

RESEARCH ARTICLE

# Simultaneous Determination of Atenolol and Amlodipine Using Second Derivative Spectroscopy

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## ABSTRACT

The present study describes employing second derivative spectrophotometry for the simultaneous determination of atenolol and amlodipine in pure form and in commercial formulations. The method is simple, accurate, precise, and economic. The zero-crossing point technique was used for analysis of the drugs in the combined formulation. The method was found to be linear in the concentration range of 5.0–50.0 µg/ml of atenolol at 251 nm and 5.0–45.0 µg/ml of amlodipine at 264 nm. The proposed method was successfully applied to determine atenolol and amlodipine in combined dosage as well as in a separate dosage. The obtained results were in good agreement with standard method.

**Keywords:** Atenolol; Amlodipine; Second derivative spectroscopy

## INTRODUCTION

Amlodipine besylate is a hypertensive drug with the chemical name of 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate (Rahman and Azmi, 2000). It is calcium-channel blocker. Amlodipine is used for treatment of hypertension and angina pectoris (Ayad et al., 2012). Amlodipine acting by relaxation of the smooth muscle in arterial wall, decreasing total peripheral resistance and thus reducing blood pressure (Bhusari and Dhaneshwar, 2012). Atenolol chemically is 4-(2-hydroxy-3-isopropylaminopropoxy)-phenyl acetamide. It is a  $\beta$ -adrenoreceptor blocking agent. Atenolol is used mainly for angina pectoris, hypertension, and myocardial infarction (Pawar et al., 2013). The important function of atenolol in the human body is to stimulate the heart to beat more rapidly (Bhusari and Dhaneshwar, 2012). A combination between amlodipine and atenolol in pharmaceutical preparations has been introduced in the markets for hypertension treatment. Literature review reveals that methods have been reported for analysis of amlodipine and atenolol by derivative spectroscopy (Prasad et al., 1998; Kasture and Rameteke, 2006), simultaneous spectroscopic estimation (Jain and Agrawal, 2000; Patil et al., 2009; Godge et al., 2017), high-performance liquid chromatography (HPLC) (Halker et al., 1988; Shimooka et al., 1989; Yeung

et al., 1991; Patki et al., 1994; Josefsson et al., 1995; Rahman et al., 2001), reversed-phase HPLC (RP-HPLC) (Ravishankar et al., 1997; Chitlange et al., 2009; Palani and Kamarapu, 2017), gas chromatography (Bresford et al., 1987), high-performance thin-layer chromatography (Agrekar and Powar, 2000), and voltammetry (Moraes et al., 2016) which were in individual formulations and combined dosage forms. Although the first derivative was used by Meghna et al., 2014 as well as the third derivative by Kasture and Rameteke, 2006 to estimate the two compounds simultaneously but the second derivative was not used for estimation of the two drugs together; therefore, the aim of this study is to develop analytical method for determination of atenolol and amlodipine in combined dose, using second derivative spectroscopy.

## MATERIALS AND METHODS

### Apparatus

A T80 + ultraviolet (UV)-visible spectrophotometer with 1.0 cm matched quartz cells was used for all spectral measurements.

### Chemicals

Atenolol and amlodipine besylate standards were supplied by Awamedica Co. (Erbil, Iraq).

### UV-derivative Spectroscopic Method

Standard solutions of amlodipine and atenolol were scanned in the wavelength range of 200–400 nm, using T80 + UV-visible spectrophotometer, with slow scan speed in absorbance measuring mode, and fixed interval 0.2 nm, where distilled water was used as a reference. The spectrophotometer was connected with the computer, and the zero-order spectrum was converted to second derivative using 21 points derivative through UVWin5 Software (V5.2.0.1104), and no smoothing was required (Rathee et al., 2010).

### Preparation of Standard Solutions

A 25 mg of atenolol standard was weighed accurately and transferred to a beaker and dissolved in little amount of distilled water, then completed to 100 ml with distilled water in a volumetric flask (250 µg/ml). A 34.665 mg of amlodipine besylate equivalent to 25 mg of amlodipine was weighed accurately and transferred to a beaker, then dissolved in little amount of distilled water and completed to 100 ml with distilled water in a volumetric flask (250 µg/ml). The solutions of the drugs were stored in the refrigerator.

### Assay of Formulations

Ten tablets of each brand were weighed and triturated into a fine powder. An amount of powder equivalent to one tablet was transferred into a beaker and dissolved with distilled water, then filtrated using Whatman no.1 filter paper. The precipitate on filter paper washed several times with distilled water and the washings were added to the filtrate. The volume of filtrate completed to 250 ml in a volumetric flask using distilled water.

## RESULTS AND DISCUSSION

The derivative spectrophotometry technique may be used for the determination of different compounds whose spectrum is overlapping with minimum error (Umapathi, 1994). The zero-order spectra of both drugs were found to be overlapping and each compound interferes with spectrophotometric determination of the other one [Figure 1]. It appears that atenolol has two maximum absorption bands at 225 nm and 274 nm, while amlodipine has one maximum absorption band at 240 nm with shoulder.

The absorption of two or more compounds in the same wavelength region which would generate inseparable interference in normal spectrophotometry can be resolved using the derivative technique. The zero-crossing point method is used for this purpose. It is defined as a particular wavelength at which one component has a response

(positive or negative), while the response of other drugs is zero. At zero crossing point, it is possible to measure the response of one component while the response of other remains zero, but for simultaneous determination of both drugs there must be two zero-crossing points at which one can be quantified while the response of the other would remain zero (Patel, 2013).

### Second Derivative Spectroscopy of Atenolol

The second derivative spectroscopy of atenolol is shown in Figure 2. Atenolol has negative peak at 225.5 nm and positive peak at 238 nm. The drug has more than one zero-crossing point, but the most useful one at 264 nm where the atenolol drug has zero absorbance for different concentrations.

### Second Derivative Spectroscopy of Amlodipine

Figure 3 shows the second derivative spectroscopy of amlodipine concentrations. Amlodipine has two negative peaks at 214 nm and 240 nm, with one positive peak at

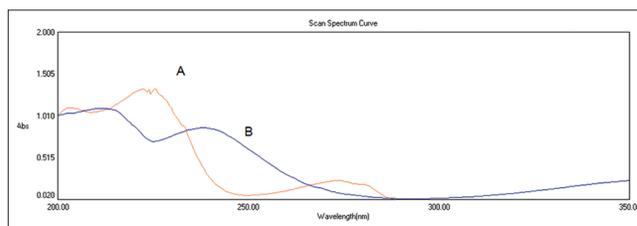


Figure 1: (A) Zero-order spectrum of 50 µg/ml atenolol (B) zero-order spectrum of 20 µg/ml of amlodipine

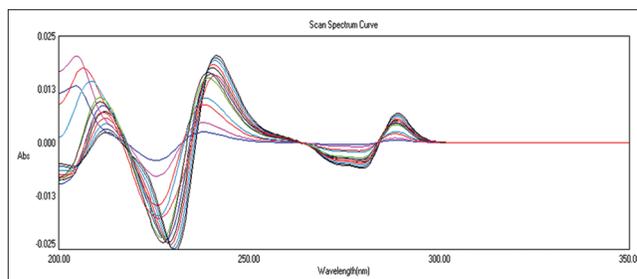


Figure 2: Second derivative spectroscopy of different concentration of atenolol (5 µg/ml–µg/ml)

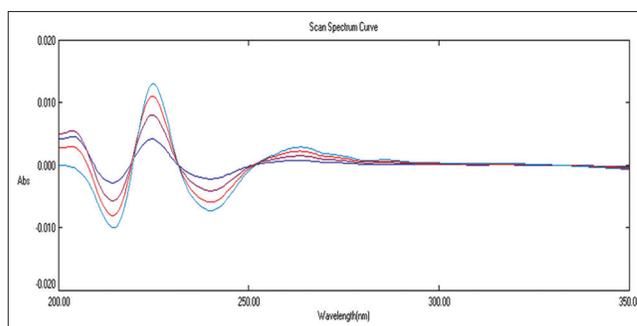


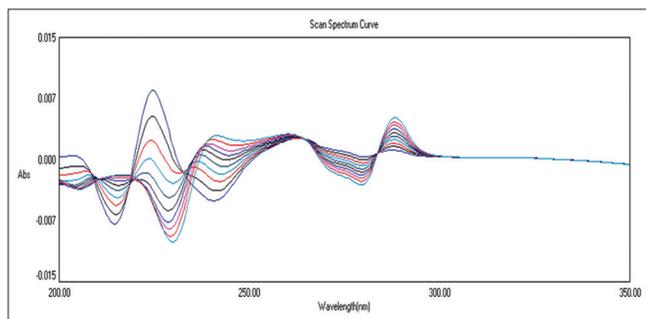
Figure 3: Second derivative spectroscopy of different conc. of amlodipine (5 µg/ml–20 µg/ml)

225 nm. These peaks could be used to determine the drug when present alone. The drug has more than one zero crossing point but the most important is 251 nm, where the  $dA^2/d^2\lambda$  is zero at this wavelength. In the zero crossing derivative method, it is necessary that zero-crossing wavelengths do not change with changing the concentrations of the test analyte. To evaluate the condition, changes in the pre-mentioned zero-crossing wavelengths for amlodipine were tested. According to the results given in Figure 3, a shift in the zero-crossing wavelengths was observed beyond the concentration of 20  $\mu\text{g/ml}$ ; hence, the determination of atenolol must be performed in the presence of 20  $\mu\text{g/ml}$  of amlodipine or less.

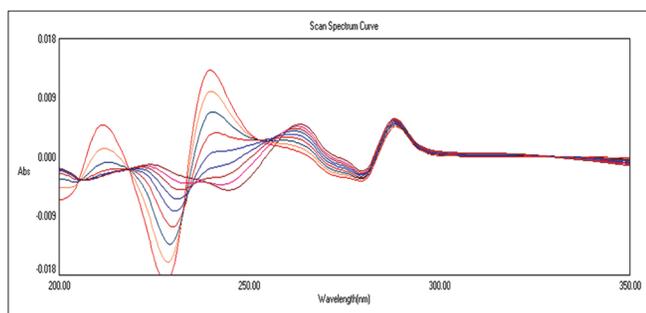
**Simultaneous Determination of Atenolol and Amlodipine**

Figure 4 shows the simultaneous determination of atenolol at 251 nm in the presence of 20  $\mu\text{g/ml}$  amlodipine, where amlodipine has zero signals; therefore, it is possible to determine atenolol in combined dose. The atenolol was also determined at 251 nm in the presence of 5.0  $\mu\text{g/ml}$  of amlodipine.

The simultaneous determination of amlodipine in the presence of 50  $\mu\text{g/ml}$  atenolol at 264 nm is shown in Figure 5. The drug was determined in the presence of 50  $\mu\text{g/ml}$  of atenolol, amlodipine may be determined even at higher concentration of atenolol because the latter zero-crossing point has not changed by the concentration, as depicted in Figure 2.



**Figure 4: Simultaneous determination of atenolol at 251 nm in the presence of 20  $\mu\text{g/ml}$  amlodipine**



**Figure 5: Simultaneous determination of amlodipine at 264 nm in the presence of 50  $\mu\text{g/ml}$  atenolol**

**Determination of Atenolol Alone using Second Derivative Spectroscopy**

For determination of atenolol, a good relation was found between the concentration of atenolol and peak amplitude at 225.5 nm and 238 nm, using peak-to-baseline in measuring peak amplitude.

**Determination of Amlodipine Alone using Second Derivative Spectroscopy**

The determination of amlodipine was performed with the aid of the peak-to-base line in measuring peak height at 214 nm and 225 nm, as shown in Figure 3.

**Calibration Graphs for Analysis of Atenolol and Amlodipine Simultaneously and Separately**

The calibration graphs were constructed between second derivative response and concentrations (Umapathi, 1994; Patel, 2013) for various series. All calibration graph parameters are tabulated in Table 1.

**Precision and Accuracy**

To check the precision of the proposed method, the pure drug solution at three different levels (different concentrations) were analyzed, each concentration being repeated 3 times. The relative standard deviation percentage was in the range of 1.19–2.62 to determine amlodipine in the presence of atenolol, and for determination of atenolol it was 0.81–4.81 in the presence of amlodipine. The accuracy of the method was calculated through the recovery value [Table 2], and it was ranged from 94.23% to 103.4%.

**Interference Study**

The influence of different compounds present in the commercial drugs on the determination of 25  $\mu\text{g/ml}$  of atenolol and 25  $\mu\text{g/ml}$  of amlodipine with second derivative spectrophotometry method were checked. The method was tolerable to 1000  $\mu\text{g/ml}$  for each of glucose, cellulose, lactose, glycogen, sucrose, maltose, KCl, and NaCl, whereas it is tolerable to 250  $\mu\text{g/ml}$  of each of fructose and starch. The relative error percentage was within  $\pm 5.0\%$ .

**Method Application**

To evaluate the validity of the proposed work, atenolol and amlodipine were determined in some commercial drugs. The results of the determination of active ingredients in the commercial drugs are presented in Table 2. Atenolol was determined at 251 nm and amlodipine at 264 nm in the combined dose drugs as in the first three samples. The atenolol and amlodipine were individually estimated, using 225.5 nm and 238 nm for atenolol and the wavelengths of 214 nm and 225nm were used for amlodipine estimation and the average contents are displayed in the next table. The results show good agreement between obtained results

**Table 1: Regression analysis of atenolol and amlodipine**

Series (λ)	Concentration (λg/ml)		DL (μg/ml)	Regression equation	Correlation coefficient
	Atenolol	Amlodipine			
A (264)	50	5–45	2.0	y=0.0001x+0.0002	0.9985
B (251)	5–50	20.0	3.0	y=7×10 <sup>-5</sup> x-0.0001	0.9968
C (251)	5–50	5.0	3.0	y=6×10 <sup>-5</sup> x-5×10 <sup>-5</sup>	0.9996
D (214)	0.0	5–20	2.0	y=0.0005x+0.0008	0.9944
E (225)	0.0	5–20	1.0	y=0.0006x+0.0017	0.9944
F (225.5)	2–25	0.0	1.0	y=0.0007x+0.0009	0.9951
G (238)	5–25	0.0	2.0	y=0.0004x+0.0004	0.9985

**Table 2: Assay results of atenolol and amlodipine in commercial formulation by the proposed method**

Commercial name	Declared content (mg)	Found by proposed method (mg)	Found by HPLC method (mg)	Recovery %
Cipla				
Atenolol	50.0	47.87	48.50	98.70
Amlodipine	5.0	5.10	4.93	103.4
Cipla				
Atenolol	25.0	24.40	24.20	100.82
Amlodipine	5.0	4.90	5.20	94.23
Microlab				
Atenolol	50.0	53.10	52.50	101.11
Amlodipine	5.0	4.90	5.17	94.77
Accord				
Atenolol	50.0	49.40	49.50	99.79
Amlong				
Amlodipine	5.0	4.75	4.86	97.73

HPLC: High-performance liquid chromatography

by proposed method and that obtained by HPLC method (British Pharmacopoeia Commission, 2009).

## CONCLUSION

The developed method for determination of atenolol and amlodipine was found to be simple, rapid, precise, and accurate. The derivative technique has succeeded in resolving the overlapped spectra and could be used for the simultaneous determination of each of atenolol and amlodipine drugs in combined dosage.

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