



## Research Article

### **SIMULTANEOUS ESTIMATION OF ALBENDAZOLE AND IT'S IMPURITIES IN PHARMACEUTICALS BY LIQUID CHROMATOGRAPHY METHOD**

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Article Received on: 08/12/18 Approved for publication: 22/01/19

**DOI: 10.7897/2230-8407.100393**

#### **ABSTRACT**

The high performance liquid chromatographic reverse phase method developed for determination of related substances or impurities of Albendazole from pharmaceutical dosage forms. The mobile phase composition of potassium dihydrogen phosphate buffer having pH 2.5, acetonitrile in the ratio of 90:10(v/v) is used as mobile phase A. The composition of acetonitrile, methanol and water in the ratio of 50:40:10(v/v) is used as mobile phase B. The separation between Albendazole and its impurities has been achieved by using column L11, 150\*4.6mm, 5µm at wavelength 254nm. The method shows well resolution between all the impurities and Albendazole peak and quantification with accuracy. After literature survey, revealed that there is no method is available in USP, EP and BP for separation of Albendazole and all the impurities in pharmaceutical formulations. This method can be used for quality control laboratory and stability study for determination of impurities of Albendazole in pharmaceutical formulations.

**Keywords:** Reverse phase, development, validation, Albendazole Dosage form, Liquid Chromatography

#### **INTRODUCTION**

Albendazole is orally administered anthelmintic drug. Chemically it is methyl 5(propylthio)-2-benzimidazolecarbamate. Molecular formula of Albendazole is C<sub>12</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>S and molecular weight is 265.34. The Chemical Structure of Albendazole shown below fig.1. Albendazole is available in the market in the form of tablets and oral suspensions. It is used for medication to relieve pain and certain infections caused by worms such as pork tapeworm and dog tapeworm. Albendazole is used in the hydatid disease. Albendazole is extensively metabolized to its active metabolite albendazole sulfoxide, which is further metabolized to the inactive albendazole sulfone. Albendazole is insoluble in water and soluble in DMF, strong acids, strong bases, ethanol, ether, chloroform<sup>1, 2, 3</sup>.

After literature survey, revealed that there is no method is available in USP, EP, BP for separation of Albendazole and all the impurities in pharmaceuticals.<sup>4, 5</sup> Especially in all the methods including USP and BP impurity B and impurity C of Albendazole are not separated and reported by addition of both the impurities. Hence developed liquid chromatographic method which separates Albendazole and all its impurities with UV detection at 254 nm. The proposed method is validated as per ICH guidelines and all the parameters can meet the specifications. Hence this method can be used for routine quality control analysis and stability studies in pharmaceutical industries.

#### **MATERIALS AND METHODS**

##### **Chemicals and reagents**

Potassium dihydrogen phosphate (GR grade), Orthophosphoric acid (GR grade), Acetonitrile HPLC grade and Methanol (HPLC grade) were purchased from Merck Fine Chemicals (Mumbai, India). Nylon 0.45µm nylon filters of Advanced Micro Devices Pvt Ltd., India are used. High purity Albendazole WS, Albendazole Oral suspension of CIPLA Ltd. was purchased from Market. Milli-Q water is used for preparation of solutions and mobile phase.

##### **Instrumentation**

The LC system equipped with Waters 2695 module is used for development. The waters system with UV/PDA detector with Empower software is used. The development and forced degradation study were performed by using PDA detector. All the development and validation activity were conducted on USP column L11, 150mm x 4.6mm, 5µm.

##### **Chromatographic Conditions for developed method**

The buffer solution of 0.02M potassium dihydrogen phosphate is prepared and adjusted to pH 2.5 with Orthophosphoric acid. The buffer solution pH 2.5, acetonitrile in the ratio of 90:10(v/v) is used as mobile phase A while mobile phase B contains the acetonitrile, methanol and water in the ratio of 50:40:10 (v/v). The gradient program used for separation of all the peaks i.e. Albendazole and its impurities shown in table 1.

The better resolution between Albendazole and its impurities achieved by using column L11, 150x4.6mm, 5µm with flow rate 1.0 mL/ min and column temperature 30°C. The injection volume 10µL is used.

**Solution-A:** Methanol: hydrochloric acid 99:1v/v

**Solution-B:** Buffer Solution pH 2.5: Methanol 40:60 v/v

**Preparation of standard solutions**

Dissolved 5 mg of Standard in 2 mL of solution-A sonicated for 5 minutes, diluted up to the volume with solution-B. Diluted 1

mL of this solution to 50mL with solution B to obtain the concentration of Albendazole (5 µg/mL).

**Preparation of sample solution**

Weighed and transferred Sample equivalent to 25mg of Albendazole into 50mL volumetric flask, added 5mL of solution-A, sonicated for 10 minutes with intermittent shaking. Diluted up to the mark with solution-B to achieve the concentration of Albendazole (500 µg/mL). Filtered this solution through Nylon syringe filter of 0.45 µm.

**Table 1. Gradient program**

Time(min)	%Mobile Phase A	%Mobile Phase B
0	100	0
13	58	42
17	80	20
30	80	20
36	100	0
40	100	0

**Table 2. Correlation coefficient, LOQ and LOD.**

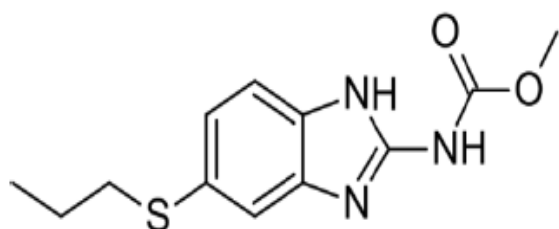
Name	Correlation coefficient	LOQ (%)	LOD (%)
Albendazole	1.00	0.080	0.030
Impurity A	1.00	0.058	0.020
Impurity B	1.00	0.053	0.020
Impurity C	1.00	0.090	0.030
Impurity D	1.00	0.053	0.020
Impurity E	0.99	0.090	0.030
Impurity F	1.00	0.030	0.010

**Table 3. Filter Study**

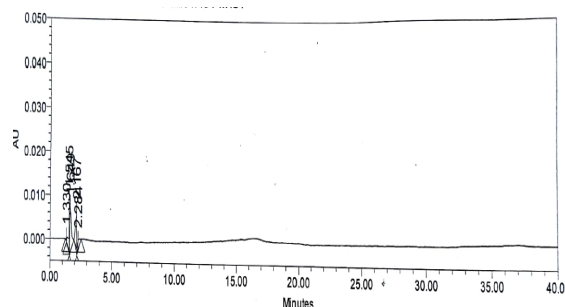
Impurities	Centrifuged		Nylon 0.45µm filter		PVDF 0.45µm filter	
	% impurities	% Difference	% impurities	% Difference	% impurities	% Difference
Total impurities	6.59	-	6.60	0.01	6.61	0.02

**Table 4. Forced degradation study**

Sample	% Assay	Total impurities (%)	Mass Balance (%)
Sample as such	104.2	0.8	---
Sample alkali hydrolysis (5mL 0.5N NaOH, 60°C for 30 min)	88.9	11.4	95.5
API as such	96.9	0.9	---
API alkali hydrolysis (5mL 0.5N NaOH, 60°C for 30 min)	92.0	6.7	100.9



**Fig.1 Structure of Albendazole**



**Fig.2. Diluent Chromatogram**

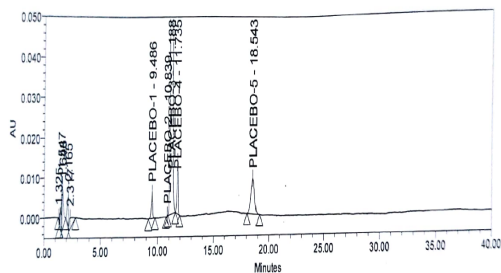


Fig.3. Placebo Chromatogram

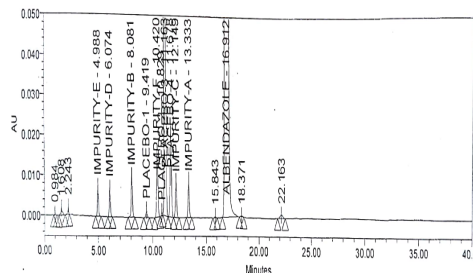


Fig.4. sample Chromatogram

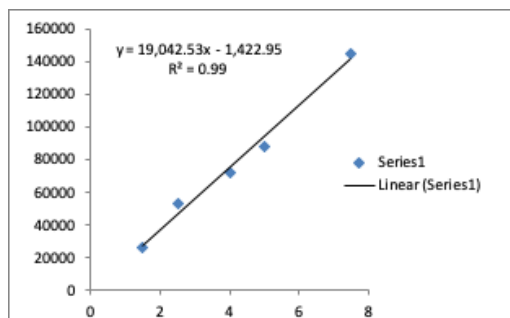


Fig.5. Linearity graph of Albendazole. (On x axis concentration in ppm & y axis Response)

**RESULTS AND DISCUSSION**

**Method development**

The HPLC method has been developed and optimized for determination of Albendazole and its impurities in Pharmaceutical suspension. The method was thoroughly evaluated and finalized after selection of mobile phase and its pH, column selection, diluent study and mobile phase composition study and different gradient program.

Solubility of Albendazole in different solvents like methanol, ethanol and methylene chloride has been performed to select the diluents for extraction of sample. Solubility also checked in water and acids like formic acid, concentrated hydrochloric acid. With the help of solubility study and extraction study acidified methanol is used as solution A and solution B as a combination Buffer solution: methanol in the ratio 40:60v/v is used as diluents. All the impurities and active peak shows optimum response at wavelength 254nm. Hence the development experiments are conducted at 254 nm wavelength.

The experiments are performed at acidic and basic mobile phase by varying the compositions of mobile phase A and mobile phase B. In acidic pH, resolution between active and impurity peaks is good. Due to this pH 2.5 buffer has been selected to avoid the compatibility issue with diluents.

Different experiments are conducted on different makes of columns KromasilC18, phenyl columns having 25cm length.<sup>6</sup> All the impurities are mid polar and after comparison with other columns in terms of separation of impurities USP column L11, Phenyl hexyl column, 150 mm x 4.6mm, 5µm has been selected.

Finally, the method was optimized for mobile phase, gradient program standard and sample concentration and validated as per ICH guidelines.<sup>7</sup> The reference chromatograms are shown in fig.2, 3, 4 and 5.

**Validation of method** done by performing the different parameter study like specificity, Reproducibility, Accuracy,

Linearity Filter study and forced degradation study as per ICH guidelines.

**Specificity**

The specificity of the method confirmed by injecting the diluent, standard solution, placebo solution and spiked samples solution into the system. It was observed that there is no any interference shows in diluent and placebo at the retention time of Albendazole and its known and unknown impurities. This shows that the developed method is specific to determine the Albendazole and its impurities without any interference.

**Linearity**

Linearity was performed by preparing the series of different concentrations of Albendazole and its impurities over the range of 5% to 150% of limits. The correlation coefficient obtained close to 1.00. The linearity graph of Albendazole is shown in below fig.5. The correlation coefficient, LOQ (Limit of Quantification) and LOD (Limit of Detection) obtained from Linearity are mentioned in below table 2.

**System Precision**

The system precision of the method was performed by injecting six injections of standard solution into the system. Calculated Relative Standard Deviation (RSD) and observed within the limit i.e. not more than 5.0%.

**Accuracy**

The accuracy of the method was performed by standard addition method i.e. spiking the known concentration of impurities in Sample and for LOQ level recovery, impurities spiked into placebo. The mean recovery values obtained for known and unknown impurities are within the limit 90% to 110%. The mean recovery for LOQ level obtained for all the impurities are within the limit 85% to 115%.

**Filter Study**

The filter study was performed by using different types of filter like Nylon 0.45µm, 0.45µm PVDF filters. Additionally the same sample solution was centrifuged. The filter selection was done by comparing the values obtained from centrifuged solution with the

values obtained from filtered sample solution. The difference between the total impurities of centrifuged sample and filtered sample should be not more than 0.05%. The values obtained from filter study are reported in table.3

#### **Forced Degradation**

The forced degradation study was performed by conducting acid, alkali and peroxide degradation for sample, Albendazole active and placebo. In acid and peroxide degradation study, degradation was not achieved. In alkali degradation, the sample shows degradation. The peak purity at each degradation passes. The summary of alkali degradation is reported in below table.4

#### **Solution stability**

Solution stability was performed by comparing the results of fresh sample with the stored samples at different time intervals. The difference between the values should be not more than 0.2%. The solution is found stable up to 24 hours at 10°C.

#### **CONCLUSION**

Literature survey and article review reveals that there is no reported method for separation of all the impurities of Albendazole. Especially there is no method is available for separation of impurity B and impurity C of Albendazole. The aim of this proposed method is to provide stability indicating HPLC method for separation of Albendazole and its impurities from each other. The proposed method is selective, accurate and precise. This simple method can be adopted easily for routine and stability study of samples in pharmaceutical formulations and bulk drugs.

#### **ACKNOWLEDGEMENT**

Authors wish to thank the Department of pharmaceutical chemistry, Y.B.Chavan College of Pharmacy, Aurangabad, Maharashtra, India for supporting for this research.

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#### **Cite this article as:**

Anna Pratima G. Nikalje and Ramesh Gadikar. Simultaneous estimation of albendazole and its impurities in pharmaceuticals by liquid chromatography method. *Int. Res. J. Pharm.* 2019;10(3):144-147 <http://dx.doi.org/10.7897/2230-8407.100393>

Source of support: Nil, Conflict of interest: None Declared

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