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Validated Ultra Violet Spectroscopic Methods For The Determination Of Levamisole Hydrochloride

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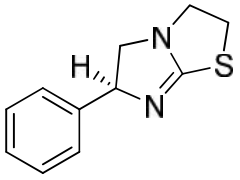
Abstract: Simple and sensitive spectrophotometric methods have been developed for the estimation of LevamisoleHydrochloride [LH] in pure and pharmaceutical dosage forms. The developed methods are validated in terms of Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantitation, Accuracy, as per International Conference on Harmonization Guidelines.UV spectroscopic determination showed absorbance maxima at 215nm using Distilled water as solvent. Beer's law is obeyed by showing linearity in the concentration ranges between 2-10 µg/ml respectively with a correlation coefficient of 0.994. The methods are applied for the determination of drugs in commercial tablets and results of analysis were validated statistically through recovery studies showing results from 98.8±0.56 to 100.03±0.71.
Keywords: LevamisoleHydrochloride, Spectrophotometric methods, Validation.

INTRODUCTION:

LevamisoleHydrochloride [LH]**(Fig 1)** is the *S*-enantiomer of tetramisole- a synthetic imidazo-thiazole derivativeacting as an anthelmintic [1]. The single enantiomer was introduced in 1969 since the other enantiomer (dexamisole) showed more adverse effects[1,2]. Levamisole proved to be also effective in combination with 5-fluorouracil as adjuvant therapy in patients with colon carcinoma and current investigations of Levamisole HCl are focused on its immunomodulatory effects. The literature survey reveals that several methods are reported for the determination of LH, by HPLC[7,8,12,15,16],LC-MS[9,13,14], GC[17], GLC[18]and few Extractive spectrophotometric methods for Tetramisole[2,3,4,5] have been reported. In this paper, we reporteda simple and sensitive UVspectroscopic method for the assay of drug. This method is established by simple UV equation method which is later validated with various parameters like linearity, specificity, precision, ruggedness, robustness, etc. The maximum absorbance was seen at 214 nm.

Fig 1: LevamisoleHydrochloride



. HCl

MATERIALS AND METHODS:

AShimadzu, UV Spec-1700, digital spectro photometer and Jasco V-630 spectrophotometer with 1 cm matched quartzcells was used for all spectral and absorbance measurements.

ASystronicssonicator was usedfor all sonication purposes. Pure drugwas procured from β-Pharmaceuticals and Research Ltd., Mangalore as a gift sample.Steam distilled water was used for all dilution purposes.throughout the work. Basic apparatus like calibrated volumetric flasks, pipette, beakers and graduated pipettes were used.

EXPERIMENTAL:

Preparation of stock solutions:
100 mg standard Levamisole Hydrochloride [LH] was weighed and transferred to a 100 ml volumetric flask and dissolved in distilled water. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000 µg/ml (Stock solution I). From this stock solution I, 10 ml solution was pipetted out and placed into 100ml volumetric flask. The volume was made up to mark with distilled water to give a solution containing 100µg/ml (Stock solution II).

Determination of [λ_{max}]
Selection of analytical wavelength: Appropriate dilutions (10µg/ml) were prepared for LH drug from the standard stock solution II and the solutions were scanned in the wavelength range of 200 - 400 nm. Absorption spectra obtained showed the absorption maxima [λ_{max}] 215 nm, which was selected as wavelength of analytical measurement for this method.

Selection of analytical concentration range:
From the standard stock solution II, appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 2 to 20 µg/ml. Absorbance for these solutions were measured at 215nm. For the standard solutions analytical concentration range was found to be 2 - 10 µg/ml and those values are reported.

Calibration curve for the LH (2 – 10 µg/ml):
Appropriate volume of aliquots from standard LH stock solution II were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with distilled water to obtain concentrations of 2, 4, 6, 8, and 10µg/ml. Absorbance of each solution against water as blank were measured at 215 nm and the graph of absorbance against concentration was plotted and is shown in Fig 1. The regression equation and correlation coefficient were determined which are presented in Table 1.

VALIDATIONOFUV SPECTROSCOPICMETHOD:

1) Linearity
Calibration curve was plotted over a concentration range of 2 – 10 µg/ml for LH. Accurately measured standard working solutions of LH (2, 4, 6, 8, and 10ml) were transferred to one set of a series of 10 ml volumetric flasks. Solutions were made up to volume with distilled water. A spectrum was recorded by placing drug solutions and diluent in sample and reference cells respectively. The absorbance was measured at 215nm (Peak maxima) and was plotted vs. concentration to give calibration curve, and regression equation and correlation coefficient (Fig 2)was calculated and presented in Table 1. The calibration curve of amplitude of absorbance against concentration of the drug showed linearity.

2) Sensitivity
The sensitivity of the proposed method for measurement of LH was estimated in terms of limit of detection [LOD] and limit of quantification [LOQ]. The LOD and LOQ were calculated by using the slope and SD of response (intercept). The mean slope value and SD of response were obtained after plotting six calibration curves. The LOD and LOQ obtained are reported in Table 11.

3) Precision
The precision of the method was established by system precision and method precision. System Precision was subjected to intraday and inter-day variation studies.

a) System Precision:
Intraday precision was determined by using three different levels of drug concentrations (2, 4, 6 µg/ml) prepared from stock solution-II and each level was analysed three times in a day. Same procedure was followed for three different days to study the Inter-day precision. Data obtained are given in the Table 3.

b) Method Precision:
Method precision was determined by using sample solution of drug concentrations 2, 4, 6, 8, and 10µg/ml) and it was analyzed six times in a day by the same analyst. Data obtained are given in the Table 4.

4) Accuracy
Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples of LH (4 µg/ml) were spiked with 80, 100 and 120 % extra LH standard and the mixture were analysed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. Data

obtained from accuracy study are given inTable 5.

5) Ruggedness

To establish ruggedness of the proposed method, assays for two different concentrations of LH tablets were performed by two different analysts. The results of assays are represented as % Recovery with SD and % RSD showing the ruggedness of the proposed method are illustrated in Table 6.

6) Reproducibility

The absorbance readings of 4µg/ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility. Data obtained are given in the Table 7.

7) Selectivity and Specificity:

Refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour. The data obtained are given in the Table 8.

STABILITY OF SAMPLE

A 10µg/ml concentration of LH was prepared as a working solution and the absorbance was measured at 30 minutes time interval and the stability of the drug was observed. The data obtained are shown in the Table 9 and Fig 2.

DETERMINATION OF LH FROM DOSAGE FORM

Sample preparation:

A tablet marketed formulation, **Dicaris (*Johnson & Johnson*)** was obtained for all analytical study. Twenty tablets each containing 100 mg of LH were weighed and powdered. The powder equivalent to 50 mg of LH was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the distilled water and sonicated for 10 min. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000 µg/ml. The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution, 0.4 ml was taken and diluted to 100 ml with distilled water to give a solution of 4µg/ml (Working Solution) and used for the estimation of LH. The data obtained are shown in the Fig 3.

Table 1: Result of calibration readings at 215 nm for LH

Concentrations (µg/ml)	Absorbance at 215 nm Mean ± S.D. (n=6)	Coefficient of variation
2	0.1841 ± 0.0027	0.014
4	0.3323 ± 0.0035	0.010
6	0.5128 ± 0.0037	0.007
8	0.6733 ± 0.0037	0.005
10	0.8545 ± 0.0026	0.003

Table 2: Statistical data for LH by UV method

Parameter	LH (at 215 nm)
Linear Range (µg/ml)	2-10
Molar absorptivity (1/mol.cm)	8.60×10^5
Regression Equation* (y)	y=bx+a
Slope (b)	0.0846
Intercept (a)	0.0033
Correlation coefficient (R^2)	0.9994
Standard deviation of slope	0.00025
Standard deviation of intercept	0.001628
Limit of Detection (µg/ml)	0.0097
Limit of Quantitation (µg/ml)	0.0295

*y = bx + a where x is the concentration of LH in µg/ml and y is the absorbance at the respective wavelength

Table 3: System Precision data for LH by spectrophotometric method

Serial No.	Concentration (µg/ml)	Inter-day Precision		Intra-day Precision	
		Mean ± S.D	RSD	Mean ± S.D	RSD
1	**2**	0.1853±0.0012	0.64	0.183±0.0016	0.89
2	**4**	0.3293± 0.0020	0.60	0.328±0.0024	0.74
3	**6**	0.513 ± 0.0024	0.60	0.5116±0.0024	0.48

Table 4: Repeatability data (Method Precision) for LH at 215 nm

Concentration	2µg/ml	4µg/ml	6µg/ml	8µg/ml	10µg/ml
Absorption	0.187	0.337	0.518	0.677	0.852
	0.185	0.333	0.517	0.673	0.859
	0.181	0.327	0.513	0.669	0.855
	0.183	0.336	0.508	0.678	0.854
	0.188	0.329	0.512	0.668	0.856
	0.181	0.332	0.509	0.675	0.851
Mean.	0.1841	0.3323	0.5128	0.6733	0.8545
Std. Dev.	0.002734	0.003543	0.003716	0.003771	0.00263
RSD	0.0148	0.0106	0.00724	0.0056	0.00307

n = 6 determination

Table 5: Determination of Accuracy, Recovery studies

Amt. of sample LH µg/ml	Amt. of Pure drug LH %	Amt. of Pure drug LH µg/ml	Amt. of drug recovered LH µg/ml	Mean % Recovery ±SD*
5	80%	4	4.02	99.5±0.81
5	100%	5	4.99	98.8±0.56
5	120%	6	5.88	100.03±0.71

Table 6: Ruggedness results for LH at 215 nm by UV Spectroscopy

Sr. No.	Concentration (µg)	Analyst I		Analyst II	
		Amount found* (µg)	(%) Recovery ± SD*	Amount found* (µg)	(%) Recovery ± SD*
1	2	1.99	98.76 ± 0.75	2.01	100.76 ± 0.71
2	4	3.93	98.41 ± 0.92	3.98	99.58 ± 0.62

Table 7: Robustness data for LH at 215 nm

Conc. µg/ml	Instrument 1	%RSD	Instrument 2	%RSD
4	0.3283 ±0.0020	0.60	0.327±0.0032	0.97

Table 8: Specificity and Selectivity study

Study	Levamisole Hydrochloride
Specificity	Specific
Selectivity	Selective

Table 9: Stability of sample

Sr. No.	Concentration of drug solution(µg/ml)	Time(min)	Absorbance at 215 nm
1	10	0	0.857
2	10	30	0.858
3	10	60	0.656
4	10	90	0.857
5	10	120	0.854
6	10	240	0.853
7	10	360	0.853
8	10	480	0.854
9	10	600	0.851

Fig 2: $\lambda_{max}$of Levamisole Hydrochloride

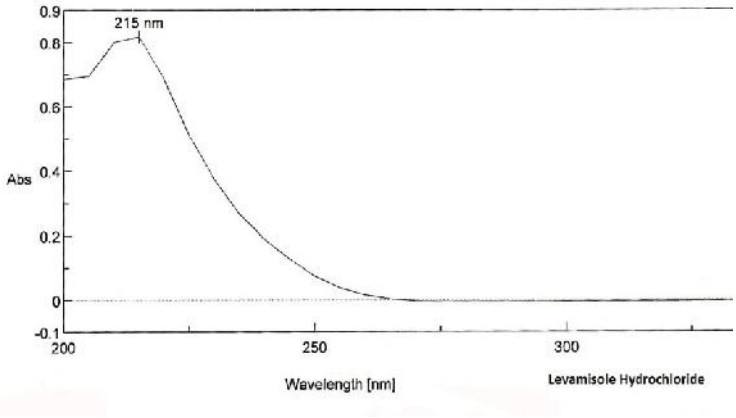


Fig 3: Calibration Curve of LH at 215 nm

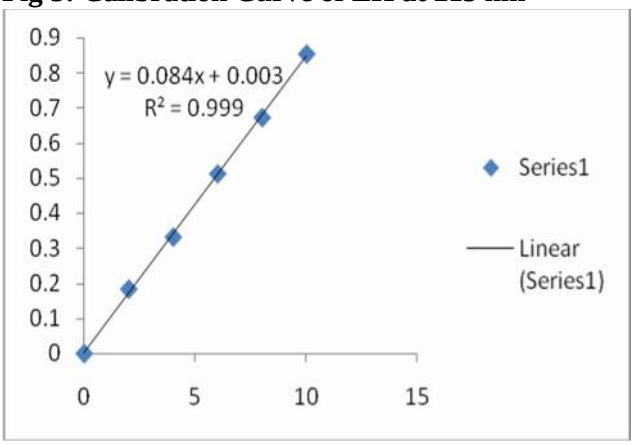


Fig 4: Stability of sample

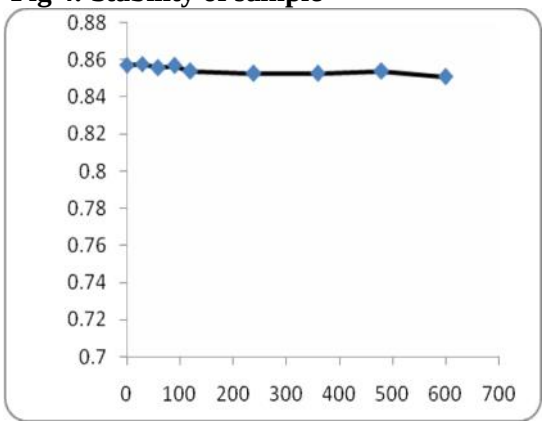


Table 10: Assay Results of Marketed Formulation

Formulation	Actual concentration of LH(µg/ml)	Amount obtained of LH (µg/ml)	%LH
Tablet	08	7.95	99.37

Table 11: Summary table of validation parameters of Levamisole Hydrochloride by spectrophotometric methods

S.R No.	Parameters	Method
1)	**Range (µg/ml)**	2-10µg
2)	**Correlation coefficient (r^2)**	0.9994
3) a) b)	**Precision:** **Method precision (n=6)** **Intermediate precision (n=3)** Intraday Inter day	0.03-0.14 0.60– 0.64 0.48-0.89
4)	**Reproducibility**	0.6-0.97
5)	**Ruggedness:** **Analyst 1** **Analyst 2**	 98.41±0.92 - 98.76±0.75 99.58±0.62 - 100.76±0.71
6)	**Accuracy (recovery, n=3)** **% Mean recovery**	**1) At Level-1 (80%)=**99.5±0.85 **2) At Level-2 (100%)=**98.8±0.56 **3) At Level-3 (120%)**100.03±0.71
7)	**LOD (µg/ml)**	0.0097
8)	**LOQ (µg/ml)**	0.0295

RESULTS AND DISCUSSION:

Specific accurate precise and simple UV spectroscopic method was developed for determination ofLevamisole Hydrochloride from their dosage form. The first method was based on Calibration curve method. In the proposed Calibration curve method, the signals were measured at 215nm. The Concentration of each drug was obtained by using the Calibration curve.

CONCLUSION:

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. LH is official in Indian Pharmacopoeia. A very few analytical methods appeared in the literature for the determination of LHincludes LC, HPLC, HPTLC and UV-Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation, UV Spectrophotometry method for the quantitative estimation of LH in bulk drug and pharmaceutical formulations has been developed. The UV methodis more sensitive, accurate and precise compared to the analytical methods appeared in the literature.The above proposed UV spectroscopic method was simple, easy to apply low cost, does not use polluting reagents and requires relatively inexpensive instruments. It may be considered as good alternative to the RP-HPLC method for estimation of Levamisole Hydrochloride in dosage forms.The reliability of the UV Method was proven by fulfilling all the validation criteria.

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