Simultaneous Determination of Amlodipine, Hydrochlorothiazide, and Valsartan in Pharmaceutical Products by a Combination of Full Spectrum Measurement and Kalman Filter Algorithm

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In this work, a new solution has been found for selecting the approximate initial value of concentration (by means of the classical least squares) and variance (calculated by the Horwitz equation) for the Kalman filter algorithm. With this solution, the Kalman method is less error-prone and has a better repeatability than the least squares method when using the full spectrum. A protocol for simultaneous determination of amlodipine (AML), hydrochlorothiazide (HYD), and valsartan (VAL) in pharmaceutical products was developed based on the spectrophotometry-chemometric method using full spectrum measurement in combination with the Kalman filter algorithm written in Microsoft Excel 2016 and Visual Basic for Applications (VBA). The method was validated on the Exforge HCT tablets with good repeatability (RSD) (varied from 2.2% to 2.3% (n = 3) for all the three studied compounds) and good recovery (90.0%–94.0% for AML, 90.3%–94.5% for HYD, and 98.5%–103.1% for VAL (n = 3)). The results were in good agreement with the measurements achieved from the high-performance liquid chromatography (HPLC) method.

1. Introduction

In the development history of analytical chemistry, chemometrics has been used and applied successfully for identification and quantification of a mixture in different matrices, particularly in pharmaceutical samples. The commonly used chemometric methods could be named as partial least squares (PLS), classical least squares (CLS), principal component regression (PCR), artificial neural networks (ANNs), derivative spectra, and Kalman filter.

Generally, each method has its own advantages and disadvantages. CLS [1] uses all the spectrum data to form a system of m equations and n unknowns (m > n), in which the transformation matrix basing on least squares principle yields acceptable results in terms of relative errors. However, if the input data contain many noises (or errors) and/or there are reactions between analytical compounds causing photometric effects to the absorbance, then this CLS method fails to reduce the noises and consequently large number of errors is expected. Meanwhile, the ANN method [1] requires long time for network formation and a number of algorithms. Therefore, to build a suitable model, each potential model developed needs to be tested until the optimal network structure is defined.

The derivative spectrum method [1, 2], in the meantime, is inapplicable when the sample contains components of overlapping or similar absorbance spectrum since it is difficult to find the suitable wavelength and their derivative spectrum still has maximum absorbance overlapping.
The Kalman filter method is able to remove maximally the noise effects, therefore, limiting the measurement errors. This method in combination with spectrophotometry was applied to analyze mixtures of metal ions and components in multicomponent pharmaceutical dosages [3, 4]. In the previous works [5–8], equations for calculating employing Kalman filter algorithm were given. However, the method for selection of initial values including initial concentrations and initial variances for the Kalman filter still has not been dealt with. Based on the pretests (data not shown), it could be addressed that the calculated results strongly depend on the method to select the initial guesses for the Kalman filter. If the selected initial guesses of concentration (\( C_{\text{init}}(0) \)) and variance (\( P_{\text{init}}(0) \)) are not suitable or selected concentration is too different from the real value, then it would cause divergent result or big errors. In general, until now, there has not been a comprehensive solution for selection of initial guesses for the Kalman filter.

From the mentioned gaps above, the three main objectives of this study are (i) to find a suitable method to define the initial guesses for the Kalman filter algorithm, (ii) to build a software based on the Kalman filter algorithm and spectrophotometry (called the Kalman method from now on) to determine simultaneously components in a mixture, (iii) to apply the developed Kalman method for simultaneous determination of the compounds with overlapping absorbance spectra in a multicomponent pharmaceutical product, specifically in this study were amlodipine, hydrochlorothiazide, and valsartan in Exforge HCT tablet.

2. Materials and Methods

2.1. Chemicals and Reagents. All the three target compounds AML (100.43%), VAL (98.38%), and HYD (99.55%) were purchased from Vietnamese Drug Quality Control Center. Stock solutions of 1000 \( \mu \text{g/mL} \) and working solutions of 50 \( \mu \text{g/mL} \), 25 \( \mu \text{g/mL} \), 10 \( \mu \text{g/mL} \), and 5 \( \mu \text{g/mL} \) of each compound were prepared in methanol (Merck, USA).

The commercial pharmaceutical product Exforge HCT tablet contains the labelled ingredients as follows: AML 10 mg, VAL 160 mg, and HYD 12.5 mg; batch number: BK917; manufactured date: 09/2016; expired date: 08/2018; registered number VN-19287-15; manufactured at Novartis Farmaceutica S.A. Ronda Santa Maria 158, 08210 Barberà del Vallès, Barcelona, Spain.

2.2. Sample Preparation. The drug sample was prepared following the method of Galande [9]: 20 tablets were balanced to calculate the average tablet weight (\( \overline{M} \)), which were then finely milled and homogenized. Take \( m \) grams, in accordance with one tablet weight (theoretically, mass of AML is 10 mg, of HYD is 12.5 mg, and of VAL is 160 mg), into a 250 mL glass bottle with cap, 50 mL of methanol was added, and the bottle was shaked well before applying ultrasonic extraction for 30 min. The solution was then filtered into a 100 mL volumetric flask and filled up with methanol. The obtained solution was diluted 100 times before measurement by using a V-630 UV/Vis Spectrometer JASCO (Japan).

Accordingly, an average mass of one tablet was \( \overline{M} = 4.1252 \text{ g} \).

Mass of an individual compound in a tablet was calculated as follows:

\[
\frac{C \cdot V \cdot K \cdot \overline{M}}{m \cdot 1000} \text{ (mg/tablet)},
\]

where \( \overline{M} \) is the average mass of one tablet (g), \( m \) is the mass of the weighted sample (g), \( C \) is the concentration of the target compound (\( \mu \text{g/mL} \)), \( V \) is the initial volume (100 mL), and \( K \) is the dilution factor (\( K = 100 \)).

Accordingly, when \( m = \overline{M} \), we have

\[
x = C \cdot 10 \text{ (mg/tablet)}. \tag{2}
\]

2.3. Kalman Filter Method. The Kalman filter is a linear parameter estimation technique. In analytical chemistry, it is used to estimate the concentrations of components in a mixture from the absorbance spectra. The initial state of concentration (at the first wavelength) is required. The next concentration state will be estimated based on the initial one. Basically, the model consists of two equations as follows:

The equation to describe the chemical system:

\[
C(k) = C(k-1) + \omega(k).
\]

And the other to describe the measurement process:

\[
A(k) = e(k)C(k) + \nu(k),
\]

where \( C(k) \) is a vector of state concentrations at point \( k \) (which is the wavelength), \( \omega(k) \) is the vector of noise contribution to the system model at point \( k \), \( A(k) \) is the measurement at point \( k \), \( e(k) \) is the state transition matrix, and \( \nu(k) \) is the corresponding measurement noise.

The Kalman filter algorithm applied in this study for multicomponent spectrophotometry analysis consists of the following equations:

(i) State (concentration) estimate extrapolation:

\[
C_{\text{pri}}(k) = C_{\text{est}}(k-1).
\]

(ii) Error covariance extrapolation:

\[
P_{\text{pri}}(k) = P_{\text{est}}(k-1).
\]

(iii) Kalman gain:

\[
K(k) = P_{\text{pri}}(k)e_T(k)(e(k)P_{\text{pri}}(k)e_T(k) + R(k))^{-1}.
\]

(iv) State estimate updation:

\[
C_{\text{est}}(k) = C_{\text{pri}}(k) + K(k)(A(k) - e(k)C_{\text{pri}}(k)).
\]

(v) Error covariance updation:

\[
P_{\text{est}}(k) = [IN - e(k)K(k)]P_{\text{pri}}(k).
\]

The above calculation steps are performed from the first wavelength to the last wavelength. Finally, the calculation program will produce the result: the concentration of each
constituent in the system and the covariance of the error. This variance is usually the smallest at the last wavelength [5–8].

2.4. Method Validation

2.4.1. Relative Error (RE %). The relative error was calculated as follows [10, 11]:

\[
RE(\%) = \frac{C - C_0}{C_0} \cdot 100, \tag{10}
\]

where \( C \) is the measured concentration (µg/mL) and \( C_0 \) is the known concentration (standard solution) (µg/mL).

2.4.2. Repeatability. Repeatability was assessed via the relative standard deviation (RSD) value [10, 11]:

\[
\text{RSD}(\%) = \frac{S \cdot 100}{X}, \tag{11}
\]

where \( S \) is the standard deviation and \( X \) is the mean concentration after \( n \) times of measurement (µg/mL).

For internal laboratory quality control, the method repeatability was approved if the obtained RSDs were lower than a half of the RSD value calculated from the Horwitz function [9–11]:

\[
\text{RSD}_{\text{Horwitz}} = 2^{(1 - 0.5 \cdot \log C)}. \tag{12}
\]

2.4.3. Method Recovery. Method recovery was calculated based on the spiked samples as follows [10, 11]:

\[
\text{Rev}(\%) = \frac{C_T - C_a}{a} \cdot 100, \tag{13}
\]

where \( a \) is the spike concentration (µg/mL), \( C_T \) is the measured concentration after spiking (µg/mL), and \( C_a \) is the measured concentration before spiking (µg/mL).

2.5. Analytical Procedure. The analytical procedure is shown in Figure 1.

The three main analytical steps are shown as follows:

Step 1. Prepare the standard solutions or samples

Step 2. Measure molecular absorbance spectra, data were recorded as .txt or .dat files

Step 3. Extract the files to the computer and run the developed Kalman-Excel program to calculate the specific concentration

3. Results

3.1. Absorbance Spectra of Standard Solutions. Absorbance spectra of four solutions, namely, AML 5 µg/mL, HYD 5 µg/mL, VAL 5 µg/mL, and mixture of AML 5 µg/mL, HYD 5 µg/mL, and VAL 5 µg/mL in methanol at the wavelength range 230–340 nm were scanned and are shown in Figure 2.

As shown in Figure 2, absorbance spectra of AML, HYD, and VAL in ethanol overlapped between 230 nm and 340 nm wavelength, causing difficulty in simultaneously determination of these compounds in mixture. This problem, however, could be solved smoothly using a combination of spectrophotometry and chemometric methods.

Within the wavelengths from 230 nm to 340 nm at 0.5 nm intervals, the measured absorbance spectrum of the standard mixture was almost fit with the theory spectrum (estimated from the additive property of absorbance); therefore, absorbance of mixture containing AML, HYD, and VAL had additive property. In other words, it was able to use full spectrum for simultaneous determination of AML, HYD, and VAL by using the combination of spectrophotometry and chemometric method.

3.2. Selection of Initial Guesses to Start the Kalman Filter. As mentioned above, the main challenge to use the Kalman filter is to choose a proper method to identify the initial guesses. A wrong selection would cause an improper calculation. In a mixture containing different substances, the initial guesses are the estimated concentrations in accordance with specific variances of individual substances. Based on the previous studies, there have been two solutions to select the initial guesses:

Group 1. Random selection of initial guesses, which means the values of concentration (C) could be randomly assigned, such as 0 or 0.5 µg/mL; and of variance (P) could be 1, etc. [5, 8].

Group 2. Assumption of initial guesses, which means either (i) C and P values are subjectively selected based on personal experience and the properties of the samples, (ii) some preliminary experiments are conducted to define the initial C and P values [1, 5], or (iii) Beer–Lambert’s law is applied at some selected wavelengths to calculate the initial C value (for individual substances in the mixture) by solving linear equation systems, while the variance P is calculated based on a specific guideline for statistical errors in analytical chemistry (i.e., applying Horwitz equation to calculate relative standard deviation (RSD), accordingly the standard deviation and variance at that concentration are defined) [5, 7, 10, 12].

In general, so far there have been no comprehensive method to select suitable initial guesses (C and P values) for the Kalman filter algorithm, raising a challenge for the analytical chemists who want to apply the Kalman filter in their studies. In this study, we investigated three different methods to select the initial C and P values, specifically based on the selection of group 1, group 2, and the proposed selection method of this study.

3.3. Random Selection of Initial Guesses. In this method, \( C_{\text{est}(0)} \) and \( P_{\text{est}(0)} \) values could be randomly selected [11]; therefore, this study chose 0.3 µg/mL for \( C_{\text{est}(0)} \) and 1 for \( P_{\text{est}(0)} \). The \( C_{\text{est}(0)} \) value of 0.3 µg/mL was delivered from the
common limit of detection (LOD) value of spectrophotometry of about 0.1 µg/mL, of which the limit of quantification would be around 0.3 µg/mL.

Apply the Kalman method for monospectral data and a mixture (AML, HYD, and VAL) of three substances (in the range of 220 nm–340 nm), and the results are shown in Table 1.

The results in Table 1 demonstrated that, in the cases of AML and HYD of mixture H1, when \( C_0 \) was close to the selected concentration (0.3 µg/mL), this method provided acceptable relative error (RE) values (20% for AML and −6% for HYD). Otherwise it brought big measurement errors with RE values fluctuated from 40% to 98% for all the target compounds in all the rest investigated mixtures. The cause of this result could be interpreted that the applied Kalman filter considered the random selected concentration of 0.3 µg/mL belonged to another distribution with a certain real value, which was different with this current distribution in accord with the real values of the studied compounds (1.00 to 9.00 µg/mL). In other words, when the initial concentration is too different from its real value, the Kalman filter using the random selection method for initial guesses will not result in convergence but divergence, causing unacceptable statistical errors.

In short, the initial guesses (\( C_{est(0)} \) and \( P_{est(0)} \)) selected in random is not suitable if the selected concentration is too different from the real value, which would cause divergent result or big errors.

### 3.4. Assumption of Initial Guesses

This assumption was applied in some previous studies [5, 7], in which a series of

| Mixture | H1 | H2 | H3 | H4 |
|---------|----|----|----|----|
| C₀ (µg/mL) | 0.250 | 0.500 | 1.000 | 5.000 |
| AML | C (µg/mL) | 0.300 | 0.300 | 0.300 | 0.304 |
| RE (%) | 20 | −40 | −70 | −94 |
| HYD | C (µg/mL) | 0.325 | 0.650 | 1.300 | 5.000 |
| RE (%) | −6 | −53 | −77 | −94 |
| VAL | C (µg/mL) | 0.301 | 0.300 | 0.300 | 0.299 |
| RE (%) | −93 | −97 | −98 | −94 |

\( C_0 \): concentration in the standard solution; C: calculated concentration.
preliminary experiments were conducted with different initial guesses \( (C_{\text{est}(0)} \text{ and } P_{\text{est}(0)}) \) to identify the suitable initial concentration and variance for the Kalman filter. Moreover, there were studies suggested automatically carrying out the preliminary experiments (employing self-written computer programs); however, the calculation speed was generally slow, which required some repeated calculations to select proper \( C_{\text{est}(0)} \) and \( P_{\text{est}(0)} \) values [1, 5].

In this study, two different solutions were tested for an assumption of \( C_{\text{est}(0)} \) and \( P_{\text{est}(0)} \) (in mixtures containing 2 or 3 compounds):

**Solution 1.** Based on the equation systems of 2 (or 3) unknowns (concentrations) at 2 (or 3) adjacent wavelengths (this is the equation showing the relationship between absorbance and concentration in a mixture with absorption coefficient \( \alpha \) (calculated from the individual spectrum of the studied compound)), concentration of each compound will be calculated which then used as \( C_{\text{est}(0)} \) for the Kalman filter. Meanwhile, the \( P_{\text{est}(0)} \) value was randomly selected, for instance by 1. The results are shown in Table 2.

Apply the Kalman method for monospectral data and a mixture of two substances (in the range of 220 nm–340 nm), and the results are shown in Tables 2 and 3.

**Solution 2.** Select 0.3 \( \mu \text{g/mL} \) as \( C_{\text{est}(0)} \) for each compound in a mixture. The initial variance \( P_{\text{est}(0)} \) was calculated based on the Horwitz equation, which resulted in a value of 0.005 in accordance with a concentration \( C = 0.3 \mu \text{g/mL} = 3 \cdot 10^{-7} \) [13]. The results are shown in Table 2.

Apply the Kalman method for monospectral data and a mixture of two substances (in the range of 220 nm–340 nm), and the results are shown in Tables 2 and 3.

**Solution 1** provided big relative errors (RE % fluctuating from 14% to 82%, except for the case of AML in mixtures H2 and H4, Table 2). This **Solution 1** required complicated steps and depended on the two initial wavelengths selected to solve the equation system, which helped us to define the initial concentration. On the contrary, the application on real samples was strongly affected by the matrix, causing big errors.

In **Solution 2**, although a different approach was applied to define the initial variance (employing the Horwitz equation), the Kalman filter in this stage brought big errors (RE % varying from 7% to 97%).

In short, the above two tested methods to identify initial guesses by either random selection or assumption failed to bring an acceptable result (judged via relative errors). The only exception resulted when the initial concentration selected was close to the real value of concentration in the mixture. It was, therefore, necessary to find a new approach in identifying the initial concentration approximately to the actual value of the analyzed compound in the mixture.

### 3.5. Selection of Approximate Initial Guesses

Based on the literature review and laboratory experiments for a mixture containing 3 substances, a new method to select initial guesses was proposed, specifically:

1. **Apply the classical least squares method to solve the system of \( m \) linear equations with \( n \) unknowns (in which \( m \) was the number of wavelengths selected to scan the absorption spectrum of the mixture and \( n \) was the number of compounds in the mixture), followed by the Gaussian elimination method to solve a system of \( n \) linear equations with \( n \) unknowns for concentrations of compounds in the mixture.** The resulting concentrations of the compounds in the mixture were selected as initial concentrations.

2. **Apply the Horwitz equation to estimate the variance corresponding to the concentration of each compound in the mixture, which was considered as the initial variance \( P_{\text{est}(0)} \).** The calculation of initial variance \( P_{\text{est}(0)} \) corresponding to the initial concentration \( C_{\text{est}(0)} \) was as follows:

From equation (11),

\[
\text{RSD}_{\text{Horwitz}} \% = \frac{S}{C_{\text{est}(0)}} \cdot 100, \tag{14}
\]

\[
\Rightarrow S = \left[ \frac{\text{RSD}_{\text{Horwitz}} \cdot C_{\text{est}(0)}}{100} \right]. \tag{15}
\]
In which \( \text{RSD}_{\text{Horwitz}} \) was calculated as (12) and \( C_{\text{est}(0)} \) represented by fractions.

\[
\text{RSD}_{\text{Horwitz}}(\%) = 2^{(1-0.5\log C_{\text{est}(0)})}, \tag{16}
\]

Finally,

\[
S^2 = P_{\text{est}(0)}, \tag{17}
\]

Noticeably, when conducting repeated measures in a laboratory, if the repeatability (represented via RSD value) smaller or equal to a half of the theory RSD value delivered from the Horwitz equation \( \text{RSD} \leq 1/2 \text{RSD}_{\text{Horwitz}} \), then the method repeatability is acceptable [10]. Accordingly, the initial variance corresponding to the initial concentration \( C_{\text{est}(0)} \) would be a quarter of the \( P_{\text{est}(0)} \) value calculated from (13).

The overall experiment was conducted as follows: apply the CLS method to define \( C_{\text{est}(0)} \) values of AML, HYD, and VAL from the spectrum data—which were the absorbance values of individual compound and mixture solutions measured from 230 nm–340 nm wavelength. From the result of \( C_{\text{est}(0)} \) calculate the specific \( P_{\text{est}(0)} \) values of AML, HYD, and VAL. Finally, provide \( C_{\text{est}(0)} \) and \( P_{\text{est}(0)} \) to the designed computer program using the Kalman filter to get the results, as shown in Table 4.

The output data provided the concentrations close to the actual values. In other words, this developed method showed good results with small relative errors (RE \( \leq 4 \% \)), compared to the two previous methods—using random selection and assumption of initial guesses.

To ensure the applicability of the developed method using selection of approximate initial guesses to identify the initial guesses for the Kalman filter, it is necessary to validate the method in both standard solutions and real samples (pharmaceutical products).

### 3.6. Relative Errors of the Method

To check the performance of the method, four different mixtures (AML/HYD/VAL, \( \mu \text{g}/\text{mL} \)) of the three target compounds were prepared, including H1: 0.250/0.325/4.000; H2: 0.50/0.65/8.00; H3: 1.00/1.30/16.00; and H4: 5.00/5.00/5.00. The absorbance spectrum of the prepared mixtures was scanned from 230 nm to 340 nm wavelength. The Kalman-Excel program was then applied to calculate the concentration of each compound in order to identify RE values. The results are shown in Table 4.

Under different mixtures, the obtained RE values of AML measurements varied from 0.4 to 2.2%, of HYD from −1.5 to 1.3%, and of VAL from −3.6 to 0.8% (Table 5). These low RE values demonstrated the high similarity of the standard concentrations and the measured concentrations of the three studied compounds. In other words, the developed method has good trueness.

### 3.7. Method Repeatability for Laboratory-Prepared Samples

The similar experiment as described in Section 3.1 was conducted, in which each mixture was prepared and analyzed in triplicate. Method repeatability was assessed based on the comparison between the calculated RSD values and 1/2 \( \text{RSD}_{\text{Horwitz}} \). The results are shown in Table 6.

The results in Table 6 show that the RSD value for both AML and VAL measurements \( (n = 3) \) in 4 different mixtures was 0.4%, for HYD fluctuated from 0.3 to 0.5%. For internal laboratory quality control, the method repeatability was approved if the obtained RSD was lower than a half of the RSD value calculated from the Horwitz function [10]. Accordingly, this developed method has good repeatability.

### 3.8. Method Repeatability and Trueness for Pharmaceutical Samples

#### 3.8.1. Repeatability

The repeatability of the procedure to simultaneously determine AML, HYD, and VAL in the pharmaceutical sample (Exforge HCT tablets, \( n = 3 \)) is described in Section 2.4.2. Accordingly, the final masses of AML, HYD, and VAL per tablet after preparation were 10.00 mg, 12.50 mg, and 160.00 mg, which were considered as the expected contents. The developed Kalman-Excel program was used for calculating the concentrations of each target compounds. The results of content of target substances and repeatability are shown in Table 7.

Average mass of AML per tablet was 9.61 mg with the RSD value of 2.3% (the expected mass was 10 mg, \( \text{RSD}_{\text{Horwitz}} \) was 8%), of HYD was 11.63 mg with RSD value of 2.2% (the expected mass was 12.50 mg, \( \text{RSD}_{\text{Horwitz}} \) was 5.5%), and of VAL was 169.17 mg with RSD value of 2.2% (the expected mass was 160.00 mg, \( \text{RSD}_{\text{Horwitz}} \) was 5.3%). Apparently, all of the RSD values were lower then the corresponding \( \text{RSD}_{\text{Horwitz}} \) implying that the developed method was successfully applied to analyze simultaneously AML, HYD, and VAL in pharmaceutical product.

#### 3.8.2. Trueness

To assess the trueness, in this study, two different approaches were considered, which were (i) define method recovery and (ii) compare the results with the ones analyzed by a validated method: high-performance liquid chromatography (HPLC).
Three replicate samples (B1, B2, and B3) were prepared from Exforge HCT tablets. Different spiked levels of AML, HYD, and VAL were added (Table 4). The absorbance spectrum was scanned from 230 to 340 nm wavelength (0.5 nm step), followed by data computing and concentration calculating by the developed Kalman-Excel program. The results of method recovery are shown in Table 8.

The average recoveries of AML, HYD, and VAL were 92.9%, 93.3%, and 101.8%, respectively (Table 8). According to AOAC, for the measured concentration from 1ppm to 10ppm, the required recovery should fluctuate from 80% to 110% [1]. Based on this, the developed method performed good trueness for all the analyzed substances, suggesting that the excipients caused almost no effects to the analytical results.

Table 6: Method repeatability.

| Mixture | AML | HYD | VAL |
|---------|-----|-----|-----|
| H1      |     |     |     |
| Rep 1   | 0.253 | 0.320 | 3.990 |
| Rep 2   | 0.252 | 0.320 | 3.980 |
| Rep 3   | 0.254 | 0.321 | 4.010 |
| Cmean   | 0.253 | 0.320 | 3.993 |
| RSDmean | 0.4   | 0.3   | 0.4 |
| 1/2 RSDHorwitz (%) | 9.9 | 6.5 | 5.9 |

Table 7: Measured concentrations of AML, HYD, and VAL in Exforge HCT tablets employing the developed Kalman-Excel program.

| Replicate samples | AML (mg/mL) | HYD (mg/mL) | VAL (mg/mL) |
|-------------------|-------------|-------------|-------------|
| B1                | 0.965       | 1.168       | 16.997      |
| B2                | 0.980       | 1.186       | 17.249      |
| B3                | 0.937       | 1.134       | 16.506      |
| Cmean (mg/mL)     | 0.961       | 1.163       | 16.917      |
| S                  | 0.022       | 0.026       | 0.378       |
| RSD %             | 2.3         | 2.2         | 2.2         |
| 1/2 RSDHorwitz (%)| 8           | 5.5         | 5.3         |

Method recovery. Three replicate samples (B1, B2, and B3) were prepared from Exforge HCT tablets. Different spiked levels of AML, HYD, and VAL were added (Table 4). The absorbance spectrum was scanned from 230 to 340 nm wavelength (0.5 nm step), followed by data computing and concentration calculating by the developed Kalman-Excel program. The results of method recovery are shown in Table 8.

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Method trueness assessment. Exforge HCT tablets were sent to the Drug, Cosmetic and Food Quality control Center of Thua Thien Hue Province for analysis using the HPLC method. AML was analyzed following Vietnam Pharmacopoeia IV guideline, USP38 was used for VAL and HYD analysis.

Student’s t-test [13, 14] was used to compare the analytical results of the two methods. The result of comparing mean values of two methods is shown in Table 9.

Table 9 shows that all the texp values were smaller than the corresponding t (0.05; f) of the three target compounds, demonstrating that the analytical results obtained from the developed Kalman–Excel method were in agreement with the ones obtained from HPLC measurements.
4. Conclusions

In this work, a new solution has been found for the first time, selecting the approximate initial value of the concentration (by means of the classical least squares) and variance (calculated by using the Horwitz equation) for the Kalman filter algorithm. This new solution allows convenient application of the chemometric-spectrophotometric method using the Kalman filter algorithm (Kalman method) to simultaneously determine two or three substances in their mixture with an UV-Vis absorption spectrophotometer. The Kalman method is less error-prone and has a better repeatability than the least squares method when using the full spectrum.

A computer program that uses the Visual Basic for Applications programming language written on the basis of Microsoft software Excel 2016 based on the Kalman filter algorithm has been written, which allows quick and convenient calculation when applied on practical testing of pharmaceutical products in laboratories.

First, the process of simultaneous analysis of three active ingredients, i.e., amlodipine, hydrochlorothiazide, and valsartan, was established in multicomponent pharmaceutical formulation by the Kalman method using full spectrum without any separation technique. The process exhibited good repeatability and trueness for all the three analyzed compounds with RSD <2.5% (n = 3), recovery varied from 93 to 102%, and the received analytical results were identical with ones of HPLC method. The process was not only simple to implement but also reduced the cost of analysis compared to the standard method of high-performance liquid chromatography (HPLC).

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table 8: Method recovery for Exforge HCT tablet samples.

| Replicate samples | AML Cspike (µg/mL) | AML Cmea (µg/mL) | REV (%) | HYD Cspike (µg/mL) | HYD Cmea (µg/mL) | REV (%) | VAL Cspike (µg/mL) | VAL Cmea (µg/mL) | REV (%) |
|-------------------|-------------------|-------------------|---------|-------------------|-------------------|---------|-------------------|-------------------|---------|
| B1                | 0                 | 0.965             | 0       | 1.168             | 0                 | 16.997  | 0                 | 1.168             | 0       |
|                   | 0.25              | 1.200             | 94.0    | 1.451             | 94.3              | 4.0     | 21.112            | 102.9             |
|                   | 0.50              | 1.415             | 90.0    | 1.710             | 90.3              | 8.0     | 24.876            | 98.5              |
| B2                | 0                 | 0.980             | 0       | 1.186             | 0                 | 17.249  | 0                 | 1.186             | 0       |
|                   | 0.25              | 1.214             | 93.6    | 1.469             | 94.3              | 4.0     | 21.363            | 102.9             |
|                   | 0.50              | 1.450             | 94.0    | 1.753             | 94.5              | 8.0     | 25.497            | 103.1             |
| B3                | 0                 | 0.937             | 0       | 1.134             | 0                 | 16.506  | 0                 | 1.134             | 0       |
|                   | 0.25              | 1.171             | 93.6    | 1.416             | 94.0              | 4.0     | 20.603            | 102.4             |
|                   | 0.50              | 1.397             | 92.0    | 1.689             | 92.5              | 8.0     | 24.567            | 100.8             |
| REVmean (%)       | 92.9              | 93.3              | 101.8   |

Table 9: Comparison of AML, HYD, and VAL contents in Exforge HCT tablet measured (n = 3) by using the developed method and HPLC method.

| AML | HYD | VAL |
|-----|-----|-----|
| Kalman | HPLC | Kalman | HPLC | Kalman | HPLC |
| Xmean (mg/tablet, n = 3) | 9.61 | 9.51 | 11.66 | 11.63 | 169.17 | 167.66 |
| S (mg/tablet, n = 3) | 0.22 | 0.09 | 0.26 | 0.19 | 3.78 | 1.24 |
| Fexp/F(0.05; 2; 2) | 5.30/19 | 1.9/19 | 9.32/19 |
| Sp | 0.16 | 0.34 | 0.10 |
| texp/t(0.05; f) | 0.53/4.3 | 0.06/4.3 | 0.71/4.30 |
| P | 0.65 | 0.96 | 0.55 |

Kalman: the Kalman-Excel program combined with spectrophotometry method; Fexp: the experimental variance of the corresponding method; F(0.05; 2; 2): F distribution at alpha 0.05 and the respective degrees of freedom of numerator and denominator; Sp: pooled variance; texp: experimental t value; t(0.05; f = 4): t value at alpha 0.05 and 4 degrees of freedom.
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