Forest Chemicals Review
www.forestchemicalsreview.com
ISSN: 1520-0191
September-October 2022 Page No. 1186 – 1195
Article History: Received: 06 April 2022, Revised: 28 April 2022, Accepted: 04 May 2022, Publication: 15 May 2022

Development of Method and Validation for Related Substances in Docetaxel Injection Formulation with HPLC

Korupolu Nagu*[1], B. Vinu[1], B. S. A. Andrews*, V D N Kumar Abbaraju[2], Kancharla Vijayalakshmi[3,] and G T Jyotesh Kumar[4]

,[1] Department of Chemistry, GIS, GITAM University, Visakhapatnam – INDIA – A.P. – 530045.

[1] Dr.B.R.Ambedkar University, Srikakulam, Andhra Pradesh, INDIA - 532410

[2]Department of Environmental Sciences, GIS, GITAM University, Visakhapatnam – INDIA – A.P. – 530045

[3]Divis Laboratories Ltd., Gachibowli, Hyderabad, Telangana - 500081

[4] Reddy Laboratories Ltd., Visakhapatnam, Andhra Pradesh, INDIA – 530049

Author for Correspondence
andrewsugc@gmail.com

ABSTRACT

A validated HPLC method is developed to determine Docetaxel (DTX) in pharmaceutical formulation. Isocratic elution at a flow rate of 1.00ml/min is employed on Zorbax XDB C18 150mm × 4.6 mm, 5µ Column, or equivalent. Exactly transferred 1100 volumes of water and 900 volumes of Acetonitrile as a mobile phase. U.V. detection wavelength is set at 230nm. An injected sample is 10.0µl. Run time is 22 minutes for Sample, Blank, Placebo, and regular. The approximate retention time identified to DTX is 6.0 min. % R.S.D DTX is identified. Mean Percentage recovery to DTX is identified within the specification limit. This work is validated by using rules and regulations given by ICH. Therefore, the proposed HPLC process should successfully be applied to routine quality control analysis formulations. This process developed is simple and better than that of different processes reported in earlier literature. Values permit application for proposed stability-indicating HPLC methodology in syrup dose forms.

Keywords: RP-HPLC Refractive index detector, DTX, flow rate, column, ICH Guidelines, USP reference.

INTRODUCTION

The molecular formulae for Docetaxel (DTX or DXL) are C43H53NO14. This drug is commercially available under the brand name Taxotere along with others. Types of cancers were denoted as breast cancer, head as well as neck cancer, stomach cancer, prostate cancer along with non-small-cell lung cancer. [1]. Along with other chemo medications; this drug used to cure the cancer disease as an injection. [2] DTX induced neurotoxicity is maybe very well recognized affect those to be well-identified in a time interval and treated latter withholding of drug. [3] In 1986, this DTX was patented and approved in medicine in 1995. This drug acts as a chemotherapy medication which is utilized to treat various types of cancer diseases[4]. Data from clinical trials were revealed that this DTX consists of activity over cytotoxic nature breast, colorectal, lung, ovarian, prostate, liver, renal, gastric, also head as well as neck cancers and melanoma. [5] Dong WukKim[6] et al., proposed DTX and

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Curcumin in rat plasma. This author used a C8 column (4.6 mm × 150 mm, 5 µm). Acetonitrile 40 v/v and triple distilled water 60v/v acted as a mobile phase. The flow rate is 1.0 ml/min measured. To determine DTX and Curcumin, estimated the wavelength at 230 nm. The samples used in this method show stability at a temperature of 48Hours. Haitao Yin[7] et al., Curcumin is denoted as a Cum. The turmeric rhizome Curcuma longa, the basic polyphenolic curcuminoid, has high potential antitumor effects in vitro and in vivo. Burin V.M[8] et al., employed on C(18) column. The mobile phase used as water acidified acetic acid with pH 2.6 as 20% and Acetonitrile with 80%. The value RSD is less than 2.6% representing the precision is very good, and the recovery obtained is about 80-120%. P A Philip et al. [9] proposed this method in plasma DDS and MADDS. In this method procedure for the extraction and standard internal methods are represented. Abir Abdalla Ahmed Ali et al. [10] proposed DOX and Lisinopril dehydrate named LID using the spectrophotometric method. With the concentration range 0.5–3 for ATE, 0.4–8 for DOX, and 5–50 LID for µg/mL, Beer's law is obeyed. 0.11 for ATE, 0.12 for DOX, and 1.16 for LID µg/mL are the limits for detection. Linear regression correlation coefficients are 0.9993 for ATE, 0.9998 for DOX, and 0.9997 for LID, and the recovery area in the range from 98.25–102.57for ATE, 97.20–100.57 for DOX, and 97.83–101.80for LID respectively.

EXPERIMENTAL

In this problem, the L.C. 20AT pump and UV-Visible detector with a flexible wavelength program and Rheodyne injector are used. Zorbax C18 150mm × 4.6 mm, 5µ, is used for this chromatography analysis. From the local market, the reference sample of DTX is purchased. Acetonitrile is A.R. grade, Glacial acetic acid, and water collected and used. Mobile Phase as 1100 volumes of water, 900 volumes of Acetonitrile into 2 liters bottle and sonicated for 5 minutes. System suitability solution was prepared by taking about 2 mg Transferred into 2 mL regular flask subjected to dissolution with diluent and marked to volume with diluent. DTX Regular Stock is designed by taking 16 mg Transferred into 10 mL regular flask subjected to dissolution with 4mL of Acetonitrile and marked to volume with diluent. 20mg/ml sample solution is prepared by taking 2.4 g of DTX sample solution is taken, then transferred into 25 ml regular flask and subjected to dilution with 5 mL of Acetonitrile and marked to volume with diluent. Transfer 4 mL of solution into a 10 mL ordinary flask and subject to dissolve and draw to volume with a suitable diluent. This total preparation finally gives 0.8 mg/mL of DTX.

METHOD DEVELOPMENT

The peak of wavelength 230nm showed spectra of DTX. Identified Required separation and peak appearances were on Zorbax XDB C18 150 mm × 4.6 mm, 5µ Column, or equivalent. Blended the mobile phase by transferring exactly 1100 mL of water and 900 mL of Acetonitrile in a 2000 mL regular flask. To measure the reaction course, between 0.50 – 1.50 mL/min is applied for optimum separation. From the observation of the response, a 1.5mL/min flow rate is acceptable for effective separation of the analyte.

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VALIDATION OF PROPOSED METHOD AND REQUIREMENTS

System Suitability:

Injected Blank (as one injection), system suitability, and Regular solution (as five injections) into chromatography and recorded different chromatograms. By using the below values, finally, it is decided that the proposed method is more suitable for the validation of the approach. Obtained results are tabulated in Table 1.

	Solution	DTX Results
Resolution	System suitability	3.0
Tailing factor	DTX Regular	1.0
Theoretical plates	DTX Regular	7481
% RSD	DTX Regular	0.6

Table 1: System suitability results

Specificity:

Carried out this experiment by passing Blank, placebo, regular solution, Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, Sample solution, and spiked sample solution (Sample + Impurity) into the chromatographic system and documented different retention times. There should be no interference because other peaks obtained for Blank (diluent), Placebo, Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, and Standard may not obstruct peak DTX. With the help of obtained values, finalized that there is no obstruction due to Blank (Diluent), Placebo, Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, and regular at the retention time peak of DTX. Obtained results are tabulated in Table 2.

Solution		Retention time (in Min)
Blank		-
Placebo		-
System suitability solution	Impurity A	5.134
	Impurity B	9.702
	Impurity C	11.843
	Impurity D	17.256
	Impurity E	1.456
	Docetaxel	6.004
Standard solution		6.055
Impurity E		1.466
Impurity-A		5.144
Impurity-B		9.700
Impurity-C		11.877

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Impurity-D	17.271
Sample preparation	6.044

Table 2: Specificity results

Stressed Condition Studies:

A stressed condition study shall be done for finalizing stability information or through its shelf life, or any non-persistent substance is identified that may not be combined by using DTX Injection peak. For this, authors are studied by preparing the various solutions, and the results are shown below in table 3. Finally, a sample is found to undergo degrading in alkali and acidic four conditions. In various solutions like peroxide and photostability conditions, the DTX peak slightly degrades. Various unknown impurities, known impurities and different degradation impurity peaks were subjected to separation from the DTX peak. DTX peak is purer, and this is finalized by Empower software. Hence, the Assay process is considered more specific & stable indicating.

Condition	Purity Angle	Purity Threshold	% Assay
Sample as such	0.242	0.324	99.4
1.0N HCl at 25°C 5 min.	0.192	0.331	82.8
0.1N HCl at 25°C 30 min.	0.202	0.326	93.2
1.0N NaOH at 25°C 30 min.	0.235	0.425	23.3
0.1 N NaOH at 25°C 30 min.	0.439	0.679	14.5
3.0%w/v H202	0.229	0.337	97.0
Neutral	0.237	0.320	95.8
UV Light	0.236	0.324	98.6
Sun Light	0.207	0.342	84.4
Thermal	0.242	0.319	99.3

Table 3: Stress condition results

Precision:

System Precision

The Retention time (R.T.) and area for a total of 6 determinations were calculated with that % RSD may also calculate. We recorded % RSD for R.T. and peak response for DTX from regular preparation. R.T. and peak responses are the same from obtained data, which RSD may support. (Less than 1.0% and less than 2.0%, respectively). Due to this reason, it could be finalized that the system's precision reaches the exactness of method validation. From the results obtained, it is concluded that retention time & area responses were consistent. Those are evidenced by using relative regular deviation. Due to this reason, it is finalized as S.P. parameters satisfy the requirement for validation. Obtained results are tabulated in Table 4.

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Docetaxel Injection concentrate 20mg/ml		
Injection No.	**Retention Time (Min)**	**Area Response**
1	6.151	6102057
2	6.149	6139196
3	6.163	6169278
4	6.114	6164682
5	6.109	6090233
6	6.092	6129681
Mean	**6.130**	**6132521**
%RSD	**0.5**	**0.5**

Table 4: System Precision results

Method Precision & Intermediate precision

Analyzed sample of DTX for about six times of a similar group. Calculated for Assay values of 6 determinations is NMT 2.0. For Intermediate Precision, **the s**ame process is repeated no. of times with the help of various instruments and different columns on different days. Calculated % of assay compared values obtained in method precision and Intermediate Precision and calculated % RSD for 12 determinations (Method precision and Intermediate Precision). The %RSD calculated for assay for six determinations is NMT 2.0. The %RSD calculated for assay for 6 determinations is NMT 2.0. Obtained results are tabulated from Table 5.

Docetaxel injection concentrate 20mg/ml		
Sample Set No.		**% of Assay**
Method precision	1	103.4
	2	103.4
	3	102.6
	4	102.8
	5	103.6
	6	103.4
Intermediate precision	**1**	102.9
	2	102.9
	3	104.6
	4	103.9
	5	102.5
	6	103.1
Mean		**103.2**
%RSD of 12 Determination		**0.6**

Table 5: Comparison results of precision

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Stability in analytical Solution:

The authors calculated stability with the help of injecting regular sample preparation at a specified range in regular intervals by day wise with the help of 5°C & 25°C. % Difference to DTX in regular is within ± 2.0. Solution stability at 5°C & 25 °C Regular solution is stable for 38 hours at 5°C (% difference is -1.7) & 33 hours at 25°C (% difference is -0.4). Sample Solutions is stable for 36 hours at five °C (% difference of -1.5) and 34 hours at 25°C(% difference -0.8). Obtained results at different temperatures are tabulated in Table 6.

SAMPLE SOLUTION at 5°C			SAMPLE SOLUTION at 25°C	
Time (Hrs.)	**Area Response**	**% Difference**	**Area Response**	**% Difference**
Initial	6652959	-	6624363	-
7	6604495	-0.7	6631903	0.1
9	6743763	1.4	6660864	0.6
12	6755897	1.5	6695644	1.1
14	6750956	1.5	6641810	0.3
16	6752861	1.5	6540494	-1.3
18	6746153	1.4	6595456	-0.4
20	6738425	1.3	6592033	-0.5
24	6649397	-0.1	6597371	-0.4
26	6614383	-0.6	6593656	-0.5
28	6599376	-0.8	6576803	-0.7
30	6576314	-1.2	6595876	-0.4
32	6585453	-1.0	6590979	-0.5
34	6592334	-0.9	6572676	-0.8
36	6554233	-1.5	--	--

Table 6: Sample solution stability at 5 ^{o}C and 25 ^{o}C

Linearity:

In the range between 50% and 150%, linearity of DTX is measured with working concentration and covered a minimum of five degrees from 80% to 120%. We performed linearity by using DTX. Noted area response to every range and calculated slope, intercept, and correlation coefficient. The tested intercept for statistical equivalence to zero. Performed precision at maximum ranges bypassing solution about six times into chromatographic system. We have plotted a graph of DTX as strength (PPM) and drawn a diagram by taking PPM on X-axis and area response on Y-axis. The correlation coefficient and R square are 0.998 intercepts should be within the limit of ± 2.0 of response at 100% range. Precision at Lower & higher ranges %RSD is NMT 2.0 Linearity plot, and the residual plot is shown in figure 1.

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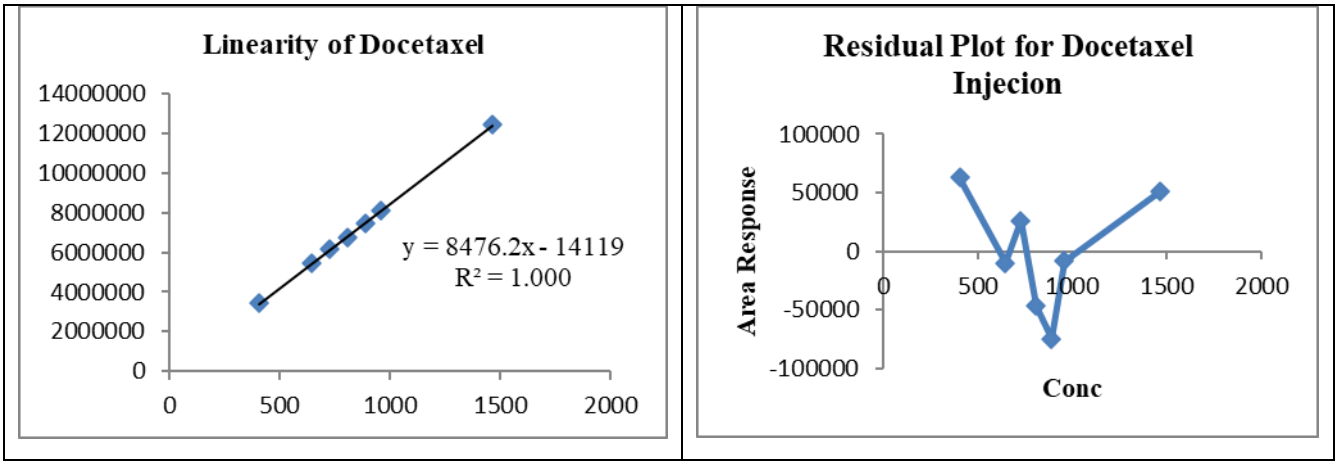
Figure: 1 Linearity and residual plot for DTX



Accuracy:

Single and mean recovery for every range lie in 50% to 150% for Known impurity. Individual and mean recovery for every content lie in 80.0% to 120.0% for DTX. Accuracy results are tabulated in Table 7.

Set	%Levels (About)	Area Response	mg added*	mg Added (Actual)	mg recovered	% recovery	Mean % Recovery	% RSD
1	50	3362235	26.85	25.1048	25.1590	**100.2**	100.0	0.9
2	50	3398580	27.01	25.2544	25.4310	**100.7**		
3	50	3342070	27.02	25.2637	25.0081	**99.0**		
1	100	6833327	54.24	50.7144	51.1326	**100.8**	101.2	0.8
2	100	6824955	54.24	50.7144	51.0700	**100.7**		
3	100	7210560	56.45	52.7808	53.9554	**102.2**		
1	150	9887817	79.18	74.0333	73.9889	**99.9**	101.0	1.2
2	150	10657742	83.40	77.9790	79.7501	**102.3**		
3	150	10085601	80.05	74.8468	75.4689	**100.8**		
(% RSD for each level and 3x3 levels)						**1.0**		

Table 7: Recovery ranges

Range:

%RSD obtained for all accuracy range determinations is NMT 2.0. The correlation and regression coefficient is NLT 0.998 for the Linearity and accuracy range parameter. Linearity with accuracy range graphs is represented in Fig 2 & 3. Finally, the range of the method is from 50% to 150% of the target strength for DTX.

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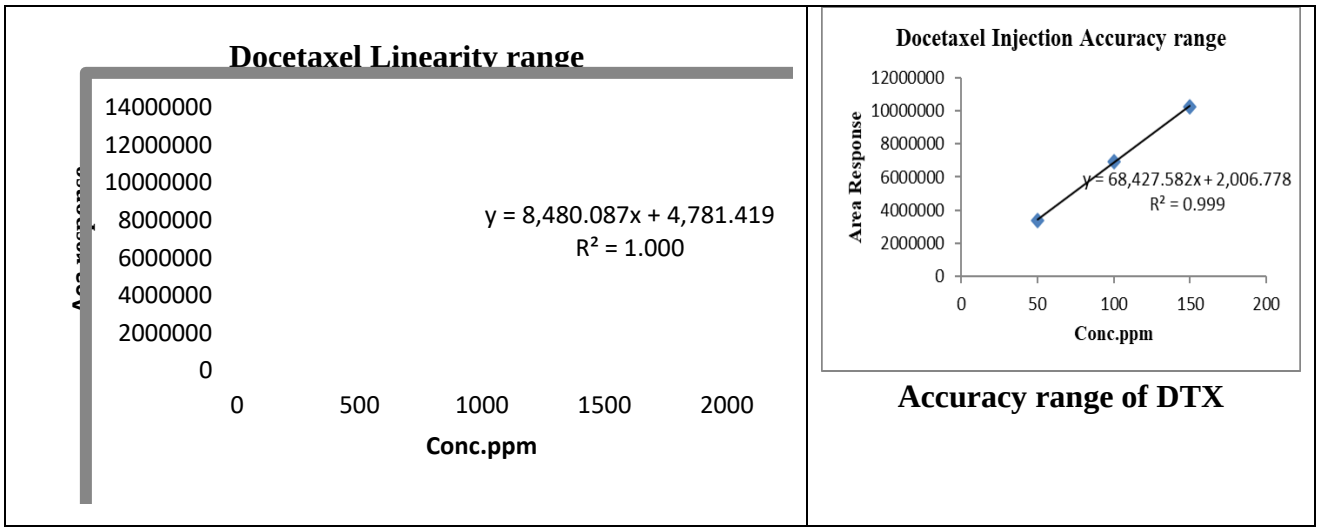
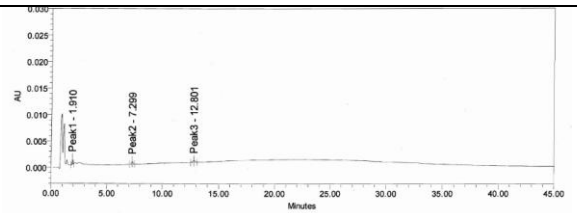
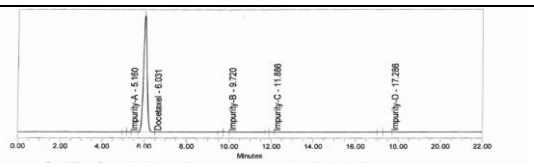


Accuracy range of DTX

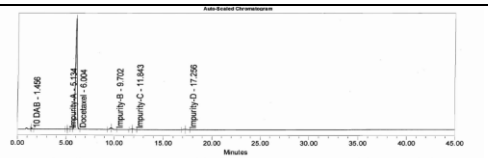
Fig: Linearity range of DTX



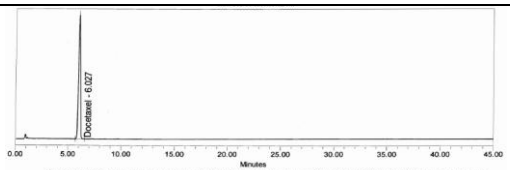
Placebo solution



System suitability solution



Spiked sample



Thermal Stressed sample

Robustness:

They changed column temperature to in and around 5°C. System Suitability parameters were obeyed for all conditions. Total impurities are known, taken off each other, and DTX peak in samples spiked with contaminants. The results confirm that the method is robust towards minor variations possible in this method. Results are tabulated in Table 8.

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Robustness	Evidence perception		**Resolution NLT 2.0**	**Tailing factor NMT2.0**	**Theoretical plate Count NLT 2000**	**%RSD NMT 2.0**
	Original Condition		2.9	1.0	7403	0.9
	Flow rate Change	-0.2 ml/min	3.0	1.0	8119	1.4
		+0.2 ml/min	2.8	1.0	6828	0.9
	Column oven Temperature	-5°C	3.0	1.0	7069	0.4
		+5°C	2.8	1.0	7830	0.3
	Change in Organic phase	+0.2 %	3.0	1.0	7454	0.2
		-0.2 %	3.0	1.0	7564	0.3
	Wave length	-5nm	2.8	1.0	7452	0.2
		+5nm	3.0	1.0	7417	0.2

Table 8: Results for Robustness

RESULTS AND DISCUSSION

Upgraded various RP-HPLC parameters, different mobile phase configurations are verified and tested. Satisfactory segregation with good peak symmetry is measured with the configurations of the Mobile phase. By transferring 1100 mL of water, 900 mL of Acetonitrile in 2000mL of regular flask treated as a mobile phase. 1.5 mL/min flow proved a better resolution and peak shape than other mixtures. The column used in this measurement is Zorbax XDB C18 (150 × 4.6) mm, 5µ, or proportionate. Specificity(Stressed condition) the peaks of Blank (diluent), Placebo, Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, and each other. There is no interference of Blank (diluent), Placebo, & known Impurities peaks with DTX Peak. Degradation products are well separated from DTX Peak, and each other purity angle is less than the peak threshold as per empower software. For System suitability, the Tailing factor is about 2.0, and resolution is not less than 2.0. Obtained results are respectively. Peak tailing of DTX in regular injection may not be maximum than 2.0 with %RSD for six replicated injections of regular may not be maximum to value 2.0. %RSD of the retention time for DTX peak measured from a total of six injections of the diluted solution may not be maximum to value as 1.0, and %RSD area of DTX peak response measured for real five injections of the diluted regular solution is NMT 2.0 respectively. %RSD of Assay for six determinations is 0.4. % % RSD for 12 measurements (Method Precision & Intermediate Precision) is NMT 2.0, which is identified as 0.6. % Difference in sample solution obtained between initial and after specified period is -1.7 at five °C & -0.4 at 25°C. Estimated HPLC method of related substance in drug product DTX Injection is validated as per ICH guidelines. The proposed process was found as specific. The method is also indicated as evidenced by stress conditions.

Acknowledgements
The authors are thankful to the Management of GITAM University for providing the necessary facilities to carry out this research work.

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Conflicts of interest

There are no conflicts of interest among the authors who were done this present work.

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