Novel Pure Component Contribution Algorithm (PCCA) and UHPLC Methods for Separation and Quantification of Amlodipine, Valsartan, and Hydrochlorothiazide in Ternary Mixture

Shereen Mowaka
British University in Egypt, Faculty of Pharmacy, Pharmaceutical Chemistry Department, 11837 El-Sherouk City, Egypt; Helwan University, Faculty of Pharmacy, Analytical Chemistry Department, Ein Helwan, 11795 Cairo, Egypt

Maha A. Hegazy
Cairo University, Faculty of Pharmacy, Analytical Chemistry Department, Kasr El-Aini St, 11562 Cairo, Egypt

Hayam M. Lutfy
Cairo University, Faculty of Pharmacy, Analytical Chemistry Department, Kasr El-Aini St, 11562 Cairo, Egypt; Future University, Faculty of Pharmaceutical Science & Pharmaceutical Industries, Pharmaceutical Chemistry Department, 12311 Cairo, Egypt

Ekram H. Mohamed
British University in Egypt, Faculty of Pharmacy, Pharmaceutical Chemistry Department, 11837 El-Sherouk City, Egypt

Two accurate and sensitive methods were developed and validated for the simultaneous determination of amlodipine (AML), valsartan (VAL), and hydrochlorothiazide (HCT) in their ternary mixture. The first method is a novel simple algorithm capable of extracting the contribution of each component from a mixture signal in which the components are partially or completely overlapped. It is based on the use of a coded function that eliminates the signal of interfering components using mean centering as a processing tool. Determination was performed at 237.6, 250.0, and 270.6 nm for AML, VAL, and HCT, respectively. Two fit values were developed and calculated for optimization of the method for each drug, one to test that the absorptivity values of the extracted spectra are within the confidence limits of the slope, and the other for correlation between the pure and extracted spectra. The fit values for AML, VAL, and HCT were α = 0.0449, 0.03981, and 0.07251, respectively, and r = 1 for each drug. The second method is an ultra-HPLC (UHPLC®) method in which separation of AML, VAL, and HCT was carried out on a UHPLC C18 column (100 × 2.1 mm, 2.2 µm) using a mobile phase of acetonitrile–methanol–phosphate buffer (pH 2.8; 25 + 50 + 25, v/v/v). The flow rate was 0.5 mL/min, and the detection was set at 255.0 nm. The proposed methods were successfully applied to the analysis of AML, VAL, and HCT in pharmaceutical formulations, without interference from the dosage-form additives. The results were statistically compared to a previously reported method, and no significant difference was found regarding accuracy or precision.

Received July 28, 2016. Accepted by JB October 17, 2016.

DOI: 10.5740/jaoacint.16-0195
Theory of the PCCA

This algorithm was developed and validated using simulated model data from Hegazy (15), who successfully applied it for the determination of binary and ternary mixtures. The following equations explain the mathematical representation involved in the following algorithm:

\[ A_m = \alpha_X C_X + \alpha_Y C_Y + \alpha_Z C_Z \]  

(1)

where \( A_m \) = the vector of the absorbance of the mixture; \( \alpha_X, \alpha_Y, \) and \( \alpha_Z \) = the molar absorptivity vectors of \( X, Y, \) and \( Z, \) respectively; and \( C_X, C_Y, \) and \( C_Z \) = the concentrations of \( X, Y, \) and \( Z, \) respectively.

By dividing by \( \alpha_Z \),

\[ B = A_m/\alpha_Z = \alpha_X C_X/\alpha_Z + \alpha_Y C_Y/\alpha_Z + C_Z \]  

(2)

\[ C = MC(B) = MC(\alpha_X C_X/\alpha_Z) + MC(\alpha_Y C_Y/\alpha_Z) \]  

(3)

\[ D = C/MC(\alpha_Y/\alpha_Z) = MC(\alpha_X C_X/\alpha_Z) + MC(\alpha_Y C_Y/\alpha_Z) \]  

(4)

\[ E = MC(D) = MC[MC(\alpha_X C_X/\alpha_Z) + MC(\alpha_Y C_Y/\alpha_Z)] \]  

(5)

\[ F = MC[MC(\alpha_X C_X/\alpha_Z) + MC(\alpha_Y C_Y/\alpha_Z)] \]  

(6)

\[ G = C_X \ast \alpha_X = \alpha_X C_X \]  

(7)

where MC = Mean centering

Equation 7 is the mathematical foundation of each component in the laboratory-prepared mixtures and pharmaceutical formulations. This calculation permits the determination of each component in the mixture (\( X \) in this equation) without interference from the other components of the ternary system (\( Y \) and \( Z \) in these equations).

As Equation 7 shows, the obtained spectra permits the determination of component \( X \) by direct measurement of the estimated absorbance value at its \( \lambda_{max} \) using the corresponding regression equation obtained by plotting the absorbance of the pure spectra of \( X \) at its \( \lambda_{max} \) versus its corresponding concentration. The pure component contribution for \( Y \) and \( Z \) could also be obtained as described for \( X \).

The above-detailed equations were scripted in sequence in MATLAB, and a function was created so that after entering all the steps, results are calculated and directly presented.

For the purpose of optimization, different divisors should be tested and compared to determine the best divisor. Two fit values were calculated for each divisor:

Fit 1: Absorptivity value (\( \alpha \)) for the estimated absorbance values at \( \lambda_{max} \)

Fit 2: Correlation coefficient (\( r \)) calculated between the estimated pure contribution of each component and its standard spectrum

The best divisor is the one that gives a fit 1 value that lies within the 95% confidence interval of the regression slope of standard substance (the absorptivity of the reference standard at its \( \lambda_{max} \)) and that gives a fit 2 value near unity, which is an indication of perfect correlation and, hence, successful extraction.

Experimental

Apparatus

(a) Spectrometer.—Spectrophotometric measurements were carried out on a JASCO V-630/Bio double-beam UV-Vis spectrophotometer (S/N C36796148), using 1.00 cm quartz cells. Scans were carried out in the range of 200.0–400.0 nm at 0.1 nm intervals. Spectra Manager II software was used. All calculations in the computing process were done in MATLAB® 6.5 (16) using a simple coded MATLAB function (17).

(b) UHPLC.—The LC system consisted of the Thermo Scientific Dionex UltiMate® 3000 UHPLC system (Thermo Fisher Scientific, United States) with a Symmetry® Acclaim RSLC 120 C18 column (100 × 2.1 mm, 2.2 μm; United States). The system was equipped with a rapid-separation diode-array detector (DAD-3000RS; Thermo Fisher Scientific) and an autosampler (WPS-3000TRS; Thermo Fisher Scientific). An Elmasonic S 60 H degasser (Germany) was used.

Reagents

(a) Acetonitrile.—HPLC grade (Sigma-Aldrich, Germany).

(b) Methanol.—HPLC grade (Sigma-Aldrich).

(c) Triethylamine.—HPLC grade (Sigma-Aldrich).

(d) Water.—Ultra-pure water (Sigma-Aldrich).

(e) Standards.—AML and HCT were kindly supplied by Sanofi-Aventis Pharmaceutical Co. (Cairo, Egypt); VAL was kindly supplied by Novartis Pharma S.A.E. (Cairo, Egypt). The purity was certified to be 99.98 ± 0.329, 99.86 ± 0.754, and
99.51 ± 0.821% according to the manufacturer certificates for AML, HCT, and VAL, respectively.

(f) Samples.—Two EXFORGE HCT® tablet dosage forms containing AML, VAL, and HCT in labeled concentrations of 5.00/160.00/25.00 mg, respectively, per tablet (batch No. S0248); and 10.00/160.00/25.00 mg, respectively, per tablet (batch No. S0092). The tablets were manufactured by Novartis Pharmaceuticals Corp. (United States) and purchased from the Egyptian market.

(g) Orthophosphoric acid.—Analytical grade (El-Nasr Pharmaceutical Chemical Co., Egypt).

(h) Potassium dihydrogen phosphate.—Analytical grade (El-Nasr Pharmaceutical Chemical Co.).

(i) Stock standard solutions.—AML, VAL, and HCT (1.00 mg/mL) in methanol were prepared for both methods.

(j) Working standard solutions.—Obtained by appropriate dilution of stock solution with methanol and mobile phase for PCCA and chromatographic methods, respectively.

Chromatographic Conditions

Chromatographic separation of the ternary mixture was performed using an isocratic elution based on using a mobile phase consisting of acetonitrile–methanol–phosphate buffer (pH 2.8; 25 + 50 + 25, v/v/v). The flow rate was 0.5 mL/min, and the detection was set at 255.0 nm. The buffer solution was filtered through a 0.25 μm membrane filter and degassed for about 30 min. Analyses were performed at ambient temperature, with an injection volume of 1 μL.

Procedure

Construction of Calibration Graphs

(a) PCCA.—Standard solutions in the range of 2.00–32.00, 4.00–40.00, and 2.00–30.00 μg/mL for AML, VAL, and HCT, respectively, were prepared and scanned in the range of 200.0–400.0 nm, values of absorbance at $\lambda_{\text{max}}$ were recorded and plotted against the corresponding concentrations, and regression parameters were calculated.

(b) UHPLC.—Aliquots equivalent to 5.00–1000.00, 10.00–1200.00, 5.00–900.00 μg AML, VAL, and HCT, respectively, were accurately transferred from their corresponding stock standard solutions into three separate series of 10 mL volumetric flasks and then diluted to volume with the mobile phase. Triplicate 1 μL injections were made for each concentration. Chromatograms were recorded at a flow rate of 0.5 mL/min at ambient temperature, and the effluent was monitored at 255.0 nm. The separation was carried out on a UHPLC C$_{18}$ column (100 × 2.1 mm, 2.2 μm) using a mobile phase of acetonitrile–methanol–phosphate buffer (pH 2.8; 25 + 50 + 25, v/v/v). The calibration graph was constructed by plotting the peak areas obtained versus the corresponding injected concentrations.

Application to Laboratory-Prepared Mixtures

(a) PCCA.—Accurate aliquots of AML, VAL, and HCT were transferred from their working standard solutions into a series of 10 mL volumetric flasks to prepare eight mixtures containing different ratios of the cited drugs. The volumes were diluted with methanol. The spectra of the prepared solutions were recorded at 200.0–400.0 nm. For mixtures containing the same ratio of the dosage form, AML was enriched via the spectrum addition technique (9) using the recorded spectrum of 5.00 μg/mL pure standard AML.

For each component, the value of the absorbance of the extracted spectra at its $\lambda_{\text{max}}$ was determined, and concentrations were obtained by using the corresponding regression equation. The claimed concentration of AML in the enriched mixture was calculated after subtracting the added concentration AML spectrum (5.00 μg/mL) analyzed by using the same procedure.

(b) UHPLC.—Accurate aliquots of AML, VAL, and HCT were transferred from their working standard solutions into a series of 10 mL volumetric flasks to prepare six mixtures containing different ratios of the cited drugs. The volumes were diluted with mobile phase. Prepared mixtures were chromatographed according to the specified conditions, and the concentration of each drug was calculated from its corresponding regression equation.

Application to Pharmaceutical Dosage Form

(a) PCCA.—Ten tablets of each EXFORGE HCT formulation were accurately weighed and finely powdered. An amount of the powder equivalent to 4.00 mg VAL was separately weighed from the 10.00/160.00/25.00 and 5.00/160.00/12.50 mg EXFORGE HCT formulations (designated A and B, respectively) and dissolved in methanol by shaking in an ultrasonic bath for about 30 min. The solutions were filtered into separate 100 mL measuring flasks, and the volume was diluted with methanol. Aliquots of 8.0 and 6.4 mL were accurately transferred from the above prepared solutions of A and B, respectively, to two separate 10 mL volumetric flasks. For series B, AML was enriched via spiking using the standard solution of 5.00 μg/mL and the spectrum addition technique by adding the D' spectrum 5.00 μg/mL pure standard AML using the software of the spectrophotometer. The concentration of each component was determined as in the Construction of Calibration Graphs section, using the specified regression equation.

(b) UHPLC.—Another portion of the above finely powdered drug equivalent to 8.00 mg VAL was separately weighed from the 10.00/160.00/25.00 mg (A) and 5.00/160.00/12.50 mg (B) EXFORGE HCT formulations. A 60.0 mL aliquot of the mobile phase was added to A and B, and the mixtures were dissolved using an ultrasonic bath for 30 min. The solutions were diluted to 100 mL with the mobile phase and then filtered using a 0.45 μm nylon membrane filter disc before use. Aliquots of 2.0 and 8.0 mL were accurately transferred from the above prepared solutions of A and B, respectively, to two separate 10 mL volumetric flasks, and the volume was diluted with the mobile phase. The concentration of each drug was calculated using the specified regression equation.

Application of Standard Addition Technique

To check the accuracy of the proposed methods, the standard addition technique was applied. The general procedures described above for each method were followed, and the concentrations of the added pure drug standard solutions were calculated from the specified regression equation.
Results and Discussion

The need for resolving overlapped spectra from multicomponent mixtures without prior separation of the individual analytes has grown dramatically. The UV absorption spectra of AML, VAL, and HCT show severe overlapping, which hinders the determination of the studied drugs using conventional spectrophotometric methods, as presented in Figure 2. In this work, a novel and very simple algorithm capable of resolution and extraction of the pure component contribution from their mixture signal, without any special requirements, was developed, optimized, and validated. PCCA introduces a simple and accurate coded function that performs the calculation with well-defined fit values and minimizes error and guarantees the best accuracy and precision. The proposed algorithm has high accuracy and sensitivity because it extracts the contribution of each compound in the mixture, which in turn, allows its determination at the corresponding \( \lambda_{\text{max}} \). The algorithm has been used for resolving binary and ternary mixtures (15). It has no limitations; in contrast to derivative methods, the proposed method eliminates the derivative steps and, therefore, the S/N is enhanced. Moreover, there is no need to have an extended spectrum over the others, which is a basic requirement of several spectrophotometric methods. The developed algorithm uses mean centering during its processing steps for the spectral resolution of severely overlapped bands. It depends on the elimination of the interfering components and so that it can extract the pure contribution of each component in a mixture. Furthermore, the method is advantageous because it allows the quantitative determination of each component at a single wavelength (\( \lambda_{\text{max}} \)), giving the highest sensitivity, accuracy, and precision results.

In addition, an accurate, fast, and economic UHPLC method was developed and validated. Both methods were successfully applied for the simultaneous determination of the studied drugs in their pure and dosage forms.

Reversed-phase UHPLC is very useful for the simultaneous determination of drugs in pharmaceutical dosage forms. This technique is widely used for higher sensitivity and selectivity. This paper describes a simple reversed-phase UHPLC method for simultaneous estimation of AML, VAL, and HCT in dosage form that is faster than the reported HPLC methods, thereby reducing cost and time dramatically.

In contrast to HPLC, UHPLC is preferred for giving faster results with better resolution, sensitivity, and efficiency. Decreasing the length of the column saves time and reduces solvent consumption, which in turn, lowers the cost (18). The UHPLC system is designed in a special way to withstand high system backpressures. Nowadays, pharmaceutical industries, as well as analytical laboratories, are in search of new ways to reduce the cost and time for analysis of drugs and to improve the quality of their products.

UHPLC, with better resolution, assay sensitivity, and high sample throughput, allows a greater number of analyses to be performed in a shorter period of time and at lower cost compared with HPLC; therefore, most QC laboratories have transferred from HPLC to UHPLC.

Methods Optimization and Development

PCCA.—For achieving maximum accuracy and precision, two fit values were calculated by the algorithm and compared between different divisors. The fit values are produced as an output from the function, along with the pure spectral contribution. The first fit value is the absorptivity at \( \lambda_{\text{max}} \) (a) the value will be accepted when it falls within the confidence limits of the regression slope of the pure standard. The second fit value is the correlation coefficient (r) between the extracted pure spectral contribution and the standard spectrum of the component; as the value reaches unity, it shows high fitness. Different divisors were tried, and the one that gave the best fit values was chosen, as presented in Table 1.

As Figure 2 shows, the absorption spectra of AML, VAL, and HCT are severely overlapped. The standard solutions of each drug were scanned separately in the region of 200.0–400.0 nm. Calibration curves were constructed by plotting the absorbance value at 237.6, 250.0, and 270.6 nm against the corresponding concentrations for AML, VAL, and HCT, respectively. All regression parameters were calculated and are presented in Table 2. To determine the AML concentration in synthetic ternary mixtures and in dosage form samples, the spectra of the mixtures shown in Figure 3 were entered as an input in the PCCA function, along with the standard divisor spectra. Simply by entering the data,
the individual pure contribution of AML, VAL, and HCT in each mixture is obtained, as presented in Figures 4, 5 and 6, respectively. The estimated absorbance values of the obtained spectra at 237.6, 250.0, and 270.6 nm are used for calculating the concentrations of AML, VAL and HCT, respectively, from the corresponding calculated regression equations.

Determination of the three compounds at their $\lambda_{\text{max}}$ value is associated with maximum sensitivity, the highest accuracy and precision, and the lowest error. Concentration ranges of the three compounds, determined using the respective regression equations, are shown in Table 2.

**UHPLC**—Chromatographic conditions were optimized through careful study of different chromatographic parameters, including wavelength detection, mobile phase composition and proportions, pH, and flow rate, in order to recognize the most suitable chromatographic system. The choice was based on the best resolution in a reasonable amount of time, as shown in Figure 7. The system suitability tests were used to verify that the conditions of the chromatographic system were adequate for the resolution and, hence, for the analysis (19). Satisfactory results were obtained for number of theoretical plates, resolution factor, tailing factor, and RSD of retention time and peak area, as presented in Table 3.

**Table 2.** Assay parameters and method validation obtained by applying the PCCA and UHPLC methods for the determination of AML, VAL, and HCT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AML</th>
<th>VAL</th>
<th>HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCCA</td>
<td>UHPLC</td>
<td>PCCA</td>
<td>UHPLC</td>
</tr>
<tr>
<td>Linearity range, µg/mL</td>
<td>2–32</td>
<td>0.5–100</td>
<td>4–40</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0426</td>
<td>1.926</td>
<td>0.0393</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0034</td>
<td>0.0163</td>
<td>0.003</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>0.9999</td>
<td>1.000</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>100.29 ± 0.50</td>
<td>100.31 ± 0.39</td>
<td>100.00 ± 0.60</td>
</tr>
<tr>
<td>Intraday RSD, %</td>
<td>0.523</td>
<td>0.433</td>
<td>0.422</td>
</tr>
<tr>
<td>Interday RSD, %</td>
<td>0.437</td>
<td>0.755</td>
<td>0.480</td>
</tr>
</tbody>
</table>

*a* Data presented as mean recovery ± SD.

*b* RSD of three concentrations of AML (8, 16, and 24 µg/mL), VAL (10, 20, and 25 µg/mL), and HCT (6, 10, and 14 µg/mL).
**Buffer and pH Effects**

Choosing suitable pH of the mobile phase was an important factor. After experimental study, a buffer with a pH of 2.8 was optimum for giving good baseline separation and optimum peak sharpness and symmetry.

Several types of buffer (phosphate, acetate, and TEA) were examined. It was found that phosphate buffer gave better peak symmetry than other types of buffers.

**Mobile Phase**

The effect of changing the ratio of organic modifiers on the retention time was investigated. At first, pure methanol was used as a single organic modifier in different ratios in the range of 40–90%. It was found that 75% was suitable for the separation of the HCT and AML peaks from the VAL peak, but the baseline for HCT and AML did not show complete separation. This problem was solved by replacing a third of the methanol with acetonitrile. Increasing the amount of acetonitrile resulted in faster elution of HCT.

Complete separation of the three peaks was obtained with a mobile phase of acetonitrile–methanol–phosphate buffer (pH 2.8; 25 + 50 + 25, v/v/v) as a mobile phase.

**Method Validation**

**Linearity**

PCCA.—Linear relationships between absorbance values at $\lambda_{\text{max}}$ of each drug and the corresponding concentrations were obtained. The regression equation for each drug was computed. The linearity of the proposed algorithm was evaluated by processing the different calibration curves on 3 different days. The analytical data of the calibration curve, including concentration ranges and calibration equation parameters, are summarized in Table 2.

UHPLC.—The linearity of the proposed chromatographic method for the determination of AML, VAL, and HCT was evaluated by analyzing a series of different concentrations of the drug. In this study, six concentrations were chosen, ranging between 0.50 and 100.00 μg/mL for AML, 1.00 and 120.00 μg/mL for VAL, and 0.50 and 90.00 μg/mL for HCT. Each concentration was analyzed in triplicate to provide information on the variation of peak area values among samples of the same concentration. Linear relationships between the area under the peak and drug concentrations were obtained. The regression equation for each drug was also computed.

The linearity of the calibration graphs is validated by the high value of the correlation coefficient and the intercept value. Characteristic parameters for regression equations of the adopted chromatographic method are given in Table 2.

**Range**

The calibration ranges for both methods were established by considering the necessary practical range according to the concentrations of AML, VAL, and HCT present in the pharmaceutical product, in order to give accurate, precise, and linear results. The calibration ranges of the proposed drugs are given in Table 2.

**Accuracy**

Accuracy of the methods was assessed using the procedure described in the Construction of Calibration Graphs section using different concentrations of pure samples along their calibration range, in triplicate. The results obtained, including mean recovery, SD, and RSD, are displayed in Table 2.

**Precision**

To judge the quality of the elaborated methods, precision was determined. For evaluation of the precision estimates, assays of three different concentrations of each of the proposed drugs were performed, in triplicate, three times in the same day (intraday), and the same selected concentrations were assayed on 3 successive days (interday), using the developed chromatographic methods and then calculating the RSD. The results shown in Table 2 indicate satisfactory precision of the proposed methods.

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**Table 3. System suitability parameters for the proposed UHPLC method according to the USP**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AML</th>
<th>VAL</th>
<th>HCT</th>
<th>USP reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time, min</td>
<td>2.351</td>
<td>3.403</td>
<td>1.543</td>
<td></td>
</tr>
<tr>
<td>Retention factor, $k'$</td>
<td>3.702</td>
<td>5.80</td>
<td>2.086</td>
<td>1–10</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>9424</td>
<td>3181</td>
<td>2060</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Height equivalent to theoretical plate</td>
<td>0.0106</td>
<td>0.0100</td>
<td>0.0485</td>
<td>The smaller the value, the higher the column efficiency</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.975</td>
<td>0.992</td>
<td>1.016</td>
<td>~1</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>4.34</td>
<td>5.14</td>
<td>&gt;1.5</td>
<td></td>
</tr>
<tr>
<td>Selectivity, $\alpha$</td>
<td>1.566</td>
<td>1.774</td>
<td>&gt;1</td>
<td></td>
</tr>
</tbody>
</table>

*USP = U.S. Pharmacopeia (19).*
Specificity

The specificity of a method is the extent to which it can be used for analysis of a particular analyte in a mixture or matrix without interference from other components. The specificity of the proposed methods was tested by analyzing eight laboratory-prepared mixtures containing different percentages of AML, VAL, and HCT at various concentrations within their linearity ranges and approximating the ratios that are found in their pharmaceutical coformulations. The laboratory-prepared mixtures were analyzed according to the procedures previously described under each of the proposed methods, and the results are presented in Tables 4 and 5. For the determination of the studied compounds in pharmaceutical formulation, the same procedure used for laboratory-prepared mixtures was applied, and the results are presented in Table 6.

Robustness (UHPLC Only)

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.3 mL/min to 0.4 and 0.5 mL/min. The organic strength was varied by ±2%, whereas pH was varied by ±0.1 units. The results presented in Table 7 indicate that the capacity of the method remained unaffected by these small, deliberate variations, providing an indication for the reliability of the proposed chromatographic method during routine work.

Table 4. Determination of AML, VAL, and HCT in laboratory-prepared mixtures by the PCCA method

<table>
<thead>
<tr>
<th>Analyte concn in laboratory-prepared mix, µg/mL</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>VAL</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>101.12 ± 1.07</td>
</tr>
</tbody>
</table>

a Average of three determinations.

b Data represent the ratio of analyte concentrations present in the pharmaceutical formulations.

c Analyte concentration after the spectrum addition and subtraction of pure standard 5 µg AML by spectrophotometer software.

Table 5. Determination of AML, VAL, and HCT in laboratory-prepared mixtures by the UHPLC method

<table>
<thead>
<tr>
<th>Analyte concn in laboratory-prepared mix, µg/mL</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>VAL</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>99.98 ± 1.14</td>
</tr>
</tbody>
</table>

a Average of three determinations.

b Data represent the ratio of analyte concentrations present in the pharmaceutical formulations.

Table 6. Determination of AML, VAL, and HCT in pharmaceutical dosage forms by the proposed methods and results obtained by the standard addition technique

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>VAL</td>
</tr>
<tr>
<td>PCCA</td>
<td>UHPLC</td>
</tr>
<tr>
<td>5.00/160.00/12.50 mg</td>
<td>100.57 ± 0.91</td>
</tr>
<tr>
<td>Standard addition</td>
<td>99.91 ± 0.95</td>
</tr>
<tr>
<td>10.00/160.00/25.00 mg</td>
<td>100.62 ± 0.80</td>
</tr>
<tr>
<td>Standard addition</td>
<td>100.59 ± 0.88</td>
</tr>
</tbody>
</table>

a Data presented as mean ± SD.

b Average of five determinations.

c Milligrams of AML/VAL/HCT.
LOD and LOQ

According to the International Conference on Harmonization recommendations (20), the approach based on both the SD and the slope of the response was used for calculating the LOD and LOQ, as presented in Table 2.

Standard Addition

The interference of excipients in the pharmaceutical formulations was studied using the proposed methods; therefore, the standard addition method was applied to the commercial pharmaceutical formulation, and the mean percentage recoveries and SDs for the proposed methods were calculated (Table 6). According to the obtained results, the excipients in pharmaceutical formulations do not interfere in the analysis of the proposed drugs in the pharmaceutical formulation.

Statistical Analysis

Table 8 presents the statistical comparison (21) of the results obtained by the proposed methods and the reported method (8). The calculated t- and F-values were less than the theoretical ones, indicating that there was no significant difference between them with respect to accuracy and precision.

Conclusions

The proposed procedures are simple and do not require sophisticated techniques. They are sensitive and selective and could be used for routine analysis of AML, VAL, and HCT in bulk powders and in their available dosage forms without prior separation.

References


(19) U.S. Pharmacopoeia Convention (2007) The U.S. Pharmacopoeia (USP 30) and National Formulary (NF 25), Rockville, MD

(20) ICHHT Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1), International Conference on Harmonization, Geneva, Switzerland