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Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF DICLOFENAC SODIUM, PARACETAMOL, AND CHLORZOXAZONE IN TERNARY MIXTURE USING CHEMOMETRIC AND ARTIFICIAL NEURAL NETWORKS TECHNIQUES

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ABSTRACT

Objective: The aim of this study is to develop and validate simple, accurate, and precise spectrophotometric methods for the simultaneous determination of diclofenac sodium (DIC), paracetamol (PAR), and chlorzoxazone (CHZ) in ternary mixture using chemometric and artificial neural networks (ANN) techniques.

Methods: Three chemometric techniques include classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS) in addition to cascade-forward backpropagation ANN (CFBP-ANN) were prepared using the synthetic mixtures containing the three drugs in methanol. In CLS, PCR, and PLS, the absorbances of the synthetic mixtures in the range 267-295 nm with the intervals $\Delta\lambda$=0.2 nm in their zero-order spectra were selected. Then, calibration or regression was obtained using the absorbance data matrix and concentration data matrix for the prediction of the unknown concentrations of DIC, PAR, and CHZ in their mixtures. In CFBP-ANN, two layers, sigmoid layer with 10 neurons and linear layer were found appropriate for the simultaneous determination of the three drugs in their ternary mixture.

Results: The four proposed methods were successfully applied to the analysis of the three drugs in laboratory prepared mixtures and tablets with good percentage recoveries in the range of 98-102%. Relative standard deviation for the precision study was found <1%.

Conclusion: The four proposed methods showed simplicity, accuracy, precision, and rapidity making them suitable for quality control and routine analysis of the cited drugs in ternary mixtures and pharmaceutical formulation containing them.

Keywords: Diclofenac sodium, Paracetamol, Chlorzoxazone, Chemometrics, Artificial neural networks.

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INTRODUCTION

Diclofenac sodium (DIC), sodium [2-(2,6-dichloroanilino) phenyl] acetate (Fig. 1a), is a derivative of phenylacetic acid classed as a non-steroidal anti-inflammatory drug, used to relieve the pain and inflammation in many conditions such as rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis [1,2]. Paracetamol (PAR), N-acetyl-p-aminophenol (Fig. 1b), is an analgesic and antipyretic agent with weak anti-inflammatory property. It is used for the management of mild-to-moderate pain and fever [1,3]. Chlorzoxazone (CHZ), 5-chlorobenzoxazol-2(3H)-one (Fig. 1c), is a centrally acting skeletal muscle relaxant with sedative properties, used as an adjunct in the symptomatic treatment of painful skeletal muscle conditions [1].

The combination of the three drugs is widely prescribed for the alleviation of the pain associated with the muscle spasm.

Literature survey reveals that several methods have been reported for the determination of DIC in pharmaceutical and biological samples. These methods include spectrophotometry and multivariate [4-6], fluorimetry [7,8], voltammetry [9,10], high-performance liquid chromatography-ultraviolet (HPLC-UV) [11-15], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [16-19], capillary zone electrophoresis [20], and densitometry [21,22]. Other methods were developed for the simultaneous determination of DIC in binary combination with PAR or CHZ that include spectrophotometry [23-25], densitometry [26], voltammetry [27], and HPLC-UV [28,29]. Some methods were reported for the simultaneous determination of ternary combination of the three drugs based on densitometry [30,31] and HPLC-UV [32,33].

The UV absorption spectra of DIC, PAR, and CHZ in methanol at concentrations corresponding to their ratio in the combined dosage form reveal strong overlap (Fig. 2). Thus, direct simultaneous spectrophotometric determination of the three drugs in the mixture is not feasible. To the best of our knowledge, no previous spectrophotometric method based on chemometric techniques and artificial neural networks (ANN) has been published for the simultaneous determination of the three drugs. Thus, the main aim of this work was to develop and validate simple and accurate spectrophotometric methods for the simultaneous determination of the three drugs in ternary mixture using chemometric and ANN techniques.

Multivariate calibrations are widely used in quantitative spectral analysis for compounds with highly overlapping spectra. These calibrations are characterized by the higher speed of data processing and minimizing of calibration models errors by measuring the absorbance at many points in the wavelength range of the zero-order and derivative spectra. Control analyses on pharmaceutical preparations using multivariate calibration methods have been proven to be a valid alternative to HPLC [34]. These calibrations have been used in many analysis methods such as spectrophotometric [35], spectrofluorometric [36], and voltammetric [37] methods. In this work, three multivariate calibration methods have been described to resolve the overlapping between DIC, PAR, and CHZ in their zero-order spectra. These methods include classical least squares (CLS), principal

component regression (PCR), and partial least squares (PLS). ANNs are computer programs designed to simulate some human brain functions using different algorithms, which can learn from experience. ANN analyses are currently perceived as an efficient and advantageous way to handle complex data and solve problems of non-linear calibration, pattern recognition, classification, prediction, and other related fields in analytical chemistry. Both linear and non-linear mapping functions can be modeled by suitably designing the network [38]. In this work, cascade-forward backpropagation ANN (CFBP-ANN) was used for the simultaneous determination of the cited three drugs in their ternary mixture with good accuracy and precision.

METHODS

Instrumentation

Shimadzu ultraviolet/visible spectrophotometer 1600 (Japan) connected to an IBM compatible computer and supported with UV probe software version 2.43 was used. The chemometric methods, CFBP-ANN, and data analysis were performed using Matlab™ software, version 7.9.0 with PLS-toolbox 2.0 and neural networks toolbox.

Reagents and reference samples

DIC, PAR, and CHZ (certified to contain 99.20%, 99.45%, and 99.64%, respectively) were kindly supplied from the central laboratory of drug control-Sana'a, Yemen. Intagesic MR® tablets nominally containing DIC (50 mg), PAR (325 mg), and CHZ (250 mg) were manufactured by G.S. Pharmaceutical limited, India. Methanol used was of analytical grade.

Standard solutions

Stock solutions

Accurately weighed 20 mg of DIC, 100 mg of PAR, and 125 mg of CHZ were transferred into separate 50 ml volumetric flasks, dissolved in, and completed to volumes with methanol to obtain standard stock solutions containing 0.4 mg/ml of DIC, 2.0 mg/ml of PAR, and 2.5 mg/ml of CHZ.

Working solutions

Accurately measured aliquots (5 ml) were transferred from each stock solution into separate 100 ml volumetric flasks. The volume was completed with methanol to obtain working solutions containing 20 µg/ml of DIC, 100 µg/ml of PAR, and 125 µg/ml of CHZ.

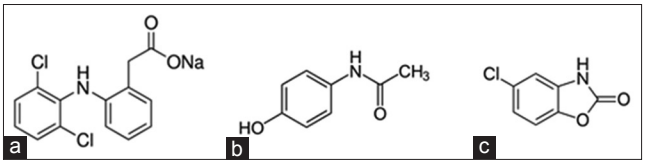

Fig. 1: Chemical structures of diclofenac sodium (a), paracetamol (b), and chlorzoxazone (c)

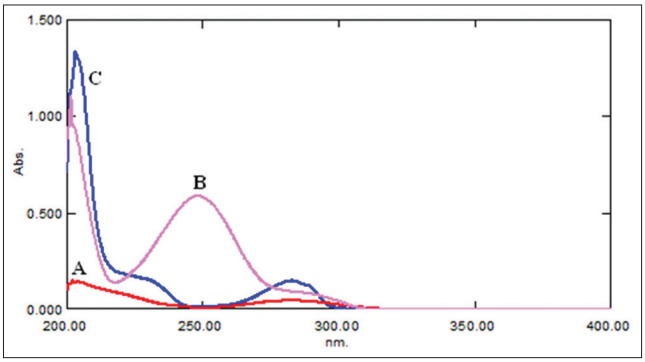

Fig. 2: Zero-order absorption spectra of diclofenac sodium (1.0 µg/ml) (a), paracetamol (6.5 µg/ml) (b), and chlorzoxazone (5.0 µg/ml) (c) in methanol

Sample preparation

Twenty tablets were accurately weighed and finely powdered using mortar and pestle. An accurate weight of the powdered tablets equivalent to 50 mg of DIC, 325 mg of PAR, and 250 mg of CHZ was transferred into a 100 ml volumetric flask. The powder was extracted with 50 ml methanol by sonication for 15 minutes, completed to the mark with methanol, and filtered through Whatman filter paper discarding the first few milliliters to produce tablet stock solution of 0.5 mg/ml of DIC, 3.25 mg/ml of PAR, and 2.5 mg/ml of CHZ. An accurately measured aliquot (5 ml) from the tablet stock solution was transferred into a 250 ml volumetric flask and completed to volume with methanol to obtain the tablet working solution of 10 µg/ml of DIC, 65 µg/ml of PAR, and 50 µg/ml of CHZ.

Construction of the training set

Twelve mixtures of DIC, PAR, and CHZ were prepared by transferring different aliquots of their working solutions into a series of 10 ml volumetric flasks and completing the volume with methanol (Table 1). The ratio of the three drugs in their combined dosage form was taken into consideration during the construction of this set. The absorbances of these prepared mixtures were then scanned between 260 and 400 nm with 0.2 nm intervals against methanol as a blank.

Construction of CLS, PCR, and PLS models

From the obtained data, three multivariate calibration models (CLS, PCR, and PLS) were constructed. The absorbance data matrix and the concentration data matrix were used for the calibration and regression. The obtained calibrations were then used for determination of the unknown concentrations of DIC, PAR, and CHZ in their ternary mixtures and in pharmaceutical dosage form. For CLS model construction, non-zero intercepts were used. The model was constructed by the feeding of the Matlab™ with the absorbance and concentration matrices of the training set, and then, K matrix was calculated. For PCR and PLS methods, PLS-toolbox 2.0 software with the training set absorbance and concentration matrices was used for the calculations.

Selection of the optimum number of factors to build the PCR and PLS models

For the selection of the optimum number of factors, cross-validation method was used leaving out one sample at a time [39]. PLS and PCR calibration on eleven calibration spectra were performed, and using this calibration, the concentration of the sample left out during the calibration process was predicted. This process was repeated 12 times until each training sample had been left out once, and all samples concentrations were predicted. The predicted concentrations of the three drugs in each sample were compared with the true concentrations in this calibration samples, and then, root-mean-square error of cross-validation (RMSECV) was calculated for each method. The optimum number of factors was selected by visual inspection.

Table 1: The concentrations of different mixtures of DIC, PAR, and CHZ used in the training set

Sample No.	DIC (µg/ml)	PAR (µg/ml)	CHZ (µg/ml)
1	1.6	9.4	8.5
2	1.6	10.4	8.0
3	1.6	11.4	7.5
4	3.2	21.8	15.5
5	3.2	20.8	16.0
6	3.2	19.8	16.5
7	4.8	30.2	24.5
8	4.8	31.2	24.0
9	4.8	32.2	23.5
10	6.4	42.6	31.5
11	6.4	41.6	32.0
12	6.4	40.6	32.5

DIC: Diclofenac sodium, PAR:Paracetamol, CHZ:Chlorzoxazone

$$RMSECV = \sqrt{\frac{PRESS}{n}} \quad (1)$$

Where PRESS is the predicted residual error sum of squares and n is the number of calibration samples [40].

$$PRESS = \sum (Y_{pred} - Y_{true})^2 \quad (2)$$

Where Y_{pred} and Y_{true} are predicted and true concentrations in µg/ml, respectively.

Construction of CFBP-ANN

The same training set (Table 1) that used in CLS, PCR, and PLS was also used for training the CFBP-ANN model created in Matlab™, version 7.9.0. CFBP-ANN which contains sigmoid layer with 10 neurons and another linear layer was found appropriate for the simultaneous determination of the three drugs in their ternary mixture (Fig. 3).

Construction of the validation set

To evaluate the prediction performance of the proposed four methods (CLS, PCR, PLS, and CFBP-ANN), nine different mixtures of DIC, PAR, and CHZ were prepared by transferring different aliquots of their working solutions into 10 ml volumetric flasks and completing the volume with methanol. The ratio of the three drugs in tablets was taken into consideration during the preparation of these mixtures. The concentrations of the three drugs in these prepared mixtures were predicted using the suggested techniques.

Analysis of DIC, PAR, and CHZ in Intagesic MR® tablets

The four methods were applied to the determination of DIC, PAR, and CHZ in commercial tablets. Further dilutions of the working tablet solution with methanol were carried out to obtain concentrations of 1.6-4.4 µg/ml of DIC, 10.4-28.6 µg/ml of PAR, and 8.0-22.0 µg/ml of CHZ. Standard addition technique was carried out to prove the accuracy of the proposed methods. The percentage recoveries of the drugs in the pharmaceutical dosage form and added standards were calculated.

RESULTS AND DISCUSSION

CLS, PCR, and PLS

The absorbance data in the wavelength range 267-295 nm with the intervals 0.2 nm were chosen as it provided the highest amount of information about the three drugs of this ternary mixture while the data below 267 nm and above 295 nm were rejected.

CLS model was constructed with non-zero intercept. The non-zero intercept allows an additional degree of freedom when k matrix is calculated. This provides an additional opportunity to adjust the effects of the extraneous substances [41]. The CLS method requires all components in the calibration samples to be known. For the PCR and PLS techniques, selection of the optimum number of factors was a very important step before constructing the models. If the number of factors retained was more than the number required, more noise would be added to the data. On the other hand, if the number retained was less than the number required, meaningful data that could be necessary for the calibration might be ignored. The optimum number of factors was selected by visual inspection. According to the Figs. 4 and 5, two factors were found suitable for both PCR and PLS methods.

CFBP-ANN

The choice of the suitable ANN was done by trying different types of ANNs that included CFBP, Elman back propagation, and radial basis networks. The CFBP-ANN was selected as it gave good results concerning accuracy and precision. Bayesian regulation training function (TRAINBR), gradient descent learning function (LEARNGD), and MSE performance were optimal for the simultaneous determination of the three drugs in their ternary mixture.

Accuracy

The accuracy was assessed by the recovery study of the three drugs in their laboratory prepared mixtures (validation set), and good mean percentage recoveries were calculated (Table 2). Accuracy of the methods was also confirmed using standard addition technique to Intagesic MR® tablets. Good mean percentage recoveries were obtained, indicating the absence of excipients interference and a good accuracy of the methods (Table 3).

Precision

The precision of the methods was assessed by studying intra- and inter-day variation using three concentrations (2.4, 3.0, and 3.6 µg/ml) of DIC, (15.6, 19.5, and 23.4 µg/ml) of PAR, and (12.0, 15.0, and 18.0 µg/ml) of CHZ, representing 80%, 100% and 120%, respectively, in triplicate during the same day and on three consecutive days. The calculated values of % relative standard deviation for the three drugs concentrations were found to be <1% for intra- and inter-day precision,

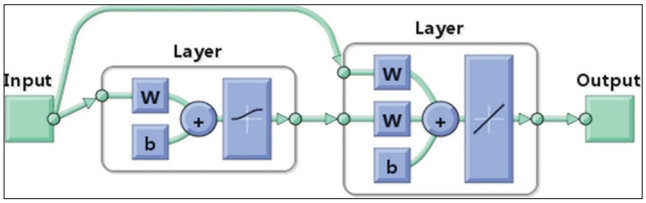

Fig. 3: Architecture of the used cascade-forward backpropagation network

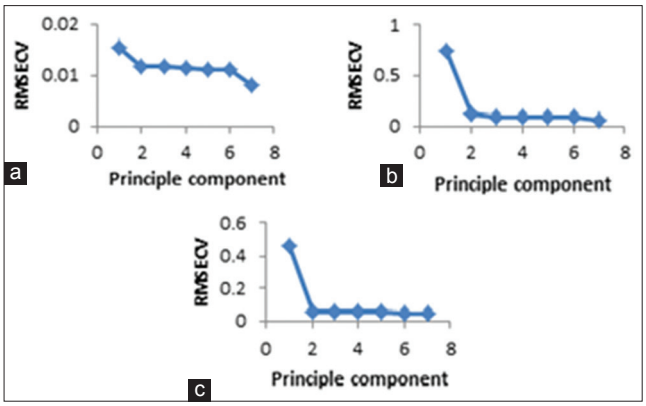

Fig. 4: Root-mean-square error of cross-validation plot as a function of the number of principle components used to construct the principal component regression model (a) diclofenac sodium, (b) paracetamol, and (c) chlorzoxazone

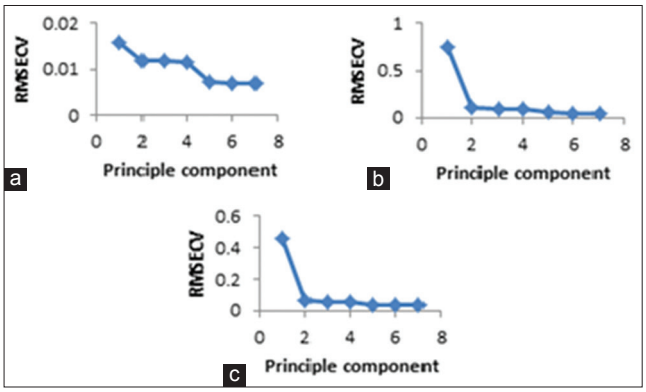

Fig. 5: Root-mean-square error of cross-validation plot as a function of the number of principle components used to construct the partial least squares model (a) diclofenac sodium, (b) paracetamol, and (c) chlorzoxazone

Table 2: Recovery results obtained of the simultaneous determination of DIC, PAR, and CHZ in synthetic mixtures (validation set) using CLS, PCR, PLS, and CFBP-ANN techniques

Concentration (µg/ml)			DIC				PAR				CHZ			
DIC	PAR	CHZ	CLS	PLS	PCR	CFBP-ANN	CLS	PLS	PCR	CFBP-ANN	CLS	PLS	PCR	CFBP-ANN
2.4	14.6	12.5	98.899	99.289	99.290	99.183	98.913	98.974	98.965	99.688	98.891	99.473	99.480	99.263
2.4	15.6	12.0	99.148	99.422	99.422	99.613	99.457	99.455	99.448	99.884	98.947	99.400	99.406	99.371
2.4	16.6	11.5	100.283	100.502	100.502	100.978	100.483	100.429	100.422	100.668	100.139	100.554	100.561	100.717
4.0	27.0	19.5	100.471	100.525	100.525	100.771	100.334	100.303	100.299	100.416	100.566	100.680	100.682	100.845
4.0	26.0	20.0	100.411	100.547	100.547	100.637	100.407	100.407	100.403	100.742	100.414	100.637	100.640	100.680
4.0	25.0	20.5	99.333	99.449	99.449	99.354	99.444	99.478	99.475	99.780	99.265	99.432	99.433	99.359
5.6	35.4	28.5	99.869	99.863	99.863	99.780	100.041	100.063	100.064	100.213	99.762	99.739	99.738	99.732
5.6	36.4	28.0	99.794	99.949	99.949	100.017	99.443	99.447	99.443	100.075	100.023	100.275	100.279	100.338
5.6	37.4	27.5	99.744	99.805	99.805	99.980	99.654	99.631	99.629	99.868	99.805	99.924	99.926	100.077
Mean±SD			99.772±0.560	99.928±0.498	99.928±0.498	100.035±0.635	99.797±0.543	99.799±0.518	99.794±0.519	100.148±0.387	99.757±0.608	100.013±0.534	100.016±0.534	100.042±0.634

SD: Standard deviation, DIC: Diclofenac sodium, CLS: Classical least squares, PCR: Principal component regression, PLS: Partial least squares, CFBP-ANN: Cascade forward back propagation artificial neural network

Table 3: The mean percentage recoveries of the simultaneous determination of DIC, PAR, and CHZ in the tablets and added standards using CLS, PCR, PLS, and CFBP-ANN techniques

Method	Tablets mean recovery% ± SD			Added mean recovery% ± SD		
	DIC	PAR	CHZ	DIC	PAR	CHZ
CLS	101.559±0.428	101.327±0.487	101.709±0.392	100.156±0.800	100.610±0.770	99.860±0.872
PCR	101.475±0.401	101.330±0.488	101.570±0.398	99.768±0.734	100.612±0.771	99.219±0.762
PLS	101.476±0.401	101.326±0.486	101.572±0.397	99.769±0.735	100.602±0.769	99.227±0.763
CFBP-ANN	101.524±0.414	100.731±0.499	101.559±0.383	99.681±0.686	99.938±0.730	99.394±0.684

SD: Standard deviation, DIC: Diclofenac sodium, CLS: Classical least squares, PCR: Principal component regression, PLS: Partial least squares, CFBP-ANN: Cascade forward back propagation artificial neural network

Table 4: Intra-day and inter-day precision results of the simultaneous determination of DIC, PAR, and CHZ using CLS, PCR, and PLS chemometric techniques

Precision	Parameters	DIC			PAR			CHZ		
		CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS
Intraday	Mean±SD	100.194-101.141± 0.045-0.118	100.114-100.782± 0.036-0.110	100.114-100.782± 0.036-0.110	100.177-101.233± 0.068-0.158	100.181-101.238± 0.068-0.158	100.177-100.391± 0.068-0.157	100.064-101.081± 0.030-0.097	99.932-100.485± 0.022-0.083	99.933-100.492± 0.022-0.083
	RSD%	0.045-0.118	0.036-0.110	0.036-0.110	0.067-0.158	0.067-0.157	0.067-0.157	0.030-0.096	0.022-0.082	0.022-0.083
Interday	Mean±SD	100.780-101.370± 0.295-0.449	100.703-101.129± 0.284-0.530	100.703-101.130± 0.284-0.530	100.962-101.692± 0.371-0.635	100.964-101.693± 0.365-0.631	100.959-101.687± 0.370-0.633	100.630-101.161± 0.413-0.475	100.503-100.762± 0.236-0.500	100.505-100.767± 0.231-0.498
	RSD%	0.291-0.446	0.281-0.526	0.281-0.526	0.365-0.629	0.359-0.625	0.364-0.627	0.409-0.470	0.234-0.497	0.229-0.495

SD: Standard deviation, RSD: Relative standard deviation, DIC: Diclofenac sodium, CLS: Classical least squares, PCR: Principal component regression, PLS: Partial least squares

Table 5: Intra-day and inter-day precision results of the simultaneous determination of DIC, PAR, and CHZ using CFBP-ANN technique

Precision	Parameters	DIC	PAR	CHZ
Intraday	Mean±SD	100.278-101.019±0.036-0.06	99.899-100.029±0.048-0.121	99.934-100.636±0.018-0.063
	RSD%	0.036-0.06	0.048-0.121	0.018-0.063
Interday	Mean±SD	100.740-101.167±0.417-0.541	100.368-100.681±0.790-0.995	100.476-100.781±0.311-0.490
	RSD%	0.412-0.537	0.787-0.990	0.309-0.487

SD: Standard deviation, RSD: Relative standard deviation, DIC: Diclofenac sodium, CFBP-ANN: Cascade forward back propagation artificial neural network

Table 6: Statistical analysis of the results obtained by applying the proposed methods and the reference methods

Statistical term	DIC					PAR					CHZ				
	Reference method[a]	CLS	PCR	PLS	CFBP-ANN	Reference method[b]	CLS	PCR	PLS	CFBP-ANN	Reference method[c]	CLS	PCR	PLS	CFBP-ANN
Mean±SD[d]	100.256±	99.841±	100.001±	100.001±	100.111±	100.438±	99.899±	99.897±	99.901±	100.236±	100.786±	99.802±	100.069±	100.065±	100.094±
	0.592	0.595	0.527	0.528	0.710	0.468	0.643	0.612	0.611	0.469	0.895	0.574	0.511	0.511	0.623
SE[e]	0.265	0.266	0.236	0.236	0.317	0.209	0.287	0.274	0.273	0.210	0.400	0.257	0.229	0.228	0.279
RSD%[f]	0.590	0.596	0.527	0.528	0.710	0.466	0.644	0.613	0.612	0.468	0.888	0.575	0.511	0.511	0.623
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
V[g]	0.350	0.354	0.278	0.278	0.504	0.219	0.413	0.375	0.373	0.220	0.801	0.330	0.261	0.261	0.388
F (2.866)[h]	0.334					0.979					1.657				
p	0.852>0.05*					0.441>0.05*					0.199>0.05*				

*No significant difference between the proposed methods and reference methods using one-way ANOVA. [a]BP: British Pharmacopoeia 2013: Potentiometric titration, [b]USP34NF29: United States Pharmacopoeia and National Formulary 2011: Spectrophotometric method, [c]USP34NF29: United States Pharmacopoeia and National Formulary 2011: Spectrophotometric method, [d]SD: Standard deviation, [e]SE: Standard error, [f]RSD: Relative standard deviation, [g]V: Variance, [h]value in parenthesis is critical F value at p=0.05

indicating that intra- and inter-day precisions of the methods as shown in Tables 4 and 5.

Selectivity

The selectivity of the four methods was evident by the good mean percentage recoveries obtained from the laboratory prepared mixtures (validation set) and from the combined dosage form (Intagesic MR® tablets) without any interference from the tablets excipients.

Statistical analysis

Statistical analysis of the recovery results obtained from the laboratory prepared mixtures using the four proposed methods and reference method for each drug was performed using one-way ANOVA. The calculated F values were < the critical one and p values were >0.05, confirming the absence of significant difference between them concerning accuracy and precision as shown in Table 6.

CONCLUSION

The four proposed methods (CLS, PCR, PLS, and CFBP-ANN) can be used for the simultaneous determination of DIC, PAR, and CHZ in synthetic ternary mixtures and pharmaceutical dosage form containing them without interference and without the need for previous physical separation of them. Multivariate calibration models were built from the spectral and concentration data matrices. Verification of the calibrations, carried out with the aid of a synthetic set of mixtures of the three drugs, produced satisfactory results showing simplicity, selectivity, and rapidity. Hence, the proposed methods can be used for quality control of the cited drugs.

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