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Research Article

RP-HPLC Method for Simultaneous Estimation of Levamisole, Mebendazole and Albendazole in Pharmaceutical Products

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ABSTRACT

Single and reproducible RP-HPLC method has been developed for the simultaneous estimation of Levamisole, Mebendazole and Albendazole in pharmaceutical products. Chromatographic separation was achieved by using Inertsil ODS-3V C18, 250 x 4.6 mm, 5µm column, mobile phase composed of sol-A: Potassium di-hydrogen phosphate (1.0 gram in 1000 ml of HPLC Water) buffer and sol-B: Acetonitrile with gradient elution (0-5min- sol-A: 80-80; 5-7min- sol-A: 80-60; 7-10min- sol-A: 60-30; 10-15min- sol-A: 30-80 and 15-20min- sol-A: 80-80). Flow rate was 1.00 ml per min and measured the absorbance at 210nm. The retention time of Levamisole, Mebendazole and Albendazole are 4.8min, 12.8min and 14.1min, respectively. The linearity of the method was evaluated from 5µg per mL to 100µg per mL for each ingredient and the correlation coefficient result was observed for each ingredient was not less than 0.999. The developed method has wide applicable in the quantification of Levamisole, Mebendazole and Albendazole in pharmaceutical dosage forms.

INTRODUCTION

Albendazole [1-6] is an anthelmintic (an-thel-MIN-tik) or anti-worm drug. 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. Side effects are llergic reactions like skin rash, itching or hives, swelling of the face, lips or tongue, changes in vision, diarrhea, difficulty breathing, fast, irregular heartbeat, fever, chills, sore throat, pain, difficulty passing urine, redness, blistering, peeling or loosening of the skin and yellowing of eyes.

Levamisole [7-11] is a cancer (antineoplastic) medication. Levamisole interferes with the growth of cancer cells and slows their growth and spread in the body. This medication stimulates the body's immune system to help treat and protect against illness. It is used in combination with other medications in the treatment of colon cancer.

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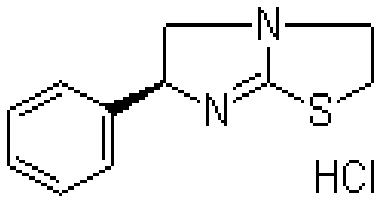
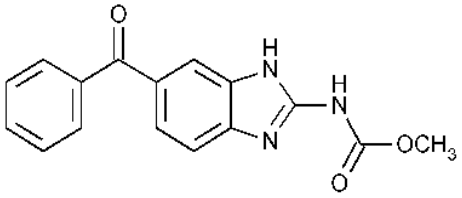
Mebendazole [12-14] is an anti worm drug and used for the treatment of pinworm (Enterobius vermicularis), round worm (Ascaris lumbricoides), common hookworm (Ancylostoma duodenale), American hookworm (Necator americanus), and whipworm (Trichuris trichiura) in single or mixed parasitic infections. Oral dosage for treatment of pinworms is 100 mg per dose, for whipworm, common roundworm and hookworm is one 100-mg tablet morning and evening for 3 consecutive days. Mebendazole side effects are transient abdominal pain, diarrhea, slight headache, fever, dizziness, exanthema, urticaria and angioedema. It is, however, contraindicated in pregnant women because it has been shown to be embryotoxic and teratogenic in experimental animals. Figure-1 represents the three active ingredients chemical structures.

All three active ingredients are available in individual and combination dosage forms [14] such as Levamisole-50mg/150mg tablets, albendazole-400mg tablets, Levamisole-150mg and Albendazole 400mg tablets, Levamisole-150mg and Mebendazole 100mg tablets etc.

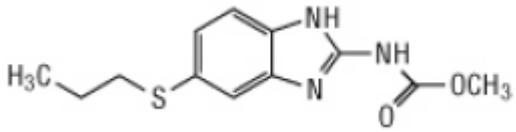
Present study is to develop a single RP-HPLC method for the determination of Levamisole, Mebendazole and Albendazole in pharmaceutical dosage forms.



Levamisole



Mebendazole



Albendazole

Figure-1: Chemical structure of active ingredients

MATERIALS AND METHOD

Reagents and Chemicals

AR grade of Potassium di-hydrogen phosphate (Merck India Pvt.Limited) and Merck HPLC Grade of acetonitrile, methanol, and water were used for analysis. All purified standard materials (not less than 98.5%) were used. All market samples were analysed with this method.

Equipments

Waters Alliance HPLC system equipped with 2695 separation module connected to 2996-Photo diode array detector and Empower-2 software and Agilent 1200 series HPLC with dual absorbance detector were used. Mettler-Toledo analytical balance, sonicator and rotatory shaker were used for this study.

Columns

Waters, Agilent, Thermo-scientific, Merck and GL-life sciences make analytical columns were used for this study.

Chromatographic conditions

Mobile phase composed of Sol-A: Potassium di-hydrogen phosphate buffer (1.0 Gram dissolved in 1000 ml of HPLC Grade Water) and Sol-B: Acetonitrile. 20µl injection volume, 1.00ml per minute flow rate with a simple gradient elution (0-5min, sol-A: 80-80; 5-7min- sol-A: 80-60; 7-10min- sol-A: 60-30; 10-15min- sol-A: 30-80 and 15-20min- sol-A: 80-80). Detection of the all three actives was achieved by using a photo diode array (PDA) detector at 210nm. Inertsil ODS-3V (GL Sciences) 250mm x 4.6mm, 5µm column is used for analysis and Methanol is used as diluent.

Standard preparation

Standard stock solution (500µg/ml) was prepared in methanol which is used as diluent. Weighed accurately 50mg of each standard (Levamisole, Mebendazole, and Albendazole) into 100ml volumetric flask and dissolved in diluent with intermediate sonication, diluted to volume with diluent and mixed. Further diluted the resulting solution to achieve a concentration of 50µg per ml.

Sample Preparation

All market samples (combination and individual formulations) were analyzed with the developed method by preparing 50µg per ml with diluent.

RESULTS AND DISCUSSION

Method development

Initial stage of method development, trials were performed with Potassium phosphate, Sodium phosphate and acetate buffers and different organic modifiers such as methanol, acetonitrile but three actives peak shape was poor. Finally the resolution and sharp peak shapes were achieved with Inertsil ODS-3V C18, 250mm, 4.6 mm ID, and 5µm column. Based on three active ingredients UV spectrum samples absorbance measured at 210nm for three active ingredients. All the three active ingredients spectrums represented in figure-2. And finally the separation was achieved by phosphate buffer and Acetonitrile with gradient program. The retention time of Levamisole is 4.9 min, Mebendazole is 12.8 min and Albendazole is 14.1 min. Blank (diluent) and standard chromatograms were represented in figure-3 and 4.

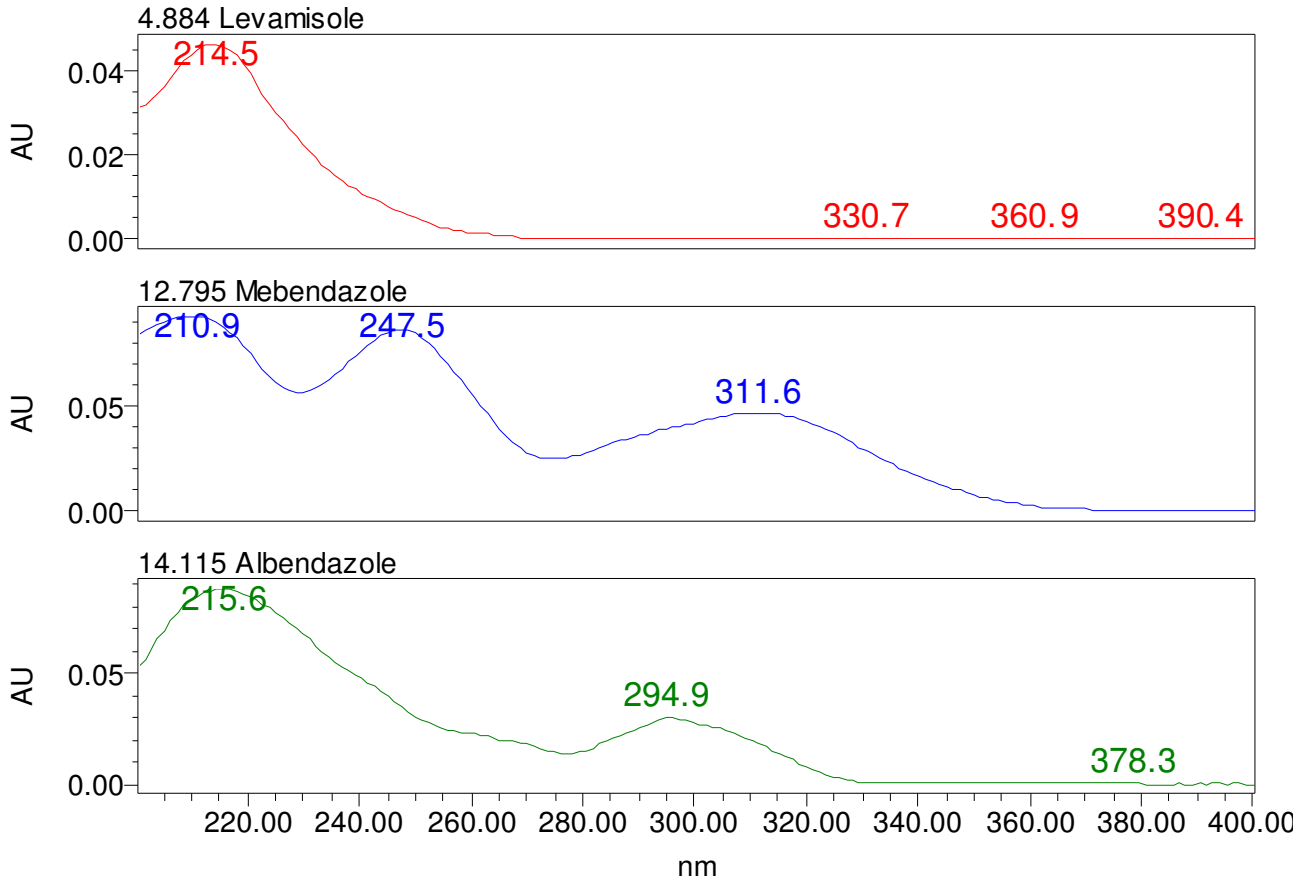


Figure 2: UV spectrum of three active ingredients

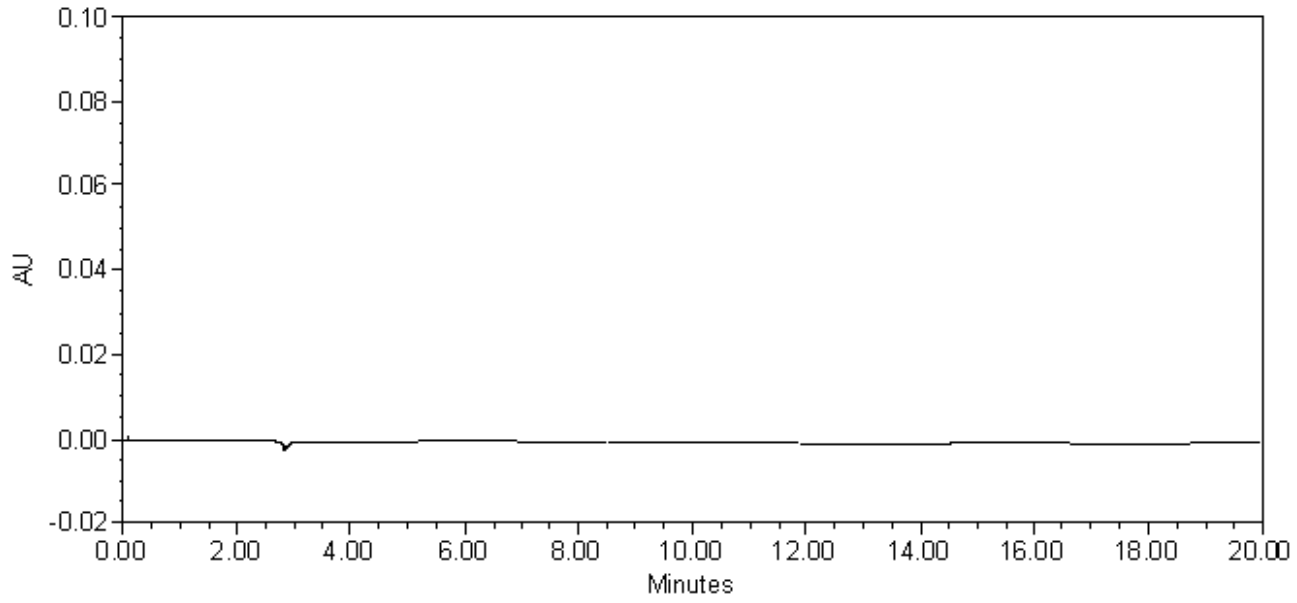


Figure 3: Blank Chromatogram

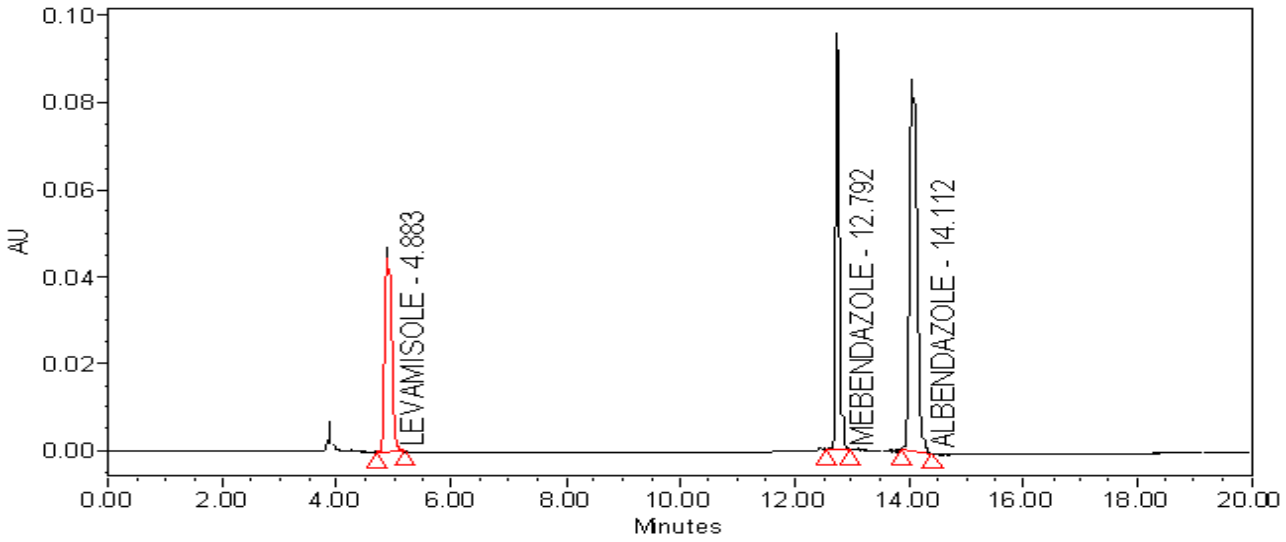


Figure 4: Standard chromatogram

Table 1: System suitability (Area %RSD)

Active Ingredient Name	Standard solution Area						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Levamisole	352882	356432	356927	355351	358664	356051	0.60
Mebendazole	499345	502347	504764	505347	504889	503338	0.50
Albendazole	740986	739072	742322	742699	741098	41098	0.19

Table 2: System suitability (Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Levamisole	4.877	4.878	4.878	4.884	4.883	4.880	0.07
Mebendazole	12.798	12.793	12.791	12.794	12.792	12.794	0.02
Albendazole	14.126	14.117	14.113	14.116	14.112	14.117	0.04

Table 3: System suitability (USP Tailing)

Active Ingredient Name	USP Tailing factor					
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average
Levamisole	1.19	1.20	1.19	1.17	1.21	1.19
Mebendazole	1.17	1.17	1.17	1.18	1.18	1.17
Albendazole	1.20	1.19	1.21	1.19	1.19	1.20

Table 4: System suitability (USP Resolution)

Active Ingredient Name	USP Resolution					
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average
Levamisole	NA	NA	NA	NA	NA	NA
Mebendazole	43.93	43.90	44.06	44.15	44.00	44.01
Albendazole	6.91	6.93	6.90	6.93	6.89	6.91

Table 5: Precision Results

Active Ingredient Name	Sample preparations						Average (%)
	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6	
Levamisole	99.81	98.77	100.10	100.21	99.35	99.63	99.65
Mebendazole	100.11	100.03	100.23	99.79	98.75	99.36	99.71
Albendazole	99.64	99.88	99.27	100.09	99.72	99.61	99.70

Table 6: Linearity Results

Linearity solutions area	Active Ingredient Name		
	Levamisole	Mebendazole	Albendazole
Level-1 (5ppm)	35049	56186	73946
Level-2 12.5ppm	87543	130442	184911
Level-3 (25ppm)	173128	258586	369985
Level-4 (37.5ppm)	261034	382081	557252
Level-5 (50ppm)	351156	504356	740458
Level-6 (62.5ppm)	432706	623956	925872
Level-7 (75ppm)	525674	751841	1109705
Level-8 (87.5ppm)	614601	868466	1284187
Level-9 (100ppm)	702543	997801	1488470
Co-relation Coefficient	0.99996	0.99997	0.99995

Table 7: Accuracy Results

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Levamisole	100.16	100.03	99.79	100.02	100.65	100.92	100.26
Mebendazole	99.89	99.86	100.31	100.44	100.54	100.59	100.27
Albendazole	99.66	98.91	99.69	100.71	100.18	99.69	99.81

Table 8: Robustness Results

Parameter		Parameter			
	Standard solution	Flow Rate		Column Oven Temperature	
	-----	+0.1mL per min	-0.1mL per min	+5°C	-5°C
Tailing factor	0.6-0.9	1.2-1.4	1.1-1.2	0.9-1.2	1.0-1.2
Percent (%) RSD	1.3-1.1	0.7-1.2	1.1-1.2	1.0-1.3	0.7-1.2

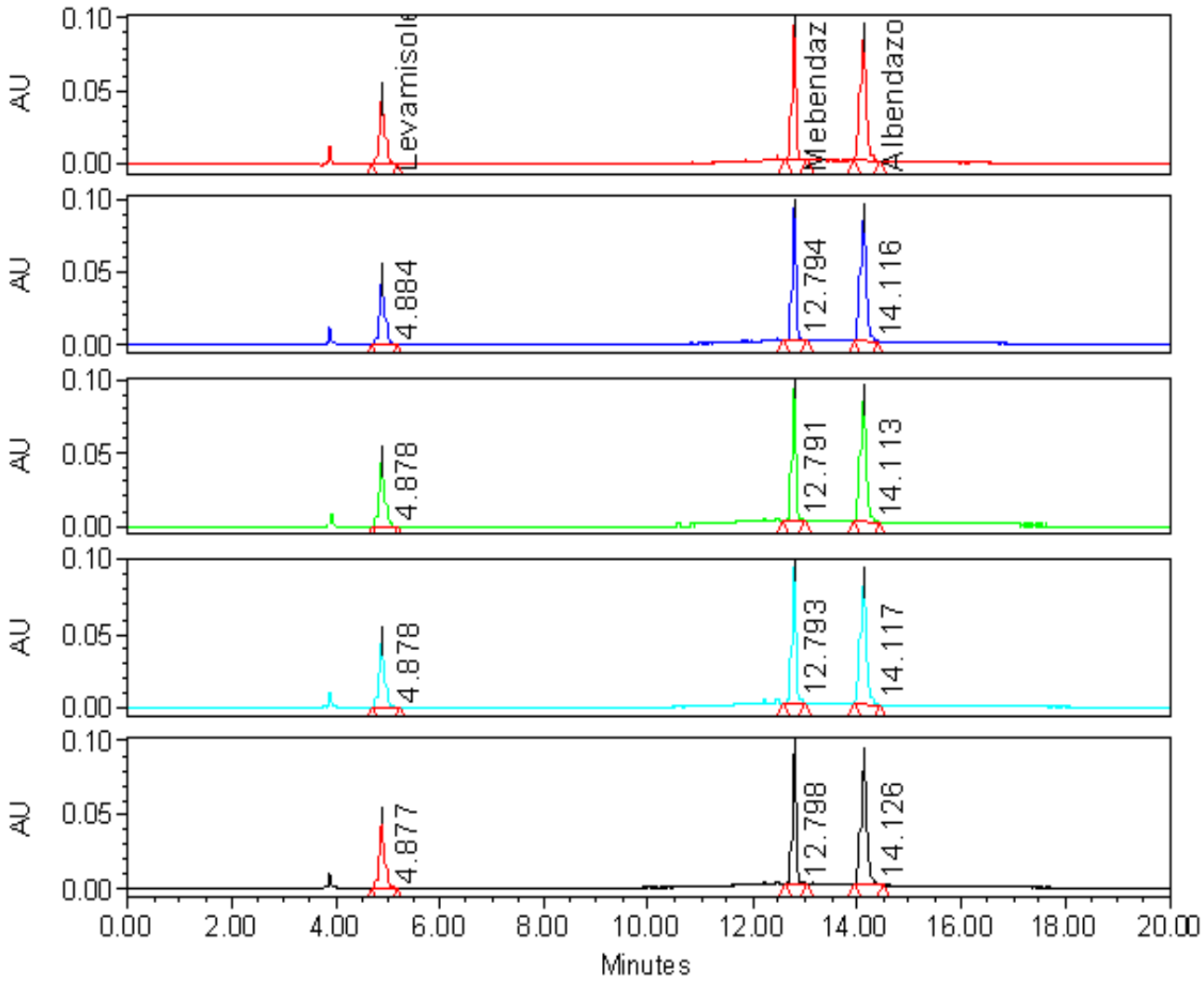


Figure 5: System suitability Solution

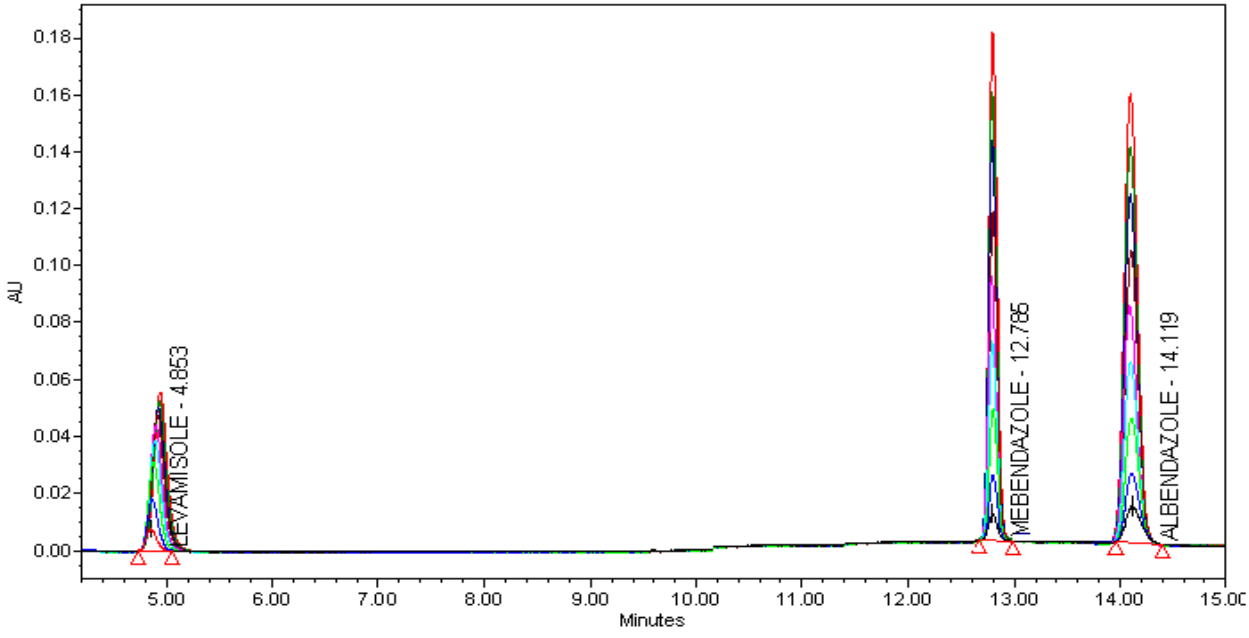
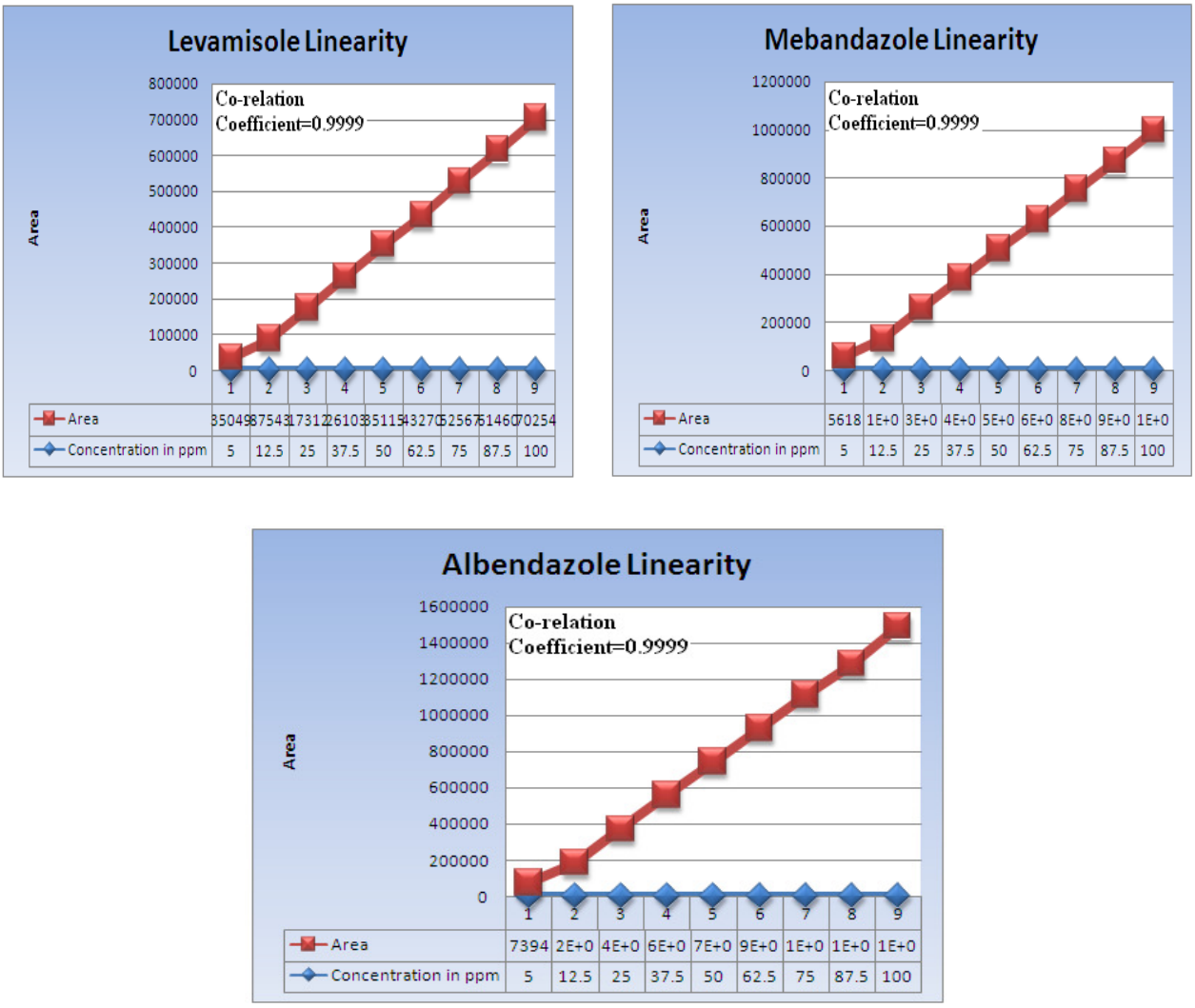


Figure 6: Linearity solutions



Graph-1: Linearity plots of three active ingredinets.

System suitability

System suitability test is an integral part of analytical method development. System suitability parameters are established by injecting the freshly prepared standard solution (each active 50ppm) in to the system for five replicate injections and calculated the percentage relative standard deviation for area and retention time, and the USP Resolution between Levamisole and Mebendazole peaks and between Mebendazole and Albendazole peaks. Results were found satisfactory. Five replicate standard solution chromatograms represented in figure-5 and tabulated the system suitability results in table-1, 2, 3 and 4.

METHOD VALIDATION

Validated the method as per ICH and FDA guidelines for parameters like specificity, precision, accuracy, linearity and range, ruggedness and robustness etc.

Specificity

Specificity of the method has evaluated for diluent and placebo interference. No interference was observed in diluent and placebo chromatograms.

Precision

Precision of the method was evaluated by carrying out six different sample preparations for all individual and combination products. Percentage relative standard deviation (% RSD) was less than 1.5% for within a day and day to day variations, which proves that the method is precise and results were tabulated in Table-5.

Linearity

Linearity was performed by analyzing nine different concentrations of the standard solution of all three active ingredients mixture. Calibration curve was constructed by plotting the area against with concentration. The results

were shown in table-6 and linearity plots represented in graph-1. The correlation coefficient value was within the limit 0.999 for all three compounds. All linearity solutions are plotted and represented in figure-6.

Accuracy

The accuracy of the method and recovery experiment was carried out. A known quantity of the pure drug was added to the placebo sample at the level of 25% to 150% of the test concentration. The recoveries of the drug product were determined and mean recoveries were in the range of 98.0-102.0 % which shows that there is no interference from excipients. Table-7 represents the recovery results.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment with different operators, different instruments and different brand columns of similar types. The percentage RSD of six different preparations assay values with two different instruments, analysts and columns were 0.32- 0.46, 0.25- 0.89 and 0.18-0.31% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate, and column temperature. It was observed that there were no marked changes in the peak shapes and the system suitability results. The robustness limit for mobile phase variation, flow rate variation and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria. Robustness results were tabulated in table-8.

CONCLUSION

The proposed single and high resolution RP-HPLC method was precise, linear and accurate. The method provides selective quantification of Levamisole, Mebendazole and Albendazole without interference from blank and placebo. The developed method has potential application for all ingredients (Levamisole, Mebendazole and Albendazole) and applicable for routine quality control analysis.

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