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Research Article

UV METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DISULFIRAM IN MARKETED TABLET PREPARATION

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ABSTRACT

Disulfiram is an alcohol deterrent drug. A simple, precise and specific spectrophotometric method was developed and validated for estimation of Disulfiram in tablet dosage form. Disulfiram showed maximum absorbance at 216 nm. The drug was derivatized in methanolic solution. Beer Lambart's law was obeyed at concentration range of 2-12 ppm. A linearity curve was calibrated in concentration versus absorbance. The regression equation of curve was calculated as Y = 0.069 X + 0.087, with correlation coefficient r^2 = 0.981. Accuracy was determined by recovery study and overall percentage recovery was found to be 96.40%.RSD values of precision were less than 1. The LOD and LOQ were calculated as 2.233 and 6.768 respectively. The developed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification, ruggedness and robustness as per ICH guidelines. The method can be successfully applied for quality control analysis of Disulfiram in pharmaceutical formulation.

Keywords: Disulfiram, Beer's lambarts law, UV- spectrophotometric method, validation.

1. INTRODUCTION

Disulfiram is an alcohol deterrent medication. Chemically it is N,N,N',N,N'-tetraethyl-thioperoxydicarbonic diamide . It blocks oxidation of alcohol at acetaldehyde stage during alcohol metabolism following DSF intake causing accumulation of acetaldehyde in blood. It has been used for the treatment of alcohol addiction.

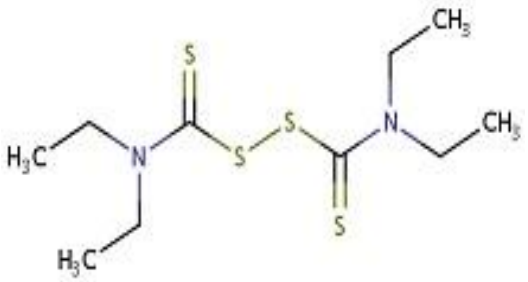

Fig. 1: Chemical structure of Disulfiram

It is official in Indian pharmacopoeia. Different methods have been reported in the literature for the assay of DSF in pharmaceuticals and it includes TLC, HPLC, and HPTLC.

On the other hand, UV spectrophotometry is still the technique of choice since it is simple, sensitive, economical, rapid and more easily manageable literature survey revealed that no stability indicating UV- spectrophotometric method has ever been reported for the quantification of DSF.

The aim of present work is to develop and validate an economical, accurate, precise and reproducible UV spectrophotometric method for the determination of DSF as in solid dosage form. Drug was found to be freely soluble in methanol which was chosen for proceeding studies.

MATERIALS AND METHODS

Instrument

Absorption spectral measurements were carried out with a UV- visible spectrophotometer (Shimadzu Model) using UV Probe software version 2 was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells)

Reagents and chemicals

The reference standard DSF pure was received as a gift sample from Unidrug Innovative Pharma Technologies Ltd, Indore, Pharmaceuticals. Methanol was used as solvent and obtained from Modern science Lab, Nashik.

Selection of absorption maxima

Solution of strength 10µg/ml was prepared for drug from standard stock solution and scanned in the wavelength range of 200-400nm. The absorption maxima was found at 216 nm which was used for further analysis.

Preparation of stock solution

10 mg of DSF was accurately weighed and dissolved in 100 ml volumetric flask. The volume was made up to the mark with methanol to give 100 µg/ml stock solutions.

Preparation of working standard solutions

Prepared stock solution was further diluted with methanol to get working standard solution of 2, 4,6,8,10 and 12 µg/ml to construct Beer's law plot for DSF. The absorbance of each solution was measured at 216 nm against methanol as blank. (**Table-1**)

Calibration curve for Disulfiram

The Appropriate volumes of aliquots from standard DSF stock solution were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 2, 4,6,8,10 and 12 µg/ml. Absorbance of each solution against methanol as a blank was measured at 216 nm. From that absorbance, regression equation, correlation coefficient was determined. The standard calibration curve for DSF was plotted by taking concentration of drug on X-axis and absorbance on Y-axis. (**Fig-2**)

2. METHOD VALIDATION

Validation parameters: The method was validated with reference to accuracy, specificity, precision, limit of detection, limit of quantification, ruggedness and robustness as per ICHQ2 (R1) guidelines.

Accuracy

Accuracy of proposed method was assessed by recovery studies at three different levels i.e 80 %, 100% and 120% .The recovery studies were carried out by adding amount of standard solution of drug to preanalysed standard solutions. The resulting solutions were then reanalysed. The results are reported in Table No.3

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra -day study was performed by analyzing three times in the same day (morning, afternoon and evening). Inter day precision was performed by analysing three different concentration of the drug for two days. Repeatability was performed by analysing same concentration of drugs. The precision of method was expressed as relative standard deviation (%RSD) and standard deviation (SD). The results are reported in Table No.4 and Table No: 5

Specificity

The specificity of DSF was evaluated by adding excipient lactose in the specific concentration. In this method working standard (100µg/ml) at 80%, 100% and 120% levels. At each level of amount triplicate determinations were performed. The percentage of concentration was calculated.

The excipient lactose did not show any effect on the estimation of DSF. Hence, the determination of DSF in tablet was considered to be free from interference due to lactose. The results are reported in Table No: 6

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot by different analysts using similar operational and environmental conditions. The results are reported in Table No.7

Robustness

Robustness of the proposed method was determined by analysis of aliquots from homogeneous slot by measuring the absorbance 211nm, 216nm and 221nm. The results are reported in Table No.8

Application of the proposed procedure for the determination of Disulfiram in tablets

The proposed method was applied for determination of DSF in tablet dosage form.

DSF content was estimated in marketed tablet (Nocohol -500 mg).Twenty tablets were weighed and average weight was calculated, crushed to fine powder.

The powder equivalent to 10 mg of DSF was transferred in 100ml volumetric flask and dissolved in 100 ml methanol solution. From above stock solution, 1 ml solution was diluted up to 10 ml with methanol. The flask was shaken and the solution was filtered through whatman filter paper. Drug solution was sonicated for 10 minutes for better dissolution and it was further used for the estimation of DSF. The result is reported in Table No. 9.

3. RESULTS AND DISCUSSION

Table 1: UV Optical characteristics and linearity data

Sr. No.	Parameter	UV Method with Methanol
1	λmax (nm)	216
2	Beer's law limits (µg /ml)	2-12
3	Regression equation (Y)	Y=0.069x + 0.087
4	Slope (b)	0.069
5	Intercept (a)	0.087
6	Correlation coefficient(r2)	0.981
7	Limit of detection (LOD) (µg/ml)	2.233
8	Limit of quantification (LOQ) (µg/ml)	6.768

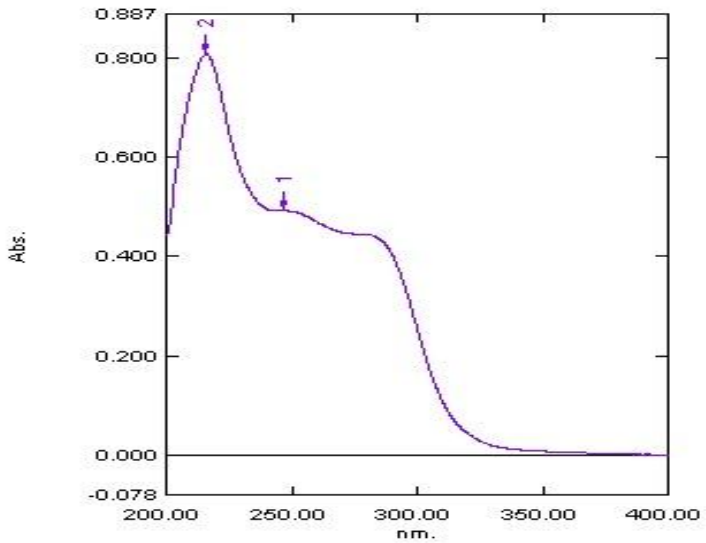

Fig. 2: UV absorption spectra of DSF standard

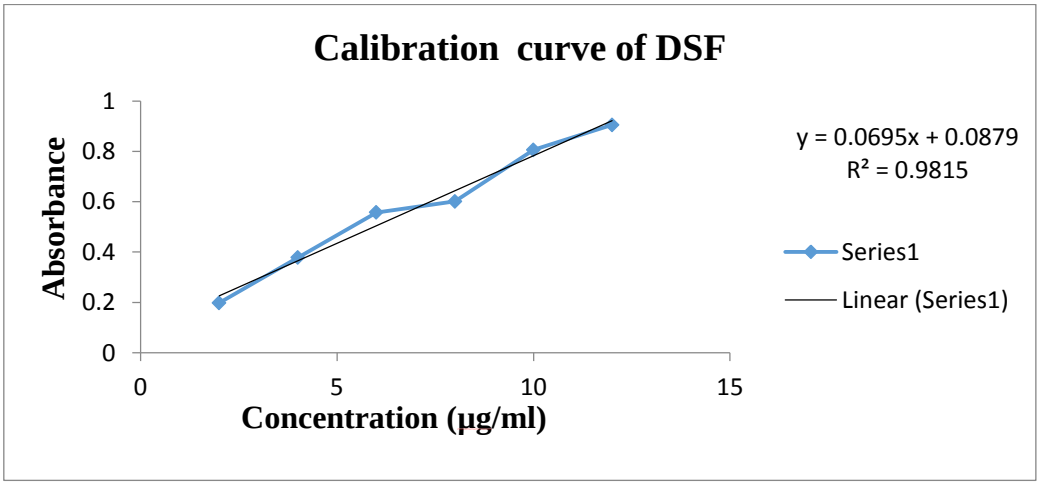

Fig. 3: UV linearity graph of Disulfiram

Validation of method

The validation of method was performed according to ICH Q2 (R1) guidelines including accuracy, precision, specificity, limit of detection, limit of quantification ruggedness and robustness.

Accuracy

Accuracy of method used for recovery study at three different levels, that is, 80%, 100% and 120%. Results are as shown in Table No.3.

Precision

Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2. Results of intraday and interday precision are shown in Table No.4 and Table No.5 respectively.

Table 3: Results of accuracy studies by UV spectroscopy

% level of addition	Concentrations (µg/ml)		Total Conc.(µg/ml)	Drug recovered	% recovery
	Standard	Standard			
80 %	4	3.2	7.2	7.00	97.22
	4	3.2	7.2	6.96	96.66
	4	3.2	7.2	6.98	96.94
100 %	4	4	8	7.64	95.50
	4	4	8	7.69	96.12
	4	4	8	7.64	95.5
120 %	4	4.8	8.8	8.47	96.25
	4	4.8	8.8	8.49	96.47
	4	4.8	8.8	8.54	97.04

Statistical validation of DSF by UV spectrophotometric method

Level of addition	% Mean recovery	S.D	% RSD	S.E
80%	96.94	0.001	0.169	0.00057
100%	95.70	0.001	0.274	0.00098
120%	96.58	0.002	0.327	0.00127

Table 4: Data for intraday and precision of DSF

Conc.(µg/ml)	Mean*	S.D	% RSD	S.E
2	0.189	0.002	0.527	0.0011
6	0.557	0.003	0.538	0.0017
10	0.814	0.002	0.307	0.0011

Table 5: Data for intraday precision of DSF

Conc.(µg/ml)	Mean*	S.D	% RSD	S.E
6	0.195	0.001	0.512	0.0005
8	0.561	0.005	0.891	0.002
10	0.805	0.002	0.328	0.001

Table 6: data for specificity of DSF

% Spike level	Concentration(µg/ml)		Absorbance	S.D	% RSD
	Standard	Excipient (lactose)			
80%	0.6	0.48	0.576	0.0472	0.389
	0.6	0.48	0.573		
	0.6	0.48	0.572		
100%	0.6	0.60	0.585	0.003	0.607
	0.6	0.60	0.581		
	0.6	0.60	0.578		
120%	0.6	0.72	0.580	0.001	0.172
	0.6	0.72	0.579		
	0.6	0.72	0.580		

Table 7: Data for ruggedness study of DSF by spectrophotometric method

Sr.No	Analyst	Conc.(µg/ml)	Mean* Absorbance	S.D	% RSD	S.E
1	Analyst I	6	0.552	0.00509	0.930	0.0029
2		6				
3		6				
1	Analyst II	6	0.547	0.00608	1.112	0.0035
2		6				
3		6				

Table 8: Data for robustness study of DSF by spectrophotometric method

Sr. No	Concentration (µg/ml)	λmax (nm)	Absorbance				S. D.	(%) RSD
			I	II	III	Mean		
1	6	211	0.509	0.509	0.508	0.508	0.001	0.1968
2	6	216	0.544	0.543	0.542	0.543		
3	6	221	0.513	0.512	0.513	0.512		

Table 9: Analysis data of tablet formulation by UV

Drug	Label claim (µg/ml)	Amount found (µg/ml)	Label claim (%)	S.D	S.E
DSF	10.00	9.96	99.62	0.0017	0.0012

DSF: Disulfiram, S.D: standard deviation, S.E: standard error and RSD: relative standard deviation

RESULTS AND DISCUSSION

Analytical method development and validation of Disulfiram formulation was the basic aim of the current research. A simple, precise and specific spectrophotometric method was developed and validated.Disulfiram showed maximum absorbance i.e.216nm. The drug was derivatized in methanolic solution.Beer's Lambarts law was obeyed at concentration range of 2-12 ppm.A linearity curve was calibrated by concentration Vs absorbane. The regression equation of curve was calculated as Y=0.069x + 0.087, Correlation Coefficient r^2 = 0.981. The accuracy was determined by recovery study and the overall percentage recovery was found to be 96.40%.The % RSD of precision was found to be less than 2. The LOD and LOQ were calculated as 2.333 and 6.768 respectively. The developed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification, robustness as per ICH guidelines. The developed and validated method was applied for estimation of Disulfiram in tablet dosage form. The method was successfully applied in quality control analysis of Disulfiram in pharmaceutical formulation.

CONCLUSION

Experimental results and discussion show that the developed method is specific and validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification, robustness as per ICH guidelines. The method can be successfully applied in the quality control analysis for the estimation of the label claim of Disulfiram in pharmaceutical formulation.

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