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A Validated UV Spectroscopic Method for determination of Levamisole HCl

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ABSTRACT: In the present study suitable UV spectroscopic method was developed and validated for Levamisole hydrochloride. The method was developed using 0.1N HCl as a solvent at wavelength of 216 nm and the parameters of validation such as accuracy, precision, LOD, LOQ, recovery study and range were evaluated as validation parameters. It was found that the developed method was accurate and precise with a regression correlation of 0.998 and can be used for routine analysis of Levamisole hydrochloride in any dosage form.

I. INTRODUCTION:

Levamisole hydrochloride chemically known as 2, 3, 5, 6–tetra hydro –6 – phenylimidazo [2,1b], thiazole hydrochloride is used to treat parasitic worm infection. The drug appears to restore depressed immune function rather than to stimulate response to above-normal levels. Levamisole can stimulate formation of antibodies to various antigens, enhance T-cell responses by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis, and increase neutrophil mobility, adherence, and chemotaxis.[1]

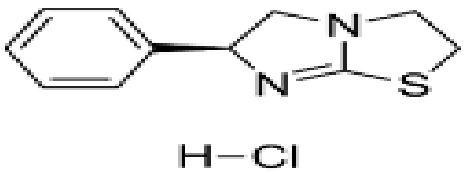

Figure 01: Structure of Levamisole HCl

The UV spectrophotometric method is one of the commonest and economical method for determination of any drug substance. The aim of this work was the development and fully validation of a new UV spectrophotometric method, which can be more economical and simpler than the official methods and with other methods published. The UV spectrophotometric method is simpler than the others studied because it does not need derivative and chemometric assistance. Moreover, this method can be used in dissolution studies because it uses its own dissolution medium as diluent [2-3].

Determination of drug substance is that the most vital facet of any drug development whether or not in bulk or together, an acceptable technique should be developed therefore on make sure that any drug either in dose type orbulk type is identified. Thetactic dev elopmentensures thatquantity of specific drug is si mply determined. The validation parameters ensure that the developed technique is precise, correct and reproducible and might be used for routine analysis of Levamisole in bulk and combined dose type.[4]

II. MATERIALS & METHODS:

Instrumentation:

A UV-Visible Spectrophotometer (UV-1600 SHIMADZU) was used for Spectrophotometric method. All weighing were

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done on electronic balance (Model Shimadzu AUW-220)

Reagents & chemicals:

Levamisole HCl was received as gift sample from Encore pharmaceutical, paithan, Aurangabad batch no "LEV 1110002". Tablet formulation manufactured by GSK was purchased from local market Dewormis containing Levamisole HCl 50mg per tablet.

Preparation of standard stock solution:

Standard drug solution of Levamisole HCl was prepared by accurately weighing 10 mg of the drug and dissolved in 0.1N HCl and the volume was made up to 100ml to obtain stock solution (100 µg/ml) [5-6].

Determination of Analytical Wavelength:

From the standard stock solution 0.8ml was pipette out into 10ml volumetric flask. The volume was made up to 10ml with 0.1N HCl. The resulting solution containing 8µg/ml was scanned between 200-400 nm [5-6].

Preparation of Calibration Curve:

Aliquots of 0.2, 0.4, 0.6, 0.8, 1, 1.2 & 1.4 ml portions of stock solutions were transferred to a series of 10ml volumetric flasks, and volume made up to the mark with 0.1N HCl. The serial dilutions in the range of 2, 4, 6, 8, 10, 12 and 14µg/ml were prepared. The absorbance was measured at λmax 216nm [5-9].

UV Method Validation

Linearity & Range:

The linearity of the response of the drug was verified at 2 to 14µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the calibration curve for Levamisole HCl was obtained [5-9].

Precision:

The accuracy of the method was determined by recovery experiments. Each solution was repeated in triplicate and the percentage recovery was calculated. The precision of the method was demonstrated by intra-day and inter-day variation studies [5-9].

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ were calculated by the equations;

LOD = 3.3σ/ S and LOQ = 10σ/S

Where S is the slope of the calibration curve and σ is the residual standard deviation.

Recovery Study:

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels, 80, 100, and 120% of Levamisole HCl standard concentration. The recovery samples were prepared in a before mentioned procedure for each recovery level. The solutions were then analyzed and the percentage recoveries were calculated from the calibration curve [7-12].

III. RESULTS & DISCUSSION:

Analytical Wavelength:

The maximum absorption was found to be at the wavelength of 216nm hence the wavelength for levamisole HCL was found to be 216nm as shown in figure: 02

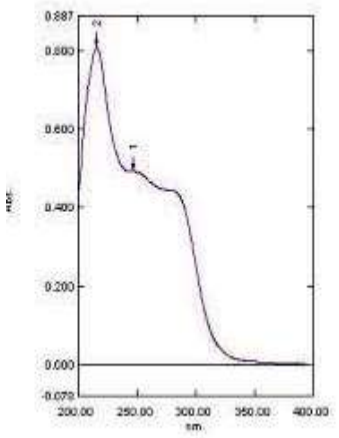

Figure 02: A typical UV Spectrum of Levamisole HCl at 216nm

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Calibration Curve:

The results of absorbance for all the prepared concentrations were plotted i.e. Concentration vs. Absorbance the method was found to be linear over the prepared concentration range with the standard equation y=0.1013x+0.0201 and Regression value was found to be 0.9987, as shown in figure: 03. From the calibration data obtained it was found that the regression coefficient was less than 1 which is within the limits of Beer lamberts' law.

Table 01: Calibration Curve Data of Levamisole HCl

Sr. No.	Concentration (µg/ml)	Absorbance ± SD
1	2	0.219 ±0.26
2	4	0.425 ±0.15
3	6	0.623 ±0.95
4	8	0.831 ±0.54
5	10	1.038 ±0.3
6	12	1.263 ±0.1
7	14	1.412 ±0.4

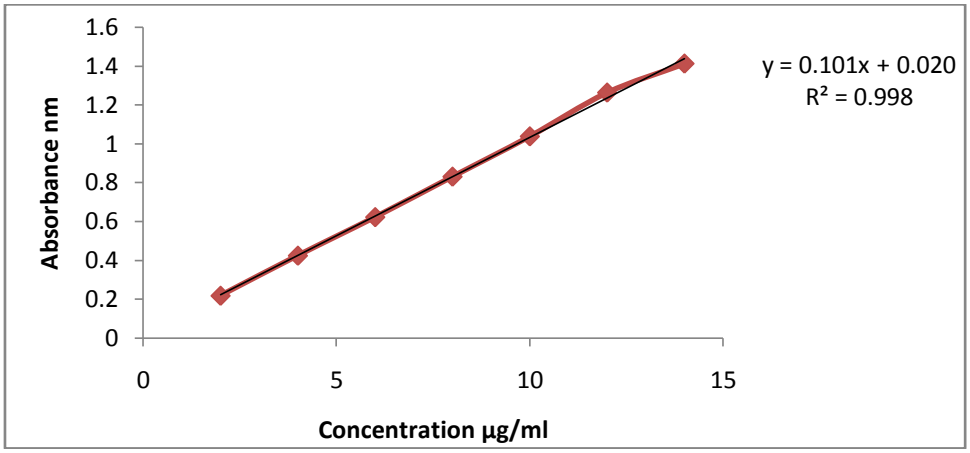

Figure 03: Calibration graph of Levamisole HCl at 216 nm

Precision:

Precision of the method was evaluated for Levamisole. The reproducibility (inter-day precision) of the method and repeatability (intra-day precision) was evaluated in the same laboratory. The values obtained were as pr Table 02 and table 03. From the data obtained in the method was found to be precise in respect of reproducibility as well as repeatability.

Table 02: Precision Determination Intra – day Precision by UV

	Absorbance		
Analyte	**0 Hr.**	**3 Hr.**	**6 Hr.**
Mean	0.4134	0.4116	0.4025
SD	0.0015	0.0006	0.0005
%RSD	0.3857	0.5117	0.1368

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Table 03: Inter – day Precision by UV

Analyte	Absorbance		
	0 Hr.	**24 Hr.**	**48 Hr.**
Mean	0.4134	0.388	0.382
SD	0.0015	0.001	0.0060
%RSD	0.3857	0.2577	1.5923

Accuracy (Recovery Study):

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels 80, 100 and 120% of Levamisole standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed and the percentage recoveries were calculated from the calibration curve. The recovery value for Levamisole HCL was 99.30±0.616 and RSD was 0.6409 which is less than 2, which shows that the method has good reproducibility.

Table 04: Recovery Study

Statistics	Level of Recovery		
	80%	**100%**	**120%**
Amount present (µg/ml)	2	2	2
Amount of standard added (µg/ml)	1.6	2	2.4
Total amount recover	3.58	4.00	4.38
%recovery	98.75	100	99.16
Mean	99.30		
SD	0.6364		
%RSD	0.6409		

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

Limit of detection is the lowest amount of analyte which can be detected but not necessarily quantified, and limit of quantification is the lowest possible concentration that can be quantified LOD and LOQ were found to be 0.39675 µg/ml & 0.95523 µg/ml respectively.

Specificity:

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components (excipients). The results were compared with the analysis of a standard Levamisole and tablet formulations. Excipients of the solid dosage form did not interfere with the analyte, which shows that the method has good specificity.

Validation parameters:

All the validation parameters as reported in table 05 were found to be within the desired range which depicts that the method was found to be reproducible with respect to all the validation parameters and can be a useful tool for routine evaluation of eletriptan in bulk and combined dosage form.

Table 05: Validation Parameters

Parameter	Results
Linearity range	2-14 µg/ml
Regression eq.	y=0.1013x+0.0201
Correlation coefficient	0.9987
Slope (m)	0.1013
Y-Intercept(c)	0.0201
Λmax	216 nm
LOD	0.39675 µg/ml
LOQ	0.95523 µg/ml
Interday precision	0.2675
Intraday precision	0.3447
Accuracy (%mean recovery)	99.30

IV. CONCLUSION:

In the present study a suitable UV Spectroscopic method was developed for Levamisole hydrochloride in 0.1 N HCl as dissolution medium for drug and method was validated for different parameters as accuracy, precision, specificity, LOD, LOQ and recovery. It can be concluded that the developed method has good reproducibility and can be routinely used for estimation of Levamisole hydrochloride in bulk and combined formulation

V. ACKNOWLEDGEMENT:

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