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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ALBENDAZOLE AND LEVAMISOLE COMBINED PHARMACEUTICAL FORMULATION BY USING REVERSE PHASE - HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT:-

Analytical Method Development and Validation for Albendazole and Levamisole in bulk and Combined Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Albendazole and Levamisole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Albendazole and Levamisole by using Symmetry ODS C18 (4.6 mm × 250 mm, 5 µm) particle size, flow rate was 1.0 ml/min, mobile phase ratio was (30:70 v/v) Methanol: TEA buffer pH 3.8 (pH was adjusted with orthophosphoric acid), detection wavelength was 250 nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower2, 996 PDA detector. The retention times were found to be 2.246 mins and 5.461 mins. The % purity of Albendazole and Levamisole was found to be 101.27% and 99.76% respectively. The system suitability parameters for Albendazole and Levamisole such as theoretical plates and tailing factor were found to be 5387, 0.97 and 5398 and 1.26, the resolution was found to be 2.97. The linearity study in Albendazole and Levamisole was found in concentration range of 30 µg-70 µg and 60 µg-140 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.14% and 100.56%, % RSD for repeatability was 0.1 and 0.5, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.56 and 1.2, and LOQ value was 1.7 and 3.6 respectively. Hence the suggested RP-HPLC method can be used for routine

analysis of Albendazole and Levamisole in API and Pharmaceutical dosage form. Keywords: Albendazole and Levamisole, Method Development, Validation, Accuracy.

I. INTRODUCTION

High Performance Liquid Chromatography

In the modern pharmaceutical industry, high- performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity, and good sensitivity. High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid- 1970 and was improved with the development of column packing material and the additional convenience of ion- line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders, and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1- 5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase. Types of Chromatography [1]

1. Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules. Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.

Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.

Application: Separation of non-ionic, non- polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

2. Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non- polar hydrocarbon chain with non-polar parts of the sample molecules. Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n- diol, etc.

Mobile phase: Methanol, Acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.

Application: Separation of non-ionic and ion forming non- polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

3. Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to anion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase. Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

Mobile phase: Methanol, Acetonitrile, buffer with added ion- pair reagent in the concentration range of 0.001 to 0.01M, etc.

Application: Ionic substances often show very poor retention in reversed-phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

4. Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase

Stationary phase:

Mobile phase: Aqueous buffer systems.

Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids. Advantages of HPLC [2]

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.

2) There ease of sample preparation and sample introduction.

3) There is speed of analysis.

4) The analysis by HPLC is specific, accurate and precise.

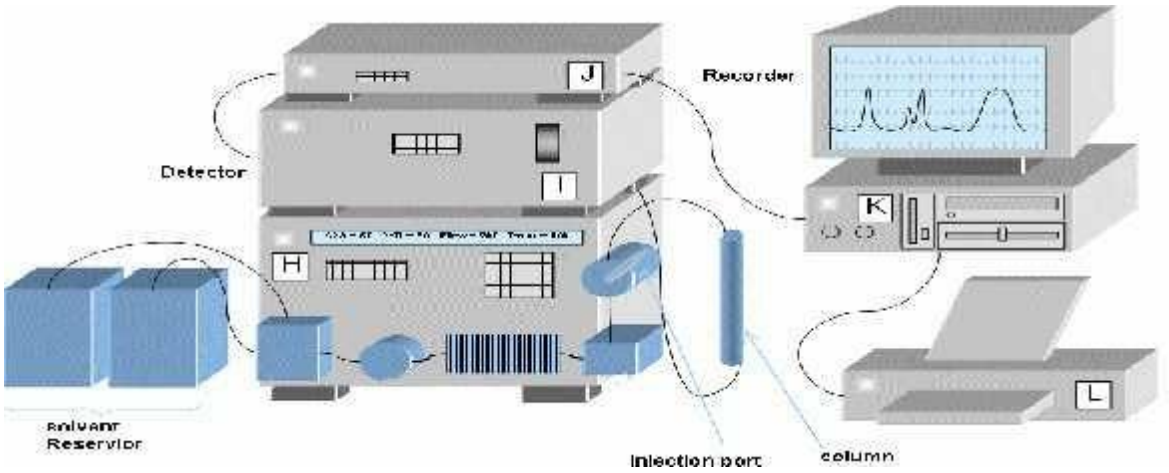
5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermolabile as well as nonvolatile substances. Applications of HPLC [2]

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analyzed by bioassay which suffers from high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins and clotrimoxazole, sulfas and peptide hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.



The basic components of HPLC are:

1. Pumping System

2. Sample Introduction Device

3. Chromatographic Column

4. Detector

5. Data handling Device

Drug Profile

DRUG PROFILE OF ALBENDAZOLE

Drug : Albendazole

Synonym : (5-(propylthio)-1H-benzimidazol-2-yl)carbamic acid methyl ester, 5- (propylthio)-2-carbomethoxyaminobenzimidazole, Albendazol, Albendazole, Albendazolum, Eskazole, O- methyl N-(5-(propylthio)-2-benzimidazolyl) carbamate and Proftril.

Drug category : Anthelmintics

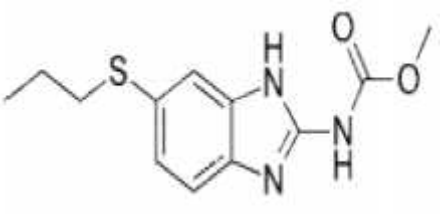
Description : A benzimidazole broad- spectrum anthelmintic structurally related to mebendazole that is effective against many diseases. (From Martindale, The Extra Pharmacopoeia, 30th ed, p38) Structure :

Chemical name/Nomenclature/IUPAC Name: Methyl N-[6-(propylsulfanyl)-1H-1,3- benzodiazol-2-yl] carbamate

Molecular Formula : C12H15N3O2S

Molecular Weight : 265.331 g/mol

PHYSICOCHEMICAL PROPERTIES:



Description (Physical State): Albendazole is a white to off-white powder.

Solubility : Albendazole was found to be soluble in dimethyl sulfoxide, strong acids, and strong bases. It is slightly soluble in methanol, chloroform, ethyl acetate, and Acetonitrile. Albendazole is practically insoluble in water.

Dosage : 400 mg, Tablet

Melting point : 208 to 208°C

pKa (strongest acidic) : 6.9

Log P : 2.7

PHARMACOKINETIC PROPERTIES:

Bioavailability : 50%

Half-life : 8 to 12 hrs

Absorption : Poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. Oral bioavailability appears to be enhanced when coadministered with a fatty meal.

Protein binding : 70% bound to plasma protein

Metabolism : Hepatic. Rapidly converted in the liver to the primary metabolite, Albendazole sulfoxide, which is further metabolized to Albendazole sulfone and other primary oxidative metabolites that have been identified in human urine. Metabolites: Hepatic. Rapidly converted in the liver to the primary metabolite, Albendazole sulfoxide, which is further metabolized to Albendazole sulfone and other primary oxidative metabolites that have been identified in human urine.

Time of peak action : 3 hr

Adverse effects/Side effects:

Nausea

Vomiting

Stomach and abdominal pain

Headache

Pharmacodynamics: Albendazole is a broad- spectrum anthelmintic. The principal mode of action for Albendazole is by its inhibitory effect on tubulin polymerization which results in the loss of cytoplasmic microtubles.

Mechanism of action: Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine- sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies.

Therapeutic efficacy/Indications:

INTERACTIONS:

Drug interactions:

3- isobutyl-1-methyl-7H-xanthine: The serum concentration of 3-isobutyl-1-methyl-7H- xanthine can be increased when it is combined with Albendazole. 3,5-diiodothyropropionicacid: The metabolism of 3,5-diiodothyropropionic acid can be decreased when combined with Albendazole.

4- hydroxycoumarin: The metabolism of 4- hydroxycoumarin can be decreased when combined with Albendazole.

Medical Uses: Albendazole is an anthelmintic (an-thel-MIN-tik) or anti- worm medication. It prevents newly hatched insect larvae (worms) from growing or multiplying in your body. Albendazole is used to treat certain infections caused by worms such as pork tapeworm and dog tapeworm.

DRUG PROFILE OF LEVAMISOLE

Name of the Drug: Levamisole

Description: Levamisole is an antihelminthic drug that was commonly used for the treatment of parasitic, viral, and bacterial infections. It was manufactured by Janssen and first used in 1969 as an agent to treat worm infestations. Levamisole was approved by the FDA in 1990 as an adjuvant treatment for colon cancer. Prior to this, Levamisole was used as an antirheumatic therapy in the 1970s and 1980s for patients with rheumatoid arthritis.

Synonyms: Levamisol, Levamisole and Levamisolum.

Chemical Structure:

IUPAC Name: (6S)-6-phenyl-2H,3H,5H,6H-imidazo[2,1-b][1,3]thiazole

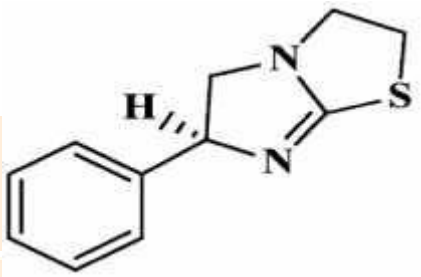
Molecular Formula: C11H12N2S

Molecular weight: 204.291 g/mol

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Verapamil is a white to pale cream crystalline powder, odourless or nearly odourless.

Solubility: Levamisole was found to be freely soluble in water; soluble in ethanol (~750 g/L), soluble in methanol and propylene glycol, slightly soluble in chloroform.



Storage Conditions: Store at room temperature and keep away from moisture and sunlight. Do not store in the bathroom.

Melting Point: 227-227.50C pKa (Strongest Basic): 6.98 Log P: 1.84 Pharmacokinetics:

Bioavailability: Not Available.

Absorption: Levamisole is rapidly absorbed (2 hours) from the gastrointestinal tract.

Volume of distribution: Not Available

Protein binding: 20-25%.

Metabolism: Primarily hepatic (extensive) with both active and inactive metabolites.

Route of Elimination: Levamisole is excreted in the urine mainly as metabolites; only a small amount (<6%) is excreted in the feces. The pharmacokinetics of Levamisole have not been evaluated in renal or hepatic disease, in children, or in the elderly.

Half Life: 4.4-5.6 hours (biphasic).

Pharmacodynamics: Levamisole is a synthetic imidazothiazole derivative that has been widely used in treatment of worm infestations in both humans and animals. As an anthelmintic, it probably works by targeting the nematode nicotinergic acetylcholine receptor. As an immunomodulator, it appears that Levamisole is an immunostimulant which has been shown to increase NK cells and activate T-cells in patients receiving this adjuvantly along with 5FU for Stage III colon cancer.

Mechanism of Action: The mechanism of action of Levamisole as an antiparasitic agent appears to be tied to its agonistic activity towards the L- subtype nicotinic acetylcholine receptors in nematode muscles. This

agonistic action reduces the capacity of the males to control their reproductive muscles and limits their ability to copulate. The mechanism of action of Levamisole as an anticancer drug in combination with fluorouracil is unknown. The effects of Levamisole on the immune system are complex. The drug appears to restore depressed immune function rather than to stimulate response to above- normal levels. Levamisole can stimulate formation of antibodies to various antigens, enhance T-cell responses by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis, and increase neutrophil mobility, adherence, and chemotaxis.

Drug Interactions:

Albendazole: The bioavailability of Albendazole can be increased when combined with Levamisole.

Ivermectin: The bioavailability of Ivermectin can be increased when combined with Levamisole.

Drug-Food Interactions: Take on an empty stomach.

Contraindications: Levamisole hydrochloride is contraindicated in patients with a known hypersensitivity to the drug or its components, in patients with pre- existing blood disorders. Caution in rheumatoid arthritis, Sjogren syndrome, epilepsy and liver disease where dose adjustment may be necessary.

Adverse effects/Side Effects: This medication causes nausea, vomiting, diarrhea, mouth sores, loss of appetite, stomach pain, change in taste and smell, muscle aches, fatigue, dizziness, headache and skin rash.

Medical Uses: Levamisole, sold under the trade name Ergamisol among others, is a medication used to treat parasitic worm infections. Specifically it is used for ascariasis and hookworm infections. It is taken by mouth. Side effects may include abdominal pain, vomiting, headache, and dizziness.

Literature Review

S. Sowjanya, et al. (2018): A reverse phase high-performance liquid chromatographic (RP- HPLC) method was developed and validated for simultaneous estimation of Levamisole and Albendazole in drug substance and in its combinational dosage form. The analysis was carried out using Inertsil ODSC18 (4.6x150mm, 5µm) column, and the separation was carried out using a mobile phase containing a buffer of pH 3.5 and Acetonitrile (70:30 v/v) pumped at a flow rate of 1.0 mL/min with variable wavelength UV- detection at 224nm. Both the drugs were well resolved in the stationary phase and the retention times were 2.350 min and 4.055 for Levamisole and Albendazole, respectively. The method was validated and shown to be linear in the concentration range of 15-45µg/ml and 40- 120µg/ml for Levamisole and Albendazole, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on standard deviation of the y- intercept and the slope of the calibration curve. LOD and LOQ values were 2.08µg/ml and 6.03µg/ml for Levamisole and 3.15µg/ml and 10.40µg/ml for Albendazole, respectively. The accuracy of the method was assessed by adding known amount of standard solution (75%, 100%, and 125% of the sample concentration) to the preanalyzed sample solution of 100% concentration. All the samples were prepared and analyzed in triplicate. The percentage mean recovery by standard addition experiments of Levamisole and Albendazole is 99.66% and 98.73%, respectively.

Purvi A. Patel, et al. (2018): The purpose of the investigation was to develop a new RP- HPLC Method for simultaneous estimation of Albendazole and Levamisole HCl in pharmaceutical dosage forms. Chromatography was carried out on an Shiseido C18 column (4.6x250mm, 5µ particle size) with a isocratic mobile phase Phosphate buffer : Acetonitrile 30:70 (v/v) (adjusted to pH 5 with 10M potassium hydroxide), at a flow rate of 1.0 mL/min and the detection was carried out using a UV detector at 217nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample & standard stock solutions, robustness and degradation studies were determined as reported in the International Conference on Harmonization guidelines. The retention times for Albendazole and Levamisole HCl were 3.177 min and 5.370 min respectively. The percentage recoveries of Albendazole and Levamisole HCl were 100.60% and 98.40% respectively. The relative standard deviation for assay of tablets was found to be less than 2%. The Method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

D. A. Jadhav, et al. (2016): There are many analytical methods available for estimation of Albendazole and Levamisole HCl separately in pharmaceutical preparations. However, no specific RP- HPLC method is available for simultaneous estimation of Albendazole and Levamisole HCl in pharmaceutical dosage form. A simple, rapid and accurate RP- HPLC method was developed and validated as per ICH Q2 (R1) guidelines for the estimation of Albendazole and Levamisole HCl in pharmaceutical dosage form. Method development was carried out on Phenomenex C18 isocratic column, (250mm×4.6mm i.d., particle size 5µm, maintained at ambient temperature) LC- 20AD Prominence Liquid chromatograph (Shimadzu, Japan) attached with SPD-20A/20AV Prominence UV/Vis detector. The mobile phase was a mixture of 0.02M potassium dihydrogen orthophosphate buffer and Methanol in ratio 40:60 v/v, the flow rate was set at 1.0 ml/min and UV detection at 219nm. In the linearity study, linearity of Albendazole was observed from 28-52µg/ml with correlation coefficient of 0.999 and linearity of Levamisole HCl was observed from 10.5-19.5µg/ml with correlation coefficient of 0.999. The developed RP- HPLC method for the quantification of Albendazole and Levamisole HCl has various advantages like good peak symmetry, less retention time and phenomenal linearity, highly sensitive, accurate, precise, simple and robust. The proposed method can be used for the routine analysis of Albendazole and Levamisole HCl in pharmaceutical dosage forms for routine application in quality control laboratories without interference of excipients.

Umangl Shah, et al. (2015): A simple, novel, sensitive and precise validated Spectrophotometric method was developed for simultaneous determination of Mebendazole (MBZ) and Levamisole Hydrochloride (LVM) in its tablet formulation. 1% H2SO4 in methanol was selected as a common solvent for estimation of MBZ and LVM. For first order derivative method, estimation of MBZ was carried out at 307nm (ZCP of LVM) and of LVM at 232.6nm (ZCP of MBZ). The linearity was obtained in the concentration ranges of 2-6µg/mL for

MBZ and 3-9µg/mL for LVM with correlation coefficient (r2) value greater than 0.995. The % RSD value for intraday & interday precision was less than 2. The detection limit and quantification limit were found to be 0.44µg/mL and 1.34µg/mL for MBZ and 0.14µg/mL and 0.42µg/mL for LVM, respectively. All the validation parameters were performed as per the ICH Q2 (R1) guidelines. The recovery study was carried out; results were 98.82–101.93% for MBZ and 100.0.

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AIM AND OBJECTIVE OF THE WORK AIM OF THE PRESENT WORK

Pharmaceutical products should be safe and efficacious on utility. Plenty of Pharmaceutical agents are available in the market in various dosage forms either as a single component or in combination with other drugs. Due to the presence of active principles, it becomes necessary to quantify the formulations in a precise manner. So the demand is insisted by Pharmaceutical regulatory agencies that the commercially available product should retain its quality, purity and potency till its expiry. In addition the regulatory agencies expect to assess the stability data that supports the expiry date of the product in the market. Quantitative analytical methods may or may not be present in Pharmacopeia for new drug components and definitely not for combined Pharmaceutical products. Therefore always it becomes necessary to develop validated analytical methods for combined Pharmaceutical product that contains two or more drug components which are precise, accurate, selective and sensitive for the routine analysis of the drug products.

OBJECTIVE OF THE PRESENT WORK

The main objective of the present work is to develop validated analytical method with the help of which we can separate and simultaneously quantitate Albendazole and Levamisole from the combined Pharmaceutical formulation. The primary objective of the study is to develop suitable liquid chromatographic method for the simultaneous estimation of Albendazole and Levamisole combined Pharmaceutical formulation.

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PLAN OF WORK

The Plan of Work involved in Method development of Selected Drugs is as follows:

- Understanding the Physicochemical Properties of Drug Molecule
- For Method development one has to study the Physicochemical properties like Appearance, Solubility, Polarity, pKa and pH of the selected drug molecules
- Collect the Literature Review of Selected Drugs
- Selection of Chromatographic Conditions
- Selection of Column
- Selection of Chromatographic Mode
- Optimization of Mobile phase

 Buffer Selection

 Effect of pH

 Effect of Organic Modifier

 Selection of Detector and Wavelength

 Developing the Approach of Analysis

 Standard Preparation

 Sample Preparation

 Diluent Preparation

 Method Optimization

 Trials for Method Development by changing different Columns and Mobile Phase ratios and different temperature modes.

 Optimization of Chromatographic Method (Optimization of Proposed Method)

 Method Validation

- System Suitability Test
- Specificity
- Linearity
- Accuracy
- Precision

 Repeatability

 Intermediate precision

 Reproducibility

- Limit of Detection (LOD)
- Limit of Quantification (LOQ)

Chemicals and instruments used:-

S.No.	Chemical	BrandNames
1	Albendazole	Sura labs
2	Levamisole	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV(MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Merck

S.No.	Instruments And Glasswares	Model
1	HPLC	WATERSAlliance2695separationmodule,Software:Empower 2,996PDAdetector.
2	pHmeter	LabIndia
3	Weighingmachine	Sartorius
4	Volumetricflasks	Borosil
5	PipettesandBurettes	Borosil
6	Beakers	Borosil
7	Digitalultrasonicator	Enertech

HPLC METHOD DEVELOPMENT: TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Albendazole and Levamisole working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer (pH-3.8) in proportion 30:70 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry ODSC18 (4.6mm×250mm,5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with autosampler and PDA Detector 996 model.

Temperature : 37ºC

Column : Symmetry ODSC18 (4.6mm×250mm,5µm) particle size

Mobile phase : Methanol: TEA Buffer (pH-3.8) (30:70 v/v)

Flow rate : 1 ml/min

Wavelength : 250 nm Injection volume : 20 µl Runtime : 10 min

METHOD VALIDATION PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Triethylamine (TEA) buffer (pH-4.2):

Dissolve 1.5 ml of Triethylamine in 250 ml HPLC water and adjust the pH 3.8. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:

Accurately measured 360 ml (36%) of Methanol and 640 ml of buffer (64%) were mixed and degassed in digital ultrasonic for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

METHOD VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution: Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Albendazole and Levamisole sample into a 10 mL clean dry volumetric flask and add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY= Sample area × 100

Standard area × Dilution of standard × Weight of sample × Purity × Dilution of sample × Weight of tablet

LABEL CLAIM

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

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Standard area × Dilution of standard × Weight of sample × Purity × Dilution of sample × Weight of tablet

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PRECISION REPEATABILITY

Preparation of Albendazole and Levamisole Product Solution for Precision:

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard in to a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard in to a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.25 ml of the Albendazole and 0.5 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard in to a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard in to a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of the Albendazole and 1.5 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Albendazole and Levamisole and calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Albendazole and 10 mg of Levamisole working standard in to a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the Albendazole and 1 ml of the Levamisole stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1 ml/min, remaining conditions are same. 20 µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer was taken in the ratio and 35:65, 25:75 instead (30:70), remaining conditions are same. 20 µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Mobilephase :Methanol: Water (60:40 v/v) Column

:X-terraC18(4.6mm×250mm)5µm

Flowrate :1.0 ml/min

Wavelength :250nm

Columntemp :40ºC

InjectionVolume :10µl

Runtime :10minutes

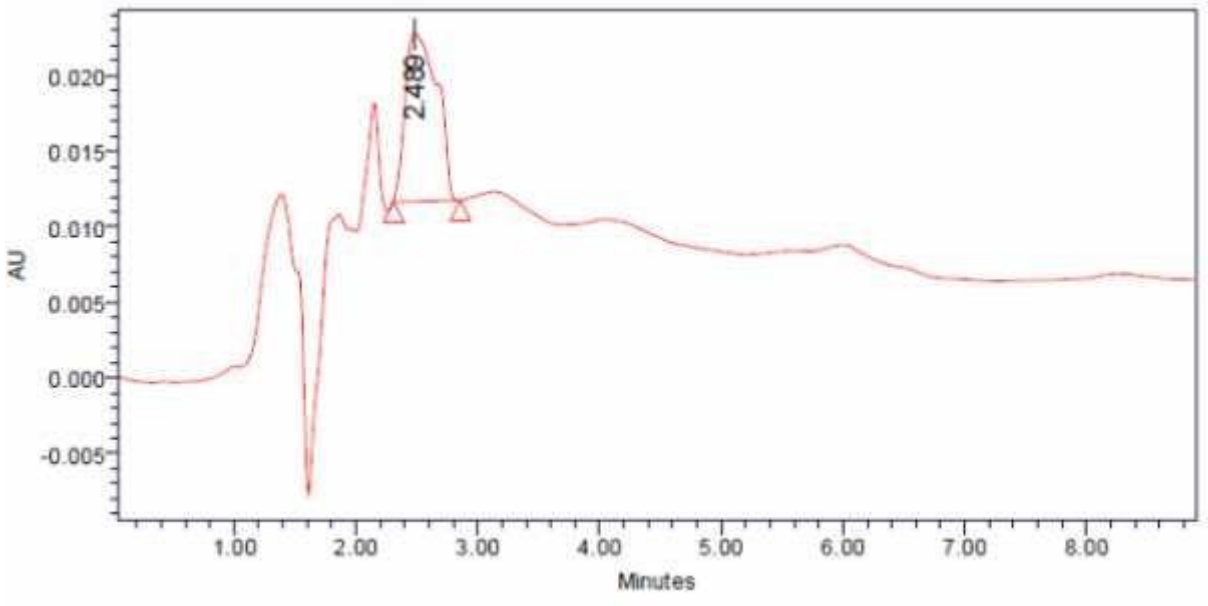

Figure-: Chromatogram for Trail 1 Table-:PeakResultsforTrail 1

S.No.	PeakName	R_t	Area	Height	USPResolution	USPTailing	USPPlatecount
1	Albendazole	2.489	2548	986		4.3	985

Observation: In a separationof Albendazole and Levamisol peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail2:

Mobilephase :Acetonitrile:Methanol(80:20%v/v)

Column :ZorbaxC18(4.6mm×150mm,5µm particlesize)Make;waters

Flowrate :0.8ml/min

Wavelength :250nm

Columntemp :36ºC

InjectionVolume :10 µlRuntime

:9minutes

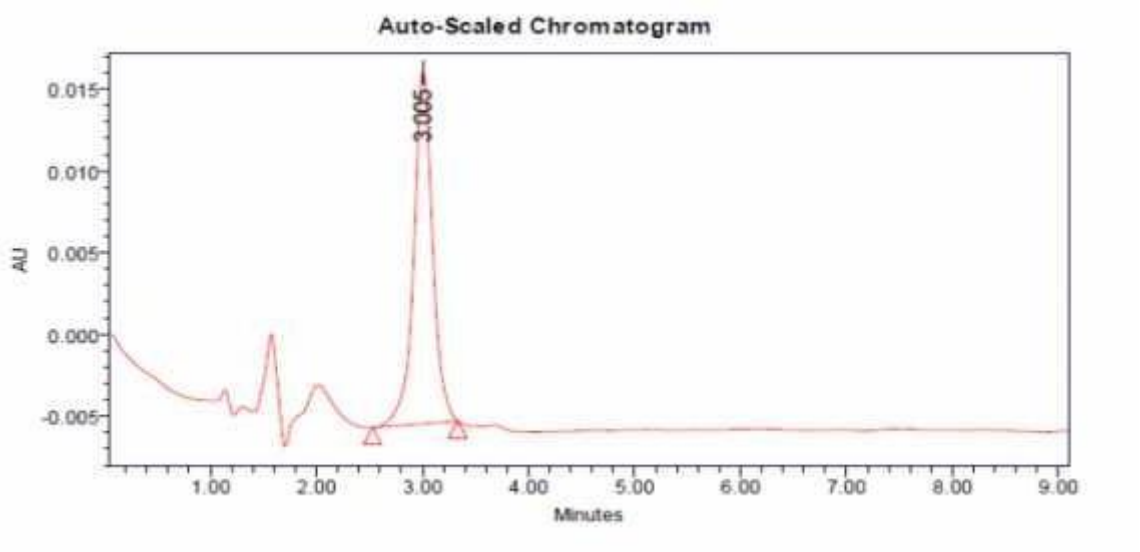

Figure-: Chromatogram for Trail 2 Table-:PeakResultsforTrail2

S.No.	PeakName	R_t	Area	Height	USPResolution	USPTailing	USPPlatecount
1	Albendazole	3.005	36528	1425		2.6	896

Observation: In a separation of Albendazole and Levamisole peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail3:

Mobilephase :Acetonitrile: Methanol (60:40 v/v) Column

:DevelosilODSC18(4.6×250mm)5µm

Flowrate :1.0 ml/min

Wavelength :250nm

Columntemp :38ºC

InjectionVolume :8µl

Runtime :10minutes

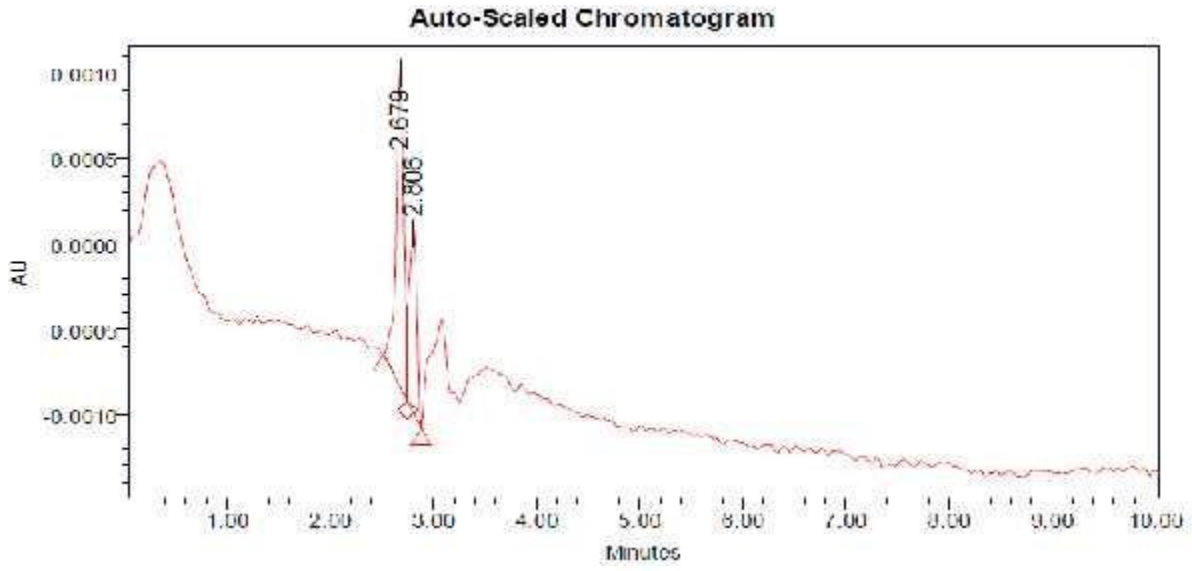

Figure:ChromatogramforTrail3 Table: Peak Results for Trail 3

S.No.	Peak name	R_t	Area	Height	USPResolution	USPTailing	USPplate count
1	Albendazole	2.679	8568	1762		0.96	256
2	Levamisole	2.806	5367	1063	1.06	2.35	687

Observation: From the above chromatogram it was observed that the void peaks are obtained and sample peaks are not separated and show less plate count in the chromatogram. So it's required more trials to obtain well peaks.

Trail4:

Mobilephase :Methanol:PhosphateBuffer(35:35%v/v)

Column :HypersilODSC18(4.6×250mm,5µm) particlesize

Flowrate :1.0ml/min

Wavelength :250nm

Columntemp :35ºC

InjectionVolume:10 µl

Runtime :14minutes

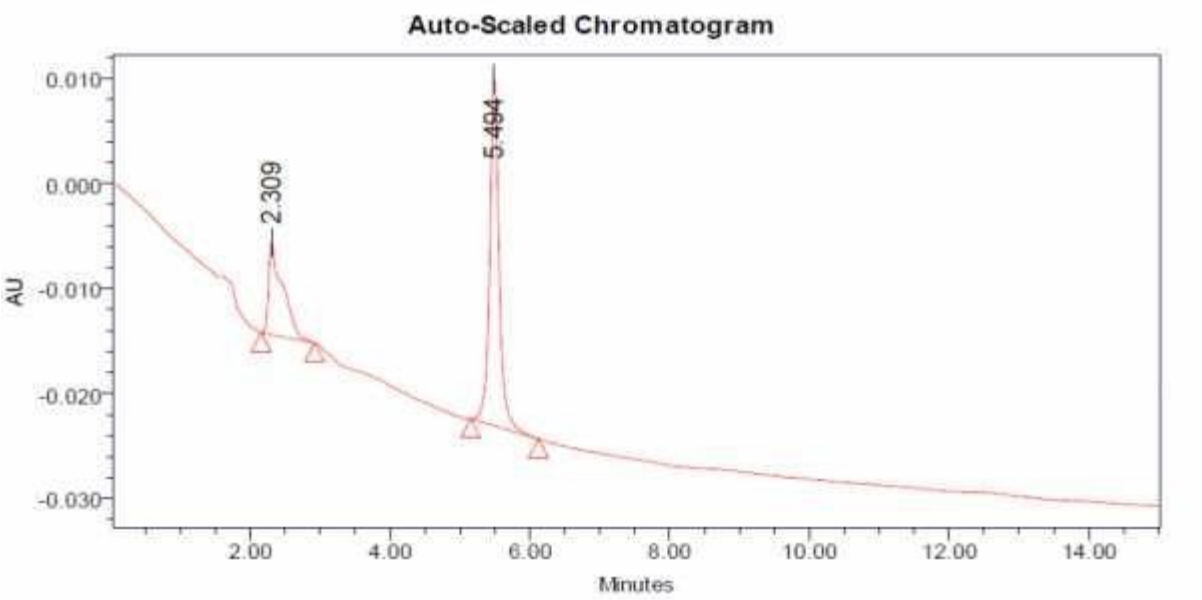

Figure: - Chromatogram for Trail 4 Table:-Peak resultsforTrail4

Observation: This trial show very less plate count and sample peaks are not well separated, so more trials were required for obtaining good peaks.

Trail5:

Mobilephase :Methanol:TEABuffer(pH-4.6)(40:60v/v)

Column :Symmetry ODSC18(4.6mm×250mm,5µm)particlesize

Flowrate :1.0 ml/min

Wavelength :250nm

Columntemp :40ºC Injection

Volume:20 µl

Runtime :10minutes

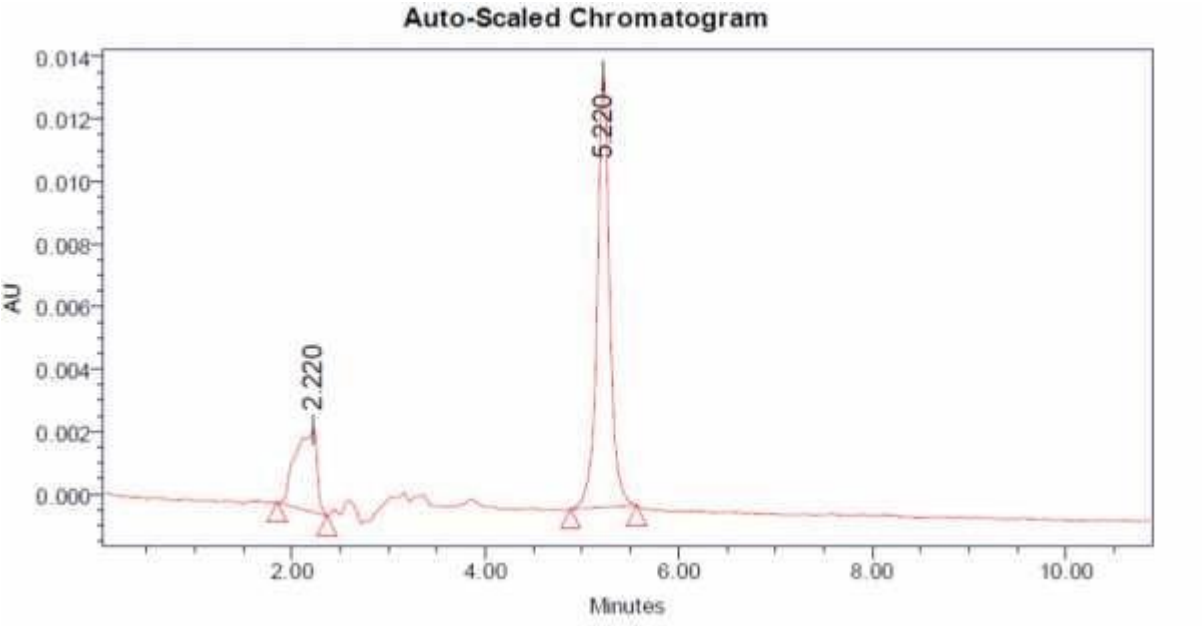

Figure:-ChromatogramforTrail 5 Table: - Peak results for Trail 5

S. No	Peakname	R_t	Area	Height	USP Resolution	USPTailing	USPplatecount
1	Albendazole	2.220	41254	3658		0.72	526
2	Levamisole	5.220	652845	14587	9.86	1.09	4568

OptimizedChromatogram(Standard)

Mobilephase :Methanol:TEABuffer (pH-3.8)(30:70v/v)

Column :Symmetry ODSC18(4.6mm×250mm,5µm)particlesize

Flowrate :1ml/min

Wavelength :250nm

Columntemp :37ºC Injection

Volume:20 µl

Runtime :10minutes

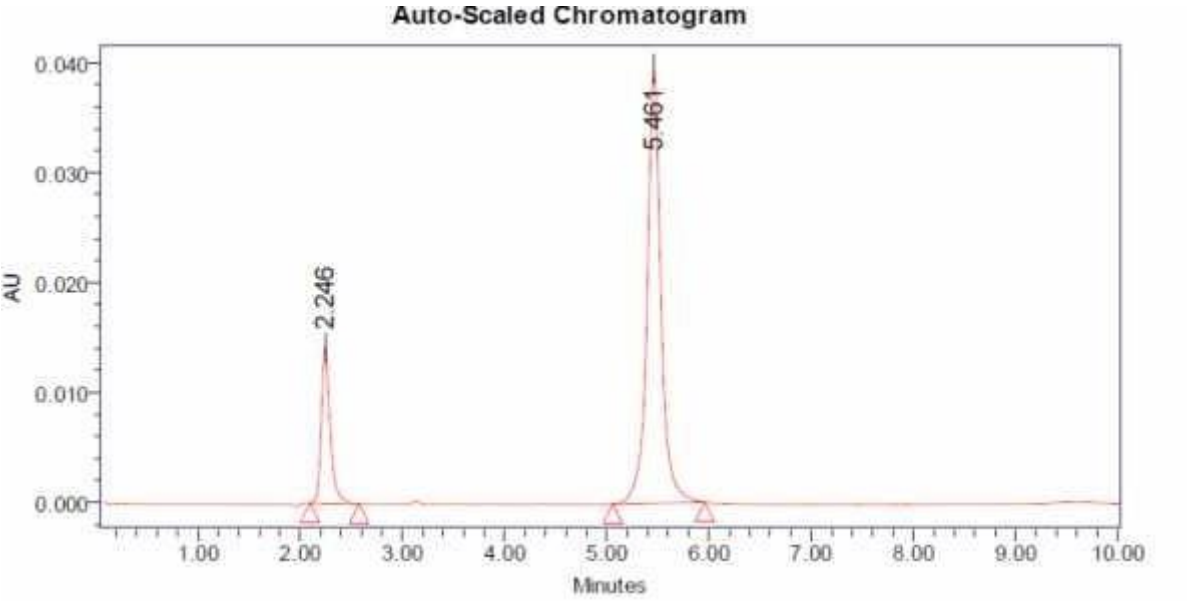

Fig-:Optimized Chromatogram

Table:- PeakResultsforOptimized Chromatogram

S.No.	Peak name	R_t	Area	Height	USPResolution	USPTailing	USPplate count
1	Albendazole	2.246	765789	69584		0.97	5587.0
2	Levamisole	5.461	2532158	190049	2.97	1.26	5398.0

Observation: From the above chromatogram it was observed that the Albendazole and Levamisole peaks are well separated and they show proper retention time, resolution, peak tail, and plate count. So it's optimized trial.

Optimized Chromatogram(Sample)

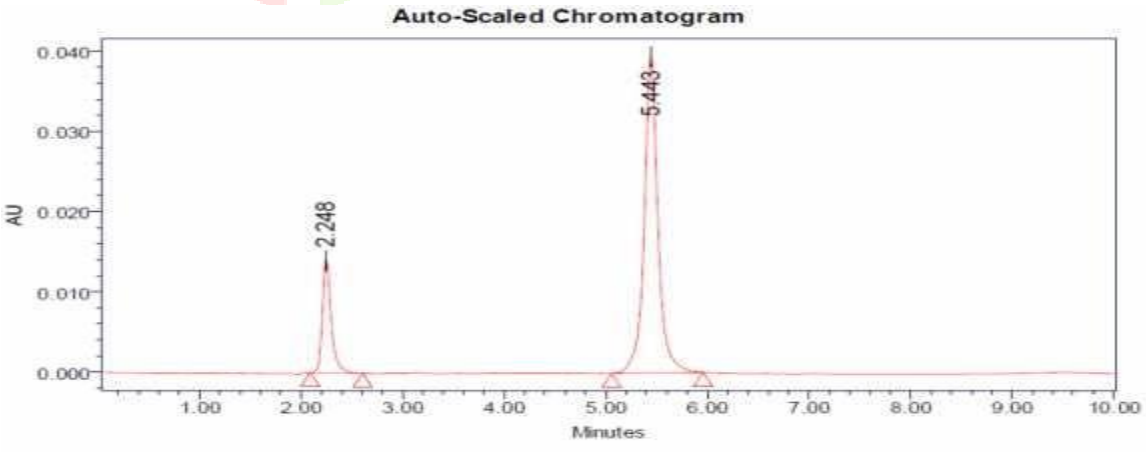

Figure-:OptimizedChromatogram(Sample) Table-:OptimizedChromatogram(Sample)

S.No.	Peakname	R_t	Area	Height	USPResolution	USPTailing	USPplatecount
1	Albendazole	2.248	775684	13124		0.99	6365.0
2	Levamisole	5.443	2658478	937405	5.06	1.23	7458.0

Acceptance criteria:

- Resolutionbetweentwodrugsmustbenotlessthan2.
- Theoreticalplatesmustbenotlessthan 2000.
- Tailingfactormust benotlessthan0.9andnot morethan2.
- Itwasfoundfrom abovedatathatallthesystemsuitability parametersfordevelopedmethodwere within the limit.

Conclusion:-

In the present investigation, a simple, sensitive, precise, and accurate RP-HPLC method was developed for the quantitative estimation of Albendazole and Levamisole in bulk drug and pharmaceutical dosage forms.Albendazole was found to be soluble in dimethyl sulfoxide, strong acids, and strong bases. It is slightly soluble in methanol, chloroform, ethyl acetate, and Acetonitrile.

Albendazole is practically insoluble in water. Levamisole was found to be freely soluble in water; soluble in ethanol (~750g/L), soluble in methanol and propylene glycol, slightly soluble in chloroform.Methanol: TEA Buffer (pH-3.8) (30:70 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2, and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate, and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Albendazole and Levamisole in bulk drug and in Pharmaceutical dosage forms.

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