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Research Article

Sequential Spectrophotometric Method for the Simultaneous Determination of Amlodipine, Valsartan, and Hydrochlorothiazide in Coformulated Tablets

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A new, simple and specific spectrophotometric method was developed and validated in accordance with ICH guidelines for the simultaneous estimation of Amlodipine (AML), Valsartan (VAL), and Hydrochlorothiazide (HCT) in their ternary mixture. In this method three techniques were used, namely, direct spectrophotometry, ratio subtraction, and isoabsorptive point. Amlodipine (AML) was first determined by direct spectrophotometry and then ratio subtraction was applied to remove the AML spectrum from the mixture spectrum. Hydrochlorothiazide (HCT) could then be determined directly without interference from Valsartan (VAL) which could be determined using the isoabsorptive point theory. The calibration curve is linear over the concentration ranges of 4–32, 4–44 and 6–20 μg/mL for AML, VAL, and HCT, respectively. This method was tested by analyzing synthetic mixtures of the above drugs and was successfully applied to commercial pharmaceutical preparation of the drugs, where the standard deviation is <2 in the assay of raw materials and tablets. The method was validated according to the ICH guidelines and accuracy, precision, repeatability, and robustness were found to be within the acceptable limits.

1. Introduction

Many analytical methods have been introduced for the analysis of mixtures among which the molecular absorption spectroscopy was the most simple, fast, and applicable in laboratories. Molecular absorption spectroscopy has been extensively used for the determination of drugs in pharmaceutical preparations with a view to the development of analytical methods. The use of this technique for pharmaceutical analyses has the inherent constraint that most active drugs absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination.

Direct spectrophotometry cannot resolve the drugs in their mixtures with impurities or other drugs, so several manipulations were performed to enable resolution of binary and ternary mixtures. Binary mixtures can be determined using different order derivatives [1], methods manipulating ratio spectra [2, 3] or dual wavelength [4–6], and isoabsorptive method [7]. For ternary mixtures, few spectrophotometric methods could resolve the overlap in their spectra, namely, Derivative Ratio Zero Crossing [8] and Double Divisor Ratio Spectra-Derivative Spectrophotometry [9] methods.

This paper describes the development and subsequent validation of a novel, simple, and rapid spectrophotometric method "*Sequential Spectrophotometry*" for simultaneous quantitation of ternary mixtures. The method was applied on a ternary mixture of AML, VAL, and HCT in bulk powder and pharmaceutical dosage forms. The linearity of response, accuracy, intermediate precision, and robustness of the described method for assay of AML, VAL, and HCT have been checked.

Amlodipine (AML), 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid 3-ethyl 5-methyl ester [10], (Figure 1), is a dihydropyridine derivative with calcium antagonist activity. It is used

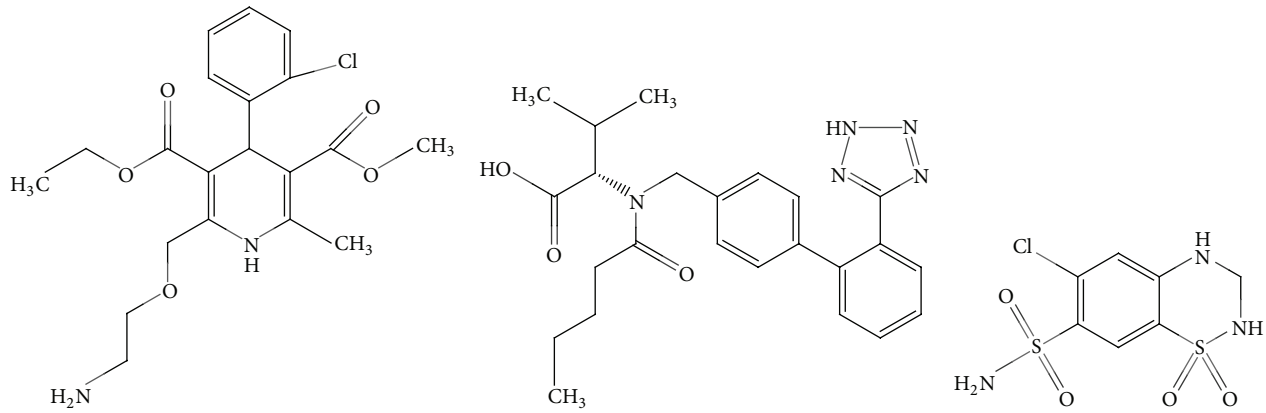

Figure 1: Structural formulae for (a) Amlodipine, (b) Valsartan, and (c) Hydrochlorothiazide.

in the management of hypertension, chronic stable angina pectoris, and prinzmetal's variant angina [11].

Valsartan (VAL) (chemically described as N-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine [10] Figure 1), 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 [12].

Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide [10] Figure 1, is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs [13].

Literature survey revealed that Amlodipine besylate and Hydrochlorothiazide are official in the British Pharmacopoeia [14], Valsartan, Hydrochlorothiazide, and their mixture are official in the United States Pharmacopoeia [15]. There are reported methods for the determination of AML, VAL, or HCT in different dosage forms [2, 4, 16–19] and in their binary mixtures [20–22]. Few methods were reported for the simultaneous estimation of AML, VAL, and HCT in their ternary mixture [23–26] and only one spectrophotometric method was developed for the determination of this mixture [27]. In previous work, the authors developed derivative and chemometric methods for the analysis of the same mixture [28].

2. Experimental

2.1. Samples

2.1.1. Pure Samples

(a) *Pure Amlodipine*. It was kindly supplied by Al-Hekma pharmaceutical Company, Cairo, Egypt; its purity was certified to be 99.89 ± 0.691.

(b) *Pure Valsartan*. It was kindly supplied by Novartis pharmaceutical Company, Cairo, Egypt; its purity was certified to be 99.69 ± 0.231.

(c) *Pure Hydrochlorothiazide*. It was kindly supplied by Al-Hekma pharmaceutical Company, Cairo, Egypt; its purity was certified to be 99.78 ± 0.364.

2.1.2. Market Samples. Three 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 the USA. market.

2.2. Reagents

Methanol. It was purchased from El-NASR, Egypt.

2.3. Instruments. SHIMADZU dual beam UV-visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and an HP1020 laserjet printer were used. 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.

2.4. Procedures

2.4.1. Standard Solutions

(a) Standard stock solutions of AML, VAL, and HCT 1 mg/mL in methanol were used.

(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 was recorded against methanol as a blank over the range of 200–400 nm.

The zero order (D_0) absorption spectrum of 16 μg/mL VAL, 16 μg/mL HCT, and a binary mixture of VAL and HCT (8 μg/mL for each) were recorded against methanol as a blank over the range of 200 to 400 nm.

2.4.3. Construction of Calibration Curves. Aliquots equivalent to 40–320 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.

For the determination of AML in presence of VAL and HCT, a calibration curve was constructed relating the absorbance of zero order spectra of AML at 359.4 nm to the corresponding concentration of AML and the regression equation was computed.

For the determination of HCT in presence of AML and VAL, a calibration curve was constructed relating the absorbance of zero order spectra of HCT at 316.4 nm to the corresponding concentration and the regression equation was computed.

For the determination of VAL in presence of AML and HCT, the isoabsorptive method was applied by measuring the absorbance of VAL and HCT at 256.8 nm (A_{iso}). Two calibration curves relating the absorbance to the corresponding drug concentrations were constructed, and the corresponding regression equations were computed.

2.4.4. Analysis of AML, VAL, and HCT in Laboratory Prepared Mixtures (Specificity). 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 were scanned from 200 to 400 nm and stored in the computer.

For the determination of AML in presence of VAL and HCT, the same procedure under linearity was applied and the concentration of AML was calculated from the corresponding regression equation.

For the determination of HCT in the presence of AML and VAL, the stored mixture spectra were divided by the spectrum of normalized AML, and the constant was subtracted from the ratio spectra followed by multiplication of the obtained spectra by the divisor. The result of these steps is the spectra of VAL and HCT binary mixture. The absorbance values at 316.4 nm (where VAL shows no interference) were recorded and the concentration of HCT was calculated from the corresponding regression equation.

For the determination of VAL in presence of HCT, the absorbance at (A_{iso}) in the binary mixture of VAL and HCT (after removal of AML) was measured, and then the total content of VAL and HCT was calculated from the corresponding regression equation. Subtraction of HCT concentration from the total concentration yields the actual concentration of VAL in the mixture.

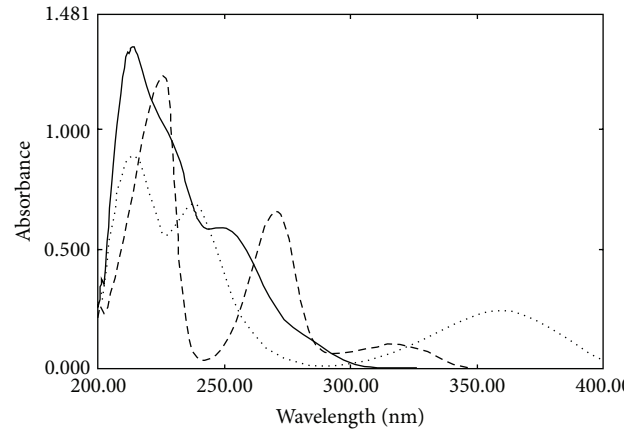

Figure 2: Zero order absorption spectra of 20 μg/mL AML (···), 20 μg/mL VAL (—), and 20 μg/mL HCT (– – –) using methanol as blank.

2.4.5. Analysis of AML, VAL, and HCT in Exforge HCT Tablets. 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 separate 100 mL measuring flasks, and the volume was completed with methanol. Five mL was transferred into 10 mL measuring flasks, suitable aliquots of AML and HCT were transferred from their standard working solutions for spiking the solution to reach concentrations of linearity range, and then volume was completed with methanol. The spectra of these solutions were scanned from 200 to 400 nm and stored in the computer. The same procedure under laboratory prepared mixtures was applied and the concentrations of AML, VAL, and HCT were calculated from the corresponding regression equations.

3. Results and Discussion

Sequential spectrophotometry depends on the presence of extended spectrum of one of the three drugs, so ratio subtraction technique can be used to remove this extended spectrum producing spectrum of the other two drugs. The second step depends on determination of the other two drugs by any spectrophotometric method that can resolve binary mixtures [6, 7, 29–33].

In this work, after direct determination of AML, we applied ratio subtraction to remove its extended spectrum producing a spectrum of HCT and VAL, where HCT was determined directly. Then the total concentration of HCT and VAL was calculated at their isoabsorptive point and VAL concentration was calculated by subtraction.

3.1. Determination of AML. The absorption spectra of the three compounds, AML, VAL, and HCT, show highly overlapped spectra in the region 200–300 nm (Figure 2). AML can be determined at 359.4 nm directly where VAL and HCT show no absorbance.

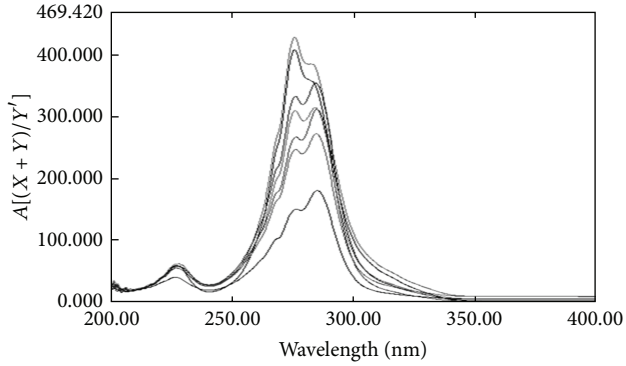

Figure 3: Ratio spectra of laboratory prepared mixtures of AML (Y) and VAL + HCT (X) using normalized AML (Y') as a divisor and methanol as a blank.

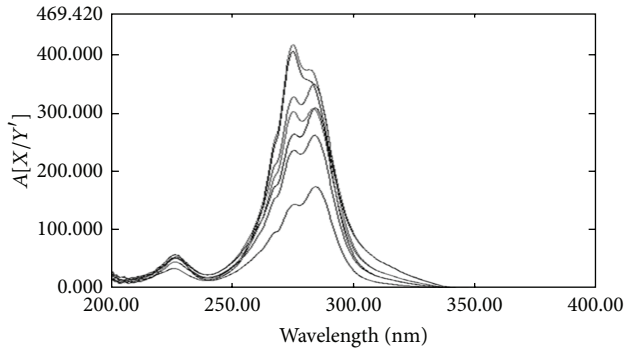

Figure 4: Ratio spectra of laboratory prepared mixtures of AML (Y) and VAL + HCT (X) using normalized AML (Y') as a divisor and methanol as a blank after subtraction of the constant.

A linear correlation was obtained between the absorbance and the corresponding concentration of AML at 359.4 nm. The regression equation was calculated as follows:

$$P_{\text{AML}} = 0.0162C + 0.0010, \qquad r = 0.9998, \qquad (1)$$

where C is the concentration of AML in μg/mL, P is the peak amplitude of the zero order spectrum of AML at 359.4 nm, and r is the correlation coefficient.

3.2. Determination of HCT. For the determination of both HCT and VAL, ratio subtraction technique was applied for removing AML spectrum.

The method depends on that when a mixture of VAL + HCT (X) and AML (Y) where the spectrum of (Y) is more extended (Figure 2), the determination of (X) could be done by scanning the zero order absorption spectra of the laboratory prepared mixtures (AML and VAL + HCT), dividing them by carefully chosen concentration of standard AML (Y' = divisor) producing a new ratio spectra that represent (X/Y') + constant as shown in Figure 3, then subtraction of the absorbance values of these constants (Y/Y') in plateau as shown in Figure 4, followed by multiplication of the obtained spectra by the divisor (Y'). Finally, the original spectra of (X) could be obtained (Figure 5), which were used for direct determination of HCT at 316.4 nm, where VAL shows no absorbance (Figure 2), and calculation of the concentration from the corresponding regression equation was done.

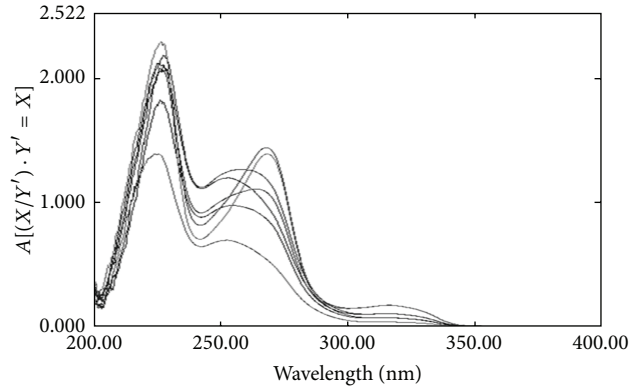

Figure 5: The zero order absorption spectra of VAL and HCT mixture obtained by the proposed ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication by the divisor (Y').

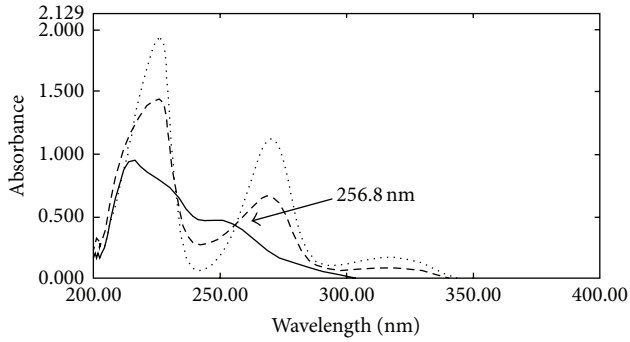

Figure 6: Zero order absorption spectra of 16 μg/mL VAL (—), 16 μg/mL HCT (···), and a mixture of 8 μg/mL VAL with 8 μg/mL HCT (– – –) showing isoabsorptive point at 256.8 nm using methanol as blank.

Table 1: Determination of AML, VAL, and HCT in laboratory prepared mixtures by the proposed method.

Concentration (μg/mL)			Proposed method recovery %[a]		
AML	VAL	HCT	AML	VAL	HCT
4[b]	40	6.25	101.01	101.38	97.78
12	40	6.25	100.91	98.20	100.74
20	40	6.25	99.53	100.04	100.74
12	20	6.25	101.37	100.16	99.26
12	4	6.25	101.55	97.34	100.74
12	20	12.5	101.22	100.12	101.48
4	4	12.5	98.26	99.82	100.74
	Mean		**100.55**	**99.58**	**100.21**
	SD		**1.208**	**1.357**	**1.262**
	RSD%		**1.201**	**1.363**	**1.260**

[a]Average of three determinations.
[b]The ratio of dosage form.

Table 2: Determination of AML, VAL, and HCT in Exforge HCT tablets by the proposed method and the reported HPLC method [23] and application of standard addition technique.

Product	Drug	Proposed method	Reported method[a]	Standard addition			
				Taken (μg/mL)	Added (μg/mL)	Found (μg/mL)	Recovery %[b]
Exforge HCT 5/160/12.5	AML	99.91 ± 1.069	100.40 ± 0.636	6	4	3.994	99.85
					6	6.074	101.23
					8	7.884	98.55
						Mean ± RSD	**99.88 ± 1.344**
	VAL	99.50 ± 0.951	100.13 ± 0.884	16	12	12.259	102.16
					16	16.14	100.88
					20	20.175	100.88
						Mean ± RSD	**101.30 ± 0.730**
	HCT	99.83 ± 1.282	100.13 ± 0.729	9.375	6.25	6.329	101.27
					9.375	9.319	99.4
					12.5	12.645	101.16
						Mean ± RSD	**100.61 ± 1.041**
Exforge HCT 5/160/25	AML	101.01 ± 1.650	99.85 ± 0.970	6	4	3.985	99.61
					6	6.121	102.02
					8	7.987	99.84
						Mean ± RSD	**100.49 ± 1.326**
	VAL	100.27 ± 1.403	100.00 ± 0.859	16	12	11.935	99.46
					16	16.379	102.37
					20	20.171	100.86
						Mean ± RSD	**100.89 ± 1.440**
	HCT	99.26 ± 1.493	99.71 ± 0.693	9.375	6.25	6.334	101.34
					9.375	9.533	101.68
					12.5	12.524	100.19
						Mean ± RSD	**101.07 ± 0.774**
Exforge HCT 10/320/25	AML	100.20 ± 1.173	100.37 ± 0.712	6	4	4.01	100.24
					6	5.934	98.91
					8	8.038	100.47
						Mean ± RSD	**99.87 ± 0.846**
	VAL	99.93 ± 0.897	100.55 ± 0.595	16	12	12.101	100.84
					16	15.949	99.68
					20	19.797	98.99
						Mean ± RSD	**99.84 ± 0.939**
	HCT	99.75 ± 1.045	100.23 ± 0.825	9.375	6.25	6.344	101.5
					9.375	9.27	98.88
					12.5	12.761	102.09
						Mean ± RSD	**100.82 ± 1.695**

[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.

The constant can be determined directly from the curve $(X + Y)/Y'$ by the straight line which is parallel to the wavelength axis in the region where (Y) is extended.

The linearity was checked between absorbance at the selected wavelength 316.4 nm and the corresponding concentration of HCT. A linear correlation was obtained and the regression equation was found to be

$$P_{\mathrm{HCT}} = 0.0108C + 0.0030, \qquad r = 0.9998, \qquad (2)$$

where C is the concentration of HCT in μg/mL, P is the peak amplitude of the zero order spectrum of HCT at 316.4 nm, and r is the correlation coefficient.

3.3. Determination of VAL. The absorbance spectra of 16 μg/mL VAL, 16 μg/mL of HCT, and a mixture containing equal concentrations of VAL and HCT (8 μg/mL of each) showed isoabsorptive point at 256.8 nm (Figure 6).

Table 3: Statistical comparison for the results obtained by the proposed spectrophotometric method and the reported method [23] for the analysis of AML, VAL, and HCT in Exforge HCT tablets.

Value	Proposed method			Reported method[a]		
	AML	VAL	HCT	AML	VAL	HCT
Mean	100.37	99.90	99.61	100.21	100.23	100.03
SD	1.252	1.015	1.141	0.733	0.730	0.693
RSD%	1.247	1.016	1.145	0.731	0.728	0.693
n	9	9	9	9	9	9
Variance	1.567	1.030	1.302	0.537	0.533	0.480
Student's t test[b]	0.343 (2.16)	0.788 (2.131)	0.927 (2.16)	—	—	—
F value[b]	2.921 (3.438)	1.933 (3.438)	2.710 (3.438)	—	—	—

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

Table 4: Assay validation sheet of the proposed spectrophotometric method for the simultaneous determination of AML, VAL, and HCT.

Parameter	Proposed method		
	AML	VAL	HCT
Accuracy (mean ± RSD)	99.93 ± 1.188	99.66 ± 0.956	100.24 ± 0.679
Precision			
Repeatability[a]	0.535	0.651	0.867
Intermediate precision[b]	1.061	1.225	1.002
Robustness[c]	0.704	0.633	0.807
Linearity			
Slope	0.0162	0.0258	0.0108
Intercept	0.0010	0.0051	0.0030
Correlation coefficient (r)	0.9998	0.9998	0.9998
Range (μg/mL)	4–32	4–44	6–20

[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.

By measuring the absorbance value at the chosen isoabsorptive point in the zero order absorption spectrum obtained from ratio subtraction method, the total content of VAL and HCT in the mixture can be calculated, while the content of HCT alone can be calculated using the zero order absorption spectrum obtained from ratio subtraction method without any interference from VAL. Thus the content of VAL can be calculated by subtraction.

By applying the isoabsorptive point, linear correlation was obtained between the absorbance values at 256.8 nm (A_{iso}) and corresponding concentrations of VAL and HCT. The regression equations were computed as follows:

$$\begin{aligned} P_{VAL} &= 0.0258C + 0.0051, \qquad r = 0.9998, \\ P_{HCT} &= 0.0254C - 0.0031, \qquad r = 0.9993, \end{aligned} \tag{3}$$

where C is the concentration of VAL and HCT in μg/mL, P is the peak amplitude of the zero order spectrum of VAL and HCT at 256.8 nm, and r is the correlation coefficient.

The proposed method was found to be valid in the range of 4–32 μg/mL, 4–44 μg/mL, and 6–20 μg/mL for AML, VAL and HCT, respectively, as shown by the small intercept and correlation coefficient approaching unity in the regression equations.

The specificity of the proposed method 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 1.

The proposed method was also applied for the determination of AML, VAL, and HCT in Exforge HCT tablets and the validity of the proposed method was further assessed by applying the standard addition technique as presented in Table 2.

Results obtained by the proposed method for the determination of the drugs in Exforge HCT tablets were statistically compared [34] to those obtained by the reported HPLC method [23]; no significant differences between the results were obtained as presented in Table 3. A validation sheet according to ICH guidelines was also presented in Table 4.

The method shows simplicity over our previous work on the same mixture [28], where the determination of the three drugs needs more steps of division and derivatization or difficult calculations as in chemometrics. Also, the method is preferred over chromatographic methods as no expensive solvents or sophisticated instruments are required.

4. Conclusion

From the previous discussion, it could be concluded that the new sequential spectrophotometry is simple, accurate, and specific and can be used for quantitation of ternary mixtures. The method was successfully applied for determination of a ternary mixture of AML, VAL, and HCT. The method is more rapid than chromatographic methods and does not need sample preparation or sophisticated techniques and instruments. It is also sensitive and selective and can be used for routine analysis of AML, VAL, and HCT in their pure powder and dosage forms.

Disclosure

The authors of the paper are academic staff in Cairo University and do not have a direct financial relationship with the commercial identities mentioned in the paper.

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