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# Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Ivermectin and Albendazole in Bulk and Tablet Dosage Form

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**Sneha Thakur*[1], Mohd. Abdul Rab[2], Keerthi Kadimcharla[3]**

[1]*Associate Professor, HOD, Department of Pharmacognosy, St. Pauls College of Pharmacy, Turkayamjal Hyd, Telangana, India*

[2]*Department of Pharmaceutical analysis, University College of Technology, Osmania University, Hyderabad, Telangana, India*

[3] *Assistant Professor, Department of Pharmaceutical analysis, University College of Technology, Osmania University, Hyderabad, Telangana, India*

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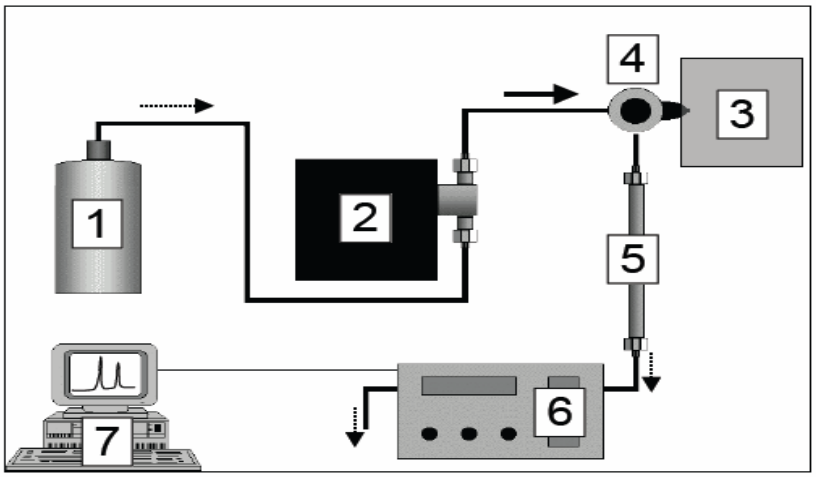
**Keywords:** Ivermectin, Albendazole. Simultaneous Estimation, RP-HPLC

**ABSTRACT**

**Background:** A new rapid, specific, simple, accurate, and precise reverse phase Liquid phase chromatographic technique using convenient and handy mobile phases for simultaneous determination of Ivermectin and Albendazole in pure and tablet dosage form has been developed. **Aim:** To develop and validate an analytical method for simultaneous estimation of Ivermectin and Albendazole in pharmaceutical formulation by Reverse phase liquid chromatography. **Material and Methods:** HPLC of Waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6 mm I.D × 250mm, 5µm) column was used for chromatographic separation. It contains a waters injector and PDA Detector (Deuterium). The mobile phase consists of Methanol: Water (85:15% v/v) and the flow rate adjusted was 1ml/min. The wavelength selected for detection was 285nm and the injection volume was 10$^{\mu}$l. **Results and discussion:** By using the developed method, the retention time of Ivermectin and Albendazole was found to be 3.2 min and 5.4 min respectively. The method has been validated for linearity, accuracy, and precision. The linearity of Ivermectin and Albendazole was in the range of 5-25 µg/ml and 20-100µg/ml respectively. The percentage recoveries obtained for Ivermectin and Albendazole were found to be in the range of 99.3 – 99.6% LOD and LOQ was found to be 3.3µg/ml and 10.1µg/ml for Albendazole 1.2 and 3.8µg/ml for Ivermectin. **Conclusion:** The developed HPLC method offers several advantages such as rapidity, usage of a simple mobile phase, and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied to the analysis of pure drug and pharmaceutical dosage forms. From the present, it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate, and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.

## INTRODUCTION

HPLC is a technique for the separation, identification, and quantification of components in a mixture. It is especially suitable for compounds that are not easily volatilized, is thermally unstable, and have high molecular weights[1]. The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream[2]. On reaching the column the sample components are selectively retained based on physicochemical interactions between the analyte molecules and the stationary phase[3]. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for the detection and quantification of the eluted components[4]. The rate of distribution of drugs between the Stationary and mobile phases is controlled by a diffusion process. If diffusion is minimized faster and effective separation can be achieved[5]. The techniques of high-performance liquid chromatography are so called because of their improved performance when compared to classical column chromatography advances in column chromatography into high speed efficient, accurate, and highly resolved method of separation[6]. A combination of drugs is mostly used to increase the spectrum of activity. Ivermectin and albendazole are one such combination that is mostly used as anthelmintic, particularly against intestinal helminthics in children. Ivermectin is a macrocyclic lactone disaccharide derived from the streptomyces avermitilis. On the other hand, albendazole is an anthelmintic[7]. The present investigation involves the development and validation of Ivermectin and Albendazole estimation of the amount of analyte present in the formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry since this method is specific, robust, linear, precise, and accurate and the limit of detection is low and also it offers the following advantages[8] as Speed (many analyses can be accomplished in 20min (or) less), Greater sensitivity (various detectors can be employed), Improved resolution (wide variety of stationary phases), Reusable columns (expensive columns but can be used for many analysis), precise and reproducible[9].




1-Reservior, 2-Pump, 3-Autosampler, 4-Injection valve, 5-Column, 6-Decetor

**Figure 1: HPLC Basic Instrumentation[6,7:]**

## MATERIALS AND METHODS

Albendazole (Pure) from Sura labs, Ivermectin(Pure) from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck

## HPLC METHOD DEVELOPMENT:

## TRAILS

### Preparation of standard solution:

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

Further pipette 0.6ml of the above Albendazole and 0.15ml of the Ivermectin stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

### Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, and note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[11,12].

**Mobile phase Optimization:**

Initially, the mobile phase tried was Methanol: Water, and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 85:15 v/v respectively.

**Optimization of Column:**

The method was performed with various columns like C18 column X- bridge column, Xterra Phenomenex Luna C 18 (4.6 x 150mm, 5 µm) was found to be ideal as it gave good peaks shape and resolution at 1ml/min flow.

**VALIDATION**

**PREPARATION OF MOBILE PHASE:**

**Preparation of mobile phase:**

Accurately measured 850ml (58%) of HPLC Methanol and 150ml of Water (15%) were mixed and degassed in a digital ultrasonicated for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:**

The Mobile phase was used as the diluents.

**RESULTS AND DISCUSSION**

**(Optimized chromatogram):**

Column: Phenomenex Luna C 18 ( 4.6 × 250MM) 5µ

Column temperature: 35ºC

Wavelength: 285nm

Mobile phase ratio: Methanol: Water(85:15 v/v)

Flow rate: 1ml/min

Injection volume: 10µl

Run time: 7 minutes

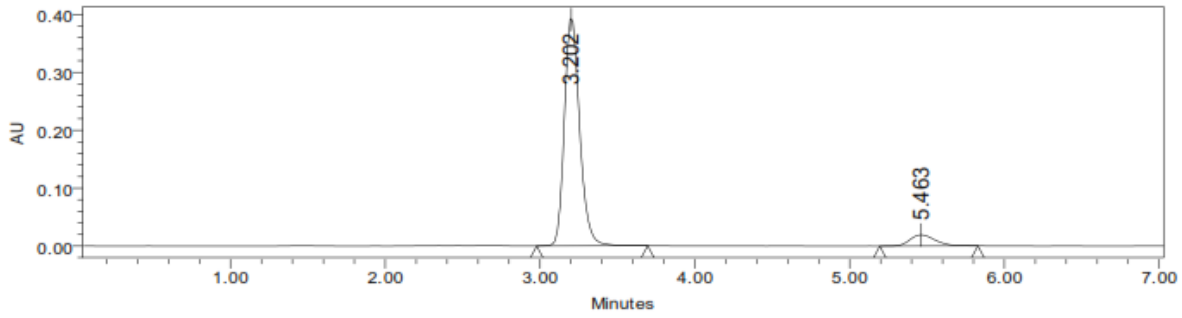



**Figure no 2: Optimized Chromatogram (Standard)**

**Table 1: Optimized Chromatogram (Standard)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.202 | 2391746 | 39726 | 1.2 | 9028 | |
| 2 | Ivermectin | 5.463 | 194627 | 8497 | 1.1 | 7398 | 7.4 |

**Optimized Chromatogram (Sample)**

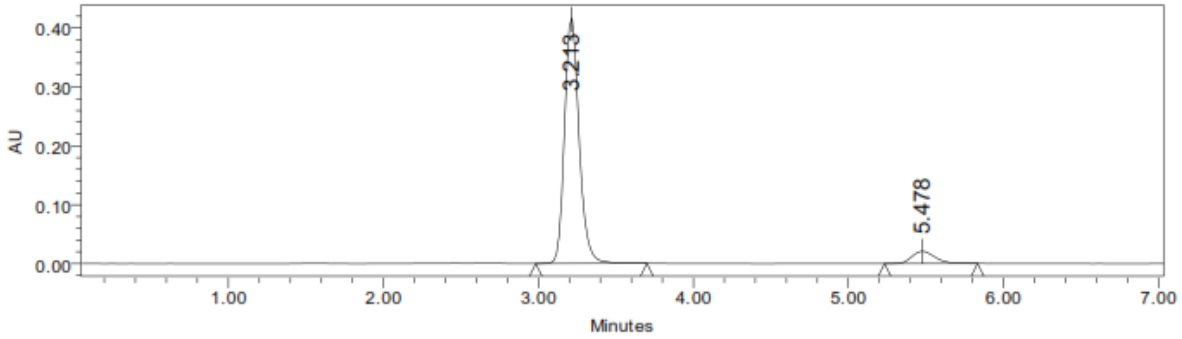



**Figure no 3: Sample Optimized Chromatogram**

**Table 2: Optimized Chromatogram (Sample)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Albendazol | 3.213 | 238164 | 391846 | 1.2 | 9472 | 7.0 |
| 2 | Ivermectin | 5.478 | 191057 | 8104 | 1.1 | 8936 | 7.5 |

**System suitability:**

**Table 3: Result of system suitability for Albendazole**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.200 | 2391746 | 394171 | 8952 | 1.2 |
| 2 | Albendazole | 3.248 | 2391647 | 381946 | 9561 | 1.2 |
| 3 | Albendazole | 3.299 | 2381647 | 391746 | 6572 | 1.2 |
| 4 | Albendazole | 3.297 | 2385631 | 386562 | 6452 | 1.2 |
| 5 | Albendazole | 3.297 | 2385635 | 389164 | 7452 | 1.2 |
| **Mean** | | | 2387261 | | | |
| **Std.Dev.** | | | 4363.771 | | | |
| **%RSD** | | | 0.182794 | | | |

**Acceptance criteria:**

- % RSD of five different sample solutions should not be more than 2.
- The % RSD obtained is within the limit, hence the method is suitable.

**Table no 4: Results of system suitability for Ivermectin**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.413 | 198362 | 7917 | 5272 | 1.1 |
| 2 | Ivermectin | 5.484 | 197486 | 7486 | 6291 | 1.1 |
| 3 | Ivermectin | 5.405 | 198354 | 7859 | 6184 | 1.1 |
| 4 | Ivermectin | 5.405 | 197352 | 7926 | 7145 | 1.1 |
| 5 | Ivermectin | 5.409 | 198453 | 7946 | 6946 | 1.1 |
| **Mean** | | | 198001.4 | | | |
| **Std.Dev.** | | | 535.1774 | | | |
| **%RSD** | | | 0.27029 | | | |

**Acceptance criteria:**

- % RSD of five different sample solutions should not be more than 2.
- The % RSD obtained is within the limit, hence the method is suitable.

**SPECIFICITY**

**Assay (Standard):**

**Table no 5: Peak results for assay standard of Albendazole**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.211 | 2397162 | 397161 | 1.2 | 9472 |
| 2 | Albendazole | 3.222 | 2394721 | 389173 | 1.2 | 9745 |
| 3 | Albendazole | 3.254 | 2389461 | 391723 | 1.2 | 8917 |

**Table no 6: Peak results for assay standard of Ivermectin**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.414 | 198462 | 7811 | 1.1 | 8492 | 7.49 |
| 2 | Ivermectin | 5.453 | 198472 | 8193 | 1.1 | 8916 | 7.52 |
| 3 | Ivermectin | 5.424 | 198735 | 7972 | 1.1 | 9372 | 7.44 |

**Assay (Sample):**

**Table no 7: Peak results for Assay sample of Albendazole**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.297 | 2391741 | 381612 | 1.2 | 9472 |
| 2 | Albendazole | 3.294 | 2389166 | 391746 | 1.2 | 8927 |
| 3 | Albendazole | 3.295 | 2361731 | 381634 | 1.2 | 9017 |

**Table no 8: Peak results for Assay sample of Ivermectin**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.435 | 198641 | 8174 | 1.1 | 9284 | 7.18 |
| 2 | Ivermectin | 5.417 | 196547 | 8942 | 1.1 | 8974 | 7.44 |
| 3 | Ivermectin | 5.434 | 194027 | 7294 | 1.1 | 9017 | 7.38 |

% ASSAY=

Sample area weight of standard Dilution of sample Purity weight of the tablet

_____ × _____ × _____×_____×_____×100

Standard area Dilution of standard Weight of sample 100 Label claim

=2380879/2393781*60/0.1731*99.8/100*1.5409/89.6*100

=98.6%

The % purity of Albendazole and Ivermectin in pharmaceutical dosage forms was found to be 98.6%

**LINEARITY**

**Table no 9: Chromatographic Data for Linearity Study of Albendazole**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 60 | 20 | 909889 |
| 80 | 40 | 1583641 |
| 100 | 60 | 2395378 |
| 120 | 80 | 3185089 |
| 140 | 100 | 3943725 |

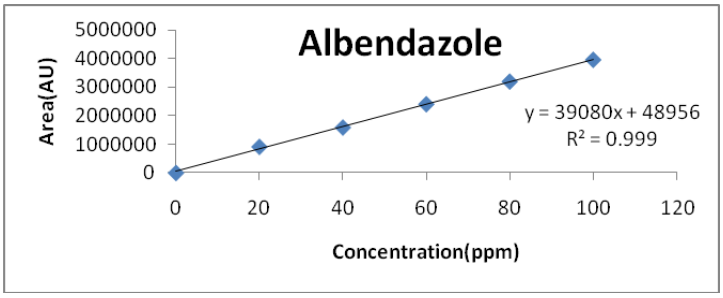



**Figure 4: Calibration graph for Albendazole**

**Table no 10: Chromatographic Data for Linearity Study of Ivermectin**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 60 | 5 | 61953 |
| 80 | 10 | 130213 |
| 100 | 15 | 198697 |
| 120 | 20 | 267002 |
| 140 | 25 | 321658 |

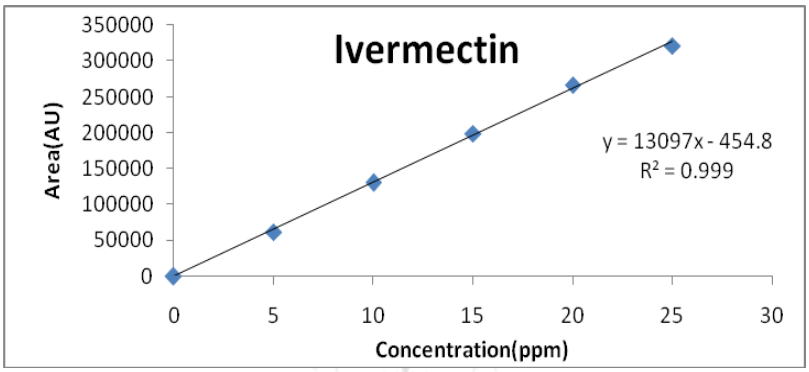



**Figure 5: calibration graph for Ivermectin**

**REPEATABILITY**

**Table no 11: Results of Repeatability for Albendazole:**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.213 | 2397164 | 381741 | 8155 | 1.2 |
| 2 | Albendazole | 3.253 | 2391741 | 371742 | 9174 | 1.2 |
| 3 | Albendazole | 3.297 | 2371846 | 391746 | 7154 | 1.2 |
| 4 | Albendazole | 3.215 | 2361748 | 391847 | 9917 | 1.2 |
| 5 | Albendazole | 3.254 | 2371649 | 384622 | 9247 | 1.2 |
| **Mean** | | | 2378830 | | | |
| **Std. dev** | | | 14958 | | | |
| **%RSD** | | | 0.628797 | | | |

**Acceptance criteria:**

- % RSD for the sample should be NMT 2.
- The % RSD for the standard solution is below 1, which is within the limits hence method is precise.

**Table no 12: Results of repeatability for Ivermectin:**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.441 | 198464 | 7291 | 6274 | 1.1 |
| 2 | Ivermectin | 5.442 | 193643 | 7219 | 6592 | 1.1 |
| 3 | Ivermectin | 5.409 | 196462 | 7194 | 6028 | 1.1 |
| 4 | Ivermectin | 5.520 | 194644 | 8174 | 6927 | 1.1 |
| 5 | Ivermectin | 5.424 | 198464 | 8653 | 5920 | 1.1 |
| **Mean** | | | 196335.4 | | | |
| **Std. dev** | | | 2190.191 | | | |
| **%RSD** | | | 1.115536 | | | |

**Intermediate precision:**

**Table no 13: Results of Intermediate precision Day 1 for Albendazole**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.211 | 2389572 | 395275 | 9375 | 1.2 |
| 2 | Albendazole | 3.211 | 2391847 | 392175 | 9275 | 1.2 |
| 3 | Albendazole | 3.210 | 2319472 | 312947 | 8265 | 1.2 |
| 4 | Albendazole | 3.212 | 2306842 | 310585 | 6254 | 1.2 |
| 5 | Albendazole | 3.211 | 2375972 | 310694 | 9028 | 1.2 |
| 6 | Albendazole | 3.297 | 2396746 | 358373 | 8928 | 1.2 |
| **Mean** | | | 2363409 | | | |
| **Std.Dev.** | | | 39730.83 | | | |
| **%RSD** | | | 1.681082 | | | |

**Acceptance criteria:**

- % RSD of six different sample solutions should not be more than 2.

**Table no 14: Results of Intermediate precision Day 1 for Ivermectin**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.411 | 197284 | 7194 | 8264 | 1.2 |
| 2 | Ivermectin | 5.410 | 197849 | 7294 | 9174 | 1.2 |
| 3 | Ivermectin | 5.420 | 196572 | 7147 | 9164 | 1.2 |
| 4 | Ivermectin | 5.423 | 195028 | 7927 | 9733 | 1.2 |
| 5 | Ivermectin | 5.419 | 199474 | 8238 | 9194 | 1.2 |
| 6 | Ivermectin | 5.409 | 197482 | 7638 | 8973 | 1.2 |
| **Mean** | | | 197281.5 | | | |
| **Std. Dev.** | | | 1466.354 | | | |
| **%RSD** | | | 0.74328 | | | |

**Acceptance criteria:**

- % RSD of six different sample solutions should not be more than 2.

**Table no 15: Result of Intermediate precision Day 2 for Albendazole**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Albendazole | 3.211 | 2389562 | 391741 | 9264 | 1.2 |
| 2 | Albendazole | 3.233 | 2381654 | 391047 | 9746 | 1.2 |
| 3 | Albendazole | 3.244 | 2381946 | 391748 | 9816 | 1.2 |
| 4 | Albendazole | 3.297 | 2391741 | 391746 | 9917 | 1.2 |
| 5 | Albendazole | 3.297 | 2386452 | 381641 | 9742 | 1.2 |
| 6 | Albendazole | 3.202 | 2374763 | 381645 | 9017 | 1.2 |
| **Mean** | | | 2384353 | | | |
| **Std. Dev.** | | | 6183.339 | | | |
| **%RSD** | | | 0.25933 | | | |

**Acceptance criteria:**

- % RSD of six different sample solutions should not be more than 2.

**Table no 16: Results of Intermediate precision Day 2 for Ivermectin**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ivermectin | 5.411 | 197486 | 7582 | 6272 | 1.1 |
| 2 | Ivermectin | 5.410 | 197486 | 7184 | 6174 | 1.1 |
| 3 | Ivermectin | 5.420 | 196746 | 7456 | 5184 | 1.1 |
| 4 | Ivermectin | 5.405 | 195862 | 7814 | 6194 | 1.1 |
| 5 | Ivermectin | 5.409 | 196582 | 7194 | 6292 | 1.1 |
| 6 | Ivermectin | 5.463 | 198463 | 7745 | 6191 | 1.1 |
| **Mean** | | | 197104.2 | | | |
| **Std. Dev.** | | | 903.542 | | | |
| **%RSD** | | | 0.458408 | | | |

**Acceptance criteria:**

- % RSD of six different sample solutions should not be more than 2.

**ACCURACY:**

**Table no 17: The accuracy results for Albendazole**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1217218 | 30 | 29.4 | 99.1 | 99.5 |
| 100% | 2397141 | 60 | 59.5 | 99.6 | |
| 150% | 3514547 | 90 | 89.7 | 99.8 | |

**Acceptance Criteria:**

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, and 150% are within the limits. Hence the method is accurate[13-14].

**Table no 18: The accuracy results for Ivermectin**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 98598.67 | 7.5 | 7.49 | 99.9 | 99.6 |
| 100% | 198359.7 | 15 | 15.0 | 100 | |
| 150% | 291512.3 | 22.5 | 22.48 | 99 | |

**Acceptance Criteria:**

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, and 150% are within the limits. Hence the method is accurate.

**LIMIT OF DETECTION**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S =Slope of the calibration curve

**Albendazole:**

**Result:**

=3.3 × 39761/39080

= 3.3µg / ml

**Ivermectin:**

**Result:**

=3.3 × 5008/13097

=1.2µg/ml

**Quantitaion limit**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ=10\times\sigma/S}$$

Where

σ =Standard deviation of the response

S =Slope of the calibration curve

**Albendazole:**

**Result:**

=10×39761/39080

=10.1µg / ml

**Ivermectin:**

**Result:**

=10×5008/13097

=3.8µg/ml

**ROBUSTNESS**

**Table no 19: Result for Robustness of Albendazole**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0mL/min | 2391746 | 3.202 | 9028 | 1.2 |
| Less Flow rate of 0.9mL/min | 2371831 | 3.639 | 7381 | 1.2 |
| More Flow rate of 1.1mL/min | 2218319 | 2.859 | 9311 | 1.1 |
| Less organic phase (about 5 % decrease in organic phase) | 2294821 | 3.460 | 7462 | 1.2 |
| More organic phase (about 5 % Increase in organic phase) | 2394811 | 3.022 | 6817 | 1.1 |

**Acceptance criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**Table no 20: Results for Robustness of Ivermectin**

| Parameter used for sample | Peak Area | Retention Time | Theoretical | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.1mL/min | 194627 | 5.463 | 7398 | 1.1 |
| Less Flow rate of 0.9mL/min | 183738 | 6.250 | 6883 | 1.1 |
| More Flow rate of 0.8mL/min | 198373 | 4.863 | 9917 | 1.2 |
| Less organic phase (about 5 % decrease in organic phase) | 178471 | 6.196 | 8372 | 1.1 |
| More organic phase (about 5 % Increase in organic phase) | 189462 | 5.010 | 7716 | 1.2 |

**Acceptance criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

## CONCLUSION

The developed HPLC method offers several advantages such as rapid, usage of simple mobile phase and easy sample preparation steps, Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied to the analysis of pure drug and pharmaceutical dosage forms. From the present study, it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate, and reproducible. The result of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose.

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