

# LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR DETERMINATION OF DOCETAXEL IN K3EDTA HUMAN PLASMA

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## ABSTRACT

A rapid and sensitive liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for determination of the Docetaxel in K3EDTA human plasma was developed and validated. Docetaxel was extracted from plasma by extraction procedure with using of MTBE as Liquid – Liquid extractive organic solvent. LC-MS/MS analysis using electro-spray ionization (ESI+) was performed on Thermo betaseal C-18 Column using as 10mM Ammonium acetate with 0.1% FA, Acetonitrile (60:40) as mobile phase. The method has a flow rate of 0.6ml/min. Retention of Docetaxel was 1.25 minutes. An excellent linearity ( $r^2$  .099) between the peak ratios and Docetaxel concentrations over the range of 0.2-100 ng/ml of plasma was studied. The lower limit of detection for Docetaxel on mass was 0.2 ng/mL.

**Keywords:** Liquid chromatography-tandem mass spectrometry, Docetaxel, MTBE extraction.

## INTRODUCTION

Docetaxel [4-acetoxy-2a-benzoyloxy-5b, 20-epoxy-1, 7b, 10b trihydroxy-9-oxotax-11-ene-13a-yl-(2R, 3S)-3-tert-butoxycarbonylamino-2-hydroxy-3-phenylpropionate] is a novel anticancer agent of the taxoid family (Figure 1A). An analogue of Paclitaxel, Docetaxel was obtained by semi synthesis from 10-deacetyl baccatian III, extracted from the needles of the European yew tree *Taxus baccata* L.<sup>1, 2</sup>. Docetaxel promotes tubulin assembly into microtubules, stabilizes microtubules and inhibits microtubules depolymerization to free tubulin. This leads to disruption of the equilibrium within the microtubules system and ultimately leads to cell death. Docetaxel has been demonstrated to be effective against wide range of tumors including breast, lung, prostate, ovarian, head and neck, gastric, pancreatic, and bladder cancers<sup>3, 4</sup>.

A literature survey revealed that only a few HPLC<sup>5</sup> and LC-MS<sup>6-14</sup> methods are available for the estimation of docetaxel in biological fluids like human, rat and mouse plasma.

Docetaxel is given with other drugs like, Cyclophosphamide, Ketoconazole and other Antineoplastic drugs which could be associated during chemotherapy (Cisplatin, Verapamil, Doxorubicin, Vinblastine, and Vincristine<sup>15-17</sup> there is a need to evaluate pharmacokinetic parameters using a validated assay, with high specificity in order that co-administered drugs and or metabolites do not interfere with the measurement of Docetaxel.

The aim of the present study was investigate the Docetaxel extracted samples, and standard samples with high sensitivity, accuracy were studied with Liquid Chromatography- tandem mass spectrometry method which is use full for Pharmacokinetic analysis.

## 2. MATERIAL AND METHODS

### 2.1 Chemicals and Reagents

Docetaxel was purchased from Sigma-INDIA, HPLC grade Acetonitrile and Methyl tertiary butyl ether (MTBE) from JT Baker, Ammonium acetate and Formic Acid from Sigma Aldrich, HPLC grade water from Merck India.

### 2.2 Solutions

Stock solution of Docetaxel was prepared by dissolving 1 mg of Docetaxel in 1ml of acetonitrile. Standard solutions were obtained by diluting this solution with acetonitrile: water (50:50) to give the final concentrations over the range of 0.2-100ng/ml for preparation of the standard calibration curve in plasma. Further concentrations required for constructing quality control samples all the solutions were stored at 2–8°C. 10mM Ammonium acetate with 0.1% FA, Acetonitrile (60:40) was prepared for mobile phase and as well for reconstitution of Docetaxel extracted sample from plasma.

### 2.3 Chromatographic condition

LC-MS/MS, API, triple quadrupole Analyst software, version 1.2 coupled with Shimadzu SIL HTC HPLC separation module. Separation was achieved using Thermo betaseal C-18 Column (150x4.60 mm-5microns). The mobile phase contains 10mM Ammonium acetate with 0.1% FA, Acetonitrile (60:40) was prepared and degassed. Chromatographic separations were performed at 40°C. The flow rate was set to 0.6ml/min. Retention time for Docetaxel is 1.25mins and Paclitaxel (Internal standard) is 1.30mins and the total run time is 5.0mins.

### 2.4 LC-MS/MS parameters

Source (Es +):- Curtain Gas: 15; Source Temperature (°C): 500; Declustering potential (DP): 100; Entrance Potential (EP): 10.

Compound: - CAD: 20; Gas1:45; Gas2:55; Collision entrance potential (CE):15; Collision exit potential (CXP):20; Ionization potential (IP): 5500.

Q1/Q3 m/z: - Docetaxel (Analyte):  
808.500/509.000, 808.500/226.000,  
808.500/282.100, 808.500/327.100,  
808.500/527.100; Paclitaxel (Internal standard):-  
854.400/286.000

### 2.5 Sample preparation and Sample extraction

Each 200µL of blank human plasma was aliquoted into each test tube and 5µL of standard solutions of Docetaxel was spiked to make the final concentration of 0.2 to 100ng/mL. These tubes are

vortex for 1mins by adding Internal standard 50 ng/ml. After adding 2ml MTBE to the test tubes, vortexed for 5 mins and the tubes were flash freeze in cold methanol for 2 mins until the aqueous layer solidify. That MTBE layer was completely removed and transferred to a clean test tube and evaporated to dryness at 45°C. These samples were reconstituted with mobile phase. LC-MS/MS system and separation is performed by reversed phase HPLC. 25µL of each sample was injected to LC-MS/MS.

### 2.6 Quantification

Calibration standards of Docetaxel were prepared by spiking 5µL of Docetaxel standard solutions to 200µL of blank human plasma to give final concentrations over the range of 0.2-100ng /mL. Calibration curves were prepared by plotting the measured peak area ratios of Docetaxel vs. concentration of standard samples. The intraday (within run) and inter day (between run) accuracy and precision of the method was determined by measuring samples on three separate days.

### 2.7 Calibration standard and quality control samples

The standard calibration samples were prepared by diluting working standard solution ( 100 µg/mL) to yield ten different concentrations over a linearity range of 0.2–100 ng/mL (i.e., 0.2, 0.4, 1.0, 2.0, 5.0, 10, 20, 50, 80 and 100 ng/mL). The quality control (QC) samples were prepared in same method as standard solutions to yield low, medium, and high concentrations (0.6, 45, and 75 ng/mL). Linearity of drug was evaluated over the concentration range of 0.2–100 ng/mL using least square linear regression analysis. Regression equations were used for determination of concentrations of QC samples containing both Docetaxel by replicate injections.

### 2.8 Linearity

Linearity was performed by analysis of ten different concentrations in the range between 0.2–100 ng/mL for both drugs. Each concentration was injected and measured in triplicate. The relation between concentration (x) and its corresponding peak area ratio (y), that is, expressed by the equation  $y = mx + c$  for both drugs (m = slope, c = intercept).

### 2.9 Inter- and intra-day accuracy and precision

A total of ten calibration standards were prepared on different days to evaluate inter- and intra-day accuracy and precision. In addition, in-house

quality control standards were run in replicates of six to evaluate intra-day and inter-day accuracy and precision. Accuracy was determined as the absolute value of the ratio of the back-calculated mean values to their respective nominal values and expressed as percentage. Precision was determined by the percentage coefficient of variation (%CV) for QC concentrations.

### 2.10 Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by replicate injections of binary mixtures. The LOD was defined as a minimum signal-to-noise of 3. The LOQ was determined by taking S/N ratio of 10.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Specificity and selectivity

Plasma samples from six different lots were tested for the presence of endogenous components, which might interfere with the detection of Docetaxel or the internal standard. These samples were pre-treated according to the sample preparation procedure, apart from addition of the internal standard solution. Chromatograms of blank plasma and plasma sample spiked with Docetaxel (0.2 ng/mL) and Paclitaxel (50.0 ng/mL) were compared to show the specificity and selectivity of the proposed procedure. The chromatograms are presented in Figs. 1, 2, 3 & 4. The retention times of Docetaxel and Paclitaxel were 1.25 and 1.3 min, respectively. No endogenous components interfering with the detection of Docetaxel and Paclitaxel were found in the chromatograms of blank plasma samples.

### 3.2 Recovery

The percentage of recovery of Docetaxel is observed as more than 65%.

### 3.3 Accuracy

The accuracy of the assay was defined as the absolute value of the ratio of the back calculated mean values of the quality control samples to their normal values, expressed as percentage. Within the batch, accuracy range from 90.54% to 112.93. Between the batch accuracy range from 97.70%-105.37%. The results are summarized in Table 1.

### 3.4 Precision

The precision of the assay was measured by the percent coefficient of variation over the concentration range of quality control samples,

respectively of Paclitaxel during the course of the study. Within batch Precision ranged from 0.04% to 15.19%. Between batch Precision ranged from 3.84% to 10.49%. The results are summarized in Table 1.

### 3.5 Stability

Short-term stability of Docetaxel was determined by comparing the mean of the area responses obtained from 3 replicate analysis of aqueous standard (45 ng /ml) at 0.0 hours and after 6.0 hours. Ratio of means of area was 102.5%. This is within the acceptance range of 90-110%.

Long-term stability of Docetaxel was determined by comparing the mean of the area responses obtained from 3 replicate analysis of aqueous standard (45 ng/ml) after 12 days and freshly prepared aqueous standard. Ratio of means of area was 101.7%. This is within the acceptance range of 90-110%.

Room temperature stability was assessed by analyte determination in five sets of MQC (45.0 ng/mL) in triplicate. Each set was left at room temperature for various times (0, 15, 30, 60, 120 min) before sample processing. Since the difference of the nominal value of Docetaxel found was lower than 7%, it was concluded that Docetaxel is stable up to 2 h at room temperature before sample processing.

Autosampler stability was estimated by analysis of QC samples (three series of LQC and HQC). Samples were analyzed at the beginning of the test and after 24 h while stored in autosampler at 4°C. The results of both sets of data differed by less than 5% from the nominal value, which proved the desired stability of the analyte during storage in auto sampler.

Freeze-thaw stability ( $-20 \pm 5$  °C) was determined as percent recovery compared to the nominal value of LQC and HQC1 (0.6 and 75 ng/mL) in triplicate. The test was carried out within 4 days in 4 runs. Every day the samples were thawed for analysis and frozen again. The difference of the nominal value varied between  $-1.3$  and  $10.9\%$  in the fourth cycle being  $8.9\%$  for LQC and  $-1.1\%$  for HQC. It was concluded that four cycles of freeze-thaw could be carried out with no loss of Docetaxel. Long-term stability at  $-20 \pm 5$  °C was performed in 4 runs over 3 months. Per-cent recovery of LQC and HQC (0.6 and 75 ng/mL) in triplicate was determined and compared to the nominal value. The obtained data showed no loss of Docetaxel. The results are summarized in Table 2.

All plasma samples for stability evaluation were prepared as per sample extraction procedure. To

verify reliability of the method, the measured concentrations were not differing by more than 15% from the nominal value. All results of stability tests implied good stability of Docetaxel over all steps of determination; therefore the method was proved to be applicable for routine analyses.

### CONCLUSION

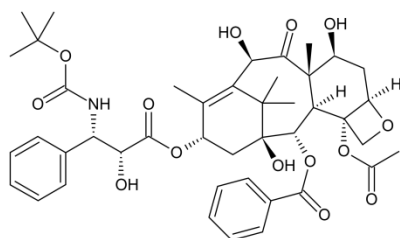
The method for the determination of Docetaxel in human K3EDTA plasma covering the concentration range 0.2–100 ng/mL, using 0.2 mL of plasma was proposed and validated. No interferences from endogenous plasma components or other sources were found and no “cross-talk” was observed in plasma samples. The assay showed good precision and accuracy. A simple preparation procedure and short retention time could allow determination of more than 250 samples per day. The analytical method presented here has been proved useful for the investigation of the characteristics of Docetaxel in human plasma in pharmacokinetic studies.

**Table 1: Precision and Accuracy of assay at three QC concentration levels of Docetaxel**

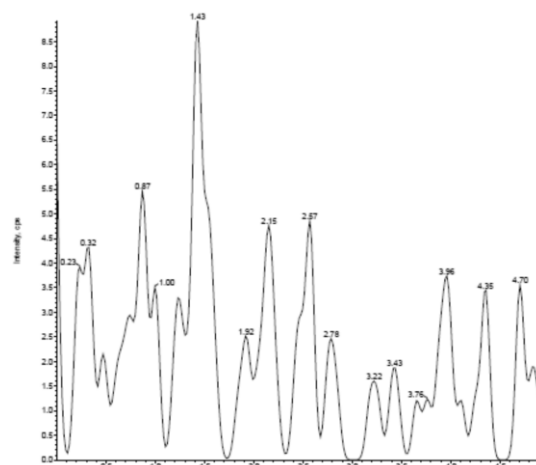
Within batch precision (%RSD) n=6	0.04% to 15.19%
Within bath accuracy(%nominal) n=6	90.54% to 112.93%
Between batch precision(%RSD)n=6	3.84% to 10.49%
Between batch accuracy (%nominal)n=6	97.70% to 105.37%

**Table 2: Stability data**

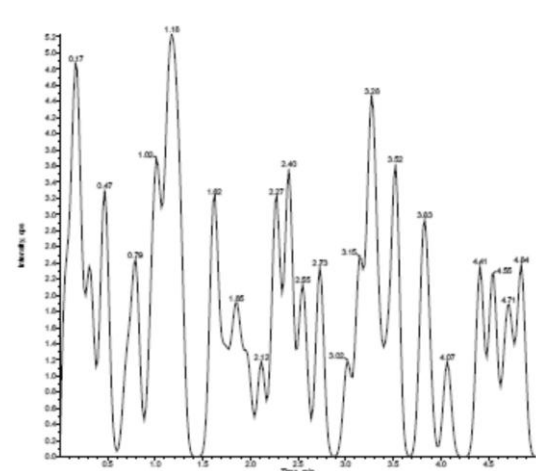
Experiment	QC level	Change %	Stability Duration
Room Temp. Stability	LQC	-6.5	2.0 Hr
	HQC	-5.0	
Auto sampler Stability	LQC	-4.3	24 Hr
	HQC	-2.7	
Freeze-thaw Stability	LQC	-1.3	4 cycles
	HQC	10.9	
Long term stability	LQC	-7.5	3 months
	HQC	-6.7	



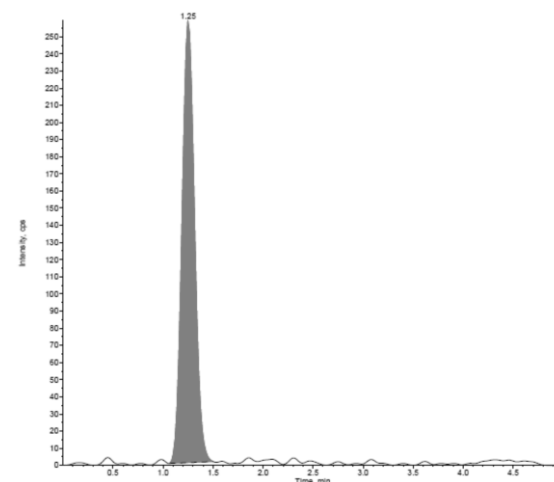
**Fig. 1A: Docetaxel**



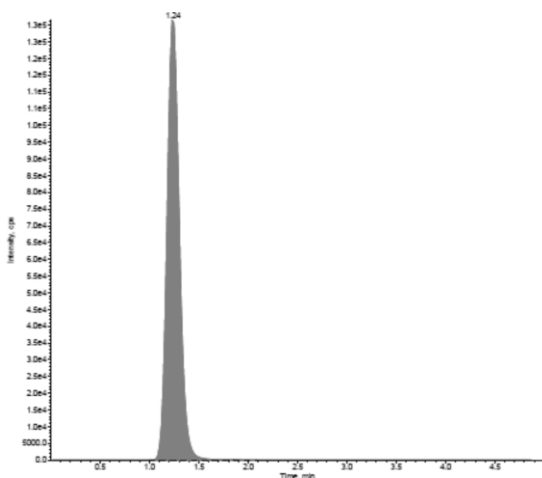
**Fig. 1: Blank plasma in Docetaxel (Analyte)**



**Fig. 2 Blank Plasma in Paclitaxel(I S)**



**Fig. 3: Docetaxel (0.2 ng/mL)**



**Fig. 4: Paclitaxel (50.0 ng/mL)**

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