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Comparative study between derivative spectrophotometry and multivariate calibration as analytical tools applied for the simultaneous quantitation of Amlodipine, Valsartan and Hydrochlorothiazide

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Abstract

Four simple, accurate and specific methods were developed and validated for the simultaneous estimation of Amlodipine (AML), Valsartan (VAL) and Hydrochlorothiazide (HCT) in commercial tablets. The derivative spectrophotometric methods include Derivative Ratio Zero Crossing (DRZC) and Double Divisor Ratio Spectra-Derivative Spectrophotometry (DDRS-DS) methods, while the multivariate calibrations used are Principal Component Regression (PCR) and Partial Least Squares (PLS). The proposed methods were applied successfully in the determination of the drugs in laboratory-prepared mixtures and in commercial pharmaceutical preparations. The validity of the proposed methods was assessed using the standard addition technique. The linearity of the proposed methods is investigated in the range of 2-32, 4-44 and 2-20 μg/mL for AML, VAL and HCT, respectively.

KeyWords: PLS; PCR; double divisor; Amlodipine; Valsartan; Hydrochlorothiazide.

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1. Introduction

Amlodipine (AML), 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid 3-ethyl 5-methyl ester) [1] (Fig. 1a) is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal's variant angina [2].

Valsartan (VAL) is chemically described as N-[p-(o-1H-Tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine [1] (Fig. 1b), is a potent and specific competitive antagonist of the angiotensin-II AT_1-receptor. It is used for treatment of hypertension, heart failure, and post-myocardial infarction [3].

Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide [1] (Fig. 1c), is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs [4].

The triple combination of AML, VAL and HCT is intended for oral administration for the treatment of hypertension and is available in several different strength combinations.

Literature survey revealed that AML and HCT are official in British Pharmacopoeia [5], VAL, HCT and their mixture are official in United States Pharmacopoeia [6]. There are reported methods for the determination of AML, VAL or HCT in different dosage forms [7-13] and in their binary mixtures [14-16]. Few methods were reported for the simultaneous estimation of AML, VAL and HCT in their ternary mixture [17-20] and only one spectrophotometric method was developed for the determination of this mixture [21].

This manuscript presents a comparative study between different derivative spectrophotometric methods and chemometric methods. The paper also describes the development and validation of the first spectrophotometric and chemometric methods for simultaneous quantitation of AML, VAL and HCT in bulk powder and pharmaceutical dosage forms.

2. Experimental

2.1. Materials and reagents

- *Pure Amlodipine;* kindly supplied by Al-Hekma pharmaceutical Company, Cairo, Egypt, its purity was certified to be 99.89 ± 0.691.
- *Pure Valsartan;* kindly supplied by Novartis pharmaceutical Company, Cairo, Egypt, its purity was certified to be 99.69 ± 0.231.
- *Pure Hydrochlorothiazide;* kindly supplied by Al-Hekma pharmaceutical Company, Cairo, Egypt, its purity was certified to be 99.78 ± 0.364.

- Exforge HCT® tablet dosage forms; labeled to contain 5(AML)/160(VAL)/12.5(HCT) mg batch number 5002125, 5/160/25 mg batch number 5002141 and 10/320/25 mg batch number 5002159, manufactured by Novartis Pharmaceuticals Corporation, USA. They were procured from U.S.A. market.
- *Methanol;* analytical grade (El-NASR Pharmaceutical Chemicals Co., Cairo, Egypt.)

2.2. Instruments

SHIMADZU dual beam UV-visible spectrophotometer (Kyoto/ Japan), model UV-1650 PC connected to IBM compatible and a HP1020 laserjet printer. The bundled software, UV- Probe personal spectroscopy software version 2.21 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

2.3. Software

All chemometric methods were implemented in Matlab® 7.0.0.19920 (R14). PCR and PLS were carried out by using PLS toolbox software version 2.1. The t-test and F-test were performed using Microsoft® Excel 2007. All calculations were performed using a Dual CPU, 1.47 GHz, 2.00 GB of RAM under Microsoft Windows Vista™.

2.4. Procedures

2.4.1. Standard solutions

a) Standard stock solutions of AML, VAL and HCT 1 mg/mL in methanol.
b) Standard working solutions for AML and VAL 80 µg/mL and for HCT 62.5 µg/mL were prepared from stock solutions by appropriate dilutions with methanol.

2.4.2. Spectral characteristics of AML, VAL and HCT

The zero-order (D_0) absorption spectrum of AML, VAL and HCT (20 µg/mL for each) solutions were recorded against methanol as a blank over the range of 200-400 nm.

2.4.3. *Construction of calibration curves for spectrophotometric methods*

Aliquots equivalent to 20-320 µg AML, 40-440 µg VAL and 20-200 µg HCT were accurately transferred from their standard working solutions into three separate series of 10-mL volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200-400 nm and stored in the computer.

2.4.3.A. Derivative ratio zero crossing (DRZC) method

For the determination of AML and HCT, The stored spectra of AML and HCT were divided by the spectrum of 4 µg/mL VAL, smoothed with $\Delta\lambda$ = 4 nm, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$ = 4 nm and scaling factor =1 was obtained. The amplitude of the first derivative peak was measured at 377.8 and 267.4 nm for AML and HCT,

respectively. Calibration graphs relating the peak amplitude at each wavelength to the corresponding concentration in µg/mL of AML and HCT were constructed.

For the determination of VAL, the stored spectra of VAL were divided by the spectrum of 20 µg/mL HCT, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$ = 4 nm and scaling factor =10 was obtained. The amplitude of the first derivative peak was measured at 283.0 nm. A calibration graph relating the peak amplitude at 283.0 nm to the corresponding concentration in µg/mL of VAL was constructed.

2.4.3.B. Double Divisor-Ratio Spectra Derivative Spectrophotometry (DDRS-DS) method

For the determination of AML, the stored spectra of AML were divided by a standard double divisor (4 µg/mL VAL + 4 µg/mL HCT), smoothed with $\Delta\lambda$ = 4 nm, then the first derivative of the ratio spectra with $\Delta\lambda$ = 4 nm and scaling factor =10 was obtained. The amplitude of the first derivative was measured at 373.2 nm. A calibration graph relating the peak amplitude at 373.2 nm to the corresponding concentration in µg/mL of AML was constructed.

For the determination of VAL, the stored spectra of VAL were divided by a standard double divisor (4 µg/mL AML + 4 µg/mL HCT), then the first derivative of the ratio spectra with $\Delta\lambda$ = 4 nm and scaling factor =10 was obtained. The amplitude of the first derivative was measured at 275.4 nm. A calibration graph relating the peak amplitude at 275.4 nm to the corresponding concentration in µg/mL of VAL was constructed.

For the determination of HCT, the stored spectra of HCT were divided by a standard double divisor (4 µg/mL AML + 4 µg/mL VAL), then the first derivative of the ratio spectra with $\Delta\lambda$ = 4 nm and scaling factor =10 was obtained. The amplitude of the first derivative was measured at 285.6 nm. A calibration graph relating the peak amplitude at 285.6 nm to the corresponding concentrations in µg/mL of HCT was constructed.

2.4.4. Analysis of AML, VAL and HCT in laboratory prepared mixtures by the spectrophotometric methods

Aliquots of AML, VAL and HCT were transferred from their standard working solutions into a series of 10-mL measuring flasks, completed to volume with methanol to prepare mixtures containing different ratios of AML, VAL and HCT. The spectra of these mixtures are scanned from 200-400 nm and stored in the computer. The same procedure under construction of calibration curves is applied and the concentrations of AML, VAL and HCT were calculated from the corresponding regression equations.

2.4.5. Experimental design for chemometric methods

A 5-level, 3-factor calibration design was performed using 5 concentration levels coded from +2 to −2 (corresponding to120% (coded +2), 110% (+1), 100% (0), 90% (−1) and 80% (−2) of the central level) for each of the 3 components to be analyzed. The design aims to span the mixture space fairly well; where there are 5 mixtures for each compound at each concentration level, resulting in 25 mixtures [22]. The central level of the design is 6 μg/mL for AML, 32 μg/mL for VAL and 9.375 μg/mL for HCT. The concentration for each level for each compound is based on the calibration range of each of the drugs, the ratio of AML, VAL and HCT in the market pharmaceutical product was involved. Table 1 represents the concentration design matrix. The 2D scores plot for the first two PCs of the concentration matrix was obtained to confirm the well position of the mixtures in space, orthogonality, symmetry and rotatability [22]. The regions from 200- 230 nm were rejected. Fifteen mixtures of this design were used as a calibration set and the other 10 mixtures as a validation set to test the predictive ability of the developed multivariate models.

2.4.6. Analysis of AML, VAL and HCT in Exforge HCT® tablets by the proposed spectrophotometric and chemometric methods

Five tablets of each Exforge HCT® formulation were accurately weighed and finely powdered. An amount of the powder equivalent to 8 mg VAL was weighed and dissolved in methanol by shaking in ultrasonic bath for about 30 minutes. The solutions were filtered into three separate 100-mL measuring flasks, and the volume was completed with methanol. Five mL aliquot from each flask was transferred into 10-mL measuring flask. Suitable aliquot of AML was transferred from their standard working solutions for spiking to reach concentrations within linearity range and then volume was completed with methanol. The spectra of these solutions were scanned from 200-400 nm and stored in the computer. Spectra obtained were analyzed by the proposed methods.

3. Results and discussion

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information [23]. It is the application of mathematical and statistical methods to design optimum procedures and to provide maximum chemical information through analysis of chemical data.

Quantitative spectroscopy has been greatly improved by the use of variety of multivariate statistical methods [24-27]. With multivariate calibrations, models are developed that relate the multiple spectral intensities from many calibration samples to the known

analyte concentrations of the samples. These models can be used in the multivariate prediction analysis of spectra of unknown samples to rapidly predict analyte concentrations.

In this manuscript, the multivariate calibration models investigated include Principal Component Regression (PCR) and Partial Least Squares (PLS). These models have frequently been used in quantitative spectral analysis to obtain very selective information from unselective data [28, 29]. The models were compared with traditional derivative spectrophotometric methods in simultaneous determination of AML, VAL and HCT in pure form and in pharmaceutical dosage form.

The absorption spectra of the three compounds, AML, VAL and HCT show highly overlapped spectral band in the region 200–300 nm (Fig. 2). It was found that only AML can be determined directly by zero order spectrophotometry, but it was determined by the proposed methods for investigational purposes. On the other hand, classical derivative spectrophotometric methods were tested (from first to fourth) for simultaneous determination of VAL and HCT in the mixture, and no satisfactory results were obtained.

In general, this study was primarily designed to present the proposed multivariate methods as attractive alternatives for classical univariate calibration methods in handling absorbance spectral data. Secondary, comparing Derivative Ratio-Zero Crossing (DRZC) versus Double Divisor-Ratio Spectra Derivative Spectrophotometry (DDRS-DS). Ultimately, analyzing AML, VAL and HCT in their pharmaceutical preparations.

3.1. Derivative Ratio-Zero Crossing (DRZC) method

Berzas et al [30], developed this method for the resolution of ternary mixtures of compounds by derivative ratio spectra-zero crossing method. The method is based on determination of two drugs in a ternary mixture after cancelling the third drug by dividing the spectrum of the selected two drugs by a ‘divisor’ (standard spectrum of the third drug in the ternary mixture) then the two ratio spectra will be derivatized. By overlaying the two derivative spectra, a point that show zero crossing of the ratio spectrum of each pure drug corresponding to the ratio spectrum of the other drug can be selected as working wavelengths for the determination of the other drug in the ternary mixture.

The main parameters that affect the shape of the ratio spectra which are wavelength, scanning speed, the concentration of the standard solution used as a divisor, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing function were carefully tested. The first derivative of the ratio spectra presented in Fig. 3 may provide a good proof for this understanding.

The spectra of 2, 10, 20 µg/mL and the normalized spectrum of HCT were used as divisors for the determination of AML, also the spectra of 4, 24, 44 µg/mL and the

normalized spectrum of VAL were tested. The spectra of 4, 12, 28 µg/mL and the normalized spectrum of AML were used as divisors for the determination of HCT, also the spectra of 4, 24, 44 µg/mL and the normalized spectrum of VAL were tested. The spectra of 2, 10, 20 µg/mL and the normalized spectrum of HCT were used as divisors for the determination of VAL. It was found that using the spectrum of 4 µg/mL VAL as a divisor for determination of AML and HCT and the spectrum of 20 µg/mL HCT as a divisor for determination of VAL provide suitable results regarding accuracy and sensitivity.

The smoothing function for the ratio spectra were tested and it was found that the ratio spectra of (AML/VAL) and (HCT/VAL) were better smoothed with $\Delta\lambda$ = 4 nm, while the ratio spectra of (VAL/HCT) did not require any smoothing. The influence of $\Delta\lambda$ for the first derivative of the ratio spectra was tested and found very appropriate to use the value of $\Delta\lambda$ = 4 nm, in the determination of the three compounds. Furthermore, the scaling factors of 1 and 10 were tested and it was found that scaling factor of 1 is suitable for determination of AML and HCT, while scaling factor of 10 is suitable for determination of VAL.

For determination of AML, there were three zero crossing points (365.8, 377.8 and 386.4 nm) as shown in Fig. 3a. For determination of VAL, there were two zero crossing points (283.0 and 294.6 nm) as shown in Fig. 3b. For determination of HCT, there were two zero crossing points (267.4 and 279.6 nm) as shown in Fig. 3c. The wavelengths were examined as working wavelengths for determination of the three drugs and best linearity and recovery were obtained at 377.8, 283.0 and 267.4 nm for determination of AML, VAL and HCT, respectively. The parameters of regression equations are shown in Table 2.

3.2. Double Divisor-Ratio Spectra Derivative Spectrophotometry (DDRS-DS) method

The method is based on determination of drugs in a ternary mixture by dividing the spectrum of the selected drug by a 'double divisor' (standard spectrum of the other two drugs in the ternary mixture) and dividing a spectrum of the ternary mixture (containing the same concentration of the selected drug) by the same 'double divisor' then the two ratio spectra will be derivatized. By overlaying the two derivative spectra, a point that shows coincidence can be selected as working wavelength for the determinations of the selected drug in the ternary mixture [31].

The main parameters that affect the shape of the ratio spectra were carefully tested. Effect of divisor concentration was tested where different concentrations of the drugs were used as divisors:

For determination of AML, the spectra of 20, 4 µg/mL and the normalized spectrum of VAL and HCT mixture were tested. For determination of VAL, the spectra of 20, 8, 4 µg/mL and the normalized spectrum of AML and HCT mixture were tested. For

determination of HCT, the spectra of 28, 20, 8, 4 μg/mL and the normalized spectrum of AML and VAL mixture were tested. The double divisors of (4 μg/mL of VAL and HCT), (4 μg/mL of AML and HCT) and (4 μg/mL of AML and VAL) gave best results regarding average recovery percent in bulk powder as well as in laboratory prepared mixtures for determination of AML, VAL and HCT, respectively.

The smoothing function for the ratio spectra was tested and it was found that smoothing the ratio spectra of (AML/VAL+HCT) was better done with $\Delta\lambda = 4$ nm, while the ratio spectra of VAL and HCT did not require any smoothing. The influence of $\Delta\lambda$ for the first derivative of the ratio spectra was tested and found very appropriate to use the value of $\Delta\lambda = 4$ nm, in the determination of the compounds. Furthermore, the scaling factors of 1 and 10 were tested and it was found that scaling factor of 10 is suitable for the three drugs.

Choice of the wavelengths was carefully studied and the coinciding points of choice were 373.2, 275.4 and 285.6 nm for the determination of AML, VAL and HCT, respectively, Fig. 4, Fig. 5 and Fig. 6. Good linearity was obtained and the regression parameters are shown in Table 2.

The specificity of the proposed spectrophotometric procedures was assessed by the analysis of laboratory prepared mixtures containing different ratios of the drugs, where satisfactory results were obtained over the calibration range as shown in Table 3. A validation sheet according to the ICH guidelines is also presented in Table 2.

3.3. Chemometric methods

Principal Component Regression (PCR) and Partial Least Squares (PLS) models have frequently been used in quantitative spectral analysis to obtain very selective information from unselective data [23, 25].

PCR and PLS models are designated to be full spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened, by discarding particularly noisy wavelengths. The wavelengths used were in range 231–400 nm in all cases. Wavelengths less than 231 nm were rejected due to the noisy content.

A calibration model is suggested, validated and then used for the prediction of unknown samples. The multivariate calibration requires a careful experimental design of the standard composition of calibration set for providing the best predictions. Multilevel multifactor design [22] was used for the construction of the calibration set.

The PCR and PLS models were constructed using training set samples of 15 mixtures containing different ratios of AML, VAL and HCT, (Table 1). PCR and PLS models were run

on the calibration data of absorption UV spectra and concentrations in validation set were calculated at the optimum number of factors.

The selection of the optimum number of factors for the PCR and PLS techniques was a very important step before constructing the models because if the number of factors retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. Different ways could be used for determining the optimum number of factors [24, 25, 32]. In this study, to select the number of factors, a cross-validation method leaving out one sample at a time [33] was employed using calibration set of 15 spectra. The predicted concentrations of the components in each sample were compared with the actual concentrations in this calibration samples and the root mean squares error of cross-validation (RMSECV) was calculated for each method as follows:

$$\mathrm{RMSECV} = \sqrt{\frac{1}{\mathrm{I}}\sum_{\mathrm{i}=1}^{\mathrm{I}}\left(c_i - \hat{c}_{i\_cv}^{A}\right)^2}$$

Where I is the number of objects in the calibration set, c_i is the known concentration for sample i and $\hat{c}_{i\_cv}^{A}$ is the predicted concentration of sample i using A components.

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both of the precision and accuracy of predictions. It was recalculated upon addition of each new factor to the PCR and PLS model. The method developed by Haaland and Thomas [25] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that results in an insignificant difference between the corresponding RMSECV and the minimum RMSECV. Three factors were found suitable for both PCR and PLS methods. As the difference between the minimum RMSECV and other RMSECV values become smaller, the probability that each additional factor is significant becomes smaller.

PCR and PLS methods were run on the calibration data using optimal number of latent variables (factors). The concentrations of the three components in calibration set were calculated. By plotting predicted concentrations of each component versus actual concentrations, a straight line is obtained. The data of the straight line for each component including slope, intercept and correlation coefficient are collected in Table 4.

In order to validate the proposed PCR and PLS models, a validation set composed of 10 synthetic mixtures of AML, VAL and HCT was analyzed with the proposed PCR and PLS models. Predicted concentrations obtained by application of PCR and PLS methods on

absorption UV spectra for simultaneous determination of AML, VAL and HCT in ternary mixtures are given in Table 5. The RMSEP was another diagnostic tool for examining the errors in the predicted concentrations. It indicated both the precision and accuracy of predictions as it played the same role of standard deviation in indicating the spread of the concentration errors [32].

The proposed chemometric and spectrophotometric methods were also applied for the determination of AML, VAL and HCT in Exforge HCT® tablets (Table 6), and the validity of the proposed methods was further assessed by applying the standard addition technique.

The results obtained for the analysis of AML, VAL and HCT in Exforge HCT® tablets by the suggested methods were statistically compared with those obtained by applying the reported HPLC method [17]. In the reported method separation was achieved on a Luna C_{18} column, (250 x 4.6mm i.d., 5 mm particle size), using a mobile phase consisting of 62% methanol and 38% phosphate buffer solution (30 mM, pH 5.5) at a flow rate of 1 mL/min and UV detection at 234 nm. The results showed no significant differences between the proposed methods and the reported one as presented in Table 7.

3.4. Chemometrics versus derivative spectrophotometry

The DRZC procedure discussed here is more simple compared to the DDRS-DS procedure; as it includes two division steps only for determination of the three drugs, while DDRS-DS requires three division steps. The analysis of laboratory prepared mixtures showed the superiority of DRZC over DDRS-DS as indicated from the selectivity results in Table 3.

This can be explained in the light of the chosen wavelengths in DDRS-DS; as searching for a coincident wavelength was a difficult step in this mixture and the most relevant wavelengths were not at a maximum or minimum point. In contrast, the zero crossing wavelengths chosen in DRZC were at maximum or minimum points which decreased $\Delta A/\Delta\lambda$ to minimum; and thus improved the accuracy, precision and robustness of the procedure as indicated in Table 2.

The PCR and PLS models showed better results, compared to those obtained from DRZC and DDRS-DS (Table 3 and Table 5). These results show the superiority of chemometrics over traditional derivative spectrophotometric methods which may be attributed to the fact that PCR and PLS are full-spectrum calibration techniques, where the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of quantitative spectral analysis.

The reported absorption correction method [21] requires tedious multiple calculations to get the concentrations of the three drugs, while the methods presented in this manuscript

are more simple and specific. Comparing these spectrophotometric methods to our previous work [20] and to the reported chromatographic methods [17-19] shows that these methods are more simple as they do not need sample preparation, buffer preparation, expensive solvents or sophisticated liquid chromatographic instruments. It could be concluded that the proposed methods are simple, sensitive and selective, thus they can be used for the routine analysis of Exforge HCT®.

4. Conclusion

From the previous discussion, it could be concluded that chemometrics show superiority over traditional derivative spectrophotometry as analytical methods for resolving ternary mixtures. DRZC shows simpler procedure than DDRS-DS for analysis of this ternary mixture. The proposed spectrophotometric and chemometric methods are precise, accurate and simple. The methods are suitable and valid for application in laboratories lacking liquid chromatographic instruments and have some advantages over reported methods.

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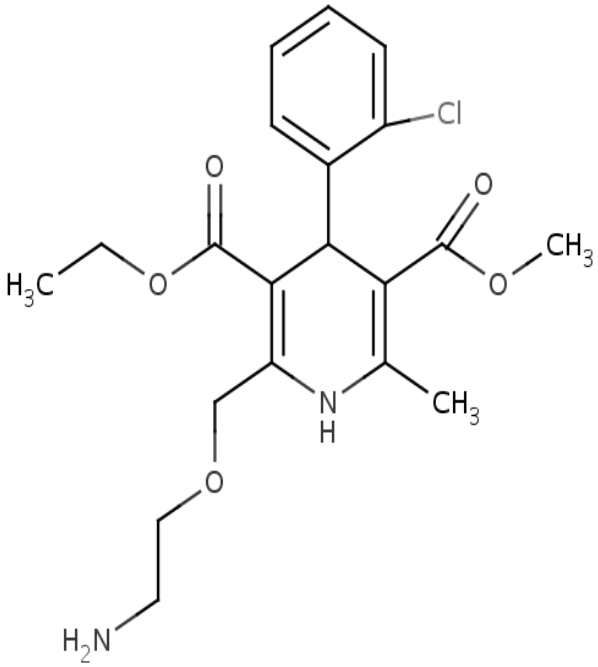
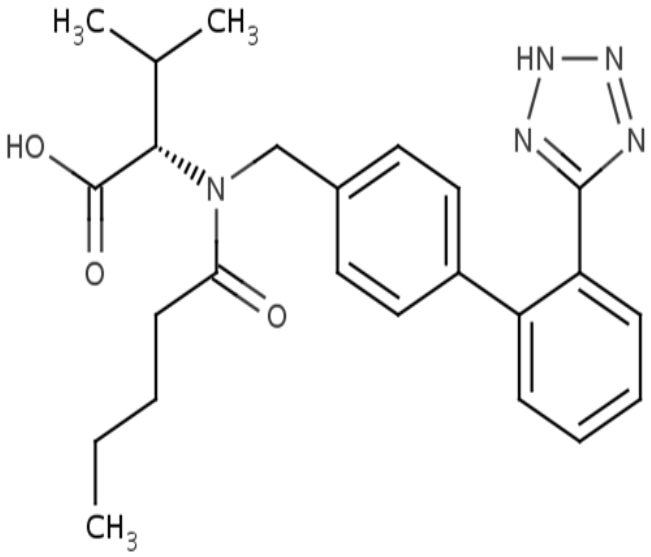


a



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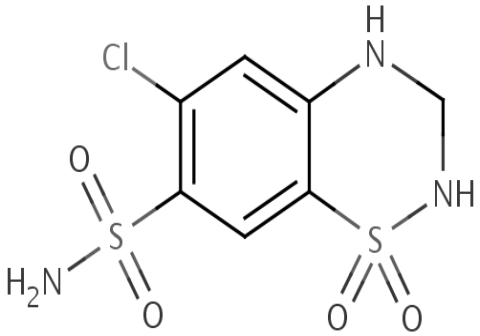

c

Fig. 1. Structural formulae for a) Amlodipine b) Valsartan c) Hydrochlorothiazide

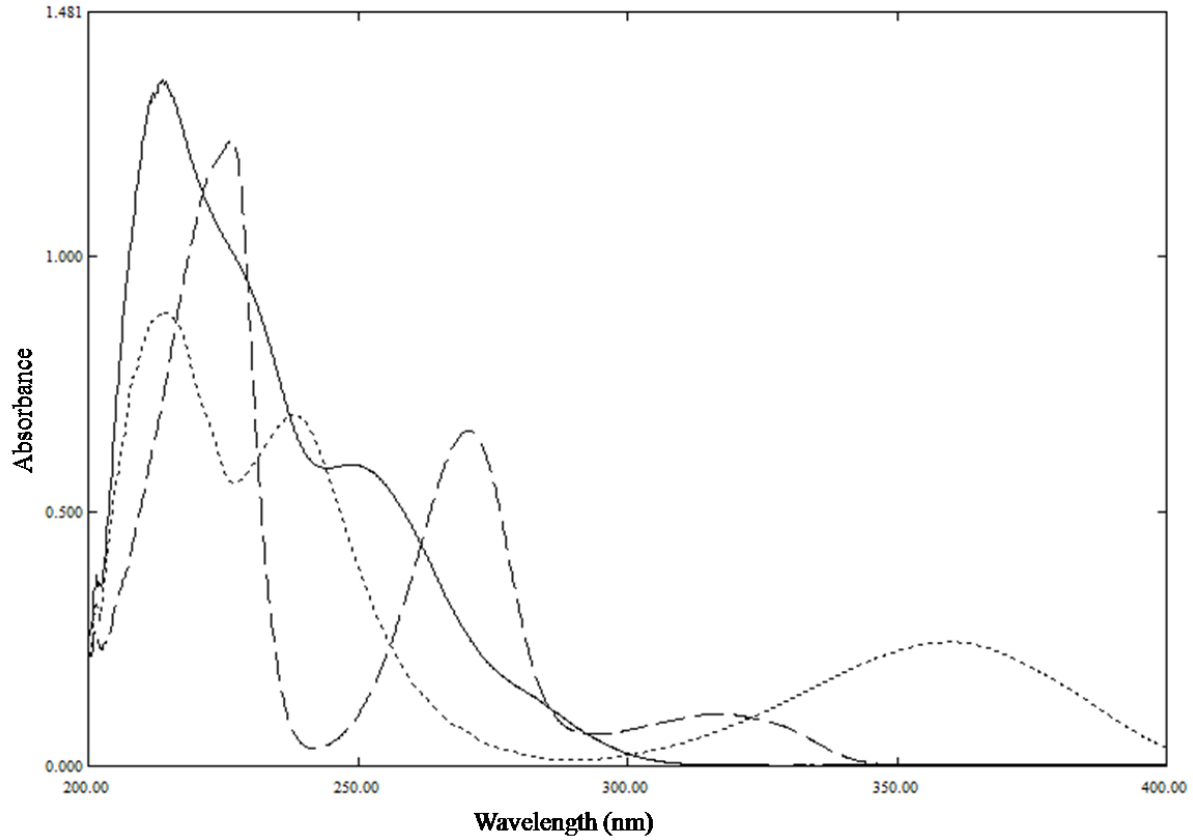

Fig. 2. Zero order absorption spectrum of 20 µg/mL AML (. . . .), 20 µg/mL VAL (—) and 20 µg/mL HCT (– – –) using methanol as blank

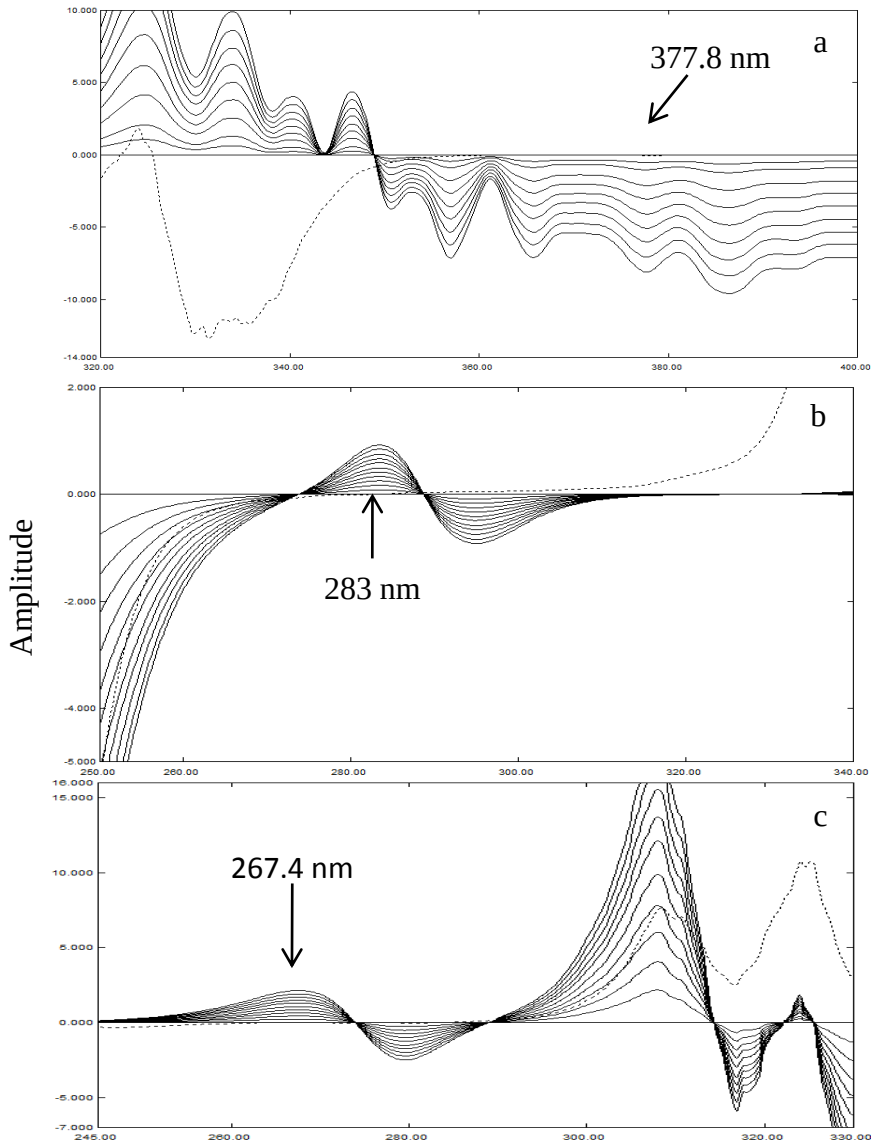

Fig. 3. First derivative of ratio spectra of:

a) 2-32 µg/mL AML (———) and 20 µg/mL HCT (- - -) using the spectrum of 4 µg/mL VAL as a divisor showing different zero crossing points for determination of AML.

b) 4-44 µg/mL VAL (———) and 20 µg/mL AML (- - -) using the spectrum of 20 µg/mL HCT as a divisor showing different zero crossing points for determination of VAL.

c) 2-20 µg/mL HCT (———) and 20 µg/mL AML (- - -) using the spectrum of 4 µg/mL VAL as a divisor showing different zero crossing points for determination of HCT.

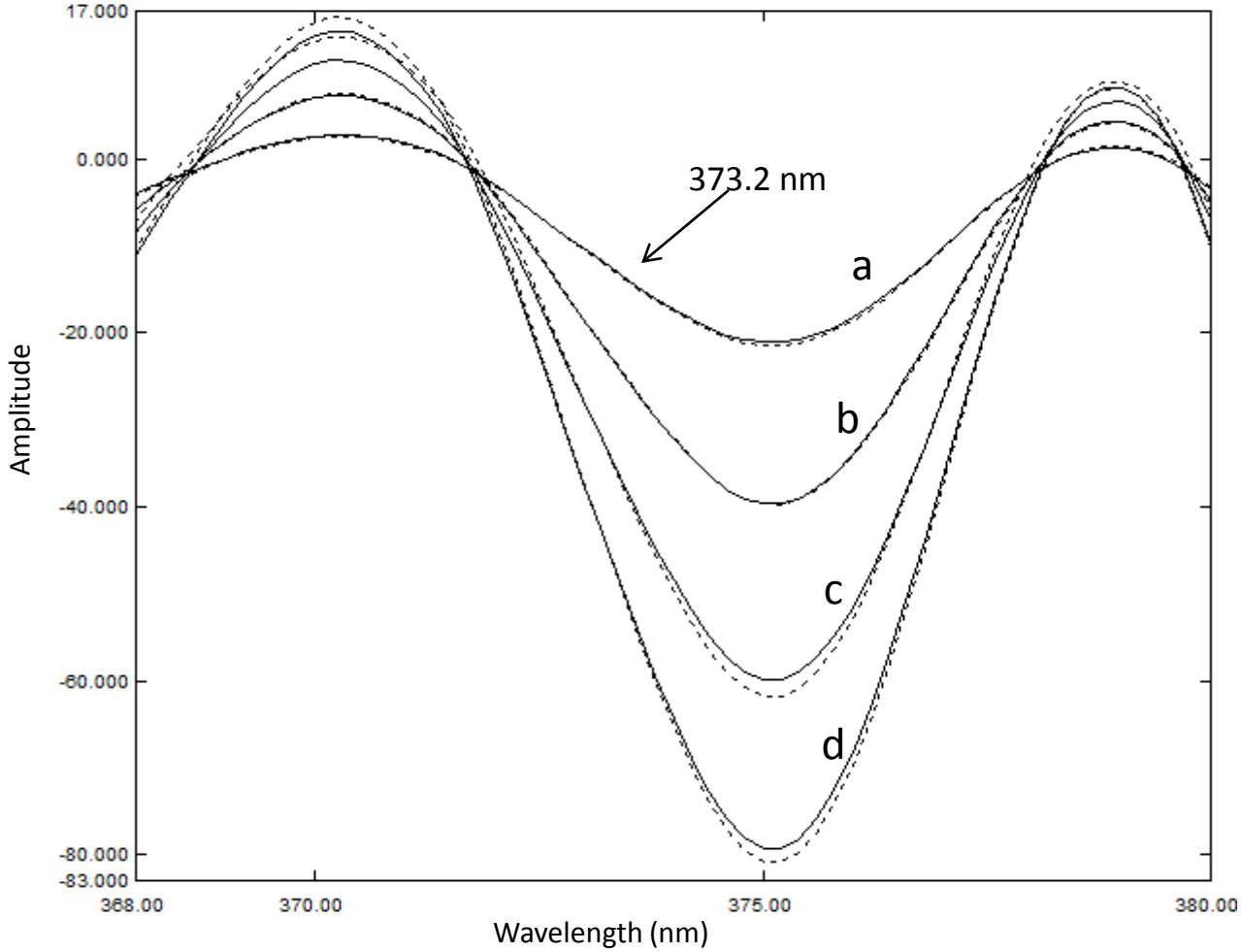

Fig. 4. The coincident spectra of the first derivative of the smoothed ratio spectra of AML (- - - -) and ternary mixture of AML, VAL and HCT (——) using the spectrum of 4 µg/mL VAL and HCT as double divisor.
(a - - -) 2 µg/mL AML, (a ——) mixture of 2, 24, 15.625 µg/mL AML, VAL HCT, respectively
(b - - -) 4 µg/mL AML, (b——) mixture of 4, 40, 9.375 µg/mL AML, VAL, HCT, respectively
(c - - -) 6 µg/mL AML, (c——) mixture of 6, 28, 6.25 µg/mL AML, VAL, HCT, respectively
(d - - -) 8 µg/mL AML, (d——) mixture of 8, 36, 6.25 µg/mL AML, VAL, HCT, respectively

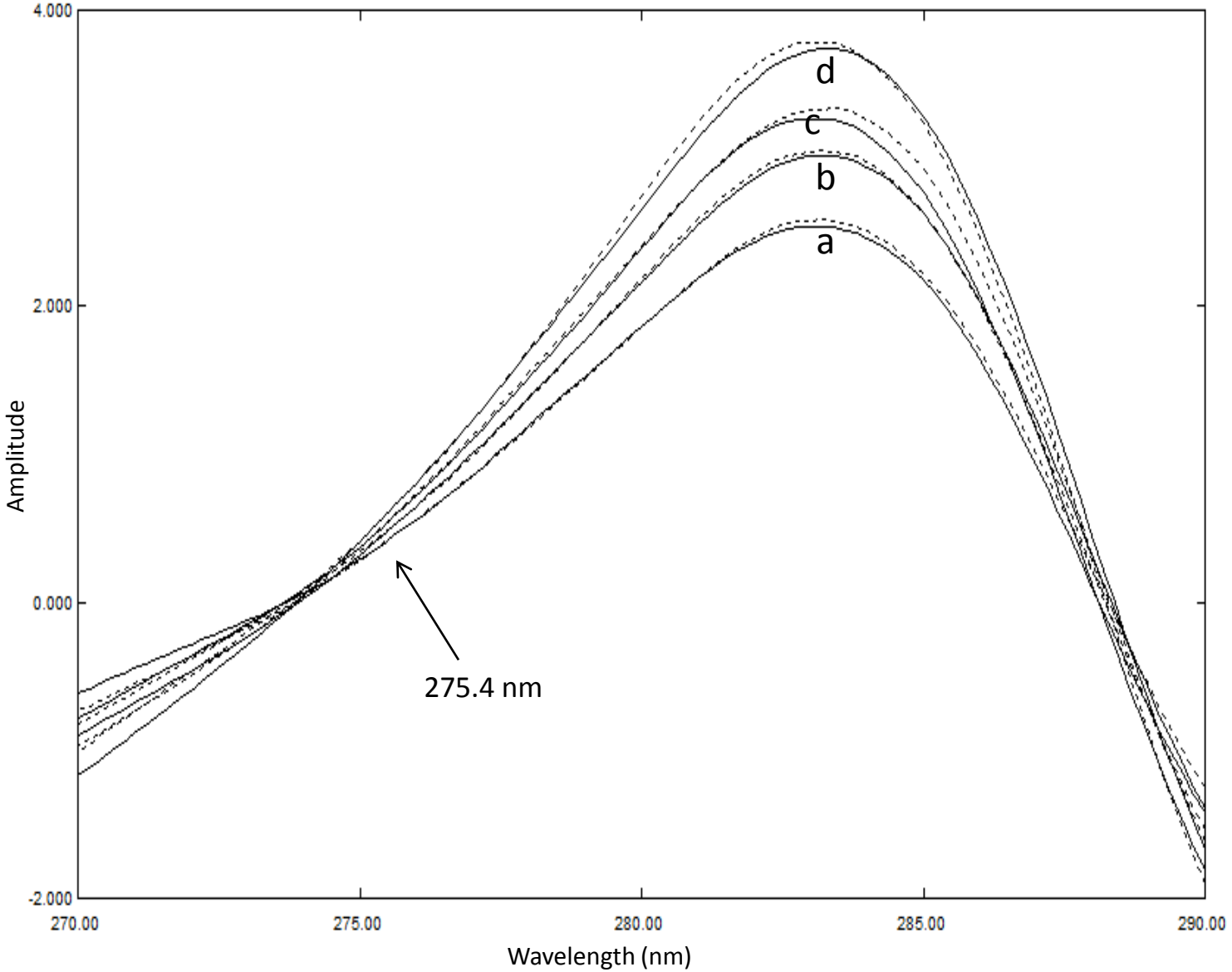

Fig. 5. The coincident spectra of the first derivative of the ratio spectra of VAL (- - -) and ternary mixture of AML, VAL and HCT (——) using the spectrum of 4 µg/mL AML and HCT as double divisor.
(a - - -) 28 µg/mL VAL, (a ——) mixture of 10, 28, 15.625 µg/mL AML, VAL, HCT respectively
(b - - -) 32 µg/mL VAL, (b——) mixture of 6, 32, 9.375 µg/mL AML, VAL, HCT, respectively
(c - - -) 36 µg/mL VAL, (c——) mixture of 8, 36, 6.25 µg/mL AML, VAL, HCT, respectively
(d - - -) 40 µg/mL VAL, (d ——) mixture of 2, 40, 6.25 µg/mL AML, VAL, HCT, respectively

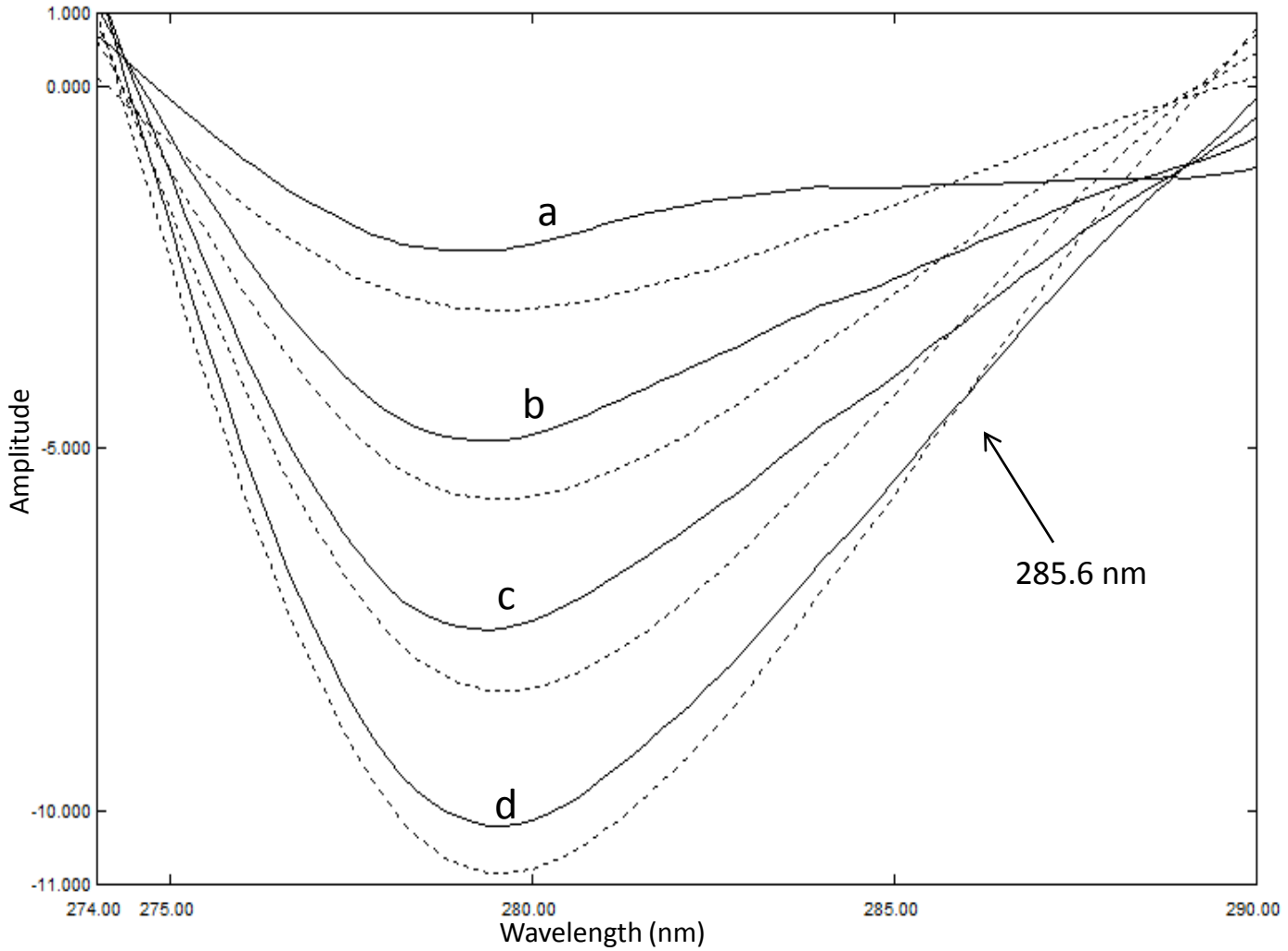

Fig. 6. The coincident spectra of the first derivative of the ratio spectra of HCT (- - - -) and ternary mixture of AML, VAL and HCT (——) using the spectrum of 4 µg/mL AML and VAL as double divisor.
(a - - -) 3.125 µg/mL HCT, (a ——) mixture of 6, 24, 3.125 µg/mL AML, VAL, HCT, respectively
(b - - -) 6.25 µg/mL HCT, (b ——) mixture of 6, 28, 6.25 µg/mL AML, VAL, HCT, respectively
(c - - -) 9.375 µg/mL HCT, (c——) mixture of 4, 40, 9.375 µg/mL AML, VAL, HCT, respectively
(d - - -) 12.5 µg/mL HCT, (d ——) mixture of 8, 40, 12.5 µg/mL AML, VAL, HCT, respectively

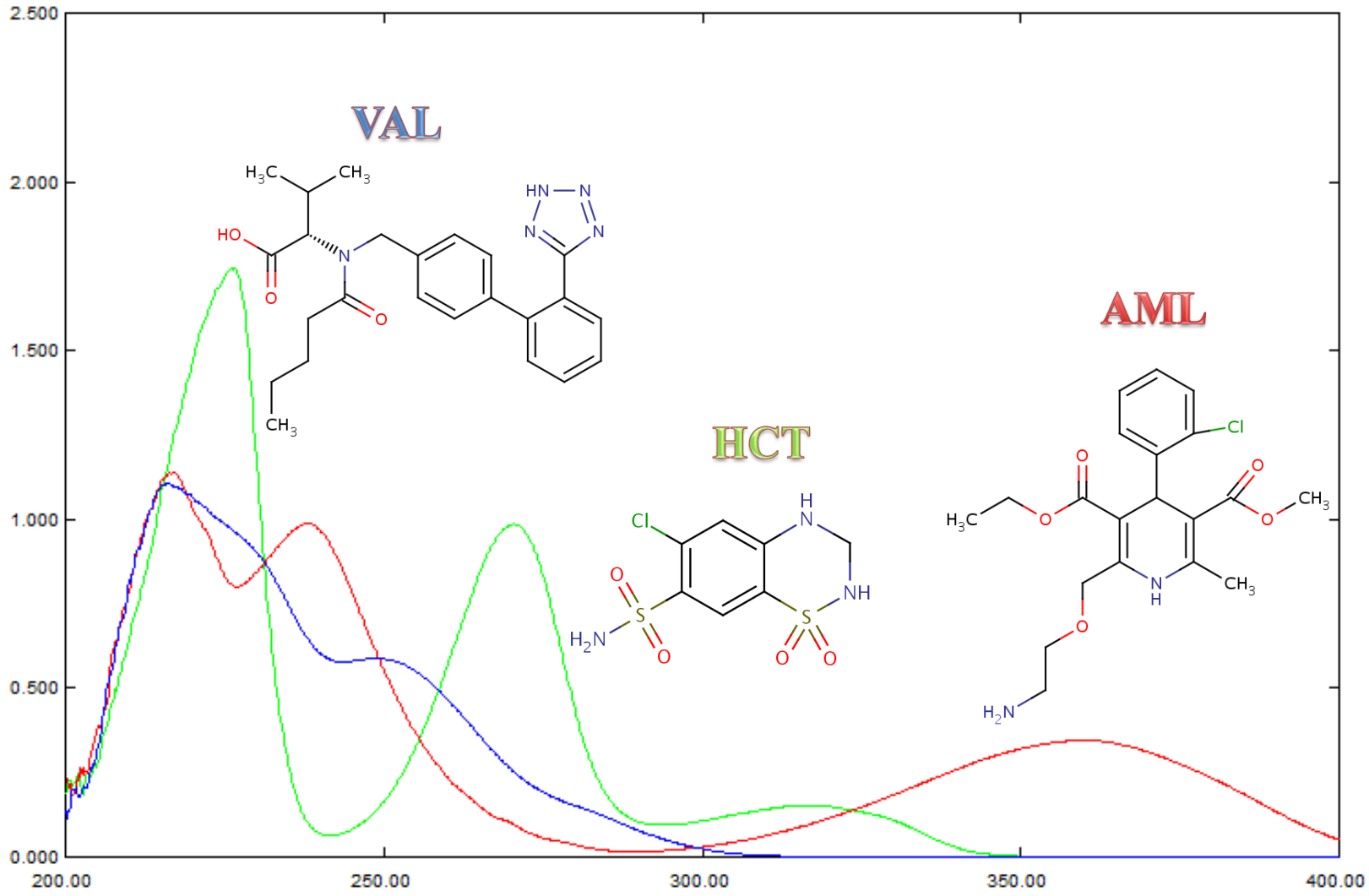
VAL
HCT
AML
2.500
2.000
1.500
1.000
0.500
0.000
200.00
250.00
300.00
350.00
400.00

Table 1. The 5-level, 3-factor experimental design shown as concentrations of the mixture components in µg/mL

Mix. No.	AML	VAL	HCT
1.	6	32	9.375
2. [a]	10	28	15.625
3.	6	24	3.125
4.	10	32	6.25
5.	2	24	15.625
6.	4	36	15.625
7.	2	40	6.25
8.	8	40	12.5
9.	4	40	9.375
10.	10	36	9.375
11.	6	28	6.25
12.	8	32	15.625
13.	4	28	12.5
14.	10	40	3.125
15.	6	40	15.625
16.	8	24	9.375
17.	10	24	12.5
18.	8	36	6.25
19.	2	36	3.125
20.	8	28	3.125
21.	2	32	12.5
22.	6	36	12.5
23.	4	24	6.25
24.	2	28	9.375
25.	4	32	3.125

[a] The shaded rows represent the validation set

Table 2. Assay validation sheet of the proposed spectrophotometric methods for the simultaneous determination of AML, VAL and HCT.

Parameter	*DRZC*			*DDRS-DS*		
	AML	***VAL***	***HCT***	***AML***	***VAL***	***HCT***
Accuracy (mean ± RSD)	99.98±1.308	99.90±1.064	100.24±1.270	100.07±1.344	100.17±1.700	100.07±1.340
Precision						
– Repeatability [a]	0.877	0.932	0.919	1.237	1.477	1.666
– Intermediate precision [b]	1.005	1.173	1.023	1.333	1.905	1.896
Robustness [c]	0.628	1.071	0.895	1.100	1.542	1.148
Linearity						
– Slope	-0.2475	0.0209	0.1059	-5.3236	0.0149	-0.3769
– Intercept	-0.1149	0.0001	1.0151	1.5170	0.0004	-0.0362
– Correlation coefficient (r)	0.9999	0.9998	0.9998	0.9999	0.9998	0.9999
Range	2-32 µg/mL	4-44 µg/mL	2-20 µg/mL	2-32 µg/mL	4-44 µg/mL	2-20 µg/mL

[a] The intraday (n = 3), average of three concentrations (12,16,20 µg/mL) for AML, VAL and HCT repeated three times within the day.

[b] The interday (n = 3), average of three concentrations (12,16,20 µg/mL) for AML, VAL and HCT repeated three times in three days.

[c] Robustness (n = 3), average of three concentrations (12,16,20 µg/mL) for AML, VAL and HCT analyzed using 75 and 70% methanol.

Table 3. Determination of AML, VAL and HCT in laboratory prepared mixtures by the proposed DRZC and DDRS-DS methods.

Concentration (µg/mL)			DRZC			DDRS-DS		
			Recovery % [a]					
AML	**VAL**	**HCT**	**AML**	**VAL**	**HCT**	**AML**	**VAL**	**HCT**
2	**40**	**3.125**	97.80	100.07	100.29	98.70	101.21	101.48
8	**40**	**3.125**	101.02	100.91	98.18	101.78	100.13	97.43
16	**40**	**3.125**	99.65	102.42	100.59	99.79	101.81	100.21
8	**20**	**3.125**	102.18	101.14	100.59	101.93	102.18	100.64
8	**4**	**3.125**	101.37	99.66	98.18	101.71	101.79	98.53
2	**40**	**6.25**	100.42	100.91	98.34	100.97	101.69	97.57
8	**40**	**6.25**	100.66	98.72	100.00	100.02	99.53	100.94
16	**40**	**6.25**	99.83	100.91	100.15	98.19	101.69	100.90
8	**20**	**6.25**	101.32	100.81	98.19	102.13	100.26	97.53
8	**4**	**6.25**	101.32	97.99	100.31	101.21	97.01	100.05
8	**20**	**12.5**	101.57	100.13	101.07	102.11	100.74	100.29
2	**4**	**12.5**	100.83	99.66	100.16	100.03	99.40	99.57
	Mean		**100.66**	**100.28**	**99.67**	**100.71**	**100.62**	**99.60**
	SD		**1.153**	**1.178**	**1.106**	**1.352**	**1.474**	**1.459**
	RSD%		**1.145**	**1.175**	**1.110**	**1.343**	**1.465**	**1.465**

[a] average of three determinations

Table 4. S**tatistical parameter values for simultaneous determination of AML, VAL and HCT using optimized PCR and PLS methods**

Parameter of interest	PCR method			PLS method		
	AML	**VAL**	**HCT**	**AML**	**VAL**	**HCT**
Concentration range (µg/mL)	2-32	4-44	2-20	2-32	4-44	2-20
No. of factors		3			3	
RMSEC [a]	0.2145	0.3963	0.0914	0.2155	0.3942	0.0914
RMSEP [b]	0.1280	0.3460	0.1313	0.1118	0.3339	0.1256
RMSECV [c]	0.2987	0.5465	0.1412	0.2996	0.5455	0.1408
Intercept [d]	0.0587	0.0923	0.0747	0.0531	-0.0136	0.0866
Slope [d]	0.9891	0.9961	0.9943	0.9861	0.9994	0.9947
Correlation coefficient (r) [d]	0.9983	0.9983	0.9995	0.9988	0.9984	0.9996

[a] Root Mean Square Error of Calibration

[b] Root Mean Square Error of Prediction

[c] Root Mean Squares Error of Cross-Validation

[d] Data of the straight line plotted between predicted concentrations of each component versus actual concentrations of calibration set.

Table 5. Determination of AML, VAL and HCT in validation set by the proposed PCR and PLS methods

Concentration (µg/mL)			PCR			PLS		
			Recovery % [a]					
AML	**VAL**	**HCT**	**AML**	**VAL**	**HCT**	**AML**	**VAL**	**HCT**
10	**28**	**15.625**	99.77	101.00	98.94	100.18	100.99	99.78
10	**32**	**6.25**	98.80	100.37	101.95	98.82	100.35	100.37
4	**36**	**15.625**	99.78	99.69	99.87	99.79	99.69	98.88
8	**40**	**12.5**	98.63	100.87	101.20	98.44	100.62	100.99
10	**36**	**9.375**	98.43	99.60	101.44	98.90	99.04	101.44
8	**32**	**15.625**	98.63	98.78	100.94	99.17	98.78	100.94
10	**40**	**3.125**	99.21	98.56	100.92	99.80	98.81	100.93
8	**24**	**9.375**	102.36	98.63	99.77	102.18	98.62	99.25
8	**36**	**6.25**	99.38	98.75	101.76	98.38	98.75	100.90
8	**28**	**3.125**	99.25	99.00	100.48	98.63	99.37	100.86
Mean			**99.42**	**99.52**	**100.73**	**99.43**	**99.50**	**100.43**
RMSEP [b]			**0.1058**	**0.3344**	**0.1088**	**0.1029**	**0.3173**	**0.0986**
RSD%			**1.143**	**0.940**	**0.952**	**1.152**	**0.874**	**0.844**

[a] average of three determinations

[b] Root Mean Square Error of Prediction

Table 6. Determination of AML, VAL and HCT in Exforge HCT® tablets by the proposed methods and the reported HPLC method [16].

Product	Drug	DRZC	DDRS-DS	PCR	PLS	Reported method [a]
Exforge	**AML**	99.61±0.999	99.57±1.488	99.69±0.986	99.89±0.917	100.40±0.636
HCT®	**VAL**	99.75±1.585	99.92±1.567	99.45±1.074	99.46±1.088	100.13±0.884
5/160/12.5	**HCT**	99.34±1.299	99.60±1.267	99.52±0.792	99.43±0.651	100.13±0.729
Exforge	**AML**	100.09±1.488	99.85±1.813	99.75±1.051	100.11±1.170	99.85±0.970
HCT®	**VAL**	100.46±0.555	99.97±1.481	99.93±1.190	100.18±1.115	100.00±0.859
5/160/25	**HCT**	100.05±1.015	99.79±.982	99.90±0.651	99.83±0.702	99.71±0.693
Exforge	**AML**	99.55±1.118	99.74±1.133	100.46±0.900	100.79±1.154	100.37±0.712
HCT®	**VAL**	100.87±1.023	100.76±1.252	99.84±0.852	99.46±0.920	100.55±0.595
5/160/25	**HCT**	101.29±0.341	101.14±1.225	100.31±1.030	100.30±1.020	100.23±0.825

[a] HPLC method using Luna C_{18} column, a mobile phase consisting of methanol – phosphate buffer (30 mM, pH 5.5) (62:38 by volume) at a flow rate of 1 mL/min and UV detection at 234 nm.

[b] average of three determinations

Table 7. Statistical comparison for the results obtained by the proposed methods and the reported method [16] for the analysis of AML, VAL and HCT in Exforge HCT® tablets

Value		Mean	SD	RSD%	n	Variance	Student's t test [a] (2.12)	F value [a] (3.44)
DRZC *Method*	***AML***	99.75	1.085	1.088	9	1.177	1.050	2.194
	VAL	100.36	1.100	1.096	9	1.209	0.296	2.269
	HCT	100.23	1.198	1.195	9	1.434	0.436	2.986
DDRS-DS Method	***AML***	99.72	1.305	1.059	9	1.702	0.976	3.172
	VAL	100.39	1.108	1.104	9	1.726	0.021	3.239
	HCT	100.38	1.157	1.153	9	1.553	0.320	3.233
PCR	***AML***	99.97	0.926	0.926	9	0.858	0.608	1.599
	VAL	99.74	0.932	0.935	9	0.869	1.237	1.630
	HCT	99.91	0.805	0.806	9	0.648	0.333	1.349
PLS	***AML***	100.26	1.027	1.024	9	1.055	0.135	1.966
	VAL	99.7	0.971	0.973	9	0.942	1.297	1.768
	HCT	99.85	0.794	0.795	9	0.631	0.490	1.313
Reported Method [b]	**AML**	100.21	0.733	0.731	9	0.537		
	VAL	100.23	0.730	0.728	9	0.533	-----	-----
	HCT	100.03	0.693	0.693	9	0.480		

[a] The values in the parenthesis are the corresponding theoretical values of t and F at P= 0.05.

[b] HPLC method using Luna C_{18} column, a mobile phase consisting of methanol – phosphate buffer (30 mM, pH 5.5) (62:38 by volume) at a flow rate of 1 mL/min and UV detection at 234 nm.

- Comparative study between chemometrics & univariate derivative spectrophotometry
- Reported methods need preparation, sophisticated instruments & expensive solvents
- First spectrophotometric & chemometric methods developed for this combination
- Wide spread of spectrophotometers makes it applicable in QC laboratories