Spectrophotometric Determination of Dapsone Using Phloroglucinol Azo Coupling Reagent

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ABSTRACT

The research was concerned with a development of spectrophotometric method for the determination of trace amounts of dapsone. The method was based on the reaction of diazotized dapsone with phloroglucinol as azo coupling reagent in basic medium, to form an intense yellow coloured, water-soluble and stable azo-dye which showed a maximum absorption at 435 nm. Beer's law is obeyed over the concentration range of 10-250 μg/25ml, i.e.,0.4-10 ppm with molar absorptivity of 4.79×10^4 $L.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.005 $\mu g.cm^{-2}$, a relative error of + 0.12 to -0.34% and a relative standard deviation of ± 3.96 to ± 0.85% ; depending on the concentration level. The method does not require temperature control or solvent extraction and has been applied successfully to the determination of dapsone in pharmaceutical preparation (Tablet).

التقدير الطيفي للدابسون باستخدام Phloroglucinol ككاشف اقتران أزوتي

الملخص

تضمن البحث تطوير طريقة طيفية لتقدير كميات متناهية الصغر من الدابسون، بالاعتماد على تفاعل الدابسون المؤزوت مع الفلوروكليسنول ككاشف اقتران في وسط قاعدي لتكوين صبغة ازوية ذات لون اصفر تعطي أعلى شدة امتصاص عند الطول الموجي 435 نانوميتر. وكانت حدود قانون بير 10 و 250 مايكروغرام/25 مللتر او 0.4 و 10 جزء/مليون جزء، وكانت الامتصاصية المولارية 4.79×10^4 لتر.مول$^{-1}$.سم$^{-1}$. ودلالة ساندل 0.005 مايكروغرام.سم$^{-2}$ والخطأ النسبي بين 0.12 + و 0.34- % والانحراف القياسي بين 3.96 ± و 0.85 ± % اعتمادا على مستوى التركيز للدواء مما يدل على أن الطريقة تمتاز بدقة ومضبوطية عاليتين. ومن محاسن الطريقة أيضا أنها لا تحتاج إلى السيطرة على درجة الحرارة ولا إلى عملية استخلاص بالمذيب. وقد تم تطبيق الطريقة المقترحة بنجاح لتقدير الدابسون في الاقراص الدوائية.

INTRODUCTION

Dapsone is widely employed as effective antibiotic for prophylaxis agent pneumocystis carinii pneumonia and an opportunistic disease in (HIV) infection. It's approved as antibiotic by food and drug administration since 1963, because it is used as therapeutic agent to treat bacterial infections in human and animals (Saillourglenisson *et al.*, 2000).

Different methods have been used for determination of dapsone. Gas chromatography has been used for the determination of dapsone in human or animal plasma (Burchfield *et al.*, 1973). Also liquid chromatography has been used for the determination of dapsone in human plasma (Shirazi *et al.*, 2001). Other chromatographic methods have been used for the determination of dapsone in pharmaceutical preparation using high performance liquid chromatography (HPLC) technique (Takla *et al.*, 1977), and high-speed gradient liquid chromatography was used to determine dapsone in serum (Luan Chen *et al.*, 2003). Spectrophotometric methods have been used to determine dapsone using different reactions: oxidative coupling reaction using different reagents such as promethazine in the presence of hypochlorite as oxidizing agent (AL-Abachi *et al.*, 1995), 4-amino-N,N-dimethylaniline in the presence of dichromate in acidic medium (AL-Talib,1997). Charg-transfere reactions is used for the determination of dapsone in dosage form using different reagent such as chloranil (Mahmood, 2000), flouranil (AL-Ghabsha *et al.*, 2004), 2,3-dichloro-5,6-dicyano-benzoquinone(DDQ) (AL-Ghabsha *et al.*, 2004). Other methods used the diazotisation of dapsone and coupling with different coupling agents such as α- naphthol in the presence of sodium carbonate (Mohammed, 1994), dibenzoylmethane in an alkaline medium to determine metoclopramide hydrochloride (MCP) and dapsone (Revanasiddappa and Manju`,2001). Iminodibenzyl in alcohol medium (Nagaraja *et al.*, 2002), 3-amino phenol in aqueous medium (Nagaraja *et al.*,2003), benzoylacetone in alkaline medium (Omran, 2005), α-naphthol in strong alkaline medium in the presence of cetavlon (AL-Ramadani, 2007).

The present method in this paper is to evaluate a spectrophotometric method for the determination of dapsone by reaction of dapsone with nitrite ion in presence of hydrochloric acid and then the coupling of diazotized dapsone with phloroglucinol in basic medium to form an intense yellow coloured, water-soluble and stable azo-dye.

EXPERIMENTAL

Apparatus:

Spectral and absorbance measurements are carriedout using Shimadzu UV-Visible Recording spectrophotometer UV-160, with 1 cm matched glass cells. pH meter type Philips PW 9420 is used for pH readings.

Reagents and solutions:

All chemicals used in this investigation are of analytical-reagent grade.

Extraction of dapsone from tablets (British Pharmacopoeia, 2000):

10 Tablets of dapsone are grand up and dissolved in acetone, then the solution is filtered and the precipitate is washed by acetone, the precipitate of dapsone is recrystalized (Ferry *et al.*, 1964) twice to yield a pure dapsone of 178ºC as a melting point (175-181 ºC).

Dapsone solution, 100 μg/ml:

This solution is prepared by dissolving 0.01 g of recrystallized dapsone mentioned above in 3ml of ethanol and completed the volume to 100 ml in calibrated flask with distilled water, the solution is kept in a brown bottle.

Hydrochloric acid solution,1N:

This solution is prepared by diluting 8.3 ml of concentrated hydrochloric acid to a 100 ml distilled water in a calibrated flask.

Sodium nitrite solution, 1%:

This solution is prepared by dissolving 1g of sodium nitrite (BDH) in 100 ml distilled water in calibrated flask. The solution is kept in a brown bottle and is stable for at least, one week .

Sulphamic acid solution, 3%:

This solution is prapred by dissolving 3 g of sulphamic acid (Flulka) in 100 ml distilled water using calibrated flask. This solution is kept in a brown bottle and is stable for, at least one week.

Sodium hydroxide solution,1N:

This solution is prepared by dissolving 10g of sodium hydroxide in distilled water. Then completing the volume to 250 ml in calibrated flask with distilled water and transferred to a plastic bottle .

Phloroglucinol solution,0.5% :

This solution is prepared by dissolving 0.5g of Phloroglucinol in 100 ml distilled water in calibrated flask. The solution is kept in a brown bottle and is stable for, at least one week.

Foreign compound solutions, 1000 μg/ml:

These solutions are prepared by dissolving 0.1 g of the compound in distilled water and the volume is completed to 100 ml in calibrated flask.

Surfactant compound solutions, 0.1%:

These solutions are prepared by dissolving 0.1g of the compound in distilled water and the volume is completed to 100 ml in a calibrated flask.

Procedure for diazotization of dapsone and calibration graph:

To a series of 25 ml calibrated flasks 0.1-2.5 ml of 100 μg.ml dapsone solution are added, then 1 ml of 1N hydrochloric acid and 1 ml of 1% sodium nitrate solution are added, the mixture is allowed to stand for 3 minutes and then 1 ml of 3% sulphamic acid solution is added with occasional shaking for another 3 minute. After that a 0.5 ml of 0.5% phloroglucinol solution and 3 ml of 1N sodium hydroxide are added.

After the volumetric flasks are completed to the mark with distilled water, the absorbance is measured at 435 nm against the reagent blank solution after 10 minute. A linear calibration graph is obtained over the concentration range of 10-250 μg/25ml

(0.4-10 ppm) dapsone and a concentration above 250 μg/25ml gives a negative deviation (Fig.1). the molar absorptivity has been found to be 4.79×10^4 L.mol^{-1}.cm^{-1}.

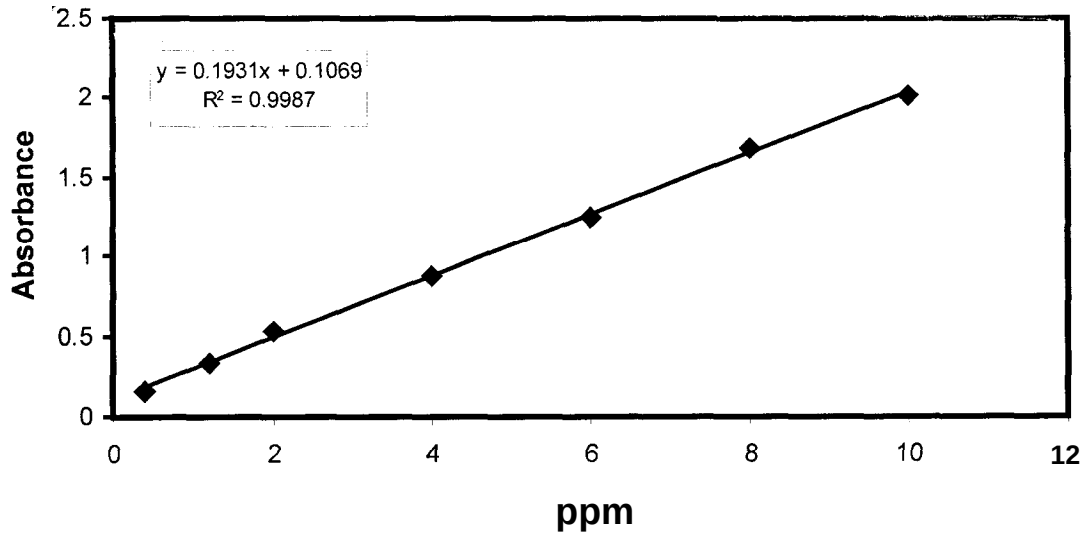


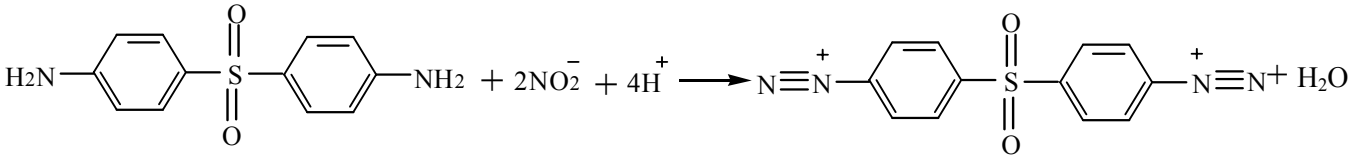
Fig. 1: Calibration graph of Dapsone determination

RESULTS AND DISCUSSION

For the subsequent experiments, 50 μg of dapsone is taken in a final volume of 25 ml.

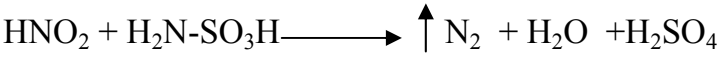
Principle of the method:

Dapsone, in acidic medium, is allowed to react with excess nitrite to form the corresponding diazonium salt:

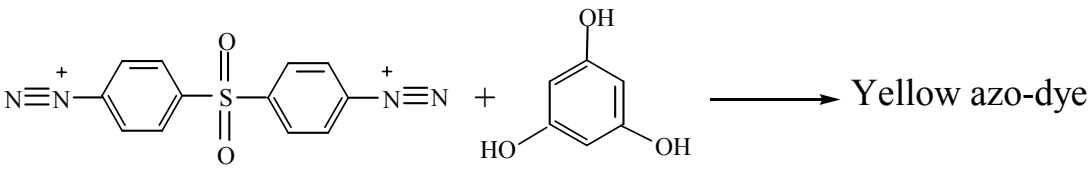


Diazotized dapsone

After removal of residual nitrite (as nitrous acid) with sulphamic acid:



The diazotized dapsone is then coupled in a basic medium with phloroglucinol to form, an intensely-yellow coloured azo dye:



Study of the optimum reaction conditions:

The effect of various parameters on the absorption intensity of the coloured azo-dye are investigated and the optimum reaction conditions have been selected.

Effect of acids:

The effect of different amounts of different acids (strong and weak) has been investigated to examin their effect on the intensity of the coloured azo-dye. The results are shown in (Table 1).

Table 1: Effect of diazotization acid on absorbance

Acid used (1N)	**Absorbance /ml of acid used for diazotization**			
	1.0	**2.0**	**3.0**	**4.0**
HCl	0.523	0.520	0.487	0.454
HNO_3	0.488	0.479	0.362	0.330
H_2SO_4	0.497	0.510	0.505	0.468
H_3PO_4	0.305	0.276	0.198	0.093
CH_3COOH	0.367	0.362	0.333	0.236
HCOOH	0.486	0.365	0.390	0.251

The results show, that 1ml of 1N hydrochloric acid solution give the best results.

Effect of sodium nitrite amount and time:

Table 2 shows that the maximum absorbance reading is obtained by adding 1ml of 1% sodium nitrite with 3 minute reaction time.

Table 2: Effect of sodium nitrite amount and time

ml of $NaNO_2$ solution(1%)	**Absorbance /minute standing time**					
	0	**1**	**2**	**3**	**5**	**10**
0.5	0.478	0.492	0.498	0.471	0.468	0.465
0.7	0.508	0.511	0.520	0.517	0.516	0.514
1.0	0.515	0.522	0.525	0.527	0.520	0.516
1.5	0.520	0.516	0.511	0.515	0.513	0.510

Effect of sulphamic acid amount and time:

The excess of nitrous acid is removed by the addition of sulphamic acid solution (Bladyga and Bourne, 1999). The effect of sulphamic acid amount and time has been studied and the results are shown in (Table 3).

Table 3: Effect of sulphamic acid amount and time

ml of sulphamic acid solution(3%)	Absorbance /minute standing time					
	0	1	2	3	5	10
0.0	0.133	0.140	0.148	0.146	0.140	0.136
0.1	0.340	0.353	0.355	0.360	0.360	0.358
0.3	0.420	0.423	0.428	0.426	0.426	0.425
0.5	0.462	0.470	0.476	0.475	0.477	0.476
0.7	0.488	0.495	0.496	0.495	0.496	0.494
1.0	0.518	0.526	0.530	0.532	0.529	0.527
1.5	0.511	0.510	0.509	0.509	0.508	0.505

The results in the above table absorved that the addition of 0.7 ml or more from 3% sulphamic acid and waiting for 3 to 5 minutes after this addition were enough to give maximum absorption of resulting azo-dye. Therefore 1ml of 3% sulphamic acid solution has been selected with 3 minutes as standing time for the reaction .

Effect of phloroglucinol amount:

The effect of different amounts of 0.5% phloroglucinol solution has been studied on the intensity of absorbance at different amounts 10-250 μg/25 of dapsone and the results are shown in (Table 4).

Table 4: Effect of coupling agent amount on absorbance

ml of 0.5% phloroglucinol solution	Absorbance / μg dapsone present in 25 ml								
	10	20	30	50	70	100	200	250	r^2
0.3	0.140	0.250	0.370	0.533	0.684	0.949	1.233	1.645	0.962509
0.5	0.122	0.231	0.334	0.535	0.738	0.977	1.909	2.172	0.995002
1.0	0.113	0.236	0.335	0.521	0.703	0.981	1.678	2.015	0.994189
2.0	0.115	0.233	0.346	0.543	0.731	0.990	1.874	2.153	0.995003
3.0	0.133	0.254	0.377	0.584	0.774	1.062	1.857	2.196	0.994284
4.0	0.119	0.243	0.359	0.590	0.792	1.056	1.876	2.493	0.995957
5.0	0.141	0.265	0.385	0.599	0.807	1.083	1.926	2.244	0.993807

Although the results in (table 4) indicate that the sensitivity increases with increasing the phloroglucinol amount, 0.5 ml of 0.5% phloroglucinol gives good sensitivity and good determination coefficient (r^2=0.995002) as well as decreases the loss in the reagent, so that it could used for the subsequent experiments.

Effect of surfactants:

Several types of surfactants (cationic, anionic and nonionic) have been studied (Table 5).

Table 5: Effect of surfactant on absorbance

Surfactant solution (0.1%)	Absorbance /ml of 0.1% surfactant			
	0	1	3	5
CTAB	0.530	0.509	0.458	0.425
SDS	0.530	0.479	0.492	0.482
Triton x-80	0.530	0.498	0.481	0.484
Tween 20	0.530	0.379	0.352	0.330
CPC	0.530	0.326	0.398	0.407

The obtained results reveal that the presence of surfactants has no effect at all. Therefore, it has been recommended to eliminate the use of surfactants in the subsequent experiments.

Effect of base amount:

This investigation showed that the azo-dye is formed in alkaline medium, therefore a different types and amounts of strong and weak bases have been studied (Table 6). The results indicate that a volume of 3 ml of 1N sodium hydroxide gives higher absorbance value (the pH of final reaction mixture is 10.36). Therefore, it has been selected for the subsequence experiments.

Table 6: Effect of base on absorbance

Base solution used (1N)	Variable	Absorbance and pH /ml of base used				
		1	2	3	4	5
NaOH	A	0.489	0.533	0.535	0.530	0.517
	pH	4.88	9.81	10.36	10.54	11.70
KOH	A	0.272	0.328	0.520	0.512	0.509
	pH	4.50	11.62	12.18	12.57	12.89
NH_4OH	A	0.294	0.312	0.441	0.490	0.469
	pH	2.63	8.47	9.32	9.61	9.80
Na_2CO_3	A	0.219	0.276	0.335	0.381	0.368
	pH	2.71	8.21	9.15	9.78	10.50
$NaHCO_3$	A	0.135	0.148	0.232	0.254	0.240
	pH	1.52	5.83	6.42	7.85	8.90
CH_3COONa	A	0.256	0.263	0.271	0.252	0.258
	pH	1.46	2.95	4.13	4.56	5.04
HCOONa	A	0.243	0.278	0.274	0.281	0.278
	pH	1.51	1.55	2.01	2.97	3.48

Effect of time:

The coloured azo-dye is developed rapidly after addition of base and exhibits maximum intensity at room temperature after 10 minuts. The colour is stable for at least 1 hour and 30 minutes and the results are given in (Table 7).

Table 7: Effect of time and dapsone amount on absorbance

μg of dapsone present	Time /(min)													
	0	5	10	20	30	40	50	60	70	80	90	100	110	120
50	0.498	0.503	0.525	0.530	0.532	0.532	0.533	0.533	0.533	0.533	0.533	0.533	0.511	0.480
100	0.864	0.866	0.876	0.878	0.878	0.878	0.878	0.878	0.879	0.878	0.878	0.878	0.868	0.832
250	1.995	1.998	2.09	2.015	2.015	2.014	2.015	2.015	2.014	2.015	2.015	2.015	1.990	1.965

From the above table the development time is 10 minuts and the stability period is at least 1 hour and 30 minutes and this is sufficient to several measurements to be performed sequentially.

Final absorption spectra:

Under the above established optimized conditions, absorption spectra of the azo-dye formed in the reaction mixture against its corresponding reagent blank and of the balnk against distilled water are recorded and shown in (Fig.2). The maximum absorption (λ_{max}) at 435 nm for the yellow azo-dye has been used.

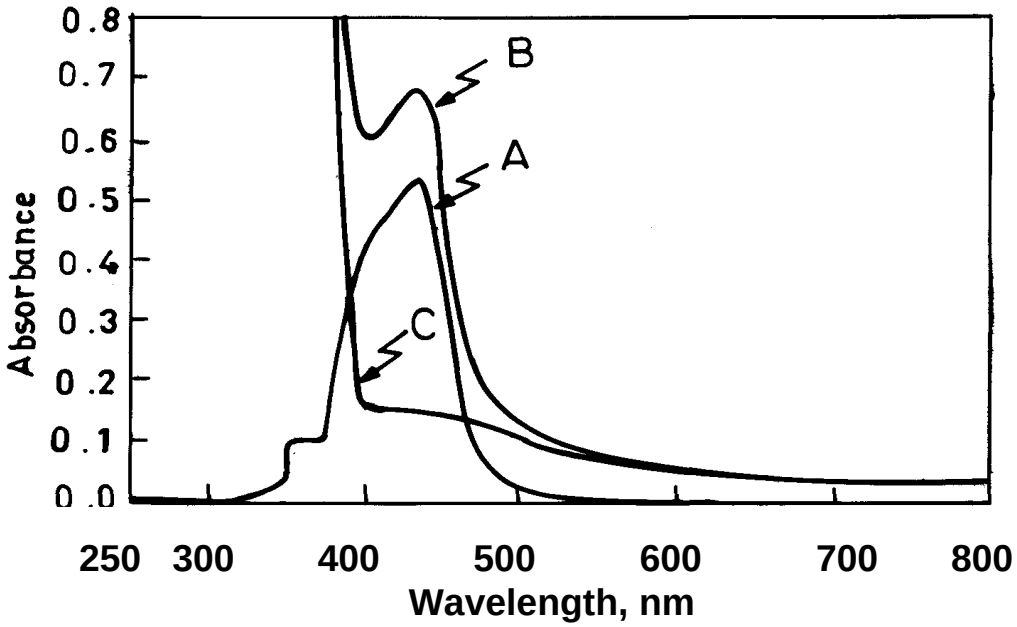


Fig. 2: Absorption spectra of 50 μg of dapsone /25 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Accuracy and precision of the method:

To check the accuracy and precision of the method, dapsone is determined at three different concentrations and the results are shown in Table 8, which indicate good accuracy and precision.

Table 8: Accuracy and precision

Amount of dapsone taken, μg/25 ml	Relative error, %*	Relative standard deviation, %*
10	+ 0.121	± 3.961
50	- 0.188	± 0.943
100	- 0.340	± 0.851

* Average of five determinations

Nature of the azo-dye:

The composition of the intense yellow azo-dye has been established using Job method (Hargis ,1988) of continuous variations this method is based on the variation of the optical densities of solution containing different ratios of the drug and reagent, while simultaneously maintaining a constant total concentration of the reactions (Fig.3).

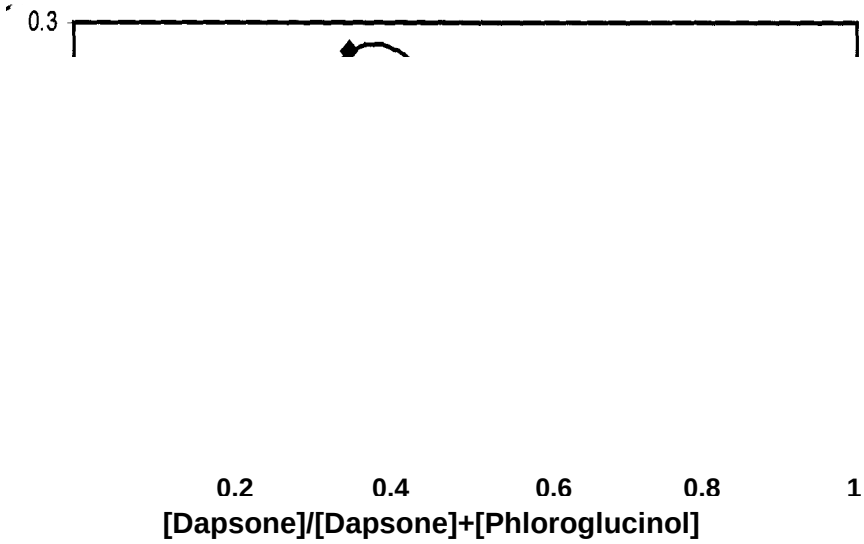


Fig. 3: Continues variation plot for diazotized dapsone-phloroglucinol azo-dye.

In order to prove the ratio of the azo-dye, the mole-ratio method has been used which included added different amount of the reagent to fixed amount of the drug with the same concentration (Fig. 4).

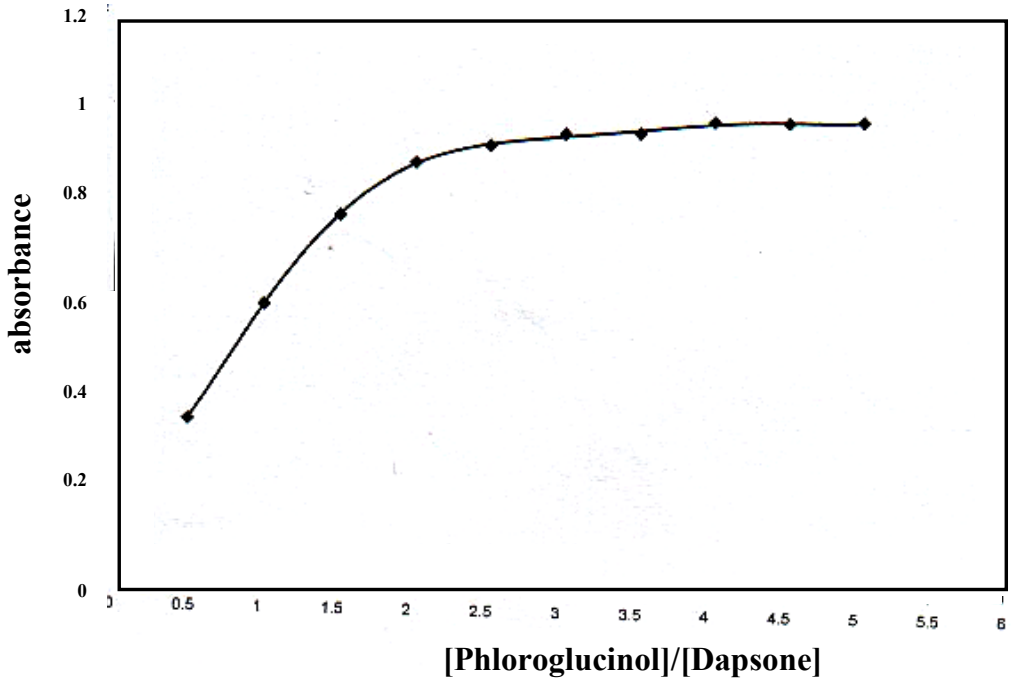
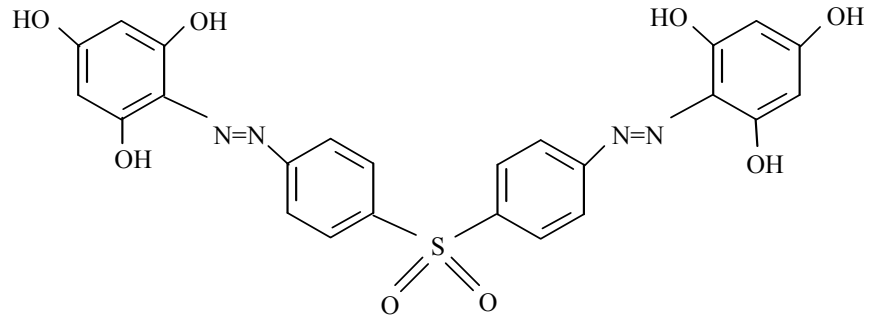


Fig. 4: Mole-ratio plote for diazotized dapsone-phloroglucinol azo- dya

The results indicate that the azo-dye has been formed in the ratio of 1:2 (dapsone: phloroglucinol), and the azo dye may have the following suggested structure:



Yellow azo-dya

The apparent stability constant of the dye formed has been calculated by: (Hargis, 1988).

$$K = \frac{1-\alpha}{4\,\alpha^3 C^2}$$

where :

K= conditional stability constant of the dye.

C= final concentration of the azo-dye.

α = degree of dissociation, and can be determined from the equation:

$$\alpha = \frac{A_m - A_s}{A_m}$$

where :

A_m= The absorbance of the solution containing excess amount of the phloroglucinol.

A_s= The absorbance of the solution containing stoichiometric amount of the diazotized dapsone and phloroglucinol.

The results are given in (Table 9)

Table 9: The stability constant of dapsone azo-dye with phloroglucinol as azo coupling reagent

ml of 2×10^{-4} M Dapsone	**Absorbance**			**K ,$L^2.mol^{-2}$**
	As	**Am**	**Δ A**	
0.5	0.093	0.232	0.139	2.915×10^{10}
1.0	0.243	0.430	0.187	2.704×10^{10}

The avarge stability constant of the dye in aqueous solution under the established experimental condition, 2.809×10^{10} $L^2.mol^{-2}$, which indicates that a stable dye products is formed.

Effect of organic solvents:

The spectrophotometric characteristics of the azo-dye in various organic solvents are given in Table 10. Water is shown to be a good medium from the point view of sensitivity and economy.

Table 10: Spectrophotometric characteristics of the azo dye in various organic solvents

Solvent*	**λ_{max} ,nm**	**ε ,L.mol^{-1}.cm^{-1}**
Acetic acid	439.5	2.275×10^4
Acetone	---	Turbid
Dimethyl sulphoxide	434.0	2.67×10^4
Ethanol	---	Turbid
Formic acid	---	Turbid
Methanol	---	Turbid
2-methoxy ethanol	---	Turbid
n-propanol	435.5	2.83×10^4
Pyridine	---	Turbid
Water	435.0	4.79×10^4

* solvent used in dilution the flask to the mark.

Study of interferences:

In order to realize the analytical application of this method, the effects of foreign compounds have been studied by carryingout the determination of 50 μg of dapsone in the presence of each of the interferent using the recommended procedure. The obtained results are shown in (Table 11).

Table 11: Effect of foreign compounds on determination of 50 μg of dapsone.

Interferes	**Recovery % of 50 μg of dapsone per μg foreign compound added**				
	100	**200**	**400**	**500**	**1000**
Acacia	96.42	97.32	99.64	100.17	97.38
Glucose	98.95	98.26	97.74	95.78	97.79
Magnesium stearate	99.47	100.52	98.09	98.95	98.47
Lactose	102.06	100.00	104.43	102.58	103.34
Starch	99.47	99.30	99.82	100.17	100.97
Sucrose	100.68	100.00	100.34	100.17	99.69
EDTA	100.00	99.15	99.66	99.70	101.35

Experimental results showed that there was no interference from excipients for the examined method up to 100-fold excess.

Application of the method:

To test the applicability of the present method, it has been applied to the determination of dapsone in pharmaceutical preparation (Tables), the results are shown in (Table 12).

Table 12: Determination of dapsone in tablet.

pharmaceutical preparation	**Certified value (mg)/ tablets**	**μg dapsone present/25 ml**	**μg dapsone found/ 25 ml**	**Recovery * (%)**
Dapsone	100	50	50.94	101.88
		100	101.02	101.02
		200	197.02	98.51

* Average of three determinations

For the reason of absence the requirements of the standard method of the determination of dapsone in british pharmacopoeia, standard addition method (Skoog *et al.*, 2000) has been used in determination of dapsone under investigation in order to prove that the proposed method is applied in the determination of dapsone without interferences (Table. 13) and (Fig. 5).

Table 13: Compartion between proposed method and standard addition method

pharmaceutical preparation	Certified value (mg)/ tablets	μg dapsone present/25 ml	μg dapsone found/ 25 ml	Recovery * (%)
Dapsone	100	50	51.0	102.0
		100	101.5	101.0

- Average of three determinations

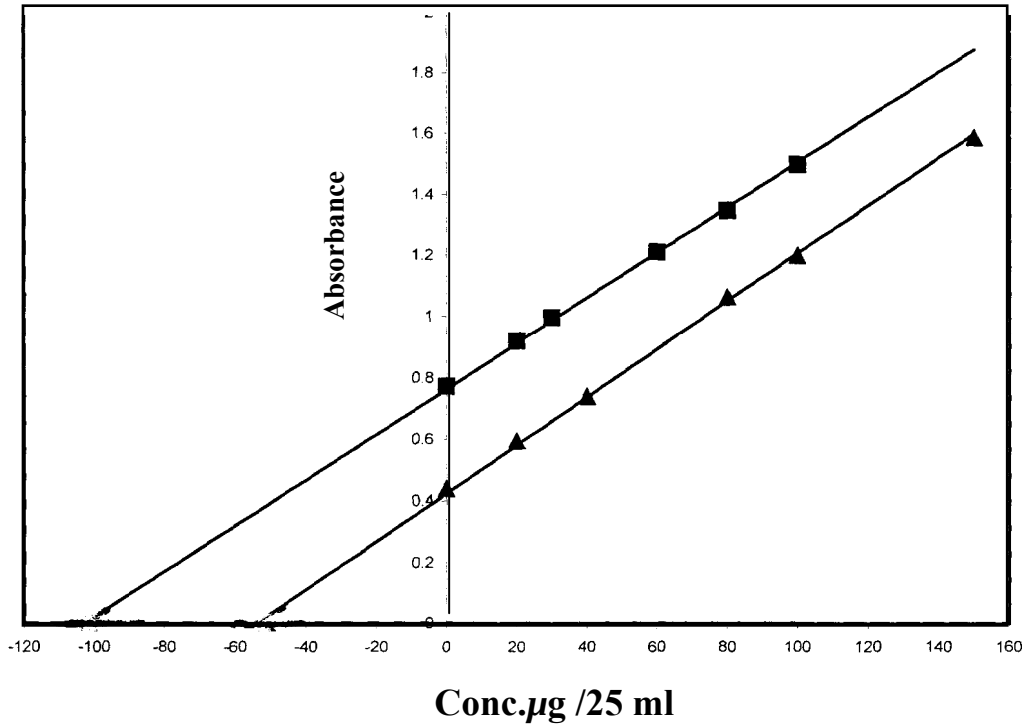


Fig 5: Graph of standard addition method for the determination of dapsone in tablets.

The results in (Table 13) and (Fig. 5) indicated that the present method to suggested for the determination of dapsone can be used with satisfactory results.

Comparison of the methods:

Table 14, shows the comparison between some of analytical variables for the present method with those of the recent spectrophotometric methods.

Table 14: Comparison of the methods.

Analytical parameters	**Present method**	**Literature method (AL-Abachi,1995)**	**Literature method (AL-Gabsha,2004)**	**Literature method (AL-Ramadani,2007)**
Type of method	Azo coupling	Oxidative coupling	CT-Complex	Azo coupling
Reagent	Phloroglucinol	Promethazine-hypochlorite	DDQ	α- naphthol
λ_{max}	435	604	344	592
Colour of the dye	Yellow	Bluish-green	Yellow	Blue
Beer's law (ppm)	0.4-10	0.2-4	0.4-12	0.2-7
Molar absorptivity (L. mol^{-1}. cm^{-1})	4.79×10^4	2.9×10^4	6.3×10^3	6.06×10^4
pH	10.36	2.5	9	11.06
Medium	Aqueous	Aqueous	Aqueous	Aqueous
Temperature	R.T	R.T	40	R.T
Development time (min)	10	---	10	5
Average recovery (%)	100.69	100.26-101.6	99.7	100.82
RSD (%)	0.85-3.96	0.27-0.54	< 2.17	< 2
Analytical application	Tablets	Tablets	Tablets	Tablets

CONCLUSION

A sensitive and simple spectrophotometric method for the assay of dapsone drug in aqueous solution has been investigated, it is based on the reaction of the diazotized dapsone with phloroglucinol as coupling reagent to form an intense yellow coloured, water-soluble and stable azo dye which exhibits maximum absorption at 435nm. The proposed method requires neither temperature control, nor solvent extraction and it can be applied successfully to the assay of drug in tablets.

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