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# Mean centering of ratio spectra as a new spectrophotometric method for the analysis of binary and ternary mixtures

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**Abstract**

In this paper a new and very simple method was developed for the simultaneous determination of binary and ternary mixtures, without prior separation steps. This method is based on the mean centering of ratio spectra. The mathematical explanation of the procedure is illustrated. After modeling procedure, the method has been successfully applied to the simultaneous analysis of binary mixtures of mefnamic acid and paracetamol and ternary mixtures of acetylsalysilic acid, ascorbic acid and paracetamol. The analytical characteristics of the method such as detection limit, accuracy, precision, relative standard deviation (R.S.D.) and relative standard error (R.S.E.) was calculated. The results showed that the proposed method is simple, rapid, accurate and precise method for analysis of binary and ternary mixtures.


*Keywords:* Mean centering; Ratio spectra; Analysis of binary and ternary mixtures

## 1. Introduction

The main problem of spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. Several spectrophotometric determination methods have been used for resolving mixtures of compounds with overlapping spectra. When these methods are compared with each other, the range of application of derivative spectrophotometry is more reliable with respect to utility and sensitivity than normal spectrophotometry [1]. Other spectrophotometric methods, such as partial least squares regression (PLSR) [2], principal component regression (PCR) [3], multi-wavelength linear regression analysis (MLRA) [4], H-point standard addition method (HPSAM) for binary [5] and ternary [6] mixtures have been utilized.

Salinas et al. [7] proposed a new spectrophotometric method, ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Their method is based on the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of a standard solution of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentration of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths.

Berzas Nevada et al. [8] developed a new method for the analysis of ternary mixtures by derivative ratio spectra-zero crossing. In this method, the simultaneous determination of three compounds in ternary mixtures is realized by the measuring of the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra. These two methods were further studied and applied for simultaneous determination of binary or ternary mixtures [9–13].

Recently, Dinc et al. [1,14–16] proposed a new spectrophotometric method for the simultaneous determination of ternary mixtures. This method was called “the double divisor-ratio spectra derivative method”. The method is based on the use of the coincident spectra of the derivative

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of the ratio spectra obtained by using a "double divisor" (sum of two spectra) and measuring at either the maximum or minimum wavelengths. But their method cannot be popularized, because it can only be used for the mixtures that the ratio of the concentrations of two interfering compounds (used as double divisor) is known. In the other words, the ratio of the concentrations of two interfering compounds should be the same in calibration, prediction and unknown samples. It is obvious that the ratio of the concentration of the analytes in real samples is always unknown.

Recently, we proposed a new spectrophotometric method for the simultaneous determination of ternary mixtures, without prior separation steps. This method is called the successive derivative ratio spectra. The method is based on the successive derivatives of ratio spectra in two steps. The method was evaluated by model data and also by application to the simultaneous spectrophotometric determination of cobalt, nicked and zinc based on their complexes with 1-(2-pyridylazo)2-naphthol in micellar media as experimental data [17].

Unfortunately, the advantages of derivative spectra are at least partially offset by degradation in signal-to-noise ratio that accompanies obtaining derivatives.

In this paper a new and very simple method was developed for the simultaneous determination of binary and ternary mixtures, without prior separation steps. This method is based on the mean centering of ratio spectra. This method eliminates derivative steps and therefore signal-to-noise ratio is enhanced. The mathematical explanation of the procedure is illustrated. After modeling procedure, the method has been successfully applied to the simultaneous analysis of binary mixtures of mefnamic acid (MEF) and paracetamol (PAR) and ternary mixtures of acetylsalysilic acid (ASA), ascorbic acid (ASC) and paracetamol (PAR).

## 2. Theoretical background

To explain the mean centering expression, let us consider a three-dimensinal vector [18]:

$$y = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix}$$

We center or mean center (MC) this column by subtracting the mean of three numbers

$$\text{calling} : \bar{y} = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix},$$

$$\text{MC}(y) = y - \bar{y} = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix} - \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} = \begin{bmatrix} +2 \\ -2 \\ 0 \end{bmatrix}$$

It could be proved that if the vector $y$ is multiplied by $n$ (a constant number), the mean centered vector is also multiplied by $n$ and also if a constant number is added to the vector $y$, the mean center of this vector is not changed.

Consider a mixture of three compounds $X$, $Y$ and $Z$. If there is no interaction among the compounds and Beer's low is obeyed for each compound, it can be written:

$$A_{\text{m}} = \alpha_X C_X + \alpha_Y C_Y + \alpha_Z C_Z \tag{1}$$

where $A_{\text{m}}$ is the vector of the absorbance of the mixture, $\alpha_X$, $\alpha_Y$ and $\alpha_Z$ are the molar absorptivity vectors of $X$, $Y$ and $Z$ and $C_X$, $C_Y$ and $C_Z$ are the concentrations of $X$, $Y$ and $Z$, respectively.

If Eq. (1) is divided by $\alpha_Z$ corresponding to the spectrum of a standard solution of $Z$ in ternary mixture, the first ratio spectrum is obtained in the form of Eq. (2) (for possibility of dividing operation, the zero values of $\alpha_Z$ should not be used in the divisor):

$$B = \frac{A_{\text{m}}}{\alpha_Z} = \frac{\alpha_X C_X}{\alpha_Z} + \frac{\alpha_Y C_Y}{\alpha_Z} + C_Z \tag{2}$$

If the Eq. (2) is mean centered (MC), since the mean centering of a constant ($C_Z$) is zero, Eq. (3) would be obtained:

$$\text{MC}(B) = \text{MC}\left[\frac{\alpha_X C_X}{\alpha_Z}\right] + \text{MC}\left[\frac{\alpha_Y C_Y}{\alpha_Z}\right] \tag{3}$$

By dividing Eq. (3) by MC($\alpha_Y/\alpha_Z$), corresponding to the mean centering of the ratio of the spectra of the standard solutions of $Y$ and $Z$ the second ratio spectrum is obtained as Eq. (4) (for possibility of dividing operation, the zero values of MC($\alpha_Y/\alpha_Z$) should not be used in the divisor):

$$D = \frac{\text{MC}(B)}{\text{MC}(\alpha_Y/\alpha_Z)} = \frac{\text{MC}[\alpha_X C_X/\alpha_Z]}{\text{MC}(\alpha_Y/\alpha_Z)} + C_Y \tag{4}$$

Now if the Eq. (4) is mean centered, since the mean centering of a constant ($C_Y$) is zero, Eq. (5) would be obtained:

$$\text{MC}(D) = \text{MC}\frac{\text{MC}[\alpha_X C_X/\alpha_Z]}{\text{MC}(\alpha_Y/\alpha_Z)} \tag{5}$$

Eq. (5) is the mathematical foundation of multi-component analysis that permits the determination of concentration of each of the active compounds in the solution ($X$ in this equation) without interfering from the other compounds of the ternary system ($Y$ and $Z$ in these equations). As Eq. (5) shows there is a linear relation between the amount of MC($D$) and the concentration of $X$ in the solution.

A calibration curve could be constructed by plotting MC($D$) against concentration of $X$ in the standard solutions of $X$ or in the standard ternary mixtures. For more sensitivity the amount of MC($D$) corresponding to maximum or minimum wavelength should be measured.

Calibration graphs for $Y$ and $Z$ could also be constructed as described for $X$.

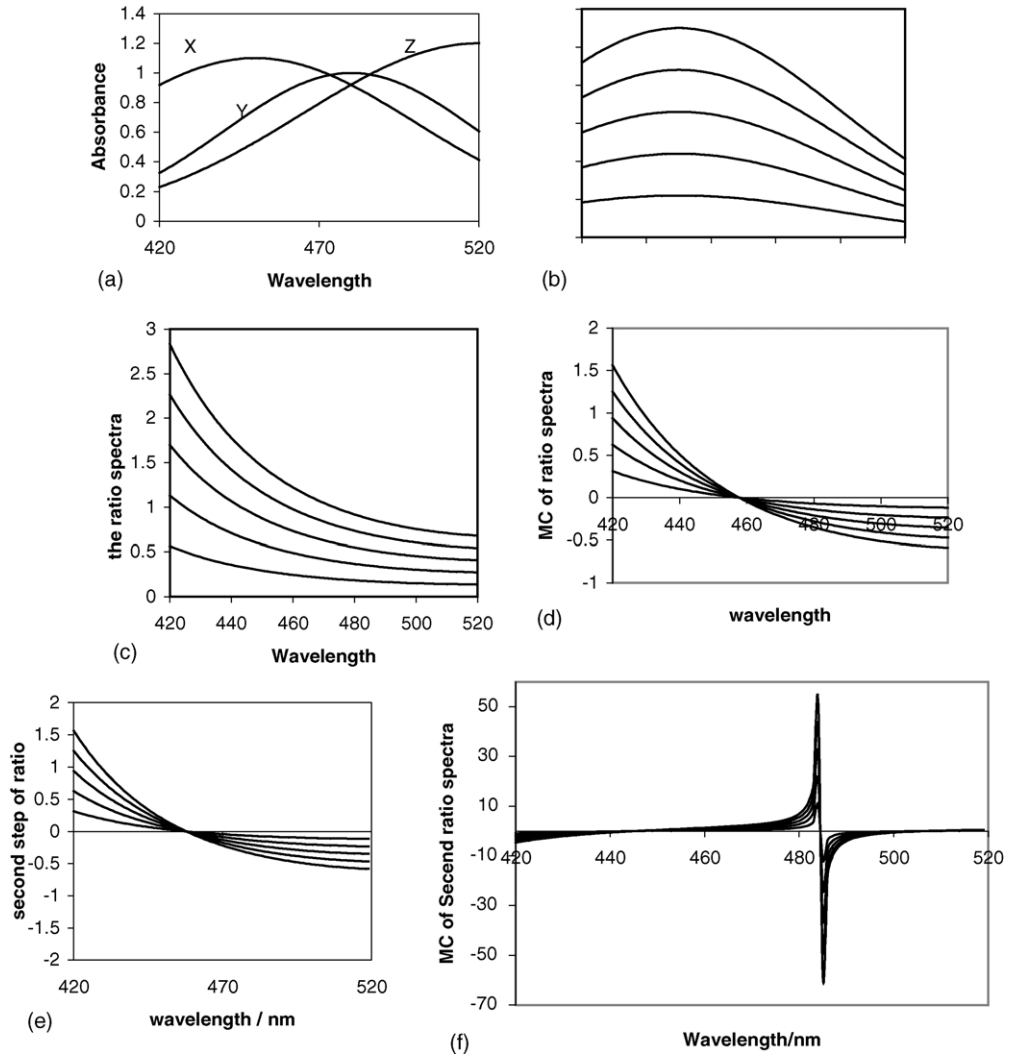


Fig. 1. The modeled zero order spectra of compounds *X*, *Y* and *Z* (a). The modeled absorption spectra of different concentration of *X* (b), the ratio spectra that were obtained by dividing spectra of *X* by the spectrum of the *Y* (c). MC of the ratio spectra (d), second ratio spectra (e) were obtained according to Eq. (4). MC of these vectors (f) were obtained according to Eq. (4).

## 3. Modeling

### *3.1. Validation of the method with model data*

To demonstrate the analytical applicability of the proposed method for the analysis of ternary mixtures, three spectra were created. The three curves form model of the overlapping spectra of three compounds *X*, *Y* and *Z* in the range 420–520 nm (Fig. 1a). The absorption spectra for different concentrations of *X* were created (Fig. 1b) and divided by the spectrum of *Y* and the ratio spectra were obtained (Fig. 1c). Mean centering (MC) of the ratio spectra was obtained (Fig. 1d). After that, these vectors are divided by MC($\alpha_Y/\alpha_Z$) corresponding to the mean centering of the ratio of the spectra of *Z* and *Y* and therefore, second ratio spectra according to Eq. (4) were obtained (Fig. 1e). Mean centering of these vectors was obtained (Fig. 1f). The concentration of compound *X* was determined by measuring the amplitude of the resulting spectra at 482 nm corresponding to the maximum wavelength shown in Fig. 1f.

The obtained model was validated with a number of 10 synthetic mixture set containing the considered components of *X*, *Y* and *Z* in different proportions that were randomly selected. The model spectra of these mixtures are shown in Fig. 2a. To predict the concentration of compound *X* in these synthetic ternary mixtures the procedure that was previously explained for calibration set was performed. Finally, mean centering of second ratio spectra for these mixtures were obtained (Fig. 2b). As can be seen from Fig. 2b the effect of the compounds *Y* and *Z* was removed from the spectra of the mixtures. The concentration of *X* in these synthetic mixtures was determined by measuring the amplitude at 482 nm corresponding to a maximum wavelength. The results are shown in Table 1.

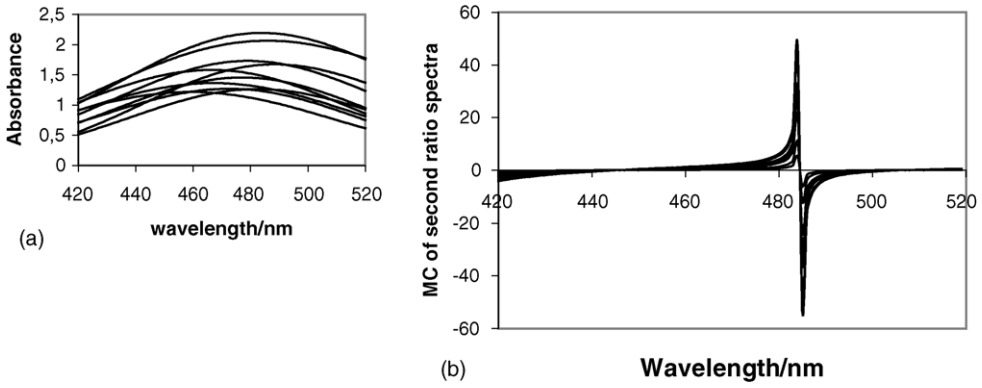


Fig. 2. The model spectra of randomly selected mixtures of the *X*, *Y* and *Z* (a) and the MC of their second ratio spectra obtained (b) according to Eq. (5) for the determination of *X*.

Table 1
Results of analysis of modeled data of *X*, *Y* and *Z* mixtures by proposed method for prediction of concentration of *X*

| Sample number | Composition of synthetic mixtures | | | Predict concentration of *X* | |
|---|---|---|---|---|---|
| | *X* | *Y* | *Z* | Noise free | ±0.003 noise |
| 1 | 9.0 | 6.0 | 1.0 | 9.0 | 8.98 |
| 2 | 2.0 | 8.0 | 3.0 | 2.0 | 2.02 |
| 3 | 6.0 | 9.0 | 8.0 | 6.0 | 6.04 |
| 4 | 5.0 | 7.0 | 1.0 | 5.0 | 5.03 |
| 5 | 9.0 | 2.0 | 1.0 | 9.0 | 9.05 |
| 6 | 8.0 | 4.0 | 2.0 | 8.0 | 7.99 |
| 7 | 5.0 | 9.0 | 4.0 | 5.0 | 5.02 |
| 8 | 1.0 | 10.0 | 6.0 | 1.0 | 0.98 |
| 9 | 8.0 | 4.0 | 10.0 | 8.0 | 8.05 |
| 10 | 4.0 | 9.0 | 2.0 | 4.0 | 4.02 |

### 3.2. Effect of random noise

Random noise was added to the generated set of artificial data in order to test the method more rigorously. In this step, after creation of the spectra of the ternary mixtures, random noise of ±0.003 absorbance unit was added to each spectrum and then the procedure was followed as explained above and the concentration of *X* in the noisy signal of ternary mixtures was predicted. For this set of data, there was a good agreement between synthesized and predicted concentrations (Table 1) that shows the applicability of the method for noisy systems.

## 4. Experimental

### 4.1. Apparatus

UV–vis absorbance spectra were recorded on a Perkin-Elmer Lambda 45 UV–vis spectrometer using quartz cells and slit width of 0.5 nm. A Metrohm model 713 pH-meter with a combined glass electrode was used for pH measurements. All calculations in the computing process were done in Matlab 6.5 and Microsoft Excel for Windows. A simple program was written for this purpose in Matlab 6.5.

### 4.2. Reagents

All chemicals were of analytical reagent grade and triply distilled water was used throughout the experiments. ASA, NaOH and HCl used were from Merck. MEF, ASA and PAR were kindly donated from Razak pharmacy factory (Karaj, Iran) and were used without any purification. Standard solutions of 500 μg mL$^{-1}$ each of MEF and PAR for binary mixture analysis were prepared by dissolving appropriate amounts of them in 0.1 M NaOH–methanol (1:9) mixture [3]. Standard solutions of 500 μg mL$^{-1}$ each of ASA, ASC and PAR for ternary mixture analysis were prepared by dissolving appropriate amounts of them in a 1:3 mixture of methanol and 0.2 M HCl [14].

### 4.3. Procedure

A calibration graph for *X* is obtained by recording and storing the spectra of standard solutions containing different concentrations of *X*, *Y* and *Z* and also by using the vector of MC($\alpha_Y/\alpha_Z$) corresponding to the mean centering of the ratio of the spectrum of the *Y* and *Z* compounds. The stored spectra of the solutions of *X* are divided by standard spectrum of *Z* according to Eq. (2). Then mean centering of these vectors with respect to wavelength are obtained according to Eq. (3) (for binary mixtures the procedure was completed here and

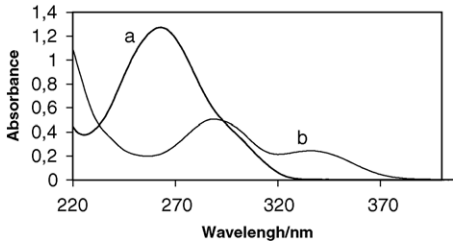


Fig. 3. The zero order spectra of paracetamol (a) and mefnamic acid (b) in 0.1 M NaOH–methanol (1:9).

the minimum or maximum of these vectors with respect to wavelength is used for the construction of calibration graph for $X$). After that residual vector is divided by MC($\alpha_Y/\alpha_Z$) according to Eq. (4). The minimum or maximum of the mean centering of later vectors with respect to wavelength is used for the construction of calibration graph for $X$. For the prediction of concentration of $X$ in synthetic ternary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of $X$. For samples with unknown matrices standard addition can be used for removing matrix effect.

The construction of calibration curves for other two active compounds and also their prediction step was performed as described for $X$.

The proposed method was used to the simultaneous determination of mefnamic acid and paracetamol as a binary system and salysilic acid, ascorbic acid and paracetamol as a ternary mixture.

## 5. Results and discussion

### 5.1. Binary mixture

As Fig. 3 shows, the absorption spectra of paracetamol (PAR) and mefnamic acid (MEF) in 0.1 M NaOH–methanol (1:9) overlapped in the wavelength region of 220–380 nm.

The absorption spectra of the standard solutions of the MEF with different concentrations were recorded in the wavelength range of 220–380 nm (Fig. 4a) and divided by the normalized spectrum of the PAR and the ratio spectra were obtained. Mean centering of the ratio spectra were obtained in the wavelength range of 249–290 nm (Fig. 4b). The concentration of MEF was determined by measuring the amplitude at 249 nm corresponding to a maximum wavelength shown in Fig. 4b. For the prediction of concentration of MEF in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of MEF.

The absorption spectra of the standard solutions of the PAR with different concentrations were recorded in the wavelength range of 220–380 nm (Fig. 5a) and divided by the normalized spectrum of the MEF and the ratio spectra were obtained. Mean centering of the ratio spectra were obtained in

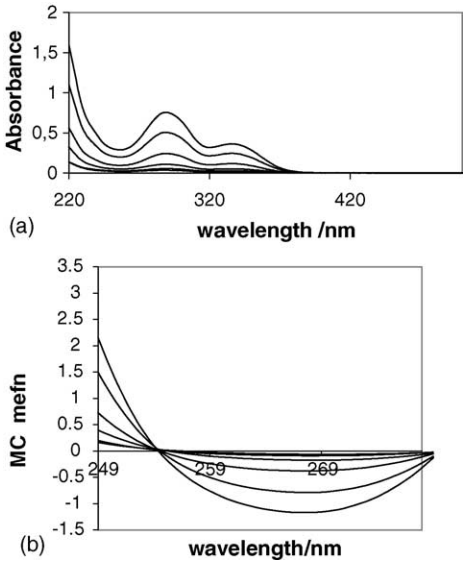


Fig. 4. The zero order spectra of different concentrations (0.5, 1, 2, 5, 10 and 15 μg mL$^{-1}$) of mefnamic acid in 0.1 M NaOH–methanol (1:9) (a) and MC of their ratio spectra obtained with according to Eq. (3).

the wavelength range of 220–300 nm (Fig. 5b). The concentration of PAR was determined by measuring the amplitude at 259 nm corresponding to a maximum wavelength shown in Fig. 5b. For the prediction of concentration of PAR in synthetic binary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of PAR.

### 5.2. Ternary mixture

As Fig. 6 shows, the absorption spectra of paracetamol (PAR), acetylsalysilic acid (ASA) and ascorbic acid (ASC)

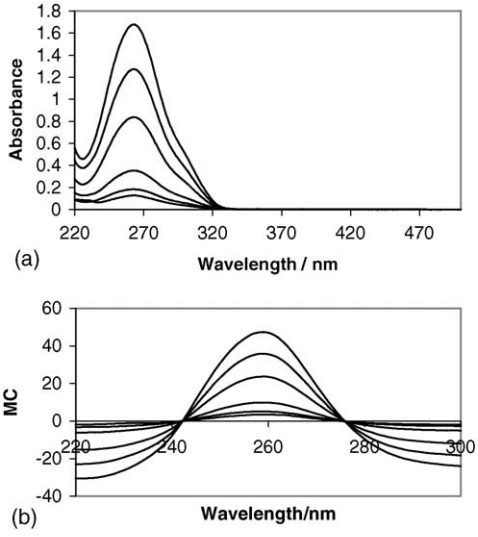


Fig. 5. The zero order spectra of different concentrations (0.5, 1, 2, 5, 10 and 15 μg mL$^{-1}$) of paracetamol in 0.1 M NaOH–methanol (1:9) (a) and MC of their ratio spectra (b) obtained according to Eq. (3).

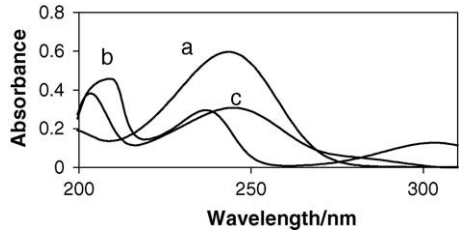


Fig. 6. The zero-order spectra of: (a) ascorbic acid; (b) salysilic acid; (c) paracetamol in methanol and 0.2 M HCl (1:3).

in methanol and 0.2 M HCl (1:3) overlapped in the region 220–310 nm.

The absorption spectra of the standard solutions of ASC with different concentrations were recorded in the wavelength range of 220–310 nm (Fig. 7a) and divided by the normalized spectrum of the PAR and the ratio spectra were obtained. Mean centering of the ratio spectra were obtained in the range 247–295 nm. After that these vectors (MC of ratio spectra) are divided by MC($\alpha_{ASA}/\alpha_{PAR}$) corresponding to the MC of the ratio of the normalized spectra of ASA and PAR and second ratio spectra (according to Eq. (4)) were obtained. MC of these vectors was obtained (Fig. 7b). The amount of ASC was determined by measuring the amplitude at 280 nm corresponding to a maximum wavelength in the MC of second ratio spectra as shown in Fig. 7b. For the prediction of concentration of ASC in synthetic ternary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of ASC.

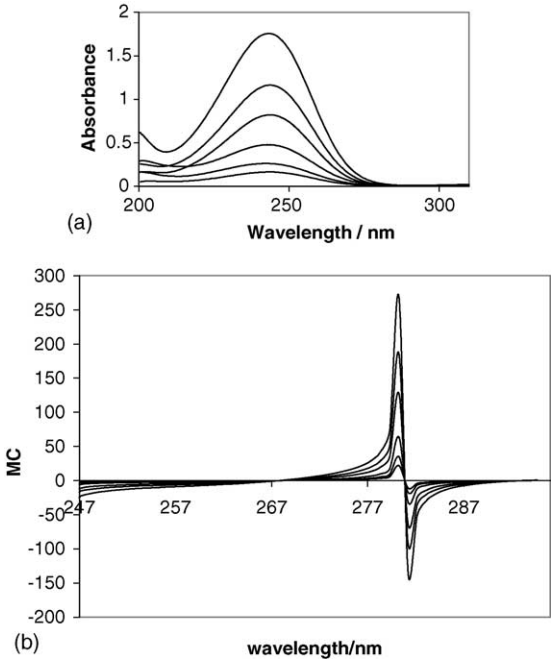


Fig. 7. The zero order spectra of different concentrations (2, 4, 8, 14, 20, and 30 μg mL$^{-1}$) of ascorbic acid in methanol and 0.2 M HCl (1:3) (a) and MC of their second ratio spectra (b) obtained according to Eq. (5).

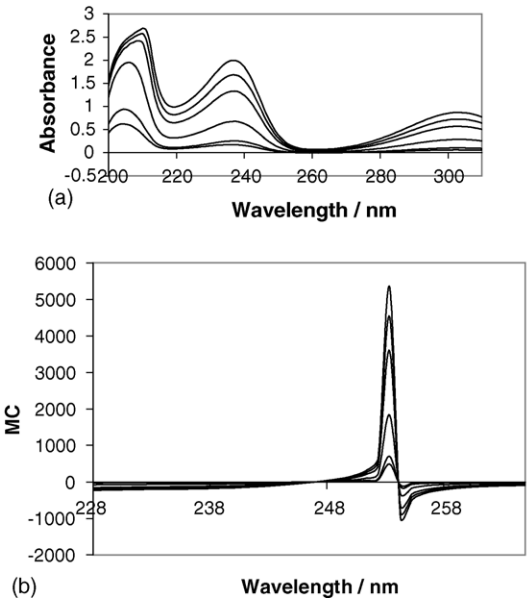


Fig. 8. The zero order spectra of different concentrations (2, 4, 10, 20, 26, and 30 μg mL$^{-1}$) of acetyl salysilic acid in methanol and 0.2 M HCl (1:3) (a) and MC of their second ratio spectra (b) obtained according to Eq. (5).

In the same way, the absorption spectra of the standard solutions of ASA with different concentrations were recorded in the wavelength range of 220–310 nm (Fig. 8a) and divided by the normalized spectrum of the ASC and the ratio spectra were obtained. Mean centering of the ratio spectra were obtained in the wavelength range of 230–265 nm. After that these vectors (MC of ratio spectra) are divided by MC($\alpha_{PAR}/\alpha_{ASC}$) corresponding to the MC of the ratio of the normalized spectra of PAR and ASC and second ratio spectra (according to Eq. (4)) were obtained. MC of these vectors was obtained (Fig. 8b). The amount of ASA was determined by measuring the amplitude at 253 nm corresponding to a maximum wavelength in the MC of second ratio spectra as shown in Fig. 8b. For the prediction of concentration of ASA in synthetic ternary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of ASA.

In the same way, the absorption spectra of the standard solutions of PAR with different concentrations were recorded in the wavelength range of 220–310 nm (Fig. 9a) and divided by the normalized spectrum of the ASC and the ratio spectra were obtained. Mean centering of the ratio spectra was obtained in the wavelength range of 230–275 nm. After that these vectors (MC of ratio spectra) are divided by MC($\alpha_{ASA}/\alpha_{ASC}$) corresponding to the MC of the ratio of the normalized spectra of ASA and ASC and second ratio spectra (according to Eq. (4)) were obtained. MC of these vectors was obtained (Fig. 9b). The amount of PAR was determined by measuring the amplitude at 272 nm corresponding to a minimum wavelength in the MC of second ratio spectra as shown in Fig. 9b. For the prediction of concentration of

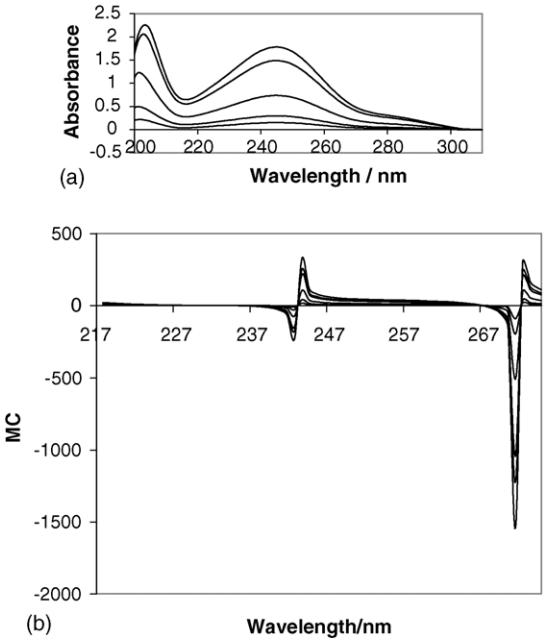


Fig. 9. The zero order spectra of different concentrations (2, 4, 10, 20 and 25 μg mL$^{-1}$) of paracetamol in methanol and 0.2 M HCl (1:3) (a) and MC of their second ratio spectra (b) obtained according to Eq. (5).

PAR in synthetic ternary mixtures and real samples the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of PAR.

### 5.3. Analytical characteristics

#### 5.3.1. Binary mixture

In the proposed method, Beer's law was obeyed in the concentration range 1–20 μg mL$^{-1}$ for PAR and 1–20 μg mL$^{-1}$ for MEF. Table 2 shows the linear regression parameters for calibration data for simultaneous determination of PAR and MEF in their binary mixtures. Limit of detection of the method for determination of PAR and MEF (defined as the concentration equivalent to three times the standard deviation of five replicate measurements of the blank) are also shown in Table 2.

To check the reproducibility of the method five replicate resolving of PAR and MEF mixtures were performed. The relative standard deviation (R.S.D.) for five replicate determinations of 10 μg mL$^{-1}$ each of MEF and PAR in binary mixtures was obtained as 2.02 and 1.39%, respectively. The mean recoveries for simultaneous determination of these species in binary mixtures were obtained as 99 and 100% for MEF and PAR, respectively.

The effect of divisor concentration on the analytical parameters such as detection limit, slope, intercept and correlation coefficient of the calibration graphs was also tested. It was observed that changing the concentration of divisors in their linear calibration range had no significant effect on the analytical parameters. Therefore, a normalized spectrum of each of the PAR and MEF was used as divisor spectrum in the proposed method. The amount of $\Delta\lambda$ had no effect on the mean centering of ratio spectra. A $\Delta\lambda$ of 1 nm was used.

Table 2
Analytical characteristics for analysis of MEF and PAR binary mixtures by the proposed method

| Analyte | Wavelength (nm) | Calibration equation | $R^2$[a] | Linear range (μg mL$^{-1}$) | LOD (μg mL$^{-1}$) |
|---|---|---|---|---|---|
| PAR | 259 | $Y = 2.3179C + 0.4029$ | 0.9999 | 1–20 | 0.08 |
| MEF | 249 | $Y = 0.3574C + 0.0039$ | 0.9996 | 1–20 | 0.45 |

[a] Squared calibration coefficient.

Table 3
Results for several experiments for analysis of MEF and PAR in binary mixtures in different concentration ratios by proposed method

| Taken (μg mL$^{-1}$) | | Found (μg mL$^{-1}$) | | Recovery (%) | |
|---|---|---|---|---|---|
| MEF | PAR | MEF | PAR | MEF | PAR |
| 1.0 | 20.0 | 0.9 | 20.2 | 92.8 | 101.24 |
| 2.0 | 15.0 | 2.0 | 15.0 | 101.9 | 100.30 |
| 3.0 | 10.0 | 2.9 | 9.7 | 96.6 | 97.46 |
| 4.0 | 7.0 | 4.1 | 7.1 | 101.5 | 101.79 |
| 5.0 | 5.0 | 5.0 | 5.1 | 99.4 | 101.88 |
| 7.0 | 3.0 | 6.8 | 3.0 | 97.4 | 99.39 |
| 10.0 | 2.0 | 9.7 | 2.0 | 97.4 | 97.61 |
| 12.0 | 1.0 | 11.9 | 1.0 | 99.3 | 95.55 |
| 15.0 | 10.0 | 14.8 | 10.0 | 98.6 | 100.00 |
| 20.0 | 1.0 | 19.9 | 1.1 | 99.3 | 110 |
| Mean recovery | | | | 98.4 | 100.5 |
| R.S.E single (%)[a] | | | | 1.43 | 1.34 |
| R.S.E.$_t$ total (%)[b] | | | | | 1.38 |

[a] Calculated by Eq. (6).
[b] Calculated by Eq. (7).

Table 4
Analytical characteristics for analysis of ASC, ASA and PAR by the proposed method

| Analyte | Wavelength (nm) | Calibration equation | $R^2$[a] | Linear range ($\mu g\,mL^{-1}$) | LOD ($\mu g\,mL^{-1}$) |
|---|---|---|---|---|---|
| ASC | 280 | $Y = 9.0393C - 0.3694$ | 0.9968 | 2–30 | 0.27 |
| ASA | 253 | $Y = 177.34C - 4.5079$ | 0.9992 | 2–30 | 0.1 |
| PAR | 272 | $Y = -50.264C + 3.6276$ | 0.9979 | 2–25 | 0.12 |

[a] Squared calibration coefficient.

Table 5
Results for several experiments for analysis of PAR, ASC and ASA in ternary mixtures by proposed method

| Taken ($\mu g\,mL^{-1}$) | | | Found ($\mu g\,mL^{-1}$) | | | Recovery (%) | | |
|---|---|---|---|---|---|---|---|---|
| ASC | ASA | PAR | ASC | ASA | PAR | ASC | ASA | PAR |
| 2.0 | 10.0 | 10.0 | 2.1 | 10.0 | 10.1 | 104.62 | 100.15 | 101.74 |
| 2.0 | 4.0 | 15.0 | 1.9 | 4.0 | 15.3 | 95.00 | 100.88 | 103.74 |
| 10.0 | 10.0 | 20.0 | 10.0 | 9.9 | 20.7 | 100.11 | 99.48 | 92.01 |
| 20.0 | 6.0 | 5.0 | 19.9 | 5.6 | 4.6 | 99.34 | 93.11 | 105.00 |
| 25.0 | 2.0 | 2.0 | 25.0 | 1.8 | 2.1 | 100.14 | 90.00 | 102.50 |
| 1.0 | 25.0 | 4.0 | 1.1 | 25.5 | 4.1 | 110.00 | 101.96 | 113.09 |
| 2.0 | 20.0 | 2.0 | 1.8 | 20.0 | 2.3 | 89.96 | 99.97 | 94.40 |
| 5.0 | 10.0 | 4.0 | 4.9 | 9.9 | 3.8 | 97.25 | 99.15 | 96.82 |
| 6.0 | 6.0 | 10.0 | 6.1 | 6.2 | 9.7 | 102.20 | 102.68 | 90.46 |
| 10.0 | 5.0 | 4.0 | 10.3 | 4.9 | 3.6 | 102.80 | 98.11 | 91.61 |
| Mean recovery | | | | | | 100.14 | 98.55 | 99.14 |
| R.S.E. single (%)[a] | | | | | | 1.25 | 1.86 | 4.03 |
| $R.S.E._t$ total (%)[b] | | | | | | | | 2.39 |

[a] Calculated by Eq. (6).
[b] Calculated by Eq. (7).

In order to obtain the accuracy and precision of the method, several synthetic mixtures with different concentration ratios of PAR and MEF were analyzed using the proposed method. The results are given in Table 3. The prediction error of a single component in the mixtures was calculated as the relative standard error (R.S.E) of the prediction concentration [19]:

$$\text{R.S.E.}\,(\%) = \left(\frac{\sum_{j=1}^{N}(\hat{C}_j - C_j)^2}{\sum_{j=1}^{N}(C_j)^2}\right)^{1/2} \times 100 \qquad (6)$$

where $N$ is the number of samples, $C_j$ the concentration of the component in the $j$th mixture and $\hat{C}_j$ the estimated concentration. The total prediction error of $N$ samples is calculated as follows:

$$\text{R.S.E.}_t(\%) = \left(\frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(\hat{C}_{ij} - C_{ij})^2}{\sum_{i=1}^{M}\sum_{j=1}^{N}(C_{ij})^2}\right)^{1/2} \times 100 \qquad (7)$$

where $C_{ij}$ is the concentration of the component in the $j$th samples and $\hat{C}_{ij}$ its estimation. Table 3 also shows the reasonable single and total relative errors for such system.

### 5.3.2. Ternary mixture

In the proposed method, Beer's law was obeyed in the concentration range 2–25 $\mu g\,mL^{-1}$ for PAR and 2–30 $\mu g\,mL^{-1}$ for ASC and 2–30 $\mu g\,mL^{-1}$ for ASA. Table 4 shows the linear regression parameters for calibration data for simultaneous determination of PAR, ASA and ASC in their ternary mixtures. Limit of detection of the method for determination of PAR, ASA and ASC in their ternary mixtures (defined as the concentration equivalent to three times the standard deviation of five replicate measurements of the blank) are also shown in Table 4.

To check the reproducibility of the method six replicate resolving of PAR, ASA and ASC in their ternary mixtures were performed. The relative standard deviation (R.S.D.) for six replicate determinations of 10 $\mu g\,mL^{-1}$ each of ASC, ASA

Table 6
Analytical characteristics for analysis of MEF and PAR by ratio derivative spectrophotometric procedures [3]

| Drug | λ (nm) | Calibration equation | | $r$[a] | Linear range ($\mu g\,mL^{-1}$) |
|---|---|---|---|---|---|
| | | Slop | Intercept | | |
| MEF | 327.5 | $1.58 \times 10^{-1}$ | $9.63 \times 10^{-3}$ | 0.9999 | 2.0–10.0 |
| MEF | 363.5 | $-1.80 \times 10^{-3}$ | $7.90 \times 10^{-3}$ | 0.9999 | 2.0–10.0 |
| PAR | 245.3 | $1.36 \times 10^{-1}$ | $-3.80 \times 10^{-3}$ | 0.9999 | 4.0–20.0 |
| PAR | 271.2 | $-1.21 \times 10^{-1}$ | $8.20 \times 10^{-3}$ | 0.9999 | 4.0–10.0 |

[a] Regression coefficient.

Table 7
Analytical characteristics for analysis of ASC, ASA and PAR by double divisor-ratio spectra derivation (DDRSD) and ratio spectra derivative-zero crossing (RSDZC) [14]

| Methods | λ (nm) | Calibration equation | $r^a$ | Linear range (μg mL$^{-1}$) |
|---|---|---|---|---|
| DDRSD | 271.8 | $Y = 1.2 \times 10^{-2} C_{ASA} - 2.5 \times 10^{-3}$ | 0.9999 | 8–28 |
| | 267.4 | $Y = 3.0 \times 10^{-2} C_{ASC} - 2.7 \times 10^{-3}$ | 0.9991 | 8–28 |
| | 241.5 | $Y = 2.5 \times 10^{-2} C_{PAR} + 5.9 \times 10^{-3}$ | 0.9998 | 8–28 |
| RSDZC | 286.1 | $Y = 2.4 \times 10^{-2} C_{PAR} + 7.2 \times 10^{-3}$ | 0.9995 | 8–28 |
| | 239.3 | $Y = 5.6 \times 10^{-2} C_{ASA} + 9.1 \times 10^{-4}$ | 0.9998 | 8–28 |
| | 292.4 | $Y = 4.7 \times 10^{-3} C_{ASA} + 8.0 \times 10^{-3}$ | 0.9980 | 8–28 |
| | 241.2 | $Y = 3.6 \times 10^{-2} C_{ASA} + 6.9 \times 10^{-3}$ | 0.9999 | 8–28 |
| | 255.1 | $Y = 9.1 \times 10^{-3} C_{ASC} + 1.9 \times 10^{-4}$ | 0.9998 | 8–28 |
| | 281.1 | $Y = 1.9 \times 10^{-3} C_{ASC} + 2.0 \times 10^{-4}$ | 0.9994 | 8–28 |

[a] Regression coefficient.

and PAR, in ternary mixtures was obtained as 2.20, 2.54 and 2.93%, respectively. The mean recoveries for simultaneous determination of these species in ternary mixtures were obtained as 99, 99 and 100% for ASC, ASA and PAR, respectively.

The effect of divisor concentration on the analytical parameters such as detection limit, slope, intercept and correlation coefficient of the calibration equations was tested. It was observed that changing the concentration of divisors in their linear calibration range had no significant effect on the analytical parameters. Therefore, a normalized spectrum of each of the PAR, ASC and ASA was used as divisor spectrum in the proposed method.

In order to obtain the accuracy and precision of the method, several synthetic mixtures with different concentration ratios of PAR, ASC and ASA were analyzed using the proposed method. The results are given in Table 5. The prediction error of a single component in the mixtures was calculated as the relative standard error (R.S.E.) of the prediction concentration according to Eqs. (6) and (7). Table 5 also shows the reasonable single and total relative errors for such system.

## 6. Conclusion

The proposed method is simple, very sensitive and easy to understand and apply. Accuracy, precision, reproducibility, sensitivity and linear rang for the proposed method are better than those reported for derivative-ratio spectra method [3,14]. Comparison of the results of Tables 2 and 4 with the results of Tables 6 and 7 proves that the analytical characteristics obtained by the proposed method for analysis of binary mixtures of MEF and PAR and ternary mixtures of ASC, ASA and PAR are significantly better than those obtained by ratio derivative methods for the same analysis [3,14].

Standard addition can be used in the proposed method and matrix effects can be removed easily. Therefore, this method can be used for resolving binary and ternary mixtures in the complex samples with unknown matrices. In contrary to the ratio derivative methods our proposed method eliminates the derivative steps and therefore the signal-to-noise ratio is not degrade. The linear ranges, relative standard deviations (R.S.D.), mean recoveries and relative standard errors show the applicability, accuracy and precision of the proposed method.

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