SMALL MOLECULE BIOMARKER ANALYSES BY LC-MS

SMALL MOLECULE BIOMARKER LC-MS ASSAYS DESIGNED TO QUICKLY DELIVER FIT-FOR-PURPOSE STUDY DATA THAT MEETS OR EXCEEDS DATA QUALITY AND EXPERIMENTAL OBJECTIVES FOR NON-REGULATED STUDIES.

Bioanalysis of biomarkers presents multiple, inherent challenges including, separation of isobaric interferences, instability/inter-conversions of analytes in biological matrices, limited sample volume for use across several assays, and quantification of concentrations below naturally occurring endogenous levels.

The scientists at Meadowhawk Biolabs are well versed in applying flexible and innovative experimental approaches to effectively address these challenges for both simple and complex assays across a wide range of matrices including plasma and tissue homogenates (Refer to Table below).

LC-MS Biomarker Assay Design Considerations

Biomarker Modulation	Experimental Approach	Blank Matrix	Calibration Standards & QCs	Assessments
Up or Down Regulation	Stripped Matrix	Authentic biological matrix modified to eliminate target endogenous compound(s) (e.g. activated charcoal)	Actual Biomarker Analytical Reference Standard(s)	• Confirm absence of analyte across multiple preparations/lots • Create matrix pool for consistency between batches and studies
	Surrogate Matrix	Target compound free substitute matrix mimicking the behavior of the biological sample matrix (e.g. Bovine Serum Albumin in buffer)	Actual Biomarker Analytical Reference Standard(s)	• Establish surrogate and authentic matrices equivalency through dilutional linearity and spike recovery experiments • Prepare QCs in surrogate, authentic or combination of matrices based on endogenous analyte levels
	Surrogate Analyte	Study sample matched, authentic biological matrix	Targeted Biomarker Stable Isotope Labeled Reference Standard	• Evaluate and balance LC-MS responses of native biomarker and stable isotope reference standard • Demonstrate parallelism of calibrants prepared by authentic and surrogate analytes
Up Regulation (≥ 3-fold change)	Standard Addition	Study sample matched, authentic biological matrix	Actual Biomarker Analytical Reference Standard(s)	• Demonstrate acceptable levels of endogenous background concentrations versus quantification range • Create matrix pool for consistency between batches and studies

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To meet study objectives at points along the drug discovery continuum, Meadowhawk offers a tiered, fit-for-purpose approach to best manage time, costs and complexity. Meadowhawk's facilities feature state-of-the-art instrumentation, including Sciex API 7500 triple quadrupole mass spectrometers paired with Shimadzu UPLC systems for ultimate sensitivity and maximum method versatility.

Additionally, for our clients in both the Boston and San Francisco Biohubs, Meadowhawk provides free, same day courier services to simplify logistics and conserve time.

Count on the team at Meadowhawk to flexibly and creatively support your small molecule biomarker bioanalytical needs supported with exceptional customer service.

Meadowhawk biolabs

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Biomarker Assay Tiering (LC-MS Platform)				
Attribute		**Exploratory**	**Optimization**	**Pre-IND**
Research Objective		To qualitatively monitor fold-changes in up or down regulation of potential biomarkers	To provide quantitative data supporting efficacy studies, PK/PD correlations and other assessments	To provide definitive quantitative data to support the design and execution of IND-enabling and clinical studies
Turnaround Time		+	++	+++
Experimental Rigor		++	+++	++++
Cost		+	++	+++
Biomarker Assay Parameters				
Precision/Accuracy Criteria		Relative Quantification	±20% (±25% LLOQ)	±15% (±20% LLOQ)
Analytical Reference Standard(s)	**Reference Material**	Preferred if available (used for confirmation of peak identification)	Required	Required
	Salt Form	Required	Required	Required
	Purity Information	Not required, assume 100% in absence	Not required, assume 100% in absence	Required
	Quantity Required	Pre-weighed or ≥ 2 mg (if applicable)	Pre-weighed or ≥ 2 mg	Pre-weighed or ≥ 10 mg
	Weighings	Single (if applicable)	Single	Triplicate
	Stock Solution Checks	No	No	Yes (equivalency within 5%)
Method Qualification		No	No	Required
Calibration Standards		Relative quantification	Predefined linear range	Customized range for biomarker/study
Quality Control Samples		No	Independently prepared at 3-4 concentration levels (extracted in triplicate)	Independently prepared at 3-4 concentration levels (extracted in triplicate)
Linearity of Dilution		Not Applicable	Independent dilution QC (2-3X ULOQ) processed and analyzed at highest dilution factor used in batch	Independent dilution QC (≥ 3X ULOQ) qualified at highest dilution factor utilized during sample processing
Internal Standard		Cocktail Internal Standard or Structural Dialog	Structural Analog or Stable Isotope Label	Structural Analog or Stable Isotope Label
Sample Dilutions		Not Applicable	Based on historical data (if available) and/or dilution into predefined matrices	
Samples Above Quantitation Limit		Not Applicable	Fit-for-purpose data extrapolation or post-extraction dilution with reanalysis	Sample reprocessing with required dilution for quantification
Parameters Evaluated		Optional estimation of concentrations by single point calibration standard (if reference material available)	Parallelism; Dilution Linearity; Endogenous Concentrations; Spike Recovery; Short-Term Matrix Stability; Freeze/Thaw Matrix Stability	
Data Deliverable		Standard Excel Tabular Summary Additional options: Bioanalytical Word Report		

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