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Spectrophotometric Determination of Primaquine by Coupling with Diazotized *p*-Nitroaniline Application to Pharmaceutical Formulations

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Abstract

A simple spectrophotometric method for the determination of primaquine phosphate has been developed. The method is based on the coupling reaction of primaquine phosphate in acidic medium with diazotized *p*-nitroaniline, to form a yellow-orange water-soluble azo dye that shows maximum absorption at 482 nm. Beer's law is obeyed over the concentration range 5-300 μg of primaquine phosphate in a final volum of 25 ml, i.e., 0.2-12 ppm with a molar absorptivity of 6.6×10^4 l mol^{-1} cm^{-1} and Sandell's sensitivity index of 0.0068 $\mu g.cm^{-2}$, a relative error of -0.63 to +0.32% and a relative standard deviation of ±0.18 to ±2.8%, depending on the concentration level. Interferences due to excipients have been examined. The proposed method has been applied to the assay of primaquine phosphate in pharmaceutical formulations (tablets).

Keywords: Primaquine; diazotized p-nitroaniline; spectrophotometry.

Introduction

Primaquine [N-(6-methoxy quinoline-8-ly) Pentane-1,4-diamine] is an antimalarial drug that is very important for the radical cure of relapsing vivax or ovale malaria and it eliminates tissue infection. It is a member of the 8-aminoquinoline group of drugs and is often administered in combination with chloroquine [1, 2].

There are various analytical procedures for the assay of Primaquine, an HPLC [3, 4], facilitating metabolic and pharmacokinetic studies [5]. A capillary electrophoresis method has been developed that allows the separation and estimation of primaquine enantiomers in pharmaceutical formulations [6]. Other methods such as voltammetry [7], gas chromatography-mass spectrometry [8], and ultra-performance liquid chromatography [9] (UPLC) have also been proposed. The British Pharmacopoeia method [10] involves the dissolution of the sample in anhydrous acetic acid with gentle heating. When cooled, the sample is titrated against perchloric acid [11], and the end point is determined potentiometrically.

Many spectrophotometric methods have been described for the determination of primaquine phosphate. Some of these methods are comprised of coupling of primaquine phosphate with diazotized reagents [12, 13] to form an intensely-coloured azo dye. Other colorimetric methods are based on the reaction of primaquine with chloanily, 3-methyl-2-benzothiazolinone hydrazone or tetracyanoethylene [14, 15, 16] are reported. Some of these methods are time-consuming [12, 14] less sensitive [16], require organic solvents [12], buffer solution and pH control [12, 14].

Therefore, another simple spectrophot-

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ometric method for the determination of primaquine has been worked out, depending on coupling of primaquine with diazotized *p*-nitroaniline to form a highly coloured dye that has proved successful for the determination of primaquine in pharmaceutical preparations.

Experimental

Apparatus

Absorption spectra are carried out using Shimadzu UV-Visible recording Spectrophotometric UV-160, with 1-cm glass cells.

Spectrophotometric measurements are carried out on a single beam Spectrophotometer UNICO-1100.

The pH measurements are performed on Philips PW 9420 pH-meter with a combined glass electrode.

Reagents

- Chemicals used are of analytical reagent grade.
- Working primaquine phosphate solution, 100 µg/ml.
- Sodium nitrite solution, 1%.
- Hydrochloric acid solution, 1 M.

Diazotized *p*-nitroaniline reagent solution, 5mM: A 0.1727 g of *p*-nitroaniline is dissolved in about 50ml distilled water. Then 20 ml of 1 M HCl is added and solution is heated, the clear mixture is then transferred to a 250 ml volumetric flask and is cooled to 0-5^{o}C in an ice-bath. 8.65 ml of 1% $NaNO_2$ is added and the mixture is stirred vigorously. After 5 min the solution is made up to volume in 250 ml volumetric flask with cold water. The solution is stored in a brown bottle in a refrigerator and is stable for at least one week.

Primaquine tablets solution: Four tablets were powdered accurately weighted and the weight of the powder equivalent to one tablet was dissolved and transferred into a 100 ml calibrated flask and made up to volume with water. The solution was filtered and the clear filtrate used for the determination. An appropriate volume of the sample solution was diluted further with water so that the concentration of primaquine phosphate in the final solution was within the working range (100 µg).

Procedure

An aliquot of standard solution containing 5-300 µg of primaquine phosphate was transferred to a 25 ml volumetric flask. 2 ml of diazotized *p*-nitroaniline (5 mM) solution is added followed by 2 ml of 0.2 M acetic acid solution and diluted to the mark. The solution was mixed thoroughly and the absorbance was measured at 482 nm against the reagent blank within 2 h using 1-cm cells.

Results and Discussion

Absorption spectra

An intense yellow-orange coloured azo dye formed as a result of mixing a dilute aqueous solution of primaquine phosphate and diazotized *p*-nitroaniline in acidic medium. The azo dye formed shows maximum absorption at 482 nm (Fig. 1), in contrast to the reagent blank. The colour is formed immediately and is stable for at least 120 minutes. The calibration graph is linear over the range 0.2 - 12 ppm. The apparent molar absorptivity has been found to be 6.6×10^4 l mol^{-1} cm^{-1}.

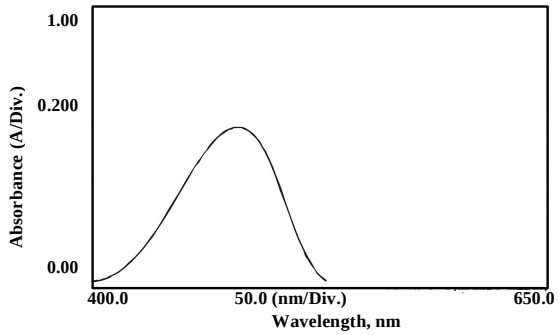


Figure 1. **Absorption spectra.**

Effect of surfactants

The effect of different types and amounts of surfactants (cetyltrimethylammonium bromide (cationic surfactant), sodium dodecyl sulfate (anionic surfactant) and Tween 80 on the colour intensity of the yellow dye has been examined and the results showed that the addition of surfactants gives no significant effect. Therefore, it has been recommended to eliminate the use of surfactants in the subsequent experiments.

Effect of acid added

Different amounts (1-10 ml of 0.1 M) of different acids (HCl, $HClO_4$, HNO_3, H_2SO_4, H_3PO_4, CH_3COOH and HCOOH) have been examined for their effect on the colour intensity of the azo dye. The experimental order of the acid (sample+acid+diazotized reagent or sample + diazotized reagent + acid) added have no effect on the intensity of the coloured azo dye Table 1.

Table 1. **Effect of different amounts of acids on absorbance of Azo-dye.**

0.1 M of Acid	Absorbance/ ml of Acid used				
	1	3	5	7	10
HCl	0.608	0.610	0.606	0.613	0.602
pH	2.16	1.78	1.60	1.48	1.37
HNO_3	0.598	0.599	0.597	0.595	0.596
pH	2.10	1.83	1.77	1.60	1.48
H_2SO_4	0.602	0.600	0.602	0.599	0.599
pH	2.14	1.88	1.76	1.66	1.53
H_3PO_4	0.600	0.601	0.602	0.599	0.604
pH	2.25	2.17	2.09	2.03	1.95
CH_3COOH	0.598	0.604	0.596	0.592	0.591
pH	2.32	2.31	2.32	2.31	2.31
HCOOH	0.602	0.603	0.597	0.598	0.600
pH	2.31	2.30	2.31	2.29	2.27
$HClO_4$	0.596	0.598	0.595	0.592	0.597
pH	2.17	1.93	1.80	1.68	1.56

Effect of base

Different amounts (1-10 ml of 0.1 M) of various bases (NaOH, KOH, Na_2CO_3, $NaHCO_3$, NH_4OH, CH_3COONa and HCOONa) have been investigated for their effect on the colour intensity of the azo dye. The colour of the azo dye changes from yellow-orange in acidic media to orange then to yellowish-brown and also becomes turbid as the amount of base increases (then pH of final reaction mixture is nearly five or more). In addition the blank changes from colourless in acidic media to yellow in basic media Table 2.

Acidic medium is preferred over basic medium because of strong colour contrast and better expected selectivity of determination because most compounds are activated towards coupling in basic medium.

2 ml of 0.2 M CH_3COOH (which equals to 4 ml of 0.1 M of the acid) is selected for the subsequent experiments.

Table 2. **Effect of different amounts of bases on absorbance of Azo-dye..**

0.1 M of Base	Absorbance /ml of Base used				
	1	3	5	7	10
Na_2CO_3	0.299	0.27	0.208	0.196	0.189
pH	6.60	9.84	10.18	10.35	10.46
$NaHCO_3$	0.604	0.27	Turbid	Turbid	Turbid
pH	3.16	6.55	6.96	7.15	7.33
CH_3COONa*	0.603	0.598	0.590	0.590	0.571
pH	2.67	4.24	4.80	5.0	5.22
NH_4OH pH	Blank and Samples are red coloured				
KOH*	0.595	0.202	0.199	0.197	0.176
pH	2.70	11.40	11.91	12.15	12.29
NaOH	0.593	0.203	0.200	0.196	0.185
pH	3.11	11.35	11.90	12.13	12.30

Choice of diazotized reagent and amount

Diazotized *p*-nitroaniline reagent has been selected in this study because of following reasons; its the strongest diazonium electrophile ever used (due to the presence of the strong electron-withdrawing nitro group), the strongest colour contrast observed in its azo dye production (due to the presence of the *p*-nitro resonating group), the most sensitive diazo-coupling reaction diazotized *p*-nitroaniline reagent can give with aromatic components [17, 18] and the colour of the diazotized reagent solution is faint yellow thus giving lower blank values. The effect of the amount of diazotized *p*-nitroaniline reagent on the maximum absorbance of the dye formed has been investigated and the results are illustrated in Table 3.

Table 3. **Effect of amount of diazo reagent solution on absorbance.**

Primaquine phosphate μg	Absorbance/ ml (5 mM) diazotized reagent				
	0.5	1.0	2.0	3.0	5.0
10	0.046	0.048	0.051	0.052	0.059
30	0.145	0.149	0.166	0.165	0.173
50	0.242	0.247	0.278	0.285	0.287
70	0.356	0.358	0.403	0.410	0.414
100	0.502	0.505	0.605	0.606	0.608
150	0.758	0.767	0.866	0.870	0.879
200	0.992	0.994	1.138	1.150	1.165
300	1.437	1.444	1.709	1.715	1.720
r^2	0.9987	0.9985	0.9994	0.9994	0.9989

2 ml of 5 mM diazotized reagent has been selected for the procedure, since it gives the highest value of determination coefficient (r^2=0.9994).

Effect of time on color development

The effect of time with different amounts of primaquine phosphate has been investigated for maximum production of the dye and longer stability period Table 4. The maximum absorbace reading is given immediately and remains constant for at least 120 minutes, independent of primaquine phosphate amounts.

Table 4. **Effect of time on colour development.**

Time, min	Absorbance/ μg of primaquine phosphate		
	10	100	200
0	0.052	0.611	1.140
5	0.057	0.612	1.138
10	0.055	0.610	1.136
15	0.054	0.609	1.135
20	0.054	0.608	1.134
25	0.055	0.607	1.135
30	0.057	0.608	1.134
35	0.056	0.609	1.134
40	0.054	0.609	1.134
45	0.054	0.609	1.134
50	0.054	0.609	1.135
55	0.054	0.608	1.135
60	0.054	0.609	1.135
120	0.055	0.609	1.136

Effect of organic solvents

The effect of organic solvents on the optical properties of the azo dye has been tested. The reaction mixture has been diluted using different organic solvents and the data are shown in Table 5.

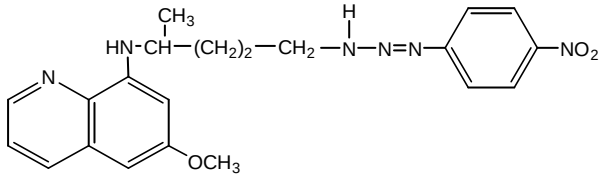
Table 5. **Effect of various organic solvents on the absobance of the azo dye.**

Solvent	Absorbance	λ_{max}
Acetone	0.666	477.5
DMF	0.577	483.0
Formic Acid	0.709	482.5
Acetic Acid	0.615	482.5
Ethanol	0.620	480.5
THF	0.600	480.0
Propanol	0.632	480.5
1,4-Dioxan	0.760	481.0
D.W.	0.607	481.5

Water is shown to be a good medium and economical as well.

Nature of the dye

Job's method of continuous variations indicates that the dye has a composition of 1:1 primaquine to diazotized *p*-nitroaniline reagent, the structure of the azo dye is represented in scheme 1.



Scheme-1. Yellow-orange azo dye

Accuracy and precision

To check the accuracy and precision, primaquine has been determined at three different concentrations Table 6. The low value of relative error and relative standard deviation shows that the accuracy and precision of the method is satisfactory.

Table 6. **Accuracy and precision of the method.**

Amount of primaquine Phosphate taken (μg)	Relative* error%	Relative* standard deviation%
10	-0.63	±2.8
100	+0.3	±0.3
200	+0.2	±0.18

***Average of five determinations**

Effect of interferences

In order to assess the possible analytical applications of the present proposed method, the interfering effect of foreign substances on the determination of 100 μg of primaquine phosphate is shown in Table 7. It is evident from the table that the proposed method is quite selective.

Table 7. **Effect of excipients on the determination of different amount of primaquine phosphate.**

Interferent	Amount added μg	Recovery %
Glucose	500	99.24
Lactose	500	99.54
Sucrose	500	100.76
Starch	500	100.46
Gum acacia	500	99.54
$CaCO_3$	500	101.82
Isonicotinic acid hydrizide	500	102.27
NaH_2PO_4	500	99.24
Na_2HPO_4	500	100.45
Chloroquine	1000	99.83

Application of the method

To test the applicability of the present method it has been applied to the determination of primaquine phosphate in to drugs Table 8.

Table 8. **Determination of primaquine phosphate in tablets.**

Drug	Manufacturing company	μg Primaquine phosphate		Recovery* %
		Present	*measured*	
Primaquine[a] phosphate	Weifa Noreay	5	4.9	98.71
		50	48.8	97.60
		100	98.88	98.88
Primaquine[b] phosphate	Medipharm-India	5	5.06	101.25
		50	50.50	101.01
		100	99.26	99.26

* Average of three determinations

Each pharmaceutical product (a and b) contains primaquine phosphate 26.3 mg which is equivalent to 15 mg primaquine base.

From the results, it can be shown that good agreement has occurred between the amount of primaquine phosphate present and that measured by the proposed procedure.

Comparison of the methods and t-test

A comparison between the present method and British pharmacopeia standard method [10] for the determination of primaquine phosphate in two drugs, is based on the t-test to show the ability of using the present method in the determination of investigated drugs Table 9.

Table 9. **Comparison of the methods and experimental t-test values.**

Drug (tablets)	Recovery %		±t. exp
	Present method	***British pharmacopeia method***	
Primaquine phosphate (Weifa)	98.64	99.63	1.087
Primaquine phosphate (Medopharm)	100.51	101.91	0.567

The results show no significant difference between the present method and the standard method.

Table 10 shows the comparison between some of analytical variables obtained from the present method and another spectrophotometric method [14].

Table 10. **Comparison of the methods.**

Analytical parameters	Present method	Literature method*
Temperature (^oC)	At room temperature	65
Development time (minutes)	Directly	20
λ_{max} (nm)	482	305
Medium of reaction	Aqueous	Aqueous
Reagent used	Diazotized *p*-nitroaniline	Chloranil
Beer's law range (ppm)	0.2-12	0.4-40
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	6.6×10^4	2×10^4
Stability of the colour (minutes)	120 (at least)	Over night
Colour of the dye	Yello-orange	-
Nature of the dye	1:1	1:1
Application of the method	Pharmaceutical preparations	-

* Sulaiman S.T. and Amin D., Inteern. *J. Environ. Anal. Chem.18* (1985) 1.

The proposed method has an advantage over the published methods that it occurs at room temperature and not specific for a particular.

Conclusion

The proposed method permits rapid, precise, and accurate determination of primaquine phosphate with an application to pharmaceutical analysis. The short analysis time and low costs are the main advantages of this method for routine analysis in quality control.

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