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An Alternative Approach to the Selection of Optimum Wavelengths for Bivariate Calibration Method

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ABSTRACT

This work aimed at comparing the effect of analytical wavelengths selection approach on the accuracy and precision of the bivariate calibration method. The combination of paracetamol and chlorzoxazone was chosen as a model for this study since their spectra are extensively overlapping over the range of 230 – 300 nm. In this study Kaiser method and sensitivities ratio method were used as wavelengths selection approaches. Kaiser method suggested 240 nm and 260 nm as optimum analytical wavelengths while the sensitivities ratio proposed 260 nm and 290 nm. Application of the bivariate method for the analysis of the two drugs mixture at the wavelength pair proposed by each method showed good method accuracy as the obtained actual concentrations of both analytes were close to theoretical ones and having percent relative standard deviations < 2%. The precision of the method at the selected wavelengths by each approach also showed percent relative standard deviations < 2%. Statistical comparison of the accuracy and precision results obtained, using t-test indicated that no significant difference between the results obtained by either of the two wavelength selection approaches. Based on these findings it can be concluded that sensitivities ratio approach is a good alternative for Kaiser method for analytical wavelengths selection for bivariate calibration method.

Keywords: *Bivariate calibration; Kaiser method; Sensitivities ratio method; Spectrophotometry.*

INTRODUCTION

Multicomponent spectrometric techniques focus on analyzing multiple compounds simultaneously within a mixture. They are widely employed for examining drugs in mixtures, pharmaceutical formulations, and clinical samples. The primary objective of multicomponent spectrometric analysis is to create a calibration model that correlates the readings from multivariate spectrometers with the composition or characteristics of the substances being analyzed. Multicomponent spectrometric presents analytical challenge as most of these analytes absorb light in the same spectral region with consequent overlapping of the spectra. When the overlapping between the analytes is not extensive, spectrophotometric methods based on simple mathematical manipulation of the spectral data are used (Beckett and Stenlake, 2001), complete spectra for the determination of all the components in the mixture is however used when extensive overlapping exists (Esbensen and Swarbrick, 2018).

The accuracy of determination can be improved by a careful selection of wavelength ranges, which results in a collinearity or spectral overlap reduction (Rossi and Pardue, 1985). The decision of how many data points and which wavelengths should be included is usually based

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on certain criteria (Mark, 1988). Various criteria have been developed to allow for wavelength selection (Mark, 1988; Lindberg et al. 1983; Jolliffe, 1986; Jochum et al. 1981; Juhl et al. 1986; Juhl et al. 1988; Otto and Wegscheider, 1986; Otto and George 1987; Frans and Harris, 1985; Bergmann et al. 1987; Kaiser, 1972; Ebel et al 1982**;** Junker and Bergmann 1974; Junker and Bergmann 1976; Honigs et al. 1983; Thijssen et al. 1985; Morgan, 1977; Warren et al. 1987; Smeyers-Verbeke et al. 1986; DiTusa and Schilt, 1985; Dinc et al. 2008; Lopez-de-Alba et al. 1997; Attia et al. 2016). Among the proposed methods, the determinant and the condition number of calibration matrix are the most preferred criteria for prediction of the best wavelength combination for exactly determined systems (Mark, 1988; Lindberg et al. 1983; Jolliffe, 1986; Jochum et al. 1981; Juhl et al. 1986; Juhl et al. 1988; Otto and Wegscheider, 1986; Otto and George 1987; Frans and Harris, 1985; Bergmann et al. 1987; Kaiser, 1972; Ebel et al 1982**;** Junker and Bergmann 1974; Junker and Bergmann 1976; Honigs et al. 1983; Thijssen et al. 1985; Morgan, 1977; Warren et al. 1987; Smeyers-Verbeke et al. 1986; DiTusa and Schilt, 1985). The matrix determinant technique developed by Kaiser (Kaiser, 1972) is commonly used for prediction of the optimum wavelengths pair to be used in bivariate calibration spectrophotometric technique (Lopez-de-Alba et al. 1997; Attia et al. 2016; Hegazy et al. 2013; López-de-Alba et al. 1997; Dinc et al. 2008), the technique involves cumbersome mathematical manipulation that requires advanced level of mathematical knowledge and computation. Di Tusa and co-workers (Di Tusa and Schilt, 1985) developed a simpler approach proposed to tackle this problem.

The intention of this work was to compare the effect of Kaiser and Di Tusa wavelengths selection criteria on the accuracy and precision of bivariate calibration method. From our previous studies [Dinc et al. 2008; ICH, 2005) we recognized the existence of extensive overlapping in the spectra of chlorzoxazone (CHL) and paracetamol (PAR), accordingly the tablet formulation combining the two drugs was selected as a model for the current study (Fig. 1**)**.

EXPERIMENTAL

Chemicals and Instruments

Paracetamol and Chlorzoxazone working standards were kindly provided by Blue Nile Pharmaceutical Company-Sudan. Relaxone capsules (Jamjoom Pharma –Kingdom of Saudi Araia): labeled to contain 500 mg of paracetamol and 300 mg of chlorzxoazone were purchased from Kingdom of Saudi Arabian., UV-Visible spectrophotometer UV 1800 (Shimadzu-Japan).

Diluting Solvent

Sodium hydroxide 0.1M was prepared by dissolving 4.0 gm of sodium hydroxide pellets in 1000 ml volumetric flask using distilled water.

Standards and Solutions

Standards Stock Solutions

Standard stock solutions of paracetamol (150 µg/mL) and Chlorzoxazone (150 µg/mL) were prepared separately by dissolving 15 mg each into 100 ml volumetric flask using 0.1M NaOH as a solvent.

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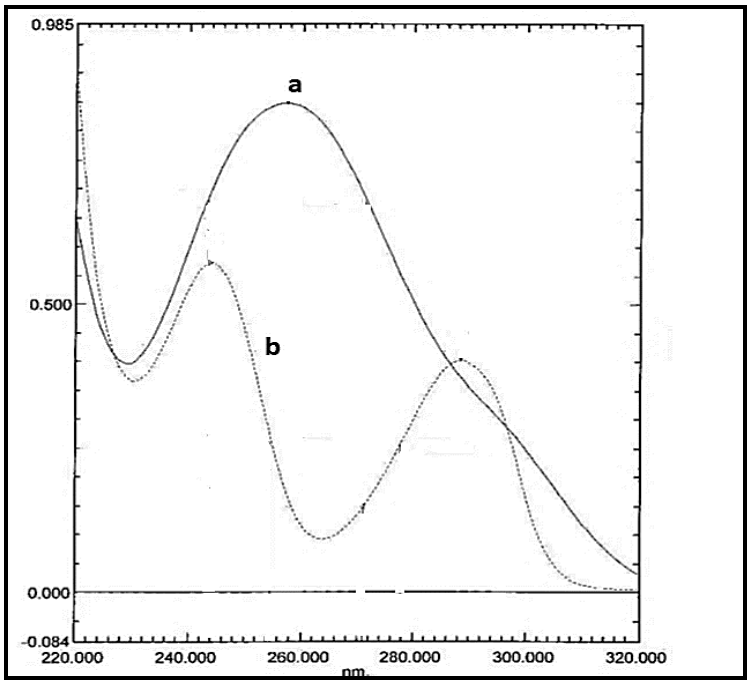

Figure 1: *Overlay spectra of a) paracetamol and b) chlorzoxazone 15 µg/ml each in 0.1 M NaOH*

Linearity Standards

Separate linearity standards sets of the two analytes were prepared by diluting aliquots from their corresponding stock standard solutions with 0.1M NaOH to give concentrations in the range of (2 -15 µg/mL) of each analyte.

Laboratory Synthetic Mixtures

Nine laboratory synthetic mixtures each containing different concentrations of paracetamol and chlorzoxazone were prepared by mixing different aliquot volumes from their stock solutions; according to multilevel multifactor approach (Brereton, 1997) in nine separate 50 mL volumetric flasks and making the volumes of the flasks to the mark with the solvent mixture. The scheme for preparation of the mixtures is given in Table 1.

Table 1. Laboratory Synthetic Mixtures Preparation Scheme

Mixture	PAR	CHL
1	0	0
2	-1	1
3	1	1
4	1	0
5	0	1
6	1	-1
7	-1	-1
8	-1	0
9	0	-1

-1 = low concentration, +1 = High concentration and 0 = intermediate concentration

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Sample Preparation

The content of twenty capsules was accurately weighed and mixed well. A quantity of the fine powder; equivalent to about 100 mg paracetamol was accurately weighed and transferred into a 100 mL volumetric flask, 50 mL 0.1 M NaOH were added and the mixture was sonicated for 5 minutes then the volume was made to the mark with 0.1 M NaOH, the solution was filtered using 0.45 µm nylon filter. Five mL of the clear filtrate were transferred into 50 mL volumetric flask and the volume was completed to the mark with 0.1 M sodium hydroxide, further 5 mL of this solution were transferred into 50 mL volumetric flask and the volume was completed to the mark with 0.1 N sodium hydroxide

Theoretical Background

According to the principle of bivariate calibration; obtaining the concentration of two components C_A and C_B existing in a mixture is carried out by solving the following equations

$$C_B = [m_{A2} (A_{AB1} - e_{AB1}) + m_{A1} (A_{AB2} - e_{AB2})] / m_{A2} m_{B1} - m_{A1} m_{B2} \quad (1)$$

$$C_A = [A_{AB1} - e_{AB1} - m_{B1} C_B] / m_{A1} \quad (2)$$

where, m_{Ai}, m_{Bi} are the slope values of linear regressions at two wavelengths, C_A, C_B are the concentrations of both components, e_{Ai}, e_{Bi} are the intercept values and e_{AB1}, e_{AB2} are the sum of the intercepts of linear calibration at two wavelengths. The two wavelengths should be selected to assure the best sensitivity and selectivity of the determination. Upon application of Kaiser method [14] for the selection of the optimum wavelengths, a series of sensitivity matrices, K, is created for each binary mixture:

$$k = \begin{bmatrix} m_{A_1} & m_{A_2} \\ m_{B_1} & m_{B_2} \end{bmatrix}$$

The values of the linear regression calibration slope evaluated for each component at λ_i, as the sensitivity factor. The determinants of these matrices are calculated and the obtained values used as the optimization criterion: A maximum sensitivity corresponds to a determinant with large diagonal elements and small off-diagonal elements.

The absorptivities ratio method (Di Tusa and Schilt, 1985) is based on the additive property of Beer's law for multicomponent mixture (equation 3).

$$A_{XY} = \varepsilon_X b C_X + \varepsilon_y b C_Y \quad (3)$$

The following set of equations (with b =1) can then be solved simultaneously for the concentrations of x and y:

$$A_1 = a_{x1} C_X + a_{y1} C_Y \quad (4)$$

$$A_2 = a_{x2} C_X + a_{y2} C_Y \quad (5)$$

If the absorbance at λ_1 is monitored as the concentration of species x is changed, and the second term in the above equation is kept constant, the following relationship results:

$$dA_1/dC_x = a_{x1}$$

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Similarly, if the absorbance at λ_2 is monitored as the concentration of species y is changed and the first term is kept constant, then

$$dA_2/dC_y = ay_2$$

In order to keep the transmitted light measurable (not too strongly absorbed) at λ_1 as well as at λ_2 for a broad range of concentrations of both x and y, the following conditions must hold:

$$ay_1 << ax_1$$

$$ax_2 << ay_2$$

i.e., the absorbance should he primarily determined by x at one wavelength and by y at the other wavelength. To find the two wavelengths that best satisfy all of these conditions, the plot absorptivitieis ratio of absorptivitieis ax/ay versus wavelengths should give a maximum at wavelength 1 and a minimum at wavelength 2. Thus, if the two wavelengths are selected on the basis of the above expressions, the concentrations of both x and y can be determined with optimum precision.

RESULTS AND DISCUSSION

Linearity and Calibration

The absorbance values of the calibration solutions of the two analytes were measured over the range of 230-300 nm at 10 nm intervals. The calibration curves were constructed for the absorbance values of the calibration solutions measured over the range of 230-300 nm at 10 nm intervals; versus their corresponding analyte concentration. from the standard drugs concentrations versus the absorbance values of the individual drugs at the selected wavelengths were linear with correlation coefficient values > 0.990, the residuals were spread uniformly and at random around the regression lines the and the confidence intervals of the intercepts contained the zero, confirming method linearity. The regression analysis data is shown in Tables 2 and 3.

Table 2. Paracetamol Linearity Data

Wavelength (nm)	**230**	**240**	**250**	**260**	**270**	**280**	**290**	**300**
Concentration	**2 – 15 (µg/mL)**							
Slope	0.0029	0.0049	0.0063	0.0066	0.0057	0.0039	0.0029	0.0020
Intercept	0.0186	0.0042	0.0179	0.0022	0.0106	0.1083	0.0023	0.0146
Correlation coefficient	0.9956	0.9998	0.9998	0.9997	0.9998	0.9992	0.9996	0.9960
LOD (µg/mL)	0.985	0.199	0.192	0.254	0.191	0.417	0.277	0.938
LOQ (µg/mL)	2.980	0.603	0.581	0.768	0.577	1.263	0.840	2.842

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Table 3. Chlorzoxazone Linearity Data

Wavelength (nm)	**230**	**240**	**250**	**260**	**270**	**280**	**290**	**300**
Concentration				**2 -15 (µg/ml)**				
Slope	0.0030	0.0047	0.0043	0.0011	0.0011	0.0028	0.0035	0.0014
Intercept	0.0029	0.0033	0.0175	0.0061	0.0120	0.0096	0.0229	0.0134
Correlation coefficient	0.9996	0.9996	0.9990	0.9991	0.9966	0.9988	0.9998	0.9993
LOD (µg/mL)	0.298	0.279	0.462	0.446	0.866	0.516	0.214	0.394
LOQ (µg/mL)	0.904	0.847	1.400	1.352	2.625	1.654	0.650	1.195

Selection of Optimum Wavelengths

The slopes (sensitivities) of the regression lines at the wavelengths over the range studied were used to form the sensitivity matrices for the determination the optimum wavelength pair. According to Kaiser's method the optimum wavelength pair was found to be 240 and 260 nm which represent the determinant with large diagonal elements and small off-diagonal elements as shown in Table 4.

Table 4. Values of Determinants of Sensitivity Matrices

λ (nm)	**230**	**240**	**250**	**260**	**270**	**280**	**290**	**300**
230	0	101.235	660.95	1658.3	1379.4	376.33	-131.38	198
240		0	902.78	**2592.68**	2149.77	502.67	-328.29	266.6
250			0	2139.17	1734.56	-74.08	-971.77	-25.1
260				0	-112.51	-1403.84	-2012.92	-703.8
270					0	-1142.21	-1683.30	-572
280						0	-568.023	7.9
290							0	296.1
300								0

The sensitivities ratios over the studied wavelengths range were calculated to determine the suitable wavelength pair, the values of the calculated ratios against their corresponding wavelengths are given in Table 5. The optimum wavelength pair according to this method was found to be 260 nm and 290 nm.

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Table 5. Sensitivities Ratio (230 – 300 nm)

Λ (nm)	PAR	CHL	Ratio
230	0.0029	0.0030	1.05
240	0.0049	0.0047	0.97
250	0.0063	0.0043	0.68
260	0.0066	0.0011	**0.17**
270	0.0057	0.0011	0.20
280	0.0039	0.0028	0.71
290	0.0029	0.0035	**1.20**
300	0.0020	0.0014	0.70

Accuracy

The accuracy of the method was tested by analyzing nine laboratory prepared synthetic mixtures containing different concentrations of PAR and CHL. The mixtures were analyzed according to bivariate method at the wavelength pair obtained according to Kaiser's method or the sensitivities ratio method.

Kaiser's Method

The results of the determination of the two analytes showed good agreement between the theoretical and actual concentrations of the two analytes. The average recoveries and relative standard deviations of PAR and CHL were 100.82 %, 1.97 % and 99.72, 1.60 %, respectively. The small relative standard deviations (<2 %) support the accuracy of the method according to the requirements of the International Conference on Harmonization (ICH). The accuracy study results are summarized in Tables 6.

Table 6. Accuracy and Precision Data According to Kaiser's Method

Parameter	PAR	CHL
Precision (n= 6)		
Repeatability (n= 6) ± (%RSD)	98.15% ± 0.60	97.18% ± 0.79
Intermediate precision (n= 12) ± (%RSD)	100.30% ± 0.73	98.21% ± 0.88
Accuracy (n = 9)		
% Recovery ± (%RSD)	100.82 ± 1.97	99.72 ± 1.60

Sensitivities Ratio Method

Using the information obtained from the linear regression equations of the two analytes at the selected wavelength pair, good results were obtained for the determination of the two analytes good agreements between the theoretical and actual concentrations of the two analytes. The average recoveries and percent relative standard deviations of PAR and CHL were 100.81 %, 1.97 % and 99.58 %, 1.35 % respectively. The small relative standard

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deviations (<2 %) satisfies the requirement for accuracy according to ICH [30]. The accuracy study results are summarized in Tables 7.

Table 7. Accuracy and Precision Data According to Sensitivities Ratio Method

Parameter	PAR	CHL
Precision (n= 6)		
Repeatability (n= 6) ± (%RSD)	97.56% ± 0.59	102.24% ± 1.02
Intermediate precision (n= 12) ± (%RSD)	100.12% ± 0.67	99.25% ± 1.88
Accuracy (n = 9)		
% Recovery ± (%RSD)	100.82 ± 1.93	99.58 ± 1.35

Further statistical comparison of the effect of wavelength selection approach on percent recovery indicated that there no difference in the results when either of the two approaches was used for the analytical wavelengths selection, as the calculated t- values were less than tabulated ones (n = 9, p = 0.05) as shown in Table 7.

Table 8. Comparison of Effect of Wavelengths Selection on Accuracy

Analyte	mean % ± RSD %	t - calculated	t – tabulated (n = 9 , p = 0.05)
Paracetamol	100.02 ± 1.97* 100.92 ± 1.93 **	0.095	2.12
Chlorzoxazone	99.52 ± 1.60* 99.58 ± 1.35**	0.098	

* Kaiser's method, ** Sensitivities ratio

Precision

The precision of the method was evaluated by analyzing the six independent samples from the commercial product on two different days. The percent relative standard deviations (%RSD) of the assay repeatability on two different days were less than 2% and the % RSD of the combined assay values (intermediate precision) from both days were less than 3.0%. The two precision parameters were found to be within the range indicated by the ICH; hence confirming good precision of the proposed approach [30]. The results are summarized in Tables 6 and 7.

Comparison of the effect of wavelength selection approach on the bivariate method precision was performed using Student t-test. The results indicated that there no significant difference when either of the two approaches was used for the analytical wavelengths selection, as the calculated t- values were less than tabulated ones (n = 6, p = 0.05) as shown in Table 8.

CONCLUSION

Statistical comparison of the accuracy and precision results obtained by using the two criteria indicated that no significant difference exists between the two criteria for wavelength

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selection; although each criteria revealed different wavelength pair. This finding strongly suggest that the sensitivities ratio can serve as simple approach which can be used with confidence for the selection of the analytical wavelengths for bivariate method as an alternative to Kaiser method.

Table 9. Comparison of Effect of Wavelengths Selection on Precision

Analyte	mean % ± RSD %	t - calculated	t – tabulated (n = 6 , p = 0.05)
Paracetamol	100.30 ± 0.73* 100.12 ± 0.67 **	0.440	
Chlorzoxazone	98.21 ± 0.89* 99.25 ± 1.88**	1.24	2.23

** Kaiser method, ** Sensitivities ratio*

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