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Research article

Different ratio spectra methods for simultaneous determination of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate

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Abstract

New, simple and specific spectrophotometric methods involving Direct spectrophotometric method ,derivative ratio spectra-zero-crossing method [DRSZ] double divisor ratio spectra derivative method [DDRD], and successive ratio spectra were developed and validated in accordance with ICH guidelines for the simultaneous estimation of Olmesartan Medoxomil [OLM], Amlodipine Besylate [AMLB], and Hydrochlorothiazide [HCTZ] in their combined pharmaceutical dosage form. First, Amlodipine [AMLB] was determined by direct spectrophotometry at 356.0 nm alone without any interference from OLM, and HCTZ. In the DRSZ method OLM, HCTZ, and AMLB were determined at 259.0nm, 279.0nm, and 358.0nm respectively, in the DDRD method OLM, HCTZ, and AMLB were determined at 266.0nm, 317.0nm, and 363.0nm respectively, and in the successive ratio spectra OLM, HCTZ, &AMLB were determined at 239.0nm, 276.0nm, and 375.0nm respectively. The calibration curves are linear over the concentration range of 5-50 µg mL^{-1} for OLM, HCTZ, and AMLB respectively for all methods. The proposed methods were successfully applied for the assay of the three analytes with excellent recoveries in Laboratory prepared mixtures. No interference was observed from common pharmaceutical additives. The methods are validated according to the ICH guidelines where accuracy, precision and repeatability are found to be within the acceptable limit.

Key words: Derivative ratio spectrum zero-crossing method [DRSZ], Double divisor ratio spectra derivative method [DDRD], Successive ratio spectra method.

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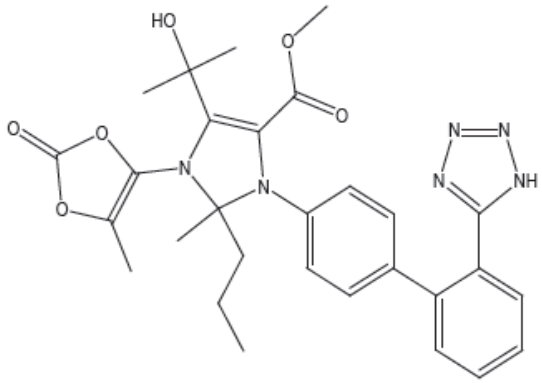
1. Introduction

Olmesartan Medoxomil [OLM] [Figure 1 a] is chemically known as N-[p-[o-1H-Tetrazol-5-ylphenyl] benzyl]-N-valeryl-L-valine [1]. It is a potent and selective angiotensin AT1 receptor blocker [2], It has been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a Medoxomil ester moiety which is cleaved rapidly by an endogenous esterase to release the active Olmesartan [3].

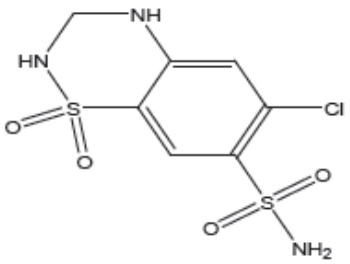
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Hydrochlorothiazide [HCTZ] [Figure 1 b] is chemically known as 6-chloro- 3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide [1]. It is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which inhibits Na Cl transport.

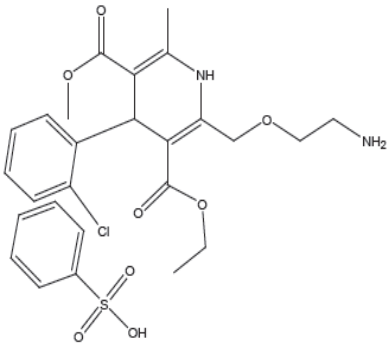
Amlodipine Besylate [AMLB] [Figure 1 c] is chemically known as 2-[[2-aminoethoxy] methyl]-4-[2-chlorophenyl]-1, 4-dihydro-6-methyl-3, 5-pyridine carboxylic acid 3-ethyl 5-methyl ester] [1].



[a] Structure of Olmesartan Medoxomil [OLM]



[b] Structure of Hydrochlorothiazide [HCTZ]



[c] Structure of Amlodipine Besylate [AMLB]

Figure 1. Chemical structures of [a] OLM, [b] HCTZ, and [c] AMLB.

It is a dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris [4].

Recently, OLM has been marketed in combination with AMLB and HCTZ in tablet dosage form [TRIBENZOR® tablets]. The triple combination of OLM, AMLB and HCTZ is intended for oral administration for the treatment of hypertension.

AMLB is official in British Pharmacopoeia [6], HCTZ is official in British Pharmacopoeia [6] and United States Pharmacopoeia [7].

Olmesartan, Amlodipine and Hydrochlorothiazide combination is not official in any pharmacopoeia so no official method is available for estimation of these drugs in Literature survey reveals that few methods are available for the simultaneous analysis of OLM, AMLB and HCTZ combination. There is one reported stability indicating UPLC method for the determination of OLM, HCTZ, and AMLB in pharmaceutical dosage form[8], HPLC [9-11] and spectrophotometry [12-16].

Therefore, the aim of this work was directed to the development of simple, sensitive, selective and validated spectrophotometric methods for the simultaneous determination of OLM,

AMLB and HCTZ in their combined dosage form.

Theoretical back ground

The main problem of spectrophotometric multi-component analysis is the difficulty of simultaneous determination of two or more active compounds in the same mixtures without preliminary separation. Several spectrophotometric determination methods have been used for resolving mixtures of compounds with overlapping spectra, such as classical derivative spectrophotometry [17–21], Vierordt's method[22]and its modified version [23], orthogonal function method [24], Fourier functions method [25-26] dual wavelength spectrophotometry [27-28], pH-induced differential spectro-photometry [29], and least square method [30], multi-component analysis program [31-32], principle component regression [PCR] [33], multi-wavelength linear regression analysis [MLRA] [34] and H-point standard addition method [HPSAM] [35-36].

However, sometimes the treatment of the normal absorbance data cannot cope with the level of interference especially when the spectra are strongly overlapped, thus special data treatment is required. Recently, the treatment of absorbance ratio spectra has been the basis of some analytical procedures so as to generate signals for the mixture depending only on a single analyte.

Examples of these methods are the ratio-derivative spectrum method, derivative ratio spectra-zero-crossing method [DRSZ] [37-40], Salinas et al. proposed the spectrophotometric method termed ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures, Berzas Nevado et al. [41] developed a method for the analysis of ternary mixtures by derivative ratio spectra-zero-crossing technique. In this method, the simultaneous determination of three compounds in ternary mixtures is realized by measuring of the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra. These two methods were further studied and applied for simultaneous determination of binary or ternary mixtures [37-42].

Double divisor ratio spectra derivative method [DDRD] [39-43], Recently, Dinc et al. [44-45] has proposed a new spectrophotometric method for the simultaneous determination of ternary mixtures. This method is called "the double divisor ratio spectra derivative method". This method is based on the use of the coincident spectra of the derivative of the ratio spectra obtained by using a "double divisor" [sum of two spectra] and measuring at either the maximum or minimum wavelengths. Successive derivative ratio spectra [46] and a method based on the mean centering of ratio spectra [47].

Fourier function-ratio spectrum method [48-49]. Unfortunately, the advantages of derivative spectra are at least partially offset by degradation in signal-to-noise ratio that accompanies obtaining derivatives. Recently, Wahbi et al. introduced the Fourier function-ratio spectrum method for determination of binary mixtures [50-51].

2. Materials and Methods

Apparatus

Spectrophotometric measurements were recorded using a Jasco [UV-530] –UV is a double beam spectrophotometer with two matched 1 cm Quartz cells and connected to personal computer loaded with [Jasco]-[spectra manager] software.

Pure drugs

Olmesartan Medoxomil [99.9%], Hydrochlorothiazide [99.9%] %], and Amlodipine Besylate [99.9%] Nutra Specialties private Limited; all were gifted from Chemipharm –Egypt.

Reagents &Materials

Acetonitrile [analytical grade], Starch, Microcrystalline cellulose, Magnesium stearate, and Silicon dioxide all were purchased from [Sigma Aldrich].

Stock Standard Solutions

Stock standard solution was prepared by dissolving OLM in acetonitrile to obtain solution having a concentration of 1.00 mg. mL^{-1}. One mL of this solution was transferred into 10 mL volumetric flasks, completing to the volume with acetonitrile to obtain stock standard solution of OLM having a concentration of 100.0 µg.mL^{-1}. The solution was stable for one week when refrigerated at 4°C.

In the same manner prepare each HCTZ, and AMLB stock standard solution with a concentration of 100.0 µg.mL^{-1}.

Working Standard Solutions

Working OLM standard solutions were prepared by accurately transferring different volumes of stock standard OLM solution into 10ml volumetric flask, completing to volume with ACN to obtain solutions in the concentration range of [5-50 µgmL^{-1}]

In the same manner prepare HCTZ, and AMLB solutions.

Laboratory prepared mixture

Synthetic mixture simulating to tablet was prepared and an aliquot equivalent to one tablet of [40mg of OLM, 13.9mg of AMLB [equivalent to 10 mg of AML base], and 25 mg of HCTZ] with excipients [140 mg of Starch, 156.322 mg of Microcrystalline cellulose, 1.6 mg Magnesium stearate, and 3.19 mg of Silicon dioxide] were dissolved in 100 ml ACN with the aid of sonication and filtered.

5ml of the filtrate was transferred into 50-ml volumetric flask and diluted with ACN then the final solution of the laboratory prepared mixture was assayed using the proposed method.

In the same manner we prepare another solution representing dosage form with different ratio of [OLM 20mg -HCTZ 12.5mg - AMLB 6.95mg] with excipients [70 mg of Starch, 78.161 mg of microcrystalline cellulose, 0.8 mg Magnesium stearate and 1.595 mg of Silicon dioxide].

Procedures

Direct spectrophotometric method for determination of AMLB

AMLB can be determined at 356.0 nm directly from zero order spectra where OLM and HCTZ show no absorbance. A linear calibration curve relating the absorbance at 356.0 nm to the corresponding concentration of AMLB was constructed and regression equation was computed [Figure 2].

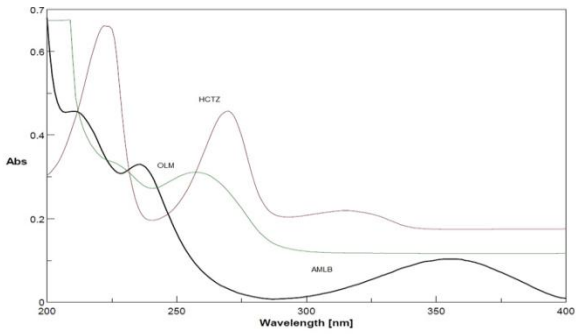


Figure 2. The absorption spectra of OLM [40 µg/ml], HCTZ [25 µg/ml], and AMLB [13.9 µg/ml] in acetonitrile

Derivative ratio spectrum zero-crossing method [DRSZ]

The recorded absorption spectra of OLM, AMLB and their ternary mixture with HCTZ were divided by a standard spectrum of HCTZ [10µg/ml] and the first derivative of the ratio spectra was calculated with Δλ= 3

nm in the range 200–400 nm. In the ternary mixture, OLM and AMLB can be determined by measuring the first derivative signals at 259.0 and 358.0 nm, respectively, similarly, the stored spectra of HCTZ and AMLB and their ternary mixture were divided by a standard spectrum of OLM [10 µg/ml] and the first derivative of the result was plotted with Δλ= 3 nm from 200 -400 nm. The concentration of HCTZ can be determined at 279.0 nm.

Double divisor ratio spectra derivative method [DDRD]

The absorption spectra of the solutions prepared at different concentrations of OLM and of the ternary mixture were recorded and divided by the sum of the absorption spectra of solutions of HCTZ and AMLB [10µg/ml each in ACN] as "double divisor" and the ratio spectra [I] were obtained. First derivatives of the ratio spectra were plotted with Δλ= 3 nm. The amount of OLM was determined by measuring the amplitude at 266.0 nm corresponding to a minimum in the first derivative of the ratio spectra in the spectral region selected [200–400 nm].

The absorption spectra of the solutions prepared at different concentrations of HCTZ and of the ternary mixture were recorded and divided by the sum of the absorption spectra of solutions of OLM and HCTZ [10µg/ml each in ACN] as "double divisor" to get the ratio spectra [II] and their first derivatives were plotted with Δλ= 3 nm from the ratio spectra. The content of HCTZ was determined from the first derivative of the ratio spectra between 200 and 400 nm and measuring the signals at 317.0 nm, corresponding to a minimum.

In the same way, the absorption spectra of the solutions prepared at different concentrations of AMLB and of the ternary mixture were stored and divided by the spectrum of the standard mixture solution of OLM and HCTZ [10 µg/ml each in ACN] as "double divisor" to give the ratio spectra [III] then their first derivatives were traced with Δλ= 3 nm from the ratio spectra.

The concentration of AMLB was determined by measuring the first derivative of the ratio spectra between 200 and 400 nm and measuring the signals at 363.0 nm, corresponding to a minimum.

Successive derivative ratio spectra method

The absorption spectra of different concentrations of OLM were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg mL^{-1} of HCTZ and the ratio spectra were obtained. First derivative of the ratio spectra was obtained with Δλ=3 nm . These vectors [first derivative of the ratio spectra] were then divided by [d/dλ][AMLB/HCTZ] corresponding to the derivative of the ratio of the spectra of 10 µg mL^{-1} of each and then the second ratio spectra were obtained. The first derivative of these ratio spectra obtained from which OLM was determined by measuring the peak amplitude at 239.0 nm.

In the same way, the absorption spectra of the solutions prepared at different concentrations of HCTZ were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg mL^{-1} of AMLB and ratio spectra were obtained. First derivative of the ratio spectra was plotted with Δλ= 3 nm .After that these vectors [first derivative of ratio spectra] are divided by [d/ dλ][OLM/AMLB] corresponding to the derivative of the ratio of the spectra of 10 µg mL^{-1} of each of OLM and AMLB and then second ratio spectra were obtained. The concentration of

HCTZ was determined by measuring the peak amplitude at 276.0 nm in the first derivative of ratio spectra.

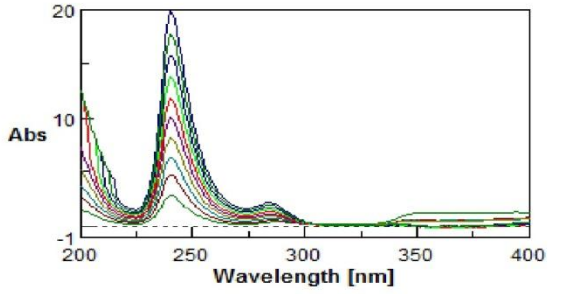
By the same way, the concentrations of AMLB were determined by measuring the peak amplitude of the first derivative of the first ratio spectra at 357.0 nm using the

spectra of 10 µg mL^{-1} of each of OLM and HCTZ to obtain the second derivative of ratio spectra.

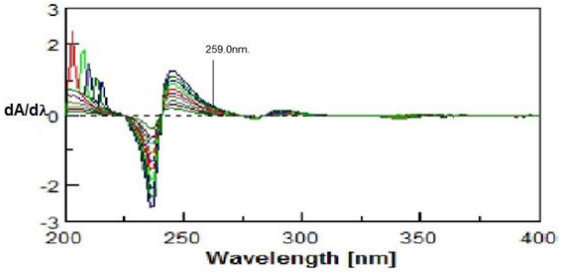
3. Results and discussion

DRSZ Method

In this method, the absorption spectra of the ternary mixture solutions of OLM, HCTZ and AMLB in ACN were divided by the spectrum of the standard solution of 10 µg/ml HCTZ in the same solvent and the ratio spectra of OLM–AMLB were obtained. Figure 3 shows the first derivative of the ratio spectra plotted with intervals of Δλ= 3 nm. The concentrations of OLM and AMLB in the ternary mixture were determined by measuring the signals at 259.0 nm for OLM [the wavelength which corresponds to the zero-crossing point of AMLB] and 358.0 nm for AMLB [the wavelength which corresponds to the zero-crossing point of OLM] Figure 4**.**

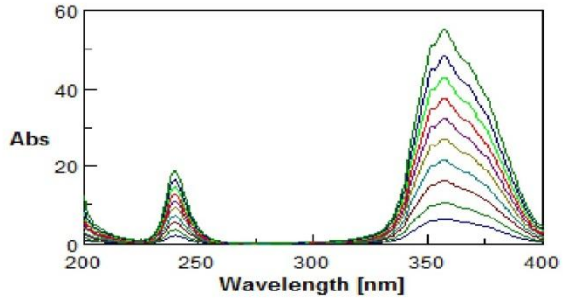


[a]

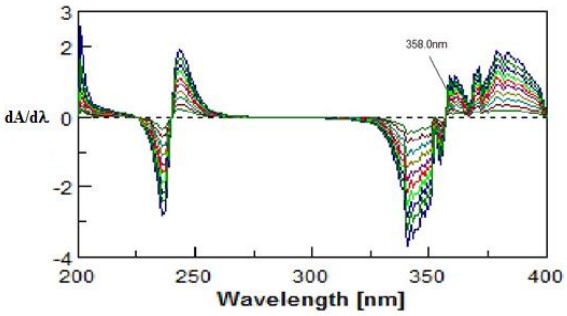


[b]

Figure 3. [a] The Ratio spectra of OLM [5-50µg/ml]in acetonitrile using HCTZ [10µg/ml] as a divisor [Δ λ=3],and [b] The first derivative of Ratio spectra



[a]



[b]

Figure 4. [a] The Ratio spectra of AMLB [5-50µg/ml] in acetonitrile using HCTZ [10µg/ml] as a divisor [Δ λ=3], and [b] The First derivative of the Ratio spectra

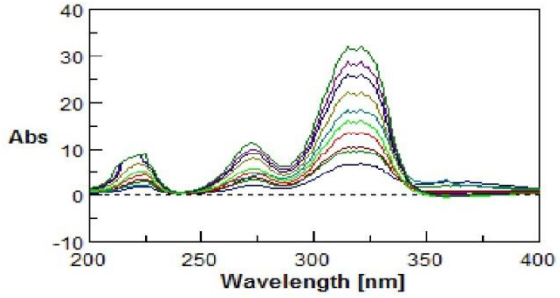
In a similar manner, the absorption spectra of the ternary mixture solutions of OLM, HCTZ and AMLB in ACN were divided by the spectrum of the standard solution of 10µg/ml OLM in the same solvent and the ratio spectra of HCTZ–AMLB were obtained by following the same procedure as above**.** Figure 5 shows the first derivative of the ratio spectra plotted with intervals of Δλ=3nm from the ratio spectra. The content of HCTZ in the ternary mixture was determined by measuring the signal at 279.0 nm for HCTZ [wavelength corresponding to zero-crossing point for AMLB].

Method optimization

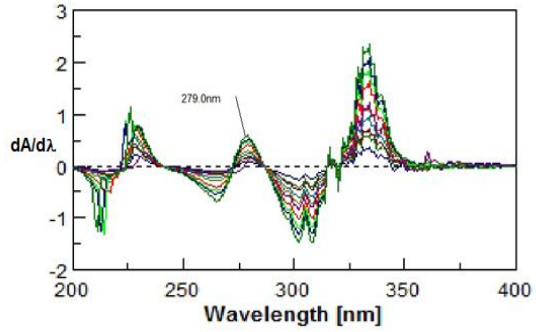
The influence of the wavelength interval ,Δλ, for obtaining the first derivative of the ratio spectra was tested and a value of Δ λ = 3nm was considered suitable for OLM, HCTZ and

AMLB determinations maximum recovery was obtained using the selected Δλ.

The effect of divisor concentration on the calibration graphs was studied. It affects only the slope of the standard curves while the maxima and minima remain at the same wavelengths. The standard solutions of 10µg/ml of HCTZ for determining OLM and AMLB and of 10µg/ml of OLM for the determination of HCTZ in their ternary mixtures and in the ratios present in their pharmaceutical preparations were found suitable.



[a]

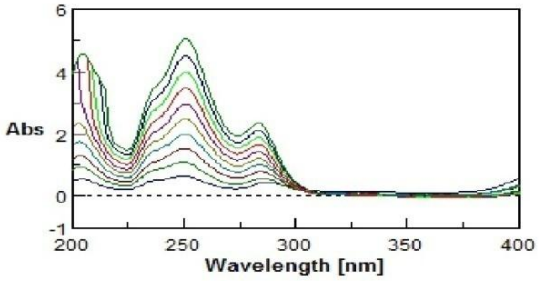


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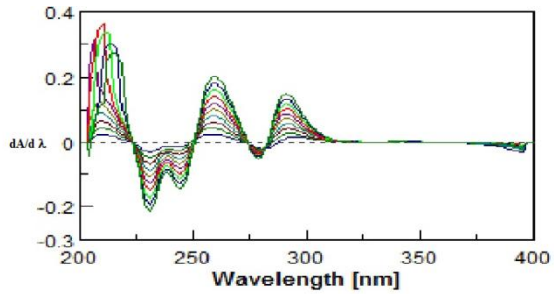
Figure 5. [a] The Ratio Spectra of HCTZ [5-50µg/ml] in acetonitrile using OLM [10µg/ml] as a divisor [Δ λ=3], and [b]The First derivative of the ratio spectra.

DDRD method

The absorption spectra of the solutions of OLM in ACN were recorded in the range 200–400 nm and divided by the double divisor [10µg/ml HCTZ and 10µg/ml AMLB] to obtain their corresponding ratio spectra Figure 6a. First derivatives of the ratio spectra thus obtained were calculated with interval of Δλ=3nm as shown in Figure 6b. The concentration of OLM was determined by measuring the amplitude at 266.0 nm corresponding to a minimum point.



[a]



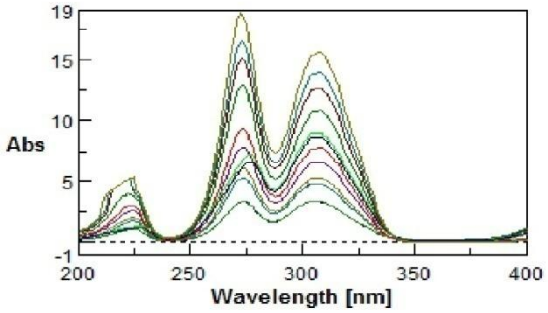
[b]

Figure 6. [a] The Ratio spectra of OLM [5-50µg/ml] in acetonitrile using HCTZ [10µg/ml]+ AMLB[10µg/ml] as double divisor [Δλ=3], and [b] The first derivative of the Ratio spectra.

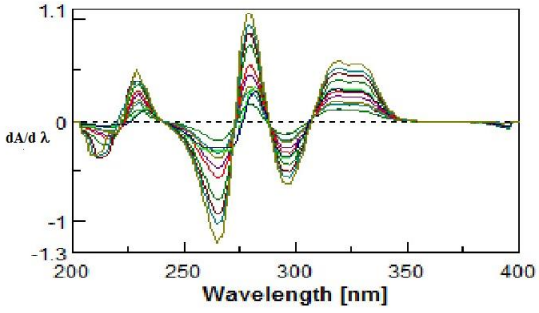
The absorption spectra of the solutions prepared at different concentrations of HCTZ in ACN were recorded between 200 and 400 nm and divided by the spectrum of the standard mixture solution of 10µg/ml OLM and 10 µg/ml AMLB The ratio spectra were thus obtained Figure 7a and their first derivatives were traced with Δλ= 3 nm. The concentration of HCTZ was determined by measuring the amplitude at 317.0 nm corresponding to a minimum point Figure 7b.

Similarly, the absorption spectra of the solutions prepared at different concentrations of AMLB in ACN in the range

200-400 nm were stored and divided by the double divisor [10µg/ml OLM and 10µg/ml HCTZ]. The ratio spectra thus obtained Figure 8a were differentiated with interval of Δλ=3 nm to the corresponding first derivatives Figure 8b. The concentration of AMLB was determined by measuring the amplitude at 363.0 nm corresponding to a minimum point.

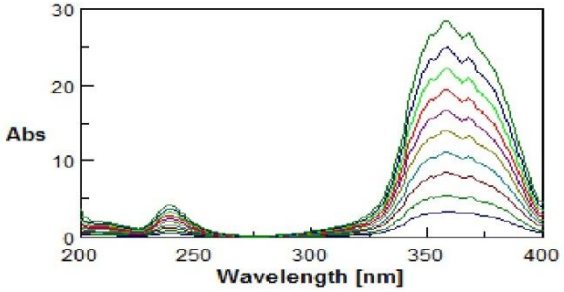


[a]

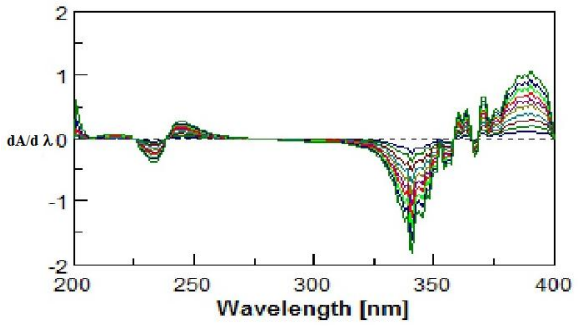


[b]

Figure 7. [a] The Ratio spectra of HCTZ[5-50µg/ml] in acetonitrile using OLM [10µg/ml]+ AMLB [10µg/ml]as double divisor [Δʎ=3], and [b] The first derivative of Ratio spectra.



[a]



[b]

Figure 8. [a] The Ratio spectra of AMLB[5-50µg/ml] in acetonitrile using {OLM [10µg /ml] + HCTZ[10µg/ml]} as double divisor [Δλ=3], and [b]The first derivative of the Ratio spectra.

These coinciding points of the derivative of the ratio spectra were selected as working wavelengths, as mentioned above, for the determinations of the subject compounds in the ternary mixture.

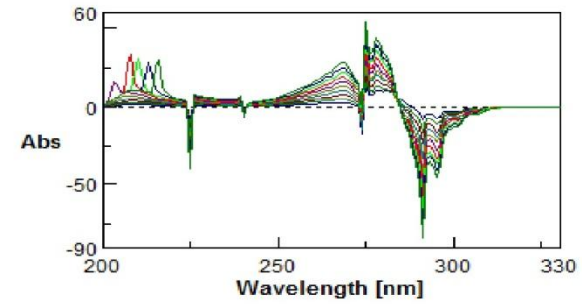
Method optimization

The main instrumental parameter conditions were optimized for reliable determination of the active compounds. For selecting the standard binary mixture as double divisor at appropriate concentrations, a very important factor in practice, some double divisor concentrations were tried. The sum of the spectra of 10µg/ml HCTZ and 10µg/ml AMLB as a 'double divisor' for determining OLM; 10µg/ml OLM and 10µg/ml HCTZ as a 'double divisor' for determining HCTZ and of 10 µg/ml OLM and 10 µg/ml HCTZ as a 'double divisor' for determining AMLB were found suitable for the determination of the three drugs in the ratios present in their pharmaceutical preparations.

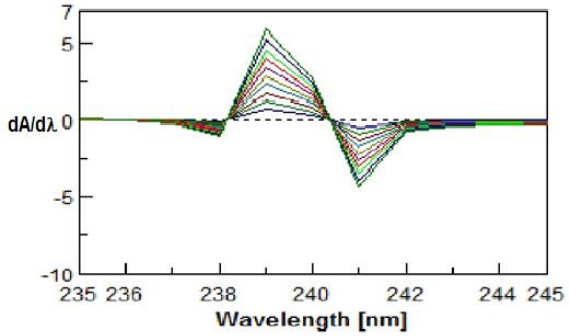
Furthermore, the influence of Δλ for the first derivative of the ratio spectra was studied. It was found appropriate to use the values of Δλ= 3 nm that gives optimum recovery for the determination of OLM, HCTZ and AMLB, respectively.

Successive derivative ratio spectra method

The absorption spectra of different concentrations of OLM were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg mL^{-1} of HCTZ and the ratio spectra were obtained. First derivative of the ratio spectra was obtained with Δλ =3 nm. These vectors [first derivative of the ratio spectra] were then divided by [d/dλ][AMLB/HCTZ] corresponding to the derivative of the ratio of the spectra of 10 µg mL^{-1} of each and then the second ratio spectra were obtained. The first derivative of these ratio spectra was obtained from which OLM was determined by measuring the peak amplitude at 239.0 nm Figure 9.



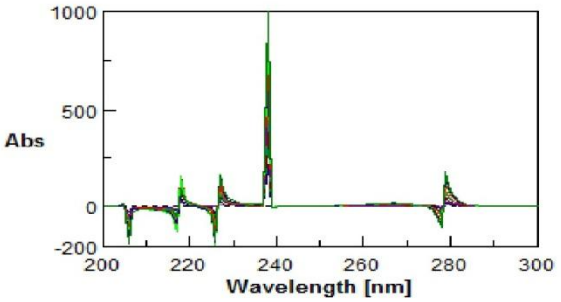
[a]



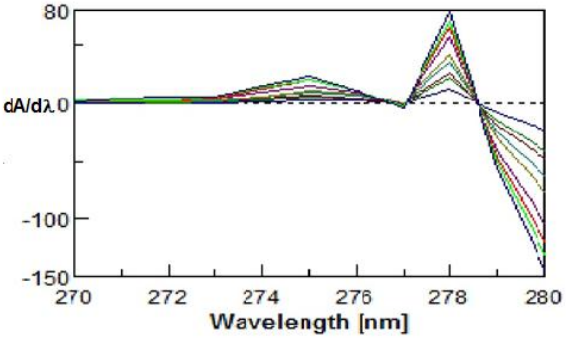
[b]

Figure 9. [a] The second ratio spectra of different concentrations [5-50µgmL⁻¹] of OLM / AMLB divided by {HCTZ/AMLB [10 µg/ml EACH] µg/ml]} in acetonitrile, and [b] the first derivative of their second ratio spectra obtained with Δλ = 3 nm.

In the same way, the absorption spectra of the solutions prepared at different concentrations of HCTZ were recorded in the range of 200–400 nm and divided by the spectrum of the standard solution of 10 µg .mL^{-1} of AMLB and ratio spectra were obtained. First derivative of the ratio spectra was plotted with Δλ= 3 nm. After that these vectors [first derivative of ratio spectra] are divided by [d/dλ] [OLM/AMLB] corresponding to the derivative of the ratio of the spectra of 10 µg mL^{-1} each of OLM and AMLB and then second ratio spectra were obtained. The concentration of HCTZ was determined by measuring the peak amplitude at 276.0 nm in the first derivative of ratio spectra Figure 10.



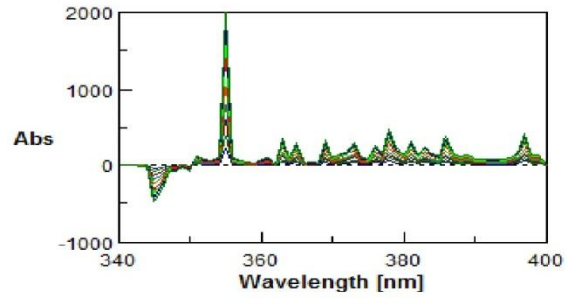
[a]



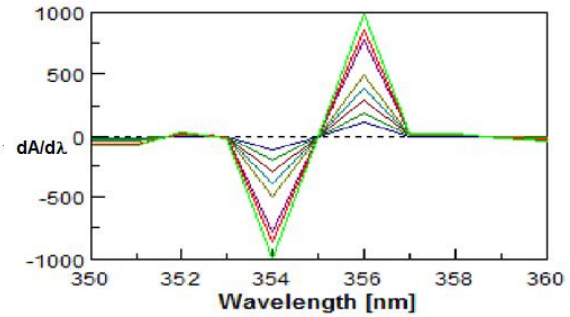
[b]

Figure 10. [a] The second ratio spectra of different concentrations [5-50µgmL⁻¹] of HCTZ/ AMLB divided by {OLM/AMLB [10 µg/ml EACH] µg/ml]} in acetonitrile, and [b] the first derivative of their second ratio spectra obtained with Δλ = 3 nm.

By the same way, the concentrations of AMLB were determined by measuring the peak amplitude of the first derivative of the first ratio spectra at 357.0 nm using the spectra of 10 µg mL $^{-1}$ of each of OLM and HCTZ to obtain the first derivative of ratio spectra Figure 11.



[a]



[b]

Figure 11. [a] The second ratio spectra of different concentrations [5-50µgmL^{-1}] of AMLB/OLM divided by {HCTZ/OLM [10 µg/ml EACH] µg/ml]} in acetonitrile, and [b] the first ratio derivative of their second ratio spectra obtained with Δλ = 3 nm.

Validation of Method

As per ICH guideline the method is validated and following parameters were evaluated.

Linearity

Under the above described experimental conditions, the graphs obtained by plotting the first derivative absorbance ratio[DRSZ method], first derivative double divisor absorbance ratio [DDRD method] and first derivative of successive ratio spectra versus concentration for OLM, HCTZ and AMLB show linear relationships in the range of [5-50 µg ml^{-1}] for the three drugs. Using the method of least squares, regression equations, correlation coefficients, limits of detection and quantitation were calculated for each of the three drugs and the results are summarized in Table 1. The high values of the correlation coefficients with negligible intercepts indicate the good linearity of the calibration graphs.

Precision

Separate determinations at different concentration levels were carried out for each drug to test reproducibility of measured analytical signals. The percentage relative standard deviations [R.S.D. %] were found to be less than 2% as shown in tables [2,3,&4].

Accuracy

In order to assess the accuracy of the three previously discussed methods, several synthetic mixtures, covering the linearity ranges, with different ratios of OLM, HCTZ and AMLB were analyzed. The percentage recovery for each mixture is calculated as shown in [Table 5]. It is obvious that the three methods could be applied to resolve the three drugs in their ternary mixtures with good accuracy.

The mixture solutions of OLM, HCTZ and AMLB combination in the concentration ratios simulating tablets ratios, 40:25:13.9 and 20:12.5:6.95 [OLM: HCTZ: AMLB], were prepared in six replicates.

Each mixture solution was resolved independently for its OLM, HCTZ and AMLB, by measuring its analytical signals, each at selected wavelengths. The proposed methods successfully determine the three drugs in the dosage form without previous separation or extraction procedures as shown in [Table 5].

Table 1. Calibration data for the determination of Olmesartan Medoxomil [OLM], Hydrochlorothiazide[HCTZ], & Amlodipine Besylate [AMLB] by the proposed methods

	λ[nm]	Linearity [µg/ml]	Regression Equations	r	Sa	Sb	Sy/x	LOD [µg/ml]	LOQ [µg/ml]
Direct spectrophotometric method for determination of AMLB	356	5-50	Y=0.0054x+0.001	0.9999	0.002	0.00005	0.00005	0.990	3.002
DRSZ									
OLM	259	5-50	Y=0.0075x+0.004	0.9992	0.002	0.00008	0.00001	1.054	3.190
HCTZ	279	5-50	Y=0.0149x+0.0692	0.9999	0.015	0.0004	0.001	0.534	1.621
AMLB	358	5-50	Y=0.016 x+0.0449	0.9992	0.044	0.001	0.026	0.896	2.170
DDRD									
OLM	266	5-50	Y=0.0028x-0.001	0.9989	0.001	0.00004	0.00002	1.35	4.100
HCTZ	317	5-50	Y=0.011x+0.019	0.9984	0.006	0.0001	0.0002	2.069	3.610
AMLB	363	5-50	Y=0.0089x-0.001	0.998	0.004	0.0001	0.0002	1.650	5.00
Successive Ratio:									
OLM	239	5-50	Y=0.115x+0.303	0.9989	0.369	0.102	2.301	2.941	3.66
HCTZ	276	5-50	Y=0.2225x+1.576	0.998	0.840	0.109	4.362	1.624	4.921
AMLB	357	5-50	Y=0.4145x+0.468	0.9999	0.450	0.033	3.337	0.263	3.442

Sa =standard error of the intercept, Sb=standard error of the slope, S y/x=residual standard deviation of regression line LOD=limit of detection, LOQ=limit of quantitation

Table 5. Assay results for the determination of OLM, HCTZ and AMLB in synthetic mixtures [in presence of excipients] using the proposed methods

Concentration of mixture component [OLM:HCTZ:AMLB] [µg/ml]	**Mean % Recovery* OLM**			**Mean % Recovery* HCTZ**			**Mean% Recovery* AMLB**			
	DRSZ 259nm	**DDRD 266nm**	**Successive 239nm**	**DRSZ 279nm**	**DDRD 317nm**	**Successive 276nm**	**Direct spectro-photometric 356.0nm**	**DRSZ 358nm**	**DDRD 363nm**	**Successive 357nm**
OLM40:HCTZ25:AMLB13.9	104	99.00	95.66	100.08	97.06	100.08	98.5	102.15	102.15	99.28
±SD	0.05	0.90	2	1.7	0.692	1.7	1.502	0.85	0.816	1.96
OLM20:HCTZ12.5:AMLB6.95	104.5	100.00	97.41	96.00	95.20	97.732	102	95.68	100.0	102.15
±SD	0.05	0.51	1.43	0.05	0.1	1.9	2	0.057	0.5196	1.68

*n=6

Table 2. Results of precision study for determination OLM, HCTZ, &AMLB using the proposed DRSZ method:

Concentration taken		Intra-day precision			Inter-day precision		
		Mean % Recovery*	S.D.	% R.S.D.	Mean % Recovery *	S.D.	% R.S.D.
OLM	20	103	0.208	0.202	103.2	0.585	0.568
	40	104.2	0.915	0.878	103.7	1.654	1.595
	40	102.7	1.038	1.011	102.96	0.96	0.933
	40	104	0.398	0.383	103.466	1.086	1.05
	40	104.5	2.07	1.988	102.166	1.941	1.9
HCTZ	12.5	99	1.514	1.533	99.5	1.184	1.19
	12.5	100.6	0.857	0.852	100.6	0.642	0.638
	25	98.8	0.172	0.175	99.4	1.114	1.125
	12.5	99.1	1.962	1.98	98.9	1.165	1.178
	25	99.1	1.02	1.03	99.033	0.955	0.965
AMLB	6.95	97.27	0.663	0.682	98.11	1.132	1.154
	6.95	97.70	0.458	0.469	98.2	1.882	1.917
	6.95	104.4	0.25	0.24	102.91	1.106	1.075
	13.9	102.7	1.499	1.46	102	1.323	1.297
	13.9	100.97	1.442	1.429	100.64	1.82	1.809

***n=3**

Table 3. Results of precision study for determination OLM, HCTZ, &AMLB using the proposed DDRD method:

Concentration taken		Intra-day precision			Inter-day precision		
		Mean % Recovery *	S.D.	% R.S.D.	Mean % Recovery *	S.D.	% R.S.D.
OLM	20	96.4	0.482	0.501	98.8	0.614	0.621
	40	95.2	0.599	0.63	98.6	0.972	0.986
	40	100.3	0.609	0.608	100.4	0.888	0.885
	40	95.1	0.998	0.105	95.1	0.155	0.163
	40	98.1	1.442	1.470	98.41	1.387	1.41
HCTZ	12.5	100.1	0.961	0.961	98.476	1.309	1.329
	12.5	97.97	1.101	1.124	99.59	1.043	1.048
	25	100.7	2.01	2	99.33	0.864	0.869
	12.5	98.26	0.216	0.22	98.14	0.229	0.233
	25	98.49	1.335	1.356	98.54	0.969	0.983
AMLB	6.95	101.08	1.876	1.856	100.28	1.452	1.448
	6.95	104.16	1.442	1.385	103.25	1.401	1.357
	6.95	103.1	1.428	1.386	102.4	1.6305	1.592
	13.9	101.5	0.548	0.540	101.75	1.1	1.081
	13.9	100.9	0.49	0.486	102.1	1.276	1.25

***n=3**

Table 4. Results of precision study for determination OLM, HCTZ, &AMLB using the proposed Successive Ratio Spectroscopy method:

Concentration taken		Intra-day precision			Inter-day precision		
		Mean % Recovery *	S.D.	% R.S.D.	Mean % Recovery *	S.D.	% R.S.D.
OLM	20	101.16	0.5476	0.539	99.442	1.275	1.283
	40	101.533	0.941	0.9269	101.187	1.294	1.279
	40	95.16	0.288	0.303	95.43	0.501	0.525
	40	95.233	0.404	0.424	95.422	1.525	1.598
	40	95.976	1.496	1.559	96.293	1.59	1.651
HCTZ	12.5	98.5	1.97	2	99.966	1.904	1.905
	12.5	104.5	0.499	0.478	101.576	0.385	0.379
	25	103.1	1.249	1.212	100.756	1.184	1.175
	12.5	96.33	0.584	0.606	98.976	0.878	0.887
	25	98.15	0.785	0.799	99.626	1.179	1.184
AMLB	6.95	98.1	1.905	1.942	98.91	1.675	1.693
	6.95	98.866	1.962	1.985	97.808	1.501	1.535
	6.95	95.06	0.109	0.115	97.275	0.549	0.565
	13.9	96.733	1.553	1.605	97.031	1.507	1.553
	13.9	97.26	0.7504	0.771	97.385	1.008	1.035

***n=3**

Conclusion

The proposed methods are simple, sensitive and easy to be applied for simultaneous determination of OLM, HCTZ, and AMLB, in their ternary mixture with good precision.
These methods don't need tedious extraction procedures or sophisticated instrumentation such as HPLC.

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