Colorimetric and Kinetic method for determination of Dapsone in bulk and medicinal preparations

Safaa Mohamed Abdel Alhamid*
National Organization for Drug Control and Research

*Corresponding Author: E-mail: safaa.hoballah@gmail.com; Mobile phone: 002-02-01090276660

ABSTRACT

Simple, rapid, accurate, and sensitive method for determination of dapsone in bulk and pharmaceutical preparation the method was based on reaction of 4-nitro phenol with dapsone in presence of acid medium and heating, forming yellow complex, having absorption maximum at 460 nm. Beer's law was obeyed in the concentration range of (3-50) µg/ml with correlation coefficient equal to 0.9999 and relative standard deviation (RSD) less than 1.0%, the recovery was found to 99.99%. The method can be applied successfully to quality control laboratories and can be applied to pharmaceutical preparation.

Key words: colorimetric, Kinetic, Dapsone, 4-nitro-phenol, validation.

INTRODUCTION

Dapsone (diamino-diphenylsulfone, DDS) is a white or slightly yellowish-white crystalline powder. It discolors on exposure to light but this is not accompanied by a significant decomposition (Moffat, 2003).

Dapsone is widely employed as effective antibiotic for prophylaxis against Pneumocystis carinii pneumonia and opportunistic disease in (HIV) infection; it's approved as antibiotic by food and drug administration since 1963 (Sailourglenisson, 2000). Dapsone is a medication most commonly used in combination with rifampicin and clofazimine as multidrug therapy (MDT) for the treatment of mycobacterium lepres infections (Leprosy), and with pyrimethamine in the treatment of malaria (Croft, 2007).

Among the various methods available for the estimation of dapsone are electrophoresis, gas chromatography and HPLC. Spectrophotometry is still preferred technique due to its simplicity, several spectrophotometric methods are available for the estimation of dapsone using diazotization and coupling with α-naphthol in the presence of sodium carbonate (Mohammed, 1994), dibenzoylmethane in an alkaline medium (Revanasiddappa, 2001) N- (1-naphthyl) ethylenediaminedihydrochloride (N-NEO) in a hydrochloric acid (Nagaraja, 2001), aminodibenzyl in alcohol medium (Nagaraja, 2002), 3-amino phenol in aqueous medium (Nagaraja, 2003), sodium 1,2-maphthaquinone-4- sulfonic as the chromogenic reagent (Wang, 2004), benzylacetone in alkaline medium (Omran, 2005), α-naphthol in strong alkaline medium in the presence of ceatovln (AL-Ramadani, 2007) and phoroglucinol in basic medium (Daood, 2008), charge transfer reactions are used for the determination of dapsone using different reagents such as chloranil (Mahmoud, 2000), flouranil (Al-gbabasha, 2004), 2,3-dichloro-5,6-dicyano benzoquinone (DDQ) (Al-gbabsha, 2004). Other methods used oxidative coupling reaction with different reagents such as permethazine in the presence of hypochlorite as oxidizing agent (Al-Abachi, 1995), 4-amino-N, N-diethyl aniline in the presence of dichromate in acidic medium (Al-Abachi, 1997), by using the same method, replaced the reagent with 4-amino N, N-dimethyl aniline (Al-Talib, 1997) and N,N-diethyl-p-phenylenediaminesulphate in the presence of KIO₄ was used (Nagaraja, 2010), liquid chromatography has been used for the determination of dapsone in human plasma (Shirazi, 2001). In meat and milk (Hadjigeorgiomic, 2009) other chromatographic methods have been used for the determination of dapsone in human plasma using HPLC technique (Queiroz, 1997) and HPLC with ultraviolet (Kwadidijk, 2002) and high-speed gradient liquid chromatography in serum (Luan chen, 2003). Finally, the potentiation of dapsone induced methemoglobunemia by N-acetyl cysteine in rats (Moraes, 2008).

This paper describes simple, accurate and sensitive method for determination of dapsone in pure and pharmaceutical dosage, the method depends on condensation reaction of primary aromatic amino group of dapsone with phenolic group in 4-nitro phenol, leading to formation of yellow colored Schiff's base complex, by this method, it could be study kinetic of reaction. From experimental data, the reaction was pseudo-first order reaction.

MATERIALS AND METHODS

Chemicals and reagents: Dapsone reference standard was supplied by PHARCO pH RMA Ltd, Cairo, Egypt, and tablets were obtained from local market. All chemicals used were of HR grade and water used was doubly distilled.
Instrumentation: UV-Visible double beam instrument with 1-cm path length cell.

Solutions: 0.1% 4-nitro-phenol in methanol. 1M Hydrochloric acid in methanol.

Standard preparation: Stock solution of dapsone (mg/ml) was prepared by dissolving appropriate amounts of dapsone in methanol. A series of working standard solutions were prepared by the appropriate diluting of the above stock solution to get concentration range of (5-50) µg/ml solution of dapsone was found to be stable during analysis time.

Sample preparation: Ten tablets, of dapsone were accurately weighed and powdered. Average weight of a tablet was determined. Quantity of dapsone tablet powder equivalent 25 mg of dapsone was dissolved in methanol and made up to volume in a 50-ml volumetric flask, the solution was sonicated for 15-minutes, a series of diluted solutions of concentration range for (5-50) µg/ml were prepared and calibration curve was made as for standard sample.

Method: Into a series of 10-ml test tubes, an aliquots of standard solution equivalent to concentration ranges of (5-50) µg/ml were transferred and 2.0 ml of 0.1% 4-nitrophenol was added, followed by 3.0 ml of 1 M hydrochloric acid in methanol, all test tubes were heated in water bath for 20-minutes at temperature equals to 55 C°, then the test tubes were cooled and contents of test tubes were transferred carefully into 10-ml volumetric flasks and completed to the mark with methanol and measure absorbance in the range of (300-500) nm, the reaction has yellow color and absorption maximum at 460 nm, all measurements were made against a blank prepared similarly omitting drug.

RESULTS AND DISCUSSION

Method Development: The reaction between dapsone and 4-nitrophenol forms yellow Schiff’s base complex with absorption maximum at 460 nm and this reaction was considered as condensation reaction between aromatic amino group in dapsone and phenol group in nitrophenol in Methanolic hydrochloric acid, with formation of yellow complex and libration of water molecule, to optimize reaction conditions, different factors must be studied, these factors are:

Effect of type of acid: This factor was done by using different acids like hydrochloric, sulphuric, nitric and acetic acid, the experimental data show that hydrochloric acid was the most suitable.

Effect of concentration and volume of hydrochloric acid: By using different molarities and different volumes of acid ranging from (0.5-5.0) ml the results revealed that 3.0ml of 1M hydrochloric acid was the best.

Effect of volume of 4-nitrophenol: By using different volumes of nitrophenol ranging from (0.5, 1.0, 1.5, 2.0, 2.5, 3.0/ml of 0.1%, the experimental data exhibit that 2.0 ml of 0.1% nitro phenol were given the highest color intensity and highest absorbance.

Effect of temperature and heating time: By heating reaction mixture at different temperatures at 30, 40, 50, 60 C° for different time intervals ranging from (5-40) minutes, the best conditions were found that heating reaction mixture at temperature equals 55C° for 20 minutes as it was given highest color intensity of formed complex and highest absorbance.

Stoichiometry of the formed complex: This factor was done by using molar ratio and continuous variation
methods. The studies revealed that the complex between dapsone and 4-nitrophenol was formed by the ratio of 1:1.

**Calibration graph:** Under the above mentioned experimental conditions, a linear relationship was established by plotting concentration of dapsone against corresponding absorbance value. The respond was linear in the concentration range of (5-50) µg/ml of dapsone with correlation coefficient of 0.9999 with slope and intercept equals to 0.228 and 0.00111, respectively under the experimental conditions, the values of LOD and LOQ. Were found to be equal to 7.23 and 24.12, respectively.

![Graph](Figure2.png)

**Figure 2. Beer's law of reaction of dapsone and 4-nitrophenol**

**Validation of the method:**

**Precision:** The intraday and interday precision values were calculated for three concentrations of dapsone. The RSD values were less than 1.0% demonstrating that the method was precise.

**Accuracy:** The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of dapsone ranges from 99.99%-100.11% with mean recovery of 99.98% and % RSD of 0.98%. The recovery of dapsone by proposed method is satisfactory as % RSD in not more than 2.0% and mean recovery between 99.99%-100.11%.

**Ruggedness and Robustness:** The method’s robustness and ruggedness was determined by changing the following parameters: volume of 4-nitrophenol, effect of heating time, volume of hydrochloric acid, concentration of hydrochloric acid and temperature range and measure corresponding values of absorbance, the experimental studies revealed that no significant change in values of absorbance.

**Analysis of marketed formulation:** The drug content was found to be 99.97% with % RSD of 0.37, it was found that no degradation had occurred in the marketed formulation that were analyzed by this method. The low RSD value indicated the suitability of this method for routine analysis of dapsone in pharmaceutical dosage forms.

**Kinetic study of the reaction:** The rate of reaction was found to be dependent on the concentration of dapsone. The rate of reaction was followed at temperature equal to 55°C keeping volumes of 4-nitrophenol and hydrochloric acid are constant, it is clear that the rate of reaction increases as concentration of dapsone increase, which indicating that rate of reaction obey the following equation.

$$\text{Rate} \propto [\text{drug}]$$

$$\Delta A \over \Delta t = K [\text{drug}]^n$$ (1) →

Where K= the rate constant, n= order of reaction, from studies, it is clear that reaction follows the pseudo first-order. The rate of reaction may be estimated by the variable time method measured as $\Delta A / \Delta t$ where A= the absorbance and t= the time in minutes taking logarithms of rate and concentration, the equation (1): was transformed into.

$$\log \text{rate} = \log k + n \log [\text{drug}]$$ (2)

$\Delta A \over \Delta t$ From equation (2), by plotting relation between log k against log [drug], straight line was obtained with slope equals to order of reaction and intercept equals to log rate constant, from the above equation, it is clear that the reaction rate is a function of concentration at fixed temperature, this functional relation between rate and concentration is sometime, called an inhibition or autocatalysis depending on the effect of the product concentration of the reaction rate.
If the substance, which is neither a reactant nor a product, affects the reaction rate, this called an inhibitor, retarder or catalyst, the relation between the rate of reaction and temperature is interpreted by thermodynamic parameters. The reagent molecules are supposed to be in a thermodynamic equilibrium with the transition state, since the energy of the transition state is necessarily higher than that of the reagent, it concentration will increase with temperature and so reaction rate will increase and this is the principle of Arrhenius equation and its empirical expression is a follow.

\[ K = A e^{-\frac{E_a}{RT}} \]  \hspace{1cm} (3)

Where \( A = \) the frequency factor, \( E_a = \) the activation energy (cal/mol) \( A \) and \( E_a \) can be extrapolated from the plot of \( \ln k \) versus \( 1/T \). \( K = \) the rate of reaction. \( R = \) gas constant = 8.314 (Joul/mol.cal). \( T = \) absolute temperature.

Arrhenius equation may be written in logarithmic form.

\[ \ln K = -\frac{E_a}{RT} + \ln A \]  \hspace{1cm} (4)

From equation (4), Arrhenius curve was plotted as \( \ln K \) VS \( 1/T \) which gives a linear relation with a slope equal to \(-E_a/R\) and intercept equals to \( \ln A \), from this relation activation energy can be calculated. By using Eyring equation heat of reaction (\( \Delta H \)) and entropy (\( \Delta S \)) can be calculated. The empirical formula of Eyring equation was as follow.

\[ \ln \frac{K}{T} = (-\Delta H^*/R) \left( \frac{1}{T} \right) + \ln K_g/h + \frac{\Delta S^*}{R} \]  \hspace{1cm} (5)

By plotting \( \ln K/T \) against \( 1/T \), gives straight line with a slope equals to \(- \Delta H^*/R\), \( R = \) gas constant has value equals 8.314 (joul/mole.cal), from slope heat of reaction (\( \Delta H^* \)) can be calculated, from intercept (\( \Delta S^* \)) can be calculated as follow, intercept = \( \ln K_g/h + \frac{\Delta S^*}{R} \)

Where: \( R = \) gas constant = 8.314 Joul / mol.cal.
\( K_g = \) Boltzman constant = 1.38x10\(^{-23}\)
\( h = \) blank constant = 6.626 x 10\(^{-34}\)

From Gibb’s equation, heat content (\( \Delta g^* \)) can be calculated as follow:

\[ \Delta G^* = \Delta H^* - T\Delta S^* \]

Effect of excipients: The effect of different excipients which were found in tablets like talc powder, magnesium stearate, glucose, starch, cellulose on the proposed method has been studied, the data revealed that no significant effect of these excipients on the proposed method, indicating that this method is suitable for determination of dapsone in pure form as well as in pharmaceutical preparation.

Table 1. Analytical parameters for reaction of dapsone with 4-nitrophenol

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>460 nm</td>
</tr>
<tr>
<td>Beers law range</td>
<td>(3-50) ( \mu )g/ml</td>
</tr>
<tr>
<td>Ring bom range</td>
<td>(7-45) ( \mu )g/ml</td>
</tr>
<tr>
<td>Molar absorptivity ( \epsilon ) (Lmol(^{-1}), cm(^{-2}))</td>
<td>3.58X10(^3)</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.228</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.00115</td>
</tr>
<tr>
<td>Standard deviation (SD)</td>
<td>0.55</td>
</tr>
</tbody>
</table>
Table 1. Analytical parameters for reaction of dapsone with 4-nitrophenol continuation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative standard deviation (RSD)</td>
<td>0.74</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>7.23</td>
</tr>
<tr>
<td>Limit of quantitation (LOQ)</td>
<td>24.12</td>
</tr>
<tr>
<td>Inter day recovery %</td>
<td>99.99</td>
</tr>
<tr>
<td>Intra-day recovery %</td>
<td>98.97</td>
</tr>
</tbody>
</table>

Table 2. Statistical results for determination of pharmaceutical preparation by the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proposed method (reaction with 4-nitrophenol)</th>
<th>Official method (HPLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery ±SD</td>
<td>99.99% + 67</td>
<td>98.98% + 58</td>
</tr>
<tr>
<td>Variance</td>
<td>0.321</td>
<td>0.142</td>
</tr>
<tr>
<td>t-test (2.03)</td>
<td>1.15 (2.03)</td>
<td>(0.142)</td>
</tr>
<tr>
<td>F-test (6.05)</td>
<td>(3.36) (6.05)</td>
<td></td>
</tr>
</tbody>
</table>

N= No. of experiments
(2.03) = Tabulated value of t-test
(6.05) = tabulated value of F-test

Overall reaction order is determined from $T_{1/2}$ and initial concentration of drug, so order of reaction was carried out by measuring absorption of reaction mixture at different time intervals, the equation.

$$\log T_{1/2} = \log \text{constant} - (n-1) \log a \quad (1)$$

A plot between $\log T_{1/2}$ and $\log (a)$ gives a straight line with intercept equals log constant and slope equals (n-1), from which, order of reaction (n) can be calculated, from Arrhenius equation.

$$K = Ae^{Ea/RT} \quad (2)$$

Where $k$= rate of reaction equals to $\frac{\Delta A}{\Delta t}$

$$\ln k = -\frac{Ea}{RT} + \ln A \quad (3)$$

A plot between $\ln K$ and, a straight line was obtained with slope equals $-Ea/R$ and intercept equals $\ln A$ from slope and value of $R$ (gas constant), activation energy can be calculated. By using Eyring equation.

$$\frac{\ln K}{T} = \left(\frac{-\Delta H}{R}\right) + \ln K_g/h + \Delta S^* \quad (4)$$

A plot between $\ln K/T$ and, gives a straight line with slope equals $(\Delta H^*/R)$ and intercept equals $\ln K_g/h+\Delta S^*$ from slope value and value of gas constant, heat of reaction $(\Delta H^*)$ can be calculated and from value of blank constant and value of Boltzmann constant, value of entropy $(\Delta S^*)$ can be calculated from value of intercept, from the following equation, heat content $(\Delta g^*)$ can be calculated

$$\Delta g^* = \Delta H^* - T \Delta S^* \quad (5)$$

$$\text{Scheme 1. Reaction of dapsone with 4-nitrophenol}$$

$$\text{Schiff's base dapsone}$$
REFERENCES


Moffat, A.C; Osselton, MD; Widdop, B, Clarke’s Analysis of drugs and poisons” 3th publication division of the Royal pharmaceutical society of great British, London, (internet), 2003.


Nagaraja P, Yahirajan H.S; Raju, C-R Vasantha, R.A, Nagendra, P, Hemantha Kumar, MS, 3-Aminophenol as novel coupling agent for spectrophotometric determination of sulphonamides derivatives, IL Farmaco 58, 2003, 1295-1309.


Sailourglesionsson F, Chene, G; Salmi, L.R; Hafner R; Salamon, Effect of dapsone on survival in HIV infected patient: ameta-analysis of finished trials, Rer Epidemiol santepublique, 48, 2000, 17-30.
