An Ecofriendly and Stability-indicating RP-HPLC Method for Determination of Dapsone: Application to Pharmaceutical Analysis

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ABSTRACT

A green and stability-indicating RP-HPLC method was developed for determination of dapsone in pharmaceutical preparations. The separation was based on a C_{18} analytical column. The mobile phase consisted of formic acid solution (pH = 3):ethanol (90:10, v/v). In forced degradation studies, the drug was subjected to oxidation, hydrolysis, photolysis and heat. Among the different stress conditions, the exposure to light (UV-C and sunlight) was found to be an important adverse stability factor. The drug exhibited degradation in both irradiation experiments affording a common photoproduct. The applied procedure was found to be linear in concentration range of 0.2-50 µg/mL ($r^2 = 0.9999$). Precision was evaluated by replicate analysis in which % relative standard deviation values for areas were found to be below 2.0. The recoveries obtained (99.50-101.38%) ensured the accuracy of the developed method. The peak of dapsone was well resolved from the photoproduct as well as the pharmaceutical excipients. Accordingly, the proposed validated and sustainable procedure was proved to be proper for routine analyzing and stability studies of dapsone in pharmaceutical formulations.

Keywords: dapsone, photoproduct, green analytical chemistry, aniline

1. INTRODUCTION

Dapsone (4,4′-diaminodiphenylsulfone, Figure 1) was discovered in 1908 [1]. It has had medical applications for more than 7 decades for treating various medical conditions including dermatitis herpetiformis, leprosy, and malaria [2]. Although dapsone has a dual therapeutic activity and demonstrates both antimicrobial and anti-inflammatory properties, its oral administration for treatment of acne has been discontinued because of its potential for systemic toxicity. Whereas, topical hydrogel of dapsone (5%), which has been marketed since late 2008, minimizes the risk of toxicity associated with systemic exposure and offers documented efficacy for the reduction of both inflammatory and non-inflammatory acne lesions [3,4].
In view of dapsone novel wide use, validated and stability-indicating methods should be available to determine this drug in topical preparations. The United States Pharmacopeia (USP) presents a Normal-phase (NP) HPLC method for assay of dapsone in raw material and tablet formulation. In addition, no method was compiled in the United States and British Pharmacopoeias for the analysis of this medicine in topical preparations. Moreover, literature survey provides little information about the stability-indicating methods for determination of dapsone in topical preparations. Also in all of the previously reported HPLC methods designed for pharmaceutical and biological analysis, acetonitrile or methanol has been used as a part of mobile phase. It is worth mentioning that these solvents are ranked by US Environmental Protection Agency (EPA) as hazardous solvents [5] and because of their inherent toxicity [6], safe detoxification of the waste solvents is essential, which may lead to high to very high disposal costs.

Taking ICH guidelines into consideration, the present study describes a simple, validated and stability-indicating analytical method for determination of dapsone in pharmaceutical preparations, which meets the green aspects in analytical chemistry. Also, the calculation of the measurement uncertainty which is based on the validation of the analytical procedures in a laboratory is presented. Moreover, the performances of the method were evaluated and its potential for the determination of dapsone in pharmaceutical preparations was investigated.

2. MATERIALS AND METHODS

2.1 Apparatus

The HPLC method was carried out on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan), set to recycle the mobile phase and was equipped with an SCL-10AVP system controller, LC-10 ADVP pump, DGU-14A degasser, and a SPD-M10AVP PDA detector. The peak areas were integrated automatically by computer using a Shimadzu Class VP software program. A 20µl volume of sample was introduced into a Rheodyne model 7725i injector.

2.2 Chromatographic Conditions

The elution was carried out on a C₁₈ column (150mm×4.6mm, 5µm particle size) from Teknokroma (Barcelona, Spain). All analyses were performed at the column temperature of 30°C ±1 under isocratic conditions with a mobile phase of formic acid solution (pH = 3.0):ethanol (90:10, v/v), and a flow-rate of 1.5 mL/min, using PDA detection at 210 nm.

2.3 Reagents

Dapsone reference material (98.4%), which is required for validation of the method [7] and dapsone as raw material were supplied by Sigma-Aldrich (Munich, Germany), and Merck (Darmstadt, Germany), respectively. All solvents and reagents were of gradient and analytical grade, respectively and were purchased from Merck (Darmstadt, Germany). HPLC grade water was obtained through a Milli-Q system (Millipore, Milford, MA, USA) and was used to prepare all solutions. The pharmaceutical formulations (topical gel 5%) [8] and the corresponding placebos (mixture of all the excipients) were prepared in our laboratory.

2.4 Preparation of Standard Solutions

Stock standard solution of dapsone was prepared in methanol at a concentration of 10mg/mL. This solution was found to be stable for at least one month, when stored at
2-8 °C. Freshly prepared working standards at concentration levels of 0.2, 1, 5, 10, 20, and 50µg/mL were obtained from stock solution by the appropriate dilution with HPLC-grade water.

2.5 Preparation of Test Solutions
A 0.25 g portion of gel (equivalent to 12.5 mg of dapsone) was transferred into a 50 mL volumetric flask. The volume was adjusted to the mark with absolute ethanol to provide a theoretical concentration of 250 µg/mL of dapsone. The solution was diluted with HPLC-grade water to make final concentration of 10µg/mL. The experiment was performed in triplicate. These samples were assayed using calibration curves of working standard solutions. The same procedure was applied to placebo to be sure about the selectivity of the method.

2.6 Method Development
A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of dapsone in pharmaceutical preparations. The suitability of mobile phase was decided on the basis of green analytical chemistry principles, selectivity and sensitivity of the assay, stability studies and separation of dapsone from the impurities formed during forced degradation studies. Different wavelengths were also investigated.

2.7 Forced Degradation Studies
The stability-indicating capability of the method was determined by subjecting dapsone solutions (standard and pharmaceutical preparations) at the concentration level of 100µg/mL to accelerated degradation by acidic, basic, heat, oxidative, and photolytic conditions to evaluate the interferences in the quantitation of dapsone. Sample solutions prepared in 1 M hydrochloric acid and 1 M sodium hydroxide, were used for the acidic and basic degradation, respectively. Both solutions were kept at 80 °C for 12 h, and then neutralized with basic or acidic solutions, as necessary. For evaluating the heat condition, the solutions heated at 80 °C for 12 h. For oxidative degradation, sample solutions were prepared in solutions of hydrogen peroxide (3%) and (30%), kept at ambient temperature for 4 h and five days, respectively, while protected from light. For photodegradation, dapsone solutions were exposed to solar light (Tehran, May-June 2012) and UV-Lamp at a wavelength of 254 nm placed in a wooden cabinet. The solutions were diluted with HPLC-grade water to final concentration of 10 µg/mL and were injected into chromatograph.

2.8 Method Validation
The developed method was validated as per the requirements of the ICH guidelines[9-12]. Linearity was evaluated by determining six working standard solutions at a concentration range of 0.2–50 µg/mL. Five sets of such solutions were prepared. Each set was analyzed to plot a calibration curve. Slope, intercept and coefficient of determination ($r^2$) of the calibration curves were calculated to ascertain linearity of the method. The limit of quantification (LOQ) was defined as the lowest concentrations with the RSDs lower than 5% and accuracies within ±5%, considering at least ten times the response compared to that of the blank. In order to check the robustness, the effect of small but deliberate variations in the chromatographic conditions was evaluated. The conditions studied were flow rate (altered by ±0.2 mL/min), column temperature (altered by ±2 °C) and pH of formic acid solution (altered by ±0.2). These chromatographic variations were evaluated for % assay of the drug. For method repeatability, assay of working standard solutions (0.2, 1, 10 and 50 µg/mL) was repeatedly performed five times on the same day (intra-day). For reproducibility, freshly prepared solutions at aforementioned concentration levels were
analyzed at different days (inter-day) and results were statistically evaluated in terms of % RSD. For recovery studies, 0.25 g portions of pre-assayed dapsone gel 5% were spiked with extra 250, 500 and 1000 µL of stock standard solution. These samples were firstly diluted in 50 mL of absolute ethanol and then in 50 mL HPLC-grade water to make final target levels of 6, 7 and 9 µg/mL. The concentrations were calculated using calibration curves. Accuracy was calculated as the deviation of the mean from nominal concentration. To assess accuracy, freshly prepared placebo of the dapsone pharmaceutical formulations was spiked with various amounts of dapsone to obtain the concentration levels of 0.2, 1, 10 and 50 µg/mL. Each solution was injected in triplicate. Selectivity of this method was indicated by the absence of any excipient interference at retention times of the peaks of dapsone. The absence of interfering peak was evaluated by injecting a blank sample consisting of diluent and placebo. The double check of the lack of interferences of the resulting by-products with the elution of the peaks of dapsone was done by calculating the F factor, meaning the ratio of the UV molar absorption coefficients of dapsone at the 295 (peak) and 230 (valley) nm, respectively, using the Eq.(1):

$$ F = \frac{A(295)}{A(230)} $$

where A(295) and A(230) are the dapsone peak areas obtained at 295 and 230 nm, respectively. The resulting F factors were compared with those of the standards [13]. Moreover, the UV-spectrum of each dapsone peak was acquired during the appearance of the peak in the chromatogram.

2.9 Estimation of the Uncertainty of the Measurements

An expanded uncertainty budget was constructed for dapsone in pharmaceutical preparations by the RP-HPLC-PDA method according to previously reported procedures [14]. Four individual sources including uncertainties associated to the measurement standard, calibration curve, precision and accuracy were taken into account to assess the expanded uncertainty.

3. RESULTS AND DISCUSSION

3.1 Optimization of the Chromatographic Conditions

The HPLC procedure was optimized with a view to develop a green stability-indicating assay method. Green analytical techniques aim to minimize or eliminate the hazardous waste associated with analytical methods. In this context, it is reasonable to use formic acid instead of more toxic additives like trifluoroacetic acid and to make ethanol-water as the mobile phase of choice for many RP-HPLC applications [15, 16]. Therefore, acetonitrile was replaced by ethanol and in order to remove the amine, formic acid solution (pH=3) was used instead of the mixture of glacial acetic acid and triethylamine in mobile phase previously reported for determination of dapsone in human plasma containing water-acetonitrile-glacial acetic acid-triethylamine (80:20:1.0:0.5 v/v/v/v) with the flow rate of 1 mL/min [17]. The usage of this mobile phase resulted in appearance of symmetrical peak of dapsone within 5 min. Injection of light brown photodegraded sample revealed a drastic decrease in dapsone concentration but no impurity was observed until 60 min. Ethanol content of mobile phase was decreased. Subsequently, another peak was observed at the wavelength of 210 nm and mobile phase composition of formic acid solution (pH=3):ethanol (90:10, v/v/v). Even though, the peak shapes and retention times were not still satisfactory. Increasing the flow rate to 1.5 mL/min reduced the retention times and sharpened the peaks. According to these preliminary results, the detection wavelength of
210 nm, flow rate of 1.5 mL/min and the mobile phase of formic acid solution (pH=3):ethanol (90:10, v/v), were finalized. Under such chromatographic condition, the unknown impurity and dapsone separated adequately and appeared at 1.7 and 9.5 min, respectively (Figure 2.(f)). Before fully implemented in the quantitative determination of drug substance and pharmaceutical preparation, this method was thoroughly validated according to ICH guidelines.

**Figure 2.** Typical chromatograms and UV-spectrums of: (a) dapsone working standard solution (10 µg/mL) and after degradation under: (b) acidic condition (c); heat condition; (d) basic condition; (e) oxidative condition: peak 3 = hydrogen peroxide; (f). photolytic condition: Peak 1 = dapsone; peak 2 = aniline.
3.2 Forced Degradation Studies

Stability-indicating method is defined as an analytical method that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities [18]. The forced degradation studies in heat condition, resulted in non-significant decrease of the area (Figure 2.(c)). However, under UV-C light at 254 nm and sunlight, dapsone exhibited a significant decrease, in that only 27.90% of dapsone was recovered after 4h exposure to UV-C. Total degradation of dapsone after 6h exposure to sunlight has been recently reported [19]. It is worth mentioning that one additional hydrophilic peak was detected at 1.7 min in both irradiation conditions (Figure 2.(f)). The acidic and basic conditions also exhibited decrease of the areas to 93.11% and 93.54%, respectively, without any detectable eluting degradation product (Figures 3.(b) and (d)). Under the oxidative condition of 3% of hydrogen peroxide, the drug content decreased to 96.88%, without any additional peak (Figure 3.(e)), indicating that the degradation products did not elute in the HPLC method or may have been degraded to nonchromophoric products. However, when dapsone was exposed to hydrogen peroxide 30%, the recovered amount of dapsone after 120 h was around LOQ. The degradation products of the parent compound were found to be similar for both the pharmaceutical and standard solutions. All the degradation studies are summarized in Table 1.

**Table 1. Summary of stress degradation studies of dapsone.**

<table>
<thead>
<tr>
<th>Stress condition/media/duration</th>
<th>Recovered dapsone (%)</th>
<th>No. of observed impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photolytic/H₂O/254 nm/4h</td>
<td>27.90</td>
<td>1</td>
</tr>
<tr>
<td>Acidic/1.0 N HCl/80°C/12 h</td>
<td>93.11</td>
<td>0</td>
</tr>
<tr>
<td>Neutral/H₂O/80°C/12 h</td>
<td>99.88</td>
<td>0</td>
</tr>
<tr>
<td>Oxidative/3.0% H₂O₂/4 h</td>
<td>96.88</td>
<td>0</td>
</tr>
<tr>
<td>Basic/1.0 N NaOH/80°C/12 h</td>
<td>93.54</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 3.** Typical chromatogram of (a) standard solution of aniline (10µg/mL) and its UV-spectrum, (b) standard solution of dapsone after irradiation and the UV-spectrum of the impurity; Peak 1=dapsone, peak 2: unknown impurity; peak 3= aniline.
3.3 Identification of the Unknown Impurity

Considering the structure of dapsone, the UV-spectrum of the photoproduct and its high polarity, it was concluded that the unknown impurity could be aniline. Therefore, a standard solution of aniline was injected into the chromatograph. The peak of aniline appeared at the same retention time (1.7 min) of that of unknown impurity. Moreover, its UV spectrum was the same as that of aniline standard. The chromatogram of a 10 µg/mL solution of aniline, its UV-spectrum and UV-spectrum of the unknown impurity are shown in Figure 3.

Aniline is rather toxic, with formation of methaemoglobin dependent on exposure-dose. Secondary consequences due to hypoxaemia include seizures, weakness, possibly death and anaemia as symptom of chronic intoxication. Moreover, an increased bladder cancer risk in workers potentially exposed to aniline at chemical manufacturing plants has been previously reported. In addition, aniline penetration into the skin has been documented [20-22]. As most of the previously designed formulations have water as the main excipient [1,8], they could be susceptible to photodegradation. Furthermore, formation of aniline during usage when one is exposed to sunlight could be possible. Consequently, due to the aforementioned marked toxicity of aniline, the pharmaceutical preparations with an aqueous medium should be protected against photodegradation. Moreover, photodegradation of dapsone in aquatic environment by sunlight has been recently investigated and total degradation of dapsone after 6 h has been reported [19]. Considering the results of this study regarding formation of aniline due to photodegradation and its toxicity, the proper handling of the waste in the pharmaceutical companies seems to be of paramount importance.

3.4 Method Validation
3.4.1 Specificity and selectivity

Specificity is the ability of the method to unequivocally assess the analyte response in the presence of its potential impurities that was illustrated by the complete separation of dapsone from degradation products as shown in Figure 2. Furthermore, the decrease observed in the drug content in photostability study, when the degradation product “aniline” appeared, proved the specificity of the method (Table 1). Also, the F factors of dapsone in stress solutions (4.98-5.08) show the same values of that of the standard (5.03). The UV-spectrum obtained during the appearance of the peaks, also confirms their purity. Consequently, the forced degradation studies documented the stability indicating power and specificity of the proposed method.

The application of the whole procedure to placebo samples in order to verify the method selectivity demonstrates that no interferences were detected (Figure 4. (b)).

3.4.2 Linearity, precision and LOQ

Linearity was determined by constructing five independent calibration curves each one with six calibration points of dapsone, including the LOQ, in the range of 0.2–50 µg/mL. The peak areas of the drug against the respective concentrations were used for plotting the graph and the linearity was evaluated by the least square regression analysis. The linearity curve was defined by the following equation y = 236912x + 13001. The value of the determination coefficient calculated \( r^2 = 0.9999 \) indicated the linearity of the calibration curve for the method. Moreover, the relative standard error of slope can be used as a parameter with respect to the precision of the regression, as a general acceptance criterion for the linearity performance of the analytical procedure [23]. This parameter should be comparable to the calculated RSD in the evaluation of the precision. In this study,
the results obtained for the RSD of the slopes was 1.36%, which is comparable to that of the maximum precision value of 1.70% (Table 2).

The results from the validation of method are summarized in Table 2. The method proved to be precise, as the intra- and inter-day precision ranged from 1.38% - 1.68% and 1.57% - 1.70%, respectively. These values fulfill the validation criteria of an analytical method designed for quality control of pharmaceutical preparations for which RSD values <2% are acceptable [23].

The LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. The LOQ of dapsone was determined to be 0.2µg/mL, considering the mean accuracy value of 103.56% and maximum RSD value of 3.77% (Table 2).

### Table 2. Precision, accuracy and recovery data for the proposed method.

<table>
<thead>
<tr>
<th>Dapsone concentration (µg/mL)</th>
<th>Precision (RSD,%)</th>
<th>Accuracy (n=3) mean (RSD,%)</th>
<th>Recovery (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraday (n=5)</td>
<td>Interday (n=5)</td>
<td>Target Concentration (µg/mL)</td>
</tr>
<tr>
<td>0.20</td>
<td>1.19</td>
<td>3.77</td>
<td>103.56 (0.69)</td>
</tr>
<tr>
<td>1.00</td>
<td>1.38</td>
<td>1.60</td>
<td>99.98 (0.61)</td>
</tr>
<tr>
<td>10.00</td>
<td>1.48</td>
<td>1.57</td>
<td>100.83 (0.97)</td>
</tr>
<tr>
<td>50.00</td>
<td>1.68</td>
<td>1.70</td>
<td>99.66 (0.30)</td>
</tr>
</tbody>
</table>

#### 3.4.3 Robustness

The percent of assay was not significantly affected with the slight changes in the chromatographic conditions like alteration in flow rates, pH of the aqueous solution of mobile phase and column temperature. Analysis was carried out in triplicate and only one parameter was changed in the experiments at a time. The calculated assays were 99.70%, 99.65% and 99.98% for the aforementioned variations, respectively. The results of the experimental variables evaluated were within the acceptable deviation (RSD <2%), indicating that the proposed method is robust under the conditions tested.

Figure 4. A chromatogram obtained from analyzing of the pharmaceutical dosage form. The solution contains the target dapsone concentration of 10µg/mL. (b) blank.
3.4.4 Recovery and accuracy

The accuracy was evaluated applying the proposed method to the analysis of the in-house mixture of the gel excipients with known amounts of the drug, to obtain solutions at concentrations of 0.2, 1, and 10 and 50 µg/mL. The accuracy was assessed from three replicate determinations and calculated as the percentage of the drug recovered from the formulation matrix. The means and RSD% obtained for dapsone accuracy are shown in Table 2 with a range of 99.66-100.83% and 0.30-0.97, respectively. These data demonstrate that the method is accurate within the desired range. Also, the results obtained from the analysis of pre-assayed pharmaceutical formulations spiked with different amounts of dapsone stock solution, revealed acceptable recoveries with the mean values of 100.52% %RSDs<1.84. These values document a high recovery in this method.

3.4.5 The uncertainty of the method

The expanded uncertainty of the method for quantification of dapsone in pharmaceutical preparations was calculated. It seems that all of the evaluated sources of error have the same contribution to the expanded uncertainty of the measurement.

3.4.6 Application of the method

The optimized and validated method was applied to the determination of dapsone in a topical gel. The amount of dapsone in gel formulations was calculated using calibration curve method. Typical chromatogram obtained following the assay of the pharmaceutical dosage form is shown in (Figure 4. (a)). The results of the assay undertaken and the calculated uncertainties are shown in Table 3. The value of 99.8% of label claim indicates that the method is selective for the analysis of dapsone without interference from the excipients used to formulate and produce these gels.

**Conclusion**

The aim of this study was to develop a green and specific method for determination of dapsone in topical preparations. The method was designed to be specific, selective, sensitive, robust, reproducible, accurate, inexpensive and easy to perform. The principal advantage of the method is the use of available environmentally friendly solvents and reagents for LC-analyzing to follow the first principle of green chemistry which emphasizes on waste prevention instead of remediation [6]. To the best of our knowledge, this is the first method which is thoroughly green and reports

<table>
<thead>
<tr>
<th>Uncertainties</th>
<th>Dapsone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{\text{standard (%)}}$</td>
<td>0.93</td>
</tr>
<tr>
<td>$U_{\text{calibration (%)}}$</td>
<td>0.87</td>
</tr>
<tr>
<td>$U_{\text{precision (%)}}$</td>
<td>0.90</td>
</tr>
<tr>
<td>$U_{\text{accuracy (%)}}$</td>
<td>0.96</td>
</tr>
<tr>
<td>$U_{\text{expanded (%)}}$</td>
<td>3.66</td>
</tr>
<tr>
<td>$U_{\text{expanded (µg/mL)}}$</td>
<td>0.37</td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>9.98</td>
</tr>
</tbody>
</table>
the metrological parameters in quantification of dapsone in pharmaceutical dosage forms. Moreover, the toxic photodegraded impurity of dapsone was separated and identified using this method.

In conclusion the newly developed method was successfully performed to analysis of dapsone in pharmaceutical preparations and it can thus be used for routine analysis, quality control and for studies of the stability of pharmaceutical formulations containing dapsone.

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REFERENCES