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Spectrophotometric Indirect Determination of Captopril through Redox Reaction with n-bromosuccinimide and RB dye in Pharmaceutical Products

Dashne M. Kokhasmail[1], Tara F. Tahir[2] and Kurdistan F. Azeez[1]

[1]Department of Chemistry, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq

[2]Department of Medical Microbiology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq

***Abstract*—A simple, accurate, and sensitive method for the spectrophotometric determination of captopril in bulk and dosage forms is reported. The method is based on the bromination of captopril with excess solution of n-bromosuccinimide (NBS) in HCl acid medium. The excess NBS is pursued by the assessment of the residual NBS based on its ability to bleach the rhodamine B dye and measuring the absorbance at 555 nm. The amount of NBS reacted coincides to the drug content. The different experimental parameters influencing the development and stability of the color are precisely studied and optimized. Beer's law is valid within a concentration range of 0.3–1.0 μg/mL with a correlation coefficient R^2 = 0.991. The limit of detection 0.169 μg/mL is attained and relative standard deviation values for five replicated measurements of 0.3, 0.7, and 1.0 μg/mL captopril were between 0.53% and 2.03%. No interference is detected from prevalent additives found in pharmaceutical preparations. The proposed method is profitably put on to the determination of captopril in the tablet formulations with mean recoveries 98.91–101.27% and the results were statistically confronted with those of a reference method by applying Student's t-and F-test.**

***Index Terms*—Captopril, Indirect determination, n-bromosuccinimide, Rhodamine B, Spectrophotometer.**

I. Introduction

Hypertension takes place with two-fold the recurrence in the diabetic community in comparison with the non-diabetic community, and more than 50% of patients with type 2 diabetes mellitus develop into hypertensive (Parving, et al., 1983). Besides, enduring a large risk cause for atherosclerosis in large blood vessels, hypertension in diabetes contributes to mini-vessel disease and is a danger cause for diabetic nephropathy and probably for diabetic retinopathy. Investigations have presented that angiotensin-converting enzyme (ACE) inhibitors can sluggish the development of diabetic nephropathy in patients with type 1 or type 2 diabetes with microalbuminuria or macroalbuminuria (Phillip and Hall, 2006).

A main thing whither toward preserve the kidney from the complexity of diabetes is to manage elevated blood pressure destructively, despite the antihypertensive drug types utilized. Literature stated that patients with type 1 diabetes and antihypertensive drugs such as diuretics, beta-blockers, and hydralazine were taken, demonstrating that decreasing blood pressure diminishes proteinuria and sluggish the dismiss of renal work (Lewis, et al., 1993). A predominance of clue records that ACE inhibitors preserve the kidney more than undertake other blood pressure suppressing medications, apparently because ACE inhibitors particularly reduce the intrarenal pressure.

Patients with type 1 diabetes, albuminuria, and mildly impaired creatinine clearance were exposed to a milestone study. The ACE inhibitor captopril was diminished the risk of a decline in renal function more effectively than did other antihypertensive regimens (Lewis, et al., 1993). It has been found that captopril is powerful in lowering blood pressure and also lowering the risk of macrovascular end points. It was confirmed that captopril does not develop any important differences in the blood levels of ionized calcium or phosphorous ions and that it does not modify the serum levels of parathyroid hormone (PTH) and metabolites of Vitamin D (Florentin, et al., 2004).

Captopril (1-[(2S)-2-methyl-3-sulfanylpropanoyl]-L-proline) is an inhibitor of ACE, acting directly on the adrenal gland to activate the release of aldosterone (Fig. 1). It inhibits elevated blood pressure by preventing the enzymatic alteration of angiotensin I to angiotensin II. It is a white crystalline powder ($C_9H_{15}NO_3S$, molar mass 217.29 g/mol)

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Corresponding author's e-mail: tara.fuad@koyauniversity.org

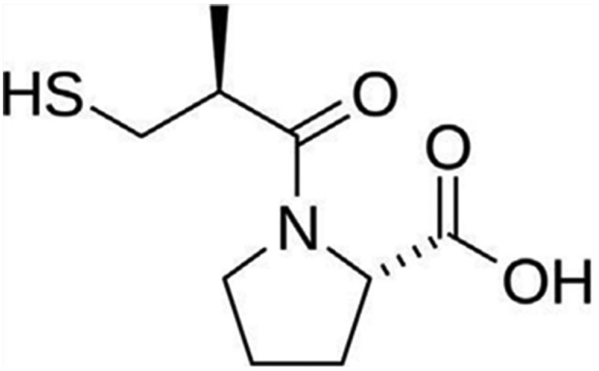

Fig. 1. Chemical structure of captopril.

having an essence sulfide-like odor and a melting point between 105 and 108°C. Captopril is plainly soluble in water or diluted solutions of alkali hydroxides, in alcohols, in methylene chloride, or in chloroform (Milan, et al., 2015).

Many methods were available for the determination of captopril in various samples including pharmaceutical products. The first approaches to captopril determination in biological matrix were made by liquid chromatography (LC) and mass spectrometry (Salem, et al., 2005, Vancea, et al., 2009).

Captopril is determined in blood, plasma, and urine using an investigate method of high-performance LC (HPLC). Nevertheless, determination methods of low concentration levels of captopril based on oxidation reaction are pursued three different oversights: Apply of extra sensitive detection method as an alternative to ultraviolet (UV) method such as electrochemical method without any derivatization of the analyte; detection of a derivatized product of captopril that is identified before or after chromatographic separation using the UV or fluorescent method; and utilize of pre-concentration techniques such as liquid-liquid extraction and multicolumn switching setup (Florentin, et al., 2004).

Despite the fact that captopril is non-toxic, but appropriate caution must be taken because it can cause hypotension when overdosed. This result is only expected following ingestion of quantities >450 mg/day. Taking medicines of captopril level above the accepted value can be harmful to the patient (Jia, et al., 2001). Accordingly, there is a sustaining demand for the development of new analytical procedures for the determination of captopril in pharmaceutical products (Lima, et al., 2016).

HPLC is recognized as a favored analytical technique for identification and quantification of most pharmaceutical products including captopril (Iqbal, et al., 2015). Disregarding that the advantage of HPLC as it is a well-built technology in the pharmaceutical field, offering sensitivity and specificity, it has defects that include high operation costs, the demand for large amounts of samples and solvents, and the formation of hazardous wastes such as organic solvents that are costly to relinquish and can have significant environmental impacts (Safila, et al., 2013).

In the published works, different methods were reported for the determination of captopril which include atomic absorption spectrometry, spectrophotometry, chemiluminescence, LC, amperometry, electrophoresis, and HPLC techniques (El-reis, et al., 2000; El-Shanawany, et al., 2014; Zhaofu, et al., 2017; Fu, et al., 2017, Shafi, et al., 2015; Marcolino-Junior, et al., 2009; Tomas, et al., 2006; Iqbal, et al., 2015, Zhang, et al., 2020).

The use of spectrophotometry provides a simple and inexpensive technique for the determination of drugs in pharmaceutical products. Sensitivity is another important characteristic of molecular absorption technique. Besides, the large linear concentration ranges make the method interesting and versatile for routine analysis of drugs in quality control laboratories (Rao, et al., 2012). Most of the reported methods were based on the redox reaction of captopril with an excess of an oxidizing agent (El-Didamony and Erfan, 2010; Skowron and Ciesielski, 2011).

The aim of the present work was, therefore, to use a convenient, low cost but sensitive analytical method as spectrophotometer for quantification of captopril in pharmaceuticals based on redox reaction with n-bromosuccinimide (NBS) and rhodamine B dye (RB). The results were compared with the recommended method described in the literature.

II. EXPERIMENTAL

A. Apparatus

Molecular absorption spectra were measured using UV-1000 CECIL 1021 spectrophotometer, UK, with a glass cell of 1.0 cm optimal path length. LW Scientific Digital Water Bath, US, was used to control the temperature of the solution during the studies. The studies were accomplished using HPLC system of Agilent 1100 controlled by ChemStation Data System. A G 1311A quaternary pump and UV detector (VWD-G1314 A) were supplied with the system. A reverse phase C18 column (Kromasil 100-5-Phenyl®, 300 mm × 4.6 mm, 5 μm) was handling at 25°C and the mobile phase of 0.1 % v/v trifluoroacetic acid and acetonitrile at ratio (80:20 v/v) was provided during the investigation. The flow rate of 1.5 mL/min elute was advised at wavelength 290 nm.

B. Material and Reagent

Captopril was collected from Awamedica Pharmaceutical Company (Kurdistan region, Iraq), used as received, the purity of which was 99.9%. A solution of 100 μg/mL of NBS (from Fluka) was prepared by dissolving 0.1 g of NBS ($C_4H_4BrNO_2$) in small amount of warm water, then diluted to 1000 mL with distilled water, and kept in 5°C refrigerator until further usage. A dye solution of 100 μg/mL of RB (from RIEDEL-DEHAEN) was prepared by dissolving 0.1 g of RB in distilled water and diluted to 1000 mL with distilled water (the solution is stable for at least 2 weeks). Hydrochloric acid, sulfuric acid, nitric acid, and acetic acid (from Scharlau) of 1 mol/L solution were prepared, individually. Interfering solutions of 500 μg/mL of fructose (from BDH), glucose (SCP), lactose (BDH), starch (from Difco), and sucrose (from Difco) were prepared individually by dissolving 0.1 g of solid compound in 100 ml distilled water.

C. Preparation of Standard and Sample Solution

A stock solution of standard captopril 1000 μg/mL was prepared by dissolving 0.1 g of standard captopril powder in

20 mL distilled water with stirring carefully, then the volume was completed to 100 mL volumetric flask with the same solvent and kept in 5°C refrigerator to remain stable for 28 days (Pereira and Tam, 1992). Working standard solutions were prepared daily by proper dilution of the stock standard solution with the same solvent.

All pharmaceutical products of captopril in the local medical store are containing 25 mg and two different companies were used for the determination (captopril Awa, Erbil, Iraq, and Rilcapton, Medochemie-Cyprus). Ten tablets of captopril were weighed and crushed for each pharmaceutical company, and the sample powder of the two companies was accurately weighed individually and placed in a 50.0 mL beaker, then dissolved in 30.0 mL of distilled water. The solution was stirred for 10 min to increase solubility. Insoluble excipient was removed by filtration using Whatman No. 41 membrane filter paper. The filtered solution was diluted to 100 mL in a volumetric flask with the same solvent.

D. Analytical Procedure

In 25.0 mL volumetric flask, 2.5 mL of NBS (100 μg/mL), 0.8 mL of HCl (1 mol/L), and adequate captopril standard or sample (0.3–1.0 μg/mL) of 100 μg/mL solution were added. This mixture was shacked thoroughly and left to stand for 10 min at 25 ± 2°C. Finally, 2.5 mL of RB (100 μg/mL) was added and directly diluted to the mark with distilled water. The absorbance was measured against reagent blank prepared in similar conditions without captopril at 555 nm.

III. Results and Discussion

Captopril is acting as a reducing agent due to the presence of thiol group (- SH) in its structure and the literature explains that captopril in aqueous solution undergoes oxidative degradation at its thiol function to yield captopril-disulfide (Chenl, et al., 1995). In this work, the reaction involves two steps (Fig. 2):

(i) Oxidation of the drug (captopril) by excess of NBS reagent, generated in sedentary by the action of HCl acid solution on captopril-disulfide
(ii) Determination of unreacted oxidant NBS by bleaching the color of RB dye in acidic medium (Abdel-Hady, 2013).

The absorption spectrum of the yielded bleaching 2.5 mL of (100 μg/mL) RB demonstrated the maximum absorbance at 555 nm against the blank solution after 10 min (Fig. 3a). The absorbance of individual 0.7 μg/mL captopril showed dropping at the same wavelength (Fig. 3b), whereas no significant absorbance was recorded for the blank reagent of NBS and RB dye (Fig. 3c).

A. Optimum of Experimental Conditions

The reaction conditions along with the different experimental parameters influencing the color development and stability of the dye were laboriously examined and optimized for the quantitative determination of captopril in bulk and the tablet dosage forms.

Selection of type and acid concentration

The reaction of 0.7 μg/mL captopril with 2.5 mL of (100 μg/mL) solution of each of NBS and RB dye was tested in 1 mol/L of HCl, HNO_3, H_2SO_4, and CH_3COOH solutions, individually. The results demonstrated that the reaction is suitable in hydrochloric acid medium (Fig. 4a). A 1 mol/L HCl solution was found to be sufficient for the oxidation of captopril as well as the bleaching of RB dye. The variation in HCl volumes indicated that highest absorbance was observed with 0.8 mL of 1 mol/L HCl, subsequent studies were performed with this volume and concentration of HCl (Fig. 4b).

Sequence of addition

The sequence of addition of 2.5 mL (100 μg/mL) NBS oxidant, drug solution, 2.5 mL (100 μg/mL) RB dye, and 0.8 mL (1 mol/L) HCl was studied through bleaching the color of RB dye and measuring its absorbance at 555 nm (Fig. 5). Best absorbance was accomplished when the sequence was in the order; drug, NBS, HCl, and then RB. This study is compatible and confirmed with the mechanism of reaction in Fig. 2.

Reagent and dye concentrations

Elementary experiments were achieved to fix the upper limits of RB dye that could be determined spectrophotometrically in acid medium and this was found to be 2.5 mL of 100 μg/mL of RB. This concentration gave stable and high intensity that was the reasonable concentration for this procedure.

A high concentration of oxidant NBS >100 μg/mL was found to destroy the color of RB dye. Under the experimental conditions, different volumes between 0.5 and 3.0 mL of

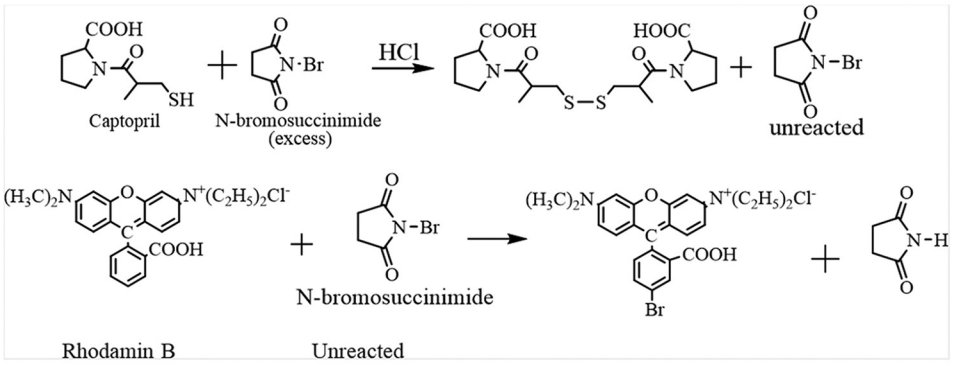

Fig. 2. Mechanism of oxidation of captopril by excess NBS and the latest reaction with RB dye.

100 µg/mL NBS were examined (Table I). Therefore, 2.5 mL of NBS was adopted in the recommended procedure.

Effect of temperature and heating time

The redox reaction of 0.7 µg/mL captopril, 2.5 ml (100 µg/mL) NBS solution, and 2.5 mL (100 µg/mL) RB dye in 0.8 mL (1 mol/L) of HCl acidic medium was examined at various temperatures and heating times through measuring the absorbance (Table II).

TABLE I
AMOUNT OF OXIDANT NBS SOLUTION FOR BLEACHING COLOR OF RB DYE VERSUS ABSORBANCE

Volume of NBS (mL)	Absorbance
0.5	0.982
1.0	0.730
1.5	0.337
2.0	0.183
2.5	0.095
3.0	0.005

NBS: n-bromosuccinimide, RB: Rhodamine B

TABLE II
EFFECT OF TEMPERATURE AND HEATING TIME ON THE ABSORBANCE OF RB DYE

Temperature°C	Absorbance					
	10 min	20 min	30 min	40 min	50 min	60 min
5	0.371	0.372	0.373	0.372	0.375	0.377
10	0.449	0.447	0.445	0.445	0.447	0.449
25	0.587	0.587	0.584	0.588	0.586	0.585
50	0.457	0.459	0.453	0.451	0.450	0.449

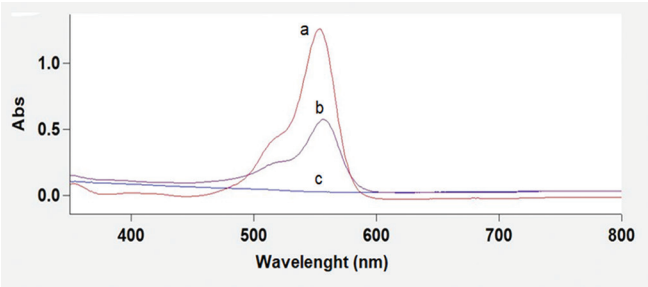

Fig. 3. Absorption spectra of (a) 2.5 mL of (100 µg/mL) rhodamine B, (b) 0.7 µg/mL captopril, 2.5 mL (100 µg/mL) of NSB and RB dye (c) blank reagent of 2.5 mL (100 µg/mL) of NSB and RB solution against distilled water.

As a general rule, increasing the temperature (5–25°C) will increase the reaction rate (for exothermic and endothermic) reactions simply because it means more energy available in the system. However, for the reversible exothermic reaction, there is a range of temperatures where this might not be true (Leenson, 1999). The absorbance was decreased as the temperature rose to 50°C. The results obtained in Table II may be understood in terms of increased quantum yield of the RB dye as the temperature is reduced (Ali, et al., 1991). A 25°C was then applied for further investigation.

Reaction time and stability of dye color

Time is a denoting factor on completing the redox reaction of 0.7 µg/mL captopril with 2.5 mL (100 µg/mL) NBS solution in 0.8 mL (1 mol/L) of HCl acidic medium and bleaching of 2.5 mL (100 µg/mL) RB dye. The maximum absorbance was attained after 10 min at 25 ± 2°C (Fig. 6). The color intensity of RB dye was stable after 25 min for at least 24 h at room temperature.

Interference studies

The effects of common excipients added in pharmaceutical preparations in the form of tablets and capsules were tested for their possible interferences in the assessment of captopril under optimum conditions (El-Didamony and Erfan, 2010). The excipient solutions (500 µg/mL) of lactose, fructose, glucose, starch, and sucrose were mixed with 0.7 µg/mL pure captopril in the final volume of 25 mL, individually. The spectra obtained were compared with the spectrum of 0.7 µg/mL captopril standard solution. A level of interference was considered to be acceptable when the error is not higher than ±5%. No significant levels of interferences were observed in the determination of captopril in the presence of the common excipients (Table III).

B. Analytical Figures of Merit

Determination of captopril was investigated under optimum experimental conditions, when the relative standard deviation (RSD%) was 2.33% as obtained from five replicated measurements of three different concentrations of captopril. In agreement with IUPAC guidelines of the validation of analytical method, the limit of detection value (LOD) = 3.3 SD/P was adopted, in which SD is the standard deviation of

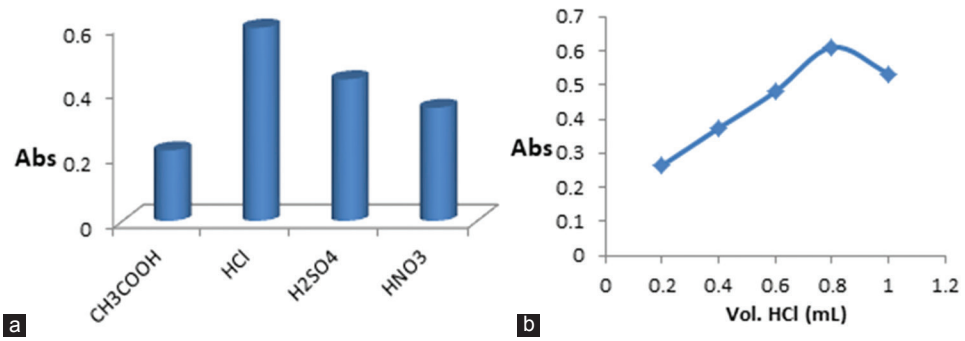

Fig. 4. (a) Absorbance vs. solutions that contain 0.7 µg/mL captopril, 2.5 mL of (100 µg/mL) solution of each of NBS and RB dye with 1 mol/L of CH3COOH, HCl, H2SO4 and HNO3, individually; (b) Absorbance vs. solutions that contain 0.7 µg/mL captopril, 2.5 mL of (100 µg/mL) solution of each of NBS and RB dye with different volumes between (0.2-1.0 mL) of 1 mol/L HCl solution.

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five reagent blank measurements and P is the gradient of the calibration curve (Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization (ICH), 2005, Ana, et al., 2014, Tahir, et al., 2019). The linear range was 0.3–1.0 µg/mL with correlation coefficient R^2 = 0.991 and molar absorptivity of 2333 L/mol/cm (Fig. 7). The LOD and limit of quantification were 0.169 µg/mL and 0.304 µg/mL, respectively. The regression equation of standard solutions was Y = 1.0665x - 0.1766 in which x is in µg/mL.

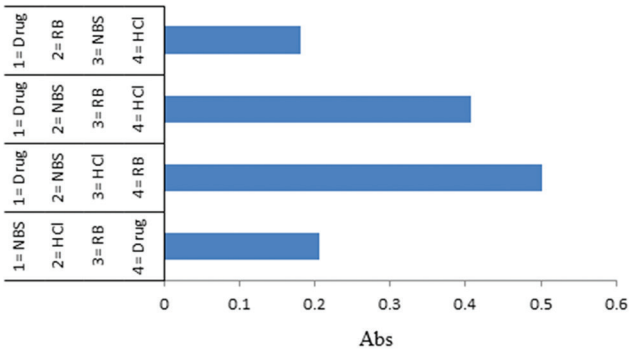

Fig. 5. Absorbance of different sequence of mixing captopril, 2.5 mL (100 µg/mL) NBS, 2.5 mL (100 µg/mL) RB and 0.8 mL (1 mol/L) HCl solution.

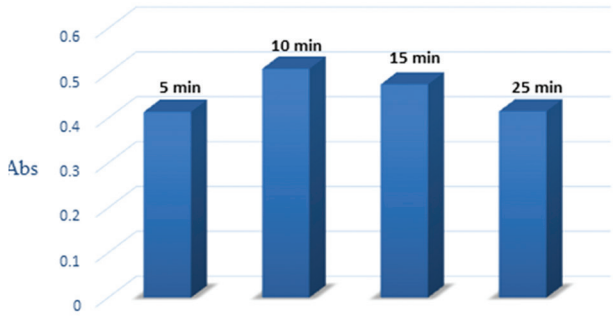

Fig. 6. Absorbance of 0.7 µg/mL captopril, 2.5 mL (100 µg/mL) NBS and 2.5 mL (100 µg/mL) RB solution in 0.8 mL (1 mol/L) of HCl acidic medium at different reaction times.

The accuracy and precision of the proposed procedure was provided by measuring the absorbance of RB dye after bleaching through excess amount of oxidant NBS that was remained after oxidation of three different concentrations of standard captopril (0.3, 0.7, and 1.0 µg/mL), individually in five replicate measurements (Table IV). The values of RSD % and E % were between 0.53%–2.03% and -1.42%–2.00%, respectively, indicating that the proposed procedure is valid and applicative.

C. Application and Comparison

The proposed procedure was successfully bestowed for the determination of captopril in pharmaceutical tables. The ingredients in the pharmaceutical tablets did not interfere in the quantification of captopril. The applicability of the proposed procedure for the analysis of captopril in pharmaceutical formulations was examined by investigating two pharmaceutical tablets and the results are shown in Table V which were compared to the standard captopril assay using HPLC method. HPLC has the capability to detach and determine compounds that are exist in any sample that can

TABLE III
INTERFERENCE STUDIES OF CAPTOPRIL QUANTIFICATION TOWARD SOME COMMON EXCIPIENTS PRESENTED IN THE PHARMACEUTICAL PRODUCTS

Coexisting materials	Allowance concentrations (µg/mL)	E %*
Lactose	500	−1.90
Fructose	500	2.60
Glucose	500	−2.20
Starch	500	2.30
Sucrose	500	2.60

* Average of three determinations

TABLE IV
ACCURACY AND PRECISION DATA FOR THE PROPOSED SPECTROPHOTOMETRIC INDIRECT DETERMINATION OF CAPTOPRIL

Contained amount (µg/mL)	Found by proposed method (µg/mL)	SD	RSD%	E%*
0.3	0.305	0.002	2.030	1.66
0.7	0.690	0.005	0.920	-1.42
1.0	1.020	0.005	0.530	2.00

*Average of five determinations

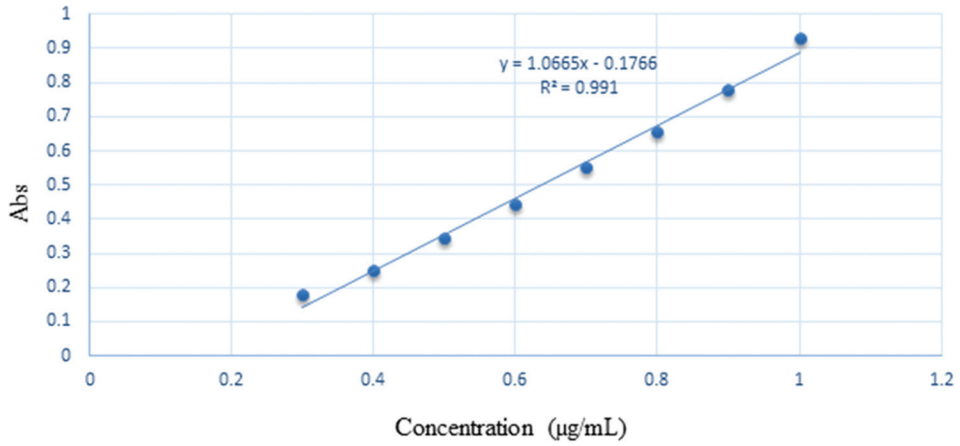

Fig. 7. Calibration curve of spectrophotometric indirect quantitation of captopril via oxidant NBS and RB dye in acidic medium under optimum condition.

TABLE V
Analysis of captopril in pharmaceutical formulations using the proposed method and standard HPLC method

Pharmaceutical tablets	Observed values (mg)	Values from proposed procedure	Values from HPLC	Recovery %	E %*	t and F values**
Captopril awa	25	25.34±1.13	25.02±0.14	101.27	1.27	t = 1.36, F = 1.78
Rilcapton	25	24.71±0.17	24.98±0.08	98.91	-1.08	t = 1.02, F = 1.98

*Average of five determinations, **Theoretical calculation of t and F at 95% confidence level (n=5) was 2.78 and 6.39, respectively, ***The values ± are the standard deviation of the five replication of each sample

TABLE VI
Comparison of determination of captopril using different redox reactions through spectrophotometric technique and the proposed method

Method based on	Ref.	LR (mol/L)	LOD (mol/L)
Reduction of ammonium molybdate	Ribeiro, et al., 2010	4.60×10^{-4} - 1.84×10^{-3}	7.31×10^{-6}
Reduction of potassium dichromate	Moldovan, et al., 2012	3.68×10^{-7} - 1.61×10^{-5}	1.10×10^{-7}
Reaction of O-phthalaldehyde	El-Shanawany, et al., 2014	9.20×10^{-6} - 1.15×10^{-5}	-
Proposed work		1.38×10^{-6} - 4.6×10^{-6}	7.77×10^{-7}

LR: Linear range, LOD: Limit of detection

be dissolved in a liquid in trace concentrations as low as parts per trillion (Iqbal, et al., 2015).

The performance of the proposed procedure was refereed by calculating the Student's t- and F-values. At 95% confidence level, the calculated t- and F-values did not overstep the theoretical values as noticeable from Table V. Consequently, it was concluded that there is no significant difference between the proposed method and the standard method. Moreover, the spectrophotometric method for the determination of captopril in pharmaceutical tablets addressed in this work is simple, fast, inexpensive, precise, and accurate and it may be suitable for routine analysis and quality control laboratories.

In the literature, captopril was quantified using different oxidizing agents through spectrophotometric technique. Table VI views the optimization results of the determination of captopril using different reagent reactions and the proposed method. Despite that some analytical method have lower LOD value and wider linear range, but the proposed method does not need pre-extraction of the sample beside the easy and short time of the reaction.

IV. Conclusion

The proposed method has the expediency of simplicity and rapidity for the determination of captopril in both bulk and dosage forms and interferences free from common tablet excipients. The investigation method comprises less rigorous control of experimental parameters such as the stability of the colored reagent dye, time of reaction, and temperature independence. The reagents applied in the proposed method are low-priced, easily accessible and the procedure does not comprise any laborious sample preparation. These preferences boosted the application of the proposed method in routine quality control of captopril in industrial laboratories.

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