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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OFLEVAMISOLE IN PURE AND DOSAGE FORM

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Abstract: A reverse phase isocratic HPLC was developed and validated for the determination of levamisole in pure and tablet dosage forms. Method development was carried out on YMCC18isocratic column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature), Shimadzu LC–sol 2010 Prominence Liquid Chromatograph. The mobile phase was a mixture of Acetonitrile: Water 80:20 v/v, and the flow rate was set at 0.7 ml/min and UV detection at 217 nm. Validation parameters were evaluated for the method according to the ICH guidelines. In the linearity study, linearity was observed from 10-50 µg/ml with correlation coefficient of 0.9999 and regression coefficient of 0.999. The limit of detection and limit of quantitation for the method were 0.204µg/ml and 0.673µg/ml, respectively. The statistical analysis shows that the method was found to be accurate, reliable, simple and reproducible. The intra and inter assay precisions were satisfactory; the values of relative standard deviations did not exceed 2%. The accuracy of the method was proved; the recovery of levamisole was 98.8% -101.1%. The chromatographic retention time of proposed method was 6.5 min and the assay of content was found to be 98.9%-100.8%. The proposed method was successfully applied for the quantitative determination of levamisole in pure form and could be used for routine analysis with phenomenal accuracy and precisions.

Keywords: RP-HPLC, Levamisole, Validation, ICH guidelines.

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INTRODUCTION

Levamisole(LMS) is (S)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole (Figure 1). LMS is a pharmaceutical with anthelminthic and immunomodulatory properties[1]that was previously used in both animals and humans to treat inflammatory conditions and cancer.LMS is the levorotatory isomer of tetramisole. Levamisole has been used in humans to treat parasitic worm infections, and has been studied in combination with other forms of chemotherapy for colon cancer, melanoma, and head and neck cancer[1,2,3].

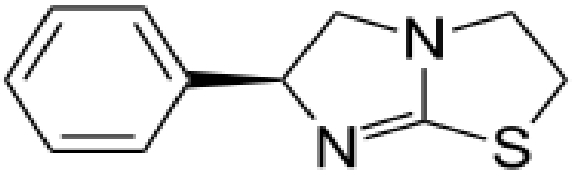

Figure 1: Structure of Levamisole

Literature survey reveals few analytical methods were reported for the determination of LMS in pure and pharmaceutical preparations and in biological fluids by spectrophotometry[2],High Performance Liquid Chromatography[3,4,5,6]. However most of the available methods have limitations such as a long runtimes, low sensitivity, uneconomical and have poor symmetry. Keeping in view of these, an attempt has been made to develop a simple, accurate, precise and reliable RP-HPLC method for the estimation of levamisole in pharmaceutical dosage forms. In fact the established method was validated with respect to specificity, linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines (ICH, 1997)[7,8,9,10].

MATERIALS AND METHODS

Chemicals and Reagents an analytically pure sample of LMS standard was procured as gift sample from Cipla Ltd., Mumbai, India. All the chemicals were analytical grade. HPLC grade acetonitrile and water used were of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of LMS formulation was procured from local pharmacy. LEVOMOL tablets containing LMS with labeled amount of50mg per tablet are manufactured by Cipla Ltd., Mumbai, India.

INSTRUMENTS AND CHROMATOGRAPHIC CONDITIONS

The HPLC analysis was performed on Shimadzu LC-sol2010 Prominence Liquid Chromatograph YMC C18 column (4.6 mm X 250 mm, 5 micron particle size). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was equipped with data N2000 software. The mobile phase consists of a mixture of acetonitrile and

water in ratio of 80:20 % v/v. The mobile phase was set at a flow rate of 0.7 mL/min. Elute was monitored at 217 nm[11,12,13].

PREPARATION OF REAGENTS AND STANDARDS

MOBILE PHASE

The mobile phase was prepared by mixing of acetonitrile and water (all of HPLC grade) in the ratio of 80:20, v/v. It is filtered through 0.45 µm nylon membrane filter and then sonicated for degassing. Stock and Working Standard Solutions Accurately 10 mg of LMS was weighed and transferred to a 10 mL clean, dry volumetric flask and mobile phase was added and sonicate to dissolve. The volume was made up to the mark with mobile phase. This is stock standard solution of LMS with concentration of 1000 µg/mL. Prepare five working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with mobile phase. The concentrations of LMS are 10, 20, 30, 40&50µg/mL respectively.

TABLET SAMPLE PREPARATION

Weigh accurately not less than 20 tablets of LMS and determine average weight. Grind the tablets of LMS (LEVOMOL) into fine powder. Weigh accurately an amount of tablet powder equivalent to 50 mg of LMS and transfer into 50 mL volumetric flask. Add 40 mL mobile phase and place in an ultra-sonication bathuntil dissolution is complete. Add mobile phase to bring up the volume to 50 mL. Pipette out 1.0 mL of the sample solution into a 10 mL volumetric flask and dilute with mobile phase up to the mark. Mix well. The resulting solution was filtered using 0.2 µm filter and degassed by sonication. The resulting solution is further diluted to give a concentration of approximately 20µg/mL.

SELECTION OF DETECTION WAVELENGTH

The UV spectrum of diluted solutions of various concentrations of LMS in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was observed at 217nm. This wavelength was used for detection of LMS.

CALIBRATION CURVE FOR LEVAMISOLE

20 µl of each calibration standard solutions (10, 20, 30, 40 &50µg/mL) were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded. Linearity curve was constructed by plotting concentration of LMS on X-axis and average peak areas of standard LMS on Y-axis and regression equations were computed for LMS. The linearity range was found to be 10-50 µg/mL. The results were presented in Table 1. The standard chromatogram of LMS calibration standard have been depicted in Figure 2.

Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range. The regression graph for LMS is presented in Figure 3. The data of analysis is presented in Table 2.

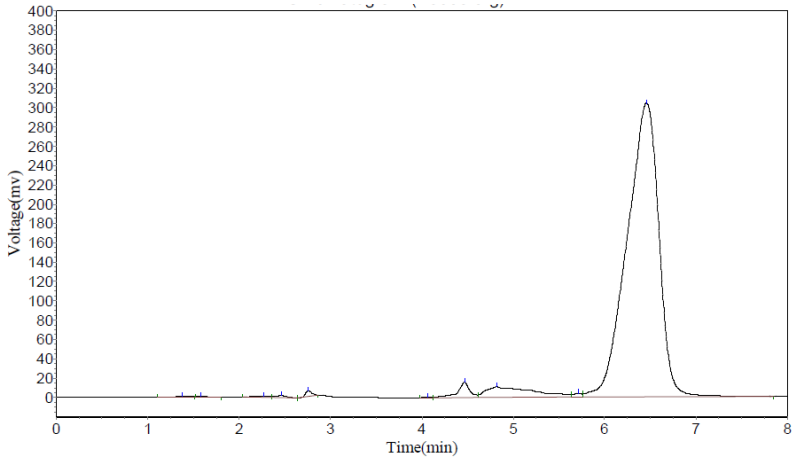

Figure 2- Standard Chromatogram of LMS

Table 1- Linearity

Concentration of levamisole (µg/ml)	Levamisole Peak Area (mV.sec)
10	1447520
20	3269722
30	5327993
40	7045261
50	8718995

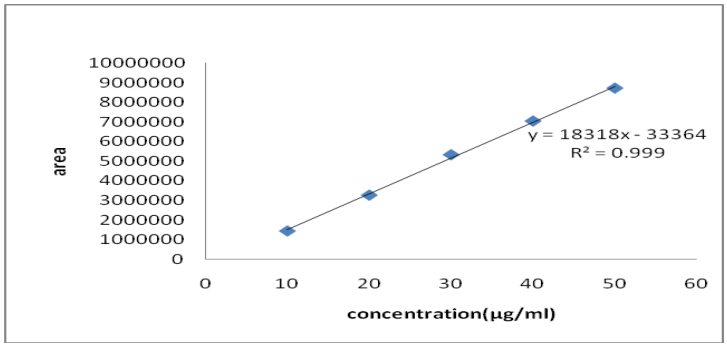

Figure 3- linearity graph

ASSAY OF LEVAMISOLE TABLETS

The developed method was applied to the assay of LMS tablets. The drug content was calculated as an average of six determinations and assay results were shown within limits. The results were very close to the labeled value of commercial tablets.

Table 3 - Data of Analysis

Linearity range	**10-50 (µg/ml)**
Regression equation	y =18318x – 33364
Slope	18318
Intercept	-33364
Correlation coefficient	0.999
System precision (% RSD)	1.81
Method precision (% RSD)	1.40
% Recovery	100.0 – 101.16 %
LOD	0.204 µg/ml
LOQ	0.673 µg/ml
% Assay	98.9%-100.8%.

RESULTS AND DISCUSSION

The present study was aimed at developing a precise, sensitive, rapid and accurate HPLC method for the analysis of LMS in pure drug and in pharmaceutical dosage forms. In order to achieve phenomenal retention time and peak asymmetry, a C18 stationary phase column (250mm X 4.6mm, 5 µm particle size) and mobile phase composed of acetonitrile and HPLC grade water in a ratio of 80:20, v/v, at a flow rate of 0.7mL/min was selected. The retention time for LMS was found to be6.5 min. UV spectra of LMS showed that the drug absorbed maximum at 217 nm, so this wavelength was selected as the detection wavelength. The correlation coefficient (0.9999) of regression was found almost equal to1 in the range of 10-50 µg/mL which states that the method was linear to the concentration versus peak area responses. On slight variation in the mobile phase ratio of upto ±5 %, the change in the peak asymmetry, plate count and retention time are within the limits which indicated that the method is robust and also indicating lack of influence on the test results by operational variable for the proposed method. This shows that the method is having phenomenal system suitability parameters under given conditions. The comparison of chromatograms of placebo, standard and sample, there was no interference observed from the peaks of placebo, standard and sample. The accuracy of the method was found to be good with the overall % RSD for recovery at 80%, 100% and 120% levels were all within the limits.This indicates that the proposed

method was found to be accurate. Method validation following ICH guidelines indicated that the developed method had high sensitivity with LOD of 0.204 µg/mL and LOQ of 0.673µg/mL. The assay results of tablets by applying the HPLC method was found to be within the pharmacopoeial limits and the assay values were found to be 98.9%-100.8%.

CONCLUSION

The developed RP-HPLC method for the quantification of LMS has various advantages like less retention time, good peak symmetry and phenomenal linearity, highly sensitive, simple, precise, accurate and robust. The mobile phase can be easily prepared and diluent is economical and readily available and it does not need sample preparation with sophisticated techniques or instruments. These attribute the high quality of the method. The proposed method can be used for the routine analysis of LMS in pure preparations of the drug and in pharmaceutical dosage forms for routine application in quality control laboratories without interference of excipients.

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