Application of Multivariate Methods to the Simultaneous Fourier Transform Infrared (FT-IR) Spectrometric Determination of Stereo-Isomers; Quinine and Quinidine

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The two stereo-isomers; quinine and quinidine have been determined in their mixtures in the IR region using chemometric multivariate methods, principal component regression (PCR) and partial least squares (PLS). A training set of thirty synthetic binary mixture solutions in the possible combinations containing 0.0—4.0 and 4.0—0.0% w/v quinine and quinidine, respectively in chloroform was used to develop the multivariate calibrations. A validation set containing thirty synthetic binary mixtures of variable ratios in the range of 0.2—4.0 and 4.0—0.2% w/v for quinine and quinidine, respectively in chloroform was used to validate the developed calibrations. The results of analysis of the validation synthetic mixtures were found to be 100.5±0.44% (R.S.D.%=0.44) and 100.5±0.38% (R.S.D.%=0.38) for quinine and 100.1±0.67% (R.S.D.%=0.67) and 100.1±0.68% (R.S.D.%=0.68) for quinidine using PCR and PLS models, respectively.

Key words fourier transform infrared spectrometry; multivariate method; chemometric; stereo-isomer; quinine; quinidine

Quinine $C_{20}H_{24}N_2O_2$, $3H_2O$, (−) (8*S*,9*R*)-6′-methoxycinchonan-9-ol trihydrate, Mol. Wt. 378.5 (Fig. 1) and quinidine $C_{20}H_{24}N_2O_2$, $2H_2O$, (+) (8*R*,9*S*)-6′-methoxycinchonan-9-ol dihydrate, Mol. Wt. 360.5 (Fig. 2) are the chief quinoline alkaloids of various species of Cinchona bark. They are stereo-isomers of which quinine is the levorotatory isomer (−) and quinidine is the dextrorotatory one (+). Quinine is used mainly as an antimalarial drug. The precise mechanism of action of quinine is unclear but it may interfere with lysosome function or nucleic acid synthesis in the malaria parasite.[1] Quinidine is an antiarrhythmic drug. It has also antimuscarinic and alpha-adrenoceptor blocking properties.[1]

Some methods have been described for the determination of quinine and quinidine. These include: HPLC with fluorescence detection,[2] stereoselective non aqueous capillary electrophoresis,[3] TLC and HPTLC with densitometric evaluation,[4] liquid chromatography with diode-laser polarimetric detection,[5] fluorescence life-time determination based on modulation measurements by ion-pair diastereomeric complexation[6] and circular dichroism.[7] First derivative spectrophotometry has been applied to the determination of the sum of quinine and quinidine.[8] FT-IR has been used for spectroscopic characterization of complexes of carbamoylated quinidine with *R* and *S* enantiomers of *N*-derivatized leucine as an evidence of intermolecular interactions.[9]

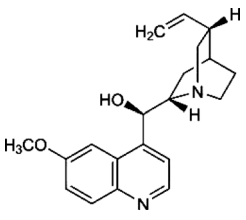

Fig. 1. Chemical Structure of Quinine

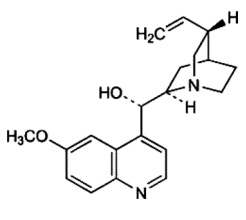

Fig. 2. Chemical Structure of Quinidine

The present work deals with the simultaneous FT-IR spectrometric determination of two stereo-isomers; quinine and quinidine using chemometric multivariate methods principal component regression (PCR) and partial least squares (PLS). This is the first application of multivariate methods to the determination of stereo-isomers. Stereoselective columns and other sophisticated tools used to separate and quantify two stereo-isomers may be routinely not available in quality control laboratories.

Experimental

Instrumentation FT-IR Spectrophotometer: A Perkin Elmer Spectrum RXIFT-IR system, manually filled liquid cell, deuterated triglycine sulfate (DTGS) detector. The device was connected to a deskjet 640 C printer.

Sonicator: Julabo USR 3 sonicator.

Rotary Evaporator: Heidolp Rotavbap Model Laborata 4000 for distillation under reduced pressure.

Balance: Sartorius Analytical Balance for weighing the solid materials.

Software The PCR and PLS analyses were carried out using the chemometrics Toolbox 3.02 software[10] for use with MATLAB 6.5.

Materials, Reagents and Pharmaceutical Formulations All materials and reagents were of analytical grade. Quinine hydrochloride was supplied by El-Nasr Co., Egypt. Quinidine sulfate was supplied by Amoun Pharmaceutical Co. S.A.E. El-Obour City, Cairo, Egypt.

Preparation of Solutions Quinine and quinidine bases were extracted from aqueous solutions of quinine hydrochloride and quinidine sulfate, respectively. Aqueous solution was made alkaline to litmus paper using 5 ml 1.0 N NaOH. The free base was extracted with three successive 25 ml portions of chloroform. The organic extract is filtered over anhydrous sodium sulfate to remove water residuals, evaporated to dryness using vacuum rotary evaporator then cooled to obtain the corresponding base. Stock solutions of 8% w/v of quinine and quinidine bases were prepared in chloroform. Suitable dilutions were made from both stocks using chloroform to prepare the solutions of the training set containing different concentration ratios of quinine and quinidine (Table 1). Solutions of the validation set (Table 1) have been prepared similarly using independently prepared quinine and quinidine stocks.

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A training set of thirty synthetic binary mixture solutions in the possible combinations containing 0.0—4.0 and 4.0—0.0% w/v quinine and quinidine, respectively was used to develop the multivariate calibrations. A validation set containing thirty synthetic binary mixtures in the range of 0.2—4.0 and 4.0—0.2% w/v quinine and quinidine, respectively was used to validate the developed calibrations.

IR Spectrometric Measurements The IR absorption spectra of solutions of quinine and quinidine in chloroform were recorded over the wavenumber range of 4000—440 cm^{-1} using 64 accumulated scans with scan speed of 1 scan/4 s and a resolution of 4 cm^{-1}.

Conditions The absorbances measured at both the absorption bands region (900—797) cm^{-1} and the stretching C–H band region (2893—2845) cm^{-1} were selected for the multivariate analysis.

Results and Discussion

Figure 3 shows the IR absorption spectra of 1.0% w/v quinine and 1.0% w/v quinidine in chloroform at the fingerprint and the non specific regions of the spectra. At the fingerprint region, quinine possesses an absorption band maximum at 855 cm^{-1} while quinidine possesses an absorption band maximum at 862.5 cm^{-1}. At the non specific region, the C–H stretching absorption band appears at 2869 cm^{-1} in quinine spectrum and at 2875 cm^{-1} in quinidine spectrum.

The composition of the training set was orthogonally designed in order to obtain maximum information on each drug from the calibration procedure. A training set was prepared as shown in Table 1. The absorbance data matrix for this training set was obtained by selecting the absorbances within the wavenumber range 900—797 and 2893—2845 cm^{-1}. The multivariate calibrations were computed with the PCR and PLS algorithms using the correlation for the absorbance data matrix and the corresponding concentration data matrix of the training set.

In order to validate the developed calibrations, an independent set of validation synthetic mixtures containing quinine and quinidine in the different compositions given in Table 1, was prepared and analyzed. The mean percentage recoveries were found to be 100.5±0.44% (R.S.D.%=0.44) and 100.5±0.38% (R.S.D.%=0.38) for quinine and 100.1±0.67% (R.S.D.%=0.67) and 100.1±0.68% (R.S.D.%=0.68) for quinidine using PCR and PLS models, respectively (Table 2). These results contributed to the high accuracy and precision of the developed multivariate methods.

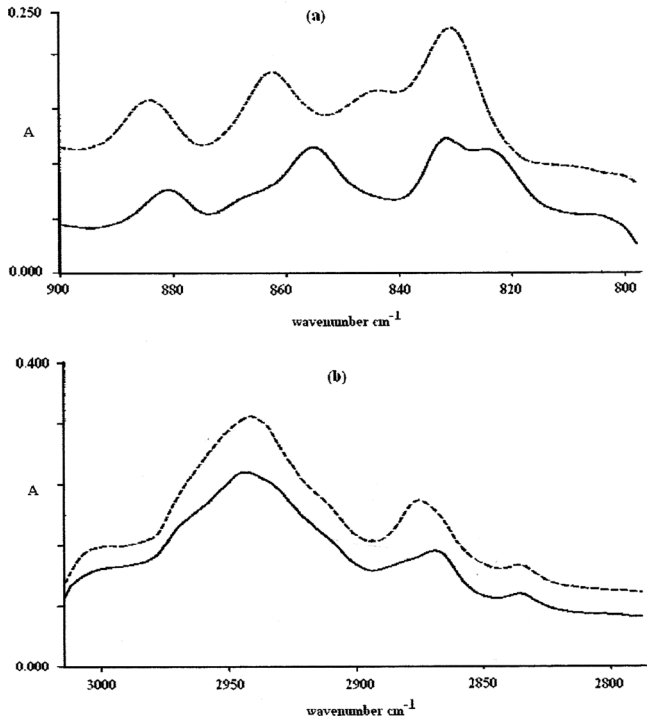

Fig. 3. IR Absorption Spectra of 1.0% w/v Quinine (——) and 1.0% w/v Quinidine (– – –) in Chloroform at the Fingerprint Region (a) and the Non Specific Region (b)

Statistical Analysis Determining how many factors to be used in the calibration is a key step in factor based techniques (PCR and PLS). Only those factors that contain analytical information must be kept. The discarded factors should contain only noise.[11,12] The Chemometrics Toolbox 3.02 Software offers several indicator functions which could be used for determining the optimum number of factors (rank). These include PCR-REV, PCR-FRAC, PCR-FIT, PCR-FITV, PCR-CROSS and PCR-PRESS for the PCR model[10] (Fig. 4); and PLS-REV, PLS-CROSS and PLS-PRESS for the PLS model[10] (Fig. 5).

The REV indicator calculates the reduced eigenvalues (REV) according to the method of Malinowski.[11] Each eigenvalue is proportional to the amount of variance in the data that the corresponding eigenvector accounts for. If a set of eigenvectors only span random noise, the corresponding eigenvalues will be approximately equal if they are properly reduced (corrected for degrees of freedom).[11] Since eigenvalues are not calculated for PLS, PLS-REV creates its own "pseudo eigenvalues" for each absorbance and concentration factor by evaluating the amount of variance in the data modeled by each factor.[10,11] Figures 4a and 5c show that the

Table 1. Composition of the Training and Validation Sets of Quinine and Quinidine

Solution No.	Concentration % w/v			
	Training set		Validation set	
	Quinine	Quinidine	Quinine	Quinidine
1, 2, 3	0.2	0.0	4.0	0.2
4, 5, 6	0.4	0.0	2.4	0.2
7, 8, 9	0.0	0.2	3.2	0.4
10, 11, 12	0.0	0.4	2.4	0.6
13, 14, 15	0.2	4.0	3.2	1.6
16, 17, 18	0.4	4.0	1.6	3.2
19, 20, 21	0.6	2.4	0.8	3.2
22, 23, 24	2.4	0.6	0.4	3.2
25, 26, 27	4.0	0.4	0.2	2.4
28, 29, 30	4.0	0.2	0.2	4.0

Table 2. Determination of Quinine and Quinidine in the Validation Set Mixtures

	Percentage recovery			
	Quinine		Quinidine	
	PCR	PLS	PCR	PLS
Mean[a)]	100.5	100.5	100.1	100.1
S.D.	0.44	0.38	0.67	0.68
R.S.D.%	0.44	0.38	0.67	0.68

a) Mean percentage recoveries for thirty determinations.

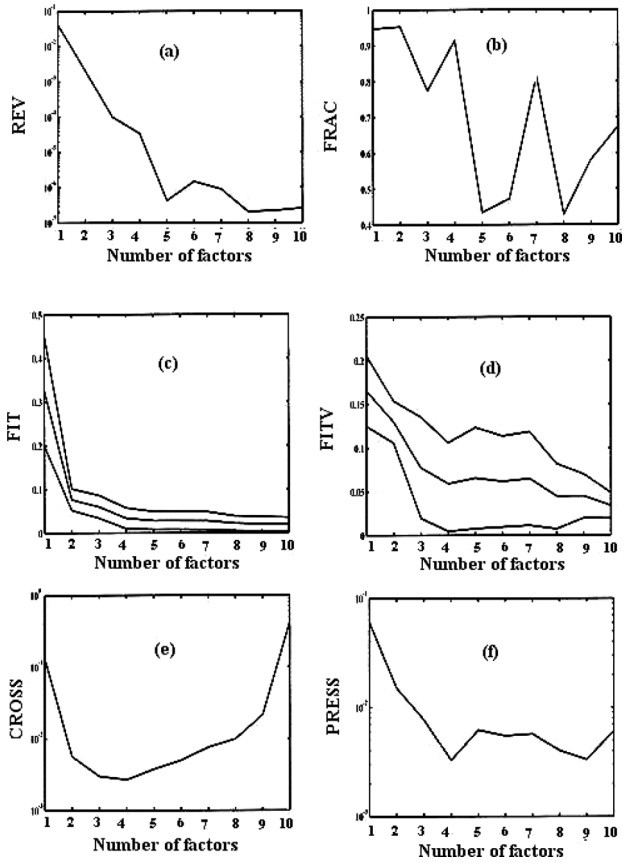

Fig. 4. REV (a), FRAC (b), FIT (c), FITV (d), CROSS (e), PRESS (f) *versus* Number of Factors for the PCR Model of Quinine–Quinidine

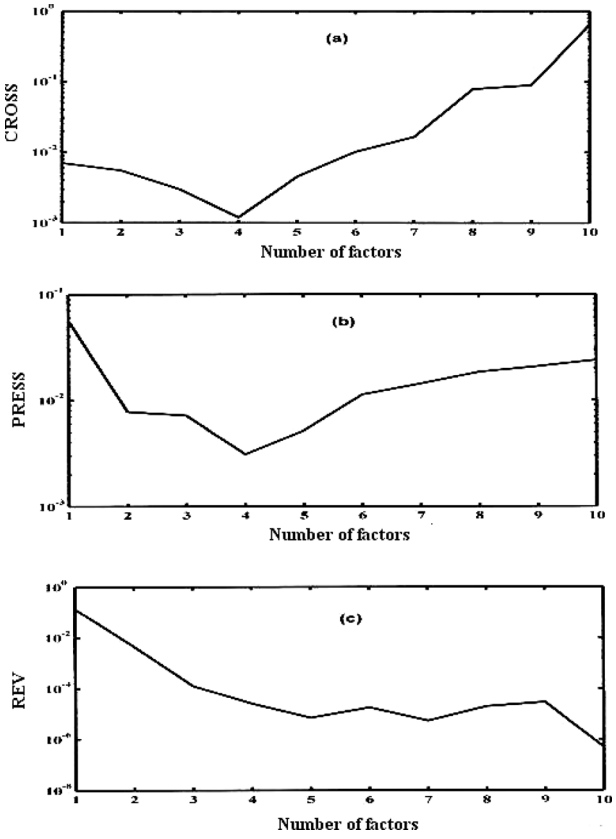

Fig. 5. CROSS (a), PRESS (b) and REV (c) *versus* Number of Factors for the PLS Model of Quinine–Quinidine

fourth reduced eigenvalue is significantly higher than those of higher rank. Therefore in this case, the optimum system rank is four according to the REV indicator.[10)]

PCR-FRAC is an empirical function based on the eigenvalues.[11)] It is related to PCR-REV and predates it.[10)] According to the FRAC rule, the optimum system rank is one less than the rank where a minimum occurs in the plot.[10)]

PCR-FIT determines how much error is present when a calibration matrix is used to predict the known concentrations of the training set as a function of the rank (number of factors) used in making the calibration.[10)] Figure 4c shows that the errors drop at rank four and that they go nearly to zero at the highest rank. This is because the first four factors contain all of the meaningful analytical information. All subsequent factors simply fit the residual noise better and better until all of the data are fit exactly when the complete set of factors is used.[10)]

PCR-FITV works like PCR-FIT except it generates calibrations with the training set and checks the fit to the validation set. It is a more reliable test than PCR-FIT, but it requires validation data.[10)]

The CROSS function performs a cross-validation procedure leaving out one sample at a time.[11,13)] It simulates a validation set by leaving out all possible combinations of one spectrum from the training set. The excluded spectrum is treated as an independent validation sample. The predicted residual error sum-of-squares, PRESS is calculated for each developed calibration.

$$\mathrm{PRESS} = \sum_{i=1}^{n} (C_i^{\mathrm{True}} - C_i^{\mathrm{Predicted}})^2$$

Where C_i^{True} represents the true concentration, $C_i^{\mathrm{Predicted}}$ denotes the predicted concentration and n is the total number of validation samples. Then the PRESS for each of the calibrations is examined and the one that gives the best results is selected. The number of factors used in that calibration is the optimum rank of the system.[10,11)] Figures 4e and 5a show that for this set of data, errors are minimized when four factors are used.

The PRESS function involves the generation of a calibration for every possible rank. Each calibration is used to predict the concentrations for a set of independently measured, independent validation samples. Then the PRESS value for each calibration is calculated. The calibration that provides the best results is selected. The number of factors used in that calibration is the optimal rank for that system.[11)] Figures 4f and 5b show that for this set of data, errors are minimized when four factors are used.

The studied indicator functions demonstrate that a rank of four factors is the optimum system rank for both the PCR and PLS calibrations. The first two factors are suggested to be due to quinine and quinidine as the main factors. The third factor is suggested to be due to base-line contribution from the instrument and the solvent. The fourth factor may be due to the steric factor.

The constructed PCR and PLS models would span nearly all the data leaving only negligible residuals. The range of residuals not spanned with the four factors was found to be

Table 3. Effect of Rank Selection on the Determination of Quinine and Quinidine in Their Validation Set Using the PCR and PLS Methods

	Percentage recoveries											
	Quinine						Quinidine					
	PCR rank			PLS rank			PCR rank			PLS rank		
	3	4	5	3	4	5	3	4	5	3	4	5
Mean[a]	95.3	100.5	101.3	95.5	100.5	101.2	97.7	100.1	101.2	97.7	100.1	101.2
S.D.	0.84	0.44	0.47	0.86	0.38	0.51	0.72	0.67	0.56	0.72	0.68	0.49
R.S.D. %	0.88	0.44	0.47	0.90	0.38	0.50	0.74	0.67	0.55	0.74	0.68	0.49
F test[b]	3.64	1.14		5.12	1.80		1.15	1.43		1.12	1.93	
t-test[b]	30.04	6.81		29.13	6.03		13.37	6.90		13.27	7.19	

a) Mean percentage recoveries for thirty determinations. *b*) Theoretical $F(0.05)=1.84$ and $t(0.05)=2.04$.

Table 4. Statistical Parameters of the Validation Synthetic Mixtures of Quinine and Quinidine Using PCR and PLS Methods

Parameter	Quinine		Quinidine	
	PCR	PLS	PCR	PLS
s^2 ($\times10^4$)	0.88	0.72	0.58	0.58
SEP ($\times10^3$)	9.37	8.51	7.60	7.63
MSEP ($\times10^5$)	9.78	8.21	5.38	6.65
RMSEP ($\times10^3$)	9.89	9.06	7.34	8.16
r	0.999990	0.999990	0.999989	0.999986
a	0.000945	0.00191	−0.002563	−0.002641
Lower 95%[a]	−0.007477	−0.006503	−0.011625	−0.011625
Upper 95%[a]	0.009367	0.010324	0.006499	0.006499
b	1.003106	1.002255	1.002086	1.001537
Lower 95%[b]	0.999393	0.998546	0.998244	0.998244
Upper 95%[b]	1.006819	1.005965	1.005928	1.005928
S_a	3.65×10^{-3}	3.64×10^{-3}	3.93×10^{-3}	4.36×10^{-3}
S_b	1.61×10^{-3}	1.61×10^{-3}	1.67×10^{-3}	1.85×10^{-3}

s^2 (variance of prediction)$=\sum_{i=1}^{n}(C_i^{\text{Predicted}}-C_i^{\text{True}}-\text{bias})^2/(n-1)$. SEP (standard error of prediction)$=[\sum_{i=1}^{n}(C_i^{\text{True}}-C_i^{\text{Predicted}})^2/(n-1)]^{1/2}$. MSEP (mean squared error of prediction)$=\sum_{i=1}^{n}(C_i^{\text{True}}-C_i^{\text{Predicted}})^2/n$. RMSEP (root mean standard error of prediction)$=[\sum_{i=1}^{n}(C_i^{\text{True}}-C_i^{\text{Predicted}})^2/n]^{1/2}$. Where C_i^{True} is the true concentration, $C_i^{\text{Predicted}}$ is the predicted concentration and n is the total number of validation samples. *a*) Lower and upper confidence limits for the intercept at 95% confidence level. *b*) Lower and upper confidence limits for the slope at 95% confidence level.

-8×10^{-3} to 8×10^{-3} and -25×10^{-3} to 20×10^{-3} for the PCR and PLS models, respectively.

Table 3 shows the effect of rank selection on the determination of quinine and quinidine in their validation set using the PCR and PLS models constructed using three, four and five rank systems. The mean percentage recoveries of the investigated drugs in the validation set are shown in the Table. The results obtained at different ranks were compared statistically using t-test and F test between rank 4 and each of rank 3 and rank 5, at 95% confidence level. Results of F and t-tests confirm that a rank of four factors is the optimum system rank and that the results obtained using a system rank higher than the optimum one are better than those obtained using a system rank lower than the optimum one.

The predictive ability of the proposed multivariate calibration models for FT-IR determination of quinine and quinidine could be defined using some validation diagnostics such as variance of prediction (s^2), standard error of prediction (SEP), mean squared error of prediction (MSEP) and root mean standard error of prediction (RMSEP).[11,12]

The MSEP and RMSEP characterize both the accuracy and precision of prediction.[12] The numerical values for these validation diagnostics using the PCR and PLS calibration models are shown in Table 4. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed methods.

Another way to validate the calibration models and to examine the results is the predicted versus true concentration plot. Points in this plot are expected to fall on a straight line with a slope of one and a zero intercept.[12] The correlation coefficient, r, is calculated for each calibration to indicate the quality of fit of all data to a straight line. The regression analysis for this linear relationship has been carried out and the results are shown in Table 4. The absence of bias has been proved by determining the confidence limits for the intercept, a, and the slope, b, at 95% confidence level.[14] The upper and lower confidence limits are shown in Table 4. For both quinine and quinidine using the PCR and PLS models with a rank of four factors, the 95% confidence interval of the intercept includes the ideal value of zero and that of the slope includes the ideal value of one. This indicates no problems with the models neither bias nor lack of fit. The absence of bias confirms the trueness of the studied models. Furthermore, there do not appear to be any sample(s) that are unusually far from the line than the rest of the data.

Conclusion

Techniques used to separate and quantify two stereo-isomers are usually sophisticated and may be routinely not available. In the infrared region, differences between the spectra of the two stereo-isomers can lead to solve this problem using chemometric multivariate methods.

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