VALIDATED BIOANALYTICAL METHOD

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Director: Dr. James Mohler

Gemcitabine in Heparinized Human Plasma

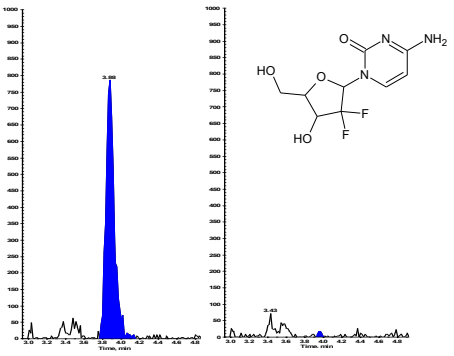
(Sensitivity: 1.00 ng/mL)

BMPK has validated a highly sensitive liquid chromatographic tandem mass spectral assay (LC-MS/MS) for the analysis of gemcitabine and its inactive uracil metabolite, 2',2'-difluorodeoxyuridine (dFdU) in heparinized human plasma. Gemcitabine is converted *in vivo* into the active metabolites gemcitabine diphosphate and triphosphate, which decrease the deoxynucleotide pool available for DNA synthesis and inhibit the cell cycle through phase boundaries.[1] The validated method has been used to support a clinical trial conducted at Roswell Park Comprehensive Cancer Center entitled "*Phase I Study of Ceritinib (LDK378), a Novel ALK Inhibi-*

Specifications and Validation Performance

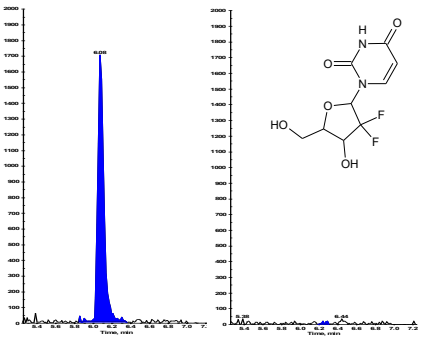
Matrix (Anticoagulant):	Human plasma (lithium heparin) collected in tubes containing THU (tetrahydrouridine, a cytidine deaminase inhibitor).
Required Volume:	100 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	Phenyl-hexyl
Mobile Phase:	10.0 mM Ammonium Acetate\Acetonitrile containing Acetic Acid
Flow Rate:	250 µL/min
Detection Type:	Tandem Mass Spectral (MS/MS)
Calibration Ranges:	1.00 - 2,000 ng/mL for Gem 5.00 - 10,000 ng/mL for dFdU
Calibrator Accuracy:	99.9% (95.6 - 103%; n=5) for Gem 100% (96.1 - 102%; n=5) for dFdU
Calibrator Precision:	2.28% RSD (1.19 - 4.53%; n=5) for Gem 2.40% RSD (1.45 - 4.88%; n=5) for dFdU
QC Concentrations:	4.00, 75.0, 1,500 ng/mL for Gem 20.0, 375, 7,500 ng/mL for dFdU
QC Accuracy:	93.9% (92.8 - 95.8%; n=18) for Gem 99.2% (95.2 - 101%; n=18) for dFdU
QC Precision:	2.77% RSD (2.64 - 2.87%; n=18) for Gem 2.75% RSD (2.47 - 3.15%; n=18) for dFdU

Gemcitabine (Gem)



LLOQ (S/N = 44) Matrix Blank

2',2'-Difluorodeoxyuridine (dFdU)



LLOQ (S/N = 27) Matrix Blank

Human Pharmacokinetic Parameters of Gemcitabine[1]

Recommended Dose:	1,000 - 1,250 mg/m^2 IV infusion over 30 min on Days 1, 8, and/or 15 of a 21 or 28-day cycle depending on the indication and combination therapy.
Terminal Phase $t_{1/2}$:	1.7 - 19.4 hours for Gemcitabine Triphosphate
Clearance:	Clearance of gemcitabine is affected by age and gender; lower clearance rates in geriatric patients and women can result in higher concentrations for any dose.
Indications:	Advanced ovarian, metastatic breast, non-small cell lung, and pancreatic cancer.

[1]Gemzar® Patient Information, Lilly USA, LLC , Revised 12/2018.

BMPK offers a wide range of bioanalytical and PK/PD modeling services to assist investigators in 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.

ROSWELL PARK COMPREHENSIVE CANCER CENTER