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RESEARCH PAPER

# Two different spectrophotometric methods for simultaneous determination of binary mixture of atenolol and amlodipine in commercial formulation

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**A B S T R A C T:**

For the simultaneous determination of a binary mixture of amlodipine (AM) and atenolol (AT) in tablets, two approaches were accessible. The first method is the second derivative of the ratio spectra ($^2$DD) that obtained via dividing the absorption spectra of binary mixtures by a standard spectrum of one of the components and then the second derivative of the ratio spectra were calculated. The extended ratio subtraction method (EXRSM) is the second approach, which launches with the ratio subtraction method coupled with 4$^{th}$ derivative spectrophotometry. The calibration curve is linear over the concentration range (2-45) µg/mL and (5-60) µg/mL for amlodipine and atenolol successively. The presented methods have been successfully applied to estimate binary mixtures of amlodipine and atenolol tablets simultaneously and also was satisfactory compared with standard method (HPLC).

KEY WORDS: Amlodipine, Atenolol, Second derivative of ratio spectra, Extended ratio subtraction method.
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## 1.INTRODUCTION :

Amlodipine (AM), 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid 3-ethyl, 5-methyl ester (Darwish et al., 2013). Amlodipine is a dihydropyridine-class long-acting calcium channel blocker used as an antihypertensive and in the treatment of angina pectoris, and its chemical structure is illustrated in (Figure 1A)(Moussa et al., 2013). The classical method for determining amlodipine alone and beside with another drugs in dosage form are high-performance liquid chromatography (Osman and Elbashir, 2017, Çelebier et al., 2010) and spectrophotometric method (Alizadeh and Hemati, 2014, Darwish et al., 2011).

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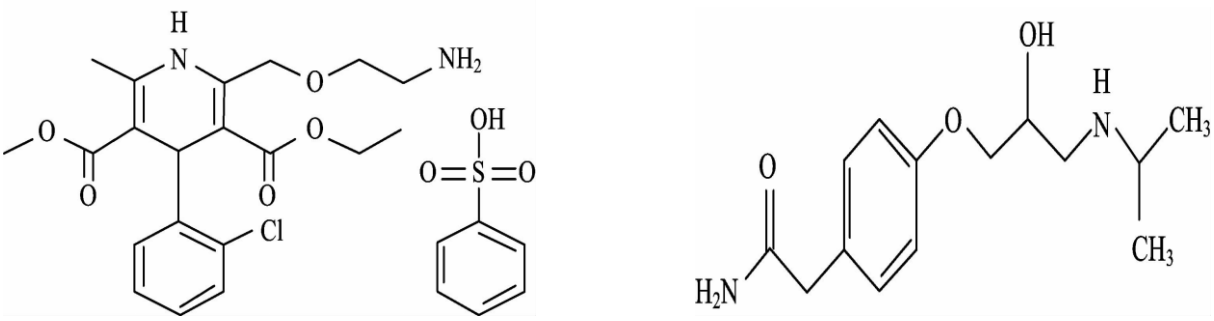
It is broadly used medicine in conjunction with atenolol. Atenolol (AT), 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy) benzeneacetamide (Prashanth and Basavaiah, 2012). The atenolol chemical structure is demonstrated in (Figure 1B), it is a cardio-selective beta-blocker that is commonly used to treat hypertension and angina pectoris (Vaikosen et al., 2020). Many analytical methods are available to determine atenolol including high-performance liquid chromatography (Elgawish et al., 2011, Spanakis and Niopas, 2013, NEELIMA et al., 2016), gas chromatography (Yilmaz, 2010) and liquid chromatography (Lawson et al., 2012). Whilst numerous analytical techniques have been reported for the simultaneous determination binary mixture of amlodipine and atenolol like liquid chromatography (Kannappan and Mannemala, 2016), spectrophotometric methods (Lamie, 2015b, Lamie, 2015a, Suresh et al., 2015, Kasture and Ramteke, 2006, Pawar et al., 2013)

and derivative spectroscopy (Mohammad et al., 2019).




(A) (B)

Figure 1: Chemical structure of (A) Amlodipine and (B) Atenolol

Blanco et al. (Blanco et al., 1987) were the first to use ratio spectra for spectral analysis, the spectrophotometric resolution of overlapped spectra was then achieved by combining most signal processing techniques with ratio spectra. For analyzing binary and ternary mixes, Salinas and colleagues modified Blanco's original equations and devised the first derivative of ratio spectra approach (Salinas et al., 1990, Nevado et al., 1992). The second derivative of the ratio spectra was chosen over the first derivative for a better resolution of the ratio spectra and for more precise and correct results (El-Gindy et al., 2001). The main parameters influencing the shape of the ratio spectra which are smoothing function and the wavelength scanning speed should test cautiously. It was noticed that noisy spectra were obtained at high speed while the noise was reduced at low scanning speed, but the measurements required a longer time (Elzanfaly et al., 2015). Furthermore, the derivative of the ratio spectra was influenced by $\Delta\lambda$. When the $\Delta\lambda$ values rise, the noise level decrease slightly (Omer and Fakhre, 2019). The capability to make measurements in the correspondence of peaks is one of the benefits of the derivative ratio spectra approach over the zero-crossing derivative method, As a result, the derivative ratio spectra approach has a possibly higher sensitivity and accuracy, as well as an easier calculation at separate peaks and no requirement to operate merely at zero-crossing point as with derivative methods (Issa et al., 2011).

Another spectrophotometric method was introduced by Lotfy and Hagazy (2012), this method begins with the ratio subtraction method (RSM), it is termed an (extended ratio subtraction method) which has benefit that the extended compound in the mixture might be estimated at its $\lambda_{max}$ which is impossible to do using the formerly established ratio subtraction approach (El-Bardicy et al., 2008), it has only been used to assess unextended compounds. Therefore, these two procedures being complementary to one another as a result these two components could be determined in the mixture (Ali et al., 2013). The correct option of the concentration of the devisor is important in the ratio subtraction method. If the devisor concentration is decreased or increased, the resulting constant value will be increased or decreased proportionately (Darwish et al., 2015).
Due to the absence of any methods for estimating these medications in combination simultaneously by second derivative of the ratio spectra and extended ratio subtraction method; hence, this paper tries to establish approaches to develop two techniques for determining the binary mixture of medicines simultaneously without previous separation.

## 2. Experimental

### 2.1. Apparatus

A T80 UV-visible spectrophotometer with 1.0 cm matched quartz cells was utilized for all spectrum measurements. To record zero order spectra and

collected data on the absorption spectra for each of amlodipine besylate and atenolol with their mixture solutions, a computer was linked to a double beam spectrophotometer. Matlab 6.5 and Microsoft Excel were used to do all calculations.

**2.2. Chemical & Materials**

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

**2.3. UV-derivative Spectroscopic Method**

Amlodipine besylate and atenolol standard solutions were scanned in the 200-400 nm wavelength range, using T80 + UV-visible spectrophotometer, with slow scan speed in absorbance measuring mode, and fixed interval 0.2 nm, where distilled water was utilized as a reference.

**2.4. Standard Solutions Preparation**

A 25 mg of atenolol standard was weighed precisely and transported to a beaker contain little amount of distilled water and dissolved, then accomplished to 100 ml using distilled water in a volumetric flask (250 µg/ml). A 34.665 mg of amlodipine besylate equivalent to 25 mg of amlodipine was weighed correctly and moved to a beaker, later dissolved in little amount of distilled water and diluted to 100 ml with distilled water in a volumetric flask (250 µg/ml). The solutions of the drugs were stored in the refrigerator.

**2.5. Assay of Formulations**

Each brand's ten tablets were weighed and grinded into a fine powder. An amount of powder corresponding to one tablet was moved into a beaker and dissolved with distilled water. Then, using Whatman no. 1 filter paper, it was filtered. The precipitate on filter paper was rinsed with distilled water several times and the washings were added to the filtrate. The volume of filtrate completed to 250 ml in a volumetric flask using distilled water.

**2.6. Calibration Graph**

**2.6.1. Second derivative of the ratio spectra**

To measure AM in occurrence of AT, the second derivative of the ratio spectra ($^2$DD) was achieved through dividing the absorption spectra of AM to the 25 µg/mL standard spectrum of AT, which smoothed with an interval of $\Delta\lambda$= 4 nm. For the quantification of amlodipine in a binary mixture the amplitude at 240 nm ($^2DD_{240}$) and 246 nm ($^2DD_{246}$) had been chosen.

The same idea was applied for determination of AT in the existence of AM, the second derivative of the ratio spectra ($^2$DD) was attained through dividing the absorption spectra of AT to the 20 µg/mL standard spectrum of AM, then smoothed using an interval of $\Delta\lambda$= 4 nm. The concentration of AT were determined at the amplitude 276 nm ($^2DD_{276}$) and 284 nm ($^2DD_{284}$) in a binary mixture.

**2.6.2. Extended ratio subtraction method coupled with 4$^{th}$ derivative spectrophotometry**

The ratio subtraction method (RSM) is the starting point for the extended ratio subtraction method (EXRSM), which uses a mixture of AM and AT to display overlapping spectra, AT representing unextended spectrum and AM representing extended spectrum. The ratio subtraction method may be used to calculate AT via dividing mixes (AT with AM) by a standard spectrum of AM′ (10 µg/mL) as a divisor, resulting in a new curve that depicts AT/AM′+AM/AM′ (constant). The values of these constants in the plateau area are being measured at (310-400 nm), next these constant values were removed, then multiplying the resulting curve by the (10 µg/mL) of AM′ as a standard spectrum after subtraction these constant values, which is the identical divisor employed, yields the AT zero-order absorption spectrum. The regression equation which describes the linear relationship between the absorbance at its $\lambda_{max}$ versus the corresponding concentration of AT used for determination the concentrations of AT.

AM was calculated by dividing the attained zero order spectrum of AT by a (30 µg/mL) of AT′ as a standard spectrum to gain the (AT/AT′) constant value, whereas AM was estimated through dividing mixtures of (AT with AM) by a (30 µg/mL) of AT′ as a standard spectrum which is the same divisor resulted in a new curve that represents AM/AT′+AT/AT′ (constant), afterward these constant values were removed, then multiplying the resulting curve by (30 µg/mL) of AT′ as a standard spectrum after subtraction these constant values, which is the same divisor employed, yields the AM zero-order absorption spectrum.

For AT to be determined by 4$^{th}$ derivative spectrophotometry and the AT concentrations were estimated via measuring the signal at 277.80nm and 281.50nm (zero-crossing point of AM). The solutions were recorded in the range of 200-400nm.

**3. Results and discussion**

AM and AT are two medicines that their typical UV absorption spectra in the wavelength region of 200 to 400nm are fully overlapped (Figure 2). As a result, using traditional spectrophotometry to resolve a combination spectrum and determining two medicines in a binary mixture at the same time is difficult. To minimize interference and resolve the overlapping spectra, two alternative methods were utilized.

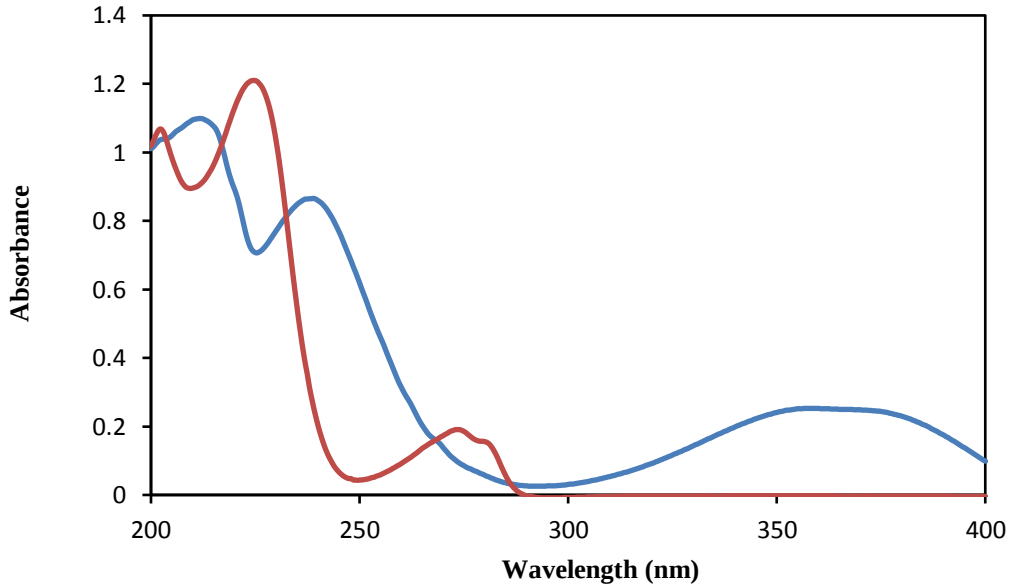



Figure 2: Zero-order absorption spectrum of 20 µg/mL amlodipine ( ), and 40 µg/mL of atenolol ( )

### 3.1. Second derivative of the ratio spectra

The absorption spectra of AM solutions at various concentrations in binary mixtures were recorded in the wavelength range of 200-400 nm and then divided by the standard spectrum of AT 25 µg/mL to get the ratio spectra displayed in Figure (3a). As revealed in Figure (3b), from ratio spectra, the second derivative was calculated and traced with an interval of Δλ= 4 nm. The concentration of AM in a binary mixture was measured and the amplitude at 240 nm ($^2DD_{240}$) and 246 nm ($^2DD_{246}$) were employed. Also Figure (4a) shows the ratio spectra of AT that attained through dividing the absorption spectra of AT at various concentrations in a binary mixture by a 20 µg/mL of AM standard spectrum to obtained the ratio spectra. Next from ratio spectra, the second derivative was calculated and traced with an interval of Δλ= 4 nm as illustrated in Figure (4b). The concentration of AT in a binary mixture was measured by using the amplitude at 276 nm ($^2DD_{276}$) and 284 nm ($^2DD_{284}$). Table (1) illustrates the results of statistical data of the calibration curves using second derivative ratio spectra method including correlation coefficients, linear range of calibration graph and limit of detection for determination each of AM and AT in binary mixture simultaneously.

The most significant parameters that needed to be optimized is the divisor concentrations, for this purpose various divisor concentrations were tested. It was found out that the standard solution of AT 25 µg/mL had the best signal-to-noise ratio therefore suitable for estimating AM in a binary mixture. As well as, for the determination of AT in a binary mix, a 20 µg/mL standard solution of AM was used as the divisor. Also, another factor required to be tested is Δλ and Δλ= 4 nm regarded to be appropriate.

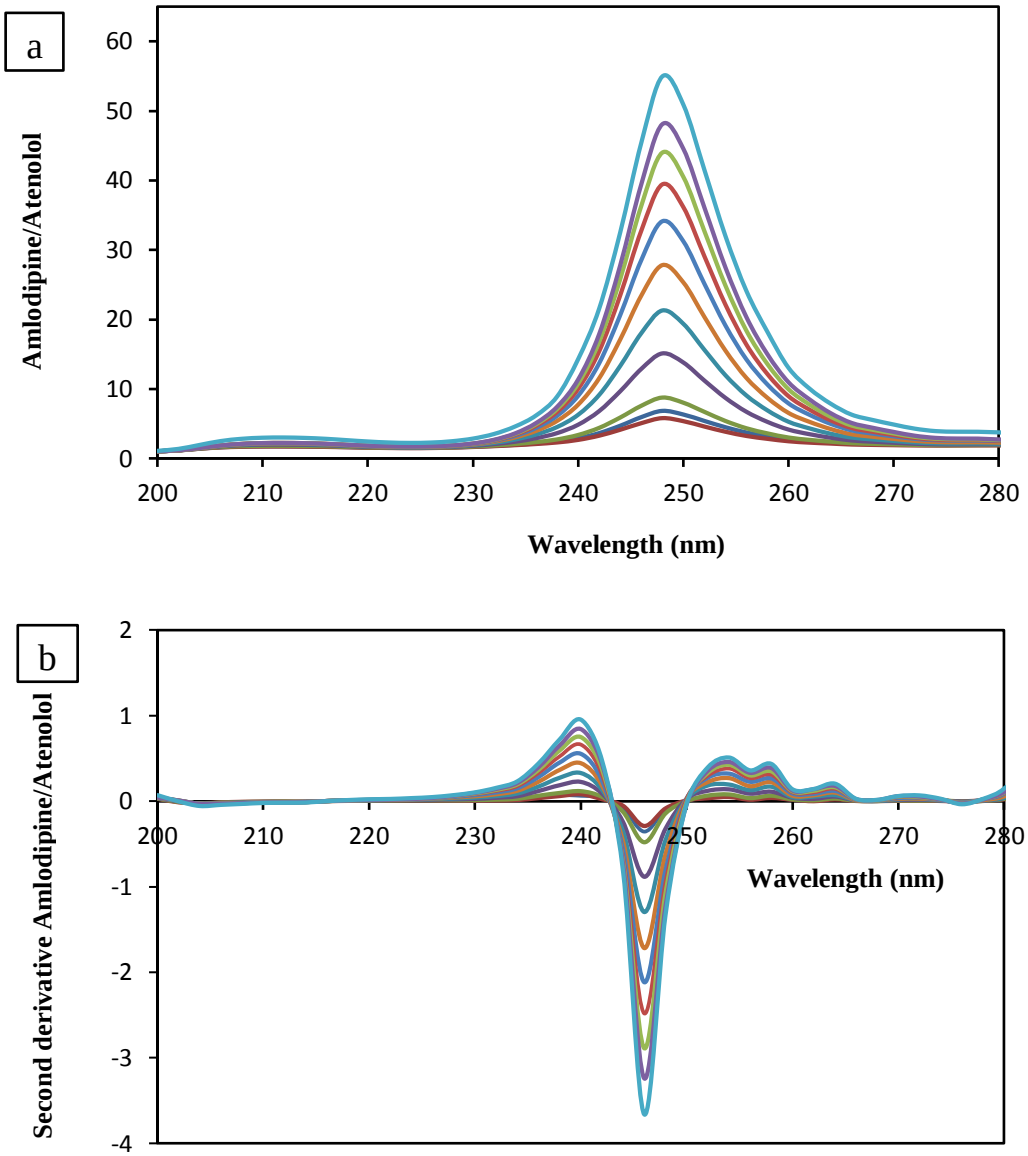
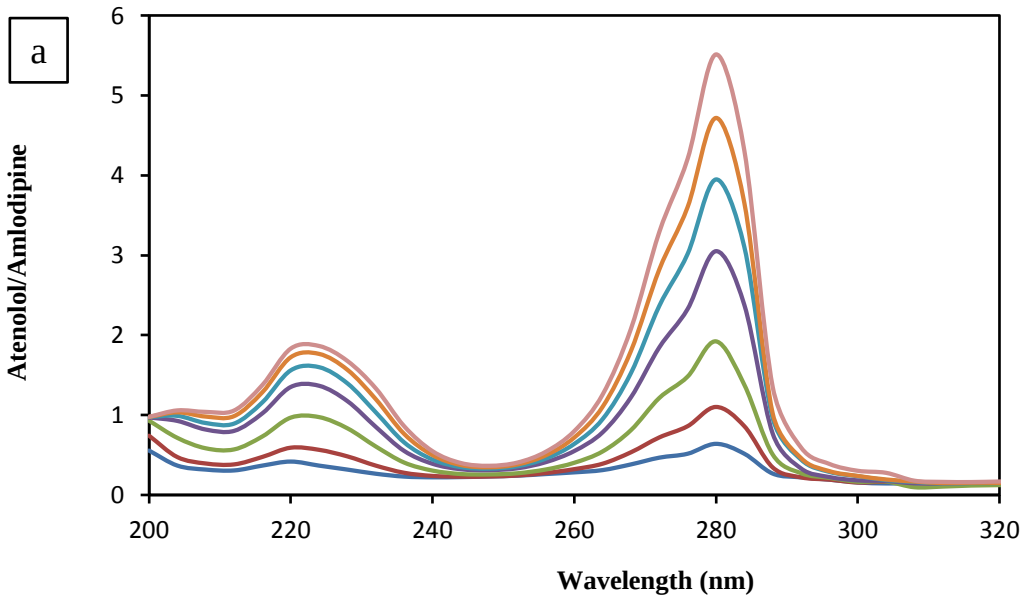



Figure 3: Ratio spectra (a) and second derivative ratio spectra (b) of AM (2, 3, 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg/mL) when AT was utilized as the divisor at 25 µg/mL



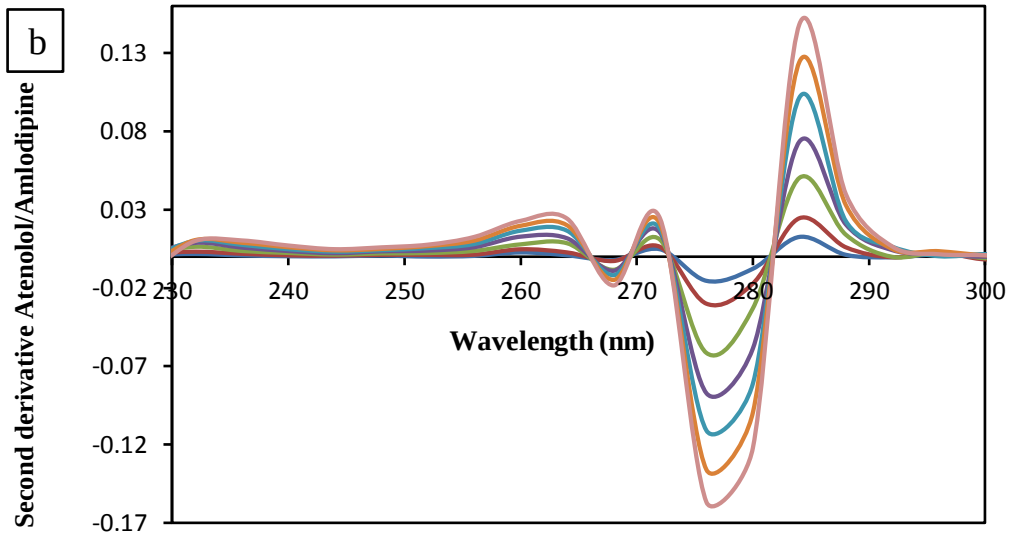



Figure 4: Ratio spectra (a) and second derivative ratio spectra (b) of AT (5, 10, 20, 30, 40, 50 and 60 µg/mL) when 20 µg/mL of AM was used as the divisor

### 3.2. Extended ratio subtraction method coupled with 4th derivative spectrophotometry

The extended ratio subtraction method launches after applying the ratio subtraction method. The ratio subtraction technique first scans the zero-order spectrum of the prepared standard solution of AT in water and then validates the linear relationship between the absorbance at the chosen wavelength of 226 nm and the corresponding concentration of AT. The method based on that, when a mixture of AT and AM in which the spectrum of AM is more extended than AT as displayed in Figure (2). The estimation of AT conducted by scanning and dividing the absorption spectra of the laboratory-prepared mixtures (AT and AM) by a standard spectrum of AM′ (10 µg/mL) as a divisor a new ratio spectrum was produced which represents AT/AM′ + (AM / AM′) as demonstrated in Figure (5). Then subtraction of the values of these constants (AM / AM′) from the total mention value in the plateau region (310-400 nm) to produce new spectra as shown in Figure (6), then the obtained spectra multiplied by the divisor AM′ (10µg/mL) as illustrated in Figure (7). Eventually, the original spectra of AT were gained and it can directly use for determination of AT at 226 nm. The determination of AM has been done using the extended ratio subtraction method through dividing these attained spectra of AT by chosen the concentration of standard AT′ (30µg/mL) to produce the ratio spectra that represents the constants AT/AT′ in plateau (266-284 nm) as given in Figure (8). The zero order absorption spectra of the laboratory prepared mixtures (AT and AM) that previously scanned were divided by a spectrum of AT′ (30µg/mL) as a divisor the new ratio spectrum was produced which represent AM/AT′ + (AT/AT′) as presented in Figure (9). Then these constants values (AT/AT′) were subtracted from the total mention value in the plateau region (266-284 nm) to produce new spectra as given in Figure (10). Next the obtained spectra were multiplied by the divisor AT′ (30 µg/mL) as displayed in Figure (11). Lastly, the original spectrum of AM was achieved and it can be used for direct determination of AM at 240 nm.

To determine AT in the occurrence of AM, the measurements were made at the zero-crossing point of AM, at 281.50 nm and by peak to baseline at 277.80 nm as shown Figure 12, that could permit their quantitation, and the corresponding calibration graphs were at 281.50 nm and 277.80 nm for the quantitation of AT as demonstrated in Figures 13. Table (1) demonstrates the outcomes of statistical data of the calibration curves using extended ratio subtraction method and 4th derivative spectrophotometry comprising linear range of calibration graph, correlation coefficients and limit of detection for each of AM and AT determined simultaneously in binary mixture.

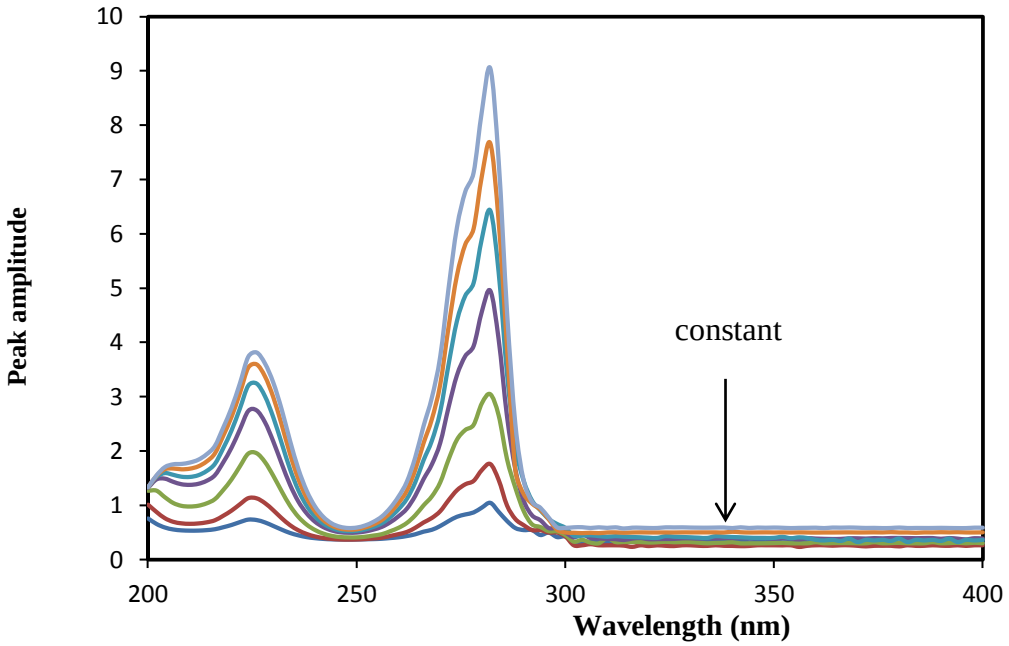



Figure 5: Ratio spectra of laboratory prepared mixture of AT (5, 10, 20, 30, 40, 50 and 60) µg/mL and 20 µg/mL of AM using 10 µg/mL of AM as a divisor

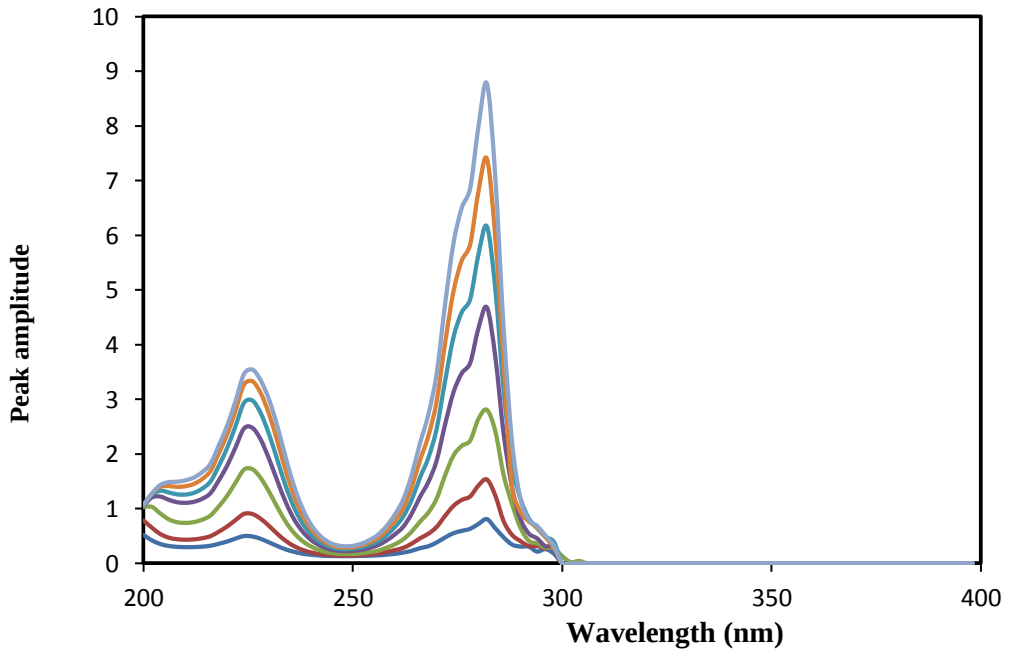



Figure 6: Ratio spectra of laboratory prepared mixture of AT (5, 10, 20, 30, 40, 50 and 60) µg/mL and 20 µg/mL of AM using 10 µg/mL of AM as a divisor after subtraction of the constant

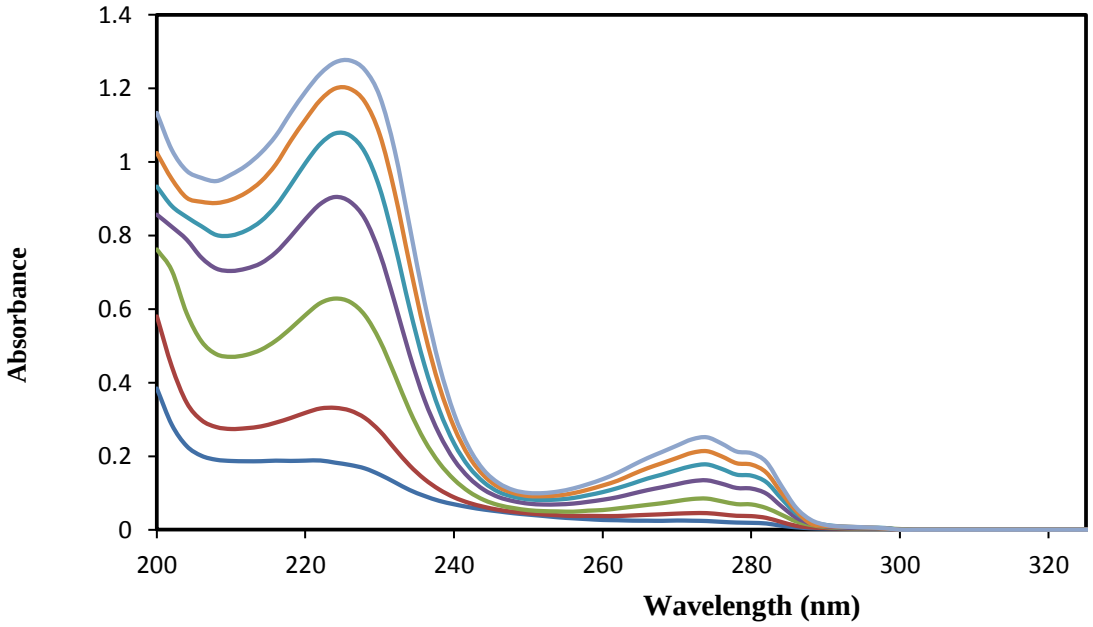



Figure 7: Zero order absorption spectrum of AT (5, 10, 20, 30, 40, 50 and 60) µg/mL after subtracting the constant and multiplying by the divisor spectrum of 10 µg/mL of AM

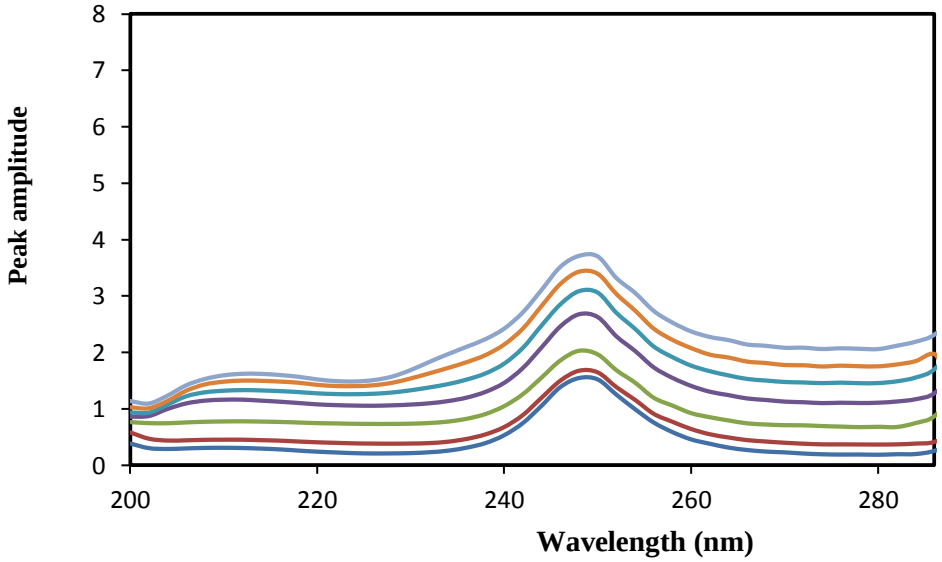



Figure 8: Ratio spectra of AT (5, 10, 20, 30, 40, 50 and 60) µg/mL using the spectrum of 30 µg/mL of AT as divisor

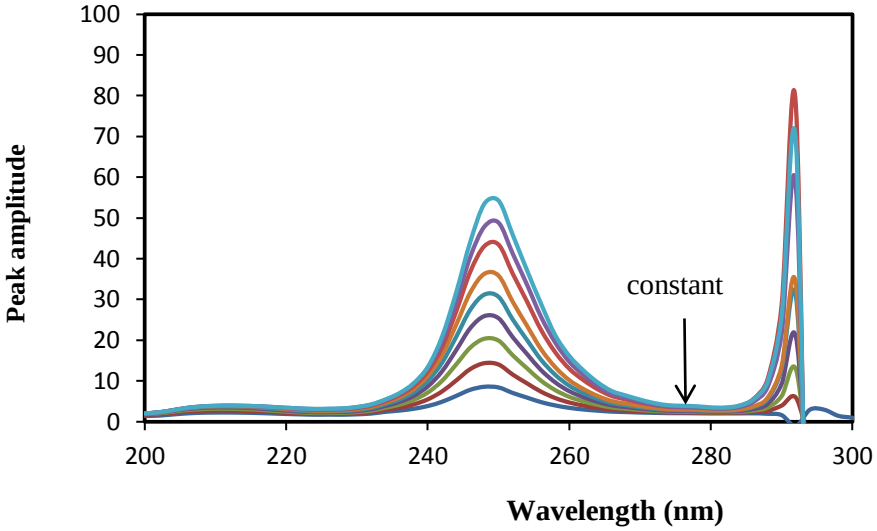



Figure 9: Ratio spectra of laboratory prepared mixtures of AM (5, 10, 15, 20, 25, 30, 35, 40 and 45) µg/mL and 50 µg/mL of AT using 30 µg/mL of AT as a divisor

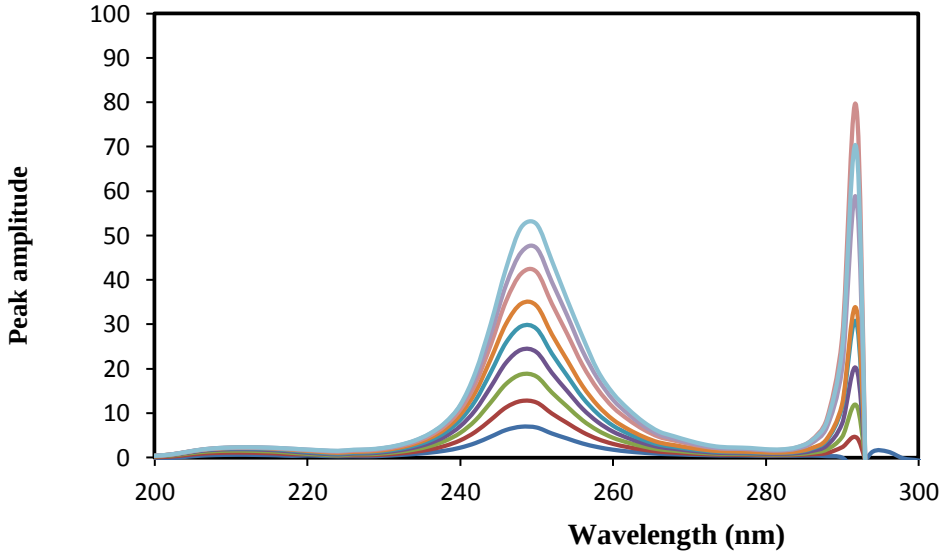



Figure 10: Ratio spectra of laboratory prepared mixtures of AM (5, 10, 15, 20, 25, 30, 35, 40 and 45) µg/mL and 50 µg/mL of AT using 30 µg/mL of AT as a divisor after subtraction of the constant

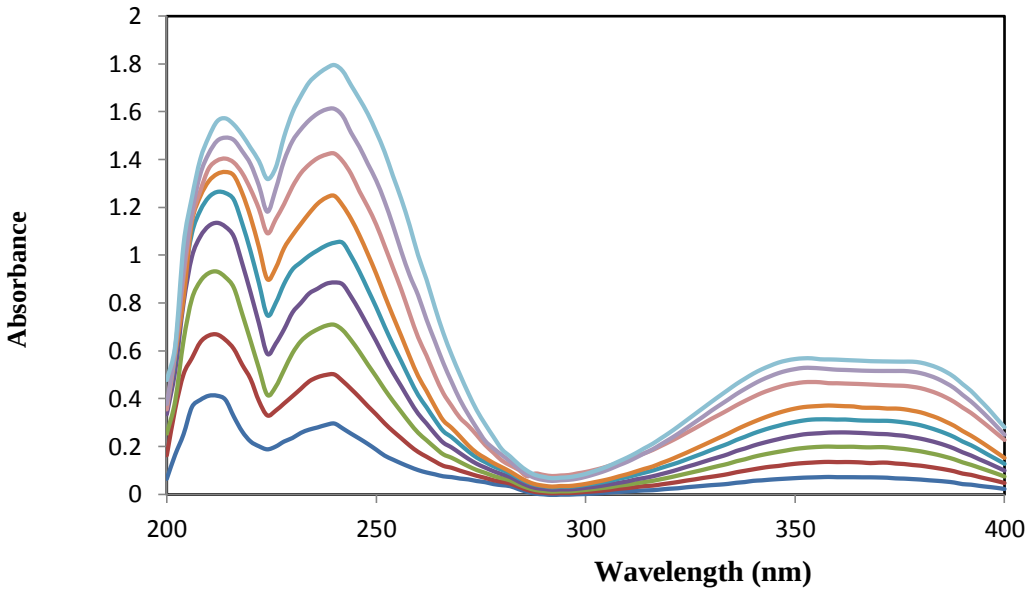



Figure 11: The zero order absorption spectra of AM (5, 10, 15, 20, 25, 30, 35, 40 and 45) µg/mL gained by the proposed extend ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication by the divisor AT

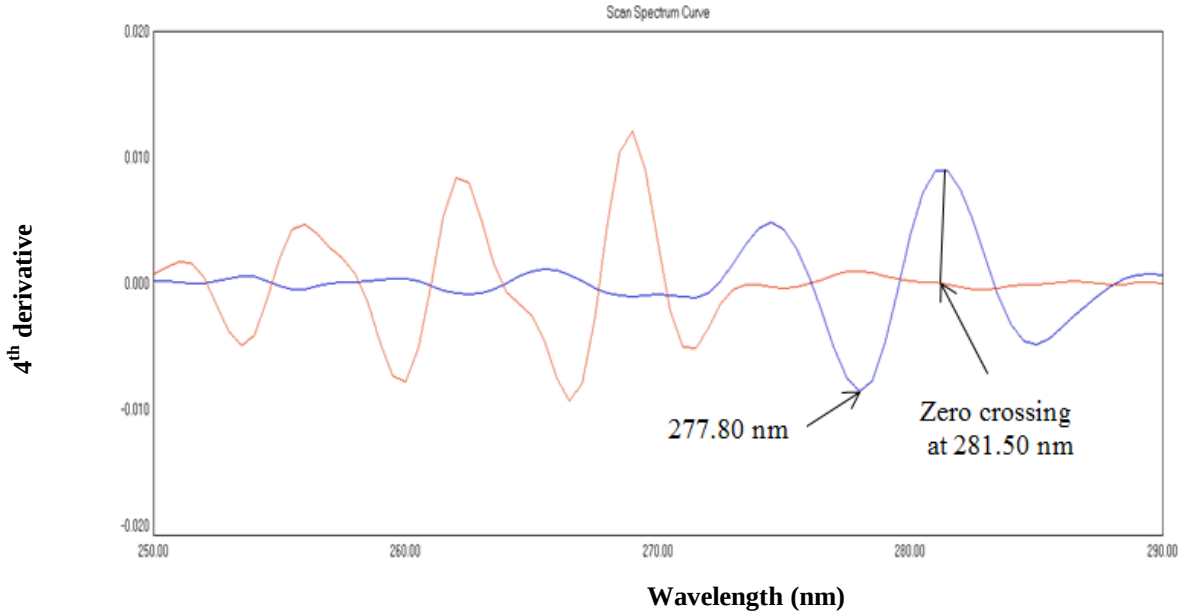



Figure 12: Fourth derivative spectra of 20 µg/mL AM, and 30 µg/mL of AT

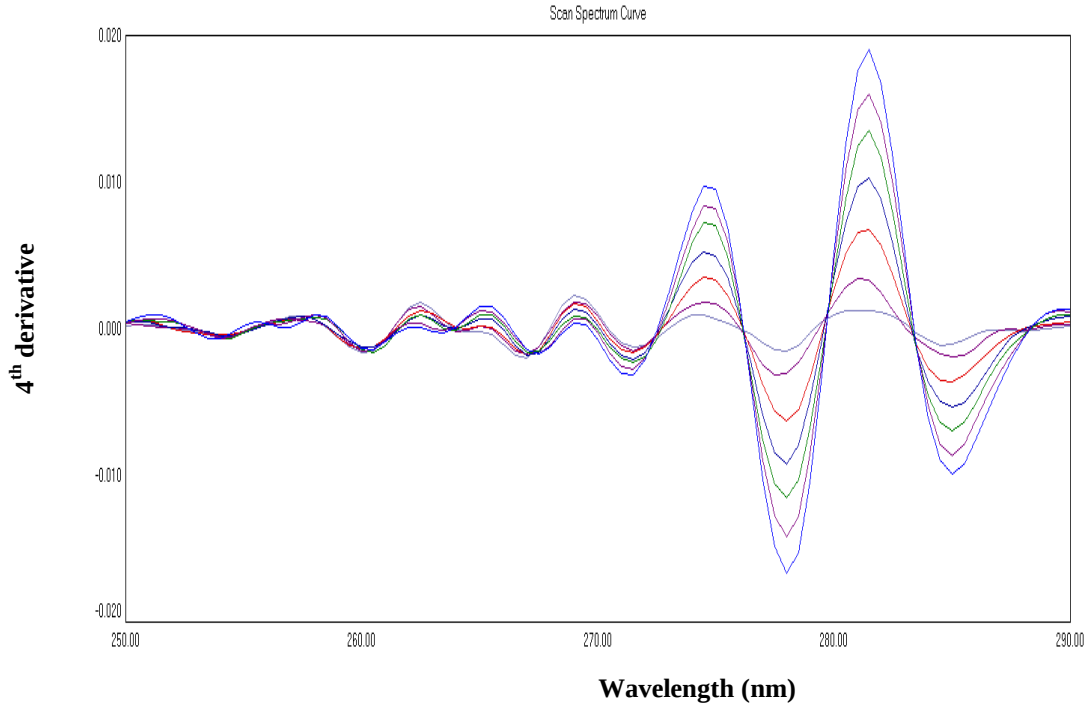



Figure 13: Fourth derivative spectra of binary mixture (5, 10, 20, 30, 40, 50 and 60 µg/mL) of AT in the presence of 20 µg/mL of AM

### 3.3. Accuracy and Precision

The pure drug solution was measured at three various concentrations with five replications for each concentration to assess the precision of the suggested methods. The results depend on the value of the relative standard deviation percentage which was in the range of 0.41-2.73 to determine amlodipine in the occurrence of atenolol, and for determination of atenolol it was in the range of 1.39-4.07 in the presence of amlodipine. The accuracy of the proposed methods was calculated through the percentage recovery value (Table 2), and it was ranged from 94.39% to102.83 %.

### 3.4. Interference Study

The influence of different compounds present in the commercial drugs on the determination of 25 µg/ml of atenolol and 25 µg/ml of amlodipine with two different spectrophotometry techniques were studied. The method was tolerable to 500 µg/ml of each of fructose and starch, whereas it is tolerable to 1000 µg/ml for each of glucose, cellulose, lactose, glycogen, sucrose, maltose, KCl, and NaCl. The relative error percentage was within ±5.0%.

### 3.5. Application of the Methods

The two methods were applied excellently for the measuring of atenolol and amlodipine in some available drugs in the markets. The outcomes of the estimation of active ingredients in the commercial drugs are showen in Table 2. amlodipine was determined at 246 nm and 240 nm while atenolol was determined at 284 nm, 281.50 nm and 277.80 nm in the combined dose drugs. The results confirme good agreement between obtained results by proposed method and that obtained by HPLC method (Commission, 2008).

**Table 1:** The statistical parameter for amlodipine and atenolol estimation using the proposed methods

| methods | Compounds | λmax (nm) | Linearity µg/mL | Regression equation | $r^2$ | LOD µg/mL |
|---|---|---|---|---|---|---|
| $^2$DD | Amlodipine | 240 | 2-45 | y = 0.0209x + 0.0198 | 0.9993 | 0.57 |
| | | 246 | | y = 0.0797x + 0.0884 | 0.9995 | 0.46 |
| | Atenolol | 276 | 5-60 | y = 0.0026x + 0.0062 | 0.9961 | 1.34 |
| | | 284 | | y = 0.0025x + 0.0001 | 0.9996 | 1.26 |
| EXRSM | Amlodipine | 240 | 5-45 | y = 0.0371x + 0.1314 | 0.9994 | 1.04 |
| 4$^{th}$ Derivative | Atenolol | 277.80 | 5-60 | y = 0.0003x + 0.0003 | 0.9937 | 2.24 |
| | | 281.50 | | y = 0.0003x - 0.0004 | 0.9979 | 2.24 |

$^2$DD: second derivative of the ratio spectra
EXRSM: extended ratio subtraction method

**Table 2:** Percentage recovery of amlodipine and atenolol in commercial formulation by proposed methods

| Commercial name | Declared content (mg) | Found by $^2$DD method (mg) | Found by (EXRSM& 4$^{th}$ Derivative) method (mg) | Found by HPLC method (mg) | Recovery% $^2$DD method | Recovery% (EXRSM& 4$^{th}$ Derivative) method |
|---|---|---|---|---|---|---|
| Cipla | | | | | | |
| Amlodipine | 5.0 | 4.91 | 5.07 | 4.93 | 99.59 | 102.83 |
| Atenolol | 50.0 | 49.06 | 47.50 | 48.50 | 101.15 | 97.93 |
| Cipla | | | | | | |
| Amlodipine | 5.0 | 5.09 | 4.94 | 5.20 | 97.88 | 95.0 |
| Atenolol | 25.0 | 24.22 | 23.75 | 24.20 | 100.08 | 98.14 |
| Microlab | | | | | | |
| Amlodipine | 5.0 | 4.88 | 4.91 | 5.17 | 94.39 | 94.97 |
| Atenolol | 50.0 | 53.81 | 51.66 | 52.50 | 102.49 | 98.40 |

## 4. Conclusions

In this study, the second derivative of the ratio spectra ($^2$DD) and the extended ratio subtraction method (EXRSM) were used to determine a binary mixture of atenolol and amlodipine. These two approaches produced good results when used to resolve a binary mixture and do not necessitate the use of sophisticated or costly equipment. Also, they are easy and quick when compared with other methods. Hence, these two recommended methods are usually appropriate for the determination each of atenolol and amlodipine drugs in combined dosage.

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