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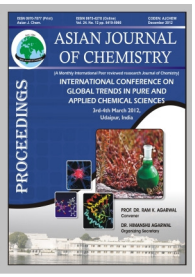

Spectrophotometric Determination of Escitalopram Oxalate in Bulk and Pharmaceutical Formulations Using 7,7,8,8-Tetracyanoquinodimethane†

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The present work describes a simple and sensitive charge transfer spectrophotometric method for the determination of escitalopram oxalate in bulk and in its pharmaceutical preparations using 7,7,8,8-tetracyanoquinodimethane (TCNQ). The method is based upon a charge transfer of *n*-electrons from the donor moiety (7,7,8,8-tetracyanoquinodimethane) to the acceptor moiety (escitalopram). The spectrophotometric measurements were recorded by measuring the absorbance at 845 nm. The different experimental parameters were carefully studied to determine the optimal conditions for the assay procedure. Then, the method was used for the determination of escitalopram oxalate over the concentration range 4-20 µg/mL. The method was successfully applied for the determination of escitalopram oxalate in its tablets. The proposed method was compared with the reference method and was found to be equally accurate and precise.

Key Words: Escitalopram oxalate, Spectrophotometry, Validation, Pharmaceutical preparations.

INTRODUCTION

Escitalopram (S-(+)-1-[3-(dimethylamino)propyl]-1-(*p*-fluorophenyl)-5-phthalancarbonit-rile oxalate), the S-enantiomer of racemic citalopram, is a selective serotonin reuptake inhibitor and belongs to a group of medicines known as antidepressants. These medicines help to normalize the levels of serotonin in the brain. Disturbances in the serotonin system of the brain are key factors in the development of depression and related disorders[1].

Several analytical methods have been reported for the determination of escitalopram oxalate in pure form, in pharmaceuticals and in biological fluids. Most of the reported methods are chromatographic methods and no official methods have been reported for the determination of escitalopram oxalate.

The reported methods include high performance thin layer chromatography (HPTLC)[2-4], high-performance liquid chromatography with tandem mass spectroscopy (HPLC/MS/MS)[5-7], high performance liquid chromatography with ultraviolet detection (LC-UV)[8-10], high performance liquid chromatography with mass spectroscopy[8,11,12], capillary electrophoresis method[13] and spectrofluorometric methods[2,14].

The literature survey revealed that no spectrophotometric method has been reported for the determination of escitalopram. Only a single absorbance correction method was developed for the simultaneous spectrophotometric estimation of escitalopram oxalate and clonazepam in combined tablet dosage form[10].

The present investigation describes the use of 7,7,8,8-tetracyanoquinodimethane for simple and rapid spectrophotometric method for the determination of escitalopram oxalate in raw materials and in certain dosage forms. The absorbance of the reaction products was measured at 845 nm. The molar ratio and stability constant of the complex was calculated and a proposal scheme of the reaction pathway was postulated.

EXPERIMENTAL

An UV-VIS spectrophotometer model ultrospec 2100-Pro (Biochrom, England) with matched 1 cm quartz cells was used for all measurements.

All chemicals used were of analytical reagent grade and the solvents were of spectroscopic grade. Pure escitalopram oxalate was kindly supplied by Saudi Pharmaceutical Industries and Medical Appliances Corporation (SPIMACO), Al-Qassim Pharmaceutical Plant). The available pharmaceutical dosage forms used in the present investigation are cipralex tablets. Cipralex tablets (B. No. 2175341) are labeled to contain 10 mg escitalopram per tablet.

7,7,8,8-Tetracynoquinodimethane was an aqueous solution of 0.2 % 7,7,8,8-tetracynoquinodimethane (Aldrich), prepared

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by dissolving 0.2 g in 100 mL of acetonitrile (Winlab laboratory chemicals). Escitalopram oxalate (Spimaco Al-Qassim Pharmaceutical, Saudi Arabia.

Standard solution: Weigh accurately 50 mg of escitalopram oxalate, transfer into a 60 mL separator, dissolve in a small volume of distilled water and make alkaline with ammonia solution. Extract the liberated base with five 15 mL portions of chloroform. Wash the combined chloroformic extracts with distilled water several times. Dry with anhydrous sodium sulphate for 5 min and then filter through dry filter-paper into a 100 mL voltumetric flask; rinse the sodium sulphate and the filter with chloroform and dilute the combined filtrates and washings to 100 mL with chloroform. For preparation of working solution in acetonitrile, an appropriate volume of solution corresponding to the required concentration is evaporated to dryness with a stream of nitrogen and the residue is dissolved in acetonitrile.

1×10^{-3} M solution of escitalopram was prepared as described above.

Construction of charge-transfer calibration graph: Transfer aliquots of a solution of the drug in acetonitrile in the concentration range (40-200 μg) into separate 10-mL volumetric flasks. Add 4 mL of 7,7,8,8-Tetracynoquinodimethane solution. Mix well and heat at 60 ºC for 25 min in a water bath. Complete to volume with acetonitrile. Measure the absorbance at 845 nm against a reagent blank prepared simultaneously. Construct the calibration graph by plotting the absorbance against the concentration of the drug and calculate the corresponding regression equation.

Application of the proposed method to the analysis of escitalopram oxalate in its tablets: Weigh and powder 10 tablets. Transfer an accurately weighed amount of the powder equivalent to 10 mg of escitalopram (12.77 mg escitalopram oxalate) into a 60-mL separator, dissolve in 10 mL of water, make alkaline with ammonia and proceed as described previously under standard solution beginning with extract the liberated base. Transfer an aliquot of the chloroformic extract in the concentration range (40.0-200.0 μg) into a 10-mL volumetric flask. Evaporate to dryness using a stream of nitrogen, dissolve in acetonitrile and proceed as described in general procedure. Calculate the nominal content either from the previously plotted calibration graph or by using the regression equation.

RESULTS AND DISCUSSION

Structurally, escitalopram oxalate contains tertiary amine and nitrile-functional groups and hence acts as *n*-electron donors (Lewis bases). It reacts with 7,7,8,8-Tetracynoquinodimethane as a π-electron acceptor (Lewis acid) to give a bluish-green chromogen exhibits strong absorption maxima at 845, 824, 762 and 744 nm (major) and 665 and 681 nm (minor) (Fig. 1). These bands may be attributed to the formation of the 7,7,8,8-tetracynoquinodimethane radical anion, which is formed by the complete transfer of *n*-electrons from the donor moiety to the acceptor moiety in a polar medium[15].

$$D^{\cdot\cdot} + A \rightarrow [D^{\cdot\cdot} \rightarrow A] \rightarrow D^{\hat{}+} + A^{\hat{}-}$$

Donor Acceptor Radical anion

The dissociation of the DA complex was promoted by the high ionizing power (dielectric constant) of acetonitrile solvent[16].

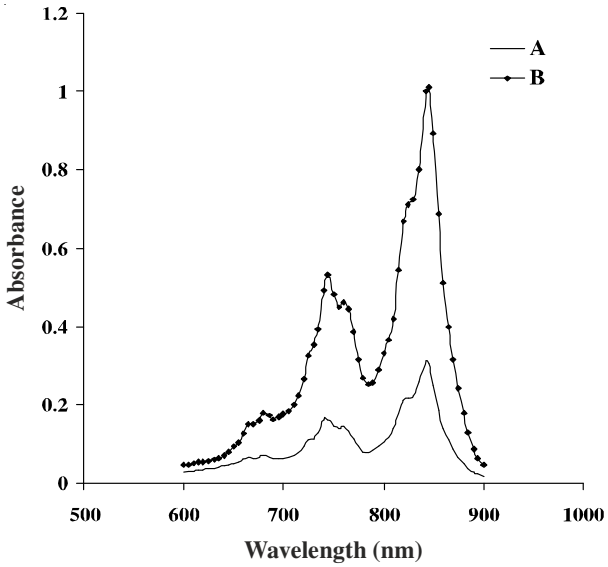


Fig.1. Absorption spectra of: (A) 7,7,8,8-tetracyanoquinodimethane (TCNQ) (0.08 % w/v), (B) The reaction product of escitalopram (20.0 μg/mL) with TCNQ

Optimization of the reaction conditions: The spectrophotometric properties of the coloured species as well as the different parameters affecting the colour development were extensively studied to determine the optimal conditions for the assay procedure.

The reaction was studied as a function of the volume of the 7,7,8,8-tetracynoquinodimethane, nature of the solvent, reaction time and stability.

The effect of 7,7,8,8-tetracynoquinodimethane concentration on the absorbance of the reaction product was studied using increasing volumes of 0.2% of 7,7,8,8-tetracynoquinodimethane solution. The maximum absorbance was attained using 4 mL, after which 7,7,8,8-tetracynoquinodimethane has no effect on the absorbance. Thus 5 mL of 7,7,8,8-tetracynoquinodimethane was used for further studies. The spectra of the radical formed between 7,7,8,8-tetracynoquinodimethane and escitalopram was recorded individually in acetone, acetonitrile, methanol, chloroform, diethyl ether and dimethyl formamide. Acetonitrile is considered to be an ideal solvent for the colour reaction as it offers excellent solvent capacity for 7,7,8,8-tetracynoquinodimethane and gives the highest yield of the radical[15].

Reaction time is determined by following the colour development at different time intervals at 25, 60 and 80 ºC. Maximum absorbance was attained by heating at 60 ºC for 25 min and at 80 ºC for 15 min, after which heating time has no effect on the absorbance. Thus heating time of 30 min at 60 ºC was used for further studies, scince the boiling point of acetonitrile was 80 ºC.

The stability of the formed complex was studied at room temperature at different time intervals and it was found to be stable for more than 3 h.

Stoichiometry and reaction mechanism: The stoichiometry of the reaction was studied by the molar ratio method[17] and Job's method of continuous variation[18]. They show that the reaction proceeds in a ratio 1:2 (donor : acceptor). This finding is anticipated by the presence of tertiary amine and

nitrile groups in escitalopram which act as electron-donating centers. Thus, the reaction pathway is proposed to proceed as described in **Scheme-I**.

Stability constant of the complex: The stability constant of the complex is calculated according to the equation[19]:

$$K_f = (A/Am) / [(1-A)/ Am]^{n-1} C^n n^n$$

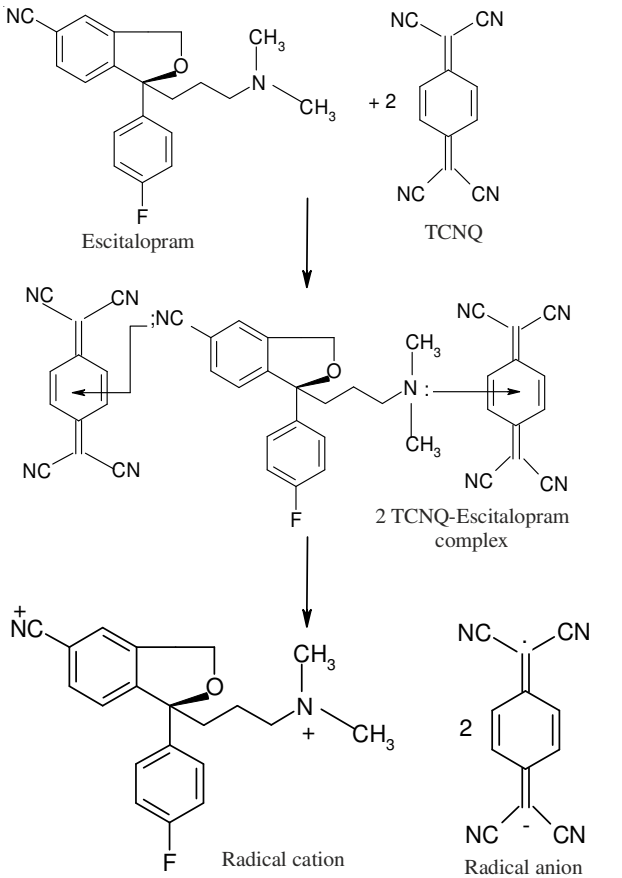
where, A: Maximum absorbance of the continous variation curve; Am : Absorbance corresponding to intersection of the two tangents of the continuous variation curve; n : Number of moles of the reagent in the reaction product; C: Molar concentration of the drug; K_f: Formation constant of the complex.

The formation constant of the escitalopram-7,7,8,8-tetracynoquinodimethane complex is 4.6430×10^5. This high value indicates stable reaction product.

The Gibbs free energy change of the reaction ΔG was also calculated adopting the following equation[18]:

$$\Delta G = -2.303 \text{ RT log } K_f$$

where ΔG: Gibbs free energy change of the reaction (kJ/mol); R: Universal gas constant (8.314 J/k mol); T: Absolute temperature (273 + 25 ºC); K_f: Formation constant of the complex.



Scheme-I: Proposed pathway for the reaction of escitalopram with TCNQ

The free energy change of the reaction between escitalopram and 7,7,8,8-tetracynoquinodimethane is -3.2334×10^4 k J/mol. The negative value of the ΔG point out to the spontaneous nature of the reaction.

Validation

Linearity and range: After optimizing the reaction conditions, calibration graph was constructed by plotting the absorbance of 7,7,8,8-tetracynoquinodimethane radical anion at 845 nm *vs*. escitalopram concentration. A straight line was obtained over the concentration range 4-20 μg/mL.

Linear regression analysis of the data using the method of least squares[20] gave high value of the correlation coefficient (r) and small values of standard deviations of intercept (S_a) and slope (S_b). These data proved the linearity of the calibration graph. Table-1 shows the performance data for the determination of escitalopram oxalate with 7,7,8,8-tetracynoquinodimethane.

Accuracy and precision: To prove the accuracy of the proposed method the % error was determined over the concentration range 4-20 μg/mL. The % errors were -0.90-0.62 % at 845 nm (Table-2). The results of the assay of escitalopram in pure form were compared with those of the published spectrophotometric method[10]. Statistical analysis[20] of the results obtained by the proposed and comparison methods using student's *t*-test and variance ratio F-test, showed no significant difference between them regarding accuracy and precision, respectively (Table-2).

TABLE-1
ANALYTICAL PERFORMANCE DATA FOR THE DETERMINATION OF ESCITALOPRAM OXALATE WITH TCNQ

Parameters	Results
Regression equation	A = 0.1144 + 0.0443C
Correlation coefficient (r)	0.9999
Standard deviation of the intercept (S_a)	0.0026
Standard deviation of the slope (S_b)	2.1305×10^{-4}
Relative standard deviation (% RSD)	0.4036
Detection limit (μg/mL)	0.1967
Quantification limit (μg/mL)	0.5959
Molar ratio (drug/reagent)	1:2
Molar absorbtivity (ε)	2.4669×10^4
Formation constant (K_f)	4.6430×10^5
Gibbs free energy change (ΔG)	-3.2334×10^4

TABLE -2
ANALYSIS OF ESCITALOPRAM OXALATE IN PURE FORM BY THE PUBLISHED METHOD AND THE PROPOSED METHOD USING TCNQ

Conc. taken (μg/mL)	Conc. found (μg/mL)	Error[a] (%)	Found[b] (%)	
			Proposed method	Published method[10]
4	3.96	-1.00	99.68	
5	5.00	0.00	100.06	
10	9.97	-0.30	99.69	100.12
15	15.09	0.60	100.62	99.78
20	19.95	-0.25	99.73	99.45
Mean ± S.D.			99.96 ± 0.40	99.78 ± 0.33
Student's *t*-value			0.641(2.447)[c]	
Variance ratio *F-test*			1.457 (19.2)	

[a]Calculated as [(measured value – true value)/(true value)] × 100.
[b]Each result is the average of three separate determinations.
[c]The figures between parentheses are the tabulated values of *t* and *F* at P = 0.05[20].

Intraday and interday precisions were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable, repeatability and intermediate precision of the proposed methods.

Selectivity: The selectivity of the optimized procedure for the assay of escitalopram was examined in presence of the

excipients present in cipralex tablets[1] *e.g*. talc, croscarmellose sodium, microcrystalline cellulose/colloidal silicon dioxide and magnesium stearate. The film coating contains hydroxypropyl methyl cellulose, titanium dioxide and polyethylene glycol. It was found that there is no significant interference from excipients. Accordingly, the proposed procedure can be considered as a selective method.

Limit of detection and limit of quantitation: Limit of detection and limit of quantitation were determined according to the ICH[21] guidelines. Limit of detection was determined by establishing the minimum level at which the analyte can reliably be detected, using the relation 3.3 $(S_a)/b$[21], limit of quantitation is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions, using the relation 10 $(S_a)/b$[21]. The results were illustrated in Table-1.

Ruggedness: To examine the ruggedness of procedure, the intraday and interday precisions were evaluated. The precision of the proposed method was found to be fairly high, as indicated by the low values of S.D.

Robustness: The robustness of the method was demonstrated by the constancy of the absorbance intensity with minor changes in the experimental parameters such as 5 ± 0.2 mL of 0.2 % 7,7,8,8-tetracynoquinodimethane and heating time of 30 ± 5 min at 60 ºC. These minor changes that may take place during the experimental operations did not affect the absorbance intensity.

Application on the analysis of dosage form: In order to evaluate the analytical usefulness of the proposed spectrophotometric method, escitalopram oxalate was determined in its tablets.

The recoveries of different concentrations of escitalopram were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained by the published spectrophotometric method[10] (Table-3). Statistical analysis[20] of the results obtained by the proposed and the comparison methods shows no significant differences between the two methods as regards to accuracy (*t*-test) and precision (F-test).

TABLE-3
ANALYSIS OF ESCITALOPRAM OXALATE IN COMMERCIAL TABLETS BY THE PUBLISHED METHOD AND THE PROPOSED METHOD USING TCNQ

Preparations	Concentration taken (μg mL^{-1})	Found[b] (%)	
		Proposed method	Published method [10]
Cipralex® tablets (10 mg scitalopram/ tablet)[b]	4.0	101.71	
	5.0	99.10	
	10	98.41	100.0
	15	99.85	98.38
	20	100.34	100.61
Mean ± S.D.		99.88 ±1.26	99.66 ± 1.15
Student's *t*-value		0.210 (2.447)[c]	
Variance ratio *F-test*		1.183 (19.2)	

[a]The average of three separate determinations.
[b]Products of H. Lundbeck A/S, Denmark.
[c]The figures between parentheses are the tabulated values of *t* and *F* at P = 0.05 [20]

Conclusion

The results obtained from the present study indicate that n-π complex formation between the escitalopram and 7,7,8,8-tetracynoquinodimethane was applied in the spectrophotometric assay of escitalopram oxalate in its dosage forms. The proposed method can be used for the routine quality control of the pure drug and in tablets without fear of interference caused by the excipients expected to be present in tablets. The method has been also applied successfully to the determination of the active constituent in a commercial pharmaceutical. The proposed method has the advantages of easy operation, high recovery and minimal use of organic solvent.

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