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Gradient RP-HPLC method development and validation for simultaneous estimation of paclitaxel and albendazole

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***Abstract*---**The goal of this research is to provide an accurate and exact technique for estimating Albendazole and Paclitaxel simultaneously utilizing gradient RP-HPLC (high performance liquid chromatography). A UV detector fixed at 230 nm was used to estimate albendazole and paclitaxel simultaneously. As the solvent system, acetonitrile and water in the ratio of 50:50 was used in the presence of 0.1 % trifluoroacetic acid. Mobile phase was run on a Inertsil ODS 3V 150 4.6 mm or equivalent column in Gradient mode at a flow rate of 1.0 mL/min. By this method albendazole and paclitaxel showed 6.199 and 9.684 retention time (Rt) respectively with continuous run upto 25 min. In the prescribed concentration range, the calibration curves for each analyte were determined to be linear (r2 > 0.994). The percentage of recovery was found to be within the limit at each level (98.0 %to 102.0 %). All validation parameters were determined to be acceptable, including system precision, method precision, linearity, range, accuracy, ruggedness, robustness, solution, and mobile phase stability findings. This suggests that the HPLC simultaneous approach for determining albendazole and paclitaxel assays is accurate. Several analytical parameters, including linearity, accuracy, precision, specificity, limit of quantification, limit of detection, robustness, ruggedness, solution stability, and mobile phase stability, were assessed using the ICH criteria, and all results were within the range.

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Keywords---albendazole, paclitaxel, simultaneous estimation, gradient HPLC.

Introduction

A combination drug treatment is always favored over single-drug therapy for cancer treatment because they act by different mechanism on targeted sites and thus overcome drug resistance.[1] Dual drug loaded drug delivery system enhanced synergistic & additives effect of drugs as well as reduced toxicity.[2] Paclitaxel (PTX) is an FDA approved anticancer drug which is widely used for treating different types of tumor cells.[3] Paclitaxel is the most successful anti-neoplastic drug and is primarily used in the cancer like ovarian, breast & lung. In addition to treating malignancies of the breast and ovary, it is also used to treat Kaposi's sarcoma, which is a malignancy associated with HIV.[4] It is BCS class-IV drug. Albendazole is basically anti-helmintic drug derived from benzimidazole family.[5] The mechanism of laction of albendazole (ABZ) is related to microtubule inhibition and preventing glucose absorption, which causes depletion of glycogen reserves and decreases ATP generation in sensitive parasites during the larval and adult stages.[6] Albendazole is also reported a potent inhibitor of VEGF and hypoxia-inducible factor 1-α. An increase in hypoxia-inducible factor 1-α in tumor growth also increased the expression of VEGF in the tumor site.[7] As this combination of drugs is successfully used in cancer therapy, there is a need to establish some unique method for estimating albendazole and paclitaxel. Despite the fact that these two medications are more effective when used jointly in cancer therapy, no High performance liquid chromatography (HPLC) technique innovations for these drugs have been revealed. The goal of this research is to create a cost-effective RP-HPLC method for simultaneous estimation of ABZ and PTX that uses less organic solvent. The current study provides a particular, simple, sensitive, reproducible, and cost-effective gradient HPLC approach for simultaneous estimation of paclitaxel and albendazole molecular structure shown in Figure 1.

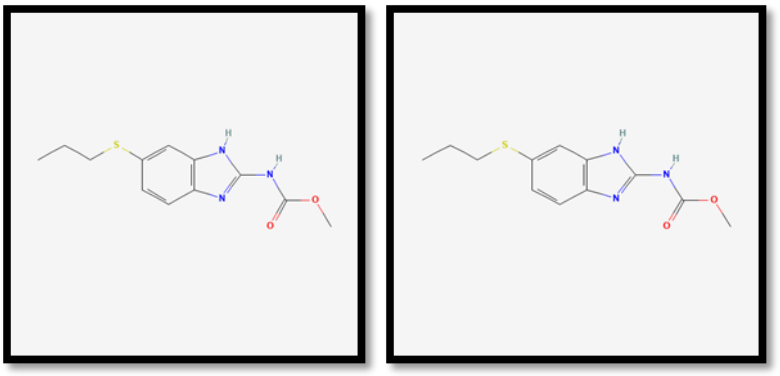

Figure 1. Chemical structure of (a) Albendazole (b) Paclitaxel

Materials and Methods

Materials

Cipla Pvt Ltd provided a gift sample of Paclitaxel; Albendazole was recieved from Sequent Pharma, Mumbai as a gift sample. HPLC grade water and acetonitrile were procured from Merck pharmaceuticals. All chemicals used were of AR grade.

Methods
Instrumentation

A Shimadzu HPLC model prominence LC-2030C 3D system was used for the analysis. For method development and data processing, a High Performance Liquid Chromatography system with quaternary gradient pumps, a variable wavelength PDI detector connected to a data recorder, and integrator software was employed. The samples were separated using a Inertsil ODS 3V 150 X 4.6 mm or equivalent column at a wavelength of 230 nm. The temperature of the column was maintained at 40 °C, the injection volume was 10 ul, and the flow rate was maintained at 1.0 mL/min. The total duration was 25 minutes. Water:Acetonitrile were used as diluents with 0.1% TFA. For preparation of mobile phase Milli Q water, Trifluroacetic acid (AR Grade) & HPLC grade Acetonitrile were used. The Mobile phase was filtered by using 0.45 µm Millipore nylon filter paper and degassed by sonication before used. All measurements were taken at room temperature.

Preparation of Mobile phase

Mobile phase A (0.1 % TFA + water) was prepared by dissolving 100 ml TFA in the 1000 mL measuring cylinder and 500 mL of Milli-Q Water. Final volume was made up with Milli-Q Water then filtered and degassed through 0.45 µ membrane filter paper. Acetonitrile was used as mobile phase B. Table 1 showed gradient program set for method development.

Table I
Gradient programs

Time (min.)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0.01	80	20
10.0	10	90
20.0	80	20
25.0	80	20

Preparation of Standard Solution
Preparation of PTX & ABZ stock solution

Weighed accurately 25 mg of PTX, as reference standard and transferred to 50 ml volumetric flask and dissolved it in 30 ml of mobile phase. After mixing, volume was made up 50 ml with mobile phase. Final concentration of stock solution was 500 ppm.

Preparation of solution mixture

Standard or stock solutions mixture was prepared by dissolving equivalent amount of drug as in the formulation in ratio 1:1 of PTX and ABZ.

Validation of analytical method

This approach was verified in accordance with USP and ICH requirements. In this study, performed all the validation parameters as per ICH guidelines like range, accuracy, linearity, precision, specificity, sensitivity (LOD & LOQ), robustness and ruggedness and all the results was found within a specified range.

Precision

When a technique is repeated on many samples, the degree of agreement among individual test findings is called precision.

System and method Precision (repeatability)

Repeated evaluations of standard and sample solution were taken independently to determine system and method precision (repeatability). The same day, five injections of the standard were used to determine system & method precision. The % RSD for the major peak area was obtained after injecting the blank and the Standard Solution in five repetitions.

Linearity & range

The linearity of the technique employed to test Albendazole and Paclitaxel was evaluated using the standard curve of detector response (peak area) versus analyte concentration. The range of an analytical technique is the range of analyte concentrations in the sample between the upper and lower bounds. [8] Linearity is established throughout the procedure's range. A series of sample preparations of ABZ and PTX was prepared at 25, 50, 75, 100, 125 and 150 ppm with respect to the target test concentration of ABZ and PTX. Each solution was injected in triplicate and calculated for correlation coefficient (r^2) of the sample.

Accuracy

The degree of agreement between the values regarded as conventional true values or an accepted reference value and the value discovered is defined as the accuracy of an analytical procedure.[9] A series of ABZ and PTX sample preparations were made at 50%, 100%, and 150% of the target test concentration and injected in triplicate into the HPLC using the standard assay protocol.

Robustness and Ruggedness

A robustness test is an experimental setting used to evaluate the robustness of a method. Robustness is described as the ability to duplicate the (analytical) procedure in numerous laboratories or under different conditions without unexpected changes in the acquired result(s). Ruggedness is defined as the degree

of repeatability of test results obtained in a variety of regular test circumstances, such as different laboratories, analysts, equipment, lots of reagent, elapsed assay times, assay temperatures, days, and so on. The method's ruggedness/robustness was tested by purposefully changing the following parameters: mobile phase composition, mobile phase flow rate, injection volume, column temperature, and detector wavelength which are described previously.[10] The parameters of chromatographic separation [retention time, RRT, resolution, and number of plates] should not alter.

Mobile phase stability

The both drugs albendazole and paclitaxel were weighed in 1:1 ratio to check mobile phase stability at different time intervals 6, 12, 24 and 48 h.

Results and Discussion

Chromatographic Separation

Chromatographic separation was done by using RP-HPLC gradient mode. Table 1 shown gradient program was used for both drugs elution. Nonetheless, the drugs were eluted in 25 minutes; the run was continued for 6 minutes to ensure complete elimination of drug residues from the column and re-equilibration of the system to starting conditions. Figure 2 depicts the entire chromatogram obtained during 25 minutes, which displays the peaks of both drugs. Albendazole had a retention time of 6.199 minutes, which was considerably separated from the peak of Paclitaxel, which had a retention period of 9.684 minutes.

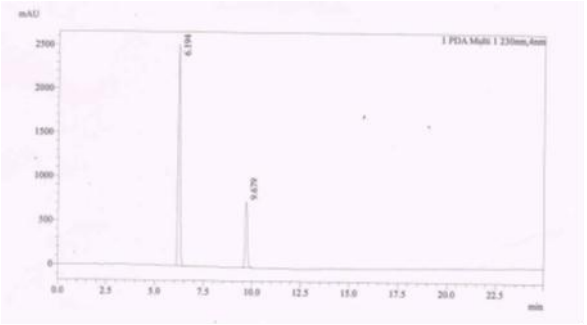

Figure 2. Chromatograph of Albendazole and Paclitaxel showing retention time at 6.194 and 9.679 respectively

Precision

The % RSD for all five replicate injections should not be more than 2.0 %. The theoretical plates from standard injections are not less than 5000, and the tailing factor is not greater than 2.0. The % RSD for the primary peak region following five injections of Standard Solution should not exceed 2.0. Table 2 shown all the parameters of system precision meet acceptance criteria and observed % RSD are in limit, hence the system is suitable.

Table 2
System precision

Standard Solution		
Sr.No	Area of ABZ	Area of PTX
Inj.1	16596783	5751765
Inj.2	16649591	5767483
Inj.3	16617467	5784546
Inj.4	16925523	5801658
Inj.5	16961001	5769000
Avg.	16750073	5774890.4
SD	177800.54	18934.661
%RSD	1.06	0.33

Method Precision

When you inject six preparation of same batch sample in to your evaluated system, obtained six results should be precise. Then the method used for quantification is correct. Simply the method precision is one of the testing parameter to check the method used for the test is reproducible of results or not. The % RSD for the assay content in six sample solutions should not be more than 2.0 %. Table 3 shown the % RSD for the assay content in six sample solutions is found to be less than 2. Hence the method for the determination of ABZ and PTX assay is precise.

Table 3
Method precision

Sample prep.	ABZ wt.(mg)	PTX wt.(mg)	Sample Area ABZ	Sample Area PTX	% Assay ABZ	% Assay PTX
Sample prep 1	25.1	25.1	16637889	5721763	99.1	99.4
Sample prep 2	25.3	25.3	17029578	5747484	98.4	98.8
Sample prep 3	25.1	25.1	17029578	5774548	98.7	98.6
Sample prep 4	24.72	25.41	16921261	5801619	99.3	99.1
Sample prep 5	24.72	25.37	16664355	5768546	98.5	98.7
Sample prep 6	24.72	25.62	17059572	5772846	98.9	99.3
	Mean				99	98.9
	SD				0.4	0.3
	%RSD				0.4	0.3

Linearity & range

Table 4 shown a series of sample preparations of ABZ and PTX was prepared at 25, 50, 75, 100, 125 and 150 ppm with respect to the target test concentration of ABZ and PTX. The samples were injected in triplicate and correlation coefficient for the sample was calculated. Correlation coefficient should not be less than 0.99. Correlation coefficient is 0.99**.** This shows that the HPLC procedure for determining ABZ and PTX assays is linear as shown in Figure 3.

Table 4
Linearity

Concentration of ABZ and PTX (ppm)	Area of ABZ	Area of PTX	Avg. Area of ABZ	Avg. Area of PTX
	1514783	578750		
50	1514787	578762	1514783	578750
	1514779	578638		
	2167361	865054		
75	2167385	865066	2175021	868125
	2177339	865078		
	2900028	1101110		
100	2900056	1151118	2900028	1157500
	2900916	1151828		
	3688040	1387268		
125	3588018	1387268	3625035	1346875
	3688058	1387262		
	4349009	1744848		
150	4349009	1744844	4350042	1736250
	4349009	1744840		
Correlation coefficient			0.99	0.99

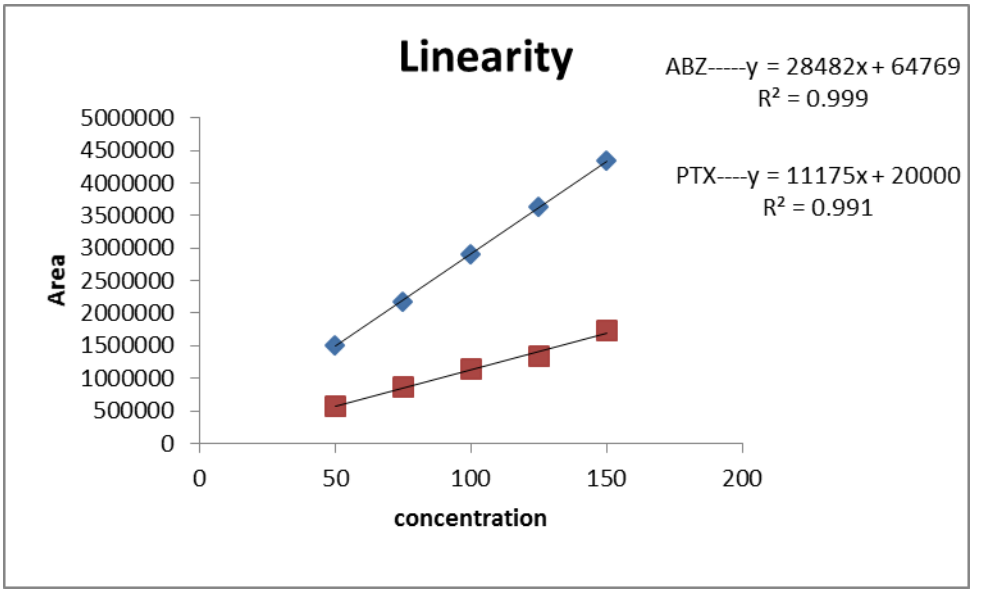

Figure 3. Calibration curves of ABZ & PTX

Accuracy

The % recovery should not be less than 98.0 and should not be more than 102.0. The % recovery at each level found within the limit (98.0% to 102.0%). As per shown in Table 5, good recoveries were obtained at various concentrations of the

ABZ (98.6 to 99.7) and PTX (98.6 to 99.7). This indicates that the HPLC method for the determination of assay of ABZ and PTX is Accurate.

Table 5
Accuracy of Paclitaxel and Albendazole

Sample prep.	Avg. Area	Amount Added	Amount Recovered	% Recovery	Avg. Recovery
Accuracy of Albendazole					
Control-1	16617452	25.32	24.47	-	
Control -2	16652147	25.12	24.48	-	-
Control- 3	16654692	25.21	24.50	-	
At 50% level -1	8462761	12.56	12.47	99.3	
At 50% level -2	8426594	12.68	12.51	98.6	99.5
At 50% level -3	8435416	13.26	13.35	100.7	
At 100% level -1	16925553	25.32	24.92	98.4	
At 100% level -2	16926528	25.26	24.99	98.9	98.6
At 100% level -3	16925896	25.32	24.92	98.4	
At 150% level -1	25388284	37.52	37.30	99.4	
At 150% level -2	25385692	37.35	37.27	99.8	99.7
At 150% level -3	25384625	37.46	37.39	99.8	
Accuracy of Paclitaxel					
Control-1	5784536	25.16	24.42	-	
Control -2	16652254	25.15	24.38	-	-
Control- 3	16654628	25.21	24.06	-	
At 50% level -1	8462654	12.34	12.22	99.3	
At 50% level -2	8426238	12.52	12.48	98.6	99.5
At 50% level -3	8435416	13.19	13.36	100.7	
At 100% level -1	16925526	25.22	24.86	98.4	
At 100% level -2	16925622	25.35	24.95	98.9	98.6
At 100% level -3	16924698	25.17	24.87	98.4	
At 150% level -1	25388249	37.32	37.29	99.4	99.7

At 150% level -2	25385435	37.28	37.25	99.8
At 150% level -3	25384752	37.17	37.29	99.8

Robustness

At each variable condition, system suitability should be fulfilled according to the test procedure. The assay content should be no less than 98.0 % and not greater than 102.0 %. The difference between the assay results obtained under control and variable circumstances should not be more than 1.0 %. The findings show that the test technique is robust for all of the variable circumstances like change in mobile phase composition, pH, flow rate and column temperature shown in Table 6.

Table 6
Robustness study (comparison of all parameters)

Description		%Assay of precision		%Assay		Difference in Assay	
	Sample	ABZ	PTX	ABZ	PTX	ABZ	PTX
+5% Mobile phase	1	99.9	99.9	99.1	99.2	0.8	0.1
	2	98.3	98.5	98.4	98.5	0.1	0.0
-5% Mobile phase	1	99.2	99.2	99.1	99.3	0.1	0.1
	2	98.4	98.6	98.4	98.5	0	0.1
+0.2% pH	1	99.1	99.2	99.1	99.3	0	0.1
	2	98.7	98.5	98.4	98.6	0.3	0.1
-0.2% pH	1	99.1	99.2	99.3	99.1	0.2	0.1
	2	98.4	98.2	98.7	98.5	0.3	0.2
1.1ml/min	1	99.6	99.2	99.1	99.2	0.5	0
	2	98.4	98.5	98.9	98.5	0.5	0
0.9ml/min	1	99.2	99.2	99.1	99.2	0.1	0
	2	98.4	98.1	98.6	98.5	0.2	0.1
Column Temp. 40 °C	1	99.3	99.2	99.3	99.1	0	0.1
	2	98.4	98.2	98.4	98.7	0	0.5
Column Temp. 50 °C	1	99.3	99.2	99.7	99.1	0.4	0.1
	2	98.4	98.5	98.4	98.6	0	0.1

Ruggedness

At each variable condition, system suitability should be fulfilled according to the test procedure. The assay content should not be less than 98.0% and should not be more than 102.0 %. The difference between the assay results should not be more than 1.0% for the results obtained at control and variable conditions. Table 7 shown the results meet all the acceptance criteria and indicate that the test

method is rugged for all variable conditions like different column, different system and different analyst considered for ruggedness study.

Table 7
Ruggedness comparison table

Description		Assay (%) (Precision Study)		Assay (%)		Difference in Assay	
Different Column		% Assay PTX	% Assay ABZ	% Assay PTX	% Assay ABZ	PTX	ABZ
	CL/08/F02	99.1	99.5	99.4	99.2	0.3	0.3
	CL/08J05	98.5	98.6	98.8	98.4	0.3	0.2
Different System		% Assay PTX	% Assay ABZ	% Assay PTX	% Assay ABZ	PTX	ABZ
	System ID: QC-HPLC-l04	99.1	99.2	99.4	99.1	0.3	0.1
	System ID: QC-HPLC-l03	98.6	98.7	98.8	98.4	0.2	0.3
Different Analyst		% Assay PTX	% Assay ABZ	% Assay PTX	% Assay ABZ	PTX	ABZ
	Aruna	99.1	99.2	99.4	99.1	0.3	0.1
	Udayasri	98.4	98.5	98.8	98.4	0.4	0.1

Mobile Phase Stability

The Acetonitrile and water mobile phase was selected on the basis of data obtained by UV spectral analysis and solubility of drugs**.** Table 8 shown the study of mobile phase stability.

Table 8
Mobile phase stability

Duration		Sample Area	Avg.Area	Assay (as such)
M.P Stability-6hr	ABZ	2727592	2724778	96.4
	PTX	2721961		96.2
M.P Stability-12hr	ABZ	2723963	2731109	96.3
	PTX	2738256		96.4
M.P Stability-24hr	ABZ	2762973	2758559	96.3
	PTX	2754143		96.5
M.P	ABZ	2759096	2763168	96.8

Stability-48hr	PTX	2767245		96.1

Conclusion

A method for simultaneous estimation of albendazole and paclitaxel was developed and validated. The results of various validation parameters were within the range. Different subjected conditions and slight chromatographic changes had no effect on the stability and repeatability of the established procedure. Based on the results, the developed RP-HPLC approach is appropriate for simultaneously estimating the Assay of ABZ and PTX.

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