Research Article

Development and Validation of a Novel HPLC Method for the Determination of Docetaxel in Pharmaceutical Dosage Forms

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ABSTRACT

Objective: To develop a quick, exact, linear, focused, and accurate determination of active content by the RP-HPLC method and simultaneously validate the assay of Docetaxel in bulk drug and pharmaceutical dosage form products. The developed method was validated per the ICH Guidelines Q2(R1).

Methodology: The Chromatographic condition was achieved using 15 cm x 4.6 mm, C18(5µm) with a flow rate of 1.5 mL/min, and an isocratic mobile phase prepared using Acetonitrile and Water with the ratio of 45% and 55%, respectively. The chromatographic peak was observed at a wavelength of UV 232nm.

Results: The developed method was validated in terms of Specificity, Interference Study, Solution Stability, Linearity and Range, Precision, Repeatability, Intermediate Precision, Accuracy, Robustness and System Suitability. The RT of the Docetaxel was found at 9.76 mins with a run time of 20 mins, and the linearity range determination was done from 100 PPM to 300 PPM. The correlation coefficient was found to be 1.0. The method's accuracy was found between 99.70% to 100.56%. All parameter limits were found to be within the limit concerning ICH Guidelines Q2(R1).

Conclusion: The developed method is simple, accurate and precise for estimating Docetaxel in bulk drug and pharmaceutical dosage forms that can be used in any pharmaceutical company as a part of an in-process clearance check.

Keywords: Docetaxel, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Docetaxel is a taxoid drug that has anti-cancer properties. It is made using a semi-synthetic process that starts with a precursor obtained from the biomass of renewable yew needles. Both anhydrous and trihydrate forms of Docetaxel are sold in the market. Aventis Pharmaceuticals created Docetaxel, also known by the brand name Taxotere, to treat a particular type of cancer. The medication is currently authorised in 90 nations for treating advanced lung cancer and 70 countries for treating advanced non-small cell lung cancer.

All drugs must undergo stability testing using an assay method in accordance with current good manufacturing practices before being released. Developing a straightforward, accurate, and precise HPLC method for the simultaneous determination of Docetaxel is, therefore, worthwhile. Thus, an RP-HPLC method was created in the current study and used for the simultaneous determination of Docetaxel and drug assay.

The objective of the current study was to develop a docetaxel RP-HPLC assay method that could ease the approval of batch manufacture of dosage forms in industries. The developed RP-HPLC assay method was validated according to ICH Q2(R1) guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Docetaxel API was a gift sourced from Cipla Ltd., Bengaluru, Karnataka, India. Chemicals like Acetonitrile, glacial Acetic Acid and Methanol were purchased from Rankem.

Instrument and Conditions

All the analytical Studies were performed using HPLC Infinity II series 1260 (Make: Agilent Technologies) driven by openlab chemstation 2.1 software, and Stainless steel column was used with the size of 15 cm x 4.6 mm, packed with Octadecylsilane (5 µm) (Mfg. by Shimadzu). HPLC used is Agilent.

Solution Preparation

Preparation of Diluent

100 mL of Acetonitrile was added to 100 mL of Water along with 0.1 mL glacial Acetic Acid.

Preparation of Mobile Phase

Acetonitrile : Water :: 45% : 55%.

Preparation of Standard Solution

Accurately 20 mg sample of Docetaxel working standard was weighed into 100 mL clean & dry volumetric flask,

about 5 mL of methanol was added and sonication was done for 2 min., allowing equilibrating to room temperature and diluting to volume with above diluent & mixed.

Preparation of Placebo Solution

Weight equivalent to 1 mL placebo was added into 100 mL clean & dry volumetric flask, about 5 mL of Methanol was added and sonication was done for 5 min., allowing equilibrating to room temperature and diluting to volume with above diluent, mixed and filtered through 0.45µn nylon syringe filter.

Preparation of Sample Solution

Weight equivalent to 1 mL of Docetaxel Injection 20 mg/mL, which is equivalent to 20 mg of Docetaxel, was added into 100 mL clean & dry volumetric flask, about 5 mL of Methanol was added and sonication was done for 5 min. allowing equilibrating to room temperature and diluting to volume with above diluent, mixed and further filtered through 0.45µn nylon syringe filter.

Chromatographic Conditions

Docetaxel was analysed using the HPLC system HPLC Infinity II series 1260 (Make: Agilent Technologies) driven by openlab chemstation 2.1 software. Separation was carried out using a stainless steel column of 15 cm x 4.6 mm, packed with Octadecylsilane (5 µm) (Mfg. Shimadzu). Chromatographic separation was monitored at UV 232 nm. The chromatographic run time was finalized at 20 min. The chromatographic condition for pump mode, flow rate, Injection volume, column temperature and sample temperature were mainitained at Isocratic, 1.5 mL/min, 20 µL, 25 ºC and 10ºC, respectively.

VALIDATION METHOD BY RP-HPLC:

As per guidelines ICH, Q2(R1), the method developed was validated for determining Specificity, Solution stability, Linearity and Range, Precision and Accuracy.

Specificity

The blank, placebo, sample and standard preparation were prepared as described in the methodology and injected into the HPLC system. The acceptance criteria for validating Specificity were no Peak should be observed due to blank & placebo at the retention time of analyte peak/s, and peak/s of interest should be well resolved from adjacent peak/s (blank and impurity). The peak purity of analyte peak/s in standard and sample determined by PDA detector should be not less than 0.95 (Purity match Value).

Solution Stability

Analytical Solution Stability: Standard and sample preparation were prepared as described in the methodology and stored at 10°C. Analyse the standard and sample solution at periodic intervals up to 36 hrs. The acceptance criteria for validating specificity is for standard Solution: System suitability shall meet as per the acceptance criteria at each time interval. For Sample Solution: Absolute difference of assay value was not more than 2.0 in each time interval.

Mobile Phase Solution Stability: The mobile phase and standard were prepared as described in the methodology and evaluated the system suitability criteria per methodology at pre-defined time intervals of 48 hours. The acceptance criteria for validating specificity is for Mobile Phase Solution Stability: System suitability shall meet the acceptance criteria mentioned in the methodology, and the Mobile phase shall be free from any particulate matter. No turbidity or no opalescence was observed.

Linearity and Range

Linearity: Prepared and analyzed a series of linearity solutions of the stock solution of the primary standard to obtain a solution at 50%, 80%, 100%, 120% and 150% of the target sample concentration.

Range: Six replicates were injected, each of lower and higher concentration levels, calculated the mean and relative standard deviation, recorded the concentration levels over which the results were linear. The acceptance criteria value of % RSD of the peak area should not be more than 2.0.

Precision

Repeatability: **S**ix samples were analysed as per methodology. Blank, standard and sample were prepared as described in the methodology and injected into the HPLC system. Mean, standard deviation, relative standard deviation and 95% confidence interval of the % assay results from the six preparations were determined. The acceptance criteria for validating the repeatability % RSD of six results should not be more than 2.0.

Intermediate Precision: The experiment was performed under repeatability with typical variation including days, analyst, instrument, column (different Lot or make) etc. Blank, standard and sample were prepared as described in the methodology and injected into the HPLC system. The mean, standard deviation, relative standard deviation and 95% confidence interval of the % assay results from the six preparations were determined. The mean, standard deviation, and relative standard deviation were determined % assay results obtained from twelve preparations of repeatability and intermediate precision. Then, the absolute difference in the % assay results obtained in repeatability (mean value of six results) and intermediate precision (mean value of six results) were calculated. The acceptance criteria for validating the Intermediate Precision The % RSD of six results should not exceed 2.0. The absolute difference between the value of the average results obtained in the repeatability and intermediate precision should not be more than 2.0. Overall %RSD of repeatability and intermediate precision results should not be more than 2.0.

Accuracy (Recovery)

Recovery solutions were prepared to obtain the solutions by covering 50% of the lowest and 150% of the highest test concentration of the sample. The recovery solutions at all three concentration levels in triplicate were prepared. The acceptance criteria for validating the accuracy of all the individual recoveries should be within 97.0 to 103.0% and %RSD of the assay at each level and overall levels should not be more than 2.0.

Robustness

A robustness study was performed on standard solutions as per process illustrated in the methodology section by altering the chromatographic conditions.

- Changing the column oven temperature (20°C and 30°C)
- Changing the wavelength of the UV detector (230 nm and 234 nm)
- Changing the flow rate of the mobile phase (1.6 mL and 1.4 mL)

System Suitability

System suitability is performed before performing any validation parameter.

RESULTS AND DISCUSSION

Specificity

Based on the results obtained, it was concluded that no interference observed due to blank solution at the same retention time of the main peak as in the standard solution and sample solution chromatograms. The peak purity value of both the corresponding peaks was 1.0. The obtained results are presented in Table 1.

Table 1: Results of the Interference study

Name of Solution	Retention Time (minute)	Peak Purity
	Docetaxel	Docetaxel
Blank	No interference	NA
Placebo	No interference	NA
Standard Solution	9.787	1.00
Sample Solution	9.764	1.00

Solution Stability

The % relative standard deviation for the standard solution when stored for 36 hours at 10°C was within the acceptance criteria of not more than 2.0. The absolute difference in the assay for sample solution when stored for 24 hours at 10°C was within the acceptance criteria of not more than 2.0. No turbidity, particulate matter and opalescence were observed in a mobile phase when stored for 36 hours at 25°C. Based on below table No. 2, the standard solution can be used up to 36 hours after preparation when stored at 10°C. Likewise, the sample solution can be used up to 36 hours after preparation when stored at 10°C. The mobile phase was also stable for 47 hours after preparation when stored at 25°C. The obtained results are presented in the following Table 2.

Linearity and Range

To obtain concentrations at 50% to 150% of the working samples, a series of solutions were prepared quantitatively by dilution of the main drug standard stock solution. After injecting each solution, the peak area was measured, the slope, Y-intercept and regression correlation were computed. The value of concentration, peak area and corrected concentration are presented in Table No.3. A graph of peak area v/s corrected concentration (PPM) has been plotted in Figure 1.

Table 2: Solution Stability of Docetaxel

Time (hours)	Standard Solution 10°C		Time (hours)	Sample Solution 10°C		
	Area	% RSD		Area	% Assay	Absolute Difference with respect to Initial
Initial	2470.8229	NA	Initial	2605.5894	100.87	NA
03	2472.5647	0.23	06	26033548	100.78	0.09
05	2471.5684	0.23	12	2601.3547	100.70	0.16
08	2463.2560	0.26	18	2559.4658	99.08	1.79
10	2472.2684	0.23	24	2557.4165	99.00	1.86
14	2473.2548	0.23	36	2555.2684	98.92	1.95
15	2463.3854	0.26				
21	2474.2648	0.24				
27	2480.2158	0.28				
36	2483.2188	0.31				
47	2486.0186	0.34				

Table 3: Linearity area of Docetaxel

Level %	Theoretical Concentration (ppm)	Corrected Concentration (ppm)	Area
		Docetaxel	Docetaxel
50%	100.0	100.0	858.928
80%	160.0	160.0	1799.799
100%	200.0	200.0	2476.497
120%	240.0	240.0	3158.532
150%	300.0	300.0	4200.541
Slope			167
Y-intercept			– 850
Correlation Coefficient			1.00
Range: 50% to 150% of working concentration (i.e. 100 ppm to 300 ppm)			

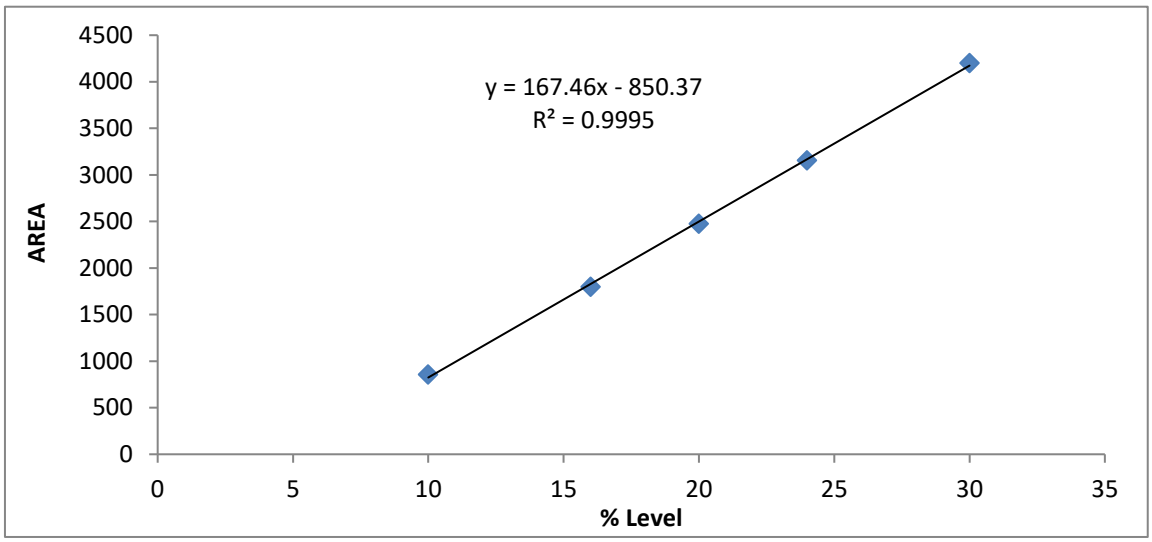

Figure 1: Linearity curve of Docetaxel

Table 4: Range area of Docetaxel

Injection No.	AREA	
	50%	150%
	Docetaxel	Docetaxel
1	858.614	4215.253
2	859.2833	4216.357
3	858.416	4215.713
4	857.4688	4216.683
5	858.2827	4217.198
6	859.7379	4216.72
Mean	**858.6338**	**4216.3210**
% RSD	**0.09**	**0.02**

The correlation coefficient was within acceptance criteria of not less than 0.999. For range relative, standard deviation at lower and higher concentrations were found within the acceptance criteria of not more than 2.0%. Hence it can be concluded that the method as linear in the range of 50 to 150%.

Precision

Repeatability: The relative standard deviation of the assay results for six individual sample preparations was within the acceptance criteria of not more than 2.0%. Therefore, based on the below results in Table 5, it was concluded that the proposed method for assay by HPLC to be precise.

Table 5: Precision of Docetaxel

Sample No.	Weight of Sample (g)	Area	% Assay
		Docetaxel	Docetaxel
1	1.1912	2607.7761	100.71
2	1.1880	2610.0337	101.06
3	1.1780	2608.2634	101.85
4	1.1800	2609.6187	101.73
5	1.1901	2612.7639	101.00
6	1.1813	2608.7083	101.59
Mean Assay			**101.323**
%RSD			**0.46**
95% Confidence Interval			**0.368**

Table 6: Intermediate Precision of Docetaxel

Sample No.	Weight of Sample (g)	Area	% Assay
		Docetaxel	Docetaxel
1	1.1801	2597.1568	101.24
2	1.1894	2604.2542	100.72
3	1.1842	2599.4666	100.98
4	1.1814	2605.2648	101.44
5	1.1856	2601.8493	100.95
6	1.1847	2606.9859	101.23
Mean Assay			**101.093**
%RSD			**0.26**
95% Confidence Interval			**0.208**

Table 7: Comparative results of RSD of Precision

Assay	Sample No.	% Assay
		Docetaxel
Repeatability	1.	100.71
	2.	101.06
	3.	101.85
	4.	101.73
	5.	101.00
	6.	101.59
Intermediate Precision	1.	101.24
	2.	100.72
	3.	100.98
	4.	101.44
	5.	100.95
	6.	101.23
Mean		**101.2083**
%RSD		**0.37**

Intermediate Precision: Different analysts analyzed on a different day, using a different HPLC and column. The obtained results for % assay and overall comparative data are presented in the following Tables 6.

Table 8: Comparative results of Repeatability and Intermediate Precision

Parameters	% Assay
	Docetaxel
Mean Assay in Repeatability	101.323
Mean Assay in Intermediate Precision	101.093
Absolute Difference	0.23

The relative standard deviation of the assay results for six individual sample preparations was within the acceptance criteria of not more than 2.0%. The relative standard deviation of the assay obtained from 12 sample preparations (Repeatability and Intermediate precision) has been within the acceptance criteria of not more than 2.0%. The absolute difference between the mean assay result obtained in repeatability and intermediate precision has been within the acceptance criteria of not more than 2. Based on the above results, it was concluded that the proposed method for Assay by HPLC is rugged.

Accuracy

Recovery solutions were prepared by spiking the drug substances diluent in the concentration level 50%, 80%, 100% and 150% of the working concentration of the sample in triplicate. The % recovery was calculated for each recovery solution, and the mean recovery was determined. The results are presented in the following Table 9. The % recovery at 50%, 80%, 100% and 150% levels were determined to be within the acceptance criteria between 97.0% to 103%. Therefore, based on the below-obtained recovery results, it was concluded that the method for assay by HPLC as accurate.

Table 9: Accuracy results of Docetaxel

Level	API spiked (mg)	Actual Recovered (mg)	% Recovery
	Docetaxel	Docetaxel	Docetaxel
50%	10.03 mg	10.00 mg	99.70
	10.04 mg	10.07 mg	100.29
	10.01 mg	10.03 mg	100.19
80%	16.07 mg	16.09 mg	100.12
	16.03 mg	16.00 mg	99.81
	16.00 mg	16.09 mg	100.56
100%	20.00 mg	20.01 mg	100.05
	20.07 mg	19.99 mg	99.60
	20.03 mg	20.01 mg	99.90
150%	30.01 mg	30.00 mg	99.96
	30.03 mg	30.07 mg	100.13
	30.00 mg	30.00 mg	100.00
Mean Recovery			**100.03**

Robustness

The % relative standard deviation for 5 replicate injections of standard solution, tailing factor, and theoretical plate was not significantly changed with the altered condition. Hence the method has been considered to be robust to the specified changes, i.e. detector wavelength (±2 nm), column oven temperature and mobile phase flow rate.

System Suitability

The standard solution was prepared as per the methodology and injected into the HPLC system before starting every validation parameter. The percentage relative standard deviation for 5 replicate injections, tailing factor and theoretical plates of standard solution. The system's suitability complies as per the methodology.

Table 10: Robustness results of Docetaxel

Condition	Retention Time	Tailing Factor	Theoretical Plate
	Docetaxel	Docetaxel	Docetaxel
Normal (Unaltered) (Repeatability)	9.787	0.98	6133
Column oven temperature (20°C)	10.102	0.99	6400
Column oven temperature (30°C)	8.594	1.00	7225
Wavelength for detection (223 nm)	9.725	0.99	6776
Wavelength for detection (227 nm)	9.648	1.02	6869
Flow rate of Mobile Phase (1.4 mL/min)	10.007	1.01	7577
Flow rate of Mobile Phase (1.6 mL/min)	8.648	0.99	6525

Table 11: System Suitability results of Docetaxel

Condition	Retention Time	Tailing Factor	Theoretical Plates
	Docetaxel	Docetaxel	Docetaxel
Specificity	9.787	0.98	6133
Solution stability	9.787	0.98	6133
Linearity and Range	9.787	0.98	6133
Repeatability	9.787	0.98	6133
Intermediate Precision	9.784	1.02	6157
Accuracy	9.787	0.98	6133

Tentative Sequence for Assay

Standard solution- Theoretical plates calculated in the Docetaxel peak was not less than 2000, with the tailing factor calculated in the Docetaxel peak not more than 2, and the relative standard deviation for 5 replicate injections as not more than 2.0%.

Table 12: Tentative Sequence for Assay

Sl.No.	Sequence Pattern	No. of Injection
1.	Blank Saturation	1
2.	Blank	1
3.	Standard Solution	5
4.	Sample Solution	2
5.	Standard Solution Bracketing	1

Chromatogram of Blank, Placebo, Standard & Sample

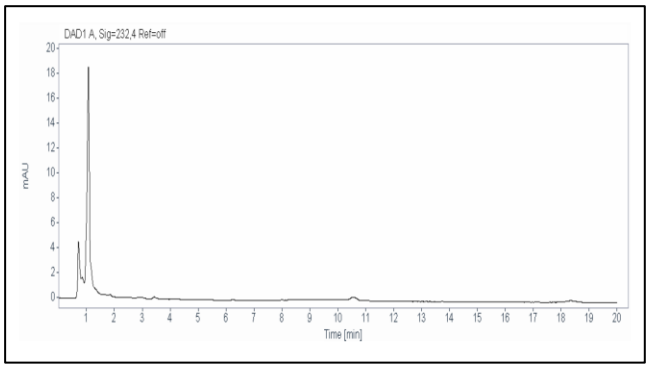

Figure 2: Chromatogram of Placebo

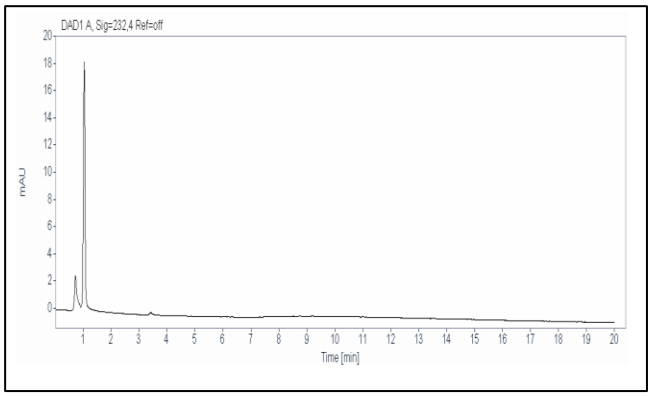

Figure 3: Chromatogram of Blank

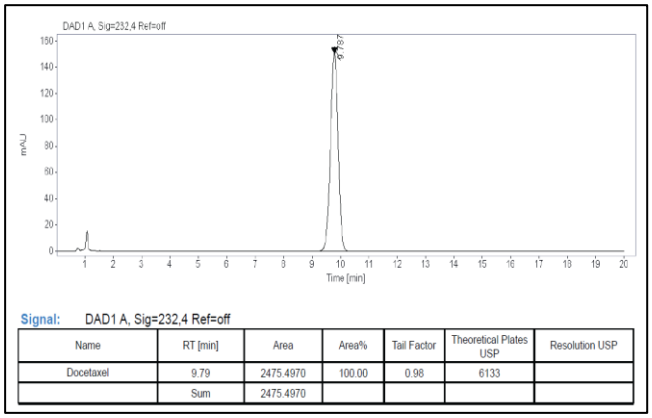

Figure 4: Chromatogram of Standard

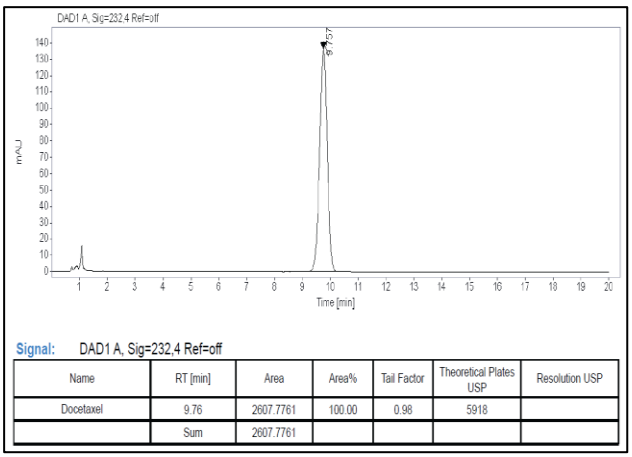

Figure 5: Chromatogram of Sample

CONCLUSION

In accordance with ICH guidelines, the suggested method was validated. The proposed approach ensures a quick, exact, linear, focused and accurate determination of active content by HPLC in pharmaceutical products. A simple and precise method was developed to make the analytical approach acceptable and economical, with quality characteristics for standard laboratory analysis. ICH Q2(R1) validation of the developed method has also been completed.

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AVAILABILITY OF DATA

The Raw data and the support of the findings of this study are available from the corresponding author, upon request.

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