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A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF CAPTOPRIL IN PHARMACEUTICAL PREPARATIONS USING AMMONIUM MOLYBDATE

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Abstract: A simple, rapid and sensitive spectrophotometric method for the determination of captopril (CPT) in pharmaceutical formulations is proposed. This method is based on the reduction reaction of ammonium molybdate, in the presence of sulphuric acid, for the group thiol of CPT, producing a green compound (λ_{max} 407 nm). Beer's law is obeyed in a concentration range of 4.60 x 10^{-4} – 1.84 x 10^{-3} mol l^{-1} of CPT with an excellent correlation coefficient (r = 0.9995). The limit of detection and limit of quantification were 7.31 x 10^{-6} e 2.43 x 10^{-5} mol l^{-1} of CPT, respectively. The proposed method was successfully applied to the determination of CPT in commercial brands of pharmaceuticals. No interferences were observed from the common excipients in the formulations. The results obtained by the proposed method were favorably compared with those given by the official reported method at 95 % confidence level.

Keywords: Captopril, spectrophotometric determination, ammonium molybdate.

Introduction

Captopril, 1 - [(2S)- 3 - mercapto -2 -methylpropionyl]-L-proline (Figure 1), (CPT) is an angiotensin-converting enzyme inhibitor, which reduces peripheral resistance end lowers blood pressure. It is extensively used for the treatment of hypertension [1] and congestive failure [2].

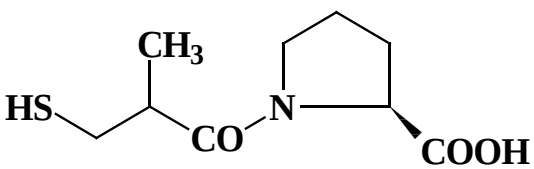

Figure 1. Chemical structure of captopril.

In order to assure the quality of CPT containing pharmaceutical formulations, several methods have been developed for its determination, including batch fluorimetry [3], chemiluminescence [4 – 7], AAS [8, 9], high-performance liquid chromatography (HPLC) [10 – 17], GC [18], differential pulse polarography [19], amperometry [20 – 22], volumetric titration [23], potentiometric titration [24 – 28], capillary electropho-

resis [29], conductometry [30], coulometry [31], voltammetry [32] and potentiometry [33].

However, batch methods are generally time-consuming and laborious. In addition, chromatographic methods are slow and require expensive and complicated instrumentation, features that make them unattractive to routine analysis. Titrimetric method has suffered from a lack of specificity and sensitivity, under certain circumstances, such as the presence of unsaturated organic compounds.

Obviously, because of its low operating costs, simple equipment, as well as the widespread use of common laboratory, spectrophotometry has been an important analytical method to the chemical workers of analysis. It is very significant to find a rapid, accurate and simple method to determine captopril in the researches of the clinical medicine. Thus, spectrophotometric methods have also been described for the determination of CPT in the pure form and in pharmaceutical formulations [27, 34 – 42]. A UV spectrophotometric procedure has been used for the determination of CPT in bulk drug and tablets in the presence of iodine, where the indirect quantization of the product was carried at 351 nm [34] This method present low selectivity, as all unsaturated compounds display one or more bands in that region of the spectrum. The CPT has been determined in the area of the visible after the reaction with iodine [34], ferric chloride in presence of 2,2'–bipirydil [34] and potassium ferricyanide [35], Pd(II) [27, 36, 37], Co(II) in presence of 2,2'-dipyridil-2-pyridylhydrazone [38], N–(1–naphtyl) ethylenediamine in acid media (nitrous acid) [39], Folin-Ciocalteu reagent [40, 41] and molybdophosphoric acid [42]. This last procedure can only be used for the determination of CPT in pure forms, because the presence of the most common excipients in pharmaceutical formulations (glucose, fructose, lactose, sucrose, starch) they interfere seriously in this method. Such interferences, no studied by the authors, they were confirmed starting from preliminary tests accomplished at our research laboratory. However, no spectrophotometric method for determination of CPT based on the reduction reaction of ammonium molybdate has been reported.

Salts of molybdenum (VI) have been used as oxidizing agents in the spectrophotometric determinations of a number of substances of pharmaceutical interest. Tetracyclines have been assayed by using sodium molybdate [43, 44]. Molybdophosphoric acid has been applied in the determination of cephalosporins [45, 46], levodopa, carbidopa, α-methyldopa, isoniazid and acetaminophen [47]. The same reagent has also been applied successfully for the determination of phenothiazines [48, 49].

In this work, we report a novel, simple, rapid, cost-effective, precise, sensitive and accurate spectrophotometric method that is ideal for routine analysis of CPT in pharmaceuticals. Additionally, the proposed technique was ascribed to the fact that they are easily and widely used in laboratory analysis in addition to being economical in terms of their implementation and maintenance.

The proposed method is based on reaction of reduction of ammonium molybdate for the group thiol of CPT in acid media. The measurement of absorbance is made spectrophotometrically at 407 nm. The results agreed fairly well with those obtained by the USP standard procedure [23] at 95% confidence level. In this method, the CPT has been determined by volumetric titration, where the oxidation of the thiol group through iodometric titration.

Experimental

Apparatus

A HP 8453 spectrophotometer with 1 cm matched silica cells was used for all absorbance measurements. Volume measurements were made with plunger-operated pipetters (25–250 µL and 100–1000 µL) and Metrohm model 665 automatic burettes. All experiments were performed in a thermostated room (25±1) °C.

Reagents and solutions

For the preparation of the solutions and samples, deionised water (conductivity > 1µS cm^{-1}) or chloroform and grade A glassware were used throughout. Analytical-reagent or pharmaceutical grade chemicals were used.

Captopril (standard substance) was purchased from Purifarma, Brazil (purity > 99.9%). Its characteristic was consistent with the USP [23].

A 4.60 x 10^{-2} mol l^{-1} (1000 mg l^{-1}) captopril stock was prepared daily by dissolving 50.0 mg of the drug in 5.0 ml of chloroform. Working standard solutions were obtained by appropriate dilution of this stock solution with the same solvent.

The sulphuric acid (Mallinckrodt, Xalostoc, Mexico) solution 8.73 mol l^{-1} was prepared in the usual way, from the concentrated acid (96%).

The ammonium molybdate [$(NH_4)_6.Mo_7.O_{24}.4H_2O$] (Mallinckrodt, Xalostoc, Mexico) aqueous solution 2% (m v^{-1}) was prepared daily.

Recommended procedure

Procedure for the calibration curve

Transfer 300 µL of captopril working standard solutions (comprising 4.60 x 10^{-4} – 1.84 x 10^{-3} mol l^{-1} of the drug) into each series of 5.0 ml graduated flasks. Add to each graduated flask 3.00 ml 8.73 mol l^{-1} H_2SO_4 followed by 1.000 ml 2% ammonium molybdate (under stirring). The graduated flask are loosely stoppered to room temperature (25±1 °C) for 30 min. After the time, the contents were diluted to the mark with deionised water and mixed well. The blank solution is prepared in a similar way, but omitting captopril. Immediately, the absorbance each flask was measured at 407 nm, against corresponding reagent blank. Calibration graphs prepares by plotting absorbance against drug concentration. These graphs or the corresponding linear least squares equations are used to convert absorbance into captopril concentration, for any analyzed sample.

Procedure for the assay of CPT in pharmaceutical samples

For the determination of CPT in pharmaceutical samples, sixteen tablets were weighed to calculate the average tablet weight. They were finely powdered and homogenized. Equivalent to about 41.7 mg of captopril of the powder was accurately weighed and transferred into a 10.0 ml standard flask dissolved with approximately 7.0 ml of chloroform and, by sonicating for 20 min in an ultrasonic bath, and the volume completed with the same solvent. Aliquots of 300 µl of supernatant liquid of this resulting mixture were analyzed according to the recommended procedure.

Results and discussion

Captopril, as all thiols was expected to undergo to some extent oxidative degradation such as the formation of disulphide [50] and this suggests the investigation of an analytical procedure based on the reactivity of the thiol group, with regard to obtaining a stability indicating assay method.

It has long been know that a colour solution is obtained by reduction of an acidified solution of Mo (VI). The substances responsible for this colour are compounds in which the mean oxidation state of Mo is between 4+, 5+ and 6+. These compounds contain both oxides and hydroxides appear to be an entire series of "genotypic" compounds (i.e. having the same basic structure but differing in the charges on cations and anions) with $MoO(OH)_2$ (green colour) as one limit and MoO_3 as the other.

So, the tone of the colour of a reduced solution of Mo (VI) changes as a function of the variation of the concentration of each absorbent particle, which derived from the reduction of the Mo (VI), the concentration and of the reducing strength of the used reducing agent. The captopril has been used as reducing agent due to reactivity of the thiol group of this compound.

The method involves the reaction of CPT with molybdate ions, in acidic media, to produce green product. The absorption spectrum of the reaction product (Figure 2) shows that the best analytical wavelength is located at 407 nm.

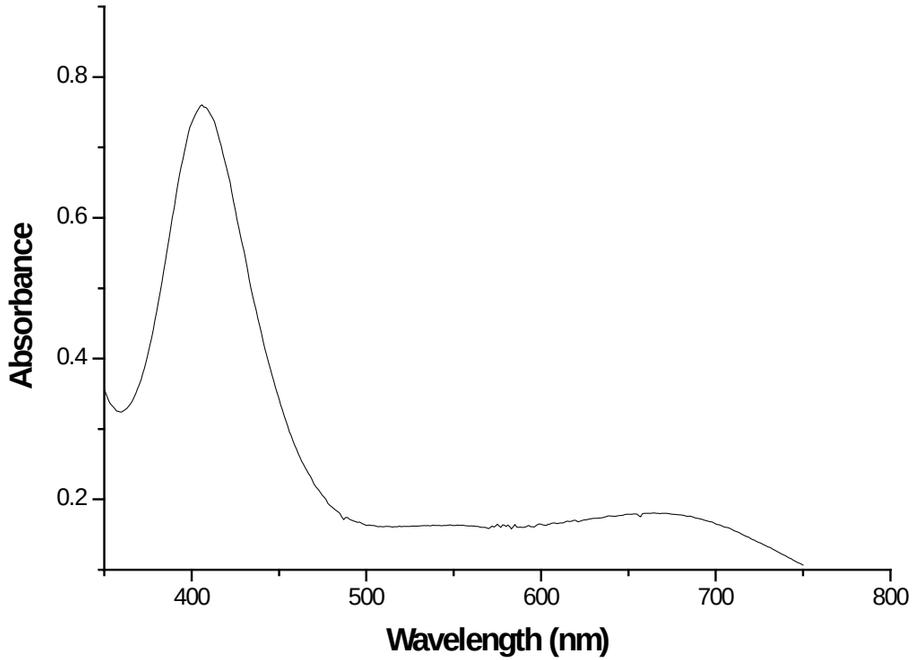

Figure 2. Absorption spectrum of the reaction product. Captopril final concentration = 1.61 x 10^{-3} mol l^{-1}; optical path = 1 cm. Measurements taken at 25 °C against the reagent blank after stoppered to room temperature for 30 min, as described in the recommended procedure.

Optimization of different experimental parameters and stability

The optimum conditions were established based on the development of maximum colour intensity and stability on variation of parameters affecting captopril oxidation and the coupled colour reaction with ammonium molybdate.

Using different concentrations of H_2SO_4, it was found that maximum colour intensity and stability were obtained by developing the reactions in 8.73 mol l^{-1} H_2SO_4, as described in the recommended procedure. At higher concentrations of H_2SO_4, the absorbance was found to decrease, whereas, below of this concentration the colour became unstable and the colour intensity diminished. Other acids were also studied for production of colour and it was found that no colour reaction was produced with acids like acetic acid, phosphoric acid and nitric acid, whereas, with hydrochloric acid a very light yellow colour was obtained which was unstable.

Ammonium molybdate was used as a colour producing reagent. The adopted ammonium molybdate concentration (2%) was found to be sufficient for providing maximum and repeatable colour intensity, when the concentration of this reagent was above or below of this concentration the absorbance was found to decrease.

The order of addition of the reactants recommended in the general procedure produced quantitative results. Any other order was found to produce deviant results and the colour intensity diminished.

The Figure 3 show that a stoppered to room temperature (25±1 °C) time of 30 min is required for full colour development and the resulting chromogen is stable for at least 1 day at room temperature. Thus, below this time (30 min) the colour intensity and the sensitivity of method diminished.

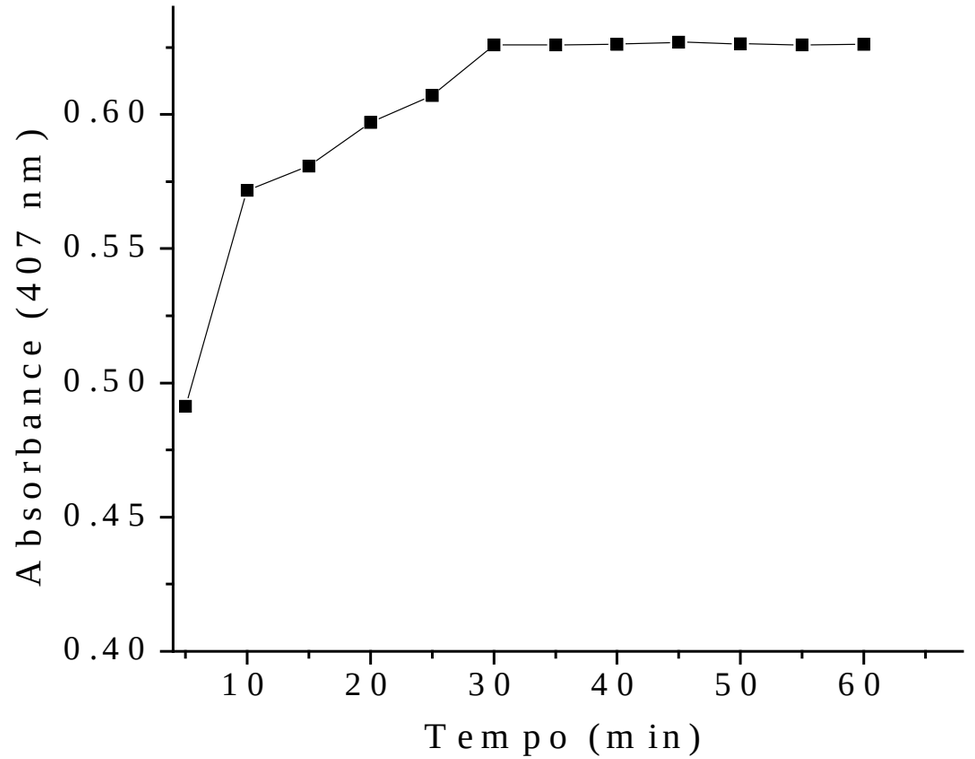

Figure 3. Effect of stoppered to room temperature (25±1 °C) time on the reaction. [a] Captopril concentration: 1.38 x 10^{-3} mol l^{-1}. [b] Measurements taken at 407 nm against the reagent blank for reactants after stoppered to room temperature (25±1 °C) time (as described in the recommended procedure). [c] The absorbance remains unchanged standing for 1 day at 25 °C.

Analytical curves and sensitivity

The analytical curve (Figure 4) was obtained by the method of least squares from eleven points, each of which was the average of three determinations. The Beer's law is obeyed within 4.60 x 10^{-4} – 1.84 x 10^{-3} mol l^{-1} of CPT, in the final solution, with an excellent correlation coefficient (r = 0.9998; slope = 471.72 ± 1.18 l mol^{-1} cm^{-1} and intercept = -0.0162 ± 0.0003). The limit of detection ($3.SD^{blank}$/slope of curve) and limit of quantification ($10.SD^{blank}$/slope of curve) were 7.31 x 10^{-6} e 2.43 x 10^{-5} mol l^{-1} of CPT, respectively [50].

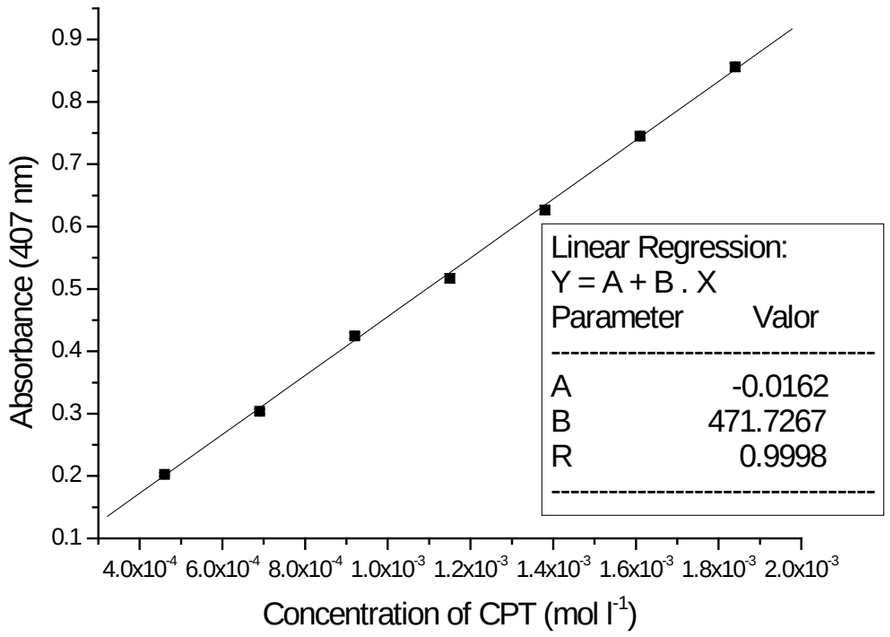

Figure 4. Analytical curve for determination of captopril.

Effected of interferents

To assess the use fullness of the proposed method, the effect of the common components (additives, adjuvants and excipients) witch often accompany captopril in tablet dosage formulations (lactose, microcrystalline cellulose, croscarmellose sodium, starch and magnesium stearate) were investigated using the developed method. The ratios of the concentrations of CPT to those excipient substances were fixed at 1.0 and 10.0. No interferences were observed in the presence of the substances tested.

Analytical applications, recovery and repeatability studies

In order to assess the utility of the presently developed method it was applied to the estimation of captopril in several pharmaceutical forms. The samples were prepared using the developed method. Then, the proposed method was successfully applied for CPT determination in six tablet formulations. The results, presented in Table 1, compare favorably with the official method of the United States Pharmacopoeia [23] at 95% confidence level. The results were subjected to a paired comparison test [52], the data of *t* and *F* ratios show no significant differences between the results of the proposed and the official methods, indicating very good accuracy and precision.

Table 1. Determination of CPT in commercial pharmaceutical preparations

Sample	Label value [a]	Proposed method				Official method [23]	
		Found [b]	R.S.D (%) [c]	*t*-value (2.45) [d]	*F*-value (9.28) [d]	Found [b]	RSD (%) [c]
A	25.0	26.2±0.8	3.0	0.40	2.56	26.0±0.5	1.9
B	25.0	26.3±0.6	2.3	0.92	4.00	26.2±0.3	1.1
C	25.0	26.5±0.6	2.2	0.56	2.25	26.3±0.4	1.5
D	25.0	25.6±0.3	1.2	0.61	9.00	25.6±0.1	0.4
E	25.0	25.0±0.5	2.0	1.37	6.25	26.6±0.2	0.8
F	12.5	12.6±0.2	1.6	1.18	4.00	12.8±.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

For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed method. The recovery studies were carried out after adding known quantities (100.0, 125.0 and 150.0 mg l^{-1}) of the standard substance (pure drug) to the six preanalyzed formulations. 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 0.1 – 1.0. These results point out the accuracy and precision of the method and the absence of significant matrix effects on spectrophotometric measurements.

Table 2. Recovery data for captopril spiked to pharmaceuticals

Sample	Added (mg l $^{-1}$)	Found (mg l $^{-1}$)	Recovery (%) [a]
A	100.0	99.9	99.9
	125.0	127.3	101.8
	150.0	151.8	101.2
			μ [a] = 100.9 ± 1.0
B	100.0	101.1	101.1
	125.0	126.0	100.8
	150.0	151.4	100.9
			μ [a] = 100.9 ± 0.2
C	100.0	100.1	100.1
	125.0	125.2	100.2
	150.0	150.4	100.3
			μ [a] = 100.2 ± 0.1
D	100.0	100.9	100.9
	125.0	125.9	100.7
	150.0	149.3	99.5
			μ [a] = 100.4± 0.8
E	100.0	100.2	100.2
	125.0	126.3	101.0
	150.0	148.9	99.3
			μ [a] = 100.2 ± 0.8
F	100.0	100.0	100.0
	125.0	125.1	100.1
	150.0	151.0	100.7
			μ [a] = 100.3 ± 0.4

[a] Average ± standard deviation (SD) of three determinations.

To examine the repeatability of the procedure, replicate (n = 10) determinations were made on the same solution containing equivalent to 1.38 x 10^{-3} mol l^{-1} of CPT (300 mg l^{-1}). The relative standard deviation (RSD) at this concentration level was 1.2. This is good evidence of repeatability of the proposed method.

Conclusion

The proposed method results a simple, sensitive, inexpensive, precise and accurate analytical technique to determine captopril in commercial pharmaceutical preparations with satisfactory recoveries. Statistical comparison for the results of the proposed method with the official reported method indicates that there is no significant difference, at 95% confidence level, with regard to accuracy and precision. Additionally, it fulfills all the main demands of routine analysis as it is robust, has low instrumentation and operational cost in comparison to chromatographic methods and it doesn't request pretreatment of the sample.

When applied to the assay of various tablet dosage forms, its advantage is in that it does not require the removal of usual excipients since they were found not to interfere with the determination of captopril.

Artigo
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Acknowledgements

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Resumo: Um método simples, rápido e sensível para a determinação espectrofotométrica de captopril (CPT) em formulações farmacêuticas é proposto. Este método é baseado na reação de redução do molibdato de amônio, na presença de ácido sulfúrico, pelo grupo tiol do CPT, produzindo um composto verde (λ_{max} 407 nm). A lei de Beer é obedecida na faixa de concentração de 4,60 x 10^{-4} a 1,84 x 10^{-3} mol L^{-1} de CPT com um excelente coeficiente de correlação (r = 0,9995). O limite de detecção e o limite de quantificação foram de 7,31 x 10^{-6} e 2,43 x 10^{-5} mol L^{-1} de CPT, respectivamente. O método proposto foi aplicado com sucesso na determinação da CPT em amostras comerciais de produtos farmacêuticos. Não foram observadas interferências dos excipientes comumente encontrados nas formulações farmacêuticas. Os resultados obtidos pelo método proposto foram comparados favoravelmente com aqueles obtidos pelo método oficial relatado em um nível de 95% de confiança.

Palavras-chave: Captopril, determinação espectrofotométrica, molibdato de amônio.

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