WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 19, 1250-1258. Research Article ISSN 2277–7105

TO DEVELOP AND VALIDATE A NEW CHROMATOGRAPHIC METHOD FOR SIMALTANEOUS ESTIMATION OF LEVAMISOLE & MEBENDAZOLE BY USING RP-HPLC

Afra Khanam*, Sujatha Palatheeya[1] and Raju Badhavath[2]

Palamuru University (Dept. of Pharmaceutical Sciences) Bandameedipally, Mahabubnagar, Telangana, India, 509001.

Article Received on
19 August 2024,

Revised on 09 Sept. 2024,
Accepted on 30 Sept. 2024

DOI: 10.20959/wjpr202419-34111

***Corresponding Author**
Afra Khanam
Palamuru University (Dept. of Pharmaceutical Sciences) Bandameedipally, Mahabubnagar, Telangana, India, 509001.

ABSTRACT

A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for the simultaneous estimation of Levamisole and Mebendazole is a bulk drug and in a synthetic mixture. The analysis was carried out using Inertsil ODS-3V, C18(150x4.6 & 5µm) column. The separation was executed by using a mobile phase containing a buffer of pH 3.2, Acetonitrile and Mixed phosphate buffer (60:40 v/v), pumped at a flow rate of 1.0 mL/min with UV-detection at 245 nm and runtime 20min. Both the drugs were well separated with good resolution on the stationary phase and the retention times were around 4.34 minutes for Levamisole and 9.08minutes for Mebendazole. Determined the quantitative assay of levamisole and mebendazole was 98 to 102%. The method was validated and shown to be linear for Levamisole and Mebendazole. The correlation coefficients for Levamisole and Mebendazole are 0.999 and 1.000 respectively. Obtained the accuracy of drug product by this method was within the acceptance limits 98 to 102% and the % RSD of assay was below 2.0%.

KEYWORDS: Levamisole, Mebendazole, Chromatography, Reverse phase, Validation, RPHPLC.

1. INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals and

manufactured exclusively in accordance with the formulae mentioned in authoritative books. Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish the composition of a substance. It is done to determine the presence of a compound or substance in each sample or not. The various tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc.

2. MATERIALS AND METHOD

Table 1: Chemicals & Reagents.

S. No	Chemical name	Make	Grade
1	Potassium dihydrogen	MERCK	EMPARTA
2	Acetonitrile	MERCK	HPLC
3	Methanol	MERCK	HPLC
4	Orthophosphoric acid	MERCK	EMPLURA
5	Water	NA	Milli-Q

Table 2: Instruments.

S. no	Instrument name	Make	Model
1	High performance liquid chromatography	SHIMADZU	LC-2050
2	Dissolution Apparatus	LAB INDIA	14000+
3	Analytical balance	SORTORIUS	QUINTUX224-10IN
4	pH Meter	METTLER TOLEDO	FP20

HPLC Column

The HPLC column is referred to as the heart of the process. The stationary phase of the column is used in separating the individual fractions of a sample mixture by using various physical and chemical parameters. The column used for execution of this study is Inertsil ODS-3V (150X4.6 & 5.0µm).

Table 3: Optimized instrument method parameters.

Mobile phase	Phosphate buffer: ACN: 65:35
Buffer pH	3.20
Column	Inertsil ODS-3V
Flow rate	1.0mL/min
Column temperature	30°C
Sample temperature	25°C

Wavelength	243nm
Injection volume	20µL
Run time	10min
Diluent	Water: Acetonitrile (30:70)

Gradient program

Time	0	4	6	10	14	15	20
%A	80	60	60	20	20	80	80
%B	20	40	40	80	80	20	20

Preparation of mixed standard solution

Weighed accurately 30mg of Levamisole and 20mg of Mebendazole in 20 ml of volumetric and added 14mL of diluent and sonicated for 5min to dissolve completely and made up to the volume with diluent. From the above stock solution transferred 5.0mL into 100mL volumetric flask and diluted volume with diluent.

Preparation of test solution

Transferred intact 5 tablets of sample into a 500mL volumetric flask. Add 30mL of water, shake on mechanical shaker for 10 minutes to disperse the tablets completely. Added 70mL of acetonitrile and 250mL diluent and sonicate for 30 minutes with intermediate shaking, allowed to equilibrate at room temperature and diluted volume up to the mark with diluent, and mixed well. Centrifuged at 5000 RPM for 5minutes and filtered this supernatant solution through 0.45µm nylon syringe filter by discarding initial 3mL of the filtrate.

Transferred 5.0mL of above supernatant solution into 100mL volumetric flask and diluted volume with diluent.

Calculation for assay

The amount of Levamisole & Mebendazole present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{At}{As} \times \frac{Ws}{Ds} \times \frac{Dt}{N} \times \frac{P}{LC} \times 100$$

Where,

At = Mean area of sample peak in sample preparation

As = Mean area of standard peak in five injections of standard-1 preparation

P = Potency of Levamisole and mebendazole standard in % (On as is basis)

Ws = Weight of standard in “mg”

Ds = Dilution steps for standard preparation

Dt = Dilution steps for sample preparation

LC = Label claim of Levamisole and mebendazole tablet in “mg”

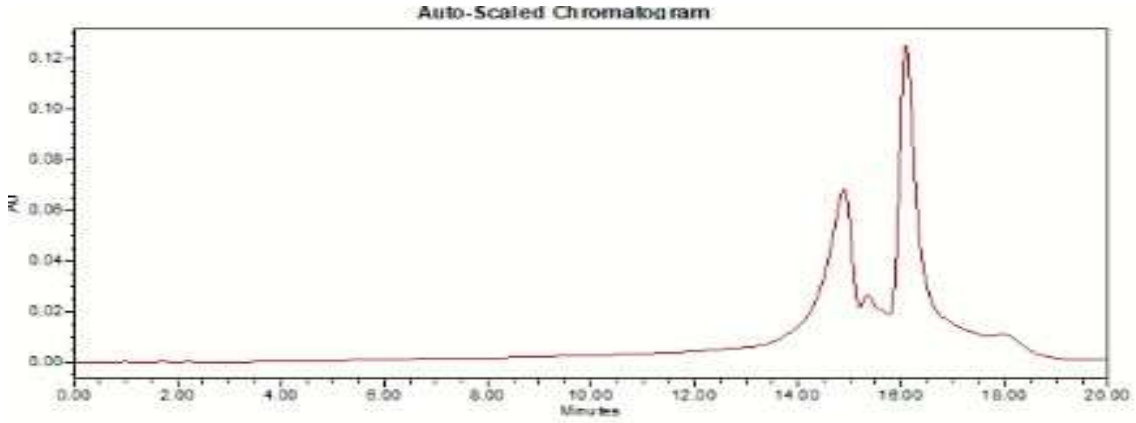
N = Number of tablets.

Similarly calculated the % assay for Levamisole and mebendazole.

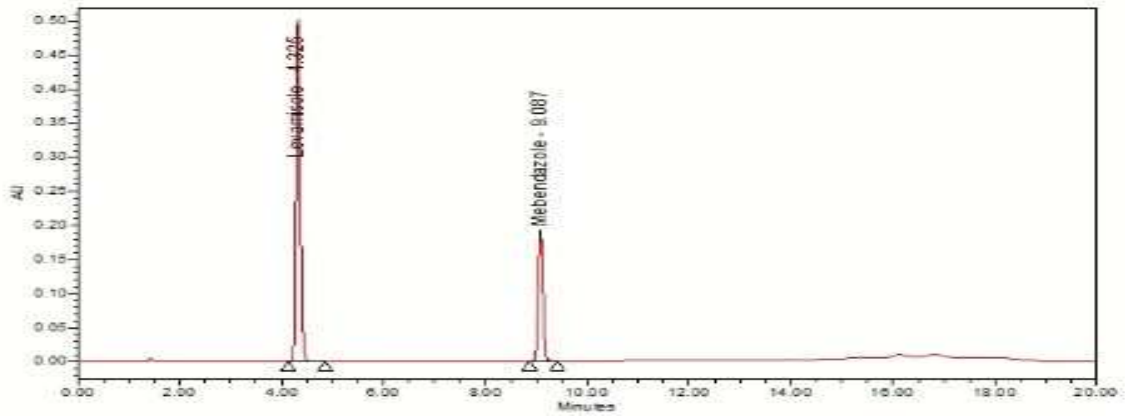
The Optimized method conditions were applied to perform the method validation activities; hence the method needs to be proven as suitable for the analysis of levamisole and mebendazole drug substance and drug product.

Reference chromatograms

1. Blank chromatograms



2. Standard Chromatograms



Quantitative % assay of levamisole and mebendazole

S. No	Preparation	%Assay of levamisole	% Assay of mebendazole
1	Sample_1	99.5	99.9
2	Sample_2	99.3	101
3	Sample_3	100.1	100.3
AVG		99.6	100.4
%RSD		0.4	0.6

Method Validation (ICH Guidelines)

1. Specificity: is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix. Typically, these might include impurities, degradants, matrix, etc. it is a measure of the degree of interference from such other things such as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only. The system precision acceptance criterion for RSD should not be more than 2.0%.

2. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure. The accuracy studies for drug substance and drug product are recommended to be performed at 50%-150%level of the test concentration. The acceptance criterion for accuracy is the Relative Standard Deviation (RSD) for all the recovery values should not be more than 2.0%.

3. Precision: The method precision is an analytical procedure to access the closeness of agreement between a series of measurements obtained by same multiple sampling of same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The relative standard deviation (RSD) for the assay of six sample preparations should not be more than 2.0%.

4. Linearity and Range: Linearity is an analytical procedure ability of the method to elicit test results that are directly proportional to the analyte concentration within the given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method. The range is normally expressed in the same units as the test results obtained by the method.

A minimum of five concentrations levels, along with certain minimum specified ranges are to be determined. The correlation coefficient should be not less than 0.9999.

5. Robustness: Robustness is the capacity of an analytical method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated varying method parameters such as percent organic solvent, pH, Flow rate and temperature. The %RSD for standard and samples under deliberately modified chromatogram should not be more than 2.0%. The above highlighted parameters shall be validated for the optimized new analytical procedure.

3. RESULTS AND DISSCUSSIONS

Table 4: System precision.

S. No	Levamisole	Mebendazole
1	2915805	1248128
2	2917805	1249773
3	2862258	1225808
4	2923456	1250777
5	2906140	1246277
6	2879932	1241762
AVG	2900899	1243754
SD	24400.94	9349.60
RSD	0.84	0.75

Table 5: Recovery of levamisole.

S. No	Stock Conc	Accuracy-Level	Added ppm	Found ppm	% Recovery	%RSD
1	1510	50%	37.75	37.3	98.81	0.43
2	1510	100%	75.5	75.21	99.62	
3	1510	150%	113.25	112.6	99.43	

Table 6: Recovery of mebendazole.

S. No	Stock Conc	Accuracy-Level	Added ppm	Found ppm	% Recovery	%RSD
1	1020	50%	25.5	25.19	98.78	0.22
2	1020	100%	51	50.47	98.96	
3	1020	150%	76.5	75.9	99.22	

Table 7: Method precision for levamisole.

S. No	Preparation No	Average % Assay	%RSD
1	Preparation_1	98.9	0.54
2	Preparation_2	99.9	
3	Preparation_3	100.3	
4	Preparation_4	100.1	
5	Preparation_5	100.0	
6	Preparation_6	99.3	

Table 8: Method precision for mebendazole.

S. No	Preparation no	Average % Assay	%RSD
1	Preparation_1	99.2	0.59
2	Preparation_2	99.4	
3	Preparation_3	100.6	
4	Preparation_4	99.9	
5	Preparation_5	100.5	
6	Preparation_6	99.5	

Table 9: Linearity peak area of levamisole.

S. No	Linearity %	peak response
1	50	1396141
2	75	2086760
3	100	2780548
4	125	3469896
5	150	4162041

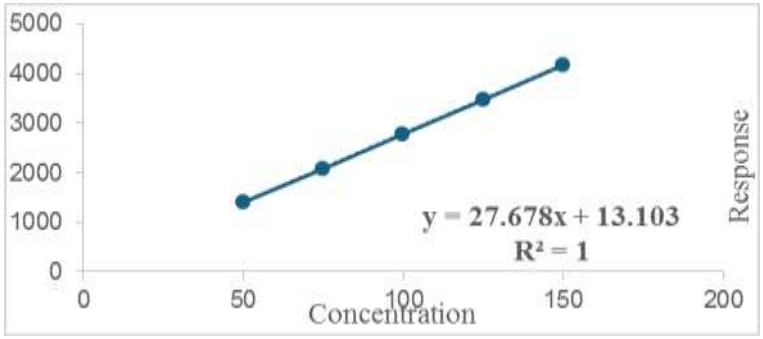
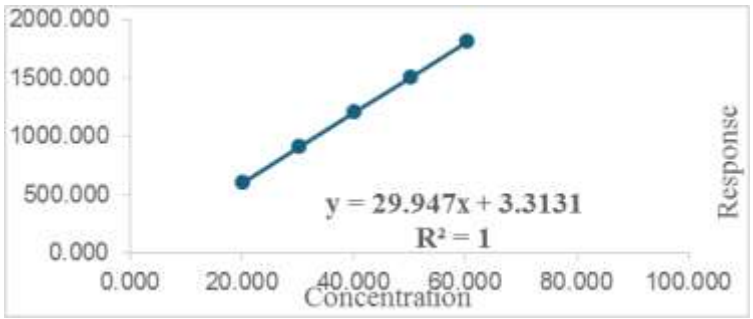


Table 10: Linearity peak area of mebendazole.

S. No	Linearity %	peak response
1	50	606279
2	75	907370
3	100	1209406
4	125	1509194
5	150	1812373



DISCUSSIONS

A simple and selective liquid chromatography method is described for the determination of Levamisole and Mebendazole dosage forms. Chromatographic separation has been achieved on a C18 column using mobile phase consisting of a mixture of 20Mm Phosphate buffer (KH2PO4) pH: 3.2 Acetonitrile (60:40v/v/v), with detection of 245 nm. The study has been carried out based on the current ICH guidelines, the required method parameters were validated, and the results found to be within the acceptance criteria. Method has met the specificity criterion and there was no interference observed by analyte peak of levamisole and mebendazole in blank solution. The system suitability for standard injections for levamisole and mebendazole % RSD were below 2.0%. The Accuracy results of the three replicate preparation of test sample from 50% to 150% level were found 98-102% and % RSD is below 2.0%. The method is linear over concentration ranges from 50% to 125% and correlation coefficient (R2) found to be 1.000.

All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

4. CONCLUSION

The reverse phase high performance liquid chromatographic method for analysis of levamisole and mebendazole was found to be accurate and precise. The current proposed method has been validated successfully according to ICH guidelines; satisfactory results were obtained. Hence the developed method can be useful for further research of levamisole and mebendazole.

By the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Levamisole and Mebendazole was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, biopharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

5. ACKNOWLEDGMENT

I would like to thank my guide, Dr. P. SUJATHA, for her constant inspiration, valuable advice, help and encouragement throughout the course of the project.

I am greatly thankful to Gali Srinath Reddy (Sr. Analytical Manager) Neo vantage analytical lab, hyderabad and Raju Badhavath for giving the opportunity and providing all requirements during the project work.

In addition, I would like to express my deepest thanks and appreciation to all my teachers for their tremendous support and guidance during the completion of my project.

6. REFERENCES

1. Simultaneous estimation of Cefpodoxime proxetil and Ofloxacin in table dosage form using RP-HPLC. Annadi Chiranjeevi and Medidi Srinivas, J. appl. pharm. sci, 2014.
2. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Domperidone and Naproxen in Tablet Dosage Form..Md. Shozan Mondal, J. appl. pharm. sci, 2011.
3. Development and validation of a stability-indicating RP-HPLC method for estimation of Lefamulin in pure and pharmaceutical drug products..Muhammad Usman, Future Journal of Pharmaceutical Sciences, 2023.
4. Bakshi M., Singh B., Singh A., Singh S. The ICH guidance in practice: stress degradation studies on ornidazole and development of a validated stability-indicating assay, Journal of Pharmaceutical and Biomedical Analysis, 2001; 26: (pg. 891-897).
5. Shaikh B. Development and Validation of A Liquid-Chromatographic Method for the Determination of Furosemide, A Diuretic, in Bovine-Milk, Journal of Agricultural and Food Chemistry, 1995; 43: 8(pg. 2117-2121).
6. V. R. Ram, P. N. Dave, H. S. Joshi. Development and Validation of a Stability-Indicating HPLC Assay Method for Simultaneous Determination of Spironolactone and Furosemide in Tablet Formulation. Journal of Chromatographic Science, 2012; 50(8): 721-726.