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Research Article

Resolution of Ternary Mixture of Aspirin, Atorvastatin, and Clopidogrel by Chemometric-Assisted UV Spectroscopic and Liquid Chromatography Methods

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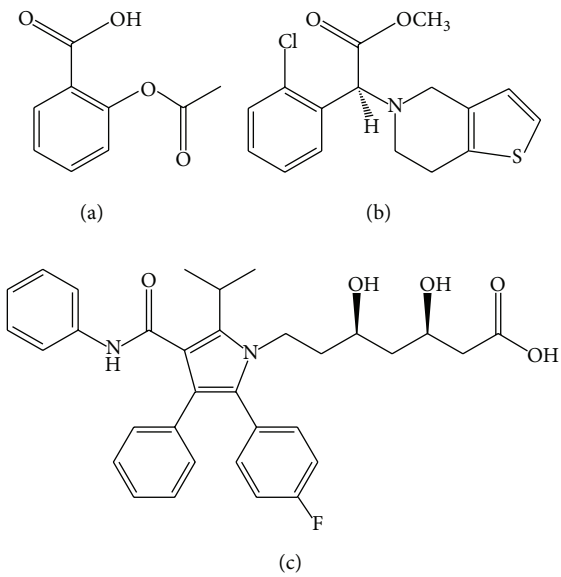
Two chemometrics-assisted UV spectrophotometric methods were proposed for the resolution of ternary mixtures without any chemical pretreatment. The first method is based on modification of H-point standard addition method which permits simultaneous analysis of three species from a unique calibration set by making the simultaneous addition of the three analytes. Quotient between the spectra of aspirin, atorvastatin, and clopidogrel was obtained and the results showed that simultaneous determination of aspirin, atorvastatin, and clopidogrel can be obeyed in the linear range 2.5–20 μg mL^{-1} of aspirin, 2.5–17.5 μg mL^{-1} of atorvastatin, and 2.5–20 μg mL^{-1} of clopidogrel in ternary mixture. The second method is based on the combination of the first derivative spectra and Cramer's matrix rule. In the matrix calculation, clopidogrel has zero crossing point at 316.8 and 212 nm, while for atorvastatin the zero crossing point at 250 nm where the matrix is greatly simplified and easily solved. The linear concentration ranges were 2.5–20 μg mL^{-1} aspirin, 2.5–17.5 μg mL^{-1} atorvastatin and 2.5–20 μg mL^{-1} clopidogrel in ternary mixtures. The results proved that the simultaneous determination of aspirin, atorvastatin, and clopidogrel could be obeyed. Both methods were applied for capsules containing the three ingredients and results were in good concordance with alternative liquid chromatography.

1. Introduction

Aspirin (ASP, acetylsalicylic acid) is used as an analgesic, antipyretic, anti-inflammatory medication and an antiplatelet agent. For clopidogrel bisulphate (CLOP), chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3, 2-c]pyridine-5(4H)acetate sulfate (1 : 1) and is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Atorvastatin calcium (ATOR), [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ,-dihydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenylamino)carbonyl]-lH-pyrrole-1-heptanoic acid, calcium salt (2 : 1) trihydrate is an inhibitor of 3 hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Chemical structures of ASP, CLOP, and ATOR are shown in Scheme 1.

A combination of ASP, CLOP, and ATOR drugs is available in the market as capsules. The ternary combination is used for atherosclerotic patients suffering from various heart diseases. Clinical trials showed that combination therapy when used in dyslipidaemic patient with coronary heart disease reduced cardiovascular events.

The official monographs describe the procedure for individual assay of ASP [1, 2], CLOP [1], and ATOR [2–4]. The literature survey revealed very few analytical methods such as spectrophotometry [5], HPTLC [6], HPLC [7, 8], and LC



Scheme 1: Aspirin (a), clopidogrel (b), and atorvastatin (c).

[9, 10] which have been reported for the determination of ASP, CLOP, and ATOR in the ternary combination.

Simultaneous analysis of a ternary mixture is difficult to perform by classical spectrophotometric method due to overlapping spectra. Chemometrics is one of the mathematical and statistical techniques used for the resolution of overlapping spectra of multicomponent mixtures. Three developed methods for resolution of a ternary mixture have been shown. Salinas et al.'s method [11] is based on the use of the derivative ratio spectra for a binary mixture. Nevado et al.'s method [12] is based on the measurements of the amplitude at the zero crossing points in the derivative spectrum of the ratio spectra. In Dinç and Onur's [13] method, the simultaneous analysis of a ternary mixture is based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the mixture.

Recently, Hajian and Afshari [14] developed a new method for the simultaneous analysis of a ternary mixture by combination of double divisor-ratio spectra derivative and H-point standard addition method (HPSAM). The fundamentals of the HPSAM were outlined by Campins-Falco et al. [15, 16]. It is a modification of the standard addition method and has been frequently applied in spectrophotometry [17–23]. It was also developed for resolution of binary mixture with simultaneous addition of both analytes [24]. In 2006, Hasani et al. proposed a modification for resolution of ternary mixture by using a single calibration graph with simultaneous standard additions of three analytes [25].

In this work, the HPSAM with simultaneous standard addition of three analytes and combination of first derivatives spectra and Cramer's matrix rule has been applied for the simultaneous analysis of ASP, ATOR, and CLOP in their ternary mixture. The matrix calculation based on Cramer's rule for a system of equations with three unknowns can be used for simultaneous determination of these compounds in the same ternary mixture and pharmaceutical formulations [13, 26]. To the best of our knowledge, this is the first report on the simultaneous determination of ASP, ATOR, and CLOP using these methods. As an alternative method, HPLC method was used for simultaneous determination of these compounds in the same ternary mixture and pharmaceutical formulation [7].

2. Theory

2.1. HPSAM with Simultaneous Addition of Three Analytes. The absorbance of a ternary mixture (A_m) consisting of compounds ASP, ATOR, and CLOP at λ_{317} and λ_{231} is

$$\begin{aligned} A_{m,317} &= A^0_{\mathrm{ASP},317} + A^0_{\mathrm{ATOR},317} + A^0_{\mathrm{CLOP},317}, \\ A_{m,231} &= A^0_{\mathrm{ASP},231} + A^0_{\mathrm{ATOR},231} + A^0_{\mathrm{CLOP},231}, \end{aligned} \tag{1}$$

where λ_{317} and λ_{231} are selected according to

$$\frac{A_{\mathrm{ATOR},317}}{A_{\mathrm{ATOR},231}} = \frac{A_{\mathrm{CLOP},317}}{A_{\mathrm{CLOP},231}} = K. \tag{2}$$

Hence,

$$\frac{M_{\mathrm{ATOR},317}}{M_{\mathrm{ATOR},231}} = \frac{M_{\mathrm{CLOP},317}}{M_{\mathrm{CLOP},231}} = K, \tag{3}$$

where M is the slop due to the addition of species of ASP, ATOR, and CLOP in the line obtained at λ_{317} or λ_{231}. The required data to apply the method are the absorbance of the mixture and the absorbance of the mixture spiked with known amount of ASP, ATOR, and CLOP at λ_{317} and λ_{231}; therefore

$$\begin{aligned} A_{m,317} = {} & A^0_{\mathrm{ASP},317} + A^0_{\mathrm{ATOR},317} + A^0_{\mathrm{CLOP},317} \\ & + M_{\mathrm{ASP},317}C_{\mathrm{ASP}} + A_{\mathrm{ATOR},317} + A_{\mathrm{CLOP},317}, \end{aligned} \tag{4}$$

$$\begin{aligned} A_{m,231} = {} & A^0_{\mathrm{ASP},231} + A^0_{\mathrm{ATOR},231} + A^0_{\mathrm{CLOP},231} \\ & + M_{\mathrm{ASP},231}C_{\mathrm{ASP}} + A_{\mathrm{ATOR},231} + A_{\mathrm{CLOP},231}. \end{aligned} \tag{5}$$

If (5) is multiplied by K, the following equation can be obtained:

$$\begin{aligned} KA_{m,231} = {} & KA^0_{\mathrm{ASP},231} + A^0_{\mathrm{ATOR},317} + A^0_{\mathrm{CLOP},317} \\ & + KM_{\mathrm{ASP},231}C_{\mathrm{ASP}} + A_{\mathrm{ATOR317}} \\ & + A_{\mathrm{CLOP},317}. \end{aligned} \tag{6}$$

At the intersection (H-point), $A_{m,317} = KA_{m,231}$; consequently, the ASP concentration can calculate as

$$-C_{\mathrm{H}} = C_{\mathrm{ASP}} = \frac{A^0_{\mathrm{ASP},317} - KA^0_{\mathrm{ASP},231}}{KA_{\mathrm{ASP},231} - M_{\mathrm{ASP},317}}, \tag{7}$$

where the superscript zero denotes the sample solution.

For analysis of ASP, the quotient between ATOR and CLOP spectra is applied and the wavelength pairs that show the same value are selected. Although this relation depends on the concentration of ATOR and CLOP, it will be equal at the two wavelengths. The concentration of ATOR and CLOP is determined by analogous procedure.

2.2. Combination of First Derivatives and Cramer's Matrix Calculation Method. Molar absorptivity (ε) values were calculated by using the absorbance measured at 212 nm, 250 nm, and 316.8 nm for first-order spectra for each compound in the ternary mixture. The wavelength values were selected because CLOP has zero-crossing point at 316.8 and 212 nm and zero-crossing point of ATOR at 250 nm, in which the matrix was greatly simplified and easily solved. By using (ε) values, a system of equations with three unknowns can be written for the compounds in the ternary mixture as follows:

$$\begin{aligned} A_{m,212} &= \varepsilon_{\mathrm{ASP},212}C_{\mathrm{ASP}} + \varepsilon_{\mathrm{ATOR},212}C_{\mathrm{ATOR}} \\ &\quad + \varepsilon_{\mathrm{CLOP},212}C_{\mathrm{CLOP}}, \\ A_{m,250} &= \varepsilon_{\mathrm{ASP},250}C_{\mathrm{ASP}} + \varepsilon_{\mathrm{ATOR},250}C_{\mathrm{ATOR}} \\ &\quad + \varepsilon_{\mathrm{CLOP},250}C_{\mathrm{CLOP}}, \\ A_{m,316.8} &= \varepsilon_{\mathrm{ASP},316.8}C_{\mathrm{ASP}} + \varepsilon_{\mathrm{ATOR},316.8}C_{\mathrm{ATOR}} \\ &\quad + \varepsilon_{\mathrm{CLOP},316.8}C_{\mathrm{CLOP}}, \end{aligned} \tag{8}$$

where A_m denotes the absorbance of the ternary mixture and (ε) represents the values of molar absorptivity for the calculated ASP, ATOR, and CLOP, respectively, at 212, 250 nm, and 316.8 nm. C is the molar concentration of ASP, ATOR, and CLOP.

The matrix simplifies and solves the system of equations with three unknowns as follows:

$$\begin{bmatrix} \varepsilon_{\mathrm{ASP},212} & \varepsilon_{\mathrm{ATOR},212} & \varepsilon_{\mathrm{CLOP},212} \\ \varepsilon_{\mathrm{ASP},250} & \varepsilon_{\mathrm{ATOR},250} & \varepsilon_{\mathrm{CLOP},250} \\ \varepsilon_{\mathrm{ASP},316.8} & \varepsilon_{\mathrm{ATOR},316.8} & \varepsilon_{\mathrm{CLOP},316.8} \end{bmatrix} \begin{bmatrix} C_{\mathrm{ASP}} \\ C_{\mathrm{ATOR}} \\ C_{\mathrm{CLOP}} \end{bmatrix} = \begin{bmatrix} A_{m,212} \\ A_{m,230} \\ A_{m,316.8} \end{bmatrix}. \tag{9}$$

This matrix can be solved and each compound was determined by solving the following operations:

$$\begin{aligned} \Delta &= \begin{bmatrix} \varepsilon_{\mathrm{ASP},212} & \varepsilon_{\mathrm{ATOR},212} & \varepsilon_{\mathrm{CLOP},212} \\ \varepsilon_{\mathrm{ASP},250} & \varepsilon_{\mathrm{ATOR},250} & \varepsilon_{\mathrm{CLOP},250} \\ \varepsilon_{\mathrm{ASP},316.8} & \varepsilon_{\mathrm{ATOR},316.8} & \varepsilon_{\mathrm{CLOP},316.8} \end{bmatrix}, \\ \Delta_1 &= \begin{bmatrix} A_{m,212} & \varepsilon_{\mathrm{ATOR},212} & \varepsilon_{\mathrm{CLOP},212} \\ A_{m,230} & \varepsilon_{\mathrm{ATOR},250} & \varepsilon_{\mathrm{CLOP},250} \\ A_{m,316.8} & \varepsilon_{\mathrm{ATOR},316.8} & \varepsilon_{\mathrm{CLOP},316.8} \end{bmatrix}, \\ \Delta_2 &= \begin{bmatrix} \varepsilon_{\mathrm{ASP},212} & A_{m,212} & \varepsilon_{\mathrm{CLOP},212} \\ \varepsilon_{\mathrm{ASP},250} & A_{m,230} & \varepsilon_{\mathrm{CLOP},250} \\ \varepsilon_{\mathrm{ASP},316.8} & A_{m,316.8} & \varepsilon_{\mathrm{CLOP},316.8} \end{bmatrix}, \\ \Delta_3 &= \begin{bmatrix} \varepsilon_{\mathrm{ASP},212} & \varepsilon_{\mathrm{ATOR},212} & A_{m,212} \\ \varepsilon_{\mathrm{ASP},250} & \varepsilon_{\mathrm{ATOR},250} & A_{m,230} \\ \varepsilon_{\mathrm{ASP},316.8} & \varepsilon_{\mathrm{ATOR},316.8} & A_{m,316.8} \end{bmatrix}, \end{aligned} \tag{10}$$

where

$$C_{\mathrm{ASP}} = \frac{\Delta_1}{\Delta}, \qquad C_{\mathrm{ATOR}} = \frac{\Delta_2}{\Delta}, \qquad C_{\mathrm{CLOP}} = \frac{\Delta_3}{\Delta}. \tag{11}$$

3. Experimental

3.1. Apparatus. A Shimadzu (Kyoto, Japan) UV-1650 PC, UV-Visible double-beam spectrophotometer with two matched 1 cm path-length quartz cells was used. The subsequent statistical manipulations were performed by transferring the spectral data to Microsoft Excel 2010 program and processing them with the standard curve fit package and matrix calculation.

A waters breeze 1515 chromatograph (Waters, USA), equipped with an isocratic pump, UV detector, and an rheodyne injector was used. The separation was achieved on a symmetry C_{18} column (3.5 μm particle size, 75 × 4.6 mm i.d.) with a mixture of acetonitrile: phosphate buffer pH 3.0; at a flow rate of 1.2 mL/min and the detection was monitored at 235 nm.

3.2. Pharmaceutical Formulations. Commercial product ASPTORGREL capsules (produced by Middle East pharm, Palestine, Batch no. 39712 containing 75 mg of aspirin, 10 mg of atorvastatin, and 75 mg of clopidogrel per capsule) were analyzed.

Aspirin, atorvastatin, and clopidogrel were kindly donated by Middle East pharm. All chemical and reagents used (methanol, acetonitrile, and orthophosphoric acid) were of HPLC grade (Merck).

3.3. Reagents. Stock solutions of 75 mg/100 mL ASP, 10 mg/100 mL ATOR, and 75 mg/100 mL CLOP were dissolved in methanol, separately. Standard solutions were prepared by serial dilution with methanol. Synthetic mixtures were prepared by mixing known amount of ASP, CLOP, and ATOR as shown in Table 1. Standard solutions were used in preparation of calibration graphs and for spectral measurements.

3.4. Procedure for the Assay of the Pharmaceutical Formulation. Five capsules of ASPTORGREL were accurately weighed and the content mixed thoroughly together; an amount equivalent to one capsule was dissolved in methanol in a 100 mL calibrated flask. After shaking, the solution was filtered into a 100 mL calibrated flask. The residue was washed with methanol, then the volume was completed to the mark. All the methods were applied to the solutions, thus were prepared.

4. Application of Methods

4.1. HPSAM with Simultaneous Addition of Three Analytes. The absorption spectra of ASP, ATOR, and CLOP (5 μg mL^{-1} each in methanol) and their ternary mixture were recorded in wavelength range 200–400 nm and saved as a text file. Synthetic samples containing different concentration ratios of ASP, ATOR, and CLOP were prepared and standard additions of them were made. Simultaneous determination of ASP, ATOR, and CLOP with HPSAM was performed by measuring the absorbance at 231.0, 317.0, 275.8, 329.2, 235.6, and 293.6 nm for each sample solution. The concentration ranges of ASP, ATOR, and CLOP for construction of HPSAM calibration curve were 2.5–20, 2.5–17.5, and 2.5–20 μg mL^{-1}, respectively.

Table 1: Recovery data obtained for different synthetic mixtures by using the HPSAM method with simultaneous addition of three analytes.

Mixture No.	ASP			ATOR			CLOP		
	Added (μg)	Found (μg)	Recovery%	Added	Found	Recovery%	Added	Found	Recovery%
1	2.5	2.488	99.5	7.5	7.501	100.0	5.0	4.987	99.7
2	5.0	5.005	100.1	7.5	7.303	97.4	5.0	4.891	97.8
3	7.5	7.444	99.3	7.5	7.499	99.9	5.0	5.015	100.3
4	10.0	9.92	99.2	7.5	7.511	100.1	5.0	5.010	100.2
5	15.0	14.66	97.7	7.5	7.407	98.8	5.0	5.065	101.3
6	20.0	20.04	100.2	7.50	7.505	100.1	5.0	5.080	101.6
			$\overline{x}$ = 99.3 RSD = 0.92						
1	10.0	10.12	101.2	2.5	2.509	100.4	5.0	5.035	100.7
2	10.0	10.08	100.8	5.0	4.999	100.0	5.0	5.090	101.8
3	10.0	9.92	99.2	7.5	7.611	101.5	5.0	5.010	100.2
4	10.0	9.75	97.5	10.0	9.85	98.5	5.0	4.888	97.8
5	10.0	9.96	99.6	12.5	12.22	97.8	5.0	4.921	98.4
6	10.0	10.0	100.0	17.5	17.34	99.1	5.0	4.999	100.0
						$\overline{x}$ = 99.6 RSD = 1.35			
1	10.0	10.31	103.1	7.5	7.438	99.2	2.5	2.486	99.4
2	10.0	9.92	99.2	7.5	7.511	100.1	5.0	5.010	100.2
3	10.0	10.22	102.2	7.5	7.502	100.0	7.5	7.333	97.8
4	10.0	9.86	98.6	7.5	7.547	100.6	10.0	10.22	102.2
5	10.0	9.99	99.9	7.5	7.444	99.2	15.0	13.03	100.2
6	10.0	9.97	99.7	7.5	7.400	98.7	20.0	19.90	99.5
									$\overline{x}$ = 99.9 RSD = 1.43

4.2. Combination of First Derivative Spectra and Cramer's Matrix Calculation. In this method, the first derivative spectra of ASP, ATOR, and CLOP and of different concentrations of ternary mixture were recorded from the data in the first method. The molar absorptivity ($mol^{-1}\,L\,cm^{-1}$) values were calculated by measuring the absorbance at 212, 250, and 316.8 nm for first-order spectra for each of the compounds in the ternary mixture. By using the molar absorptivity values, a system of equations with three unknowns can be obtained, which can be solved by means of Cramer's rule, and the concentration of ASP (2.5–20 $\mu g\,mL^{-1}$), ATOR, (2.5–17.5 $\mu g\,mL^{-1}$), and CLOP (2.5–20 $\mu g\,mL^{-1}$) was determined.

4.3. HPLC Method. The concentration of ASP, ATOR, and CIOP can be determined simultaneously by using Londhe et al.'s method [7]. Separation was achieved on an isocratic reversed-phase high-performance liquid chromatography with inertsil ODS analytical column (75 × 4.6 mm; 3.5 μm) with a mixture of acetonitrile phosphate buffer pH 3.0 as mobile phase and at a flow rate of 1.2 mL/min. UV detection was performed at 235 nm.

5. Results and Discussion

5.1. HPSAM with Simultaneous Addition of Three Analytes. The absorption spectra of ASP, ATOR, and CLOP and their ternary synthetic mixture are shown in Figure 1. As can be seen, the spectra overlapped in the region 200–400 nm. In order to resolve the mixture, the quotient between the spectra of ASP, ATOR, and CLOP must be obtained. Figure 2 shows these quotients; hence, the wavelength pairs that show the same ratio of absorbance value in order to calculate the analyte concentration can be obtained. The three wavelength pairs, 231.0–317.0, 275.8–329.2, and 235.6–293.6 nm, were chosen for determination of ASP, ATOR, and CLOP, respectively. Figure 3 shows the H-point standard addition plots for calculation of ASP, ATOR, and CLOP concentration from one calibration set.

Various mixture compositions of ASP, ATOR, and CLOP were prepared and tested between 2.5–20 $\mu g\,mL^{-1}$ ASP, 2.5–17.5 $\mu g\,mL^{-1}$ ATOR, and 2.5–20 $\mu g\,mL^{-1}$ CLOP in ternary mixture as shown in Table 1. Mean recoveries and relative standard deviation (n = 5) of the method were obtained as 99.3 and 0.92% for ASP, 99.6 and 1.35% for ATOR, and also 99.9 and 1.43% for CLOP, respectively. Limits of detection were 0.28, 0.17, and 0.33 $\mu g\,mL^{-1}$, respectively for

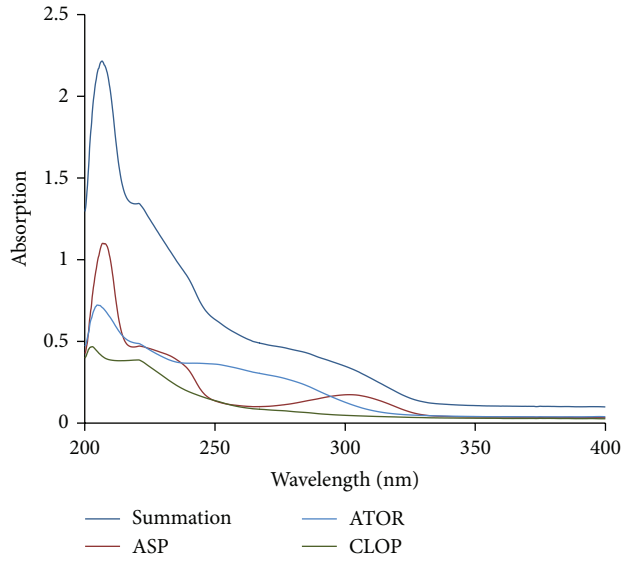


Figure 1: The absorption spectra of (a) the mixture of ASP, ATOR, and CLOP, (b) 5 μg mL^{-1} ASP (c) 5 μg mL^{-1} ATOR (d) 5 μg mL^{-1} CLOP.

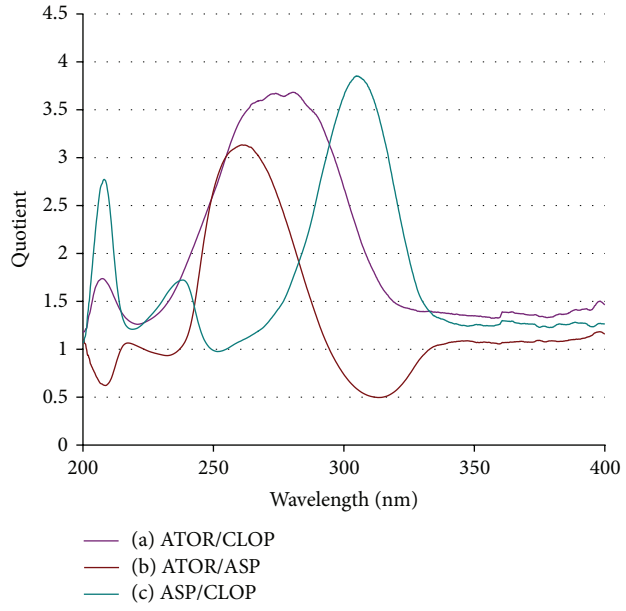


Figure 2: Quotient between the spectra of (a) ATOR/CLOP (b) ATOR/ASP and (c) ASP/CLOP.

ASP, ATOR, and CLOP, which were calculated as LOD = C_H + 3SD$_H$, where C_H and SD$_H$ are the mean and standard deviation for five replicate measurements of blank sample using HPSAM [27].

5.2. Combination of First Derivative and Cramer's Matrix Calculation Method. As seen in Figure 4, the spectra of the first derivative of the three compounds, ASP, ATOR, and CLOP, overlapped in the region 200–400 nm. The wavelength values of ASP, ATOR, and CLOP are chosen as 316.8, 250,

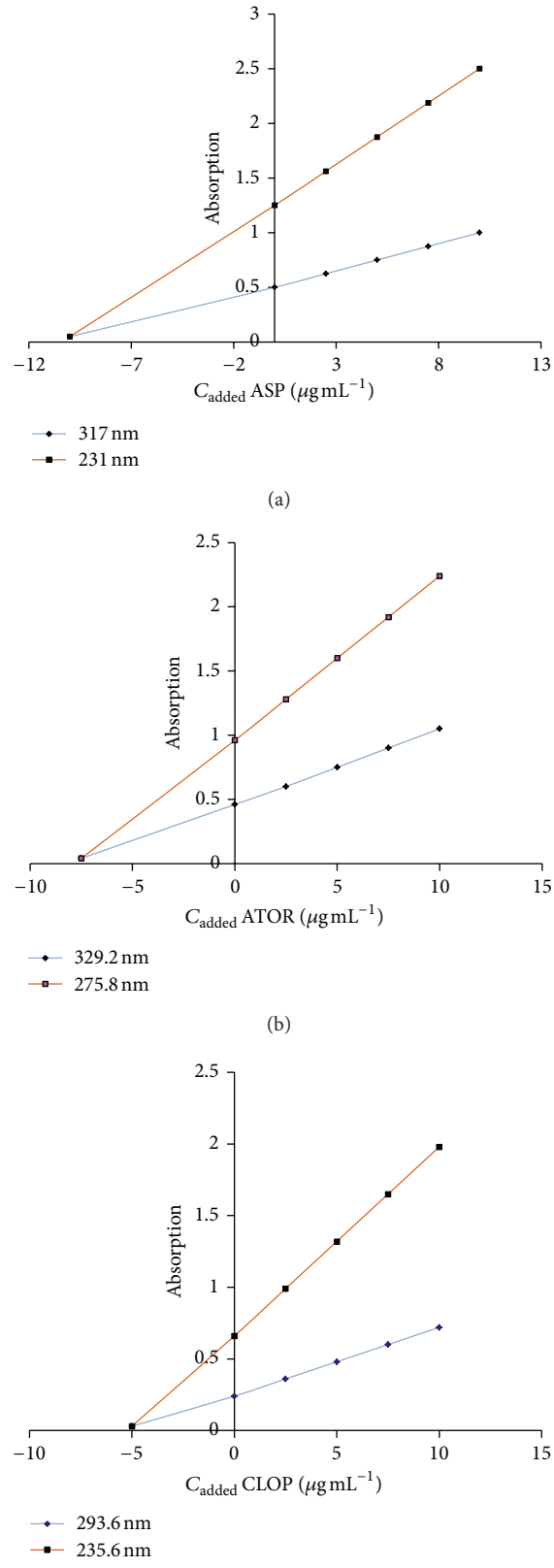


Figure 3: HPSAM plots for calculation of the ASP (10 μg mL^{-1}), ATOR (7.5 μg mL^{-1}) and CLOP (5 μg mL^{-1}) concentrations from one calibration set.

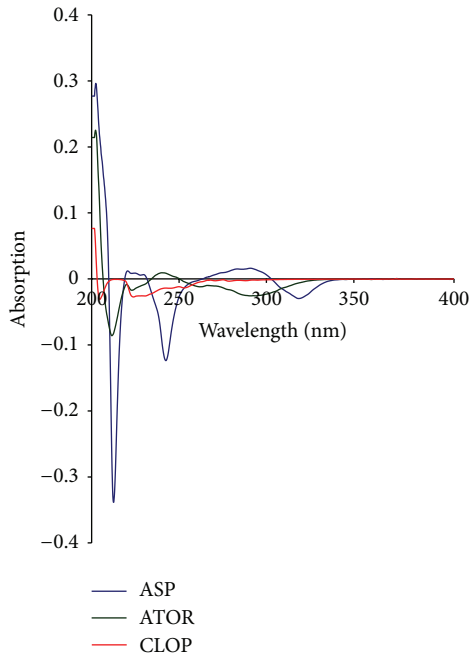


Figure 4: The first-order derivative spectra of (a) 20 μg mL^{-1} ASP (b) 20 μg mL^{-1} ATOR and (c) 20 μg mL^{-1} CLOP.

and 212 nm, respectively. In application of matrix calculation, CLOP has zero crossing point at 316.8 and 212 nm, whilst zero crossing point of ATOR is at 250 nm. A system of equations with the three compounds (5.0 μg mL^{-1} of each in methanol) can be written as

$$\begin{bmatrix} 3001 & 2237 & 0.000 \\ 1144 & 0.000 & 201.1 \\ 253.2 & 201.1 & 0.000 \end{bmatrix} \begin{bmatrix} C_{\text{ASP}} \\ C_{\text{ATOR}} \\ C_{\text{CLOP}} \end{bmatrix} = \begin{bmatrix} 0.1033 \\ 0.0349 \\ 0.0088 \end{bmatrix}. \quad (12)$$

By using the matrix calculation, the concentration is 4.88, 4.90, and 5.16 μg mL^{-1} for ASP, ATOR, and CLOP, respectively. Various mixture compositions of ASP, ATOR, and CLOP were prepared and tested between 2.5–20 μg mL^{-1} ASP, 2.5–17.5 μg mL^{-1} ATOR, and 2.5–20 μg mL^{-1} CLOP in ternary mixtures as shown in Table 2. Mean recoveries and relative standard deviations ($n = 5$) of the method were obtained as 100.0 and 1.44% for ASP, 99.8 and 1.35% for ATOR, and also 100.9 and 1.55% for CLOP, respectively.

Limits of detection were 0.23, 0.29, and 0.21 μg mL^{-1}, respectively, for ASP, ATOR, and CLOP, which were calculated as LOD = 3.3(SD/b), where b is the slope and SD is the standard deviation of the regression line.

5.3. HPLC Method. The chromatogram at 235 nm showed a complete resolution of all peaks (Figure 5). The retention times were 1.32, 4.040, and 8.855 min, for ASP, ATOR, and CLOP, respectively. The calibration graph was linear over the range of 5–30 μg mL^{-1} ATOR and 30–105 μg mL^{-1} for ASP and CIOP. Mean recoveries and relative standard deviation ($n = 5$) for the HPLC method were found to be 99.59

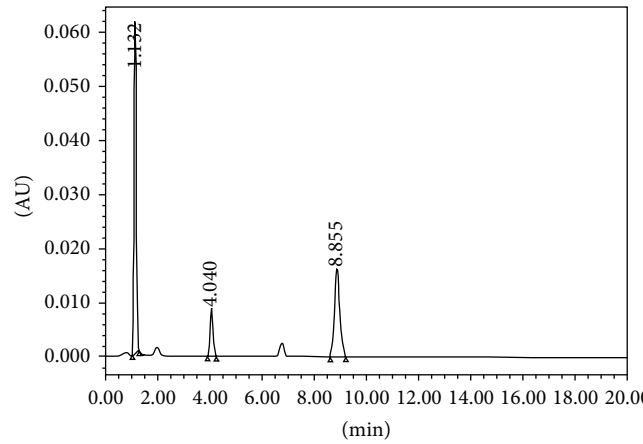


Figure 5: HPLC chromatogram of ASP, CLOP and ATOR capsules.

and 0.56% for ASP, 99.73 and 0.69% for ATOR, and 100.17 and 0.25% for CLOP, respectively, in the synthetic mixture prepared from known amounts of ASP, ATOR, and CLOP.

5.4. Application. To evaluate the applicability of the proposed methods, the two proposed methods were applied to simultaneous determination of ASP, ATOR, and CLOP in commercially available capsules. Table 3 shows a good agreement between the obtained results which indicates the successful applicability of the methods described in this work.

6. Conclusion

Chemometric methods essentially reduce the solvents used and the duration of analysis by doing most of the works on the front desk using microcomputers with appropriate softwares on the primary data generated in the laboratory work. The proposed methods have been successfully applied to the simultaneous determination of ASP, ATOR, and CLOP in capsule dosage of synthetic samples. A comparative study of the use of HPLC and chemometrics-assisted methods for the resolution of ternary mixture of ASP, ATOR, and CLOP has been accomplished, showing that the proposed methods provide, with adequate software support, a good pattern of the high resolving power of this technique. HPSAM is a modification of the previously described HPSAM that permits the resolution of three species from a unique calibration set by making the simultaneous addition of the three analytes. The principal advantage of the method is using a single calibration curve with simultaneous standard additions of three analytes instead of individual standard addition for every analyte and a calibration curve for each analyte. Based on the results obtained, it has been shown that the combination of first derivative and Cramer's matrix calculation method calculates the analyte concentrations, ASP, ATOR, or CLOP, in the presence of each other. The principal advantage of the method is using the zero crossing point in which the matrix is greatly simplified and easily solved by means of Cramer's rule.

Table 2: Recovery data obtained for different synthetic mixtures by using the matrix calculation method.

Mixture No.	ASP			ATOR			CLOP		
	Added (μg)	Found (μg)	Recovery%	Added	Found	Recovery%	Added	Found	Recovery%
1	2.5	2.488	99.5	5.0	5.161	103.3	5.0	4.973	99.5
2	5.0	4.875	97.5	5.0	4.900	98.0	5.0	5.165	103.3
3	7.5	7.563	100.8	5.0	5.012	100.2	5.0	4.899	98.0
4	10.0	10.11	101.1	5.0	5.008	100.2	5.0	4.901	98.0
5	15.0	15.21	101.4	5.0	5.014	100.3	5.0	4.890	97.8
6	20.0	19.96	99.8	5.0	4.982	99.6	5.0	5.065	101.3
			$\overline{x}$ = 100.0 RSD = 1.44						
1	5.0	5.000	100.0	2.5	2.502	100.0	5.0	5.111	102.2
2	5.0	4.875	97.5	5.0	4.900	98.0	5.0	5.165	103.2
3	5.0	4.998	100.0	7.5	7.442	99.2	5.0	5.001	100.0
4	5.0	5.009	100.2	10.0	10.19	101.9	5.0	5.005	100.0
5	5.0	4.875	97.5	12.5	12.58	100.6	5.0	4.962	99.2
6	5.0	5.00	100.0	17.5	17.36	99.2	5.0	4.900	98.0
						$\overline{x}$ = 99.8 RSD = 1.35			
1	5.0	4.999	100.0	5.0	4.981	99.6	2.5	2.505	100.2
2	5.0	4.875	97.5	5.0	4.900	98.0	5.0	5.165	103.3
3	5.0	5.010	100.2	5.0	4.999	100.0	7.5	7.468	99.6
4	5.0	5.008	100.2	5.0	4.991	99.8	10.0	9.920	99.2
5	5.0	5.002	100.0	5.0	5.005	100.0	15.0	15.301	102.0
6	5.0	4.900	98.0	5.0	5.012	100.2	20.0	20.221	101.1
									$\overline{x}$ = 100.9 RSD = 1.55

Table 3: Assay results for ASPTORGREL capsules.

ASPTORGREL capsule	Certified amounts (mg/capsule)	*Mean recovered amount (mg/capsule) ± SD		
		HPSAM method	Combination method	HPLC
ASP	75	75.9 ± 0.72	74.7 ± 0.66	75.3 ± 0.69
ATOR	10	9.92 ± 0.13	10.1 ± 0.09	9.87 ± 0.10
CLOP	75	75.6 ± 0.85	75.1 ± 0.77	75.0 ± 0.71

*Mean of five measurements (n = 5).

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