VALIDATED BIOANALYTICAL METHOD

# Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

## 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 Inhibitor, in Combination with Gemcitabine-Based Chemotherapy in Patients with Advanced Solid Tumors*".**

### Specifications and Validation Performance

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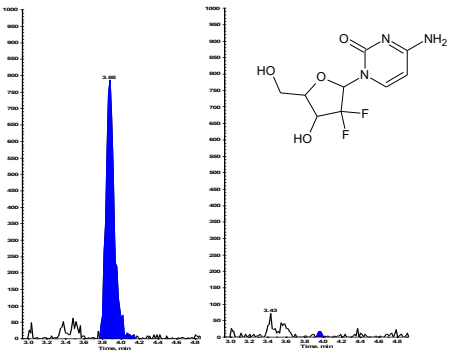
|---|---|
| **Matrix (Anticoagulant):** | Human plasma (lithium heparin) collected in tubes containing THU (tetrahydrouridine, a cytidine deaminase inhibitor). |
| **Required Volume:** | 100 µL |
| **Preparation Procedure:** | Protein Precipitation |
| **HPLC Column:** | Phenyl-hexyl |
| **Mobile Phase:** | 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<br>5.00 - 10,000 ng/mL for dFdU |
| **Calibrator Accuracy:** | 99.9% (95.6 - 103%; n=5) for Gem<br>100% (96.1 - 102%; n=5) for dFdU |
| **Calibrator Precision:** | 2.28% RSD (1.19 - 4.53%; n=5) for Gem<br>2.40% RSD (1.45 - 4.88%; n=5) for dFdU |
| **QC Concentrations:** | 4.00, 75.0, 1,500 ng/mL for Gem<br>20.0, 375, 7,500 ng/mL for dFdU |
| **QC Accuracy:** | 93.9% (92.8 - 95.8%; n=18) for Gem<br>99.2% (95.2 - 101%; n=18) for dFdU |
| **QC Precision:** | 2.77% RSD (2.64 - 2.87%; n=18) for Gem<br>2.75% RSD (2.47 - 3.15%; n=18) for dFdU |

**Gemcitabine (Gem)**



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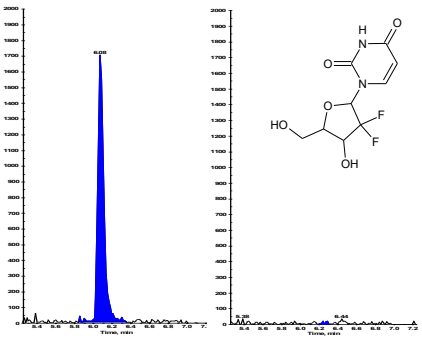
**2',2'-Difluorodeoxyuridine (dFdU)**



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### Human Pharmacokinetic Parameters of Gemcitabine[1]

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|---|---|
| **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 Joshua Prey, MS, Research Project Administrator at (716) 845-3313 or Joshua.Prey@RoswellPark.org.**

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