

A Simple and Environmentally Friendly Reflectometric Method for the Rapid Quantitative Analysis of Captopril in Pharmaceutical Formulations

Paulo Roberto S. Ribeiro^{a*}, Leonardo Pezza^b and Helena R. Pezza^b

^a Centro de Ciências Sociais, Saúde e Tecnologia – UFMA, CEP 65900-000, Imperatriz, MA, Brazil.

^b Instituto de Química – UNESP, P.O. Box 355, CEP 14801-970, Araraquara, SP, Brazil.

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Abstract

This paper describes a simple, environmentally friendly and rapid quantitative spot test procedure for the determination of captopril (CPT) in bulk drug and in pharmaceutical formulations by using diffuse reflectance spectroscopy. The proposed method is based on the reflectance measurements of the orange compound (λ_{\max} 490 nm) produced by the spot test reaction between CPT and *p*-chloranil (CL). Under optimal conditions, calibration curves were obtained for CPT by plotting the optical density of the reflectance signal (A_R) vs. the log of the mol L⁻¹ concentration, from 6.91×10^{-3} to 1.17×10^{-1} , with a good coefficient of determination ($R^2 = 0.9992$). The common excipients used as additives in pharmaceuticals do not interfere in the proposed method. The method was applied to determine CPT in commercial pharmaceutical formulations. The results obtained by the proposed method are compared favorably with those obtained by an official procedure at 95% confidence level. The method validation results showed that the sensitivity and selectivity of the methods were adequated for drug monitoring in industrial quality control laboratories.

Keywords:

Captopril; charge transfer complex; diffuse reflectance; pharmaceutical formulations

1. Introduction

Captopril (CPT), 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline, is an oral active inhibitor of the angiotensin-converting enzyme. It is widely used for the treatment of hypertensive diseases [1]. This compound (Figure 1) can also be used to treat congestive heart failure [2].

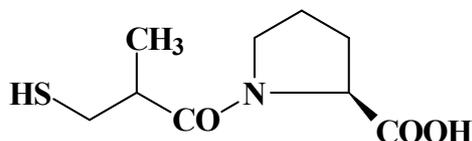


Fig. 1. Chemical structure of captopril

Several methods have been reported for the quantitative determination of CPT, including batch fluorimetry [3], chemiluminescence [4–7], atomic absorption spectroscopy (AAS) [8, 9], high-performance liquid chromatography (HPLC) [10–17], gas chromatography (GC) [18], differential pulse polarography [19], amperometry [20–22], volumetric titration [23], potentiometric titration [24–28], capillary electrophoresis [29], conductometry [30],

* Corresponding Author

E-mail: pauloufma@ufma.br

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coulometry [31], voltammetry [32], and spectrophotometry [27, 33-40]. However, batch methods often have a variety of disadvantages: they can be sophisticated, time-consuming, and laborious or require expensive and complicated instrumentation, features that make them unattractive to routine analysis. Titrimetric method has a lack of specificity and sensitivity, under certain circumstances, such as the presence of unsaturated organic compounds. Spectrophotometric methods based on UV absorption present low selectivity, because all unsaturated compounds display one or more bands in that region of the spectrum.

From the above consideration, the need for a fast, low-cost and selective method seems clearly apparent, especially for routine quality control analysis of pharmaceutical products containing captopril. In the search for such a method, our attention was attracted to a quantitative spot test by diffuse reflectance spectroscopy, from which measurements are fast, easy to conduct and involve low consumption of reagents. Moreover, the reflectance measurements can be performed in locus by using a very simple homemade reflectometer or a portable diffuse reflectance spectrophotometer, which is small, lightweight, inexpensive and battery operated, highly attractive characteristics for many applications in any location by almost everyone [41]. .

The measurement of the radiation reflected from a mat surface constitutes the area of spectroscopy known as diffuse reflectance spectroscopy. It has been found that when drops of solution come into contact with a suitable reagent on filter paper, colored products are produced on the surface of this paper, producing distinct flecks or rings within a circle wetted by water. Furthermore, the degree of color spot was found to be proportional to the concentration of the test analyte. The optical sensing of chemical species is based on their interaction with light [42].

Some methods reported in literature showed that the appropriate use of diffuse reflectance spectroscopy could yield reliable results evidencing the potential of this technique for quantitative analysis [43-59]. To the best of our knowledge, there are no reports on the use of the reflectance analytical methods for the determination of captopril in pharmaceuticals.

The present paper reports a new, simple, rapid, portable, environmentally friendly and inexpensive reflectometric method for quantitative analysis of CPT in bulk drug and in pharmaceutical formulations. Also, this method does not involve any pre-treatment procedure or heating steps. In this work, attempts were made to determine CPT (*n* donor) through charge transfer complexation with *p*-chloranil (CL) (π acceptor). The method is based mainly on charge transfer complexation reaction of CPT with CL. A quantitative analysis carried out by measuring the reflectance of the colored product developed in this reaction occurring on filter paper surfaces. The colored product was quantified at 490 nm.

The results obtained by the proposed method were in excellent agreement with those given by the official method [23], at 95% confidence level, proving that the method is a reliable alternative for the analysis of CPT in pharmaceutical formulations.

2. Material and Methods

2.1. Apparatus

Volume measurements were made with “Eppendorf” plunger-operated pipetter (10 – 100 μ L). A Labsphere RSA-HP-8453 reflectance sphere integrator (76 mm diameter, 5W halogen source) coupled to a Hewlett Packard HP 8453A diode array spectrophotometer was used for all reflectance measurements. All experiments were performed in a thermostated room ($25\pm 1^\circ\text{C}$).

2.2. Reagents and Solutions

Reagents or pharmaceutical grade chemicals were used. For the preparation of the solutions and samples, deionised water and grade A glassware were used throughout. Whatman 41 filter paper was used as solid support.

Captopril (standard substance) was purchased from Purifarma, Brazil (purity > 99.9%). Characteristic properties were consistent with the USP [23]. A $13.81 \times 10^{-2} \text{ mol L}^{-1}$ captopril stock was prepared daily by dissolving 150.0 mg of the drug in 5.0 mL of methanol. Working standard solutions were obtained by appropriate dilution of this stock solution with the same solvent.

The CL (Sigma-Aldrich) solution (0.08 mol L^{-1}) was prepared daily by dissolving 98.4 mg in 5.0 mL of 1,4-dioxane (Mallinckrodt – p.a. grade).

Six commercial samples (A – F) of CPT containing 12.5 and 25 mg CPT per unit were purchased from local drugstores in Araraquara, Brazil.

2.3. Procedures

2.3.1. Recommended Procedure for the Calibration Curve

For the spot reaction, 20 μL of captopril working standard solutions (comprising 6.91×10^{-3} to $1.17 \times 10^{-1} \text{ mol L}^{-1}$ of the drug) were spotted onto 1 cm^2 Whatman 41 filter paper followed by addition of 20 μL of the reagent solution. The solutions were dropped onto the paper with a micropipette fixed in a holder according to procedure described by Tubino et al. [45]. Then after the paper was placed in another holder with the aid of a tweezers where the measurements were performed. The blank solution is prepared in a similar way, but omitting CPT. Record the reflectancies at 490 nm, against the reagent blank. Calibration graphs prepared by plotting the optical density of the reflectance signal (A_R) vs. the log of the mol L^{-1} drug concentration. These graphs or the corresponding linear least squares equations are used to convert reflectance into CPT concentration, for any analysed sample.

2.3.2. Procedure for the Assay of CPT in Pharmaceutical Samples

For the determination of CPT in pharmaceutical samples, twenty tablets were weighed to calculate the average tablet weight. They were finely powdered and homogenized. A portion of this powder, equivalent to 120.0 mg of CPT was accurately weighed and dissolved in 8.0 mL of methanol by shaking for 15 min in a mechanical shaker. The resulting mixture was filtered and transferred into 10.0 mL graduated flask, the volume completed with methanol. Aliquots of 20 μL of this solution were analyzed according to the recommended procedure described in Section 2.3.1.

2.3.3. Study of Interferences

Since the aim of this study was to determine CPT in pharmaceuticals, the effects of the most commonly used excipients were carefully examined. The excipients studied were sucrose, glucose, lactose, fructose, talc, poly(ethylene glycol), polyvinylpyrrolidone, microcrystalline cellulose, croscarmellose sodium, starch and magnesium stearate. For this study, solutions containing CPT and each of the excipients taken separately in concentrations equal or ten times greater than that of CPT were shaken with water in a magnetic mixer for 15 minutes, diluted and analysed under the same conditions described in Section 2.3.1.

3. Results and Discussion

The CPT is the nitrogenous compound that acts as n -donors to the π acceptors. These acceptors react with the basic nitrogenous compounds to form charge transfer complexes or radical anions according to the polarity of the solvent used [60]. Hence CL used in the proposed methods is selective reagent for the determination of the CPT. In this procedure a coloured product appeared on the surface of the filter paper indicating the formation of chromogen.

To optimize the concentration of the reagent 0.04; 0.06; 0.08; 0.10 and 0.12 mol L⁻¹ of the CL in methanol were used for color development and 1.17 x 10⁻² mol L⁻¹ solution of the CPT reference material. Absorption values of less than 2% variation were obtained for the different concentrations. However the solution of 0.08 mol L⁻¹ of CPT was adopted because it provided higher absorbance values making the proposed method more sensitive. The reflectance spectrum of the reaction product shows that the maximum wavelength is located at 490 nm (Fig.2).

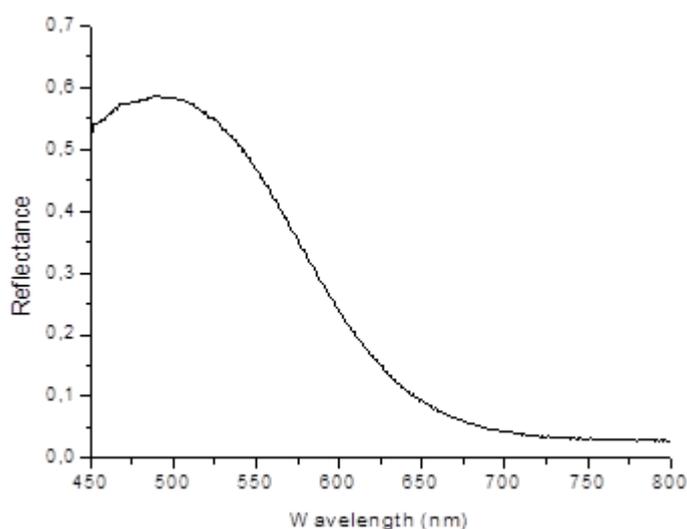


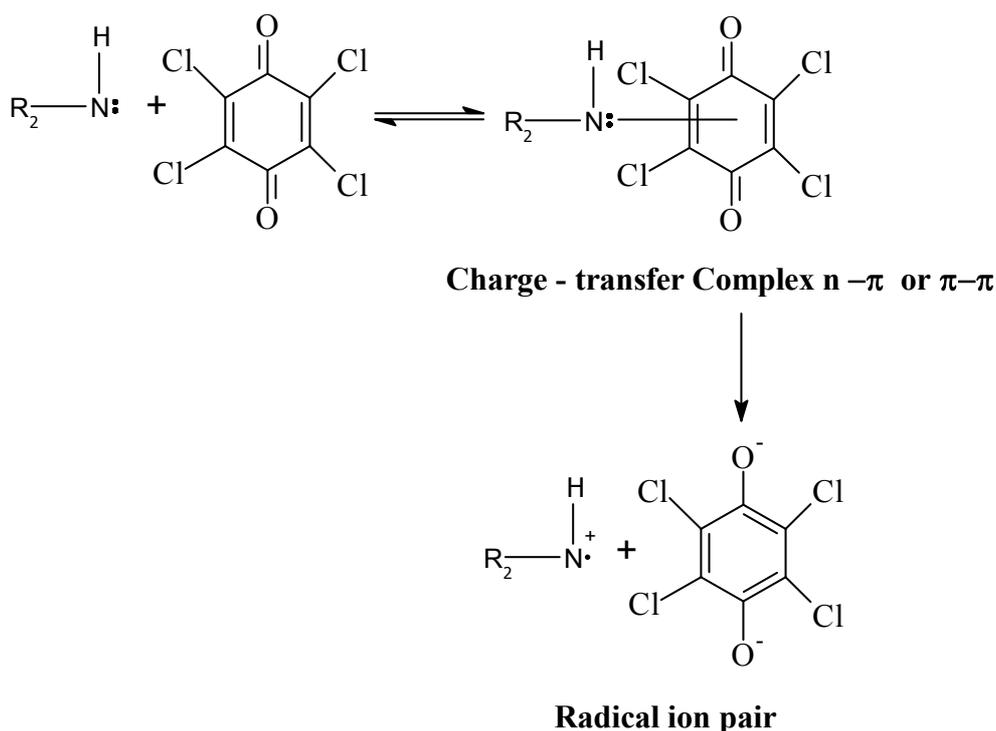
Fig. 2. Reflectance spectrum of the reaction product

The probable mechanism for the reaction between CPT and CL involves the formation of radical ion pairs [61] as shown in Scheme 1.

3.1. Analytical curves and stability

Under optimized experimental conditions, a series of standard solutions was analyzed to test the linearity. The calibration curve (Fig.3) was found to be linear in the 6.91 x 10⁻³ to 1.17 x 10⁻¹ mol L⁻¹ concentration range ($A_R = -0.2508 + 0.4039 \times C$, where A_R is the reflectance measurement at 490 nm and $C = \log(10^3 [\text{CPT}]) \text{ mol L}^{-1}$ [45]). The factor 10³ was used in plot (Fig.3) in order to obtain an adequate form of the calibration graph with log values higher than zero, with a good coefficient of determination ($R^2 = 0.9992$).

In almost all colored and precipitated reactions conducted by spot tests, the product of particular reaction is proportional to the concentration of the analyte and the limit of detection can be defined when a visible color appears on the filter paper or on a spot plate [42]. Thus, the reported visual qualitative detection limit is about 1.78 x 10⁻³ mol L⁻¹. In this work we found, from the graph of the Fig. 3, the LOD (3.SD_{blank}/slope of analytical curve) and LOQ (10.SD_{blank}/slope of analytical curve) were 2.64 x 10⁻⁴ mol L⁻¹ and 8.81 x 10⁻⁴ mol L⁻¹, respectively [62].



Scheme 1. the probable mechanism for the reaction between CPT and CL involve the formation of radical ion pairs.

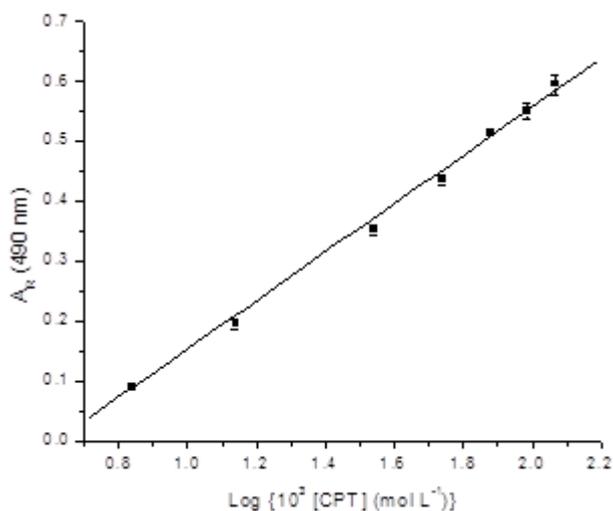


Fig.3. Calibration curve for captopril

The Fig.4 show that the color development is immediate at room temperature ($25\pm 1^\circ\text{C}$). The resulting chromogen is stable for at least 1 day at room temperature. In this manner, all intensity reflectance values were taken immediately after of the addiction of reagents in the filter paper.

3.2. Determination of stoichiometric relationships

For the determination of stoichiometric relationships equimolar concentrations of CPT and CL, $1.64 \times 10^{-2} \text{ mol L}^{-1}$ each were prepared. Molar ratio of the reactants (drug: reagent) in the charge transfer complex was determined by the continuous variation method (Job's

method) [63] and it was found to be 1:1 for the drug (Fig.5). These ratios may be due to the presence of donating center, the nitrogen of molecule of CPT (Fig. 1).

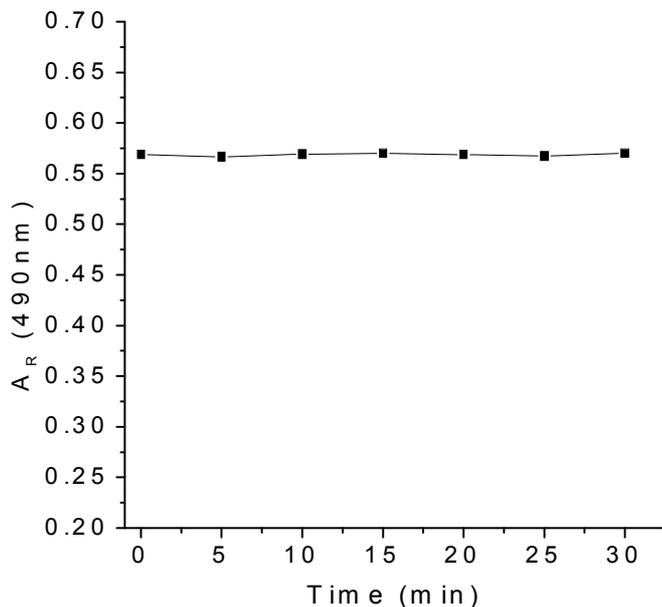


Fig.4. Reflectance measurements to room temperature ($25\pm 1^\circ\text{C}$) time on the reaction.

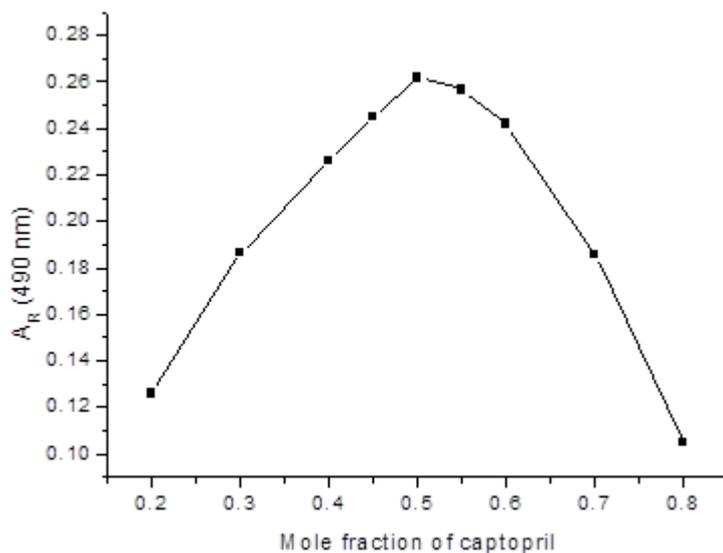


Fig. 5. Continuous variation plot for CPT with CL.

3.3. Effect of interferences

The effects of common excipients, which often accompany captopril in commercial pharmaceutical formulations (sucrose, glucose, lactose, fructose, talc, poly(ethylene glycol), polyvinylpyrrolidone, microcrystalline cellulose, croscarmellose sodium, starch and magnesium stearate) were carefully examined. For this study, solutions containing captopril and each one of the excipients taken separately in concentrations equal or 10 times greater than that of captopril were shaken with deionized water in a magnetic mixer for 5 minutes, diluted, filtered where necessary, and analyzed under the same conditions described in the recommended procedure. The effect of each excipient was considered to be an interference

when the signal showed an error over or equal to 3.0% in the determination of the drug. No interferences were observed in the presence of the substances tested.

3.4. Analytical applications, recovery and repeatability studies

The proposed method was applied to the determination of captopril in commercial tablets. The samples were prepared using the developed method. Then, the proposed method was successfully applied for CPT determination in marketed products. Four replicate determinations were made of each sample. Satisfactory results were obtained (Table 1).

Table 1. Determination of CPT in commercial pharmaceutical preparations

Sample	Label value ^a	Proposed method				Official method [23]	
		Found ^b mg unit ⁻¹	RSD (%) ^c	<i>t</i> -value (2.45) ^d	<i>F</i> -value (9.28) ^d	Found ^b mg unit ⁻¹	RSD (%) ^c
A	25.0	24.9±0.3	1.2	1.53	9.00	24.9±0.1	0.4
B	25.0	24.5±0.1	0.4	0.62	4.00	24.6±0.2	0.8
C	25.0	25.1±0.1	0.4	2.30	4.00	24.8±0.2	0.8
D	25.0	25.2±0.3	1.2	0.33	1.00	25.2±0.3	1.2
E	12.5	12.4±0.2	0.8	0.09	1.00	12.4±0.1	0.8
F	12.5	12.2±0.2	1.6	1.92	4.00	12.0±0.1	0.8

^a Label to content for tablets: mg unit⁻¹.

^b Average value ± standard deviation (SD) of four determinations.

^c Relative standard deviation (RSD) of four determinations.

^d The figures between parentheses are the theoretical values of *t* and *F* at *P* = 0.05.

The results of the proposed method were statistically compared with those obtained by official method of the United States Pharmacopoeia [23] at 95% confidence level. The Table 1 shows that the calculated *F*- and *t*-values [64] are less than the theoretical ones, confirming very good accuracy and precision at the 95% confidence level.

Moreover, to check the validity of the proposed method, the standard addition method was applied by adding known quantities (150.0, 226.0 and 300.0 mg L⁻¹ corresponding to levels of 50, 75 and 100%, respectively) of the standard substance (pure drug) to the five previously analyzed tablets. The recovery of drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results presented in Table 2 show that the percentage average recoveries were found to be close to 100.0% of CPT from six commercial pharmaceutical preparations samples; the relative standard deviation (RSD) were within 0.3 – 2.1%. These results point out the accuracy and precision of the method and the absence of significant matrix effects on reflectometric measurements, therefore it is suggested that there is no interference from any excipients, which are present in tablets.

The measurement precision was determined by performing 10 replicate measurements of the method concentration (sample solution containing equivalent to 5.52 x 10⁻² mol L⁻¹ of CPT). The relative standard deviation (RSD) at this concentration level was found to be 1.6%. These results of accuracy and precision showed that the proposed method has good repeatability.

4. Conclusion

In the present study, we have demonstrated the potential of diffuse reflectance spectroscopy for the analysis of CPT in bulk drug and in tablets. The results obtained from

this work showed good performance of this technique, suggesting its use as an advantageous alternative for quantitative analytical purposes.

Table 2. Recovery data for captopril spiked to pharmaceuticals

Sample	Added (mg L ⁻¹)	Found (mg L ⁻¹)	Recovery (%) ^a
A	150.0	451.6	101.1
	226.0	528.0	100.9
	300.0	595.5	98.5
			$\mu^a = 100.2 \pm 1.4$
B	150.0	449.4	99.6
	226.0	527.6	100.7
	300.0	601.5	100.5
			$\mu^a = 100.3 \pm 0.6$
C	150.0	449.8	99.9
	226.0	524.0	99.1
	300.0	596.7	98.9
			$\mu^a = 99.3 \pm 0.5$
D	150.0	452.6	101.7
	226.0	527.4	100.6
	300.0	594.7	98.2
			$\mu^a = 100.2 \pm 1.7$
E	150.0	448.5	99.0
	226.0	526.7	100.3
	300.0	609.7	103.2
			$\mu^a = 100.8 \pm 2.1$
F	150.0	451.8	101.2
	226.0	527.8	100.8
	300.0	604.5	101.5
			$\mu^a = 101.2 \pm 0.3$

^a Average \pm standard deviation (SD) of three determinations.

The developed method represents an advantageous alternative compared to other available methods, because it is inexpensive, simple, portable, precise, accurate, and allows rapid determination at low operating costs, and it also requires minimum amounts of samples and reagents/solvents (environment friendly analytical method). Moreover, the method has the advantages of simplicity without involving heating and it does not require the removal of usual excipients present in pharmaceutical formulations since they were found not to interfere with the determination of CPT. Thus, these advantages demonstrate the utility of this method for routine use.

Green analytical chemistry is in its initial stage of development, but there is a clear trend towards to fast and consistent growth. Soon, analytical methods showing high performance, but which are not environmentally friendly, tend to be unacceptable for stimulating the development of cleaner methods. This will contribute to the development of

an essential environmental conscience for the future. This paper is a contribution towards to a green chemistry.

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