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DEVELOPMENT AND VALIDATION OF RP-LC METHOD FOR DAPSONE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Dapsone in tablet formulations. The separation was achieved by using column YMC pack ODS AQ C18 (150×4.6mm, 3µm), in mobile phase consisted of acetonitrile and pH 4.5 ammonium acetate buffer, adjusted to pH 4.5 with the help of dilute acetic acid in the ratio of (75:25, v/v). The flow rate was 1.0 mL/min^{-1} and the separated Dapsone was detected using UV detector at the wavelength of 254 nm. Column temperature 25°C and sample temperature ambient and injection volume 10µl. The retention time of Dapsone, was noted to be 7.05 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

KEYWORDS: Liquid Chromatography; Dapsone, Validation.

1.0 INTRODUCTION

Dapsone, also known as diaminodiphenyl sulfone (DDS),[1] is an antibiotic commonly used in combination with rifampicin and clofazimine for the treatment of leprosy[2] 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.[3] Additionally, it has been used for acne, dermatitis herpetiformis, and various other skin conditions. Dapsone is available both topically and by mouth.[4]

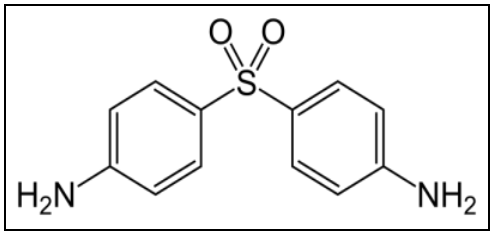

Fig. 4.01: Dapsone structural formula.

Literature survey reveals that few analytical methods have been reported for the estimation of Dapsone in pharmaceutical dosage form including UV-Vis spectroscopy,[5] high performance liquid chromatography (HPLC).[7-8] and TLC.[6] Although reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast. Hence we tried to develop stability indicating HPLC method for Dapsone. The present work describes a simple, stability indicating HPLC method for the determination of Dapsone in bulk and tablet dosage form according to ICH guidelines.[9-10]

2.0 EXPERIMENTAL

2.1. Chemicals and Reagents

Analytical-grade Ammonium acetate, acetic acid, were from Merck Chemicals Mumbai, India. Acetonitrile and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India.

2.2. Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower^2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) Centrifuge Eppendorf 5810 were use in the present assay.

2.3 Preparation phosphate Buffer pH 2.5: Accurately measured quantity of 7.7 gm of Ammonium acetate in 1000 ml of HPLC Grade water and pH was adjusted to 4.5 with dilute acetic solution and degassed. The solution was filtered through 0.45µ filter paper and degassed.

2.4 Mobile phase preparation

Mixed Ammonium acetate buffer (pH4.5) and Acetonitrile in the ratio of 75:25 % V/V.

2.5 Diluent preparation

Mix Acetonitrile and Water in the ratio of (50:50 %v/v) and Sonicated for about 5 minutes for degas the diluent.

2.6 Standard preparation

Weighed accurately and transferred about 40mg of Dapsone working standard into a 200ml volumetric flask and dissolved in 150ml diluent by sonicating 10 min. and was made up to the volume with diluent. Further transfer 2ml of this solution into a 20ml and was made up to the volume with diluent. The solution was filtered through 0.45 µm PVDF membrane filter. (20µg/mL).

2.7 Sample preparation

Weighed accurately a quantity of the mixed contents of 5 tablets equivalent to about 200mg of Dapsone and transferred into a 200ml volumetric flask. Added about 150ml of diluent and sonicated for about 30min and diluted to the volume with diluent. The solution is centrifuged at 400RPM for about 5 minutes. Further diluted 2ml of the above solution to 100ml with diluent and mixed.

2.8 Chromatographic conditions

Chromatographic analysis was performed on YMC pack ODS AQ C18 (150×4.6mm, 3µm) (Make: YMC) column. The mobile phase consisted of pH 4.5 ammonium acetate buffer and Acetonitrile in the ratio of 75:25%v/v. The flow rate was 1.0 mL/min, column oven temperature 25°C, the injection volume was 10µL, and detection was performed at 254 nm using a photodiode array detector (PDA).

3.0 RESULTS AND DISCUSSION

Method development

Spectroscopic analysis of compound Dapsone showed that maximum UV absorbance (λmax) at 254 nm respectively. To develop a suitable and robust LC method for the determination of Dapsone, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Agilent Zorbax AQ C18 with the following different mobile phase compositions like that Buffer and acetonitrile in the ratio of 40:50 v/v 50:50 v/v & 55:45. It was observed that when Dapsone was injected, Peak Tailing, not satisfactory.

For next trial YMC pack ODS AQ C18 (150×4.6mm, 3µm) column used and the mobile phase composition were changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 75:25 v/v. respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 254nm. The retention time of Dapsone is 7.05 minutes and the peak shape was good.

The chromatogram of Dapsone standard using the proposed method is shown in **(Fig: 1.2)** system suitability results of the method are presented in **Table-1.1.**

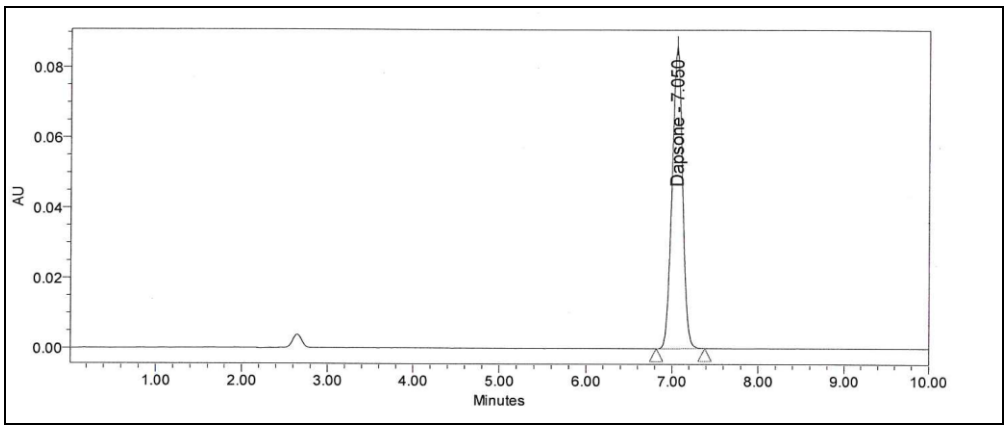

Figure 1.2: Chromatogram showing the peak of Dapsone.

4.0 Method validation

The developed RP-LC method extensively validated for assay of Dapsone using the following parameters.

4.1 Specificity

Preparation of blank solution

Mix Acetonitrile and Water in the ratio of (50:50 %v/v) and Sonicated for about 5 minutes for degas the diluent.

Preparation of Placebo solution

Placebo solution was prepared in duplicate by weighing the equivalent amount of excipients present in the finished drug product and analysed as per proposed method. Interference due to placebo was evaluated for each of the placebo preparations.

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic

conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig: 1.3)** showed no peak at the retention time of Dapsone peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Dapsone in Dapsone tablets. Similarly chromatogram of placebo solution **(Fig: 1.4)** showed no peaks at the retention time of Dapsone peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Dapsone in Dapsone tablets.

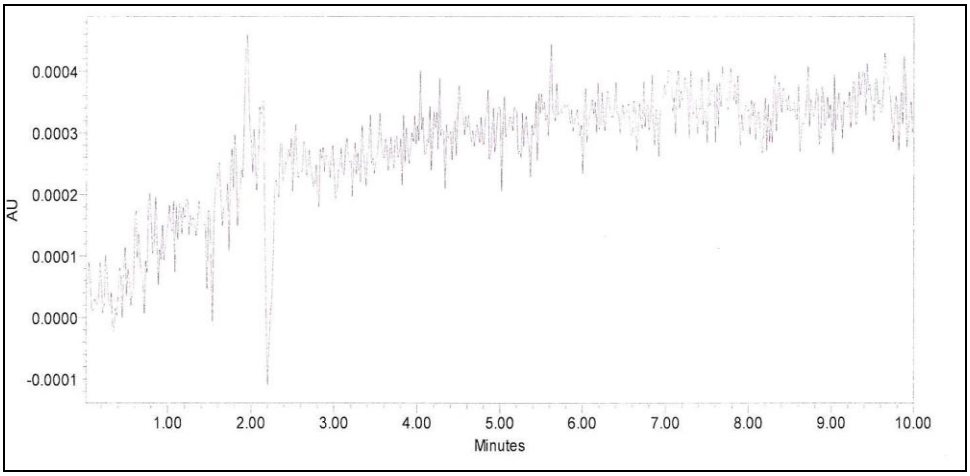

Fig: 1.3 Chromatogram showing the no interference of diluent for Dapsone.

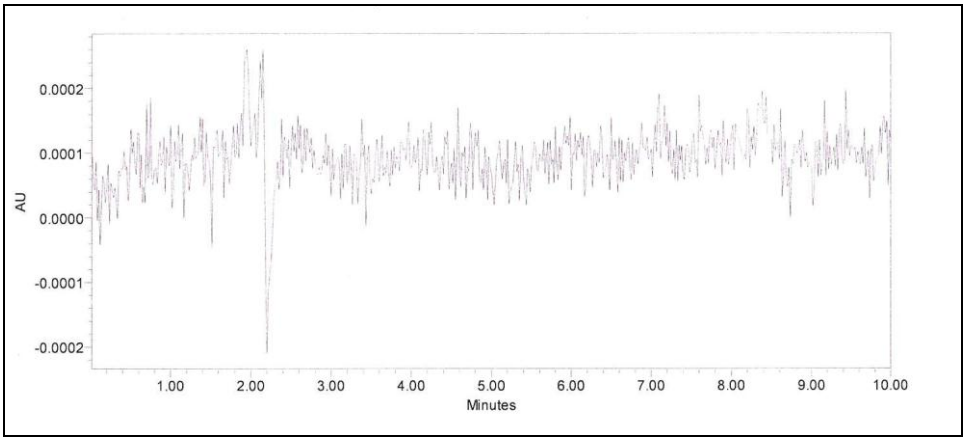

Fig. 1.4: Chromatogram showing the no interference of placebo for Dapsone.

Table 1.1: System suitability parameters for Dapsone by proposed method.

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Dapsone	7.05	15483	1.1

4.2 Method precision

The precision of test method was evaluated by doing assay for six samples of Dapsone tablet as per test method. The content in mg and % label claim for Dapsone for each of the test

preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table: 1.2.**

Table: 1.2 Method precision data for Dapsone.

No. of injections	Dapsone
	Percentage assay
Preparation 1	98.9
Preparation 2	99.9
Preparation 3	100.7
Preparation 4	100.7
Preparation 5	101.7
Preparation 6	99. 0
Average	**98.9**
%RSD	**0.5**

4.3 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Dapsone was determined in the range of 5% to 150% (1, 5, 10, 15, 20, 25 and 30µg/ml for Dapsone). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r2] was found to be 1.000.Therefore the HPLC method was found to be linear standard curve were calculated and given in **Figure: 1.5** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.3** the method was found to be linear within the proposed range.

Table: 1.3: Linearity studies for Dapsone by proposed method.

S. No.	Dapsone		
	Linearity concentration	Concentration (µg / ml)	Average area response
1	5%	1	75059
2	25%	5	376926
3	50%	10	757263
4	75%	15	1113806
5	100%	20	1490162
6	125%	25	1857921
7	150%	30	2222507
Correlation coefficient:			1.000
Slope (m):			74096
Intercept (y):			4926

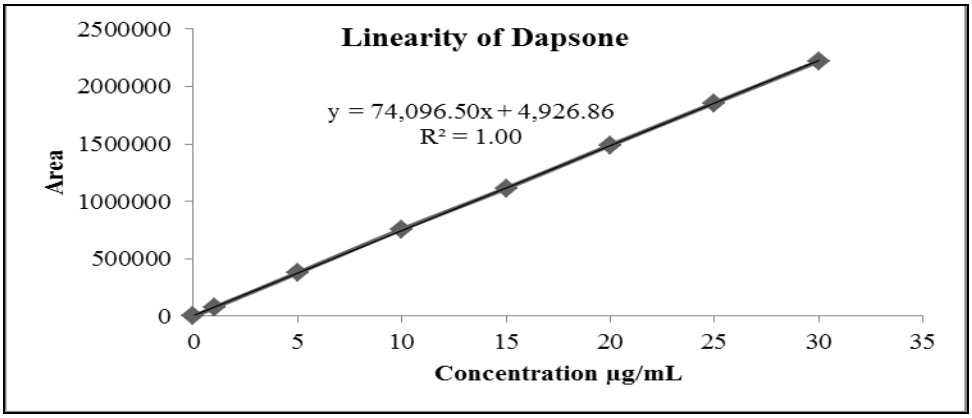

Figure: 1.5 Calibration curve for Dapsone

4.4 Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Dapsone, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level was found to be 99.5, 99.3 and 98.5. %RSD was found to be 0.2, 0.1 and 0.6 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of assay of Dapsone in formulation was found to be accurate. The data obtained which given in **Table: 1.4** the method was found to be accurate.

Table: 1.4 Recovery studies for Dapsone by proposed method.

S. No.	% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Mean recovery
1.	50	20.69	20.04	99.4	99.5
2.		20.43	20.75	99.3	
3.		20.04	20.65	99.7	
1.	100	40.06	40.71	99.3	99.3
2.		40.15	40.74	99.3	
3.		40.87	40.45	99.3	
1.	150	60.02	60.97	99.3	98.5
2.		60.33	60.11	98.2	
3.		60.49	60.07	98.8	

4.5 Robustness

4.5.1 Effect of variation in flow rate

As the flow rate in the proposed method was 1ml/min, the flow rate was changed between 0.9 ml/min to 1.1 ml/min. After equilibration of mobile phase with stationary phase, standard

solution was injected and the chromatograms were recorded. The results were shown in **Table: 1.5.**

Table: 1.5 System suitability data for Flow rate variation.

System suitability parameters	Parameters and Results		(% Assay)	
	(0.9 ml/min)	(1.1 ml/min)	Dapsone	%Diff
% RSD for area count of five replicate injections of standard.	0.1	0.2	100.5	N/A
Tailing factor	1.1	1.2	100.4	0.1
Theoretical plates	16031	15184	100.4	0.1

4.5.2 Effect of variation in pH

Prepared and injected standard and check standard solution as per the test method into HPLC system with PH variation of ± 0.5 units and evaluated system suitability parameters. The results were shown in **Table: 1.6.**

Table: 1.6 System suitability data for pH variation.

System suitability parameters	Parameters and Results		(% Assay)	
	(pH 4.0)	(pH 5.0)	Dapsone	%Diff
% RSD for area count of five replicate injections of standard.	0.2	0.2	100.5	N/A
Tailing factor	1.2	1.2	100.3	0.3
Theoretical plates	17368	17524	99.8	0.7

4.5.3 Effect of variation in mobile phase composition

Prepare two Isocratic programs, injected standard and check standard solutions as per the test method and evaluated system suitability parameters. System suitability parameters are within the specified limits as per test method. The results were shown in **Table: 1.7.**

Table: 1.7 System suitability data for Mobile phase variation.

System suitability	Parameters and Results		(% Assay)	
Parameters	Mobile phase variation (67.5:32.5)	Mobile phase variation (77.5:22.5)	Dapsone	%Diff
% RSD for area count of five replicate injections of standard.	0.1	0.4	101.2	N/A
Tailing factor	1.2	1.2	100.8	0.4
Theoretical plates	120422	13866	94.5	1.1

5.0 CONCLUSION

An RP-HPLC method for estimation of Dapsone was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Dapsone in bulk drugs and formulations. The optimized method consists of mobile phase pH 4.5 ammonium acetate buffer and Acetonitrile in the ratio of 75:25%v/v with YMC pack ODS AQ C18 150×4.6mm, 3µm column. The retention time of Dapsone was found to be 7.05min. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 1.0µg/ml to 30.0µg/ml, the correlation coefficient was found to be 1.000. Relative standard deviation for method precision was found to be 0.5.

We have developed a fast, simple and reliable analytical method for determination of Dapsone in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Dapsone. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Dapsone in its different pharmaceutical dosage forms.

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