After elapsing 60 minutes of dissolution, collected about 10.0 mL, filtered through filter paper, pipetted 3.0 mL and transferred to a 25.0 mL volumetric flask and completed with NaOH 0.01M.

Filter and Stability Study

The filter study was conducted by analysis of standard solutions and sample (222 µg/mL) before and after the process filtration using the following dissolution medium (H₂0, HCl 0.1N, Acetate Buffer pH: 4.5, Phosphate Buffer pH: 6.8 H₂0 + Sodium Lauryl Sulfate 0.5% + Sodium Lauryl Sulfate 0.75% + Sodium Lauryl Sulfate 1.0%). The stability was evaluated keeping standard solutions and sample (222 µg/mL) stored under light at room temperature (n = 3). After 24 h, readings were performed the same solutions against standard solutions nimesulide (222 µg/mL) newly prepared (n = 6) was determined percentage of responses.

Study of the dissolution medium

Various conditions were tested for dissolution test of the tablets, which are indicated in table I aiming to determining the most suitable conditions. For each condition was carried out the dissolution profile of withdrawing aliquots at times of 5, 10, 15, 30, 45 and 60 minutes. The rates collected were filtered through milex 0.45µm discarding the first 5 mL. The concentration of drug in the dissolution medium was determined employing a spectrophotometer (Blank: NaOH 0.01M and UV detection at 390 nm).

Table 1. Conditions evaluated in the development of the dissolution test for tablets of nimesulide β-cyclodextrin.

Dissolution Medium	Water	HCl 0,1N	Ammonium Acetate Buffer pH 4.5	Potassium Phosphate Buffer pH 6.8	Sodium Lauryl sulfate 0.5%	Sodium Lauryl sulfate 0.75%	Sodium Lauryl sulfate 1.0%
Volume (mL)	500/900	500/900	500/900	500/900	500/900	500/900	500/900
Apparatus	Basket/Paddle	Basket/Paddle	Basket/Paddle	Basket/Paddle	Basket/Paddle	Basket/Paddle	Basket/Paddle
Rotation	50/75/100	50/75/100	50/75/100	50/75/100	50/75/100	50/75/100	50/75/100

Method Validation

The proposed method was validated according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines Q2 (R1) [13]. The primary endpoints are adequate, specificity, linearity, precision, accuracy and robustness.

Adequation

It was prepared standard solution of nimesulide. We conducted a sweep in the ultraviolet and visible (200-900 nm). It was determined by the wavelength where the absorbance is maximal. From there took place five readings. The standard deviation was calculated between the relative standard solution readings.

Specificity

To determine the selectivity/specificity have been prepared standard solutions of nimesulide, sample and placebo. After preparation of these solutions was held 3 standard solution readings and second readings of other solutions. It carried out also in parallel a scan of each of the preparations in the wavelength range 200-900 nm to verify the interference of other components (impurities and placebo) with the active ingredient.

Linearity

The linearity of the method was examined by linear regression and analysis of the calibration curves. Calibration curves were constructed over the graphical representation of absorbance and nimesulide β-cyclodextrin compared their corresponding concentrations. From the standard solution, a calibration curve at seven different levels of concentrations (45%, 55%, 65%, 75%, 85%, 95% and

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105%) was constructed and evaluated the correlation coefficient (r) RSD values.

Precision

The accuracy of the methodology developed was determined at two levels: repeatability (intra-run) and intermediate precision (inter-run).
Repeatability was determined after the preparation of six sample solutions. After preparation the reading is carried out of the solutions and used to calculate the mean and standard deviation of the six preparations.
Intermediate precision was determined as the repeatability test, but the test was performed on different days with different analysts and different equipment.

Accuracy

For the determination accuracy was prepared under the same conditions, analytical solutions of the sample in triplicate concentrations in the theoretical work corresponding to 80% (0.01066 mg/mL nimesulide equivalent to 0.04264 mg/mL nimesulide beta-cyclodextrin), 100% (0.01333 mg/mL nimesulide equivalent to 0.05332 mg/mL nimesulide beta-cyclodextrin) to 120% (0.015996 mg/mL nimesulide equivalent to 0.063984 mg/mL nimesulide beta-cyclodextrin). Was prepared by a standard solution yet, the readings were made and if the calculated average amount recovered (accuracy in %) at each concentration level.

Robustness

The robustness of the method was determined after preparation of standard solutions and samples. Was performed 5 consecutive readings of the standard solution and the sample solution 2 readings varying the following analytical parameters:

- Wavelength (390 nm, 396 nm and 400 nm);

- Filter paper brand.

It is also determined the stability of the prepared analytical solutions. For this purpose, standard solutions were prepared and the sample and held at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours two consecutive readings of each solution.

3. RESULTS AND DISCUSSION

Filter and Stability Test

The filter test study was to evaluate the adequation of the membrane filters of 0.22 µm and 0.45 µm used in the dissolution test of nimesulide beta-cyclodextrin drug 400 mg tablet, and to evaluate the interference of the excipients in the quantification of active, so discarding the first 5 ml and without neglecting initial volume. Performed this procedure in the following dissolution media (H_2O, HCl 0.1 N, Acetate Buffer pH: 4.5, Phosphate Buffer: pH 6.8 + Sodium Lauryl Sulfate 0.5% + Sodium Lauryl Sulfate 0.75% + Sodium Lauryl Sulfate 1.0%), and thus it was found that 0.45 µm membrane, and the first 5 ml are discarded results were presented within the specified ($\pm$ 2,0%) in all the proposed dissolution media.

It was used and validated analytical methodology indicative of stability it possible to quantify the drug solubilized in the dissolution media in the presence of their possible degradation products. Variations exceeding $\pm$ 2% show that the drug does not have stability in dissolution medium. The nimesulide β-cyclodextrin drug is stable for 24 hours in all tested dissolution media (H_2O, HCl 0.1 N, Acetate Buffer pH: 4.5, Phosphate Buffer pH: 6.8 H_2O + Sodium Lauryl Sulfate 0.5% + Sodium Lauryl Sulfate 0.75 % + Sodium Lauryl Sulfate 1.0%), once in every time (0h, 6h, 12h, 18h and 24h) area of variation is less than $\pm$ 2%.

Study of the dissolution medium

The nimesulide β-cyclodextrin 400mg tablet product is classified as immediate release dosage form and drug nimesulide belongs to class 2 biopharmaceutical classification, the drug has low solubility and high permeability. The FDA (1997) [15] recommended for immediate release dosage forms must release 85% of the drug between 30 and 45 minutes. The first dissolution profiles of nimesulide beta-cyclodextrin 400mg tablet was performed in water, HCl 0.1N, buffer pH 4.5 and in buffer pH 6.8. Evaluation of solubility of nimesulide β-cyclodextrin drug indicated that such means would not be appropriate and discriminative for the dissolution study. The completion of the dissolution profiles in these media only confirmed what was expected. It is further noted that the 20% level of the active release has not been achieved in any of these dissolution media.

The dissolution medium water showed the

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highest amount of nimesulide β-cyclodextrin dissolved between resources without surfactant. Therefore, continuation of the dissolution study occurred with the addition of increasing concentrations of the surfactant sodium lauryl sulfate in water. Were adopted concentrations of 0.5, 0.75 and 1.0% lauryl (w/v) for performing the next profiles. As the aim is to evaluate the medium, the initial dissolution conditions were maintained, or used to rotation 50 rpm paddle apparatus and 900 mL of dissolution medium. Based on the results, the dissolution medium containing 1.0% sodium lauryl sulfate (w/v) was chosen for further study because it showed a higher release of the active and lower coefficients of variation of 20% for the first two points (5:10 minutes) and less than 10% for other collection times (15, 30, 45, 60 and infinity test).

Once defined the dissolution medium was held dissolution profiles using paddle apparatus, 900 ml of aqueous solution of sodium lauryl sulfate 1.0% (w/v) in each tub to determine the most appropriate rotation. To this end, we investigated the release of the active at increasing speeds of 50, 75 and 100 rpm as the technical note No. 3/2013 of ANVISA [16] and the recommendations of the United States Pharmacopeia (USP 37) [14]. Based on the obtained results, it was possible to define the most appropriate speed for the drug dissolution analysis nimesulide β-cyclodextrin 400 mg tablet was the rotation of 100 rpm, as this has been possible to verify the more gradual release of the active and there was formation plateau. It is also noted that the release was minimal difference between the collection time of 60 minutes (99.08% of the active release) and the condition of infinity test (100.37%). Furthermore, the variation coefficients were 20% smaller than the sampling times of 5 and 10 minutes and less than 10% for the collection times of 15, 30, 45 and 60 minutes. Once set, the dissolution medium and the most suitable rotation, two dissolution profiles were performed using as medium an aqueous solution of sodium lauryl sulfate, 1.0% (w / v) with 900 ml in each vessel, and rotation 100 rpm to define the most suitable apparatus. To this end, we investigated the release of the active shovel and basket apparatus.

The comparison between the spade and basket apparatus showed that the basket is not feasible to analyze the drug nimesulide β-cyclodextrin 400 mg, for its use significantly affected the release of the asset. It was observed that was not reached even 80% of the active release apparatus with the basket 60 min

dissolution. As for the paddle apparatus was the release of practically all of the active ingredient within 60 minutes of dissolution (97.33%) and the release difference to infinity test conditions was approximately 3%. The coefficients of variation for the paddle apparatus for times of 5 minutes and 10 form less than 20% and for the periods of 15, 30, 45 and 60 minutes were less than 10%. Thus, the blade apparatus had superior performance in the evaluation of the dissolution of nimesulide β-cyclodextrin drug in relation to the basket apparatus. After the dissolution medium is defined, and rotating apparatus most suitable for the development of discriminating dissolution methodology investigated the influence of the volume of dissolution medium added to the vat in the active principle release. This evaluation was carried out two profiles dissolution using an aqueous solution of sodium lauryl sulfate, 1.0% (w/v) as the medium, 100 rpm and rotating paddle apparatus. The data of the solubility test indicated that the volumes 500 and 900 ml can be used for the dissolution profile test, since a minimum of 3 times the volume amount necessary to prevent formation of a saturated solution is satisfied (condition sink). From the data obtained it was found that the volume of the dissolution medium affected the release of the active ingredient. The volume of 500 mL, although to achieve sink conditions, negatively affecting the dissolution of the drug, for 60 minutes to dissolve only 85.16% of the active has been released. According Gibaldi (1991) [17] excipients present in a pharmaceutical formulation can promote or hinder the dissolution of the drug and consequently the speed and the amount of it that is available to be absorbed. Since the data obtained for the volume of 900 mL indicate that this volume was ideal as it released nearly all assets in 60 minutes of dissolution (97.33%). Furthermore, the coefficients of variation for the volume of 900 mL were approved and the values found for 5 times and 10 minutes make up less than 20% and for the periods of 15, 30, 45 and 60 minutes were less than 10%, as recommended by RDC No. 31 of 2010 [8]. Thus, the volume of 900 mL showed superior performance in assessing the dissolution of nimesulide beta-cyclodextrin medicinal product in relation to the volume of 500 mL (Table 2).

Method Validation

After the development of the analytical methodology dissolution proceeded to validate it according to the parameters set in the RE No. 899 of

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ANVISA [18].

Table 2. Optimum conditions for the dissolution test nimesulide β-cyclodextrin 400 mg tablet.

Parameters	
Dissolution medium	Solution of sodium lauryl sulfate 1% (w/v)
Trappings	Paddle
Volume (mL)	900
Rotation speed (rpm)	100
Quantification method	Spectrophotometry – 390nm (Blank: NaOH 0,01M)

Adequation

It conducted a sweep in the ultraviolet and visible (200-900 nm) of the electromagnetic spectrum and found that the wavelength ideal for quantifying the asset was 390 nm.

It found that the spectrophotometric system was inappropriate as relative standard deviation (RSD) between 5 readings was less than 2%.

Specificity

The specificity/selectivity were determined by scanning in the range of 200 to 900 nm of the standard solution, sample, diluent and placebo. The analysis of the scan spectra showed that the diluent and placebo solutions do not interfere with the quantification of analyte nimesulide β-cyclodextrin at a wavelength of 390 nm (length at which shows maximum absorption).

Linearity

The study evaluated the linearity of the method in the range 45-105%. From the absorbance values obtained for each concentration plotted the graph. Data from the analytical curve were fitted by linear regression analysis using the method of least squares to minimize the sum of squared residuals of the regression.

The mathematical equation that describes the linear relationship between absorbance and concentration (Figure 2) was $y = 0.00074 + 0,6093x$.

The obtained correlation coefficient was 0.9995, which means that 99.95% of the total variation around the mean is explained by linear regression. The obtained correlation coefficient

fulfilled the minimum acceptance criterion of 0.99 set by ANVISA in the RE No. 899 of 2003 [18]. In addition, he pointed out that there was a very strong linear relationship between absorbance and concentration variables.
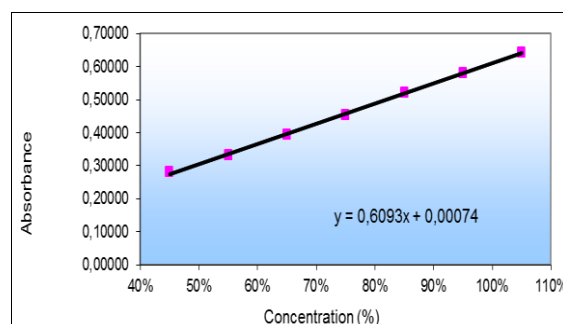
Figure 2. Equation of the line obtained by linear regression.

Precision

Precision was evaluated by the proximity of the results of six sample preparations made by different analysts in different days. Resolution RE No. 899 of ANVISA [18] does not admit values greater than 5% relative standard deviation to analyze precision of an analytical methodology. The repeatability and intra-run precision was determined on the first day by one analyst using the spectrophotometer 1. The standard deviation for this test was 1.07%, so the method has repeatability. As for the determination of intermediate precision, the second analysts trained over six samples were read in spectrophotometer 2. The relative standard deviation of the six samples obtained by the second analyst in second day was 0.71%.

Accuracy

The limits have been adopted for this test were 98- 102% [13]. The accuracy was verified by the degree of recovery for three levels (80%, 100% and 120%). The experimental data revealed that the mean analyte nimesulide beta-cyclodextrin recovery was 100.17% and that the greatest relative standard deviation value was 0.4% in concentration of 120%. It was found that in all experimental concentrations used (80, 100 and 120%) average recovery levels are within the specified limits, which indicates adequate accuracy of the method.

Robustness

The robustness of the method was assessed by adapting the system and the variation of the content of samples subjected to conditions purposely changed in the analytical method. The studies revealed that the change in wavelength has not changed the performance of the method, as the results obtained at different wavelengths were statistically similar. This growth was less than 1%.

The analytical solution stability test was performed by comparing the initial absorbance of the standard solution and freshly prepared sample (zero time) with the absorbance of the same solution after a predetermined time. The standard solution was stable for 24 hours while the sample solution for 18 hours. During these periods the changes in absorbance were less than ± 2.0% over the initial absorbance.

4. CONCLUSION

A physical mixture of nimesulide with β-cyclodextrin was an important pharmacological strategy to circumvent the problem of low solubility of the nimesulide and improve the dissolution rate and consequently oral bioavailability. In implementing the solubility tests it was observed that the membrane was 0.45 µm most suitable for quantification of active therefore results presented within specification for all solutions tested and in all filter conditions. The nimesulide β-cyclodextrin drug was stable for 24 hours in water, HCl 0.1N, acetate buffer pH 4.5, phosphate buffer pH 6.8 and aqueous solution of sodium lauryl sulfate 1.0 % (w/v). There was no significant change in pH between the beginning and end of the study solubility.

The most discriminative conditions for the implementation of the dissolution test were 60 minutes for the length of the dissolution test, 900 mL for the volume of dissolution medium, aqueous solution of sodium lauryl sulfate 1.0 % (w/v) into the medium dissolution, 100 rpm for rotation and shovel for the apparatus.

The validation of the methodology confirmed and secured his suitability to use, precision, accuracy, robustness, specificity and selectivity. Therefore, the proposed analytical methodology for analysis of the drug dissolution of nimesulide beta-cyclodextrin 400 mg tablet assured reliable results and met the guidelines for registration.

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