International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 406-412
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Development and Validation of Stability Indicating UV Spectroscopic Method for Estimation of Dapsone

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Received: 15 Jan 2019 / Accepted: 12 Mar 2019 / Published online: 1 Apr 2019
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Abstract

A stability-indicating UV Spectrophotometric method has been developed for analysis of the Dapsone in the presence of the degradation products and is validated as per ICH Q2 R1 guidelines. Dapsone in methanol shows maximum absorbance at 295 nm. The data of linear regression analysis indicated a good linear relationship over the range of 2-20 µgml^{-1}concentrations with a correlation coefficient (R^2) of 0.984. The LOD and LOQ were found to be 0.066 µg ml^{-1} and 1.200 µg ml^{-1}, respectively. Percentage assay of Dapsone tablets was found to be in the range of 98.36 - 101.49 %. Dapsone was subjected to different stress testing conditions. Degradation of Dapsone was mainly found in alkaline and acidic condition. The developed method was found to be simple, accurate and precise for analysis of Dapsone and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

Keywords

Dapsone, Method development, Stability indicating method, Ultraviolet spectroscopy, Validation.

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INTRODUCTION:

Dapsone (DAP) chemically bis (4-aminophenyl) sulphone[1],is an antibiotic commonly used in combination with rifampicin and Clofazimine the treatment of leprosy. It is a second-line medication for the treatment and prevention of pneumocystis pneumonia and for the prevention of toxoplasmosis in those who have poor immune function. Additionally, it has been used for acne, dermatitis herpetiformis and various other skin conditions [2]. Literature survey reveals that few analytical methods have been reported for the estimation of Dapsone in pharmaceutical dosage form including UV-Vis spectroscopy [3,4], high performance liquid chromatography (HPLC) [2,4-9], TLC [5], LC-MS/MS [10] and HPTLC [11].Present work describes a simple, stability indicating UV spectroscopic method and validation for the determination of Dapsone in bulk and tablet dosage form according to ICH guidelines.

MATERIALS AND METHODS

Reagents and chemicals

Dapsone Tablets I.P labeled to contain Dapsone 100 mg was procured from local market. Methanol (AR grade), was purchased from S.D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), acetic acid (CH_3COOH), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Preparation of standard stock solution:
Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg ml^{-1}. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg ml^{-1}. Further 1 ml of this solution was diluted to 10 ml with methanol to get concentration of solution 10 µg ml^{-1}.

Selection of detection wavelength:
From the standard stock solution (1000 µg ml^{-1}) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra were obtained. It was observed that the drug showed linear, stable and considerable absorbance at 295 nm. Representative UV spectrum of Dapsone is shown in Fig. 1.

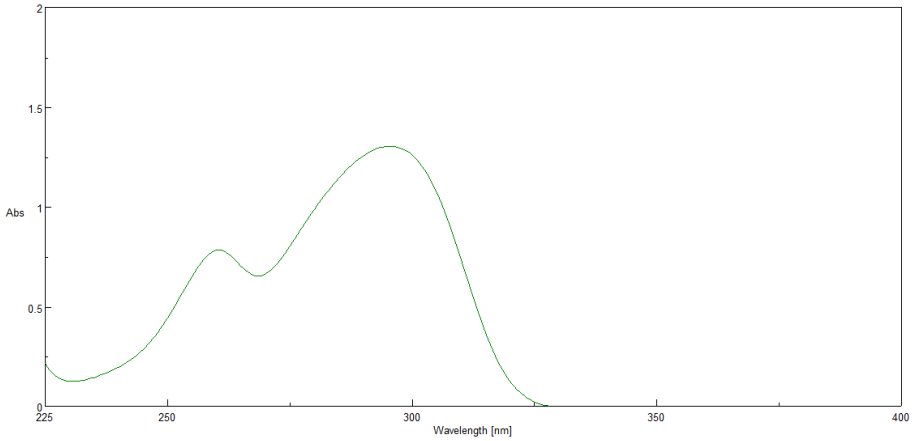

Figure 1: UV Spectrum of Dapsone (10 µg ml^{-1})

Preparation of sample solution:
Twenty tablets were weighed and finely powdered. A quantity of tablet powder equivalent to 10 mg of Dapsone (label claim: 100 mg Dapsone per tablet) was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. This solution was further diluted with methanol to get solution having concentration of 4 µg ml^{-1}.

Stress degradation studies of bulk drug:
Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies were carried out at concentration level of 10 µg ml^{-1}.

Alkaline hydrolysis:
To 1 ml 100µg ml^{-1} solution of Dapsone, 1 ml of 0.1 N NaOH was added. The above solution was kept for 24 hours at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of solution 10 µg ml^{-1}. Resulting solution was taken in cuvette and absorbance was recorded. Under alkaline hydrolysis, percent recovery obtained for Dapsone was 51.73 %. The representative UV spectrum is shown in Fig. 2.

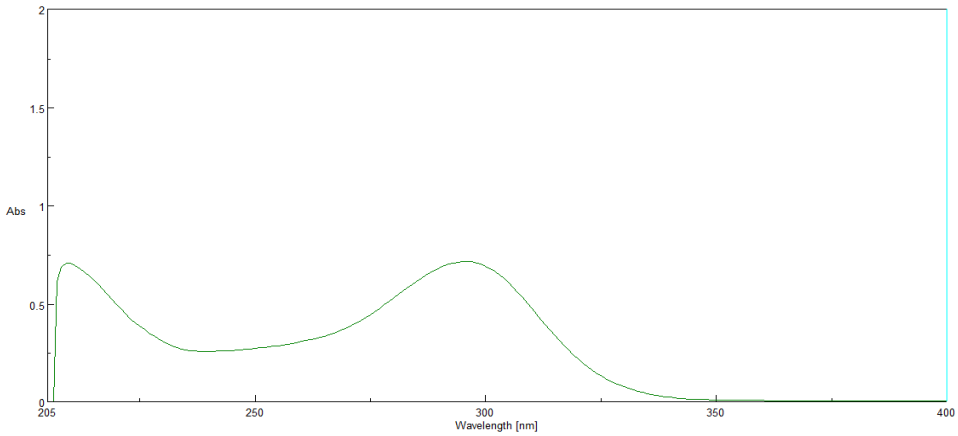

Fig. 2: UV spectrum of Dapsone after alkaline degradation.

Acid hydrolysis:
To 1 ml 100 µg ml^{-1} solution of Dapsone, 1 ml of 0.1 N HCl was added. The above solution was kept for 24 hours at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of solution 10 µg ml^{-1}. Resulting solution was taken in cuvette and absorbance was recorded. Under acid hydrolysis, percent recovery obtained for Dapsone was 46.71%. The representative UV spectrum is shown in Fig. 3.

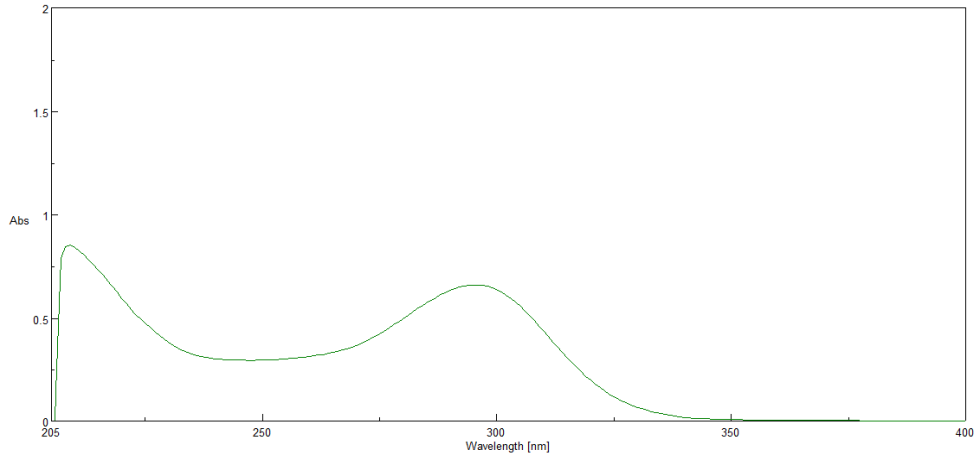

Fig 3: UV spectrum of Dapsone after acidic degradation.

Degradation under oxidative condition:
To 1 ml 100 µg ml^{-1} solution of Dapsone, 1 ml of 30% H_2O_2 was added. The above solution was kept for 24 hours at room temperature. After exposure the volume was made up to 10 ml with methanol to get the concentration of solution 10 µg ml^{-1}. Resulting solution was taken in cuvette and absorbance was recorded. Under oxidative degradation, percent recovery obtained for Dapsone was 84.23%. The representative UV spectrum is shown in Fig. 4.

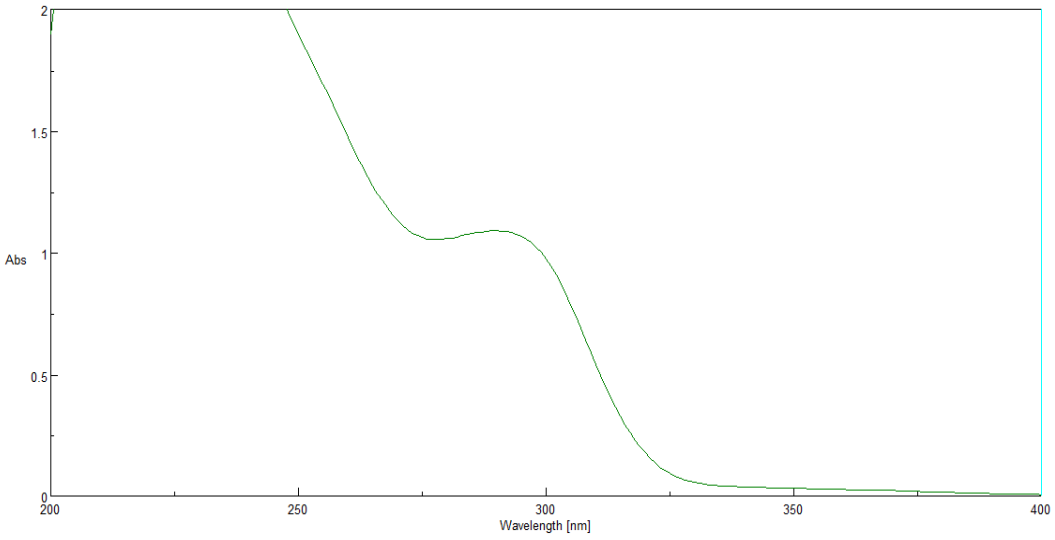

Fig. 4: UV spectrum of Dapsone after oxidative degradation.

Degradation under dry heat:
Dry heat studies were performed by keeping drug sample in oven (80^0C) for a period of 48 hours. A 10 mg of sample was weighed and transferred to 10 ml volumetric flask then dissolved in 10 ml of methanol to get concentration of solution 1000 µg $ml^{-1.}$ From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg ml^{-1}. From this solution, 1 ml was taken and added to 10 ml volumetric flask and volume was made up with methanol to get 10 µg $ml^{-1}$and absorbance was recorded. Under dry heat degradation condition, percent recovery obtained for Dapsone was 98.61%. The representative UV spectrum is shown in Fig. 5.

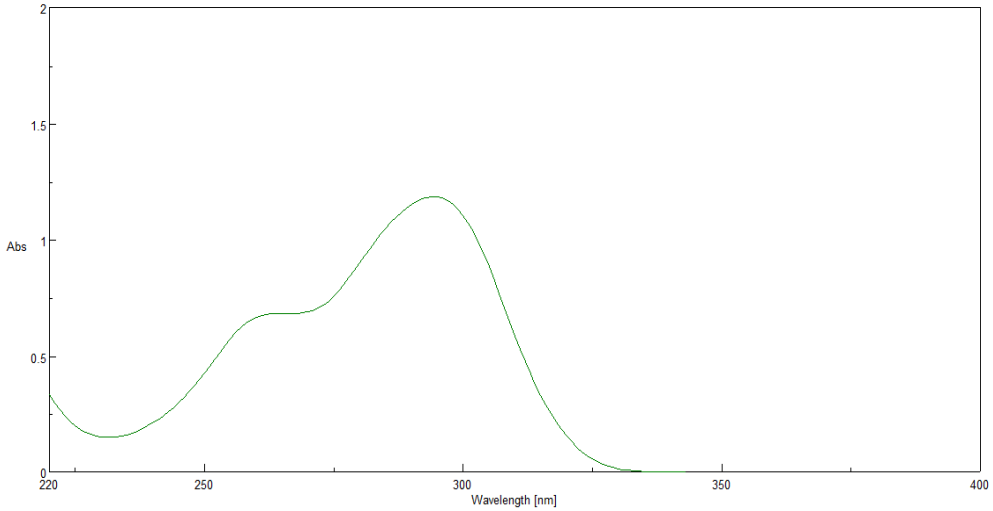

Fig. 5: UV spectrum of Dapsone after dry heat degradation.

Photo-degradation studies:

1.UV illumination for solid drug sample:

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200-watt hr/m^2. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain concentration of solution 1000 µg ml^{-1}.From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg ml^{-1}.From this solution, 1 ml was taken and added to 10 ml volumetric flask and volume was made up with methanol to get 10 µg ml^{-1} and absorbance was recorded. Under photo degradation study by UV light, percent recovery obtained for Dapsone drug solid sample was 81.38%. The representative UV spectrum is shown in Fig. 6.

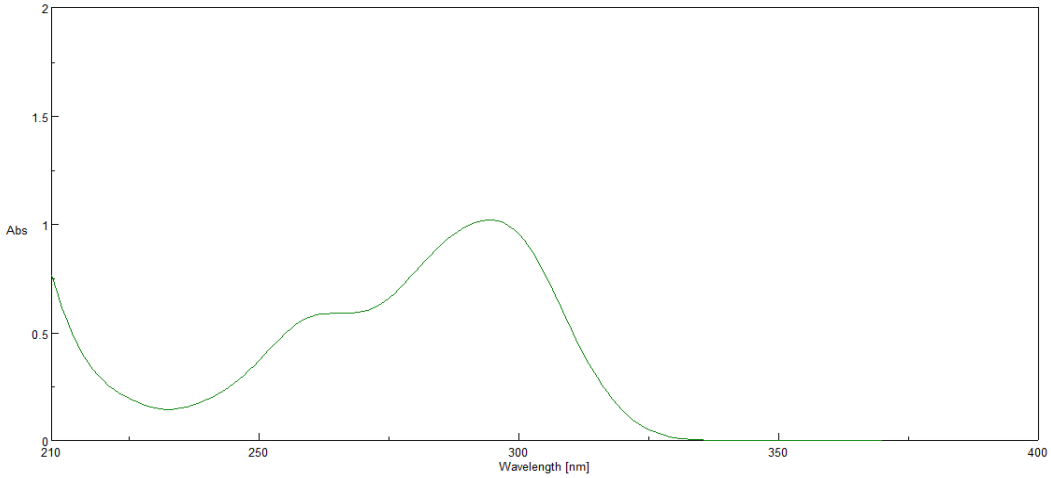

Fig. 6: UV spectrum of Dapsone after photolytic UV degradation of solid drug sample.

2.Fluroscent light:

The photo degradation study of the drug was studied by exposing the drug to fluroscent light providing illumination of NLT 1.2×10^6 Lux hr of fluroscent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain concentration of solution 1000 µg ml^{-1}. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg ml^{-1}. From this solution, 1 ml was taken and added to 10 ml volumetric flask and volume was made up with methanol to get 10 µg $ml^{-1}$and absorbance was recorded. Under photo degradation study by fluroscent light, percent recovery obtained for Dapsone was 87.47%. The representative UV spectrum is shown in Fig. 7.

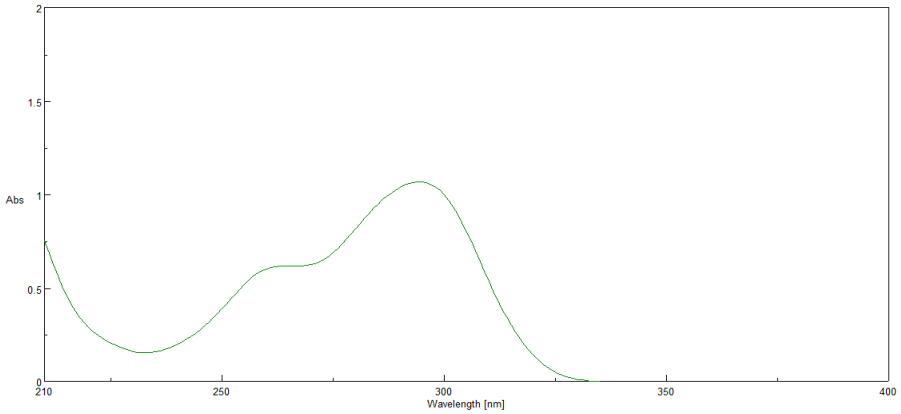

Fig. 7: UV spectrum of Dapsone after photolytic fluroscent light degradation.

VALIDATION OF ANALYTICAL METHOD:[12]

Linearity:

From the standard stock solution (1000 µg ml^{-1}) of Dapsone, solution was prepared containing 100 µg $ml^{-1}$of Dapsone in methanol. This solution was further diluted with methanol to get range of solution containing different concentrations 2-20 µg ml^{-1}. Absorbance was taken at λ_{max}295 nm. The linearity (relationship between absorbance and concentration) was determined by analyzing six solutions over the concentration range of 2-20 µg ml^{-1}. The equation of calibration curve was found to be y = 0.111x + 0.138. The absorbance of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 8.

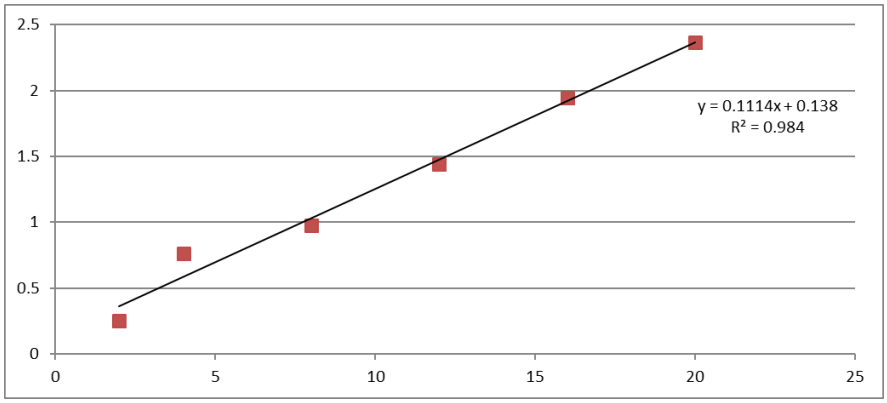

Fig. 8: Linearity curve of Dapsone (2-20 µg ml^{-1}).

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1.

Table 1: Intra-day and Inter-day variation studies data for Dapsone (Precision)

Concentration	Intra-day precision			Inter-day precision		
Conc. (µg ml^{-1})	Conc.	% recovery	%RSD	Conc.	% recovery	%RSD
4	3.819	95.47		4.008	100.20	
	3.912	97.80	1.66	3.872	96.79	1.78
	3.943	98.59		3.912	97.80	
8	7.179	89.73		7.724	96.54	
	7.289	91.11	1.01	7.633	95.41	1.48
	7.319	91.48		7.498	93.73	
16	16.534	103.34		15.943	99.64	
	16.601	103.75	0.21	15.755	98.47	0.84
	16.592	103.70		16.017	100.11	

Limit of detection (LOD) and limit of quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ were found to be 0.066 µg $ml^{-1}$and 1.200 µg ml^{-1}, respectively.

Assay:

Dapsone Tablets (labeled to contain Dapsone I.P. 100 mg) formulation analysis was carried out as mentioned under section preparation of sample solution. Sample solution was taken in cuvette and absorbance was recorded. Basic concentration of sample chosen was 4 µg ml^{-1} from tablet solution. Procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 2.

Table 2: Assay of marketed formulation.

	Absorbance	Amount Recovered (µg ml^{-1})	% Recovery
1	0.5763	3.9344	98.36
2	0.5902	4.0592	101.48
3	0.5851	4.0134	100.33
4	0.5784	3.9533	98.83
5	0.5767	3.9380	98.45
6	0.5789	3.9578	98.94
Mean	0.5809	3.9760	99.40
SD	0.0055	0.0496	1.2421

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard solution to tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 4 µg ml^{-1} of Dapsone. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Table 3: Accuracy of Dapsone.

Level	Amount of sample taken (µg ml^{-1})	Amount of standard taken (µg ml^{-1})	Absorbance	Recovered concentration	% Recovery
50%	4	2	0.798 0.813 0.7871	5.9300 6.0592 5.8268	98.97%
100%	4	4	1.023 1.041 1.012	7.9470 8.1059 7.8456	99.57%
150%	4	6	1.2434 1.2456 1.2398	9.9228 9.9425 9.8905	99.18%

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength (± 2 nm) was altered and the effects on the absorbance was noted. The method was found to be robust.

RESULTS AND DISCUSSION:

The developed method was found to be simple, sensitive, specific, accurate and precise for analysis of Dapsone in bulk and pharmaceutical dosage form without any interference from the excipients. Specificity of the UV method was cross checked by analyzing the same degradation samples by HPLC method [Neosphere C18, 150*4.6 mm, 3.5 µ Column; Mobile Phase – Ammonium acetate buffer (pH 3 adjusted with acetic acid): Methanol 60:40; Retention time – 2.707]Although sample get degraded under acidic, alkaline and oxidative conditions (seen from change in UV spectral pattern) and decrease in percentage purity; but no additional peak observed in HPLC for degradants. This proves that degradants are non absorbing at quantification wavelength 295 nm and developed UV method is specific. The results indicated the suitability of the method to study stability of Dapsone under various forced degradation conditions. Summary of results has been given in Table 4.

Table 4: Summary of Validation Parameters.

Sr. No.	Validation parameters	Dapsone
1.	Detection wavelength (nm)	295
	Linearity equation	y = 0.111x + 0.138
2.	R^2	R^2 = 0.984
	Range	2-20µg ml^{-1}
	Precision	(%RSD)
3.	Intra-day	0.96
	Inter-day	1.36
4.	Assay	98.36-101.49 %
5.	Accuracy	Mean ± %RSD
	50	98.83-100.99%
	100	98.07-101.32%
	150	98.90-99.43%
6.	Limit of detection	0.066 µg ml^{-1}
7.	Limit of quantitation	1.200 µg ml^{-1}
8.	Robustness	Robust

CONCLUSION:

A simple, precise, accurate and stability indicating UV spectroscopic method without interference from the excipients or from degradation products has been developed and validated for the determination of Dapsone as bulk drug and in tablet dosage form. Drug found to be degraded under acidic condition evident from change in UV spectrum pattern of drug. The developed method can be used for quantitative analysis of Dapsone in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

ACKNOWLEDGEMENT:

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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