VALIDATED BIOANALYTICAL METHOD

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Director: Dr. James Mohler

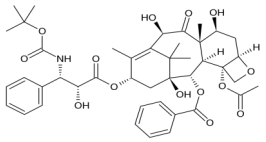
Docetaxel in Heparinized Human Plasma

(Sensitivity: 0.200 ng/mL)

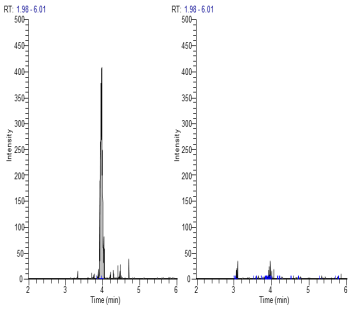
BMPK has validated a highly sensitive HPLC assay with tandem mass spectrometric detection (LC-MS/MS) for the analysis of docetaxel (Taxotere®) in heparinized human plasma. Docetaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells, which is essential for mitotic and interphase cellular functions.[1] Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. *In vitro* drug interaction studies have shown that docetaxel is metabolized by the CYP3A4 isoenzyme and its metabolism can be inhibited by CYP3A4 inhibitors, such as ketoconazole, erythromycin, troleandomycin, and nifedipine.[1] Based on these *in vitro* findings, it is likely that CYP3A4 inhibitors and/or substrates may lead to substantial increases in docetaxel blood concentrations. Currently, it is approved alone or in combination with other agents for locally advanced or metastatic breast cancer, non-small cell lung cancer, hormone refractory prostate cancer, gastric adenocarcinoma, and squamous cell carcinoma of the head and neck cancer.

Specifications and Validation Performance

Matrix (Anticoagulant):	**Human Plasma (Sodium Heparin)**
Sample Volume:	**200 µL**
Preparation Procedure:	**Liquid / liquid extraction**
HPLC Column:	**C18**
Mobile Phase:	**Acetonitrile with Ammonium Acetate**
Flow Rate:	**200 µL/min**
Detection Type:	**Tandem Mass Spectral Analysis (MS/MS)**
Calibration Range:	**0.200 - 400 ng/mL**
Calibrator Accuracy:	**100% (93.6 - 108%; n=5)**
Calibrator Precision:	**2.56% CV (1.42 - 4.84%; n=5)**
QC Concentrations:	**0.750, 15.0 and 300 ng/mL**
QC Accuracy:	**105% (104 - 105%; n=18)**
QC Precision:	**4.45% CV (3.75 - 5.13%; n=18)**



Docetaxel
Formula: $C_{43}H_{53}NO_{14}$
MW: 807.88 g/mol



Human Pharmacokinetic Parameters of Docetaxel[1,2,3]

Single Agent Recommended Dosing	**60-100 mg/m^2 IV qw followed by 7 day rest; dependent on disease type and prior treatment**
Single Agent Maximum Tolerated Dose (MTD)	**>125 mg/m^2 IV qw followed by 7 day rest, dependent on disease type and prior treatment**
Active Metabolites	**None**
Metabolism	**75% excreted in feces and 6% in urine after 7 days as oxidized metabolites (>8% as unchanged drug)**
Plasma Protein Binding	**94% *in vitro*, 97% *in vivo***
Overall Exposure by Area Under the Curve (AUC)	**Dose proportional from 70-115 mg/m^2 using a three-compartment pharmacokinetic model**
Major Adverse Reactions	**Hepatoxicity, neutropenia, hypersensitivity, fluid retention**

[1]Patient Information Leaflet for Taxotere, Aventis Pharmaceuticals, Inc., Revision. May 2004; [2]CDER Application Number NDA 20-449/S-035, Approved March 22, 2006; and [3]Clinical Pharmacokinetics of Docetaxel, Clin Pharmacokinet, 45 (3), 2006.

BMPK offers a wide range of bioanalytical and PK/PD modeling services to assist investigators with their basic research, preclinical, and clinical study objectives. For information on services and pricing, contact Wenjuan Zha, Ph.D., Associate Director at (716) 845-3258 or Wenjuan.Zha@RoswellPark.org.

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