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Original Article

DEVELOPMENT AND VALIDATION OF SMART SPECTROPHOTOMETRIC-CHEMOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF CHLORPHENIRAMINE MALEATE AND ETILEFRINE HYDROCHLORIDE IN BULK POWDER AND IN DOSAGE FORM COMBINATIONS

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ABSTRACT

This paper describes threesensitive, accurate and precise chemometric spectrophotometric methods for the simultaneous determination of chlorpheniramine maleate (CPM) and etilefrine hydrochloride (ETF) in bulk powder and capsules without prior separation.

Multivariate calibration chemometric methods are proposed for simultaneous determination of CPM and ETF. The chemometric methods applied are classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS). These approaches are successfully applied to quantify both drugs using the information included in the absorption spectra of appropriate solutions. In these multivariate methods, calibration sets of standard samples composed of different mixtures of CPM and ETF have been designed. The methods were validated according to the International Conference on Harmonization (ICH) guidelines. The specificity of the proposed methods was tested using laboratory-prepared mixtures. The developed methods were successfully applied for the determination of CPM and ETF in bulk powder and dosage form combination.

Keywords: Antihistaminic drugs; Chemometric, Chlorpheniramine maleate; CLS, Etilefrine hydrochloride, CLS, PCR, PLS

INTRODUCTION

Chlorpheniramine maleate (CPM) is chemically designated as γ-(4-Chlorophenyl)- N,N-dimethyl-2-pyridinepropamine maleate [1] (Fig. 1a). It is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. Several procedures are reported for quantitative determination of CPM including spectrophotometry [2-5], TLC [6], HPLC [7-9], chemiluminescence [10, 11], capillary electrophoresis [12, 13] and chemometry [14].

Etilefrine hydrochloride (ETF) is chemically designated as alpha-[(Ethylamino)methyl]-3-hydroxy benzenemethanol hydrochloride [1] (Fig. 1b).

It is a cardiac stimulant used as an anti-hypotensive. It is a sympathomimetic amine of the 3-hydroxy-phenylethanolamine series used in treating orthostatic hypotension of neurological, cardiovascular, endocrine or metabolic origin. Reported methods of analysis of etilefrine hydrochloride include spectrophotometric [15-17], capillary electrophoresis [18], GC-MS [19], HPLC [20], (PMR) spectroscopy [21], glass capillary column GC [22], and ion exchange [23] methods.

Under computer-controlled instrumentation, multivariate calibration methods are playing a very important role in the multi-component analysis of mixtures by UV–VIS spectrophotometry[24-28]. These approaches are useful for the resolution of band overlapping in quantitative analysis.

Multivariate calibration has been found to be the method of choice for complex mixtures [29, 31]. In order to avoid time-consuming procedures, attempts to resolve overlapping spectra by using various chemometric methods have been done. Multivariate statistical analysis methods presume that there is a linear relationship between absorbance and component concentrations. Each method has a calibration step in which the relationship between the spectra and the component concentrations is elucidated from a set of reference samples (calibration set). This step is followed by a prediction step in which the results of the calibration are used to calculate the component concentrations from an "unknown" sample spectrum (Validation set) [32].

Reviewing the literature in hand, there are no reported determination methods for this combination except a derivative spectrophotometric method [33] and the one developed by the manufacturer [34].

The multivariate calibration methods investigated in this manuscript include the three most common inverse least square (ILS) methods. These are classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS).In this work, multivariate calibration methods were applied to the determination of CPM and ETF.

The proposed procedures were successfully applied for determination of CPM and ETF in bulk powder and in its pharmaceutical dosage form (capsules).

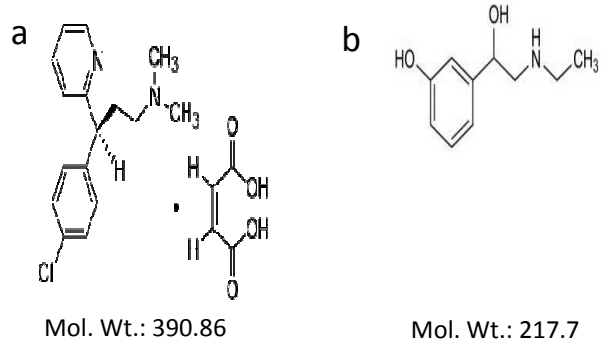

Fig. 1: Chemical Structure of (a) chlorpheniramine maleate and (b) etilefrine hydrochloride.

MATERIALS AND METHODS

Instrumentation

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) using a pair of 1 cm matched quartz cells. The spectrophotometer is connected to an IBM PC with an HP inkjet printer. The bundle software, UV-Probe spectroscopy software version 2.1 (Shimadzu, Kyoto, Japan), was used to process absorption. The data points for the zero-order spectra were then collected every 0.1 nm.

pH meter: Oakton EcoTestr pH 2 Waterproof pH Tester (Model No.:WD-35423-10)

RotaVap: BUCHIKRVR 65/45 ROTAVAPOR-R MIXER (Rotavap w/ condenser, bath evaporating flask)

Software

Microsoft Excel was used for handling and storing absorbance data.

The computations were made using the PLS-Toolbox 2.1 under Matlab™, Version 5.3 [35].

Materials

Pure samples

Pure drug samples of CPM and ETF were kindly supplied by EIPICO Pharmaceuticals, 10th of Ramadan City, Egypt. Their purity was checked and found to be 99.42 ± 0.672 % and 99.09 ± 0.764 % according to the BP [36] for CPM and ETF, respectively.

Pharmaceutical dosage form

Balkis capsules (EIPICO Pharmaceuticals) Batch No. 1102490, labeled to contain 6 mg chlorpheniramine and 20 mg etilefrine per capsule both bound to an ion exchange rresin were purchased from local pharmacies.

Solvent

Double distilled water.

Stock and working standard drug solutions

Stock standard solutions

CPM and ETF stock standard solutions (both are 1 mg ml^{-1}), prepared by dissolving 100 mg of CPM and ETF, each, in a few milliliters of double distilled water into two 100-ml volumetric flasks and then completing to the mark with the same solvent.

Working standard solutions

CPM working standard solution (0.25 mg ml^{-1}), prepared by transferring 25 ml of CPM stock standard solution into a 100-ml volumetric flask and completing to the mark with the same solvent.ETF working standard solution (0.5 mg ml^{-1}), prepared by transferring 50 ml of ETF stock standard solution into a 100-ml volumetric flask and completing to the mark with the same solvent.

Procedure

Spectral characteristics and wavelengths selection

The absorption spectra of 30 µg ml^{-1} of CPM, 100 µg ml^{-1} of ETF and a mixture of both containing the same previous concentration of each drug over the wavelength range of 200–300 nm were recorded.

Linearity and construction of calibration curves

Construction of training and validation set

Multilevel multifactor design was used for the construction of 25 binary mixtures. A five level two-factor design was used [37] in which 1.5, 2, 2.5, 3 or 3.5 ml aliquots of CPM working standard solution and 3, 3.5, 4, 4.5 or 5 ml aliquots of EFT working standard solution were combined in 25 ml volumetric flasks and completed to the mark with double distilled water. Final concentration ranges were 15-35 µgml^{-1} and60-100 µgml^{-1} for CPM and ETF, respectively. The ranges of concentrations were selected in order to ensure that the total absorbance will not exceed the linear range of the spectrophotometer. From the 25 samples, 17 samples were chosen for the construction of the calibration set, while 8 samples were used as an external validation set. Figure 2 shows the relation between different samples. Concentrations of the two compounds in both calibration and validation sets are presented in Table (1).The absorbance of these mixtures were measured between 200 and 300 nm at 0.1 nm intervals against double distilled water as blank.

Pre-processing the data

For the three methods, the regions from 200 to 229.9 nm and above 290.0nmwere rejected to end up with 601 wavelengths between 230 and 290 nm. For CLS, three wavelengths (240, 260 and 280 nm) were chosen to construct the absorbance matrix. For PCR and PLS, seven wavelengths (230, 240, 250, 260, 270, 280 and 290 nm) were chosen to construct the absorbance matrix.

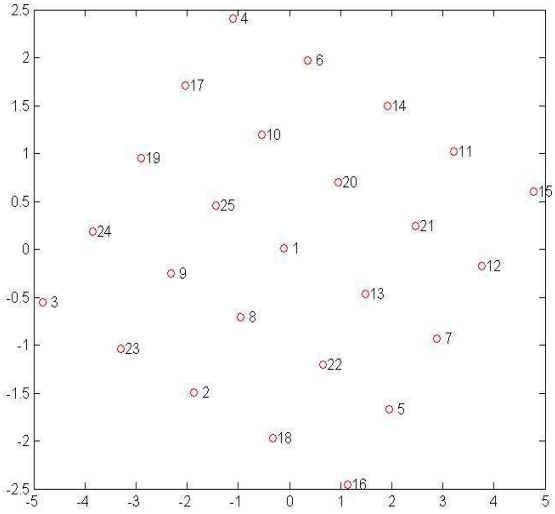

Fig. 2: The first two latent variables of the concentration matrix showing the relation between different samples.

Constructing the models

For the three techniques, the absorbance data matrix for the training set concentration matrix(Table 1) was obtained by the measurement of absorbances between 200.0 and 300.0 nm in the intervals of 0.1 nm then performing the pre-processing as mentioned above. In these techniques, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for prediction of the unknown concentrations of CPM and ETF in their binary mixtures and pharmaceutical formulation. For CLS method, CLS model was constructed with non-zero intercept. To build the CLS model, the computer was fed with the absorbance and concentration matrices for the training set. The calculations to obtain the K matrix were carried out. For the PCR and PLS models, the training set absorbance and concentration matrices together with PLS-toolbox 2.0 software were used for calculations.

Selection of the optimum number of latent variables to build the PCR and PLS models

The cross validation method was used, leaving out one sample at a time, to select the optimum number of latent variables (LVs). Given a set of seventeen calibration samples, PCR and PLS calibrations were performed, and using this calibration, the concentration of the sample left out was predicted. The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated. The maximum number of LVs used to calculate the optimum RMSECV was selected to be twelve. The RMSECV indicates both the precision and accuracy of predictions. It was recalculated upon addition of each new LV to the PLS and PCR models.

Table 1: Concentrations of chlorpheniramine maleate and etilefrine hydrochloride in the calibration and validation sets

Sample No.	CPM (µg ml^{-1})	ETF (µg ml^{-1})
1	25	80
2	25	60
3	15	60
4	15	100
5	35	70
6	20	100
7	35	80
8	25	70
9	20	70
10	20	90
11	30	100
12	35	90
13	30	80
14	25	100
15	35	100
16	35	60
17	15	90
18	30	60
19	15	80
20	25	90
21	30	90
22	30	70
23	20	60
24	15	70
25	20	80

The shaded samples are those of the validation set.

Assay of laboratory prepared mixtures containing different ratios of chlorpheniramine maleate and etilefrine hydrochloride

Solutions containing different ratios of CPM and ETF were prepared by transferring accurately measured aliquots of CPM and ETF from their corresponding working standard solutions into a series of 25-ml volumetric flasks and the volume was completed to the mark with double distilled water. The final concentration ranges were 5 - 60 µg ml^{-1} for CPM and 10 - 140 µg ml^{-1} for ETF. Zero order absorption spectra of these different laboratory prepared mixtures were recorded using double distilled water as blank and the procedure under linearity for the three methods was then followed. Concentrations of CPM and ETF in the prepared samples were calculated from the computed corresponding regression equations or models.

Application to pharmaceutical preparation

The following procedures were carried out as a modification to the manufacturer method of analysis obtained by personal communication [34]. To determine the content of etilefrine and chlorpheniramine in commercial Balkis capsules (each capsule labeled to contain 20 mg etilefrine and 6 mg chlorpheniramine), the following procedure was followed in the dark (using aluminum foil to cover all flasks).

The contents of 10 capsules were carefully emptied and weighed. A portion of the contents equivalent to the average weight of two capsules was accurately weighed and transferred to a 250-ml conical flask. This portion was washed twice with 100 ml of warm double distilled water using a magnetic stirrer for 5 minutes each. Each washing was decanted after complete sedimentation of the resin quantitatively, keeping the resin in the flask.

Extraction was carried out using the following procedure

Extracting solution was prepared as follows: 4.6 g of sodium chloride were dissolved in 200 ml of distilled water, and then mixed with 20 ml of 25% hydrochloric acid and 700 ml methanol.

i- 100 ml of the extracting solution (see above) were added to the 250-ml conical flask, the flask was then stoppered (to prevent evaporation of the methanol during extraction) and stirring was done for 1½ hours using a magnetic stirrer.

ii- the extracting solution was collected, in a 1000-ml evaporating flask, by decantation, keeping the resin in the extracting vessel.

iii- Steps i and ii were repeated three more times (each 1½ hours) and the extracting solution was collected into the same 1000-ml evaporating flask.

iv-The extraction was repeated using 100 ml of the extracting solution but the extraction was continued overnight (12 hours).

v- The extracting solution was transferred by the same method to the evaporating flask.

The pH was adjusted to 7 using 10% W/V sodium hydroxide solution. Methanol was evaporated using a rotary evaporator (Rotavap). The remaining solution was transferred quantitatively to a250-ml volumetric flask, then was completed to volume using double distilled water, then was mixed and filtered through 0.5µm Whatman filter paper. From the above prepared solution, further dilutions were prepared in the linearity range using double distilled water. The analysis was done in triplicates.

For chlorpheniramine determination: the general procedure described above under each method to determine the concentration of chlorpheniramine di-hydrochloride in the prepared dosage form solution from the corresponding *modified* computed model was followed. The *modified* computed models were obtained by multiplying the concentration of CPM used to obtain the models by 0.8897 then rebuild each model to get the *modified* computed model. The found chlorpheniramine di-hydrochloride percentage obtained from the previous step was multiplied by 0.7901 to obtain the found chlorpheniramine percentage. For etilefrine determination: the general procedure described above under each method to determine the concentration of ETF in the prepared dosage form solution from the corresponding computed model was followed. The found ETF percentage was multiplied by 0.8323 to obtain the found etilefrine percentage.

RESULTS AND DISCUSSION

The zero order absorption spectra of CPM and ETF show severe overlapping that prevents the use of direct spectrophotometry for their analysis without preliminary separation (Fig. 3). No spectrophotometric procedure was found in literature for the simultaneous determination of CPM and ETF.

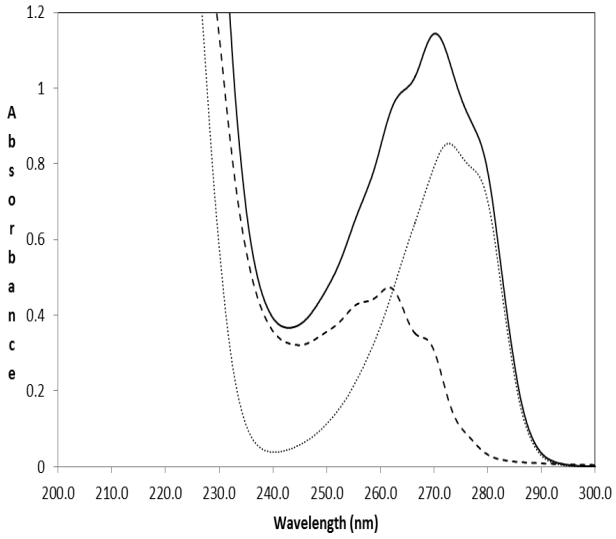

Fig. 3: Zero order absorption (^{0}D) spectra of 30 µg ml^{-1} chlorpheniramine maleate (-------), 100 µg ml^{-1} etilefrinehydrochloride (·····) and mixture of both (_____) using double distilled water as solvent.

With the aim of finding spectrophotometric methods for the analysis of CPM and ETF, several chemometric approaches were evaluated. Multivariate calibration is useful for spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of single wavelength greatly improves the precision and predictive ability [38]. The full-spectrum methods have the ability to achieve improved precision since there is a signal averaging effect when many or all the spectral intensities are included in the analysis making it less susceptible to noise in the spectra.

Haal and and Thomas [29] made a comparison of the different multivariate calibration methods for quantitative spectral analysis. They concluded that it is difficult to generalize about the superiority of one method over another, because the relative performance of the methods is often dependent on particular data set being analyzed. CLS method requires that all components in the calibration samples must be known regarding number of constituents and concentration of every constituent. For PCR and PLS methods, unlike CLS all overlapping spectral components do not have to be known.

A convenient method for resolving mixtures, which can in principle be applied to the present case, is the least square analysis. The simplest of them is the classical least squares (CLS). It should certainly be preferred when the selection of variables is simple. In such cases, the regression coefficients for different selected collinear wavelengths may have relatively little meaning for interpretation purposes, but the model performs well, both in the calibration and predictions tages, provided that the model having linearity between responses and concentrations and the prediction is performed within the calibration domain. In addition, the baseline effects and noise are probably non-significant or of very low significance.

The wavelength range 230.0–290.0 nm with 0.1 nm intervals was chosen as it provides the greatest amount of information about the mixture components. 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 [39].

Selection of the optimum number of latent variables for PCR and PLS methods

Selection of the optimum number of LVs for the PCR and PLS techniques was a very important step before constructing the models. If the number of LVs retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was less than the required, meaningful data that could be necessary for the calibration might be ignored. To select the optimum number of LVs for PCR and PLS methods, a cross-validation method using leave one out, was used [39]. Given the set of 17 calibration spectra corresponding to the samples listed in Table 1,the PCR and PLS models were constructed using 16 calibration spectra samples. The concentration of the sample left-out during calibration was predicted. This process was repeated 17 times until each calibration sample had been left-out once. The predicted concentration of the compound in each sample was compared with the actual known concentration of the drug. The RMSECV was calculated in the same manner each time. The method described by Haal and and Thomas [32] was used for selecting the optimum number of LVs. The method used an F-test to compare RMSECV values from cross-validation. The procedure starts by finding the smallest RMSECV value, RMSECV(k*) then all the models with fewer LVs (k < k*) are compared with the model with k*LVs.

F(k) = RMSECV(h)/RMSECV(k*)

Where, k = 1, 2, 3, 4,…….k*

The number of LVs chosen (k) will be the minimum number having F (k) <$F_{d,m,m}$ where d is the level of significance and m is the number of calibration samples As the difference between the minimum RMSECV and other RMSECV values become smaller, the probability that each additional LV is significant becomes smaller [30]. The maximum number of LVs used to calculate the optimum RMSECV was selected as twelve. Three and two LVs were found suitable for PCR and PLS, respectively, as in Figures4 and 5. The results predicted by the multivariate methods for the training set model are summarized in table 2.

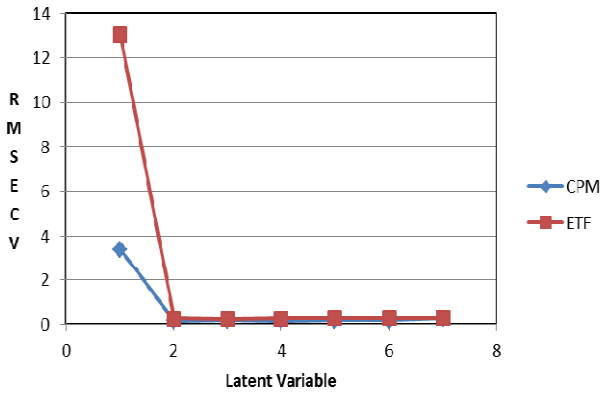

Fig. 4: RMSEC plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the PCR calibration.

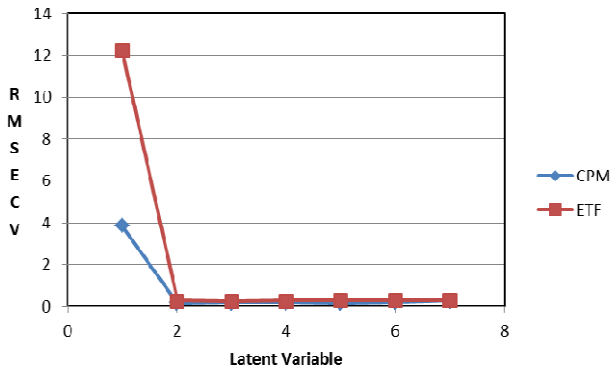

Fig. 5: RMSEC plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the PLS calibration.

Selection of the optimum number of wavelengths for model building and sample recovery for CLS, PCR and PLS methods

The absorption spectra of training and validation sets for CPM and ETF mixtures were recorded over the wavelength range of 200–300 nm at an interval of 0.1 nm as shown in figure3. Wavelength selection was carried out in two successive steps:

a- Pre-processing the data: which takes place by eliminating the regions from 200.0 to 229.9 nm and above 290.0 as it has either very high absorbance or showing almost zero absorbance, respectively.

b- Manual wavelength selection: The original data comprises 601 wavelengths (230.0 - 290.0 nm recorded every 0.1 nm). Several trials were done to select the optimal number of wave lengths to build the models. The trials include:

i- 601 wavelengths (the original spectrum between 230 to 290 nm recorded every 0.1 nm)

ii- 61 wavelengths (the original spectrum between 230 to 290 nm recorded every 1 nm)

iii- 7 wavelengths (the original spectrum between 230 to 290 nm recorded every 10 nm, namely 230, 240, 250, 260, 270, 280 and 290)

iv- 3 wavelengths (by dividing the spectrum from 230 to 290 nm into 3 equal sections and selecting the middle wavelength of each section, this will result in choosing 240, 260 and 280 nm)

Satisfactory results were obtained from all the 4 selections mentioned above, showing almost the same recovery average and standard deviation. For CLS, three wavelengths were selected while for PCR and PLS, seven wavelengths were selected for building the models as they show a very slight improvement in standard deviation. But again, any of the four above mentioned selections can be successfully used for the determination of both drugs.

Table 2: Results obtained by applying CLS, PCR and PLS calibration methods to validation set of chlorpheniramine maleate and etilefrine hydrochloride

			CLS (3 λ)				PCR (7 λ)				PLS (7 λ)			
Mixture	Actual (µg ml^{-1})		Found (µg ml^{-1})		Found %		Found (µg ml^{-1})		Found %		Found (µg ml^{-1})		Found %	
number	CPM	ETF	CPM	ETF	CPM	ETF	CPM	ETF	CPM	ETF	CPM	ETF	CPM	ETF
1	25	60	25.15	60.25	100.58	100.41	25.06	60.02	100.23	100.03	25.09	59.90	100.35	99.84
2	35	80	34.94	80.00	99.82	100.00	35.01	79.98	100.03	99.97	35.02	79.93	100.06	99.92
3	20	70	20.24	70.78	101.22	101.11	20.09	70.58	100.44	100.83	20.09	70.59	100.44	100.84
4	20	90	20.08	89.93	100.41	99.92	20.11	89.91	100.57	99.90	20.12	89.90	100.58	99.89
5	25	100	25.12	100.12	100.49	100.12	25.25	100.28	101.00	100.28	25.25	100.27	101.00	100.27
6	15	80	15.14	80.37	100.93	100.47	15.00	80.34	100.00	100.43	14.99	80.38	99.94	100.47
7	30	90	30.35	89.62	101.17	99.58	30.37	89.79	101.24	99.77	30.36	89.84	101.20	99.82
8	30	70	30.40	70.47	101.32	100.67	30.30	70.41	100.99	100.58	30.29	70.43	100.98	100.61
Average					100.74	100.28			100.56	100.22			100.57	100.21
S.D.					0.513	0.480			0.473	0.370			0.458	0.397

All results are average of three determinations

Comparison of the results from the proposed methods

Table 3 shows the actual and predicted amounts± the standard deviations (%) of the studied drugs as given by the CLS, PCR and PLS of the spectral data. These spectral data were obtained experimentally in the calibration range of each drug at the wavelength range from 230 to 290 nm. The results confirm the considerable degree of agreement between the three techniques and indicate that these methods are suitable for this analysis in the given calibration domain for each drug if compared with the official methods. The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations. The results are obtained in Table 4.

Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating adequate models as shown in Figures 6–8. The RMSECV was used as a diagnostic test for examining the error in the predicted concentrations. RMSECV indicates both the precision and accuracy of predictions. RMSECV plays the same role of standard deviation in indicating the spread of the concentration errors [40]. In Table 4, the RMSECV, slope and intercept of predicted Vs. true concentrations are obtained. As can be seen, the results are satisfactory and indicate good predictive abilities of the developed models. The chemometric methods were applied successfully to the analysis of CPM and ETF in Balkis capsules. The interfering species (additives) were not included in calibration samples, but were present during capsules determination.

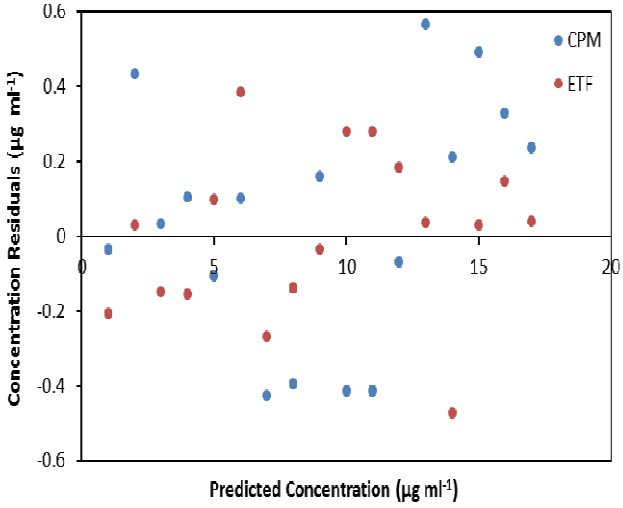

Fig. 6: Concentration residuals vs. predicted concentration of CPM and ETF using CLS (with non-zero intercept).

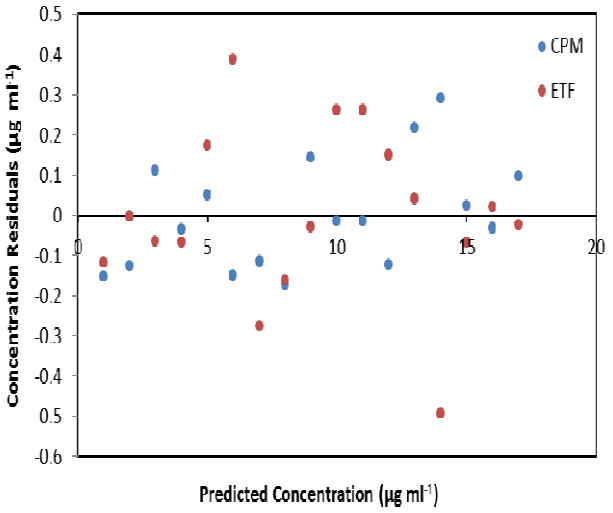

Fig. 7: Concentration residuals vs. predicted concentration of CPM and ETF using PCR.

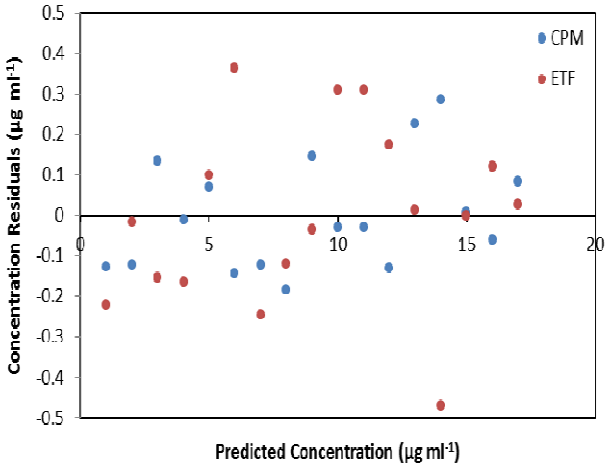

Fig. 8: Concentration residuals vs. predicted concentration of CPM and ETF using PLS.

Determination of chlorpheniramine and etilefrine in Balkis capsules

Extraction optimization

Determination of chlorpheniramine and etilefrine in Balkis capsules was the most challenging part of this work. Chlorpheniramine and etilefrine are present in the capsules as chlorpheniramine resinate and etilefrine resinate, respectively. Chlorpheniramine and etilefrine bases are linked to anion exchange resin to provide the sustained release action. Several unsuccessful trials to extract the two bases from the resins were performed. The following common solvents were tried using a magnetic stirrer to extract the bases: double distilled water, 0.1 N HCl, 0.1 N NaOH, methanol and Benzene. Unsatisfactory recovery percentages were obtained using all the previous solvents even when the extraction time was extended from 30 minutes to 1 hour and then to 2 hours.

The manufacturer method of analysis obtained by personal communication [34] included extraction by the specified extraction solution previously mentioned under 2.5.4. The extraction time was one and half hour followed by another 12 hours. Then the extract was analyzed utilizing HPLC and the results obtained were compared to the results obtained from the extraction of specific weights of standard etilefrine resinate and standard chlorpheniramine resinate. Although the recovery percentages were improved applying this extraction regimen, but still did not provide satisfactory recovery percentages. A modification to the previous extraction method was to extend the extraction period by 1.5, 3, 4.5 and 6 hours divided into 1.5 hours intervals showed gradual improvement in recovery percentage. The best and optimum results were found by extraction of the capsules content for the original 1.5 hours stated by the manufacturer then adding three 1.5 hour extraction periods each with 100 ml of the extraction solutions. This was followed by the 12 hours stated by the manufacture.

Calculation optimization

Chlorpheniramine is present in the capsule as chlorpheniramine resinate. After extraction, it will be available in the final extraction solution as chlorpheniramine di-HCl. Models were built and calculated according to the authentic Chlorpheniramine maleate (CPM). So, models have to be rebuilt by multiplying all the chlorpheniramine maleate (CPM) concentrations used in them by 0.8897 to convert the chlorpheniramine maleate (CPM) concentration to chlorpheniramine di-HCl concentration.

Chlorpheniramine conversion factor 1 =

(Mol. weight of chlorpheniramine di − HCl)/(Mol. weight of chlorpheniramine maleate) = 347.8/390.9 = 0.8897

Then, to convert the chlorpheniramine di-HCl found percentage to chlorpheniramine found percentage, it should be multiplied by the conversion factor of 0.7901.

Chlorpheniramine conversion factor 2 =

$(Mol.\ weight\ of\ chlorpheniramine)/(Mol.\ weight\ of\ chlorpheniramine\ di-HCl)$ = 274.8/347.8 = 0.7901

Etilefrine is present in the capsule as etilefrine resinate. After extraction, it will be available in the final extraction solution as etilefrine HCl (ETF). Models were built and calculated according to the authentic etilefrine HCl (ETF). So, to convert the etilefrine HCl (ETF) found percentage to etilefrine found percentage, it is multiplied by the conversion factor of 0.8323.

Etilefrine conversion factor = $(Mol.\ weight\ of\ etilefrine)/(Mol.\ weight\ of\ etilefrine\ HCl)$ = 181.2/217.7 = 0.8323

Method Validation

Validation of the proposed methods was assessed according to ICH guidelines [41].

Linearity

Linearity of the proposed methods was verified by analyzing five different concentrations in the range of 10-60 µg ml^{-1} for CPM and 10-140 µg ml^{-1} for ETF. Each concentration was made in triplicate. The assay was performed according to the experimental conditions previously mentioned.

Range

The calibration range was established through consideration of the practical range necessary according to adherence to Beer's law and the concentration of CPM and ETF present in the pharmaceutical preparations to give accurate, precise and linear results. Assay parameters are declared in table 4.Although the concentration ranges used to build the models are 15-35 and 60-100 µg ml^{-1}for CPM and ETF, respectively, yet the 3 methods were able to detect concentration ranges of 10-60 and 20-140 µg ml^{-1}for CPM and ETF, respectively. This not only illustrated the resolving power of the 3 methods but also the suitability of these methods for the CPM and ETF mixture.

Accuracy

The accuracy of the proposed methods was performed by applying the suggested procedures for determination of the validation

samples as well as different blind samples of CPM and ETF.The concentrations were obtained from the corresponding model, from which the percentage recoveries suggested good accuracy of the proposed methods. Results are shown in table 4.

Table 3: Recovery percent average and standard deviation for calibration and validation set for chlorpheniramine maleate and etilefrine hydrochloride using CLS, PCR and PLS using 601, 61, 7 and 3 wavelengths for construction of the model

Technique	Number of wavelengths used for building the model	CPM				ETF			
		Calibration set		Validation set		Calibration set		Validation set	
		Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
CLS	601 wavelengths	100.28	1.062	100.90	0.585	100.03	0.271	100.21	0.393
	61 wavelengths	100.29	1.090	100.90	0.604	100.03	0.266	100.21	0.394
	7 wavelengths	100.40	1.352	101.01	0.837	100.01	0.259	100.22	0.404
	3 wavelengths	100.18	0.885	100.74	0.513	100.06	0.319	100.28	0.480
PCR	601 wavelengths	99.99	0.604	100.58	0.466	100.00	0.243	100.18	0.334
	61 wavelengths	99.99	0.600	100.57	0.480	100.00	0.238	100.19	0.330
	7 wavelengths	99.99	0.588	100.56	0.473	100.00	0.246	100.22	0.370
	3 wavelengths	100.00	0.528	100.30	0.706	100.00	0.248	100.25	0.409
PLS	601 wavelengths	99.99	0.625	100.59	0.545	100.00	0.249	100.18	0.364
	61 wavelengths	99.99	0.618	100.58	0.542	100.00	0.246	100.18	0.366
	7 wavelengths	99.99	0.602	100.57	0.458	100.00	0.256	100.21	0.397
	3 wavelengths	99.99	0.645	100.54	0.559	100.00	0.253	100.22	0.390

All results are average of three determinations.

Table 4: RMSECV and statistical parameter values for chlorpheniramine maleate and etilefrine prediction using multivariate calibration methods

Parameter	CPM			ETF		
	CLS	PCR	PLS	CLS	PCR	PLS
RMSECV	0.2256	0.1984	0.1962	0.3847	0.3069	0.3146
Range µg ml-1	10 - 60	10 - 60	10 - 60	20 - 140	20 - 140	20 - 140
Intercept	0.1701	-0.0539	-0.0602	1.4906	0.6807	0.5160
Slope	1.0003	1.0081	1.0085	0.9838	0.9935	0.9955
Correlation coefficient (r)	0.9997	0.9998	0.9998	0.9997	0.9998	0.9997
Accuracy	100.74 ± 0.513	100.56 ± 0.473	100.58 ± 0.542	100.28 ± 0.480	100.22 ± 0.370	100.21 ± 0.397
Repeatability[a]	100.63 ± 0.715	100.42 ± 0.612	100.46 ± 0.671	100.38 ± 0.591	100.31 ± 0.467	100.29 ± 0.513
RSD%[a]	0.711	0.609	0.668	0.589	0.466	0.512
Intermediate precision[b]	100.51 ± 0.821	100.49 ± 0.745	100.53 ± 0.786	100.56 ± 0.784	100.36 ± 0.591	100.40 ± 0.643
RSD%[b]	0.817	0.741	0.782	0.780	0.589	0.640

a: Intra-day (n=3), average of three concentrations of CPM (10, 30 & 50 µg ml-1) and ETF (40, 80 &120 µg ml-1) repeated within the same day.
b: Inter-day (n=3), average of three concentrations of CPM (10, 30 & 50 µg ml-1) and ETF (40, 80 &120 µg ml-1) repeated in three consecutive days.

Table 5: Determination of chlorpheniramine maleate and etilefrine in its dosage form by the proposed spectrophotometric methods

Recovery* % ± SD of the claimed mean (mg tablet-1)														
	CPM							ETF						
		CLS		PCR		PLS			CLS		PCR		PLS	
Pharmaceutical Formulation	Label Content mg	Found mg	Recovery %	Found mg	Recovery %	Found mg	Recovery %	Label Content mg	Found mg	Recovery %	Found mg	Recovery %	Found mg	Recovery %
Balkis Capsules Batch No. 1102490	6.00	5.91	98.50	5.93	98.83	5.95	99.17	20.00	19.61	98.05	19.86	99.30	19.91	99.55
		5.83	97.17	5.90	98.33	5.91	98.50		19.54	97.70	19.97	99.85	19.92	99.60
		5.89	98.17	5.88	98.00	5.90	98.33		20.13	100.65	20.03	100.15	20.07	100.35
	Mean		97.94		98.39		98.67	Mean		98.80		99.77		99.83
	± S.D.		0.694		0.419		0.441	± S.D.		1.612		0.431		0.448
	% RSD		0.708		0.426		0.447	% RSD		1.631		0.432		0.449

Table 6: Statistical comparison of the results obtained by the proposed methods and the reference methods for the determination of chlorpheniramine maleate and etilefrine in pure powder form

Parameter	CPM				ETF			
	Manufacturer method*	CLS	PCR	PLS	BP method**	CLS	PCR	PLS
Mean	99.42	99.63	99.72	99.66	99.09	100.08	99.70	99.71
S.D.	0.672	0.643	0.454	0.475	0.764	0.680	0.402	0.398
n	5	5	5	5	5	5	5	5
Variance	0.451	0.413	0.206	0.225	0.583	0.462	0.162	0.158
Student t (2.306)***		0.621	0.426	0.528		0.063	0.169	0.163
F (6.388)***		1.093	2.195	2.002		1.263	3.606	3.681

*: Manufacturer method is a non-aqueous potentiometric titration method.
**: BP method is a non-aqueous potentiometric titration method.
***: Figures in parenthesis are the corresponding tabulated values at P = 0.05

Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of CPM and ETF within the linearity range, including the ratio present in the pharmaceutical dosage form. Satisfactory results were obtained as shown in table 4.

Precision

Repeatability

The intra-day precision of the developed method was evaluated by analyzing samples of three different concentrations of CPM (10, 30, and 50 µg ml^{-1}) and ETF (40, 80, and 120 µg ml^{-1}) in triplicates on the same day. The relative standard deviations were calculated (Table 4).The relative standard deviations were found to be below 2% and the method proved to be repeatable.

Reproducibility (Intermediate precision)

The previous procedures were repeated inter-daily on three different days for the analysis of the three chosen concentrations. The relative standard deviations were calculated (Table 4). The relative standard deviations were found to be below 2% and the method proved to be reproducible.

Stability

CPM and ETF working standard solutions in double distilled water showed no spectrophotometric changes up to 2 weeks when stored at room temperature.

Application of the method in assay of capsules

The proposed spectrophotometric multivariate calibration methods were applied for the determination of CPM and ETF in their combined pharmaceutical formulation (Balkis Capsules) as shown in table 5. It shows that the developed methods are accurate and specific for determination of the cited drugs in presence of dosage form excipients.

Statistical analysis

Results obtained by the proposed methods for the determination of pure samples of CPM and ETFwere statistically compared to those obtained by the official methods (BP methods)[36]. The calculated t and F values were found to be less than their corresponding theoretical ones confirming good accuracy and excellent precision (Table 6).

CONCLUSION

In this manuscript, three chemometric techniques have been investigated to determine which technique is the most suitable for the simultaneous determination of CPM and ETF without the use of preliminary separation step. The good recoveries obtained in all cases as well as the reliable agreement with the reported procedures proved that the proposed procedures could be applied efficiently for determination of the studied drugs simultaneously in their binary mixtures as well as in the commercial dosage forms with satisfactory precision. The proposed methods are simple, sensitive, accurate, precise and economical. They could be easily applied in quality control laboratories for the routine analysis of the studied drugs in pure bulk powder and dosage form without any preliminary separation step. The most striking features of the methods are their simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision, sensitivity and stability.

It is noteworthy to mention that using double distilled water as a solvent, besides being cheap; it is extremely safe to the environment (Green Analytical Chemistry). The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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