For Research Use Only. Not for use in diagnostic procedures.

Highly Sensitive and Selective Bioanalytical Quantitation Method for Docetaxel in Human Plasma using 4000 QTRAP® LC/MS/MS System

Santosh Kapil, Aman Sharma, Jeyan A, Anoop Kumar, Manoj Pillai *SCIEX, 121, Udyog Vihar, Phase IV, Gurgaon, Haryana, India*

INTRODUCTION

Docetaxel **(Figure 1)** is an important antineoplastic agent used in various cancer therapies. It is used mainly for the treatment of breast, ovarian, prostate and non–small cell lung cancer. It is a semi synthetic analog of taxol which inhibits microtubule disassembly and inhibits cell replication. Inhibition of these structures ultimately results in cell death. Docetaxel has high protein binding nature which results in improper assay results. Docetaxel was the first drug of cancer with the name of Texotere in France. Docetaxel is given intravenously, on its own or in combination of other chemotherapy drugs. We now report a rapid, specific, highly sensitive and specific LC/MS/MS method capable of quantifying low level of docetaxel from 0.2 to 2000 ng/ml in human plasma using paclitaxel **(Figure 2)** as internal standard.

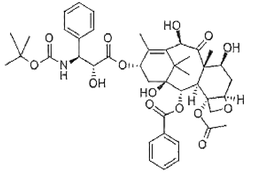


Figure 1: Structure of Docetaxel

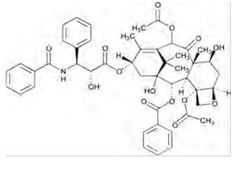


Figure 2: Structure of Paclitaxel

To provide robust support for its pharmacokinetic studies, a rapid and sensitive LC-MS/MS method was developed and validated for the analysis of docetaxel in plasma samples.

Key Features of the Method using SCIEX 4000 QTRAP® System

Sensitivity & Selectivity In Human Plasma – Low level of quantification in plasma samples with Low detection limits of 0.2ng/mL in plasma.

Dynamic Range – High plasma concentrations (Cmax) requires Quantitation over a wide range.

High throughput Analysis – Fast sample preparation with excellent reproducibility and recovery results.

Excellent Precision And Accuracy – Data quality (LLOQ QC, LQC, MQC and HQC levels) met USFDA bioanalytical method validation criteria.

Features of SCIEX 4000 QTRAP® System

The Turbo V™ Source having efficient ionization, enhance gas dynamics, improve quantitation levels and decreases chemical noise

Curtain Gas™ interface - Pristine ceramic interface having Proven Curtain Gas™ interface technology combined with improved gas dynamics

LINAC® collision cell- Patented LINAC high-pressure collision cell technology to ensure maximum ion transfer from the interface to the detector.

Digital pulse counting detector provides consistent quantitative results over a wide dynamic range

MATERIALS AND METHODS

Sample Preparation:

An aliquot of 490µL of human plasma samples were spiked (2%) with Docetaxel standard and 25 µL internal standard (Paclitaxel) solution. The samples were vortexed and 2mL of Tertiary butyl methyl Ether (TBME) was added to it for liquid-liquid extraction and kept for vortexing followed by centrifugation at 5ºC, 4000 rpm for 10 min. The upper layer was transferred to another glass tube and evaporated to dryness under a stream of nitrogen at 40ºC and reconstituted with 500 µL methanol: water (4:1, v/v) for quantitation in 4000 QTRAP® LC-MS/MS system.

HPLC Conditions:

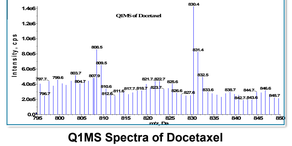
A GL Sciences LC 800 System was used, The chromatography was achieved on Phenomenex Gemini 5µ C18 110 ˚ A (150 x 4.6 mm, 5 µm) column at the flow rate of 0.8 mL/min. Isocratic elution was carried out using 0.1% formic acid in water (Mobile Phase A) and 0.1% formic acid in methanol (Mobile Phase B) in the ratio of (1:4 v/v). The total run time was 6.5 min. The injection volume was set to 20µL. The rinsing solution was Methanol: Water (50/50) mixture.

MS Conditions:

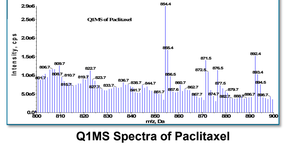
Analyte	MRM	Dwell Time (ms)	DP	EP	CE	CXP
Docetaxel +Na	830.4/549.1	200	156	10	35	16
Paclitaxel +H	854.4/286.2	200	156	10	35	8

Curtain	20
CAD	8
ISV	5500
Temp	650
GS1	50
GS2	50

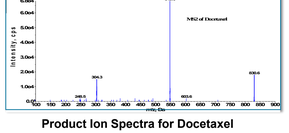
The optimization was performed for ion source dependent parameters for Docetaxel and Paclitaxel. figure below shows the presence of ions of interest.



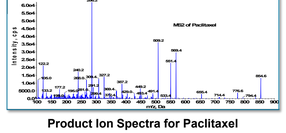
Q1MS Spectra of Docetaxel



Q1MS Spectra of Paclitaxel



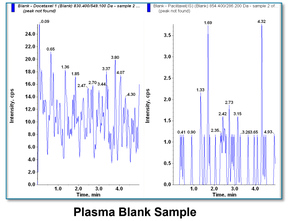
Product Ion Spectra for Docetaxel



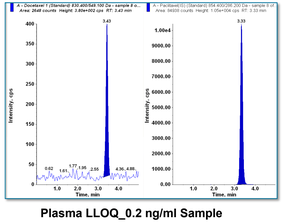
Product Ion Spectra for Paclitaxel

RESULTS

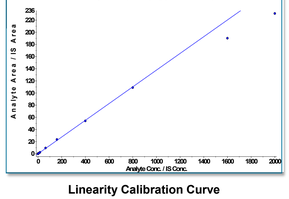
Docetaxel standards extraction from human plasma gave a linear calibration curve over the dynamic range from 0.2 to 2000 ng/mL. At LLOQ (0.2 ng/mL) signal to noise ratio was 47.3. **Figure below** shows representative peaks for blank extract, plasma spiked with 0.2 ng/mL docetaxel. The calibration curve for docetaxel in human plasma shows excellent linearity over 4.0 orders of magnitude concentration range with an r value of >0.99.
Paclitaxel was adopted as internal standard because of similarity of ionization. Analyte retention time and internal standard peak retention times were consistent, docetaxel eluting at 3.4 min and paclitaxel at 3.3 min. QC samples were run in replicates of six at concentration of 0.200ng/mL (LLOQ QC), 0.590ng/mL (LQC), 160.666ng/mL(MQC), 803.334ng/mL (M1QC) and 1606.666 ng/mL (HQC). The calculated concentration correlates well with the actual spiked-analyte concentration in plasma matrix with a per cent recovery of 87% for all concentrations of analyte and 81% for internal standard.
The below chromatograms are Plasma blank, LLOQ, Internal Standard, and the linearity curve



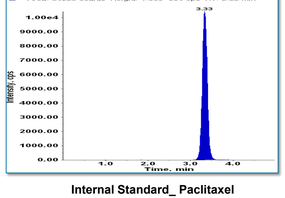
Plasma Blank Sample



Plasma LLOQ_0.2 ng/ml Sample



Linearity Calibration Curve



Internal Standard_ Paclitaxel

The peak shape for internal standard (paclitaxel) used for the assay of the docetaxel in human plasma and showed no interference in the plasma extraction method.
The % CV for Internal standard in extracted samples from PA BATCH is 4.40%. it shows good reproducibility of the results. The Total run time for each sample was 6.5 min, using a flow rate rapid enough for high-throughput analysis showed good sensitivity and reproducibility at all levels of concentration.

Table: The QC concentrations at different ranges was injected to check the repeatability of docetaxel in plasma matrix

Precision and accuracy of docetaxel replicates measured as a single group					
Docetaxel Sample	Nominal Concentration (ng/mL)				
	LLOQ QC	LQC	MQC	M1QC	HQC
	0.2	0.59	160.666	803.334	1606.666
1	0.196	0.629	169.746	769.587	1536.367
2	0.204	0.641	174.528	815.202	1447.4
3	0.198	0.606	159.033	729.601	1392.062
4	0.177	0.667	179.577	859.353	1360.105
5	0.188	0.589	181.631	752.074	1360.636
6	0.197	0.621	161.546	801.094	1342.197
7	0.203	0.616	164.902	702.431	1402.695
8	0.188	0.579	165.38	737.469	1512.053
9	0.201	0.666	173.164	735.564	1356.372
10	0.191	0.675	153.29	727.962	1553.487
11	0.207	0.652	148.642	723.775	1429.144
12	0.216	0.658	153.184	722.839	1394.066
13	0.197	0.559	161.027	792.935	1371.495
14	0.202	0.581	162.645	795.618	1437.971
15	0.217	0.561	156.833	807.537	1391.122
16	0.224	0.612	164.258	742.44	1353.737
17	0.226	0.642	161.05	789.438	1401.414
18	0.213	0.501	161.44	779.739	1448.229
Mean	0.2	0.61	163.99	765.81	1416.14
S.D (+/-)	0.013	0.046	8.943	41.582	63.435
C.V. (%)	6.5	7.54	5.45	5.43	4.48
% Nominal	100	103.39	102.07	95.33	88.14
N	18	18	18	18	18

CONCLUSIONS

- A higher sensitivity and high throughput LC-MS/MS method of docetaxel was validated to quantify docetaxel concentration for the bioequivalence study in human plasma on the 4000 QTRAP® System
- Lower limit of quantification for docetaxel was achieved 0.2 ng/mL and analyte recovery is 87%, even under high-throughput conditions.

REFERENCES

1. Wang, R, Wu, XY, Xie,H, Zhang,JH, Ma, Men,XD, Wang, J, Zhang,JL, Li, WB and Jia, ZP "The pharmacokinetics evaluation and bioequivalence of new docetaxel injections and taxotere using healthy rats." Journal of Bioanalysis & Biomedicine. 2010; 2 (1): 023-027.
2. Terish, JD, Kumar S, Ramesh N and Sasi jith SL "Simultaneous estimation of docetaxel and vehicle polysorbate 80 in human plasma by liquid chromatography / tandem mass spectrometry in injectable dosage forms". International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2(3): 1215-1219

TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures.
AB Sciex is doing business as SCIEX. The trademarks mentioned herein are the property of Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.
© 2016 AB Sciex.
Document number: RUO-MKT-10-4079-A