

Development and validation of a dissolution method using HPLC for diclofenac potassium in oral suspension

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The present study describes the development and validation of an *in vitro* dissolution method for evaluation to release diclofenac potassium in oral suspension. The dissolution test was developed and validated according to international guidelines. Parameters like linearity, specificity, precision and accuracy were evaluated, as well as the influence of rotation speed and surfactant concentration on the medium. After selecting the best conditions, the method was validated using apparatus 2 (paddle), 50-rpm rotation speed, 900 mL of water with 0.3% sodium lauryl sulfate (SLS) as dissolution medium at $37.0 \pm 0.5^\circ\text{C}$. Samples were analyzed using the HPLC-UV (PDA) method. The results obtained were satisfactory for the parameters evaluated. The method developed may be useful in routine quality control for pharmaceutical industries that produce oral suspensions containing diclofenac potassium.

Uniterms: Dissolution method/development. Dissolution method/validation. Diclofenac potassium/release in oral suspensions. Oral suspension/quality control. High performance liquid chromatography/qualitative analysis.

O presente estudo descreve o desenvolvimento e validação de um método de dissolução *in vitro* para avaliação da liberação de diclofenaco potássico suspensão oral. O teste de dissolução foi desenvolvido e validado de acordo com as diretrizes internacionais. Parâmetros como linearidade, especificidade, precisão e exatidão foram avaliados, bem como a influência da velocidade de rotação e a concentração de tensoativo no meio. Depois de selecionar as melhores condições, o método foi validado usando o aparato 2 (pás), velocidade de rotação de 50 rpm, 900 mL de água com 0,3% de lauril sulfato de sódio (LSS) como meio de dissolução a $37,0 \pm 0,5^\circ\text{C}$. As amostras foram analisadas pelo método de CLAE-UV (PDA). Os resultados obtidos foram satisfatórios para os parâmetros avaliados. O método desenvolvido pode ser útil na rotina de controle de qualidade para as indústrias farmacêuticas que produzem suspensões orais contendo diclofenaco potássico.

Unitermos: Método de dissolução/desenvolvimento. Método de dissolução/validação. Diclofenaco potássico/liberação em suspensão oral. Suspensão oral/controle de qualidade. Cromatografia líquida de alta eficiência/análise qualitativa.

INTRODUCTION

Aqueous solubility of any drug is a key property that determines dissolution and absorption, and thus bioavailability *in vivo*. The most widely used test to establish the rate of drug release is the dissolution

test, which is a very important tool to demonstrate bioequivalence from batch-to-batch as well as ensuring the quality of products and performance after certain changes, for instance in the formulation and manufacturing process (Azarmir *et al.*, 2007; Frost, 2004; Modi, Tayade, 2007).

For drugs belonging to Biopharmaceutical Classification System Class 2, dissolution is the limiting step for drug absorption and the dissolution profile must be clearly defined and highly reproducible (Oliveira *et al.*, 2009). From a biopharmaceutics point of view, a more

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discriminating dissolution method is preferred because the test will indicate possible changes in product quality (Vaucher, 2009).

In order to demonstrate that the method is appropriate for dissolution test purposes, the parameters of linearity, specificity, precision, accuracy, stability and influence of the filter type should be evaluated (FDA, 1997; The United States Pharmacopeia, 2012).

Diclofenac potassium (DP), benzenecetic acid 2-[(2,6-dichlorophenyl)amino], is a non-steroidal drug with anti-inflammatory, analgesic and antipyretic properties. It is used for rheumatoid arthritis, degenerative disease, chronic pain associated with cancer and kidney stones and endodontic procedures (Janbroers, 1987; The United States Pharmacopeia, 2012).

DP is readily absorbed orally, but the presence of food reduces its plasma concentration (C_{max}) from about 40% to 60%. Unlike sodium salt which has a delayed release, DP is formulated to achieve dissolution under acidic conditions in the stomach. When compared to other salts, it has higher solubility (Jijun *et al.*, 2011; Olson *et al.*, 1997). In the past few years several studies have been published concerning the determination of DP in pharmaceutical formulations (Elkady, 2010; Souza, Tubino, 2005; Naidoo *et al.*, 2009; Scallion, Moore, 2009; Sparidans *et al.*, 2008). The USP 2012, presents the official dissolution test method *in vitro* for DP in tablets, where the dissolution medium utilized is simulated intestinal fluid without enzyme, however the dissolution test *in vitro* for DP in oral suspension is not listed in any pharmacopoeia and no dissolution test for oral suspension has been reported in the literature).

The present study aims to develop and validate a dissolution test for DP in oral suspension using a high performance liquid chromatographic method (HPLC) to determine the release rate of this drug.

MATERIAL AND METHODS

Chemicals and Reagents

Diclofenac potassium reference standard with 99.8% purity, was supplied by Farmacopeia Brasileira (2012). Two batches, Z0047 and Z0049A, of oral suspension (Cataflam[®]) containing 2 mg/mL of diclofenac potassium were obtained from the commercial market. The excipients of the pharmaceutical formulation were citric acid, sorbic acid, deionized water, strawberry flavor, microcrystalline cellulose, sodium cyclamate, hydroxyethyl cellulose, propylparaben, methylparaben, propylene glycol, glyceryl polyoxyethylene glycol stearate, saccharin sodium. All of them were obtained from different local distributors.

Water was purified using the Millipore[®] system. All other reagents were of analytical grade.

Instrumentation and chromatography conditions

The dissolution test was performed in a PHARMA TEST[®], PTWS-3E (Hainburg/Germany), digitally controlled heater/circulator and multibath ($n=8$), in accordance with general methods (United States Pharmacopeia, 2012). The LC system consisted of a Shimadzu[®] (Kyoto, Japan), provide with an LC-20AT pump, SIL-20A ht auto sampler, CTO-20AC column oven, SPD-M20A PDA detector, CBM-20A system controller, and LC solution software was used to quantify the samples. The Ultra Basic Denver potentiometer was used to determine the pH of all solutions.

Chromatographic separations were achieved using a Phenomenex[®] Luna C8 (150 x 4.6 mm, 5 μ m) column at $30.0 \pm 0.5^\circ\text{C}$. The mobile phase contained a mixture of methanol: buffer phosphate pH 2.5 (70:30 v/v), flow rate of 1.0 mL/min, PDA detection at 275 nm. The injection volume was 20 μ L.

Determinations of sink conditions

The selection of a dissolution medium may be based on the solubility data and dosage range of the drug product. The sink conditions were determined in different solvents, such as: phosphate buffer (pH 6.8 and 7.4) and water with sodium lauryl sulphate (0.3%, 0.5%, 1.0% and 1.5%). An amount of drug equivalent to the highest dose that can be administered was added in 250 mL of each medium. After stirring for 24 hours, an aliquot was transferred to a volumetric flask and diluted with dissolution medium. The drug solubility in each medium was determined in duplicate.

In vitro drug release studies

The dissolution test study with USP apparatus 2 (50/75 rpm, paddles) was tested to evaluate the best conditions. The following procedure was performed for all tests to develop this method: an equivalent amount of 10.0 mg of DP was added to each vessel containing medium that was selected based on the solubility data. Syringes were utilized to add the product to each vessel. The syringes were weighed before and after adding the product, and the weight difference was related to product density.

Sample aliquots were withdrawn at 5, 10, 15, 30 and 60 minutes and replaced with an equal volume of fresh medium to maintain a constant total volume. The percentage of drug dissolved was determined using the HPLC method.

Dissolution method validation

Specificity

It was evaluated by preparing a placebo sample of oral suspension at the usual concentration. This sample was transferred to a vessel ($n = 3$) with dissolution medium and stirred for 2 hours at 150 rpm using paddle and a temperature of $37.0 \pm 0.5^\circ\text{C}$. Aliquots of this solution were filtered and analyzed using the HPLC method.

Linearity

Aliquots of a $100 \mu\text{g/mL}$ solution of DP reference standard prepared in a dissolution medium were transferred to 25 mL volumetric flasks to obtain the final concentrations of 2.0, 6.0, 10.0, 14.0 and $18.0 \mu\text{g/mL}$. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, using analysis of variance (ANOVA).

Accuracy and precision

Accuracy was evaluated by recovering the amount of DP reference substance added to the placebo. Aliquots of 0.36, 1.8 and 3.24 mL of the standard solution (5 mg/mL) were added to the vessel containing placebo equivalent to a usual concentration and dissolution medium at $37.0 \pm 0.5^\circ\text{C}$, and agitated for 60 minutes with a paddle at 50 rpm. The final concentrations were 2.0, 10.0 and $18.0 \mu\text{g/mL}$. The analysis was done in duplicate on three days. Repeatability and intermediate precision were evaluated based on RSD from the recovery data.

Stability and influence of filter

Dissolution of the sample ($n=3$) was carried out for analysis of stability, under previously selected conditions. Samples were collected after 2 hours and injected into the HPLC system at 0, 12 and 24 hours. For determination of filter interference, samples ($n=3$) of DP were submitted to a dissolution test. After a predetermined time the samples were collected and filtered using F. Maia filter (Sample A) and centrifuged (sample B). Sample B was used as a standard value. This study aims to evaluate whether the drug will adsorb on polymeric membrane filter. The acceptance criterion for loss by adsorption is a maximum of 5.0% (Linderberg *et al.*, 2005).

Evaluation of dissolution profiles

The dissolution profiles were compared using the model independent method. The model independent approach includes the difference factor (f_1) and the similarity factor (f_2) using equations 1 and 2 respectively:

$$f_1 = \{[\sum_{i=1}^n |Rf - Tf|] / [\sum_{i=1}^n Rf]\} \times 100 \quad (\text{Equation 1})$$

$$f_2 = 50 \times \log\{[1 + (1/n)\sum_{i=1}^n (R_i - T_i)^2]^{-0.5} \times 100\} \quad (\text{Equation 2})$$

where n is the number of time points, R_i and T_i are the percent dissolved of the reference and test product, respectively.

The percent of error is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles (Polli *et al.*, 1996). According to the FDA, 1997, two dissolution profiles are declared similar if f_1 is between 0 and 15 and f_2 is between 50 and 100.

RESULTS AND DISCUSSION

Solubility determination

DP BCS (Biopharmaceutical Classification System) is class II, and showed low solubility and high permeability. Therefore, drugs of this class may show dissolution rate as the limiting factor in the absorption process (Fortunato, 2005; Qureshi, 2006). The solubility test showed that DP was soluble in phosphate buffer (pH 6.8 and 7.4) and water with sodium lauryl sulphate (0.3%, 0.5%, 1.0% and 1.5%), Table I, suggesting that the sink condition was satisfied. The term sink condition is defined as the volume of medium at least greater than three times that required, forming a saturated solution of a drug (The United States Pharmacopeia, 2012).

TABLE I - Solubility test of diclofenac potassium in different fluids

Dissolution medium	% Drug Dissolved after 24 hours ^a	RSD (%)
Buffer solution phosphate (pH 6.8)	98.07	0.34
Buffer solution phosphate (pH 7.4)	99.93	0.72
Water + 0.3% SLS	98.41	0.41
Water + 0.5% SLS	98.05	0.91
Water + 1.0% SLS	98.49	0.37
Water + 1.5% SLS	98.96	0.68

^a Mean of two analysis; RSD – Relative Standard Deviation

Dissolution profiles of DP in oral suspension

Dissolution tests using a paddle at 75 rpm and phosphate buffer (pH 6.8 and 7.4) and water with sodium lauryl sulphate (0.3%, 0.5%, 1.0% and 1.5%) were evaluated (Figure 1 and 2, respectively).

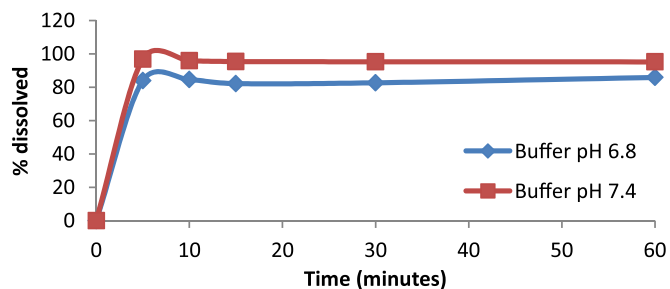


FIGURE 1 – Mean dissolution profile of Cataflam® oral suspension ($n=12$) using phosphate buffer pH 6.8 and 7.4 and paddle rotating at 75rpm.

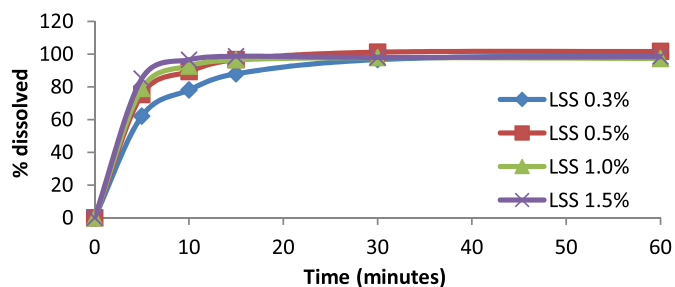


FIGURE 2 – Mean dissolution profile of Cataflam® oral suspension ($n=12$) using water with sodium lauryl sulphate SLS (0.3%, 0.5%, 1.0% and 1.5%) and paddle rotating at 75 rpm.

For assays utilizing buffer pH 6.8 and 7.4, a very rapid drug release was found in the first 5 minutes, 83.98% and 96.96% respectively, however for the dissolution media using water with SLS at concentrations of 0.3%, 0.5%, 1.0% and 1.5%, we found that the release rate of the drug increases as the surfactant concentration increases in the dissolution media.

A rotation speed of 50 rpm (Figure 3) was used to retard drug release in dissolution medium containing water with SLS 0.3% and 0.5%.

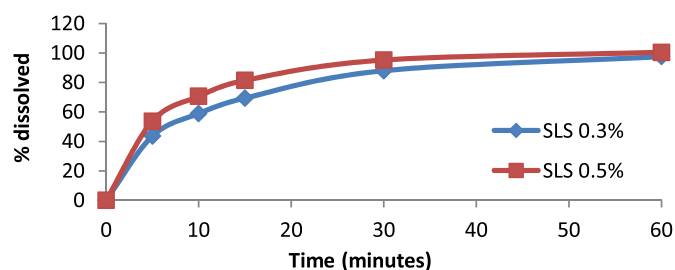


FIGURE 3 – Mean dissolution profile of Cataflam® oral suspension ($n=12$) using water with sodium lauryl sulphate 0.3% and 0.5%, apparatus rotating at 50rpm.

For both dissolution mediums tested the agitation speed of the apparatus significantly influenced the rate of drug release at each sampling time.

Using water with sodium lauryl sulphate 0.3% and apparatus rotating at 50 rpm, a slower, reproducible and discriminating release profile was obtained between batches when compared with the dissolution medium containing water with SLS 0.5%. FDA (1997) recommends using the smallest possible amount of surfactant in the dissolution medium, because the higher the concentration of the surfactant, the greater the difficulty of obtaining a correlation. Therefore, the dissolution medium containing water with 0.3% SLS was selected to develop the method.

Method validation

Specificity

Specificity was observed by analyzing a placebo solution. The chromatograms showed that no interference of excipients was observed for the drug retention time (about 7.2 min) Figure 4.

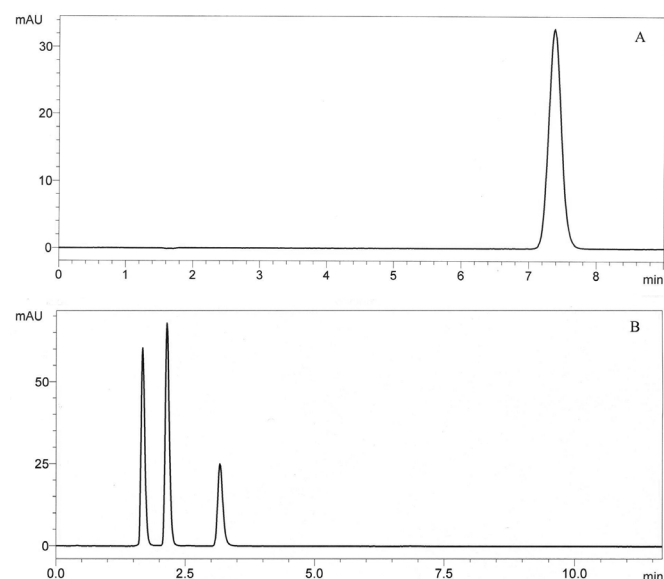


FIGURE 4 - The specificity of the method shows a peak of DP (A) and excipients solution (B) in the dissolution medium water with 0.3% SLS.

Linearity and Range

The linearity was tested in the concentration range of 2.0 – 18.0 $\mu\text{g/mL}$ of drug substance. The results demonstrate method linearity with a correlation coefficient of 0.9999, the slope and y -intercept obtained were 42580 and 6257, respectively. The analysis by ANOVA showed significant regression and non-significant linearity deviation ($P < 0.05$) for the relation between the area of the peaks of substance and its concentrations. The results were considered acceptable and the linearity curves were used to calculate *in vitro* drug release studies.

Accuracy and Precision

The recovery measured is typically 95.0-105.0% of the drug amount added (FROST, 2004). The accuracy of this method was considered adequate in the range of 97.85-100.08% for DP (Table II), corroborating the accuracy of the method. The intermediate precision and repeatability were evaluated at three different concentrations of 2.0, 10.0 and 18.0 µg/mL over three days, by different analysts. The RSD values are shown in (Table III).

TABLE II - Accuracy of the dissolution method for DP in oral suspension

Standard solution (µg/mL)	Recovery (%) (<i>n</i> = 6)
2	97.85
10	99.99
18	100.08
Mean recovery (%) ± SD (<i>n</i> = 18)	99.31 ± 1.26 (95.43 – 103.14)

SD – Standard Deviation

TABLE III - Intermediate precision and repeatability of the dissolution method for DP in oral suspension

Intraday (<i>n</i> = 6)	Mean value (%) ± SD	RSD (%)
day I	101.13 ± 2.49	2.46
day II	97.82 ± 0.94	0.96
day III	98.98 ± 2.04	2.06
Interday (<i>n</i> = 3)	99.31 ± 1.68	1.7

SD – Standard Deviation; RSD – Relative Standard Deviation

Stability of drug in solution and influence of filter type

After testing the stability of drug in dissolution medium, the drug remained stable in solution for 24 hours when stored at room temperature. All results obtained during analysis times were very close to the initial value, according to (Table IV).

TABLE IV -Stability of drug after dissolution test

Dissolution medium	% Drug Dissolved after test			RSD (%)
	Time zero	After 12 hours	After 24 hours	
Sample in solution (<i>n</i> = 3)	99.65	99.29	99.52	0.18

RSD – Relative Standard Deviation

The filter used was unable to interfere in the quantitative analysis of the drug. The sample filtered using F. Maia filter (*sample A*) presented an average content of 99.42% and the centrifuged sample (*sample B*), an average content of 97.71%, which proves the noninterference of the filter used in the tests.

Discrimination power of selected dissolution method

Evaluation of dissolution profiles is an important tool when it is necessary to know the behavior of two products before submitting them to the Study on Bioavailability/Bioequivalence, for post-registration changes, waiver of *in vivo* studies of smaller dosages as well as optimizing the pharmaceutical process to develop new formulations (Brasil, 2010). The profiles were evaluated accordingly (Brasil, 2010), using points 10, 15 and 30 minutes. Factors f_1 and f_2 were 2.55 and 72.65 respectively, coefficient variation was less than 20.0% for the first points and less than 10.0% for the other points, confirming that the profiles obtained between batches of pharmaceutical products are similar, according to (Figure 5). The results of dissolution profiles for both products are given in Table V.

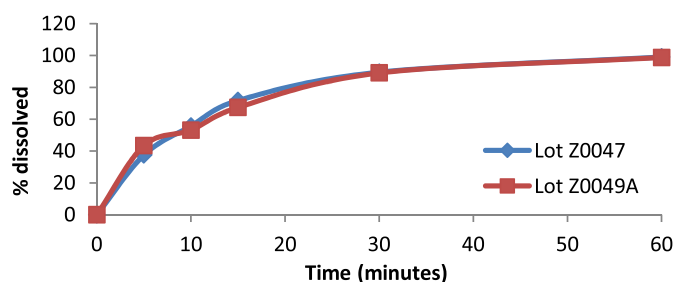


FIGURE 5 – Comparison of profiles of Cataflam® oral suspension (*n* = 12) batches Z0047 and Z0049A, using water with sodium lauryl sulphate 0.3% and apparatus rotating at 50 rpm.

CONCLUSION

A quick, simple dissolution method was successfully developed for the evaluation of the *in vitro* release of DP

TABLE V - Comparison of *in vitro* drug release profiles of two batches of DP oral suspension

Time (minutes)	% drug release ^a			
	Lot Z0047	RSD (%)	Lot Z0049A	RSD (%)
0	0	-	0	-
5	43.48	2.05	43.26	3.49
10	58.91	2.23	53.76	1.09
15	69.74	1.41	67.44	2.18
30	88.16	1.49	89.73	2.54
60	98.89	1.38	99.06	1.81

^a Mean of two analysis; RSD – Relative Standard Deviation

in oral suspension. The conditions used were USP type II apparatus at 50 rpm, containing 900 mL of water with 0.3% SLS, at 37.0 °C ± 0.5. Drug stability was maintained under the developed dissolution conditions, and therefore the method could be used in routine quality control of DP in oral suspension.

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