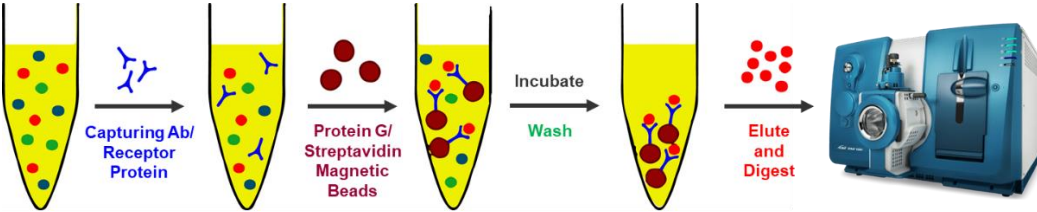


Kansas City Discussion Group

Speaker:	Dr. Nitish Mishra, Scientist II, Principal Investigator, KCAS Bio
Topics:	A Next-Gen Workflow for Dual Quantification of TAb and ADC via Hybrid Immunoaffinity LC-MS/MS
Location:	Cinzetti's; 7201 W. 91st Street, Overland Park, KS 66212; phone: 913-642-0101
Date:	August 28, 2025
Time:	Gather and register: 5:30 pm, Dinner: 6:00 pm, and Speaker after: ~6:30 pm
Menu:	Buffet style and choice of water, tea, or soft drink
Reservations:	Please contact Dr. Paul Haney at phaney@mriglobal.org or 816-326-5476. The requested deadline for reservations or cancellations is August 26, 2025.
Cost:	\$20 cash or check, payable at the door (\$10 for students with Academic ID).
Abstract:	Ligand-binding assays (LBAs) have long been the standard for large-molecule bioanalysis. However, the rise of hybrid liquid chromatography–tandem mass spectrometry (LC-MS/MS) platforms—especially those with immunoaffinity enrichment—has introduced highly specific, sensitive, and multiplexed options that can complement or even replace traditional LBAs. Here, we introduce a new high-throughput hybrid LC-MS/MS method that uses a 1.5 immunoprecipitation (IP) workflow to enable the simultaneous quantification of total antibody (TAbs) and antibody-drug conjugate (ADC) from a single sample extract. The method utilizes a biotinylated capture antibody (biotinylated Anti-2D12 Ab) to bind to the analyte, enabling selective immunocapture from preclinical and clinical samples. Magnetic streptavidin-coated beads facilitate enrichment, followed by automated washing and elution on the KingFisher™ Flex platform. The enriched analyte is then divided into two parallel workflows: one undergoes trypsin digestion to release a signature peptide from the complementarity-determining region (CDR) for TAb measurement. At the same time, the other is digested with papain to free the cytotoxic payload. Both components are analyzed using reversed-phase LC-MS/MS on Sciex 6500 and 7500 systems. This unified extraction approach offers several benefits: • Reduced sample volume and fewer freeze-thaw cycles due to less processing. • Better consistency between TAb and ADC measurements • Increased throughput and reproducibility • Less dependence on critical reagents

	A case study will be shown demonstrating the successful use of this platform in both nonclinical and clinical settings for ADCs. Overall, this 1.5 IP hybrid LC-MS/MS strategy offers a scalable, efficient, and robust solution for dual-analyte quantification, meeting the pharmacokinetic and bioanalytical needs of complex biotherapeutic agents.  Keywords: Hybrid LCMS/MS, TAB qualification, ADCs, 1.5 immunoprecipitation assay, Immunoaffinity enrichment, Sciex QTRAP 6500s/7500s.
Biography:	Dr. Nitish Mishra is a Scientist II and Principal Investigator at KCAS Bio, where he leads method development efforts within the Biopharma LC-MS/MS method development team. With over 12 years of research experience spanning medicinal chemistry, bioanalytical chemistry, protein science, microbiology, and drug formulation, he brings a multidisciplinary approach to solving complex analytical challenges in support of drug discovery and development. Dr. Mishra holds a Bachelor of Pharmacy, two Master of Science degrees, and a Ph.D. in Pharmaceutical Sciences. During his doctoral training at the University of Missouri–Kansas City (UMKC) School of Pharmacy, he managed the institution’s mass spectrometry core facility. His research included method development for small molecules, untargeted and targeted metabolomics, biomarker analysis, protein–ligand interaction studies, high-throughput screening, and antibacterial drug discovery. At KCAS Bio, Dr. Mishra leads the development and pre-validation of hybrid LC-MS/MS assays for the quantitation of peptides, proteins, monoclonal antibodies, antibody-drug conjugates (ADCs), oligonucleotides, lipids, and small molecules in complex biological matrices. His work employs advanced sample preparation strategies—such as solid-phase extraction, protein precipitation, and immunocapture—and he is proficient with Q-ToF and Sciex triple quadrupole mass spectrometry platforms. He has received multiple awards for academic and research excellence and has authored first-author publications. Dr. Mishra is passionate about advancing science that improves human health and continues to contribute to the growth of translational bioanalytical technologies through collaborative, innovation-driven research.
Reminder:	Non-members are welcome. Please select Kansas City Discussion Group (KCDG) membership on your AAPS application or dues notices. Thank you!