VALIDATED BIOANALYTICAL METHOD

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Director: Dr. James Mohler

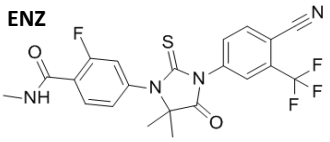
Enzalutamide in EDTA Human Plasma

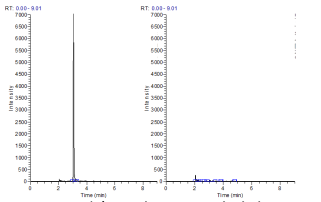
(Sensitivity: 10.0 ng/mL)

BMPK has validated a highly sensitive liquid chromatographic tandem mass spectral assay (LC-MS/MS) for the analysis of enzalutamide (ENZ) and its active metabolite, N-desmethyl enzalutamide (DM-ENZ), in EDTA plasma. Enzalutamide (MDV3100) is approved in the US for patients with metastatic and non-metastatic castration-resistant prostate cancer (CRPC). Enzalutamide is an androgen receptor (AR) signaling inhibitor. It induces CYP3A4, which may result in increased metabolism of some concomitantly administered drugs such as sorafenib. The validated method was applied to a clinical trial conducted by Memorial Sloan Kettering for treatment of advanced hepatocellular carcinoma with Roswell Park participating as a satellite site. The assay has also been qualified in woodchuck plasma for preclinical use.

Specifications and Validation Performance	
Matrix (Anticoagulant):	Human Plasma (Dipotassium EDTA)
Required Volume:	50.0 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	C18
Mobile Phase:	Methanol with Formic Acid
Flow Rate:	300 µL/min
Detection Type:	Tandem Mass Spectral (MS/MS)
Calibration Ranges:	10.0 - 20,000 ng/mL for ENZ and DM-ENZ
Calibrator Accuracy:	100% (93.2 - 104%; n=5) for ENZ
	100% (94.4 - 104%; n=5) for DM-ENZ
Calibrator Precision:	2.00% CV (1.23 - 2.86%; n=5) for ENZ
	2.15% CV (1.26 - 3.60%; n=5) for DM-ENZ
QC Concentrations:	30.0, 675, 15,000 ng/mL for ENZ and DM-ENZ
QC Accuracy:	102% (95.3 - 107%; n=18) for ENZ
	102% (95.3 - 106%; n=18) for DM-ENZ
QC Precision:	2.61% CV (2.21 - 3.04%; n=18) for ENZ
	2.66% CV (2.23 - 3.07%; n=18) for DM-ENZ

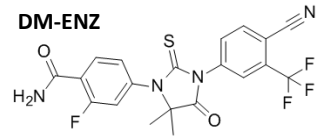
ENZ

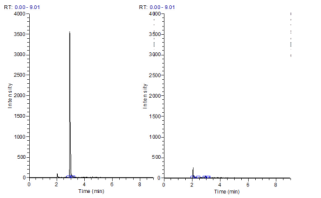




ENZ LLOQ (S/N = 157) Matrix Blank

DM-ENZ





DM-ENZ LLOQ (S/N = 179) Matrix Blank

Human Pharmacokinetic Parameters of Enzalutamide[1]	
Recommended Dose	160 mg once daily (oral gelatin capsule)
Maximum Tolerated Dose (MTD)	240 mg once daily
Bioavailability	84.2%
Active Metabolite	N-desmethyl enzalutamide
Metabolism	CYP2C8 and CYP3A4/5
Plasma Protein Binding	97 - 98% (constant from 0.05 - 25 µg/mL)
Maximum Plasma Concentration (C_{max})	3.36 µg/mL
Time to Maximum Plasma Concentration (T_{max})	1 - 2 hours post dose
Terminal Half-Life ($t_{1/2}$)	5.82 days (range: 2.8 - 10.7 days)

[1]Astellas Investigator's Brochure (Edition 7).

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.

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