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Simultaneous spectrophotometric determination of drugs lacking peak maxima in their zero-order profiles by graphical or statistical representation of data

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RESEARCH ARTICLE

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ABSTRACT

Trandolapril has no sharp peak in its zero-order spectrum, therefore it is difficult to be measured by direct spectrophotometry. In this study, direct univariate spectrophotometric methods were developed and validated for determination of Trandolapril and Verapamil combination in pure and tablet dosage forms. The first method for measuring both Trandolapril and Verapamil is Absorbance Subtraction (AS), this method depends on the presence of iso-absorptive point in the zero-order curve at 217 nm. It has the advantage of measuring the concentration of both Trandolapril and Verapamil from unified regression equation at the iso-absorptive point. The second, third and fourth methods were applied on the first order spectra of the studied drugs. Second method is Derivative Subtraction (DS) for Trandolapril and Derivative subtraction followed by spectrum subtraction (DS-SS) for Verapamil. The third and fourth methods are constant value and concentration value methods. In the concentration value method, the concentration of the drugs is determined from the graphical representation without the use of regression equations. All the developed methods were validated as per International Conference on Harmonization guidelines and the results proved that the developed methods are simple, accurate, and selective. Moreover, a statistical comparison between the developed methods and a reference method was done. Also, One-way ANOVA statistical test was done between all the proposed spectrophotometric methods and results showed no significant differences.

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1. Introduction

Trandolapril (TR) is used for patients with left ventricular systolic dysfunction as it is classified as an angiotensin converting enzyme (ACE) inhibitor [1,2]. Trandolaprilat is the active form, where trandolapril, as a prodrug, is converted to its active metabolite in the liver by esterase enzymes [3]. It has the longest duration of action among the anti-hypertensive drugs developed so far [3,4]. Trandolapril has been approved by the FDA 1996 and is available as a single drug or in combination with verapamil (Tarka®, Abbott Laboratories) which is an immediate release formulation of Trandolapril and a slow release formulation of verapamil [5].

Verapamil (VER) is categorized as calcium channel blocker. It regulates the movement of calcium across the cell membrane of the arterial smooth muscle and in contractile myocardial cells [6,7]. Combination of Trandolapril and Verapamil demonstrated better compliance and more effectiveness than monotherapy [8,9].

Many methods were reported for the analysis of this combination or one of its components in pharmaceuticals or body fluids. These include spectrophotometry [10], HPLC with spectrophotometric detection [11,12], high performance thin layer chromatography [13,14] and LC-MS/MS [15,16]. In 2014, Vijayalakshmi *et al.* suggested a colorimetric method for determination of Trandolapril and Flucloxacillin in pharmaceutical formulation, where the Trandolapril color complex was measured at 430 nm [17]. Amir *et al.* suggested a colorimetric method in 2016 using Bromothymol Blue and bromocresol green for determination of Trandolapril [18]. The main problem is that, Trandolapril has no sharp peak in zero order spectrum. So, the key challenge was the determination of Trandolapril by direct spectrophotometry without the use of coloring reagents. The aim of the present study was to develop and validate direct univariate spectrophotometric methods for determination of Trandolapril and Verapamil combination without the aid of external reagents as chromogens.

1.1. Theoretical background

1.1.1. Constant value (CV)

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Constant value is a simple method used for resolving overlapped binary mixtures, so in a mixture of X+Y, where Y is more extended than X, by dividing the zero order spectrum of the mixture by zero order normalized spectrum of a divisor of the more extended compound Y; which obtained by dividing certain spectrum of Y component by its concentration (1 µg/mL concentration); therefore the constant obtained from the plateau region at the extended part is related only to the concentration of the extended compound, and by the same way for getting the concentration of the less extended compound X, after resolving from Y, its concentration could be obtained by dividing its spectra by the normalized spectrum of a divisor of X (1 µg/mL concentration), therefore, the constant obtained from the plateau region is related to the concentration of X. The calibration curve is constructed between the concentration and constant obtained by dividing each spectrum by its normalized one [19]

1.1.2. Concentration value

Concentration value is a new spectrophotometric approach depending upon graphical representation of the spectra, where the concentration value of the drug is obtained directly from the spectral chart and represents the actual concentration of the drug without the need of regression equations. It is conducted by dividing the zero order absorption spectrum of the mixture by zero order normalized spectrum of the divisor of the more extended component (1 µg concentration); therefore the constant obtained from the plateau region represents the concentration of the more extended component without the need of any equation or calculation steps, therefore the recoveries calculated directly from the values of the constant as it is considered to be equal to the concentration of the drug in the mixture. The less extended component can be also obtained by resolving it first from the more extended one by any resolution technique, and then dividing the obtained spectra by zero order of the normalized spectrum of the divisor of the less extended component (1 µg concentration); so plateau region represents the concentration of the less extended component [20,21].

1.1.3. Absorbance subtraction method

This method is utilized for the analysis of a binary mixture with severe overlapping, and intersects at iso-absorptive point, and one spectrum is extended than the other. At iso-absorptive point (λ_{iso}) the absorbance (A_{iso}) is equal for both X and Y and the absorbance (A_2) at another selected wavelength (λ_2) in the extended part is only for the extended component Y. So, the absorbance factor which is a constant representing the ratio of the absorbance values at λ_{iso} (A_{iso}): to those at λ_2 (A_2) at the extended part [$F = A_{iso}/A_2$] is calculated, and then the factor is multiplied by the Absorbance at λ_2 (A_2) to get the absorption of the extended component alone at the iso-absorbtive point, after that the absorption of the less extended drug at iso-absorptive point can be obtained easily by subtraction of the absorption of the more extended one at λ_{iso} from the total Absorption of both at λ_{iso}. By this simple manipulation step, the absorbance value corresponding to X and Y could be obtained easily and separately at λ_{iso}. So, the concentration of each component could be obtained via the iso-absorptive point regression equation without any need for a complementary method or resolution step.

This smart method enables quantitative estimation of both X and Y in their binary mixture (X + Y) through the same unified regression equation and by using simple mathematically calculated factor. The unified regression equation obtained simply by plotting the absorbance values of the zero order spectra of either X or Y at iso-absorptive point (λ_{iso}) against the corresponding concentrations of X or Y, respectively.

The following equations explain:

$$\text{Absorbance of Y in the mixture at } \lambda_{iso} = F \times A_2 \quad (1)$$

$$\text{Absorbance of X in the mixture at } \lambda_{iso} = A_{iso}\,(X+Y) - (F \times A_2) \quad (2)$$

where A_2 is the absorbance at a selected wavelength (λ_2) in the extended part and only represent Y [22].

1.1.4. Derivative subtraction

DS applies the same principal for Ratio Subtraction (RS) but on the first derivative spectra. It is used for binary mixture where the first derivative (D1) of one component of the mixture is more extended than the other. DS can solve either the extended or the less extended spectrum. By eliminate the spectra of one compound leaving the other alone on its D1 profile [23].

1.1.5. Derivative subtraction spectrum subtraction

This resolution technique is similar to Ratio Subtraction-Spectrum Subtraction (RS-SS) but on first derivative profile. It Measure the studied drugs in the first order profile. It has the advantage of being more sensitive as the extended part of the mixture becomes more obvious. Moreover, we can enhance the sensitivity further more by calculating the concentration of the component using the difference between maximum and minimum amplitude ($P_{max-min}$) of first derivative. It is used for binary mixture where the first derivative (D^1) of one component of the mixture is more extended than the other. It can determine either the extended or the less extended spectrum [24] as follows:

$$(X+Y) / Y` = X/Y` + Y/Y`, \text{ were } Y/Y` = \text{constant} \quad (3)$$

$$X/Y` + \text{constant} - \text{constant} = X/Y` \quad (4)$$

$$X/Y` * Y` = X \quad (5)$$

$$(X+Y) - X = Y \quad (6)$$

2. Experimental

2.1. Materials and reagents

Trandolapril and Verapamil hydrochloride reference standards were kindly supplied by Abbott laboratories for pharmaceuticals and chemical industries (USA). The purity of the standards was certified to be 99.85 and 99.87% for Trandolapril and Verapamil, respectively. Structures of the compounds are shown in Figure 1. Tarka® commercial tablets labeled to contain 2 mg Trandolapril and 180 mg Verapamil hydrochloride were purchased from local Egyptian market. HPLC grade Acetonitrile supplied from Sigma Aldrich (Germany). Double distilled water was used throughout the study and is indicated by the word water.

2.2. Instrumentation and conditions

A double-beam UV/Visible spectrophotometer model J-760, Jasco, Japan was used. The absorption spectra of the standard and the tested solutions were recorded in 1.0 cm quartz cells over the range 200-400 nm at room temperature using Spectramanager software.

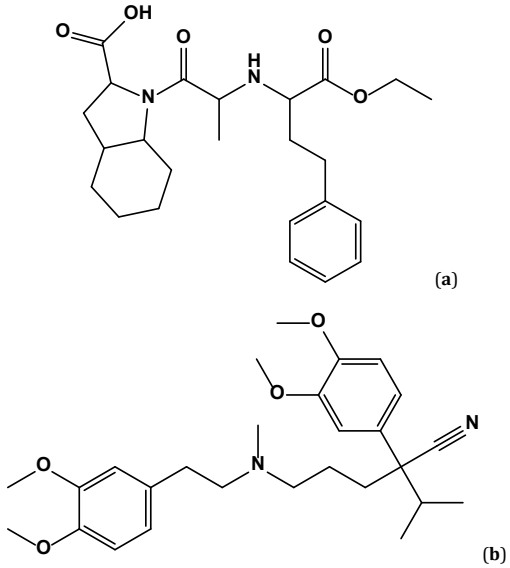

Figure 1. Chemical structures of a) Trandolapril and b) Verapamil.

2.3. Spectral characteristics and wavelength selection

Zero-order (D^0) absorption spectra of both Trandolapril and Verapamil (10 µg/mL), were scanned against a blank (200-400 nm), overlaid using the Spectramanager software to detect the spectral characteristics, extent of overlap, and to predict the best methods for resolution of the mixture.

2.4. Procedures

2.4.1. Preparation of standard stock and working solutions

Primary stock solutions of standard Trandolapril and Verapamil were separately prepared in 100 mL volumetric flasks by dissolving 20 mg of each standard powder in the least amount of acetonitrile and completed to the volume by water. Primary stocks solutions of Trandolapril and Verapamil were diluted with water to prepare standard working solutions (100 µg/mL).

2.4.2. Preparation of pharmaceutical dosage form

To determine Trandolapril and Verapamil in commercial tablets (Tarka®), 10 tablets were finally powdered, then, a portion of the powder equivalent to one tablet was weighted accurately and transferred to a 100 mL beaker. 50 mL of acetonitrile was added, stirred using a magnetic stirrer for 15 min and filtered through 0.5 µm Whatman filter paper into a 100-mL volumetric flask. The residue was washed three times each time with 10 mL of acetonitrile and the solution was completed to the mark with water.

2.4.3. Validation

The proposed methods were Validated according to International Conference on Harmonization (ICH) guidelines [25].

2.4.3.1. Linearity and construction of calibration curves

Accurately measured aliquots of Trandolapril and Verapamil were transferred from their working solutions into two separate series of 10 mL volumetric flasks and the volumes were completed to the mark with water to prepare standard solutions for the calibration samples consist of six concentrations covering a concentration range 1-30 µg/mL for Trandolapril and 2-50 µg/mL for Verapamil. Samples scanned from 200-400 nm and the obtained zero-order D^0 spectra saved on the computer.

2.4.3.1.1. For absorbance subtraction method of both TR and VER

Unified regression equation obtained from the calibration curve relating the absorbance at the iso-absorptive point of the scanned zero-order (D^0) spectra of TR or VER at 217 nm to the corresponding concentrations.

2.4.3.1.2. For derivative subtraction of Trandolapril

The first derivative (D^1) of the stored zero-order (D^0) absorption spectra are computed and stored. Regression equation for the calibration curve relating the peak amplitude of the first derivative spectra of Trandolapril at 217 nm to the corresponding concentrations was computed.

2.4.3.1.3. For derivative subtraction coupled with spectrum subtraction method of Verapamil

The first order (D^1) of the stored zero-order D^0 absorption spectra were computed and stored. Regression equation for the calibration curve relating the difference between the maximum and minimum amplitudes ($P_{max-min}$) of the first derivative (D^1) spectra (amplitudes in the first order 238.5-223.5) of Verapamil versus the corresponding concentrations was computed.

2.4.3.1.4. For constant value of both TR and VER

By dividing the first-order spectrum of the more extended component VER by First order normalized spectrum divisor of VER (1 µg concentration), the constant was obtained from the extended plateau region which is related to the concentration. So, the calibration curve was constructed relating the concentration to the constant. For TR resolution technique DS-SS to eliminate VER spectrum was done first, then dividing the First-order spectrum of TR by First order normalized spectrum divisor of TR (1 µg concentration), the constant was

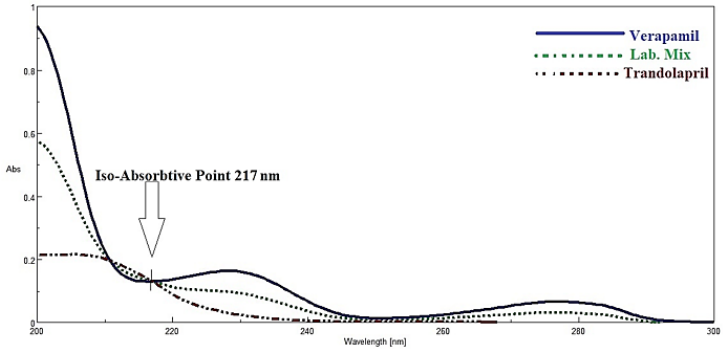

Figure 2. Zero-order absorption UV spectra of 5 µg /mL of Trandolapril (Green) (-----) and Verapamil (Blue) (____), separately in acetonitrile, and binary of a mixture of Trandolapril and Verapamil (Red) 2.5 µg /mL of each (- - -) in Acetonitrile showing iso-absorbtive point at 217 nm.

obtained which is related only to TR concentration; So, the calibration curve was constructed relating the concentration to the constant.

2.4.3.2. Determination in laboratory prepared mixtures (selectivity)

From the previously prepared stock solutions of Trandolapril and Verapamil, different mixtures were prepared by mixing accurate portions of both analytes and transferred to a series of 10 mL volumetric flasks. The final volume is completed by water.

2.4.3.3. Accuracy

Three replicates of different concentrations of Trandolapril and Verapamil were used for checking accuracy of the developed methods. The concentrations were obtained from the corresponding regression equation for each method, from which the percentage recoveries suggested good accuracy of the proposed methods.

2.4.3.4. Repeatability and Intermediate precision

Three concentrations of Trandolapril and Verapamil were analyzed intra-daily for three times using the proposed methods. The relative standard deviations were calculated.

The previous procedures were repeated inter-daily on three different days for the analysis of the three chosen concentrations. The relative standard deviations were calculated.

2.4.3.5. Limit of quantitation (LOQ) and limit of detection (LOD)

According to ICH recommendations, several approaches for determining the quantitation and detection limits are possible. The standard deviation of the intercept and the slope approach was used to calculate LOD and LOQ, where:

LOD = 3.3 x SD of intercept / slope coefficient (7)

LOQ = 10 x SD of intercept / slope coefficient (8)

2.4.4. Application to pharmaceutical dosage form

From the previously prepared stock solutions of pharmaceutical formulation further dilutions were prepared in the obtained linearity range using water. The stock was diluted to the concentration of 45 µg/mL Verapamil and 0.5 µg/mL Trandolapril in 100 mL volumetric flask, then, a portion from Trandolapril standard stock solution equivalent to 1.5 µg was added and then the solution completed to the mark with water. The Standard addition of Trandolapril is to increase its concentration to be within the linearity range, so the final concentration of dosage form will be 45 µg/mL Verapamil and 2.0 µg/mL Trandolapril.

The validity was further assessed by the standard addition technique, by preparing another two dilutions of the dosage form; one for TR with concentration within its linearity range and another one for Verapamil with concentration within its linearity.

3. Results and discussion

Several reported chromatographic methods were found for determination of the mixture. Also, two different colorimetric methods were found for the mixture [16,17]. But during the literature survey, there were no direct spectrophotometric methods for determination of Trandolapril and Verapamil combination. Trandolapril, in zero order profile, lacks a sharp peak which could be used for its direct determination. So, the challenge was to develop a spectrophotometric method for Trandolapril determination in a mixture with good accuracy and precision. In our present study, different univariate spectrophotometric methods were developed and validated for determination of Trandolapril and Verapamil combination. As shown in Figure 2, it is obvious that the spectra of the two drugs are severely overlapped. It was found that only Verapamil can be determined directly by zero order spectrophotometry at 277 nm. But unfortunately, the method will be insensitive as Verapamil here will be determined at its lowest peak. So, there was a need for a sensitive method for Verapamil determination at its λ_{max}.

3.1. Methods development

3.1.1. Absorption subtraction

Absorbance subtraction on the zero order absorption spectrum, where a unified regression equation is constructed at iso-absorptive point 217 nm as shown in Figure 2, and a factor is calculated by dividing VER zero order absorbance at 217 nm by its absorbance at 275 nm (A 217 nm / A 275 nm = 2.01491). By multiplication of the factor by the free peak absorbance at λ 275 nm, the absorbance due to VER at 217 nm is determined, Subtracting VER absorbance at 217 nm from the total absorbance of the lab mixture at 217 nm, the absorbance of TR is determined; and by substituted of both VER absorbance and TR absorbance in the unified regression equation; both VER and TR concentration is determined.

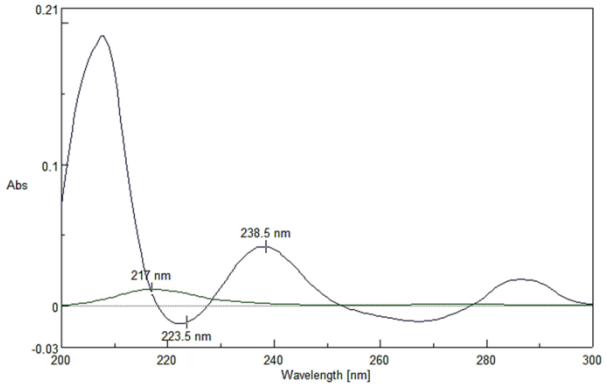

Figure 3. First order absorption UV spectra of 20 µg/mL Verapamil (-----) and 5 µg/mL Trandolapril (——) in acetonitrile.

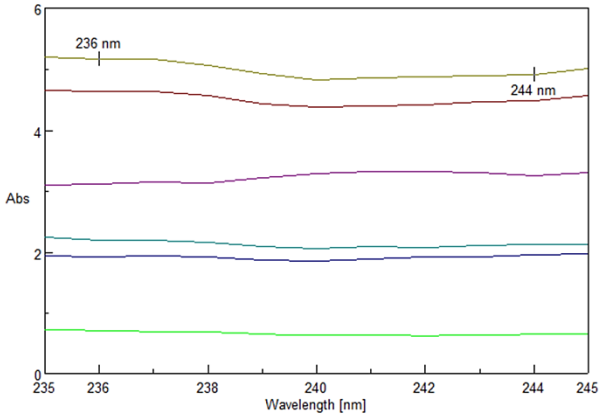

Figure 4. The division of first order spectra of laboratory prepared mixture of Verapamil and Trandolapril by 10 µg /mL of Verapamil as a divisor showing the constants.

3.1.2. Derivative subtraction for determination of TR and derivative subtraction followed by spectrum subtraction for VER

By obtaining the first order spectrum of both VER and TR, where TR sharp peak appeared at 217 nm as shown in Figure 3; so, the spectrum of the mixture of Trandolapril and Verapamil was divided by 10 µg/mL Verapamil as a divisor. After subtraction of the constant which is shown in Figure 4, multiplication by the divisor gives the 1st order spectrum of Trandolapril. After that; VER only could be obtained by Spectrum Subtraction (SS) method. This could be summarized by the following equations:

$$TR + VR / VR' = TR/VR' + VR/VR' = TR/VR' + Constant - Constant = TR/VR' \quad (9)$$

$$TR/VR' \text{ X } VR' = TR \text{ first order curve} \quad (10)$$

$$TR+VER - TR = VER \text{ first order curve} \quad (11)$$

Then, Trandolapril could be measured at 217 nm in its 1st order curve, while the concentration of the extended component VER is calculated using the difference between maximum and minimum amplitude ($P_{max-min}$) of first derivative spectra, thus decrease the error and increase the sensitivity of the method. The difference between maximum and minimum amplitude (P_{max} and P_{min}) used were 238.5 nm and 223.5 nm, respectively, as shown in Figure 3.

3.1.3. Constant value and concentration value for VER

VER is extended more than TR in its first derivative spectra as shown in Figure 3. So, when the spectra of the mixtures of VER and TR are divided by the spectrum of normalized divisor of VER, a constant at the plateau region on the extended part 240 to 400 nm is obtained as shown in Figure 5.

This constant is equivalent to the concentration of VER as it is resulting from dividing the spectra of the mixture by the normalized spectra of VER and in the extended part it's related only to VER concentration according to the following equations:

$$TR + VER / VER` = TR/VER` + VER/VER` \quad (12)$$

$$VER + TR / VER` = TR/VER` + constant \quad (13)$$

While VER` is a normalized divisor (1 µg/mL concentration) so that Constant is equal to the concentration of VER at this extended part where there is no contribution of TR.

So for constant value we construct a calibration between that constant and corresponding concentration to correct any error as explained before and the concentration of VER is obtained from that calibration curve regression equation, for concentration value we directly get the concentration from the spectra plateau region using that constant. The results of both methods were compared and both have good recoveries.

Table 1. Validation parameters of the proposed spectrophotometric methods, determination of the studied drugs in the laboratory prepared mixtures, dosage form and application of standard addition technique.

Method	**First derivative (D^1)**			**Zero order (D^0)**
	Derivative Subtraction (DS)	**Constant value**	**Concent. value**	**Abs. subtraction**
Drug	Trandolapril			
Range (µg/mL)	1-30			2-30
Regression Equation	y = 0.003x + 0.0002	y = 1.0004x - 0.0146		y = 0.0256x +0.0023
Correlation coefficient (r)	r^2 = 0.9999	r^2 = 1		r^2 = 0.9999
Accuracy [a]	99.59±0.471	99.367±0.696	99.89±0.421	99.189±0.764
Repeatability [b]	99.771	99.995	100.305	99.384
RSD%	0.775	0.5	1.051	1.961
Intermediate precision [c]	99.159	99.368	100.305	101.054
RSD%	0.148	0.663	0.529	0.265
LOQ (µg/mL)	1			2
LOD (µg/mL)	0.33			0.67
Laboratory prepared mixtures n = 5	100.146±0448	99.846±0.359	99.973±0.459	99.8515±0.309
Recovery of pharmaceutical dosage form	100.871±1.342	100.022±1.920	99.958±1.549	100.313±1.489
Recovery of standard additions	100.410±0.990	101.109±1.906	100.097±1.091	99.508±1.110
Method	**First derivative (D^1)**			**Zero order (D^0)**
	Derivative subtraction spectrum subtraction DS-SS	**Constant value**	**Concent. value**	**Absorbance subtraction**
Drug	Verapamil			
Range (µg/mL)	2-50	2-30		
Regression Equation	$P_{max-min}$ = 0.0024x + 0.0012	y = 0.997x + 0.009		y = 0.0256x +0.0023
Correlation coefficient (r)	r^2 = 0.9999	r^2 = 1		r^2 = 0.9999
Accuracy [a]	100.304±0.529	99.985±1.028	100.013±0.619	100.42±0.547
Repeatability [b]	100.712	100.63	100.73	100.02
RSD%	0.806	0.789	0.797	0.984
Intermediate precision [c]	100.680	100.867	100.773	100.820
RSD%	0.132	0.066	0.033	0.017
LOQ (µg/mL)	2			
LOD (µg/mL)	0.67			
Laboratory prepared mixtures n = 5	99.735±0.620	100.0381±0.171	100.228±0.110	99.907±0.333
Recovery of pharmaceutical dosage form	100.44±1.651	100.06±0.0798	100.144±1.557	99.589±1.035
Recovery of standard additions	99.159±0.896	99.842±1.690	99.552±1.647	100.021±0.902

[a] Mean±SD.
[b] Intra-day (n = 3), Average of three concentration of the analytes (5, 10 and 20 µg/mL) repeated three times within the same day.
[c] Inter-day (n = 3), Average of three concentration of the analytes (5, 10 and 20 µg/mL) repeated three times in three different days.

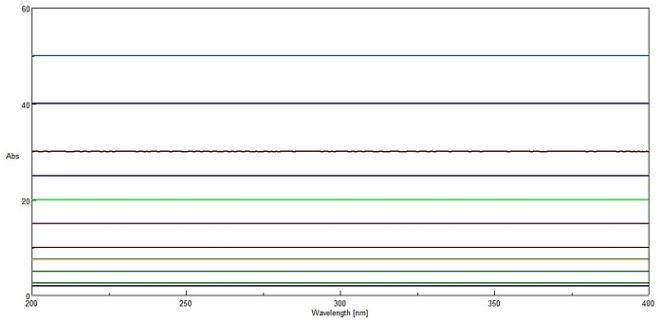

Figure 5. The constant value obtained after division of zero order spectra of Verapamil concentrations (2-50 µg/mL) by the spectrum of Normalized 1 µg/mL devisor of Verapamil.

3.1.4. Derivative subtraction constant value and derivative subtraction concentration value of TR

In first order curve (D^1) profile; First TR resolved from VER by Derivative Subtraction (DS) according to the following equations:

(TR+VER)/VER` = TR/VER`+ VER/VER`,
were VER/VER`= constant (14)

TR/VER`+ constant – constant = TR/VER` (15)

TR/VER`* VER` = TR (16)

After that we divide the obtained spectra by a normalized spectrum divisor of TR. So, the constant obtained in the plateau region which shown in Figure 6 is related to TR concentration. So, for constant value, a calibration curve between that constant and corresponding concentration is constructed to correct any error as explained before and the concentration of TR is obtained from that calibration curve regression equation. While for concentration value, the concentration is directly obtained from the spectra plateau region using that constant. The results of both methods were compared and both have good recoveries.

3.2. Validation

Validation of the proposed methods was assessed according to International Conference on Harmonization (ICH) guidelines [25]. Validation was done relative to linearity and range, accuracy, precision, selectivity, LOQ, and LOD. All the validation parameters are shown in Table 1.

The proposed progressive and successive spectrophotometric methods were statistically compared with the reported HPLC method [12] and the results are tabulated in Table 2. It was found that there is no significant difference between developed methods and the reference method.

Table 2. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of Trandolapril and Verapamil in bulk powder.

Parameter	Drug	Method		Mean	S.D	RSD%	N	Variance	Student's t-test (2.23) [a]	F-test (5.05) [a]
Developed method	Verapamil	First Derivative D^1	DS-SS	100.304	0.529	0.527	6.000	0.280	0.625	1.286
			Constant value	99.985	1.028	1.017	6.000	1.057	0.237	2.936
			Conc. value	100.013	0.619	0.613	6.000	0.383	0.247	1.064
		Zero order D^0	Absorbance subtraction	100.042	0.547	0.545	6.000	0.299	0.175	1.204
	Trandolapril	First Derivative D^1	DS	99.590	0.471	0.475	6.000	0.222	1.467	1.094
			Constant value	99.367	0.696	0.700	6.000	0.484	1.812	2.384
			Conc. value	99.890	0.421	0.425	6.000	0.177	0.358	1.146
		Zero order D^0	Absorbance subtraction	99.189	0.764	0.771	6.000	0.584	2.185	2.877
Reported method [b]	Verapamil			100.10	0.600		6.000	0.360		
	Trandolapril			99.98	0.450		6.000	0.203		

[a] The values in parenthesis are the corresponding theoretical values of t and F at p = 0.05.
[b] Method [12].

Table 3. Results of ANOVA (single factor) for comparison of the proposed methods for the determination of Trandolapril and Verapamil in pure powder form.

Source of variation		SS [a]	df [b]	Variance	F [c]	P-value	$F_{critical}$ [d]
Trandolapril	Between groups	1.6457	3	0.5486	1.4956	0.2462	3.098
	Within groups	7.3360	20	0.3668			
	Total	8.9817	23				
Verapamil	Between groups	0.3899	3	0.1300	0.2575	0.8551	
	Within Groups	10.0950	20	0.5047			
	Total	10.4850	23				

[a] Sum of squares.
[b] degree of freedom between and within groups.
[c] Calculated F.
[d] Critical (tabulated) value for F at p = 0.05.

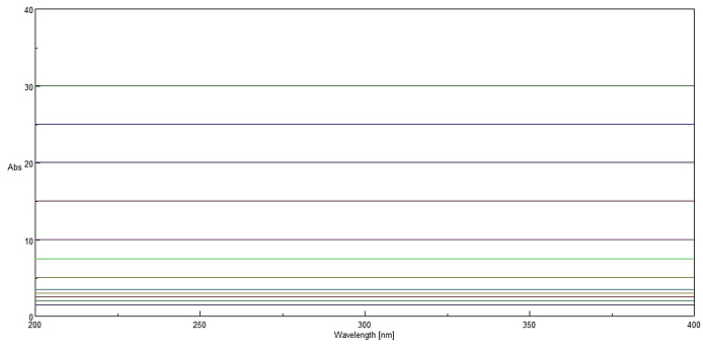

Figure 6. The constant value obtained after division of Zero order spectra of Trandolapril concentrations (1-30 µg/mL) by the spectrum of Normalized 1 µg/mL devisor of Trandolapril.

The results of proposed spectrophotometric methods were also compared statistically using One-way where there was no significant difference as shown in Table 3.

4. Conclusion

Trandolapril lacks a sharp peak in its zero-order curve. So, its determination by direct spectrophotometric method with acceptable accuracy and precision was a challenge. So, a new method is developed for the determination of TR in the mixture without depending on its shoulder peak. Furthermore, a new sensitive method developed for the determination of VER. All the spectrophotometric methods were developed and validated for the determination of Trandolapril and Verapamil combination in both laboratory prepared mixtures and marketed dosage forms and was found to be accurate, reproducible, and selective. All the developed methods showed no significant difference with each other and with the reference HPLC method. The developed methods could be used in quality control laboratories for fast determination of the cited drugs.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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