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A new spectrophotometric method to estimate atenolol, amlodipine, and furosemide in pharmaceutical dosages

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Atenolol, amlodipine, and furosemide are the most important medications that have been used to treat cardiovascular diseases. Due to this fact, we suggested a new method to estimate these three drugs in their pure form and their pharmaceutical dosages. The suggested spectrophotometric method was based on redox reaction of the mentioned drugs at the selected wavelength 516 nm of the resulting complex from the reaction of these three drugs in step I with cerium (IV) ions as an oxidizing agent in acidic medium, step II which is occurred between unreacted cerium (IV) with the reagent safranin. The linearity of this method was obeyed Beer's law from (0.8-34), (1.6-40), and (2-40) μg.ml^{-1} of atenolol, amlodipine, and furosemide respectively, with molar absorptivity (2.66×10^4, 2.15×10^4, and 1.57×10^4) L.mol^{-1}.cm^{-1} of atenolol, amlodipine, and furosemide, respectively. The limit of detection (LOD) is 0.915, 1.098, and 1.372 μg.ml^{-1} for atenolol, amlodipine, and furosemide, respectively. The limit of quantification (LOQ) is 3.05, 3.66, and 4.57 μg.ml^{-1} for atenolol, amlodipine, and furosemide, respectively. The method reveals a good accuracy and precision and it was applied successfully to the determination of these three drugs in pharmaceutical preparations. No interferences were observed from common additives found in pharmaceutical preparations. The Reddish-wine color of the product was highly stable and did not show a significant change in absorbance up to 60 min.

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KEYWORDS

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Introduction

Patients with cardiovascular disease are commonly managed based on their medical conditions by clinicians by prescribing a cocktail of medications. Atenolol, amlodipine, and furosemide on their chemical structures depicted in Figure 1 are mostly involved as components of these cocktails [1]. These drugs have been used to treat cardiovascular diseases such as angina and hypertension. The first medication is atenolol, chemically named as (4-(2-hydroxy-3-isopropylaminopropoxy) phenyl-cetamide); it is commercially known as Tenormin. The second medication is amlodipine, or 2-[(2-aminoethoxy) methyl] -4-(2-chlorophenyl), which is trade named as Norvasc. Finally, furosemide or (4 chloro-N- [2-furyl methyl] -5-sulfamyl-anthranilic acid), the commercially name of furosemide is known as Lasix [1].

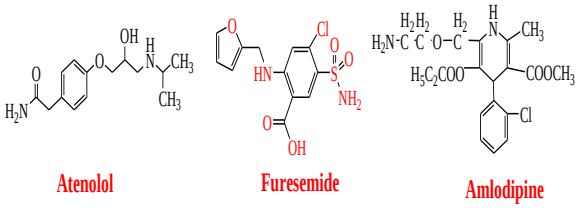


FIGURE 1 Chemical structures of the studied three drugs

Many methods depending on various techniques were fixed in literature for estimating these three drugs, such as kinetic spectrophotometric method for estimating atenolol in dosage forms [2-7], a complex formation between atenolol and chloranilic acid depending on charge transfer reaction [8,9], or using sodium nitroprusside [10]. The other spectrophotometric method was based on dual wavelengths [11]. Flow-injection chemiluminescence was also used for estimated atenolol [12]. Furthermore, new techniques were utilized based on electrical method to assay atenolol [13,14]. Finally, chromatographic technique was used to determine atenolol in a dosage form [15].

Estimation of amlodipine in human plasma by HPLC, Liquid chromatography-mass, RP-HPLC, LC, and HPTLC method has been reported [7,11,16-24]. In addition, spectrophotometry technique have been used for estimating amlodipine via binary complex formation with eosin [25], or oxidation with cerium (IV) [26], charge-transfer complex formation with p-chloranilic acid [27], and 2, 3-dichloro 5,6-dicyano 1,4-benzoquinone and ascorbic acid [28]. The other methods were used for the determination of amlodipine in pure forms and tablet [29,30]. Furthermore, chromatographic methods have been reported to estimate amlodipine such as UHPLC method [31], HPLC [32,33], LC method [34], HPLC/MS/MS [35], HPTLC method [36], and RP-HPLC method [37].

Spectrophotometric method was studied for determination of furosemide depending on the use of charge transfer complex reaction [38], first order derivative [39], or the use of principal component regression [40].

Safranin is the reagent used to determine atenolol, amlodipine, and furosemide medications, as displayed in Figure 2. Safranin is a basic biological dye commonly used as a counter-stain in some staining protocols like gram staining. It is a crystalline solid with a characteristic green metallic luster and easily soluble in water [41].

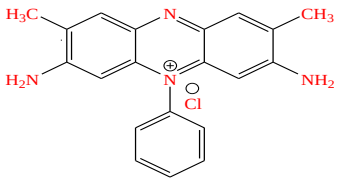


FIGURE 2 Chemical backbone of safranin reagent

In this paper, an indirect visible method was suggested to determine the studied drugs in pure and dosages form. These drugs were oxidized with cerium (IV) which was proportional inversely with safranin reagent, so that, the addition of an excess amount of cerium (IV) to the atenolol, amlodipine, and furosemide solutions in the presence of acidic medium, followed by the reaction of residual amount of cerium (IV) safranin reagent reduces the absorption of safranin dye which was measured at 516 nm.

Experimental

Apparatuses: All spectrophotometric measurements were recorded in JASCOV-360 digital spectrophotometer equipped with 1-cm glass cells. Gilson micropipette with disposable tips was used to add samples, and A HANNA pH211 pH meter was employed to monitor the pH, and a BEL-sensitive balance was also employed to perform the appropriate weighing procedures.

Preparation of chemicals

Chemical solutions of a high degree of purity were used, atenolol, amlodipine, and

furosemide drugs solutions have been prepared at 1000 µg/mL (SDI). When 0.1 g of these three drugs were dissolved in distilled water using a 100 mL volumetric flask, a simple dilution process of this solution was used to prepare working solution at 100 µg/ml, and then 0.002% of safranin reagent solution (BDH) was prepared when 0.002 g of safranin was dissolved in 100 ml distilled water. Oxidizing agent solution was prepared when 0.1353 g of ammonium cerric sulphate (Fluka) in 3 mL of concentrated sulphuric acid, and then the volume to the mark was completed with distilled water by using a 100 ml volumetric flask. Appropriate dilution of concentrated sulphuric acid with distilled water was used in a 100 mL volumetric flask to prepare 2N sulphuric acid solution. Finally, the preparation of dosage form [(Vascoten tablet) Edochemi LTD-Cyprus, Amloneer 5mg Tablet/Pioneer Co., Iraq, and furosemide 40mg/tab] was carried out by weighing ten tablets of each drug, and then it was grinded into a fine powder. After dissolving and filtering through filter paper, the solution was diluted to obtain 100 µg/mL as a concentration suitable for analysis [43].

Discussion of the results

To study the optimal conditions for the oxidation-reduction reaction between atenolol, amlodipine, and furosemide with an oxidizing agent and reagent, a 100 µg /25 mlof the mentioned three drugs have been used. The optimum condition of this reaction was studied and selected as follows:

Sulphuric acid

Sulphuric acid was selected after studying several types of acids, (acetic acid, hydrochloric acid, and sulphuric acid), which were prepared at a concentration of 2N, various amount (0.1-5.0) mL of sulphuric acid was studied, as depicted in Figure 3.

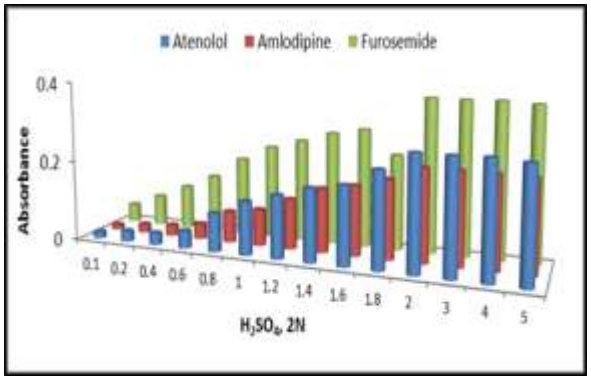


FIGURE 3 Effect of H_2SO_4 amount

The practical results indicated in Figure 3, revealed that 2 mL of 2N sulphuric acid was adopted, depending on the highest absorbance value.

Oxidizing agent and time of oxidation process

Via the optimum amount of Ce(IV) ions prepared at 300 µg/ml, the optimum amount of Ce(IV) was estimated by taking a different quantity (1-4) mL of Ce(IV) solution in volumetric flasks of 25 mL containing different quantities of the studied drugs (50-700) µg, and then 2 mL of sulphuric acid was added at a concentration of 2N. After that, this mixture was left for 20 minutes (as an optimum time for completing oxidation process, Figure 4), 0.002% of safranin reagent was added, the experimental results proved that 1.5 mL of the oxidizing agent Ce (IV) gave the best value for absorption and correlation coefficient (0.9997), therefore 1.5 ml of Ce (IV) was selected for the subsequent experiments [6].

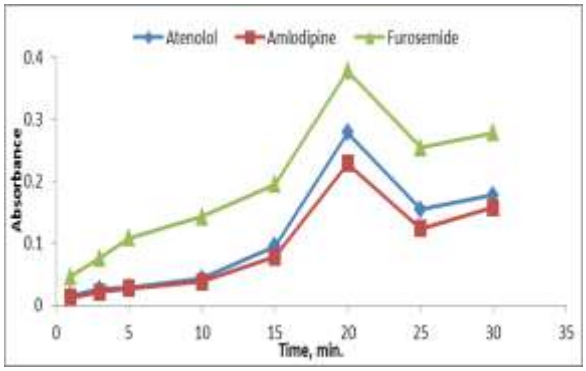


FIGURE 4 Time for oxidation process

Optimum quantity of safranin

To determine the optimum amount of safranin used, Volumetric flasks of 25 mL were used containing different quantities ranging from (50-700) µg of each drug, and then sulfuric acid and the Ce(IV) ions were added in their optimal quantities, followed by the addition of various volumes (1-3) mL of 0.002 % safranin reagent, the measurements of the absorption intensity were carried out at the selected wavelength 516 nm after waiting 20 min to complete the oxidation step. The experimental results revealed that 2 ml of safranin reagent gave the best value for absorbance and correlation coefficient (0.9993). Therefore, 2 mL of 0.002 % safranin reagent was selected for the subsequent experiments [41].

Surfactants and addition sequence

The effect of adding different types of surfactants (positive, negative, and neutral) was studied with changing the sequence of addition, where the experimental results showed that the addition of all kinds of surfactants did not clear any effect on the intensity of absorption of the resulted visible complex, as represented in Table 1. Therefore, this study was neglected for the subsequent experiments [42].

TABLE 1 The effect of surfactants with changing reaction component sequences

No.	Sequences	Absorbance									
		S=CPC,(ml)			S=SDS,(ml)			S=Triton X-100, (ml)			With out surfactant
		2	4	6	2	4	6	2	4	6	
		Atenolol									
I	At+H+O+R+S	0.245	0.242	0.241	0.221	0.209	0.188	0.255	0.249	0.231	0.273
II	At+O+H+S+R	0.205	0.145	0.110	0.195	0.187	0.145	0.214	0.203	0.201	
III	At+R+S+H+O	0.105	0.115	0.106	0.118	0.111	0.102	0.189	0.174	0.172	
IV	At+S+R+O+H	0.112	0.101	0.091	0.106	0.089	0.068	0.197	0.181	0.173	
		Amlodipine									
I	Am+H+O+R+S	0.182	0.165	0.161	0.112	0.109	0.101	0.190	0.183	0.178	0.221
II	Am+O+H+S+R	0.197	0.191	0.169	0.082	0.072	0.077	0.197	0.191	0.184	
III	Am+R+S+H+O	0.166	0.162	0.161	0.113	0.098	0.083	0.176	0.173	0.165	
IV	Am+S+R+O+H	0.172	0.172	0.164	0.108	0.104	0.101	0.179	0.170	0.170	
		Furosemide									
I	Fu+H+O+R+S	0.289	0.278	0.271	0.223	0.212	0.203	0.292	0.291	0.292	0.367
II	Fu+O+H+S+R	0.247	0.238	0.231	0.239	0.217	0.211	0.297	0.283	0.278	
III	Fu+R+S+H+O	0.211	0.209	0.200	0.203	0.189	0.169	0.272	0.267	0.235	
IV	Fu+S+R+O+H	0.168	0.165	0.162	0.194	0.183	0.177	0.264	0.251	0.249	

At=Atenolol, Am=Amlodipine, Fu=Furosemide, H=Acid, O=Oxidant, R=Reagent, and S=Surfactant.

Stabilization of the resulting complex

The effect of time on the intensity and stability of the resulted complex was studied for atenolol, amlodipine, and furosemide at 100 µg.ml^{-1} and 516 nm under the optimal conditions and for different periods of time at room temperature, as displayed in Figure 5, which indicates that the resulting colored complex is stable after 5 minutes and remained stable up to 2 hours.

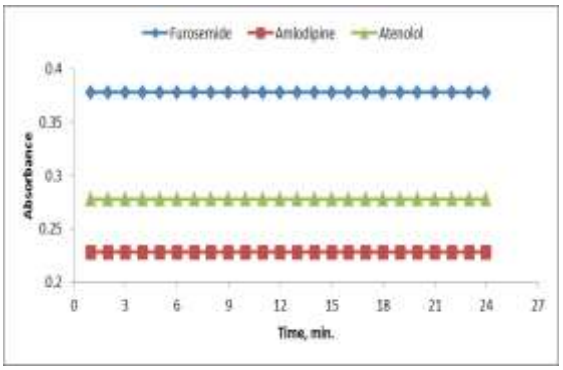


FIGURE 5 Stability of the colored complex

Calibration curve and final spectrum

The present method was studied to determine the mentioned drugs under optimal conditions. Different quantities (25-1000) µg of these drugs were added to a series of 25 mL volumetric flasks, and then sulphuric acid, oxidizing agent, and reagent were added in their optimal quantities. After the dilution of all the flasks to the mark with distilled water, the measurements were carried out as shown in Figure 7 [10,30].

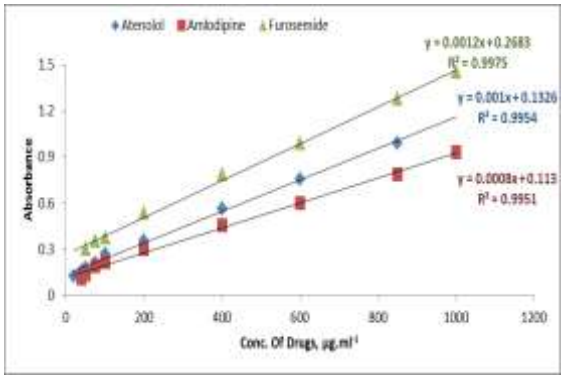


FIGURE 6 Calibration curve atenolol, amlodipine, and furosemide

The proposed method follows Beer's law within at (20-850), (40-1000), and (50-1000) µg/25 mL of atenolol, amlodipine, and furosemide, respectively, as reveled in Figure 6, while Table 2 shows the values of the analytical variables recorded at the selected wavelength for the present work.

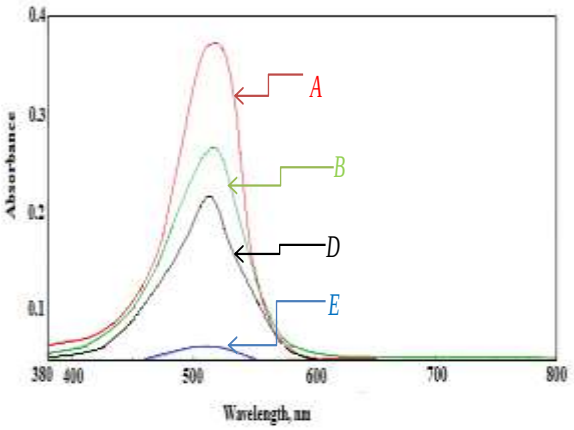


FIGURE 7 Final spectrum for 100 µg of A= furesemide, B= atenolol, D= amlodipine measured against reagent blank, and E=Blank measured against distilled water

TABLE 2 Analytical variables for the present method

analytical variables	values
Beer's law, µg.ml^{-1}	Atenolol = 0.8-34 Amlodipine = 1.6-40 Furosemide = 2-40
ε, l.mol^{-1}.cm^{-1}	Atenolol = 2.66×10^4 Amlodipine = 2.15×10^4 Furosemide = 1.57×10^4
Sandell's index, µg.cm^{-2}	Atenolol =0.01 Amlodipine = 0.019 Furosemide = 0.021

Accuracy and precision measurements

The different concentrations of atenolol, amlodipine, and furosemide were studied for the purpose of verifying the accuracy and compatibility of the current method, as listed in Table 3 which indicates that the present method has a good accuracy and compatibility [42].

TABLE 3 Accuracy and precision

Drugs	Amount of drugs taken, µg/25ml	RSD*, %	Recovery*, %
Atenolol	100	0.473	99.98
	300	0.371	99.94
Amlodipine	100	0.323	98.96
	300	0.270	98.97
Furosemide	100	0.327	100.09
	300	0.279	100.13

*Average of 5 determinations

Continuous variations method

The reaction ratio of the studied drugs to the oxidizing agent and the oxidizing agent to the reagent ratio were studied by using the Job's method (continuous variables method), as shown in Figure 8 [12,38].

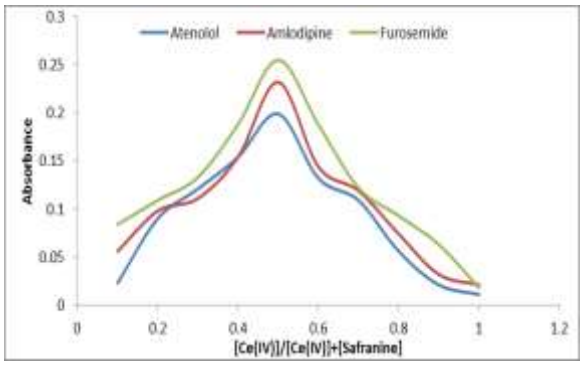
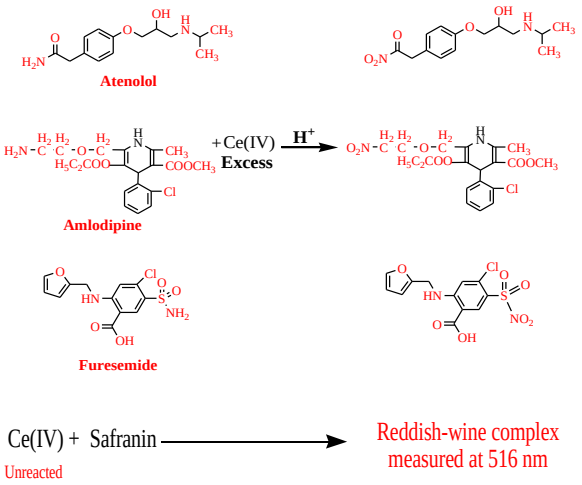


FIGURE 8 Reaction ratio of tenolol, amlodipine, and furosemide with Ce(IV)

The results in Figure 5 indicate the reaction ratio between the cerium ions quaternary and the oxidizing agent is 1:1 depending on this fact the suggested equations, as shown below:



Medicines additives

Several chemical additives have been added to drugs in specific proportions to improve the smell, taste, or appearance of the pharmaceuticals, such as (gum acacia, starch, lactose, and gelatin). The effect of these substances on the proposed method was studied, as represented in Table 4, which indicated that the studied foreign substances do not interfere with the current method [9,40].

TABLE 4 Study effect of Interferences chemicals (gum acacia, starch, lactose, and gelatin) on atenolol, amlodipine, and furosemide

Drugs	Recovery(%) of 100 µg drugs / µg of added interference											
	Acacia			Starch			Lactose			Gelatin		
	100	500	1000	100	500	1000	100	500	1000	100	500	1000
Atenolol	99.98	99.91	99.96	99.98	99.99	99.90	99.89	99.91	99.95	99.86	99.88	99.84
Amlodipine	100.01	100.21	100.09	100.08	100.11	100.16	100.09	100.04	100.12	100.13	100.08	100.05
Furosemide	99.92	99.99	99.91	99.98	99.98	99.95	99.93	99.95	99.92	99.87	99.83	99.81

Application of the current method

The present work was applied for estimating the studied drugs (atenolol, amlodipine, and furosemide) in their pharmaceuticals, as indicated in Table 5.

TABLE 5 Application of the present method

Amount of Drugs, µg	Recovery(%) of Drugs*		
	Vascoten 100 mg Tablet	Amloneer 5mg Tablet	Furosemide 40mg Tablet
100	99.84	100.13	99.83
300	99.76	100.07	99.92

The results shown in Table 5 reveal that the proposed method has good recoveries for the mentioned drugs. The t-test calculations of the present method when it compared with the literature methods indicate that t-test did not exceed the theoretical values at the 95% confidence level for five degrees of freedom, as represented in Table 6 [42].

TABLE 6 The results of t-test analysis

Drug	t-test	Tabulated t-test
Vascoten 100 mg Tablet	1.702	2.571
Amloneer 5mg Tablet	1.522	
Furosemide 40 mg Tablet	1.621	

Comparison of the method:

The current method has been compared with literature methods to determine atenolol, amlodipine, and furosemide, as depicted in Table 7 [38,42,43].

TABLE 7 Comparison of the present method with the literature

Analytical parameters	Present method	Literature method [42]	Literature method [43]	Literature method [38]
Reaction	Oxidation-reduction	Oxidation-reduction	Charge-transfer reaction	Charge-transfer reaction
λ_{max} (nm)	516	540	745	450
Reagent	Safranin	Meta-cresol purple	7,7,8,8-tetracyanoquinodimethane	(2,3 –dichloro-5,6-dicylano-1, 4-benzoquinone
Beer's law, µg.ml^{-1}	Atenolol = 0.8-34 Amlodipine = 1.6-40 Furosemide = 2-40	1-20	20–110	20-160
Molar absorptivity, l.mol^{-1}.cm^{-1}	Atenolol = 2.66×10^4 Amlodipine = 2.15×10^4 Furosemide = 1.57×10^4	1.20×10^4	2.73×10^3	2.0847
Color	Reddish-wine	Red	Blue	Reddish pink
Sandell's index, µg.cm^{-2}	Atenolol =0.01 Amlodipine = 0.019 Furosemide = 0.021	----	0.14	0.00208
R.S.D. (%)	0.270-0.473	≤3.42%	0.94	0.3240
Application of the method	Pharmaceutical dosages	Pharmaceutical preparation	Pharmaceutical preparation	Pharmaceutical preparation

Table 7 indicates that the present method was sensitive and can be successfully applied for determining atenolol, amlodipine, and furosemide in their pharmaceuticals.

Conclusion

In this work, the most important drugs which were used to treat cardiovascular diseases have been studied by using a visible method, This method was depend on oxidation reduction reaction between atenolol, amlodipine, and furosemide and ceric (IV) ions in the presence of sulfuric acid and safranin dye. The reduced color of safranin was proportional to the concentration of the oxidizing agent and to the concentration of these three drugs, and thus has been measured at 516 nm. This method was applied in a very successful way to estimate atenolol, amlodipine, and furosemide in pure and pharmaceutical dosages forms.

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