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Spectrophotometric Determination of Doxycycline Hyclate in Pharmaceutical Preparations Using Oxidative coupling reaction

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method for determination of microgram amounts of Doxycycline Hyclate in aqueous solution is described. The method is based on the Oxidative coupling reaction between Doxycycline Hyclate and 4-(Methyl amino) phenol sulfate (metol) in the presence of sodium persulphate and sodium hydroxide to form an intense green colored product with maximum absorption at 626 nm. Beer's law is obeyed over the concentration range of(2 – 44) μg.cm^{-1} with molar absorptivity of 1.6669 x10^{4} l.mol^{-1}.cm^{-1} and Sandell`s sensitivity of 0.030 μg.cm^{-2}.The detection limit was 0.581 μg.cm^{-1}. The optimum conditions for all colour development are described and the proposed method has been successfully applied for the determination of Doxycycline Hyclate in bulk drug and pharmaceutical Preparations (Doxydar and doxycycline). The common excipients and additives did not interfere in this method.

Keywords: Doxycycline Hyclate, Oxidative coupling Spectrophotometric determination.

INTRODUCTION

Doxycycline Hyclate (Doxy), chemically known as (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene- 2-carboxamide hydrochloride hemi ethanol hemihydrate figure 1.

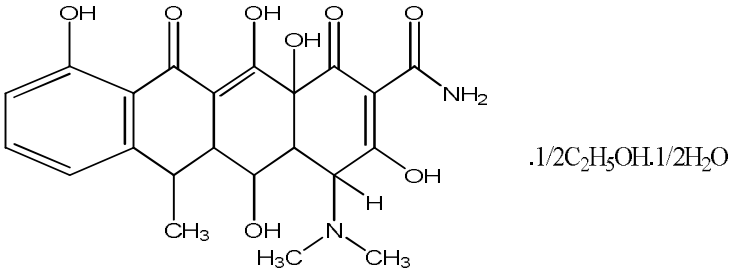

Figure1. The chemical structure of Doxycycline Hyclate (Doxy)

It is one of the tetracycline derivatives is a broad spectrum antibiotic, with activity against a wide range of gram-positive and gram-negative bacteria [1]. It is a bacteriostatic inhibiting the bacterial protein synthesis due to the disruption of transfer RNA and messenger RNA at the ribosomal sites [2]. Doxy is frequently used to treat chronic prostatitis, sinusitis, syphilis, chlamydia, pelvic inflammatory disease, acne, rosacea, and rickettsial infections [1]. It is also used for malaria treatment. Doxy is widely used in medicine and veterinary practice. As a result, Doxy residue can occur in food products of animal origin [3]. The drug is official in the British Pharmacopoeia (BP)[4], the United States Pharmacopoeia (USP)[5]and Merck index [6].which describes HPLC methods for the determination of Doxy either in raw material or in pharmaceutical formulations. Chromatographic techniques are the most widely used ones. Although these procedures are specific, most of the described methods are time consuming and require multistage extraction fluorimetry[7],Phosphorimetry[8],Liquid chromatography[9- 13],thinlayer chromatography[14], sequential injection chromatography[15], ion selective electrodes based potentiometry[16] and capillary electrophoresis[17]. Few visible spectrophotometric methods based on the different reaction mechanisms are found in the literature for the assay of doxy. These include FIA-spectrophotometry with copper carbonate [18]. chloramine-T[19] and 4aminophenazone/potassium hexacyanoferrate(III)[20] and also based on colour reactions with thorium (IV)[21], sodium cobalt nitrite[22] and uranyl acetate[23]. Besides, kinetic spectrophotometry using Cu(II)/H_2O_2[24] and multivariate calibration method[25] have also been reported by different authors. A complexometric titration method has been reported for the determination of Doxy .The method employs spectrophotometric titration of doxy with Mg^{2+} and Ca^{2+} in aqueous buffer. The titrations were performed at fixed pH values the consistent sets of UV-Visible absorption and fluorescence spectra were recorded at various times but the method utilized buffers, pH is very crucial and a sophisticated instrument to measure fluorescence response is required. In this paper described a newly, simple, speed and sensitive procedure for the determined of micrograms amounts of Doxycycline Hyclate (doxy) depending on the oxidative coupling reaction (reaction to the color generator). and through its reaction reagent Para -(Methyl amino) phenol sulfate(metol) in the presence of sodium persulphate as oxidant in the midst of a strong base and study the optimum conditions for this reaction .The application of the method on some different types of pharmaceutical preparations which is containing (Doxy) compound was obtained by different doses and forms with high accuracy and precision.

MATERIALS AND METHODS

Apparatus

- All spectral and absorbance measurements were carried out on applied UV-Visible 160 digital double - beam recording spectrometer.
- pH meter , Jenway 3020 .
- Heating-cooling water bath (Haake, Fe3).
- Sensitive balance (Sartorius BL 210S).

Reagents: All Chemicals used were a high degree of purity they were prepare by the following -

1. Doxycycline Hyclate standard powder was provided from the state company for drug industries and medical appliances Samara –Iraq (SDI). The standard stock solution of (Doxy) at a concentration (500) $\mu g.cm^{-1}$ by dissolving (0.05)gm of pure material in(100)ml Distilled water. This solution is stable for at least a month after saving well away from the light.
2. Sodium persulphate (0.01) M: - it was provided from (BDH Chemicals Ltd, Laboratory reagent) company by dissolving (0.238) gm of pure material in (100) ml Distilled water.
3. Sodium hydroxide (1M):- it was provided from merck company and the purity was 98%.it was prepare by dissolving (4) gm in (100) ml Distilled water.
4. Para-(Methyl amino) phenol sulfate (metol) (0.005) M: - it was provided from (BDH Chemicals Ltd, Laboratory reagent) company by dissolving (0.030) gm of pure material in(50 ml) ethanol from(BDH Chemicals Ltd,%99.9) .

Recommended Procedure: In a series of volumetric flasks of 25-ml, aliquots of standard solutions of, doxycycline hyclate with concentrations of (2-44) $\mu g.cm^{-1}$, respectively in final volume were added separately, followed by addition of (1ml) Para -(Methyl amino) phenol sulfate(metol) (0.005) M and(2 ml) of Sodium persulphate (0.01) M, and addition after that(1ml)of sodium hydroxide (1 M). The contents were diluted to the mark with distilled water, the solutions were heated for 20 min in a water bath adjusted at 45°C and the absorbance was measured at (626 nm), respectively against reagent blank and a calibration curve was constructed.

Procedure for Assay of doxycycline hyclate in Pharmaceutical Preparations: A number of preparations doxycycline hyclate containing (Doxy) as active ingredient were taken and it It included the following -

1. DuraDox capsules (100) mg - they were provided from. Julphar company (U.A.E) which were containing (100) mg (Doxy).
2. Vibramycin tablets (100) mg - they were provided from Pfizer PGM,France company, which were containing (100)mg of doxycycline monohydrate.
3. BIDOX Doxycycline capsules (100) mg - they were provided from Ajanta pharma limited company,India which were containing (100)mg (Doxy).
4. Doxydar capsules (100) mg - they were provided from Dar Al Dawa company, Jordan which were containing (100) mg (Doxy).
5. doxycycline capsules (100)mg - they were provided from Actavis,Bamstaple company, UK, which were containing (100)mg (Doxy).

Procedure for capsules [4]: Twenty capsules were weighed and finely mixed from each type of capsules separately. An accurately weighed portion of the powder equivalent to(0.05)gm of (Doxy) Which depends on the type of capsules that be used .It was dissolved in distilled water and transferred into a 100mL volumetric flask and diluted to 100mL with same solvent The solution was filtered into a 100mL volumetric flask, the residue was washed and diluted to volume with the same solvent to obtain 500 ppm of (Doxy) . We then take the suitable amount of each record solution and treated in the same conditions that were used in the based way of working was to find a concentration depending on a calibration curve.

Procedure for tablets [5]: Twenty tablets were weighed and finely powdered from type of tablets. An accurately weighed portion of the powder equivalent to (0.05) gm of (Doxy).it was dissolved in (5 ml) of distilled water and (20 ml) of hydrochloric acid (0.1M) with heating and after that filtering to separate the non-dissolved components. It was transferred into a 100-ml calibrated flask and diluted to the final volume with distilled water. Then we calculated the concentration depending on the standard calibration curve.

RESULTS AND DISCUSSION

Study the optimum conditions for reaction: - Different conditions were studied which are affecting on the intensity the absorption of the dye formed so as to in order to improve.

1. Effect of Reagent volume: The effect of Reagent volume on the intensity absorption were studied .it was taken from (0.5 – 4) ml of reagent at concentration (0.005M) with Presence (2ml) of oxidizing agent and (1ml) from basic solution. It was found that (1 ml) is the best volume of the reagent that gives the highest absorption, which was used in the following experiments.

2. Effect of oxidizing agent volume: The effect of oxidizing agent volume on the intensity absorption were studied. .it was taken from (0.5 – 6) ml of Sodium persulphate at concentration (0.01M) with Presence (1ml) of the reagent and (1ml) from basic solution. It was found that (2 mL) is the best volume of the oxidizing agent that gives the highest absorption, which was used in the following experiments.

3. Effect of base: It was found that the presence of base led to increase the intensity of the produced product, therefore some bases such as NaOH, NH_4OH, KOH and some basic salt like Na_2CO_3 and CH_3COONa are examined. It was found that all these bases gave the absorbance the color product, so; NaOH was selected which was found that (1 ml) of this base give high sensitivity which selected in subsequent experiments.

4. Effect of Order of Addition: It was found that the best order of addition that gives the highest absorption(R+D+O+B) where (R= Reagent, D=drug substance, O= oxidizing agent and A=basic solution) which selected in subsequent experiments.

5. Effect of Temperature: The resulting product of the proposed method were studied at different temperatures. The results indicate that the absorbance values increased in the temperature range (0-80ºC), whereas, at higher temperatures the absorbance value increase, indicating that stability of the product was increasing by heating. The coloured product was stable at temperature (45ºC) which was giving the highest absorbance. This temperature was selected in this method and using in subsequent experiments.

6. Effect of Reaction Time: The colour intensity reached its maximum after the drug (doxy) had been reacted immediately with the Reagent in the presence of sodium periodate and basic solution became stable after 20 minutes with the heating by a water bath adjusted at 45°C, therefore 20 minutes development time was selected as optimum in the general procedure. The colour obtained was stable for at 140 minutes.

7. Absorption Spectra: The spectral scan was conducted to obtain the Greater wavelength absorption of resulting compound resulting after installing the optimum conditions for reaction.a gainst blank solution that was containing the Reagent, oxidizing agent and the using base. Figure.2 shows maximum absorption at 626 nm where (A) spectrum represents compound product from the reaction and (B) is giving the spectum of blank.

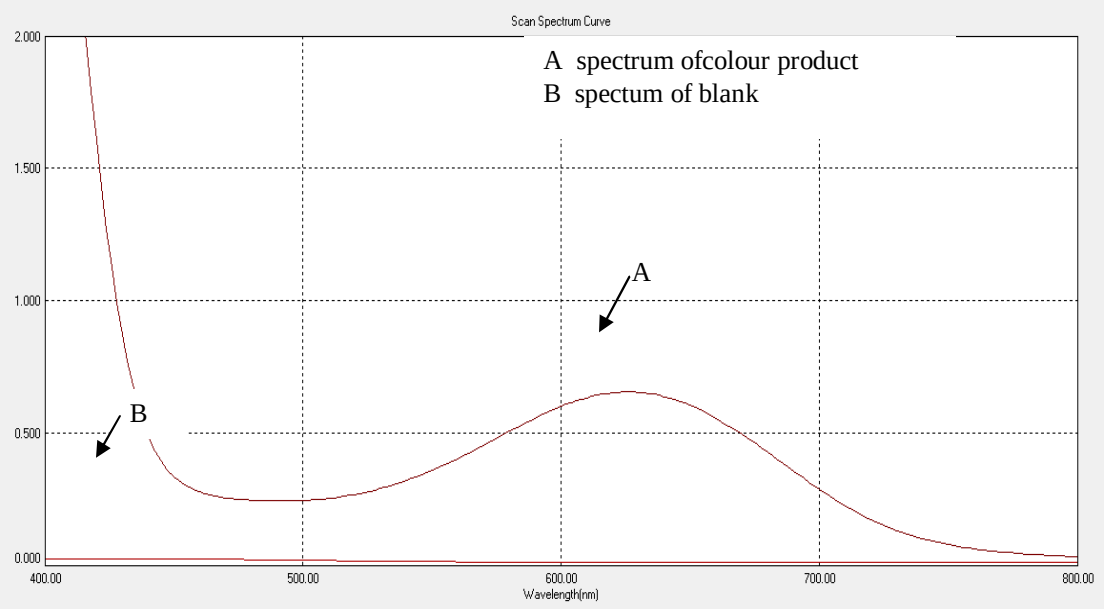

Figure 2. The spectra of green product formed (A) and of the blank (B).

8. Calibration curve: Employing the conditions described in the procedure, a linear calibration curve for doxycycline hyclate is obtained (figure 3), which shows that Beer's law is obeyed over the concentration range of (2 - 44) $\mu g.cm^{-1}$ with correlation coefficient of 0.9996 and an intercept was 0.0060 and the slope

of curve was 0.0325.The conditional molar absorptivity of the red product formed was found to be 1.6669 x10^4 L.mol^{-1}.cm^{-1}. And Sandell's sensitivity was 0.030 (µg.cm $^{-2}$).The detection limit (LOD) was 0.581 µg.ml^{-1} .

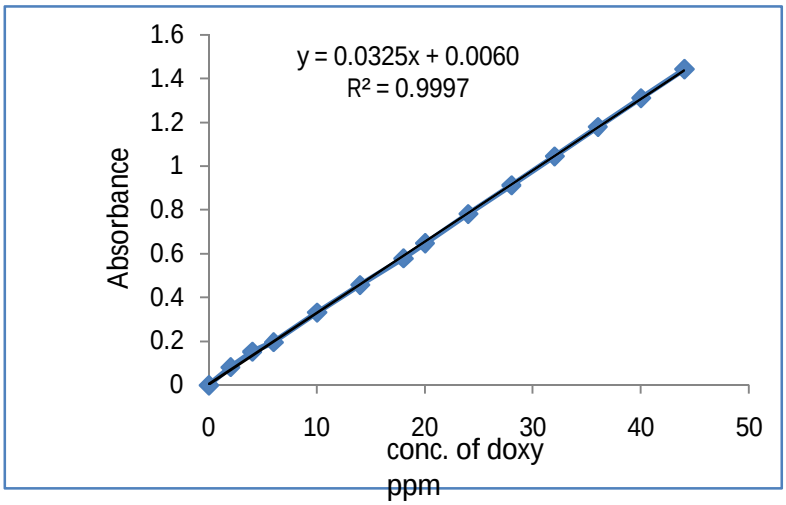

Figure 3. The Calibration curve of (DOXY)

9. Accuracy and precision: The accuracy and precision of the method were checked by determining Doxycycline Hyclate at three different concentrations. The results represented in table 1 indicate that the method is satisfactory and have good accuracy and precision (For five replicates of each concentration of Doxy).

Table 1. Accuracy and precision of the proposed method.

No	Conc. Of Doxy (µg/ml)		Error%	Recovery%	R.S.D □ %
	Present	Found			
1	6	5.920	- 1.333	98.667	1.042
2	18	18.091	+ 0.505	100.505	0.882
3	30	30.177	+ 0.590	100.590	0.692

10. Stoichiometry of reaction: The stoichiometry of the reaction between doxycycline hyclate and the R reagent was investigated using Job's method and mole ratio method; the results obtained (figure 4) show that 1:1 drug to reagent complex was formed at 626nm. The product formed was water soluble, the stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of Doxycycline Hyclate and the reagent with that of solution containing the optimum amount (1ml of 9.74×10^{-4} M).and other solution reagent solution at five times the concentration of the original concentration. The average conditional stability constant of the colour product in water under the described experimental conditions was 6.34×10^5 l^2.mol^{-2}.

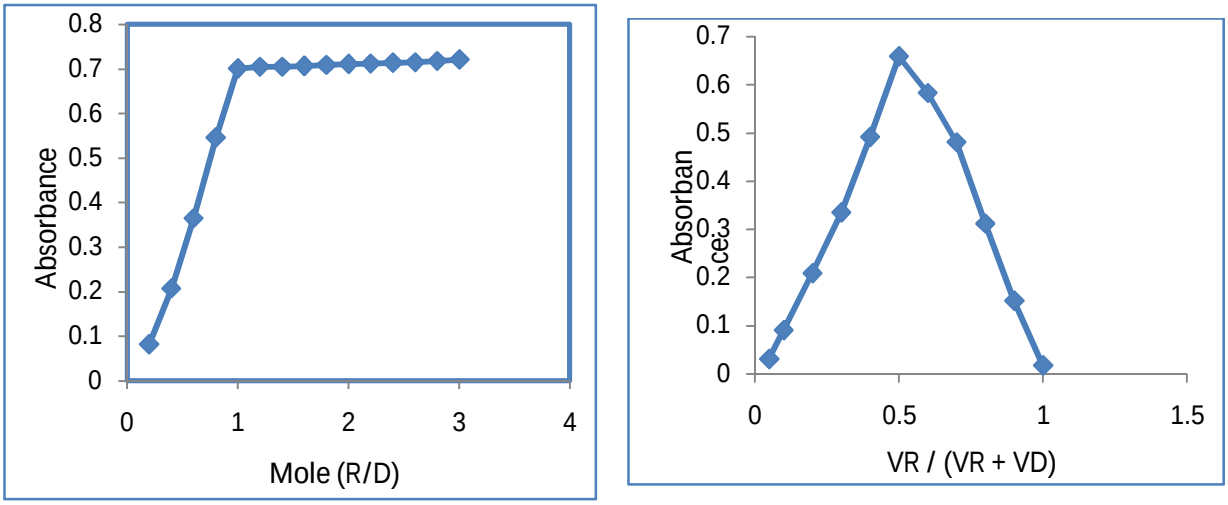

Figure 4 .Mole ratio and continuous variation plots for reaction of (doxy)with reagent in the presence of NaS_2O_8 and NaOH**.**

The formation of the colour product between (Doxy) and reagent in the presence of NaS_2O_8 and NaOH was suggested at the scheme of reaction probably occurs as the following equation[27] figure 5.

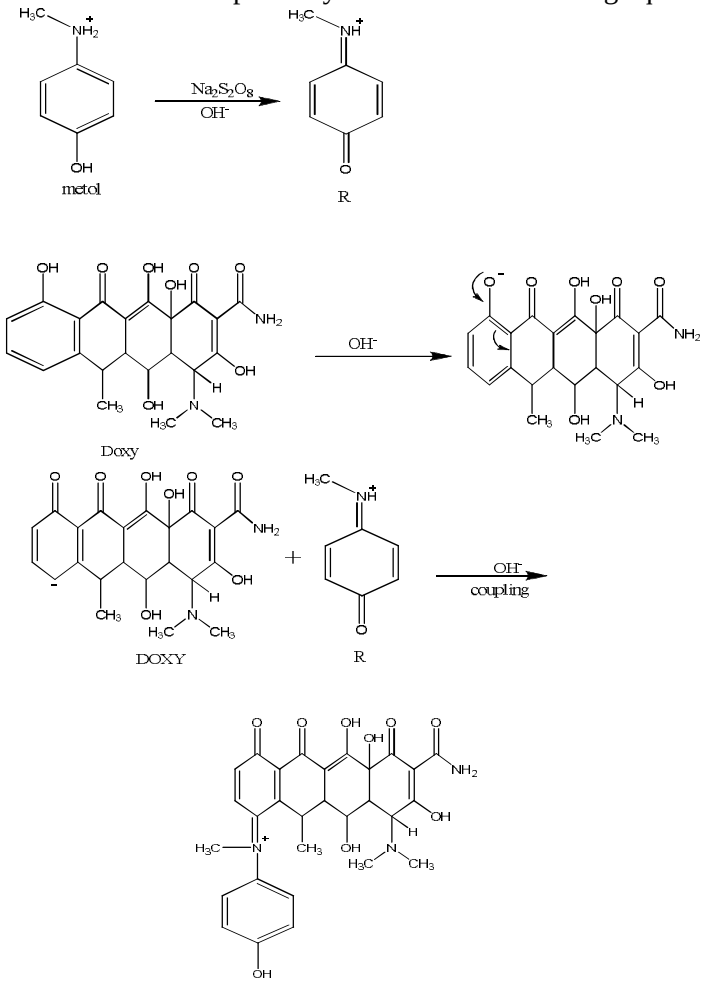

Figure 5. Scheme of the oxidative coupling reaction.

11. Effect of organic solvents: The spectrophotometric characteristics of the colour product in various organic solvents are given in table 2. Water is shown to be a good medium from the point view of sensitivity and economy. (Solvent used in dilution to the mark of (25ml) conical flask).

Table 2.Spectrophotometric characteristics of the colour product in various organic solvents

Solvent	**λ_{max} ,nm**	**ε ,L.mol^{-1}.cm^{-1}**
Acetone	580	3.176×10^3
chloroform		Two layers
2- propanol	522	2.546×10^3
Acetic acid	460	1.321×10^3
Dimethyl sulphoxide	524	5.821×10^3
CCl_4		Two layers
Dioxane	476	2.970×10^3
Dimethyl formamide	530	5.732×10^3
Ethanol	494	6.142×10^3
Benzene		Two layers
Methanol	442	6.323×10^3
Teri butyl alcohol		Two layers
Formic acid		Turbid
Pyridine		Turbid
Di ethyl ether	476	1.428×10^3

12. Interference: The excipients studied were - lactose, talc, starch**,** Acacia, Sucrose, Glucose, magnesium stearate, polyvinylpirrolidone (PVP), Benzoic acid and Cross povidone. For this study, solution was containing (Doxy) and each one of the excipients was taken separately in concentrations ten-times greater than that of (Doxy) were analyzed under the same procedure in the Calibration curve. Level of interference was considered to be acceptable if the error was not higher than ± 2% relative to the expected No interferences were observed in the determination of (Doxy) in the presence of the excipients studied(Average of three determinations) presented in Table 4.

Table4.Determination of (20ppm) Doxycycline Hyclate in the presence of excipients.

Interference	**% Error**	**% Recovery**
Lactose	- 1.340	98.660
Talc	- 0.595	99.405
Starch	- 1.050	98.950
Acacia	+ 0.660	100.66
Sucrose	- 0.440	99.560

Glucose	- 0.550	99.450
magnesium stearate	- 0.250	99.750
PVP	+ 0.545	100.545
Benzoic acid	- 1.010	98.990
Cross povidone	- 0.615	99.385

13. Evaluation of the Proposed Method: For evaluating the competence and the success of the proposed method the results obtained were compared with those obtained by standard BP method for same pharmaceutical preparations of (Doxy). The results obtained by the two different methods table 5 were statistically compared using the t-test and variance ratio F-test at 95% confidence level .the t-test value was(0.750) .the theoretical value was(2.29) for six samples.and the F-test value was(0.148) .the theoretical value was(5.05) for six samples. The calculated t- and F-values did not exceed the theoretical values which indicated that there was no significant difference between either methods in accuracy and precision in the determination of (Doxy) in pharmaceutical preparations.

APPLICATIONS

The applicability of the method for the assay of pharmaceutical formulation was examined. The result of assay for available formulations of Doxycycline Hyclate drugs are summarized in table 5.

Table 5: Assay of Doxycycline Hyclate in bulk and dosage forms.

Pharmaceutical preparations containing (DOXY)	Average recovery %	
	Proposed method	Standard method
Pure (Doxy)	99.920	99.863
DuraDox capsules (100)mg (U.A.E)	100.301	99.838
Vibramycin tablets (100)mg France	99.931	99.265
Bidox Doxycycline capsules (100)mg India	99.977	98.431
Doxydar capsules (100)mg Jordan	99.988	100.118
doxycycline capsules (100)mg UK	100.517	99.011

Where the average of three determinations and the standard method were taken from British Pharmacopoeia (2009). The results were reproducible and the assay of formulations was cross checked by the Standard method.

CONCLUSIONS

A simple, rapid, precise and sensitive spectrophotometric method has been developed for the determination of trace amounts of doxycycline hyclate in aqueous solution based on its oxidative coupling reaction with para-(Methyl amino) phenol sulfate (metol) and sodium persulphate in the presence of sodium hydroxide. The proposed method does not require the solvent extraction step; the method was applied successfully for the determined of small amounts commercial (Doxy) drug.

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