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Research Article

# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF ORGANIC IMPURITIES OF DOCETAXEL IN PARENTERAL FORMULATION OF DOCETAXEL USING UV DETECTOR

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**ABSTRACT**

**Objective:** The objective is to develop a novel, rapid, simple, precise, accurate, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method for quantitative estimation of organic impurities of docetaxel (DTX) parenteral formulation through high-performance liquid chromatography (HPLC).

**Methods:** Finalized chromatographic conditions were used for a reversed-phase C18 column with particle size of 3 µm and dimension of 4.6×150 mm, water as mobile phase-A and acetonitrile mobile phase-B. The flow rate is 1.2 mL/min with gradient elution and ultraviolet (UV) detection at 232 nm. Acetonitrile: water:glacialcetic acid in the ratio of 100:100:0.1 (v/v/v) as diluent.

**Results:** Analytical test method for the quantitative determination of organic impurities of DTX in parenteral formulation of DTX using HPLC with UV detector was verified and found to be linear over the tested concentration range for all impurities (10-deacetylbaccatin: 0.032–0.466 µg/mL; DTX: 0.015–0.151 µg/mL; 6-oxodocetaxel: 0.023–2.080 µg/mL; 4-epidocetaxel: 0.022–1.380 µg/mL; 4-epi-6-oxodocetaxel: 0.021–0.673 µg/mL). The calibration charts plotted were linear with a regression coefficient of $R^2$>0.999. Method-precise results were found to be within the acceptance criteria. Limit of detection and limit of quantification for the active ingredients and their impurities were established with respect to test concentration.

**Conclusion:** It was concluded that the method is simple, sensitive, precise, and accurate and hasthe ability to separate the drug from degradation products and excipients found in the dosage form.

**Keywords:** Docetaxel, Organic impurities, Forced degradation, Validation, High-performance liquid chromatography, Lung cancer.

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## INTRODUCTION

Docetaxel (DTX) is a clinically well-established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer [1,2]. Based on the available literature, survey reveals that there is no sophisticated method for estimation of organic impurities of DTX in parenteral dosage form in common laboratories [3,4]. Hence, the present study is related to the development of a new analytical method for the estimation of organic impurities of DTX in DTX concentrate for solution for infusion 40 mg/mL in fill volume-based parenteral dosage forms [5,6]. Validation of the proposed analytical method was done as per International Conference on Harmonization (ICH) guidelines [7,8]. The chemical formula for DTX is C43H53NO14. The molecular weight of DTX is 807.8792 g/mol [9,10]. The structure of DTX is shown in Table 1. The DTX-related impurities are 10-deacetylbaccatin, 6-oxodocetaxel, 4-epidocetaxel, and 4-epi-6-oxodocetaxel which are shown in Table 1 [11,12].

## METHODS

The DTX reference standard and its related impurities received from EP CRS. DTX n samples and placebo were used for the research work and received from perfomic analytical laboratories. Glacial acetic acid AR grade was purchased from Merck Germany; aacetonitrile high-performance liquid chromatography (HPLC) grade was purchased from Finar; and ethanol HPLC grade was purchased from Hayman. HPLC grade water.

### Instrument and chromatographic condition

Chromatographic analysis for the detection of DTX was performed using the HPLC (Waters and Agilent) equipped with Empower software, Waters 2695 ultraviolet (UV), and Agilent 1260 VWD detector and YMC Pack ODS-A C18 analytical column with particle size of 3 µm and dimension of 4.6 × 150 mm. The mobile phase was composed of water is mobile phase-A and acetonitrile is mobile phase-B. The analysis was performed at 45°C of Column oven temperature and 10°C of autosampler temperature by Gradient elution mobile phase-B, 0.0 min-28, 9.0 min- 28, 39.0 min-72, 39.1 min-100, 49.0 min-100, 49.1 min-28, 60.0 min- 28 at a flow rate of 1.2 mL/min, with UV- detector set at 232 nm and injection volume of 20 µL. The diluent is a mixture of acetonitrile, water, and glacial acetic acid in the ratio of 100:100:0.1 (v/v/v).

### Preparation of solutions

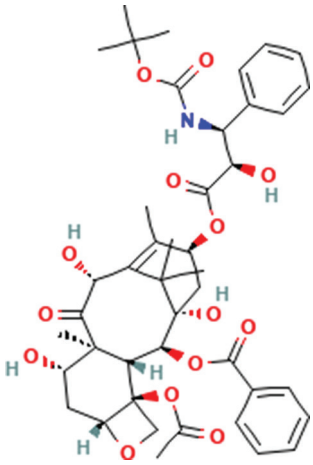
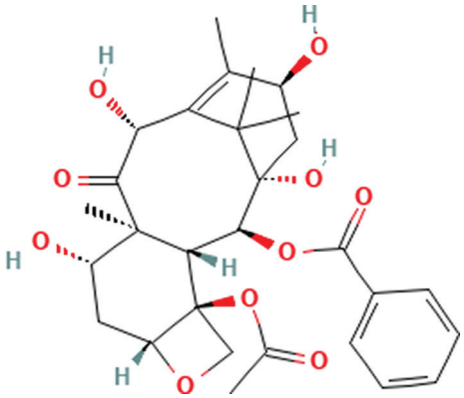
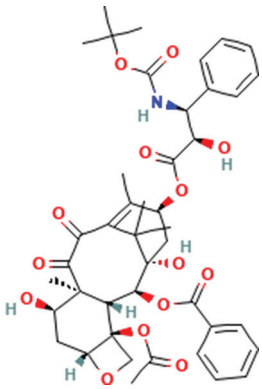
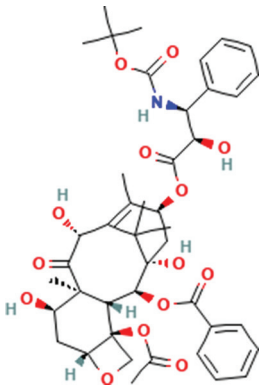
*Preparation of system suitability solution*

Weigh and transfer about 10 mg of DTX for identification standard into a 10 mL volumetric flask. Add about 5 mL of diluent, dissolve, and dilute to the volume with diluent and mix well.

*Preparation of standard solution*

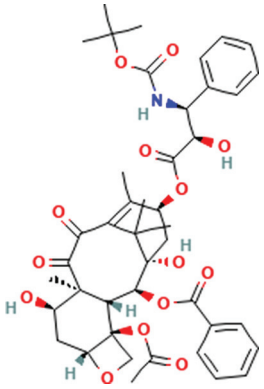
Weigh and transfer about 21.4 mg of DTX trihydrate standard into a 100 mL volumetric flask. Add 5 mL of ethanol dissolve and dilute to the volume with diluent and mix well.

**Table 1: Docetaxel and related compound structures**

| S. No | Name of Compound | Structure |
|---|---|---|
| 1 | Docetaxel |  |
| 2 | 10-Deacetylbaccatin |  |
| 3 | 6-Oxodocetaxel |  |
| 4 | 4-Epidocetaxel |  |

*(Contd...)*

**Table 1:** ***(Continued)***

| S. No | Name of Compound | Structure |
|---|---|---|
| 5 | 4-Epi-6-oxodocetaxel |  |

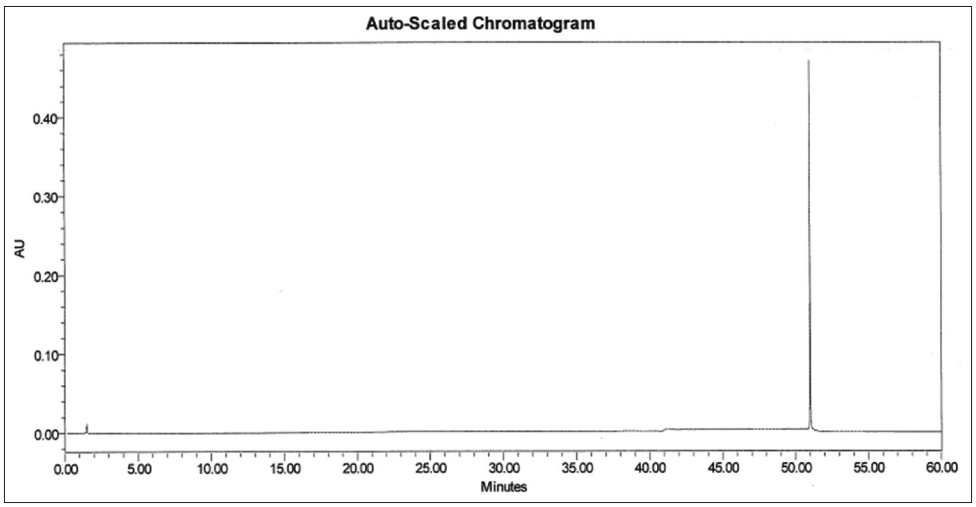



**Fig. 1: Blank solution**

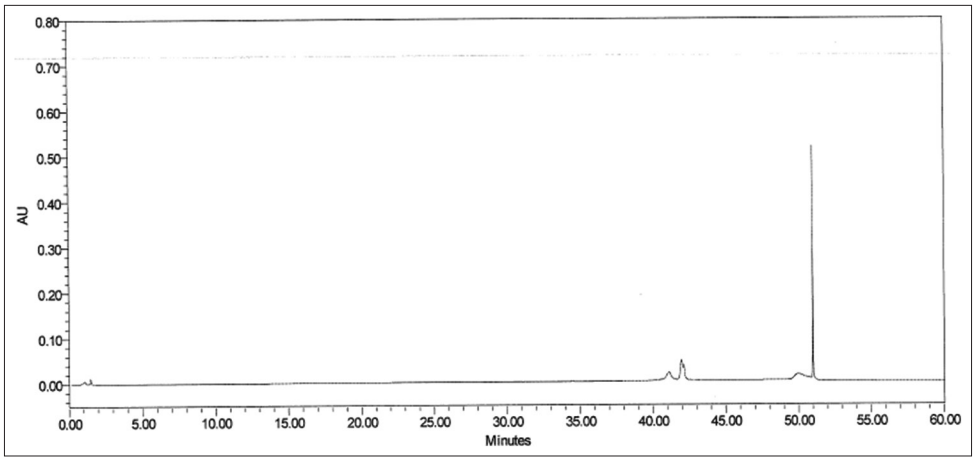



**Fig. 2: Placebo solution**

*Preparation of sensitivity solution*

Transfer 1.0 mL of standard solution into a 100 mL volumetric flask, dilute to volume with diluent and mix well. Transfer 1.0 mL of the obtained solution into a 10 mL volumetric flask, dilute to volume with diluent and mix well.

*Preparation of placebo solution*

Transfer 1.0 mL of placebo into 200 mL volumetric flask and dilute with 70 mL diluent and mix well, then dilute to volume with diluent and mix well.

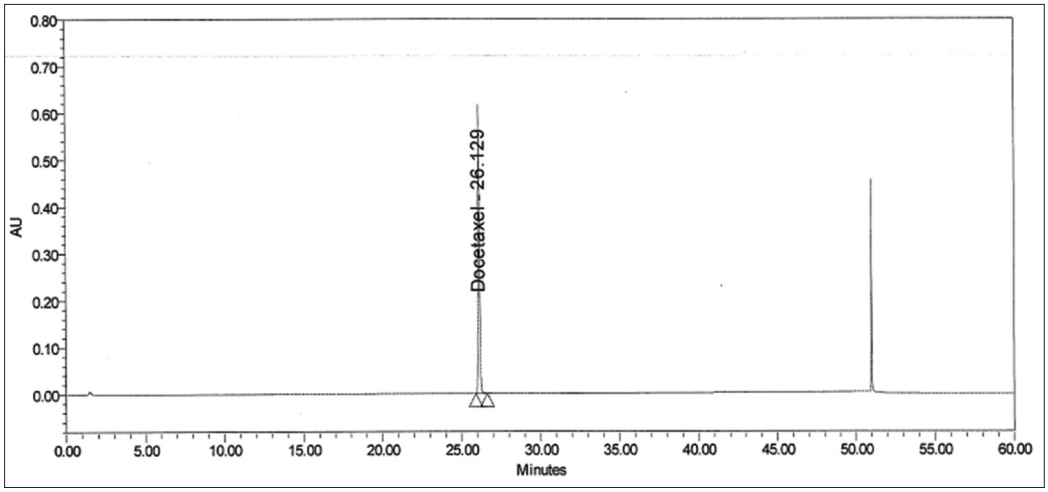



**Fig. 3: Standard solution**

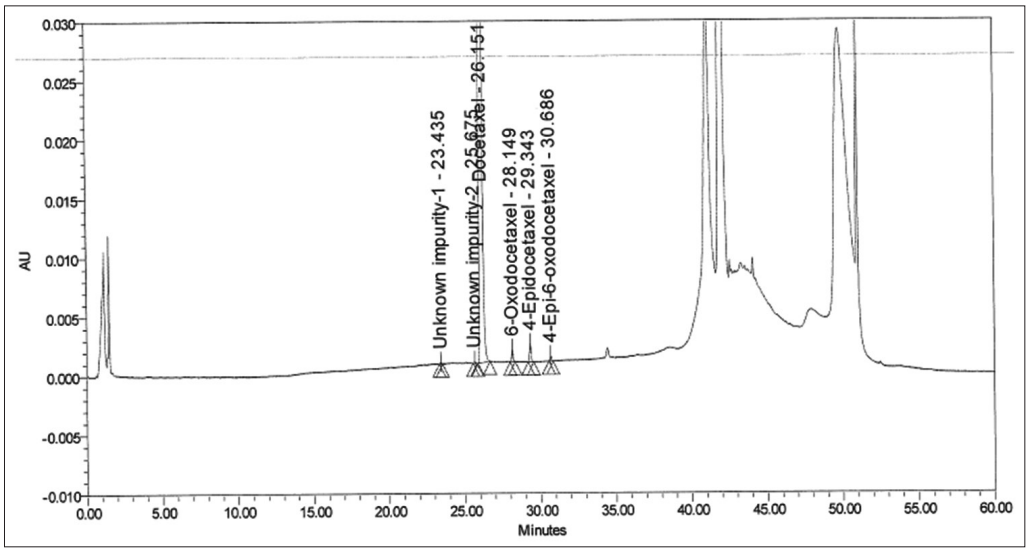



**Fig. 4: Sample solution**

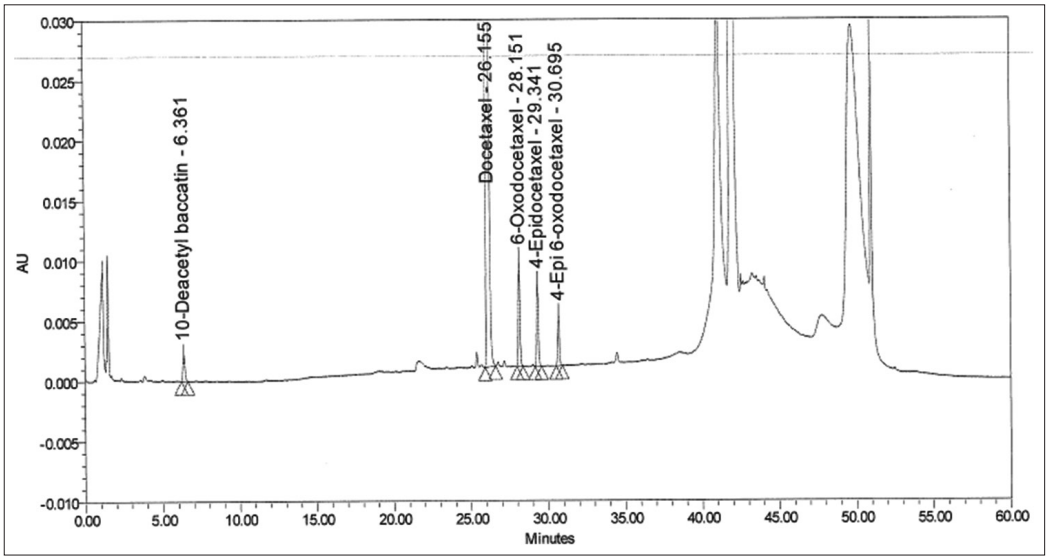



**Fig. 5: Spiked solution**

*Preparation of test solution*

Prepare a pooled sample solution by mixing 5 vials. Dilute 1.0 mL of pooled sample solution to 200 mL with diluent and mix well.

**Validation of method**

The method was evaluated according to the ICH requirements with system suitability, linearity, accuracy, method precision, sensitivity Limit of quantification (LOQ) and limits of detection (LOD), robustness, and a forced degradation study among the validation parameters [13,14].

**Table 2: Results of system suitability**

| S. No. | Standard solution | | |
|---|---|---|---|
| | Peak area | Theoretical plates | Asymmetry |
| 1 | 3456756 | 12140 | 1.0 |
| 2 | 3434167 | 12342 | 1.0 |
| 3 | 3476232 | 12336 | 1.0 |
| 4 | 3464536 | 12325 | 1.0 |
| 5 | 3423429 | 12405 | 1.0 |
| Average | 3451024 | NA | NA |
| standard deviation | 21774.5 | NA | NA |
| % relative standard deviation | 0.6 | NA | NA |

## RESULTS AND DISCUSSION

### Method validation

*System suitability*

System suitability was demonstrated by preparing a blank solution, system suitability solution, and standard solution as per the test method and injecting the same into the HPLC system. The System suitability was evaluated by computing theoretical plates, % relative standard deviation (RSD), and tailing factor. The observations are tabulated in Table 2.

*Acceptance criteria*

The RSD from the areas of each of the DTX peaks in the chromatogram of the standard solution should be not more than 5.0%.

*Conclusion*

The obtained system suitability results are found satisfactory.

**Specificity**

Injected the blank solution, placebo solution, sensitivity solution, system suitability solution, standard solution, unspiked sample solution, and spiked sample solution and analyzed as per the test method. The observations are tabulated in Table 3.

**Table 3: Results of specificity**

| S. No | Name of the Solution | Retention time (Min) | Purity angle | Purity threshold | Peak Purity |
|---|---|---|---|---|---|
| 1 | Blank solution | ND | NA | NA | NA |
| 2 | Placebo solution | ND | NA | NA | NA |
| 3 | Standard solution-Docetaxel | 26.33 | 0.19 | 0.23 | Pass |
| 4 | Individual known impurities | | | | |
| | 10-Deacetylbaccatin | 6.25 | 7.23 | 9.34 | Pass |
| | 6-Oxodocetaxel | 28.32 | 2.12 | 2.45 | Pass |
| | 4-Epidocetaxel | 29.56 | 3.23 | 4.32 | Pass |
| | 4-Epi-6-oxodocetaxel | 30.72 | 6.13 | 7.56 | Pass |
| 5 | Spiked sample solution | | | | |
| | 10-Deacetylbaccatin | 6.42 | 7.21 | 12.11 | Pass |
| | Docetaxel | 26.25 | 0.23 | 0.35 | Pass |
| | 6-Oxodocetaxel | 28.23 | 2.12 | 3.52 | Pass |
| | 4-Epidocetaxel | 29.42 | 2.14 | 4.24 | Pass |
| | 4-Epi-6-oxodocetaxel | 30.75 | 3.63 | 6.11 | Pass |

**Table 4: Results of the forced degradation study**

| S. No | Samples name | % assay | Total impurities in (%) | Peak purity for Docetaxel |
|---|---|---|---|---|
| 1 | Control sample | 100.6 | 0.63 | Pass |
| 2 | Acid stress sample | 93.2 | 6.84 | Pass |
| 3 | Peroxide stress sample | 94.2 | 5.42 | Pass |
| 4 | Thermal stress sample | 99.1 | 0.92 | Pass |
| 5 | Ultraviolet stress sample | 101.1 | 0.72 | Pass |
| 6 | Humidity stress sample | 100.5 | 0.69 | Pass |
| 7 | Photolytic stress sample | 101.0 | 0.70 | Pass |
| 8 | Base stress sample | 98.3 | 2.23 | Pass |

**Table 5: Results of method precision**

| S. No | 10-Deacetylbaccatin | 6-Oxodocetaxel | 4-Epidocetaxel | 4-Epi-6-oxodocetaxel | Any unspecified impurity | Total impurities |
|---|---|---|---|---|---|---|
| 1 | Not detected | 0.18 | 0.58 | 0.29 | 0.13 | 1.2 |
| 2 | Not detected | 0.19 | 0.57 | 0.27 | 0.13 | 1.1 |
| 3 | Not detected | 0.18 | 0.59 | 0.31 | 0.12 | 1.2 |
| 4 | Not detected | 0.18 | 0.54 | 0.28 | 0.13 | 1.2 |
| 5 | Not detected | 0.17 | 0.58 | 0.30 | 0.13 | 1.1 |
| 6 | Not detected | 0.19 | 0.59 | 0.29 | 0.13 | 1.2 |
| Average | NA | 0.18 | 0.58 | 0.29 | 0.13 | 1.17 |
| Standard deviation | NA | 0.01 | 0.02 | 0.01 | 0.00 | 0.05 |
| % relative standard deviation | NA | 4.1 | 3.3 | 4.9 | 3.18 | 4.43 |

**Table 6: Results of establishment of LOD and LOQ**

| S. No | Name | LOD | | LOQ | |
|---|---|---|---|---|---|
| | | Concentration (%) | S/N | Concentration (%) | S/N |
| 1 | Docetaxel | 0.014 | 2.7 | 0.015 | 11.6 |
| 2 | 10-Deacetylbaccatin | 0.015 | 3.0 | 0.032 | 11.9 |
| 3 | 6-Oxodocetaxel | 0.011 | 2.9 | 0.023 | 12.1 |
| 4 | 4-Epidocetaxel | 0.013 | 2.8 | 0.022 | 10.4 |
| 5 | 4-Epi-6-oxodocetaxel | 0.01 | 3.0 | 0.021 | 10.9 |

LOD: Limit of detection, LOQ: Limit of quantification

**Table 7: Results of precision at the limit of quantification**

| S. No | 10-Deacetylbaccatin | 6-Oxodocetaxel | 4-Epidocetaxel | 4-Epi-6-oxodocetaxel | Docetaxel |
|---|---|---|---|---|---|
| 1 | 1726 | 1232 | 1342 | 1167 | 1543 |
| 2 | 1639 | 1178 | 1387 | 1098 | 1498 |
| 3 | 1567 | 1287 | 1298 | 1165 | 1562 |
| 4 | 1646 | 1198 | 1412 | 1094 | 1454 |
| 5 | 1667 | 1206 | 1467 | 1132 | 1412 |
| 6 | 1551 | 1259 | 1398 | 1068 | 1538 |
| Average | 1633 | 1227 | 1384 | 1121 | 1501 |
| Standard deviation | 64.9 | 40.8 | 58.4 | 40.6 | 58.3 |
| % relative standard deviation | 4.0 | 3.3 | 4.2 | 3.6 | 3.9 |

**Table 8: Results of linearity for 10-Deacetylbaccatin**

| % Level | Area response | Concentration (µg/mL) |
|---|---|---|
| Limit of quantification | 1547 | 0.032 |
| 50 | 9600 | 0.152 |
| 100 | 19478 | 0.293 |
| 125 | 25298 | 0.372 |
| 150 | 29873 | 0.466 |
| Correlation coefficient | | 0.999 |
| Slope | | 71896 |
| Intercept | | −428.6 |
| % Y-intercept | | −2.3 |

**Table 9: Results of linearity for docetaxel**

| % Level | Area response | Concentration (µg/mL) |
|---|---|---|
| Limit of quantification | 1135 | 0.015 |
| 50 | 1914669 | 0.053 |
| 100 | 3896738 | 0.113 |
| 125 | 5001182 | 0.125 |
| 150 | 5930850 | 0.151 |
| Correlation Coefficient | | 0.999 |
| Slope | | 39969.4 |
| Intercept | | −33260.5 |
| % Y-intercept | | −1.8 |

**Table 10: Results of linearity for 6-Oxodocetaxel**

| % Level | Area response | Concentration (µg/mL) |
|---|---|---|
| Limit of quantification | 568 | 0.023 |
| 50 | 29746 | 0.683 |
| 100 | 59464 | 1.399 |
| 125 | 75541 | 1.758 |
| 150 | 89558 | 2.080 |
| Correlation coefficient | | 0.999 |
| Slope | | 43102.1459 |
| Intercept | | 20.9051 |
| % Y-intercept | | 0.0 |

*Acceptance criteria*

- Blank and placebo should not show any interference at the retention time of analyte and known impurities.
- The retention time of the 10-Deacetyl baccatin,6-Oxodocetaxel, 4-Epidocetaxel, and 4-Epi-6-oxodocetaxel solutions in individual solution should match with retention time of 10-Deacetyl

**Table 11: Results of linearity for 4-Epidocetaxel**

| % Level | Area response | Concentration (µg/mL) |
|---|---|---|
| Limit of quantification | 1135 | 0.022 |
| 50 | 1914669 | 0.453 |
| 100 | 3896738 | 0.936 |
| 125 | 5001182 | 1.148 |
| 150 | 5930850 | 1.380 |
| Correlation coefficient | | 0.999 |
| Slope | | 4348901.2 |
| Intercept | | −95534.7 |
| % Y-intercept | | −2.5 |

**Table 12: Results of linearity for 4-Epi-6-Oxodocetaxel**

| % Level | Area response | Concentration (µg/mL) |
|---|---|---|
| Limit of quantification | 821 | 0.021 |
| 50 | 11018 | 0.224 |
| 100 | 22503 | 0.449 |
| 125 | 28163 | 0.561 |
| 150 | 32698 | 0.673 |
| Correlation coefficient | | 0.999 |
| Slope | | 49243.9 |
| Intercept | | 10.54 |
| % Y-intercept | | 0.8 |

**Table 13: Results of % recovery for docetaxel**

| % Level | % Recovery | Mean % Recovery | % relative standard deviation |
|---|---|---|---|
| LOQ Level-1 | 117.4 | 102.6 | 13.3 |
| LOQ Level-2 | 100.0 | | |
| LOQ Level-3 | 90.5 | | |
| 100% Level-1 | 99.1 | 97.3 | 2.0 |
| 100% Level-2 | 97.6 | | |
| 100% Level-3 | 95.3 | | |
| 150% Level-1 | 97.5 | 99.2 | 2.2 |
| 150% Level-2 | 98.5 | | |
| 150% Level-3 | 101.6 | | |

LOQ: Limit of quantification

**Table 14: Results of % recovery f or10-deacetyl baccatin**

| % Level | % Recovery | Mean % Recovery | % relative standard deviation |
|---|---|---|---|
| LOQ Level-1 | 100.0 | 100.0 | 4.0 |
| LOQ Level-2 | 104.0 | | |
| LOQ Level-3 | 96.0 | | |
| 100% Level-1 | 93.0 | 92.9 | 0.9 |
| 100% Level-2 | 93.6 | | |
| 100% Level-3 | 92.0 | | |
| 150% Level-1 | 89.5 | 88.9 | 0.6 |
| 150% Level-2 | 88.8 | | |
| 150% Level-3 | 88.4 | | |

LOQ: Limit of quantification

**Table 15: Results of % recovery for 6-oxodocetaxel**

| % Level | % Recovery | Mean % Recovery | % relative standard deviation |
|---|---|---|---|
| LOQ Level-1 | 82.1 | 83.3 | 2.5 |
| LOQ Level-2 | 82.1 | | |
| LOQ Level-3 | 85.7 | | |
| 100% Level-1 | 117.8 | 117.9 | 0.1 |
| 100% Level-2 | 118.0 | | |
| 100% Level-3 | 117.9 | | |
| 150% Level-1 | 97.7 | 97.2 | 0.2 |
| 150% Level-2 | 97.1 | | |
| 150% Level-3 | 97.1 | | |

LOQ: Limit of quantification

**Table 16: Results of % recovery for 4-epidocetaxel**

| % Level | % Recovery | Mean % Recovery | % relative standard deviation |
|---|---|---|---|
| LOQ Level-1 | 107.7 | 109.0 | 2.0 |
| LOQ Level-2 | 107.7 | | |
| LOQ Level-3 | 111.5 | | |
| 100% Level-1 | 93.4 | 93.4 | 0.1 |
| 100% Level-2 | 93.5 | | |
| 100% Level-3 | 93.4 | | |
| 150% Level-1 | 95.4 | 95.2 | 0.2 |
| 150% Level-2 | 95.1 | | |
| 150% Level-3 | 95.1 | | |

LOQ: Limit of quantification

**Table 23: Results of unspecified impurity**

| % Level | % Recovery | Mean % Recovery | % relative standard deviation |
|---|---|---|---|
| LOQ Level-1 | 119.2 | 114.1 | 3.9 |
| LOQ Level-2 | 111.5 | | |
| LOQ Level-3 | 111.5 | | |
| 100% Level-1 | 107.6 | 107.4 | 0.2 |
| 100% Level-2 | 107.4 | | |
| 100% Level-3 | 107.2 | | |
| 150% Level-1 | 108.6 | 108.3 | 0.3 |
| 150% Level-2 | 108.4 | | |
| 150% Level-3 | 108.0 | | |

LOQ: Limit of quantification

**Table 18: Results of standard solution stability**

| Hours | % Recovery (Standard solution) | | |
|---|---|---|---|
| | Benchtop | Hours | Refrigerator (10°C) |
| Initial | NA | Initial | NA |
| 23 h | 100.7 | 24 h | 100.1 |
| 38 h | 100.9 | 39 h | 100.5 |
| 59 h | 100.9 | 60 h | 100.4 |

**Table 19: Results of 10-deacetyl baccatin**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % Difference |
| Initial | ND | NA | Initial | NA | NA |
| 13 h | ND | 0 | 14 h | ND | 0 |
| 29 h | ND | 0 | 30 h | ND | 0 |
| 49 h | ND | 0 | 50 h | ND | 0 |

**Table 20: Results of 6-oxodocetaxel**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % Difference |
| Initial | 0.17 | NA | Initial | 0.17 | NA |
| 13 h | 0.17 | 0 | 14 h | 0.17 | 0 |
| 29 h | 0.17 | 0 | 30 h | 0.16 | 0.01 |
| 49 h | 0.17 | 0 | 50 h | 0.22 | −0.05 |

**Table 21: Results of 4-epidocetaxel**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % Difference |
| Initial | 0.29 | NA | Initial | 0.29 | NA |
| 13 h | 0.29 | 0 | 14 h | 0.29 | 0 |
| 29 h | 0.29 | 0 | 30 h | 0.29 | 0 |
| 49 h | 0.28 | 0.01 | 50 h | 0.29 | 0 |

**Table 22: Results of4-epi-6-oxodocetaxel**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % difference |
| Initial | 0.08 | NA | Initial | 0.08 | NA |
| 13 h | 0.08 | 0 | 14 h | 0.08 | 0 |
| 29 h | 0.08 | 0 | 30 h | 0.08 | 0 |
| 49 h | 0.08 | 0 | 50 h | 0.08 | 0 |

**Table 17: Results of % recovery for 4-epi-6-oxodocetaxel**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % Difference |
| Initial | 0.03 | NA | Initial | 0.03 | NA |
| 13 h | 0.03 | 0 | 14 h | 0.03 | 0 |
| 29 h | 0.03 | 0 | 30 h | 0.03 | 0 |
| 49 h | 0.03 | 0 | 50 h | 0.03 | 0 |

**Table 24: Results of total impurities**

| Hours | Benchtop | | Refrigerator (10°C) | | |
|---|---|---|---|---|---|
| | Impurity (% w/w) | % Difference | Hours | Impurity (% w/w) | % Difference |
| Initial | 0.57 | NA | Initial | 0.57 | NA |
| 13 h | 0.50 | 0.07 | 14 h | 0.57 | 0 |
| 29 h | 0.51 | 0.06 | 30 h | 0.58 | −0.01 |
| 49 h | 0.58 | −0.01 | 50 h | 0.57 | 0 |

baccatin,6-Oxodocetaxel,4-Epidocetaxel, and 4-Epi-6-oxodocetaxel solutions in spiked sample solution.

- The purity angle should be less than the purity threshold as per empower software.

**Table 25: Results of spiked sample solution stability**

| Hours | % recovery | | | | |
|---|---|---|---|---|---|
| | 10-Deacetyl baccatin | | 6-Oxodocetaxel | | |
| | Benchtop | Refrigerator (10°C) | Hours | Benchtop | Refrigerator (10°C) |
| Initial | 96.0 | 96.0 | Initial | 106.8 | 106.8 |
| 14 h | 92.7 | 92.7 | 15 h | 106.1 | 105.4 |
| 30 h | 96.0 | 92.7 | 31 h | 105.4 | 104.7 |
| 50 h | 96.0 | 96.0 | 51 h | 107.6 | 106.1 |
| **Hours** | **4-Epidocetaxel** | | **4-Epi-6-oxodocetaxel** | | |
| | **Benchtop** | **Refrigerator (10°C)** | **Hours** | **Benchtop** | **Refrigerator (10°C)** |
| Initial | 112.1 | 112.1 | Initial | 108.6 | 108.6 |
| 14 h | 111.1 | 114.3 | 15 h | 106.3 | 106.3 |
| 30 h | 114.3 | 114.3 | 31 h | 106.3 | 106.3 |
| 50 h | 113.2 | 113.2 | 51 h | 106.3 | 108.6 |
| **Hours** | **10-Deacetyl baccatin** | | | | |
| | **Benchtop** | | **Refrigerator (10°C)** | | |
| | **%Impurity found** | **%Difference** | **Hours** | **% Impurity found** | **% Difference** |
| Initial | 0.29 | NA | Initial | 0.29 | NA |
| 14 h | 0.28 | 0.01 | 15 h | 0.28 | 0.01 |
| 30 h | 0.29 | 0.00 | 31 h | 0.28 | 0.01 |
| 50 h | 0.29 | 0.00 | 51 h | 0.29 | 0.00 |
| **Hours** | **6-Oxodocetaxel** | | | | |
| | **Benchtop** | | **Refrigerator (10°C)** | | |
| | **% Impurity found** | **% Difference** | **Hours** | **% Impurity found** | **% Difference** |
| Initial | 1.65 | NA | Initial | 1.65 | NA |
| 14 h | 1.64 | 0.01 | 15 h | 1.63 | 0.02 |
| 30 h | 1.63 | 0.02 | 31 h | 1.62 | 0.03 |
| 50 h | 1.66 | 0.01 | 51 h | 1.64 | 0.01 |
| **Hours** | **4-Epidocetaxel** | | | | |
| | **Benchtop** | | **Refrigerator (10°C)** | | |
| | **% Impurity found** | **% Difference** | **Hours** | **% Impurity found** | **% Difference** |
| Initial | 1.32 | NA | Initial | 1.32 | NA |
| 14 h | 1.31 | 0.01 | 15 h | 1.34 | −0.02 |
| 30 h | 1.34 | −0.02 | 31 h | 1.34 | −0.02 |
| 50 h | 1.33 | −0.01 | 51 h | 1.33 | −0.01 |
| **Hours** | **4-Epi-6-oxodocetaxel** | | | | |
| | **Benchtop** | | **Refrigerator (10°C)** | | |
| | **% Impurity found** | **% Difference** | **Hours** | **% Impurity found** | **% Difference** |
| Initial | 0.56 | NA | Initial | 0.56 | NA |
| 14 h | 0.55 | 0.01 | 15 h | 0.55 | 0.01 |
| 30 h | 0.55 | 0.01 | 31 h | 0.55 | 0.01 |
| 50 h | 0.55 | 0.01 | 51 h | 0.56 | 0 |

*Conclusion*

The specificity results are found satisfactory and found no interference from the blank and placebo solution at the retention times of DTX and its impurities. Furthermore, peak purity has been passed for all peaks in spiked sample solutions as well as individual known impurities.

**Forced degradation**

Performed acid hydrolysis stress study, base hydrolysis stress study, peroxide oxidation stress study, thermal stress study, humidity stress study, fluorescent, and UV-light stress study as per the test method. The observations are tabulated in Table 4.

*Acceptance criteria*

- The peak should be homogeneous and there should be no co-eluting peaks.
- The purity angle should be less than the purity threshold as per Empower software.

*Conclusion*

The forced degradation parameter was established and the results were found satisfactory.

**Method precision**

Method precision was demonstrated by preparing six samples of DTX concentrate for solution for infusion 40 mg/mL as per test method and injected into the chromatographic system. The precision of the method was evaluated by calculating the impurities found and % RSD for impurities found for each set of samples. The results of the precision study are tabulated in Table 5.

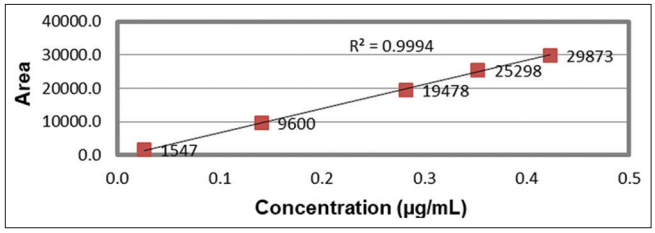



**Fig. 6:**

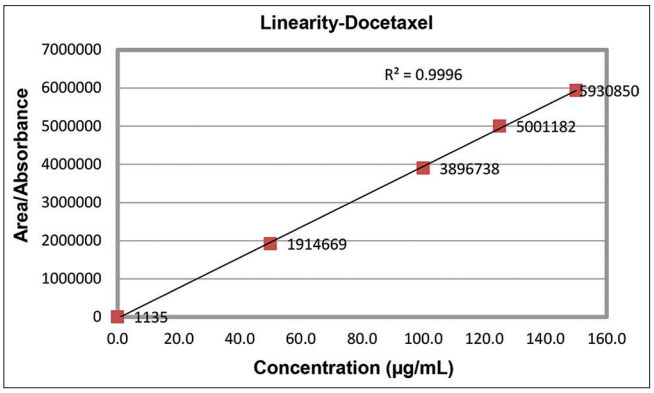



**Fig. 7:**

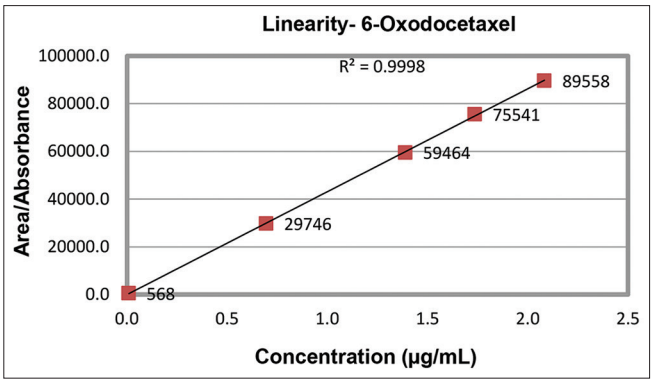



**Fig. 8:**

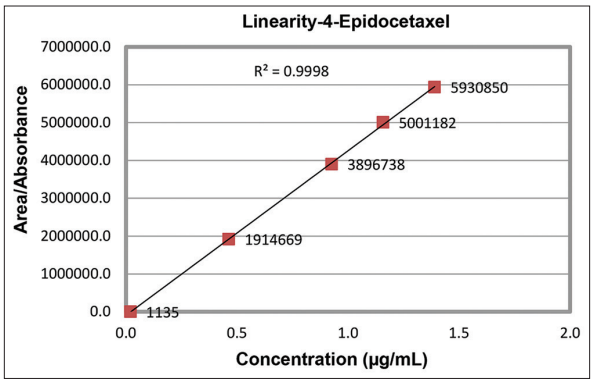



**Fig. 9:**

*Acceptance criteria*

For spiked six sample solutions, the % RSD for % impurities of 10-Deacetyl baccatin, 6-Oxodocetaxel, 4-Epidocetaxel, and 4-Epi-6-oxodocetaxel should be not more than 10.0.

*Conclusion*

The method precision parameter has been established and results are found satisfactory.

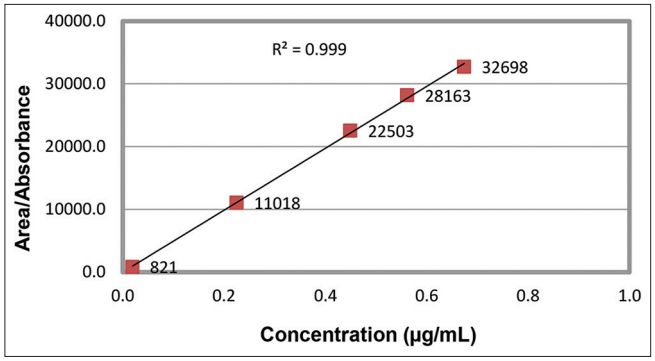



**Fig. 10:**

**LOD and LOQ**

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ precision was demonstrated by preparing LOQ solution as determined concentration and chromatographed the same into the HPLC system in six replicated injections. The LOQ precision was evaluated by computing the % RSD for the peak area of these standard injections. The observations are tabulated in Table 6.

*For LOQ precision*

Acceptance criteria

- The concentration is acceptable as LOD if the signal-to-noise ratio is between 3 and 2:1 (range should be between 2 and 6).
- The concentration is acceptable as LOQ if the signal-to-noise ratio is 10:1 (range should be between 10 and 20).
- The % RSD for six replicate injections of LOQ solution should be not more than 10.0.

Conclusion

The LOD and LOQ parameters were established as mentioned in the protocol and results were found satisfactory.

**Linearity**

The linearity of detector response was demonstrated by preparing solutions over the range of LOQ to 150% of the specification limit with respect to the sample concentration of DTX. These solutions were injected into the HPLC system and the responses of the same were recorded. A plot of concentration versus peak area was done. The coefficient of determination between concentration and response and % Y intercept was evaluated. The observations are in Table 8.

*Acceptance criteria*

- The coefficient of determination ($r^2$) should not be <0.98.
- The %Y intercept should be within ± 5.0 of the response corresponding to the target concentration.

**Accuracy**

The accuracy of the test method was demonstrated by preparing recovery samples at LOQ, 100%, and 150% of the target concentration level. The recovery samples were prepared in triplicate for each concentration level. The above samples were injected and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of the recovery at each level by computing the % relative standard deviation of triplicate recovery samples results tabulated in Table 13.

*Acceptance criteria*

- For the LOQ level, the % Recovery should be between 75.0 and 125.0
- For the above LOQ level, the % Recovery should be between 80.0 and 120.0

- The % RSD for the LOQ level should not be more than 15.0
- The % RSD for above LOQ levels should be not more than 10.0.

*Conclusion*

The accuracy parameter was established as mentioned in the protocol and results were found satisfactory.

**Range**

The range of test methods was established through the determination of linearity, accuracy, and precision from LOQ to 150% of the specification limit with respect to the concentration of DTX in the sample solution. The analytical method for the determination of organic impurities has shown suitable, accuracy, and linearity in the interval between LOQ to 150% level.

**Solution stability and mobile phase stability**

*Solution stability*

The stability of solutions such as standard solution and sample solutions was established at various conditions such as benchtop condition and autosampler (10°C) condition. The response of these was compared with respect initial standard solution and sample solution. Results are tabulated in Table 18.

*Standard solution stability*

*Sample solution stability*

*Acceptance criteria*

- The % recovery for standard solutions at regular intervals should be between 95.0 and 105.0.
- The % of impurities found should meet the specification limit.
- Difference of impurity in % w/w for 10-Deacetyl baccatin, 6-Oxodocetaxel, 4-Epidocetaxel, 4-Epi-6-oxodocetaxel, and any unspecified impurity should not be more than 0.05 and 0.2 for total impurities for unspiked sample solution

## CONCLUSION

The solution stability parameter was established as mentioned in the protocol. The standard solutions are stable up to 59 h in benchtop condition and 60 h stable in refrigerator (10°C) condition, and sample solutions are stable up to 49 h in benchtop and 50 h in refrigerator (10°C) condition.

## CONCLUSION

A sensitive and selective RP-HPLC method has been developed and validated for the analysis of Quantitative Estimation of Organic impurities of DTX in DTX concentrate for solution for infusion 40 mg/mL in fill volume-based parental dosage forms. Further, the developed RP-HPLC method has excellent sensitivity, precision, accuracy, and reproducibility. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed the suitability of the method to study the stability of DTX under various forced degradation conditions, such as acid, base, oxidative, thermal, UV, and photolytic degradations. Finally, it was concluded that the method is simple and sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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