Quantitative Determination of Citalopram Hydrobromide by Spectrophotometry and Chemometry in Presence of Its Degradation Products and Additives in Pharmaceutical Preparation

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***Abstract*—**Simple, accurate, sensitive and validated stability-indicating UV spectrophotometric and chemometric methods were developed for determination of Citalopram Hydrobromide (CT) in presence of its alkaline, oxidative degradation products and in its pharmaceutical preparation. Method (A) is a successive derivative ratio spectrophotometric one, which depends on the successive derivative of ratio spectra in two steps and measuring Citalopram Hydrobromide at 277nm and 293nm.Method (B) is mean centering of ratio spectra which depends on using the mean centered ratio spectra in two successive steps and measuring the mean centered values of the second ratio spectra at 237nm and method (C) used two chemometric techniques ; principal component regression(PCR) and partial least-squares (PLS). The proposed methods were checked using laboratory-prepared mixtures and were successfully applied for the analysis of pharmaceutical formulation containing Citalopram Hydrobromide. The proposed methods were validated according to the ICH guidelines. The obtained results were statistically compared with those obtained from the manufacturer HPLC method, showing no significant difference with respect to accuracy and precision.

***Keywords*—**Citalopram Hydrobromide; degradation; stability-indicating; successive derivative ratio; mean centering of ratio spectra; chemometrics.

I. Introduction

CITALOPRAM hydrobromide [(±)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1, 3-dihydroisobenzofuran -5-carbonitrile, hydrobromide] is an orally active selective serotonin reuptake inhibitor (SSRI) and is used in the management of depression [1].

Several analytical techniques, including chromatography [2-4] , spectrophotometry [5-7] and capillary electrophoresis [8-10], have been reported for the analysis of Citalopram hydrobromide (CT) in plasma and dosage form. Only stability –indicating HPLC methods have been described for determination of CT in presence of its degradation products [11-13].

For the simultaneous determination of two or more compounds in the same mixtures without a separation step, several spectrophotometric methods were used. The quantitative spectrophotometric resolution of the mixtures of two or more compounds having overlapped spectra is an interesting and challenging issue for analytical chemists due to its low cost, time consuming and does not require prior separation of the mixture.

This work describes the application of successive derivative of ratio spectra (SDR), mean centering of ratio spectra (MCR) and chemometric methods; principal component regression (PCR) and partial least-squares (PLS) for stability –indicating determination of CT in presence of its alkaline and oxidative degradations. The proposed methods are rapid, simple, accurate and do not require separation or pretreatment.

Afkhami and Bahram [14] introduced this successive derivative of ratio spectra (SDR) method for the simultaneous determination of three components in ternary mixtures. This method is based on several successive steps: calculating the derivative of ratio spectra, and then these derivative ratio spectra are divided by the derivative ratio spectra of a divisor of the other two components. Finally, the derivative is calculated for those obtained ratio spectra.

The mean centering of ratio spectra (MCR) is a well-established successive method in which binary and ternary mixtures could be determined without previous separation [15]. In this method the ratio spectra are obtained after which the constant is removed by mean centering of the ratio spectra, then an extra step was performed where the mean centered ratio spectra is further divided by the mean centered vector of the other two components, then the second ratio spectra was mean centered. This method eliminates derivative steps and therefore signal-to-noise ratio is enhanced.

Principal component regression and partial least squares

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regression are two related families of methods that are often used in chemometrics. The use of PLS method for chemical applications was initiated by Joreskog and Wold [16].

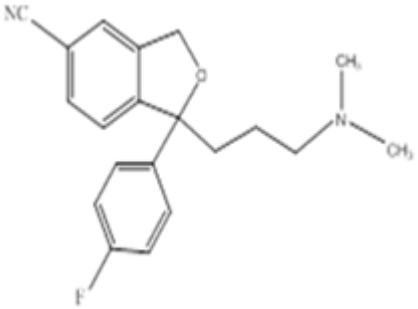

Fig.1. Chemical structure of Citalopram Hydrobromide

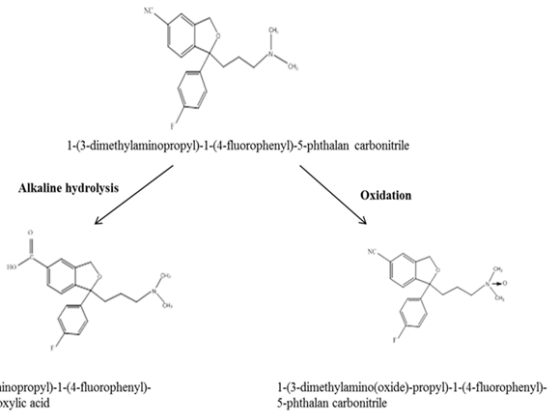

Fig.2. Degradation pathway and structures of degradation products

II. EXPERIMENTAL

2.1. Apparatus and software

Shimadzu – UV 1800 double beam UV–Visible spectrophotometer (Japan) with matched 1 cm quartz cells at 200–800 nm range were used for all absorbance measurements. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software. Matlab® version7.9 and PLS-Toolbox 2.0 software for the calculations of chemometry.

2.2. Chemicals and reagents

CT pure sample was kindly supplied by SEDICO Company for pharmaceuticals and chemical industries (Cairo, Egypt). Its purity is found to be 99.87% [4]. Spectroscopic analytical grade methanol was supplied from (S.d. fine-chem limited – Mumbai). sodium hydroxide pellets, hydrochloric acid 30-34%, ethyl alcohol absolute, hydrogen peroxide 30% (ADWIC, Egypt).

Pharmaceutical formulation: Citalo® tablets labeled to contain20 mg of CT , manufactured by Delta Pharma Company, Cairo, Egypt.

2.3. Standard solutions

Stock standard solution of CT (1.0 mg mL^{-1}) was prepared in methanol. The working standard solution (0.1 mg mL^{-1}) was freshly prepared by dilution from the stock solution using methanol.

2.4. Preparation of degradation products

2.4.1 Alkaline degradation

CT alkaline degradation product (ALD) was obtained by heating 10.0mL CT transferred from the stock solution with 10 mL 5.0 M sodium hydroxide at 80°C in oven for three hours. The resulting solution was neutralized with HCl, transferred into 100-mL volumetric flask and complete to the mark with methanol to obtain concentration of 0.1 mg $mL^{-1}$and tested for complete degradation by the thin layer chromatography (TLC) technique using ethyl acetate: formic acid: acetic acid: methanol in a ratio (12:1:1:1, v/v/v/v) as a mobile phase and detecting the spots at 254 nm. The prepared degradate was subjected to IR and mass spectrometry to confirm its structure.

2.4.2. Oxidative degradation

CT oxidative degradation product (OXD) was obtained by heating 10.0mL CT with 10 mL 30% H_2O_2 at 70°C in oven for five hours. The resulting solution transferred into 100-mL volumetric flask and completed to the mark with methanol to obtain concentration of 0.1 mg $mL^{1}$and tested for complete degradation with the same mobile phase system of TLC. The prepared degradate was subjected to IR and mass spectrometry to confirm its structure.

2.5. Procedure

2.5.1. Linearity and construction of calibration curves

2.5.1.1. For spectrophotometric methods

Different volumes (0.2-3.8mL) were transferred from the working standard solution of CT (0.1 mg mL^{-1}) into a series of 10 mL-volumetric flasks and then diluted with methanol to obtain a concentration range of 2.0-38.0 µg mL^{-1}. The absorption spectra of the prepared solutions were measured at (200–400 nm) and stored in the computer.

For successive derivative of ratio spectra (SDR), the zero order absorption spectra of different concentration of CT were divided by the spectrum of 10.0 µg mL^{-1} OXD and the ratio spectra were obtained. First derivatives of the ratio spectra were obtained with $\Delta\lambda$ = 4 and scaling factor 10. These vectors (D^1 of the ratio spectra) are divided by (d/dλ) (10.0 µg $mL^{-1}$of ALD/10.0 µg $mL^{-1}$of OXD) corresponding to the derivative of the ratio of the spectra of ALD and OXD and therefore, second ratio spectra were obtained. First derivative of these vectors were obtained using $\Delta\lambda$ = 4 and scaling factor 10. The calibration curve of CT was constructed by plotting the amplitude of the resulting spectra at 277nm and 293nm against its corresponding concentration.

For mean centering of ratio spectra (MCR), the ratio spectra of different concentration of CT drug were obtained using the same divisor (10.0 µg $mL^{-1}$of OXD), and then the obtained ratio spectra, in the range of (210–300 nm) were mean centered (MC). Those MC vectors were divided by the mean centered vector of the other two components (10.0 µg $mL^{-1}$of ALD/10.0 µg $mL^{-1}$of OXD), then those second ratio spectra were mean centered. The calibration curve was

constructed by plotting the amplitude of the resulting spectra at 237 nm for CT against its corresponding concentration.

2.5.1.2. For chemometric methods

Multilevel multifactor design was used for the construction of the calibration and validation sets [17]. A five-level, five-factor calibration design was used .The concentrations details are given in Table 1. The absorption spectra of the prepared mixtures (mixtures of CT, OXD and ALD) were recorded at 200-400nm and transferred to Matlab® for subsequent data manipulation. Seventeen mixtures were used for building the calibration model, while seven mixtures were chosen to be used as an external validation set.

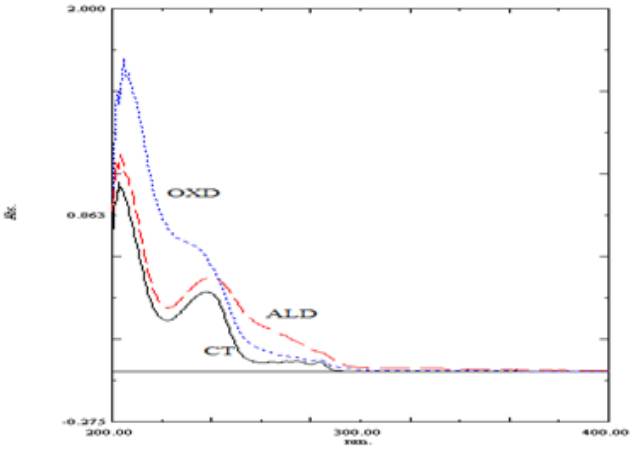

Fig.3. Zero order absorption spectra of CT (__), ALD (_ _) and OXD (….) (each 10.0µg mL^{-1}) using methanol as a blank.

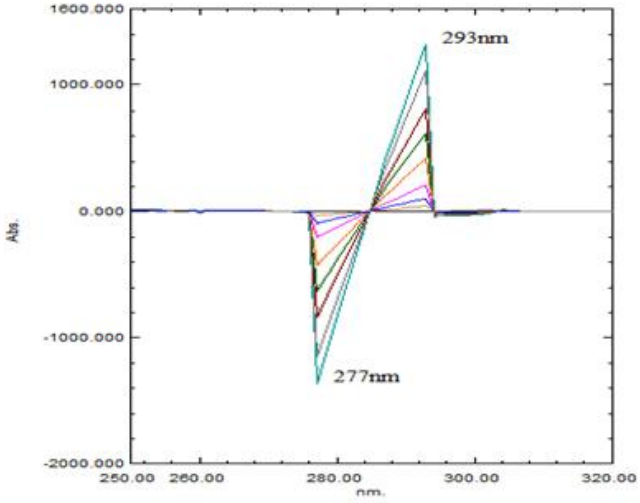

Fig.4. Successive derivative ratio spectra of 6.0-38.0µg mL^{-1} of CT, with $\Delta\lambda = 4$ and scaling factor 10.

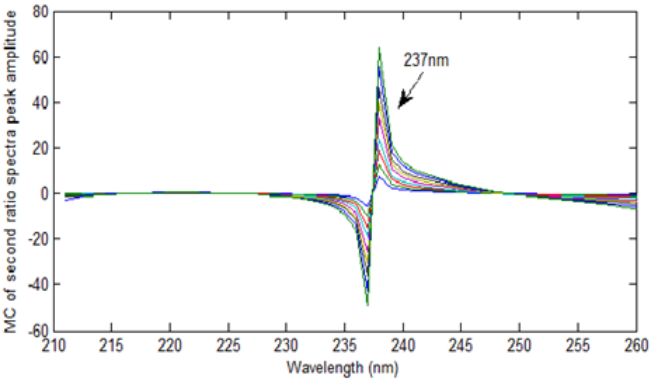

Fig.5. The mean centered vectors of the second ratio spectra obtained for CT in the range of 6.0-38.0µg mL^{-1}.

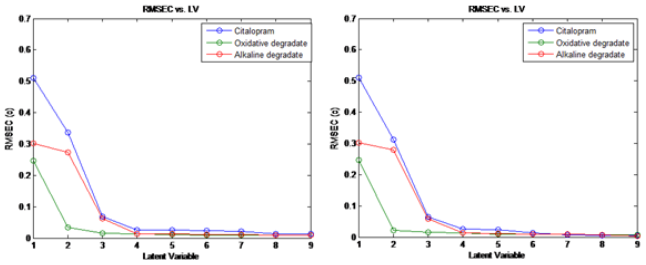

Fig.6. RMSEC of the calibration set of CT as a function of latent variables used to construct PCR (a) and PLS (b) calibration models, respectively.

TABLE I
CONCENTRATIONS OF CT, ALD AND OXD, IN THE CALIBRATION AND VALIDATION SETS.

Mix no.	CT (µg mL^{-1})	ALD (µg mL^{-1})	OXD (µg mL^{-1})
1	14.0	0.6	0.6
2	14.0	0.2	0.2
3	6.0	0.2	1.0
4	6.0	1.0	0.4
5	22.0	0.4	1.0
6	10.0	1.0	0.6
7	22.0	0.6	0.4
8	14.0	0.4	0.4
9	10.0	0.4	0.8
10	10.0	0.8	1.0
11	18.0	1.0	0.8
12	22.0	0.8	0.6
13	18.0	0.6	1.0
14	14.0	1.0	1.0
15	22.0	1.0	0.2
16	22.0	0.2	0.8
17	6.0	0.8	0.2
18*	18.0	0.2	0.6
19*	6.0	0.6	0.8
20*	14.0	0.8	0.8
21*	18.0	0.8	0.4
22*	18.0	0.4	0.2
23*	10.0	0.2	0.4
24*	6.0	0.4	0.6

* Mixtures of the validation set

2.6. Application to laboratory prepared mixtures

Into a series of 10 mL volumetric flask, accurate aliquots of CT, ALD and OXD were transferred from their working solutions to prepare eight mixtures containing up to 80% of the two degradates. The volumes were completed with methanol. The spectra of the prepared solutions were recorded and stored from 200 to 400 nm. The proposed

methods were applied and the concentration of CT was calculated by substitution in the regression equations.

2.7. Application to pharmaceutical preparation

A portion of Citalo® tablets powder equivalent to 0.01 gm CT was transferred to 50-ml beaker, extracted and sonicated with 20 ml methanol, the extraction was repeated three times and all extracts were mixed and transferred into 100-mL volumetric flask and completed to the volume with methanol to obtain concentration of 0.1 mg mL^{-1}. The solution was filtered through a Whatmann filter paper No. 41. One milliliter of the prepared solution was transferred into 10-mL volumetric flask and complete to the volume with methanol to obtain solution with a final concentration 10.0 µg mL^{-1}. The concentration of CT drug was calculated using the regression equations of the proposed methods. Also, when carrying out the standard addition technique, different known concentrations of pure standard CT drug were added to the pharmaceutical dosage form and proceeding using the previously mentioned methods.

III. Results and Discussion

The focus of the present work is to develop accurate, specific, reproducible and sensitive stability indicating methods for the determination of CT in pure form, in presence of its degradation products and in pharmaceutical formulation with satisfactory accuracy and precision.

CT was reported to degrade in alkaline medium to give 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phthalan-5-carboxylic acid and to be oxidized to give 1-(3 dimethylamino (oxide)-propyl)-1-(4-fluorophenyl)-5-phthalan carbonitrile [11,12] as shown in Fig.2. Thus these degradation products were prepared and their purity was confirmed by IR and LC/MS.

The absorption spectra of the three compounds, CT, ALD and OXD overlapped closely in the region 200–400 nm as shown in Fig. 3. For this reason, the determination of CT was not possible from direct measurements of absorbance in the zero-order spectra.

3.1. Successive derivative of ratio spectra (SDR)

The method was based on the successive derivative of ratio spectra in two steps. For determination of CT, The absorption spectra of different concentrations of CT were recorded in the range of 200–400 nm and were divided by the standard spectrum of 10.0 µg mL^{-1} of OXD and first derivative of the produced ratio spectra was then obtained. Then these vectors (first derivative of the first ratio spectra) were divided by (d/dk) (ALD/OXD) corresponding to the first derivative of the ratio spectra of 10.0 µg mL^{-1} of each to obtain the second ratio spectra and the first derivative of these ratio spectra was calculated. The concentration of CT was determined by measuring the maximum amplitude at 277 and 293 nm as shown in Fig. 4. The advantage of this method is that it can be applied for resolving ternary mixtures with no limitations, but the disadvantages of this method is the application of several derivitization steps using two divisors for the determination of each component leading to lower amplitude values and subsequently minimum sensitivity if compared to those obtained by mean centering of ratio spectra (MCR).

3.2. Mean centering of ratio spectra (MCR)

This well-established spectrophotometric method used for the ratio spectra of ternary mixture through which the constant is removed by mean centering. It depends on manipulation of the ratio spectra using Matlab software to cancel the effect of one or more components in the mixture to determine the other one. It eliminate the derivative step and therefore the signal-to-noise ratio is enhanced [18]. For determination of CT, the obtained ratio spectra (CT/OXD) as in SDR were mean centered in the wavelength range of (210–300 nm) and then divided by the mean centered vector of(ALD/OXD), then the obtained second ratio spectra were mean centered. In order to optimize the developed MCR method, the effect of divisor concentration on the selectivity of the method has been tested. Different concentrations each of OXD and ALD were tested. It was found that the divisor had a great effect on the selectivity of the method where reproducible and good results have been obtained upon using a concentration of 10.0 µg mL^{-1} each of OXD and ALD. The concentration of CT was determined by measuring the MC amplitude at 237nm corresponding to a maximum wavelength as shown in Fig.5.

The corresponding concentration ranges and calibration equations for the proposed methods were listed in Table 2. The selectivity of the proposed procedures was assessed by the analysis of laboratory prepared mixtures containing different ratios of the CT, OXD and ALK where satisfactory results were obtained as shown in Table 3. The proposed procedures were also applied for the determination of CT in tablet dosage form; and the validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients' interference. The results obtained were shown in Table 5.

The proposed spectrophotometric methods were validated in compliance with the ICH guidelines [19] as shown in Table 2.The data showed that the methods were accurate, precise and specific over the specified range.

3.3. Chemometric methods

3.3.1. Principal component regression (PCR)

The principal component regression method combines the principal component analysis (PCA) with an inverse least square (ILS) regression to create a quantitative model for complex samples. The eigenvectors resulting from the data decomposition represent the spectral variations that are common to all of the spectroscopic calibration data. Therefore, using the new data to calculate a regression model instead of straight spectral responses will produce a robust model for predicting concentrations of the desired constituents in very complex samples [20].

3.3.2. Partial least-squares (PLS)

Partial least-squares is a quantitative spectral decomposition technique that is closely related to (PCR). However, in PLS, the decomposition is performed in a slightly different fashion. In PCR method only the information in the matrix is used during data decomposition, but in the PLS method, the concentration data matrix is also used in this step. So PLS not only has the advantage of PCR, but also it produces more robust model as it removes noise from both absorbance and concentration data[21].

The first step in the determination of the cited drug by multivariate calibration methods involves constructing the calibration matrix for the ternary mixture. The calibration set was obtained by using the absorption spectra set of 17 mixtures of CT and its two degradates with different ratios of each component as shown in Table 1. The initial model was found to give bad results so the regions from 200 to 215nm and above 320nm were rejected.

Selection of the optimum number of factors is very important step before constructing the models, because if the number of factors retained is more than the required, more noise will be added to the data. On the other hand, if the number retained is too small meaningful data that could be necessary for the calibration may be discarded. In this study, the data was autoscaled as a pre-processing step, leave one out cross validation method was applied and the root mean squared error of calibration RMSEC values of different developed models were compared. The selected model was that with the smallest number of factors such that RMSEC for that model was not significantly greater than RMSEC from model with an additional factor. Four factors were found suitable for both PCR and PLS as shown in Fig.7.

The root mean square error of calibration (RMSEC) was calculated.

$$RMSEC = \sqrt{\frac{PRESS}{n - f - 1}}$$

Where, n = the number of calibration samples

f = the number of factors

PRESS = $\sum (Y_{pred} - Y_{act.})^2$

Where Y_{pred} and Y_{act} are the predicted and true concentration in µg ml^{-1}, respectively.

To assess the prediction ability of the suggested models, an external validation set was used. The two chemometric PCR and PLS methods were successfully applied for the determination of CT in Citalo® tablets and the validity of the proposed methods is further assessed by applying the standard addition technique. Table 5.

The validation of the developed PCR and PLS models was assessed using several diagnostic tools, Table 4. These tools were grouped into two categories in model diagnostic tools that are used to determine the quality of the model and sample diagnostic tools which are used to study the relationship between the samples and to identify unusual samples. The predicted concentrations of the validation samples were plotted against the true concentration values. This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero Table 4. The RMSEP was a diagnostic tool for examining the errors in the predicted concentrations; it indicates both the precision and accuracy[22]. The results in Table 4 indicate the high predictive abilities of the two models.

RMSEP was calculated from the following equation:

$$RMSEP = \sqrt{\frac{\sum (Y_{act.} - Y_{pred.})^2}{n}}$$

Where, Y_{pred} and Y_{act} are the predicted and true concentration in µg ml^{-1}, respectively and n is the number of samples.

Q^2 was another parameter which determined the variation in the samples prediction. Q^2 was calculated from the following equation:

Q^2 = 1- (PRESS/SSQ)

SSQ = $\sum$ ($Y_{pred} - Y_{mean}$)^2

3.4. Statistical analysis

Statistical comparison of the results obtained by the proposed methods and HPLC manufacturer method was shown in Table 6. The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed and the manufacturer methods with respect to accuracy and precision.

IV. Conclusion

The present work introduces two spectrophotometric and two chemometric stability-indicating methods for determination of Citalopram Hydrobromide in presence of its alkaline and oxidative degradation products without prior separation. These methods are simple, sensitive, rapid and of low cost compared with the reported HPLC methods. Thus, the proposed methods could be successfully applied for the routine analysis of CT, both in bulk powders, in dosage form and in presence of its degradation products, in quality control laboratories without any preliminary separation step.

TABLE II
ASSAY PARAMETERS AND VALIDATION SHEET OBTAINED BY APPLYING THE PROPOSED SPECTROPHOTOMETRIC METHODS

Parameters	SDR		MCR
	At 277nm	At 293nm	
Calibration range (µg mL^{-1})	6.0-38.0	6.0-38.0	6.0-38.0
Slope	58.65	56.49	1.79
Intercept	-856.07	-824.67	-5.81
Correlation coefficient (r)	0.9996	0.9995	0.9991
Accuracy [a]	99.81	99.54	100.20
Repeatability [a,b]	0.39	0.47	0.86
Inter-day precision [a,b]	0.50	1.13	1.05
LOD (µg mL^{-1})	0.76	0.91	1.65
LOQ (µg mL^{-1})	2.31	2.75	5.01

[a] Average of three experiments.
[b] Relative standard deviation of three concentrations of CT (12.0,20.0 and 30.0 µg mL^{-1}).

TABLE III
ANALYSIS OF LABORATORY PREPARED MIXTURES BY APPLYING THE PROPOSED METHODS.

Degradates %	SDR		MCR
	At 277nm	At 293nm	
10	99.54	98.67	100.65
20	99.07	98.65	99.54
30	100.47	99.83	100.74
40	101.65	99.52	99.52
50	102.32	101.87	101.25
60	100.64	102.98	100.87
70	102.21	102.53	101.34
80	102.58	102.06	101.98
Mean ± SD	101.06 ± 1.32	100.76 ± 1.78	100.73 ± 0.85

[a] Average of three experiments.

TABLE IV
SUMMARY OF RESULTS OBTAINED, BY APPLYING THE DIAGNOSTIC TOOLS FOR MODEL VALIDATION OF THE CHEMOMETRIC METHODS

Validation parameters	PCR	PLS
a) Predicted vs.known conc.plot		
1.Slope	1.01	1.00
2. intercept	0.01	0.007
3. correlation coefficient (r)	0.9992	0.9994
b) RMSEP	0.024	0.017
c) Q^2	0.998	0.998

TABLE V
APPLICATION OF STANDARD ADDITION TECHNIQUE TO THE ANALYSIS OF CITALO® TABLETS, BY THE PROPOSED METHODS

Pure added (µg mL^{-1})	SDR		MCR	Chemometry	
	At 277nm	At 293nm		PCR	PLS
	Found Recovery %[a]				
2.00	99.54	100.05	99.53	99.62	99.76
5.00	100.79	100.31	100.64	101.74	101.34
10.00	100.32	100.54	99.67	101.10	100.36
Mean ± SD	100.21±0.63	100.30±0.24	99.94±0.60	100.82±1.08	100.48±0.79

CT claimed to be 10.0µg mL^{-1}.
[a] Average of three experiments

TABLE VI
STATISTICAL COMPARISON BETWEEN THE RESULTS OBTAINED BY THE PROPOSED METHODS AND THE MANUFACTURER HPLC METHOD FOR THE DETERMINATION OF CT IN CITALO® TABLETS.

Items	SDR		MCR	Chemometry		HPLC method [4]
	277nm	293nm		PCR	PLS	
Mean %[a]	100.41	99.77	99.81	100.37	100.28	99.60
SD	0.79	0.88	0.59	0.34	0.28	0.42
n	5	5	5	5	5	5
Variance	0.62	0.77	0.34	0.11	0.07	0.17
Student's t-test [b]	0.78	0.07	0.77	0.15	0.09	
F-value [c]	2.14	1.45	2.04	1.98	1.10	

[a] Average of five determinations [4] Using C_{18} column, Mobile phase; water: acetonitrile: trifluoroacetic acid (67:33:0.2, v/v/v) and UV detection at 238nm.
[b] The corresponding tabulated values of t equals to 2.201 at P = 0.05.
[c] The corresponding tabulated values of F equals to 6.094 at P = 0.05

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