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Development and Validation of an Eco-friendly HPLC-UV-DAD Method for the Determination of Allopurinol in Pharmaceuticals with Application to *In vitro* Dissolution Studies

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Abstract: In this study, a sustainable HPLC-UV-DAD method was developed and validated for the determination of allopurinol in tablets and optimization of the dissolution test using factorial design. The separation of the analyte from the sample matrix was achieved in 3.01 minutes in a C8 column (4.6 mm X 150 mm X 5 µm), using mobile phase 0.1 mol L^{-1} HCl (25%) + ethanol (50%) + ultrapure water (25%) by UV detection at 249 nm. The method presented satisfactory analytical parameters of validation (specificity, selectivity, linearity, stability, precision, accuracy, and robustness), showing no matrix effects. The dissolution test was optimized by complete factorial design 2^3 and, the optimal conditions were: HCl 0.001 mol L^{-1}, apparatus II (paddle) and 75 rpm. The analytical procedures and dissolution tests were applied to allopurinol tablets marketed in Bahia, Brazil, to evaluate the dissolution studies. The pharmaceuticals had similar dissolution profiles and first-order dissolution kinetics. This new and sustainable HPLC-UV-DAD method is friendly to the environment and can be used for the routine pharmaceutical analysis of allopurinol in fixed dosage forms.

Keywords: allopurinol; eco-friendly HPLC-UV-DAD method; factorial design; dissolution; pharmaceutical and chemical analysis.

1. Introduction

Allopurinol is a drug used in treating idiopathic gout, lithiasis, and acute nephropathy induced by uric acid. In neoplastic or myeloproliferative diseases, after its treatment with cytotoxic agents, increase blood levels of urates. It is the natural isomer of hypoxanthine that inhibits the enzyme hypoxanthine oxidase. Its main metabolite oxypurinol decreases the levels of uric acid in plasma and urine [1]. Chemically denominated as 1H, 2H, 4H-pyrazolo [3,4-d] pyrimidin-4-one (Figure 1), allopurinol is soluble in water, ethanol, and chloroform, presenting an increment in solubilization with an increase in the pH of the solvent and also has stability in exposure to light and moisture [2,3]. There is a controversy about its classification in the biopharmaceutical classification system, is classified as class 4 (low solubility and low permeability) by the Drug Delivery Foundation (DDF) and class 1 (high solubility and high permeability) [4,5].

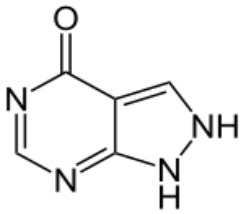

Figure 1. Molecular structure of the allopurinol.

In Brazil, allopurinol is available on the market as tablets (100 mg and 300 mg), totaling 17 presentations (02 reference and 15 generic drugs) [6]. Pharmacopeias determine the tests that must be performed on the active pharmaceutical ingredient and the pharmaceutical forms. In the absence of information in the compendiums, industries must adopt other official pharmacopeias or develop and validate methods capable of identifying and quantifying the drugs presented in the most diverse pharmaceutical forms and in their active pharmaceutical ingredients [7–9]. In addition, these methods must respect the principles of green chemistry, with the elimination and/or reduction in the use of solvents toxic to the environment, decreasing the environmental impact of pollutants' emission and contributing to a sustainable environment [10]. Furthermore, analytical techniques, such as high-performance liquid chromatography (HPLC) and molecular absorption spectrophotometry in the ultraviolet (UV) region, in combination with chemometric tools, are used for drug analysis due to its advantages and low solvent consumption [11–13]. However, there are still toxic and environmentally unsustainable solvents, such as methanol and acetonitrile [14–18].

The speed and dissolution rate of dissolution is decisive for the release and absorption of a drug from a solid oral pharmaceutical form. Therefore, the dissolution kinetics study is very important and, dissolution testing has been used primarily as a quality control test for solid oral drug products [19–24]. The dissolution test determines the percentage of the active pharmaceutical ingredient released in the dissolution medium as a function of time, using the label's quantity as a marker. Also, it demonstrates whether the product meets the criteria defined in the studied drug monograph through an *in vitro* prediction ratio of bioavailability [25,26]. To assess the quality of medicines, the dissolution profile is compared with the reference medicine. This test is performed by collecting multipoint and the dissolution test's execution to determine if the dissolution profiles of the two drugs tested are statistically comparable to evaluate the pharmaceutical equivalence [27–29].

Therefore, this study aimed to develop and validate an efficient, rapid, and sustainable HPLC-UV-DAD method, combined with chemometric tools, for the determination of allopurinol and obtain the dissolution profiles of allopurinol tablets. In addition to proposing a green and environmentally sustainable method, this new method could also be used for the routine analysis of allopurinol in fixed dosage pharmaceutical forms. The method was validated according to guidelines and applied for the assay of allopurinol tablets.

2. Materials and Methods

2.1. Reagents and materials.

All chemical reagents used in experiments were gradient grad (Quimex®, Merck, Brazil). Allopurinol reference chemical substance (RCS) was purchased from Brazilian Pharmacopoeia, through the National Quality Control Institute of the Oswaldo Cruz Foundation (FIOCRUZ, Brazil), with 100.0% purity, calculated concerning the desiccated

substance and determined by HPLC. For the preparation of all standard solutions and samples, ultrapure water (with resistivity 18 MΩ cm^{-1}) obtained from the Milli-Q Pluswater purification system (Millipore Molsheim, France) was used. All laboratory glassware was washed in a 10% (v v^{-1}) HNO_3 solution for 24 h, rinsed with high-purity water, and dried at ambient temperature. Nylon filters with 0.22 µm pores (Agilent, USA) were used to filter the samples. Allopurinol tablets, containing 100 and 300 mg (reference and generic products, designed as Z1, Z2, M1, M2, S, and P), were purchased from pharmacies located in Salvador and Santo Amaro da Purificação, Bahia, Brazil. All tests were performed on products within the expiration date.

2.2. Instruments.

A high-performance liquid chromatography (Shimadzu scientific instruments, Kyoto, Japan), outstanding model, equipped with high-pressure quaternary pump (Model LC-20AD), degasser (model DGU-20A5), diode array detector (Model SPD-20A), sampler automatic (model SIL-20A), column heating oven (model CTO-20A) and, a communication module (model CBM-20A) and operated by the LC solutions software, was used. The used column (stationary phase) C8 column (150 mm X 4.6 mm X 5.0 µm) was purchased from Shimadzu, Barueri, SP, and Brazil. The dissolution tests were performed in the dissolving equipment (Ethik, Model 299, Vargem Grande Paulista, SP, Brazil) with a manual collection of aliquots (10 mL), at the determined times and replacement of the dissolution medium. The determination of tablet weight, content uniformity, and allopurinol dosage were performed using the analytical balance (M164-AI Mark®, Piracicaba, SP, Brazil). The friability test used the friabilometer equipment (Ethik, Model HX 300-2, Vargem Grande Paulista, SP, Brazil) and, the disintegration test was performed on the disintegrator (Nova Ética, Modelo 301 / AC01, Vargem Grande Paulista, SP, Brazil).

2.3. Chromatographic conditions.

To define the chromatographic conditions and to solubilize the RCS allopurinol, 20 µL of solutions containing RCS allopurinol (1.0 µg mL^{-1}) were injected using the solvents: ethanol, HCl (0.1 mol L^{-1}), NaOH (0.1 mol L^{-1}), and methanol, with mobile phase methanol and ultrapure water (50: 50, v v^{-1}). Then, the composition of the mobile phase was evaluated: ethanol + ultrapure water (50: 50 v v^{-1}); ethanol + ultrapure water (70: 30 v v^{-1}); 0.1 mol L^{-1} HCl + ultrapure water (50: 50 v v^{-1}); ethanol + 0.1 mol L^{-1} HCl (50: 50 v v^{-1}); and, 0.1 mol L^{-1} HCl + ethanol + ultrapure water (25: 50: 25 v v^{-1}), checking which flow had the best separation between peaks. The flow rate of the mobile phase was investigated from 0.6 to 0.9 mL min^{-1}. After optimization, 20 µL of the solution containing the RCS allopurinol (1.0 µg mL^{-1}) + 1% solution (w v^{-1}) of excipients (lactose, starch, croscarmellose sodium, microcrystalline cellulose, macrogol, crospovidone, povidone, polyvinylpyrrolidone, and magnesium stearate) contained in the formulation of the tablets, were injected to assess selectivity. Retention time (Rt), retention factor (k), the number of theoretical plates (N), and chromatographic resolution (R) were calculated.

2.4. Analytical validation.

The method was validated through the analysis of specificity, selectivity, linearity, stability, precision, accuracy, limits of detection (LOD) and quantification (LOQ), and robustness, in accordance with the protocols suggested by the International Conference on

Harmonisation (ICH) and International Union of Pure and Applied Chemistry (IUPAC) recommendations [30,31].

Specificity and selectivity were evaluated by the exclusive analytical response of the analyte (allopurinol) without interference from excipients, matrix, impurities, or degradation products. The analytical signals of the mobile phase and 1% solution (w $v^{-1)}$ of excipients (placebo) were evaluated, and, therefore, well-shaped peaks also indicate the specificity of the method. Linearity was determined by the correlation coefficients of the analytical curves generated (concentrations of allopurinol versus the peak area was constructed) by the injections (triplicate) of the working solutions at five concentration levels. The solutions and samples' stabilities were evaluated by checking possible changes in the analytical signal after HPLC analysis for 24 h, at room temperature, and kept at 37 °C. Triplicate averages with a standard deviation (RSD) lower than 10% were considered satisfactory for evaluating the precision. A known amount of RCS allopurinol (0.5 µg mL^{-1}) was added to a sample solution (Z1). Recoveries (> 80%) were considered satisfactory for assessing the accuracy of the proposed method. Robustness was evaluated from the flow of the mobile phase variations (0.6; 0.7; 0.8 and 0.9 mL min^{-1}) by RSD (%) of measurements in triplicate. The limits of detection and quantification were estimated using the following equations: LOD = 3.3 × SDb/a and LOQ = 10 × SDb/a, respectively, where SDb is the standard deviation of the intercept and a is the slope of the regression line [32].

Table 1. Variables and levels were used in the experimental design and complete factorial design 2^3 with 6 repetitions of the central point.

Variables	**Levels**		
	-1	**0**	**1**
Rotation (rpm)	50	75	100
[HCl] (mol L^{-1})	0.001	0.01	0.1
Apparatus	basket	basket or paddle	paddle
Complete factorial design 2^3			
Experiment	**rpm**	**[HCl]**	**apparatus**
1	-1(50)	-1(0.001)	-1(basket)
2	+1(100)	-1(0.001)	-1(basket)
3	-1(50)	+1(0.1)	-1(basket)
4	+1(100)	+1(0.1)	-1(basket)
5	-1(50)	-1(0.001)	+1(paddle)
6	1(100)	-1(0.001)	+1(paddle)
7	-1(50)	+1(0.1)	+1(paddle)
8	+1(100)	+1(0.1)	+1(paddle)
9 (C)	0(75)	0(0.01)	0(basket)
10 (C)	0(75)	0(0.01)	0(basket)
11 (C)	0(75)	0(0.01)	0(basket)
12 (C)	0(75)	0(0.01)	0(paddle)
13 (C)	0(75)	0(0.01)	0(paddle)
14 (C)	0(75)	0(0.01)	0(paddle)

2.5. Dissolution studies.

Brazilian Pharmacopoeia [8] did not indicate methodologies for allopurinol dissolution tests. Only in 2019, with the publication of its sixth edition, methods using molecular absorption spectrophotometry in the ultraviolet region (UV) for quality control of allopurinol (identification, dissolution, and dosage tests) were introduced. Currently, the Brazilian and United State (USP) [33] pharmacopeias recommend the use of UV spectrophotometric methods for the dissolution test for allopurinol in tablets, under the following conditions: 900 mL hydrochloric acid (HCl) 0.01 mol L^{-1}; apparatus 2 (paddle), with a rotational speed of 75

rotations per minute (rpm), for 45 minutes. Therefore, this study aims to develop and validate an unedited and sustainable HPLC-UV-DAD method to evaluate this drug's dissolution. For the allopurinol dissolution test, in tablets, a complete factorial design 2^3 was carried out, with 6 central points, with the following variables studied: concentration of the dissolution medium (0.1; 0.01 and 0.001 mol L^{-1} HCl), in a volume of 900 mL; the speed of rotation of the dissolver (50, 75 and 100 rpm); and, the type of apparatus [type 1 (basket) and type 2 (paddle)] during 45 minutes and, the optimal conditions for drug determination were established (Table 1).

After optimizing the proposed dissolution test for allopurinol, the allopurinol tablets (Z1, Z2, M1, M2, S, and P) were submitted to the dissolver obtain the dissolution profiles. 10 mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 0, 1, 3, 5, 10, 15, 20, 30, 40, 45, 60, 75, and 90 minutes. The aliquots were filtered, and the concentrations of allopurinol in samples were determined by the proposed HPLC-UV-DAD method. The profiles were compared using the method based on ANOVA (factors: f_1 and f_2), dependent model method (definition of zero-order and first-order models) with the construction of time (minutes) versus the quantity of undissolved allopurinol. The correlation coefficient (r), dissolution rate constant (k), dissolution half-life ($t_{50\%}$), and dissolved amount of allopurinol were calculated after 45 minutes of dissolution test (Q_{45}). Also, the dissolution efficiency (ED%) was calculated using the trapezoid method. Weight uniformity, friability, and disintegration time were determined according to Brazilian [8] and USP [33] pharmacopeias.

3. Results and Discussion

3.1. Analytical performance and validation.

The HPLC-UV-DAD proposed method was validated to provide analytical applications for the dissolution test's rapid quality control analysis. The results showed that the proposed method met the validation requirements defined by the guidelines (Table 2), presented good linearity and homoscedasticity, showing no matrix effects. Therefore, it is suggested that this proposed method can be used to quantify allopurinol as an active pharmaceutical ingredient and finished product, respecting the limits of the working range.

Table 2. Validation of the method for determination of allopurinol (tablets) using HPLC-UV-DAD.

Figures of merit	**Values obtained (ICH)**	**Values obtained (IUPAC)**
Accuracy (%)	99.76 – 99.81	99.79 – 99.82
Precision (RSD, %)	< 0.50	< 0.50
Detection limit (LOD, µg mL^{-1})	0.06	0.05
Quantification limit LOQ, (µg mL^{-1})	0.18	0.12
Linearity regression equation	y = 507223.8x + 1022446.6	y = 538618.3x + 1022446.6
Correlation coefficient (r)	0.9961	0.9976
Determination coefficient (r^2)	0.9922	0.9952
Linear working range (µg mL^{-1})	0.18 – 0.80	0.12 -0.80
Robustness (RSD, %)	<1.90	<2.00

3.2. HPLC-UV-DAD method and applications.

Among the medium for the RCS allopurinol solubilization, ethanol and 0.1 mol L^{-1} HCl showed satisfactory results (Figure 2), at 210 and 249 nm wavelengths. In all studies consulted in the literature, the solvents used in the solubilization of allopurinol were methanol and acetonitrile. The present study proposes using an alternative solvent, friendly to the environment, through a new sustainable and efficient method. Thus, 0.1 mol L^{-1} HCl was selected, respecting the spectrograms obtained, green chemistry principles, and similarity with

gastric fluid. Rajkumar *et al*. (2014) [16] developed and validated a method for simultaneous determination of allopurinol and lipoic acid (210 and 250 nm) in tablets using reverse-phase HPLC, in India. Ammar *et al*. (2017) [14] used the wavelength of 254 nm to quantify allopurinol and salicylic acid simultaneously in Egypt. In USP [33], the allopurinol determination is carried out at a wavelength of 230 nm. In this study, the wavelength of 249 nm was selected for the determination of the allopurinol.

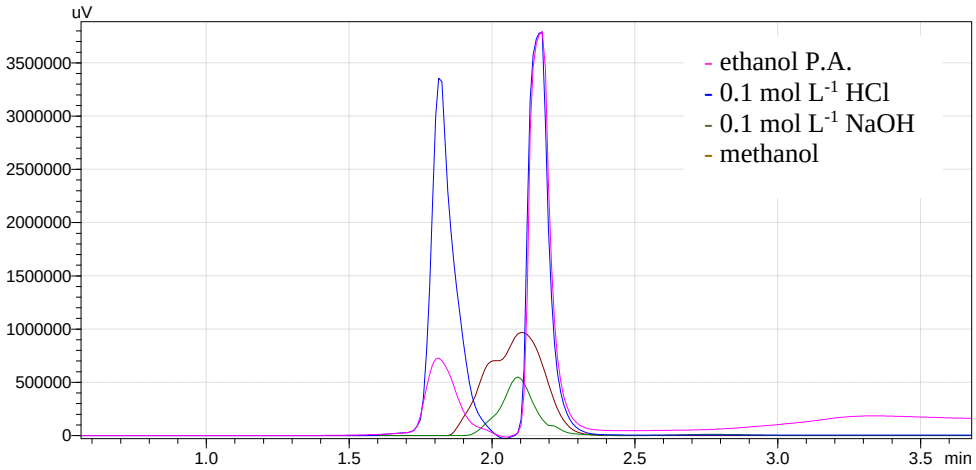

Figure 2. Ideal solubilization medium for allopurinol (RCS and tablets) determination.

The mobile phase 0.1 mol L^{-1} HCl + ethanol + ultrapure water (25: 50: 25, v v^{-1}) was selected for analysis, as it has lower consumption of pure ethanol solvent, meeting the principles of sustainability. The flow rate of the mobile phase was determined at 0.6 mL min^{-1} (Figure 3). For Jahangirian *et al*. (2017) [10], the consumption of a smaller proportion of solvents, especially toxic ones, must be one of the questions to be evaluated in the development of methods aiming at the minimum environmental impact to the ecosystem.

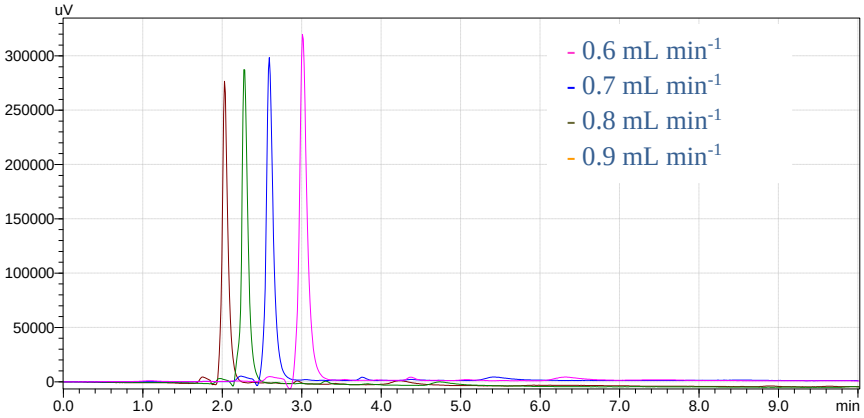

Figure 3. Selection of flow rate of the mobile phase: allopurinol RCS (1.0 µg mL^{-1}) chromatograms in 0.1 mol L^{-1} HCl.

Rajkumar *et al*. (2014) [16] used, as a mobile phase, a mixture of aqueous solution of acetic acid + sodium acetate (2.72 g L^{-1}) adjusted to pH 4.5 + acetonitrile (96: 4, v v^{-1}). Ammar *et al*. (2017) [14] used a mixture of acetonitrile + ammonium acetate buffer at pH 4.6 (50:50, v v^{-1}). The Brazilian [8] and USP [33] Pharmacopoeias recommend as media, a mixture of 0.25% (w v^{-1}) monobasic potassium phosphate aqueous solution + methanol (70: 30, v v^{-1}) and (50: 50, v v^{-1}), respectively. In the literature, no methods were found using this mobile phase to determine allopurinol, being, therefore, one of the innovations of this study.

The retention time of allopurinol (3.01 minutes), the retention factor (5.20), several theoretical dishes (6,442), and chromatographic resolution (2.05) were calculated, with a running time of 5.0 minutes being defined. It stands out that the running time recommended in the Brazilian [8] and USP [33] Pharmacopoeias for their determinations is 30 and 35 minutes, respectively. Based on these studied parameters, the optimum conditions for allopurinol determination (Table 3) were established.

Table 3. Optimal conditions of HPLC-UV-DAD method for allopurinol (RCS and tablets) determination.

Parameters	**Optimal conditions**
Column	C8 (4.6 mm X 150 mm X 5 µm)
Mobile phase	0.1 mol L^{-1} HCl (25%) + ethanol (50%) + ultrapure water (25%)
Flow rate (mL min^{-1})	0.6
Temperature (ºC)	30
Running time (minutes)	5
Detector	DAD (249 nm)
Retention time (Rt, minutes)	3.01

3.3. Dissolution studies.

After developing and validating the proposed HPLC-UV-DAD method, dissolution studies (test and dissolution profiles) were planned to evaluate the dissolution and release (%) of allopurinol in tablets. The Brazilian Pharmacopeia [8] indicates that no less than 75% of the drug is dissolved in 45 minutes. After experimental design and evaluation by the Pareto diagram, it was possible to observe that none variable showed a statistically significant contribution in the dissolution of allopurinol, isolated or in combination (Figure 4).

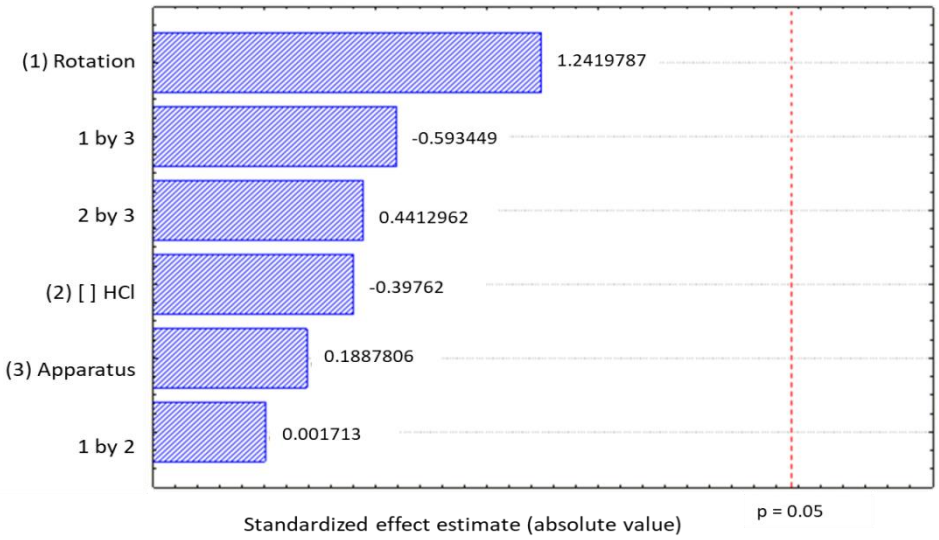

Figure 4. Pareto's diagram of the experimental design for determining the conditions for the allopurinol drug dissolution test.

Thus, the following conditions were selected: dissolution medium (900 mL, 0.001 moL L^{-1} HCl), apparatus type 2 (paddle) and 75 rpm rotation. Furthermore, 50 rpm could be used, but the 75 rpm rotation was selected. It is the same as described in USP [33] and Brazilian [8] Pharmacopoeias. According to the results obtained, this variable did not cause prejudice to the method since this change in the rotation did not interfere with the test results. Also, it is noteworthy that, in the proposed method, the HCl concentration was ten-fold lower than recommended by pharmacopeias. Multifactorial analysis for determining the optimal conditions of an experiment is extremely important since it is able to establish the best

conditions for determining an analyte, reducing the number of tests performed and, especially, when a large amount is needed of toxic and environmentally harmful solvents. Moreover, the use of smaller amounts of these solvents has been proposed by the chemical, food, pharmaceutical, and cosmetic industries [10,12,34].

The dissolution test reports, *in vitro*, the amount of allopurinol dissolved in the reaction medium and, therefore, available to be absorbed in the gastrointestinal tract. Therefore, it is an important test to be evaluated when developing a formulation and in quality control [26]. Tablets containing allopurinol released more than 80% of allopurinol in 45 minutes (Z1 = 99.92; Z2 = 99.11; M1 = 99.63; M2 = 99.93; S = 99.62 and P = 99.44 %) and, therefore, met the criteria defined by the Brazilian [8] and USP [33] pharmacopoeias.

The pharmaceutical dissolution profiles were obtained for the evaluation of pharmaceutical equivalence. The results obtained (Figure 5) demonstrated that allopurinol-containing drugs (Z1, Z2, M1, M2, S, and P) exhibited comparable dissolution profiles for the drug, according to ANOVA (Table 4). The tested drugs released ≥ 75% of the drug after 10 minutes of testing. For comparison of the dissolution profiles, the difference factor (f_1) was calculated among pairs. Similarity factors (f_2) were not calculated since when the drug has high solubility and provides an immediate release, very fast dissolving technology (dissolving more than 75% of the drug in less than 15 minutes) for both drugs, the factor f_2 loses its discriminative power and, therefore, it is not necessary to calculate it [27].

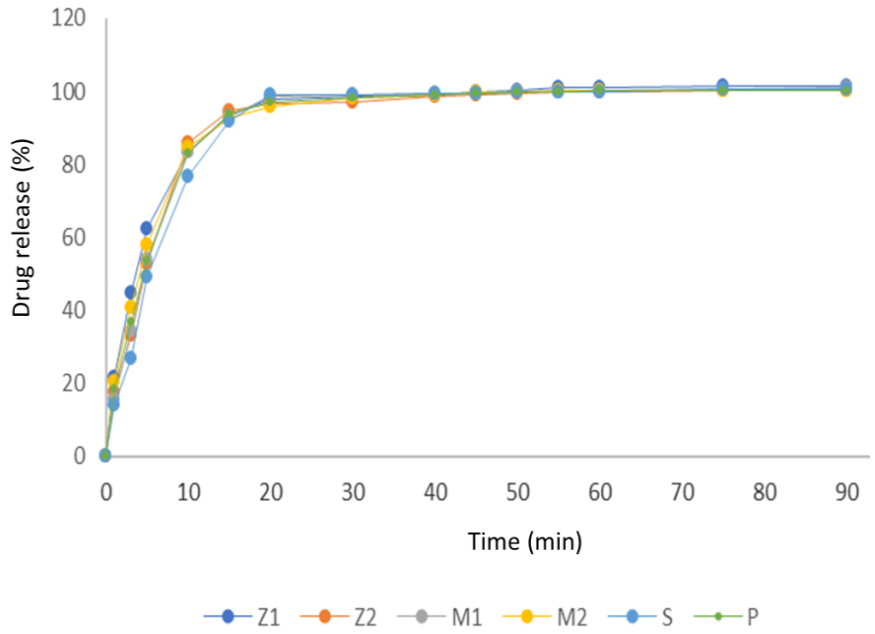

Figure 5. Dissolution profiles of allopurinol tablets (100 and 300 mg) of products Z1, Z2, M1, M2, S, and P by HPLC-UV-DAD method. (USP type 2 apparatus at 75 rpm with 900 mL 0.001 moL L^{-1} HCl at 37.0 ± 0.5 ºC for 90 minutes).

Table 4. Evaluation of variance (ANOVA) of the dissolution profiles of drugs containing allopurinol and f1 values using HPLC-UV-DAD method.

ANOVA					
Statistical analysis	**Variance between groups**	**Variance within the group**	**F (calculated)**	**F (critical)**	**p-value**
HPLC-UV-DAD method	0.008747	9.877099	0.014878	2.323126	0.999918
Samples (Drug)	**Z1 x M1**	**Z1 x S**	**Z2 x M2**	**Z2 x P**	
f_1	2.18	4.05	1.29	0.48	

Additionally, the zero and first-order kinetic models were calculated for the proposed HPLC-UV-DAD method. The first order dissolution kinetic model adjusted better to this process, showing correlation coefficients closer to 1 compared to zero order. From the model definition, it was possible to determine the parameters: dissolution rate constant (K), dissolution half-life ($T_{50\%}$) and, dissolved amount of allopurinol 45 minutes after the start of the dissolution test (Q_{45}) (Table 5). The values obtained for $T_{50\%}$ of the tested drugs were near (4.56 to 6.81 minutes), corroborating with the data from the dissolution profile analysis (ANOVA and f_1). In addition, K varied a little quantity (0.10 to 0.22), reinforcing that allopurinol's dissolution was very fast in the tablets.

Table 5. Statistical parameters of regression studies, applying models (zero and first-order) by HPLC-UV-DAD method and mean values of kinetic parameters (± standard deviations) from first-order kinetics model, across dissolution profiles of allopurinol.

Dissolution kinetic model

Drug product	**Z1** (r)	**Z2** (r)	**M1** (r)	**M2** (r)	**S** (r)	**P** (r)
Zero order	0.7041	0.7060	0.7094	0.7071	0.7270	0.7104
First order	**0.9782**	**0.9784**	**0.9682**	**0.9782**	**0.9669**	**0.9790**

Kinetic parameters (± standard deviations) from first-order kinetics model

Drug product	**$T_{50\%}$ (minutes)**	**Q_{45} (%)**	**K (minutes^{-1})**
Z1	4.99 (± 0.27)	99.82 (± 5.76)	0.14 (± 0.01)
Z2	6.81 (± 0.35)	99.07 (± 4.51)	0.10 (± 0.01)
M1	5.82 (± 0.49)	99.56 (± 9.77)	0.12 (± 0.02)
M2	4.56 (± 0.33)	99.86 (± 4.17)	0.15 (± 0.01)
S	4.75 (± 0.52)	99.49 (± 8.96)	0.15 (± 0.02)
P	5.17 (± 0.19)	99.41 (± 3.20)	0.13 (± 0.01)

r: correlation coefficient

Moghal *et al*. (2016) [35] determined the kinetic dissolution model of allopurinol in the immediate release tablets developed in Savar Dakar, Bangladesh. Of the 09 drugs tested, 07 presented the first-order model like the one that best explains the dissolution of formulated drugs, corroborating this study's findings. Ammar *et al*. (2017) [14] performed the dissolution test on four drugs using the 0.1 mol L^{-1} HCl medium and established the dissolution kinetics. Of those analyzed, two presented a first-order model for dissolution of allopurinol, one of them, the reference drug (100 mg).

Dissolution efficiency (DE,%) of allopurinol (106.03 – 107.66%) in the analyzed drugs was calculated using the trapezoids rule, according to the established criterion (≥ 75%, in 45 minutes), in Brazilian [8] and USP [33] pharmacopeias. Alghadi and Hamedelneil (2017) [5] studied the immediate release of allopurinol in 0.1 mol L^{-1} HCl, in tablets sold in Sudan. Only one drug product had a dissolution percentage below 75% of the allopurinol, in 15 minutes of testing. All other medicines showed drug release > 90% in 15 minutes. Moghal *et al*. (2016) [35] evaluated the *in vitro* release of allopurinol in the immediate release tablets developed in Bangladesh, using as excipients croscarmellose sodium, lactose, microcrystalline cellulose, crospovidone, talc, and magnesium stearate. The dissolution profiles in the 0.01 mol L^{-1} HCl medium, using molecular absorption spectrophotometry with detection at 250 nm, showed drug release > 80% within 10 minutes. Kumar *et al*. (2016) [36] in Punjab, India, formulated and evaluated nine formulations containing allopurinol with the excipients crospovidone, croscarmellose sodium, magnesium stearate and talc (also present in the formulations studied in this study), using the HCl 0.1 mol L^{-1} dissolution medium, by UV spectrophotometric method, at 250 nm and found dissolution> 75% of the allopurinol dose, in eight formulations.

Tablets with a higher amount of crospovidone had a greater dissolution of allopurinol in a shorter time.

The data obtained in this study corroborate with the authors mentioned above. These same excipients are also present in the formulations of the analyzed products, which may explain the very rapid dissolution of allopurinol in tablets tested. From the results obtained for the dissolution studies (profile, kinetics, and dissolution efficiency), it is concluded that the drugs containing allopurinol met the requirements determined by the pharmacopoeias. However, this study's innovative character stands out, as no study found in the scientific literature has performed dissolution tests using HPLC-UV-DAD methods.

Table 6 shows the results obtained, following the Brazilian [8] and USP [33] pharmacopoeias, to determine average weight, friability, disintegration time, and uniformity of unit doses allopurinol tablets.

Table 6. Average weight, friability, disintegration time, and uniformity of unit doses in allopurinol tablets.

Drug products	Average weight (mg ± SD)	Friability (Loss, %)	Disintegration time (minutes and seconds)	Uniformity of unit doses (%)
Z1	179.7 ± 1.3	0.2	5’ 35” (± 0.03)	98.7 – 100.4
Z2	543.6 ± 0.9	0.2	4’ 40” (± 0.05)	97.2 – 100.3
M1	175.8 ± 0.9	0.3	1’ 50” (± 0.12)	99.6 – 100.3
M2	513.6 ± 0.3	0.3	1’ 10” (± 0.07)	99.3 – 100.6
S	174.0 ± 1.7	0.2	4’ 50” (± 0.13)	97.8 – 101.7
P	537.8 ± 1.1	0.,2	3’ 29” (± 0.11)	99.3 – 100.3

SD: standard deviation

Disintegration is a physical phenomenon, and the faster this process, the more drug may be available to be dissolved in the reaction medium and later absorbed into the body, becoming bioavailable. M1 and M2 (generic drugs) showed the shortest disintegration times, which may be related to several technological factors of drug production, such as hardness and excipients present in the formulations, for example, disintegrating agents, favoring the decompression of powders [26]. Very wide variations in disintegration times can induce a difference in the drug dissolution profile and, therefore, must be taken into account in developing generic drugs [37]. The drugs showed uniform doses in production, and, therefore, these lots have quality for commercialization. The results obtained in this study corroborate with Kumar *et al*. (2016) [36]; Kumar and Basava Rao (2016) [38], and Moghal *et al*. (2016) [35], who performed the tests to determine weight, friability, and disintegration in tablets containing allopurinol.

4. Conclusions

A rapid and sustainable HPLC-UV-DAD method was developed and validated, in combination with chemometric tools, to determine allopurinol and obtain the dissolution profiles of allopurinol tablets. The method was validated according to guidelines (ICH and IUPAC) and applied for allopurinol tablets' assay. Allopurinol was determined in three minutes, with a total running time of 5 minutes, representing 6 times faster than a recommended race in the USP Pharmacopoeia. The allopurinol dissolution test (tablets) was optimized, reducing (10 times) the HCl concentration (from 0.01 to 0.001 mol L^{-1}), thus contributing to the principles of green chemistry. The drugs showed first-order dissolution kinetics, and it was possible to determine the dissolution half-life, constant in the dissolution rate, the amount of allopurinol dissolved in 45 minutes, and dissolution efficiency. Therefore, this new and sustainable HPLC-UV-DAD method is friendly to the environment and can be used for allopurinol's routine pharmaceutical analysis in fixed dosage forms.

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Conflicts of Interest

The authors declare no conflict of interest.

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