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VALIDATION METHODS OF DAPSONE DRUG WITH COMPARITIVE STUDY OF STANDRAD AND SAMPLE BY USING UV-SPECTROSCOPY

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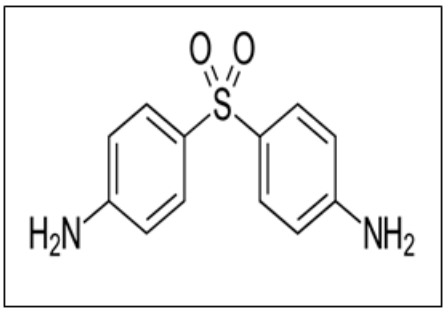
ABSTRACT

The present study of this article is validation methods of dapsone with comparative studies of crude form by using UV-Spectroscopy. During the validation process the drug will studied with comparatively. The absorbance ranges from 294 to 296 nm. UV is the most effect method for the validation studies for comparison purposes. The process which is used in the validation methods of this article will obey the beers lamberts law. The method was validated in terms of Linearity, Precision, Accuracy, Ruggedness, Robustness, LOD, LOQ and Assay. Results of analysis were validated statistically and by recovery studies. From that it was observed that there was no interference of impurities or excipients during the validation of drug in formulation. Further this method utilizes no additional chemical reagents for development of any coloring species with Dapsone as observed in some studies in literature and hence this method is very accurate, simple and less time consuming. This shows adaptability of the method for validation of dapsone in crude and dosage form.

KEYWORDS: Dapsone, validation, UV-spectroscopy, comparative study, analysis, first order.

INTRODUCTION

Dapsone is also known as Diphenylsulfone, Sulfona, 4,4ˈ-sulfonyldianiline,4-[(4-aminobenzene)sulphonyl]aniline.



Dapsone was first synthesized in 1908. Dapsone was first studied as an "antibiotic" in 1937, when two groups in England and France were the first to investigate Dapsone as an "antimicrobial" agent in the frame work of sulphonamide research. Parent sulphon Dapsone, were the first sulphones used to treat "Gonorrhea". Its use for leprosy began in 1945. Dapsone is considered to be one of the safest drugs for treating leprosy. A sulfone active against a wide range of bacteria but mainly employed for its action against "Mycobacterium leprae". It is also used with pyrimethamine in the treatment of malaria. It is second – line medicine for the treatment and prevention of pneumocystis pneumonia and for the prevention of toxoplasmosis. It is used for acne, dermatitis and various other skin conditions.

MATERIALS AND METHODS

The instrument used for the present study was genesys 10S UV-Visible spectrophotometer with quartz cell size-length 10mm, Diameter-45*12.5*12.5.

METHOD OF VALIDATION

SOLUBILITY TEST

Solubility test the drug dapsone was performed by using various solvents. The solvents include water, methanol, chloroform, and acetone. But it was found that dapsone soluble in methanol and water in the ratio of methanol: water (60:40).

DETERMINATION OF λmax

PREPARATION OF STOCK SOLUTION

Accurately weighed 10mg of Dapsone transferred 10ml and volumetric flasks; 5ml of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of Dapsone). Aliquots of standard stock solution were pipette out and suitably diluted with dilu²ent to get the final concentration of 2-14µg/ml. The solutions were scanned spectrum mode from 400nm to 200nm wavelength range.

PREPARATION OF SAMPLE STOCK SOLUTION

Twenty tablets were weighed and powdered. Powdered tablet equivalent to 10mg of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Dapsone).

VALIDATION METHOD

Linearity and Range

The calibration curve was evaluated by its correlation coefficient. The absorbance of the dapsone in the range of 2-14µg/ml was linear with a correlation coefficient (r^2) is 0.999.

Accuracy

Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using dapsone working Standard as per the test method and taken absorbance of each solution in triplicate. The recovery results showed that the proposed method has an acceptable level of accuracy for SFS which is from 80% - 120%.

Preparation of Standard stock solutions

Twenty tablets were weighed and powdered. Powdered tablet equivalent to 10mg(113.5mg powdered weight) of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Dapsone).

Preparation of 80% Spiked Solution

Powdered tablet equivalent to 8mg (90.8mg powdered weight) of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (800 µg/ml of Dapsone). From this solution 0.1ml was taken into a 10ml volumetric flask and made up to the mark with diluent.

Preparation of 100% Spiked Solution: Powdered tablet equivalent to 10mg (113.5mg powdered weight) of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Dapsone). From this solution 0.1ml was taken into a 10ml volumetric flask and made up to the mark with diluent.

Preparation of 120% Spiked Solution: Powdered tablet equivalent to 12mg (136mg powdered weight) of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1200 µg/ml of Dapsone). From this solution 0.1ml was taken into a 10ml.

Acceptance Criteria

The percentage (%) Recovery for each level should be between 98.0 to 102.

Precision

Precision of the method was verified by precision studies (Intra-day) were performed by analysis of Dapsone respectively on the same day. (Inter-day) of the method was checked by repeating analysis of Dapsone on a different day. Measurement of peak area for active compound was expressed in terms of percentage Relative Standard Deviation (%R.S.D.) for the method.

Preparation of Sample stock solutions

Twenty tablets were weighed and powdered. Powdered tablet equivalent to 10mg of dapsone weighed and transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Dapsone).

Intra-day Precision

Six working sample solutions of 10ppm are injected and the percentage Amount found was calculated and percentage relative standard deviation (%RSD) was found to be 0.56% As the limit of Precision was less than "2" the system precision was passed in this method.

Inter-day Precision

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained absorbance was mentioned in the above table. Average area, percentage relative standard deviation (% RSD) and were calculated for drug and obtained as 0.26%.As the limit of Precision was less than "2" the system precision was passed in this method.

Specificity

Checking of the interference in the optimized method. We should not find interfering peaks in blank, placebo and sample at absorbance max of these drugs in this method. So this method was said to be specific.

Limit of detection (LOD)

Sample Preparation: 0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Dapsone, were transferred to 10ml volumetric flasks and made up with the same diluents Detection limit of the Dapsone in this method was found to be 0.027µg/ml.

Limit of quantification (LOQ)

Sample preparation

0.25ml of standard stock solution was pipette out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml dapsone, were transferred to 10ml volumetric flasks and made up with the same diluents. Quantification limit of the dapsone in this method was found to be 0.082µg/ml.

Robustness

Robustness of the method was determined by carrying out the analysis under different wavelength conditions that is 296nm and 298nm by observing the 10ppm concentration solution. Percentage relative standard diveation (%RSD) of the above conditions is calculated.

Ruggedness

Six working sample solutions of 10ppm are injected and percentage standard deviation %(RSD) was found to be 0.34% as the limit of Precision was less than "2" the system precision was passed in this method.

Assay of Dapsone

Assay of the marketed formulation (Avlosulfon) was carried out Standard solution and sample solutions were observed separately into the system and Absorbance were recorded and drug present in sample was calculated using before mentioned formula.

RESULTS AND DISCUSSION

The method followed for validation of Dapsone was found to be precise as the percentage standard deviation (% RSD) values for intra-day and inter-day were found to be less than 0.56% better recoveries that is 99.56% -101.67% for first order kinetics obtained at each added concentration indicating that the method was accurate. The validation method indicated was specific which was found by the percentage recoveries ranging from 99.56% - 101.67%. The LOD and LOQ were found to be with the limits indicating sensitivity of the method. The validated method was also found to be robust and rugged indicating the percentage recovery studies less than 2% that is 0.49 and 0.34 respectively. Assay results indicated that the amount of drug was in good agreement with the label claim of the respective formulation. The results were discussed in the following tables.

LIMIT OF DETECTION

S.NO	WAVELENGTH (nm)	ABSORBENCE
01	398.20	0.023
02	369.00	0.021
03	365.80	0.029
04	361.20	0.016
05	357.60	0.016
06	355.00	0.014
07	344.00	0.010
08	338.80	0.010
09	320.40	0.010
10	317.60	0.009
11	314.00	0.009
12	309.40	0.012
13	307.00	0.008
14	305.00	0.009
15	304.20	0.017
16	299.00	0.027

LIMIT OF QUANTIFICATION

S.NO	WAVELENGTH (nm)	ABSOBENCE
01	398.20	0.013
02	372.80	0.006
03	371.80	0.006
04	359.80	0.006
05	358.20	0.016
06	357.80	0.005
07	349.00	0.011
08	348.60	0.008
09	348.00	0.006

10	344.00	0.009
11	338.80	0.005
12	337.00	0.007
13	336.00	0.008
14	329.60	0.006
15	314.00	0.004
16	297.20	0.010
17	296.80	0.082
18	202.80	0.814
19	202.80	0.855
20	202.00	0.873
21	200.60	0.980

LINEARITY

S.NO	CONCENTRATION	PIPETTE FROM THE STOCK SOLUTION	WAVELENGTH	ABSORBANE	% LINEARITY LEVEL
1	0	0	0	0	0
2	2	0.25	297.80	0.159	25
3	4	0.50	296.60	0.263	50
4	6	0.75	294.40	0.360	75
5	8	1	294.00	0.464	100
6	10	0.25	294.40	0.578	25
7	12	0.50	295.00	0.672	50
8	14	0.75	295.20	0.811	75

ACCURACY

%LEVEL	AMOUNT SPICKED(*µ*g/ml)	AMOUNT RECOVERED(*µ*g/ml)	%RECOVERY	MEAN %RECOVERY
50%	80	81.18	101.47	100.08%
100%	100	99.65	99.65	100.08%
150%	120	118.94	99.12	100.08%

PRECISION

Intra-day

S.NO	ABSORBANCE
1	0.621
2	0.628
3	0.631
4	0.626
5	0.628
6	0.630
AVG	0.627
STANDARD DEVIATION	0.004
%RSD	0.567

Interday

S.NO	ABSORBANCE
1	0.621
2	0.619
3	0.622
4	0.623
5	0.620
6	0.619
AVERAGE	0.621
STANDARD DEVIATION	0.002
%RSD	0.263

Ruggedness

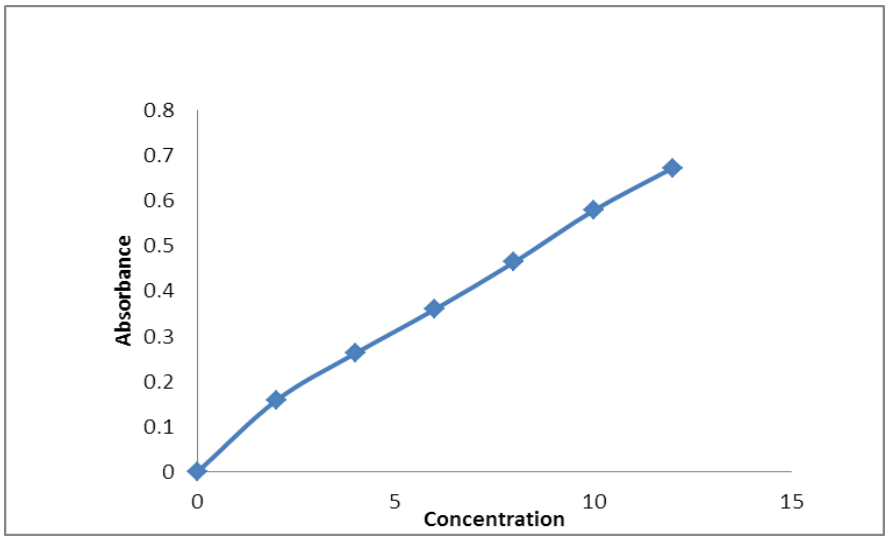
S.NO	ABSORBANCE
1	0.624
2	0.623
3	0.625
4	0.619
5	0.622
6	0.621
AVG	0.622
STANDARD DEVIATION	0.002
%RSD	0.347

Robustness

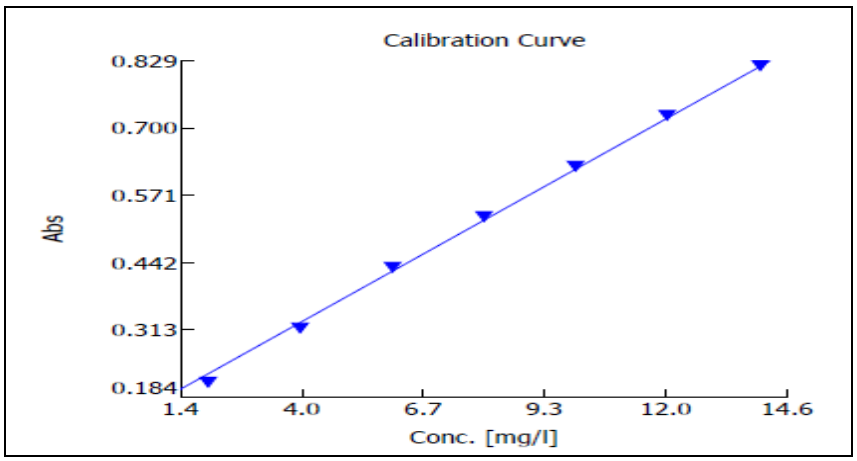
Concentration	Wavelength	Absorbance	% Amount	Standard Deviation	% Relative Standard Deviatin
10	296	0.618	98.17%	0.003	0.49
10	297	0.621	98.19%	0.0029	0.47
10	298	0.619	98.18%	0.0027	0.51

PARAMETERS	DAPSONE	LIMIIT
Linearity **:**Range (µg/ml)	2-14µg/ml	R< 1
Regression coefficient	0.999	
Slope(m)	0.0503	
Intercept(c)	0.1146	
Regression equation (Y=mx+c)	y = 0.0503.x + 0.1146	
Assay(% mean assay)	99.44%	90-110%
Specificity	Specific	No interference of any peak
System precision %RSD	0.567	NMT 2.0%
Method precision %RSD	0.263	NMT 2.0%
Accuracy %recovery	100.08%	98-102%
LOD	0.027	NMT 3
LOQ	0.082	NMT 10
Robustness		
wavelength minus	0.49	%RSD NMT 2.0

wavelength plus	0.24	
Ruggedness	0.34	%RSD NMT 2.0



λmax curve



Linearity curve

CONCLUSION

The bulk and dosage forms were validated in terms of Linearity, Specificity, Precision, Accuracy, LOD, LOQ, Robustness, Ruggedness and Assay. Results of the study were validated statistically and recovery studies. The validation results indicating that the amount of drug is in agreement with label claim of the formulation. It was observed that there was no interference of impurities or excipients during the validation of drug formulation. This study thus exploits that the possibility for determining pharmacokinetic profile of Dapsone which may be required in clinical study in near future. The proposed spectroscopic method was found to be simple, precise, highly accurate and less time cosuming. Hence it is a preferred method for analysis of Dapsone in bulk and dosage form.

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