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Validated Spectrophotometric Methods for Determination of Escitalopram Through Study of Charge Transfer and Ion Pair Complexation

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ABSTRACT

Two simple and sensitive spectrophotometric methods were developed and validated for quantitative determination of escitalopram(Esc). The first method is based on the formation of charge-transfer complexes between the drug as n-donor and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetracyanoethylene (TCNE) and *p*-chloranilic acid (*p*-CA) as π -acceptors to give highly colored complex species. The products exhibit absorption maxima at 456, 841, 413 and 518 nm in acetonitrile for DDQ, TCNQ, TCNE and *p*-CA, respectively. Beer's law was obeyed in the range of 8.28–373.00 μg mL^{-1}. Moreover Kinetic spectrophotometry was adopted for analysis of Esc with TCNE, using initial rate and fixed time methods. The second method is based on the formation of yellow ion-pair complexes between escitalopram and four sulphonphthalein acid dyes, namely; bromocresol purple (BCP), bromophenol blue (BPB), bromocresol blue (BCB), and bromocresol green (BCG) in chloroform. The formed complexes are measured at 407, 413, 415 and 416 nm for BCP, BPB, BCB and BCG, respectively. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9996 - 0.9999) are found between absorbance of the formed complexes and concentrations of escitalopram in the range of 2.07–41.44 μg mL^{-1}. Spectral characteristics and stability constants of the formed ion associates are discussed in terms of the nature of donor and acceptor molecular structures. The molar absorptivities and association constants for the colored complexes were evaluated using the Benesi-Hildebrand equation. The proposed methods were successfully applied for determination of the drug in tablets with good accuracy and precision.

1. Introduction

Escitalopram is S(+)-1-[3-(dimethylamino) propyl]-1-(4-flurophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrogen Figure 1.Escitalopram is a highly selective serotonin reuptake inhibitor antidepressant developed for the treatment of depression and anxiety disorders [1]. Preclinical studies have demonstrated that the therapeutic activity of citalopram resides in escitalopram as it is thirty folds more potent than R-enantiomer [1]. Different analytical methods were developed for quantitative determination of escitalopram in biological fluids and pharmaceutical formulations. They include HPLC with mass spectrometry detection [2], LC with ultraviolet absorption detection [3–11], voltametric [12], capillary electrophoresis [13], thin-layer chromatography (TLC) [14-16] and spectrophotometric [17-23] methods have been published.
The present work aims chiefly to study the reaction of DDQ, TCNQ, TCNE and *p*-CA reagents (electron acceptors) as first time with Escitalopram (electron donor) and using BCP, BPB, BCB and BCG as ion-pair reagents for spectrophotometric determination of the given drug in pure form and in some of its pharmaceutical preparations. Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometeric methods. The literature is still poor in analytical assay methods based on kinetics for the determination of escitalopram.

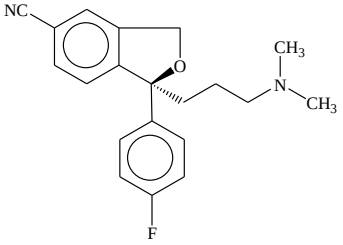

Figure 1: Molecular structure of escitalopram.

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2. Experimental

2.1. Instruments

Spectrophotometers, Shimadzu (Japan), UV-2450 and UV-1601 PC, dual-beam UV-vis spectrophotometer with matched 1 cm quartz cells, connected to computer fitted with UV Probe software was used for the measurements.

2.2. Materials and reagents

Escitalopram (Esc) was kindly supplied by Hikma Pharmaceuticals (Egypt). Its purity was found to be 99.20% according to official method [24]. The pharmaceutical dosage form used in this study was Citalopm® tablets labeled to contain 20 mg of Esc per tablet, Batch No. 570909.

DDQ, TCNQ and TCNE were of analytical reagent grade and were provided by Merck. *p*-CA was obtained from Aldrich.

Stock solution ($1.0x10^{-3}$ mol L^{-1}) of Esc was freshly prepared in acetonitrile or chloroform.

Stock solutions ($1.0 x10^{-2}$ mol L^{-1}) of DDQ, TCNE, TCNQ and *p*-CA were prepared by dissolving an appropriate weight in acetonitrile.

Stock solutions ($5.0 x10^{-3}$ mol L^{-1}) solutions of BCP, BPB, BCB and BCG were prepared by dissolving an appropriate weight in chloroform.

Working solutions were prepared by appropriate dilution of stock solution with acetonitrile or chloroform.

2.3. General analytical procedure for determination of Escitalopram

2.3.1. Method A (Charge Transfer reagents)

Into 10 mL calibrated flasks, 1 mL of $1x10^{-2}$ mol L^{-1} of each DDQ, TCNQ or TCNE reagents and 1.5 mL of $1x10^{-2}$ mol L^{-1} *p*-CA, followed by 1 mL of $1.0x10^{-3}$ mol L^{-1} Esc stock solution were added. The mixtures were allowed to stand for 20 and 35 min at 25±1°C in case of DDQ and TCNE, respectively, to obtain maximum color. For *p*-CA, the color was obtained spontaneously, while in case of TCNQ, the mixture was placed in test tube covered with aluminum foil and heated in water bath at 60 °C. After cooling the mixture was transferred into 10 mL calibrated flasks, and the volume was completed with acetonitrile. The produced color was measured using a reagent blank prepared in the same manner without addition of the drug. The method was repeated using different volumes of Esc. A calibration graph was obtained by plotting absorbance versus concentration. The concentration of the unknown samples was determined by using appropriate calibration graph.

2.3.2. Method B (Ion Pair reagents)

Into a series of 10 mL volumetric flasks, aliquots of 0.2–2.0 mL of the standard drug solutions ($5.0 x 10^{-4}$ mol L^{-1}) were transferred separately, 2.0 mL of each BCP, BPB, BCB or BCG were added. Then the volume was completed to the mark with chloroform and the reactions were allowed to proceed at room temperature. The reactions were achieved instantaneously at room temperature (25 ± 2 ◦C). The absorbance of the yellow colored ion pair complexes were measured at 407, 413, 415 and 416 nm for BCP, BPB, BCB and BCG, respectively, against corresponding reagent blank similarly prepared. The calibration graph was obtained by plotting absorbance versus concentration and the regression equations were computed.

2.3.3. Pharmaceutical dosage forms (capsules)

The content of 10 capsules of drugs under investigation was weighed. A mass of powder equivalent to $1.0x10^{-3}$ mol L^{-1} Esc was weighed accurately, dissolved in acetonitrile or chloroform, filtered through Whatman no. 41 filter paper and washed with the acetonitrile or chloroform. Then, washing solution was diluted to 100 mL with acetonitrile or chloroform in calibrated measuring flasks. Then the procedure was followed as mentioned above.

2.4. Stoichiometry

The stoichiometric ratios were determined by Job's method of continuous variation [25]. Equimolar solutions of Esc ($1.0×10^{-3}$ mol L^{-1}) and each of reagent used were prepared in acetonitrile or chloroform. A series of solutions of drug and the respective ion pair was made up by comprising different complementary proportions in 10 mL volumetric flasks with the appropriate solvent. The absorbances of the resulting solutions were measured at the wavelength of maximum absorbance against reagent blanks treated similarly.

2.5. Determination of the stability constants and standard free energy

The association constants were calculated for the interaction of escitaloram with different reagents using Benesi–Hildebrand equation [26] which depends on the presence of one of the reacting species in large excess, so that its concentration is virtually unaltered on formation of the complex. The experiment was conducted by using variable concentrations of the drug solution.

Formation constant of the CT complexes under investigation (K_f) were determined by substituting the data obtained from continuous variation method in equation derived for calculating the stability constant spectrophotometrically [27].

$$K_f = \left(\frac{A}{A_m}\right)\left[\frac{1-A}{A_m}\right]^{n+1} C^n n^n$$

where A is the maximum absorbance obtained from Job's continuous variation curve, A_m is the absorbance corresponding to the intersection of the two tangents of the continuous variation curve, C is the concentration corresponding to maximum absorbance, n is the ratio of the drug in the reaction product.

2.6. Determination of thermodynamic parameters of Esc-TCNQ complex

Into a series of 10 mL glass stoppered test tubes, aliquots of Esc solution ($1.0 x 10^{-3}$ mol L^{-1}) were transferred, followed by 1.0 mL of $1.0 x 10^{-2}$ mol L^{-1} TCNQ and 2 mL of acetonitrile. The tubes were heated in a thermostatic water bath at different temperatures (40, 50 and 60 ^{o}C) for different time intervals. At the specified time, the tubes were cooled and the content of each tube was transferred quantitatively to a 10 mL volumetric flask, then the detailed procedure under Section *2.3.1.* was followed.

2.7. Initial rate method (Esc + TCNE) for the determination of Esc

Aliquots of standard Esc solution (0.20–1.40 mL, $5x10^{-4}$ mol L^{-1}) were transferred into a series of 10 mL calibrated flasks followed by 2.0 mL of $1.0 x10^{-2}$ mol L^{-1} TCNE solution. The

volume was made up to the mark with acetonitrile. After mixing, the contents of each flask, they were immediately transferred to spectrophotometric cell and the increase in absorbance was recorded at 413 nm as a function of time between 0–35 min against reagent blank treated similarly. The rate of the reaction (*v*) at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration graph was constructed by plotting the logarithm of the initial rate (log *v*) vs the logarithm of the molar concentration of the Esc (log C). The amount of the drug was obtained either from the calibration graphs or from the regression equation.

2.8. Fixed time method (Esc + TCNE) for the determination of Esc

Aliquots of standard Esc solution (0.20–1.40 mL, $5x10^{-4}$ mol L^{-1}) were transferred into a series of 10 mL calibrated flasks followed by 2.00 mL of $1.00x10^{-2}$ mol L^{-1} TCNE solution and the volume was completed with acetonitrile. After mixing, the absorbance was measured directly at 413 nm against reagent blank treated similarly. The amount of the drug was calculated from the corresponding calibration graph or the regression equation.

3. Results and discussion

The studied seized drug has high electron density sites, so it may act as a powerful electron donor. The structure of Esc is shown in Figure 1. Esc was found to react with the dyes in acetonitrile or chloroform medium to produce an intense and stable charge transfer or ion–pair complexes. Chemically, the structure of Esc possesses an aliphatic tertiary amino group gives its basic nature. This structure suggests the possibility of utilizing CT reagents or acidic dye as chromogenic reagent.

3.1. Absorption spectra

TCNQ in acetonitrile reacts with the amino group of Esc and gives intense greenish CT complexes which absorb maximally at λ = 841 nm, as shown in Figure 2A. The absorption spectra of TCNE result in the formation of an intense yellow color product which exhibits an absorption maximum at λ = 413 nm. *p*-CA behaving as p-acceptor is able to form charge transfer complexes with a variety of drugs acting as electron donors and gives λ_{max} at 518 nm. The reaction of DDQ with Esc results in the formation of an intense orange-red product which exhibits three maxima at 456, 547, 588 nm. The band at 456 nm, having the highest absorption intensity, was selected for construction of Beer's plot.

The absorption spectra of solutions containing the Esc and acid dyes used exhibit new absorptions at longer wavelengths than that of Esc alone (λ_{max}= 238 nm). These new absorption spectra of the ion-pair complexes, formed between Esc and each of BCP, BPB, BCB, and BCG, respectively, were measured in the range 300–550 nm against the blank solution as shown in Figure 2B. The ion-pair complexes show maximum absorbance at 407, 413, 415 and 416 nm for BCP, BPB, BCB and BCG, respectively.

3.2. Effect of solvent

In order to select the suitable solvent for CT complex formation, the reaction of DDQ, TCNQ, TCNE and *p*-CA with Esc was made in different solvents. Acetonitrile, ethanol, methanol, acetone, 1.2 dichloroethan and DMSO were examined. Acetonitrile afforded the maximum sensitivity when compared with all other solvents. This is because it possesses the highest dielectric constant of all solvents examined [28], a property which is known to promote the dissociation of the original charge-transfer complexes to the radical ions. For ion pair reaction, maximum absorbance intensity and stability of the formed complexes were obtained upon using chloroform as diluting solvent.

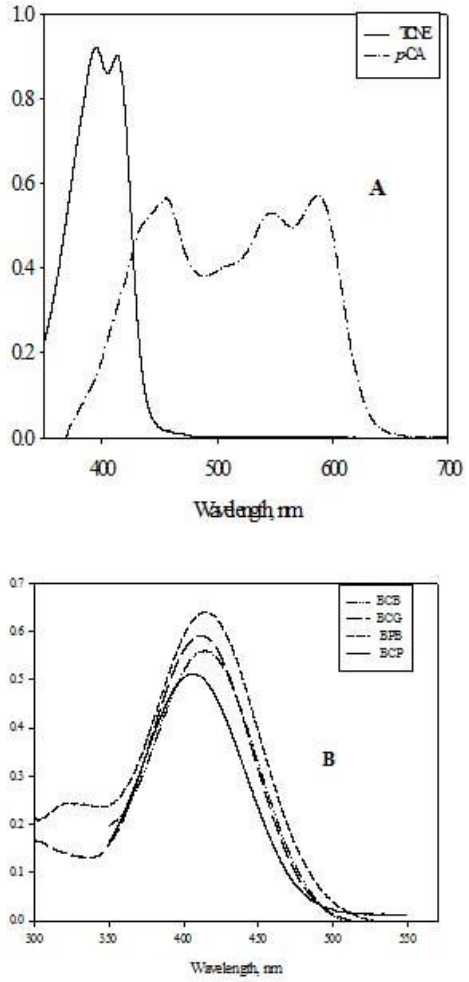

Figure 2: Absorption spectra of 41.44 µg mL^{-1} Esc using DDQ, TCNE and 24.86 µg mL^{-1} Esc with different acid dyes against reagent blank.

3.3. Effect of temperature and time

The effect of temperature on the reaction was studied in the range of 25-60 ^{o}C. All complexes, with exception of TCNQ, are formed at 25 ^{o}C and the maximum color development is recorded immediately, 20 and 35 min in case of electron acceptor *p*-CA, DDQ and TCNE, respectively. The optimum temperature for the formation of Esc-TCNQ is found to be 60 ^{o}C, thus we conclude that the absorbance increases with increase of temperature. The color products were stable for at least 24 hours except in case of DDQ, the complex was stable for about 30 min. For ion pair reaction, complete color development was attained instantaneously and remained stable for more than 24 h for all the studied reagents, thus permitting quantitative analysis to be carried out with good reproducibility.

3.4. Effect of reagents concentration

When various volumes of DDQ, TCNQ, TCNE and *p*-CA were added to a fixed concentration of Esc, it was found that a steady and maximum absorbance of CT complexes was found to occur from 0.5 to 2.5 mL of $1.0x10^{-2}$ mol L^{-1} reagent. At least 1 mL of $1.0x\ 10^{-2}$ mol L^{-1} is required for maximum color development using DDQ, TCNQ and TCNE, while 1.50 mL of $1.0x10^{-2}$ mol L^{-1} *p*-CA was the suitable volume for the production of maximum color intensity using 2 mL of $5.0x10^{-3}$ mol L^{-1} Esc. Higher concentrations of reagent did not affect the color intensity as indicated in. While for ion pair reactions, it was found that 2.0 mL of 5.0×10^{-3} mol L^{-1} of each dye was sufficient for the production of maximum and reproducible absorbance.

3.5. Stoichiometry of the CT complexes

The stoichiometric ratios were determined by Job's method of continuous variation [25] between Esc and reagents. The results show that 1:1 complexes were formed between the Esc and reagents as shown in Figure 3A, B.

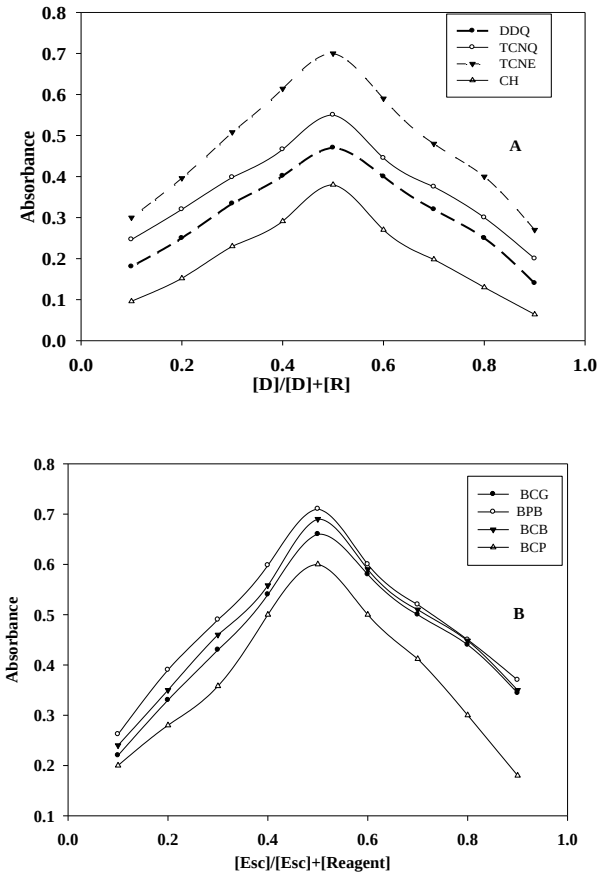

Figure 3: Continuous variation method for Esc using different reagents at respective λ_{max}.

3.6. Mechanism of reaction between Escitalopram with CT reagents or acid dyes

Since only a tertiary amine basic group exists in the chemical structure of Esc, the complex is formed by the lone pair of electron donating by Esc base as n-donor with each of DDQ, TCNQ, TCNE and *p*-CA reagent as an electron acceptor, which a partial ionic bond (D^+A^-) is assumed to be formed.

D + A → [D→A] (D^+A^-) → D^+ + A^-

Donor Acceptor Donor- Acceptor complex radical anions

While for the reaction of Esc with acid dyes, the electrostatic attraction between positive protonated Esc^+ and negative BCP^-, BPB^-, BCB^-, and BCG^-. The reaction equilibrium can be represented as follows:

$$Esc^+ + D^- \leftrightarrow Esc^+D^-$$

where Esc^+ and D^- represent the escitalopram and the anion of the dye, respectively.

3.7. The association constant and the free energy

The association constant of 1:1 complexes was determined using Benesi–Hildebraned method applying the following equation [26].

$$\frac{[A_o]}{A_\lambda^{AD}} = \frac{1}{\varepsilon^{AD}} + \left(\frac{1}{K_c^{AD}.\varepsilon_\lambda^{AD}}\right)\frac{1}{[D_o]}$$

where $[A_o]$ and $[D_o]$ are the total concentration of reagent and drug, respectively, A_λ^{AD} and ε_λ^{AD} are the absorbance and the molar absorptivity of the complex at λ_{max} and K_c^{AD} is the association constant of the complex. From the above equation, on plotting the values of $[A_o]/A_\lambda^{AD}$ versus $1/[D_o]$ a straight line was obtained with slope equals $1/K_c^{AD}.\varepsilon^{AD}$ and intercept of this line with the ordinate is $1/\varepsilon^{AD}$, Figure 4A, B.

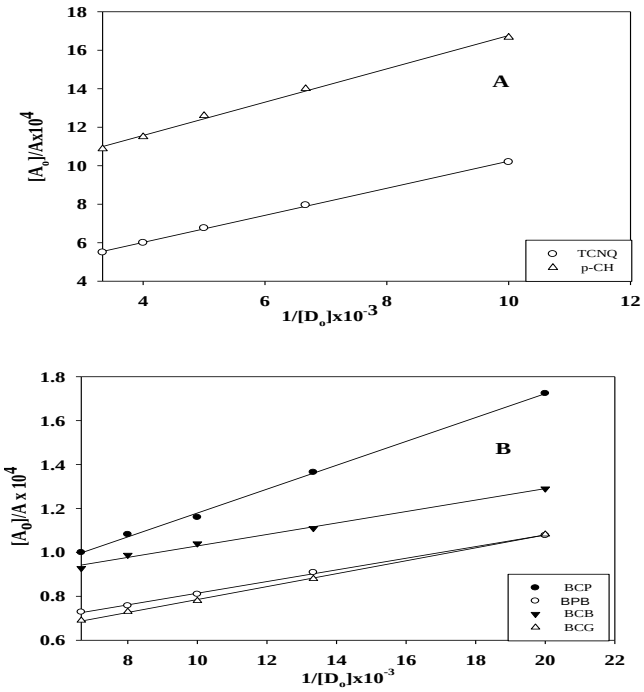

Figure 4: Benesi-Hildebrand plots for Esc with for different reagents.

However, it should be noted that ε^{AD}, which is the molar absorptivity of the complex itself should not be confused with any stoichiometric values calculated with reference to the amount of any analyte being determined. The latter is best described, as Beer's value while the former is Benesi–Hildebrand's value. The association constants K and the molar absorptivities, ε^{AD} are listed in Table 1. The stability constants (K_f) of Esc complexes with reagents were calculated (Table 1) from the continuous variation data using Eq. (1) [27].

The standard free energy of complexation ΔG˚ is related to the association or the formation constant by the following equation [29]:

$$\Delta G^{\circ} = -2.303\ RT \log K_f \qquad (3)$$

where ΔG^{o}, the free energy change of the complex; R, the gas constant 1.987 cal mol^{-1} C^{-1}; T absolute temperature, K_f is the formation constant of drug-reagent complex. The negative values of the calculated free energy indicate the stability of these complexes and their spontaneous formation, Table 1.

3.8. Kinetic and thermodynamic parameters

The kinetic studies of the reaction of Esc with TCNQ were carried out at different temperatures (40, 50 and 60 ^{o}C). The plot of ln $\{(A_i - A_t)/(A_i - A_o)\} = -kt$ as a function of time produced straight line with slope equal to -k. The equation that gave the best fit for the experimental data corresponding to first order [30] (Figure 5) and the slope represents the rate constant.

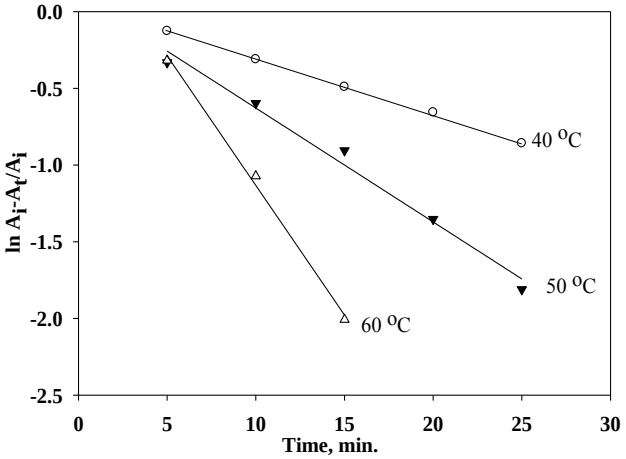

Figure 5: Pseudo first order plots for the reaction of 2.00x10^{-4} mol L^{-1} Esc using 2 mL of 1.00x10^{-3} mol L^{-1} of TCNQ at 841 nm against reagent blank.

Table 2 summaries the calculated k and $t_{1/2}$ at different temperatures, from which it is evident that as the temperature increases, the $t_{1/2}$ decreases i.e. the reaction was less time consuming.

Table 2: Kinetic data for the reaction of Esc with TCNQ at different temperatures

T (K)	k mol min^{-1}	$t_{1/2}$ min	r	TΔS* kJ/mol	ΔH* kJ/mol	ΔG□* kJ/mol
313	3.89x10^{-2}	17.81	0.9994	-9.37	60.86	70.23
323	7.40x10^{-2}	9.36	0.9987	-9.67		70.53
333	1.69x10^{-1}	4.10	0.9991	-9.97		70.83

The relationship between the reaction rate and temperature is determined by the Arrhenius equation:

$$\ln k = -E_a/RT + \ln A$$

E_a is the activation energy, R = 8.314 J/mol·K, T is the absolute temperature in Kelvins, A is frequency factor [31]. A plot of ln k versus 1/T produces a straight line with slope equals to -E_a/R and intercept equals to ln A. Activation energy (E_a) can be calculated from the slope (E_a/R) and was found to be 63.55 kJ/mol indicating that the reactions need low activation energy and frequency factor (A) from the intercept of the Arrhenius plot and found to be 1.51x10^{9}.

The other activation parameters such as enthalpy, entropy and free energy of activation were calculated using Eyring equation which was applied in the following form:

$$\ln k/T = (-\Delta H^*/R)(1/T) + \ln k_B/h + \Delta S^*/R$$

where k_B is Boltzmann's constant [1.381×10^{-23} J k^{-1}], h is Plank's constant [6.626x10^{-34} J·s] and ln k_B/h equals 10.76, ΔH* (kJ/mol) is activation enthalpy, while ΔS* (kJ/mol) is activation entropy and ΔG* (kJ/mol) is the free activation enthalpy (Gibb's free energy). The Eyring plot of ln(k/T) versus 1/T produced a straight line, its slope =ΔH*/R from which ΔH* was calculated and listed in Table 2.

3.9. Evaluation of the kinetic methods through reaction of Esc with TCNE at room temperature

Under the previously mentioned optimum conditions, the absorbance –time curves for the reaction of TCNE with Esc at room temperature were constructed, Figure 6. As the intensity of color increases with time, it was useful to develop a kinetically based method for the assay of the studied reaction. The quantitation of Esc under the optimum conditions outlined above where TCNE concentration, was higher than the initial concentration of Esc would result in pseudo first order reaction with respect to drug concentration.

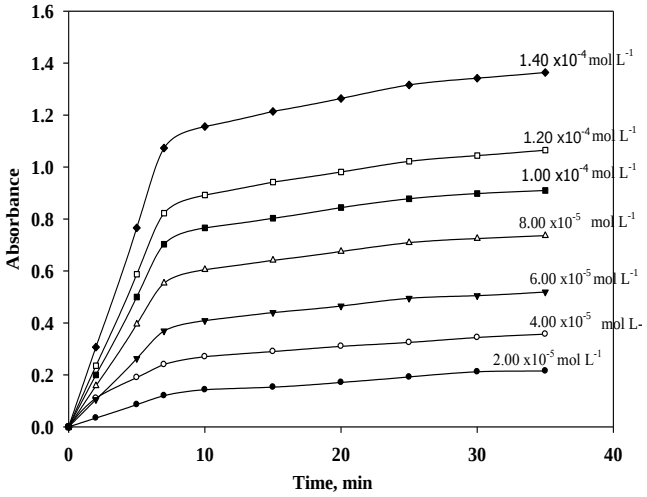

Figure 6: Absorbance–time curves for the reaction of 1 mL of 1x10^{-3} mol L^{-1} TCNE with Esc (2.00 x10^{-5}-1.40 x10^{-4} mol L^{-1}).

When the kinetic parameters were performed under pseudo first order conditions at room temperature, the rates were followed with various concentrations of the drug in the range 8.28-58.02 µg mL^{-1} Esc, keeping TCNE concentration constant at 1.00x 10^{-3} mol L^{-1}. As shown in Figure 6, the rate of the reaction was directly proportional to the drug concentration i.e. drug-dependent in a pseudo- first order, which clearly indicated that the rate obeys the following equation:

$$Rate = v = k\,[C]^n$$

where k is the pseudo first order rate constant, v is the rate of the reaction, $[C]^n$ is the molar concentration of the drug and n is the order of the reaction. This equation was the basis for several experiments, which were run to obtain drug

concentration using the rate data. Initial-rate, rate constant, variable time and fixed time methods [32-34], were tried and the most suitable one was selected taking into account applicability, sensitivity and correlation coefficient.

3.9.1. Initial-rate method

The initial rate of reaction would follow a pseudo order rate constant and obeyed the following rate equation:

$$v = (\Delta A/\Delta t) = k\,C^n$$

where v is the reaction rate, A is the absorbance, t is the measuring time, k is the pseudo first order rate constant, C is the concentration of the drug in mol L^{-1} and n is the order of the reaction. A calibration graph was constructed by plotting the logarithm of the initial rate of reaction (log *v*) *vs* logarithm of drug concentration (log C) which showed a linear relationship over the concentration range of 8.28-58.02 µg mL^{-1}, Figure 7. The logarithmic form of the above equation was written as follows:

$\log v = \log (\Delta A/\Delta t) = \log k + n \log C$

$\log v = \log \Delta A/\Delta t = 0.669 + 0.996 \log [Esc]$ $(r=0.9994)$

Thus, k= 4.67 s^{-1}, and the reaction is pseudo first order (n=0.996≈1) with respect to Esc concentration. The limit of detection (LOD) and limit of quantification (LOQ) for initial rate method were determined and were found to be 0.19 and 0.63 µg mL^{-1}, respectively.

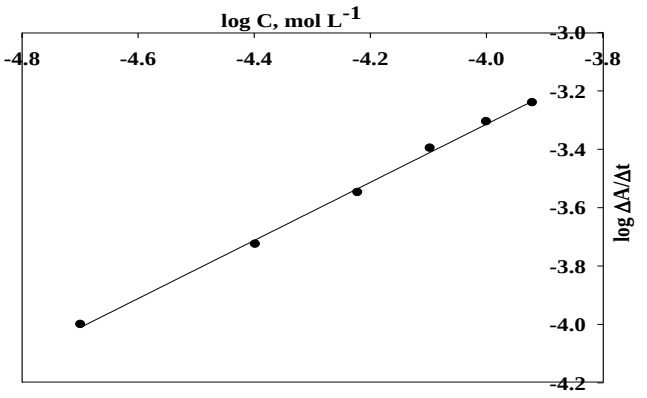

Figure 7: Calibration plot of logarithm rate of the reaction against logarithm molar concentration of Esc for initial rate method.

3.9.2. Rate constant method

Graphs of log absorbance vs. time for Esc concentration in the range of 8.28-58.02 µg mL^{-1} ($2.00x10^{-5}$-$1.40x10^{-4}$ mol L^{-1}) were plotted and all appeared to be rectilinear, Table 3. Pseudo first order rate constant (k) corresponding to different Esc concentrations were calculated from the slopes multiplied by 2.303 and are presented in Table 3. Regression of C vs. k gave the following equation:

$k = -0.0004 + 1.80\,C$ $r= 0.9120$

Table 3: Values of rate constant k

[Esc], (mol L^{-1})	k (sec^{-1})
$2.00 x10^{-5}$	$-4.61x10^{-4}$
$4.00 x10^{-5}$	$-3.34 x10^{-4}$
$6.00 x10^{-5}$	$-3.03 x10^{-4}$
$8.00 x10^{-5}$	$-3.30 x10^{-4}$
$1.00 x10^{-4}$	$-2.38 x10^{-4}$
$1.20 x10^{-4}$	$-2.30 x10^{-4}$
$1.40 x10^{-4}$	$-2.15 x10^{-4}$

3.9.3. Variable time method

Reaction rate data were recorded for different Esc concentrations in the range 8.28-58.02 µg mL^{-1}. A preselected value of the absorbance 0.20 was fixed and the time was measured in seconds, Table 4.

The reciprocal of time (1/t) vs. the initial concentration of Esc was plotted and the following equation of calibration graph was obtained:

$1/t = 0.0011 + 102.72\,C$ $r =0.9956$

The range of Esc concentrations giving the most satisfactory results was 8.28-58.02 µg mL^{-1}.

Table 4: Values of reciprocal time taken at fixed absorbance for the different rates of variable concentration of Esc at constant concentrations of TCNE

[Esc], (mol L^{-1})	1/t (sec^{-1})
$2.00 x10^{-5}$	$6.17x10^{-4}$
$4.00 x10^{-5}$	$2.78x10^{-3}$
$6.00 x10^{-5}$	$5.56x10^{-3}$
$8.00 x10^{-5}$	$7.25x10^{-3}$
$1.00 x10^{-4}$	$9.80x10^{-3}$
$1.20 x10^{-4}$	$1.11x10^{-2}$
$1.40 x10^{-4}$	$1.28x10^{-2}$

3.9.4. Fixed time method

At preselected fixed time, the absorbance of colored solution containing varying amounts of Esc was measured at 25 ^oC and 413 nm. Calibration graphs were constructed by plotting the absorbance against the initial concentration of Esc at fixed time 7, 10, 15, 20, 25, 30 and 35 min. The regression equations, correlation coefficients and linear ranges are given in Table 5.

Table 1: Analytical parameters of escitalopram with different reagents

Parameters	**Reagents**							
	BCP	BPB	BCB	BCG	DDQ	TCNQ	TCNE	*p*-CA
λ_{max} (nm)	407	413	415	416	456	841	413	518
Beer's law limits (μg/mL)	4.14-41.40	2.07-24.86	4.14-37.30	2.07-24.86	8.28-82.88	20.72-165.76	8.28-58.02	41.44-373.00
Molar absorptivity (L/mol.cm)	$1.32x\ 10^4$	$2.14x10^4$	$1.10\ x10^4$	$2.00\ x\ 10^4$	$5.50x\ 10^3$	$3.12x10^3$	$9.33x10^3$	$1.88x\ 10^3$
Sandell sensitivity (μg/cm)	0.038	0.019	0.031	0.017	0.073	0.133	0.047	0.345
Ringbom optimum concentration range (μg/mL)	8.29-31.00	4.14-20.72	8.29-29.00	4.14-22.80	16.57-70.00	20.72-145.00	16.00-49.00	82.88-372.00
Regression equation								
Slope (a)	0.0264	0.0516	0.0319	0.0481	0.0137	0.0075	0.0217	0.0029
Intercept (b)	0.014	-0.022	-0.0054	-0.013	0.0110	0.0062	0.0067	0.0143
Correlation coefficient (r)	0.9997	0.9999	0.9999	0.9996	0.9993	0.9998	0.9996	0.9994
SEE	0.015	0.018	0.016	0.017	0.015	0.009	0.021	0.015
LOD (μg/mL)	0.97	0.48	0.69	0.37	0.21	0.33	0.10	0.52
LOQ (μg/mL)	3.23	1.59	2.29	1.19	0.71	1.12	0.33	1.75
K_f	$1.35x10^4$	$3.47\ x10^4$	$4.27\ x10^4$	$2.45\ x10^4$	$2.63x10^3$	$2.57x10^3$	$5.25x10^3$	$6.92x10^3$
K_c^{AD} (L/mol)	$1.17x10^4$	$2.07x10^4$	$2.95x10^4$	$1.67x10^4$	$5.00x10^3$	$3.33x10^3$	$9.80x10^3$	$1.25x10^3$
ε_λ^{AD} (L/mol.cm)	$1.57x10^4$	$2.07x10^4$	$1.30x10^4$	$2.04x10^4$	$1.41x10^3$	$4.27x10^3$	$7.5x10^3$	$9.24x10^3$
(ΔG°) (kJ/mol)	-5.55	-5.89	-6.09	-5.76	-4.66	-4.65	-5.05	-5.22
Robustness± SD	99.78 ±0.008	99.54±0.007	100.40±0.073	99.31±0.005	98.33±0.056	99.43±0.03	98.11±0.064	99.01±0.099
R.S.D.[a]	0.004	0.071	0.003	0.070	$4.15x10^{-3}$	$1.40x10^{-2}$	$2.88x10^{-3}$	$2.51x10^{-3}$
R.S.D.[b]	0.068	0.009	0.048	0.011	$2.02x10^{-2}$	$2.40x10^{-2}$	$3.70x10^{-3}$	$1.59x10^{-3}$

R.S.D.% is the relative standard deviation of five determinations. K_c^{AD} is the association constants calculated by applying Benesi–Hildebrand method. K_f is the formation constants calculated from the continuous variation data.

[a, b] The intraday and interday (n = 5) relative standard deviations by the proposed methods.

Table 5: Regression equations for Esc at fixed time and 25 ^{o}C

Time, min	Regression equations	r	SEE	Linear range, µg mL^{-1}
7	-0.0267 +0.0170C	0.9951	0.0394	8.28-58.02
10	-0.0157+0.0180C	0.9959	0.0386	8.28-58.02
15	-0.0116+0.0190C	0.9965	0.0375	8.28-58.02
20	-0.0052+0.0200C	0.9967	0.0378	8.28-58.02
25	-0.0017+0.0207C	0.9967	0.0389	8.28-58.02
30	0.0059+0.0212C	0.9965	0.0393	8.28-58.02
35	0.0067+0.0217C	0.9996	0.0405	8.28-58.02

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient, linear range and the intercept were obtained for a fixed time of 35 min. Therefore, the fixed time of 35 min was utilized for the assay of Esc concentration. The limit of detection (LOD) and limit of quantification (LOQ) for fixed time (35 min) method were determined and found to be 0.10 and 0.33 µg mL^{-1}, respectively. For more accurate analysis, Ringbom optimum concentration range was calculated and found to be 16.00–45.00 µg mL^{-1}. Table 1 shows the values of molar absorptivity, Sandell's sensitivity and some analytical characteristics for fixed time (35 min) method.

3.10. Method validation

For methods used in drug analysis, validation report including linearity, LOD, LOQ, accuracy, precision, specificity, ruggedness and robustness has to be proved according to ICH guidelines [35].

3.10.1. Linearity and sensitivity

Beer's law was obeyed in the concentration ranges 8.28-82.88, 20.72-165.76, 8.28-58.02 and 41.44-373.00 µg mL^{-1} for DDQ, TCNQ, TCNE and *p*-CA complexes, respectively. While for using acid dyes, Beer's law plots were linear with very small intercepts and good correlation coefficients in the concentration range of 4.14-41.40, 2.07-24.86, 4.14-37.30 and 2.07-24.86 µg mL^{-1} Esc with BCP, BPB, BCB and BCG respectively, Table 1.

For accurate determination, Sandell sensitivities were calculated which reflected the sensitivity and accuracy of the proposed methods. The limits of detection and quantification were calculated according to the equation:

LOD = 3.3σ/S, where σ= the standard deviation, S=slope of calibration curves. While the LOQ =10 σ/S, the LOD and LOQ were calculated and the data are presented in Table 1.

3.10.2. Accuracy and precision

In order to study the accuracy and precision of the method, three concentration levels of Esc within the linearity range were selected. The within day precision (intra-day precision) was performed by taking five independent analyses at each concentration level within 1 day during the stability time period. The standard deviations (S.D.) and R.S.D. % were calculated, Table 1. The daily precision (inter-day precision) was measured by assaying a single sample of each concentration on five consecutive days within the stability time period. The results are summarized in Table 1, which can be considered very satisfactory.

Table 6: Application of standard addition technique for determination of Esc in Citalopm® tablets using the proposed methods

Method	Taken (µg mL^{-1})	Pure Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery*%
DDQ	20.00	20.00	19.62	98.10
		40.00	39.50	98.76
		60.00	58.80	98.00
Mean± R.S.D.				98.29±0.20
TCNQ	20.00	40.00	39.20	98.00
		60.00	58.40	97.33
		100.00	99.28	99.28
Mean± R.S.D.				98.20±0.41
TCNE	20.00	10.00	10.03	100.30
		20.00	20.40	102.00
		30.00	30.02	100.07
Mean± R.S.D.				100.79±1.03
p-CA	20.00	100.00	100.74	100.74
		200.00	199.70	99.85
		300.00	300.69	100.23
Mean± R.S.D.				100.27±0.44
BCP	5.00	5.00	4.90	98.00
		8.00	7.96	99.50
		10.00	10.10	101.00
Mean± R.S.D.				99.50± 1.50
BPB		5.00	5.00	100.00
		8.00	7.88	98.60
		10.00	10.02	100.20
Mean± R.S.D.				99.60±0.87
BCB		5.00	4.90	98.10
		8.00	8.08	101.00
		10.00	10.00	100.00
Mean± R.S.D.				99.70±1.47
BCG		5.00	4.90	98.00
		8.00	8.00	100.00
		10.00	10.01	100.10
Mean± R.S.D.				99.37± 1.18

* Average of three determinations.

3.10.3. Ruggedness and robustness

The ruggedness of the proposed methods was assessed by applying the procedures using two different instruments in two different laboratories at different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible, as RSD% did not exceed 2%. While robustness was assessed by evaluating the influence of small concentration variation of reagents (1.8-2.2 mL) and reaction time (3-5 min). The small variations in any of the variables did not significantly affect the results, Table 1.

3.10.4. Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany Esc in its dosage forms (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide and magnesium stearate) was studied. The results indicated that there is no interference from the excipients, indicating a high selectivity for determination of Esc in its dosage forms.

3.11. Analysis of pharmaceutical preparations

The obtained satisfactory validation results made the proposed procedures suitable for the routine quality control analysis of Esc. The proposed methods were applied for the determination of Esc in Citalopm® tablet applying standard addition technique using CT reagents. The obtained mean recoveries ± RSD% ranged from 98.20±0.20 to 100.79±1.03%, Table 6.

The results were statistically compared with those obtained by the reported method using F-test and t-test with five degrees of freedom at the 95% confidence level, proving no significance difference with respect to accuracy and precision, Table 7.

Table 7: Statistical analysis of the data for Esc using CT and ion pair reagents compared with official method

Statistic	DDQ	TCNQ	TCNE	*p*-CA	Official method**
Recovery%	99.00	98.56	100.99	99.66	99.10
S.D.	1.48	0.50	0.96	0.67	0.99
N	5	5	5	5	5
Variance	2.19	0.25	0.92	0.45	0.98
t (2.776)*	0.13	1.09	2.08	1.05	---------
F(6.390)*	2.23	3.92	1.02	1.46	---------
	BCP	BPB	BCB	BCG	
Recovery%	99.97	99.20	98.43	99.20	99.10
S.D.	0.017	0.009	0.004	0.011	0.091
n	5	5	5	5	5
Variance	2.19	0.25	0.92	0.45	0.98
t (2.776)	1.09	0.20	1.08	0.18	---------
F(6.390)	2.23	3.92	1.07	2.18	---------

*****Values between parentheses are the theoretical values of t and F at confidence 95%.

** Manufacture non-aqueous titration method.

4. Conclusion

Unlike GC and HPLC procedures, the spectrophotometer is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as reagent concentration. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of Esc in pure form and in pharmaceutical preparations.

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