VALIDATED BIOANALYTICAL METHOD

# Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

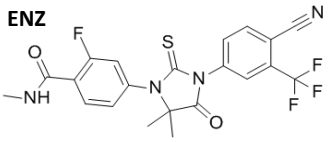
# Enzalutamide in EDTA Human Plasma

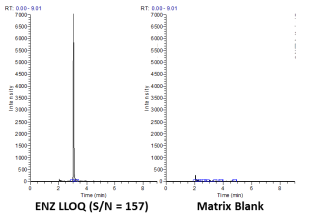
**(Sensitivity: 10.0 ng/mL)**

**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 |

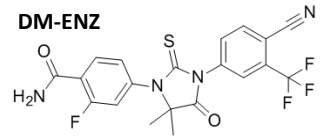
ENZ

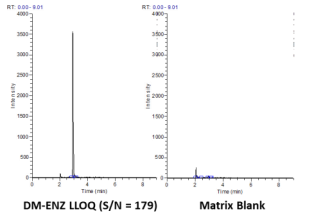




ENZ LLOQ (S/N = 157) Matrix Blank

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

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