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DEVELOPMENT AND VALIDATION OF ALBENDAZOLE AND PRAZIQUENTAL BY USING RP-HPLC

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Abstract

The present study aims to develop and validate a robust, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Albendazole and Praziquantel in pharmaceutical dosage forms. A C18 column was employed with a suitable mobile phase composed of acetonitrile and phosphate buffer in an optimized ratio, delivered at a flow rate of 1.0 mL/min. The detection was carried out using a UV detector at a wavelength of 254 nm. The method was validated in accordance with ICH guidelines for parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ). The retention times for Albendazole and Praziquantel were found to be distinct and consistent, ensuring effective resolution without interference. The method demonstrated excellent linearity over the specified concentration range, with correlation coefficients (R^2) greater than 0.999 for both drugs. Recovery studies confirmed the accuracy of the method, and %RSD values for intra- and inter-day precision studies were within acceptable limits. The developed RP-HPLC method is simple, reliable, and suitable for routine quality control analysis of Albendazole and Praziquantel in bulk and tablet formulations.

Introduction:

Parasitic infections remain a significant public health concern in many parts of the world, particularly in tropical and subtropical regions. Among the widely used anthelmintic agents, Albendazole and Praziquantel have proven to be highly effective in the treatment of a broad spectrum of parasitic infestations. Albendazole, a benzimidazole carbamate derivative, exhibits broad-spectrum activity against intestinal and tissue helminths by inhibiting microtubule synthesis, which disrupts the uptake of glucose in parasites and leads to their eventual death. On the other hand, Praziquantel is a pyrazinoisoquinoline derivative known for its efficacy against trematodes and cestodes, particularly schistosomiasis and tapeworm infections. It increases membrane permeability to calcium ions, causing muscle contraction and paralysis of the parasite. These two drugs are often used in combination therapy or fixed-dose formulations due to their complementary modes of action and broad activity profiles.[2]

The increasing use of fixed-dose combinations of Albendazole and Praziquantel in the management of co-infections necessitates the development of reliable and validated analytical methods for their simultaneous estimation. Quality control and regulatory compliance require that pharmaceutical dosage forms be analyzed with accurate, precise, and robust methods to ensure consistency, safety, and efficacy. While several analytical methods exist for the individual estimation of Albendazole or Praziquantel, there is a clear need for a simple, fast, and validated RP-HPLC method capable of estimating both drugs simultaneously in combined dosage forms.[4]

High-performance liquid chromatography (HPLC), particularly reverse-phase HPLC (RP-HPLC), is widely regarded as a powerful and versatile technique in pharmaceutical analysis due to its high resolution, reproducibility, and ease of operation. RP-HPLC is especially advantageous for separating structurally diverse compounds within the same formulation. The use of C18 columns, in conjunction with optimized mobile phases and UV detection, enables the selective separation and quantification of active pharmaceutical ingredients, even in the presence of excipients or degradation products.[7]

The present study is focused on the development and validation of a robust RP-HPLC method for the simultaneous estimation of Albendazole and Praziquantel in bulk drugs and pharmaceutical tablet dosage forms. The method is designed to be simple, accurate, and reproducible, with optimized chromatographic conditions that provide good resolution and sharp peak shapes. Method validation is performed in accordance with ICH Q2(R1) guidelines, ensuring that all critical parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ) are systematically evaluated and meet acceptance criteria.[6]

This method is expected to serve as a valuable tool for routine quality control analysis in pharmaceutical industries and research laboratories. It also supports regulatory submissions by providing a validated procedure for the simultaneous estimation of Albendazole and Praziquantel, thus ensuring the consistent quality and therapeutic performance of combination products in the market.[12]

Material and Method:

Albendazole and Praziquantel reference standards were obtained as gift samples from a certified pharmaceutical manufacturer. Commercially available combination tablets containing both Albendazole and Praziquantel were purchased from a local pharmacy for analysis. All chemicals and solvents used during the study were of either HPLC grade or analytical reagent (AR) grade. Acetonitrile and methanol (HPLC grade) were procured from Merck (India), and potassium dihydrogen phosphate and orthophosphoric acid used in buffer preparation were of AR grade. HPLC-grade water was obtained from a Milli-Q water purification system. Prior to use, all solvents and solutions were filtered through 0.45 µm membrane filters and degassed using a sonicator to ensure clarity and remove any air bubbles.[14]

The chromatographic analysis was carried out using a Shimadzu LC-20AT HPLC system equipped with a UV-visible detector (SPD-20A), a quaternary pump, and a manual injector with a 20 µL loop. The chromatographic separation was achieved using a **C18 column (250 mm × 4.6 mm, 5 µm particle size)**. The mobile phase consisted of a mixture of **acetonitrile and phosphate buffer**, optimized in the ratio of **65:35 v/v**, with the pH of the buffer adjusted to around 3.5 using orthophosphoric acid. The flow rate of the mobile phase was maintained at **1.0 mL/min**, and the elution was monitored at a **wavelength of 254 nm** using a UV detector. The column was operated at ambient temperature (25 ± 2°C).[17]

Standard stock solutions of Albendazole and Praziquantel were prepared by accurately weighing 10 mg of each drug and dissolving them separately in 10 mL of mobile phase to obtain concentrations of 1000 µg/mL. Working standard solutions were prepared by suitable dilution with the mobile phase to obtain concentration ranges of 5–50 µg/mL for both drugs. Calibration curves were plotted by injecting six different concentrations, and the peak areas were recorded and analyzed to assess linearity.[19]

For sample preparation, twenty tablets were weighed and finely powdered. An amount of tablet powder equivalent to one tablet (containing known quantities of Albendazole and Praziquantel) was transferred into a 100 mL volumetric flask. About 70 mL of the mobile phase was added, and the mixture was sonicated for 20 minutes to ensure complete extraction of the active ingredients. The solution was filtered using a 0.45 µm membrane filter and diluted appropriately with the mobile phase to bring the concentrations within the linearity range of the standard solutions.[21]

The developed RP-HPLC method was validated according to **ICH Q2(R1) guidelines**. System suitability was assessed by injecting standard solutions and evaluating parameters such as theoretical plates, tailing factor, resolution, and retention time. Linearity was evaluated by

constructing calibration plots over a defined concentration range. Accuracy was determined by recovery studies at three levels (80%, 100%, and 120%), and precision was evaluated by conducting intra-day and inter-day repeatability studies. The method's **specificity** was assessed by checking for any interference from common excipients. LOD and LOQ values were determined based on signal-to-noise ratios, and **robustness** was examined by introducing small variations in chromatographic conditions such as flow rate and mobile phase composition.[24]

Results and Discussion:

Solubility Studies

These studies are carried out at 25^0C

Albendazole:

Albendazole is a white to off-white powder. It is 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.

Praziquantel:

White or almost white, crystalline powder. It is very slightly soluble in water, freely soluble in alcohol and in methylene chloride.

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Albendazole

10 mg of Albendazole was weighed in to 100 ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1 ml to 10 ml with methanol.

Preparation of standard stock solution of Praziquantel

10 mg of Praziquantel was weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200-400 nm against methanol as blank. The resulting spectra are the absorption curve shows characteristic absorption maxima at 248 nm for Albendazole, 210 nm for Praziquantel and 221 nm for the combination.

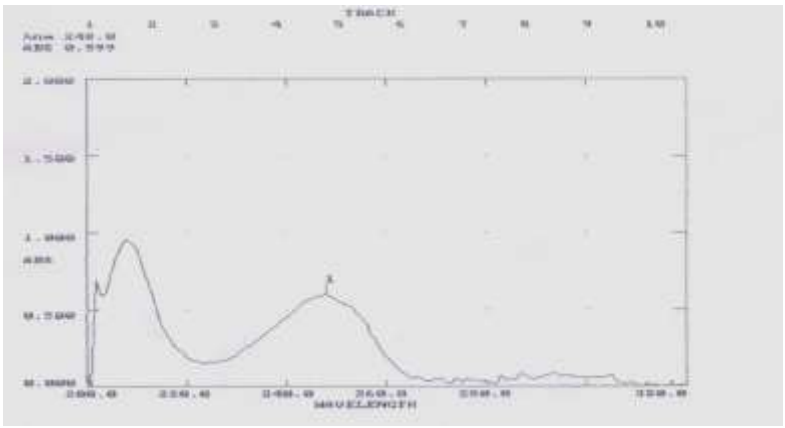

Fig. 8.1: UV spectrum of Albendazole

Observation: λ_{max} was found to be 237 nm for Albendazole shown in the Figure 8.1.

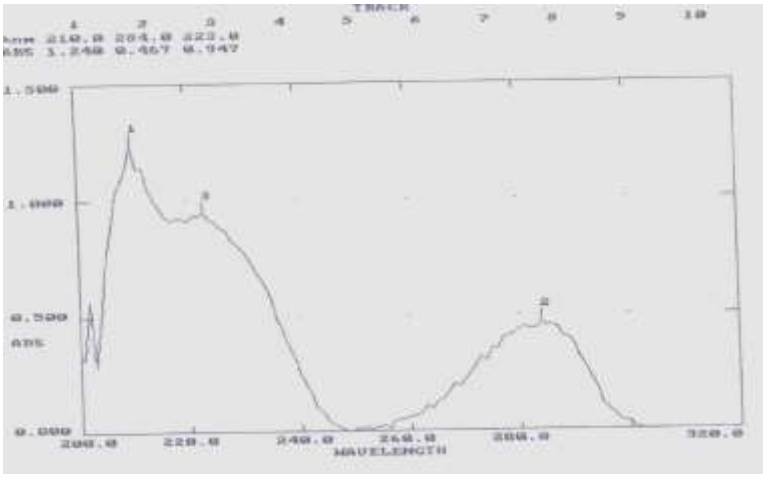

Fig. 8.2: UV spectrum of Praziquantel

Obeservation: λ_{max} was found to be 210 nm for Praziquantel shown in the Figure 8.2.

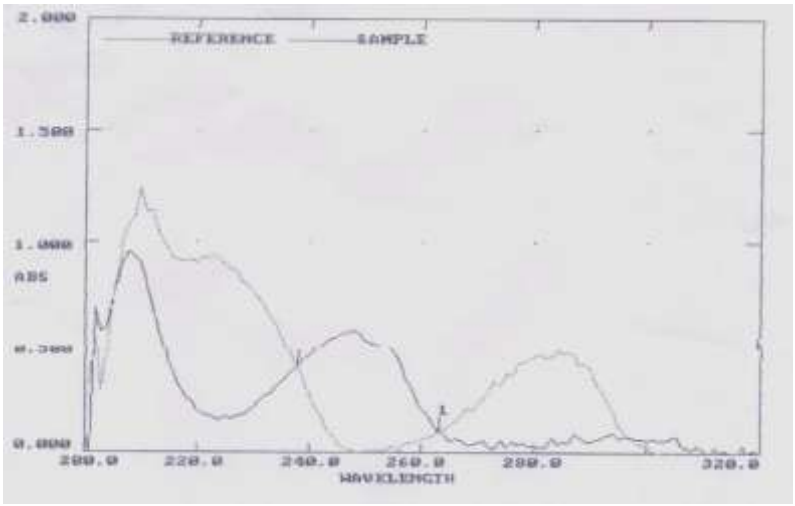

Fig. : UV-OVERLAP spectrum of Praziquantel and Albendazole

Observation: The isobestic point was found to be 238 nm for Praziquantel and Albendazole in combination and was shown in Figure 8.3.

Assay

Preparation of samples for Assay

Standard sample

Standard stock solutions of Albendazole and Praziquantel (microgram/ml) were prepared by dissolving 300 mg of Albendazole and 25 mg of Praziquantel dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min

and dilute to 50 ml with mobile phase. Further dilutions of 120 µg/ml of Albendazole and 10 µg/ml of Praziquantel was made by adding 1 ml of stock solution to 50 ml of mobile phase.

Tablet sample

20 tablets (each tablet contains 300 mg of Albendazole and 25 mg of Praziquantel) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Albendazole and Praziquantel (µg/ml) were prepared by dissolving weight equivalent to 300 mg of Albendazole and 25 mg of Praziquantel and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 120 µg/ml of Albendazole and 10 µg/ml of Praziquantel was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Praziquantel and Albendazole present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where

AS: Average peak area due to standard preparation

AT: Average Peak area due to assay preparation

WS: Weight of Praziquantel / Albendazole in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

AW: Average weight

P: Standard purity

LC: Label claim

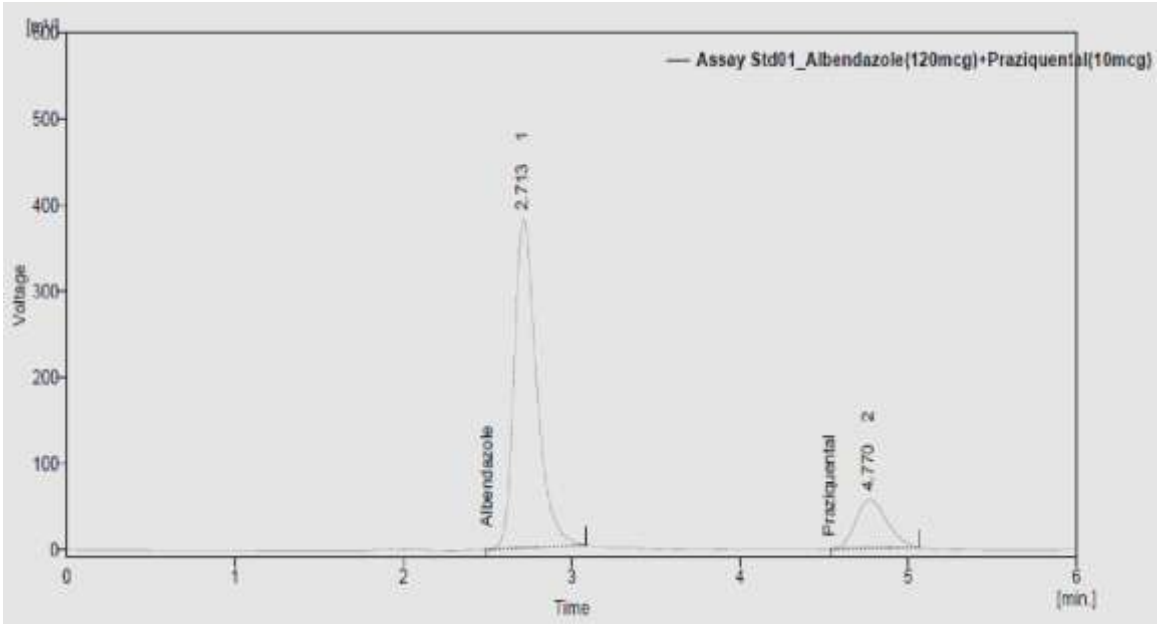

Fig. : Chromatogram of Assay standard preparation-1

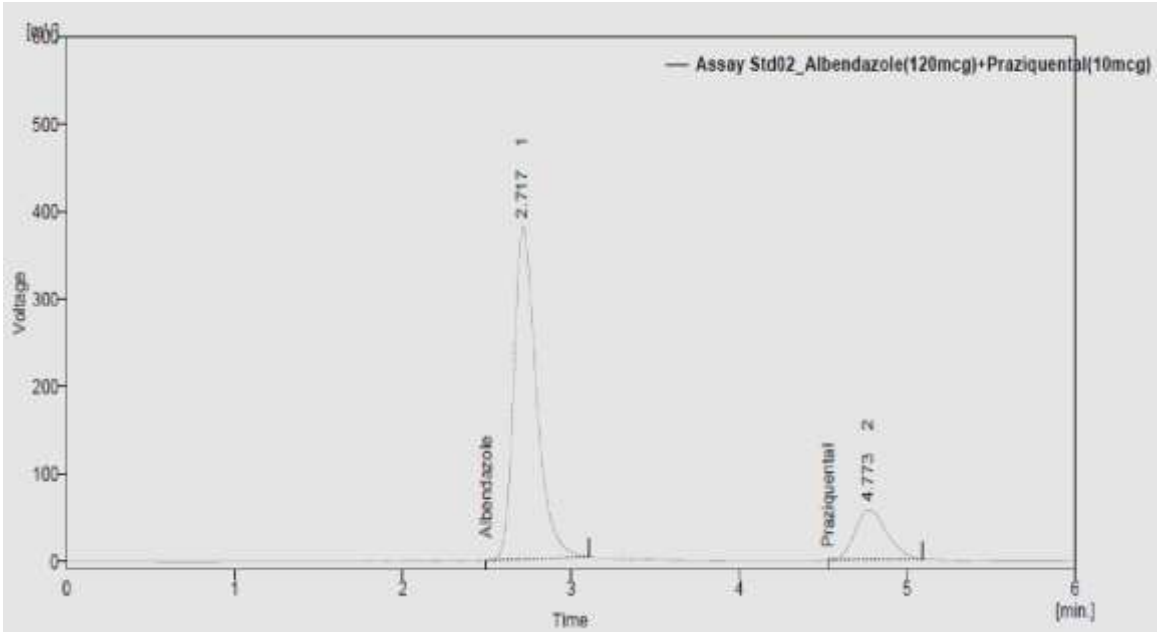

Fig. : Chromatogram of Assay standard preparation-2

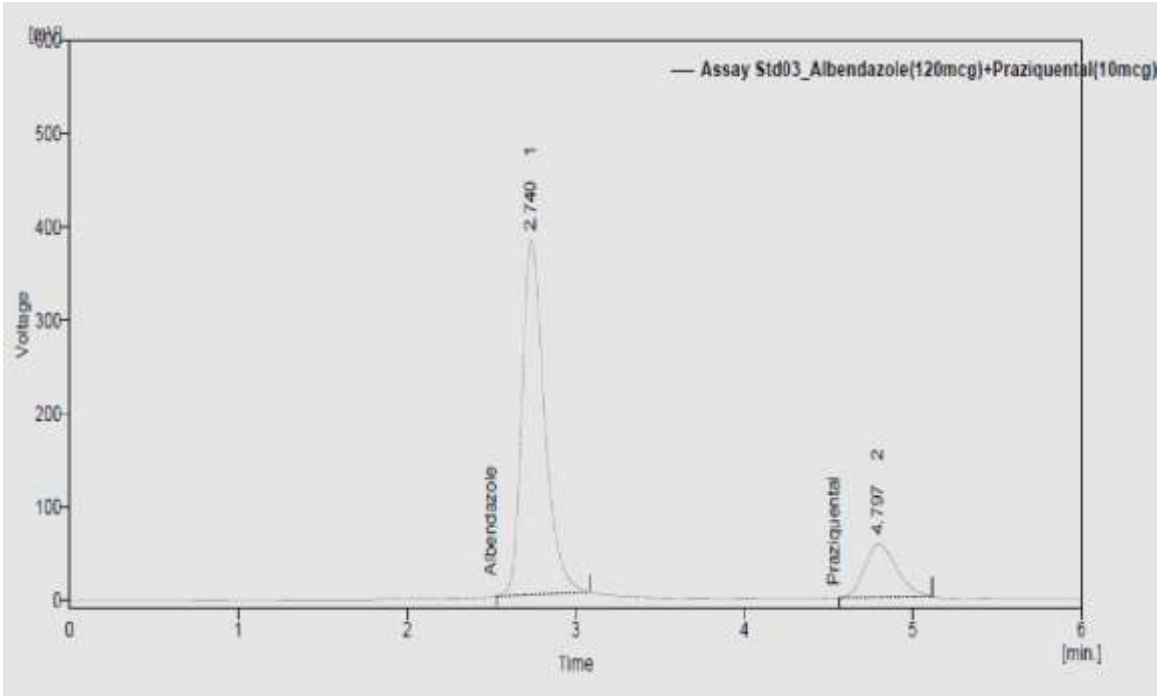

Fig. : Chromatogram of Assay standard preparation-3

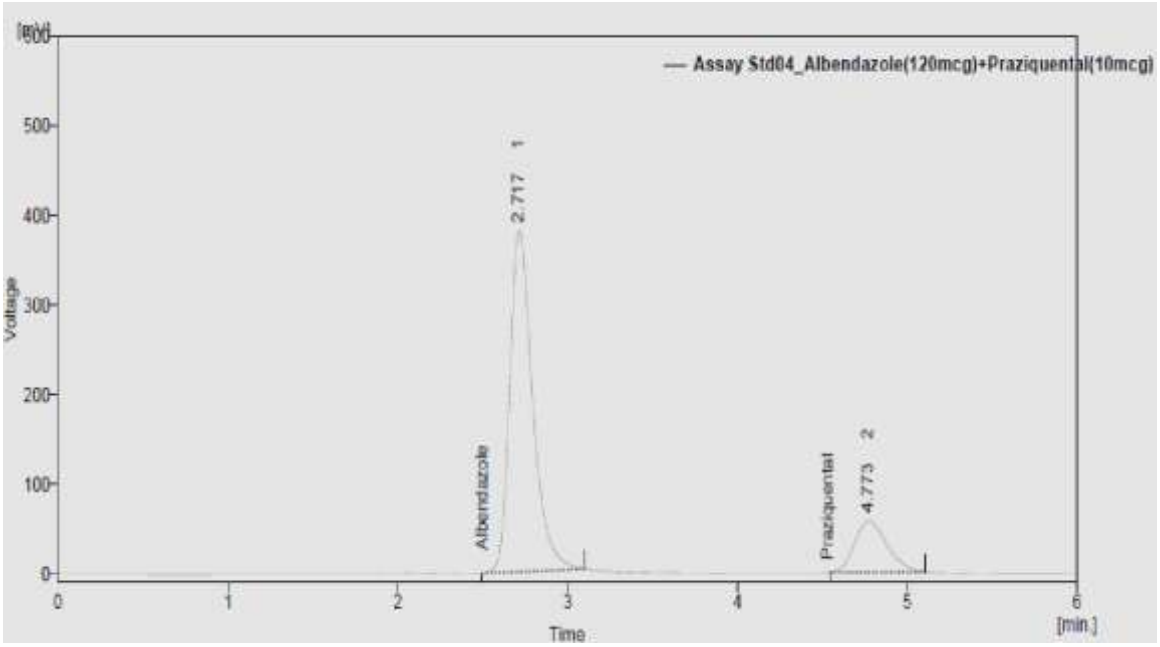

Fig. : Chromatogram of Assay standard preparation-4

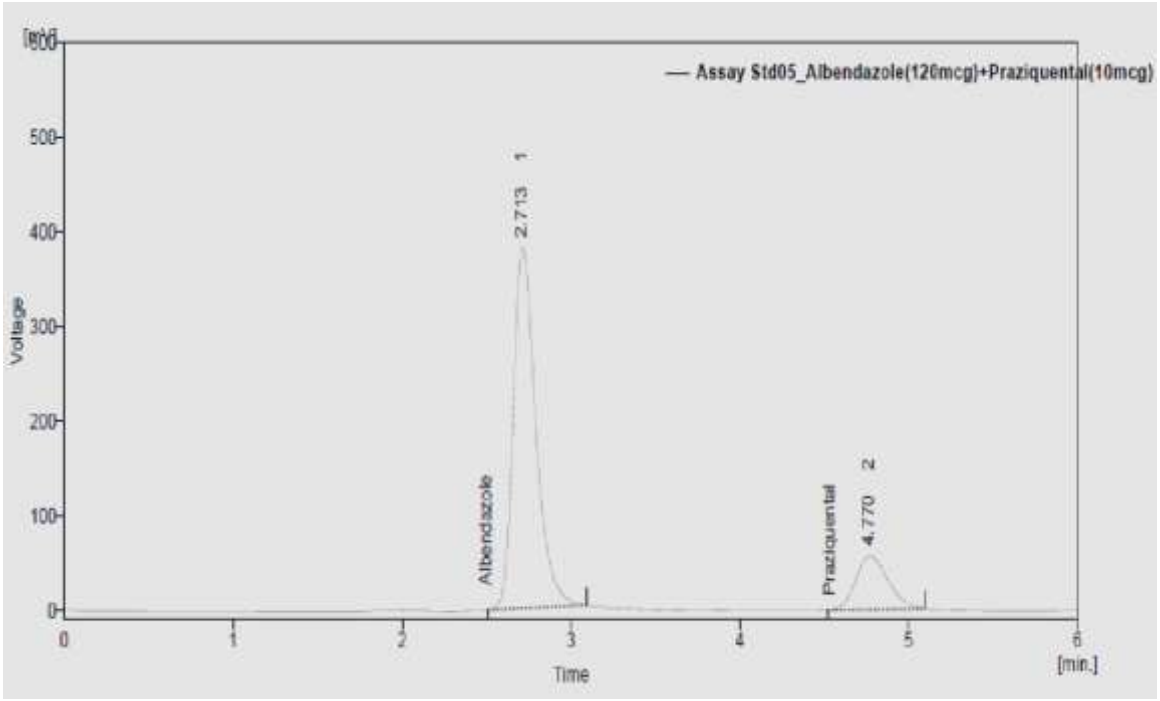

Fig. : Chromatogram of Assay standard preparation-5

Conclusion :

HPLC method development involves several essential steps, sample pretreatment, and detection of sample bands, choosing separation conditions and method validation is the process of providing documented evidence that the method does what it is intended to do. The process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use. Method validation is performed as per the ICH guidelines. Obtained study demonstrated that the HPLC method described in the protocol is valid for the determination of assay of Albendazole and Praziquantel in formulation.

Prior to my work, I provide the information regarding introduction to analytical methods which are available for analyzing analyte *viz.* spectroscopic and chromatographic, electrochemical and other conventional methods etc. emphasizing on introduction to HPLC, principle, classification, instrumentation of HPLC, characterization of chromatogram. It also includes introduction of HPLC method development, stages of method development, its

purpose, parameters and acceptance criteria of analytical method validation and drug profile of Albendazole and Praziquantel.

Literature review indicates Few analytical methods have been reported for the single and simultaneous determination of Albendazole and Praziquantel in combined pharmaceutical dosage forms using spectrophotometry40,42, HPLC43,38 and different mathematical approaches 44. But there is no method reported for simple estimation of Albendazole and Praziquantel in tablet formulation by RP-HPLC method in short run time. So the scope and objective of the present work is to optimize condition to develop estimation of Albendazole and Praziquantel by RP-HPLC.

Experimental methodology for the development of HPLC method for the assay of Albendazole and Praziquantel in Tablets contains all the trials explored for the method development are detailed in this chapter The optimized HPLC chromatographic conditions include, a stainless steel column- Inertsil ODS 3v column 250 mm x 4.6 mm, 5µm, A mixture of 20 volumes of phosphate buffer pH 4.0:70 volumes of acetonitrile and 10 volumes of methanol was prepared (filtered through 0.45 µm filter and degassed using sonicator), as the mobile phase, Flow rate of 1.0 ml per minute, a detection wavelength of 226 nm, column temperature 25ºC and injection volume 20 µl. It also includes experimental procedure of all validation parameters for validation of developed method.

The result includes responses of chromatographs blank, standard, test in system suitability and specificity. Responses of chromatographs give value of peak area, no. of theoretical plates, retention time and their asymmetry. Every validation parameter as per ICH guidelines was practically explored and the results and conclusions drawn are detailed in this chapter. The method developed was validated for linearity, system precision, method precision and ruggedness, robustness and accuracy, limit of detection and quantitation, stability of the solutions were also determined.

Five consecutive injections of the mixture of standard solution showed % RSD (% Relative Standard Deviation) less than 2 concerning retention time and peak areas for both the drugs which indicate the method developed and optimized is system precise.

Six consecutive injections of the sample showed % RSD less than 2 concerning % assay and peak areas for both the drugs which indicate the method developed and optimized is intraday precise, by the test of repeatability and hence can be understood that the method gives consistent results.

Six consecutive injections of the sample solution on the other consecutive day, showed % RSD less than 2 on different days and between days for % assay for both the drugs, which indicate the method developed and optimized is inter day precise.

A linear relationship between peak areas versus concentrations was observed for Albendazole and Praziquantel in the range of 50% to 150% concentration. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Albendazole and Praziquantel is 0.9916 and 0.9901., which meet the method validation acceptance criteria, an indication of method being linear in the range of 50% to 150%.

Accuracy studies revealed that found desirable recoveries were achieved (98-102%) as per acceptance criteria of method validation. % RSD for Albendazole HCl and Praziquantel was less than 2. Hence, the method developed and optimized is accurate.

Method developed is found to be robust as it is found that the results of peak performance parameters are:

- Resolution factor (Rs) >2.0
- Tailing factor < 2.0 and
- Number of theoretical plates (Efficiency) more than 2000, which are in acceptance criteria to method validation despite deliberate variations done concerning flow rate, % organic phase and column temperature.

The LOD for this method was found to be 6.71 µg/ml & area 157.19 for Albendazole and 0.22 µg/ml & area 13.11 for Praziquantel.

The LOQ for this method was found to be 20.32 µg/ml & area 370.99 for Albendazole and 0.67 µg/ml & area 39.73 for Praziquantel.

From all the above validation conclusions, it is very clear that the Reverse Phase HPLC isocratic method developed and validated as per ICH guidelines is sensitive, accurate, precise, linear and convenient for intended applications in any pharmaceutical industries.

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