Spectrophotometric Determination of Escitalopram Oxalate in Pure Form and its Pharmaceutical Preparation via Oxidative Coupling Reaction

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Abstract— **background: One of the techniques for spectroscopic determination are the oxidative coupling reaction, have been utilized in the estimation of compounds in many fields, such as agriculture, clinical, and pharmacological analysis.**

Aims: The aim of this studying to locate a sensitive and cheap technique for the estimation of escitalopram-oxalate (ESC) in pharmacological compositions and in pure form.

Methods and Material: The process relies on the coupling between the escitalopram oxalate with 4-aminoantipyrine (4-AAP) in a basic medium in the attend of N-Bromosuccinimide (NBS) to produce an intense red azo dye, soluble in water with max. Abs. at 528 nm against the blank solution.

Statistical analysis used: The UV–Vis spectra were exported to MS Excel software. All statistics were analyzed by using SPASS Program Version 18.

Results: The method has obeyed Beer's law in the concentration range of (1.0-20) µg/ml with a relative error of 0.16-1.30 and a relative standard deviation of 0.852-2.936 depending on the concentration level. The values of molar absorptivity(ε), Sandell's sensitivity, and limit of detection were 1.4628x104 L mole^{-1} cm^{-1}, 0.0283µg/cm2, and 0.1061µg/ml, respectively.

Conclusions The method used to synthesize azo dye is an easy, fast, and inexpensive method and gives high sensitivity and absorbency, and the wavelength of 528 is where the maximum absorption occurs. It was also discovered that the dye's color stays stable at room temperature.

Key-words: Escitalopram oxalate, Losiram, Oxidative coupling, Spectrophotometry.

Introduction

Escitalopram oxalate (ESC) is an antidepressant of the selective serotonin reuptake (SSRI)class. It is used in the treatment of major depressive disorder in adolescents and adults[1]. ESC is 1-[3-(Dimethyl amino)propyl]-1-(4-fluorophenyl)-13-dihydro-5-isobenzo furan carbo nitrile oxalate[2] (Figure 1). A white, frosted substances having a melting point of 146°C. It is soluble in alcohol, slightly soluble in acetone, and sparingly soluble in water[3]. ESC is produced as the oxalate salt for therapeutic use, the empirical formula is $C_{20}H_{21}FN_2O.C_2H_2O_4$ with a molecular weight of 414.40g/mol[4].

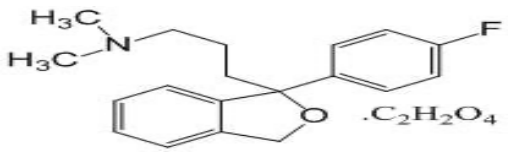

Figure 1: The structure of escitalopram oxalate

One of the techniques for spectroscopic determination is the oxidative coupling reaction, which is an important process in analytical chemistry with a variety of uses[5]. Two or more substances are paired in the existence of an oxidizing agent under the appropriate conditions, to form a colored product[6].

The oxidative-coupling reactions have been utilized in the estimation of compounds in many fields, such as agriculture, clinical, pharmacological analysis, and food by applying different analytical methods, such as spectroscopic methods[7], flow-injection methods[8], and chromatographic methods[9].

A variety of techniques have been used to determine ESC like Spectro techniques[10-13], high-performance liquid chromatography techniques[14-17], and electrochemical techniques [18]. The current method is based on the coupling of ESC with 4-AAP in the presence of NBS to form a red-colored product in an alkaline medium that has been proven successful for the quantification of ESC in pharmacological compositions as well as in pure form.

Subjects and Methods

Apparatuses

T92+Spectrophotometer double beam, china with 1.0 mm quartz-cell, was used, Jenway model 3310 pH-meter was used to check the pH of solutions, and sartorius balance BL210 SAG, Germany .

Chemicals

All chemicals used in this study were of a high degree of purity (Fluka, BDH, SDI).

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Preparation of solutions

Standard Escitalopram Oxalate solution(ESC) 250 µg/ml
It was produced by defrosting 0.0248g from ESC in distilled water(DW) and completed with D.W. in a volumetric flask of 100 ml. The Standard ESC solution was stable for approximately ten days.

4-Aminoantipyrine Reagent Solution(4-AAP) 5x10-3M

It was produced by defrosting 0.1016 g of 4-AAP in DW and completed with DW in a flask of 100ml.

N-Bromosuccinimide oxidizing agent(NBS) 3x10-3M

It was produced by defrosting 0.0534 g of NBS in 3.0 ml of acetone; then the volume was completed to 100 ml with DW.

Sodium carbonate solution, approximate 1x10-2M

It was produced by defrosting 0.1059 g in 100 ml of DW.

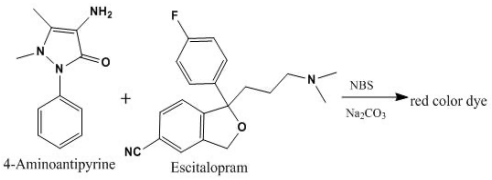
Solution of ESC pharmacological compositions (250 µg/ml)

Ten tablets (containing 20 mg of ESC) were weighed accurately. After grinding and mixing well, the weight was equal to 2.387g; after that, a weight of 0.2983 g of this crushed, equivalent to 0.025g of the drug, and dissolved in DW, the solution was mixed fine, and filtered to 100ml (volumetric-flask). Then, the volume was completed to the sign with DW.

RESULTS AND DISCUSSION:

Preliminary Study

The principle of the method is the coupling of the reagent with the ESC drug in the presence of the oxidizing agent in a basic medium where a solution of red color is formed and gives the maximum wavelength (528) nm against the blank solution (Scheme 1).



Scheme 1: The proposed mechanism to produce the red colored dye

Studying The Typical Circumstances

The sway of diverse variables on the absorption density of the azo dye was examined to determine the typical circumstances for the method.

The Influence of The Amount of Coupling Reagent

A study was carried out to establish the typical amount of reagent solution (4-AAP), which gives the maximum absorption of the colored product by adding diverse volumes (0.5-2.0 ml) of 4-AAP reagent ($5x10^{-3}$M) to the vials containing 1.0 ml (ESC), 2.0 ml (NBS, $3x10^{-3}$M), and 1.0 ml (sodium carbonate), with DW, the volume was finished at 25 ml. (Figure 2), indicating that smaller amounts resulted in incomplete complex formation. Increased concentration increases the absorbance, reaching its maximum when using a 4-AAP solution of 1.0 ml.

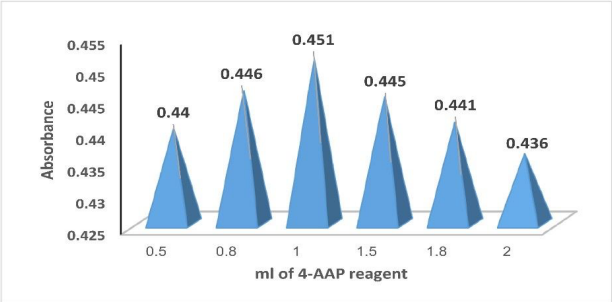


Figure 2: Effect of the amount of coupling reagent

The Impact of The Amount of NBS

A study was carried out to establish the typical quantity of NBS ($3x10^{-3}$M) by adding diverse volumes (1.0-2.5ml) of NBS to flasks containing 1.0 ml of ESC solution and 1.0 ml of 4-AAP reagent solution in the presence of 1.0ml (sodium carbonate). The volume was completed to 25 ml with DW and measured at wavelength 528 nm. The outcomes in Figure 3 show that the absorbance of the product increased with the increasing volume of NBS even when reached a constant at 2.0 ml, after which the (Abs) decreased. So, it was utilized in trailing experiments.

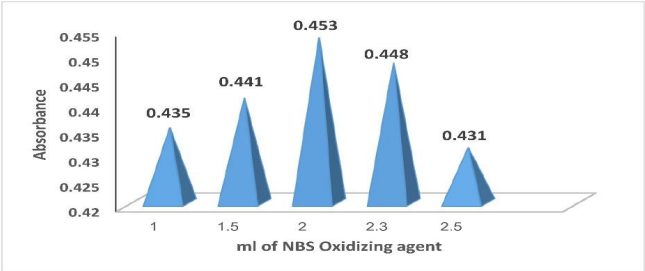


Figure 3: The impact of the volume of oxidizing agent

The Impact of The Base Type Solution

The coupling reaction between the reagent 4-AAP and the ESC occurs in an alkaline medium, so the effect of various bases and alkaline salt was investigated to determine which produced the higher absorbance. From the results in Figure 4, it is clear that sodium carbonate gives the highest absorbance. Therefore, it was utilized in trailing experiments.

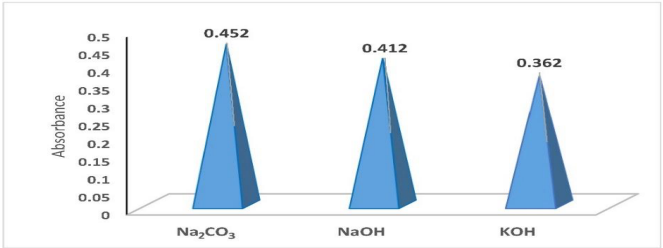


Figure 4: The impact of bases used during the oxidative coupling reaction

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The Impact of The Amount of Base Solution

A study was carried out to establish the typical amount of base solution by adding diverse volumes (0.5-2.0ml) of sodium carbonate; it was found that 1.0 ml of sodium carbonate gives the highest (Abs) at pH=10.6. So, the volume was adopted in the later experiments. The outcomes are shown in Table 1.

TABLE 1: THE IMPACT OF THE AMOUNT OF BASE SOLUTION

ml of Na_2CO_3 ($1x10^{-2}$)	Absorbance	pH
0.5	0.442	8.2
0.8	0.447	9.7
1.0	0.451	10.6
1.3	0.446	11.4
1.5	0.441	11.7
2.0	0.436	12.1

Effect Order of Addition

The effect of various orders to choose the utmost sequence in addition to the reactants, it was found from the results that the order No. 3, (E + R + O + B) is the best order to form a colored product with maximum absorbance. Therefore, was chosen in the later experiments, as shown in Table 2.

TABLE 2: EFFECT OF SEQUENCES OF ADDITION

No.	*Order of addition	Abs
1	O + R + E + B	0.420
2	O + E+ B + R	0.433
3	E + R + O + B	0.453
4	E + O + R + B	0.446
5	R + B + E + O	0.440
6	O + B + E + R	0.320

*(E) ESC, (R) 4-AAP, (O) NBS, (B) Na_2CO_3.

The impact of time on the steadily of the formed dye

The stability of the formed dye was probed by examining the effect of time on the absorbance of three different concentrations (4.0, 10, and 15µg/ml) of ESC according to the procedure of the proposed method. The results in Table 3, show that the red color dye is stable for 50 minutes.

TABLE 3: THE IMPACT OF TIME ON THE STEADILY OF THE FORMED DYE

Time(min)	Absorbance		
	4µg/ml	10µg/ml	15µg/ml
2	0.215	0.442	0.616
3	0.220	0.452	0.623
5	0.220	0.452	0.623
10	0.220	0.452	0.623
15	0.219	0.452	0.622
20	0.219	0.451	0.622
25	0.219	0.451	0.622
30	0.218	0.450	0.621
35	0.218	0.450	0.621
40	0.218	0.449	0.621
45	0.217	0.449	0.620
50	0.217	0.447	0.617
60	0.182	0.416	0.562

The Impact Of Temperature

The impact of temperature in the range (5.0-50°C)on the absorbance of the formed dye has been examined, it was found that a temperature (25°C) gave the finest absorbance. Therefore, the later experiments were carried out at this temperature, the outcomes are shown in (Table 4).

TABLE 4: THE IMPACT OF TEMP.

Temperature°C	Abs.
5.0	0.390
10	0.414
15	0.431
20	0.442
25	0.453
30	0.442
35	0.436
40	0.429
45	0.412
50	0.402

The Eventual Absorption Spectrum

The eventual absorption spectrum was measured by using 1.0 ml of escitalopram oxalate (250µg/ml), 1.0 ml of 4-Aminoantipyrine ($5x10^{-3}$M), and 1.0 ml of NBS ($3x10^{-3}$M) in the presence of sodium carbonate solution ($1x10^{-2}$) at a temperature of 25°C and the solution was left for 3.0 minutes to complete the reaction. In a volumetric flask, the volume was completed to 25 ml; the absorption was measured against the blank solution; it was found to give a higher Abs. at (528 nm), while its blank gave a little absorption at the same wavelength. The results are shown in Figure 5.

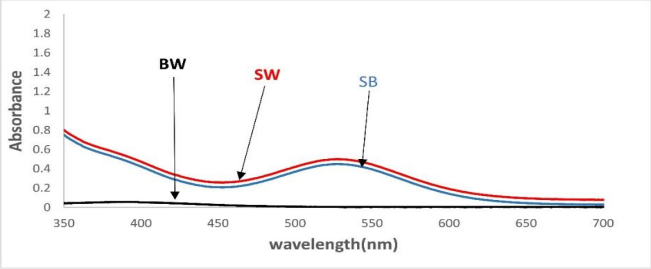

Figure 5: The eventual absorption spectrum for the determination of ESC (10µg/ml) against distilled water (SW), ESC against blank solution (SB), and Blank solution against distilled water (BW).

TABLE 5: THE OPTIMUM CONDITIONS OF THE DEVELOPED METHOD

λ max	528 nm
Amount of 4-AAP reagent ($5x10^{-3}$)	1ml
Amount of NBS oxidizing agent ($3x10^{-3}$)	2 ml
Amount of sodium carbonate ($1x10^{-2}$)	1ml
Temperature	25°C
Solvent	Distilled water

The Calibration Graph

After selecting the optimized experimental conditions shown in Table 5, ESC solution (1-20 µg/ml) was rushed to a series of flasks(25 ml), thereafter 1.0 ml of 4-AAP reagent, 2.0 ml of NBS oxidizing agent solution, and 1.0 ml of sodium carbonate,

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the solutions have been left for 3.0 minutes to complete the reaction, and then the volumes were completed to the sign with DW. The absorbance of the solutions was measured against the blank solution at a wavelength (528 nm). Figures 6 and 7 show that the calibration curve is linear over the concentration range (1- 20 µg/ml), while higher concentration shows a negative deflection from Beer's law. The molar absorption coefficient was 1.4628 x10^4 L.$mole^{-1}$.cm^{-1}, and the Sandel sensitivity value was 0.0283 µg/cm^2.

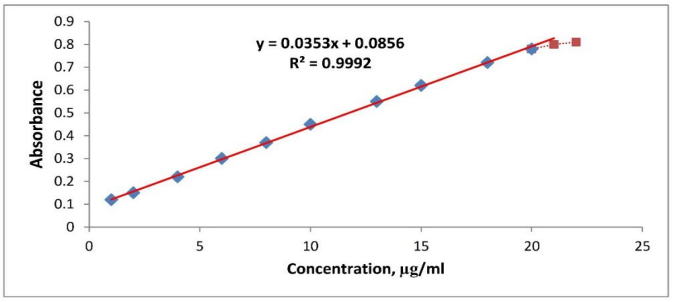

Figure 6: The calibration graph for the ESC drug

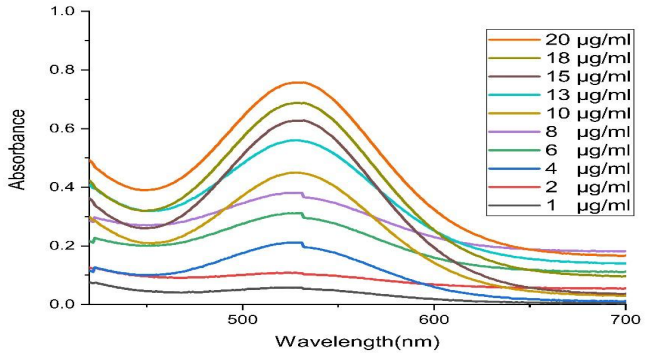

Figure 7: The calibration graph spectra for the ESC drug

The Precision And Accuracy

The precision and accuracy of the method were examined by metering the recovery%, and precision through the relative standard deviation (RSD%) and the relative error (RE%) For three different concentrations (4.0,10, and 15) µg/ml (measuring the absorbance of six times) at wavelength 528 nm for each concentration and taking the average for it. The findings in Table 6, showed that the technique for determining ESC had acceptable precision and accuracy.

TABLE 6: THE RESULTS OF PRECISION AND ACCURACY

Amount of ESC µg/ml		*RE%	*Recovery%	*RSD%
Taken	Measured			
4	4.04	1.0	101.0	0.852
10	10.06	0.60	100.60	2.936
15	15.03	0.20	100.20	1.513

*Average of six times

Limit Of Detection (LOD)

The (LOD) was calculated by measuring the absorption of the blank solution[19] at optimized condition (6 times) at wavelength 528 nm by using the following equation[20]: LOD= 3.3 SD/b. Where SD is the standard deflection and b is the slope of the calibration. LOD of the method was calculated it was found to be 0.1061µg/ml.

The type of dye that was created

To know the nature of the formed dye (stoichiometry of drug with the reagent), The continuous variation method (Job method) was applied. In this method, the concentration of the ESC solution and the 4-AAP reagent solution was equal to 6x10^{-4}M.

The Continuous Variation Method (Job Method)

In a sequence of flasks (25ml), diverse volumes of the ESC drug ranging from (1-9ml) and diverse volumes (9-1ml) of 4-AAP reagent solution were mixed, 2.0 ml (NBS), and 1.0 ml (sodium carbonate solution) was added, and then completed to the mark with DW. The (Abs) was measured at 528 nm against the blank solution. The results in Figure 8, showed that the proportion is 1:1.

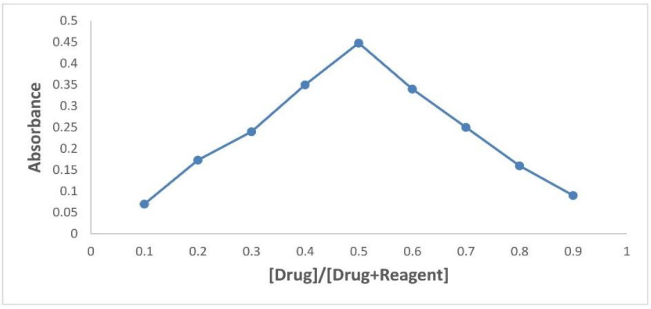

Figure 8: The continuous variation method for a reaction product ESC with 4-AAP by oxidative coupling

The proposed formula for the dye formed by the reaction of escitalopram oxalate with the reagent of 4-Aminoantipyrine is shown in Figure 9.

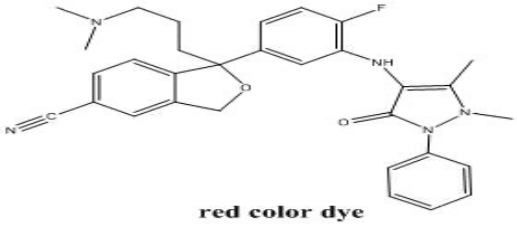

Figure 9: The proposed chemical structure of the red-colored dye

APPLICATIONS

The method was applied to a pharmaceutical formulation containing ESC drug, which is the pharmaceutical formulation (Losiram) in the form of tablets and each tablet contained (20 mg) of the ESC.

DIRECT METHOD

In this method, it was taken three different concentrations (4, 10, and 15 µg/ml) of the pharmaceutical formulation were

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conveyed to 25 ml of the volumetric flasks, the solutions transaction as in the construction of a calibration graph, and the absorbance was measured at wavelength 528 nm (six times), relative error(RE%), recovery(Rec%), and relative standard deviation were calculated. The outcomes are shown in Table 8.

TABLE 8: THE RESULTS OF THE DIRECT METHOD FOR THE IDENTIFY OF ESC IN PHARMACOLOGICAL COMPOSITIONS

Conc. µg/ml Taken	Conc. µg/ml Measured	*RE%	*Rec%	*RSD%
4	3.95	-1.25	98.75	0.560
10	10.11	1.10	101.1	0.360
15	14.77	-1.53	98.47	0.285

*Average of Six times

Standard Addition Method

To demonstrate that the method was devoid of interferences, the method of standard additions was applied to determining escitalopram oxalate in its pharmaceutical preparation. The addition of constant volumes (0.4,1.0 ml) which is equivalent to (4.0, and 10 µg/ml) of pharmaceutical solution in two series of six volumetric flasks of 25 ml, then adding increasing (0.2/ 0.4/ 0.6/ 0.8/ and 1.0 ml) of ESC solutions, and then the absorption was measured against the blank solution at the wavelength 528 nm. The findings are listed in Table 9, and Figure 10.

TABLE 9: THE STANDARD-ADDITION METHOD FOR THE IDENTIFY OF ESC IN PHARMACOLOGICAL COMPOSITIONS

Conc. µg/ml Taken	Conc. µg/ml Measured	*RE%	*Recovery %
4	3.88	-3.0	97.0
10	9.94	-0.6	99.4

*Average of six times

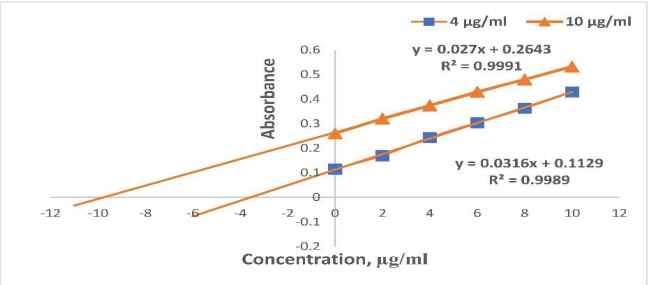

Figure 10: The standard addition curve for determination of ESC in pharmaceutical preparation

CONCLUSION

A precise spectral method was developed for the estimation of escitalopram oxalate by the oxidative coupling reaction. The method is based on the reaction of ESC with the 4-AAP reagent in the presence of oxidizing agent (NBS) in a basic medium, where a red-colored azo dye is formed, and the highest absorption is given at the wavelength of 528 nm. The method was successfully applied to determine the pharmaceutical preparation containing escitalopram oxalate (Losiram) with recovery of no less than 97.0%.

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