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Conventional univariate versus multivariate spectrophotometric assisted techniques for simultaneous determination of perindopril arginin and amlodipine besylate in presence of their degradation products

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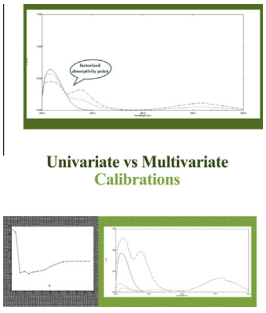
HIGHLIGHTS

- Application of smart and simple spectrophotometric methods manipulating the absorbance spectra.
- The applied methods are SIMs with high accuracy and precision and low sample preparation.
- CRACLS method can estimate the absorbance spectra of unknown components in a mixture.
- The novel AFM overcomes the limitations of isoabsorptive method in mixture analysis.
- AFM depends on an independent factor (absorptivity) rather than a dependent factor (absorbance).

GRAPHICAL ABSTRACT



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ABSTRACT

The resolving power of spectrophotometric assisted mathematical techniques were demonstrated for the simultaneous determination of perindopril arginin (PER) and amlodipine besylate (AML) in presence of their degradation products. The conventional univariate methods include the absorptivity factor method (AFM) and absorption correction method (ACM), which were able to determine the two drugs, simultaneously, but not in the presence of their degradation products. In both methods, amlodipine was determined directly at 360 nm in the concentration range of 8–28 μg mL^{-1}, on the other hand perindopril was determined by AFM at 222.2 nm and by ACM at 208 nm in the concentration range of 10–70 μg mL^{-1}. Moreover, the applied multivariate calibration methods were able for the determination of perindopril and amlodipine in presence of their degradation products using concentration residuals augmented classical least squares (CRACLS) and partial least squares (PLS). The proposed multivariate methods were applied to 19 synthetic samples in the concentration ranges of 60–100 μg mL^{-1} perindopril and 20–40 μg mL^{-1} amlodipine. Commercially available tablet formulations were successfully analysed using the developed methods without interference from other dosage form additives except PLS model, which failed to determine both drugs in their pharmaceutical dosage form.

Introduction

Perindopril is chemically designated as (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)butyl] amino]-1-oxopropyl] octahydro-1*H*-indole-2-carboxylic acid, Fig. 1. It is an angiotensin converting

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enzyme inhibitor that is used in the treatment of hypertension and heart failure [1]. Angiotensin II is a powerful circulating vasoconstrictor and inhibition of its synthesis in hypertensive patients results in a fall in peripheral resistance and a lowering of blood pressure. While, amlodipine (AML), 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester, Fig. 1. It is a dihydropyridine type long acting calcium channel blocker with slow onset of vasodilatory action. Also, it may cross the blood brain barrier and is used in cerebral ischaemia. Both ACE inhibitors and calcium channel blockers reduce the risk of stroke, coronary heart disease and cardiovascular death [2].

Perindopril arginin is co-formulated with amlodipine besylate to be superior in lowering systolic and diastolic blood pressures [3].

Amlodipine besylate is an official drug, its analysis was described in British Pharmacopoeia [4]. However, neither perindopril arginin nor its pharmaceutical combination with amlodipine besylate (Coveram®tablet) is official in any pharmacopeia. On detailed literature survey, it was found that different techniques were described for the estimation of perindopril erbumine in biological fluids and pharmaceutical formulations either in binary mixtures, enantiomeric mixtures or in presence of its metabolite such as; spectrophotometry [5,6], chromatography [7–9], selective biosensors [10–12], but no method was reported for the analysis of perindopril arginin salt.

Also, different analytical techniques have been published for the determination of amlodipine besylate either in binary mixture or enantiometric mixture depending on the theory of chromatography as HPLC [13–15], or using TLC theory [16–18], capillary electrophoresis using different back ground electrolytes and chiral selectors [19–21], and voltammetry in binary mixture with valsartan or in bulk powder [22,23]. While, perindopril in combination with amlodipine were analysed by spectrophotometric and HPLC methods [24,25] and in the presence of their degradation products [26].

General speaking, HPLC is considered more specific than spectroscopy, but spectrophotometric technique has the merits of rapidness, simplicity and validity. Therefore, it was thought worthwhile to develop and validate simple, precise and accurate spectrophotometric methods for determination of both active compounds in presence of their alkaline degradation products and their pharmaceutical formulations using conventional univariate and multivariate calibration methods and compare their abilities in the resolution of a quaternary mixture of perindopril, amlodipine along with their degradation products. According to the literatures in hands, the alkaline stress condition is the most effective one that induces complete degradation in less time than other conditions. Therefore, the alkaline degradation is the most likely and more worthy to be studied.

In this study, four spectrophotometric techniques are proposed to resolve the spectral overlap. Two different univariate methods are suggested for the determination of the spectrally overlapped perindopril and amlodipine in their binary mixture. These methods are the absorptivity factor spectrophotometric method (AFM) and the absorption correction method (ACM). Moreover, more advanced multivariate methods are developed for determination of perindopril, amlodipine and their degradation products in their quaternary mixtures. Therefore, two multivariate calibrations methods are developed, partial least squares (PLS) and Concentration residuals augmented classical least squares (CRACLS) and applied as stability indicating multivariate methods. CRACLS is a recently developed method for the resolution of complex mixtures, while PLS is a conventional chemometric method which is used for the purpose of comparison with the newly proposed CRACLS one.

Theory of absorptivity factor method (AFM) [27]

x and y are two components in mixture, where the concentration of y can be determined without any interference from x by other spectrophotometric method, the concentration of x could be determined by applying the absorptivity factor method.

When x and y have the same absorbance at certain wavelength (λ) in spite of their different absorptivities, it means that

$$A_x = A_y$$

$$\therefore a_x b_x C_x = a_y b_y C_y \quad (1)$$

where $b_x = b_y = b = 1$ cm

$$\therefore a_x C_x = a_y C_y$$

Then the absorptivity factor (F) can be calculated as follows:

$$a_x/a_y = C_y/C_x \quad (2)$$

$$\therefore a_x/a_y = F$$

$$\text{so, } a_x = F\ a_y \quad (3)$$

In a mixture of x and y, the absorbance at the selected wavelength (λ) corresponds to the total absorbance (A_T) as follows:

$$\textit{therefore } A_T = a_x b_x C_x + a_y b_y C_y$$

$$\therefore a_x = F\ a_y$$

$$\therefore A_T = F\ a_y b C_x + a_y b C_y,$$

$$A_T = a_y b(F\ C_x + C_y) = a_y b C_T \quad (4)$$

Total concentration (C_T) can be obtained using the regression equation of y which represents the linear relationship of the absorbance at the selected wavelength (λ) and the concentration. So, concentration of x (C_x) can be obtained after subtraction of C_y and multiplication by the inverse of absorptivity factor as the following:

$$C_T = F\ C_x + C_y$$

$$F\ C_x + C_y - C_y = F\ C_x$$

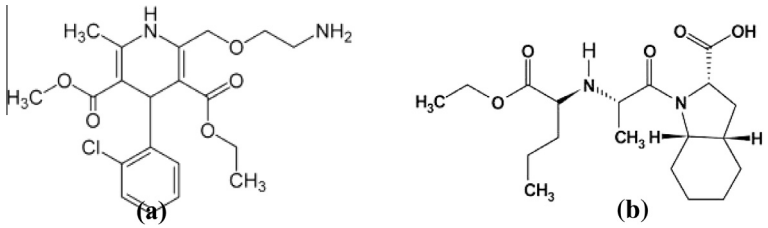

Fig. 1. Chemical structure of (a) amlodipine (b) perindopril.

$$\therefore C_x = F\ C_x x1/F \quad (5)$$

where A_x, A_y, C_x, C_y, b_x and b_y are absorbance, concentrations and pathlength of x and y, respectively .

Theory of absorption correction method (ACM) [28]

For a mixture of two components (x & y), the concentration of y can be determined clearly at λ_1

$$C_{y1} = A_{y1} \pm b_{y1}/a_{y1} \quad (6)$$

While at λ_2, the concentration is the sum of C_x and C_y,

$$C_{T\lambda 2} = C_{x\lambda 2} + C_{y\lambda 2} \quad (7)$$

where the absorbance of A_{y1} is converted to its correspondence A_{y2} as follows:

Since $C_{y1} = C_{y2}$,

$$A_{y1} \pm b_{y1}/a_{y1} = A_{y2} \pm b_{y2}/a_{y2}$$

Therefore, $A_{y2} = a_{y2}/a_{y1}(a_{y1} \pm b_{y1}) \pm b_{y2}$

So the concentration of x can be determined at λ_2 as follows:

$$C_{x2} = (A_2 - A_{y2}) \pm b_{x2}/a_{x2} \quad (8)$$

where A_1, A_2, a_1, a_2, b_1, b_2, C_1 and C_2 are the absorbance, slope, intercept, concentration at λ_1 and λ_2, respectively.

Theory of CRACLS multivariate calibration method [29]

CRACLS is a new method that estimates absorptivity and thus it does not require the condition of knowing the spectra of all components in the measured sample.

The calculation of the absorptivity from absorbance and concentration is given by:

$$A = C\hat{S} + E_A \quad (9)$$

where, A is the absorbance set, C is the concentration set, E_A is the error of regression and $\hat{S}$ is the estimated absorptivity by a process of repetitive approximation as shown in the following steps

$$\text{Step 1}: \ \hat{S} \text{ is calculated}: \ \hat{S} = (C'C)^{-1}C'A \quad (10)$$

$$\text{Step 2}: \ \hat{S} \text{ is used to predict } C': \quad C' = A\hat{S}'(\hat{S}\hat{S}')^{-1} \quad (11)$$

$$\text{Step 3}: \text{ Error in } C': \quad E = C' - C \quad (12)$$

Step 4: One vector of E is augmented to the original C (E is considered as a new component).

Step 5: Step (1) is repeated using the augmented C until no further improvement in prediction is achieved.

Experimental

Instruments

- Spectrophotometric analysis was carried out on double beam UV–visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with matched 1 cm quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption spectra. The spectral band width was 2 nm with wavelength-scanning speed of 2800 nm min^{-1}.
- Matlab® for Windows™ version 7.0.1 Mathwork Inc. 2004 was used in calculating multivariate calibrations, for CRACLS, all computation were performed by MATLAB® with previously designed codes. The PLS procedure was taken from PLS-Toolbox 2.1 Eigenvector Research, Inc.2005 created by B.M. Wise and N.B. Gallagher for use with Matlab®.
- Mass spectrometer, Shimadzu Qp-2010 (Nakagyo-ku, Kyoto, Japan), operated on EI mode at 70 eV.
- IR Spectrometer: Shimadzu 435 (Nakagyo-ku, Kyoto, Japan), sampling were undertaken as potassium bromide discs.
- pH-metre, Digital pH/MV/TEMP/ATC metre, Model-5005, Jenco Instruments (Arjons Drive, San Diego, California, USA).

Materials and reagents

Pure standard

Standard PER (99.68% ± 0.69) and AML (100.14% ± 1.14) were kindly supplied by Servier Egypt Industries limited, 6th October City, Cairo, Egypt. Their purities were assessed according to the manufacturer method and official [4] HPLC methods, respectively.

Pharmaceutical formulation

Coveram®tablet is available in several different strength combinations including 5/5 mg, 10/10 mg, 5/10 mg and 10/5 mg of PER and AML, respectively. It is manufactured by Servier (Ireland) industries limited for les laboratories Servier, France. Batch No. 77597, 376112, 73935& 59423 and were purchased from the Egyptian market.

Degraded sample

Both PER and AML were subjected to alkali degradation. Solutions were prepared by dissolving, separately, 10 mg of pure PER and AML powders in 100 mL of 0.1 M and 1 M NaOH, respectively, and refluxed for 2 hrs in case of PER and 1 h for AML. Complete degradation was confirmed by TLC using ethyl acetate-methanol-toluene-ammonia solution 33% (6.5:2:1:0.5 by volume) as developing system [26]. The solutions were neutralized by hydrochloric acid to pH 7.0 then subjected to evaporation under vacuum nearly to dryness. The residues after evaporation were extracted with 100 mL methanol four times, 25 mL for each in order to prepare stock solutions of each degradation product equivalent to 0.1 mg mL^{-1} of its respective drug.

Chemicals and reagents

Hydrochloric acid (0.1 & 1 M aqueous solutions) sodium hydroxide (0.1 & 1 M aqueous solutions) and ammonium hydroxide 33%; El-Nasr Pharmaceutical Chemicals Co., (Abu-Zabaal, Cairo, Egypt), Methanol of spectroscopic grade; S.D.Fine Chemicals Ltd. (Mumbai, India).

Standard solutions

(a) Stock standard solutions of both PER and AML (0.1 mg mL^{-1}) in methanol for ACM and AFM methods.
(b) Stock standard solutions of both PER and AML (1 mg mL^{-1}) in methanol for PLS and CRACLS methods.
(c) Stock solutions of the alkaline degradation products equivalent to 0.1 mg mL^{-1} in methanol.
(d) Working solution of AML degradation product (25 μg mL^{-1}). Transfer accurately, 25 mL of its respective stock solution (0.1 mg mL^{-1}) into 100-mL volumetric flask, and complete to mark with methanol.

All stock standard solutions were freshly prepared on the day of analysis and stored in refrigerator to be used within 24 h. Solutions containing AML or its alkaline degradation product were wrapped with aluminium foil to be light protected.

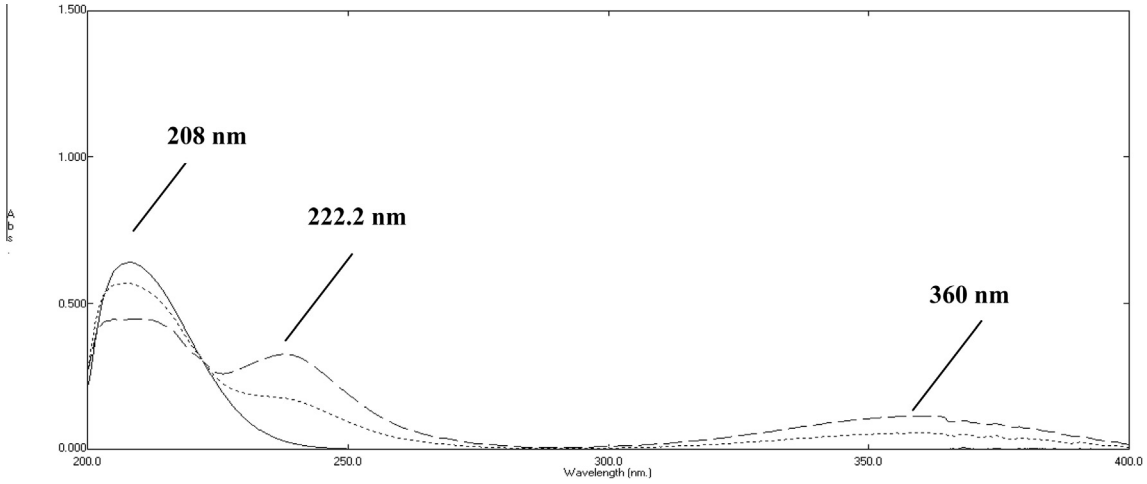

Fig. 2a. Zero order absorption spectra of 40 μg mL^{-1} of perindopril (––––), 10 μg mL^{-1} of amlodipine (– – –) and a (4:1) mixture containing 20 μg mL^{-1} of perindopril and 5 μg mL^{-1} of amlodipine (.....) using methanol as a blank.

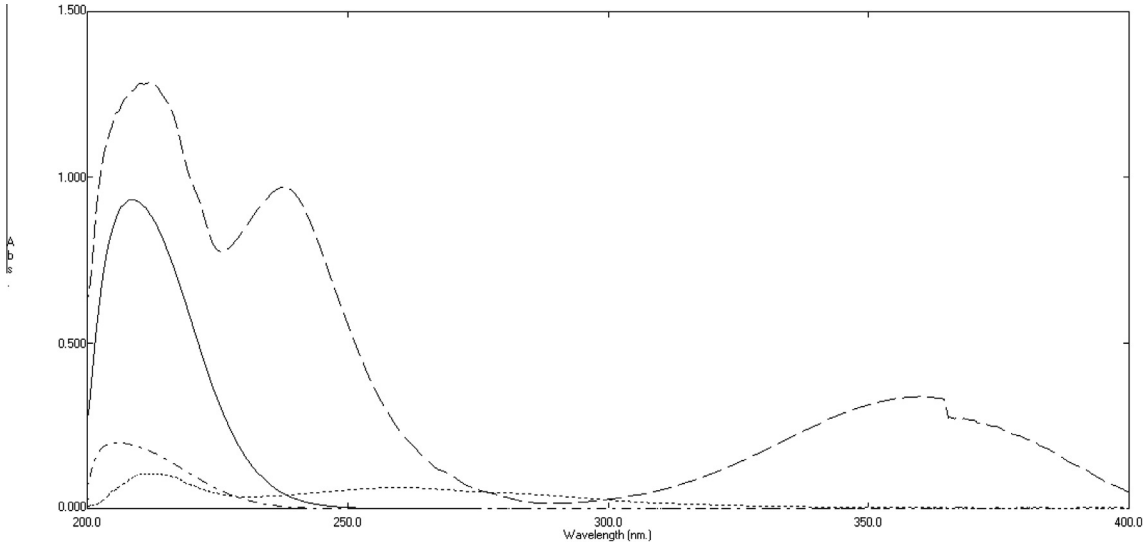

Fig. 2b. Zero order absorption spectra of 60 μg mL^{-1} of perindopril (–––––), 30 μg mL^{-1} of amlodipine (– – – –), 6 μg mL^{-1} of perindoprilate (–·–·–·–) and 6.25 μg mL^{-1} of amlodipine degradation product (.....) using methanol as a blank.

Procedure

Construction of calibration curve

For AFM & ACM univariate spectrophotometric methods. Serial dilutions of PER and AML in methanol were prepared separately from their respective stock standard solutions (0.1 mg mL^{-1}) into two sets of 10-mLvolumetric flasks in the range of 10–70 and 4–28 μg mL^{-1}, respectively. The absorption spectra of these solutions were recorded, and absorbance at λ_{max} (360 nm) for AML and at λ_{iso} (222.2 nm) for each of AML and PER were measured for AFM method. The absorbances at λ_{max} 360 nm for AML and at λ_{max} 208 nm for each of AML and PER were measured for ACM. Calibration curves relating the values of absorbance at the selected wavelength to the corresponding drug concentrations were constructed and then the regression equations were computed.

For multivariate calibration methods (PLS and CRACLS). The training (calibration) and validation set containing different concentrations of the four components (PER, AML and their degradation products) was designed according to five levels four factors design, 19 mixtures were randomly chosen and used as a calibration set. The mixtures were prepared by mixing different aliquots of PER, AML, perindoprilate stock solutions and DEG working solution in the range of 60–100, 20–40, 6–10 and 5–10 μg mL^{-1}, respectively, in a series of 10-mL volumetric flasks. The UV absorption spectra of the prepared solutions were recorded over the range 210–400 nm, then the data points of spectra were transferred to Matlab® for subsequent data analysis and construction of multivariate calibration models. All the spectral data were used for building CRACLS model, while subjected to mean centred as a pre-processing step before building PLS model.

Analysis of laboratory prepared mixtures containing different ratios of Perindopril arginin and amlodipinebesylate using the suggested methods

For conventional spectrophotometric methods (AFM & ACM). Aliquots of intact PER, AML were mixed to prepare mixtures containing different ratios of PER and AML including the market ratios. The absorbance of the resulting solutions was measured at λ_{max} 360 nm which corresponds to the concentration of AML alone,

so its concentration in each mixture was determined using its corresponding regression equation.

For AFM, absorbance of each mixture at $\lambda_{222.2}$ nm which corresponds to total concentration of AML and ¼PER in the mixture (C_{AML} + ¼C_{PER}) was measured. The total concentration of the mixture was calculated using the computed regression equation of AML at 222.2 nm, the concentration of AML was subtracted from total concentration then the result was multiplied by the inverse of the absorptivity factor ($1/F = 4$) to obtain the concentration of PER.

For ACM, absorbance of the prepared mixtures at 208 & 360 nm were recorded. The computed regression equations at λ_{max} 208 & 360 nm for AML were rearranged to obtain a relation between the absorbance of AML at these two wavelengths (208 &360 nm). Using the resulting equation, the postulated values of absorbance of AML at 208 nm were calculated. Subtraction of these values from the recorded absorbance of the mixtures at 208 nm yields the absorbance corresponding to PER whose concentration can be calculated using its computed regression equation at 208 nm.

For multivariate calibration methods (CRACLS & PLS). An external validation set was used and was randomly chosen. This set is made up of 6 mixtures containing different ratios of PER, AML and their degradation products. Aliquots of PER, AML, PER degradation product stock solutions and AML degradation product working solution were mixed in 10-mL volumetric flasks, and then the volumes were completed with methanol. The spectra of these solutions were recorded from 210 to 400 nm, and used for assessing the predictive ability of the developed models by determination of the concentration of PER and AML in each mixture.

Assay of pharmaceutical formulations (Coveram®tablets)

Ten tablets of Coveram® [5/5 mg, 10/10 mg, 5/10 mg and 10/5 mg of PER and AML, respectively] were weighed, finely powdered and mixed thoroughly. An accurately weighted portion of the powdered tablets equivalent to the weight of one tablet was transferred into four separate 100-mL volumetric flasks, 50 mL of methanol was added, sonicated for 10 min, and then 25 mL methanol was added and sonicated for further 10 min to ensure complete dissolution. Volumes were completed with methanol and then filtered. Suitable dilution was made to obtain concentrations of each of the two drugs in the range of linearity. The general procedure previously described under analysis of laboratory prepared mixtures for each method was followed to calculate concentrations of PER and AML.

Table 1
Determination of perindopril and amlodipine in laboratory prepared mixtures by the proposed spectrophotometric methods.

Perindopril:amlodipine		Recovery* %		
Ratio	Concentrations (μg mL^{-1})	Perindopril		Amlodipine
		Absorptivity factor at 222.2 nm	Absorbance correction at 208 nm	Zero order at 360 nm
1:1	15:15	98.29	100.09	101.13
1:2	10:20	98.68	99.52	100.60
2:1	24:12	100.53	98.32	100.72
1.5:1	12:8	100.04	98.51	100.20
4:1	40:10	100.90	98.41	100.80
Mean ± SD		99.69 ± 1.15	98.97 ± 0.79	100.69 ± 0.34
%RSD		1.15	0.80	0.34

* Average of three separate determinations.

Results and discussion

Direct spectrophotometric technique is a simple, reproducible and rapid technique commonly used for separation of drug mixtures with high accuracy and precision without prior separation or chemical derivatization. Therefore, it was thought worthwhile to use the conventional univariate spectrophotometric methods (AFM & ACM) as a satisfactory tool for determination of PER and AML in their binary mixture in spite of their overlapped spectra, Fig. 2a. AFM method, depends on the calculation of an independent factor (absorptivity) which is very accurate, precise and reliable. While, ACM uses the mathematical equations to transform the absorbance in a selected wavelength to its correspondence in another one. These methods failed to resolve the sever overlap of PER, AML and their degradation products complex mixture, Fig. 2b.

On the other hand, multivariate calibration spectrophotometric methods succeed for the determination of PER, AML along with their degradation products without previous chemical separation are developed. As the developed models relate the multiple spectral intensities from many calibration samples to the known analyte concentrations of the samples, succeeded in the resolution and determination of PER and AML in this multicomponent mixture and can be used as stability indicating method.

For conventional spectrophotometric methods (AFM & ACM)

The total absorbance of any mixture is a result of summation of the individual absorbance of each component in the mixture. So, if the relation between the absorptivities of the components is obtained at specific wavelength, by rearrangement we can calculate the concentration of each component [30]. Iso-absorptive point method can be considered a special case of this method at which the absorptivity factor equals the unity [31]. However; when two components with different concentrations have the same absorbance at certain wavelength, the absorptivity factor can be calculated at this wavelength as explained before in the theory and used for the calculations of their concentrations. This theory can be verified experimentally by recording the absorption spectra of 40 μg mL^{-1} PER, 10 μg mL^{-1} AML and a mixture of 20 & 5 μg mL^{-1} of PER & AML, respectively. As shown in Fig. 1a, these spectra have the same absorbance at 222.2 nm, at which the absorptivity factor was calculated and found to be a_{PER}/a_{AML} = ¼.

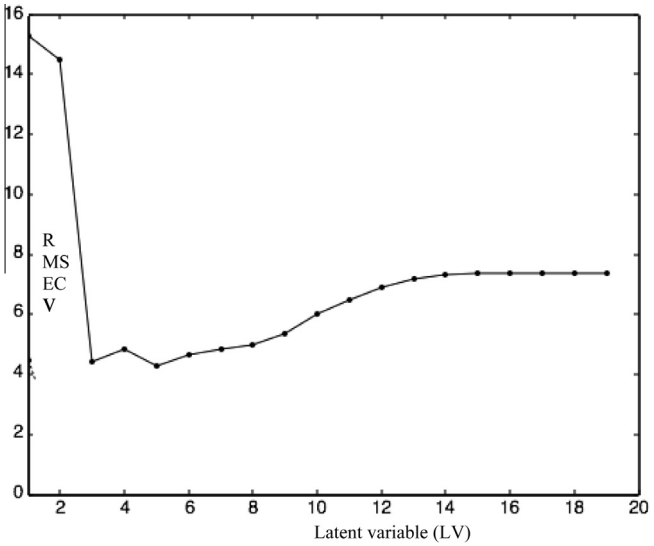

Fig. 3. RMSECV plot of the cross validation results of the training set as a function of the number of Latent variables used to construct the PLS calibration.

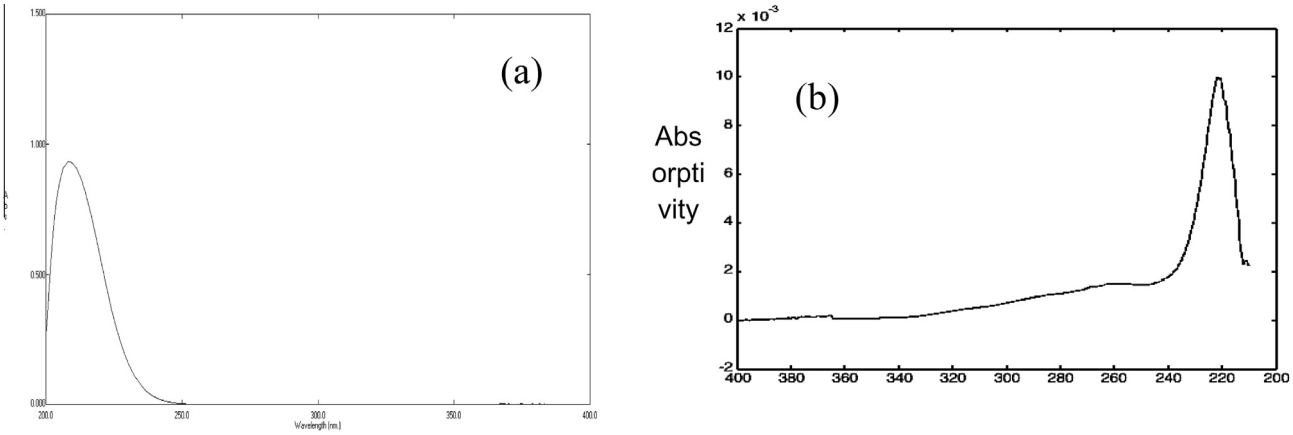

Fig. 4. Zero order absorption spectrum of perindopril (a) and its estimated spectrum by CRACLS method (b).

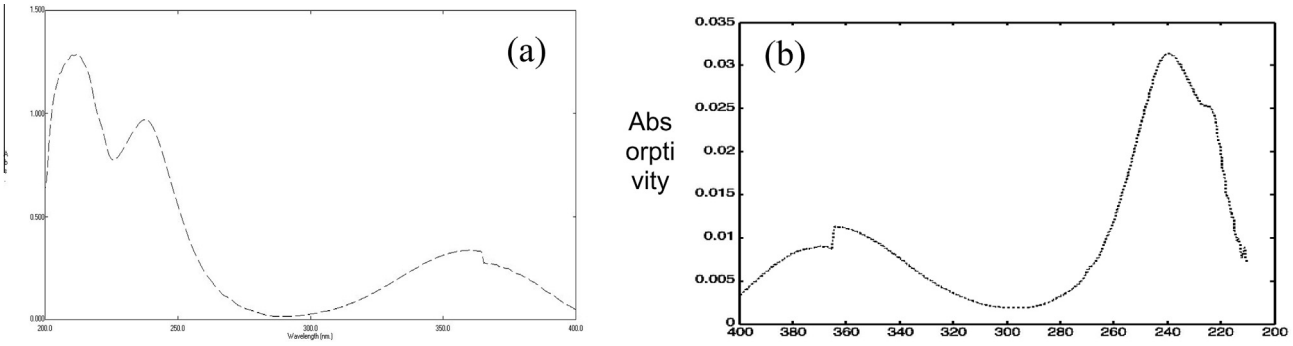

Fig. 5. Zero order absorption spectrum of amlodipine (a) and its estimated spectrum by CRACLS method (b).

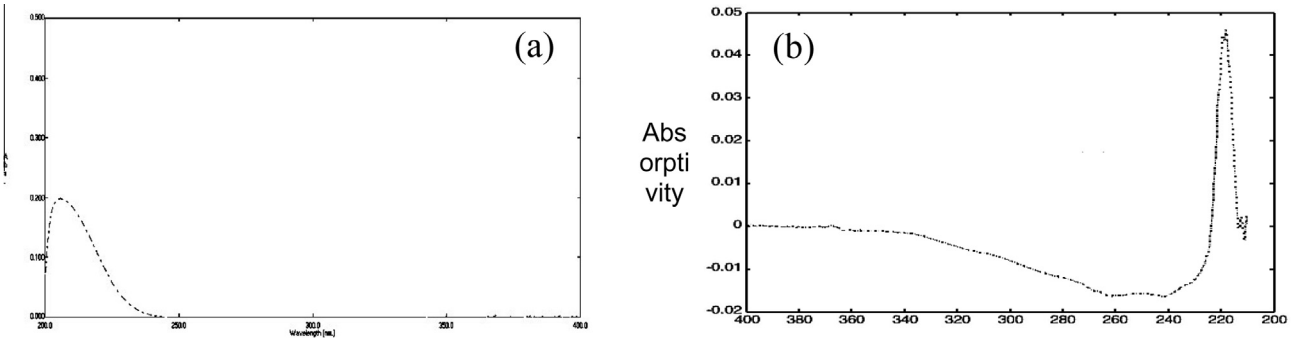

Fig. 6. Zero order absorption spectrum of perindoprilate (a) and its estimated spectrum by CRACLS method (b).

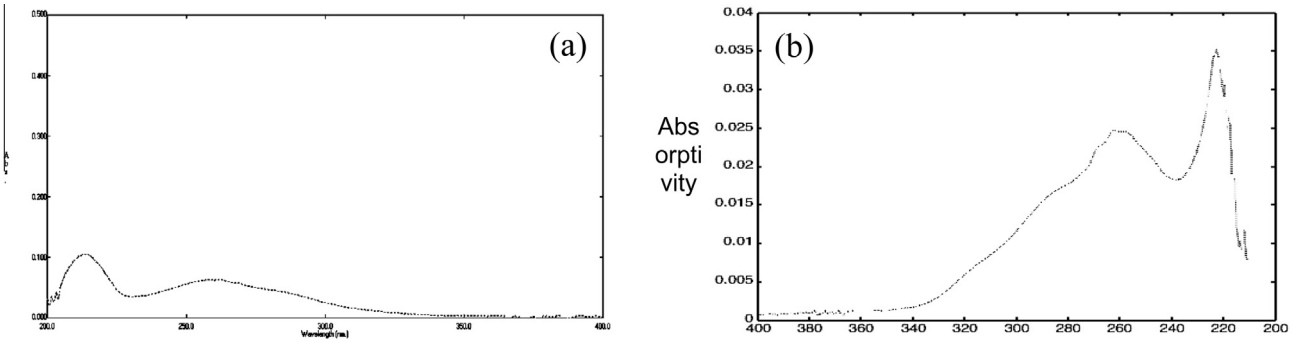

Fig. 7. Zero order absorption spectrum of amlodipine degradation product (a) and its estimated spectrum by CRACLS method (b).

Table 2
Determination of perindopril and amlodipine in the validation set by the proposed PLS and CRACLS.

Mix. No.	Recovery %			
	Perindopril		Amlodipine	
	PLS	CRACLS	PLS	CRACLS
1	101.35	99.45	99.19	99.73
2	100.24	100.66	99.74	99.44
3	99.38	100.05	100.22	100.52
4	99.62	100.78	99.25	99.43
5	101.68	99.41	101.03	100.42
6	97.52	99.65	100.63	100.47
Mean ± %RSD	99.97 ± 1.51	100.00 ± 0.60	100.01 ± 0.75	100.00 ± 0.53
RMSEP	1.48	0.518	0.258	0.202

The absorption spectra of different laboratory prepared mixtures with variable ratios were recorded, and the absorbance at 360 nm was measured and used to calculate the concentration of AML in the mixture from its corresponding regression equation. Then use the absorbance at 222.2 nm to calculate the total concentration of the mixture from the regression equation of AML at this wavelength. So, after subtraction of the concentration of AML from the total concentration then multiplying the result by the inverse of absorptivity factor ($1/F = 4$) the concentration of PER was obtained.

Calibration curves were constructed between absorbance at λ222.2 nm for PER and its concentration in the range of 10–70 μg mL^{-1} and at λ_{max} 360 and λ222.2 nm for AML and its concentration in the range of 8–28 and 4–28 μg mL^{-1}, respectively. Linear correlations were obtained from which the regression equations were calculated and found to be:

$$A_1 = 0.0075C_1 + 0.0050 \quad r_1 = 0.9999 \quad \text{at 222.2 nm for PER} \quad (13)$$

$$A_2 = 0.0303C_2 - 0.0083 \quad r_2 = 0.9999 \quad \text{at 222.2 nm for AML} \quad (14)$$

$$A_2 = 0.0114C_2 - 0.0014 \quad r_2 = 0.9998 \quad \text{at 360 nm for AML} \quad (15)$$

where A_1, A_2, C_1, C_2, r_1 and r_2 are the absorbance, the concentrations in μg mL^{-1} and the correlation coefficients of PER and AML, respectively.

Table 3
Determination of perindopril and amlodipine in Coveram®tablets by the proposed spectrophotometric methods with standard addition technique.

Pharmaceutical dosage form	Recovery* % ± RSD		
	Perindopril		Amlodipine
	Absorptivity factor at 222.2 nm	Absorbance correction at 208 nm	Zero order at 360 nm
Coveram®tablets 5(PER)/ 5(AML) mg Batch No. 77597	100.95 ± 0.96	99.43 ± 0.77	100.46 ± 0.46
Standard addition	99.55 ± 0.84	99.95 ± 1.12	100.04 ± 0.62
Coveram®tablets 10(PER)/ 10(AML) mg Batch No. 376112	100.89 ± 1.19	99.05 ± 0.71	100.61 ± 0.55
Standard addition	99.76 ± 0.90	100.45 ± 0.89	99.68 ± 0.36
Coveram®tablets 5(PER)/ 10(AML) mg Batch No. 73935	101.05 ± 1.00	98.77 ± 0.79	100.14 ± 0.56
Standard addition	99.46 ± 0.83	99.73 ± 1.01	99.84 ± 0.70
Coveram®tablets 10(PER)/ 5(AML) mg Batch No. 59423	100.99 ± 1.10	99.00 ± 0.82	99.87 ± 0.44
Standard addition	99.86 ± 0.79	100.16 ± 1.00	99.53 ± 0.56

* Average of three separate determinations.

From Eqs. (13) and (14), one can conclude that the absorptivity of PER is ¼ times that of AML, which confirms the chosen factor.

For the second method (ACM), Linear relationships was obtained between absorbance at λ_{max} 208 nm for PER and its concentration in the range of 10–70 μg mL^{-1} and at λ_{max} 208 nm for AML and its concentration in the range of 4–28 μg mL^{-1}. Linearity was obtained from which the regression equations were calculated and found to be:

$$A_1 = 0.0147C_1 + 0.0494 \quad r_1 = 1.0000 \quad \text{at 208 nm for PER} \quad (16)$$

$$A_2 = 0.0418C_2 + 0.0216 \quad r_2 = 0.9999 \text{ at 208 nm for AML} \quad (17)$$

where A_1, A_2, C_1, C_2, r_1 and r_2 are the absorbance, the concentrations in μg mL^{-1} and the correlation coefficients of PER and AML, respectively.

As previously mentioned AML has a maximum at λ_{max} 360 nm, while PER did not absorb at this wavelength. Accordingly, the absorbance at 360 nm of the mixed drugs in laboratory prepared mixture, were linearly correlated with AML concentration and not affected by the presence of PER. Then, we can use the advantage of that both AML and PER have almost the same peak at at λ_{max} 208 nm, so the absorbance of this point corresponds to total contribution of AML and PER ($A_T = A_{PER} + A_{AML}$). Thus evaluation of the contribution of AML at 208 nm can offer the opportunity for PER quantification. In fact, the absorbance of AML at 208 nm in the mixture can be calculated by rearranging Eqs. (15) and (17) to obtain Eq. (18) as follows:

$$C_{360\ nm} = (A_{360\ nm} + 0.0014)/0.0114$$

$$C_{208\ nm} = (A_{208\ nm} - 0.0216)/0.0418$$

$$\therefore C_{360\ nm} = C_{208\ nm}$$

$$\therefore A_{208\ nm} = 3.667A_{360\ nm} + 0.0267 \text{ for AML} \quad (18)$$

So, the absorbance of PER at 208 nm in the mixture could be obtained by subtraction of the calculated absorbance of AML at 208 nm from the recorded absorbance of the laboratory prepared mixture at the same wavelength and then the concentration of PER can be calculated from its regression Eq. (16).

In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by analysing laboratory prepared mixtures of PER and AML prepared in different ratios. Results obtained are shown in Table 1, such results encourage the use of the methods described for the assay of commercial tablets and the application of standard addition technique in compliance with ICH to verify the accuracy of the method for the determination of drug product.

For multivariate calibration methods (CRACLS& PLS)

The developed models were applied for the analysis of PER and AML in presence of their alkaline degradation products. A training (calibration) set was designed with nineteen quaternary samples containing different concentrations of both drugs and their alkaline degradation products. The training set was designed to give symmetric and orthogonal distribution of the four components in order to allow accurate determination. Upon optimisation of data handling, it was found that the best results were obtained when the spectra were digitized each at 0.2 nm in the range of 210–400 nm, where 950 experimental points were used in the calculations.

For the developed PLS model, the selection of the optimum number of latent variables was a very important step before constructing the model because if the number of variable retained was more than required, more noise will be added to the data.

Table 4
Determination of perindopril and amlodipine in Coveram®tablets by the suggested CRACLS and the manufacturer methods.

Pharmaceutical formulations	Recovery % ± RSD** of perindopril		Recovery % ± RSD** of amlodipine	
	CRACLS	Manufacturer method*	CRACLS	Manufacturer method*
Coveram®tablets 5(PER)/5(AML) mg Batch No. 77597	100.16 ± 0.40	100.66 ± 0.47	99.92 ± 0.25	100.22 ± 0.51
F-value (5.05)	1.38	–	4.33	–
Student's *t*-test (2.228)	1.984	–	1.299	–
Coveram®tablets 10(PER)/10(AML) mg Batch No. 376112	99.59 ± 0.58	100.06 ± 0.40	99.61 ± 0.23	99.50 ± 0.48
F-value (5.05)	2.13	–	4.60	–
Student's *t*-test (2.228)	1.626	–	0.509	–
Coveram®tablets 5(PER)/10(AML) mg Batch No. 73935	100.34 ± 0.82	99.51 ± 0.55	99.67 ± 0.32	99.09 ± 0.59
F-value (5.05)	2.23	–	3.40	–
Student's *t*-test (2.228)	2.065	–	2.094	–
Coveram®tablets 10(PER)/5(AML) mg Batch No. 59423	100.24 ± 1.18	100.19 ± 0.80	99.58 ± 0.20	99.75 ± 0.39
F-value (5.05)	2.17	–	3.75	–
Student's *t*-test (2.228)	0.086	–	0.955	–

– Figures between parenthesis are the corresponding tabulated values of *t* and *F* at *P* = 0.05.
* Manufacturer method is HPLC using C_{18} column and mobile phase consists of (66:34, v/v) acetonitrile – aqueous phase (water + 0.3% triethylamine adjusted to pH 2.5 with 35% perchloric acid) and UV detection at 215 nm.
** Average of six determinations for the proposed CRACLS method and the manufacturer method.

Table 5
Regression of the proposed methods for the determination of perindopril and amlodipine by ACM, AFM and the determination of both drugs in presence of their degradation products by multivariate calibrations in the validation set.

Method parameter	Perindopril				Amlodipine				
	ACM	AFM	CRACLS	PLS	ACM	AFM	At 360 nm	CRACLS	PLS
Wavelength (nm)	208	222.2			208	222.2	360		
Linearity range ($\mu g\ mL^{-1}$)	10–70		60–100		4–28		8–28	40–60	
Regression equation									
Intercept	0.0494	0.005	1.458	0.5155	0.0216	−0.0083	−00,014	0.5900	0.1700
Slope	0.0147	0.0075	0.9841	1.0123	0.0418	0.0303	0.0114	0.9806	1.0029
Correlation coefficient (*r*)	1.0000	0.9999	0.9990	0.9930	0.9999	0.9999	0.9998	0.9995	0.9990
Accuracy mean ± %RSD	100.11 ± 0.60	99.34 ± 0.56	100.00 ± 0.60	99.97 ± 1.51	100.00 ± 0.51	100.12 ± 0.60	99.57 ± 0.84	100.00 ± 0.53	100.01 ± 0.75
Specificity & selectivity	99.69 ± 1.15	98.97 ± 0.80	–	–	–	–	100.69 ± 0.34	–	–
Precision									
(%RSD)[a]	±0.41	±0.52	–	–	±0.45	±0.58	±0.32	–	–
(%RSD)[b]	±0.55	±0.67			±0.56	±0.79	±0.85		
LOD ($\mu g\ mL^{-1}$)	0.50	0.74	3.05	10.11	0.27	0.31	0.53	0.58	0.89
LOQ ($\mu g\ mL^{-1}$)	1.52	2.24	9.25	30.62	0.71	0.95	1.59	1.76	2.71

RSD%[a], RSD%[b]: the intra-day, inter-day, respectively (n = 3) relative standard deviation of different concentrations.

On the other hand, if the number retained was too small, meaningful data might be discarded.

Different methods could be used for the determination of optimum number of variables. In this study, the leave one out cross validation method was used and the RMSECV values of different models were compared. The selected model was that of the smallest number of variables which was found to be five for the mean centred data, Fig. 3.

The CRACLS model which was built for PER and AML in presence of their alkaline degradation products, has an advantage over PLS model. This model has the ability to estimate pure components spectra by including the components concentration and augmenting the error 19 times. Figs. 4–7 show the resemblance of the estimated spectra and the actually scanned ones.

The validation of the suggested models was done using several diagnostic tools. These tools were grouped into two categories, which were the model diagnostic tools used to determine the quality of the model and the sample diagnostic tools which are used to study the relationship between the samples and to identify unusual samples.

To test the predictive ability of the developed models (CRACLS and PLS), they were challenged with the spectra of the validation set, made up of six samples different than those of the training set, Table 2. Also, the root mean square error of prediction (RMSEP) was calculated and used as a diagnostic tool for examining the errors in the predicted concentrations; it indicates both accuracy and precision. The calculated RMSEP of CRACLS model is smaller than PLS indicating the high predictive ability of the suggested CRACLS model as shown in Table 2.

The conventional spectrophotometric methods are valid and applicable for the analysis of PER and AML in their pharmaceutical formulation. Furthermore, the validity of the proposed methods was assessed by applying the standard addition technique, which produced accurate results indicating that excipients do not adversely affect the results as shown in Table 3.

Where CRACLS model is considered to be more sensitive, accurate and precise than PLS model, its use for the analysis of both drugs in their combined dosage form was encouraged. It was successfully applied to the analysis of PER and AML in pharmaceutical formulation, Table 4, while PLS model failed to determine both drugs in their dosage form.

Linear correlations were obtained for all the proposed methods, linearity of the conventional calibrations were obtained between the absorbance of the ^{0}D absorption spectra, and the corresponding concentrations of the drugs. While in multivariate calibrations, the linear correlations were obtained between the predicted and the original concentrations of both drugs. The concentration ranges and regression parameters were shown in Table 5. The same table showed the mean recoveries and RSD values of their laboratory prepared mixtures, moreover, LOD and LOQ were also calculated

Table 6
Statistical analysis of the results obtained by applying the proposed spectrophotometric methods and the manufacturer or official method for the determination of perindopril (a) and amlodipine (b).

Method parameter	(a)-Perindopril				
	ACM	AFM	CRACLS	PLS	Manufacturer method*
Mean	100.11	99.34	100.00	99.97	99.68
%RSD	0.60	0.56	0.60	1.51	0.69
n	6	6	6	6	6
Variance	0.36	0.31	0.36	2.28	0.48
F-value (5.05)	1.33	1.55	1.33	4.75	–
Student's *t*-test (2.228)	1.194	0.971	0.842	0.426	–

Method parameter	(b)-Amlodipine					
	ACM	AFM	At 360 nm	CRACLS	PLS	Official method**
Mean	100.00	100.12	99.57	100.00	100.01	100.14
%RSD	0.51	0.60	0.84	0.53	0.75	1.14
n	6	6	6	6	6	6
Variance	0.26	0.36	0.71	0.28	0.56	1.30
F-value (5.05)	5.00	3.61	1.83	4.64	2.32	–
Student's *t*-test (2.228)	0.286	0.039	1.018	0.269	0.232	–

* Manufacturer method is HPLC using C_{18} column and mobile phase consists of (66:34, v/v) acetonitrile-aqueous phase (water + 0.3% triethylamine adjusted to pH 2.5 with 35% perchloric acid) and UV detection at 215 nm.
** Official method is HPLC method, using C_{18} column, 2.3 g L^{-1} amm. acetate-methanol (30:70, v/v) as a mobile.

to assist the validity of the proposed methods according to ICH guidelines [32].

The results obtained by applying the proposed conventional and multivariate calibrations for the determination of perindopril and amlodipine in bulk powder were statistically compared with the manufacturer HPLC method for PER and official method [4] for AML using student's *t* and *F*-values, indicating no significant difference regarding both accuracy and precision as shown in Table 6.

From the comparative view, the proposed multivariate methods provide powerful means for stability indicating determination of each drug in presence of its degradation products which is not applied in the reported ones [24,25]. Also, can predict the absorption spectra of the degradation products depending on the calculation of independent variable (absorptivity) as in CRACLS method. While the univariate AF method has the merits of high precision and reliability as it can estimate the concentration of each drug depending in its calculation on an independent variable (absorptivity) not a dependent one (absorbance) as in the published method [24].

Conclusion

Mathematically based univariate spectrophotometric methods have the advantage of being rapid and simple for resolution and determination PER and AML in their dosage form without any interference from excipients. While, when the issue of stability arises and the spectra became more complex, the need of multivariate calibration methods glow up. Accuracy, precision and high specificity along with spectral extraction of mixture components was achieved by CRACLS algorithm which was capable of determining PER and AML in the presence of their spectrally overlapped degradation products and in dosage form, while conventional PLS failed. The proposed methods was confirmed to be of comparable accuracy and precision to the reference methods.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.05.096.

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