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SPECTROPHOTOMETRIC SIMULTANEOUS ANALYSIS OF TWO-COMPONENT MIXTURE BY BIVARIATE AND MULTIVARIATE CALIBRATIONS USING THE LINEAR REGRESSION FUNCTIONS

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Spectral bivariate calibration (**SBC**) and spectral multivariate calibration (**SMC**) approaches based on the linear regression models were applied to the simultaneous determination of chlorzoxazone (**CHL**) and paracetamol (**PAR**) in tablets. **SBC** model was constructed by using four linear regression functions at two-wavelength set (220 and 244 nm) selecting two optimal wavelengths among 25 wavelength points. **SMC** model for each compound was obtained by using of 50 linear regression functions at 25 wavelength points in the spectral range 220-293 nm without selecting any optimal wavelength points. **SBC** and **SMC** was validated by using an independent validation set containing **CHL-PAR** combinations in the linear concentration range. The recovery results were found between 100.6% and 102.6% for both proposed appropaches, therefore we conclude that a successful method application was reported. The results obtained in this study clearly indicate that the proposed **SBC** and **SMC** approaches are suitable to be used for the quality control and routine analysis of the commercial tablets containing **CHL** and **PAR** compounds.

INTRODUCTION

In the last decade, several chemometric resolutions have been used for both qualitative and quantitative analysis of mixtures or commercial pharmaceutical preparations containing one or more active compounds together with a constant matrix.[1-6]

In order to deal with the quantitative analysis of complex samples the chemometric approaches have been applied to the spectrophotometric,[7-11] chromatographic[11] and electrochemical analyses.[12]

Getting the chemometric calibrations for sample analysis require data processing by using some additional related powerful softwares based on various mathematical computations, such as the abstract vector decomposition procedure. Finally, one of the main problems for an analytical chemist is to grasp and use the complexity of the mathematical approaches in chemometrics.

Despite the fact that the usual classical derivative method and its modified versions have been applied to various mixtures, it appears that this technique is unable to provide desirable results in all cases. For instance the higher order derivative diminishes the peak amplitudes and decreases the signal-to-noise ratio; therefore it is difficult to find an optimal zero-crossing point to perform the calibration graph. As shown in [13-15] the application of the derivative method to the analysis of ternary mixture does not provide reliable results for all cases. [13-15]

Based on a separation step procedure and a complex component use, HPLC and its related hyphenated versions were applied to sample analysis but they require a long time period for the optimization of the chromatographic conditions and high cost for analysis. In some particular cases, the above mentioned chromatographic methods do not provide the desirable results due to the problems of the separation procedure.

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For the spectrophotometric quantitative resolution of two-component mixtures, the bivariate calibration method was elaborated by López-de-Alba and co-workers.[16-18] Taking into account this approach a new method based on the simultaneous use of multi-liner regression functions, was worked out[18] and applied to several mixtures.[19-22]

The quantitative analysis of **PAR** combinations with other active compounds by spectrophotometry[16-19,21-27] and by HPLC[28-29] has been presented in the literature.

The goal of this paper is to introduce simple mathematical approaches for the simultaneous determination of PAR and HCL in their binary mixtures. These mathematical **SBC** and **SMC** methods are based on four linear regression functions for both compounds obtained at the selected two wavelengths (220 nm and 244 nm) and 50 linear regression functions obtained at 25 wavelength points in the spectral range of 220 and 293 nm with intervals of $\Delta\lambda$= 3nm, respectively. The validity of two mathematical approaches was performed by analyzing various synthetic mixtures of CHL and **PAR** compounds. The proposed **SBC** and **SMC** approaches can be used for the routine quality control of the tablet containing **CHL** and **PAR** compounds.

EXPERIMENTAL

Apparatus and Software

Shimadzu UV-1601 double beam UV-Visible spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E320 printer were used to record the absorption spectra and their absorbance measurements. The statistical and chemometric treatment of data were elaborated by using writing a simple algorithm in MATLAB 7.0 and Microsoft EXCEL softwares.

Commercial pharmaceutical preparation

A commercial pharmaceutical tablet (Parafon® Tablet produced by SANTA FARMA Pharm. Ind., Turkey) containing 300 mg **PAR a**nd 250 mg **CHL** per tablet was analysed.

Standard solutions

Stock solutions of **PAR** and **CHL** (50 mg/100 mL for each compound) were prepared in methanol. A standard series for both **PAR** and **CHL** in the linear concentration range of 4-14 μg/mL and 6-16 μg/mL, respectively, was obtained from the above stock solutions. An independent set consisting of 12 synthetic mixture solutions of **PAR** and **CHL** in the above concentration range were obtained by dilution of stocks. In this study 0.1 M HCI was used for all dilutions.

RESULTS AND DISCUSSION

For the mathematical approaches, **SBC** and **SMC**, the absorption spectra of **CHL** and **PAR** in the linear concentration range between 6-16 μg/mL and 4-22 μg/mL were registered and stored in the spectral region of 210-310 nm, respectively. A similar spectral registration procedure was applied to the other sample solutions. As it is shown in Fig. 1a and Fig. 1b, the UV-spectra of **CHL** and **PAR** overlap in the same spectral region of 210-310 nm. Therefore, the quantitative resolution of the mixture consisting of **CHL** and **PAR** cannot be obtain by the usual spectral techniques.

In the previous studies, the analysis of **CHL** and **PAR** was performed by using chromatographic and other spectrophotometric methods. However, these indicated methods require the use of a separation step for HPLC, a graphical treatment for derivative spectrophotometry and complex mathematical calculations for chemometric calibrations.

Having in mind to minimize or to eliminate the disadvantages of the above graphical and chromatographic analysis methods, two simple mathematical **SBC** and **SMC** models were elaborated for the simultaneous spectrophotometric determination of **CHL** and **PAR** in samples.

In this paper, the elaborated **SBC** and **SMC** contain simple mathematical contents and they are easy to be applied for the analysis of both compounds.

Spectral bivariate calibration approach

The **SBC** model requires the selection of the critical wavelengths which possess higher sensitivities in the spectral range between 210-310 nm of both compounds. 25 wavelengths with the length of interval $\Delta\lambda$=3 nm were selected and linear regression functions for each wavelength point were obtained for both compounds.

The calculated linear regression equations together with their corresponding statistical results were depicted in Table 1. The slopes of the linear regression equations shown in Table 1 provide the sensitivity matrices presented in Table 2.

The Kaiser technique [30-31] provides the selection of the best wavelength pair in order to construct **SBC** from the absolute values of the determinant of the sensitivity matrices. This calculation is based on the following formula

$$C_n^p = \frac{p!}{(p-n)!n!} \qquad (1)$$

where C_n^p denotes the number of two pairs of sensitivity matrices, p represents the number of wavelength and n indicates the number of the compounds. According to the above formula the values of the determinant of sensitivity matrices in different 300 combinations were obtained.

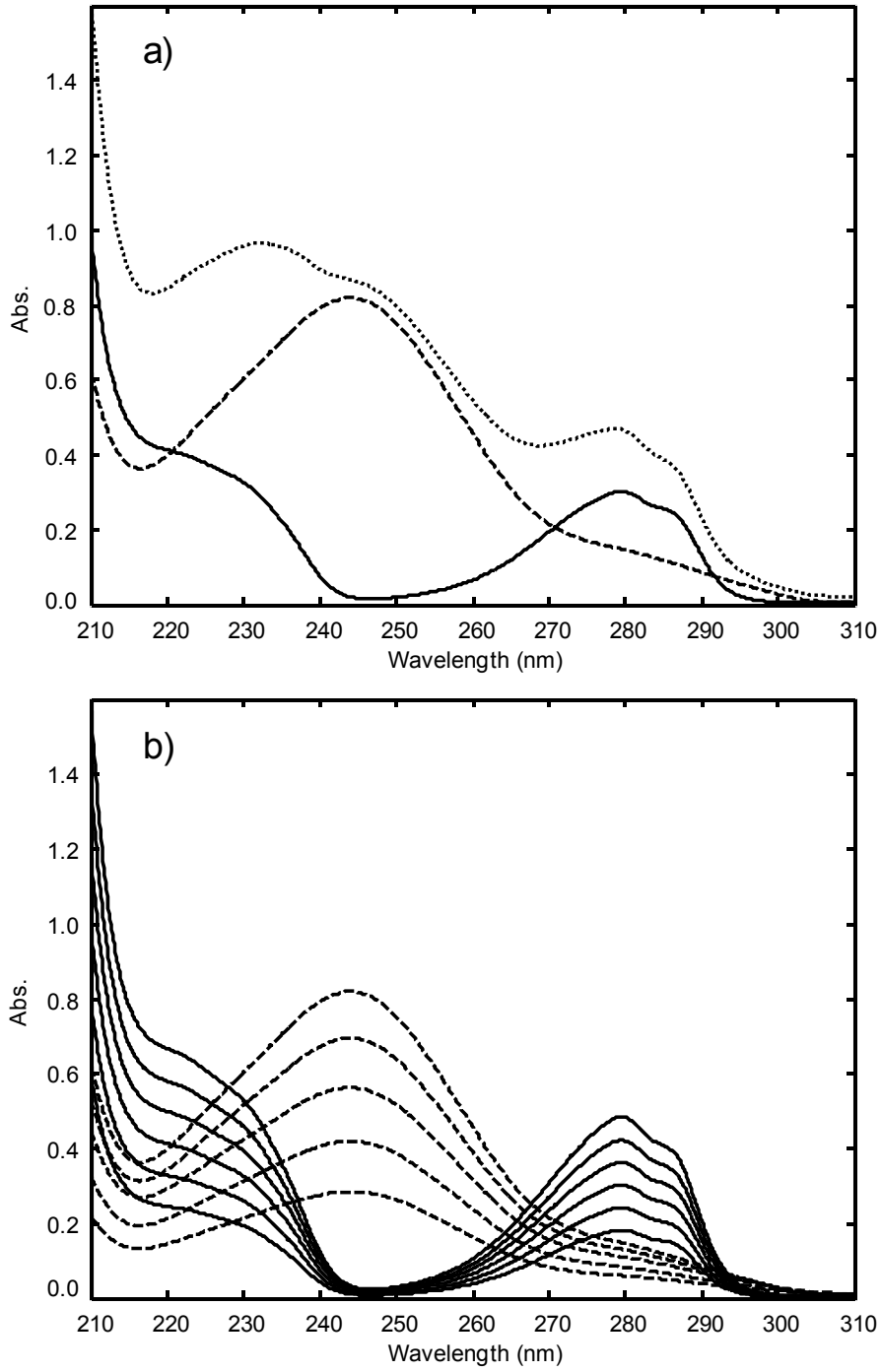

Fig. 1 – a) Zero-order spectra of **CHL** (—), **PAR** (----) and their binary mixture (.....) and b) Zero-order spectra of **PAR** and **CHL** in the concentration range of 6-16 μg/mL and 4-14 μg/mL, respectively.

Table 1

Statistical resulsts for the linear regression analysis

	CHL								PAR							
λ (nm)	m	n	r	se(m)	se(n)	se(r)	LOD	LOQ	m	n	r	se(m)	se(n)	se(r)	LOD	LOQ
220	0.0422	-0.0089	1.0000	$1.24x10^{-4}$	$1.42 x10^{-3}$	$1.04 x10^{-3}$	0.25	0.83	0.0317	0.0245	0.9981	$9.90 x10^{-4}$	$9.53 x10^{-3}$	$8.28 x10^{-3}$	2.21	7.37
223	0.0403	-0.0074	1.0000	$1.05 x10^{-4}$	$1.21 x10^{-3}$	$8.81 x10^{-4}$	0.22	0.74	0.0367	0.0232	0.9989	$8.77 x10^{-4}$	$8.44 x10^{-3}$	$7.33 x10^{-3}$	1.69	5.63
226	0.0376	-0.0069	1.0000	$1.12 x10^{-4}$	$1.29 x10^{-3}$	$9.34 x10^{-4}$	0.25	0.84	0.0421	0.0222	0.9992	$8.23 x10^{-4}$	$7.92 x10^{-3}$	$6.88 x10^{-3}$	1.38	4.61
229	0.0345	-0.0060	1.0000	$1.39 x10^{-4}$	$1.60 x10^{-3}$	$1.16 x10^{-3}$	0.34	1.13	0.0472	0.0225	0.9994	$7.85 x10^{-4}$	$7.55 x10^{-3}$	$6.57 x10^{-3}$	1.18	3.92
232	0.0299	-0.0051	0.9999	$1.51 x10^{-4}$	$1.73 x10^{-3}$	$1.26 x10^{-3}$	0.43	1.42	0.0523	0.0212	0.9996	$7.35 x10^{-4}$	$7.07 x10^{-3}$	$6.15 x10^{-3}$	0.99	3.31
235	0.0228	-0.0055	0.9999	$1.25 x10^{-4}$	$1.43 x10^{-3}$	$1.04 x10^{-3}$	0.46	1.54	0.0576	0.0196	0.9997	$7.23 x10^{-4}$	$6.96 x10^{-3}$	$6.05 x10^{-3}$	0.89	2.96
238	0.0138	-0.0042	0.9999	$7.74 x10^{-5}$	$8.91 x10^{-4}$	$6.47 x10^{-4}$	0.48	1.59	0.0625	0.0186	0.9998	$6.64 x10^{-4}$	$6.39 x10^{-3}$	$5.56 x10^{-3}$	0.75	2.51
241	0.0057	-0.0029	0.9995	$9.29 x10^{-5}$	$1.07 x10^{-3}$	$7.78 x10^{-4}$	1.38	4.61	0.0659	0.0190	0.9998	$6.37 x10^{-4}$	$6.13 x10^{-3}$	$5.33 x10^{-3}$	0.68	2.28
244	0.0022	-0.0012	0.9982	$6.55 x10^{-5}$	$7.54 x10^{-4}$	$5.48 x10^{-4}$	2.52	8.40	0.0673	0.0184	0.9998	$6.32 x10^{-4}$	$6.08 x10^{-3}$	$5.28 x10^{-3}$	0.66	2.21
247	0.0016	-0.0003	0.9967	$6.39 x10^{-5}$	$7.36 x10^{-4}$	$5.35 x10^{-4}$	3.44	11.47	0.0659	0.0178	0.9998	$5.77 x10^{-4}$	$5.55 x10^{-3}$	$4.82 x10^{-3}$	0.62	2.06
251	0.0021	0.0007	0.9981	$6.39 x10^{-5}$	$7.36 x10^{-4}$	$5.35 x10^{-4}$	2.61	8.70	0.0599	0.0181	0.9998	$6.16 x10^{-4}$	$5.93 x10^{-3}$	$5.15 x10^{-3}$	0.73	2.42
254	0.0031	-0.0003	0.9979	$1.00 x10^{-4}$	$1.16 x10^{-3}$	$8.40 x10^{-4}$	2.75	9.17	0.0530	0.0186	0.9998	$5.43 x10^{-4}$	$5.23 x10^{-3}$	$4.55 x10^{-3}$	0.73	2.42
257	0.0045	-0.0003	0.9990	$1.00 x10^{-4}$	$1.16 x10^{-3}$	$8.40 x10^{-4}$	1.88	6.27	0.0452	0.0182	0.9998	$4.33 x10^{-4}$	$4.17 x10^{-3}$	$3.62 x10^{-3}$	0.68	2.26
260	0.0065	-0.0003	0.9995	$1.00 x10^{-4}$	$1.16 x10^{-3}$	$8.40 x10^{-4}$	1.30	4.35	0.0371	0.0178	0.9997	$4.44 x10^{-4}$	$4.27 x10^{-3}$	$3.71 x10^{-3}$	0.85	2.82
263	0.0095	-0.0013	0.9998	$1.00 x10^{-4}$	$1.16 x10^{-3}$	$8.40 x10^{-4}$	0.89	2.98	0.0292	0.0183	0.9996	$4.28 x10^{-4}$	$4.12 x10^{-3}$	$3.58 x10^{-3}$	1.04	3.45
266	0.0133	-0.0008	0.9999	$1.14 x10^{-4}$	$1.31 x10^{-3}$	$9.51 x10^{-4}$	0.72	2.41	0.0228	0.0180	0.9994	$4.08 x10^{-4}$	$3.93 x10^{-3}$	$3.42 x10^{-3}$	1.27	4.23
269	0.0178	-0.0008	0.9999	$1.37 x10^{-4}$	$1.58 x10^{-3}$	$1.15 x10^{-3}$	0.65	2.17	0.0179	0.0185	0.9990	$3.95 x10^{-4}$	$3.81 x10^{-3}$	$3.31 x10^{-3}$	1.56	5.20
272	0.0223	0.0012	0.9999	$1.42 x10^{-4}$	$1.64 x10^{-3}$	$1.19 x10^{-3}$	0.54	1.80	0.0148	0.0189	0.9986	$3.86 x10^{-4}$	$3.72 x10^{-3}$	$3.23 x10^{-3}$	1.84	6.14
275	0.0264	0.0009	1.0000	$1.21 x10^{-4}$	$1.40 x10^{-3}$	$1.02 x10^{-3}$	0.39	1.30	0.0129	0.0185	0.9981	$3.95 x10^{-4}$	$3.81 x10^{-3}$	$3.31 x10^{-3}$	2.16	7.20
278	0.0295	0.0004	1.0000	$1.37 x10^{-4}$	$1.58 x10^{-3}$	$1.15 x10^{-3}$	0.39	1.31	0.0118	0.0177	0.9983	$3.45 x10^{-4}$	$3.32 x10^{-3}$	$2.89 x10^{-3}$	2.08	6.92
281	0.0294	0.0006	1.0000	$1.00 x10^{-4}$	$1.16 x10^{-3}$	$8.40 x10^{-4}$	0.29	0.96	0.0106	0.0175	0.9982	$3.19 x10^{-4}$	$3.07 x10^{-3}$	$2.67 x10^{-3}$	2.13	7.11
284	0.0260	0.0010	1.0000	$1.08 x10^{-4}$	$1.25 x10^{-3}$	$9.05 x10^{-4}$	0.35	1.17	0.0091	0.0185	0.9975	$3.19 x10^{-4}$	$3.07 x10^{-3}$	$2.67 x10^{-3}$	2.49	8.29
287	0.0232	0.0011	0.9999	$1.27 x10^{-4}$	$1.47 x10^{-3}$	$1.06 x10^{-3}$	0.46	1.55	0.0076	0.0179	0.9969	$3.00 x10^{-4}$	$2.88 x10^{-3}$	$2.51 x10^{-3}$	2.80	9.33
290	0.0130	0.0010	0.9999	$1.10 x10^{-4}$	$1.26 x10^{-3}$	$9.18 x10^{-4}$	0.72	2.39	0.0060	0.0173	0.9950	$3.01 x10^{-4}$	$2.90 x10^{-3}$	$2.52 x10^{-3}$	3.55	11.82
293	0.0047	0.0008	0.9999	$3.87 x10^{-5}$	$4.46 x10^{-4}$	$3.24 x10^{-4}$	0.70	2.33	0.0044	0.0177	0.9925	$2.70 x10^{-4}$	$2.60 x10^{-3}$	$2.26 x10^{-3}$	4.36	14.52

Table 2

Sensitivity valuse **CHL** and **PAR** compound for each linear regression function at 25 wavelength

No.	λ (nm)	$B_{CHL}x10^{-2}$	$B_{PAR}x10^{-2}$
1	220	4.22	3.17
2	223	4.03	3.67
3	226	3.76	4.21
4	229	3.45	4.72
5	232	2.99	5.23
6	235	2.28	5.76
7	238	1.38	6.25
8	241	0.57	6.59
9	244	0.22	6.73
10	247	0.16	6.59
11	251	0.21	5.99
12	254	0.31	5.30
13	257	0.45	4.52
14	260	0.65	3.71
15	263	0.95	2.92
16	266	1.33	2.28
17	269	1.78	1.79
18	272	2.23	1.48
19	275	2.64	1.29
20	278	2.95	1.18
21	281	2.94	1.06
22	284	2.60	0.91
23	287	2.32	0.76
24	290	1.30	0.60
25	293	0.47	0.44

As shown in Table 3, the highest absolute determinant value of the sensitivity matrices among 300 determinant values was found to be 220/244 wavelength pair, therefore it is considered the optimal two-wavelength set to construct the **SBC** approach.

Table 4 shows the linear regression equations for **CHL** and **PAR** at 220/244 wavelength pair. Therefore, the equation set corresponding to the **SBC** method was depicted in Table 5. The next step was to apply the **SBC** approach described in [18-22] to the equation set shown in Table 5. The analysis of **CHL** and **PAR** compounds in the synthetic mixtures given in Table 7 was performed by the obtained **SBC** approach.

Spectral multivariate calibration approach

Generalization of the **SBC** approach is called **SMC** and it contains in our specific case *25*-wavelength procedure instead of two-wavelengths. [18-22] Hence, the 25-wavelengths set, as shown in Table 1, at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range of 220-293 nm were used to construct the **SMC** model for the analysis of CHL and PAR in their mixture. As indicated in Table 1, 25 linear regression equations for each active compound were obtained by measuring the zero-order absorbance values at the wavelengths set.

The equation set corresponding to the **SMC** approach is indicated in Table 6 and it was obtained with the help of numerical values of m and n presented in Table 1. The next step is to apply the **SMC** algorithm to the equation set indicated in Table 6. Therefore, the **SMC** approach was used to the resolution of the binary mixture containing **CHL** and **PAR**.

Table 3

The optimal wavelength points by the Kaiser method for the sellection of the wavelength paire for the analysis of the **CHL-PAR** mixture

λ/λ	220	223	226	229	232	235	238	241	244	247	251	254	257	260	263	266	269	272	275	278	281	284	287	290	293
220	0.0	2.7	5.8	9.0	12.6	17.1	22.0	26.0	**27.7**	27.3	24.6	21.4	17.6	13.6	9.3	5.4	1.9	0.8	2.9	4.4	4.8	4.4	4.1	1.6	0.4
223		0.0	3.2	6.4	10.1	14.8	20.1	24.5	26.3	26.0	23.4	20.2	16.6	12.6	8.3	4.3	0.7	2.2	4.5	6.1	6.5	5.9	5.5	2.4	0.0
226			0.0	3.2	7.1	12.1	17.7	22.4	24.4	24.1	21.6	18.6	15.1	11.2	7.0	3.0	0.8	3.8	6.3	8.0	8.4	7.5	6.9	3.2	0.3
229				0.0	3.9	9.1	15.0	20.0	22.2	22.0	19.7	16.8	13.5	9.7	5.6	1.6	2.2	5.4	8.0	9.9	10.2	9.1	8.3	4.1	0.7
232					0.0	5.3	11.5	16.7	19.0	18.9	16.8	14.2	11.2	7.7	3.8	0.1	4.0	7.2	10.0	11.9	12.2	10.9	9.9	5.0	5.0
235						0.0	6.3	11.7	14.1	14.1	12.4	10.3	7.7	4.7	1.2	2.5	6.2	9.5	12.3	14.3	14.5	16.9	18.8	19.6	19.2
238							0.0	5.5	7.9	8.1	7.0	5.4	3.4	1.1	1.9	5.2	8.7	11.9	14.7	16.8	16.9	15.0	13.5	7.3	2.3
241								0.0	2.4	2.7	2.0	1.0	0.4	2.2	4.6	7.5	10.7	13.9	16.7	18.8	18.8	16.6	14.9	8.2	2.8
244									0.0	0.4	0.1	0.9	2.0	3.6	5.8	8.4	11.6	14.7	17.5	19.6	19.6	17.3	15.4	8.6	3.1
247										0.0	0.4	1.2	2.2	3.7	5.8	8.4	11.4	14.5	17.2	19.3	19.2	17.0	15.2	8.5	3.0
251											0.0	0.7	1.7	3.1	5.1	7.5	10.3	13.0	15.5	17.4	17.4	15.4	13.7	7.7	2.7
254												0.0	1.0	2.3	4.1	6.3	8.9	11.4	13.6	15.3	15.3	13.5	12.1	6.7	2.4
257													0.0	1.3	3.0	5.0	7.2	9.4	11.4	12.8	12.8	11.3	10.1	5.6	1.9
260														0.0	1.6	3.5	5.4	7.3	9.0	10.2	10.2	9.1	8.1	4.4	1.5
263															0.0	1.7	3.5	5.1	6.5	7.5	7.6	6.7	6.1	3.2	1.0
266																0.0	1.7	3.1	4.3	5.2	5.3	4.7	4.3	2.2	0.5
269																	0.0	1.4	2.4	3.2	3.4	3.0	2.8	1.3	0.1
272																		0.0	1.0	1.7	2.0	1.8	1.7	0.6	0.3
275																			0.0	0.7	1.0	1.0	1.0	0.1	0.6
278																				0.0	0.3	0.4	0.5	0.2	0.7
281																					0.0	0.1	0.2	0.4	0.8
284																						0.0	0.1	0.4	0.7
287																							0.0	0.4	0.7
290																								0.0	0.3
293																									0.0

Table 4

Linear regression equations of **CHL** and **PAR** at 220 and 244 nm

λ (nm)	**CHL**	**PAR**
220	$A = 4.22x10^{-2}xC_{CHL} - 8.86 x10^{-3}$	$A= 3.17 x10^{-2}xC_{PAR} + 2.45x10^{-2}$
244	$A=2.20x10^{-3}xC_{CHL}-1.20x10^{-3}$	$A= 6.73 x10^{-2}xC_{PAR} + 1.84x10^{-2}$

Table 5

Equation pair obtained at two-wavelength set for construction of the bivariate calibration

	λ (nm)	
1	220	$A_{m1} = 4.22x10^{-2} xC_{CHL} + 3.17 x10^{-2} {}_xC_{PAR} + 1.57x10^{-2}$
9	244	$A_{m9} = 2.20 x10^{-3}xC_{CHL} + 6.73 x10^{-2} {}_xC_{PAR} + 1.72x10^{-2}$

Table 6

Equation set for **SMC** approach

	λ (nm)	Equation set
1	220	$A_{m1} = 4.22x10^{-2} xC_{CHL} + 3.17 x10^{-2} {}_xC_{PAR} + 1.57x10^{-2}$
2	223	$A_{m2} = 4.03 x10^{-2}xC_{CHL} + 3.67 x10^{-2} {}_xC_{PAR} + 1.58x10^{-2}$
3	226	$A_{m3} = 3.76 x10^{-2}xC_{CHL} + 4.21 x10^{-2} {}_xC_{PAR} + 1.53x10^{-2}$
4	229	$A_{m4} = 3.45 x10^{-2}xC_{CHL} + 4.72 x10^{-2} {}_xC_{PAR} + 1.65x10^{-2}$
5	232	$A_{m5} = 2.99 x10^{-2}xC_{CHL} + 5.23 x10^{-2} {}_xC_{PAR} + 1.62x10^{-2}$
6	235	$A_{m6} = 2.28 x10^{-2}xC_{CHL} + 5.76 x10^{-2} {}_xC_{PAR} + 1.42x10^{-2}$
7	238	$A_{m7} = 1.38 x10^{-2}xC_{CHL} + 6.25 x10^{-2} {}_xC_{PAR} + 1.45x10^{-2}$
8	241	$A_{m8} = 5.69 x10^{-3}xC_{CHL} + 6.59 x10^{-2} {}_xC_{PAR} + 1.61x10^{-2}$
9	244	$A_{m9} = 2.20 x10^{-3}xC_{CHL} + 6.73 x10^{-2} {}_xC_{PAR} + 1.72x10^{-2}$
10	247	$A_{m10} = 1.57 x10^{-3}xC_{CHL} + 6.59 x10^{-2} {}_xC_{PAR} + 1.76x10^{-2}$
11	251	$A_{m11} = 2.07 x10^{-3}xC_{CHL} + 5.99 x10^{-2} {}_xC_{PAR} + 1.88x10^{-2}$
12	254	$A_{m12} = 3.09 x10^{-3}xC_{CHL} + 5.30 x10^{-2} {}_xC_{PAR} + 1.83x10^{-2}$
13	257	$A_{m13} = 4.51 x10^{-3}xC_{CHL} + 4.52 x10^{-2} {}_xC_{PAR} + 1.78x10^{-2}$
14	260	$A_{m14} = 6.51 x10^{-3}xC_{CHL} + 3.71 x10^{-2} {}_xC_{PAR} + 1.75x10^{-2}$
15	263	$A_{m15} = 9.51 x10^{-3}xC_{CHL} + 2.92 x10^{-2} {}_xC_{PAR} + 1.70x10^{-2}$
16	266	$A_{m16} = 1.33 x10^{-2}xC_{CHL} + 2.28 x10^{-2} {}_xC_{PAR} + 1.72x10^{-2}$
17	269	$A_{m17} = 1.78 x10^{-2}xC_{CHL} + 1.79 x10^{-2} {}_xC_{PAR} + 1.77x10^{-2}$
18	272	$A_{m18} = 2.23 x10^{-2}xC_{CHL} + 1.48 x10^{-2} {}_xC_{PAR} + 2.01x10^{-2}$
19	275	$A_{m19} = 2.64 x10^{-2}xC_{CHL} + 1.29 x10^{-2} {}_xC_{PAR} + 1.94x10^{-2}$
20	278	$A_{m20} = 2.95 x10^{-2}xC_{CHL} + 1.18 x10^{-2} {}_xC_{PAR} + 1.80x10^{-2}$
21	281	$A_{m21} = 2.94 x10^{-2}xC_{CHL} + 1.06 x10^{-2} {}_xC_{PAR} + 1.81x10^{-2}$
22	284	$A_{m22} = 2.60 x10^{-2}xC_{CHL} + 9.07 x10^{-3} {}_xC_{PAR} + 1.95x10^{-2}$
23	287	$A_{m23} = 2.32 x10^{-2}xC_{CHL} + 7.57 x10^{-3} {}_xC_{PAR} + 1.90x10^{-2}$
24	290	$A_{m24} = 1.30 x10^{-2}xC_{CHL} + 6.00 x10^{-3} {}_xC_{PAR} + 1.83x10^{-2}$
25	293	$A_{m25} = 4.69 x10^{-3}xC_{CHL} + 4.39 x10^{-3} {}_xC_{PAR} + 1.85x10^{-2}$

Method Validation

The linearity of each **CHL** and **PAR** was obtained in the range of 6-16 μg/mL and 4-14 μg/mL respectively. Correlation coefficients corresponding to linear regression equations presented in Table 1 were found more than 0.99. At the end of the linear regression analysis, the numerical values of the limit of detection (LOD) and the limit of quantitation (LOQ) were depicted in Table 1. Slopes and intercepts of each linear regression equation were used to construct the **SBC** and **SMC** approaches.

In this work, **SBC** and **SMC** calibration approaches were validated by analyzing the synthetic mixtures of **CHL** and **PAR**. To check the validity of the calibration approaches, the simultaneous resolution of the synthetic mixtures

containing of **CHL** and **PAR** in 12 different concentration levels for both compounds was performed. Numerical results of the means recoveries and the relative standard deviations of the approaches were shown in Table 7. The numerical values illustrated in Table 7 are satisfactory to validate the accuracy and precision of all calibration approaches.

Table 7

Recoveries obtained by applying **SBC** and **SMC** approaches to the synthetic binary mixtures

		Added(mg/mL)				Recovery (%)			
Mixture		**SBC**		**SMC**		**SBC**		**SMC**	
CHL	**PAR**	**CHL**	**PAR**	**CHL**	**PAR**	**CHL**	**PAR**	**CHL**	**PAR**
10	4	9.95	3.92	9.88	3.93	99.5	98.1	98.8	98.2
10	6	10.14	5.94	10.00	5.95	101.4	99.0	100.0	99.1
10	8	9.98	8.01	9.89	8.01	99.8	100.1	98.9	100.2
10	10	10.18	10.12	10.09	10.13	101.8	101.2	100.9	101.3
10	12	10.11	12.04	10.02	12.04	101.1	100.3	100.2	100.3
10	14	10.30	14.24	10.22	14.26	103.0	101.7	102.2	101.8
6	12	6.48	12.33	6.42	12.23	108.0	102.8	107.1	102.0
8	12	8.45	12.36	8.12	12.15	105.6	103.0	101.5	101.3
10	12	10.43	12.31	10.36	12.31	104.3	102.6	103.6	102.6
12	12	11.92	11.90	11.79	11.92	99.3	99.2	98.3	99.3
14	12	14.40	11.97	14.33	11.98	102.9	99.7	102.4	99.8
16	12	16.77	12.22	16.75	12.21	104.8	101.8	104.7	101.8
					Mean	102.6	100.8	101.5	100.6
					SD	2.67	1.62	2.62	1.35
					RSD	2.60	1.61	2.58	1.35

SD=Standard deviation
RSD= Relative standard deviation

Table 8

Determination results of **CHL** and **PAR** in tablets by **SBC** and **SMC** approaches

	mg/tablet			
	SBC		**SMC**	
Sample no.	**CHL**	**PAR**	**CHL**	**PAR**
1	252.0	292.6	254.0	295.3
2	260.0	295.8	254.8	296.1
3	251.0	295.3	259.0	295.8
4	258.7	304.3	256.1	305.5
5	254.9	294.0	250.4	294.6
6	264.9	293.1	254.2	297.0
Mean ($\bar{X}$)	256.9	295.8	254.7	297.4
SD	5.29	4.30	2.82	4.07
RSD	2.06	1.45	1.11	1.37
SE	2.00	1.63	1.07	1.54
CL (p=0.05)	$\bar{X}$ ±4.24	$\bar{X}$ ±3.44	$\bar{X}$ ±2.26	$\bar{X}$ ±3.26

Commercial tablet analysis

The determination results of **CHL** and **PAR** in commercial tablet formulation are shown in Table 8. As it can be seen the results of both approaches were found close to each other. The statistic parameters indicate that **SMC** and **SBC** are very suitable to determine the two compounds in tablet formulation.

CONCLUSIONS

In literature several graphical and chromatographic methods can be seen for determining the overlapping binary mixture systems. These approaches require some initial steps which show time consuming and higher cost (for HPLC). In this paper we study **CHL** and **PAR** possessing the overlapping absorption spectra in the working spectral range. Therefore, new simple and efficient approach to analyze this binary mixture should be developed. For all these reasons we proposed two calibration models, basically **SBC** and **SMC**, and we successfully apply them for the simultaneous analysis of **CHL** and **PAR** compounds. In addition of that, the proposed approaches do not require any graphical and separation procedure, are easy to applied and also very cheap. Therefore, we strongly recommend these new approaches to be used for the quality control and routine analysis of pharmaceutical tablet formulation containing both **CHL** and **PAR**.

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