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ARTICLE
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# Correspondence of captopril disulfide content with sulfur odor in 25 mg captopril tablets from public pharmacies (health clinics) and commercial drugstores

## Correspondência de teor de dissulfeto de captopril com odor de enxofre em comprimidos de captopril 25 mg, provenientes de farmácias públicas (postos de saúde) e drogarias comerciais

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## ABSTRACT

**Introduction:** Captopril (CP) is the drug of choice for the treatment of hypertension. Its degradation leads to the formation of captopril disulfide dimer (DSCP), associated with a strong odor in the drug, which can cause the patient abandonment of treatment. **Objective:** To determine DCSP, associate the olfactory perception of the sulfur odor given off by the product and carry out the evaluation of the package insert for captopril 25 mg tablets distributed in the public and private sectors. **Method:** The performance of CP and DSCP determination method of the Brazilian Pharmacopoeia 6 ed was verified by HPLC (DAD). Thirteen products of 25 mg captopril tablets were analyzed, 2 of which came from the public sector from different batches and the same manufacturer: the other 11 came from the private sector from different batches and manufacturers. The samples were analyzed regarding appearance, odor perception, identification, weight determination, CP and DSCP content (by HPLC) and package insert content. **Results:** Among the 13, the expired drug had 4.4% DSCP; the others were in accordance with the specification. Correspondence of perceptible sulfur odor was established for drugs with DSCP content above 0.5%. Considering the texts on sulfur odor in the package inserts, the findings were: none information (3 products), characteristic odor (2), slight sulfur odor (1), slight sulfur odor without decreasing effectiveness (7). **Conclusions:** The samples showed satisfactory results for the tests performed. There was a lack of homogeneity in the information in the package inserts about odor of the tablets. The patient's perception of sulfur odor, even within the tolerated limit of DSCP, can lead to non-acceptance of the drug and consequent non-adherence to the treatment of hypertension, in addition to causing damage to the SUS.

**KEYWORDS:** Captopril Disulfide; Degradation Product; Sulfur Odor; Anti-hypertensive

## RESUMO

**Introdução:** O captopril (CP) é o medicamento de escolha para o tratamento da hipertensão arterial. Sua degradação leva à formação do dímero dissulfeto de captopril (DSCP), este associado a um odor forte no medicamento, podendo causar abandono do tratamento pelo paciente. **Objetivo:** Determinar DCSP, associar a percepção olfativa de odor de enxofre desprendido do produto e realizar a avaliação de bula de comprimidos de captopril 25 mg distribuídos pelos setores público e privado. **Método:** Foi verificado o desempenho do método de determinação do CP e DSCP pela Farmacopeia Brasileira 6ª ed. por HPLC (DAD). Foram analisados 13 produtos de comprimidos de captopril 25 mg, sendo dois provenientes do setor público de lotes diferentes e mesmo fabricante e 11 do setor privado de diferentes fabricantes e lotes. Foram avaliados aspectos do comprimido quanto à percepção de odor, determinação

de peso, identificação e teor de CP e de DSCP e análise do conteúdo da bula. **Resultados:** Dentre os 13, o medicamento vencido apresentou 4,4% de DSCP, os demais estavam de acordo com a especificação. Verificou-se correspondência do odor de enxofre perceptível com teor de DSCP acima de 0,5%. Considerando os textos de bula sobre odor de enxofre, as constatações foram: nenhuma informação (três produtos), odor característico (dois), leve odor de enxofre (um), leve odor de enxofre sem diminuir a eficácia (sete). **Conclusões:** As amostras apresentaram resultados satisfatórios para os ensaios realizados. Verificou-se falta de homogeneidade nas informações das bulas sobre o odor dos comprimidos. A percepção do paciente quanto ao odor de enxofre, mesmo dentro do limite tolerado de DSCP, pode levar a não aceitação do medicamento e consequente não adesão ao tratamento da hipertensão, além de gerar prejuízos ao SUS.

Palavras-chave: Dissulfeto de Captopril; Produto de Degradação; Odor de Enxofre; Anti-hipertensivo

## INTRODUCTION

Controlling, reducing the effects, and eliminating the suffering caused by diseases have always been man's challenges since the beginning. Although the health of a population does not depend only on health services and the use of medicines, the importance of health care and the contribution of drug therapy are undeniable. Pharmaceutical Assistance constitutes a public health action and an integral part of the health system, in addition to being decisive for the solvability of care and health services, involving the allocation of large volumes of public resources. Thus, pharmaceutical assistance forms a link between the improvement of care for the drug user and public health[1].

Hypertension, popularly known as high blood pressure, is defined by a multifactorial clinical condition characterized by diastolic blood pressure permanently increased above 90 mmHg, together with systolic blood pressure above 140 mmHg. In Brazil, it affects 36 million adults, including elderly people over 60 years of age. It directly or indirectly contributes to 50% of deaths related to cardiovascular diseases, being the main cause of death in the country[2]. The drug of choice for treatment is captopril (CP) being the most used in Brazil, classified as an inhibitor of the renin-angiotensin-aldosterone system (RAAS), listed in the Brazilian National Essential Medicines List (RENAME) and available in the Unified Health System (SUS) as a drug considered essential for access to the population[3].

Captopril (D-2-methyl-3-mercapto-propanoyl-L-proline) was the first drug of the class of angiotensin-converting enzyme (ACE) inhibitors to be developed and commercialized, responsible for hydrolyzing angiotensin I to angiotensin II, reducing blood pressure by decreasing peripheral vascular resistance[3,4]. Other indications for the use of CP are in the treatment of patients with heart failure, myocardial infarction, and diabetic nephropathy.

According to Banker and Anderson[5], the therapeutic activity of a drug does not depend only on its intrinsic activity, but fundamentally on the formulation and dosage form. CP, by the oral route, is rapidly absorbed from the gastrointestinal tract, the maximum plasma concentration is reached in 1 h, having 75% of bioavailability, and the action is reduced by 25% to 30%, if administered concomitantly with food. Clearance is mainly via the kidneys, eliminating between 40%-50% in the urine as CP and the remainder as a dimer, called captopril disulfide (DSCP) and captopril-cysteine disulfide[3,5,6].

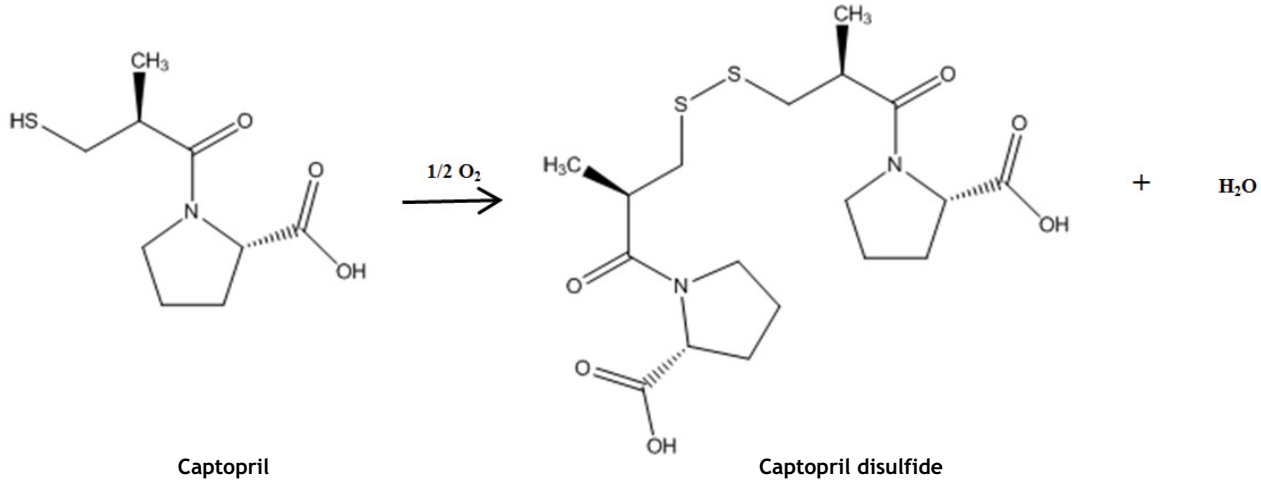
With According the COVID-19 pandemic, studies on the relationship between the use of drugs that act on the RAAS, especially ACE inhibitors, with the evolution of the infection were deepened, since the SARS-CoV-2 virus uses ACE2 receptors to access the cell. The interaction between the *Spike* protein and the ACE2 receptor may be determinant for virus transmissibility, replication, and disease severity. Now, it is recommended to maintain therapy with this class of anti-hypertensive drugs, considering the risks of discontinuing the drugs[7,8,9].

CP, $C_9H_{15}NO_3S$, is presented in the form of white or almost white crystalline powder, it is easily soluble in water, methanol, and methylene chloride, and soluble in dilute solutions of alkali hydroxides[10]. It is a diprotic acid with two dissociation constants 3.7 and 9.8, referring to the carboxylic acid and the sulfhydryl group, respectively[11,12].

CP is susceptible to degradation through the oxidative pathway, forming DSCP, which is its main degradation product, in which through the reaction involving the thiol function, 1/2 mol of oxygen is sufficient to degrade 2 mol of CP at high temperature and humidity, as illustrated in Figure 1[10].

Sealing a *blister*, which contains the pharmaceutical form, is a very critical step in the production process. The tablet must be well sealed, with the packaging made of material impermeable to oxygen and moisture, thus avoiding the formation of probable degradation products[3].

In recent years, CP tablets analyzed for DSCP content had unsatisfactory results in several batches of different pharmaceutical industries, consequently, they were suspended or banned by the Brazilian National Health Surveillance Agency (Anvisa)[14]. DSCP concentration is related to a decrease in the amount of CP[5] and an unpleasant odor and metallic taste[15]. For this reason, it was verified the importance of determining DSCP in solid pharmaceutical form - tablet, whose maximum allowed limit is 3.0%, according to the Brazilian Pharmacopoeia 6th edition (FB 6th ed.)[10].




**Captopril** **Captopril disulfide**

Source: Adapted from Freitas[13].

**Figure 1.** Formation of captopril disulfide degradation product by oxidation.

Odor is an organoleptic physical property of a chemical substance[10] and, to determine this property, sensory analysis is carried out, which is a technique widely used in food quality control. In this test, the organs of the sensory system are used as measurement "instruments" in order to assess consumer acceptability and measure perceptible changes that affect the sensory characteristics of the product[16]. Although this application is more common for foods, it has also been used for pharmaceutical products in the formulation development phase, in the correction of the palatability of bitter drugs with unpleasant taste, in the use of flavorings, and in the quality control of the final product. The CP tablet naturally exudes sulfur odor and this, added to a discreet metallic taste, makes the investigation relevant, due to the fact that it is related to the high amount of its degradation product, DSCP, which collaborates in reducing therapeutic adherence and even the abandonment of drug treatment.

The drug package insert is considered a means of communication between the manufacturer and the patient, to inform and guide the prescription, preparation, administration, warnings, among other clarifications necessary for the safe use of the drug and effective treatment[17]. The CP package insert must describe the appearance of the tablet, its color, odor, flavor, and the possibility of an accentuated or altered odor. These descriptions are important for clarify the physical integrity of the tablet, as well as its restrictions and care, thus allowing its proper use. In this work, it was verified the importance of evaluating the existence of this information in the package inserts of the different manufacturers[18].

The *Instituto Adolfo Lutz*, Official Laboratory of the Government of the State of São Paulo, through the Nucleus of Physical and Chemical Testing in Medicines (NFQM) receives products collected by the Health Surveillance that were launched on the market and presented suspicion of post-marketing non-compliance, among which, they received drug samples with changes of odor or taste of CP tablets registered by health professionals from Basic Health Units (UBS) due to complaints by patients. The analyzes carried out in HPLC followed the official compendium (FB 6th ed.) which, according to Resolution of the Collegiate Board of Directors (RDC) No. 166, of July 24, 2017, must have their analytical methodologies partially validated.

The main objectives of this work were to determine the DSCP degradation product in 25 mg CP tablets distributed by the public and private sectors in the city of São Paulo, to verify if the DSCP content corresponds to the odor given off from the tablets by means of an olfactory perception test, and to evaluate the content of the package inserts, from different manufacturers, regarding the description of the characteristics of the drug (odor), among other properties.

## METHOD

### Samples

Thirteen samples of 25 mg CP tablets were analyzed. Two samples, from the same manufacturer, collected by the Health Surveillance Coordination of the city of São Paulo for fiscal analysis, one of which was associated with the technical complaint of discontinuation of treatment with the indication of a strong odor, thus being rejected for use by the patient, and the other sample was associated with quality verification. In order to acquire the largest possible number of brands available in the private sector from the main retail chains and small pharmacies drugstores in the city of São Paulo, ten samples were acquired in the network, nine being generic drugs from different manufacturers and one is a similar drug. One sample categorized as reference drug that expired 17 years ago was also analyzed. This sample was packaged in a *blister* inside the manufacturer's original cartridge, with a preserved appearance.

### Equipment, reagents, and standards

The following were used: Unique Ultrasonic Cleaner ultrasound, GoldSun model 0411 vacuum pump, Burrell® mechanical stirrer, Binder® drying oven and Mettler Toledo analytical balance models AL204 and MT5, phosphoric acid (Merck®), HPLC grade methanol (Vetec®) and water purified by the Purilab Classic system (Elga®); chemical reference substance (CRS) of the CP (INCQS) and DSCP (USP).

To validate the method, we used: hydrochloric acid HCl (Merck®), sodium hydroxide NaOH (Synth®), hydrogen peroxide $H_2O_2$ (Dinâmica®), iron III chloride $FeCl_3$ (LabSynth®), and sodium chloride NaCl (Synth®). The placebo was formulated with the following reagents: corn starch (Adicel®), lactose monohydrate (Synth®), microcrystalline cellulose (Avicel®), croscarmellose sodium (Roquette®), silicon dioxide (Adicel®), stearic acid (Synth®).

The excipients used by the different manufacturers were verified and a placebo was prepared that contained the excipients in common.

### Analytical methodology by HPLC

A Waters Alliance 2695 liquid chromatograph (Mildford, MA, USA) was used, with degasser, column oven, quaternary pump, DAD detector, and Empower 3 control software. The chromatographic conditions followed the recommendations of the FB 6th ed.: 220 nm wavelength; Waters Spherison® chromatographic column 250 x 4.6 mm, packed with silica chemically bonded to octadecylsilane group (C18), 5 µm particles, at room temperature; mobile phase flow: 1.0 mL/min and injection volume: 20 µL. The mobile phase consisted of a mixture of a 0.1% v/v solution of phosphoric acid and methanol (45:55 v/v), the same composition and proportion used as a diluent in the preparation of standard solutions, sample, and in degradation assays.

### Standard solution preparation

A solution containing 1.00 mg/mL of CP and 0.03 mg/mL of DSCP was prepared.

### Sample solution preparation

20 tablets were weighed, pulverized, an amount of powder equivalent to 50 mg of CP was transferred to a 50 mL volumetric flask and 30 mL of diluent was added. The flask was placed in an ultrasound bath for 15 min and then on a mechanical shaker for 15 min. The flask was made up to volume with diluent and filtered (theoretical final concentration: 1 mg/mL CP).

### Validation

According to RDC No. 166/2017, the partial validation of a methodology published in an official compendium requires that the parameters of accuracy, precision, and selectivity be evaluated in order to demonstrate the suitability for the intended use. As this is an analytical method intended for the quantification of impurity, the partial validation included the limit of quantification parameter. Additionally, an evaluation of the linearity of the analytical method was carried out. To demonstrate the suitability of the method, the sample of 25 mg CP tablets that showed the lowest concentration of DSCP content (RDC No. 166 of 2017) was listed for validation[19].

### Accuracy

The accuracy was verified in terms of recovery by the standard addition method. Three concentrations (low, medium, and high) were prepared with three replicates of each level. It was weighed, in triplicate, for nine 50 mL volumetric flasks, sample at a concentration of 80%, 100%, and 120%, the equivalent of 50 mg of CP, adding 2 mL of standard CP solution (1 mg/mL) and 30 mL of the diluent, in each flask and submitted to 15 min of ultrasound and mechanical agitation for another 15 min and completed with diluent.

### Precision

The repeatability was performed with the same measurement procedure, the same analyst, the same instrument, under the same environmental conditions, as well as the intermediate precision, but with different analysts. The replicates were independent with six determinations of 1.00 mg/mL CP and fortified with standard DSCP solution at a final concentration of 0.03 mg/mL. The means of the determinations and the estimate of the relative standard deviations (RSD%) were calculated.

### Selectivity

In order to demonstrate the absence of interference from degradation products, as recommended by RDC No. 166/2017 on the validation of analytical methods, RDC No. 53, of December 4, 2015, and Guide 4 of 2015[20,21] on the forced degradation study, the sample, the placebo, and the standard were exposed to seven degradation conditions in acid and alkaline media, oxidation, metal ions heat, light, and humidity, as shown in Table 1.

The samples were weighed according to the item “Sample solution preparation” (amount of powder equivalent to 50 mg of CP), and the standard and placebo were prepared according to the item “Standard and placebo preparation”, all were individually transferred to 50 mL volumetric flask. For acid and basic hydrolysis, oxidation and metal ions conditions, 5 mL (10% of the volume of the 50 mL volumetric flask) of the degrading solutions were added: HCl 1.00 M, NaOH 0.10 M, $H_2O_2$ 3.0%, $FeCl_3$ 0.05 M. For the heat condition, the flasks were placed in an oven at 60ºC. For the sun exposure condition, the flasks were kept close to the laboratory window for three consecutive days with controlled temperature in the range of 25.0 ± 5.0ºC. For the moisture test, the flasks without lids were placed in the desiccator containing saturated saline solution (40.0% w/w of NaCl in water) at the bottom below the support, then, the lid of the desiccator was placed and transferred to an oven at a controlled temperature of 30ºC. This procedure was an alternative proposed by Connors (1986) still commonly used in the absence of greenhouses with humidity control for degradation with humid heat[23].

**Table 1.** Stressful conditions of sample degradation, standard, and placebo.

| Degradating | Degradation mode | Temperature | Exposure and collection time |
|---|---|---|---|
| Acid hydrolysis | 1.00 M HCl solution | Room | 0 h, 3, 7, and 10 (days) |
| Basic hydrolysis | 0.10 M NaOH solution | Room | 0 h, 2, 6, and 9 (days) |
| Oxidation | 3.0% $H_2O_2$ solution | Room | 0 h, 24 h, 72 h |
| Metal ions | 0.05 M $FeCl_3$ solution | Room | 0 h, 24 h |
| Heat | Stove | 60ºC | 0 h, 3, 7, and 10 (days) |
| Light | Sun exposure | Room | 0 h, 24 h, 72 h |
| Moisture | Desiccator with saturated saline solution, in a stove and 75% RH | 30ºC | 0 h, 3, 7, and 10 (days) |

Source: Elaborated by the authors, 2021.
HCl: hydrochloric acid; h: time; NaOH: sodium hydroxide; $H_2O_2$: hydrogen peroxide; $FeCl_3$: iron chloride.

The collections were performed according to the exposure and collection times presented in Table 1, except for the standard and the placebo, whose collections were performed in the non-degraded condition (time zero) and at the last collection time. 30 mL of diluent was added to all sample flasks, standard and placebo. The flask was taken to ultrasound, mechanical shaker, and made up to volume with diluent, according to the sample preparation procedure. The analysis to verify the degradation was carried out on the same day for the collection at time zero (0 h), and for the other collections the analysis was carried out on the days or times according to Table 1.

All procedural steps were performed under protection from light (except for photolytic degradation). To avoid the formation of secondary compounds, the hydrolysis samples were not neutralized, and in the oxidative degradation, 10% methanol was used[22].

The samples were analyzed against a freshly prepared standard control solution without degradation treatment. The maximum exposure times, i.e., the end-points for each stressful condition, were defined based on the WHO guide TRS 929 – Annex 5[24].

### Placebo preparation

For preparation of placebo, the limits stipulated by the US Food and Drug Administration (FDA) in the Inactive Ingredient Search for Approved Drug Products were used as a reference, in which the maximum potencies per unit dose of the excipients of a formulation are described[25]. In this way, the excipients described in the package insert were weighed at their maximum potency and mixed in a volumetric flask.

### Linearity, limits of quantification and detection

Seven CRS concentrations of DSCP (0.006; 0.021; 0.024; 0.027; 0.030; 0.033; 0.036 mg/mL) and CP (0.5; 0.7; 0.8; 0. 9; 1.0; 1.1; 1.2 mg/mL) were prepared for the evaluation of linearity. The limit of quantification was estimated using linear regression data from the calibration curve and the equation LQ = (10 x intercept/slope). The LD was calculated based on the LQ, dividing the LQ by 3.3. The linear regression line was obtained by correlation using the least squares method.

### Statistical analysis

Minitab® software was used to conduct the statistical calculations. Analysis of variance (ANOVA, $p < 0.05$) was performed, the RSD was defined as acceptable when < 5%, correlation coefficient above 0.990 and slope coefficient different from zero.

### Appearance and perception of odor

For the appearance of tablets of each sample, the following characteristics were evaluated: color, shape, concave or smooth face, grooved or not, and the description of the tablet surface.

Regarding the organoleptic test: nine laboratory analysts carried out the test, by appointment and without any interruption, in an isolated, clean, and odor-free environment, through the perception of sulfur odor exhaled from the tablets shortly after they are removed from the *blister*, their primary packaging.

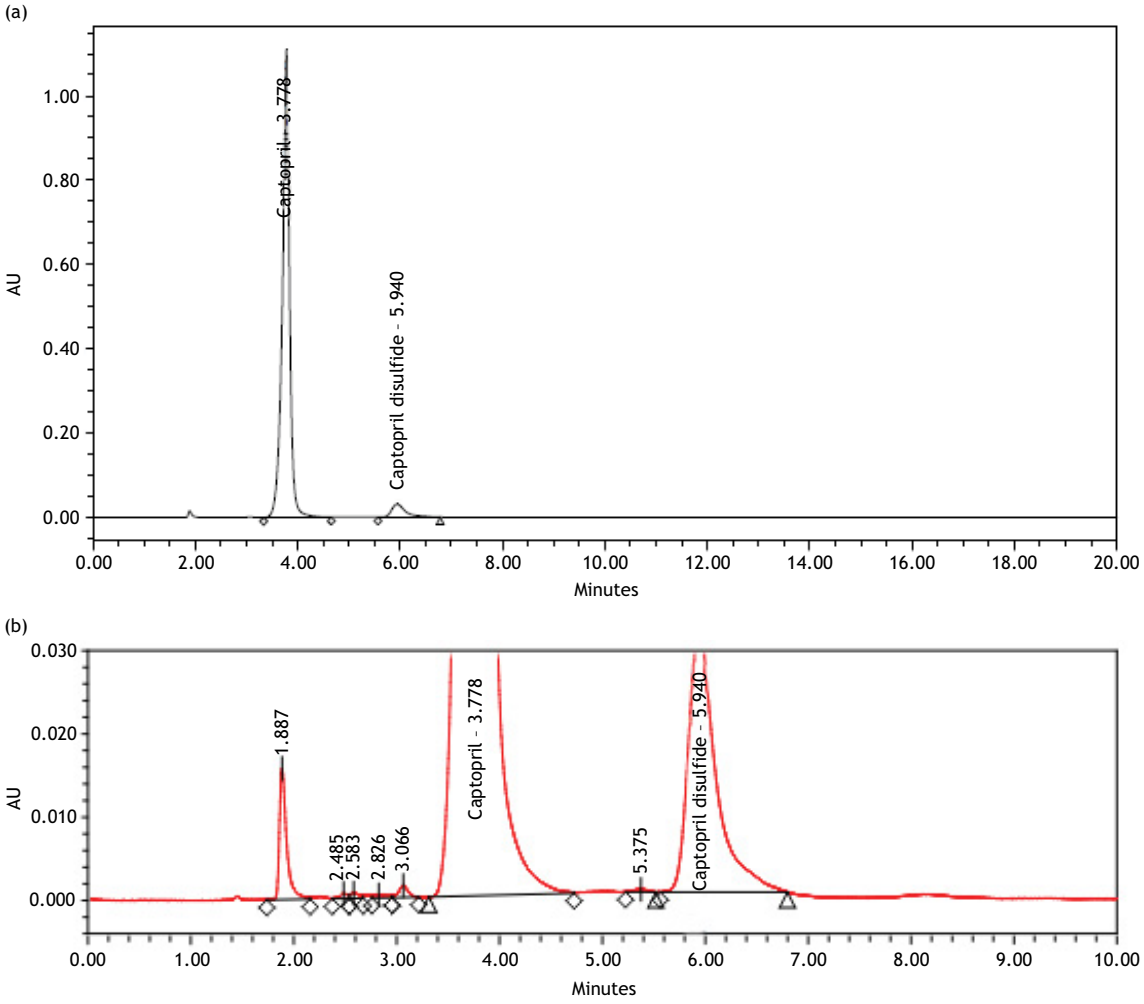
A table for the results of 13 unidentified samples was given to each analyst to record the perception of sulfur odor as: not perceptible, perceptible, or strong sulfur odor, where *not perceptible* it corresponds to no or no perception of sulfur odor in the tablet sample; *perceptible*, slight or subtle perception of sulfur odor; and *strong sulfur odor* would be the perception of sulfur odor in a more evident way, the odor is more intense than the previous item.

The package inserts of the samples were numbered from 01 to 13 and evaluated for the description of the characteristics of the drug and care in storage. The written texts of the package inserts regarding information on odor and product efficacy were compared with the results of DSCP levels obtained.

## RESULTS AND DISCUSSION

### System suitability

Five replicates of standard solution were injected at concentrations of 1.00 mg/mL of CP and 0.03 mg/mL of DSCP, with RSD of 0.14% and 0.27%, respectively, being less than DPR 2.00% as recommended by the FB 6th ed. Relative retention times were 3.778 min. for CP and 5.940 min. of DSCP (Figure 2) with a resolution between peaks of 5.37, above the 2 required by the




Source: Elaborated by the authors, 2021.

**Figure 2.** (a) System suitability chromatogram with peak standards for captopril tr = 3.778 min and captopril disulfide tr = 5.940 min; (b) Zoom of the chromatogram shown in Figure 2a.

monograph. The number of theoretical plates of 3,270 and 3,106 and asymmetry of 1.27 and 1.57 were also verified for CP and DSCP, respectively. These data demonstrate that the method provides reliable results for the analytical run, meeting the system's suitability parameters.

## Metodology validation

The accuracy of the method was carried out in order to verify the recovery of CP (CRS), which was added in known amounts to the sample solution. Results of 99.59%, 98.64%, and 96.26% were obtained for low, medium, and high concentrations, respectively. It meeting the adopted specification of 90% to 110%[10]. The found result of RSD was 0.5%, indicating no dispersion between injections of each concentration.

The results of repeatability and intermediate precision of the method were demonstrated by scattering the results, calculating the mean and RSD. It is a method for the quantification of impurities, the samples were fortified with known concentrations of the DSCP standard. The application of the *F* test in the results for CP and DSCP showed a calculated F, less than F critical and *p-value* greater than 0.05, confirming that there was no statistical difference between the repeatability and intermediary precision values (Table 2).

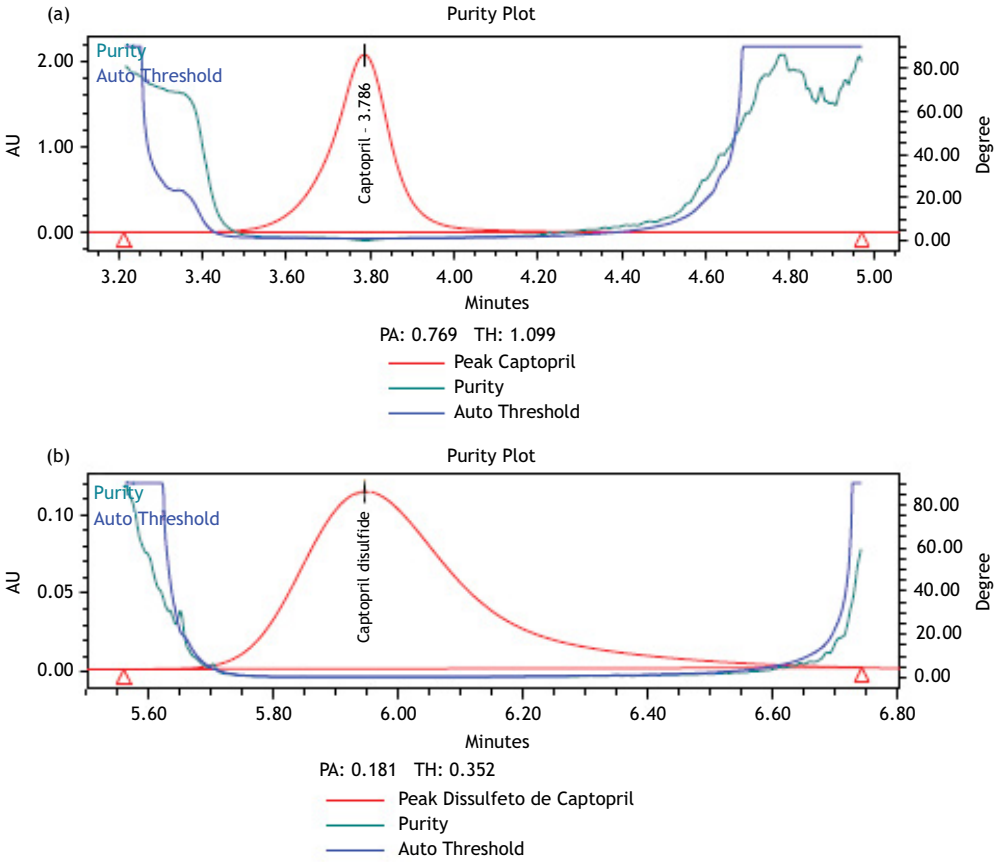
Selectivity is the method's ability to separate the compound of interest from components that are present in the sample, this demonstrates there is no coelution with the studied assets CP and DSCP. The sample was stressed in the conditions of oxidation, light, heat, acid and basic hydrolysis, humidity, and metallic ions, in order to verify if the products formed would not interfere in the identification of captopril.

The CP and DSCP peaks showed satisfactory spectral purity in the finished product, since the purity angle was lower than the purity threshold, indicating no coelution with the degradation products, as shown in Figure 3.

**Table 2.** Results of repeatability and intermediate precision, *F* tests of captopril and captopril disulfide.

| | Repeatability | | Intermediate precision | | F test | | |
|---|---|---|---|---|---|---|---|
| | mean | RSD | mean | RSD | Calculated *F* | Critical *F* | *p-value* |
| CP | 98.06 | 0.59 | 97.35 | 1.42 | 2.5081 | 4.3009 | 0.1275 |
| DSCP | 0.69 | 0.74 | 0.69 | 1.38 | 0.8745 | 4.3009 | 0.3599 |

Source: Elaborated by the authors, 2021.
CP: captopril; DSCP: captopril disulfide; RSD: relative standard deviation.




Source: Elaborated by the authors, 2021.

**Figure 3.** Captopril (a) and captopril disulfide (b) peak purity chromatograms developed in the optimized and validated method.

The selectivity was also evaluated by comparing the chromatograms obtained from the samples against those from the CRS and from the placebo previously prepared in the laboratory. The control placebo, without degradation, and the placebo after the stress test at the last collection time for each condition were injected into the system. The placebo showed no signal during the chromatographic runs under any of the conditions studied.

After 24 h of exposure to basic hydrolysis (1M NaOH), it was observed that the pH of this solution resulted in the formation of double chromatographic peaks (ionized and non-ionized species), opting for a milder degradation solution with NaOH 0.1 M. For this reason, collection times for basic hydrolysis were delayed by one day relative to the other conditions.

As can be seen in Table 3, the conditions of oxidation, metal ions and basic hydrolysis were the most significant in the degradation of CP and consequent formation of DSCP, demonstrating the existence of a fast reaction kinetics. CP was resistant to high temperatures, showing a degradation of 15% only after 10 days of exposure at 60ºC. CP can be considered stable in acidic solution, against humidity, and did not show photosensitivity. For the conditions of light, humidity, heat, and acid hydrolysis, in the first 72 h of exposure, the CP and DSCP contents remained within a narrow range, not being significant from the degradation point of view. The degradation tests did not result in chromatographic peaks that interfered with the reading of CP and DSCP.

The method showed linearity for CP in the range of 0.5 to 1.2 mg/mL with correlation coefficient R = 0.9988 and straight

**Table 3.** Results found in percentage of captopril degradation and captopril disulfide formation according to stressful conditions.

| Active content (%) | Collection time | Oxidation | Light | Acid hydrolysis | Basic hydrolysis | Heat | Moisture | Metal ions |
|---|---|---|---|---|---|---|---|---|
| Captopril (%) | 0 h | 96.17 | 94.53 | 94.77 | 100.42 | 95.84 | 103.34 | 103.34 |
| | 24 h | 73.72 | 99.29 | - | 30.71 | - | - | 44.28 |
| | 72 h | 65.48 | 97.65 | 97.20 | - | 97.87 | 105.17 | - |
| | 6 d | - | - | - | ND | - | - | - |
| | 7 d | - | - | 96.13 | - | 95.48 | 104.67 | - |
| | 9 d | - | - | - | ND | - | - | - |
| | 10 d | - | - | 95.56 | - | 80.04 | 103.40 | - |
| Captopril disulfide (%) | 0 h | 0.35 | 0.26 | 0.61 | 0.56 | 0.11 | 0.65 | 0.65 |
| | 24 h | 22.61 | 0.32 | - | 50.29 | - | - | 42.89 |
| | 72 h | 29.36 | 0.29 | 1.26 | - | 0.44 | 0.66 | - |
| | 6 d | - | - | - | 74.94 | - | - | - |
| | 7 d | - | - | 1.55 | - | 0.42 | 0.60 | - |
| | 9 d | - | - | - | 76.38 | - | - | - |
| | 10 d | - | - | 1.88 | - | 12.61 | 0.64 | - |

Source: Elaborated by the authors, 2021.
h: hours; d: days; ND: not detected.

line equation y = (1.19 x 107)x + 2.30 x 103 (considering y the peak area and x the concentration in mg/mL) in the range from 0.006 to 0.036 mg/mL of DSCP with R = 0.9981 and equation of the straight line y = (1.48 x 107)x + 1.51 x 104.

The LQ was estimated using linear regression data for each asset (LQ = 10 x intercept/slope), being 0.050 and 0.003 mg/mL for CP and DSCP, respectively. The LD calculated as LD = LQ/3.3, was 0.0152 mg/mL for CP and 0.909 µg/mL DSCP.

The validation of the methodology for determining CP and DSCP by liquid chromatography showed that the method is suitable for the analysis of samples of CP 25 mg tablets.

### Aspect

Visual inspection of the samples was performed for uniformity of coloring of the tablets, tablets missing from the *blister*, broken or cracked, and any other apparent alteration. According to the analysis, the tablets from batches 1 to 13 showed satisfactory results with a circular shape, with no broken tablets or cracks, and a smooth surface. Even the expired sample presented characteristics within the specifications. According to the FB 6th ed., the tablets must have an intact, homogeneous surface, with a characteristic, smooth, and shiny color, being devoid of defects, such as flaws, cracks, and contamination[10].

### Determination of weight and CP and DSCP contents

The weight determination test for uncoated tablets, an average weight greater than 80 mg and less than 250 mg, the maximum accepted variation is ± 7.5%[10]. This test makes it possible to verify whether the units of the same batch have weight uniformity. None of the drugs studied presented results outside the acceptance criteria for the trial.

The determination of the DSCP content was performed according to the monograph of the FB 6th ed., however, no study was found in the scientific literature that carried out the relationship between the content of this degradation product and the perception of its odor.

According to the specification of the FB 6th ed., the CP content of a drug must be between 90.0% and 110.0% of the amount declared on the label of $C_9H_{15}NO_3S$, and the maximum DSCP limit is 3.0%. 13 drugs studied showed CP content within the acceptance range. According to Table 4, it is possible to observe that the DSCP content for the expired drug (lot 1) was 4.4%, however, as this was used only as a reference to evaluate the evolution of the formation of the degradation product, it cannot be considered an unsatisfactory result. The colors in Table 4 are associated in Figure 4, both in relation to the information on the package insert and the odor per sample.

Angiotensin converting enzyme inhibitors are an important therapeutic advance in the treatment of patients with hypertension and congestive heart failure[26].

According to reports in the literature, only free CP is pharmacologically active. However, when DSCP is metabolized, it can undergo reversible interconversions, resulting in CP, which can also present pharmacological action[26,27,28], but with a different half-life from that in which the patient ingested the medication. In the long term, this may affect pharmacological treatment as it leads to increased concentrations of total CP, probably due to the accumulation of CP metabolites.[26]. At the same time,

the analysis of the DSCP content above the specified indicates that there was a greater degradation of the active substance. At high concentrations in the body, DSCP causes symptoms such as nausea, dizziness, and malaise similar to chemically similar compounds, such as carbon disulfide[29]. Given these facts, it is important to control DSCP in the drug.

### Odor perception linked to package insert information

The package inserts of the 13 samples were analyzed for information about the possible presence of sulfur odor. In seven samples, there was agreement in the information, namely: "captopril tablets may have a slight sulfur odor, which does not reduce their effectiveness". It is considered that this text would be the most complete, solving the possible doubts that patients or health professionals would have when using or providing guidance on the drug. The slight odor described in the package insert would be the same as what we categorized as "perceivable odor" for the odor perception test.

However, for the other samples, the information was incomplete or even non-existent, as for three package inserts analyzed, as shown in Figure 4. In this way, it was verified that there was no homogeneity between the information on the package inserts of the different manufacturers, on the descriptions of odor, evidencing the lack of standardization in the texts in the package inserts of the commercialized drugs. It is possible to infer that there was no requirement for the standardization of this information by the health authorities. Standardizing the contents of package inserts would certainly make a valuable contribution to the confidence established regarding the safety of this drug.

The perception test of the sulfur odor exhaled from the tablets just removed from the *blister*, each analyst individually filled in the table in which the odors were classified into: not perceptible, perceptible, and strong odor.

According to Figure 5, the results obtained for the disulfide content (%) and the odor classification are observed in the analyzed samples. There was a strong odor in the samples with DSCP content above 0.5%, indicating a correlation between the perception of odor and the increase in the concentration of the degradation product formed from the CP, the DSCP, as described by Peixoto et al.[5].

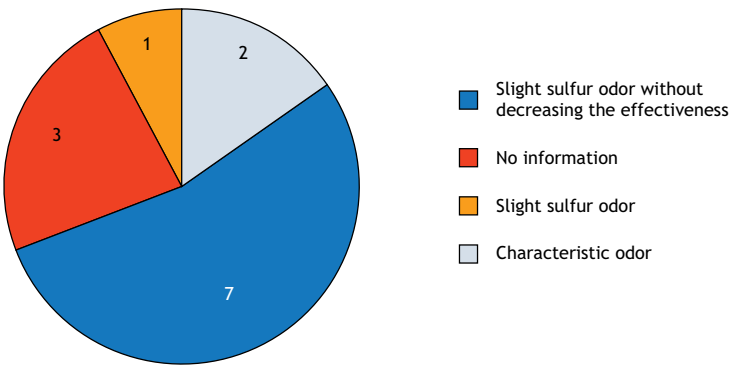
The physical and organoleptic characteristics of the pharmaceutical form such as odor, taste, appearance, hardness, uniformity, and dissolution rate in the tablets may change over time, however, the stability of pharmaceutical products must remain within the previously established specifications throughout the shelf life.

It is important to emphasize that the correlation between sulfur odor and DSCP content has, in this study, an investigative aspect. It is not expected to adopt this assay as part of the routine in quality control, although the application of the organoleptic test

**Table 4.** Captopril and captopril disulfide content results for 13 samples of CP 25 mg tablets.

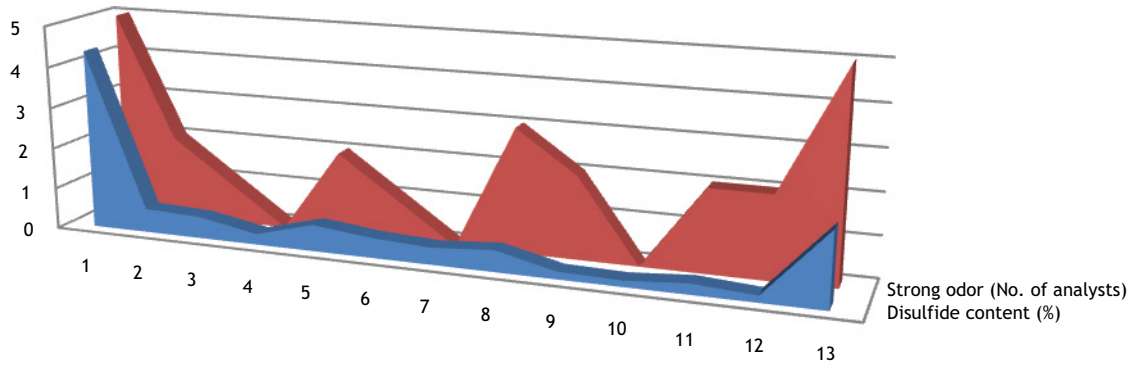
| Sample | Captopril content (%) | Captopril disulfide content(%) |
|---|---|---|
| 1 (expired) | 92.57 | 4.37 |
| 2 (Surveillance) | 96.38 | 0.58 |
| 3 (Surveillance) | 96.02 | 0.52 |
| 4 | 98.8 | 0.26 |
| 5 | 95.3 | 0.64 |
| 6 | 91.47 | 0.48 |
| 7 | 95.6 | 0.41 |
| 8 | 90.64 | 0.52 |
| 9 | 100.55 | 0.18 |
| 10 | 99.22 | 0.15 |
| 11 | 95.94 | 0.26 |
| 12 | 97.37 | 0.16 |
| 13 | 91.33 | 1.79 |

Source: Elaborated by the authors, 2021.
Specification in CP %: 90.0% and 110.0%; Maximum DSCP limit: 3.0%
S1: expired sample, S2 and S3: samples from the Health Surveillance, S4 - S13: samples acquired in the commerce.




Source: Elaborated by the authors, 2021.

**Figure 4.** Distribution of the presence of information in the package inserts analyzed regarding sulfur odor.




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Disulfide content (%) | 4.37 | 0.58 | 0.52 | 0.26 | 0.64 | 0.48 | 0.41 | 0.52 | 0.18 | 0.15 | 0.26 | 0.16 | 1.79 |
| Strong odor (No. of analysts) | 5 | 2 | 1 | 0 | 2 | 1 | 0 | 3 | 2 | 0 | 2 | 2 | 5 |

Source: Elaborated by the authors, 2021.

**Figure 5.** Correlation between the results of captopril disulfide (DSCP) content and classification as strong odor for the 13 analyzed tablet samples.

has the purpose of evaluating the sensory characteristics of sulfur odor and metallic palatability of the formulation.

Although the presence of odor cannot always indicate a change in the pharmacological effect, it is essential to monitor and maintain the concentration of the degradation product within the specified limits. Furthermore, it is known that the patient's confidence in the drug can become impaired if the physical or organoleptic characteristics, among other characteristics perceptible to the patient, are altered[30]. The patient's perception of sulfur odor, even when the concentration of the degradation product is within the tolerated limit, can lead to drug rejection due to lack of confidence, impairing the treatment of hypertension.

When the patient abandons the treatment of their illness because they do not trust the quality of the medication, it ends up causing losses to the SUS, since the public resource invested in the purchase for free distribution will be wasted, in addition to the fact that the uncontrolled disease (acute or chronic) may worsen, resulting in damage to the patient's health, requiring higher-cost treatments such as hospitalizations, disability, or necessarily reaching death.

## CONCLUSIONS

The evaluated samples presented satisfactory results regarding the tests of: appearance, weight determination, CP and DSCP content, according to official acceptance criteria. The expired sample had a DSCP content of 4.4%, above specification, as expected. It was used only as a reference, to observe the formation of the degradation product with time, excluding it from the acceptability criteria. There was a lack of homogeneity in the information on the package inserts, among the samples studied, on the description of odor in the tablets. Users of the medication as well as health professionals (doctors, pharmacists, and nurses) need to be informed through this important means of communication, "the package inserts", that the CP tablet exhales a sulfur odor, as a peculiar characteristic of the drug. It is possible to infer that the perception of sulfur odor in the tablets is directly related to the increase in DSCP concentration, considering that degradation products can result in reduced or toxic activity and that reports classified as quality deviations with patient involvement could be related to drug stability problems. One can verify the importance of monitoring the degradation product, DSCP, in CP tablets as a contribution to health promotion, protection, and recovery.

## REFERENCES

1. Conselho Nacional de Secretários de Saúde - CONASS. Assistência farmacêutica no SUS. Brasília: Conselho Nacional de Secretários de Saúde; 2007.
2. Malachias MVB, Souza WKSB, Plavnik FL, Rodrigues CIS, Brandão AA, Neves MFT et al. 7a diretriz brasileira de hipertensão arterial. Arq Bras Cardiol. 2016;107(3):1-83.
3. Rafael K, Faria MGI. Controle de qualidade dos comprimidos de captopril: uma breve revisão literária. Rev Uningá Rev. 2013;16(2):49-53.
4. Marcatto AP, Lamim R, Block LC, Bresolin TMB. Análise de cápsulas de captopril manipuladas em farmácias. Rev Ciencias Farm Basica Apl. 2005;26(3):221-5.
5. Peixoto MM, Santos Junior AF, Santos CAA, Caetité E. Avaliação da qualidade de comprimidos de captopril dispensados em Feira de Santana - BA. Infarma. 2005;16(13/14):69-73.
6. Banker GS, Anderson NR. Tablets. In: Lachman L, Lieberman HA, Kanig JL, organizadores. The theory and practice of industrial pharmacy. 3a ed. Philadelphia: Lea & Febiger; 1986. p. 293-345.

7. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with COVID-19. New Engl J Med. 2020;382(17):1653-9. https://doi.org/10.1056/NEJMsr2005760
8. Ferrari F. COVID-19: updated data and its relation to the cardiovascular system. Arq Bras Cardiol. 2020;114(5):823-6. https://doi.org/10.36660/abc.20200215
9. Singh AK, Gupta R, Misra A. Comorbidities in COVID-19: outcomes in hypertensive cohort and controversies with renin angiotensin system blockers. Diabetes Metab Syndr. 2020;14(4):283-7. https://doi.org/10.1016/j.dsx.2020.03.016
10. Agência Nacional de Vigilância Sanitária - Anvisa. Farmacopeia Brasileira. 6a ed. Brasília: Agência Nacional de Vigilância Sanitária; 2019.
11. Pereira AV, Garabeli AA, Schunemann GD, Borck PC. Determination of dissociation constant (Ka) of captopril and nimesulide: analytical chemistry experiments for undergraduate pharmacy. Quim Nova. 2011;34(9):1656-60. https://doi.org/10.1590/S0100-40422011000900029
12. National Institutes of Health - NIH. Captopril. PubChem. 2021[acesso 5 mar 2021]. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/Captopril#section=Optical-Rotation
13. Freitas JRLE. Caracterização dos produtos de degradação do captopril por espectrometria de massas de alta resolução e avaliação da toxicidade após a fotocatálise heterogênea (TIO2/UV-C) [dissertação]. Ouro Preto: Universidade Federal de Ouro Preto; [acesso 04 abr 2021]. Disponível em: https://www.repositorio.ufop.br/bitstream/123456789/4212/1/DISSERTACAO_CaracterizaçãoProdutosDegradação.pdf
14. Agência Nacional de Vigilância Sanitária - Anvisa. Busca: dissulfeto de captopril. Brasília: Agência Nacional de Vigilância Sanitária; 2021[acesso 20 fev 2021]. Disponível em: https://www.gov.br/anvisa/pt-br/search?SearchableText=dissulfeto de captopril
15. Souza JAL, Albuquerque MM, Grangeiro S, Pimentel MF, Santana DP, Simões SS. Quantification of captopril disulphide as a degradation product in captopril tablets using near infrared spectroscopy and chemometrics. Vibr Spectr. 2012;62:35-41. https://doi.org/10.1016/j.vibspec.2012.04.011
16. Jorge N. Estudo do comportamento do óleo de girassol e do efeito do dimetil polisiloxano em termoxidação e frituras. Campinas: Universidade Estadual de Campinas; 1996[acesso 16 jul 2021]. Disponível em: http://www.repositorio.unicamp.br/handle/REPOSIP/254665
17. Agência Nacional de Vigilância Sanitária - Anvisa. Perguntas e respostas sobre bulas. Brasília: Agência Nacional de Vigilância Sanitária; 2021[acesso 8 abr 2021]. Disponível em: http://antigo.anvisa.gov.br/perguntas-e-respostas-sobre-bulas
18. Agência Nacional de Vigilância Sanitária - Anvisa. Bulas e rótulos de medicamentos. Brasília: Agência Nacional de Vigilância Sanitária; 2020[acesso 13 maio 2021]. Disponível em: https://www.gov.br/anvisa/pt-br/assuntos/medicamentos/bulas-e-rotulos
19. Agência Nacional de Vigilância Sanitária - Anvisa. Resolução RDC Nº 166, de 24 de julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Diário Oficial União. 25 jul 2017.
20. Agência Nacional de Vigilância Sanitária - Anvisa. Resolução RDC Nº 53, de 4 de dezembro de 2015. Estabelece parâmetros para a notificação, identificação e qualificação de produtos de degradação em medicamentos com substâncias ativas sintéticas e semissintéticas, classificados como novos, genéricos e similares, e dá outras providências. Diário Oficial União. 7 dez 2015.
21. Agência Nacional de Vigilância Sanitária - Anvisa. Guia para obtenção do perfil de degradação, e identificação e qualificação de produtos de degradação em medicamentos. Brasília: Agência Nacional de Vigilância Sanitária; 2015[acesso 17 fev 2018]. Disponível em: http://portal.anvisa.gov.br/documents/10181/2738062/Perfil+e+produtos+de+degradação+em+medicamentos.pdf/c18a4857-9a5c-4292-a1bf-07af6cad6902?version=1.01bf-07af6cad6902
22. Baertschi SW, Alsante KM, Reed R. Pharmaceutical stress testing: predicting drug degradation. 2a ed. London: Informa Healthcare; 2011.
23. Connors KA, Amidon GL, Stella VJ. Chemical stability of pharmaceuticals: a handbook for pharmacists. 2a ed. New York: John Wiley & Sons; 1986.
24. World Health Organization - WHO. WHO expert committee on specifications for pharmaceutical preparations: WHO 39th technical report series. Brasília: World Health Organization; 2005.
25. US Food and Drug Administration - FDA. Inactive ingredient search for approved drug products. Silver Springs: US Food and Drug Administration; 2019[acesso 20 ago 2019]. Disponível em: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm
26. Kubo SH, Cody RJ. Clinical pharmacokinetics of the angiotensin converting enzyme inhibitors. Clin Pharmacokinet. 1985;10(5):377-91. https://doi.org/10.2165/00003088-198510050-00001
27. Drummer OH, Kourtis S. Bradykinin-potentiating activity of captopril disulphide dimer (SQ 14,551). Eur J Pharmacol. 1988;153(1):11-7. https://doi.org/10.1016/0014-2999(88)90582-1
28. Migdalof BH, Antonaccio MJ, Mc Kinstry DN, Singhvi SM, Lan S-J, Egli P et al. Captopril: pharmacology, metabolism, and disposition. Drug Metab Rev. 1984;15(4):841-69. https://doi.org/10.3109/03602538409041080

29. Gouvêa RM, Arruda MA, Santos R, Cunha ALC. Avaliação do teor do produto de degradação dissulfeto de captopril em comprimidos de captopril 25mg. Arq Bras Med Nav. 2009;70(1):40-6. https://doi.org/10.22239/2317-269X.00945

30. Agência Nacional de Vigilância Sanitária – Anvisa. Formulário nacional da Farmacopeia Brasileira. 2a rev. Brasília: Agência Nacional de Vigilância Sanitária; 2012.

## Author's Contributions

Farias FF – Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Trujillo LM – Conception, planning (study design), and writing of the work. Vieira EA, Gasparin LFO, Yano HM – Acquisition, analysis, data interpretation, and writing of the work. Martins VAP – Writing of the work. All authors approved the final version of the work.

## Conflict of Interests

The authors inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study.

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