Research Article

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The Journal of Biological and Chemical Luminescence

Received: 1 September 2008, Revised: 15 January 2009, Accepted: 19 January 2009, Published online in Wiley Interscience: 20 May 2009

(www.interscience.wiley.com) DOI 10.1002/bio.1129

Determination of enalapril maleate and atenolol in their pharmaceutical products and in biological fluids by flow-injection chemiluminescence

Nawal A. Al-arfaj, Eman A. Al-Abdulkareem* and Fatma A. Aly

ABSTRACT: A chemiluminescent method using flow injection (FI) was investigated for rapid and sensitive determination of enalapril maleate and atenolol, which are used in the treatment of hypertension. The method is based on the sensitizing effect of these drugs on the Ce(IV)–sulfite reaction. The different experimental parameters affecting the chemiluminescence (CL) intensity were carefully studied and incorporated into the procedure. The method permitted the determination of 0.01–3.0 μg mL^{-1} of enalapril maleate in bulk form with correlation coefficient $r = 0.99993$, lower limit of detection (LOD) 0.0025 μg mL^{-1} (S/N = 2) and lower limit of quantitation (LOQ) 0.01 μg mL^{-1}. The linearity range of atenolol in bulk form was 0.01–2.0 μg mL^{-1} ($r = 0.99989$) with LOD of 0.0003 μg mL^{-1} (S/N = 2) and LOQ of 0.01 μg mL^{-1}. In biological fluids the linearity range of enalapril maleate was 0.1–2.0 μg mL^{-1} in both urine and serum, and for atenolol the linearity range was 0.1–1.0 μg mL^{-1} in both urine and serum. The method was also applied to the determination of the drugs in their pharmaceutical preparations. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: enalapril maleate; atenolol; chemiluminescence; Ce(IV)–sulfite reaction; pharmaceuticals; biological fluids

Introduction

Enalapril maleate (*N*-{*N*-[(*S*)-1-ethoxycarbonyl-3-phenylpropyl] L-alanyl}-proline hydrogen maleate) is an active angiotensin con-verting enzyme (ACE) inhibitor that has been shown to be effective in the treatment of hypertension and congestive heart failure by dilating peripheral vascular resistance without causing significant changes in heart rate or cardiac output.[1] Enalapril acts as a prodrug of the diacid enalaprilat, its active form, which is poorly absorbed by mouth. Following oral administration about 60% of a dose of enalapril is absorbed from the gastrointestinal tract and peak plasma concentrations are achieved within about 1 h. Enalapril is extensively hydrolysed in the liver to enalaprilat; peak plasma concentrations of enalaprilat are achieved 3–4 h after an oral dose of enalapril. The half-life is about 11 h.[2] Atenolol (2-[4(2-hydroxy-3-izopropylaminopropoxy)phenyl] acetamide) is a beta-blocker, which constitute one of the most frequently prescribed groups of cardiovascular drugs. They are competitive antagonists at β-adrenergic receptor sites and are used in the management of cardiovascular disorders, such as hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. Atenolol is incompletely absorbed from the gastrointestinal tract; following oral administration about 50% is absorbed. Peak plasma concentrations are reached in 2–4 h. The half-life is about 6–7 h. Atenolol undergoes little or no hepatic metabolism and is excreted mainly in the urine.[2] The methods of analysis of the bulk drugs are officially in the *British Pharmacopoeia* a potentiometric titration method for both drugs.[3]

Several methods have been reported for the analysis of atenolol, including UV spectrophotometry,[4,5] colorimetry,[6] fluorimetry,[7] ^{1}HNMR,[8] IR,[9] voltammetry,[10,11] potentiometry,[12] TLC,[13,14] HPTLC/UV detection,[15,16] GC/MS,[17] HPGC/MS,[18] HPLC/UV detection,[19,20] HPLC–tandem mass spectrometry (HPLC/MS/MS),[21,22] ultraperformance liquid chromatography with tandem mass spectrometric (UPLC/MS/MS),[23] HPLC–fluorescence detection,[24] capillary liquid chromatography (CLC) and pressurized capillary electrochromatography (pCEC),[25] CZE,[26,27] miceller electrokinetic chromatography (MEKC)[28] and FIA-UV detection.[29] Reviewing the literature revealed that up to the present time, nothing has been published concerning the chemiluminescence determination of atenolol.

Enalapril maleate has been assayed in several methods in pure form, in pharmaceutical preparations, in biological fluids or in mixture with other drugs. These include spectrophotomety,[30–32] flow-injection spectrophotomety,[33] fluorimetry,[34] ^{1}HNMR,[35] IR,[36] amperometry,[37,38] potentiometry,[39,40] polarography,[30] TLC,[41] GC,[42] GC/MS,[43] HPLC/UV detection,[44,45] HPLC/MS,[46] HPLC/MS/MS,[47] CZE,[48] MEKC[49] and a flow-injection chemiluminescence method,[50] which is based on the CL reaction of the drug with tris(2,2′-bipyridyl)ruthenium(II), Ru(bipy)$_3^{2+}$ and acidic potassium permanganate. Calibration graphs were obtained over concentration ranges of 0.005–0.2 and 0.7–100 μg mL^{-1} with a detection limit (S/N = 2) of 0.001 μg mL^{-1}.[50] The attractiveness of tha proposed

* Correspondence to: E. A. Al-Abdulkareen, Department of Chemistry, College of Science, Women Student-Medical Studies and Science Sections, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia. E-mail: Ksu_chem@yahoo.com

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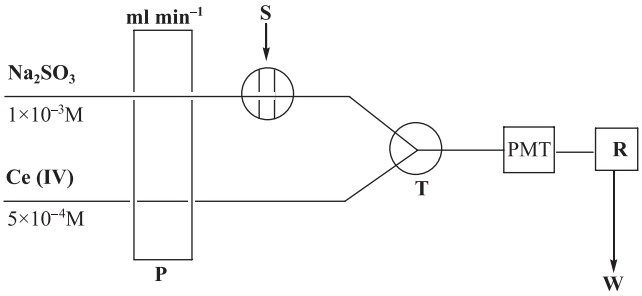

Figure 1. FI manifold for the chemiluminescence determination of the studied drugs: P, peristaltic pump; S, sample port; T, Perspex T-piece; PMT, photomultiplier tube; R, recorder; W, waste.

analytical procedure applying chemiluminescence (CL) coupled with flow-injection analysis (FIA) is not only the simplicity and sensitivity of detection but also that the reagents used here are cheaper than those reported previously.

The oxidation of sulfite by Ce(IV) in sulfuric acid medium is a well-known chemiluminescence reaction.[51–53] The emission has been attributed to the formation of excited sulfur dioxide molecules which radiate during de-excitation.[54]

The aim of the present work is to develop a sensitive and accurate flow injection CL method for the determination of enalapril maleate and atenolol in pharmaceutical preparations and biological fluids.

Experimental

Apparatus and flow system

The flow system used for the determination of enalapril maleate and atenolol with CL detection is shown schematically in Fig. 1.

A 3MP4 peristaltic pump (Gilson Minipuls) with two channels and variable speed was used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant total flow rate of 3.2 mL min^{-1} using PTFE tubing (0.06 mm i.d.). Enalapril maleate and atenolol solutions were injected through the sample injection valve which allows mixing of the sample with the sulfite solution and then combination with Ce(IV) solution just before the detector. The emitted light was measured by a photomultiplier tube (Thorn EMI, 9789QB) which was operated at 1200 V. The signal was recorded by a (Chessell Ltd) recorder. Peak heights were measured.

Reagents and materials

A stock standard solution of enalapril maleate and atenolol (Pharco Pharmaceuticals, Alexandria, Egypt), 1.0 mg mL^{-1} were dissolved in distilled water. Working standard solutions of the studied drugs were prepared immediately before use. The reagents used were: aqueous sodium sulfite (BDH Ltd, UK), 1×10^{-3} M; cerium sulfate solution (Fluka, Switzerland), 5×10^{-4} M; sulfuric acid solution (BDH Ltd, UK), 0.1 and 1×10^{-3} M; sodium hydroxid solution (Fluka, Switzerland), 0.1 and 2.0 M; hydrochloric acid solution (BDH Ltd, UK), 0.1 M. The solvents used were dichloromethane, chloroform and 2-propanol, all from Fluka, Switzerland. Tablets containing enalapril maleate or atenolol were purchased from commercial sources. Serum samples were supplied by United Diagnostics Industry K.S.A. and urine samples were obtained from healthy volunteers.

Procedures

General procedure. Working solutions of studied drugs in the range of 0.01–3.0 μg mL^{-1} of enalapril maleate, 0.01–2.0 of atenolol (Table 1) were prepared from stock solutions. A 200 μL aliquot of enalapril maleate and 150 μL of atenolol were injected

Table 1. Data element required for assay validation of studied drugs

Analytical Performance characteristics	Value	
	atenolol	Enalapril maleate
Linear calibration range (μg mL^{-1})	0.01–2.0	0.01–3.0
Regression equation	$I = 0.81 + 14.12\ C$	$I = 2.51 + 17.55\ C$
$I^a = a + b\ C$		
Correlation coefficient (r)	0.99989[b]	0.99993[b]
δ_a: standard deviation of intercept	0.013	0.016
δ_b: standard deviation of slope	0.087	0.024
LOD[c] (μg mL^{-1})	0.0003	0.0025
LOQ (μg mL^{-1})	0.01	0.01
RSD % (n = 15)	0.76	0.68
Accuracy (mean ± SD)	100.1 ± 0.87	99.8 ± 0.68
Student's t-value	0.49 (2.447)[d]	1.16 (2.447)[d]
Variance F-ratio	2.78 (6.94)[e]	1.25 (6.94)[e]

[a]Intensity (mV).
[b]Eleven data points.
[c]S/N = 2.
[d]Tabulated t-value at confidence levels 95% (57).
[e]Tabulated F-value at confidence levels 95% (57).

into a stream of 1×10^{-3} M sodium sulfite solution which was then combined with a stream of 5×10^{-4} M Ce(IV) solution and the resulting peak heights were measured. Calibration graphs were prepared by plotting the peak heights against the drug concentration.

Procedure for tablets. An accurately weighed amount of ten powdered tablets equivalent to 10.0 mg of each drug, was transferred into a 50 mL volumetric flask. Distilled water was added and completed to the mark. The contents of the flasks were sonicated for 1 h, filtered and then analyzed as described above under general procedure.

Procedure for spiked biological fluids of enalapril maleate. An aliquot of standard aqueous solution of enalapril maleate containing 100 μg was added to 1 mL of serum or urine sample in a centrifuge tube and shaken well for 3 min. 1 mL of an aqueous solution of 0.1 M NaOH was added, shaken for 1 minute and then 5 mL of dichloromethane was added. The mixture was vortex mixed at high speed for 5 min, and then centrifuged at 3000 rpm for 10 min. The resulting supernatant was transferred into a small conical flask. The extraction was repeated two times with 5 mL of dichloromethane. The combined extracts were evaporated to dryness at room temperature and the residue was dissolved in 1 mL of 0.1 M HCl. The solution was transferred into a 10 mL volumetric flask and completed to volume with distilled water, then analyzed according to the general procedure.

Procedure for spiked biological fluids of atenolol. An aliquot of 1 mL of serum or urine sample was spiked with aliquot of standard aqueous solution of atenolol containing 100 μg and alkalinized with 0.1 mL of 2.0 M NaOH, shaken for 1 min and then 5 mL of chloroform/2-propanol mixture (4:1, v/v) were added. The mixture was vortex-mixed at high speed for 20 min, and then centrifuged at 3000 rpm for 10 min. The extraction was repeated twice with 5 mL of the same mixture. The organic layers were collected and transferred to another flask. The solvent was evaporated to dryness at room temperature and the residue was dissolved in 1 mL of 0.1 M HCl. The solution was transferred into a 10 mL volumetric flask and completed to volume with distilled water, then analyzed according to the general procedure.

Results and discussion

The sensitizing effect of enalapril maleate and atenolol on the chemiluminescent reaction of the oxidation of sodium sulfite was studied using different oxidants. A very weak CL signal was obtained with potassium permanganate. Other oxidants, such as potassium dichromate and potassium bromate gave medium CL signals. Maximum CL intensity was obtained when cerric ammonium sulfate dihydrate was used as an oxidant in an acidic medium.

A weak CL signal (recorded as a baseline) appeared when both reagents were mixed and flowed in the FIA system. This signal notably increased when a solution of each of the studied drugs was injected, as a peak which height increased proportionally with the drug concentration. The maximum for each drug CL signal was obtained when the sample was injected into a stream of 1×10^{-3} M sodium sulfite and then mixed with 5×10^{-4} M Ce(IV) prior to the detector. The different experimental parameters affecting the CL intensity were studied.

Configuration designs. The flow injection configuration used for the determination of the studied drugs was so designed to provide different reaction conditions for magnifying the CL signal generated by their sensitizing effect on the oxidation of sodium sulfite by Ce(IV). CL signal was obtained only when the sample was injected into a stream of 1×10^{-3} M sodium sulfite and then mixed with 5×10^{-4} M Ce(IV) prior to the detector. When Ce(IV) and sodium sulfite are continuously mixed and introduced into the flow cell, weak CL radiation was emitted from this reaction. Then when the drug solution is injected into the sodium sulfite stream, the intensity is enhanced in proportional to its concentration. A schematic diagram of the manifold is shown in Fig. 1.

Optimization of experimental variables

The optimization of chemical variables included reagents concentrations and some physical variables, including the flow rate and the sample volume, was performd using the univariate optimization procedure by changing one variable in every turn and keeping the other at their optimum values.

Effect of sulfuric acid concentration as a solvent for cerium(IV). C e(IV) becomes stable when dissolved in sulfuric acid solution, so the effect of sulfuric acid concentration on CL intensity was studied in the range 1×10^{-2} to 0.1 M. The CL intensity was highest for both the studied drugs at 1×10^{-3} M sulfuric acid, thus, 1×10^{-3} M H_2SO_4 was used in the preparation of Ce(IV) solution.

Effect of cerium(IV) concentration. The CL intensity depends on Ce(IV) concentration, so a study was carried out in the range 1×10^{-5} to 1×10^{-2} M Ce(IV). The maximum CL signal for each drug was obtained with 5×10^{-4} M Ce(IV) which was used for further investigation.

Effect of sodium sulfite concentration. The effect of sodium sulfite concentration on the CL intensity was studied in the range of 1×10^{-5} up to 1×10^{-2} M. The optimum sulfite concentration for both drugs was 1×10^{-3} M after which the intensity started to decrease.

Effect of total flow rate. This parameter had a critical influence to the CL intensity since optimum flow rate allows the reaction to proceed for a suitable time, before the reagents enter the cell. The solutions of oxidant and sulfite were introduced into the manifold at equal flow rates. The optimum intensity was obtained on using total flow rate of 3.2 mL min^{-1} for both drugs.

Effect of sample volume. The injected sample volume was varied from 10 to 700 μL by changing the length of the sample loop connected to the injection valve. It was found that the peak height increased with increasing sample volume up to 150 μL of enalapril maleate or 200 μL of atenolol.

Effect of coil length from T to PMT. The effect of coil length from T to PMT was studied in the range 10–250 cm. The maximum CL signal was obtained on using 50 cm, which was the suitable coil length for this studty.

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Effect of some sensitizers and surfactants. The effect of some organized systems which work as sensitizers or surfactants for many reactions, including neutral surfactants (Triton X-100), cationic surfactants (cetyltrimethylammonium bromide and cetylpyridinium chloride) and anionic surfactants (sodium dodecyl sulfate), fluorophores as rhodamin 6G, rhodamin B and fluoroescein, was investigated. The CL signals of both studied drugs disappeared or decreased. Therefore this cannot be considered as a valuable parameter in the quantitative measurements.

CL mechanism

The CL mechanism can be explained depending on the fact that the studied drugs are fluorogenic compounds in aqueous solutions; therefore the possible CL mechanism by analogy is:

$$HSO_3^- + Ce^{4+} \rightarrow HSO^{\bullet}_3 + Ce^{3+}$$

$$2HSO^{\bullet}_3 \rightarrow S_2O_6^{2-} + 2H^+$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2*$$

$$SO_2* + (\text{enalapril or atenolol}) \rightarrow (\text{enalapril or atenolol})* + SO_2$$

$$(\text{enalapril or atenolol})* \rightarrow (\text{enalapril or atenolol}) + \text{Light}$$

Sulfite ion acts as the reductant and the energy from the chemical reaction is released as chemiexcitation of sulfur dioxide, which emits radiation at wavelenghths > 300 nm. Enalapril maleate or atenolol increases the weak radiation emitted during the CL oxidation of sulfite by Ce(IV) in sulfuric acid medium, so these drugs act as sensitizers.

Determination of the studied drugs

Under the described experimental conditions, a series of standard solutions of the studied drugs were pumped, each as three replicates, to test the linearity of the calibration graph. A plot of the CL intensity vs concentration of each of the studied drugs was found to be linear over the range 0.01–3.0 μg mL^{-1} of enalapril maleate with an LOD of 0.0025 μg mL^{-1} (S/N = 2), and over the ranges of 0.01–2.0 of atenolol with an LOD of 0.0003 μg mL^{-1} (S/N = 2). The LOQ for both drugs was 0.01 μg mL^{-1}. Linear regression analyses of the results are shown in Table 1.

Application of the method

Analysis of pharmaceutical preparations. In order to evaluate the analytical usefulness of the proposed chemiluminescent method, the studied drugs were determined in their pharmaceutical preparations. The results in Table 2 are in accordance with those obtained by the official method.[3] Statistical analysis of the results using Student's *t*-test and the variance ratio *F*-test showed no significant difference between the two methods as regards accuracy and precision, as illustrated in Table 2.

Analysis of spiked biological fluids. The high sensitivity attained by the proposed method allows the determination of the studied drugs in biological fluids. The extraction procedure for biological fluids was performed using dichloromethane, which was chosen as the best extraction solvent for enalapril maleate,[50] and chloroform/2-propanol mixture (4:1, v/v) as the suitable extraction solvent for atenolol.[55] Table 3 shows the performance data and the results of determination of the studied drugs in urine and serum.

Validity of the proposed method

Linearity. The proposed method was tested for linearity. The regression plot showed a linear dependence of CL intensity on enalapril maleate and atenolol concentrations. The relation between CL intensity and the studied drugs concentrations was calculated by the least-square method, and the results obtained are given in Table 1 which summarizes the data obtained in the validation of the method. The calibration range, LOD, S/N = 2 (the limit of detection was calculated according to the typically acceptable signal-to-noise ratios 2:1)[56] and LOQ as well as the slope and intercept were also clarified. Validation of the method was done by statistical analysis of the regression line regarding δ_a and δ_b.

Precision and accuracy of the method. The intra-day data was evaluated through triplicate analysis of samples containing 0.2, 0.5 and 0.9 μg mL^{-1} of the studied drugs. The inter-day data was determined by triplicate analysis of the above concentrations of the studied drugs on three consecutive days. The percentage error and %RSD are presented in Table 4. The reproducibility was investigated using 0.5 μg mL^{-1} of the studied drugs (n = 15) and the %RSD < 2, which illustrates that the results were highly reproducible.

The accuracy of the proposed method was evaluated by analyzing standard solution of the studied drugs. The percentage found of the studied drugs compared with those obtained by the official method (3) are given in Table 1. Statistical analysis[57] of the results, obtained by the proposed and the official method (3) using Student's *t*-test and variance ratio *F*-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

Robusteness of the method

The robustness of the method adopted in the proposed method was demonstrated by the consistency of the CL intensity with minor changes in the experimental parameters such as the change in the concentration of Ce(IV), $5 \times 10^{-4} \pm 1 \times 10^{-4}$ M and change in the concentration of sulfuric acid, $1 \times 10^{-3} \pm 0.1 \times 10^{-3}$ M. These minor changes that may take place during the experimental operation did not affect the CL intensity.

Conclusion

The proposed method is simple, accurate, low-cost and precise. This FIA-CL procedure was investigated for the rapid determination of enalapril maleate and atenolol in pharmaceutical preparations and biological fluids. Solutions can be analyzed at a rate of 55 samples per hour. Furthermore the proposed procedure showed clear advantages such as short period of real-time drug analysis, and a very simple extraction procedure for urine and serum samples. As a consequence, the proposed method appears convenient for therapeutic drugs since it is sensitive and requires smaller volumes of samples.

Table 2. Analysis of studied drugs in their dosage forms by the proposed and official methods (3)

Preparation	Concentration taken (μg mL^{-1})	Found (%)	
		Proposed method[f]	Official method (3)[j]
Renitec tablets[a] (5 mg enalpril maleate/tablet)	0.05	99.0	
	0.1	99.9	
	0.5	100.0	
	1.5	99.0	
	2.0	98.5	
Mean ± SD		99.4 ± 0.63	99.5 ± 0.5
Student's *t*-value		0.23 (2.447)[g]	
Variance *F*-ratio		1.59 (19.20)[h]	
Renitec tablets[a] (10 mg enalpril maleate/tablet)	0.05	99.0	
	0.1	100.1	
	1.5	99.9	
	2.0	99.0	
	2.5	99.2	
Mean ± SD		99.4 ± 0.52	99.2 ± 0.76
Student's *t*-value		0.45 (2.447)[g]	
Variance *F*-ratio		2.14 (6.94)[h]	
Renitec tablets[a] (20 mg enalpril maleate/tablet)	0.05	99.9	
	0.1	99.0	
	1.5	100.1	
	2.0	100.5	
	2.5	99.0	
Mean ± SD		99.7 ± 0.67	99.0 ± 0.6
Student's *t*-value		1.48 (2.447)[g]	
Variance *F*-ratio		1.25 (19.20)[h]	
Angiotec tablets[b] (5 mg enalpril maleate/tablet)	0.05	100	
	0.1	99.9	
	1.5	100.4	
	2.0	99.0	
	2.5	99.0	
Mean ± SD		99.7 ± 0.63	99.5 ± 0.46
Student's *t*-value		0.38 (2.447)[g]	
Variance *F*-ratio		1.88 (19.20)[h]	
Angiotec tablets[b] (10 mg enalpril maleate/tablet)	0.05	100.1	
	0.1	99.0	
	1.0	100.2	
	2.0	99.0	
	2.5	100.9	
Mean ± SD		99.8 ± 0.83	99.2 ± 0.76
Student's *t*-value		1.03 (2.447)[g]	
Variance *F*-ratio		1.19 (19.20)[h]	
Lapril tablets[c] (20 mg enalpril maleate/tablet)	0.05	100	
	0.1	100.1	
	1.0	99.9	
	1.5	98.9	
	2.5	99.5	
Mean ± SD		99.7 ± 0.49	99.6 ± 0.48
Student's *t*-value		0.36 (2.447)[g]	
Variance *F*-ratio		1.04 (19.20)[h]	
Normoten tablets[d] (50 mg atenolol/tablet)	0.025	99.9	
	0.05	99.8	
	0.1	100.0	
	0.15	98.9	
	1.0	101.5	

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Table 2. (*Continued*)

Preparation	Concentration taken (μg mL^{-1})	Found (%)	
		Proposed method[f]	Official method (3)[j]
Mean ± SD		100.0 ± 0.94	101.0 ± 0.78[i]
Student's *t*-value		1.73 (2.45)[g]	
Variance *F*-ratio		1.45 (19.20)[h]	
Normoten tablets[d] (100 mg atenolol/tablet)	0.025	100.0	
	0.05	100.8	
	0.1	100.0	
	0.5	99.9	
	1.0	99.8	
Mean ± SD		100.1 ± 0.40	99.9 ± 0.71[i]
Student's *t*-value		0.47 (2.45)[g]	
Variance *F*-ratio		3.15 (6.94)[h]	
Tenormin tablets[e] (50 mg atenolol/tablet)	0.025	100.1	
	0.1	100.0	
	0.15	99.9	
	0.5	100.0	
	1.0	98.5	
Mean ± SD		99.7 ± 0.67	100.4 ± 1.12[i]
Student's *t*-value		0.13 (2.45)[g]	
Variance *F*-ratio		2.79 (6.94)	
Tenormin tablets[e] (100 mg atenolol/tablet)	0.025	100.0	
	0.05	100.8	
	0.1	100.0	
	0.15	101.6	
	1.0	99.3	
Mean ± SD		100.3 ± 0.88	99.9 ± 0.47[i]
Student's *t*-value		0.71 (2.45)[g]	
Variance *F*-ratio		3.51 (19.2)[h]	

[a]Products of Merck Sharp and Dohme B. V. Haarlem, Netherlands.
[b]Products of The Jordanian Pharm. Mtg Co. Ltd.
[c]Products of Midpharma, Jordan.
[d]Products of Jazeera Pharmaceutical Industries, Saudi Arabia.
[e]Products of Astrazeneca UK Limited.
[f] Each result is the avarage of three separate determinations.
[g]Tabulated *t*-value at confidence levels 95% (57).
[h]Tabulated *F*-value at confidence levels 95% (57).
[i] As reported for tablets of atenolol in the *British Pharmacopoeia*.
[j]As reported for pure form for enalpril maleate.

Table 3. Performance data for the chemiluminometric determination of the studied drugs in serum and urine

Compound	Serum			Urine		
	Linear calibration range	Regression equation	Correlation cofficient[b]	Linear calibration range	Regression equation	Correlation cofficient[b]
	(μg mL^{-1})	$I^a = a + b\,C$		(μg mL^{-1})	$I^a = a + b\,C$	
Enalapril maleate	0.1–2.0	$I = 1.14 + 11.37\,C$	0.99985	0.1–2.0	$I = 1.33 + 12.41\,C$	0.99989
Atenolol	0.1–1.0	$I = 0.09 + 10.51\,C$	0.99987	0.1–1.0	$I = 0.17 + 11.32\,C$	0.99984

[a]Intensity (mV)
[b]Seven data points.

Table 4. Intra-day and intr-day data for the studied drugs

Concentration taken (μg mL^{-1})	Enalapril maleate						Atenolol					
	Intra-day			Inter-day			Intra-day			Inter-day		
	Found[a]	%Error	%RSD	Found[a]	%Error	%RSD	Found[a]	%Error	%RSD	Found[a]	%Error	%RSD
0.2	0.199	−0.5	0.64	0.198	−1.0	0.61	0.199	−0.5	0.68	0.199	−0.5	0.70
0.5	0.499	−0.2	0.62	0.498	−0.4	0.53	0.498	−0.4	0.69	0.497	−0.6	0.67
0.9	0.910	+1.1	0.88	0.890	−1.1	0.83	0.898	−0.2	1.01	0.902	+0.2	0.35

[a]Average of three determinations.

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