RESEARCH ARTICLE

Dapsone as Novel Reagent for Determination of Doxycycline *via* Diazotisation Coupling Reaction

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ABSTRACT

A sensitive, simple, accurate, and precious spectrophotometric method for the determination of doxycycline (Dox) was developed and applied in both pure and pharmaceutical form. The reaction is based on the dapsone diazotization in a hydrochloric acid solution with sodium nitrite. The salt formed in diazonium is then reacted to form yellow-orange azo- dye with doxycycline in the sodium hydroxide solution, showing a maximum absorption at 468 nm. The law of beer obeyed in the concentration range of 1-8.2µg mL^{-1} and molar absorption is 5.573×10^4 L mol^{-1} cm^{-1}. The relative standard deviation is ≤ 2, and the average recovery is 98.7%. The method was suitable for determining doxycycline in its pharmaceutical preparation, as a capsule, with no interference by widely known excipients used as additives in its commercial formulations. The finding aligns favorably with the official method.

Keywords: Dapsone, Diazotisation, Doxycycline, Spectrophotometric.

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INTRODUCTION

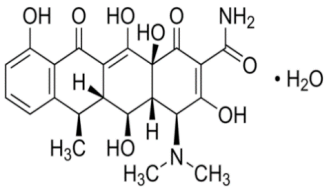
Antibiotics are probably considered the most successful family of drugs that have been developed so far for improving human health. Tetracycline is one of the most important classes of antibiotics. It has become the second most common class of drugs worldwide in terms of development and use.[1] The second generation of tetracycline, doxycycline (Dox), is available in both doxycycline monohydrate and doxycycline hydrate. Trade names include vibramycin, doryx, doxy-100, doxsig, zadorin, doxylag, periostat, adoxa, alodox, monodox, oraxyl, apo-doxy, vibra-tabs and doxycin[2] (Scheme 1). Doxycycline, as a member of the tetracycline family used in the treatment of a wide range of infections. It is effective against a wide variety of gram-positive and gram-negative bacteria.[3] It works by preventing the growth of bacteria by blocking bacterial protein synthesis.[4] It can also be used to treat respiratory tract infection (RTI), including pneumonia and chronic bronchitis, pelvic inflammatory disease, urinary tract infection, chlamydia, syphilis, mycoplasma, traveler's diarrhea, cholera, malaria, plague, melioidosis, brucellosis, Q fever, and other infections.[5-8]

For pharmaceutical formulations, several analytical methods have been used to determine Dox. Among these are spectrophotometric methods,[9-13] Flow injection analysis (FIA),[14-16] high-performance liquid chromatography (HPLC),[17-20] thin layer chromatography (TLC),[21] voltammetry[22] and electro-chemistry.[23] However, some of these methods suffer from several disadvantages such as low sensitivity, require a nonaqueous medium, needing heating, solvent extraction or used expensive instruments which require special training. The present work describes the development of the spectrophotometric method based on the determination of Dox as a coupling agent with diazotized Dapsone in an aqueous medium, to form a yellow-orange azo-dye measured at 468 nm. The procedure for determining Dox was applied as a capsule in its pharmaceutical preparation.

EXPERIMENTAL

Apparatus

OPTIMA Sp. 300 Spectrophotometer with cells matching 1 cm is used in this work. THE Philips PW 9420 pH-meter is



$C_{22}H_{24}N_2O_8 \cdot H_2O$.M.wt462.45g/mol

Scheme 1: Chemical structure of doxycycline monohydrate

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used for pH measurements, JRAD oven, and Diamond MTC 500 balance.

Preparation of Reagents

All Chemicals used were of analytical-reagent grade provided from Fluka and BDH companies. 100 μgmL^{-1}. The standard solution of doxycycline is made by dissolving 0.01 g of pure form in 5 mL ethanol and then diluted to 100 mL by distilled water in a volumetric flask. The appropriate concentrated acid dilution prepares a solution of 1N hydrochloric acid. 0.1% Dapsone is prepared by dissolving 0.1 gm of pure compound in 100 mL distilled water in a volumetric flask. 1% sodium nitrite solution, 2% sulphamic acid aqueous solution, and 1N sodium hydroxide solution are prepared by sufficient dilution of the concentrated volume with distilled water.

Recommended Procedure for Pure Doxycycline

In a series of 25 mL standard flasks, One milliliter of 0.1% solution of Dapsone taken. A 1 N hydrochloric acid solution (0.5 mL) was added to the Dapsone solutions. A 1% sodium nitrite solution (1 mL)was added to each volumetric flask and cooled in an ice bath for 7 minutes with Stirring. Then 1 mL of 2% sulphamic acid was added, then solutions had been allowed to stand at room temperature for 5 minutes. Doxycycline solution (1–8.2 μgmL^{-1}) then added and 2 mL of 1 N sodium hydroxide and 2 ml of 1% CTAB (Cetrimonium bromide) solutions. The volumetric flasks were completed with distilled water up to the mark limit and the maximum absorption measured at a wavelength of 468 nm against the blank solution, the calibration graph was developed.

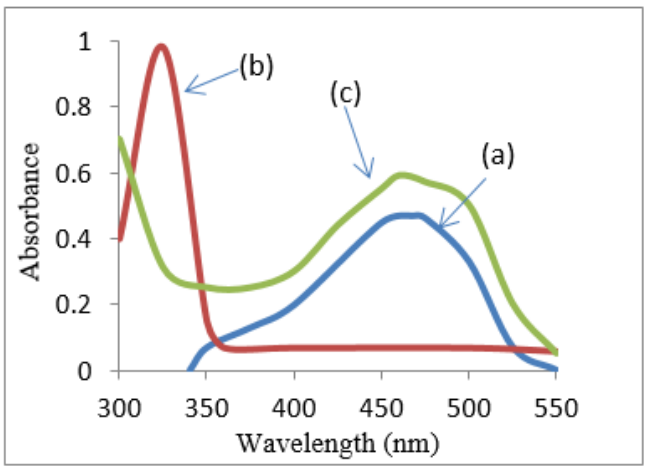


Figure 1: Absorption spectra of (a) 4 μgmL^{-1} Doxycycline with Dapsone against reagent blank, (b) Reagent blank against distilled water and (c) 4 μgmL^{-1} Doxycycline against distilled water.

Capsule

The contents of ten capsules Dox (each capsule contains 100 mg Dox) have been weighed and finely powdered. A powdered quantity equal to one capsule in water containing a few drops of diluted HCl was dissolved, then filtered. The filtrate was made up to 1L and the solution was treated with sufficient aliquots as defined in the prescribed pure sample procedure.

RESULTS AND DISCUSSION

The procedure requires dapsone diazotisation, accompanied by coupling into an alkaline medium with Dox to create a yellow-orange product.

Spectral Characteristics

Figure 1 showed the absorption spectra for the yellow-orange product with maximum absorption at 468 nm. The colorless, blank reagent is found to be practically negligible absorption at the λ_{max} of the product.

Optimum Reagent Concentrations

The effect of reagent concentrations, on the azo dye formation reaction, have been optimized by setting all parameters constant and optimizing one at a time.

As seen in Figure 2, the effect of different parameters on the absorbance of the azo dye formed was studied. It has been observed that 1 mL of solution of Dapsone (a), 0.5 mL of 1 N solution of HCl (b), 2 mL of 0.1% CTAB surfactant (c), 1 mL of 1% solution of sodium nitrite (Table 1), a 2% solution of sulphamic acid in the range of 1.5–3 mL (Table 2), and 1–3 mL of 1 N sodium hydroxide (See Table 3) solution were required to reach optimum color intensity with doxycycline. However, the above optimum amounts of reagents are used to obtain maximum sensitivity for the colored product and recommended in the general procedure. Excess nitrite during diazotization may be reduced by using a solution of sulphamic acid, whereas the increase of sulfamic acid is not affecting the intensity of color.The effect of organic solvent besides water on dilution of solutions was examined. It was found that water is the best solvent to obtain high sensitivity. Finally, the sequence of reactions must be followed the general procedure for obtaining high sensitivity.

However, the effect of temperature and developing time were examined and found that the product was formed immediately and stable for more than 1-hour. It has been found

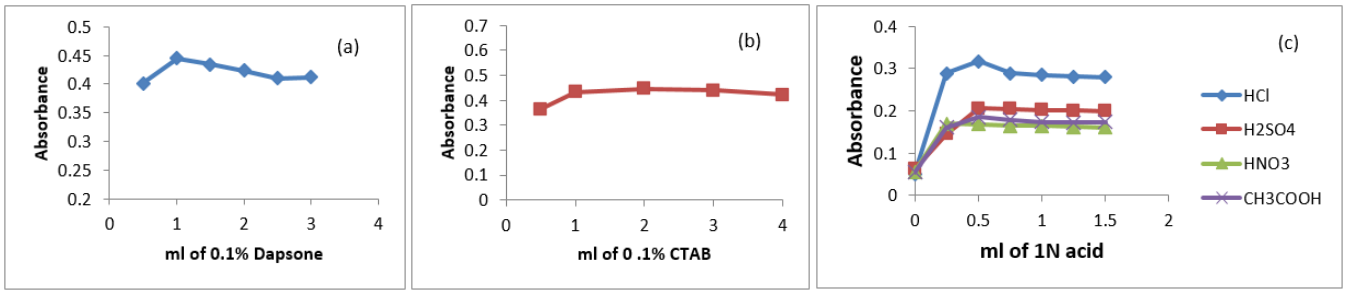


Figure 2: Optimum conditions for determination of doxycycline

there is no effect of temperature ranging from 0–50°C on the absorbance, so room temperature was chosen in this method (Table 4).

Quantitation

A linear correlation was found between absorbance at λ_{max} and concentration range of 1–8.2 µg mL^{-1}, Sandell index, molar absorptivity, limit of detection (LoD), and limit of quantification (LoQ) presented in Table 5 indicated the sensitivity of the method. The method's accuracy and precision were established by analyzing the pure drug solution at three different levels within the working range. The recovery (%), which is a measure of accuracy and %RSD a measure of precision, are summarized to reveal the high precision and accurate method (Table 5).

Specificity

The specificity of the proposed method was studied by measuring the absorption of solutions containing interference from the excipients of pharmaceutical formulations containing 4µg/mL of doxycycline and various amounts of excipients in a final volume of 25 mL, up to 20 fold-excess. It was found that the examined excipients did not interfere seriously, except Sulphanilamide shows some interference at higher concentrations (Table 6).

Table 1: Effect of Nitrite and Standing time.

mL of 1% $NaNO_2$ Solution	*Absorbance / Standing time (min)*				
	1	*3*	*5*	*7*	*9*
0.25	0.141	0.211	0.235	0.237	0.241
0.5	0.229	0.231	0.234	0.234	0.239
0.75	0.233	0.230	0.230	0.232	0.234
1.0	0.235	0.291	0.317	0.347	0.345
1.25	0.142	0.166	0.170	0.231	0.296
1.5	0.145	0.220	0.193	0.314	0.321

Table 2: Effect of Sulphamic Acid and Standing

mL of 2% Sulphamic acid	*Absorbance / Standing time (min)*			
	1	*3*	*5*	*7*
0.0	0.297	0.311	0.314	0.301
0.5	0.299	0.323	0.321	0.326
1.0	0.297	0.329	0.332	0.330
1.5	0.337	0.338	0.342	0.340
2.0	0.335	0.338	0.341	0.336
2.5	0.331	0.334	0.337	0.339
3.0	0.337	0.335	0.340	0.338

Table 3: Effect of Base.

	Absorbance / mL of base used						
Base (1N)	*0.5*	*1.0*	*1.5*	*2.0*	*2.5*	*3.0*	$\Delta\lambda^*$
NaOH	0.232	0.445	0.446	0.447	0.445	0.446	191
KOH	0.121	0.420	0.431	0.432	0.416	0.411	191
Na_2CO_3	0.016	0.019	0.242	0.433	0.299	0.293	86
$NaHCO_3$	0.012	0.019	0.233	0.321	0.278	0.289	106
NH_4OH	0.023	0.243	0.238	0.322	0.221	0.230	84

Analytical Applications

The suggested method has been applied successfully for the determination of doxycycline in its pharmaceutical formulation as a capsule, using four different concentrations. The results cited in (Table 7) indicate the method is accurate (The average recovery % within the range 101.6–103.22). The results obtained from the Doxycycline capsule were compared favorably with the official method[24] through t-test and F-test, at a confidence level of 95 % (n = 4), for accuracy and precision, respectively. The experimental t-test and F-tests were found 1.67 and 4.42, which are lower than the theoretical values (t = 4.30, F = 9.28), indicating no significant differences between the two methods (Table 7).

Reaction Mechanism and Stability Constant

In the diazotization reaction, Dapsone could be diazotized in acidic medium. The diazonium ion reacts with a molecule of

Table 4: Effect of temperature

Standing time (min)	*Absorbance / temperature (°C)*			
	0.0	*RT=25°C*	*40*	*50*
2	0.441	0.443	0.445	0.444
5	0.445	0.448	0.447	0.445
10	0.447	0.446	0.445	0.443
15	0.446	0.449	0.440	0.439
20	0.445	0.448	0.443	0.440
30	0.444	0.445	0.442	0.441
35	0.442	0.444	0.441	0.441
40	0.440	0.442	0.440	0.440
45	0.441	0.441	0.439	0.439
50	0.442	0.440	0.438	0.438
55	0.441	0.441	0.437	0.437
60	0.442	0.440	0.436	0.432

Table 5: Optical characteristics of the method

Analytical parameters	*Result*
λmax (nm)	468nm
Colour of the dye	Yellow-Orange
Bee's law (µg mL−1)	1–8.2
Molar absorptivity (L. mol-1.cm-1)	5.5727×104
Sandell's index	0.0083
Recovery (%)	98.7
Relative standard deviation (%)	< 1.9 %
Regression equation*	
Slope (b)	0.1157
Intercept (a)	0.0206
Correlation coefficient (r)	0.99594
Stability (Hrs)	< 1 hrs
LOD (µg mL−1)	0.0311
LOQ (µg mL−1)	0.1037

*Regression Equation (Y = bx + a) where x is the concentration in µg ml^{-1}

doxycycline by electrophilic substitution at the para position of the phenolic group in doxycycline structure. An investigation of the continuous molar variation of the doxycycline and Dapsone demonstrated the drug interacts at a ratio of 2:1 respectively, and a similar result has been observed with the mole ratio method,[25] (Figure 3). In Scheme 2, a mechanism of reaction based on the results above is shown. However; according to the result obtained from molar ratio, the stability constant was found to be 1.7957×10^9 L^2 mol^2, indicate the high stability of the product, which calculated from the following equations:

$$\propto = \frac{Am-As}{Am} \qquad k = \frac{1-\propto}{4\propto^3 C^2}$$

Where:

α is the dissociation constant

Am is the absorbance of the product in the presence of an optimum amount of Dapsone

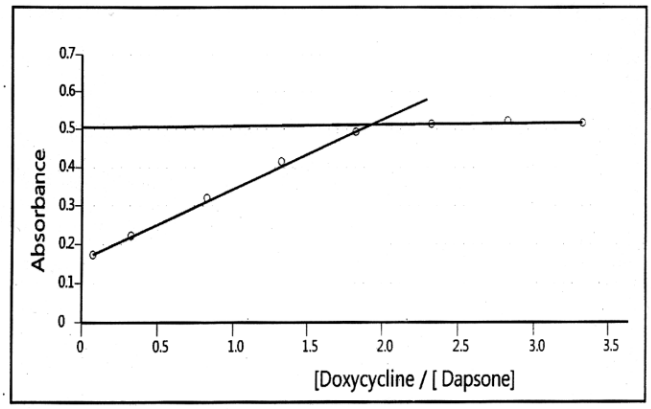
As is the absorbance of product in the presence of a stoichiometric amount of Dapsone

K is the stability constant of the product

C is the molar concentration of the product.

Table 6: Effect of pharmaceutical excipients for determination of doxycycline

	(%)Recovery of 4µg/mL Doxycycline for every fold excess excipient			
Excipient	*1*	*5*	*10*	*20*
Glucose	99.77	99.23	99.14	99.07
Lactose	98.36	98.47	98.46	99.82
Fructose	100.49	101.02	99.87	100.13
NaCl	99.23	99.46	100.25	100.34
Urea	99.76	99.14	97.98	96.29
Na_2SO_4	99.52	99.72	99.90	97.84
Starch	100.96	99.99	100.01	101.21
Arabic Gum	100.12	102.12	99.86	100.14
CH_3COONa	101.96	99.89	100.47	102.43
Benzoic acid	100.13	98.71	101.12	101.31
EDTA	99.98	99.87	99.45	100.08
Sulphanilamide	103.39	104.78	111.04	118.49
Histidine	97.93	102.32	99.48	99.36



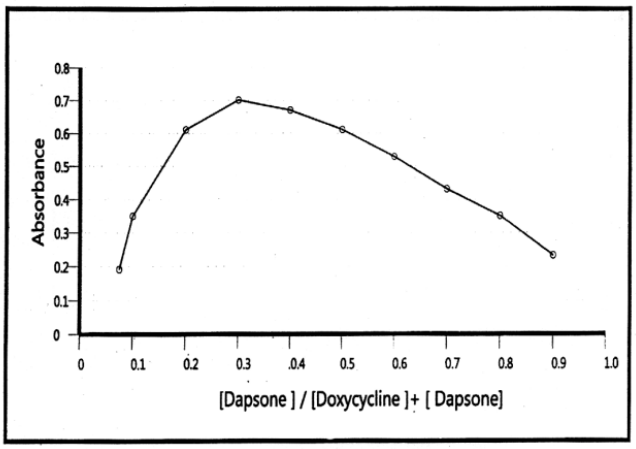
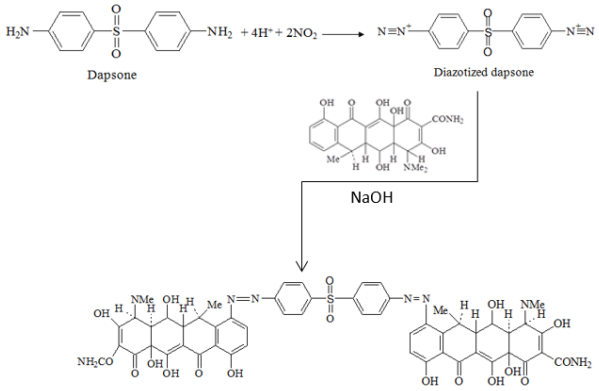


Figure 3: Continuous variation (a) and mole ratio methods (b)



Scheme 2: Reaction mechanism for diazotisation and coupling of Doxycycline and Dapsone.

Table 7: Application of the method for determination of doxycycline in capsule and comparison with the official method

Pharmac formulate	*Certified value (mg)*	*Amount present (µg /mL)*	*Recovery (%)*	*Average drug content found (mg)*	*R.S.D.%*
Doxycycline Capsule	100	0.4	101.70	102.22	1.564
		1.0	103.20		
		2.0	102.40		
		3.0	101.60		
British Pharmacopeia	100	0.4	101.54	102.28	0.776
		1.0	102.10		
		2.0	102.27		
		3.0	103.24		

Table 8: Comparison of the proposed method with published spectrophotometric methods

	Reagent			
	Present method	*Literature method*		
Analytical parameters	*Dapsone*	*Bromanil, acetonitrileto [9]*	*Fe (III),HCl [10]*	*Bromophenol blue [12]*
Type of Method	Azo-Dye	Charge transfer complex	Cloud point extraction	Chloroform extraction complex
Colour of dye	Yellow-Orange	--	Brown	--
λ_{max}(nm)	468	377	430	413
pH	Alkaline(11.2)	Alkaline(9)	Acidic(4)	--
Medium	Water	Acetonitrileto	Water	Chloroform
Temp.(°C)	RT	50	65	RT
Development time (min(	7	3	15	10
Stability period (min(	<hr	60	-	-
Beer's law (µg/mL(	1-8.2	1-50	0.2-8	5-40
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.5727×10^4	1.5725×10^4	5.85×10^4	-
Recovery (%)	98.7	97.9	98.33	98-99
RSD(%)	> 2.0	0.92	> 2.0	-
Application	Capsules	Capsules	Tablet	Tablet
Disadvantages	--	Using of organic solvent	Using of organic solvent& Need heating	Using of organic solvent and Need extraction

Comparison Dapsone Method with other Spectrophotometric Methods

The method proposed is favorably compared with other spectrophotometric methods published. As shown in Table 8, the method proposed is more sensitive than other methods and does not require heating.

CONCLUSION

A sensitive, simple, accurate, and precious spectrophotometric method was used to determine doxycycline. It depends on the diazotization coupling between dapsone and the drug. Studies showed that the suggested method is highly reproducible and accurate. Sample analysis has shown that the common excipients do not interfere. The advantages of the proposed method are simple, need no extraction and less, time consuming, and the ability to apply in a pharmaceutical preparation with success.

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