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# A novel pure component contribution algorithm (PCCA) for extracting components' contribution from severely overlapped signals; an application to UV-spectrophotometric data

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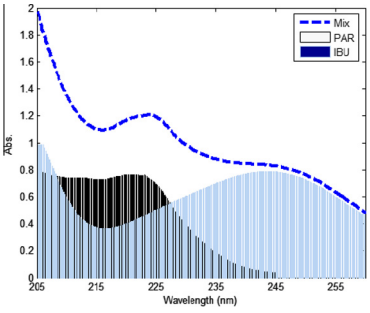
## HIGHLIGHTS

- Simple, highly accurate and sensitive pure component contribution algorithm was developed.
- Each component in the mixture is determined at its $\lambda_{max}$.
- It has been successfully applied for resolving binary and ternary mixtures.
- It does not need derivative calculations or an extended spectrum.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A novel, simple and accurate algorithm capable of extracting the contribution of each component from a mixture signal where the components are completely overlapped was developed. It is based on the development of a coded function which eliminates the signal of interfering components using mean centering as a processing tool; finally the pure contribution of each component is extracted. The algorithm allows the determination of each component as a single one. It was validated by the use of simulated data set of three overlapped signals and tested against simulated random noise. Two fit values were developed and calculated for optimization, one to test that that the absorptivity values of the extracted spectra are within the confidence limits of the slope and the other is the correlation between the pure and extracted spectra. It has been successfully applied to real UV data of binary mixture of Ibuprofen and Paracetamol and ternary mixture of Amiloride hydrochloride, Atenolol and Hydrochlorothiazide in tablets and capsules, respectively. The results were compared to previously reported separation method and no significant difference was found regarding both accuracy and precision.



## 1. Introduction

Several mathematical calculations have been reported for resolution of overlapped signals, while each has a limitation that should be considered during applications. Most of these techniques for signal resolution was applied on spectroscopic data.

Spectrophotometry is one of the simplest techniques utilizing instrument of relatively low cost and highest availability providing analytical methods which are simple, accurate and less time consuming if compared to chromatographic methods. Spectrophotometric methods are highly favorable in quality control laboratories; where a huge number of samples either raw materials, in-process or finished products have to be daily analyzed and released. While, its main problem is the low selectivity which somewhat hinders the simultaneous determination of

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multi-component mixtures. Several methods have been used for resolving mixtures of components with overlapping spectra; some are based on the spectral extension of one of the components over the others as ratio subtraction [1] and its extension [2], absorption subtraction [3], amplitude modulation [4] and absorption factor [5]. However, most of the developed methods utilize derivative calculation for spectral resolution [6]. Salinas et al. [7] proposed ratio-derivative spectrophotometry for binary mixtures and it was extended to ternary mixtures by ratio derivative zero crossing point [8–13]. Dinc et al. [6,14,15] developed the double divisor-ratio spectra derivative method. Afkhami and Bahram [16] developed successive derivative ratio spectra in which two successive derivative steps have been performed on ratio spectra. Amplitude factor [17] and derivative subtraction [18] were also developed and based on derivatization as a basic manipulation step. However, the advantages of derivative spectra are at least partially offset by degradation in signal-to-noise ratio that accompanies derivative calculations. A method for simultaneous determination of binary and ternary mixtures based on mean centering of ratio spectra was developed [19,20]. Other methods were also developed and based on the determination of the overlapped spectra without derivatization as ratio difference [2] and constant center [21] while, they are limited for determination of components in their binary mixtures.

The aim of this work was the development of simple and novel algorithm capable of resolution and extraction of the pure component contribution from their mixture signal without any requirements as either spectral extension or derivative calculation. Moreover, the suggestion of simple and accurate coded function which performs the calculation with well-defined fit values, this would minimize the error and guarantee best accuracy and precision.

For achieving this aim, pure component contribution algorithm (PCCA) was scripted in Matlab®. Mean centering was used in the calculation as a basic processing tool. The mathematical explanation of the procedure is illustrated. After modeling procedure, the method has been applied to the analysis of a binary mixture of Ibuprofen (IBU) and Paracetamol (PAR) and a ternary mixture of Amiloride hydrochloride (AML), Atenolol (ATN) and Hydrochlorothiazide (HCT) in tablets and capsules, respectively.

## 2. Algorithm development and validation

### 2.1. Theory of the proposed algorithm

Consider a mixture of three components *X*, *Y* and *Z*. If there is no interaction among them and Beer's law is obeyed for each component, it can be written:

$$A_m = \alpha_X C_X + \alpha_Y C_Y + \alpha_Z C_Z \quad (1)$$

where, $A_m$ 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)

$$B = A_m/\alpha_Z = \alpha_X C_X/\alpha_Z + \alpha_Y C_Y/\alpha_Z + C_Z \quad (2)$$

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

$$C = \mathrm{MC}(B) = \mathrm{MC}(\alpha_X C_X/\alpha_Z) + \mathrm{MC}(\alpha_Y C_Y/\alpha_Z) \quad (3)$$

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):

$$D = \mathrm{C}/\mathrm{MC}(\alpha_Y/\alpha_Z) = \mathrm{MC}(\alpha_X C_X/\alpha_Z)/\mathrm{MC}(\alpha_Y/\alpha_Z) + C_Y \quad (4)$$

Now if the Eq. (4) is mean centered, Eq. (5) would be obtained:

$$E = \mathrm{MC}(D) = \mathrm{MC}[\mathrm{MC}(\alpha_X C_X/\alpha_Z)/\mathrm{MC}(\alpha_Y/\alpha_Z)] \quad (5)$$

By dividing Eq. (5) by MC [MC($\alpha_X/\alpha_Z$)/MC($\alpha_Y/\alpha_Z$)], corresponding to the mean centering of the mean centered ratio of the spectra of the standard solutions of *X* & *Z and Y* & *Z*, constant value corresponding to $C_X$ *is obtained*, Eq. (6):

$$F = \mathrm{MC}[\mathrm{MC}(\alpha_X C_X/\alpha_Z)/\mathrm{MC}(\alpha_Y/\alpha_Z)]/\mathrm{MC}[\mathrm{MC}(\alpha_X/\alpha_Z)/\mathrm{MC}(\alpha_Y/\alpha_Z)] = C_X \quad (6)$$

By multiplying Eq. (6) by $\alpha_x$ corresponding to the spectrum of a standard solution of *x*, the absorption spectrum of X in the mixture is obtained as Eq. (7):

$$G = C_X * \alpha_X = \alpha_X C_X \quad (7)$$

Eq. (7) is the mathematical foundation of each component in the laboratory prepared mixtures and pharmaceutical formulations. This permits the determination of each component in the mixture (*X* in this equation) without interfering from the other components of the ternary system (*Y* and *Z* in these equations). As Eq. (7) shows, the obtained spectra permits the determination of component *X* by direct measurement of the estimated absorbance value at its $\lambda_{max}$ using the corresponding regression equation obtained by plotting the absorbance of the pure spectra of *X* at its $\lambda_{max}$ versus its corresponding concentration. Pure component contribution for *Y* and *Z* could also be obtained as described for *X*.

### 2.2. Algorithm coding

The previously detailed equations were scripted in sequence in matlab and a function was created in order to just by only entering all the steps will be calculated and the results directly presented (pure contribution of each compound in the mixture).

### 2.3. Algorithm optimization

In the purpose of optimization of the developed algorithm, different divisors should be tested and compared, in order to test the best divisor; two fit values were calculated for each divisor:

**Fit 1:** is absorptivity value ($\alpha$) for the estimated absorbance values at $\lambda_{max}$

**Fit 2:** is the correlation coefficient (*r*) calculated between the estimated pure contribution of each component and its standard spectrum.

The best divisor should results in:

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

### 2.4. Algorithm validation by simulated model data

The analytical applicability of the proposed algorithm was demonstrated for resolution of ternary mixtures by creating three overlapped spectra. The three curves form a model of the overlapping spectra of three components *X*, *Y* and *Z* in the range 200–300 nm (Fig. 1(a)). The created spectra for each component were used for construction of calibration curves representing the absorbance of each component at its $\lambda_{max}$ versus the corresponding concentration; the regression parameters for each component were calculated.

The method was proposed using 10 synthetic mixtures containing the considered components; *X*, *Y* and *Z* in different proportions