VALIDATED BIOANALYTICAL METHOD

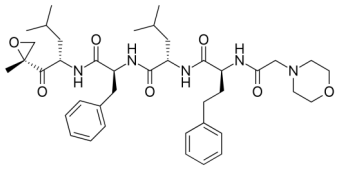
Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Carfilzomib in Heparinized Human Plasma

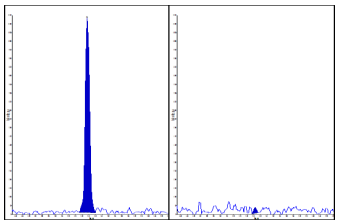
(Sensitivity: 400 pg/mL)

BMPK has validated a highly sensitive liquid chromatographic tandem mass spectrometric assay (LC-MS/MS) for the analysis of carfilzomib in sodium heparinized human plasma. Carfilzomib is a tetrapeptide epoxyketone proteasome inhibitor that irreversibly and selectively binds to N-terminal threonine-containing active sites of the 20S proteasome, the proteolytic core particle of the 26S proteasome. Carfilzomib (Kyprolis™, Amgen) was initially approved by the FDA in 2012 and is indicated for use as a single agent or in combination with dexamethasone, or lenalidomide plus dexamethasone, for the treatment of patients with relapsed or refractory multiple myeloma, who have received one or more lines of therapy (1). The validated method has been used to support an ongoing Roswell Park clinical trial entitled "Phase I/II Study of Carfilzomib plus Rituximab plus Ifosfamide plus Carboplatin plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-cell Lymphoma (DLBCL)".

Specifications and Validation Performance	
Matrix (Anticoagulant):	Human Plasma (Sodium Heparin)
Required Volume:	100 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	C18
Mobile Phase:	Acetonitrile with NH4 Formate / Formic Acid
Flow Rate:	600 µL/min
Detection Type:	Tandem Mass Spectral (MS/MS)
Calibration Ranges:	0.400 - 1,000 ng/mL
Calibrator Accuracy:	100% (97.8 - 101%; n=5)
Calibrator Precision:	2.23% CV (0.942 - 3.97%; n=5)
QC Concentrations:	1.20, 30.0 and 750 ng/mL
QC Accuracy:	104% (102 - 106%; n=18)
QC Precision:	3.11% CV (2.47 - 3.70%; n=18)



Carfilzomib
$C_{40}H_{57}N_5O_7$
MW: 719.922



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Human Pharmacokinetic Parameters of Carfilzomib[1,2]	
Recommended Dosing	20/27, 20/56 mg/m^2 twice weekly or 20/70 mg/m^2 once weekly administered as an intravenous infusion over 10-30 minutes
Maximum Tolerated Dose (MTD)	20/56 mg/m^2 twice weekly
Mechanism of Action	Irreversibly binds to 20S proteasome; anti-proliferative and proapoptotic activity *in vitro* in solid and hematologic tumors
Active Metabolites	None
Metabolism	Rapidly metabolized by peptidase and epoxide hydrolysis; ~25% of dose excreted in urine as metabolites in 24 hours
Plasma Protein Binding	97% in humans over the range of 0.40 to 4.0 µM
Maximum Plasma Concentration (C_{max})	Dose-dependent increase in C_{max} and AUC_{inf} at doses between 20 and 70 mg/m^2 administered as a 30 min infusion
Plasma Terminal Half-Life ($t_{1/2}$)	0.4 - 1.2 hours

[1]Kyprolis Package Insert, 09/2018 and [2]Carfilzomib, Investigator's Brochure, 13 October 2016.

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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