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Sensitivity of levamisole in different solvents by UV method

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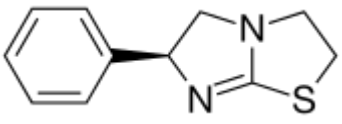
Abstract

Levamisole was originally used as an anthelmintic to treat worm infestations in both humans and animals. Two simple UV spectrophotometric methods have developed for determination of levamisole in bulk and formulations. The absorption maxima (λmax) of levamisole was found to be 216nm in 0.1 N HCl and in double distilled water observed at 215 nm and Beer-Lambert's law was obeyed over the concentration range 2-10µg/ml.LOD and LOQ values of levamisole was found to be 0.125 µg/ml, 0.416 µg/ml and 0.098 µg/ml, 0.326µg/ml in 0.1 N HCl and double distilled water respectively. The validation parameters were treated statistically with 't' test and significant differences were observed. The study clearly revealed that the solvents influence the determination of levamisole. The methods developed were rapid and easily can be applied for the estimation of levamisole in bulk and pharmaceutical formulations.

Keywords: Levamisole; Hydrochloric acid; Double distilled water; UV Spectrophotometry

1. Introduction

Levamisole used to treat parasitic worm infections, specifically ascariasis and hookworm infections.[1] levamisole has been used to treat a variety of dermatologic conditions, including skin infections, leprosy, warts, lichen planus, and aphthous ulcers.[10] Choice of solvent can shift peaks to shorter or longer λ depends on their nature of the interaction of the particular solvent with the environment of the chromophore in the excited state of the molecule. Solvents can affect the fine structure of absorption curves as well as the intensities and wavelengths of maxima. There are various methods are available for the estimation of levamisole. Among these methods High performance liquid chromatography (HPLC), spectrophotometric methods and LC/MS reported for the quantification of levamisole. The effect of solvents for the determination of levamisole has not been reported .In the present study two UV spectrophotometric methods were developed for estimation of levamisole in bulk and pharmaceutical formulations among these the sensitive one can be reported by calculating limit of detection (LOD) and limit of quantification (LOQ) as per ICH guidelines.



. HCl

Figure 1 Structure of levamisole hydrochloride

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2. Material and methods

Absorbance measurements were made on double beam UV-Visible spectrophotometer with spectral band width of 0.5nm and wavelength accuracy of ± 0.3nm with 10 mm matched quartz cuvettes (ELICO SL 244) were employed. Levamisole HCl reference standard was gifted by Aurochem laboratories Pvt. Ltd, Mumbai. Hydrochloric acid used was of analytical grade purchased from National scientific Laboratories Vijayawada.

2.1. Preparation of standard stock solutions of levamisole hydrochloride

50 mg of levamisole hydrochloride pharmaceutical grade was accurately weighed two times separately and transferred into two different 50 ml volumetric flasks and dissolved in 0.1N HCl, double distilled water volume was made up to the mark with the same solvents the stock solution obtained was 1000ppm(1mg/ml).

2.2. Determination of λ max

The two different stock solutions were suitably diluted with 0.1 N HCl and double distilled water to get a concentration of10 ppm (10µg/ml) and scanned in the UV region ranges from 200nm-400nm. The wave length at which maximum absorbance observed was noted. The absorbance of the standard solutions containing the hydrochloric acid and double distilled were observed at 216 nm and 215 nm respectively.

2.2.1. Beer's Lambert law

The two different stock solutions were suitably diluted to get concentration range from 2-10µg/ml and their absorbance were measured at λmax 216 nm against hydrochloric acid as blank and λmax 215 nm against double distilled water as blank. Calibration curve constructed for levamisole in different solvents by taking concentration (µg/ml) on x-axis and their absorbance on y-axis.

The proposed methods are validated for the following parameters

- Linearity

Linearity ranges of the proposed UV methods were found out. In order to find out the linearity range of proposed UV methods a curve was constructed by plotting absorbance obtained for the analyte against its concentrations in 0.1 N HCl and double distilled water. A series of 2(µg/ml), 4(µg/ml), 6(µg/ml), 8(µg/ml), and 10(µg/ml) were prepared for standard calibration curve and absorbance were observed. The results were subjected to regression analysis by the least squares method to calculate slope (m), intercept (c) and regression coefficient (R^2) and the values are depicted in Table No.1&2.

- Precision

Precision of method was determined in terms of repeatability (within run precision), intermediate precision and reproducibility (between run precision).

- Repeatability

Repeatability of the method was determined by analyzing three samples of 4(µg/ml), 6(µg/ml), 8(µg/ml) concentration in two different solvents and the %RSD and SE were calculated.

2.3. Intermediate precision

2.3.1. AIntraday precision

It was calculated by analyzing six test samples of levamisole on the same day ,and the intraday precision of the method was determined by evaluating the samples of levamisole on different days or and on two different spectrophotometers in the same laboratory.

2.3.2. Reproducibility

The sample solutions were prepared and analyzed in different labs sta

2.3.3. Sensitivity

The sensitivity of the proposed UV method was measured in terms of limit of detection (LOD) and limit of quantification (LOQ).The LOD and LOQ were calculated using formula:

$$LOD=3.3\sigma/s\& \ LOQ=10\sigma++/s$$

Where σ=standard deviation of Y-intercepts of regression lines. S=slope of the calibration curve. The values are depicted in Table No.3&4.

2.3.4. Sandell's sensitivity and Molar absorption coefficient

It is calculated by using the following formula

$$S=\epsilon.\ p$$

Where S=sandell's sensitivity ϵ=specific extinction coefficient P=concentration of substance in mg/liter.

2.3.5. Robustness

To determine the robustness of the method, the experimental conditions were altered and assay was evaluated. Sample solutions were prepared and absorbance were observed at ± 5nm from absorption maxima.

2.3.6. Accuracy

Accuracy of the methods were confirmed by studying recovery at three different concentrations for 80, 100, 120% of these expected, in accordance with ICH guidelines by replicate analysis, Standard drug solutions were added to a pre analyzed sample solution and %drug content was measured.

2.3.7. Analysis of tablet formulations

Twenty tablets from each brand were weighed and grained into a fine powder using a Pestle and Mortar. An amount of tablet powder equivalent to 25 mg of LVS was taken and dilutions were made with respective solvents, filtered using Whatmann No.42 filter. Analyze these solutions by using proposed UV methods.

2.3.8. Recovery study

To further ascertain the accuracy and reliability of the proposed methods, recovery experiments were performed via standard-addition procedure. Pre-analyzed tablet powder was spiked with pure LVS at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The percent recovery of pure LVS added was within the permissible limits indicating the absence of inactive ingredients in the assay. These results are depicted in Table.No.6

3. Results and discussion

The wavelength maxima obtained for levamisole in two different solvents were 216 nm in 0.1 N HCl and in double distilled water observed at 215 nm. The developed UV spectrophotometric methods followed beer's law in the range of 2-10µg/ml. The relative standard deviation values were observed less than '1' indicates precision of the method, the lower standard error value indicates the accuracy of the method. The molar extinction coefficient, sandell's sensitivity, and LOD & LOQ values were calculated as per ICH guide lines, the values are depicted in table No.5. Based on LOD and LOQ values, the solvents were ranked 0.1N HCl > double distilled water. The study clearly revealed the sensitivity of the method improved in the presence of double distilled water than in hydrochloric acid. Double distilled water was found to be more sensitive as it offered lowest LOD and LOQ values. LOD and LOQ values are subjected to statistically and data shown in Table.No:3&4. Significant differences were observed between the solvents used for the determination of drug. Lowest LOD and LOQ values were observed with the solvent i.e. double distilled water. The LOD and LOQ values of levamisole in 0.1 N HCl and double distilled water were subjected to t-test. The calculated 't value compared with t-table value, the observed value was greater than the table value indicated that the significant differences between the LOD and LOQ values of levamisole in two different solvents.

Table 1 Linearity of levamisole HCl in 0.1N HCl

Sr. No.	Concentration(ppm)	Absorbance
1.	2	0.211
2.	4	0.442
3.	6	0.643
4.	8	0.825
5.	10	1.055

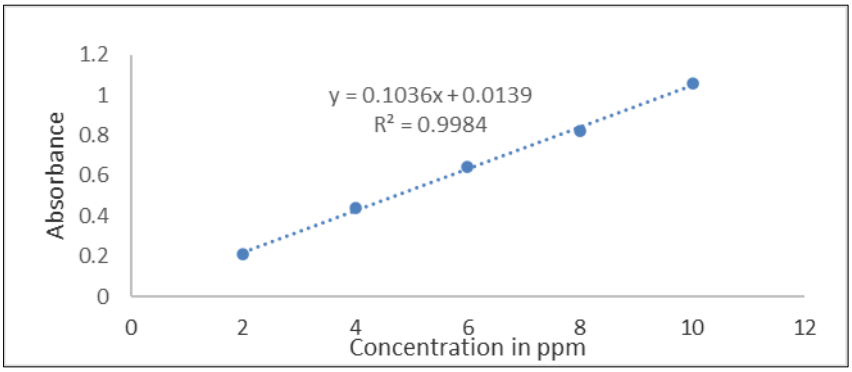

Figure 2 Calibration curve of levamisole HCl in 0.1N HCl

Table 2 Linearity of levamisole HCl in double distilled water

Sr. No.	Concentration(ppm)	Absorbance
1.	2	0.183
2.	4	0.376
3.	6	0.554
4.	8	0.734
5.	10	0.925

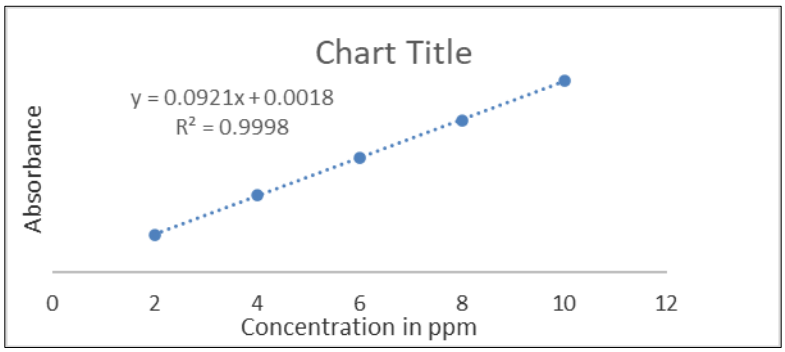

Figure 3 Calibration curve of levamisole HCl in double distilled water

Table 3 Limit of Detection (LOD)

S.No	0.1N HCl LOD±S.D(n=6)	Double distilled water LOD±S.D(n=6)	tCal	tTab	D.F
1.	$0.125\pm1.13X10^{-4}$	$0.098\pm2.32X10^{-4}$	5.43	2.228	10

D.F=Degrees of freedom, tCal-Calculated t value, tTab–t table value

Table 4 Limit of Quantification (LOQ)

S. No	0.1 N HCl, LOQ±S.D(n=6)	Acetic acid LOQ±S.D(n=6)	tCal	tTab	D.F
1.	$0.416\pm2.34X10^{-4}$	$0.326\pm1.56X10^{-4}$	11.23	2.228	10

D.F=Degrees of freedom, tCal-Calculated t value, tTab–t table value

Table 5 Validation Parameters of Proposed UV methods

S.No	Parameters	0.1 N HCl	Double distilled water
1.	λmax	216 nm	215 nm
2.	Range	2-10µg/ml	2-10µg/ml
3.	Regression equation	Y=0.1036X+0.0139	Y=0.0921X+0.0018
4.	Slope	0.1036	0.0921
5.	Intercept	0.0139	0.0018
6.	$A^{1\%}_{1cm}$	1064	924
7.	R^2	0.9984	0.9998
8.	Molar absorption coefficient(litre/mole.cm-1)	1.142×10^4	$1.123X10^4$
9.	LOD	0.125µg/ml	0.098µg/ml
10.	LOQ	0.416µg/ml	0.326µg/ml
11.	Sandell's sensitivity(µg/cm2/0.001absorbanceunit)	0.01214	0.01067

Table 6 Results of recovery study by standard addition method

Brad Name of Tablets	0.1 N Hydrochloric acid				Double distilled water			
	LVS i ntablets (µg/ml)	Pure LVS added (µg/ml)	Total Found (µg/ml)	Pure LVS recovered percent ±S.D(n=3)	LVS in tablets (µg/ml)	Pure LVS added(µg /ml)	Total found (µg/ml)	Pure LVS recovered percent ±S.D(n=3)
Vermisol-50	10	2	11.78	98.16± 1.13	10	2	11.86	98.83±1.24
	10	4	14.02	100.1±1.19	10	4	13.98	99.86±1.33
	10	6	15.92	99.5±0.86	10	6	16.08	100.5±0.92
Vitilex-50	20	2	22.07	100.3± 1.26	20	2	21.91	99.59±1.08
	20	4	23.97	99.88±0.78	20	4	24.09	100.4±0.78
	20	6	26.14	100.5±1.35	20	6	25.95	99.81±1.23

Mean value of three determinations, LVS: Levamisole, S.D: Standard deviation

4. Conclusion

The proposed methods were easy to perform and applicable for estimation of levamisole HCl in bulk and pharmaceutical formulations in Quality control laboratories

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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