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Efficient processing of Single and Multiple Spectral Variables for Resolution and Quantitation of Paracetamol, Chlorzoxazone and Diclofenac

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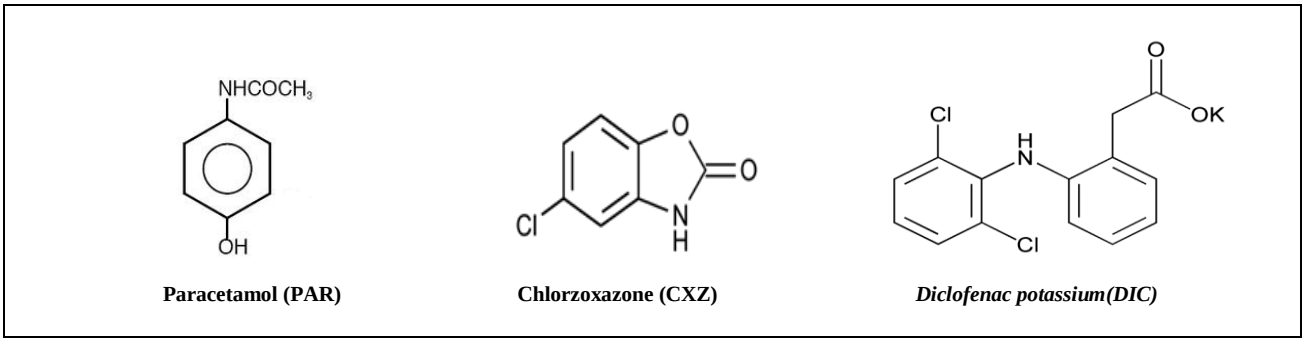
ABSTRACT

Objectives: The main aim of this study is to apply smart, simple, rapid and accurate methods for simultaneous determination of Paracetamol (PAR), Chlorzoxazone (CXZ) and Diclofenac potassium (DIC), in their bulk powder and tablet dosage form. **Methods:** Potent processing methods were used which were based on the application of continuous wavelet transform (CWT) and Savitsky–Golay derivatization (SAVGOL) as univariate spectrophotometric methods, partial least squares (PLS) and synergy interval partial least squares (siPLS) as multivariate methods. **Results:** PAR, CXZ and DIC were determined in the concentration ranges of 2–30, 2–50 and 2–30 µg mL^{-1}, respectively. The regressing and validation parameters of the proposed methods revealed the efficiency of the methods. The results obtained for the analysis of those drugs by the proposed methods were statistically compared to those obtained by applying a reported high performance liquid chromatographic method. Statistical comparison was done, and no significant difference was found between the proposed methods and the reported one. **Conclusion:** Successful determination of ternary mixture containing PAR, CXZ and DIC was achieved with no need for tedious sample separation or pre-treatment derivatization which is considered a great benefit in quality control laboratories.

Keywords: *Chlorzoxazone; CWT; Diclofenac; SAVGOL, siPLS; Paracetamol; PLS*

INTRODUCTION

A major goal of pain management is to provide pain relief that is clinically meaningful, sustained, and associated with minimum and reversible adverse effects. Many recent studies show superior efficacy of co formulated drugs versus Monotherapy in management of different types of pain, one of these commonly prescribed drug combinations include fixed-dose formulation of paracetamol (PAR) combined with, muscle relaxants (CXZ) plus NSAIDs (DIC) for treatment of painful muscle spasm[1,2]. PAR is a para-aminophenol derivative, has analgesic and antipyretic properties and weak anti-inflammatory activity. It is often the analgesic or antipyretic of choice in asthmatic patients, those with a history of peptic ulcer, and in children[2,3]. Recently Literature survey has revealed some analytical methods for the determination of PAR either individually or in other combination such as spectrophotometry[4,5], voltametry [6] and HPLC [7,8]. Chlorzoxazone (CXZ) is 5-Chloro-2(3*H*)-benzoxazolone a centrally active muscle relaxant. Used to decrease muscle tension and thus to relieve the painful muscle spasm associated with musculoskeletal disorders [2,3]. Recent methods for the determination of CXZ in single form or with other combination include spectrophotometry[9,10] and chromatography [11,12]. Diclofenac potassium (DIC) is [2-[(2, 6-dichlorophenyl)amino]phenyl]acetate is a nonsteroidal anti-inflammatory drug (NSAID) applied to reduce inflammation and as an analgesic for reducing



Paracetamol (PAR) **Chlorzoxazone (CXZ)** ***Diclofenac potassium(DIC)***

Figure 1. Chemical structure of the studied drugs

pain in certain conditions [2-3]. Several analytical methods recently found in the literature for determination of DIC either alone or with other combination, including the spectrophotometry[13-15], spectrofluorimetry[16], potentiometry[17,18], voltammetry[19,20] and chromate-graphy[21-23]. The three drugs (**Figure 1**) are official in the United States Pharmacopeia (USP) [3].

Literature review revealed that the three cited components were simultaneously determined in ternary mixture by HPLC, HPTLC [24-29], and by simultaneous equation methods [30-31]. To our knowledge, chemometric techniques for simultaneous determination of these drugs have not been reported.

So, the aim of this work is primarily to simultaneously determine the three components in bulk powder and tablet dosage form without prior separation or pre-treatment derivativezation which considered a great benefit in quality control laboratories and to compare the efficiency of single and multiple variables regression methods for such resolution and quantitation. Thus, spectral resolution and quantitation of the selected components was achieved using single variable continuous wavelet transform (CWT) and Savitsky–Golay derivatization (SAVGOL) methods and multiple variables synergy interval partial least squares (siPLS)[32], and normal partial least squares (PLS) methods.

Theoretical background

Continuous wavelet transformation (CWT)

Continuous Wavelet Transform (CWT) is one of the recent mathematical techniques for signal processing in which the data cuted up into different frequency components, and then study each component with a resolution matched to its scale where a spectrum of a chemical species decomposed into simpler, fixed building blocks at different scales and positions[33,34]. Recently, the combined use of CWT and zero-crossing technique with a mathematical model for the resolution of multi-component overlapping signals has been formulated by Dinc and Baleanu[35, 36]. Continuous Wavelet Transform (CWT) combined either with a zero-crossing technique [37, 38] or ratio spectra [39] was used for simultaneous determination of chemical species in binary and ternary mixtures. For quantitation of multicomponent mixtures using CWT, calibration of each analyte in the mixture can be performed by modelling CWT-signal against concentration at zero-crosses of the other component.

Savitzky-Golay derivatization technique (SAVGOL)

This technique is based on established mathematical procedures applied to a set of digital data points for the purpose of smoothing the data and improving the signal-to-noise ratio without distorting the signal. It was first developed by Savitzky and Golay who presenting an alternative and simplified method of determining the new value of each data point [40], and published tables of convolution coefficients for various polynomials and subset sizes[41]. Some errors. in the tables have been corrected[42, 43]. The method has been extended for the treatment of 2-and 3-dimensional data.

PLS method

It is the conventional chemometric algorithm applied for separation and resolution of complex mixture, its theory was well established and based on factor analysis[44].

siPLS method

The main principle of this method is to develop PLS regression model in smaller selected spectral wavelength region using the same or less number of latent variables. Many algorithms have been proposed for the selection of characteristic wavelength, However, among the different types of utilization algorithms, the iPLS method has gained much attention due to its high efficiency and ability to represent results in a graphical manner, focusing on models with specified intervals and interpretation [32]. In recent years, many successful applications of iPLS based methods have been reported in the literature [45,46].

Table 1. Regression and validation parameters of CWT and SAVGOL methods for the determination of PAR, CXZ and DIC in pure form.

Parameter	CWT			SAVGOL		
	PAR	CXZ	DIC	PAR	CXZ	DIC
Linearity range (µg mL^{-1})	2-30	2-50	2-30	2-30	2-50	2-30
slope	0.0274	0.034	0.0064	2.2289	2.0491	0.4196
Intercept	0.0042	0.0006	0.0006	0.3028	1.1262	0.2855
Mean %	100.16	100.28	100.87	100.27	100.69	99.96
SD	0.584	0.602	0.529	0.908	0.859	0.901
Accuracy(Mean ± SD)	98.25 ± 1.32	100.60 ± 0.658	100.19 ± 0.624	99.30 ± 1.45	100.30 ± 0.402	99.44 ± 0.739
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
R^2	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
(LOD) (µg mL^{-1})	0.247	0.443	0.273	0.265	0.573	0.425
(LOQ) (µg mL^{-1})	0.914	1.48	0.910	0.883	1.91	1.41
SE of intercept	0.001	0.003	0.0004	0.137	0.265	0.048
SE of slope	0.00008	0.0001	0.00002	0.008	0.009	0.0025
Repeatability (RSD %)	0.301	0.706	0.674	0.218	0.522	0.569
Intermediate precision (RSD %)	1.05	1.72	0.927	0.713	0.682	1.44
residuals standard deviation (Sy/x)	0.0023	0.005	0.0005	0.197	0.392	0.059

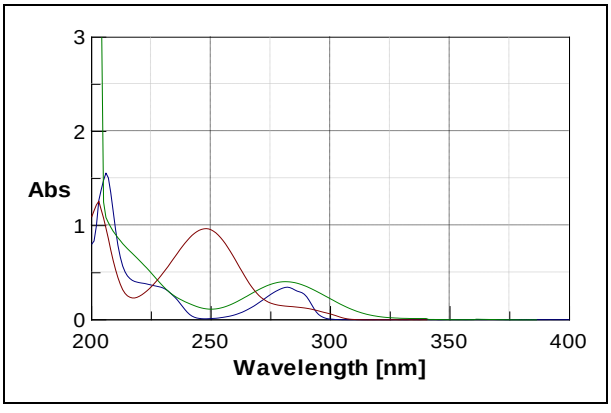


Figure 2. Absorption Spectrum of 10 µg mL^{-1} of each of PAR(___), CXZ(___) and DIC(___)

MATERIALS AND METHODS

Experimental

Instruments and software

All absorbance measurements were carried out using Jasco (V-530) double beam UV-Visible spectrophotometer (Japan), with 1 cm matched quartz cell. Spectra were automatically obtained by Jasco UV-Probe (VWS-580 Spectra Manager software). The spectra were scanned from 200-400 nm using 0.1 nm interval. All computations were performed in Matlab® for Windows TM version 6.5. The PLS and siPLS procedure were taken from PLS and si PLS Toolboxes 2.0 for use with Matlab® 7.9.

Chemicals and solvents

Pure samples

PAR and CXZ in pure form were kindly supplied by EVA Pharma Medical Company, Giza, Egypt. DIC was kindly supplied by Adwia Pharmaceuticals, 10th of Ramadan city, Egypt. The samples purity were found to be 99.69%, 99.59%, and 100.22 % for PAR, CXZ and DIC, respectively according to reported method [27].

Market samples

Myospaz fort® Tablet dosage forms was purchased form the local market, Hifenac-MR® tablet Batch No. FG9416 (labeled to contain 325 mg PAR, 500 mg CXZ, and 50mg DIC per tablet) is manufactured by WIN Medicare Pvt Ltd, Pharmaceuticals, India.

Solvents

Methanol of spectroscopic grade (sdfine-chem limited, Industrial state, Mumbai).

Standards

Standard solutions containing 100 µg mL^{-1} of each of PAR, CXZ and DIC were prepared by dissolving 10.00 mg of each drug in 100 mL methanol.

Procedure

Univariate regression.

Construction of calibration curves for univariate methods

Aliquots equivalent to 0.2-3.0 mL of PAR, 0.2-5.0 mL of CXZ and 0.2-3.0 mL of DIC were accurately and separately transferred from their corresponding standard solutions (100.00 µg mL^{-1}) using calibrated micro pipettes to a series of 10-mL volumetric flasks. Each flask was completed to volume with methanol to reach a final concentration range of 2.00-30.00 µg /mL for PAR, 2.00-50.00 µg mL^{-1} for CXZ and 2.00-30.00 µg/ mL for DIC. The spectra of the prepared standard solutions were scanned from 200 - 400 nm with 0.1 nm interval.

CWT univariate method

The zero order spectra were transformed by CWT technique using the 2nd coefficient for CXZ and DIC and the 3_{rd} coefficient for PAR. The calibration curves were constructed relating the peak amplitudes at 270 nm, 292 nm and 317 nm to the corresponding concentrations of PAR, CXZ and DIC, respectively and then The, regression equations were computed.

Savitzky-Golay (SAVGOL) univariate method

The first, second and third derivative of the zero order spectra was calculated by SAVGOL technique, and the amplitude of the 2nd derivative values were then plotted versus the corresponding concentrations at wavelengths of 223 nm, 299 nm and 320 nm for PAR, CXZ and DIC, respectively and then the regression equations were computed.

Assay of laboratory prepared mixtures

Combining different aliquots of PAR, CXZ and DIC standard solutions (each 100 µg mL^{-1}) were accurately transferred into a series of 10 ml volumetric flasks. They were completed to volume with methanol to prepare different Mixtures containing different ratios of PAR, CXZ and DIC including the ratio of the dosage form. And then scanned from 200-400 nm.

For Continuous wavelet transforms (CWT)

The scanned spectra of the laboratory prepared mixture were subjected to 2nd coefficient for CXZ and DIC and the 3_{rd} coefficient for PAR. The amplitude values of the obtained spectra were recorded at 270 nm, 292 nm and 317 nm to the corresponding concentrations of PAR, CXZ and DIC, respectively Then the concentrations of the drugs were calculated from the corresponding computed regression equations.

For Savitzky-Golay (SAVGOL)

The second derivative of the scanned spectra of the laboratory prepared mixtures was calculated by SAVGOL method. The amplitude values of the obtained spectra were recorded at 223 nm, 299 nm and 320 nm for PAR, CXZ and DIC, respectively. Then the concentrations of the drugs were calculated from the corresponding computed regression equations.

Multivariate regression

Construction of calibration and validation sets for PLS and siPLS

For application of multivariate methods, five levels three factor experimental design [44] was applied to prepare mixtures of PAR, CXZ and DIC. Twenty- five mixtures were prepared, 18 of them were used for building the calibration model, while seven mixtures

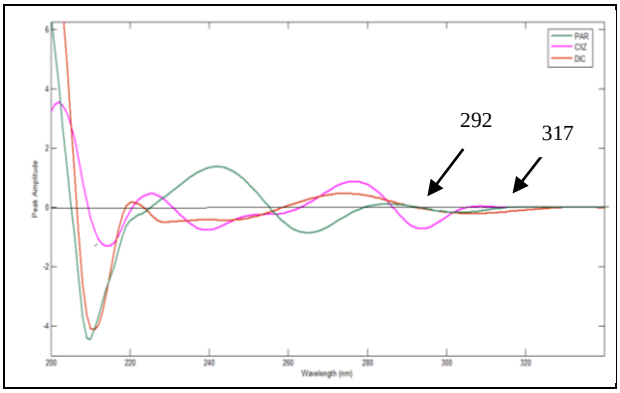


Figure 3. Second coefficient of CWT using 20 µg mL^{-1} of PAR, CXZ and DIC for determination of CXZ at 292 nm and DIC at 317 nm.

Table 2. Determination of PAR, CXZ and DIC in laboratory prepared mixtures using CWT method.

Mixture No.	PAR	CXZ	DIC	PAR	CXZ	DIC
	Conc. µg mL^{-1}			Recovery %		
1	4	10	10	101.70	99.44	102.34
2	13	20	2	99.50	98.60	102.89
3	14	4	6	100.49	99.70	102.81
4	10	14	20	100.95	99.18	100.00
5	20	8	14	100.75	99.49	100.67
Mean ± SD				100.68 ± 0.798	99.40 ± 0.472	101.74 ± 1.32

Mix 2: The ratio of the lab mixture present in Myospaz Fort® tablets

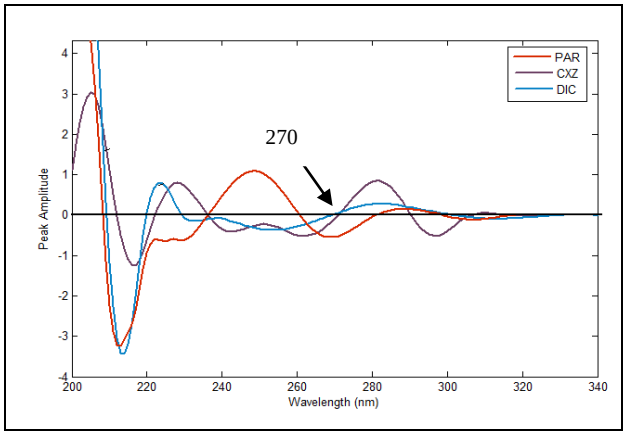


Figure 4. Third coefficient of CWT using 20 µg mL^{-1} of PAR, CXZ and DIC for determination of PAR at 270 nm.

randomly chosen and used as an independent validation set. The prepared mixtures contained the three drugs with different ratios and within the concentration range of 2.00-20.00 µg/ mL for each of PAR, CXZ and DIC.

The absorption spectra of the prepared mixtures were recorded in the range 200 - 400 nm at 0.1 nm intervals using methanol as blank. For construction of the calibration models **(**PLS) and (siPLS), the absorbance and concentration matrices for the training set were used. An external validation set was used to assess the validity of the developed PLS model.

Assay of pharmaceutical formulations

Ten tablets of **Myospaz fort®** tablets was accurately weighed and finely powdered. An accurate weight of the powdered tablets equivalent to 325 mg PAR, 500 mg CXZ and 50 mg DIC, was sonicated with 60.0 mL methanol for 20 min, filtered into a 100-mL volumetric flask and completed to volume with methanol. The prepared solutions had the following concentrations, 3.25, 5.0 and 0.5 mg mL^{-1} of PAR, CXZ and DIC in a respective order. The prepared solutions were further diluted to have concentrations within the linearity ranges of the applied methods.

RESULTS AND DISCUSSION

Resolving the overlapped spectra of multi component mixtures using advanced spectrophotometric methods and other chemometric spectral calibration techniques, which are very easy to apply, very rapid, sensitive and yet very cheap for analysis of mixture without prior separation of the constituent analytes, was rather a crucial task for analytical studies, related to the quality control and routine analysis of commercial products in the research or industry laboratories. In the last few years, the development of methods for the resolution of such mixtures has grown dramatically. Where these methods considered preferable over other sophisticated analytical instrumentations or techniques which always require optimization of conditions such as pH, temperature, flow rate, However, lower selectivity is considered a main disadvantage of spectrophotometric methods. To increase the selectivity; numerical and graphical techniques were introduced for treatment of spectral data where advanced spectrophotometric techniques and chemometric algorithms were used i.e. chemometric techniques has brought a new, rapid, easy to apply methodology and yet very cheap for the determination of analytes in samples[48].

Table 3. Determination of PAR, CXZ and DIC in laboratory prepared mixtures using SAVGOL method.

Mixture No.	PAR	CXZ	DIC	PAR	CXZ	DIC
	Conc. µg mL^{-1}			Recovery %		
1	4	10	10	100.79	99.55	101.21
2	14	8	6	99.73	100.51	99.88
3	10	20	20	102.71	99.58	100.68
4	20	14	14	98.88	101.20	99.66
5	13	20	2	101.47	99.35	101.73
Mean ± SD				100.72 ± 1.49	100.04 ± 0.79	100.64 ± 0.87

Mix 5: The ratio of the lab mixture present in Myospaz Fort® tablets

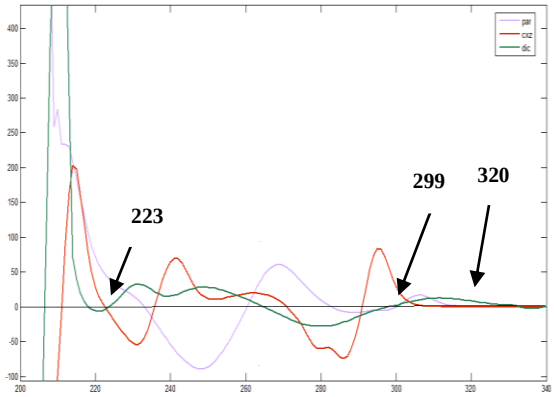


Figure 5. Second derivative of SAVGOL using 20 µg mL^{-1} of PAR, CXZ and DIC for determination of these drugs at the selected wavelengths.

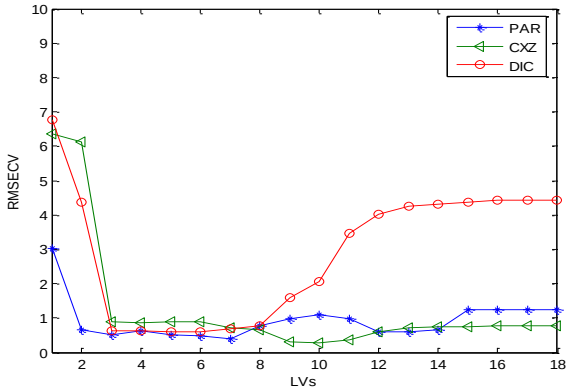


Figure 6. RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model

In this work, two spectrophotometric methods CWT, SAVGOL derivatization techniques and two chemometric methods PLS, siPLS were developed to resolve the strongly overlapped spectra of PAR, CXZ and DIC **Figure 2**, and thus simultaneously determine these compound in their bulk powders and pharmaceutical dosage forms.

*Continuous wavelet transforms (**CWT**)*

Recently, the combined use of CWT and zero crossing technique with a mathematical model for the resolution and signal processing of multicomponent overlapping signals has been successfully employed to simultaneously determined binary and ternary mixture, this owing to its efficiency and high speed in data treatment.

Various wavelet families with different scales were tested with the purpose of finding the optimal signal processing settings to obtain desirable calibration graphs as well as reliable determination of the studied drugs. The spectral data points were transformed by CWT method with 2nd coefficient for CXZ and DIC and the 3$_{rd}$ coefficient for PAR as observed in **Figures 3** and **4**. The calibration graphs of PAC, CXZ and DIC for the wavelet methods were constructed by plotting the transformed signals at 317 nm, 270 nm and 292 nm for PAC, CXZ and DIC respectively versus the concentration at the zero-crossing points of the other three compounds.

Table 4. Concentrations of PAR, CXZ and DIC in the calibration and validation set using PLS and siPLS methods.

Mix. No.	**PAR** (µg mL^{-1})	**CXZ** (µg mL^{-1})	**DIC** (µg mL^{-1})
1	10	10	10
2	10	4	2
3	4	4	10
4	4	20	6
5	20	8	20
6	8	20	10
7	20	10	6
8[a]	10	8	6
9 [a]	8	8	14
10 [a]	8	14	20
11[a]	14	20	14
12[a]	20	14	10
13	14	10	20
14	10	20	20
15	20	20	2
16	20	4	14
17	4	14	2
18 [a]	14	4	10
19	4	10	14
20	10	14	14
21 [a]	14	14	6
22	14	8	2
23	8	4	6
24	4	8	10
25	8	10	2

[a] *Mixtures of validation set*

Table 5. Summary of the results obtained by applying the diagnostic tools for model validation of PLS and siPLS chemometric methods.

Validation parameters	**PLS**			**siPLS**		
a) **Predicted vs known conc. Plot**	**PAR**	**CXZ**	**DIC**	**PAR**	**CXZ**	**DIC**
1-slope	1.0545	0.9734	0.9649	0.9494	1.0059	1.0187
2-Intercept	-0.5023	0.2563	0. 3029	0.5957	-0.0597	-0.1573
3-Correlation coefficient	0.9997	0.9995	0.9997	0.9998	1	0.9998
b) **RMSEP**	0.333	0.246	0.280	0.2436	0.1135	0.1604
c) **Recovery of validation set (Mean ±SD)**	101.86 ± 1.51	100.07 ± 1.84	100.53 ± 2.13	100.66 ± 1.25	99.56 ± 1.09	100.62 ± 1.81

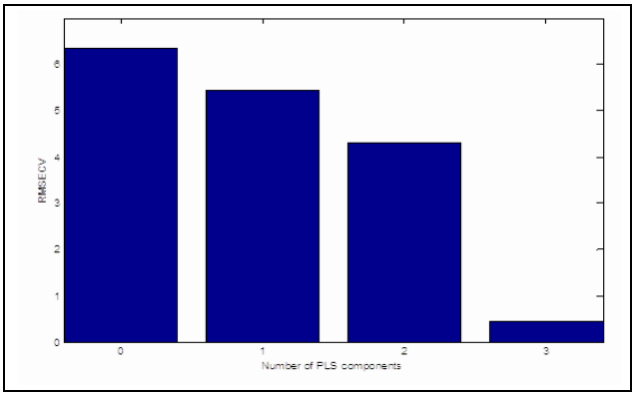


Figure.7 RMSECV versus PLS components for model on interval of 5 :9:10

The results were summarized in **Table 1**. The obtained correlation coefficients of equations along with other statistical parameters were in acceptable range, **Table 1**.

*For Savitzky-Golay (**SAVGOL**)*

An SAVGOL is an advanced technique that can be applied to a set of data points for the purpose of smoothing the data. second derivative of the overlaped spectra of PAC, CXZ and DIC were calculated by SAVGOL technique, **Figure 5**. PAR, CXZ and DIC were determined at 223 nm, 299 nm and 320 nm respectively which correspond to the zero crossing points.

The results were summarized in **Table 1**. The obtained correlation coefficients of equations along with other statistical parameters were in acceptable range, **Table 1**.

Methods validation

Univariate methods.

Method validation was performed according to ICH guidelines [49] for both CWT and SAVGOL methods

Linearity

The linearity of the proposed CWT and SAVGOL methods was evaluated by analyzing different concentrations of each of PAR, CXZ and DIC ranging between 2.00-30.00, 2.00-50.00 and 2.00-30.00 µg mL^{-1}, in order. Each concentration was repeated three times, the results are represented in **Table 1**. The high values of the correlation coefficient (r) and small values of residuals standard deviation (Sy/x) indicate good linearity of the calibration graphs.

Accuracy

To study the accuracy of the proposed methods, procedures construction of calibration curves for PAR, CXZ and DIC were repeated three times for the determination of five different concentrations of pure PAR, CXZ and DIC. The accuracy expressed as percentage recoveries and percent relative error is shown in **Table 1**. Good accuracy of the developed method is indicated by the results obtained.

Precision

The repeatability and intermediate precision were evaluated through replicate analysis of PAR, CXZ and DIC using three different concentrations and each concentration was measured three successive times intra and inter-daily, respectively. The percentage relative standard deviation was calculated, the results are summarized in **Table 1**. The precision of the proposed method is fairly high, as indicated by the low values of % RSD.

The approach based on the SD of the response and the slope was used for determining the detection and quantitation limits, **Table 1**.

Selectivity

Selectivity of the proposed method was achieved by the analysis of different laboratory prepared mixtures of PAR, CXZ and DIC within the linearity range. Satisfactory results are shown in **Table 2 and Table 3**.

Solution stability

The prepared solutions of the studied drugs exhibited no absorbance changes for one day when kept at room temperature and for about 1 week when stored refrigerated at 2-8°C.

Multivariate methods

PLS method

PLS model is considered as the conventional algorithm in quantitative spectral analysis [50, 51]. It was found that spectral bands of the cited active compounds are highly overlapped **Figure 2**, which hinders their direct determination. The concentration details of the prepared mixture solutions were given in **Table 4**. Five level three factor design was used for preparation of calibration and validation sets. The region below 230 nm and more than 300 nm were rejected due to the noisy content. In order to construct PLS calibration model, the raw data of the calibration samples were mean centered as a pre-processing step and the 'random subsets' cross-validation method was used. The number of factors should account as much as possible for the experimental data without resulting in over fitting. Various criteria have been developed to select the optimum number [52]. The root mean squares error of cross-validation (RMSECV) was calculated for examining the errors in the predicted concentrations. Seven latent variables were found optimum for the mean centered data for the mixture of PAC, CXZ and DIC using PLS, **Figure 6**. In order to assess the

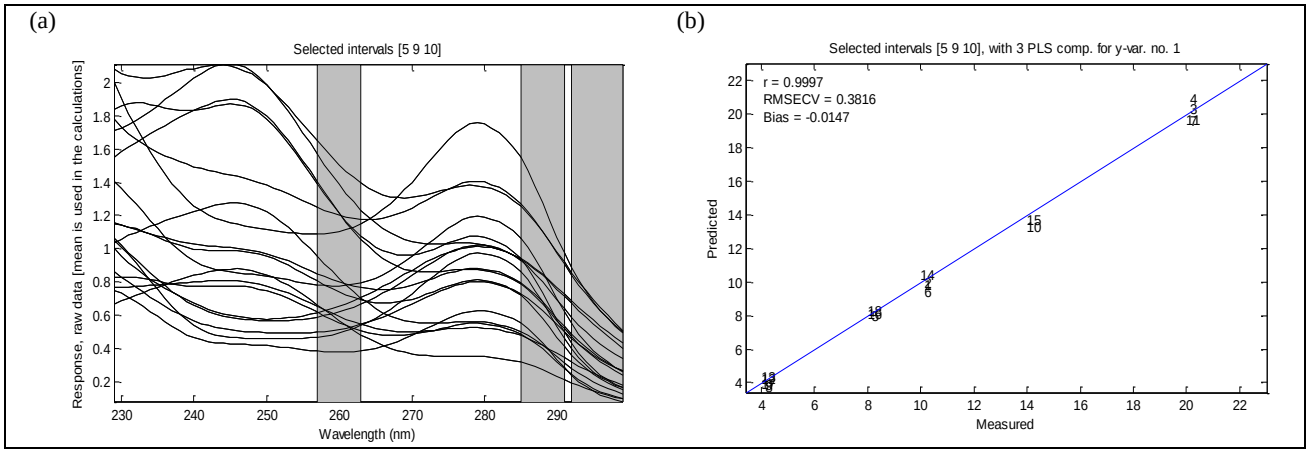


Figure 8. Spectral regions selected to build the models and results: (a) siPLS model by combination of subintervals 5, 9 and 10 for quantification; (b) average content of the three components (µg mL^{-1}) vs. the predicted values by cross-validation for the siPLS model with 3 LVs

Table 6. Statistical results of siPLS model for PAR, CXZ and DIC.

	intervals			
pls com	PAR	CXZ	DIC	RMSE
3	10	9	6	0.4492
3	10	9	5	0.4493
4	10	6	5	0.4574
4	10	9	4	0.4582
4	10	6	3	0.4614
5	10	6	4	0.4696
4	10	5	3	0.4705
4	10	6	4	0.4752
4	9	7	6	0.4824
4	10	8	5	0.4909

predictive ability of the developed PLS model, it was applied on an external validation set for the determination of the three compounds. The predicted concentrations of the validation samples were plotted against the true concentrations. This was used to determine whether the model has accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero. The root mean square error of prediction (RMSEP) was also calculated **Table 5**. The RMSEP was used as a diagnostic tool for examining the prediction errors; it has indicated both accuracy and precision. The mean recoveries and standard deviations obtained by the proposed PLS method for the determination of the ternary mixture were summarized in **Table 5**. Also, the regression equations parameters were shown.

siPLS model

It has been demonstrated that wavelength selection is potentially able to improve the prediction ability by finding out an optimized combination of the informative regions. In this work,
the spectrum region was divided into 10 equidistant subintervals by the siPLS algorithm. For all the 10 subintervals, a calibration model based on PLS using different numbers of latent variables was developed. The RMSECV was calculated as a critical value for comparison of these models in relation to the whole spectrum model. **Figure 7** shows the RMSECV for best combined intervals selected (bars) and latent variables for this model. the minor RMSEP values which was better than the full-spectrum PLS ones shown in **Table 6**., Different combinations of intervals were tested by means of the siPLS algorithm. The statistical results of different combinations are shown in **Table 6**. As can be seen in the table, the combination of subintervals 5, 9 and 10 gives the lowest RMSECV that are better than the other subintervals. The siPLS algorithm avoids the loss of relevant spectral region that will improve the performance of the calibration model. A graphic test of model constructed by the synthetic subintervals 5, 9 and 10, also the average content of the three components (µg/mL) vs. the predicted values by cross-validation for the siPLS model with 3 LVs is shown in **Figure 8**.

The suggested methods were successfully applied for the analysis of PAC, CXZ and DIC in

Table 7. Determination of PAR, CXZ and DIC in Myospaz fort® film coated tablet by the proposed CWT and SAVGOL methods and application of standard addition technique.

Dosage form		CWT			SAVGOL		
		PAR	CXZ	DIC	PAR	CXZ	DIC
Myospaz fort® tablet B.N. FG9416 325mg PAR 500mg CXZ 50mg DIC	%Found[a] (± SD)	101.72 ± 1.07	99.34 ± 0.45	101.46 ± 0.95	99.61 ± 1.77	101.72 ± 1.58	100.22 ± 1085
	Standard addition[b] (mean ± SD)	100.70 ± 1.31	102.33 ± 1.41	101.40 ± 1.26	101.80 ± 1.45	99.27 ± 0.576	98.58 ± 0.377

[a] *Mean of five determination*
[b] *Mean of three determination*

Table 8. Determination of PAR, CXZ and DIC in Miospaz fort® film coated tablet by the proposed PLS and siPLS chemometric methods and application of standard addition technique.

Dosage form		PLS			siPLS		
		PAR	CXZ	DIC	PAR	CXZ	DIC
Myospaz fort® tablet B.N.FG9416 325mg PAR 500mg CXZ 50mg DIC	%Found[a] ± SD)	100.83± 0.81	101.19 ± 0.21	100.20 ± 0.56	100.48 ± 0.37	99.93 ± 0.88	99.92 ± 0.43
	Standard addition[b] (mean ± SD)	100.88 ± 0.757	101.34 ± 1.45	100.96 ± 0.937	100.42 ± 0.372	101.44 ± 1.14	99.91± 0.343

[a] *Mean of five determination*
[b] *Mean of three determination*

Table 9. Statistical comparison of the results obtained by the CWT and SAVGOL spectro-photometric methods and the reported HPLC method[27] for PAR , CXZ and DIC.

Method	CWT			SAVGOL			Reported[27]		
	PAR	CXZ	DIC	PAR	CXZ	DIC	PAR	CXZ	DIC
Mean[a] %	99.83	100.28	100.87	100.45	100.51	99.96	99.69	99.59	100.22
SD	0.339	0.602	0.530	1.001	0.818	0.901	0.621	0.666	0.677
N	6	7	6	6	5	6	5	5	5
Variance	0.115	0.363	0.2804	1.002	0.669	0.8118	0.386	0.443	0.458
F test	3.359 (7.39)	1.222 (6.23)	1.63 (7.39)	2.60 (9.36)	1.50 (9.60)	1.77 (9.36)			
t test	0.489 (2.26)	1.88 (2.23)	1.79 (2.26)	1.476 (2.26)	1.95 (2.31)	0.529 (2.26)			

[a] *The obtained results are the average of three determinations*
[27] *Is the reported method using C_{18} column (250 × 4.6 mm, 5 µm). Mobile phase dilute orthophosphoric acid) and acetonitrile (45: 55, v/v), at 220 nm*
Values between parentheses are the tabulated t test and F test values at probability 0.05

Table 10. Statistical comparison of the results obtained by the PLS and siPLS chemometric methods and the reported HPLC method[27] for PAR, CXZ and DIC

Method	PLS			siPLS			Reported[27]		
	PAR	CXZ	DIC	PAR	CXZ	DIC	PAR	CXZ	DIC
Mean[a] %	99.75	99.83	100.09	99.09	100.31	100.71	99.69	99.59	100.22
SD	0.589	0.707	0.513	0.500	0.366	0.853	0.621	0.666	0.677
N	5	5	5	5	5	5	5	5	5
Variance	0.347	0.499	0.263	0.250	0.134	0.728	0.386	0.443	0.458
F test	1.11 (9.61)	1.13 (9.61)	1.74 (9.61)	1.54 (9.61)	3.30 (9.61)	1.59 (9.61)			
t test	0.191 (2.31)	0.579 (2.31)	0.336 (2.31)	1.68 (2.31)	2.12 (2.31)	1.006 (2.31)			

[a] *The obtained results are the average of three determinations*
[27] *Is the reported method using sing C_{18} column(250 × 4.6 mm, 5 µm). Mobile phase dilute orthophosphoric acid) and acetonitrile(45: 55, v/v), at 220 nm*
Values between parentheses are the tabulated t test and F test values at probability 0.05

Myospaz fort® tablets. The validity of the proposed method is further assessed by applying the standard addition technique. The results obtained are shown in **Tables 7** and **8**.

Statistical comparison

When results obtained by applying the proposed CWT, SAVGOL derivatization technique PLS and siPLS methods for analysis of pure PAC, CXZ and DIC were compared to those obtained by applying the reported method[27], they showed no significant difference regarding accuracy and precision represented by Student's t-test and Variance ratio F-test respectively[53]. The results are shown in **Table 9 and Tables 10**.

CONCLUSION

CWT, SAVGOL derivatization technique PLS and siPLS methods have provided a smart solutions for almost all chemistry problems. They were ideal methods for the spectral resolution and prediction of multi-mixtures in the presence of original overlapping signals. These provided approaches considered an accurate, economic, rapid and precise methods for analysis of pure PAC, CXZ and DIC or in its pharmaceutical formulation without excipients interference and thus successfully could be used for the quality control and routine analysis of commercial products in laboratories especially which lacking liquid chromatographic instruments.

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Conflict of Interest

All authors want to declare that there is no conflict of interests regarding the publication of this paper. And all data concerning this study are available in presented table and available for any researcher.

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