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DETERMINATION OF THE ANTIHYPERTENSIVE DRUGS CAPTOPRIL, ATENOLOL, AND METOPROLOL IN DOSAGE FORMS

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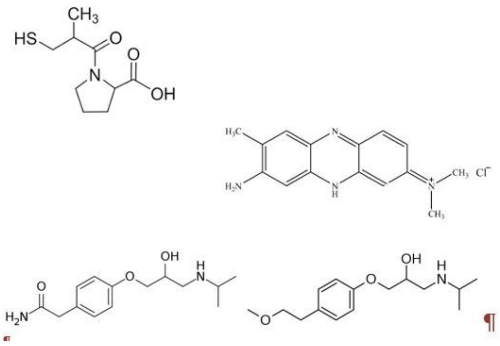
Graphical abstract



Abstract

Three antihypertensive drugs Captopril (CPL), Atenolol (ATL), and Metoprolol (MTL) have been estimated sensitively and selectively by a single spectrophotometric method using neutral red dye NLR. The method is based on oxidation of the drugs by known excess of N-bromosuccinamide (NBS) in the first reaction step followed by the oxidation of NLR dye with the exceed ed amount of NBS, as the concentration of the drug compounds increase, the color of the dye increase, the dye exhibits absorbance peak at 523 nm. The linearity range was from 1 to 10 µg/ml for the three drugs, the molar absorptivity values were 2.1164 × 105, 1.9202× 104, and 57.934× 104 l.mol^{-1}.cm^{-1} for CPL, ATL, and MTL respectively, the sensitivity index of Sand'll were 0.0102, 0.0138, and 0.01182 µg/cm^2 for CPL, ATL, and MTL respectively. LOD values for the three drugs ranged from 0.00405 to 0.00547 and LOQ values were ranged from 0.0135 to 0.0182 respectively, the method exhibits good applicability to the dosage forms and high confidence as compared with standard method and standard addition method.

Keywords: Captopril, Atenolol, Metoprolol, neutral red, bleaching reaction

1.0 INTRODUCTION

Captopril (CPL) is 1-[(2S)3-Mercapto–2–methyl–1–oxo propyl]-l-proline (C9H15NO3S = 217.3 g/mole). Solid captopril compound is a white or off-white crystal powder with 106°C melting and two dissociation steps at pKa 3.7 and 9.8. It is soluble in water, ethanol, methanol, and in dilute alkaline solution [1, 2]. Captopril is used for the treatment of hypertension and heart failure. The absorption of the drug begins in the mouth, excess of 60 to 70% of the dose reaches within one hour to the maximum concentration in the plasma.

The drug crosses the placenta and may be excreted in breast milk, while 40 to 50% of the oral dose is excreted unchanged in the urine. Approximately 3% of captopril is excreted as disulfide and approximately 30% as polar metabolites [3, 4]. Captopril has been determined by flow injection [5, 6] spectrophotometric via oxidation with potassium iodate in acidic [7] or spectrophotometric coupled with cloud-point extraction [8] and by chromatographic methods [9, 10].

Atenolol (ATL) is 4-[2-Hydroxy–3-[(1–methylethyl) amino] propoxy] benzeneacetamide (C14H22N2O3 =266.3 g/mole). Solid atenolol is crystal powder with 146° to 148° C melting and one dissociation step with pKa 9.6. It is freely soluble in methanol but slightly soluble in water and very slightly soluble in acetone [1, 2]. Atenolol is beta–adrenoceptor blocking agent

used for treatment of high blood pressure and heart-associated chest pain [11]. The oral atenolol dose is rapidly absorbed, distributed, and excreted in the urine and faeces within 24 hours in the form of 2-hydroxyatenolol and atenolol glucuronide as metabolites of the drug [12]. Atenolol has been determined by flow injection analysis [13] spectrophotometry [14, 15], and chromatographic methods [16, 17, 18, 19].

Metoprolol (MTL) is 1-[4-(2-Methoxyethyl) phenoxy]-3-[(1–methylethyl) amino]-2–propanol (C15H25NO3 = 267.4 g/mole). Solid metoprolol is a white crystalline powder with 120°C melting and one dissociation step with pKa 9.7. Metoprolol is very soluble in water, ethanol and chloroform [1, 2]. Metoprolol is beta–adrenoceptor blocking agent also used for treatment of high blood pressure and heart-associated chest pain. The oral metoprolol is absorbed after administration of the oral dose to be distributed across the blood-brain and placental barriers. About 95% of the commission was paid for the pilgrimage within 48 hours and two-thirds of this percentage was excreted as the active metabolite, 4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetic acid, and about 10% as another inactive metabolite, 2-hydroxy-3 [4- (2-methoxyethyl)-phenoxy] propionic acid. While between 1-10% is excreted as, a-hydroxymetoprolol and O-desmethylmetoprolol [20, 21]. Metoprolol has been determined by spectrophotometric [22, 23, 24] and chromatographic methods [25].

Neutral red (NLR) is 3- amino-7-dimethylamino-2-methylphenazine hydrochloride (C15H17N4 288.78 g/mole), it is a tissue dye used to stain fetal tissues and the Golgi apparatus in neurons and granules. It may be used as indicator for the acid-base medium because it appears in red color at pH 6.8 and yellow at pH 8 [26].

In this article neutral red dye is used for determination of the three ant hypertension drugs using one simple procedure based on bleaching reaction of the dye.

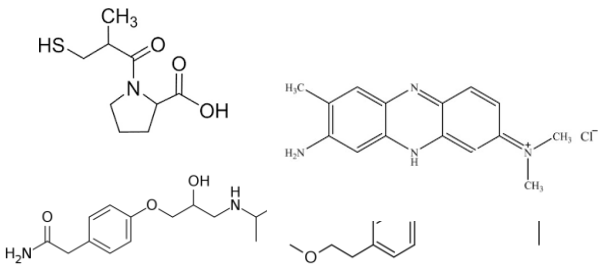


Figure 1 The chemical structure of the drugs and the neutral red dye

2.0 EXPERIMENTAL

2.1 Instruments

Absorption spectra were measured on double-beam Jasco V- 630 spectrophotometer with 1.0 cm matched glass cells, and pH measurements were measured on HANNA 301 pH meter.

2.2 Chemicals

A high percentage of purity of all chemicals and reagents were used.

CPL Solution (50 µg.ml^{-1})

0.0050 g of the pure CAP powder has been dissolved in 15 ml of distilled water with stirring and diluted up to the mark with distilled water in a100 ml volumetric flask.

ATL Solution (50 µg.ml^{-1})

0.0050 g of the pure CAP powder has been dissolved in 15 ml of distilled water with stirring and diluted up to the mark with distilled water in a100 ml volumetric flask.

MTL Solution (50 µg.ml^{-1})

0.0050 g of the pure CAP powder has been dissolved in 15 ml of distilled water with stirring and diluted up to the mark with distilled water in 100 ml volumetric flask.

NLR Solution (100 µg.ml^{-1})

The solution was prepared by dissolving 0.010g of dye in 100 ml of distilled water, completing the volume to the mark in a 100 ml volumetric flask with distilled water and placing the solution in a dark flask.

NBS Solution (177.98 µg.ml^{-1})

The solution was prepared by dissolving 0.01779 g of the pure NBS powder in 100 ml of distilled water, completing the volume to the mark in a 100 ml volumetric flask with distilled water and placing the solution in a dark flask. The solution is daily prepared.

Hydrochloric Acid Solution (1 M)

The solution was prepared by diluting 8.5 ml of concentrated acid (sp.gr. 1.18, 36%) to 100 ml with distilled water.

Solutions of Surfactants (1 x 10^{-3} M)

SDS, CPC and CTAB solutions are prepared dissolving 0.0288, 0.0339, and 0.0364 g respectively in 100 ml of distilled water.

3.0 RESULTS AND DISCUSSION

3.1 Study the Optimal Reaction Conditions for Oxidation and Bleaching Color of Neutral Red Dye NLR

Figure 2 shows the absorption spectrum of NLR which exhibits a maximum at 523 nm. NLR dye has not been

used by other researchers yet. In order to get the suitable amount of the dye, (0.1-2) ml of 100 µg.ml^{-1}of NLR has been mixed with 0.5 ml of 1 M HCl, diluted to make 10 ml and measured. Figure 3 shows the calibration graph of NLR and linear relation from 1 to 16 µg.ml^{-1}of NLR with 0.9987 correlation coefficient (Figure 3). One milliliter has been selected.

3.2 Preliminary Study

First of all, the spectrum of NLR dye must be fixed in acidic medium, the dye NLR (100 µg.ml^{-1}) has been diluted to 10 ml in the calibrated flasks after mixing with 0.5 ml of 1 M HCl and measured versus the blank solution. Figure 2 shows the absorption spectrum of the NLR dye and its maximum absorbance at the wavelength of 523 nm, the calibration curve of the dye at 523 nm has been prepared to detect the best amount (within the linearity range) of the dye that can be used. Figure 3 shows that the absorbance of NLR exhibits linear relation against 1-16 µg.ml^{-1} concentration,10 µg.ml^{-1} has been selected.

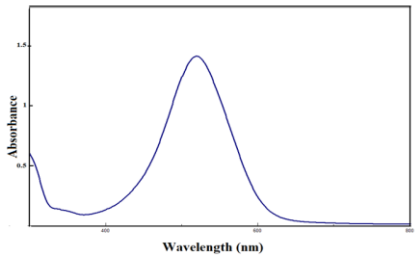


Figure 2 The absorption spectrum of 10 µg.ml^{-1}of NLR

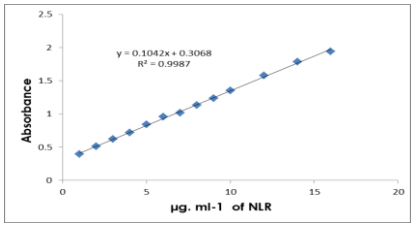


Figure 3 The calibration curve of NLR

3.3 Selection of the Oxidizing Agent

N-Bromosuccenimde (NBS),potassium periodate, chlorosuccenimde (NCS), and sodium hypochlorite has been examined by adding 1 ml of each oxidant at concentration 1x10^{-3} M to the 1 ml of the dye NLR (100 µg.ml^{-1})in the presence of 0.5 ml of 1 M HCl and dilute to make 10 ml in volumetric flasks, and read the absorbances of each solution against blank at 523 nm. Figure 4 show higher oxidation and subsequent bleaching of red-NLR color when NBS and sodium hypochlorite were used, NBS has been selected in post-experiments, the best amount of NBS which give the powerful bleaching effect has been cheeked to be 1.2 ml as shown in Figure 5

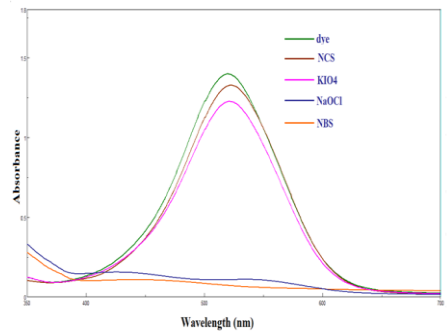


Figure 4 Absorption spectrum of NLR in the presence of different oxidizing agent

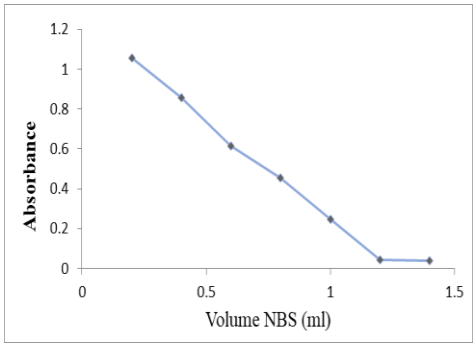


Figure 5 Select the amount of NBS which give the best bleaching effect

3.4 Select the Suitable Acid for the Reaction and Its Suitable Volume of it

Three series of the calibrated flasks contain CPL(1 ml of50 µg.ml^{-1}), MTL (1 ml of50 µg.ml^{-1}), and (0.5 ml of 50 µg.ml^{-1}) of ATL solution, followed by 0.5 ml of 1 M of different acids to the three series and 1.2 ml of NBS oxidant, standing period 15 minutes, then 1 ml of the dye NLR (100µg.ml^{-1}), diluted to 10 ml and measured at the wavelength of 523 nm versus the blank solution. As Table 1 shows, hydrochloric acid gives the best absorbance value, while Table 2 show that 0.5 ml is the best amount of the acid.

Table 1 Effect of acids on reaction

ml of 0.5 1M acid	*Absorbance*			Final pH		
	CPL	*ATL*	*MTL*	*CPL*	*ATL*	*MTL*
HCl	**0.768**	**0.528**	**0.754**	**1.81**	**1.86**	**1.79**
H_2SO_4	0.433	0.1582	0.229	1.89	1.92	1.85
HNO_3	0.518	0.237	0.458	1.98	2.05	2.15
CH_3COOH	0.537	0.131	0.583	2.43	2.85	2.93

Table 2 Select the amount of HCl

ml of 1 M HCl	Absorbance		
	CPL	*ATL*	*MTL*
0.3	0.592	0.422	0.589
0.5	**0.771**	**0.530**	**0.759**
1.0	0.694	0.502	0.711
1.5	0.683	0.491	0.692
2.0	0.674	0.452	0.679
2.5	0.653	0.433	0.650

3.5 The Oxidizing Periods

The above procedure has been applied but with period of time for oxidation of the drugs and period for oxidation of NLR dye, from Table 3 we can conclude that 25 min is required for complete oxidation of CPL, 20 min for ATL, and for MTL, while 5 min is sufficient to oxidize NLR.

Table 3 The oxidizing periods

Standing time before addition NLR (min)	Absorbance / Standing time after addition of NLR and before dilution (min.)						
	0	5	10	15	20	25	30
CPL (5 µg / ml)							
0	0.410	0.442	0.478	0.481	0.484	0.481	0.482
5	0.536	0.541	0.543	0.549	0.551	0.552	0.551
10	0.583	0.586	0.588	0.590	0.587	0.584	0.582
15	0.615	0.644	0.648	0.643	0.649	0.645	0.642
20	0.678	0.684	0.689	0.685	0.681	0.685	0.687
25	0.770	**0.773**	0.771	0.769	0.768	0.770	0.767
30	0.685	0.688	0.681	0.684	0.680	0.679	0.681
ATL (2.5 µg / ml)							
After addition	0.316	0.3551	0.3556	0.3549	0.3551	0.3554	0.3548
5	0.402	0.433	0.438	0.434	0.436	0.432	0.431
10	0.455	0.463	0.459	0.461	0.458	0.460	0.462
15	0.504	0.538	0.535	0.533	0.534	0.531	0.530
20	0.610	**0.621**	0.615	0.618	0.614	0.619	0.613
25	0.563	0.571	0.567	0.570	0.568	0.571	0.569
MTL (5 µg / ml)							
After addition	0.335	0.347	0.350	0.348	0.351	0.347	0.342
5	0.476	0.485	0.489	0.483	0.485	0.485	0.485
10	0.511	0.542	0.545	0.543	0.540	0.537	0.539
15	0.622	0.645	0.648	0.647	0.649	0.651	0.648
20	0.745	**0.762**	0.758	0.756	0.755	0.753	0.754
25	0.678	0.685	0.681	0.683	0.687	0.684	0.680

3.6 Effect of Temperature

The prepared samples have been left at 10, R.T (23°C), and 40°C for many periods of time, the figures below (6) show that R.T is the optimum in all cases.

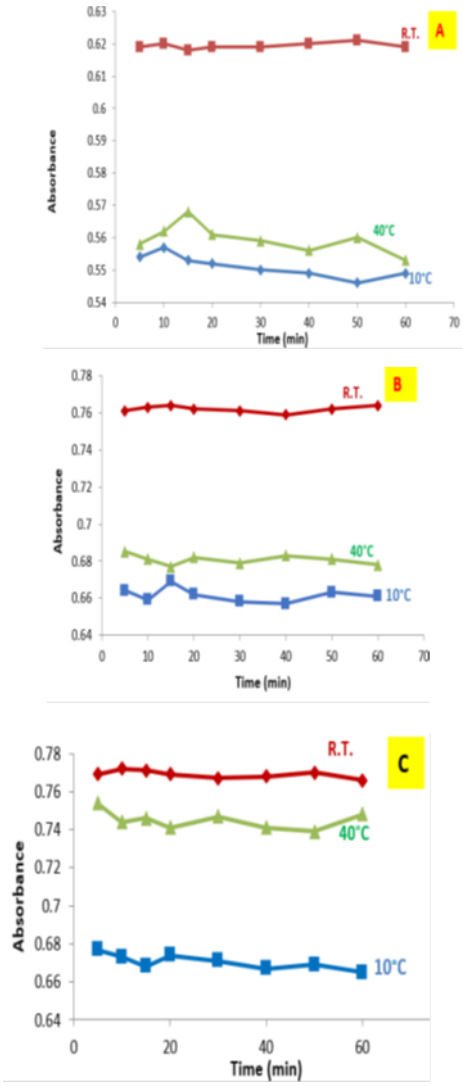


Figure 6 Effect of temperature on reaction process of; A:ATL, B:MTL and C:CPL

3.7 The Sequence of Chemicals

2.5 μg.ml^{-1} of ATL has been followed in this study as the example of other drugs. Table 4 shows the sequence D (drug), A (acid), OX (oxidant), NLR is preferred and it is used in pre-and post-experiments.

Table 4 Sequence of chemicals

Sequence number	Reaction component	Absorbance
I	**D+A+OX+NLR**	**0.629**
II	D+OX+A+NLR	0.613
III	A+OX+D+NLR	0.617
IV	NLR+D+A+OX	0.123
V	NLR+A+OX+D	0.021
VI	OX+A+NLR+D	0.043

Absorption Spectra and Calibration Curves

Under the selected reaction conditions, the absorption spectra of NLR in the presence of the three drugs compounds has been scanned as shown in Figure 7. The criteria for calibration curves have been done for the three drugs by adding increasing concentration of CPL, ATL, and MTL into three series of 10 ml calibrated flask, followed by 0.5 ml of 1 M HCl, 1.2 ml NBS(1x10^{-1}M), Shake and left for 25min (CPL)and 20 min (ATL,MTL), then 1 ml of NLR (100 μg/ml), 5 min. standing period at R.T. (23°C), dilution to the mark, and measure at 523 nm. Figure 8 show the linearity ranges were from one to ten μg/ml of the three CPL, ATL, and MTL.

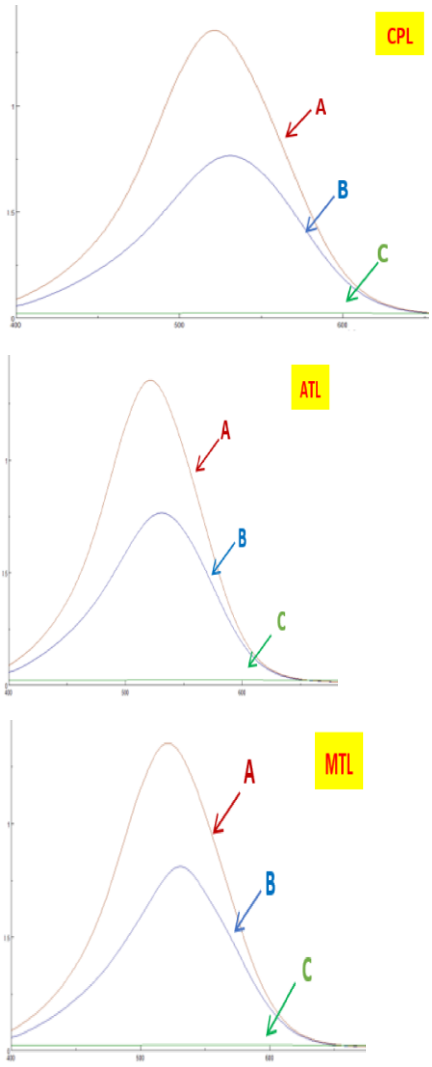


Figure 7 The absorption spectra of NLR in the presence of the three drugs compounds

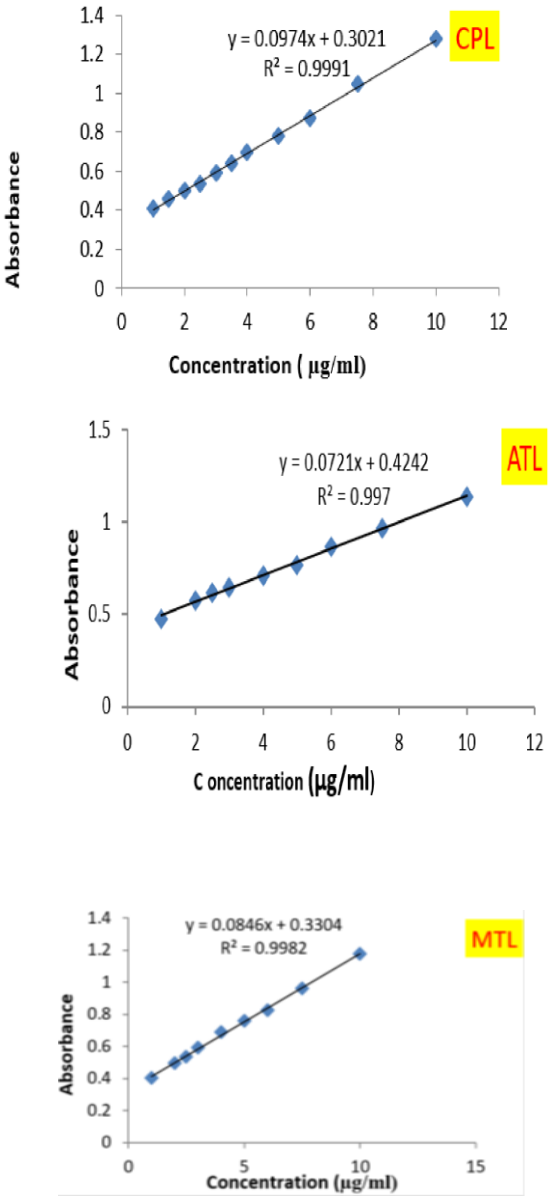


Figure 8 Calibration curves for estimation of CPL, ATL, and MTL

LOD and LOQ Evaluation

The calculated values of limit of determination and quantitation has been derived by the equation of σ_B for ten repletion of the blank preparation, where σ_B is:

$\sigma_B = \sqrt{\frac{\sum Xi-\bar{X})^2}{n-1}}$ Where Xi is the blank readings, $\bar{X}$ is the mean of readings, n is the number of measurements, while LOD and LOQ can be estimated by: LOD $= \frac{3\,\sigma}{slope}$, LOQ $= \frac{10\,\sigma}{slope}$.

Table 5 shows the summary of the measured, selected and calculated data.

Table 5 the summary of selected conditions of the reaction with the values of the calibration curves

Parameters	Value / Drug		
	CPL	*ATL*	*MTL tartrate*
λ max	523	523	523
Oxidation period (min.)	25	20	20
Linearity range (µg/ml)	1-10	1-10	1-10
slope	0.0974	0.0721	0.0846
intercept	0.3021	0.4242	0.3304
Coefficient of determination	0.9991	0.997	0.9982
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	21.164×10^4	1.9202×10^4	57.934×10^4
Sandell's sensitivity index ($\mu g/cm^2$)	0.0102	0.0138	0.01182
LOD*	0.00405	0.00547	0.00466
LOQ*	0.0135	0.0182	0.0153

*Average of ten replications of blank preparations.

Accuracy and Precision of the Calibration Curve

Accuracy and precision of the calibration curve has been estimated by replication of three graduate concentrations within the calibration (2.5, 5, and 7.5). Table 6 shows good precision between readings (ranged from 0.134 to 0.778 %) and good accuracy in compared with standards (ranged from -1.55 to +0.7%).

Table 6 Precision and accuracy of the standard curve of three drugs

Drug	Amount taken (µg/ml)	Amount found (µg/ml)	Recovery % *	Relative standard deviation % *	Relative error % *
CPL	2.5	2.48	99.25	0.1886	- 0.75
	5	4.94	98.85	0.258	- 1.15
	7.5	7.43	98.53	0.778	- 1.46
ATL	2.5	2.46	99.37	0.134	- 0.63
	5	4.96	99.14	0.151	- 0.86
	7.5	7.45	98.48	0.73	- 1.52
MTL tartrate	2.5	2.46	98.45	0.476	-1.55
	5	5.03	100.7	0.589	0.7
	7.5	7.48	99.7	0.47	-0.24

* Average of three determinations

Chemical Reactions

The reaction steps can be summarized by formation of disulfide product of CPL as in scheme -1 and amide product of ATL as in scheme -2, MTL undergo same ATL reaction.

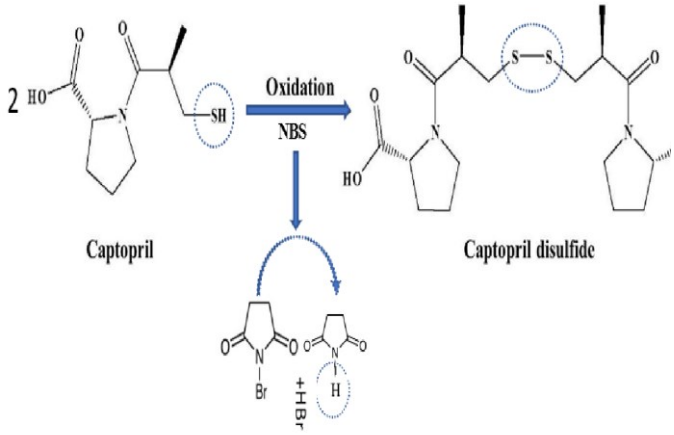


Figure 9 Oxidation of CPL

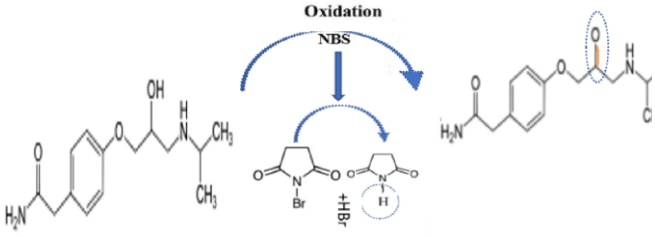


Figure 10 Oxidation of ATL

Determination of the Three Drugs Compounds in the Pharmaceutical Preparations

Pharmaceutical Solutions, (50 µg.ml^{-1})

CPL pharmaceutical solutions (Aceprotin 50 mg manufacturer medochemieltd limassol, Cyprus): Five tablets (Aceprotin 50 mg/tablet were weighed separately from the various original companies, were pestled and mixed well, taken as equivalent to 0.005 g and dissolved in distilled water and completing the volume up to 50 ml of distilled water, followed by filtration and washing the filter paper several times to get rid of suspended matter. The volume was completed in a 100 ml volumetric flask with distilled water and the solution was obtained at a concentration of 50 µg.ml^{-1} for each pharmaceutical product separately, then it was diluted by taking volumes of the aforementioned solutions to prepare three different concentrations according to the selected reaction conditions.

ATL pharmaceutical solutions (vascoten 100 mg/tablets (manufacturer medochemie, Cyprus, Novaten 100 mg/tablets (manufacturer ajanta pharma limited) and Tenormin 50 mg/tablets (manufacturer Astra Zeneca) were weighed separately from the various original companies, were pestle and mixed well, taken as equivalent to 0.005 g and dissolved in distilled water and completing the volume up to 50 ml of distilled water, followed by filtration and washing the filter paper several times to get rid of suspended matter. The volume was completed in a 100 ml volumetric flask with distilled water and the solution was obtained at a concentration of 50 µg.ml^{-1} for each pharmaceutical product separately, then it was diluted by taking volumes of the aforementioned solutions to prepare three different concentrations according to the reaction conditions.

MTL pharmaceutical solution) Metoprolol tartrate 50 mg/tablets (manufacturer Accord Healthcare Limited), Metoprolol tartrate 50 mg/tablets (manufacturer Bristol laboratories Ltd). were weighed separately from the various original companies, were pestle and mixed well, taken as equivalent to 0.005 g and dissolved in distilled water and completing the volume up to 50 ml of distilled water, followed by filtration and washing the filter paper several times to get rid of suspended matter. The volume was completed in a 100 ml volumetric flask with distilled water and the solution was obtained at a concentration of 50 µg.ml^{-1} for each pharmaceutical product separately, then it was diluted by taking volumes of the mentioned solutions to prepare three different concentrations according to the reaction conditions.

Table 7 show high recovery of the determination of the three drugs ranged from 97.7 to 101.3%.

Table 7 Determination of the three drugs compounds in the pharmaceutical preparations

Drugs	Pharmaceutical preparation	Certified value	Amount taken (µg/ 10 ml)	Recovery * (%)
CPL	Aceprotin tablet (cyprus)	50 mg	2.5	98.3
			5	97.9
			7.5	101.3
ATL	Vascoten tablet (cyprus)	100 mg	2.5	99.6
			5	100.4
			7.5	98.9
	Novaten tablet (Ajanta)	100 mg	2.5	99.8
			5	100.08
			7.5	98.8
	Tenormin tablet (Astra Zenca)	50 mg	2.5	98
			5	98.5
			7.5	101
MTL tartarat	Metoprolol tartrate (health care)	50 mg	2.5	98.1
			5	99.6
			7.5	97.7
	Metoprolol tartrate) Bristol)	50 mg	2.5	98.5
			5	100.7
			7.5	99.6

*average of four replications

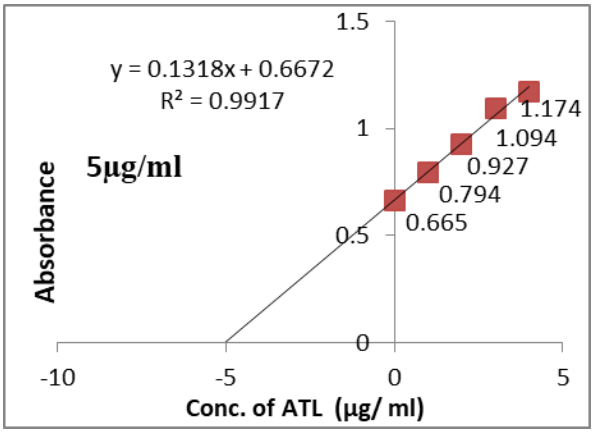
Statistical Study

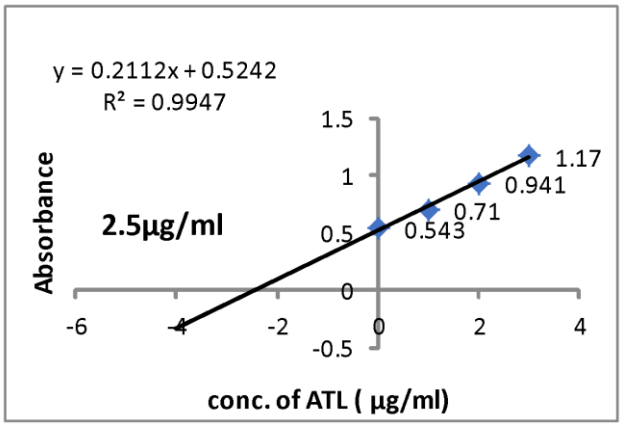
The present procedure for determination of three drugs in tablets has been applied and compared with the standard method approved in the British pharmacopeia (potentiometric titration with perchloric acid) [28]. The confidence of the method has been derived using t-test ([29] calculations of replications as shown in Table 8 which exhibits good trustability of the method. Figure 11 and Table 9 show the standard addition-test of the three drugs at two concentrations which indicate high ability to determine drug compounds in pharmaceutical preparations without interfering by the ingredients.

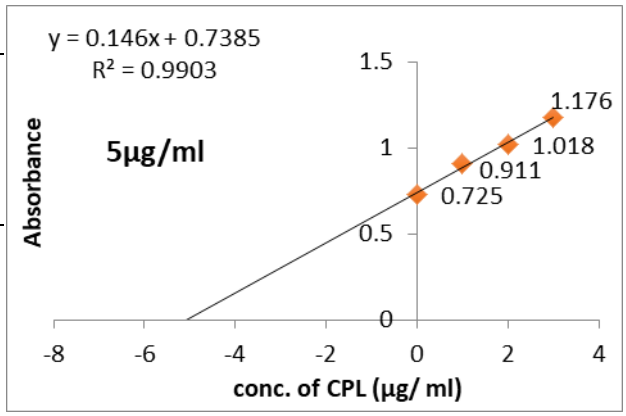
Table 8 The confidence test of the method

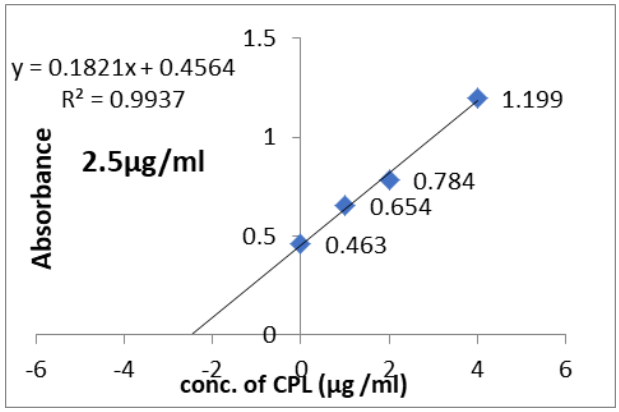
Drug	Pharmaceutical preparation	Recovery * %		t-exp
		Present method	British pharmacopeia method	
ATL	Aceprotin tablet 50 mg(cyprus)	99.2	98.3	0.685
	Tenormin tablet 50 mg(Astra Zenca)	99.63	97.5	1.272
MTL tartrate	Metoprolol tartrate tablet 50 mg (Bristol)	99.6	98.12	0.967

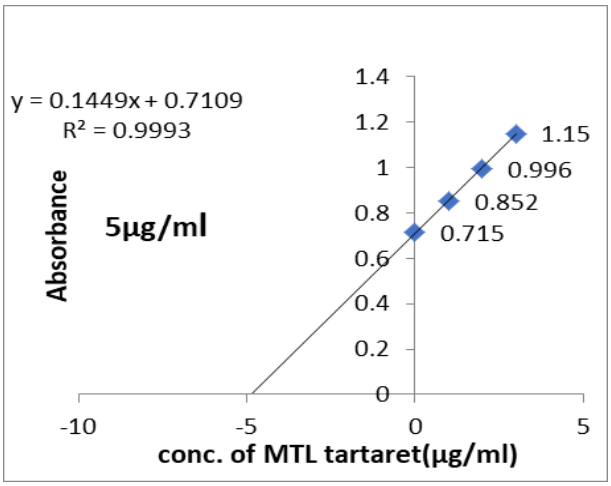
* average of three replications of each method











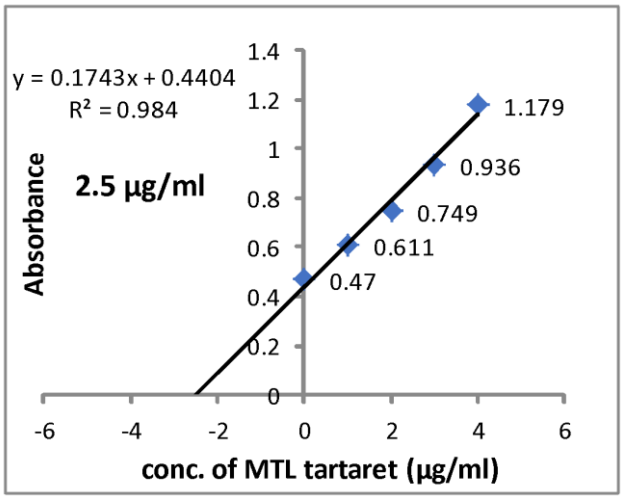


Figure 11 Calibration curves of the three drugs via standard addition methods

Table 9 Calculations of standard addition method

Drug	Pharmaceutical preparation	Amount Present (µg/10 ml)	Amount measured (µg/10 ml)	Recovery(%)
Captopril	Aceprotin tablet 50 mg(cyprus)	2.5	2.506	100.1
		5	5.05	101.16
Atenolol	Tenormin tablet 50 mg(Astra Zenca)	2.5	2.48	99.28
		5	5.06	101.2
Metoprolol tartrate	Metoprolol tartare tablet 50 mg (Bristol)	2.5	2.52	100.8
		5	4.9	98.1

4.0 CONCLUSION

One simple procedure has been used for the determination of three ant hypertension drugs, captopril, atenolol, and Metoprolol. The antihypertensive drugs have been estimated via oxidation by NBS in the first reaction step followed by oxidation of NLR dye by the exceed amount of NBS, as the concentration of the drug compounds increase, the color of the dye increase. The linearity range was from 1 to 10 for the three drugs with excellent precision ranged from 0.134 to 0.778 % and high accuracy ranged from -1.55 to +0.7 %, the requirements of the reaction are very simple, smooth principal technique, no heat, no need to use buffers, the three drug have been determined in their dosage forms with recovery ranged from 97.7 to 101.3%.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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