Article

Simultaneous Determination of Paracetamol, Ibuprofen, and Caffeine in Tablets by Molecular Absorption Spectroscopy Combined with Classical Least Square Method

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Abstract: In this paper, the classical least square (CLS) method with molecular absorption spectrophotometric measurement was used to determine simultaneously of paracetamol (PAR), ibuprofen (IBU), and caffeine (CAF) in tablets. The absorbance spectra of the standard solutions and samples were measured over a wavelength from 220 to 300 nm with a 0.5 nm step. The concentration of PAR, IBU and CAF in the sample solutions were calculated by using a program called CLS-Excel written in Microsoft Excel 2016 and Visual Basic for Applications (VBA). The method and CLS-Excel program were tested on mixed standard laboratory samples with different PAR, IBU, and CAF concentration ratios, and they showed small errors and satisfying repeatability. An analytical procedure for tablets containing PAR, IBU, and CAF was developed. The reliability of the procedure was proved via the recovery and repeatability of the analysis results with an actual tablet sample and comparing the mean contents of active substances in the tablets obtained from the analytical procedure with the HPLC method. The procedure is simple with a reduced cost compared with the HPLC standard method.

Keywords: paracetamol; ibuprofen; caffeine; classical least-square; simultaneous; spectroscopy

1. Introduction

Paracetamol (PAR), ibuprofen (IBU), and caffeine (CAF) are the main active ingredients widely used in multi-component pharmaceuticals. PAR is a common pain reliever and fever reducer. IBU is a non-steroidal anti-inflammatory drug with good analgesic and antipyretic effects. CAF is a methylated xanthine that stimulates the central nervous system, reduces feelings of fatigue and drowsiness, increases brain excitement and sensory perception, thereby helping humans to work more effectively. The combination of these ingredients in tablets enhances the healing effect.

The quality control of multi-component pharmaceutical products requires fast and reliable analytical techniques. The UV-Vis spectroscopy method is commonly used in laboratories due to its simplicity and low equipment cost. However, the quantitative analysis of pharmaceutical products containing many components having overlap spectra is often difficult. To analyze them by conventional UV-Vis method, we must often extract specific substance or mask substances which interfere in analytical procedure. So the procedure becomes complicated, consuming much time, chemicals, solvent and especially reliability is not good. Currently, numerous UV-Vis spectroscopy methods combined with chemometrics were developed to analyze simultaneously substances with overlapping absorption spectra. These methods often use the entire spectrum data and computer programs to calculate, eliminate measurement errors, and statistically assess a large amount of data to give reliable and useful information. In particular, they allow to calculate the concentrations of substances in multi-component solutions with high accuracy without separation or masking. This advantage enables researchers to design simple, low-cost,

short analytical procedures with high reliability. The UV-Vis molecular absorption spectrophotometric method coupled with chemometrics used for simultaneous determination of substances in multi-component pharmaceuticals include the classical least square (CLS) [1,2], partial least square (PLS) [2-4], principal component regression (PCR) [2,4], artificial neural network (ANN) [3,4], derivative [5-7], Kalman filter [8] methods, etc. As we know, absorption spectra of PAR, IBU, and CAF overlap much in ultraviolet region. For the determination of PAR, IBU and CAF in multi-component drugs, there have been various methods, such as standard methods [9,10], spectroscopy [3,5,6,7], chromatography [11]. To the best of our knowledge, there is no published paper concerning to using CLS method with full spectrum to simultaneously determine PAR, IBU, and CAF in drugs. So, in this paper, we apply CLS method for the full spectrum to simultaneously determine PAR, IBU, and CAF in drugs using a self-written program called CLS-Excel written on Microsoft Excel 2016 and Visual Basic for Applications (VBA).

2. Experimental

2.1. Apparatus and chemicals

2.1.1. Apparatus

A Cary 60 UV-Vis spectrophotometer was used to rapid spectral scanning in a wavelength range of 190–990 nm, connected to a computer with Cary WinUV software for storing spectral data as an excel spreadsheet. Other equipment and instrument include a Precisa XB 2204 analytical balance with an accuracy of 0.0001 g; a Quartz Double Distiller, micropipettes 100 μL, 1000 μL of HTL, pipettes, volumetric flasks, beakers, triangular flasks with sandblasted stoppers, glass rods, filter papers, glass funnels, etc.

2.1.2. Chemicals

Paracetamol 99.9%, ibuprofen 100.1%, and caffeine 99.9% conforming with Vietnamese pharmaceutical standards were supplied from the Central Institute for Drug Testing, Vietnam.

Ibuparavic, as a drug sample, contains paracetamol (300 mg/tablet), ibuprofen (200 mg/tablet), and caffeine (20 mg/tablet) was purchased from Thanh Nam Pharmaceutical Manufacturing and Trading Co., Ltd., Vietnam. The production batch number is 601119, produced on January 11, 2019 and expired by January 10, 2022. A box has 10 blisters with 10 hard capsules each, and the registration number is GC 318-19.

Distilled water and methanol (Merck) are also used.

Paracetamol, ibuprofen, and caffeine standard solutions

First, stock solutions with a 50 μg/mL concentration were prepared as follows: precisely 12.5 mg of each preparation was placed into a 250 mL volumetric flask with methanol, appropriately shaken, and made up to the mark. Then, 50 mL of each solution was transferred to a 100 mL volumetric flask and made up to the mark with methanol to obtain a 25 μg/mL working solution. Finally, 10 mL of the working solution was placed into a 25 mL volumetric flask and made up to the mark with methanol to a 10 μg/mL PAR and IBU standard solution. For preparing a 5 μg/mL CAF standard solution, 5 mL of the working solution was used.

Mixed experimental solutions

The working solution of PAR, IBU, and CAF was mixed with different volume ratios. The standard and working solutions were used to verify the reliability of the method.

2.2. Analytical procedure

The theoretical basis of the classical least-squares method is as follows:

For multicomponent systems, the absorbance is cumulative. We use Beer's law for a system of n components and m wavelengths ($m > n$). Let $e_i = \varepsilon_i \times b$, $Y_i = A_i$, and $x_i = C_i$, where ε_i is the molecular absorptivity of the i-th component; C_i is the concentration of the i-th component in the mixture; A_i is the absorbance of the mixed solution measured at the i-th wavelength. A system of linear equations is obtained with m equations and n unknowns as follows:

$$\begin{cases} Y_1 = e_{11}x_1 + e_{12}x_2 + \ldots + e_{1i}x_i + \ldots + e_{1n}x_n \\ Y_2 = e_{21}x_1 + e_{22}x_2 + \ldots + e_{2i}x_i + \ldots + e_{2n}x \\ \vdots \\ Y_j = e_{j1}x_1 + e_{j2}x_2 + \ldots + e_{ji}x_i + \ldots + e_{jn}x_n \\ \vdots \\ Y_m = e_{m1}x_1 + e_{m2}x_2 + \ldots + e_{mi}x_i + \ldots + e_{mn}x_n \end{cases} \quad (2.1)$$

The molecular absorbance measured at the jth wavelength is y_j. This parameter is often erroneous, and it is different from the actual value Y_j by a value s_j, where s_j is the measurement residual:

$$s_j = y_j - Y_j \quad (2.2)$$

The function representing the total squared error S is

$$S = \sum_{j=1}^{m}(y_j - Y_j)^2 = \sum_{j=1}^{m}[y_j - (e_{j1}x_1 + e_{j2}x_2 + \cdots e_{ji}x_i + \cdots + e_{jn}x_n)]^2 \quad (2.3)$$

For S to be minimized, the derivative of S with respect to x_i must be 0. If we take the derivative of S with respect to x_1 and let the derivative equal 0, we get the following equation:

$$\frac{dS}{dx_1} = 2\sum_{j=1}^{m}\left[y_j - \left(e_{j1}x_1 + e_{j2}x_2 + \cdots e_{ji}x_i + \cdots + e_{jn}x_n\right)\right].\left(-e_{j1}\right) = 0$$

Transforming this equation, we get

$$\sum_{j=1}^{m}e_{j1}^2x_1 + \sum_{j=1}^{m}e_{j1}e_{j2}x_2 + \cdots + \sum_{j=1}^{m}e_{j1}e_{ji}x_i + \cdots + \sum_{j=1}^{m}e_{j1}e_{jn}x_n - \sum_{j=1}^{m}e_{j1}y_j = 0 \quad (2.4)$$

Similarly, we also take the derivative S with respect to the remaining x_i and let these derivatives equal 0. Combining this equation with Eq. (2.4), we get the following system of equations:

$$\begin{cases} x_1\sum_{j=1}^{m}e_{j1}^2 + x_2\sum_{j=1}^{m}e_{j1}e_{j2} + \cdots + x_i\sum_{j=1}^{m}e_{j1}e_{ji} + \cdots + x_n\sum_{j=1}^{m}e_{j1}e_{jn} - \sum_{j=1}^{m}e_{j1}y_j = 0 \\ x_1\sum_{j=1}^{m}e_{j1}e_{j2} + x_2\sum_{j=1}^{m}e_{j2}^2 + \cdots + x_i\sum_{j=1}^{m}e_{j2}e_{ji} + \cdots + x_n\sum_{j=1}^{m}e_{j2}e_{jn} - \sum_{j=1}^{m}e_{j2}y_j = 0 \\ x_1\sum_{j=1}^{m}e_{j1}e_{ji} + x_2\sum_{j=1}^{m}e_{j2}e_{ji} + \cdots + x_i\sum_{j=1}^{m}e_{ji}^2 + \cdots + x_n\sum_{j=1}^{m}e_{ji}e_{jn} - \sum_{j=1}^{m}e_{ji}y_j = 0 \\ x_1\sum_{j=1}^{m}e_{j1}e_{jn} + x_2\sum_{j=1}^{m}e_{j2}e_{jn} + \cdots + x_i\sum_{j=1}^{m}e_{ji}e_{jn} + \cdots + x_n\sum_{j=1}^{m}e_{jn}^2 - \sum_{j=1}^{m}e_{jn}y_j = 0 \end{cases} \quad (2.5)$$

Let

$$a_{ki} = \sum_{j=1}^{m} e_{ji}\, e_{jk};\ b_k = \sum_{j=1}^{m} e_{jk}\, y_j \tag{2.6}$$

Where: $i = \overline{1..n};\ k = \overline{1..n}$

The system of equations can be summarized as follows:

$$\begin{cases} a_{11}.x_1 + a_{12}.x_2 + \ldots + a_{1i}x_i + \ldots + a_{1n}.x_n = b_1 \\ a_{21}.x_1 + a_{22}.x_2 + \ldots + a_{2i}x_i + \ldots + a_{2n}.x_n = b_2 \\ \vdots \\ a_{k1}.x_1 + a_{k2}.x_2 + \ldots + a_{ki}x_i + \ldots + a_{kn}.x_n = b_k \\ \vdots \\ a_{n1}.x_1 + a_{n2}.x_2 + \ldots + a_{ni}x_i + \ldots + a_{nn}.x_n = b_n \end{cases} \tag{2.7}$$

The values of a_{ki} and b_k in the system of equations (2.7) are calculated from the initial experimental values of e_{ji} by using equation (2.6). The system of equations (2.7) is a system of linear equations consisting of n equations with n unknowns. Solving this system of equations with the Gaussian reduction method, we have the concentration of the components x_i. The concentration of the components in the sample solution was calculated by using the CLS-Excel program.

The advantage of this method is that it uses all spectral data to create a system of linear equations with more equations than unknowns. Then, by transforming this system of equations with the least-squares technique, we obtain a system of an equal number of equations and the unknowns. As a result, the error becomes minimal and thus enhancing the accuracy. The concentration of the substances in the sample solution is determined rapidly, thanks to the program. The method can be applied to the substances in the mixtures with the components' complex absorption spectra overlapping.

The steps for measuring and calculating the concentration of substances are as follows:

- Preparing standard solutions of each component to be determined and the sample solutions containing their mixtures.
- Scanning the spectrum of the solutions at an appropriate wavelength range to obtain csv files in the form of an excel spreadsheet.
- Running the CLS-Excel program for the data from the excel files to calculate the concentration of components in the mixed solution and their relative error.

2.3. Statistical parameters

2.3.1. Relative error

The relative error between the determined concentration and the preparation concentration (RE%) was calculated according to Eq. (2.8)

$$RE(\%) = \frac{(C - C_0).100}{C_0} \tag{2.8}$$

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

2.3.2. Repeatability

The repeatability was assessed by using the relative standard deviation value RSD%:

$$RSD(\%) = \frac{S.100}{C_{mean}} \tag{2.9}$$

with S is the standard deviation, and C_{mean} is the mean concentration after n measurements (μg/mL). For in-laboratory quality control, method repeatability is satisfactory when the RSD% values obtained are less than 1/2$RSD_{Horwitz}$ [12, 13]

$$RSD_{Horwitz} = 2^{(1-0.5\times \lg C)} \tag{2.10}$$

where C is the concentration expressed as a power. (for example, C = 5 μg/mL = 5 ×10^{-6})

2.3.3. Accuracy

The method's accuracy was determined from the recovery or comparing the mean concentration value with that of the HPLC standard method.

a. Recovery

The recovery of the method was calculated, based on the standard addition according to Eq. (2.11)

$$Rev(\%) = \frac{(C_2 - C_1).100}{C_{\text{add}}} \tag{2.11}$$

with C_2 (µg/mL) is the determined concentration of the sample solution after standard addition; C_1 (µg/mL) is the determined concentration of the sample solution before standard addition; and C_{add} (µg/mL) is the standard addition concentration [14].

b. Comparison of the proposed method with the standard method

According to [15], to determine the method's accuracy, we analyze the same sample repeatedly with the proposed method and the standard method. Then, we compare the two sample mean values by using the student test.

$$t_{\text{exp}} = \frac{\left|\overline{X}_{\text{A}} - \overline{X}_{\text{B}}\right|}{\sqrt{\left(\frac{s_{\text{A}}^2}{n_{\text{A}}}\right) + \left(\frac{s_{\text{B}}^2}{n_{\text{B}}}\right)}} \tag{2.12}$$

where t_{exp} is the experimental student value; $\overline{X}_A$ and $\overline{X}_B$ are the mean value of methods A and B; n_A and n_B are the number of repeat measurements of methods A and B; s_A^2, s_B^2 are the variance of the two methods.

If the variances are identical ($F_{exp} < F_{(\alpha, n_1-1, n_2-1)}$, the common variance s_{pool} and t_{exp} are calculated from formula (2.13)

$$t_{\text{exp}} = \frac{\left|\overline{X}_{\text{A}} - \overline{X}_{\text{B}}\right|}{s_{\text{pool}}\sqrt{1/n_{\text{A}} + 1/n_{\text{B}}}} \tag{2.13}$$

In which s_{pool} is calculated from formula (2.14)

$$s_{\text{pool}} = \sqrt{\frac{(n_{\text{A}} - 1)s_{\text{A}}^2 + (n_{\text{B}} - 1)s_{\text{B}}^2}{n_{\text{A}} + n_{\text{B}} - 2}} \tag{2.14}$$

where $n_A + n_B - 2 = \nu$, and ν is the number of degrees of common freedom to the two methods.

If the variances are not identical, the common number of degrees of freedom is calculated according to formula (2.15) with the value ν rounded:

$$\nu = \frac{\left[(s_{\text{A}}^2/n_{\text{A}}) + (s_{\text{B}}^2/n_{\text{B}})\right]^2}{\left[\frac{(s_{\text{A}}^2/n_{\text{A}})^2}{n_{\text{A}} + 1}\right] + \left[\frac{(s_{\text{B}}^2/n_{\text{B}})^2}{n_{\text{B}} + 1}\right]} - 2 \tag{2.15}$$

Finally, we compare the t_{exp} value with the theoretical student value $t(\alpha, \nu)$, where α is the significance level (usually taken as 0.05), and ν is the degrees of freedom determined above. If $t_{exp} < t(\alpha,\nu)$, the mean values of the two methods are not significantly different.

3. Results and Discussion

3.1. Accuracy and repeatability of analytical method on laboratory samples

From the working standards of PAR 25 µg/mL, IBU 25 µg/mL and CAF 25 µg/mL, prepare the solutions in a 25 mL volumetric flasks: The standard solutions of PAR, IBU, and CAF and their mixtures were prepared as described in section 2.1.2. The standard

solutions and the mixtures were measured three times. The solutions were spectroscopically scanned in the range of 220–300 nm with a 0.5 nm intervals. The relative error between the determined concentration and the preparation concentration of PAR, IBU, and CAF in the mixed solutions was calculated according to the CLS-Excel program, and the corresponding relative standard deviation (RSD%) of the analytical results was also calculated. The absorption spectra of the standard solutions and laboratory mixture solutions are illustrated in Figure 1. The concentrations of PAR, IBU, and CAF in the mixtures and statistic data are presented in Table 1.

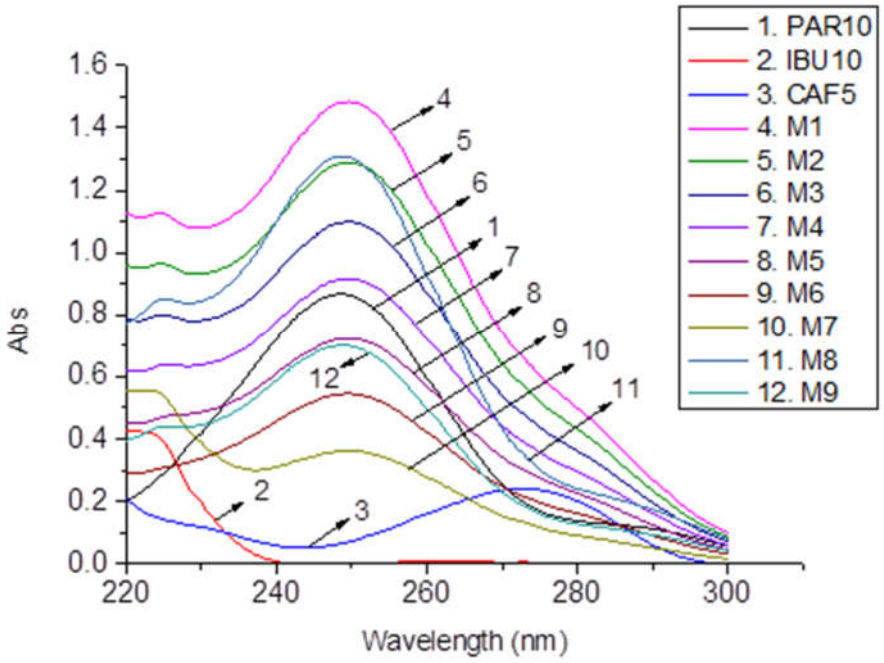

Figure 1. UV absorption spectra of standard solutions and laboratory mixture solutions with different concentration ratios (Standard solution (μg/mL): PAR 10, IBU 10, CAF 5; PAR/IBU/CAF mixed solution (μg/mL): M1 (16:12:7); M2 (14:10:6); M3 (12:8:5); M4 (10:6:4); M5 (8:4:3); M6 (6:2:2); M7 (4:10:1); M8 (15 :10:1); M9 (8:5:0.5)).

Table 1. Concentration of PAR, IBU, and CAF in the mixture and their RE and statistics *.

Sample	Conc. ratio (μg/mL) PAR/IBU/CAF	Run order	PAR			IBU			CAF		
			C_{PAR} (μg/mL)	RE (%)	Statistics	C_{IBU} (μg/mL)	RE (%)	Statistics	C_{CAF} (μg/mL)	RE (%)	Statistics
M1	16:12:7	1 2 3	16.091 16.179 16.161	0.57 1.12 0.82	C_{mean} = 16.144 RSD (%) = 0.288 1/2RSD$_H$ = 5.270 RE$_{mean}$ (%) = 0.84	11.858 11.892 11.885	–1.18 –0.93 –0.96	C_{mean} = 11.878 RSD (%) = 0.151 1/2RSD$_H$ = 5.504 RE$_{mean}$ (%) = – 1.02	6.989 6.989 7.000	–0.16 –0.16 0.00	C_{mean} = 6.993 RSD (%) = 0.091 1/2RSD$_H$ = 5.969 RE$_{mean}$ (%) = –0.11
M2	14:10:6	1 2 3	13.992 14.059 14.013	–0.06 0.42 0.09	C_{mean} = 14.021 RSD (%) = 0.244 1/2RSD$_H$ = 5.378 RE$_{mean}$ (%) = 0.15	10.004 9.981 10.002	0.04 –0.19 0.02	C_{mean} = 9.996 RSD (%) = 0.127 1/2RSD$_H$ = 5.657 RE$_{mean}$ (%) = – 0.07	5.991 5.980 5.990	–0.15 –0.33 –0.17	C_{mean} = 5.987 RSD (%) = 0.102 1/2RSD$_H$ = 6.109 RE$_{mean}$ (%) = –0.22
M3	12:8:5	1 2 3	11.943 12.008 11.964	–0.47 0.07 –0.30	C_{mean} = 11.992 RSD (%) = 0.277 1/2RSD$_H$ = 5.504 RE$_{mean}$ (%) = –0.23	8.023 7.996 7.989	0.29 –0.05 –0.14	C_{mean} = 8.003 RSD (%) = 0.224 1/2RSD$_H$ = 5.850 RE$_{mean}$ (%) = 0.03	4.957 4.958 4.974	–0.86 –0.84 –0.52	C_{mean} = 4.963 RSD (%)= 0.192 1/2RSD$_H$ = 6.279 RE$_{mean}$ (%) = –0.71
M4	10:6:4	1 2 3	9.979 10.035 10.004	–0.21 0.35 0.04	C_{mean} = 10.006 RSD (%) = 0.280 1/2RSD$_H$ = 5.657 RE$_{mean}$ (%) = 0.06	6.040 6.052 6.044	0.67 0.87 0.73	C_{mean} = 6.045 RSD (%) = 0.101 1/2RSD$_H$ = 6.109 RE$_{mean}$ (%) = 0.76	3.961 3.959 3.969	–0.98 –1.03 –0.78	C_{mean} = 3.963 RSD (%) = 0.134 1/2RSD$_H$ = 6.493 RE$_{mean}$ (%) = – 0.91

M5	8:4:3	1 2 3	7.899 7.937 7.909	–1.26 –0.79 –1.14	C_{mean} = 7.918 RSD (%) = 0.247 1/2RSD$_H$ = 5.850 RE$_{mean}$ (%) = –1.02	4.023 4.024 4.014	0.58 0.60 0.35	C_{mean} = 4.020 RSD (%) = 0.129 1/2RSD$_H$ = 6.493 RE$_{mean}$ (%) = 0.50	2.971 2.967 2.971	–0.97 –1.10 –0.97	C_{mean} = 2.970 RSD (%) = 0.089 1/2RSD$_H$ = 6.781 RE$_{mean}$ (%) = –0.93
M6	6:2:2	1 2 3	5.973 6.027 6.007	–0.45 0.45 0.12	C_{mean} = 6.002 RSD(%)= 0.455 1/2RSD$_H$ = 6.109 RE$_{mean}$ (%) = 0.04	1.998 2.002 1.992	–0.10 0.10 –0.40	C_{mean} = 1.997 RSD (%) = 0.252 1/2RSD$_H$ = 7.207 RE$_{mean}$ (%) = –0.13	2.003 1.999 2.003	0.15 –0.05 0.15	C_{mean} = 2.002 RSD (%) = 0.115 1/2RSD$_H$ = 7.207 RE$_{mean}$ (%) = 0.08
M7	4:10:1	1 2 3	4.009 4.012 4.010	0.23 0.30 0.25	C_{mean} = 4.010 RSD (%)= 0.038 1/2RSD$_H$ = 6.493 RE$_{mean}$ (%) = 0.26	10.053 9.989 10.035	0.53 –0.11 0.35	C_{mean} = 10.026 RSD(%) = 0.329 1/2RSD$_H$ = 5.657 RE$_{mean}$ (%) = 0.26	1.006 0.997 1.005	0.60 –0.30 0.50	C_{mean} = 1.003 RSD (%) = 0.492 1/2RSD$_H$ = 8.000 RE$_{mean}$ (%) = 0.27
M8	15:10:1	1 2 3	14.929 15.060 14.876	–0.47 0.40 –0.83	C_{mean} = 14.955 RSD (%)= 0.633 1/2RSD$_H$ = 5.322 RE$_{mean}$ (%) = –0.30	9.984 10.010 9.915	–0.16 0.10 –0.85	C_{mean} = 9.970 RSD (%) = 0.492 1/2RSD$_H$ = 5.657 RE$_{mean}$ (%) = 0.10	1.008 0.997 0.986	0.80 –0.30 –1.40	C_{mean} = 0.997 RSD (%) = 1.103 1/2RSD$_H$ = 8.000 RE$_{mean}$ (%) = –0.30
M9	8:5:0.5	1 2 3	8.004 7.981 8.044	0.05 –0.24 0.55	C_{mean} = 8.010 RSD (%)= 0.398 1/2RSD$_H$ = 5.849 RE$_{mean}$ (%) = 0.12	4.974 5.023 4.981	–0.52 0.46 –0.38	C_{mean} = 4.993 RSD (%) = 0.531 1/2RSD$_H$ = 6.280 RE$_{mean}$ (%) = –0.15	0.507 0.497 0.498	1.40 –0.60 –0.40	C_{mean} = 0.501 RSD (%) = 1.099 1/2RSD$_H$ = 8.877 RE$_{mean}$ (%) = 0.133

Note: The number of decimal places is taken to represent the calculation result.

Table 1 shows that at different concentration ratios, the errors of the concentrations of PAR, IBU, and CAF determined with the CLS method are from –1.40 to 1.12%, and the RSD% values are also small (RSD%$_{max}$ = 1.103) and less than 1/2RSD$_{Horwitz}$. Therefore, the method's accuracy and repeatability are satisfactory for the mixed laboratory solutions with different concentration ratios.

3.2. Actual sample analysis

3.2.1. Sample treatment

Twenty tablets from the same production batch were weighed, and the average weight was determined (M). Then, the tablets were ground to fine powder in an agate mortar. An amount of powder equal to 0.7 to 1.0 of the average tablet weight was placed into a 250 mL beaker containing 150 mL of methanol. The content of the beaker was sonicated for 30 min and quantitatively transfered to a 250 mL volumetric flask, made up to the mark with methanol and thoroughly mixed. The solution was then filtered through blue-band filter paper; the first 10 mL of the filtrate was discarded. Next, 10 mL of the filtrate was transferred to a 100 mL volumetric flask, made up to the mark with methanol, and thoroughly mixed (solution 1). Again, 10 mL of solution 1 was diluted to 100 mL with methanol to obtain solution 2. Finally, solution 2 was subjected to UV-Vis absorption determination, and the CLS-Excel program was used to calculate the concentration of the active substances. The concentration of the active ingredients from another 20 pills from the same batch was determined simultaneously with the HPLC method.

3.2.2. Calculation of the content of substances

The content of active ingredients in one tablet was determined from formula (3.1)

$$H \text{ (mg/tablet)} = C_m \times 100 \times (100/10) \times (250/10) \times (1/1000) \times (M/m) = 25 \times C_m \times (M/m) \quad (3.1)$$

where C_m (μg/mL) is the concentration of each active ingredient determined in the sample solution; m is the weight of the sample (mg); M is the average tablet weight (mg).

3.2.3. Simultaneous quantification of PAR, IBU, and CAF in drug samples

The ibuparavic tablets characteristics were described in Section 2.1.2 with the average tablet weight: 0.5265 g.

The samples were treated as described in Section 3.2.1 with precisely 526.5 mg of powder. The entire spectrum of the sample solution was scanned in the wavelength range 220–300 nm, with a 0.5 nm step. The concentration of PAR, IBU, and CAF in sample solutions was determined with the CLS-Excel program, and their content was calculated from formula (3.1).

The absorption spectra of standard solutions and sample solutions of ibuparavic are presented in Figure 2, and the content of the active ingredient is displayed in Table 2.

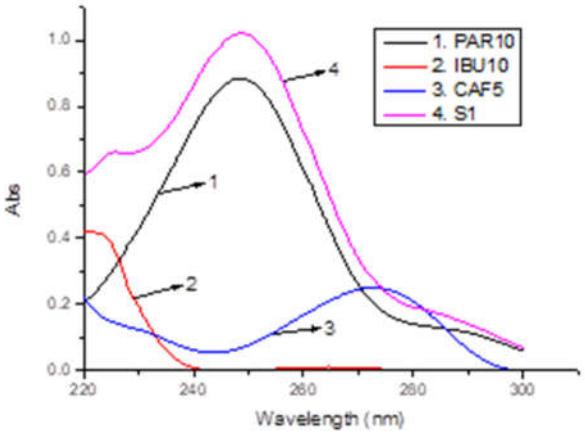

Figure 2. Absorption spectra of standard solutions and sample solutions of ibuparavic (standard solution 10 μg/mL: PAR (1), IBU (2); standard solution 5 μg/mL: CAF (3); S1: Ibuparavic drug sample solution (4)).

Table 2. Concentration of PAR, IBU, and CAF in sample solutions and their drug content in Ibuparavic tablets.

Sample	PAR		IBU		CAF	
	C_{PAR} (μg/mL)	content (mg/tablet)	C_{IBU} (μg/mL)	content (mg/tablet)	C_{CAF} (μg/mL)	content (mg/tablet)
S1	11.467	286.68	7.765	194.13	0.812	20.30
S2	11.503	287.58	7.749	193.73	0.795	19.88
S3	11.463	286.58	7.825	195.63	0.802	20.05
Mean	11.478	286.95	7.780	194.50	0.803	20.08
RSD%	0.192		0.515		1.052	
$1/2RSD_H$	5.541		5.875		8.269	
%H*	95.65		97.25		100.40	

Note: %H*: % Active inredient compared with labelled content.

The data show that the method is highly reproducible with all three components (RSD% < 1.2). The content of each substance in the ibuparavic tablets is as follows: PAR: 286.95 ± 1.37 mg, IBU: 194.50 ± 2.49 mg and CAF 20.08 ± 0.52 mg. This content is consistent with that reported on the label of these tablets and also agrees with the quality standards required by Vietnam's Ministry of Health: PAR 300 mg ± 5% (285–315 mg), IBU 200 mg ± 5% (190–210 mg), and CAF 20 mg ± 5 % (19–21 mg).

3.3. Accuracy verification

3.3.1. Recovery

Four batches of the sample powder equal to 0.7 times the average tablet weight were weighed. No standard addition was carried out for the first batch. The remaining three batches were added with PAR, IBU, and CAF with increasing amounts of standard. The samples were treated as described in Section 3.2.1. The spectra of standard solutions PAR 10 μg/mL, IBU 10 μg/mL, and CAF 5 μg/mL, the sample solution without standard (S0), and sample solutions after adding standards (S1, S2, S3). The concentration of PAR, IBU, and CAF in the standard and sample solutions was calculated with the CLS-Excel program. The spectra of the standard solutions and the analyte are shown in Figure 3. The concentration of the standard additions and that of the sample without and with the added standard are presented in Table 3. The Rev% recovery of the concentration of the standard addition solutions was calculated from Eq. (2.11).

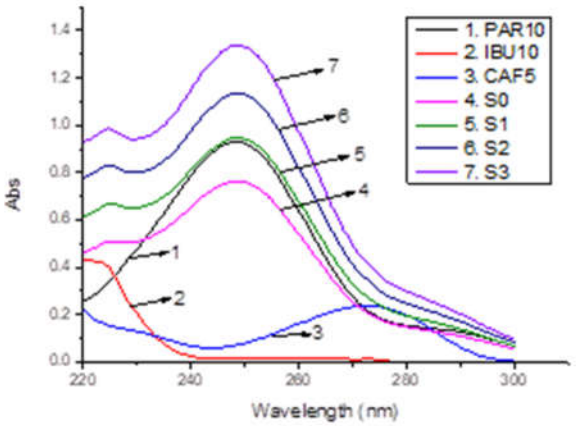

Figure 3. Absorbance spectra of standard solutions, sample solution and standard addition solutions (standard solution (μg/mL): PAR 10, IBU 10, CAF 5; S0: drug sample without standard S0 (m = 0.7 $M_{tablets}$); PAR/IBU/CAF mixed solution (μg/mL): S1 (2:2:0.5); S2 (4:4:1); S3 (6:4:1.5)).

Table 3. Recovery of ibuparavic with CLS method.

Repeated sample	PAR			IBU			CAF		
	C_{added} (μg/mL)	$C_{measured}$ (μg/mL)	Rev (%)	C_{added} (μg/mL)	$C_{measured}$ (μg/mL)	Rev (%)	C_{added} (μg/mL)	$C_{measured}$ (μg/mL)	Rev (%)
S_{01}		8.045			5.462			0.562	
S_{02}	0	8.000	–	0	5.502	–	0	0.568	–
S_{03}		7.987			5.483			0.564	
Statistics	$C_{measured\ (mean)}$ = 8.011 RSD% = 0.380			$C_{measured\ (mean)}$ = 5.482 RSD% = 0.360			$C_{measured\ (mean)}$ = 0.565 RSD% = 0.540		
S_{11}		9.901	92.80		7.475	100.65		1.035	94.60
S_{12}	2.000	9.916	95.80	2.000	7.543	102.05	0.500	1.057	97.80
S_{13}		9.944	98.30		7.564	104.05		1.064	100.00
Statistics	$C_{measured\ (mean)}$ = 9.920 RSD% = 0.22 Rev_{mean} (%) = 95.63			$C_{measured\ (mean)}$ = 7.527 RSD% = 0.61 Rev_{mean} (%) = 102.25			$C_{measured\ (mean)}$ = 1.052 RSD% = 1.44 Rev_{mean} (%) = 97.47		
S21		11.824	94.48		9.551	102.23		1.578	101.60
S22	4.000	11.713	92.83	4.000	9.538	100.90	1.000	1.585	101.70
S23		11.711	93.10		9.536	101.33		1.582	101.80
Statistics	$C_{measured\ (mean)}$ = 11.749 RSD% = 0.55			$C_{measured\ (mean)}$ = 9.542 RSD% = 0.09			$C_{measured\ (mean)}$ = 1.582 RSD% = 0.22		

	Rev_{mean} (%) = 93.47			Rev_{mean} (%) = 101.48			Rev_{mean} (%) = 101.70		
S31		13.893	97.46		11.247	96.42		2.074	100.80
S32	6.000	13.777	96.28	6.000	11.246	95.73	1.500	2.093	101.67
S33		13.774	96.45		11.238	95.92		2.093	101.80
Statistics	$C_{measured\ (mean)}$ = 13.815 RSD% = 0.49 Rev_{mean} (%) = 96.73			$C_{measured\ (mean)}$ = 11.238 RSD% = 0.04 Rev_{mean} (%) = 96.02			$C_{measured\ (mean)}$ = 2.087 RSD% = 0.52 Rev_{mean} (%) = 101.42		

Note: The number of decimal places is taken to represent the calculation result.

Table 3 shows that the method's recoveries satisfactory: 92.80–98.30% for PAR, 95.73–104.05% for IBU, and 94.60–101.80% for CAF. All recovery values are within the allowable range required by AOAC [13].

3.3.2. Comparison between CSL-Excel and HPLC methods

To objectively evaluate the accuracy of our method, we compared the content of the active ingredients in Ibuparavic tablets with those determined with the standard HPLC method performed by the Centre for Drug, Food, and Cosmetic Testing in Thua Thien Hue, Vietnam. The comparison was carried out statistically [15] (Table 4).

Table 4. Comparison between CLS-Excel and HPLC methods.

N_0	Content (H, mg/tablet)					
	PAR		**IBU**		**CAF**	
	CLS	**HPLC**	**CLS**	**HPLC**	**CLS**	**HPLC**
1	286.68	287.98	194.13	193.02	20.30	20.37
2	287.58	285.26	193.73	195.90	19.88	20.19
3	286.58	289.18	195.63	195.69	20.05	19.98
H_{mean}	286.95	287.47	194.50	194.87	20.08	20.18
t_{cal}	0.438		0.342		0.622	
$t_{theory}(0.05;4)$	2.78		2.78		2.78	
p	0.68		0.75		0.57	

The results in Table 4 shows that the calculated t-values are smaller than the t-theory values, indicating that the CSL-Excel and HPLC methods are statistically identical at α = 0.05. So we can say that the method has a satisfying accuracy.

4. Conclusions

An analytical procedure for simultaneous determination of PAR, IBU, and CAF in tablets was developed by using the molecular absorption spectrophotometric method with the entire spectrum, coupled with the classical least square technique. The analytical procedure has satisfactory repeatability with an RSD% less than or equal to 1.052. The recoveries obtained for PAR, IBU, and CAF ranged from 92.80 to 98.30, 95.73 to 104.05, and 94.60 to 101.80%, respectively. The content of PAR, IBU, and CAF in the drug sample Ibuparavic analyzed with the the procedure are consistent with those of HPLC method at the 0.05 significance level.

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.

Acknowledgments: This paper was funded by Vietnam Ministry of Education and Training with code no. B2020-DHH-16.

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