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Development and Validation of a UPLC Method for the Determination of Albendazole Residues on Pharmaceutical Manufacturing Equipment Surfaces

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***Abstract*—**In Pharmaceutical industries, it is very important to remove drug residues from the equipment and areas used. The cleaning procedure must be validated, so special attention must be devoted to the methods used for analysis of trace amounts of drugs. A rapid, sensitive and specific reverse phase ultra performance liquid chromatographic (UPLC) method was developed for the quantitative determination of Albendazole in cleaning validation swab samples. The method was validated using an ACQUITY HSS C_{18}, 50 x 2.1mm, 1.8µ column with a isocratic mobile phase containing a mixture of 1.36g of Potassium dihydrogenphosphate in 1000mL MilliQ water, 2mL of triethylamine and pH adjusted to 2.3 ± 0.05 with ortho-phosphoric acid, Acetonitrile and Methanol (50:40:10 v/v). The flow rate of the mobile phase was 0.5 mL min^{-1} with a column temperature of 35^0C and detection wavelength at 254nm using PDA detector. The injection volume was 2µl. Cotton swabs, moisten with acetonitrile were used to remove any residue of drug from stainless steel, teflon, rubber and silicon plates which mimic the production equipment surface and the mean extraction-recovery was found to be 91.8. The selected chromatographic condition was found to effectively elute Albendazole with retention time of 0.67min. The proposed method was found to be linear over the range of 0.2 to 150µg/mL and correlation coefficient obtained is 0.9992. The proposed method was found to be accurate, precise, reproducible and specific and it can also be used for routine quality control analysis of these drugs in biological samples either alone or in combined pharmaceutical dosage forms.

***Keywords*—**Cleaning validation, Albendazole, residues, swab analysis, UPLC.

I. Introduction

MEDICINES are primarily intended to promote good health; however, when residual compounds remain in the manufacturing process, potential for side effects from toxic levels of contaminants increases. Cross-contamination with extraneous residues of any kind presents a safety risk to patients consuming any drug product. For this reason, the FDA has recognized, with greater importance, that effective cleaning and sanitizing protocols are a proactive measure in preventing cross-contamination in pharmaceutical and cosmetic production.

As 21 CFR sect 211.67 states [1], "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements." The objective of the cleaning validation [2] is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives, excipients and/or cleaning agents so that the analytical monitoring may be reduced to a minimum in the routine phase.

Albendazole is chemically described as Methyl [5-(proplylsulphanyl)-1H-benzimidazol-2-yl] carbamate (Fig. 1). Literature survey reveals various analytical methods for the determination of Albendazole by HPLC [3]-[6].

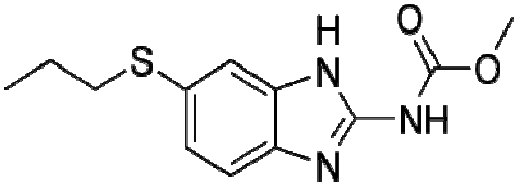

Fig. 1 Albendazole

II. Materials and Methods

A. Chromatographic Conditions

WATERS Acquity UPLC with PDA detector and Empower 2.0 software was employed for present study. The chromatography determination performed by using ACQUITY HSS C18, 50 x 2.1mm, 1.8µ column with a isocratic mobile phase containing a mixture of 1.36g of Potassium dihydrogenphosphate in 1000mL MilliQ water, 2mL of triethylamine and pH adjusted to 2.3 ± 0.05 with ortho-phosphoric acid, Acetonitrile and Methanol (50:40:10 v/v). The flow rate of the mobile phase was 0.5mL min-1 with a column temperature of 35^0C and detection wavelength at 254nm using PDA detector. The injection volume was 2µl.

B. Preparation of Stock Solutions

1. Preparation of Standard Solution

The 1000 µg/ml of Albendazole were prepared using Acetonitrile. The subsequent dilutions of this solution were

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made with Acetonitrile to get concentration range of 0.2 to 150µg/mL. The standard calibration curve for Albendazole (Fig. 4) are constructed with the series of working standards taking the concentration on X-axis and their respective peak areas on Y- axis.

2. Preparation of Sample Solution

The Cleaned swab was taken and sampling was done according to sampling procedure on the surface of the equipment where sampling has to be done (Fig. 2). The sampled swab was dipped into 10 ml of diluent and was sonicated for about 5 minutes. The solution was filtered through 0.2µm Nylon filter and the sample solution was analyzed using HPLC. The Standard chromatogram of Albendazole was shown in Fig. 3.

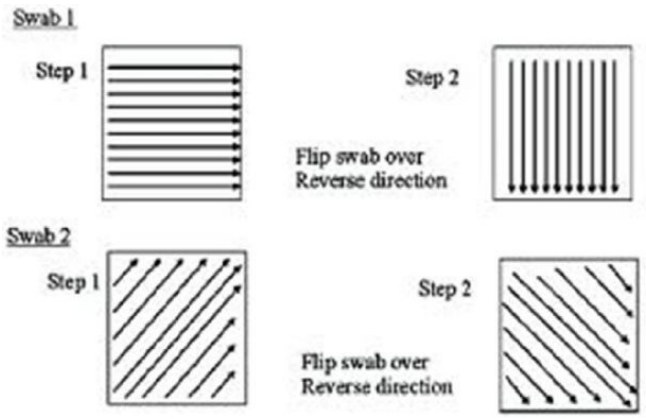

Fig. 2 Structure of Swabbing Pattern

3. Method Validation

The method was validated in accordance with USP & International Conference of Harmonization (ICH) Guidelines [7].

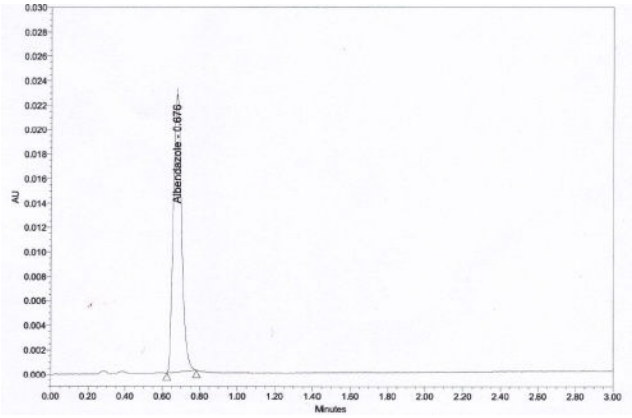

Fig. 3 Standard chromatogram of Albendazole

III. Results and Discussion

A. System Suitability

System suitability tests are used to verify the reproducibility of the chromatographic system.

TABLE I
System Suitability

Sl. No.	System Suitability Parameter	Observations	Proposed Acceptance Criteria
1	% RSD for six replicate injections of analyte peak in standard solution	0.5	Should be not more than 5.0%
2	Tailing factor for analyte peak in standard solution	1.2	Should be not more than 2.0
3	USP plate count for analyte peak in standard solution	1214	Should be not less than 1000

B. Limit of Detection and Limit of Quantification

TABLE II
Peak Results for Limit of Detection and Limit of Quantification

Concentration	Area response
0.05	473
0.1	637
0.2	1274
0.3	1837
0.4	2573
0.5	3427
0.6	4279
0.7	5014
0.8	5627
1.0	7194
Slope	6571.27
RSD	126.31
LOD	0.06
LOQ	0.19

C. Linearity and Range

The method was found to be linear in the concentration range of 0.2µg/ml to 150µg/ml and Correlation coefficient obtained is 0.9992.

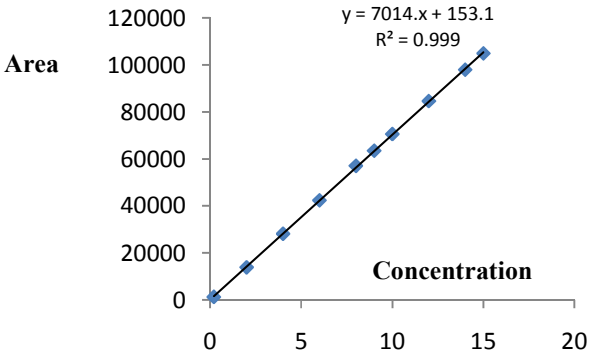

Fig. 4 Calibration graph of Albendazole

TABLE III
PEAK RESULTS FOR LINEARITY

Linearity level Conc. (%)	Area Response
LOQ	1274
20	13860
40	28125
60	42345
80	57045
90	63482
100	70556
120	84583
140	97945
150	104852

D. Precision at Lower and Higher Concentrations

This was carried out to check reproducibility of results at lower level and higher levels of linearity.

TABLE IV
PEAK RESULTS FOR PRECISION AT LOWER AND HIGHER CONCENTRATIONS

Sl. No.	Area Responses	
	Lower level (LOQ)	Higher level (150%)
1	1104	104852
2	1034	103503
3	1113	104282
4	1062	106038
5	1082	103738
6	1192	104292
Average	1097	104450
SD	54.30	909.9
% RSD	4.9	0.8

E. Precision

1.System Precision

TABLE V
PEAK RESULTS FOR SYSTEM PRECISION

Injection No	Area Response
1	70458
2	70748
3	69703
4	70395
5	70030
6	70194
7	68028
8	69309
9	69308
10	70295
Average	69847
SD	791.7
% RSD	1.2

2. Method Precision

Method precision indicates whether a method is giving consistent results for a single material.

TABLE VI
PEAK RESULTS FOR METHOD PRECISION

Spl. No.	Inj.	RT (min)	Area
1	1	0.70	69586
	2	0.70	69384
2	1	0.71	70194
	2	0.72	69930
3	1	0.70	69294
	2	0.70	69248
4	1	0.69	68295
	2	0.71	68943
5	1	0.71	69038
	2	0.72	68327
6	1	0.70	67285
	2	0.71	67194
	Mean		68893.6
	SD		959.8
	%RSD		1.4

F. Specificity

On the basis of these chromatograms we can say that there is no interference of blank swab (Fig. 5) and placebo (Fig. 6).

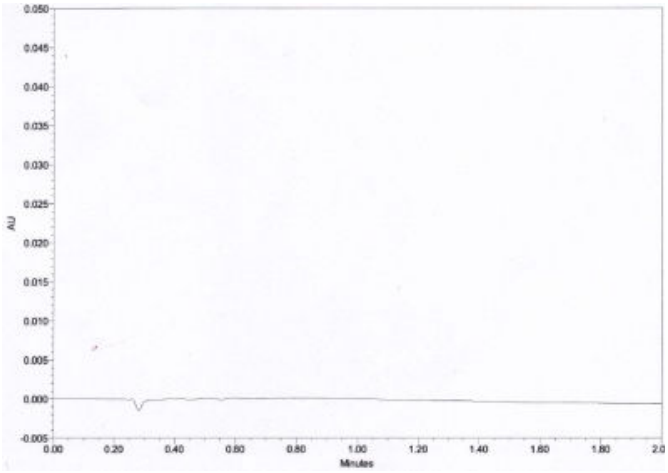

Fig. 5 Chromatogram of blank

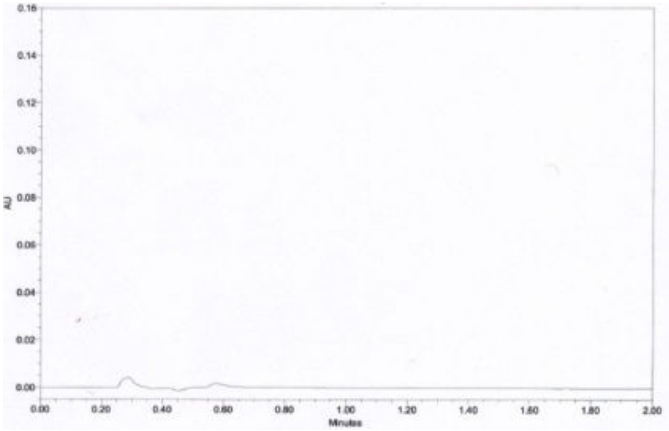

Fig. 6 Chromatogram of placebo

G. Ruggedness

Ruggedness is a measure of reproducibility of test results

under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst.

TABLE VII
RUGGEDNESS RESULTS

Sr. No.	Albendazole in ppm			
	SET I	SET II	SET III	SET IV
1	9.8	9.9	10.0	9.9
2	9.9	10.1	9.9	9.9
3	9.8	10.1	9.9	9.8
4	9.9	10.0	10.1	10.0
5	9.9	9.9	10.1	9.9
6	9.9	9.9	10.0	10.1
Average	9.9	9.9	10	9.9
SD	0.05	0.09	0.08	0.10
% RSD	0.5	0.9	0.9	1.0
Overall Average			9.9	
Overall % RSD			0.8	

SET – I : Method precision
SET – II : Variability due to HPLC system
SET – III : Variability due to HPLC column
SET – IV: Variability due to analyst

H. Accuracy

TABLE VIII
RECOVERY RESULT OF ALBENDAZOLE

Recovery level	% Recovery of Albendazole					
	Stainless steel plate	Teflon plate	Rubber plate	Silicon plate	Average recovery (Particular level)	% RSD (Particular level)
LOQ	86.4	92.6	88.5	86.9	88.6	3.17
50%	90.2	92.2	90.4	93.4	91.5	1.66
100%	90.7	96.2	93.6	87.1	92.4	3.4
150%	89.3	95.4	90.2	94.2	92.3	3.2
Average Recovery	89.15	94.1	90.67	90.4	Overall Recovery 91.2	
% RSD	2.15	2.12	2.34	4.35	Overall RSD 2.8	

I. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

TABLE IX
PEAK RESULTS FOR ROBUSTNESS

Parameter	Condition	USP tailing	USP Plate Count
Flow rate by ± 10%	0.4 ml	1.6	1245
	0.5 ml	1.2	1214
	0.6 ml	1.2	1175
Column Oven temperature by ± 5°C	30°C	1.3	1032
	35°C	1.2	1214
	40°C	1.2	1474
pH of Buffer solution by ± 0.2 units	2.1	1.1	1495
	2.3	1.2	1214
	2.5	1.7	954
Wavelength of analysis ± 5nm	- nm	1.5	1204
	* nm	1.2	1214
	+ nm	1.2	1213
Organic composition of mobile phase by ± 5%	525:385:90	1.4	896
	500:400:100	1.2	1214
	475:415:110	1.1	1423

*Wavelength selected for specific drug

J. Solution Stability

The standard and sample solutions of Albendazole were found to be stable for 36 hrs and no additional peak was observed in the solution.

TABLE X
SOLUTION STABILITY FOR STANDARD AND SAMPLE SOLUTION

	Standard solution				Sample solution			
Time (Hrs.)	Inj.1	Inj.2	Average area	% Difference	Inj.1	Inj.2	Average area	% Difference
Initial	69765	69675	69720	NA	67884	67452	67668	NA
6hrs	70175	70532	70354	0.9	68395	68792	68594	1.4
12hrs	70456	70246	70351	0.9	68030	68146	68088	0.6
20hrs	70987	70257	70622	1.3	68304	68394	68349	1.0
26hrs	70975	70794	70885	1.7	68013	68359	68186	0.8
30hrs	71492	70137	70815	1.6	68363	68624	68494	1.2
36hrs	70345	71359	70852	1.6	68674	68954	68814	1.7

K. Filter Interference

TABLE XI
FILTER INTERFERENCE FOR STANDARD AND SAMPLE SOLUTION

For Standard			For Sample		
Filtration Method	Unfiltered	0.2µm SY25NN	Filtration Method	Centrifuged	0.2µm SY25NN
Area (Inj. 1)	70839	70193	Area (Inj. 1)	68959	68795
Area (Inj. 2)	71385	70493	Area (Inj. 2)	69894	68498
Avg. Area	71112	70343	Avg. Area	69427	68647
% Difference		1.1	% Difference		1.1

IV. CONCLUSION

Although various methods have been reported for the estimation of Albendazole it was found that the methods proposed were time taking and results in delay of batch clearance for the next production batch to start. The cleaning validation procedures for Albendazole with UPLC were not reported. Hence an attempt has been made to develop simple and accurate methods for the estimation of Albendazole by UPLC.

Results of analysis of the swab samples revealed that the proposed method is suitable for their analysis with no interference from the swab and recovery is found to be acceptable. The method was found to be linear, precise, accurate, specific and all proved to be sensitive, convenient and effective for the determination of Albendazole in swab samples.

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