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SENSITIVE AND VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF PROTON PUMP INHIBITOR DEXLANSOPRAZOLE IN PURE FORM AND PHARMACEUTICAL FORMULATIONS USING ALIZARIN DERIVATIVES

El Sheikh Ragaa [1], Amin Alaa S [2], Gouda Ayman A [1,3]*, Negeda Ola S [1]

[1] Chemistry Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt

[2] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

[3] Faculty of Public Health and Health Informatics, Umm Al Qura University, Makkah, Saudi Arabia

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***Corresponding author**

E-mail: aymangouda77@gmail.com

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ABSTRACT

Two simple, sensitive, accurate, and precise spectrophotometric methods have been developed and validated for the determination of proton pump inhibitor dexlansoprazole (DXL) in pure form and pharmaceutical formulations. The method was based on the formation of charge transfer complex between DXL and quinalizarin (Quinz) and alizarin red S (ARS) as chromogenic reagents in methanol which showed an absorption maximum at 558 and 542 nm using Quinz and ARS, respectively. The optimization of the reaction conditions such as the type of solvent, reagent concentration and reaction time were investigated. Under the optimum conditions, beer's law is obeyed in the concentration ranges 1.0-12 and 1.0-16 µg mL^{-1} using Quinz and ARS, respectively with good correlation coefficient ($r^2 \geq 0.9994$) and with a relative standard deviation (RSD% ≤ 1.11). The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. The methods were successfully applied to the determination of DXL in its pharmaceutical formulations and the validity assesses by applying the standard addition technique. Results obtained by the proposed methods for the pure DXL and commercial tablets agreed well with those obtained by the reported method.

Keywords: Dexlansoprazole, Spectrophotometry, Quinalizarin, Alizarin red S, Charge transfer reaction, Dosage forms.

INTRODUCTION

Dexlansoprazole (DXL), Chemically is known as (R)-(+)2-([3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl]methylsulfinyl)-1H-benzimidazole (Figure 1). DXL is a new-generation proton pump inhibitor as an R-enantiomer of lansoprazole. DXL is used to treat gastroesophageal reflux disease and exhibiting high efficacy in the treatment of symptoms and lesions associated with erosive esophagitis caused by gastroesophageal reflux disease. DXL allow the esophagus to heal and prevent further damage to the esophagus. DXL works by decreasing the amount of acid made in the stomach. [1, 2]

Literature survey reveals that few methods were developed for determination of enantiomers of lansoprazole in the biological fluids using liquid chromatography. [3-7] Two methods were reported for determination of DXL using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [8] in human plasma and high-performance liquid chromatographic method [9] in in bulk and capsule dosage form. These methods require long and tedious pre-treatment of the samples and laborious clean up procedures prior to analysis.

A through literature search has revealed that only few spectrophotometric methods available for determination of DXL in pure and dosage forms. [10, 11] However, many of the above methods suffered from one or other disadvantage like poor sensitivity, require high cost solvents in addition to elaborate treatment, need tedious extraction procedures, measurements done at shorter wavelengths, heating or cooling step, use of expensive chemical and/or complicated experimental set-up.

Spectrophotometric technique, because of simplicity and low cost, sensitivity and selectivity, significant accuracy and precision and broad availability and applicability for pharmaceutical analysis. In the present work, we developed simple, sensitive, rapid, accurate and validated spectrophotometric method for the determination of DXL in pure and dosage forms. The proposed method involves the formation of charge transfer complex between DXL and two alizarin derivatives; quinalizarin (Quinz) and alizarin red S (ARS) as chromogenic reagents.

MATERIALS AND METHODS

Apparatus

All the absorption spectral measurements were made using Varian double beam UV-VIS spectrophotometer (Tokyo, Japan) equipped with 10 mm matched quartz cells.

Materials and Reagents

All employed chemicals and solvents (dimethyl sulfoxide, methanol, acetonitrile, acetone and ethanol) were of analytical-reagent grade and high-purified water was used throughout the study.

Pure DXL drug and pharmaceutical formulations

Pharmaceutical grade DXL was received from Delta Pharmaceutical Industries, Cairo, Egypt. The commercial pharmaceutical formulations (Doxirazol tablets (Hikma Pharmaceutical Co., Amman, Jordan), labeled to contain 30 and 60 mg DXL per tablet) were purchased from local market were subjected to the analytical procedure.

Stock standard Solutions

A standard stock solution of DXL containing 100 µg mL^{-1} was prepared by dissolving 10 mg of pure drug in 20 mL methanol and was further diluted to 100 mL with the same solvent to obtain the working concentration. The standard solution was kept in the refrigerator and was found to be stable for at least one week if they had been stored in a cool (< 25 ºC) and dark place.

Reagents

Quinalizarin 1,2,5,8-tetrahydroxy-anthraquinone (Quinz) and alizarin red S, 3,4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (ARS) were Sigma-Aldrich products and used without further purification. A stock solution 1.0 x 10^{-3} mol L^{-1} was prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of methanol, then completed to the mark with methanol in 100 mL volumetric flask. This solution was stable for one week.

General procedures

Aliquots of the standard working solution of DXL in the concentration ranges (1.0-12 µg mL^{-1}) and (1.0-16 µg mL^{-1}) using Quinz and ARS, respectively were transferred into a set of 10 mL volumetric flasks. To each flask 2.0 mL of (1.0 x 10^{-3} mol L^{-1}) Quinz or ARS solution was added. Then the mixture was shaken to promote the reaction and the volume was completed to the mark with methanol. The absorbance of the resulting solutions was measured at 558 and 542 nm using Quinz and ARS, respectively against a reagent blanks prepared simultaneously. The calibration graph was constructed by plotting the absorbance versus the final concentration of DXL. The corresponding regression equation was derived.

Assay procedure for tablets

The content of ten tablets each containing 30 and 60 mg DXL was finely powdered using an agate mortar and weighed accurately. An accurately weighed quantity of the powder equivalent to 50 mg DXL were transferred into 100 mL calibrated flask and dissolved in 25 mL methanol. The content of the flask was shaken and sonicated for about 10 min, mixed well and then filtered using Whatman No.42 filter paper. The first portion of the filtrate was rejected, and the solution was then completed to volume with methanol to prepare a stock solution of 500 µg mL^{-1}. This solution was further diluted with the same solvent as appropriate to obtain the working concentration ranges. Aliquots covering the working concentration ranges for each method were transferred into a series of 10 mL volumetric flasks and the proposed methods were applied. The nominal content of the tablets was determined using the corresponding regression equations or the calibration graphs.

Stoichiometric relationship

The stoichiometric ratios of the charge transfer complexes formed between DXL and reagents were determined by applying the continuous variation method attributable to Job [12] and modified by Vosburgh and Coober [13] at the optimum wavelengths of maximum absorbance. Job's method of continuous variation was employed, a 1.0 × 10^{-3} mol L^{-1} standard solution of DXL and 1.0×10^{-3} mol L^{-1} solution of reagent were used. A series of solution were prepared in which the total volume of drug and reagent was kept at 2.0 mL. The reagents were mixed in various proportions with drug and diluted to volume in a 10-mL calibrated flask with methanol following the above-mentioned procedures

RESULTS AND DISCUSSION

Absorption spectra

Solutions of reagents in methanol exhibits an absorption bands with a well-defined maximum at 490 and 422 nm for Quinz and ARS, respectively, while DXL solution in methanol showed no absorption in the 400-700 nm range. At optimum conditions, the addition of DXL to reagent solution in methanol caused an immediate change in the absorption spectrum with the appearance of a new characteristic band for the radical anion (absorbing species) with maximum absorption at 558 and 542 nm using Quinz and ARS, respectively (Figures 2, 3). The high difference between maximum wavelength of the reagent and the charge transfer product absorption bands ~ 68 and 120 nm using Quinz and ARS, respectively, allowed the measurement of the charge transfer products with only a small contribution of the reagents that was added in excess in the medium.

Optimization of the experimental conditions

The effect of the solvent nature

Charge transfer reaction was tested in DMSO, methanol, acetonitrile, acetone and ethanol solvents. Although the highest dielectric constant of DMSO and acetonitrile, best sensitivity was achieved with methanol, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Then, methanol was chosen for further experiments (Figure 4).

Effect of the reagent concentration

In order to achieve this objective, an experiment was performed when various volumes of reagents solutions (1.0 x 10^{-3} mol L^{-1}) in the range of 0.2-3.0 mL were added to a fixed DXL concentration (12 µg mL^{-1}) (Figure 5). The results are shown that 2.0 mL of (1.0 x 10^{-3} mol L^{-1}) Quinz or ARS reagent solution was enough to develop the color to its full intensity and gave the highest and constant absorbance values.

Effect of the reaction time and temperature

The optimum reaction time was determined by following the color development at laboratory ambient temperature (25±2°C). Complete color development was attained after 2.0 min for DXL with both reagents. On raising the temperature, the absorbance of the charge transfer complex was decrease with a hypochromic shift, until decayed at 50 °C.

Sequence of additions

The most favorable sequence of addition is " DXL -reagent-solvent" for complete color development, highest absorbance and stability at the recommended wavelength. Other sequences needed longer time in addition to lower stability. The complexes with this sequence remain stable for at least 12 h.

Stoichiometric ratio

The molar ratio of DXL to reagent (Quinz or ARS) in the charge transfer complex was determined by Job's method [12] of continuous variations, keeping the sum of the molar concentrations of the investigated DXL and reagent fixed. As shown in Figure 6, the molar ratio which gave maximum absorbance was found to be (1:1) (DXL: reagent).

According to literature review in molecular charge-transfer complexes are formed in non-polar solvents while radical anion species are predominant in polar solvents. [14-19] Also, it is believed that the addition of basic compounds that contains a lone pair of electrons, such as DXL, results in the formation of charge-transfer complexes of n-π type. This kind of complexes can be considered an intermediate molecular-association compound that forms a corresponding radical anion in polar solvents. In this case, radical anions results from the total transfer of charge (Scheme 1).

Validation of the proposed methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to International Conference on Harmonization (ICH) [20] guidelines.

Linearity, detection, and quantification limits

By using the above procedures, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response in the two methods to the concentration of DXL over the ranges cited in Table 1. Linear regression analysis of the data gave the following equations. For Quinz, A = - 0.0051+ 0.0515C, r^2= 0.9998 and A= 0.001 + 0.0316C, r^2= 0.9994 using ARS, where A is the absorbance, C is the concentration of DXL (µg mL^{-1}), and r^2 is the correlation coefficient.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH. [20] The results are shown in Table 1. The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected, and the results are also summarized in Table 1. LOQ and LOD were calculated according to the following equations:

LOQ=10s /b

LOD=3.3s /b

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte. b: is the slope of the calibration curve.

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, intraday and inter-day determination of DXL at three different concentrations for each method were prepared and analyzed. The intraday studies were performed in one day and inter-day studies in five days (for each level n = 6). The accuracy and precisions expressed as percent relative error (RE%) and relative standard deviation (RSD%) values, respectively and found to be within -1.0-0.60% and 0.50–1.60%, respectively for intraday analysis and within -1.10-0.30% and 0.35-1.30%, respectively for interday analysis (Table 2). The data proved good accuracy precision for the developed methods.

Ruggedness and robustness

The ruggedness of the proposed method was assessed by applying the procedures using two different instruments in two different laboratories at different times and two different analysts. Results obtained from laboratory-to-laboratory and analyst-to- analyst variation were found to be reproducible because the RSD did not exceed 2.0%.

Robustness of the proposed method was assessed by evaluating the influence of small variation of experimental variables, i.e., concentrations of reagent and reaction time, on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. The recovery values ± %RSD were 98.80 - 100.80 ± 0.40 - 1.80% for Quinz and 99.10 - 101.20 ± 0.50 - 1.70% for ARS. This indicated the reliability of the proposed method during its routine application for the analysis of DXL.

Specificity and effect of excipients

The specificity of the proposed method was investigated by observing any interference encountered from the common capsules excipients. The standard addition method was applied by adding known amounts of pure DXL to a previously analyzed tablet solution. The recovery of the added DXL was calculated by comparing the concentration of the spiked mixtures with that of the previously found value. As can be seen from Table 3, satisfactory results better than the reported spectrophotometric methods were obtained. The high recovery values of the proposed methods indicated that the excipients did not interfere with the proposed methods indicating the high selectivity of the proposed methods.

Analysis of the pharmaceutical preparations

The proposed method was applied to the determination of DXL in pharmaceutical formulations (Doxirazol tablets, 30 mg DXL per tablet) and (Doxirazol tablets, 60 mg DXL per tablet). The method was tested for linearity, specificity, accuracy, repeatability, and precision according to ICH recommendations. The results of the proposed methods were statistically compared with those obtained using the reference methods. [10] Recovery ± SD values were obtained. Statistical analysis of the results, using Student's t-test and the variance ratio F-test at 95% confidence level revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 4). [21] It is evident from these results that the proposed methods is applicable to the analysis of DXL in its dosage forms with comparable analytical performance.

Table 1: Analytical parameters for the determination of DXL by the proposed methods.

Parameters	Quinz	ARS
λ_{max}	558	542
Conc. Range (µg mL^{-1})	1.0-12	1.0-16
Molar absorptivity ε, (L mol^{-1} cm^{-1}) x 10^4	0.966	1.1854
Sandel sensitivity, (µg cm^{-2})	38.24	31.16
Regression equation [a]		
Intercept (a)	-0.0051	0.001
Slope (b)	0.0515	0.0316
Correlation coefficient (*r*)	0.9998	0.9994
Mean ± SD [b]	99.10 ± 1.10	99.40 ± 0.90
Relative standard deviation; RSD%	1.11	0.91
Relative error, RE%	1.15	0.96
Variance	1.21	0.81
Detection limits, (LOD) (µg mL^{-1})	0.29	0.26
Quantification limits, (LOQ) (µg mL^{-1})	0.97	0.87
Calculated t-value [c]	0.77	0.30
Calculated F-value [c]	2.47	1.65

[a] $A=a + bC$, where C is the concentration in (µg mL^{-1}), A is the absorbance, a is the intercept and b is the slope.
[b] Mean of six determinations.
[c] Theoretical values of *t* (2.57) and *F* (5.05) for five degrees of freedom and 95 % confidence level at p = 0.05.

Table 2: Evaluation of intra-day and inter-day precision and accuracy for DXL obtained by the proposed methods.

Method	Added (µg mL^{-1})	Intra-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
Quinz	4.0	99.20	0.70	-0.80	3.97 ± 0.03
	8.0	99.50	0.90	-0.50	7.96 ± 0.08
	12	100.60	1.20	0.60	12.07 ± 0.15
ARS	5.0	99.00	0.50	-1.0	4.95 ± 0.03
	10	99.60	1.40	-0.40	9.96 ± 0.15
	15	99.10	1.60	-0.90	14.87 ± 0.25
		Inter-day			
Quinz	4.0	98.90	0.35	-1.10	3.96 ± 0.015
	8.0	99.70	0.70	-0.30	7.98 ± 0.06
	12	99.50	1.10	-0.50	11.94 ± 0.14
ARS	5.0	99.10	0.50	-0.90	4.96 ± 0.026
	10	100.30	0.85	0.30	10.03 ± 0.09
	15	100.20	1.30	0.20	15.03 ± 0.21

[a] Mean of six determination, RSD%, percentage relative standard deviation; R.E%, percentage relative error.
[b] Mean ± standard error.

Table 3: Application of the standard addition technique for the determination of DXL in pharmaceutical preparations using the proposed methods

Sample	Taken (µg mL^{-1})	Added (µg mL^{-1})	Doxirazol tablets (30 mg DXL /tab.)		Doxirazol tablets (60 mg DXL /tab.)	
			Total found (µg mL^{-1})	Recovery %	Total found (µg mL^{-1})	Recovery %
Quinz	4.0	2.0	5.952	99.20	5.946	99.10
		4.0	8.04	100.50	7.96	99.50
		6.0	9.96	99.60	9.98	99.80
Mean ± SD				99.77 ± 0.67		99.47 ± 0.35
V [b]				0.44		0.123
RSD%				0.67		0.35
SE				0.38		0.20
ARS	6.0	3.0		99.40		99.30
		6.0		99.00		99.60
		9.0		99.20		99.70
Mean ± SD				99.20 ± 0.20		99.53 ± 0.21
V [b]				0.04		0.04
RSD%				0.20		0.21
SE				0.12		0.12

[a] The average of at least three determinations.
[b] V= variance; RSD%= percentage relative standard deviation; SE= standard error.

Table 4: Application of the proposed method to the determination of DXL in dosage forms

Samples	References method [10]	Proposed methods	
		Quinz	ARS
Doxirazol tablets (30 mg DXL /tab.)			
X ± SD [a]	99.40 ± 0.38	99.60 ± 0.50	99.10 ± 0.47
t-value (2.57) [b]		0.71	1.10
F-value (5.05) [b]		1.73	1.53
Doxirazol tablets (60 mg DXL /tab.)			
X ± SD [a]	99.20 ± 0.40	99.40 ± 0.48	99.50 ± 0.60
t-value (2.57) [b]		0.72	0.93
F-value (5.05) [b]		1.44	2.25

[a] Average of six determinations.

[b] Theoretical values of t *and F* for five degrees of freedom and 95 % confidence level at p = 0.05.

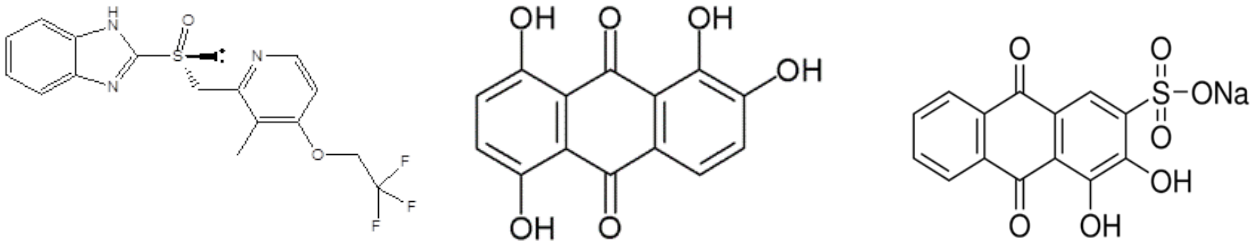


Figure 1: The chemical structures of DXL and alizarin reagents

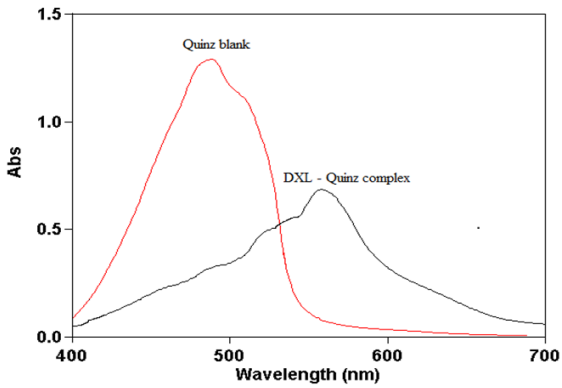


Figure 2: Absorption spectra of charge transfer complexes of 15 µg mL^{-1} DXL with (1.0 x 10^{-3} mol L^{-1}) Quinz in methanol solvent obtained against Quinz reagent blank solution prepared in the same solvent.

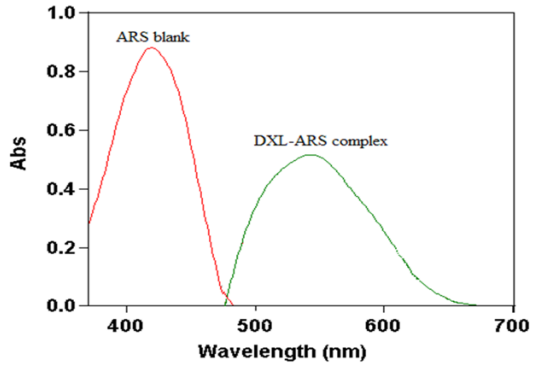


Figure 3: Absorption spectra of charge transfer complexes of 12 µg mL^{-1} DXL with (1.0 x 10^{-3} mol L^{-1}) ARS in methanol solvent obtained against ARS reagent blank solution prepared in the same solvent

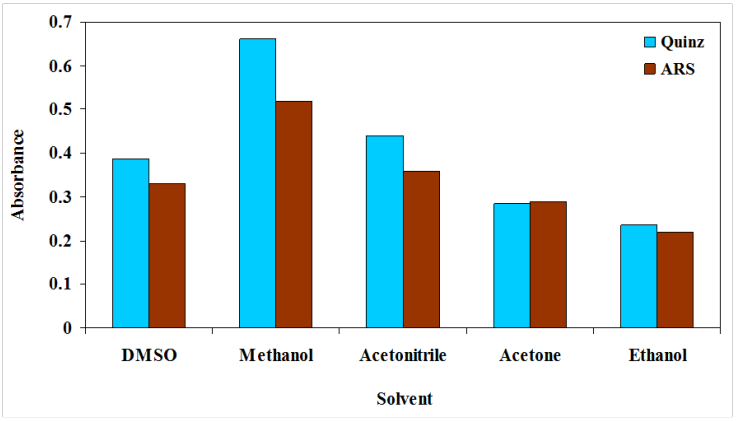


Figure 4: Effect of different solvents on the charge transfer complex of DXL-reagent solution obtained against (1.0 x 10^{-3} mol L^{-1}) reagent solution prepared in different solvents. DXL concentration = 12 µg mL^{-1}.

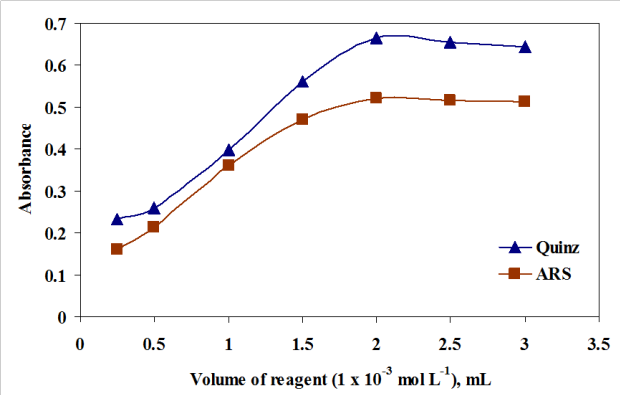


Figure 5: Effect of (1.0 x 10^{-3} mol L^{-1}) reagent volume on the absorbance of charge transfer complex. DXL concentration = 12 µg mL^{-1}

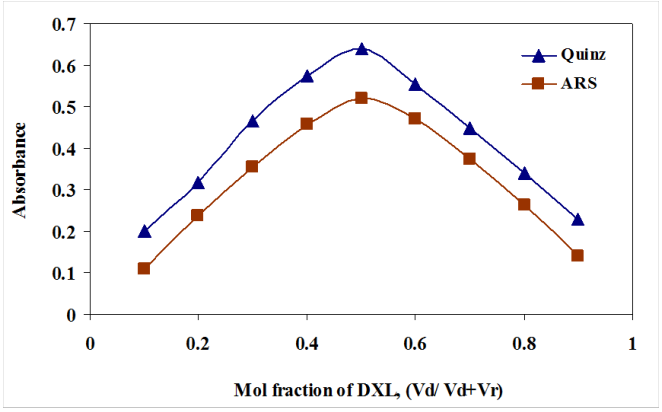
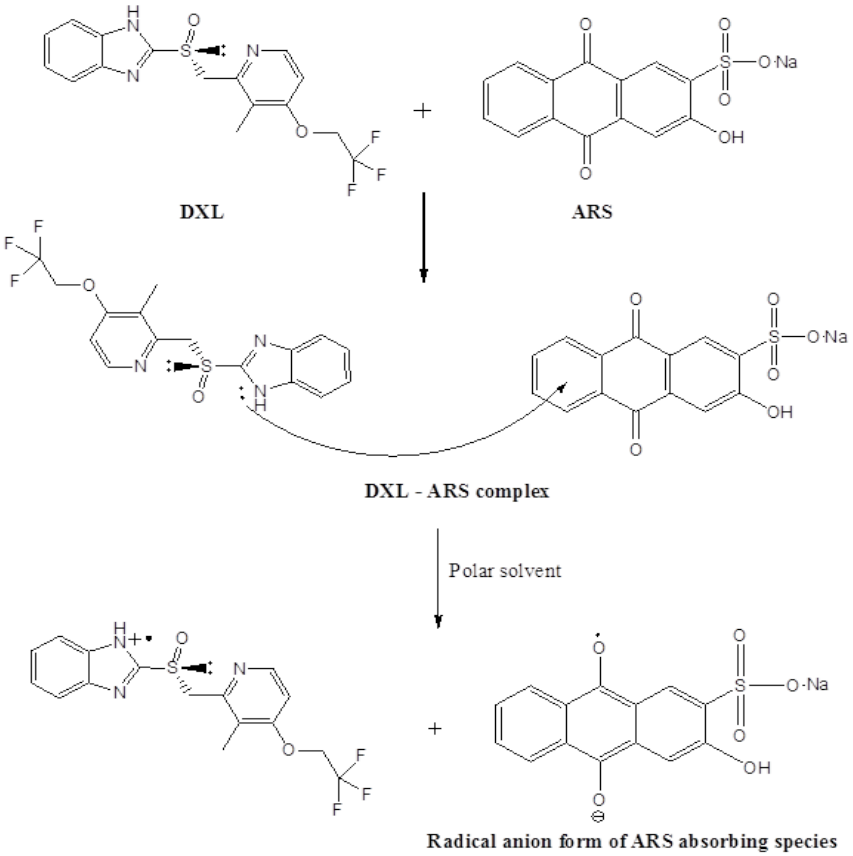


Figure 6: Application of Job's method to the reaction between reagents and DXL at optimum wavelength



Scheme 1: Possible mechanism of radical anion formation from ARS and DXL reaction

CONCLUSION

The developed two methods are simple and rapid, sensitive, accurate, robust, and economic. It does not require extraction, heating, or pH adjustment. The chromophore formed is quite stable. These characteristics make the proposed methods very suitable for routine analysis of DXL in quality control laboratories.

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