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تعیین همزمان آتنولول و پروپرانولول در نمونه‌های دارویی به روش اسپکتروفتومتری مشتقی مرتبه سوم

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Simultaneous Determination of Atenolol and Propranolol in Pharmaceutical Formulations by Third Derivative Spectrophotometric Method

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چکیده
در این مطالعه، یک روش اسپکتروفتومتری مشتقی مرتبه سوم براساس روش صفر متقابل برای تعیین همزمان آتنولول و پروپرانولول توصیف شده است. عوامل مؤثر شامل دما و pH، بررسی و بهینه‌سازی شدند. تحت شرایط بهینه تجربی (درجه حرارت: ۱۰ ^{o}C و pH = ۸-۱۰)، منحنی‌های درجه‌بندی بین گستره‌های غلظتی ۰/۸ تا ۱۲/۹ $\mu g/mL$ برای آتنولول و ۰/۳ تا ۶/۴ $\mu g/mL$ برای پروپرانولول خطی بودند. حد تشخیص ۰/۲ $\mu g/mL$ و انحراف استاندارد نسبی کمتر از ۳٪ بدست آمد. روش پیشنهادی کاربرد رضایت‌بخشی برای تعیین همزمان دو دارو در فرمولاسیون‌های دارویی دارد. بازیابی قابل قبولی، در گستره ۹۷ تا ۱۰۷ درصد بدست آمد.

واژه‌های کلیدی
آتنولول؛ پروپرانولول؛ اسپکتروفتومتری مشتقی؛ صفر متقابل.

Abstract
In this study, a third derivative spectrophotometric method based on zero-crossing technique is developed for the simultaneous determination of atenolol and propranolol. Effective parameters including temperature and pH were investigated and optimized. Under optimum experimental conditions (temperature: 10 ^{o}C and pH: 8-10), calibration curves were linear between concentration ranges of 0.8 to 12.9 μg mL^{-1} of atenolol and 0.3 to 6.4 μg mL^{-1} of propranolol. The limit of detection was 0.2 μg mL^{-1} and relative standard deviation was lower than 3%. The proposed method was applied for the simultaneous determination of both drugs in the pharmaceutical formulations. Acceptable recoveries of the analytes, ranging from 97 to 107%, were obtained.

Keywords
Atenolol; Propranolol; Derivative Spectrophotometry; Zero-Crossing.

1. INTRODUCTION

Atenolol [4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide, Fig. 1] is used in treatment of hypertension. It is also used to treat the patients who have cardiac pain because of coronary insufficiency. Atenolol acts via blocking nerve impulses to some part of body, so it is known as a *β*-blocker. By blocking these impulses, atenolol may help heart to work more affective and regular, so its workload reduces. Unlike propranolol this drug does not pass through the blood–brain barrier thus, avoid various central nervous system side effects [1-2]. Propranolol [1-(1-methylethyl) amino)-3-(1-naphthalenyloxy)-2-propanol, Fig. 1] is a *β*-adrenergic blocking drug widely prescribed for the treatment of cardiac arrhythmia, sinus tachycardia, angina pectoris and hypertension [3].

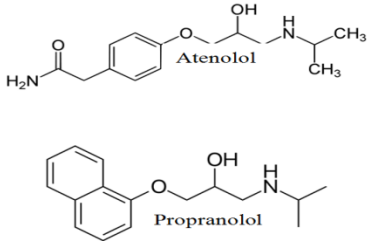

Fig. 1. Chemical structures of atenolol and propranolol.

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It has also been suggested for using in a number of other conditions including dysfunctional labour and anxiety. When this drug is administered over a long period of time, it reduces mortality caused by hypertension and lengthens survival in patients with coronary heart disease [4]. Propranolol is available in generic form as propranolol hydrochloride and about 90% of drug absorbed through digestive tract [5]. These days, the fraudulent consumption of atenolol and propranolol is quite common; therefore they have been included in the list of forbidden substances by the International Olympic Committee [6]. Therefore, the simultaneous determination of them can be attractive for clinical aims. According to the literature survey it was found that few analytical methods such as HPLC [7-9], GC/MS [10-11], fluorimetry [12], and voltammetry [13] were reported for this purpose. However, these methods usually suffer from long analysis time, expensive equipments and large volume of solvents. Therefore, it is still necessary that easier and faster methods to develop for the simultaneous determination of atenolol and propranolol.

Derivative spectrophotometry has some advantages over the conventional spectrophotometric methods, including discrimination of the sharp spectral features over the large bands and the improvement of the resolution of the overlapping spectra [11-12]. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. This technique rapidly gained ground in application for the analysis of pharmaceutical formulations. In the quantitative analysis of mixture substances with highly overlapping spectra, the zero-crossing technique is frequently used. This method is based on the measurement of the absolute value of the derivative spectrum of the mixture at wavelength where the intensity of one of the substances of the mixture goes to zero. At this wavelength, the intensity is proportional to the other substance [14-24].

In this study, a third derivative spectrophotometric method based on zero-crossing technique was developed for the simultaneous determination of atenolol and propranolol in the binary mixtures of pharmaceutical formulations. This method exhibited a precise, accurate and cost effective assay for these drugs.

.2. EXPERIMENTAL

2.1. Apparatus

A double-beam Shimadzu UV-Vis spectrophotometer (160-A, Japan) with 1-cm matched quartz cell was used to record the absorption spectra. The derivative spectra were obtained by data processing on normal absorption spectra (using UV-Vis instrument). A thermostated water bath (Heidolph, Germany) was used to keep the temperature of all solutions at the working temperature. A pH meter (ISTEK Inc., South Korea) was applied to measure the acidity of solutions. Solutions of NaOH and HCl (0.1 mol L^{-1}) were used to adjust pH.

2.2. Reagents

Atenolol and propranolol hydrochloride were kindly obtained from Abidi Co. (Iran). The freshly prepared aqueous solutions of the pure drugs (100 μg mL^{-1}) were used as the standard solutions for analytical purposes. All other chemicals and solvents were analytical grade. Deionised water was used to prepare solutions.

2.3. Pharmaceutical formulation samples

The different volumes of the two standard solutions (atenolol and propranolol, 100 μg mL^{-1}) were poured to a volumetric flask and made up to the mark with distilled water. The concentrations of atenolol and propranolol were determined by measuring the absorbance at zero-crossing points.

2.4. Selection of the zero-crossing wavelengths

Initially, the first, second, third and fourth-orders of derivative spectra were obtained within 200-400 nm for appropriate standard solutions of analytes. The third derivative spectrum was selected because of the spectral characteristics, good selectivity and sensitivity. The main instrumental parameters that affect the shape of the derivative spectra such as the scan speed and the wavelength increment ($\Delta\lambda$) were optimized. A scan speed of 200 nm/min and $\Delta\lambda$=6 were selected. By employing the zero-crossing technique, the wavelengths of third derivative spectra were found at which no interference of measured quantities is observed in drugs solutions. The zero points of atenolol and propranolol were 239 and 232 nm, respectively. At 239 nm atenolol showed a zero absorbance but propranolol had a considerable absorbance and similarly, at 232 nm only atenolol had absorbance. The measurements in these points exhibited the best response and the least effect by the other drug.

3. RESULT AND DISCUSSION

In this work, the derivative spectrophotometry method was applied for the simultaneous determination of atenolol and propranolol with satisfactory results. Measurements made at the zero-crossing point of one of the two drugs were a function only of the concentration of the other substance.

3.1. Preliminary studies

The normal absorption of atenolol and propranolol showed that the spectra of these two compounds completely overlapped and each compound interfered in the spectrophotometric determination of other one. Therefore, to overcome this problem a suitable technique such as derivative spectrophotometry can be used. After initial studies, it was found that third derivative spectra were suitable for the simultaneous determination of atenolol and propranolol (Figs. 2 and 3). The zero-crossing technique was employed in measurements; using wavelengths λ=232 *nm* and λ=239 *nm* for the determination of atenolol and propranolol, respectively.

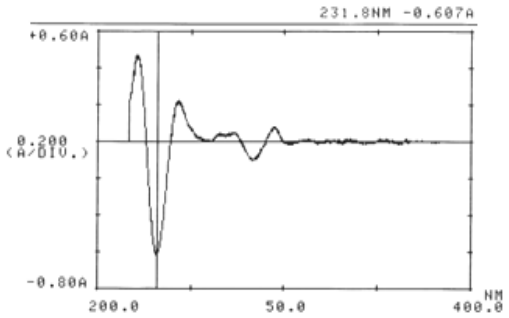

Fig. 2. The third derivative spectrum of atenolol.

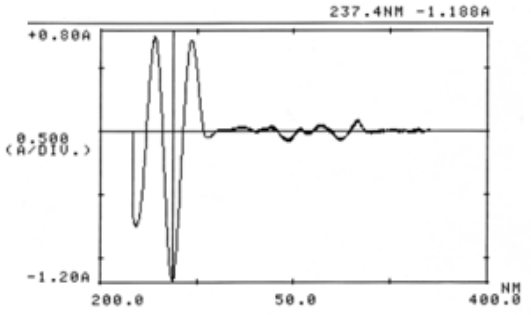

Fig. 3. The third derivative spectrum of propranolol.

3.2. Optimization of effective parameters

In order to obtain the best sensitivity, effective parameters including pH (3-10) and temperature (10-50 ^{o}C) were investigated and optimized (Figs. 4 and 5). According to these Figs., pH=8-10 and temperature 10 ^{o}C were found as optimum values for the simultaneous determination of both drugs.

3.3. Analytical parameters

Under optimum conditions, calibration curves for atenolol and propranolol were plotted from the third derivative spectra by measuring the height of peaks at 232 nm and 239 nm for atenolol and propranolol, respectively. The linear ranges of 0.8

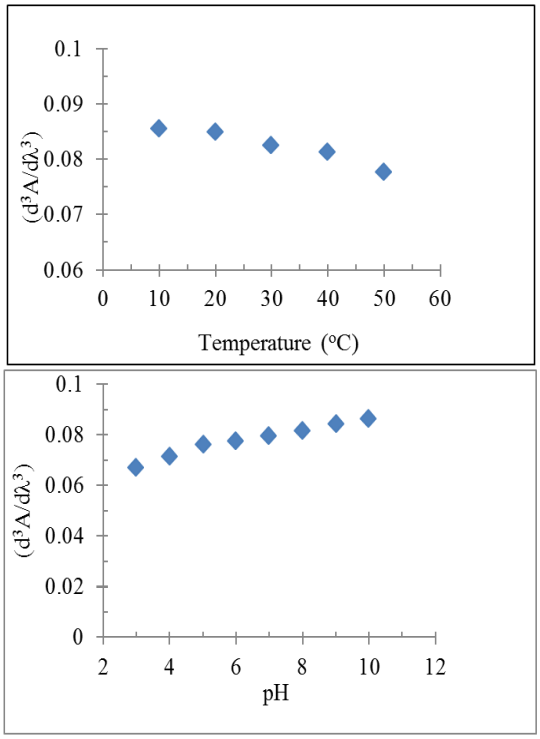

Fig. 4. Effect of temperature and pH for the atenolol determination.

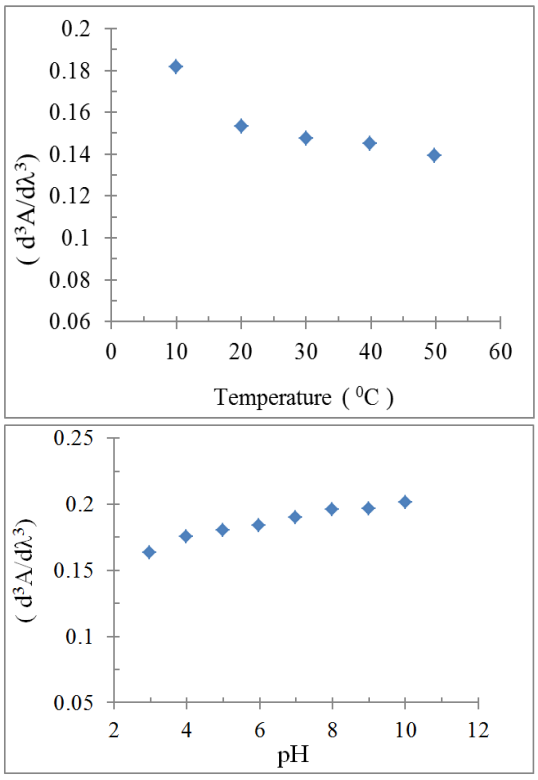

Fig. 5. Effect of temperature and pH for the propranolol determination.

to 12.9 µg mL^{-1} (R^2=0.9928) of atenolol and 0.3 to 6.4 µg mL^{-1} (R^2=0.9872) of propranolol were obtained (Figs. 6 and 7).

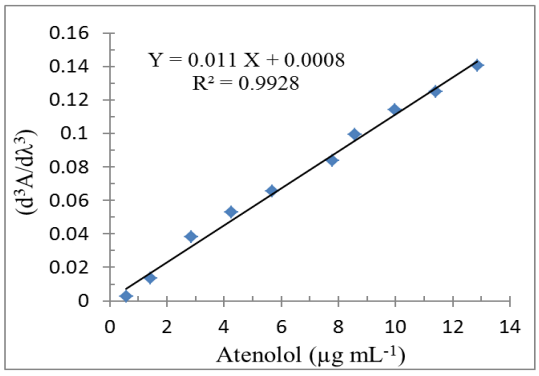

Fig. 6. Calibration curve of atenolol.

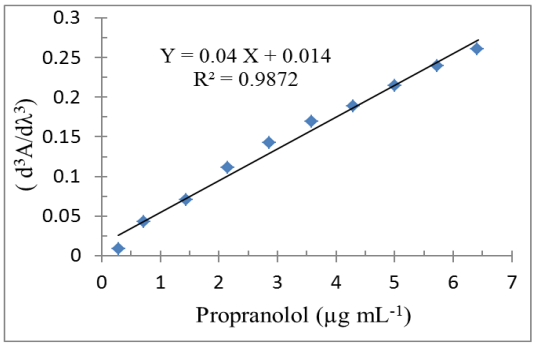

Fig. 7. Calibration curve of propranolol.

The limit of detection was 0.2 µg mL^{-1} for two drugs and also the relative standard deviations of developed method for the determination of atenolol and propranolol were 1.0 and 2.3%, respectively. The analytical parameters of the proposed method were comparable to those of other methods [7-13] used for the simultaneous determination of atenolol and propranolol (Table 1).

For studying of selectivity, the effects of foreign species on the third derivative spectrophotometric determination of atenolol or propranolol were investigated individually under the selected conditions. The tolerance limit was set as the concentration required to cause a 3% error in the determination. According to Table 2, many foreign species did not interfere, even in a 1-fold the drugs.

3.4. Application of the proposed method in pharmaceutical formulations

In order to evaluation of capability of the developed method in the simultaneous determination of two drugs, the different pharmaceutical formulations of atenolol and propranolol were prepared and determined (Table 3). According to the results reported in this Table, the developed method showed a good accuracy. Thus, it can be applied as an alternative and attractive method for the simultaneous determination of atenolol and propranolol.

Table 1. Comparison of the analytical parameters of the proposed method with other methods.

Method	LOQ	RSD	LDR	Ref.
RP-HPLC-UV	15[a] 5.0[p]	3.1[a] 1.3-3.1[p]	15-120[a] 5.0-60[p]	7
RP-HPLC	0.078[a] 0.15[p]	0.04-3.08	1.25-40	8
Fluorimetry	-	-	0.01-0.40[a] 0.006-0.20[p]	12
Voltammetry	0.25[a] 0.05[p]	1.2-3.4[a] 0.4-4.6[p]	0.5-10.9[a] 0.05-2.3[p]	13
Derivative spectrophotometry	0.6	1.0[a] 2.3[p]	0.8-12.9[a] 0.3-6.4[p]	This work

[a] atenolol, [p] propranolol.
Concentration is as µg mL^{-1}.

Table 2. Tolerance limit of foreign species on the determination of atenolol or propranolol.

Foreign species	Tolerance limit (atenolol, propranolol)
K^+	(0.3, 0.8)
Na^+	(0.4, 0.8)
NH_4^+	(0.2, 0.7)
Cl^-	(0.6, 1.4)
ClO_4^-	(0.3, 1.4)
NO_3^-	(0.3, 1.5)
Glucose	(0.2, 0.1)
Saccarose	(0.2)
Urea	(0.5)

Table 3. Simultaneous determination of atenolol and propranolol (µg mL^{-1}) in pharmaceutical formulations.

Sample	Atenolol added	Atenolol found (Recovery%)	Propranolol added	Propranolol found (Recovery%)
1	1.4	1.5 (107)	0.70	0.70(100)
2	2.8	3.0 (107)	2.1	2.2 (104)
3	4.3	4.4 (102)	2.8	2.9 (103)
4	5.7	5.6 (98)	3.6	3.7 (102)
5	8.6	9.0 (104)	4.3	4.2 (97)

4. CONCLUSION

This paper demonstrated the potential of third derivative spectrophotometric method as an accurate, rapid and simple analytical technique for the simultaneous determination of atenolol and propranolol in the pharmaceutical formulations. The proposed method eliminated the need of separation step prior to analysis, saving both time and cost. The analytical parameters of developed

method were acceptable and comparable to those of other methods for the simultaneous determination of two drugs.

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