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A Newer RP - HPLC Method for the Estimation of Dapsone in Bulk and In Pharmaceutical Formulations

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method is developed and validated for the estimation of Dapsone in tablet dosage form. The expected separation and peak shapes were obtained on Luna C18, 15 cm x 4.6 mm (5 µm) column. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol and water with or without different buffers indifferent combinations were tested as mobile phases on a Luna C18, 15 cm x 4.6 mm (5 µm) column. A mixture of Methanol:Water in the ratio of 40:60 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing. The flow rate was 1.0ml/min and effluents were monitored at 260 nm. The retention time for Dapsone was ± 2.4 min. The method was validated for accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Dapsone in bulk and in pharmaceutical formulations without interference and with good sensitivity. And recovery of Dapsone from tablet formulation was found to be 93%. The proposed method was successfully applied for the quantitative determination of Dapsone in tablet formulation.

Keywords: Dapsone, HPLC, Linearity, Validation, 260 nm

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INTRODUCTION

The Molecular formulae for Dapsone is $C_{12}H_{12}N_2O_2S$. It is a white or slightly yellowish-white, crystalline powder, very slightly soluble in water, freely soluble in acetone, sparingly soluble in alcohol. It dissolves freely in dilute mineral acids. Dapsone is an odorless white to creamy-white crystalline powder with a slightly bitter taste, used in combination with pyrimethamine in the treatment of malaria.[1,2] Dapsone is commercially available in both topical and oral formulations. Topical dapsone is available in a 5% gel formulation that is sold under the brand name Aczone and is produced by Allergan. Oral dapsone is also available but is less commonly used than other sulfonamide antibiotics, many of which have a lower incidence of adverse effects.[3] It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.[4] It is used prophylactically to prevent Pneumocystis pneumonia and toxoplasmosis in patients unable to tolerate trimethoprim with sulfamethoxazole.[5] Dapsone is also used to treat Brown recluse spider bites[6] In presumed cases of brown recluse spider bites, dapsone is often used effectively, but clinical trials do not demonstrate similar effectiveness;[7] however, dapsone may be effective at treating many "spider bites" because many such cases are actually misdiagnosed microbial infections.[8] Anjan De1 et.al.,[9] developed that the drug was found to be soluble in 60% methanol. Standard stock solution was prepared in methanol: water (60:40). λ max was found to be 293nm &310nm for zero order &first order. Calibration curves were prepared.

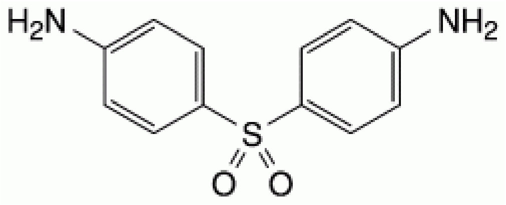

Figure : 1Dapsone

The proposed method obeys Beer's law in the range of 2-6µg/ml. Absorption maxima was determined with 4µg/ml by scanning in the range of 200-400nm. % Recovery studies were in the range of 98.88%-100.25% &101.13%-102.75% for zero order & first order respectively. This shows the adaptability of the method for routine estimation of dapsone in bulk & dosage form. This study thus exploits the possibility for determining Pharmacokinetic profiles of dapsone in rat plasma which may be required in clinical study in near future. Venkata Raveendra Babu Vemula et.al.,[10] proposed a simultaneous estimation of sulphadoxine and pyrimethamine in tablet formulation. The chromatographic analysis was carried out on Xbridge C18column (10.0×4.6 mm, 5µ) with Phosphate buffer: Acetonitrile (80:20 %v/v) as mobile phase, at a flow rate of 1.0 ml/min and with detection wave length 223nm. The retention times for sulphadoxine and Pyrimethamine were 4.3 min and 6.3 min respectively. The calibration plots were linear over the concentration range of 250-1500 µg/ml for sulphadoxine and 12.5 - 75µg/ml for pyrimethamine. The percentage recoveries for sulphadoxine and pyrimethamine were 100.2 % and 99.99 %, respectively. S.L. Bonde et.al.,[11] developed reversed-phase high performance liquid chromatographic/Tandem mass spectrometeric(LC/MS-MS) method for simultaneous Determination of Dapsone andN-Acetyl Dapsone in human plasma using Dapsone D8 as internal standard (IS). The plasma samples were subjected to Liquid-liquid extraction using tertiary butyl methyl ether.Seperation was achieved on a Chromolith C18 Hi-resolution column (100mm×4.6mm ID) using Acetonitrile and 2mM Ammonium acetate as a mobile phase by isocratic elution at a flow rate of 0.8 mL/min. Detection was performed using electrospray ionization in positive ion multiple reaction monitoring (MRM) mode by monitoring the ion transitions from m/z 249.3→156.1(Dapsone), m/z 291.1→156.0(N-Acetyl Dapsone) and m/z 257.3→160.0 (IS). Calibration curves were linear in the concentration range of 0.50–2,500.00 ng/mL for Dapsone, and 0.25–20.00 ng/mL for N-Acetyl Dapsone. Abdalla A et.al., described a[12] simple and sensitive spectrophotometric method for the quantitative analysis of pyrimethamine in pharmaceutical formulations has been described. The method is based on the formation of colored product between PYM and 1,2-naphthoquinone-4-sulfonate (NQS) at 60 °C. The reaction is followed spectrophotometrically by measuring the increase in absorbance at 483 nm as a function of time. Under the optimized reaction condition, Beer's law correlation for the absorbance (A) with PYM concentration (C) was obeyed in the range 12–40 µg mL^{-1} the regression equation for the calibration data was $A = 0.704 + 0.0132C$, with correlation coefficient (0.996). The molar absorptivity (ε) was 5.8×10^3 L mol^{-1} cm^{-1}. The limits of detection and quantification were 3.25 and 10.83 µg mL^{-1}, respectively. The results were in good agreement with those obtained with the official high performance liquid chromatography (HPLC) method.

MATERIALS AND METHOD

Instrumentation:

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Luna C18, 15 cm x 4.6 mm (5 µm) column. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

Chemicals and Solvents:

The reference sample of Dapsone was obtained from the local market. Methanol, water and Acetonitrile used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

The mobile phase:

A mixture of Methanol: Water in the ratio of 40: 60 v/v was prepared and used as mobile phase.

Preparation of the Solutions

The Standard solution of the Drug:

Accurately weigh 100 mg of Dapsone standard into a 100 ml volumetric flask, dissolve in and make up to volume with solvent. Dilute 10 ml of this solution to 100 ml with solvent. Dilute 10 ml of this solution to 100 ml with solvent. Further dilute 10 ml of this solution to 100 ml with solvent. Filter through a 0.45 µm filter.

Preparation of Sample (tablet) solution:

Place swab in 10 ml of Solvent (volume accurately determined). Sonicate for 5 minutes.
Squeeze swab out well. Filter sample through a 0.45 µm filter. Inject the Blank, standard and sample preparation according to SOP ELN_PR_LAB_0006 to test the system
suitability to the following criteria:

Preparation of Standard solution (Stock Solution)

Accurately weigh 100 mg of Dapsone reference standard and quantitatively transfer into a 100 ml volumetric flask (1 mg/ml). Add 60 ml of methanol and sonicate for 15 minutes, cool and make up to volume with solvent. Filter through a 0.45 µm filter before use, discarding the first few milliliters of filtrate. The range of standard solutions from 0.000312 mg/ swab to 1.0 mg/swab was also injected twice and the average result was used in treatment of results.

METHOD DEVELOPMENT

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength:

The spectrum of 10ppm solution of the Dapsone in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Dapsone were showed maximum absorbance at 260nm.

Choice of stationary phase and Mobile Phase:

Finally the expected separation and peak shapes were obtained on Luna C18, 15 cm x 4.6 mm (5 µm) column. A mixture of Methanol : water in the ratio of 40 : 60 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Flow rate:

Flow rates of the mobile phase were changed from 0.5 – 1.5 mL/min for optimum separation. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions

Chromatographic conditions as optimized above were shown in Table: 1 These optimized conditions were followed for the determination of Dapsone in bulk samples and in its Formulations.

Table 1: Optimized chromatographic conditions for estimation Dapsone

Mobile phase	Methanol : Water 40:60v/v
Pump mode	Isocratic
Diluent	Mobile phase
Column	Luna C18, 15 cm x 4.6 mm (5 µm)
Column Temp	Ambient
Wavelength	260 nm
Injection Volume	20 µl
Flow rate	1.0 mL/min
Run time	6 min
Retention Time	± 2.4 minutes

VALIDATION OF PROPOSED METHOD AND REQUIREMENTS:

The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:

The specificity of method was performed by comparing the chromatograms of blank, standard and sample (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are for blank No peak was observed and for Dapsone 2.790 was the retention time in Minutes. The results must show that the solvent solution (solution 1) and placebo solution (solution 2) must not contain any components which co-elute with the active compound peak (solution 3 & 4). Each of the degradation products and impurities must be well resolved from the active compound peak (at least baseline resolution > 1.5) and must elute within the specified assay run time. Determine the peak purity for each of the active peaks in at least the solutions 3 and 4 above. The purity angle must be less than the threshold angle. No components are seen to co-elute with the Dapsone peak, and the peak Purity results indicate that Dapsone peak can therefore be considered spectrally pure. The method employed is specific for the API Dapsone in the product. The solutions listed below (Table :1) were injected using the conditions specified in the method of analysis. The chromatograms obtained are shown in the Figure: 2 to 6. And the Peak purity results are shown in the Figure:7 to Figure:8.

Table: 2

1. Solvent - Methanol (Solution1)
2. Placebo at working concentration - (Solution 2)
3. API at working concentration - (Solution 3)
4. Product at working concentration -(Solution 4)
5. Detergent solution - (Solution 5)

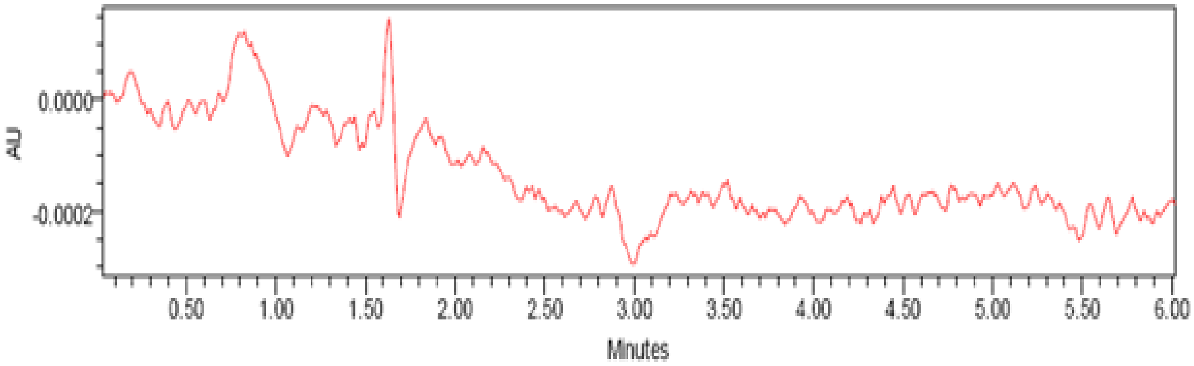

Figure:2 Solvent – no significant peaks detected

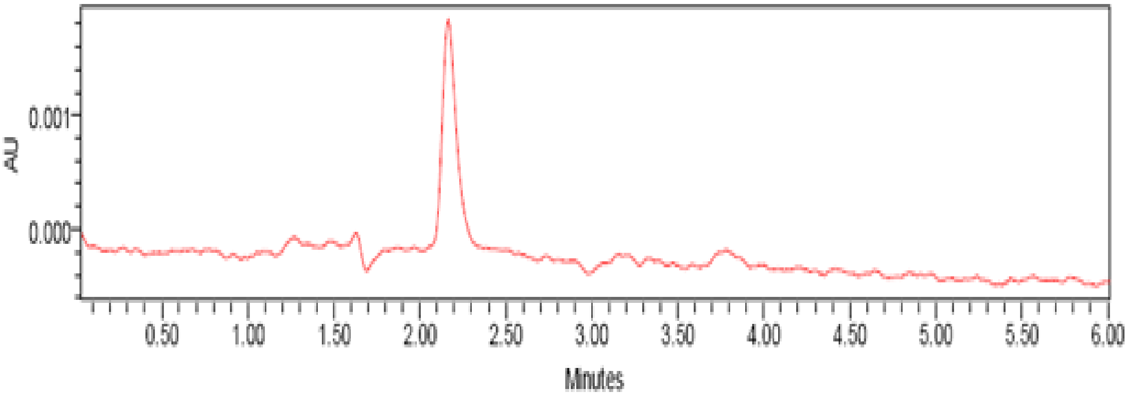

Figure:3 Placebo – no significant peaks detected

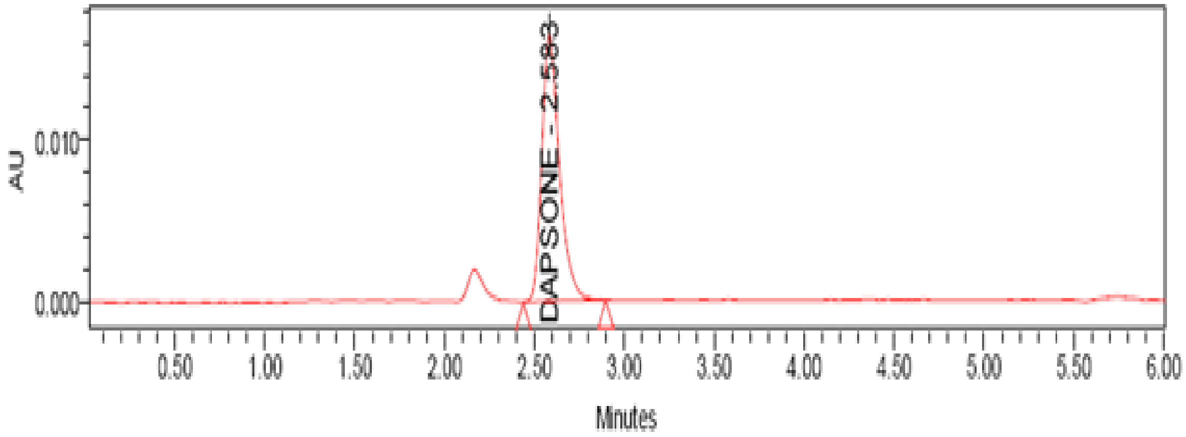

Figure:4 Peak due to Hydrochlorothiazide eluted at about 1.8 min

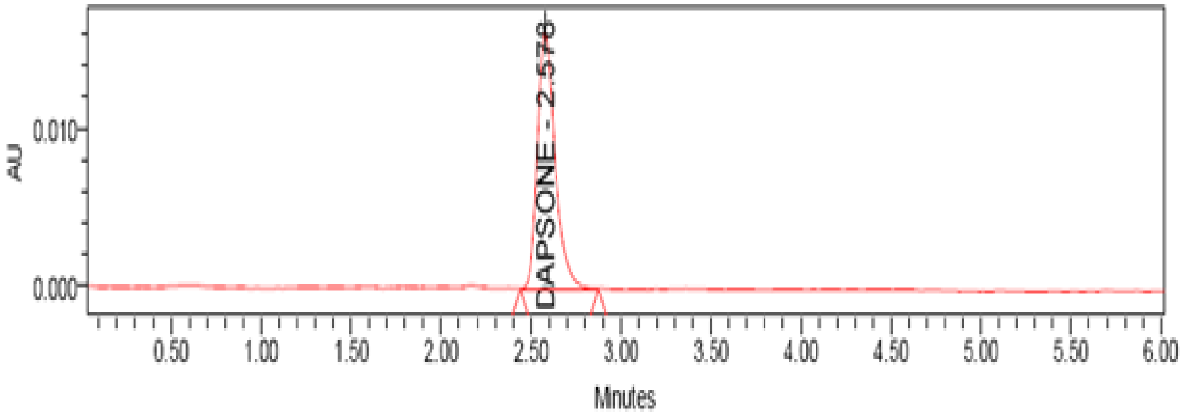

Figure:5 Product - peak due to Hydrochlorothiazide eluted at about 1.8 min

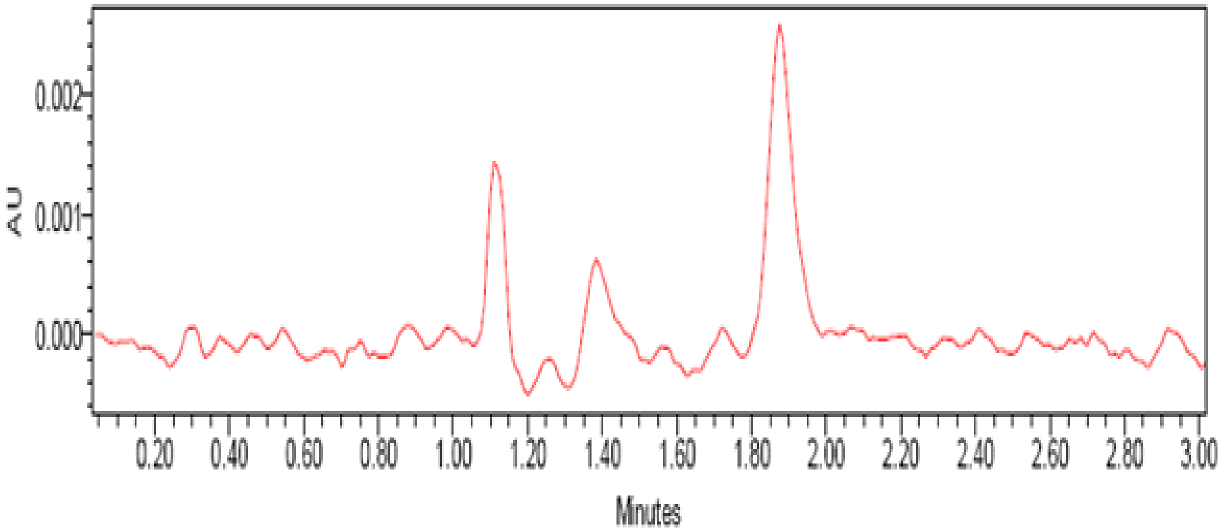

Figure:6 Detergent - no significant peaks detected

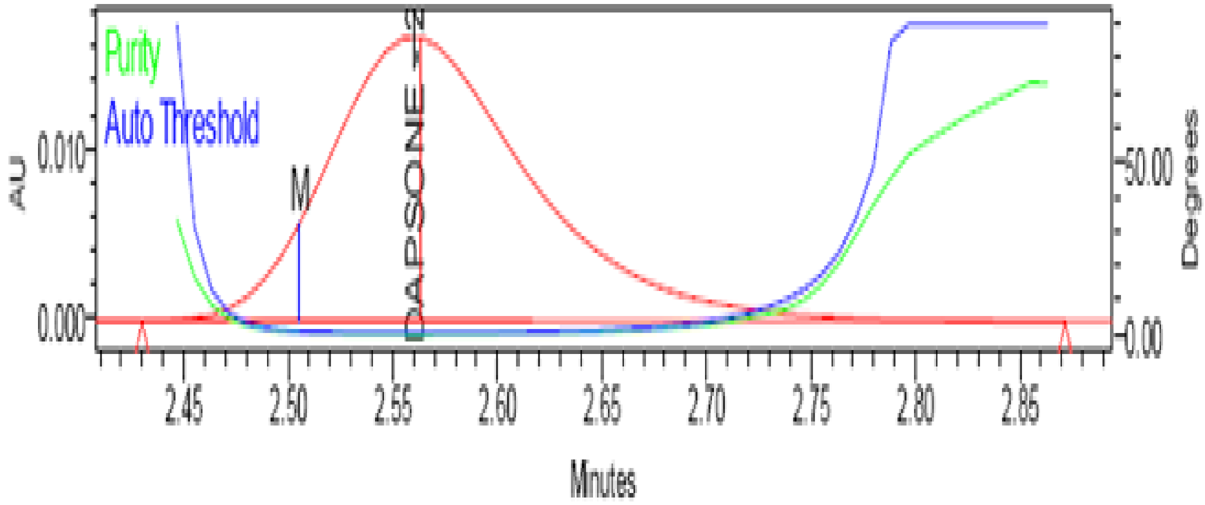

Figure:7 Peak purity Angle <Threshold (0.199 < 0.458)

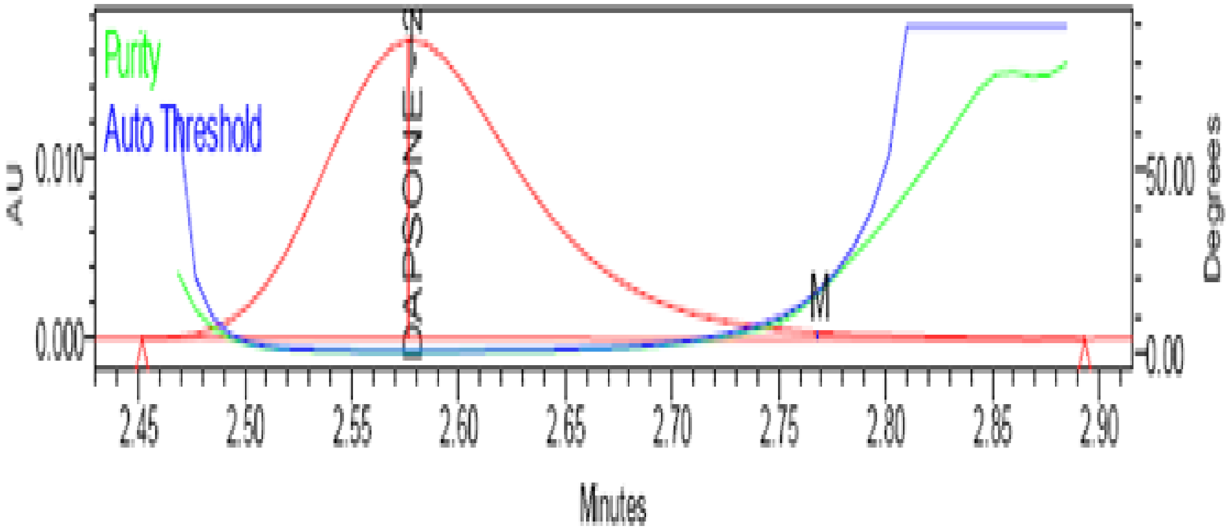

Figure:8 Peak purity Angle <Threshold (0.216 < 0.461)

Linearity

Linearity was performed by preparing mixed standard solutions of Telmisartan at different concentration levels including working concentration mentioned in experimental condition i.e. 12 ppm. The response was read at 256 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Fig 1.

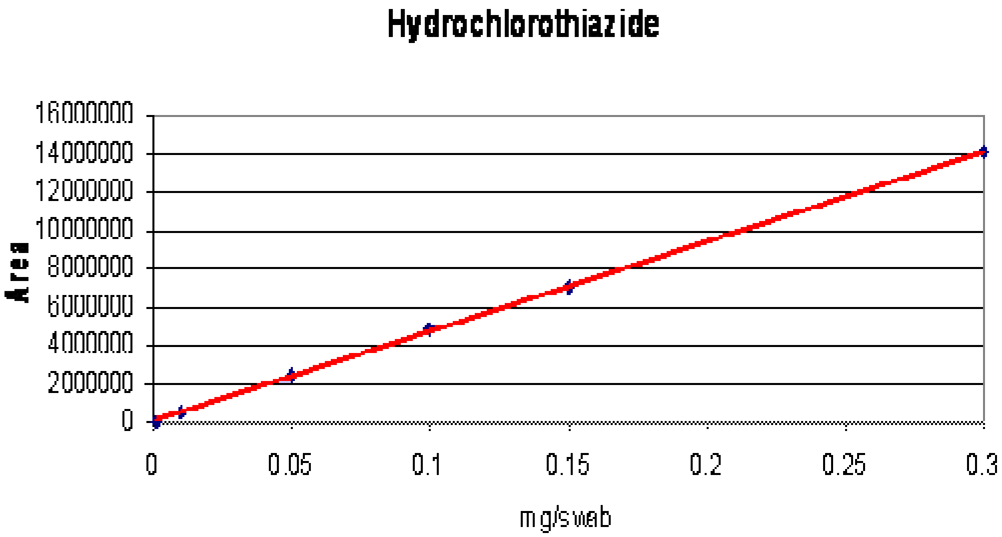

Figure: 2 Linearity Curve

System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to the Dapsone for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability. The results are tabulated in the Table:3.

Table:3 System suitability results

Sample	Dapsone Area
1	7667
2	7641
3	7613
4	7568
5	7534
6	7562
Mean	7598
% RSD	0.7

Detection Limit

The limit of detection by definition is a parameter of a limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates that analyte concentration is above or below a certain level. The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected. The maximum allowable carryover of Dapsone is 0.304 mg as determined in the Cleaning Validation Matrix.

Preparation of Standard solution (Stock Solution)

Accurately weigh 100 mg of Dapsone reference standard and quantitatively transfer into a 100 ml volumetric flask (1 mg/ml). Add 60 ml of methanol and sonicate for 15 minutes, cool and make up to volume with solvent. Filter through a 0.45 µm filter before use, discarding the first few milliliters of filtrate.

Preparation of Standard solutions (range)

From (1 mg/ml) stock solution below, a series of standard solutions were prepared as follows:

1.0 mg/swab : dilute 10 ml of 1 mg/ ml solution into 100 ml with solvent
0.10 mg/swab : dilute 10 ml of 1 mg/ swab solution into 100ml with solvent
0.01 mg/swab : dilute 10 ml of 0.1 mg/ ml solution into 100ml with solvent
0.005 mg/ swab : dilute 10 ml of 0.01 mg/ swab solution into 20ml with solvent
0.00125 mg/ swab : dilute 5 ml of 0.005 mg/ swab solution into 20 ml with solvent
0.000312 mg/ swab : dilute 5 ml of 0.00125 mg/ swab solution into 20 ml with solvent

Procedure

The range of standard solutions above was also injected twice and the average result was used in treatment of results. Six solutions containing 10.0, 1.0, 0.1, 0.01, 0.005, and 0.00125 mg/swab of Dapsone, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed. The results are tabulated in the Table: 4. And the Curve was shown in the Figure: 2.

Table: 4

Conc (mg/swab)	Area 1	Area 2	Average
10.0	7513407	7419090	7466249
1.0	743479	740795	742137
0.1	74170	74967	74569
0.01	7516	7516	7516
0.005	3825	3829	3827
0.00125	1064	1043	1054

Method Precision

The percentage of a test procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision % recovery of a known amount of API in the sample after swabbing. An amount of material (predetermined limit) is placed on a specific surface area (stainless steel) and swabbed as outlined in the Cleaning Validation SOP using the specified solvent and specified material. The precision of the analytical method is determined by assaying the swabs and calculating the % Recovery of the API results. The precision will entail repeated testing of six samples prepared in the following manner. Six replicate injections of API MAC working standard solutions were injected according to the method of analysis. The percentages Recovery for the peak responses were determined. The analytical system complies with the requirements specified by the method precision.

Standard solution

Accurately weigh 200 mg of Dapsone reference standard into a 50 ml volumetric flask. Add 30 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. (Solution 1 to be use for sample preparation) Dilute 10 µl to 10 ml with solvent. Filter through a 0.45 µm filter.

Sample Preparation

Place 10 µl of solution 1 onto a specific surface area of stainless steel plate. Swab the surface area; take the swab stick and place into a 10 ml volumetric flask, Add 10ml of solvent and sonicate for 10 minutes. Filter sample through a 0.45 µm filter.

Sample	% Recovery
1	91
2	86
3	85
4	108
5	95
6	94
Mean	**93**

CONCLUSION

The Cleaning Validation method is proven to be valid and the validation test results show that the method complies with the validation requirements. The method is therefore acceptable as valid.

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