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Simultaneous Estimation of Telmisartan and Amlodipine by Second Derivative Spectrophotometric Method and First Derivative Ratio-Spectrophotometric Method

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ABSTRACT

Two simple, specific, precise and accurate spectrophotometric methods have been developed for the simultaneous estimation of Telmisartan (TEL) and Amlodipine besylate (AML) involving: second derivative Spectrophotometric method (I) and first derivative Ratio -Spectrophotometric Method (II). Method (I) is based on measurement of amplitude of second derivative spectrum absorbance at two wavelengths; 329 nm and 368 nm for Telmisartan and Amlodipine besylate respectively. The latter (method II) depends on measurement of amplitude of the first derivative of the ratio spectrum at two wavelengths, 319 nm and 288 nm for Telmisartan by using 2 µg/mL of AML as a divisor and 393 nm for Amlodipine besylate by using 4 µg/mL of TEL as a divisor. Beer's law obeyed in concentration range of 1 - 35 µg/ mL and 2- 16 µg/ mL for Telmisartan and Amlodipine besylate respectively for both methods. The proposed methods are recommended for routine analysis since they are rapid, simple and specific. The described UV methods were successfully employed for the analysis of each drug in their combined dosage form. For method (I), the mean% recoveries were found to be 100.49±0.15 for Amlodipine besylate and 98.99±1.83 for Telmisartan. For method (II), the mean% recoveries were found to be 99.55±0.92 and 100.48±1.69 for Telmisartan at 319 nm and 288 nm respectively and 99.92±1.69 for Amlodipine besylate at 393 nm. The validation of methods was carried out utilizing ICH guidelines.

Keywords: Telmisartan, Amlodipine, second derivative, first derivative ratio-spectroscopy.

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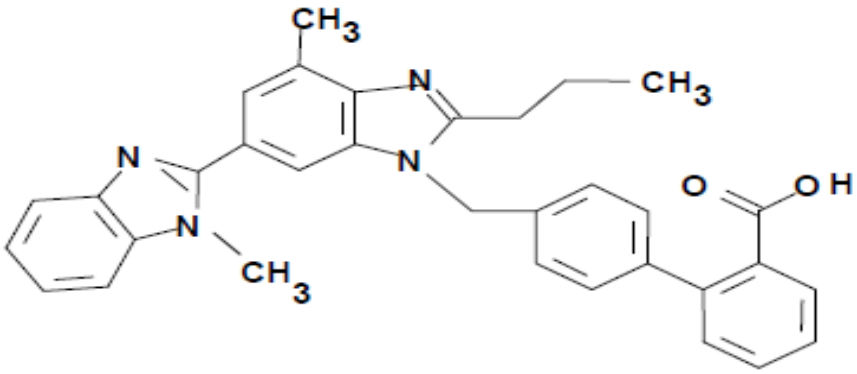
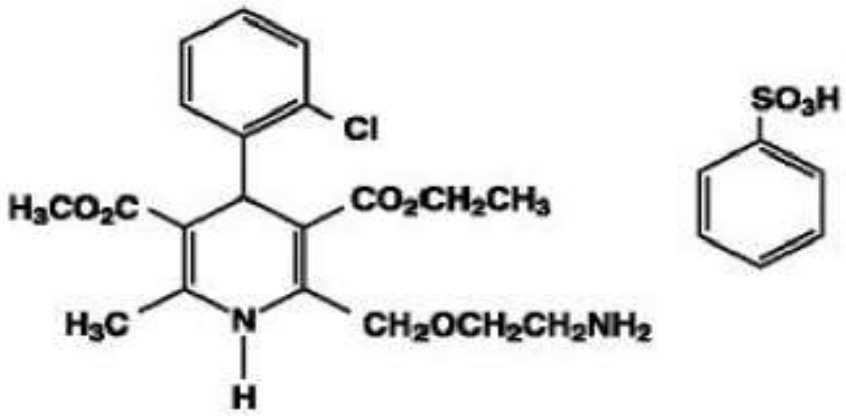
INTRODUCTION

Telmisartan (TEL): is chemically described as 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. Its empirical formula is: $C_{33}H_{30}N_4O_2$



Telmisartan is new highly selective, non peptide angiotensin II type I (AT I)-receptor antagonist[1]. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and angiotensin II circulating levels do not overcome the effect of telmisartan on blood pressure. Telmisartan lowers blood pressure through blockade of the renin –angiotensin -aldosterone system (RAAS) and is widely used in the treatment of hypertension[2] and is official in British pharmacopoeia[3].

Amlodipine besylate's (AML): chemical name is 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate. Its empirical formula is: $C_{20}H_{25}ClN_2O_5 \bullet C_6H_6O_3S$.



Amlodipine is a dihydropyridine calcium channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular

resistance and a reduction in blood pressure[1]and is official in British pharmacopoeia[4]. There are several publications describing analytical methods for the determination of AML and TEL individually or with other drugs as combination. TEL is official in both USP 34[5] and BP 2009[3]. Several analytical methods have been reported for determination of TEL either in dosage forms involve; HPTLC[6, 7], UV/Vis spectrophotometery[8, 9] and HPLC[10] or in biological fluid such as UV/Vis spectrophotometery[11], HPLC with UV detector[12, 13] and liquid chromatographic–electrospray ionization mass spectrometric method[14]. AML is official in both USP 34[15] and BP 2009[4]. Different methods are reported for estimation of AML in dosage forms such as UV Spectrophotometric method [16], HPLC method[17-20] and HPTLC[21-24] or in biological fluid including UV spectrophotometric method[25], LC and Capillary electrophoresis[26] and GC method[27]. Literature survey reveals few analytical methods for simultaneous determination of both drugs (AML & TEL) involving Zero-order UV spectrophotometric methods by measuring TEL at λ 298 nm and AML at λ360 nm[28,29]. First Derivative Synchronous Spectrofluorimetric method, TEL was determined at emission wavelength of 675 nm (zero-crossing wavelength point of AML) and AML was measured at 458 nm (zero-crossing wavelength point of TEL)[30].Two simple methods have been developed for determination of AML and TEL by simultaneous equation method based on measurement of absorbance at 367 nm and 292 nm for AML and TEL respectively and first order derivative spectrophotometric method using glacial acetic: water (20: 80 v/v) as a solvent and measured at 270 nm and 295 nm for AML and TEL respectively[31]. HPLC methods using UV detector[32-35], By HPLC method measured at 210 nm and UV spectrophotometric method measured at 297nm (λmax of Telmisartan) and 362nm (λmax of Amlodipine)[36], HPTLC[37] and LC–MS/MS[38]. In all UV and HPLC methods which reported they couldn't determine all the ratios present in marketed combinations of them. The goal of this work is the development of a new, rapid, sensitive and fully validated method for the direct and simultaneous determination of AML and TEL in raw materials and in their laboratory prepared mixtures with excipients. This method can be used for quality control of these drugs in formulation products.

MATERIALS AND METHOD

Instruments

The development of the new methods was carried out on JASCO spectrophotometer, model no. V-530 with 1cm matched quartz cells. The absorption spectra of reference and test solution were carried out in a 1cm quartz cells over the range of 200- 450 nm. Spectrophotometer connected to a personal computer loaded with [Jasco]-[spectra manager] software.

Chemicals and Reagents for both methods

Pure drugs

Telmisartan (99.70%) and Amlodipine besylate (99.80%) were kindly supplied from Sigma Company for Pharmaceutical Industries, Quesna, Menofia, Egypt. All chemicals (analytical grade) were used.

Preparation of standard stock solutions for both methods

AML and TEL were weighed (25 mg each) and transferred into two separate 25 mL volumetric flasks and dissolved in 25 mL of methanol to obtain solutions with concentration 1.0 mg/mL of AML and TEL, respectively.

Preparation of working solutions for both methods

Accurately measured volumes of stock standard solution of AML or TEL were transferred into a series of 10 mL volumetric flasks and diluted appropriately with methanol to obtain working standard solutions of AML and TEL with concentrations 2-16, 1-35 µg/mL respectively.

Construction of Calibration curves

Method (I)

For Amlodipine

The zero order spectra were recorded for each of the prepared working solutions using methanol as blank. Then the second derivative spectra were calculated. Calibration curves were obtained by plotting the amplitude of the peak of second derivative spectra measured at 368 nm for AML versus concentration of drug. Then regression equation was calculated.

For Telmisartan

The zero order spectra were recorded for each of the prepared working solutions using methanol as blank. Then the second derivative spectra were calculated. Calibration curves were obtained by plotting the amplitude of the peak of second derivative spectra measured at 329 nm for TEL versus concentration of drug. Then the regression equation was calculated.

Method (II)

For Amlodipine

The zero order spectra were recorded for each of the prepared working solutions using methanol as blank. Then the ratio spectra were obtained using 4 µg/mL of TEL as a divisor. Calibration curves were obtained by plotting the amplitude at 393 nm ($1DD_{393}$) in the first derivative of the ratio spectra versus concentration of AML. Then the regression equation was calculated

For Telmisartan

The zero order spectra were recorded for each of the prepared working solutions using methanol as blank. Then the ratio spectra were obtained using 2 µg/mL of AML as a divisor. Calibration curves were obtained by plotting the amplitude at 319 nm ($1DD_{319}$) or at 288 nm ($1DD_{288}$) in the first derivative of the ratio spectra versus concentration of TEL. Then the regression equations were calculated.

Preparation of Laboratory prepared mixtures

TWYNSTA® is a medicine that contains two active substances, telmisartan and amlodipine. It is available as blue and white, two-layered oval tablets contains both drugs in different ratios (40 mg telmisartan/10 mg amlodipine, 40 mg telmisartan/5 mg amlodipine, 80 mg telmisartan/10 mg amlodipine and 80 mg telmisartan/5 mg amlodipine). Mixtures simulated to this dosage form was prepared by mixing a weight of each component equivalent to one tablet content involving 40mg of TEL, 5mg of AML, 2mg povidone, 2 mg magnesium stearate, 2mg starch, 2mg silica, 0.2mg %NaOH and 46.8 mg avicel. Then this mixture was transferred to 100 mL volumetric flask and sonicated for 15 min for complete dissolution of both drugs and made up to volume with methanol. The solution was filtered and the first 10 mL of the filtrate was discarded. 1.0 mL of the filtrate was taken and diluted with methanol to obtain working laboratory prepared mixtures of AML and TEL with ratios as in the dosage form: (1:8) then the procedures repeated with different weight of TEL and AML to prepare mixtures in concentration (1:4, 1:16). Each drug concentration was calculated from its corresponding regression equation either for method (I) or method (II).

Validation of the Developed Methods

The validity of the method was studied regarding linearity, specificity, accuracy, and precision according to ICH guidelines[39].

Linearity

The method was found linear over a concentration range of 2-16 µg/mL for AML and 1-35 µg/mL for TEL, Regression equations were calculated. The results of slope, intercept, standard deviation about the slope and intercept are summarized in tables (1& 2) for both methods. The linearity of the calibration curve was indicated by the high value of the correlation coefficient.

Accuracy&precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses three times for interday and intraday for three different concentrations covered the linearity range.

Three replicate standard solutions at three different concentrations for both drugs (AML:TEL) at three different ratios 1:4, 1:8 and 1:16 as in the dosage forms marketed were prepared on single day and on three separate days. The concentrations found and recoveries were calculated from corresponding regressions equations. Accuracy was expressed as percentage recovery and precision was calculated as R.S.D. The results obtained were summarized in tables (3-10) indicating good accuracy and precision of the methods.

Specificity

The methods were determined as specific by percent recovery obtained from analysis of laboratory prepared mixtures. As there is no marketed formulation available, laboratory prepared mixture with expected excipients was made at three different ratios. The mean % recoveries and R.S.D were calculated as shown in tables (11-13) indicating no interferences from the expected excipients.

Detection Limit

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as:

$LOD = 3.3\ \sigma / S$

Where

σ= Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as:

$LOQ = 10\ \sigma / S$

Where

σ= Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

RESULTS AND DISCUSSION

Derivative spectrophotometry [40,41] (DS) is one of the simple spectrophotometric techniques. It is based on so called derivative spectra[42] which are generated from parent zero-order ones. The differentiation of zero-order spectrum can lead to separation of overlapped signals and elimination

of background caused by presence of other compounds in a sample. The mentioned properties can allow quantification of one or few analytes without initial separation or purification. The method (I) deals with development of new spectrophotometric method for simultaneous determination of TEL & AML in their dosage form. Although several methods are available for determination of AML and TEL, few methods are reported for their simultaneous determination in their combined dosage form (Twynsta ®) in the ratios (1:4, 1:8, 1:16). This research succeeds in development of simple, time saving and cheap methods for their determination. The overlain zero-order spectra of both drugs revealed that TEL can be determined in presence of AML but the determination of AML is difficult in presence of TEL as shown in figure(1). The first order derivative also failed to solve this problem as revealed in figure (2).The second derivative spectra allowed their simultaneous determination as shown in figures (3-5). It was found that TEL can be measured at λ 329 nm while AML measures zero. In the same time AML can be measured at λ 368 nm while TEL measures zero (zero-crossing point).

To explain the principle of ratio derivative spectrophotometry (method II): Consider a mixture of two compounds A and B. The absorption spectrum of the mixture is given by the equation:

$$A_{M,\lambda_1} = \varepsilon_{A,\lambda_1} C_A + \varepsilon_{B,\lambda_1} C_B \qquad (1)$$

Where $A_{M,\lambda 1}$ is the absorbance of mixture at wavelength λ_1, $\varepsilon_{A,\lambda 1}$ and $\varepsilon_{B,\lambda 1}$ are the molar absorbivity of A and B at λ_1 , C_A and C_B are the concentrations of A and B in the mixture. Eq. (1) is divided by the absorbance at λ1 of a standard solution of A whose concentration is C^0_A i.e. $\varepsilon_{A,\lambda 1}\ C^0_A$ then Eq. (1) becomes:

$$\frac{A_{M,\lambda_1}}{\varepsilon_{A,\lambda_1} C_A^0} = \frac{C_A}{C_A^0} + \frac{\varepsilon_{B,\lambda_1} C_B}{\varepsilon_{A,\lambda_1} C_A^0} \qquad (2)$$

This can be simplified to:

$$\frac{A_{M,\lambda_1}}{\varepsilon_{A,\lambda_1}} = C_A + \frac{\varepsilon_{B,\lambda_1}}{\varepsilon_{A,\lambda_1}} C_B \qquad (3)$$

Differentiating Eq. (3) with respect to λ gives

$$\frac{d}{d\lambda}\left(\frac{A_{M,\lambda_1}}{\varepsilon_{A,\lambda_1}}\right) = C_B \frac{d}{d_\lambda}\left(\frac{\varepsilon_{B,\lambda_1}}{\varepsilon_{A,\lambda_1}}\right) \qquad (4)$$

Equation (4) indicates that the derivative ratio spectrum of the mixture is dependent only on the values of C_B and is independent of the value of C_A in the mixture. The advantages of the derivative ratio spectra method over the zero-crossing derivative method, is the possibility of performing

measurements in correspondence of peaks, hence, a potentially greater sensitivity and accuracy, also in the derivative ratio method the easy measurement on the separate peaks and no need to work only at zero-crossing point as in case of derivative methods[43, 46]. In the method (II), the first derivative of ratio spectra where TEL can be measured at λ 319 nm and λ 288 nm by using 2 µg /mL of AML as a divisor as revealed in figure (6) and AML can be measured at λ 393 nm by using 4 µg /mL of TEL as a divisor as shown in figure (7).

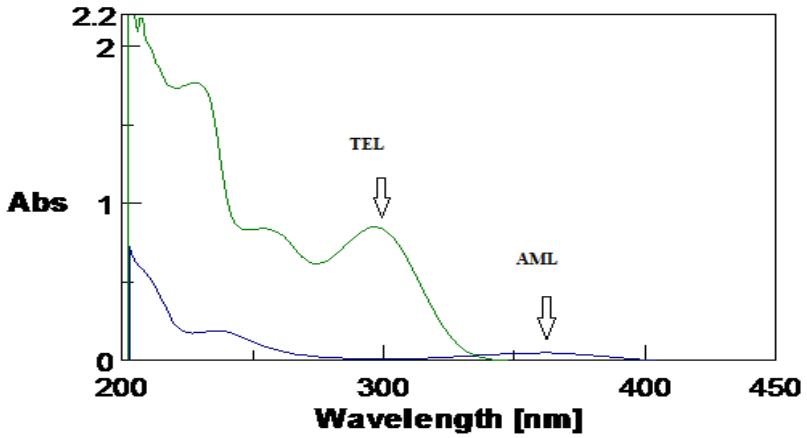


Figure 1: Overlay Zero order UV spectra of 4 µg/mL AML and 16 µg/mL TEL in methanol.

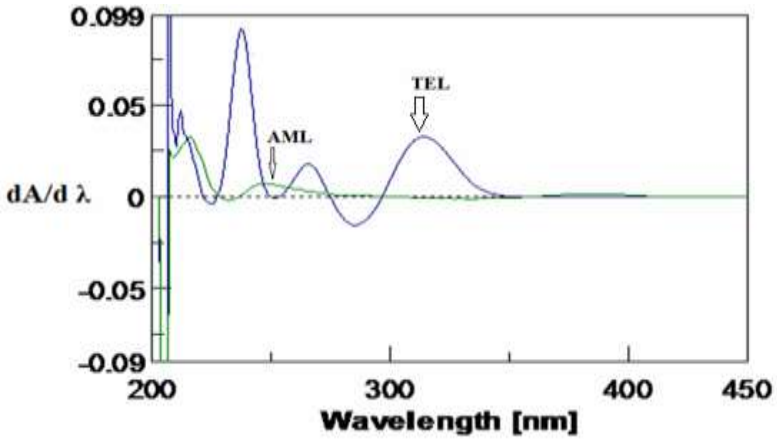


Figure 2: Overlay First derivative UV spectra of 4 µg/mL AML and 16 µg/mL TEL in methanol.

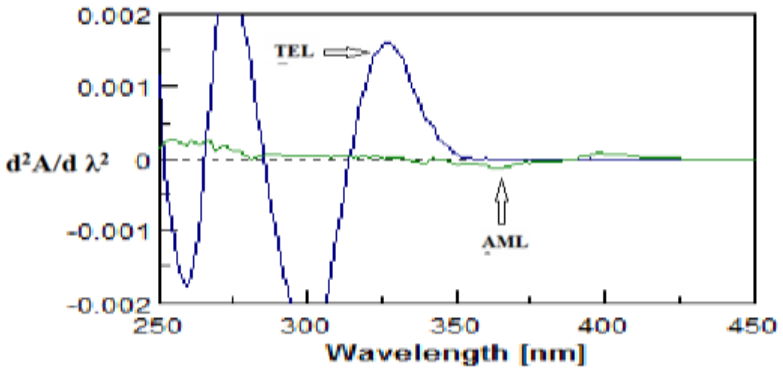


Figure 3: Overlay Second derivative UV spectra of 4 µg/mL AML and 16 µg/mL TEL in methanol.

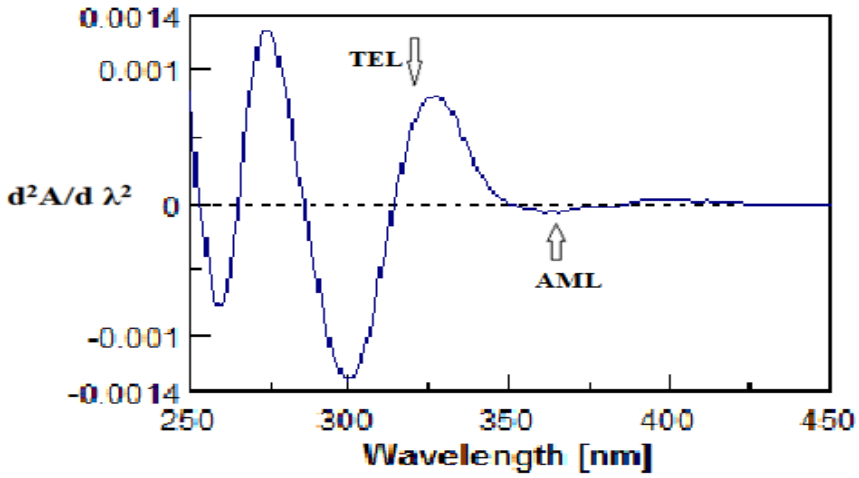


Figure 4: The second derivative spectrum of laboratory prepared mixture with expected excipients with concentration of amlodipine 2 µg/mL and telmisartan 8 µg/mL.

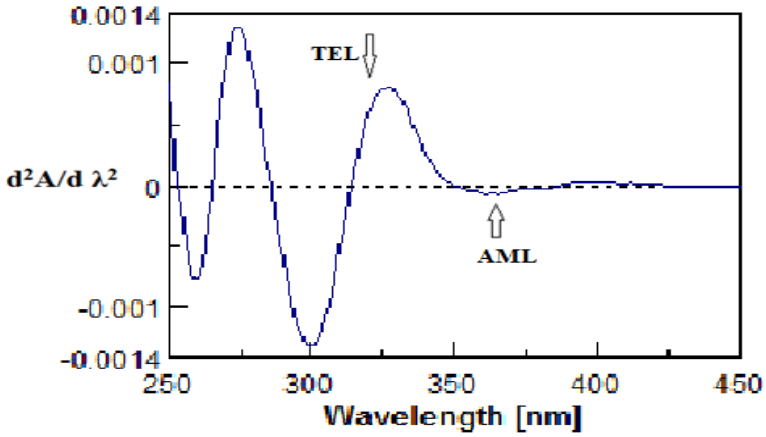


Figure 5: The second derivative spectrum of laboratory prepared mixture with concentration of amlodipine 2 µg/mL and telmisartan 8 µg/mL.

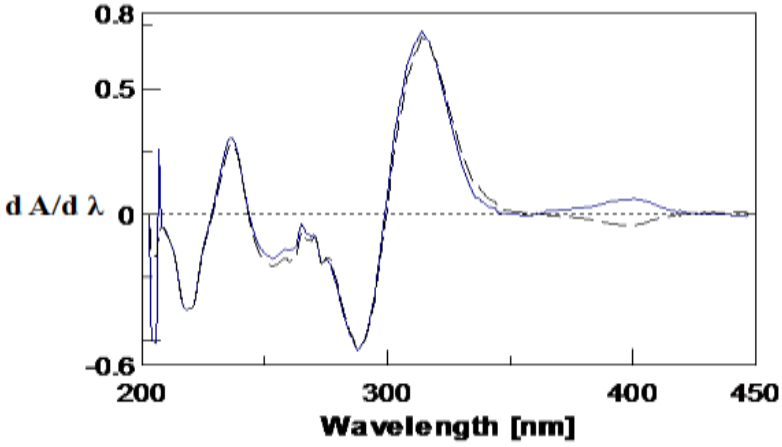


Figure 6: Overlay the first derivative ratio spectroscopy for TEL 8 µg/mL (---) and TEL: AML (2:8) (____) using AML 2 µg/mL as a divisor.

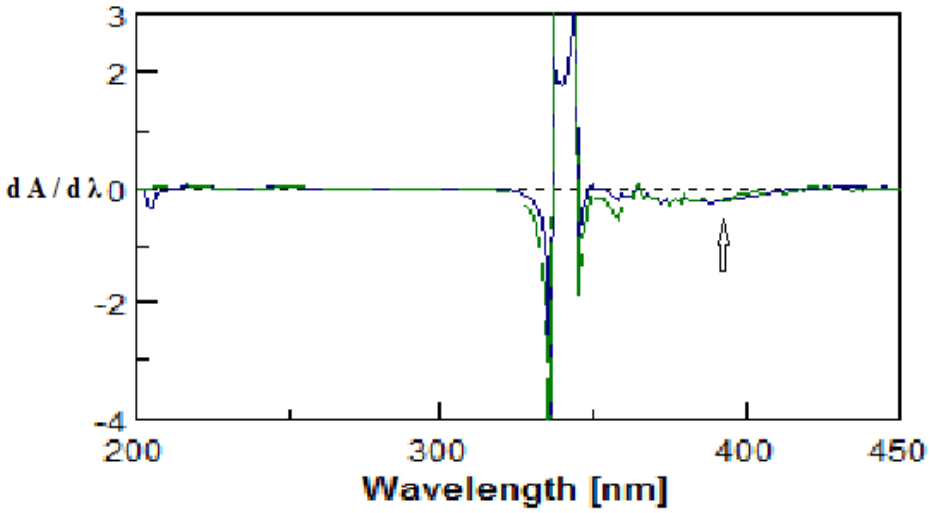


Figure 7: Overlay the first derivative ratio spectroscopy for AML 2 µg/mL (---) and TEL: AML (2:8) (____) using TEL 4 µg/mL as a divisor.

Table 1: Quantitative parameters for the determination of amlodipine besylate and telmisartan with the proposed second derivative spectrophotometric method

Compound	Linearity range(µg/mL)	r	b	a	$S_{y/x}$	S_b	S_a	LOD (µg/mL)	LOQ (µg/mL)
Amlodipine besylate	2- 16	0.9996	1.92E-05	2.87E-06	2.34E-06	2.03E-07	2.12E-06	0.36	1.10
Telmisartan	1- 35	0.9999	0.0001	1.60E-05	1.26E-05	4.25E-07	6.07E-06	0.20	0.61

r: correlation coefficient.

b: slope.

a: intercept.

$S_{y/x}$: standard deviation of residual.

S_b: standard deviation of slope.

S_a: standard deviation of intercept.

LOD: limit of detection.

LOQ: limit of quantitation.

Table 2: Quantitative parameters for the determination of telmisartan and amlodipine besylate with the ratio spectra derivative spectrophotometric method

Compound	Linearity range (µg/mL)	r	a	b	S_a	S_b	LOD (µg/mL)	LOQ (µg/mL)	$S_{y/x}$
TEL $^1DD_{319}$	1-35	0.9997	9.89 E-03	7.35 E-02	6.98 E-03	4.00 E-04	0.31	0.95	0.02
TEL $^1DD_{288}$	1- 35	0.9993	9.78 E-03	6.20 E-02	7.27 E-03	3.94 E-04	0.39	1.17	0.01
AML $^1DD_{393}$	2-16	0.9999	1.83E-02	9.32E-02	0.53E-02	0.57E-03	0.19	0.57	6.63E-03

r: correlation coefficient.

b: slope.

a: intercept.

$S_{y/x}$: standard deviation of residual.

S_b: standard deviation of slope.

S_a: standard deviation of intercept.

LOD: limit of detection.

LOQ: limit of quantitation.

Table 3: Repeatability of different concentrations of amlodipine besylate in laboratory prepared mixtures with telmisartan using the second derivative spectrophotometric method

Taken TEL (µg/mL)	Taken AML (µg/mL)	Concentration found* ± S.D AML (µg/mL)	RSD
Intra-day			
8.00	2.00	2.03±0.02	1.16
16.00	2.00	2.07±0.04	1.98
32.00	2.00	2.06±0.02	1.17
Inter-day			
8.00	2.00	2.01±0.02	0.85
16.00	2.00	2.01±0.04	1.99
32.00	2.00	2.01±0.04	1.89

*** The concentration found is the mean of three determinations.**

Table 4: Repeatability of different concentrations of telmisartan in laboratory prepared mixtures with amlodipine using the second derivative spectrophotometric method

Taken AML (µg/mL)	Taken TEL (µg/mL)	Concentration found*± S.D TEL (µg/mL)	RSD
Intra-day			
2.00	8.00	8.16±0.09	1.12
2.00	16.00	16.30±0.05	0.32
2.00	32.00	32.01±0.14	0.43
Inter-day			
2.00	8.00	8.04±0.13	1.67
2.00	16.00	16.03±0.19	1.24
2.00	32.00	31.40±0.43	1.37

*** The concentration found is the mean of three determinations.**

Table 5: Repeatability of different concentrations of telmisartan at $^{1}DD_{319}$ in laboratory prepared mixtures using the ratio spectra derivative spectrophotometric method

Taken AML (µg/mL)	Taken TEL (µg/mL)	Concentration found* ±S.D TEL (µg/mL)	RSD
Intra-day			
2.00	8.00	7.89±0.07	0.85
2.00	16.00	15.76±0.01	0.05
2.00	32.00	31.43±0.05	0.14
Inter-day			
2.00	8.00	7.79±0.09	1.25
2.00	16.00	15.68±0.26	1.68
2.00	32.00	31.06±0.26	0.85

*** The concentration found is the mean of three determinations.**

Table 6: Repeatability of different concentrations of telmisartan at $^{1}DD_{288}$ in laboratory prepared mixtures using the ratio spectra derivative spectrophotometric method

Taken AML (µg/mL)	Taken TEL (µg/mL)	Concentration found *± S.D TEL (µg/mL)	RSD
Intra-day			
2.00	8.00	8.13±0.03	0.32
2.00	16.00	16.24±0.01	0.08
2.00	32.00	32.33±0.04	0.12
Inter-day			
2.00	8.00	7.92±0.15	1.93
2.00	16.00	16.05±0.19	1.19
2.00	32.00	31.98±0.45	1.40

*** The concentration found is the mean of three determinations.**

Table 7: Repeatability of different concentrations of amlodipine at $^1DD_{393}$ in laboratory prepared mixtures using the ratio spectra derivative spectrophotometric method

Taken AML (µg/mL)	Taken TEL (µg/mL)	Concentration found * ±S.D AML (µg/mL)	RSD
Intra-day			
2.00	8.00	1.98±0.02	1.01
2.00	16.00	2.01±0.01	0.49
2.00	32.00	2.04±0.02	0.98
Inter-day			
2.00	8.00	1.97± 0.03	1.44
2.00	16.00	1.98±0.03	1.52
2.00	32.00	2.04±0.04	1.96

*** The concentration found is the mean of three determinations.**

Table 8: Recovery of amlodipine and telmisartan in their laboratory prepared mixtures by the proposed second derivative spectrophotometric method

Amlodipine besylate			Telmisartan		
Taken (µg/mL)	**Found* (µg/mL)**	**%Recovery**	**Taken (µg/mL)**	**Found* (µg/mL)**	**%Recovery**
2.00	2.01	100.50	8.00	8.04	100.50
2.00	2.02	101.00	16.00	16.03	100.19
2.00	1.99	99.50	32.00	31.40	98.13
Mean% recovery ± S.D.		100.33±0.62	Mean% recovery ± S.D.		99.61±1.06

*** The concentration found is the mean of three determinations.**

Table 9: Recovery of telmisartan at $^1DD_{319}$ and $^1DD_{288}$ in laboratory prepared mixtures by the proposed ratio spectra derivative spectrophotometric method

Taken TEL(µg/mL)	Taken AML(µg/mL)	Found* TEL (µg/mL)	%Recovery
$^1DD_{319}$			
8.00	2.00	7.79	97.38
16.00	2.00	15.68	98.00
32.00	2.00	31.06	97.06
Mean% recovery ± S.D.			97.48±0.39
$^1DD_{288}$			
8.00	2.00	7.92	99.00
16.00	2.00	16.05	100.31
32.00	2.00	31.98	99.94
Mean% recovery ± S.D.			99.75±0.55

*** The concentration found is the mean of three determinations.**

Table 10: Recovery of amlodipine at $^1DD_{393}$ in laboratory prepared mixtures by the proposed ratio spectra derivative spectrophotometric method

Taken AML(µg/mL)	Taken TEL (µg/mL)	Found* AML (µg/mL)	%Recovery
2.00	8.00	1.97	98.50
2.00	16.00	1.96	98.83
2.00	32.00	2.02	101.83
Mean% recovery ± S.D.			99.44±1.49

*** The concentration found is the mean of three determinations.**

Table 11: Recovery of amlodipine and telmisartan in laboratory prepared mixtures with expected excipients by the second derivative spectrophotometric method

Amlodipine besylate			Telmisartan		
Taken (µg/mL)	Found* (µg/mL)	%Recovery	Taken (µg/mL)	Found* (µg/mL)	%Recovery
2	2.02	100.83	8	7.73	96.63
2	2.01	100.50	16	16.07	100.44
2	2.01	100.50	32	31.74	99.20
3	3.01	100.33	12	11.59	96.57
3	3.02	100.50	24	23.87	99.46
4	4.02	100.42	16	15.78	98.61
4	4.02	100.38	32	32.67	102.08
Mean% recovery ± S.D.		100.49±0.15	Mean% recovery ± S.D.		98.99±1.83

*** The concentration found is the mean of three determinations.**

Table 12: Recovery of telmisartan at $^1DD_{319}$ and $^1DD_{288}$ in laboratory prepared mixtures with expected excipients by the proposed ratio spectra spectrophotometric method

Taken AML (µg/mL)	Taken TEL(µg/mL)	Found* TEL(µg/mL)	%Recovery
$^1DD_{319}$			
2.00	8.00	8.05	100.63
3.00	24.00	23.61	98.38
2.00	32.00	31.56	98.63
Mean% recovery ±S.D.			99.55±0.92
$^1DD_{288}$			
2.00	8.00	8.22	102.75
3.00	24.00	23.69	98.71
2.00	32.00	31.99	99.97
Mean% recovery ±S.D.			100.48±1.69

*** The concentration found is the mean of three determinations.**

Table 13: Recovery of amlodipine at $^1DD_{393}$ in laboratory prepared mixtures with expected excipients by the proposed spectrophotometric method

Taken AML (µg/mL)	Taken TEL (µg/mL)	Found*AML (µg/mL)	%Recovery
2.00	8.00	2.02	101.00
2.00	16.00	1.99	99.50
3.00	12.00	3.08	102.67
4.00	16.00	4.01	100.25
3.00	24.00	2.91	97.00
4.00	32.00	3.94	98.50
2.00	32.00	2.01	100.50
Mean% recovery ±S.D.			99.92±1.69

*** The concentration found is the mean of three determinations.**

CONCLUSION

The developed methods were validated as per ICH guidelines and were found to be within the prescribed limit. It concludes that the developed methods are simple, accurate, robust, sensitive and precise and suitable for both authentic and tablet dosage form.

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