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Original Article

ADVANCED AMPLITUDE CENTERING AS AN INVIGORATING MANIPULATION FOR UNIFIED WAVELENGTH SPECTRAL RESOLUTION OF TERNARY MIXTURES

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ABSTRACT

Objective: This work presented the novel spectrophotometric approach namely, advanced amplitude centering (AAC). The method was applied for the resolution of ternary mixtures with partially or completely overlapped spectra.

Methods: Advanced amplitude centering was based on the determination of ternary mixtures using single divisor where the concentrations of the components are determined through progressive manipulation performed on the same ratio spectrum. The centered amplitude at unified wavelength was resolved and applied for the determination of three components with partially and severely overlapped spectra. The work discussed the applications and advantages of the novel univariate advanced amplitude centering compared to the chemometric model, partial least square (PLS).

Results: The specificity of the proposed methods was checked using laboratory-prepared mixtures of amlodipine (AML), valsartan (VAL) and hydrochlorothiazide (HCT) and was successfully applied for the analysis of two pharmaceutical formulations. The validity of results was assessed by applying the standard addition technique.

Conclusion: The results obtained were found to agree statistically with those obtained by a reported method, showing no significant difference with respect to accuracy and precision.

Keywords: Amplitude centering, Severely overlapping spectra, Amlodipine, Valsartan, Hydrochlorothiazide, Resolution of mixtures

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INTRODUCTION

Analysis of complex mixtures containing three or more components represented a challenge for analysts. For resolving such mixtures, Analysts preferred using hyphenated instrumentations such as LC-MS, GC-MS, LC-NMR [1, 2] , which consume lots of time and money. Unfortunately, the spectrophotometric methods, which provided a solution for complex ternary mixtures, were tedious, require several manipulating steps and exhibited several limitations [3-5]. Although chemometric models are cheap, but they require special software and a large number of synthetic mixtures [6, 7].

The components of interest were amlodipine (AML), valsartan (VAL) and hydrochlorothiazide (HCT). Several analytical techniques, including chromatography, spectrophotometry and electro-chemistry, were reported for the analysis of each the components individually: AML [8-12] , VAL [13-16] and HCT [17-19] The binary mixture of AML and VAL was determined using spectrophotometric [20] and HPLC methods [21] while the mixture containing AML and HCT was determined using spectrophotometric method [22] and capillary electrophoresis [23] The binary mixture of VAL and HCT was determined using spectroscopic, TLC and HPLC methods [24].

The determination of the ternary mixture of AML, VAL and HCT was reported using spectrophotometric methods which utilised multi-step manipulation using several equations and wavelengths [25] but with certain limitations as the existence of iso-absorptive point [26] Advanced chemometric tools were also reported [27, 28] which required certain decoding. TLC and HPLC chromatographic methods were reported for the analysis too [29].

The concept of amplitude centering was firstly introduced as a progressive determination of three components in their ternary mixture where their spectra were partially overlapped [30] This was done through the same ratio spectrum via their corresponding amplitudes using single divisor.

The aim of the presented work was to introduce a modification on amplitude centering method which allows the simultaneous determination of any ternary mixture either with partially or completely overlapping spectra using single divisor by unified wavelength spectral resolution. The resolution efficiency of the proposed technique was checked by the analysis of the ternary mixtures' spectra AML, VAL and HCT and their pharmaceutical formulations.

Unlike all the reported methods, the advanced amplitude centering (AAC) was selective, less expensive, time-saving and could be applied for the analysis of any ternary mixture with no limitations or sophisticated instrument. Additionally, this procedure had comparable precision and accuracy to the PLS chemometric ones but with more simplicity, as there is no need for special software (Matlab®) and this advantage permits its wider application in solving analytical problems.

Theory of advanced amplitude centering (AAC)

This novel approach of advanced amplitude centering (AAC) which was introduced by Saleh *et al.* [30] The approach depended on the progressive determination of three components in their ternary mixture through the same ratio spectrum via their corresponding amplitudes using single divisor. It could be applied for a ternary mixture of X, Y and Z with partially or completely overlapped spectra. This novel approach was based on amplitude difference, which is the first step of the constant center spectrophotometric method [31] in order to resolve the centered amplitude recorded at a single wavelength on the ratio spectrum of the mixture to amplitudes corresponding to each component in the ternary mixture.

Approach for partially overlapping spectra

For a mixture of three drugs (X+Y+Z), at λ_1 and λ_2: X and Y spectra show overlapping where Z is extended. This method consisted of

three steps complementary to each other namely; amplitude calculation and amplitude factor then finally amplitude subtraction.

By dividing the spectrum of the mixture by a known concentration of Z as a divisor (Z'). The division will give a new spectrum that can be summarized as follows:

$$\frac{X+Y+Z}{Z'} = \frac{X}{Z'} + \frac{Y}{Z'} + \frac{Z}{Z'} = \frac{X}{Z'} + \frac{Y}{Z'} + \text{Constant} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The amplitude calculation step for component Z where the amplitude Z/Z' constant can be accurately determined at the extended region parallel to the wavelength axis,

Then subtract this constant value Z/Z' to get the ratio spectrum of X and Y

$$= [\frac{X}{Z'} + \frac{Y}{Z'} + \frac{Z}{Z'}] - \frac{Z}{Z'} = \frac{X}{Z'} + \frac{Y}{Z'}$$

The obtained ratio spectrum of a binary mixture of X and Y $(\frac{X}{Z'} + \frac{Y}{Z'})$ which exhibit complete overlapped ratio spectra at two wavelengths λ_1 and λ_2.

$$P_1 = P_{X1} + P_{Y1} \text{ at } \lambda_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$P_2 = P_{X2} + P_{Y2} \text{ at } \lambda_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where P_1 and P_2 is the amplitudes of the ratio spectrum of the mixture at λ_1 and λ_2 and Px_1, Px_1 and P_{Y1}, P_{Y1} are the corresponding amplitudes of X and Y at the two chosen wavelengths.

The amplitude factor is calculated in order to cancel the effect of Y at the two selected wavelengths, the equality factor of pure Y (F_Y) is calculated which is the amplitude ratio of the different concentration of pure Y at λ_1 and $\lambda_2$is calculated:

$$F_Y = P_{Y1}/P_{Y2}. \; P_{Y1} = F_Y \; P_{Y2}$$

Then substitute P_{Y1} at equation (3) as follows:

$$P_1 = P_{X1} + F_Y \; P_{Y2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

By multiply equation (4) by F_Y

$$F_Y P_2 = F_Y \; P_{X2} + F_Y \; P_{Y2} \quad \ldots\ldots\ldots\ldots (6)$$

The difference between the two equation (5) and (6), $F_Y \; A_{Y2}$ will be cancelled as follows:

$$\Delta P \; (P_1 - F_Y \; P_2) = P_{X1} - F_Y \; P_{X2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Therefore, $\Delta P \; (P_1 - F_Y \; P_2)$ of the mixture is dependent only on the values of C_X, and is independent of the value of C_Y in the mixture.

The amplitude calculation step for X in the mixture starts with amplitude difference method where a regression equation was computed representing the linear relationship between the difference of ratio amplitudes (ΔP) $(P_{X1} - F_Y \; P_{X2})$ of different concentration of pure X at λ_1 and λ_2 using a the previously used concentration of Z' as a divisor versus the corresponding ratio amplitude P_{X1} at λ_1; therefore

$$(P_{X1} - F_Y \; P_{X2}) = \text{slope } P_{X1} \pm \text{intercept} \quad \ldots\ldots\ldots\ldots (8)$$

The postulated amplitude value related to component X only at λ_1 ($P_{(x) \text{ postulated}}$) in the mixture of (X+Y) represents P_{X1} or (X/Z')1 can be calculated via the previously computed regression equation (8) using $\Delta P \; (P_1 - F_Y \; P_2)$ of the mixture at the two selected wavelengths where (F_Y) is the equality factor of pure Y at these wavelengths.

Finally, the amplitude subtraction step was applied where the amplitude (y/z') corresponding to Y can be calculated by subtracting the recorded amplitude of the ratio spectrum of the resolved binary mixture(X+Y) (P_{recorded}) at (λ_1), and postulated amplitude corresponding to component X only ($P_{(X) \text{ postulated}}$) in the mixture at the same wavelength (λ_1).

$$\frac{Y}{Z'} = (\frac{X}{Z'} + \frac{Y}{Z'})1 - (\frac{X}{Z'})1 \quad \ldots\ldots\ldots\ldots (9)$$

The concentration of X or Y or Z is calculated using the regression equation representing the correlation between the amplitudes of ratio spectra $\frac{X}{Z'}$ or $\frac{Y}{Z'}$ or $\frac{Z}{Z'}$ at λ_1 and the corresponding concentration of pure X or Y or Z.

Approach For completely overlapping spectra

This Amplitude centering consisted of two steps complementary to each other namely; amplitude calculation and amplitude subtraction. For a mixture of three drugs (X+Y+Z) with no extension of Z. The ratio spectra of the mixture is obtained by dividing the spectrum of the mixture by a known concentration of Z as a divisor (Z'). The division will give a new spectrum that can be summarized previously in equation (1).

Amplitude calculation step starts with amplitude difference method which was applied twice on the ratio spectra of different concentrations of pure Y and X using Z' as a divisor separately at λ_1 andλ_2, where X shows two equal amplitudes at these chosen two wavelengths and at λ_1 and λ_3 where Y shows two equal amplitudes at these chosen two wavelengths, so the difference will represent component Y only or X only. Two regression equations were computed representing the linear relationships between the difference of amplitudes ($\Delta P = \{P1 - P2\}$) of different concentrations of pure Y at λ_1 and λ_2 or ($\Delta P = \{P1 - P3\}$) of different concentrations of pure X at λ_1 and λ_3 versus the corresponding ratio amplitude of Y or X at λ_1 this can be summarized as follows:

$$\{P1 - P2\} = \text{slope } (\frac{Y}{Z'})1 \pm \text{intercept} \quad \ldots\ldots\ldots (10)$$

$$\{P1 - P3\} = \text{slope } (\frac{X}{Z'})1 \pm \text{intercept} \quad \ldots\ldots\ldots (11)$$

Where, $\{P1 - P2\}$ represent $(\frac{Y}{Z'})1$ and $(\frac{Y}{Z'})2$ or $\{P1 - P3\}$ represent $(\frac{X}{Z'})1$ and $(\frac{X}{Z'})2$ is the amplitude difference at λ_1 and λ_2 or λ_1 and λ_3 and $(\frac{Y}{Z'})1$ or $(\frac{X}{Z'})1$ is the postulated amplitude corresponding to component Y or X at λ_1.

The postulated amplitude value which corresponding to component Y or X in the mixture ($P_{(Y) \text{ postulated}}$) or ($P_{(x) \text{ postulated}}$) was obtained by substitution in previously computed regression equations using amplitude difference between two wavelengths (λ_1 andλ_2) in case of Y Eq (10) or (λ_1 andλ_3) in case of X Eq (11), where the constant Z/Z' will be cancelled along with those corresponding to X or Y respectively which show two equal amplitudes at these chosen two wavelengths.

Finally, apply amplitude subtraction where the amplitude corresponding to the constant Z/Z' can be calculated by subtracting the recorded amplitude of the mixture (P_{recorded}) at (λ_1), and the previously calculated postulated amplitude ($P_{\text{postulated}}$) corresponding to component Y and X in the mixture separately atλ_1.

$P_Z = (P_{\text{recorded}}) - (P_{\text{postulated of X and Y}})$

$$\frac{Z}{Z'} = [\frac{X}{Z'} + \frac{Y}{Z'} + \frac{Z}{Z'}] - [\frac{X}{Z'} + \frac{Y}{Z'}] \quad \ldots\ldots\ldots (12)$$

The concentration of X or Y or Z is calculated using the regression equations representing the correlation between the amplitudes of ratio spectra $\frac{X}{Z'}$ or $\frac{Y}{Z'}$ or $\frac{Z}{Z'}$ at the same wavelength λ_1 and the corresponding concentration of X or Y or Z.

MATERIALS AND METHODS

Apparatus and software

Shimadzu-UV 1800 double beam UV-Visible spectrophotometer (Japan) with matched 1 cm quartz cells at 200-800 nm range were used for all absorbance measurements. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

Chemicals and reagents

Pure samples

\Amlodipine (AML), valsartan (VAL) and hydrochlorothiazide (HCT) were supplied by Sigma–Aldrich (USA). Their purity was found to be 99.04±0.65, 99.32±0.91 and100.11±0.91 for AML, VAL and HCT by the official methods [32] respectively.

Market sample

Exforge HCT® film coated tablets. Formulation (A) labeled to contain 10 mg of AML, 160 mg of VAL and 25 mg of HCT per tablet (batch number: S0092). Formulation (B) labeled to contain 5 mg of AML, 160 mg of VAL and 12.5 mg of HCT per tablet (batch number: S0250). Both formulations were manufactured by Novartis pharmaceutical cooperation, USA.

Solvents

Analytical grade methanol (SD fine-chem limited-Mumbai) and distilled water.

Standard solutions

Stock solutions

AML, VAL and HCT were prepared in a solvent mixture of methanol: water (50:50 v/v), of concentration 1 mg/ml. This solvent mixture ratio completely dissolved the three drugs quantities with the least amount of methanol to economize the usage of organic solvent, which is cost effective and eco-friendly.

Working solutions

They were freshly prepared by dilution from the stock solutions with the solvent mixture to obtain a concentration 40 µg/ml for AML and VAL; and 20 µg/ml for HCT.

Procedure

Linearity and construction of calibration curves

Aliquots equivalent to (4–36 µg/ml), (2–42 µg/ml) and (2–20 µg/ml) of AML, VAL and HCT were prepared separately in a solvent mixture of methanol: water (50:50 v/v). The absorption spectra of the prepared solutions were measured at (200 – 400 nm) and stored in the computer.

Advanced Amplitude centering (AAC)

The stored zero order absorption spectra of the three drugs were divided by the spectrum of 24 µg/ml of AML.

For partially overlapped spectra

The peak amplitudes of the obtained ratio spectra at 239.5 and 275.5 nm were recorded. Equality factor (F) was calculated for VAL ratio spectra at 239.5 and 275.5 nm ($P_{275.5}/P_{239.5}$). The amplitudes of HCT at 239.5 nm of the stored spectra were multiplied by the equality factor (F) of pure VAL. The amplitude difference (ΔP) of the ratio spectra of HCT at 275.5 nm and 239.5 nm, after multiplying the later by F, was calculated.

A linear regression was constructed between this amplitude difference ($\Delta P_{275.5-239.5}$) of HCT against its corresponding amplitude at 275.5 nm and the regression equation was computed. Two calibration curves were constructed between the amplitudes at 275.5 nm against the corresponding concentration of both HCT and VAL and the regression equations were computed. A calibration curve was constructed between the constant amplitudes at plateau region (350-380 nm) against the corresponding concentration of AML and the regression equation was computed.

For completely overlapped spectra

The amplitude difference (ΔP) of the ratio spectra of HCT at 275.5 nm and 289.5 nm was calculated and linear regression was constructed between this amplitude differences ($\Delta P_{275.5-289.5}$) of HCT against its corresponding amplitude at 289.5 nm where the regression equation was computed. Meanwhile, the amplitude difference (ΔP) of the ratio spectra of VAL at 289.5 nm and 264.5 nm was calculated and linear regression was constructed between this amplitude differences ($\Delta P_{289.5-264.5}$) of VAL against its corresponding amplitude at 289.5 nm where the regression equation was computed. Calibration curves were constructed between the amplitudes at 289.5 nm against the corresponding concentration of each of AML, VAL and HCT and the regression equations were computed.

Construction of calibration set for PLS model

Multilevel fractional factorial design [33, 34] was used for the construction of the calibration and validation sets. A five-level, five-factor calibration design was used. Fifteen mixtures were used for constructing the calibration set. The laboratory prepared mixtures of each of AML, VAL and HCT were prepared within their corresponding concentration ranges. The absorption spectra of the prepared mixtures were recorded in the range of (200-400 nm); and transferred to Matlab® (version 7.9), with PLS toolbox 2.0, for subsequent data manipulation.

Application to laboratory prepared mixtures (validation set for PLS)

Into a series of 10 ml volumetric flask, accurate aliquots of AML, VAL and HCT were transferred from their working solutions to prepare seven mixtures containing different ratios of the cited drugs. The volumes were completed with the solvent mixture. The spectra of the prepared solutions were recorded at (200-400 nm). The concentration of each drug was calculated by substitution in the corresponding regression after applying the corresponding manipulating steps for each method.

Application to pharmaceutical formulations

Five tablets of each exforge HCT® formulations were accurately weighed and finely powdered. An amount of the powder equivalent to 6.4 mg VAL was weighed and dissolved in the solvent mixture by shaking in an ultrasonic bath for about 30 min. The working solutions were filtered into separate 100 ml measuring flasks, and the volume was completed with a solvent mixture. For each formulation, five ml was transferred from its working solution into 10 ml measuring flasks. Then formulation (A) was spiked with 60 µg of AML, while formulation (B) was spiked with 40 µg of AML from its working solution and completed to volume with a solvent mixture. The spectra of these solutions were scanned from 200 to 400 nm and stored in the computer. The same procedure under AAC was applied and the concentrations of AML, VAL, and HCT were calculated from the corresponding regression equations. The claimed concentration of AML in both formulations was calculated after subtraction of the added concentration (standard solution of AML 4 µg/ml analyzed by using the same procedure). When carrying out the standard addition technique, different known concentrations of the pure standard of each drug were added to the pharmaceutical formulations before proceeding in the previously mentioned methods.

RESULTS AND DISCUSSION

Exforge HCT® is present in the market as two formulations with different ratios of the drugs AML, VAL and HCT. Formulation (A) with ratio 10/160/25 of AML/VAL/HCT while formulation (B) with ratio 5/160/12.5 of AML/VAL/HCT, respectively. Advanced amplitude centering (AAC) was applied for the determination of this ternary mixture using centered amplitudes at a single wavelength. In the first formulation, higher concentrations of AML is present which suggested the approach of the partially overlapping spectrum; while the second one, lower concentrations of AML is present which suggested the approach of the completely overlapping spectrum. The results of both approaches of AAC were compared to PLS model.

The overlapping zero-order absorption spectra (D_0) of AML, VAL and HCT is shown in fig. 1.

Advanced amplitude centering (AAC) via partially overlapped spectra approach

This novel approach was applied to resolve the components of the ternary mixture progressively using single divisor, the spectrum of AML is extended than both VAL and HCT and eliminate the extended spectrum of AML the resolved binary mixture of VAL and HCT exhibit complete overlapped ratio spectra at two wavelengths λ_1 and λ_2 where amplitude difference of HCT aren't equal zero between those two wavelengths λ_1 and λ_2., so the well-established constant center method [30] cannot be applied.

The spectra of the laboratory prepared mixture of the three components were divided by 24 µg/ml AML' as a divisor to obtain the ratio spectra (VAL/AML')+(HCT/AML')+(AML/AML'), fig. 2, where (AML/AML') represented a constant that can be measured accurately at the plateau region in the range of (350-380 nm) at concentrations higher than or equal to 6 µg/ml of AML.

The constant amplitude was measured at the plateau region and substituted in the corresponding regression equation to calculate the concentration of AML. By subtracting this constant, we obtain the ratio spectra of the binary mixture of VAL and HCT.

The concentration of HCT was determined using amplitude difference at λ_1 and λ_2, where $\lambda_1$equals to 275.5 nm which exhibits maximum amplitude for HCT ratio spectrum. The amplitude of the interfering substance (VAL) between those two selected wavelengths at λ_1 and λ_2 (275.5 and 239.5 nm) weren't equal (amplitude difference does not equal zero), so the conventional constant centre method could not be applied. To cancel the effect of VAL, its equality factor (F) was calculated at the two selected wavelengths ($P_{275.5}/P_{239.5}$).

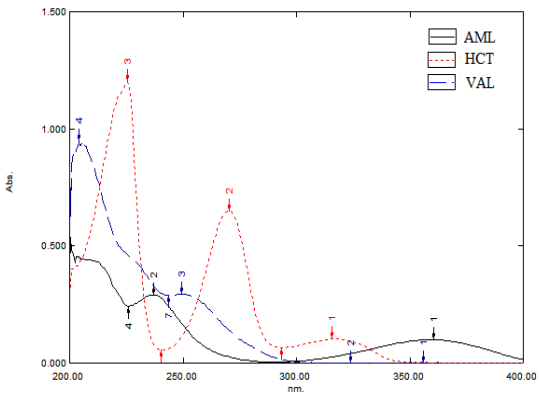

Fig. 1: UV spectra of 10 µg/ml of each of ML, VAL and HCT in in a solvent mixture of methanol: water (50:50 v/v)

The amplitude difference (ΔP) at (275.5 239.5 nm) of the ratio spectra of HCT was calculated after multiplying the later by F. The postulated amplitude value (HCT/AML') [$P_{postulated}$] at 275.5 nm can be calculated by substitution in the equation representing the linear relationship between the differences of ratio amplitude ($\Delta P_{275.5-239.5}$) versus the corresponding ratio amplitudes at 275.5 nm.

$$\Delta P = 0.9613\ (HCT/AML') + 0.0141\ (r = 0.9994)\ \ldots\ldots\ldots\ (13)$$

The amplitude of VAL at the same point (275.5 nm) could be measured by calculating the difference between the recorded amplitude of the mixture [$P_{recorded}$] and postulated amplitude of HCT [$P_{postulated}$] at the same point 275.5 nm via amplitude difference method.

The concentrations of each of VAL and HCT were calculated using its corresponding regression equation representing the linear relationship between their amplitude values of ratio spectra at 275.5 nm versus their corresponding concentration.

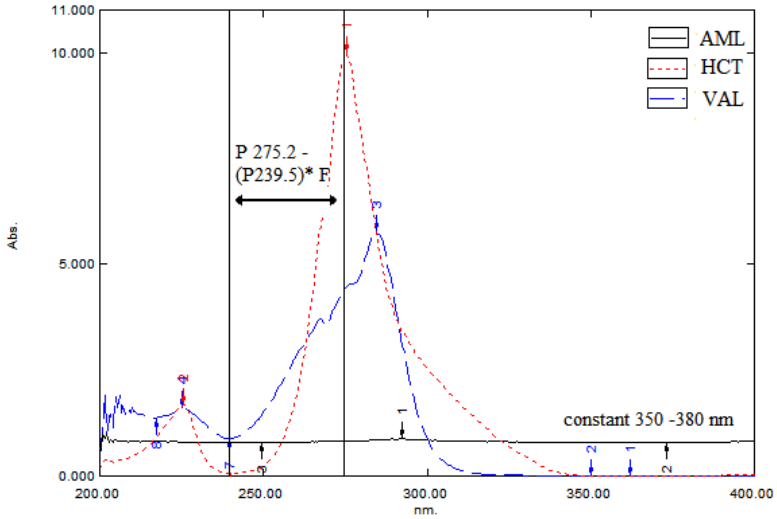

Fig. 2: Ratio spectra of 20 µg/ml of each of AML, HCT and VAL using AML' divisor (24 µg/ml)

Advanced Amplitude centering (AAC) via completely overlapped spectra approach

This approach would be applied in the case of severely overlapped spectra of the three components in a mixture using single divisor. It starts with dividing the spectrum of the laboratory prepared mixture by 24 µg/ml AML' as a divisor to obtain the ratio spectra (VAL/AML')+(HCT/AML')+(AML/AML'), fig. 3. At concentrations lower than 6 µg/ml of AML, the constant amplitude of (AML/AML') cannot be measured accurately at the plateau region in the range of (350-380 nm) due to high noise to signal ratio. So the three components would be progressively determined using the centred amplitude at a single wavelength.

The concentration of HCT was determined using amplitude difference at λ_1 and λ_2, (275.5 and 289.5 nm), where the amplitude difference for the interfering substance (VAL) and the amplitude difference for AML constant between those two selected wavelengths would be equal to zero; so the amplitude difference would only be related to HCT. The postulated amplitude value

(HCT/AML') [P $_{postulated}$] at 289.5 nm could be calculated by substitution in the equation representing the linear relationship between the difference of ratio amplitude (ΔP) at (275.5– 289.5 nm) versus the corresponding ratio amplitudes at 289.5 nm.

$$\Delta P = 2.2504(HCT/AML') - 2.2861 \ (r = 0.9992) \ldots\ldots\ldots (14)$$

The concentration of VAL was determined using amplitude difference at λ_1 and λ_2, (289.5 and 264.5 nm), where the amplitude difference for the interfering substance (HCT) and the amplitude difference for AML constant between those two selected wavelengths would be equal to zero; so the amplitude difference would only be related to VAL. The postulated amplitude value (VAL/AML') [P $_{postulated}$] at 289.5 nm could be calculated by substitution in the equation representing the linear relationship between the differences of ratio amplitude (ΔP) at (289.5–264.5 nm) versus the corresponding ratio amplitudes at 289.5 nm.

$$\Delta P = 0.3205 \ (VAL/AML') - 0.3288 \ (r = 0.9993) \ldots\ldots\ldots\ldots (15)$$

The constant amplitude of AML at the same point (289.5 nm) could be measured by calculating the difference between the recorded amplitude of the mixture [P $_{recorded}$] and postulated amplitudes of both HCT and VAL [P $_{postulated}$] at the same point 289.5 nm via amplitude difference method.

The concentrations of each of AML, VAL and HCT were calculated using its corresponding regression equation representing the linear relationship between their amplitude values of ratio spectra at 289.5 nm versus their corresponding concentration.

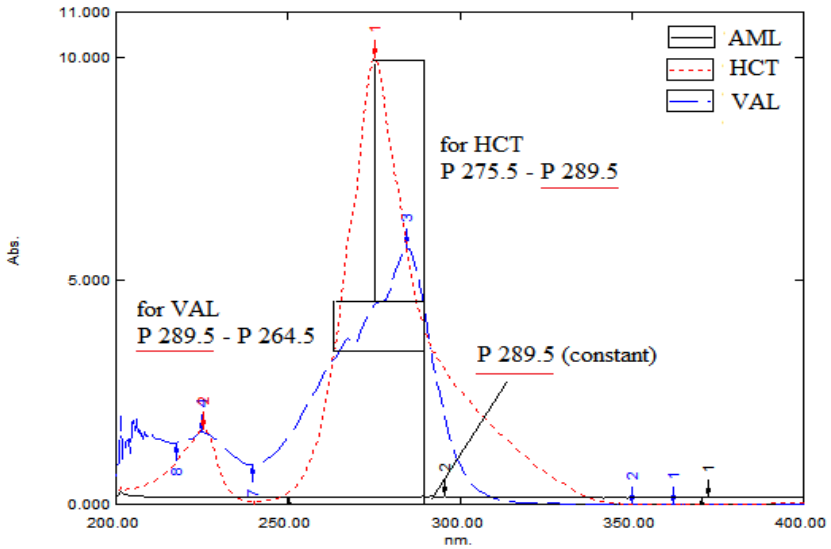

Fig. 3: Ratio spectra of 4 µg/ml of AML and 20 µg/ml of each of HCT and VAL using AML' divisor (24 µg/ml)

The selected divisor for both approaches was chosen to compromise between minimal noise and maximum sensitivity and gave the best results regarding average recovery percent when used for the analysis the laboratory prepared mixtures. The validation parameters for the proposed approaches were listed in table 1.

This technique was introduced as a novel progressive technique for resolving complex mixtures where two components were completely overlapped, but they were more extended than the third one (first approach) or with severely overlapped spectra of the three components (second approach).

The advantage of this novel technique over the other manipulating ratio spectra methods applied for ternary mixtures (as successive derivative ratio and mean centering of ratio spectra) was requiring one divisor only and utilizing the centered amplitude at single wavelength for the progressive resolution and determination of the three components, so it saved time and avoided several manipulations.

Table 1: Assay parameters and validation sheet obtained by applying the proposed AAC approaches

Parameters	AAC via partially overlapped spectra approach			AAC via severely overlapped spectra approach		
	AML	VAL	HCT	AML	VAL	HCT
Wavelength (nm)	350-380	275.5		289.5		
Calibration range [a] µg/ml	6-32	2-36	2–20	4-32	6-36	2–20
Slope	0.0431	0.1711	1.1533	0.4360	0.1875	0.3556
Intercept	-0.0093	0.0246	-0.0946	-0.0238	-0.0192	0.0409
Correlation coefficient (r)	0.9994	0.9997	0.9999	0.9997	0.9991	0.9998
Mean [a]	100.16	99.81	100.22	100.32	99.95	99.88
RSD %	1.381	1.224	0.906	1.060	1.248	0.946
Accuracy [ab]	100.51 ±0.87	100.75 ±0.65	99.11 ±0.74	99.99 ±0.75	99.68 ±1.05	100.21 ±0.85
Repeatability [ac]	0.621	1.002	0.623	0.788	0.911	0.778
Inter-day precision [ac]	0.744	1.133	0.775	0.817	0.963	0.798
Robustness [acd]	0.411	0.963	0.825	1.003	0.755	0.933

[a]Average of three experimen12, [b]mean±standard deviation of 3 concentrations of each drug (8, 10 and 12 µg/ml), [c]Relative standard deviation of 3 concentrations of each drug (8, 10 and 12 µg/ml), [d]Robustness were checked by testing the effect of solvent (45, 55, 60 % methanol).

PLS model

The calibration set was constructed using the absorption spectra set of 15 mixtures, as listed in table 2. The initial model was found to give bad results so the regions above 350 nm were rejected. Cross-validation methods leaving out one sample at a time was employed. The root means squares error of cross-validation (RMSECV) was calculated which was used as a diagnostic test for examining the errors in the predicted concentrations. It indicated both precision and accuracy of predictions. The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with an additional factor. A number of factors of 4 were found to be optimum for the mixture, as shown in Fig.4. To assess the prediction ability of the suggested models, an external validation set was used.

The predicted concentrations were compared with the true concentrations of the compounds in each sample. The root mean squared errors of prediction (RMSEP) and the regression equations for the predicted versus actual concentration, were listed in table 3 as diagnostic tools for model validation.

The selectivity of the proposed procedures was assessed by the analysis of laboratory prepared mixtures (validation set for PLS) containing different ratios of the cited drugs, where satisfactory results were obtained as shown in table 4.

Table 2: Concentrations of AML, VAL and HCT in the calibration set (PLS model)

Experiment no.	Levels			Conc (µg/ml)		
	AML	VAL	HCT	AML	VAL	HCT
1	0	0	0	20	22	10
2	-2	2	-1	4	2	6
3	2	-1	2	36	42	18
4	2	0	-1	36	42	6
5	0	-1	-1	20	22	6
6	-1	1	2	12	12	18
7	1	2	1	28	32	14
8	1	0	2	28	32	18
9	0	2	2	20	22	18
10	2	-2	1	36	42	14
11	-2	1	-2	4	2	2
12	-2	0	1	4	32	14
13	0	1	1	20	32	14
14	1	-1	-2	28	2	2
15	-2	-1	0	4	22	10

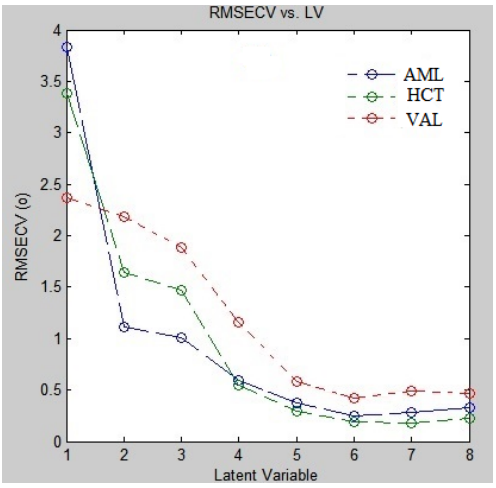

Fig. 5: RMSECV of the calibration set of AML, HCT and VAL as a function of latent variables

Application for pharmaceutical formulations

Formulation (A) with ratio 10/160/25 of AML/VAL/HCT, where concentrations of AML is higher than or equal 6 µg/ml, was analyzed using the approach of partial overlapping spectrum of Advanced amplitude centering (AAC); while formulation (B) with ratio 5/160/12.5 of AML/VAL/HCT, where concentrations of AML is lower than 6 µg/ml, was analyzed using the approach of sever overlapping spectrum of Advanced amplitude centering (AAC). Both formulations were analyzed using PLS model and the results were compared to AAC approaches where no difference was observed. The validity of the proposed approaches was further assessed by applying the standard addition technique showing no excipients' interference. The results obtained were shown in table 5.

Table 3: Summary of results obtained by applying the diagnostic tools for model validation of the PLS model

Parameters	AML	VAL	HCT
Slope	1.0521	0.9996	0.9705
Intercept	-0.2828	0.0657	0.1029
Correlation coefficient (r)	0.9997	0.9990	0.9991
RMSEP	0.343	0.102	0.053

Table 4: Analysis of laboratory prepared mixtures by applying the proposed spectrophotometric methods

AML: VAL: HCT in (µg/ml)	AAC via partially overlapped spectra approach			AAC via severely overlapped spectra approach			PLS		
	AML	VAL	HCT	AML	VAL	HCT	AML	VAL	HCT
	Recovery %[a]								
10: 10: 10	101.71	99.88	100.11	101.36	99.56	100.95	101.22	99.66	100.15
20: 10: 10	99.96	99.25	100.67	99.36	99.34	101.06	99.64	98.96	100.35
6: 6: 18	99.63	100.67	100.34	99.46	101.07	99.66	99.39	101.22	99.55
10: 30: 10	99.38	99.38	99.87	99.35	99.36	98.79	99.57	100.58	100.79
12: 4: 4	100.98	99.88	99.54	98.85	99.87	99.63	99.32	100.89	100.44
8: 32: 5 [b]	100.64	100.64	98.86	100.95	101.22	99.21	101.08	99.36	100.27
5: 32: 2.5 [c]	96.21	99.47	99.05	101.02	99.37	100.58	100.38	98.88	101.08
Mean	99.79	99.88	99.78	100.05	99.97	99.98	100.09	99.94	100.38
±SD	1.77	0.58	0.67	1.02	0.82	0.89	0.81	0.95	0.49

[a]Average of 3 experiments, [b]ratio present in exforge ® formulation (A), [c]ratio present in exforge ® formulation (B)

Table 5: Application of standard addition technique to the analysis of exforge® formulations by applying the proposed methods

Methods	AAC via partially overlapped spectra approach	AAC via severely overlapped spectra approach	PLS	PLS
Exforge®	Formulation (A) [a]	Formulation (B)[a]	Formulation (A)[a]	Formulation (B)[a]
AML	100.25±0.69	98.36±0.61	100.61±0.04	99.15±0.82
Standard addition	99.64±0.54	99.11±0.44	99.71±0.99	99.05±0.49
VAL	101.52±0.87	100.87±0.62	101.88±0.71	99.52±0.73
Standard addition[b]	99.99±0.78	100.67±0.82	100.87±0.76	100.96±0.34
HCT	102.00±0.89	99.15±0.32	101.54±0.79	99.52±0.41
Standard addition[b]	99.73±0.96	101.08±0.44	99.88±0.39	101.45±0.63

[a]mean±SD for six experiments, [b]Average of three experiments (pure added equivalent to 8, 10, 12 µg/ml of AML, and 6, 7, 8 µg/ml of VAL and HCT)

Table 6: Statistical comparison between the results obtained by the proposed methods and the reported methods for the determination of AML, VAL and HCT in pure powder form

Items	AML				VAL				HCT			
	AAC (partial)	AAC (Severe)	PLS	Reported method[a] [9]	AAC (partial)	AAC (Severe)	PLS	Reported method[a] [12]	AAC (partial)	AAC (Severe)	PLS	Reported method[a] [14]
Mean %	100.16	100.32	100.09	100.31	99.81	99.95	99.94	100.30	100.22	99.88	100.38	100.32
Variance	1.9057	1.1216	0.6495	0.3913	1.4802	1.5578	0.9082	1.5274	0.8201	0.8943	0.2361	0.7472
n	7	7	7	5	7	7	7	5	7	7	7	5
Student's *t*-test [b]	0.224	0.010	0.523		0.691	0.472	0.581		0.186	0.764	0.153	
F value [cd]	4.870 [c]	2.866 [c]	1.660 [c]		1.032 [d]	1.020 [c]	1.682 [d]		1.098 [c]	1.197 [c]	3.165 [d]	

[a]Reported methods for AML and VAL are chromatographic methods, while for HCT, it is a chemometric method, [b]the corresponding tabulated values of *t* equals to 2.228 at P=0.05, [c]the corresponding tabulated values of *F* equals to 6.094 at P=0.05, [d]the corresponding tabulated values of *F* equals to 4.120 at P=0.05

The proposed spectrophotometric methods were validated in compliance with the ICH guidelines [35] as shown in table 1. The data showed that the methods were accurate, precise, robust and specific over the specified range. Statistical comparison of the results obtained by the proposed methods and reported ones was shown in table 6. The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

CONCLUSION

This work introduced the novel approaches of advanced amplitude centering (AAC) which could be applied for solving overlapped spectra of the components in ternary mixtures either partially or severely overlapped. The advantage of the novel advanced amplitude centering approaches was the determination of three components in their ternary mixture with minimum absorbance data manipulation by unified wavelength resolution using single divisor. The resolution efficiency of the results were compared with those obtained by chemometric method PLS, where satisfactory results were obtained. The proposed method AAC was simpler than other reported spectrophotometric methods as it did not require sample preparation, buffer preparation, expensive solvents or sophisticated liquid chromatographic instruments. As a final conclusion, the proposed univariate method was superior over other reported univariate methods since it had very high-resolution power when applied to ternary mixtures with partially or severely overlapped spectra without any limitations and with good accuracy and precision; therefore it could be considered as an alternative to chemometric methods with minimum sample preparation so they could be easily applied in quality control laboratories for the simultaneous determination of amlodipine (AML), valsartan (VAL) and hydro-chlorothiazide (HCT).

CONTRIBUTION OF AUTHORS

Both authors had equal contributions in the presented work.

CONFLICT OF INTERESTS

Authors declared no conflict of interests.

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