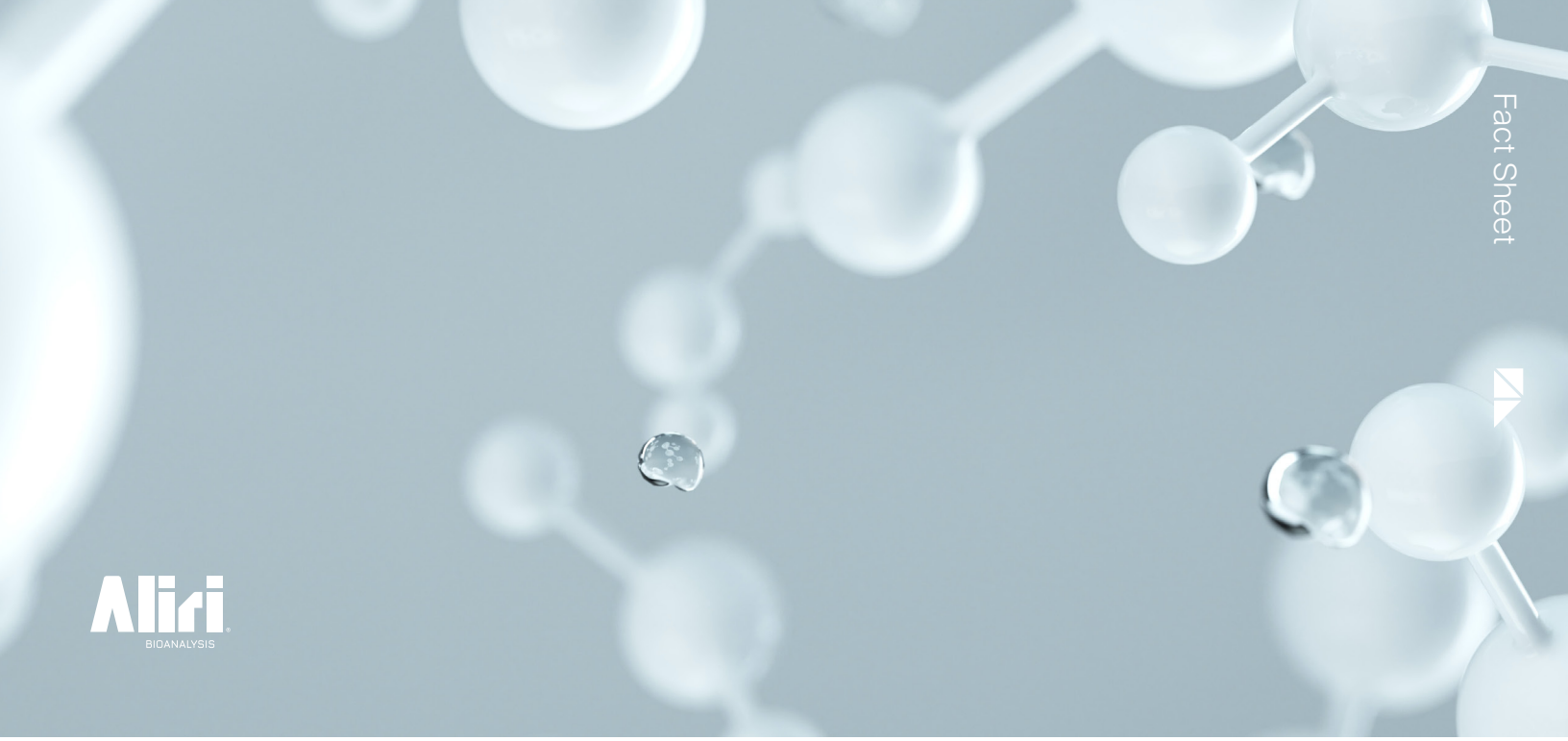

OPTIMIZING SMALL MOLECULE DRUG DEVELOPMENT STRATEGIES:

A step by step approach to a rugged method

Developing a rugged method for optimizing small molecule drug development strategies is pivotal in the journey toward effective therapies. A methodic approach not only ensures consistency and reliability in data, but also empowers you to make informed decisions, enhancing efficiency and success of your project. We've outlined a robust approach below, including 7 crucial steps, to help you navigate the complexities and uncertainties that come with advancing small molecules for drug development.

STEP 1
MS tuning
	• Optimize sensitivity for at least two separate MRM transitions per analyte/internal standard
	• Conduct MS tuning using:
	  • Acidic and basic pH values
	  • Two different organic solvent modifiers
	  • Positive and negative ionization modes
	  • ESI and APCI sources

STEP 2
HPLC separation
	• Explore characteristics as a function of:
	  • Mobile phase acidity/basicity
	  • Function of organic modifier
	• Determine chromatographic "mode" behavior
	• Separate compounds of interest from endogenous matrix effectors (phospholipids)

STEP 3 **Sample preparation**	• Protein precipitation (Dirty but fast) • Support liquid exchange of liquid/liquid extraction (Clean and fast) • Complete solid phase extraction (Highly specific)

STEP 4
Stability
	• Assess stability in both the sample matrix and stock/spiking solution diluent
	• Assess stability in the sample matrix
	  • Benchtop and Freeze Thaw
	  • Exposure to light
	  • Oxidation
	• Assess stability in solution diluent
	  • Start early
	  • Don't confuse stability with adsorption
	  • Don't confuse stability with solubility
	• If matrix treatment is required use the following guide
	  • Make sure the treatment volumes for the samples lend to accurate pipetting
	  • If treating preclinical samples you should try to establish a treatment procedure that could be used in clinical trials

STEP 5 **Matrix effects**	• Consider species sample volume when deciding on preparation scheme • Mouse and Rat will most likely have limited volume and may require separate individuals for both the high and low concentration tests • Test Hemolysis with more than one lot of hemolyzed plasma • Test Hyperlipidemia with more than one lot of matrix • If matrix effects fail due to precision it may be due to matrix effects issues • If matrix effects fail due to accuracy it may be due to preparation issues
STEP 6 **Moving from method development into method validation**	• Establish robustness by handing off validation to another scientist • Map out the planned analytical experiments • Establish solution stability as soon as possible • Keep method development engaged while validating the assay • QC in real time • Have an established guide to determine next steps if a test fails to meet the acceptance criteria

STEP 7
Moving from method validation into sample analysis
	• Conduct a method validation to sample analysis meeting to review the following:
	• Accuracy and precision data
	  • Update the method to include established stability
	  • Update method using the validation scientist as a guide to adjust any of the wording for the sample extraction method
	  • Determine reasonable batch size for sample analysis
	  • If you are treating samples for stability the sample concentrations for the study should reflect the untreated concentrations
	  • Keep the method validation and method development scientist engaged through early sample analysis
	  • Conduct ISR as early as possible even if there is a small number of qualifying samples
	• Neither method development or method validation are complete until ISR meets the acceptance criteria

COMMON TROUBLE SHOOTING

- You cannot determine accuracy until you have precision
- If you have poor precision, you must first determine if it is extraction related or instrument related
- If precision is failing due to the extraction you should extract some blanks and post spoke and analyte to determine if it is related to recovery
- If accuracy is failing, you should ensure the preparation scheme for both the analytical curve and QCs are similar in design
    - To ensure it is not a stock solution issue, you can always prepare test QCs from the spiking curve vs using the QC spiking solution, if the “curve” QVs pass but the actual QVs fail reprepare the solutions for both curve and QCs
- If sensitivity is dropping during the course of the run but returns back to normal with a different column you should consider the addition of a forward or backward flush to re-equilibrate the column from injection to injection

Contact us to learn more about our small molecule drug development workflows.