INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 1 | Jan - Mar -2017
Journal Home page: www.ijpar.com

Research article *Open Access*

RP-HPLC method development and validation of levamisole in pure and pharmaceutical formulation

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ABSTRACT

A simple, fast, precise, selective and accurate RP-HPLC method was developed and validated for the determination of levamisole from pharmaceutical formulation. Chromatographic separation was achieved on a YMC C18 column (250 x 4.6mm, 5 µ particle size) using a mobile phase acetonitrile and water in the ratio of 80:20%V/V. The flow rate was 0.7ml / min and effluent was detected at 217nm. The retention time of levamisole was found to be 6.2min. Linearity was observed in the concentration range of 10 -50µg / ml .The method was validated according to ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of levamisole.

Keywords: Levamisole, RP-HPLC

INTRODUCTION

Levamisole is chemically (*s*)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*] [1,3]thiazole used as anti helmenthic.

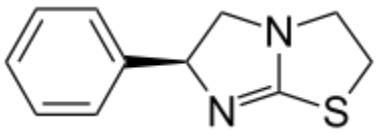

Figure 1: Chemical structure of levamisole

Several HPLC, GC and LC/MS-MS methods have been reported for the analysis of levamisole in plasma that suffer from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard.

The objective of this study was to develop reverse phase high performance liquid

chromatography method for the estimation of levamisole in pure and pharmaceutical dosage form without any derivatization and having short retention time. This method was found to be linear, precise, accurate, sensitive, specific, and robust, and therefore suitable for routine analysis.

MATERIALS AND METHOD

HPLC Instrumentation and Chromatographic conditions

The analytical separations were carried out on a waters 2487 HPLC system equipped with Photo Diode Array detector. The output of signal was monitored and integrated using LC – solutions 2000 software. The analytical column was YMC C18 (250 × 4.6mm, 5µ). Mobile phase consisted Acetonitrile and water in the ratio of 80:20% v/v. Mobile phase was mixed, filtered through 0.45µ membrane filter and degassed under ultrasonication. The mobile phase was used as diluent. The flow rate was 0.7 ml/min and runtime was 8 minute. The column was maintained at ambient temperature. UV detection was measured at 217 nm and the volume of sample injected was 20 µl.

Preparation of standard stock solution

100mg of levamisole was weighed accurately and dissolved in 100ml of mobile phase to get the concentration of 1000 µg/ml. Resultant solution was filtered through Whatman filter paper. The standard chromatogram for levamisole (20µg/ml) was shown in figure 2.

Preparation of working standard solution

Working standard solutions of levamisole were prepared by accurately transferring the (0.1,0.2,0.3,0.4, and 0.5ml) aliquots of the standard stock solution into a series of five 10 ml volumetric flasks. The volume was made up to mark with mobile phase to obtain concentration range of 10 – 50 µg/ml.

Preparation of sample solutions

25mg of tablet powder was weighed accurately and was taken into 25mL volumetric flask and then the sample was diluted to 25ml with mobile phase to get concentration of 1000µg/ml and used for analysis.

RESULTS AND DISCUSSION

HPLC method development and optimization

To optimize the chromatographic conditions, different columns, mobile phases, flow rates etc., were tested. Acetonitrile and water in the ratio of 80:20%v/v was preferred as mobile phase because it resulted in a greater response to levamisole after several preliminary investigatory runs compared with the different mobile phase combinations. The effect of the flow rate was studied in the range 0.5 to 1ml/min and 0.7ml/min was preferred to be effective. Under these conditions, the analyte peak obtained was well-defined and free from tailing. The retention time was found to be 6.2min. The optimized chromatographic parameters were listed in table 1

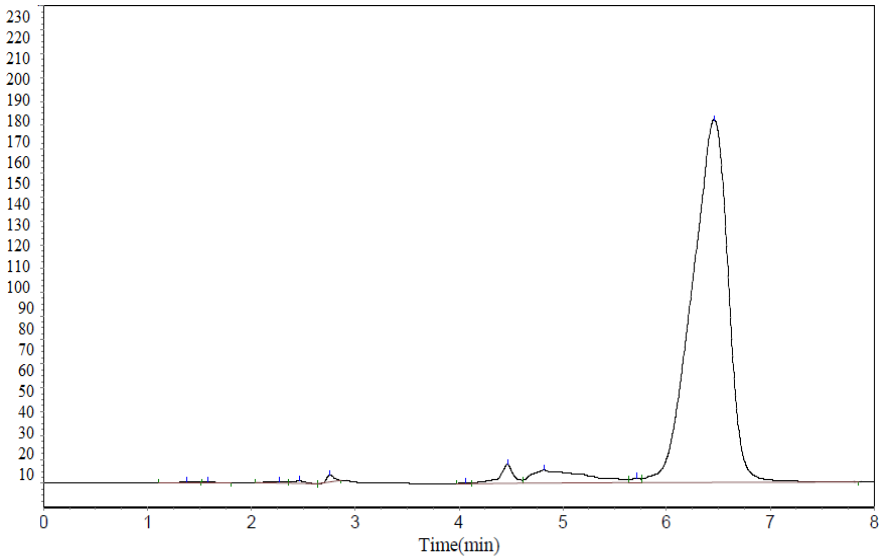

Table 1: Optimized chromatographic parameters

Elution	:	Isocratic
Mobile phase	:	Acetonitrile: water (80:20)
Column	:	YMCc18 column
Flow rate	:	0.7ml/min
Detection	:	217nm
Injection volume	:	10µl
Temperature	:	Ambient
Retention time	:	6.2min
Run time	:	8min
Concentration	:	10 - 50µg/ml

Validation of the method [8], [11],[12]

When method development and optimization are complete, it is necessary to accomplish method validation. The validation studies include linear range (correlation coefficient), method precision (RSD, %), method accuracy (% recovery and RSD, %), sensitivity studies (LOD & LOQ), and robustness.

System suitability studies [8],[11],[12]

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), tailing factor (T), and peak asymmetry (AS) were evaluated. The system suitability test was performed using five replicate injections of standards before analysis of samples. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. System suitability parameters were shown in table 2.

Table 2: System suitability parameters

Parameters	Values
Tailing factor	1.02
Theoretical plates	3800
Retention Time	6.2 min
%RSD	0.2359

Linearity [8],[11],[12]

The linearity of the method was evaluated by preparing six series of standard solutions of levamisole in the range of 10 – 50 µg/ml in mobile phase and injecting the solutions into the HPLC system. Excellent correlation between levamisole peak area and concentration was observed with R^2 = 0.999 (Figure.3). The regression equation was found to be Y=18318x-33364. Statistical data are presented in table 3 and the calibration curve was shown in figure 3.

Table 3: Linearity results for levamisole

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

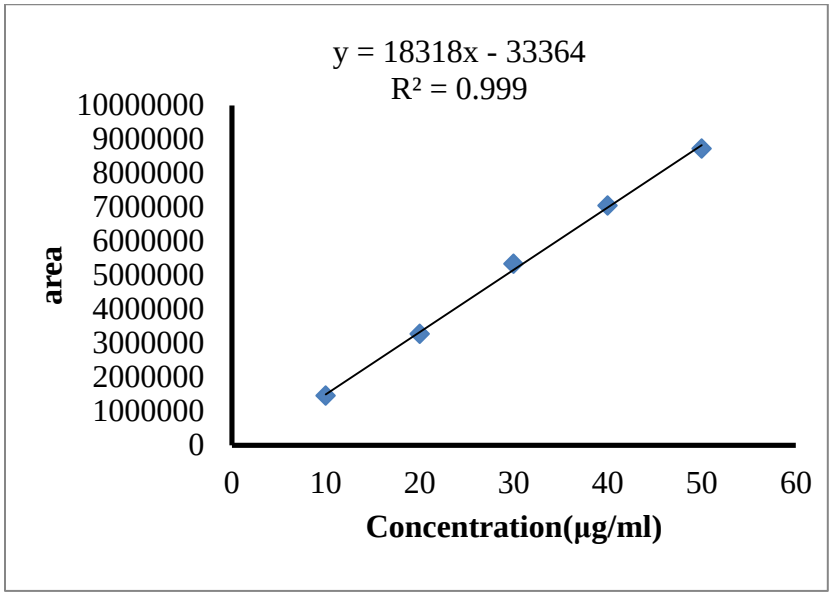

Figure 3: Calibration curve for Levamisole

Precision [8],[11],[12]

System precision [8],[11],[12] (Repeatability)

To study precision, five replicate standard solutions of levamisole (20µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4: Results of system precision for levamisole

Sl.no	Retention time (min)	Peak area
1	6.567	3269721
2	6.546	3278563
3	6.545	3389672
4	6.524	3232761
5	6.435	3268544
Mean	6.5234	3287852
SD	0.051704	59561.22
%RSD	0.792592	1.811554

Method precision [8], [11], [12] (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses 5 times on the same day and on different days for concentration of sample solutions of 20µg/ml. The result was reported in terms of relative standard deviation (% RSD). Results of method precision studies were shown in table 5.

Table 5: Results of Method precision for levamisole

S.no	Standard area =3307922	
	Area	% Labelled Claim
1	3345817	98.8
2	3325894	99.4
3	3369841	98.1
4	3258974	101.5
5	3374281	98.3
Mean	3334961	99.22
S.D	46735.33	1.370036
%RSD	1.401375	1.380807

Intermediate precision

The intermediate precision of the proposed method was determined by performing the method by two analysts (Analyst 1 and Analyst 2) for concentration of sample solutions 20µg/ml. The percent relative standard deviation (% RSD) for peak responses was calculated. The results for intermediate precision were shown in table 6.

Table 6: Results of Intermediate precision for levamisole

	S.no	ANALYST – 1		ANALYST – 2	
		Retention time	Area (Mv.sec)	Retention time	Area (Mv.sec)
Levamisole	1	6.56	3269722	6.52	3458124
	2	6.54	3278562	6.32	3584691
	3	6.52	3349672	6.51	3412576
	4	6.54	3232761	6.31	3412589
	5	6.51	3368892	6.49	3487569
	MEAN	6.534	3299922	6.43	3471110
	S.D	0.0164	57251.26	0.0381	63547.01
	% RSD	0.250995	1.734928	0.592535	1.830741

Accuracy [8],[11],[12]

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at 2 different concentrations 20µg/mL and 40µg/mL of these expected, in accordance with ICH guidelines, by replicate analysis (n=2). Known amount of standard drug solution (20µg/ml) was added to a pre analyzed sample solution (20, 40µg/ml) and percentage drug content was measured. The closeness of obtained value to the true value indicates that the proposed method is accurate.

%Recovery = [(Ct –Cpa)/ Cs] × 100.

Where, Ct = Total concentration of analyte

Cpa = Concentration of pre-analysed sample

Cs = Concentration of standard added to pre-analyzed sample.

Robustness [8],[11],[12]

The robustness study was performed to evaluate the influence of small but deliberate variation in the

chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength

- Change in the detection wavelength by ± 2nm (215nm and 219nm)
- Change in flow rate by ± 0.1 ml/minute (0.6 ml/min and 0.8 ml/minute)

After each change, sample solution was injected and % assay with system suitability parameters were checked.

Robustness values were given in table 7

Table 7: Results of Robustness for levamisole

Parameter	Rt(min)	Area(mvsec)
Flow rate(ml/min)		
0.6	6.86	3312458
0.8	6.85	3239874
Wavelength(nm)		
219	6.87	3365478
215	6.86	3356984

Limit of Detection and Quantitation [8],[11],[12]

Detection and Quantitation limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

Limit of Detection = σ × 3.3/S

Limit of Quantitation = σ × 10/S

Where σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Results of LOD & LOQ were found as 0.2040 µg/ml and 0.6120 µg/ml.

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions were compared in order to provide an indication of specificity of the method.

Assay of pharmaceutical formulation [8],[11],[12]

The proposed validated method was successfully applied to determine levamisole in their pharmaceutical dosage form and the % Assay results were shown in table 8.

Table 8: Results of % assay by using RP – HPLC method

Drug	Sl.no	Amount found(mg)	Test area	Standard area	%Assay (A_T /A_S*100)
levamisole	1	149.6735	3300722		99.78234
	2	149.766	3302761	3307922	99.84398
	3	149.5756	3298562		99.71704

CONCLUSION

A simple, rapid, accurate, and precise RP-HPLC method for the analysis of levamisole in pure and in pharmaceutical dosage forms had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed is cost-effective due to short retention time which enabled analysis of levamisole samples with a small amount of mobile phase. From the % RSD values of precision and recovery studies the method was found to be precise and accurate. The low detection and quantification limits achieved indicate the method is very sensitive. The robustness data gathered during method validation showed that the method is not susceptible to small changes in chromatographic conditions. The proposed RP-HPLC method developed by the author is suitable for routine analysis and quality assessment of levamisole in pharmaceutical products.

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