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Uni and Simplex Optimization for the Spectrophotometric Determination of Erythromycin ethylsuccinate Drug via Charge-Transfer Complex Formation

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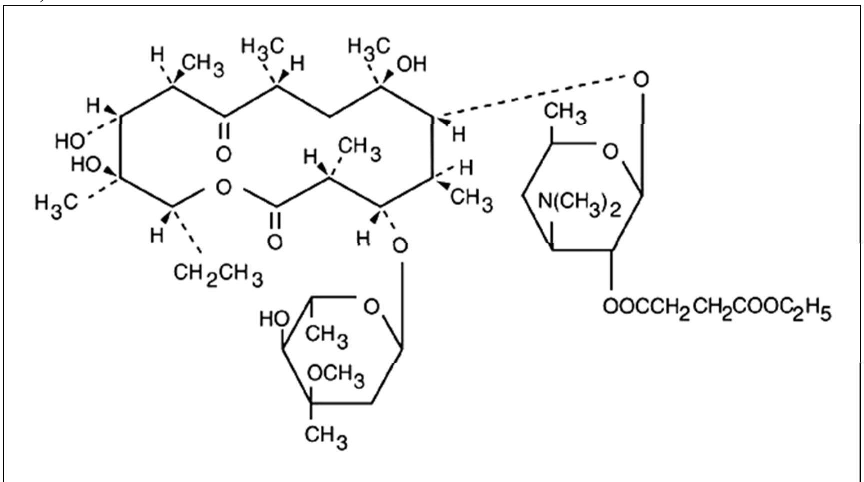
Abstract

Charge transfer complex formation method has been applied for the spectrophotometric determination of erythromycin ethylsuccinate, in bulk sample and dosage form. The method was accurate, simple, rapid, inexpensive and sensitive depending on the formed charge- transfer complex between cited drug and, 2,3-Dichloro-5,6-dicyano-p- benzoquinone (DDQ) as a chromogenic reagent. The formed complex shows absorbance maxima at 587 nm against reagent blank. The calibration graph is linear in the ranges of (10 - 110) $\mu g.mL^{-1}$ with detection limit of $0.351 \mu g.mL^{-1}$. The results show the absence of interferences from the excipients on the determination of the drug. Therefore the proposed method has been successfully applied for the determination of erythromycin ethylsuccinate in pharmaceutical preparations.

Keywords: Simplex, Spectrophotometric, Erythromycin ethylsuccinate , Charge- transfer.

Introduction

Erythromycin is the most employed macrolide antibiotic for treating a myriad of infections caused by gram-positive bacteria such as anthrax, tonsillitis, otits media and syphilis [1,2], it is often prescribed as an alternative for patient allergic to penicillin [2,3]. It has been also employed a as part of therapeutic cocktails together with amino glycoside antibiotics that covers gram-negative microorganisms [3]. Erythromycin is available in several forms including estolate, ethysuccinate and stearate [2,4]. The chemical structure of erythromycin ethysuccinate is given in (Scheme 1).



Scheme 1: The chemical structure of erythromycin ethylsuccinate

Several methods have been reported for determination of erythromycin ethylsuccinate in bulk and pharmaceutical dosage forms, these methods include liquid chromatography [5-11], high performance liquid chromatography[12-15], liquid and solid extraction[16,17], Potentiometry [18], voltammetry[19] and Spectrofluorimetry[20,21].

Spectrophotometry [22-25] are most convenient techniques because of their inherent simplicity, adequate sensitivity, low cost and wide availability in all quality control laboratories.

In experimental chemistry, the optimization of technical system is the process of the adjusting of the control variables to find the levels that achieve the best optimization. Usually, many conflicting response must be optimized simultaneously. In lack of systematic approaches the optimization is done by trial and error, or by changing one control variable at a time while holding the rest constant, such methods requires a lot of experiments to be carried out.

Simplex optimization of experimental parameters was first introduced by Spendley [26], and then modified by Nelder [27] and Aberg [28].

Simplex is a geometric figure in which there are n $_{+}$1 vertices, where (n) represents the number of variables [29], the method found a lot of applications in field of analytical chemistry [30-32], because it offers the capability of optimizing several factors simultaneously depending on a statistical design search to find out the maxima or minima of response, by rejecting the point producing the worst response and a replacement of it by the new point which is obtained statistically.

The present work describes the utility of 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) reagent for spectrophotometric determination of erythromycin ethylsuccinate in pure form as well as in these dosage forms. In addition, the optimization of chemical dependent variables of affecting absorbance has been studied by using modified simplex method via computer program.

Apparatus

A cintra 5 spectrophotometer with 1 cm quartz cells were used for absorbance measurements. PH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (DELL) was used for data processing.

Experimental

Material and reagents

All chemicals used were of analytical reagent grad unless otherwise is mentioned, erythromycin ethylsuccinate, standard powders (purity 99.8%) were kindly provided by the State Company for Drug Industries and Medical Appliances, Samara-Iraq (SDI).

DDQ 0.1 %(w/v) solution, was prepared by dissolving 0.01 g of the DDQ in 5 mL of acetonitrile and then the solution was diluted to a final volume 10 mL with acetonitrile. Working solutions were freshly prepared by subsequent dilutions. This solution is prepared daily using red- glass volumetric flask because it is a light sensitive reagent.

Standard drugs solutions

Erythromycin ethylsuccinate stock solution (1000 $\mu g.mL^{-1}$), was prepared by dissolving 100 mg of erythromycin ethylsuccinate in 10mL methanol and diluting to 100mL in a volumetric flask with acetonitrile. Working solutions were freshly prepared by subsequent dilutions.

General recommended procedure

In a series of 5 mL volumetric flasks, 0.2 mL of 0.1 % of DDQ solution was added to aliquot volumes of the standard erythromycin ethylsuccinate stock solution containing (50-550 µg). The resulted mixtures were diluted to volume with acetonitrile. The absorbance of each solution was recorded at the λ_{max} of the formed charge-transfer complex (585.5 nm) against reagent blank which prepared by the same manner, but without addition of erythromycin ethylsuccinate.

Analysis of Erythromycin ethylsuccinate in pharmaceutical preparations

The content of 10 capsules were mixed well and a certain amount of fine powder was accurately weighted to give an equivalent to 250 mg for capsules and dissolve in 50 mL of methanol, swirled, leaved to stand for 5 mints and diluted to 100mL in a volumetric flask with acetonitrile. The solution then was filtered by using Whatman filter paper No.41 to avoid any suspended or undissolved material before use, and the first portion of the filtrate was rejected, Working solutions were freshly prepared by subsequent dilutions with acetonitrile and analyzed by the recommended procedure.

Results and discussion

Spectrophtometric procedures are popular for their sensitivity in the assay of drugs and hence, charge-transfer complex formation has received considerable attention for the quantitative determination of many pharmaceutical compounds [33-36].

Erythromycin ethylsuccinate react with DDQ to give yellow color charge-transfer complex, which exhibits absorption maxima at 585.5 nm against their reagent blank (Figure1). The some bands may be attributed to the formation of DDQ radical anion, which probably resulted from the dissociation of the donor-acceptor complex in relatively high polar solvents like acetonitrile [36]. Therefore, in order to avoid the maximum interference from the reagent blank, the absorbance measurements were carried out at 585.5 nm in the subsequent work.

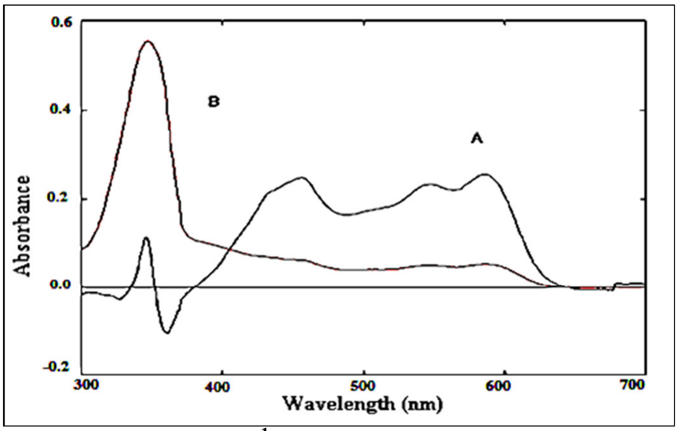


Figure1: Absorption spectra of (A) 50 µg.mL^{-1} erythromycin ethylsuccinate-DDQ charge-transfer complex, (B) the blank solution under the recommended procedure

Optimization of experimental variables

I. Univariable method

The experimental variables affecting the development and stabilities of charge-transfer complex formation were achieved through a number of preliminary experiments. Such factors include reagent volume, reaction time, temperature, and the type of organic solvent. For this reason, a variable was modified while maintaining the other variables at their constant values, then by maintaining that variable at its optimized value, another was modified; all variables were optimized via this method.

Effect of reagent volume

In order to predict the optimum required amount of DDQ for quantitative reaction with erythromycin ethylsuccinate, different volumes (0.1 – 1.0 mL) of 0.1% solutions of DDQ were tested. The results shown in Figure 2 indicate that increasing the volume of DDQ has a positive effect on the absorption signal of the formed complex up to 0.2 mL, while larger volumes of reagent solution have reverse effect. This may be attributed to the possibility of formation of new species upon the reaction of erythromycin ethylsuccinate with relatively higher amounts of the reagent, which may absorb radiation at different wavelengths. Therefore, 0.2 mL of 0.1 % solutions of DDQ was found to be the optimum amount, since it results in maximum color intensity with minimal blank reading.

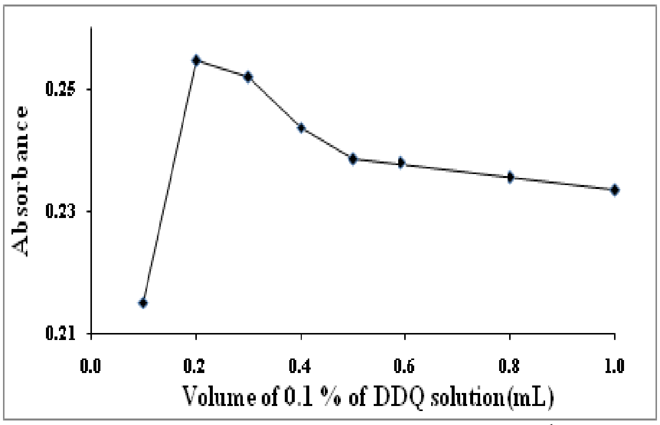


Figure 2: Effect of reagent amount on the absorbance of 50 µg.mL^{-1} erythromycin ethylsuccinate

Effect of reaction time

The optimum reaction time is determined by following the color development at ambient temperature (25 ± 2 °C). It was found that the reaction of erythromycin ethylsuccinate with DDQ, under the conditions of the study, is instantaneous, and the formed complex attained maximum absorbance immediately after mixing. The developed color remained strictly unaltered for at least 2 hours in dark place.

Effect of temperature

The optimum reaction temperature was determined by following the color development at ambient temperature in the range from (25 - 50 ± 2°**C**). It was found that. The value of the absorbance starts to decrease considerably when reaction temperature raised, this may be due to decomposition of the formed charge transfer complex.

According to the obtained results, room temperature (i.e. 25 ± 2°C) was selected as an optimum temperature for maximum color production (Figure 3).

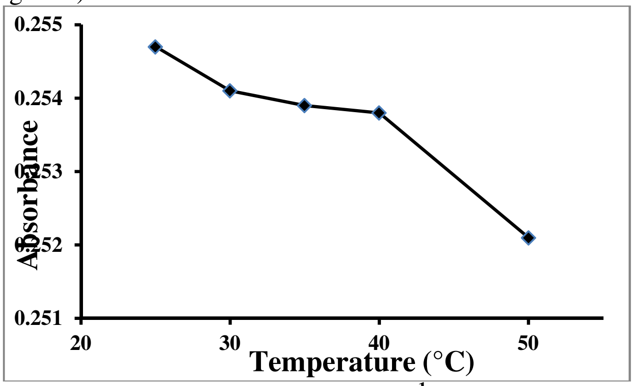


Figure 3: Effect of temperature on the absorbance of 50 μg.mL^{-1} erythromycin ethylsuccinate; 0.1% DDQ

Effect of organic solvent

Several organic solvents, namely acetonitrile, dichloromethane, chloroform, methanol, benzene, 1,2-dichloroethane, in addition to water, were examined for their ability to solvate the reaction constituents and to results in maximum absorbance for erythromycin ethylsuccinate – DDQ charge transfer complex. Acetonitrile was found to be the most suitable solvent e to achieve quantitative recovery of erythromycin ethylsuccinate complex (Table 1)

Table 1: Effect of different types of solvent on the determination of 50 μg.mL^{-1} erythromycin ethylsuccinate; 0.1% DDQ

Solvent	Absorbance
Acetonitrile	0.255
Dichloro methane	0.224
Chloroform	0.155
Methanol	0.116
Benzene	0.062
1,2-Dichloro ethane	0.021
Water	0.042

II. Simplex method

Simplex method used to optimize the required reagent volume, reaction time and the reaction temperature. After choosing the convenient boundary conditions for each of the mentioned control variables (Table 2).

Table 2: Boundary of Simplex indeprndent variables for determination of erythromycin ethylsuccinate

Variable	Range	Step size
Reagent volume (mL)	0.1-0.5	0.1
Reaction Time (min.)	0-20	5.0
Temperature (°C)	25-45	5.0

Four arbitrary experimental conditions should were carried out and when the results were entered to the Multi-simplex program points (1 to 4), the Simplex program starts to reflect the worst point through the centroid of other points to obtain a new point 5. An experiment was then performed utilizing the variable setting as a reflected point; because this value was better than that at point 1, the latter was rejected and replaced by point 5. A measured absorption signal was feeding again to the program and the process is repeated successively until the optimum conditions are obtained and were similar to those obtained by the univariate method. (Table 3).

Table 3: Multivariate experiments (Simplex optimization) of the experimental condition for the determination of 50 µg.mL^{-1} erythromycin ethylsuccinate

Exp. No.	Reagent volume (mL)	Reaction time (min.)	Temperatur (°C)	Abs.	Operation
1	0.1	0	25	0.215	
2	0.3	5	30	0.248	
3	0.4	15	35	0.224	
4	0.5	10	45	0.221	
5	0.5	15	45	0.212	R
6	0.2	5	30	0.251	C
7	0.1	5	25	0.210	R
8	0.4	10	40	0.234	C
10	0.1	0	30	0.211	R
11	0.3	10	35	0.249	C
12	0.1	0	25	0.215	R
13	0.2	10	35	0.242	R
14	0.2	0	30	0.253	C
15	0.2	0	25	0.255	E
16(12)	0.1	0	25	0.215	E
17(12)	0.1	0	25	0.215	C
18	0.3	0	25	0.252	C
19(12)	0.1	0	25	0.215	R
20(2)	0.3	5	30	0.248	C
21	0.1	5	30	0.198	R
22(12)	0.1	0	25	0.215	C
23	0.4	5	30	0.246	R

Calibration graph

Employing the optimum experimental conditions, a linear calibration graph for the determination of erythromycin ethylsuccinate, by charge-transfer complex formation with DDQ, was obtained (Figure 4), which shows that Beer's law was obey in the concentration range of (10 -110) µg.mL^{-1}, with a correlation coefficient (R= 0.9993) and detection limit of 0.351 µg.mL^{-1}.

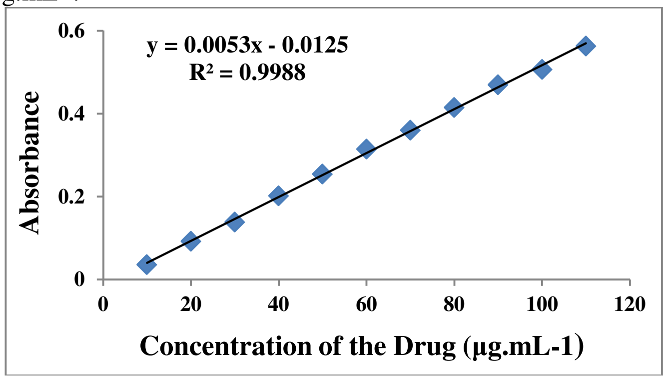


Figure 4: Calibration graph of erythromycin ethylsuccinate under optimum experimental conditions

Spectral characteristics of the proposed method

Under optimum experimental conditions of the proposed method, the regression plot showed linear dependence of absorbance signals on the concentrations of the studied drug in the range given. The regression equations, correlation coefficients, molar absorptivities, detection limits and sandell sensitivity in addition to other parameters are given in Table 4.

Table 4: Spectral characteristics and statistical data of the regression equation for determination of erythromycin ethylsuccinate via charge transfer formation

Parameter	Value
λ_{max} (nm)	585.5
Color	yellow
Linearity range ($\mu g.mL^{-1}$)	10.0-110.0
Molar absorptivity($L.mol^{-1}.cm^{-1}$)	4568.918
Regression equation	A = 0.0053 [Cim. $\mu g.mL^{-1}$] + 0.0125
Calibration Sensitivity	0.0053
Sandell's Sensitivity ($\mu g.cm^{-2}$)	188.679
Correlation of Linearity (R^2)	0.9988
Correlation coefficient (R)	0.9993
Detection limit ($\mu g.mL^{-1}$)	0.351

Stoichiometry of the complex

To establish structure of the complex formed between erythromycin ethylsuccinate and DDQ, slope analysis method Figures (5 and 6) and Job's method of continuous variation (Figure 7) have been. The results showed that the formed complex constructed with ratio of 1:1 (erythromycin ethylsuccinate: DDQ) .A proposed structure for the formed complex could be represented as in (Scheme 2). The possible mechanism for the reaction based on the formation of an original donor-acceptor (DA) complex through the interaction between tertiary amine group of erythromycin ethylsuccinate (electron donor) and DDQ (π - acceptor). On the other hand, the dissociation of DA complex is promoted by the high ionizing power of the solvent where a complete electron transfer from the donor to the acceptor moiety takes place, followed by formation of the DDQ radical anions as a predominant chromogen [25].

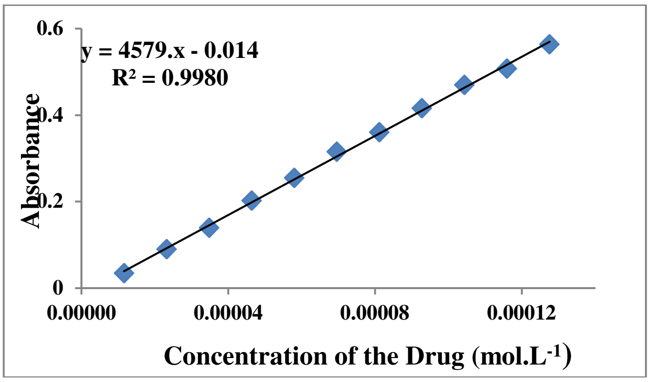


Figure 5: Results obtained for the slop ratio method, with variable concentrations of Erythromycin ethylsuccinate; ($1.762x10^{-4}$MDDQ)

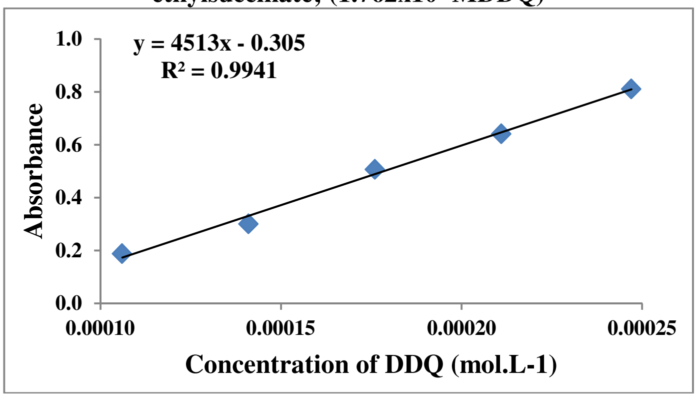


Figure 6: Results obtained for the slop ratio method, with variable concentrations of DDQ; ($1.160x10^{-4}$ M erythromycin ethylsuccinate)

Ratio = Slope R / Slope D =4513 / 4579 = 0.986 ≈1

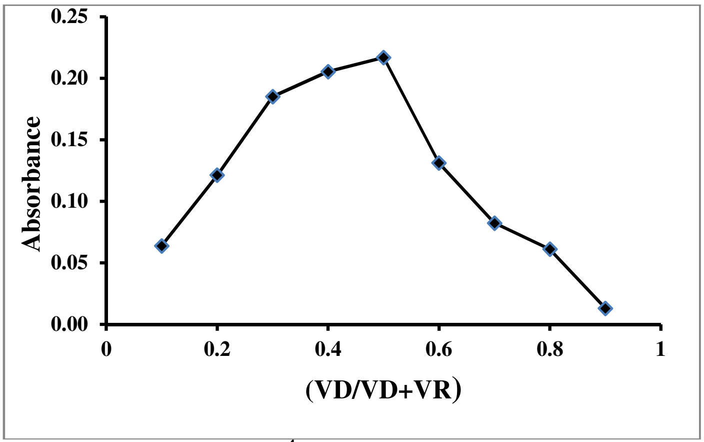
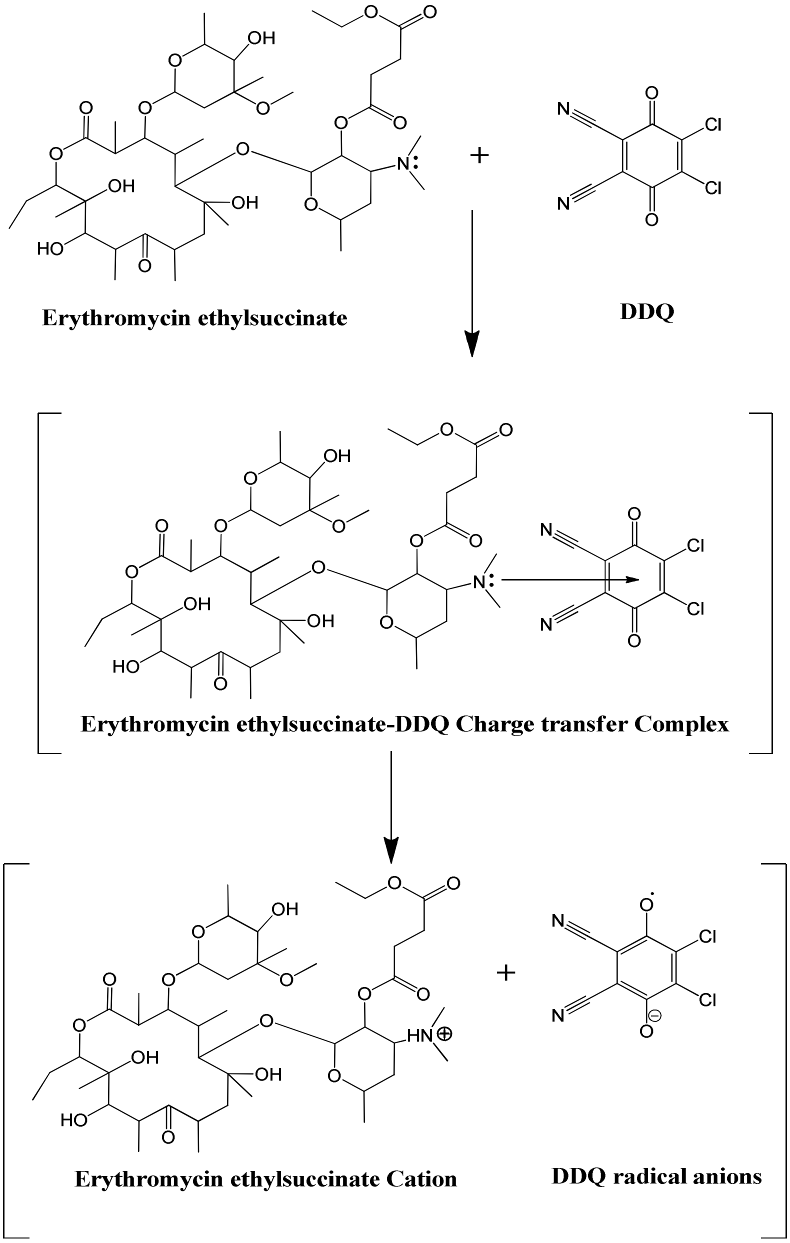


Figure 7: Continuous variation of 5.80 x10^{-4} M erythromycin ethylsuccinate; 5.80 x10^{-4} M DDQ



Scheme 2: The proposed structure of the formed complex illustrating the possible charge transition between donor and acceptor

Accuracy and precision
The accuracy and precision of the proposed method was checked by analyzing three replicates of three different concentration levels of the drug (within Beer's law range). The accuracy was determined by calculating the relative error percentage, while the precision was tested by calculating the percentage relative standard deviation (%RSD). The results indicated good accuracy with reasonable precision of the proposed method (Table 4).

The proposed method was advantageous when compared statistically with other methods found in the literature in having good sensitivity and the results are shown in Table 5.

Table 4: Evaluation of accuracy and precision for the determination of erythromycin ethylsuccinate

Conc. ((µg.mL^{-1})		Relative Error %	R.S.D.* %
Taken	Found*		
40	39.400	-1.500	1.635
70	69.570	-0.614	0.824
100	98.906	-1.694	1.367

*Average of three determinations.

Table 5: Analytical parameters for the analysis of erythromycin ethylsuccinate by the proposed and others methods

Ref. No.	methods	Linear range µg.mL^{-1}	ε L.mo^{-1}. cm^{-1}	Correlation Coefficient (R)	Recovery %	RSD%
23	Spectrophotometric Direct UV	3.0-15.0	37.43	0.9836-0.9892	97.6	0.48
	1st Derivative	3.0-15.0	44.03	0.9917-0.9967	106.5	0.65
37	Spectrophotometric Ion-Pair	2.0-61.0	-	-	98.4-103.6	1.4-4.4
6	High-pressure L.C.	60.0-120.0	-	-	99.9	Less than 1.0 %
38	Extraction	0.4.0-56.0	-	-		1.3
25	Charge transfer	1.724-129.3	8500		98.3	
39	Spectroflurimetric	0.0426-1.2	-	-	98.3-100.8	0.014-0.058
40	Charge transfer	1.0-80.0	9910	-	97	-
-	Proposed method	10.0-110	4568.918	0.9993	98.500-99.386	0.824-1.635

Interferences study
The results showed that no interferences were found in the presence of up to 500 µg.mL^{-1} of the studied excipients (lactose, sucrose, starch, glucose, magnesium stearate and sodium citrate) in the determination of cimetidine (Table 6).

Table 6: Percent recovery for 40 µg.mL^{-1}of erythromycin ethylsuccinate in the presence of 500 µg.mL^{-1}of excipients

Excipients	Erythromycin Ethylsuccinate Conc. Taken (40 µg.mL^{-1})	
	Conc. Found (µg.mL^{-1})	%Recovery
Lactose	40.012	100.030
Sucrose	40.104	100.260
Starch	39.746	99.3650
Glucose	39.672	99.180
Magnesium Stearate	39.818	99.545
Sodium Citrate	40.204	100.510

*Average of three determinations.

Analysis of dosage forms
The applicability of the proposed method for the determination of erythromycin ethylsuccinate in commercial dosage form was examined by analyzing of their content of the active ingredient by the proposed method (charge-transfer complex formation). The results given in Table 7, reveal that the recoveries were in the range of, reflecting high accuracy and precision of the proposed method as indicated by low percentage relative standard deviation value. The recommended method was statistically compared with other methods, no significant

differences were found between the calculated and theoretical values of t and F- test at 95% confidence limit (Table 8).

Table 7: Spectrophotometric determination of erythromycin ethylsuccinate in pharmaceutical preparations via charge-transfer complex formation with DDQ

Sample	Labeled amount (mg)	Found amount (mg)	Conc. taken ($\mu g.mL^{-1}$)	Conc.* Found ($\mu g.mL^{-1}$)	Recovery %	R.S.D* %
Erythrosam Erythromycin Ethyl Succinate **250mg/ Cap. SDI/Iraq**	250	252.263	40	40.362	100.905	1.105
		252.240	70	70.627	100.896	1.089
Erythronin Erythromycin Ethyl Succinate **250mg/ Cap. NDI/Iraq**	250	252.625	40	40.420	101.050	1.299
		252.55	70	70.713	101.020	0.899
Erythrodar Erythromycin Ethyl Succinate **250mg/ Cap. Jordan**	250	256.62	40	41.059	102.648	1.293
		256.350	70	71.778	102.540	0.928

*Average of three determinations.

Table 8: t- and F-values for analysis of erythromycin ethylsuccinate in pharmaceutical compounds(S.D.I)

Proposed Method,	T-Values[a]	F-values[b]	Other Methods			Ref. No.
			N	$\bar{x}$	S.D	
N=3 **S.D** = 0.434 **μ** = 10.112 **T**[c] = 0.447	0.369	10.426	(N=9)	9.800	1.400	37
	1.105	1.457	(N=8)	9.83	0.359	25
	2.241	9.400	(N=6)	9.700	0. 140	40

a- Theoretical values for t at 95% confidence limit were N=10(2.228), 9(2.262) and 7(2.365) respectively,
b-Theoretical values for F at 95%were N=(8,2)(19.372), N=(2,7)(4.738) and N = (2,5)(5.786) confidence limit respectively,
C- Theoretical values for t at 95% confidence limit were N=2(4.303).

Conclusions

The utility of DDQ reagent for the spectrophotometric determination of erythromycin ethylsuccinate. was established. The method based charge-transfer complex formation between the cited drug and DDQ as a chromogenic reagent. The proposed method was found to be accurate, simple and sensitive. It was satisfactorily applied to the determination of erythromycin ethylsuccinate in pharmaceutical product samples.

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