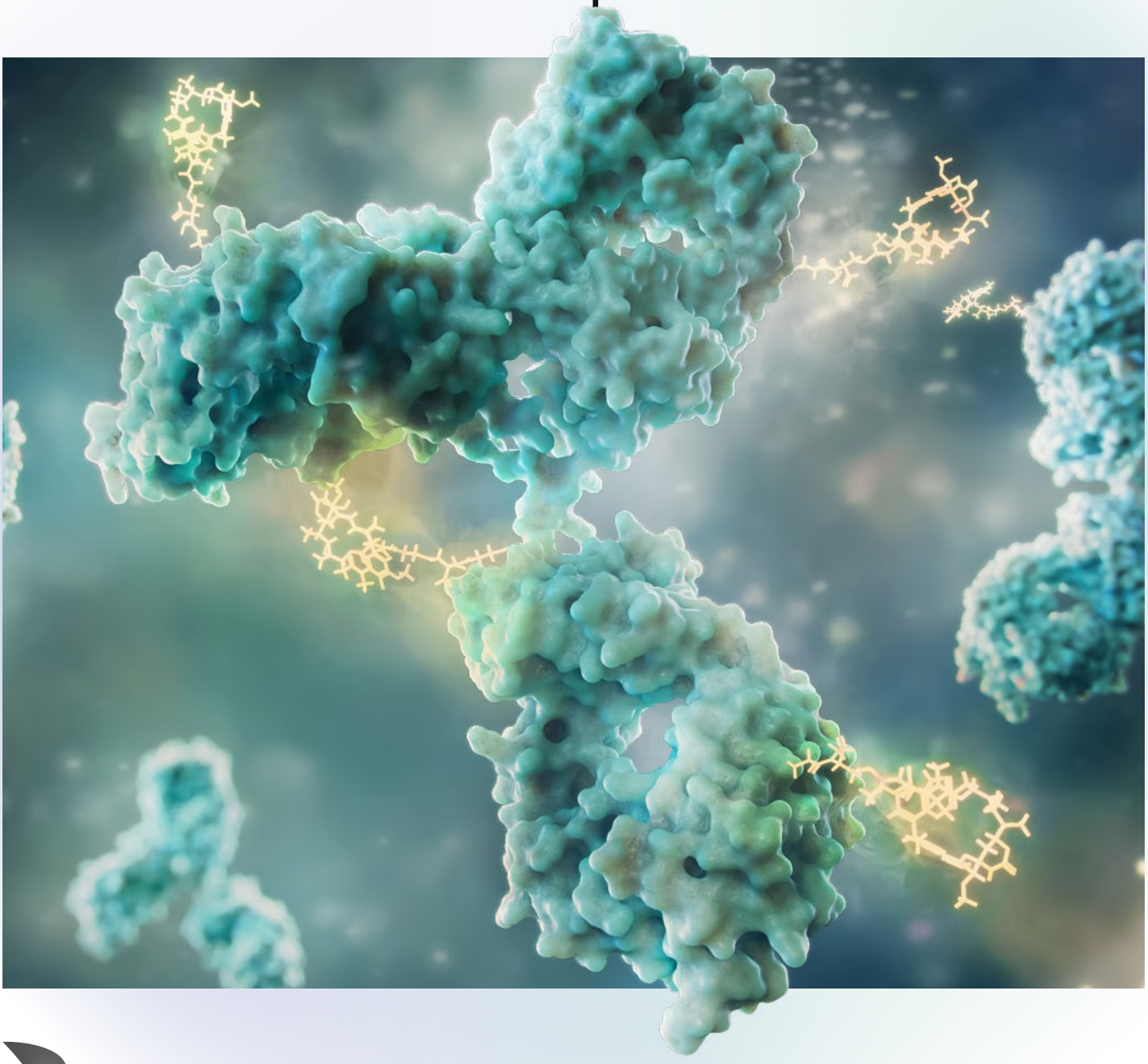
SCIEX

From complexity to confidence:

Analytical solutions for ADC development



danaher

The power of precision

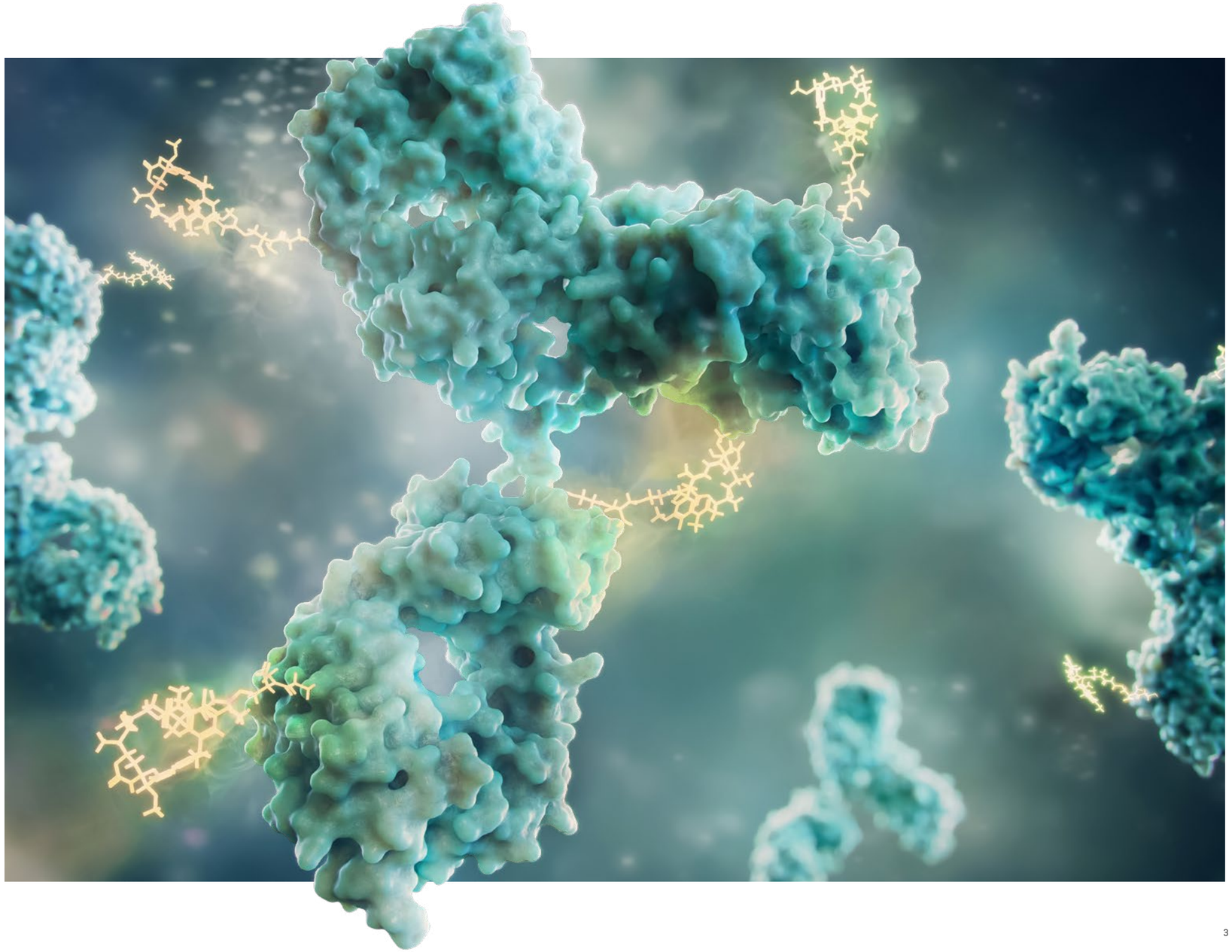
Turn ADC complexity into decision-ready insight

Antibody–drug conjugates (ADCs) are inherently complex, combining an antibody, linker, and payload into a single molecule. This creates multiple, interconnected sources of heterogeneity, including but not limited to changes in DAR, charge variants, post-translational modifications, and conjugation sites that must be both characterized, and understood.

To support confident development decisions, analytical strategies must:

- Quantify ADC components to assess stability, safety, and efficacy
- Interpret structural differences and quality attributes with specificity
- Resolve charge variants and structural isoforms with confidence
- Be able to monitor consistency across development and manufacturing
- Support deep characterization and routine testing

This requires workflows that deliver both structural understanding and quantitative insight, allowing ADC attributes to be consistently tracked throughout development.



Turn analytical development into a lifecycle advantage

Analytical strategies for ADC development must do more than characterize molecules, they must generate reliable data to support decisions across the entire product lifecycle.

From early development through manufacturing, analytical methods need to deliver consistent, and transferable insight into ADC heterogeneity. In this e-book explore the SCIEX platforms and workflows that can help you turn ADC complexity into decision-ready insight.

- **icIEF UV/MS (Intabio ZT system)** Direct linkage of charge separation to mass detection for more interpretable process insights
- **LC MS + EAD (ZenoTOF 7600+ system and ZenoTOF 8600 system)** Deep structural characterization to support root cause analysis and comparability
- **LC-MS/MS (SCIEX 7500+ systems)** Highly sensitive and reproducible quantitative analysis
- **Capillary electrophoresis (BioPhase 8800 system, PA 800 Plus system)** Reproducible, high-resolution methods for structural integrity and charge well suited for development and lot release

How orthogonal workflows strengthen ADC characterization

Zoe Zhang
SCIEX

Q1 How do orthogonal workflows provide more insight than single techniques?

Given the inherent structural complexity of ADCs, it is challenging for a single analytical technique to analyze all product quality attributes [PQAs]. Orthogonal workflows improve data confidence by enabling robust correlation of charge, structure, and identity into actionable insights.

ADC characterization requires understanding multiple, interconnected PQAs, including charge heterogeneity, DAR, and post translational modifications [PTMs]. Traditionally, these attributes are assessed using separate assays across multiple platforms, increasing complexity and slowing decision-making.

This orthogonal workflow combines icIEF UV/MS with subsequent EAD-based LC MS peptide mapping, enabling ADCs to be characterized from complementary perspectives. With the Intabio ZT system, icIEF UV/MS provides charge separation and peak identification at the intact level. While the EAD-based LC MS method provides amino acid-level characterization.

Together, this provides multi-level insight into ADC heterogeneity, rather than a single analytical view.

Q2. How does icIEF UV/MS change charge variant analysis?

Charge heterogeneity analysis becomes much more powerful when you can assign identity to each peak - otherwise you're only seeing part of the story.

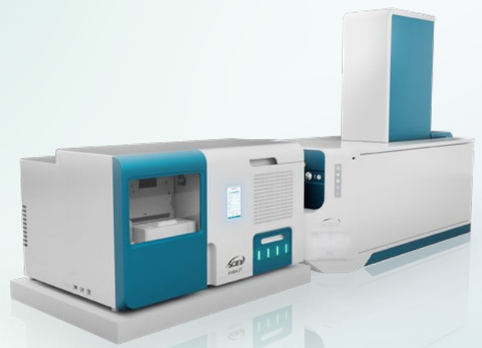
The icIEF UV/MS workflow enables high-resolution separation and sensitive detection of charge variants, while also providing mass-based identification of proteoforms associated with each peak. Being able to do this 95% faster than fractionation methods means the icIEF-UV/MS workflow to be used throughout development, including during scale up and in-process control. The specificity of the workflow also enables quality attribute localization at the intact level.

This allows scientists to move from complex charge profiles to confident interpretation, linking observed variation directly to underlying structure.

Q3. What additional insight does EAD-based peptide mapping provide?

EAD fundamentally changes ADC characterization by preserving fragile payloads and PTMs during fragmentation, enabling confident localization, disulfide mapping, and isomer differentiation in a single, reproducible LC-MS workflow. Allowing you to localize what matters, without losing important information like sequence coverages and positional isomers.

This supports confident interpretation of ADC and structural insight in a single injection.



In-depth charge heterogeneity analysis of antibody-drug conjugates with a streamlined icIEF-UV/MS and EAD-based peptide mapping workflow

This technical note demonstrates the unique capabilities of imaged capillary isoelectric focusing (icIEF)-UV/MS and electron activated dissociation (EAD)-based peptide mapping workflows for rapid charge heterogeneity analysis and comprehensive characterization of a GlyCLICK®-generated antibody-drug conjugate (ADC). These orthogonal, streamlined workflows provide multi-level information about product quality attributes (PQAs) of ADCs, facilitating important decision-making early in the development pipeline.

Read technical note

Resolve ADC charge complexity with clarity

The role of icIEF-UV/MS in ADC development

Move from simply resolving heterogeneity to understanding its origin. icIEF-UV/MS provides a powerful bridge between separation and identification, enabling more interpretable, lifecycle-ready insights that support development, process optimization, and long-term product control.

The challenge

Charge heterogeneity analysis for ADCs is complicated by:

- Conjugation variability and post-translational modifications
- Limited ability to identify and localize unknown species
- Dramatic profile changes between mAb and conjugated species

Traditional approaches can separate variants but not explain them directly, making root-cause analysis difficult and time-consuming.

How icIEF-UV/MS addresses the challenge

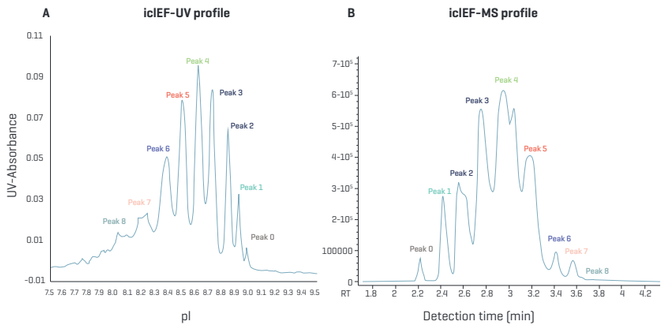
icIEF-UV/MS combines charge separation with mass identification on a single platform to deliver:

- High-resolution charge separation (icIEF) with simultaneous UV detection
- Direct mass identification of charge variants (MS)
- Accurate DAR measurement

Why use the Intabio ZT system

The Intabio ZT system brings together icIEF-UV and MS in a single, integrated platform to help make sense of ADC complexity. By combining high-resolution separation with simultaneous UV and MS detection, it connects charged species with mass identification, making results easier to interpret.

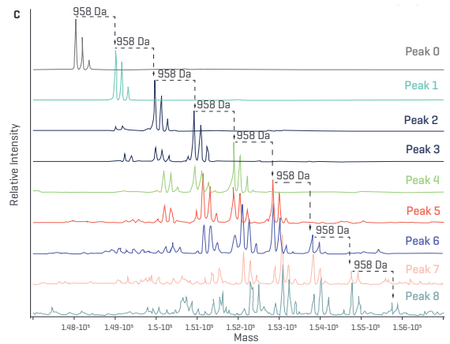
Charge variant identification and DAR measurement in a single injection:



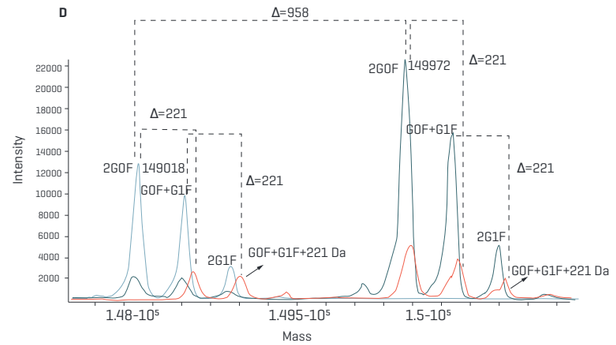
Directly link charge separation to mass detection for more interpretable insights across the lifecycle

Gain charge variant separation of major species and corresponding intact MS base peak electropherogram.

Intact protein separation, detection and deconvolution of T-DM1 using the icIEF-UV/MS workflow. (A) An icIEF-UV profile demonstrating charge variant separation of 9 major species of T-DM1. (B) icIEF-MS profile (intact MS base peak electropherogram (BPE)) of TDM1 charge variants detected by the ZenoTOF 7600 system. (C) Stacked view of deconvoluted MS spectra of intact T-DM1 with various numbers of the payload separated by icIEF. A mass difference of +958 Da was observed for the adjacent peaks. (D) Zoomed-in view of the deconvoluted spectrum showing 3 major glycoforms with DARs of 1–2. A mass difference of +221 Da was observed for these major glycoforms. The +221 Da species correspond to the antibody conjugated with the linker but no coupled payload.

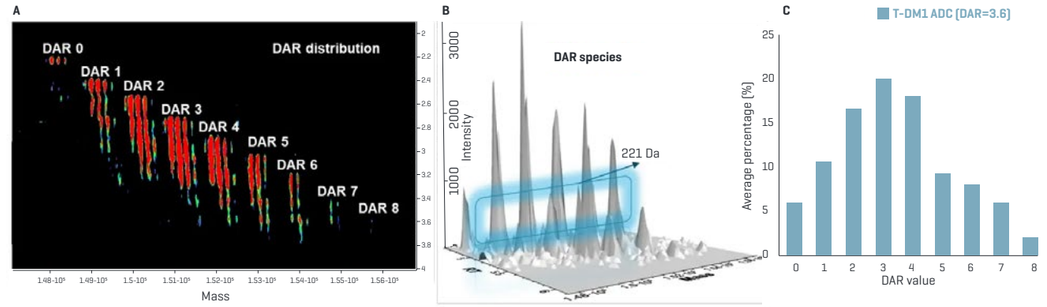


View deconvoluted MS spectra of intact ADC to identify variations in payloads linked to antibody.



Zoom in on deconvoluted MS spectra to identify major glycoforms and identify reason for mass differences.

Intuitive data visualization of intact deconvolution and DAR measurement



Intuitive data visualization of intact deconvolution and DAR measurement of T-DM1 using Biologics Explorer software. (A) MS ion map view of the identified major peaks of T-DM1 with DAR distribution. (B) 3D view of the identified different DM1 payloads providing confident information about the detected payload, DAR distribution along with lower intensity peaks (+221 Da mass shift) identification. (C) An average DAR value of 3.6 was automatically determined by Biologics Explorer software for the intact T-DM1.

Intabio ZT system

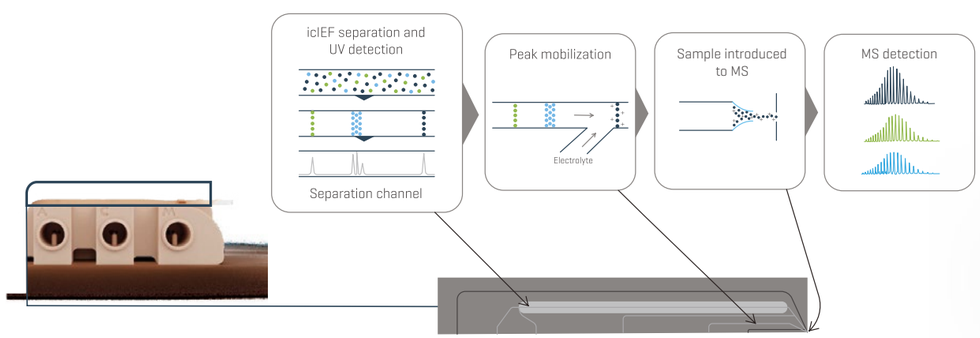
The Intabio ZT system couples icIEF separation and UV detection to high-resolution mass spectrometry on the ZenoTOF 7600 system. One integrated workflow with a single, accessible dataset containing the required information to make the right decisions, fast.

Learn more

Microfluidic technology integrates key analytical functions

The Intabio ZT system cartridge integrates key analytical functions using proprietary microfluidic chip technology for seamless icIEF-UV/MS analysis.

- Separation of charge variants with imaged capillary isoelectric focusing (icIEF)
- Quantitation with real-time UV detection
- Integrated on-chip electrospray ionization for MS identification



Microfluidic chip

Achieve comprehensive proteoform ID in minutes, not weeks

Leverage key analytical functions with microfluidic chip-based integrated icIEF-UV/MS technology

Eliminate the guesswork with the ZenoTOF 7600 system

Advancing charge variant analysis for ADCs

Jingwen Ding

Q1: What limitations do traditional charge variant analysis workflows face?

When separation and identification are disconnected, you lose both time and confidence in your interpretation. Separating charge variants is only part of the solution. Identifying each component is where traditional workflows fall short.

Conventional methods, such as icIEF or IEX, often require fraction collection followed by subsequent LC-MS analysis, which increases the analysis time, sample requirements, and the risk of artifacts. This disconnect makes it difficult to maintain alignment between separation and identification, slowing interpretation and decision-making.

The icIEF-UV/MS workflow enables high-resolution charge-based separation and sensitive mass detection, providing mass-based ID of proteoforms associated with each charge variant. This allows scientists to move from complex charge profiles to confident interpretation, linking observed variation directly to underlying structure.

Q2. What specific insights does this workflow provide for ADC characterization?

Compared to traditional intact LC-MS analysis of complex ADC molecules, where different DAR species are detected simultaneously, charge-based separation prior to MS analysis simplifies complex ADC spectra.

This enables identification of proteoforms with different payload distributions and supports accurate DAR determination at the intact level. With the capability to identify proteoforms, icIEF-UV/MS provides deeper understanding and clarity of ADC complexity at the intact level.

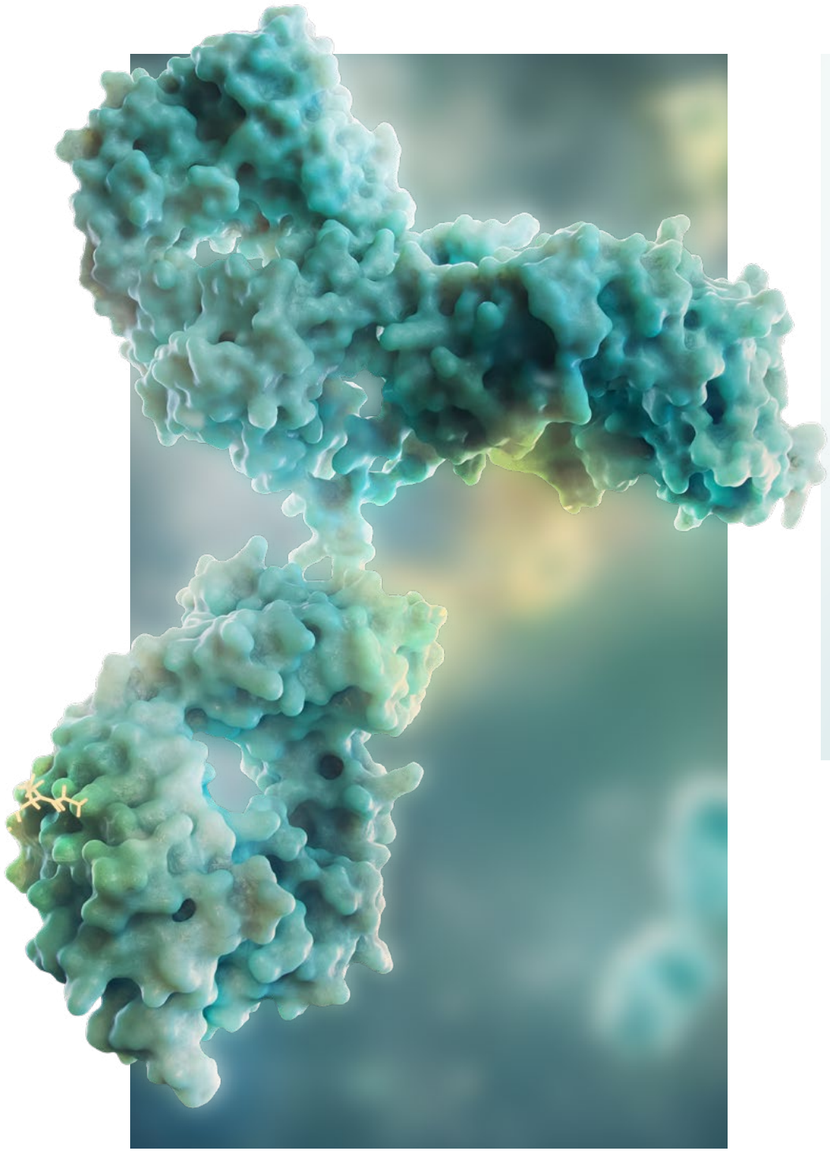
Q3. How does this workflow support ADC development and lifecycle analytics?

By combining charge-based separation, quantitation, and identification in a single assay, this integrated workflow enables faster and more confident decision-making earlier in ADC development. Compared to fractionation-based approaches, it's 95% faster, easier to implement, and more practical for analysis across development process.

This supports early characterization, comparability assessments, and ongoing monitoring, providing a more consistent analytical foundation across the development lifecycle.

95%

faster than fractionation-based approaches



Charge variant analysis of antibody-drug conjugates using an icIEF-UV/MS workflow

This technical note demonstrates a novel integrated workflow using the Intabio ZT system for charge variant analysis of antibody-drug conjugates (ADCs). This innovative system offers direct chip-based integration of icIEF with mass spectrometry (MS), achieving separation and confident identification of proteoforms with different payloads, reliable quantitation of charge variants and determination of drug-to-antibody ratios (DARs). Further, the streamlined icIEF-UV/MS platform significantly reduces acquisition and analysis time compared to ion exchange chromatography (IEX) with fraction collection, eliminating multiple weeks of effort.

Read technical note

Confirm ADC structure with confidence

High-resolution MS workflows provide deep structural confirmation for actionable insight.

Deep structural characterization to support root-cause analysis and comparability

ADC complexity extends beyond what can be resolved by charge or size alone. Variability in DAR, conjugation sites, sequence, and PTMs can all impact product quality.

Separation techniques detect differences, but structural confirmation requires molecular-level insight. High-resolution mass spectrometry workflows provide the depth necessary to characterize ADC structure with confidence.

The challenge

ADC heterogeneity extends beyond charge and size to include:

- Variability in DAR and conjugation sites
- PTMs and sequence variants
- Formation of aggregate species and higher-order structures

While separation-based methods can resolve these differences, they cannot fully explain their structural origin. Understanding ADC heterogeneity requires analytical approaches that provide direct product insight.

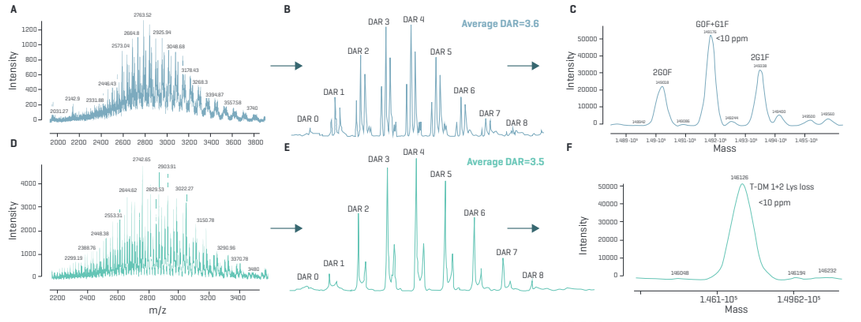
How high-resolution MS workflows address the challenge

High-resolution LC-MS and native SEC-MS workflows provide complementary structural insight, enabling confirmation of ADC heterogeneity across multiple levels:

- Accurate DAR determination and distribution analysis
- Intact, subunit, and peptide-level analysis to characterize ADC primary structure
- Direct identification of PTMs, sequence variants, and glycoforms
- Isomer differentiation for more confident structural interpretation

These workflows enable confident structural confirmation and root-cause analysis, linking observed heterogeneity to underlying molecular features across development and comparability studies.

Observe charge state distributions of glycosylated and deglycosylated forms of intact ADC, and calculate average DAR distributions.



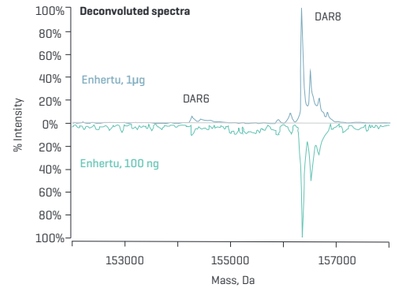
Intact protein deconvolution and DAR measurement of the glycosylated and deglycosylated forms of T-DM1 using Biologics Explorer software. (A and D) Charge state distributions of the glycosylated and deglycosylated forms of T-DM1. (B and E) Deconvoluted mass spectra of the glycosylated and deglycosylated forms of T-DM1 that reveal the DAR distribution of the detected peaks. (C) Zoomed view of the deconvoluted spectrum of the glycosylated form of T-DM1 with 3 major glycoforms annotated. (F) Zoomed view of the deconvoluted spectrum of the deglycosylated form of T-DM1.

Why use a ZenoTOF system

The ZenoTOF 7600+ system and ZenoTOF 8600 system enable comprehensive, multi-level characterization of ADCs, supporting native, intact, subunit, and peptide-level analysis within a single mass spec platform.

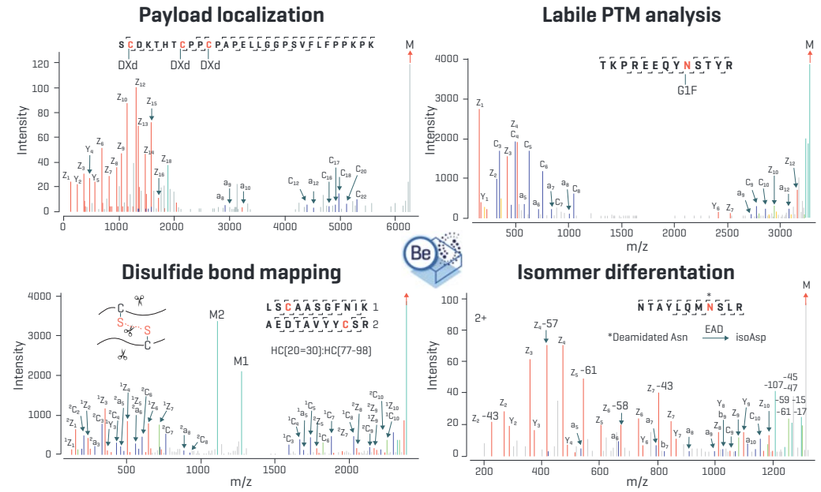
Enhanced sensitivity enables intact analysis for mass confirmation and preservation of non-covalent complexes for aggregate detection and accurate DAR assessment. Advanced fragmentation capabilities with EAD enable confident localization of payloads, PTMs, and sequence variants.

Confirm major proteoforms and DAR distribution. Enhanced sensitivity enables detection of low abundant species.



Sensitive native SEC-MS analysis of Enhertu-a Cys-linked ADC with consistent detection of the DAR8 and low-abundant DAR6 species at sample loads of 1ug and 100ng.

Gain high sequence coverage, DAR determination, accurate PTM and payload localizations, confident disulfide bond mapping and clear isomer differentiation with EAD-based peptide mapping



EAD-based reduced and non-reduced peptide mapping workflows enable comprehensive characterization of Cys-linked ADCs such as trastuzumab deruxtecan (Enhertu, T-DXd).

ZenoTOF 8600 system

The ZenoTOF 8600 system is designed to deliver reproducible, interpretable data across demanding workflows, helping scientists evaluate results with evidence as studies evolve and scale.

Learn more

Designed to protect data quality under demanding conditions

Mass Guard technology with an optical detector is designed to support operation at higher ion currents while helping protect the system from contamination

Flexible ionization across LC flow regimes

The OptiFlow Pro ion source is designed for flexibility when switching between nano-, micro-, and analytical-flow regimes, supporting a range of experimental strategies.

Fragmentation options for structural confidence

High-energy electron-activated dissociation (EAD) enables acquisition of high-quality spectra on timescales comparable to and in parallel with CID, enabling confident structural interpretation of protein modifications in a single injection.

High-speed DIA, designed to improve selectivity at scale

ZT Scan DIA 3.0 supports scan speeds up to 1342 Hz, enabling narrow, continuously sliding isolation windows to improve precursor selectivity while maintaining dense MS/MS.

Sensitivity options for low-abundance features

The Zeno trap operation accumulates ions before pulsing them into the TOF, enabling confident analysis of low-level signals.

Designed to evolve with your science

Flexible front-end options, acquisition modes, and processing solutions enable researchers to adapt their methods to evolving experimental needs while maintaining data quality.

Advancing native MS for ADCs and aggregate characterization

Haichuan Liu
SCIEX

Q1. What insight does native MS provide for ADCs and aggregates?

Seeing intact complexes and aggregates gives you a much clearer picture of the ADC and all its components.

Key attributes of ADCs, such as aggregation and complex formation, depend on noncovalent interactions that are often disrupted in traditional MS workflows. Native MS enables measurement of these species by maintaining non-covalent interactions, which is especially important for direct DAR measurement of cysteine-linked ADCs, detection of intact assemblies and non-covalent complexes, and sensitive identification of low-abundance aggregates, such as dimers.

When you preserve non-covalent structure, you move beyond composition to understanding how these molecules exist in solution.

Q2. What has limited the routine use of native MS in biopharmaceutical development?

Native MS has always been powerful but has been constrained by low sensitivity and the need for large sample amounts, making it difficult to apply broadly across development.

Due to high sensitivity capabilities, the ZenoTOF 8600 system enables native MS with low sample loads, around 100 ng, while improving detection of intact complexes and other species in low abundance.

This means that improved sensitivity isn't just a performance metric. It expands what native MS can detect, how easily it can be applied, and enables the detection of more low-level species that may impact product quality.

Inside the technical note: Native mass spectrometry analysis of biotherapeutics and aggregates with enhanced sensitivity

Native mass spectrometry [MS] enables accurate mass measurement and rapid impurity assessment of intact biotherapeutics under native conditions. It was demonstrated that the ZenoTOF 8600 system provided a >3X MS sensitivity improvement for intact mass analysis compared to the previous platform. This technical note focuses on native MS analysis of monoclonal antibodies [mAbs], a cysteine [Cys]-linked antibody-drug conjugate [ADC], and mAb aggregates with enhanced sensitivity (Figure 1), which reduces the sample consumption required to generate high-quality data under native conditions.

Read technical note

Q3. How does this workflow support development and comparability?

When you can detect subtle structural changes early, you make better decisions and avoid surprises later.

By enabling detection of low-level aggregates and intact structural variants, native MS supports impurity assessment, stability evaluation, and comparability analysis, while reducing sample requirements and increasing workflow accessibility.

More sensitive, intact-level analysis means earlier and more confident assessment of structural changes, bringing higher-order structural insight into routine analysis.

Go deeper with confident structural insight: a bit more on EAD

Preserve what matters in ADC characterization

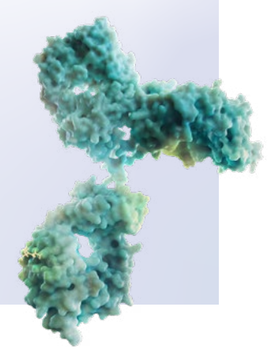
ADCs present a unique analytical challenge: the very features that define their function-payloads, linkers, PTMs, and disulfide linkages-are often chemically fragile and difficult to characterize. Traditional fragmentation approaches can disrupt these features, limiting confidence in structural interpretation.

EAD fragmentation preserves labile features while delivering rich structural insight. By maintaining payloads and modifications on signature fragment ions, EAD enables more accurate localization and interpretation of key structural attributes.

EAD workflows are designed to fit within modern pipelines. Single-injection methods run as a complement to conventional CID, enabling comprehensive characterization without adding complexity.

Enable deeper insight while maintaining the molecular information needed for confident analysis with EAD-based LC-MS workflows:

- **Confident payload localization**
  Preserves payload-bearing fragments to directly assign conjugation sites
- **High-confidence PTM characterization**
  Enables localization of labile modifications critical to CQA assessment
- **Improved disulfide mapping**
  Supports structural integrity assessment in cysteine-linked ADCs
- **Detection of subtle structural variants**
  Enables differentiation of isomers and low-level heterogeneity



Quantifying ADC attributes with confidence

The role of quantitative LC-MS in ADC Development

Quantitative LC-MS workflows are essential for ADC development because they provide the data needed to understand exposure, stability, and payload release. Reliable results enable confident decisions about safety, efficacy, and toxicity.

Quantitative LC-MS workflows support:

- Accurate quantitation of antibody, free payload, and conjugated antibody
- Detection and measurement of low-level variants and impurities
- Pharmacokinetic and pharmacodynamic insight for exposure and payload release

The challenge

Quantitative ADC analysis must balance:

- Sensitivity to detect low-abundance species
- Accuracy for reliable attribute measurement
- Reproducibility for cross-study comparison
- Throughput to support large datasets

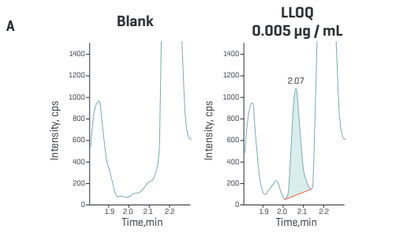
Traditional approaches may provide measurement, but without sufficient sensitivity or consistency, quantitation can introduce variability rather than clarity.

How quantitative LC-MS addresses the challenge

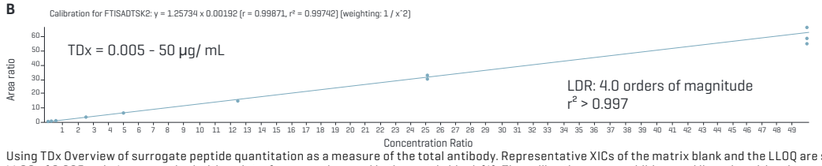
SCIEX quantitative workflows enable:

- High-sensitivity detection of the ADC, its components, and metabolites
- Reliable, robust, and reproducible linearity
- Consistent performance across samples and runs
- Scalable analysis for lab-to-lab and scientist-to-scientist transferability

Meet critical quantitative performance criteria with accuracy and reproducibility

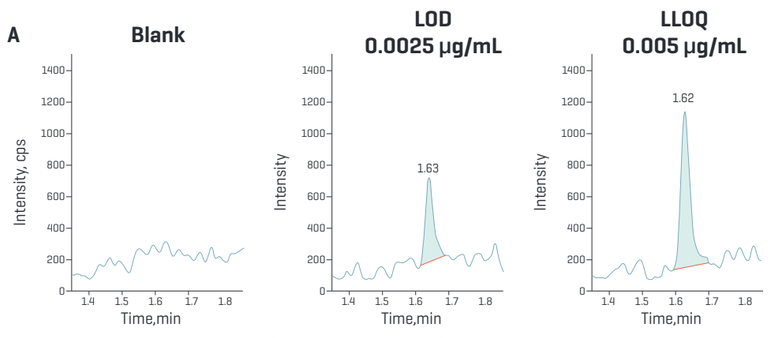


Actual C...	Mean	Standard D...	Percent CV	Average Accuracy.
0.005	0.005	0.000	3.05	99.5
0.025	0.026	0.001	4.23	104.
0.050	0.048	0.001	3.09	96.8
0.125	0.125	0.001	0.85	99.6
0.250	0.266	0.009	3.31	107.
0.500	0.507	0.008	1.49	101.
2.500	2.550	0.055	2.14	102.
5.000	5.021	0.027	0.52	100.
12.500	11.702	0.218	1.86	93.6
25.000	25.161	0.900	3.58	101.
50.000	47.864	4.595	9.60	95.7

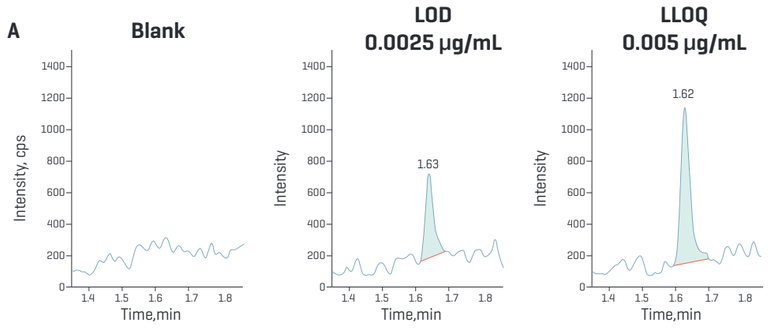


Using TDx Overview of surrogate peptide quantitation as a measure of the total antibody. Representative XICs of the matrix blank and the LLOQ are shown.An LLOQ of 0.005 µg/mL was reached with no interferences observed in the matrix blank (A). The calibration curve exhibits good linearity with a dynamic range of 4 orders of magnitude (B). The quantitative performance of the total antibody assay demonstrates acceptable accuracy (±7%) and precision (%CV <10) (C).

Detect low levels of free payload and conjugated payload



Using trastuzumab deruxtecan (TDx) for free payload assay. Representative extracted ion chromatograms (XICs) of the matrix blank, limit of detection (LOD, 0.0025 ng/mL) and the LLOQ are shown. An LLOQ of 0.005 ng/mL was reached with no interferences observed in the matrix blank



Using TDx for payload-based ADC quantitation. XICs of the matrix blank,LOD (0.0025 µg/mL) and the LLOQ are shown. An LLOQ of 0.005 µg/mL was reached with no interferences observed in the matrix blankwas reached with no interferences observed in the matrix blank

Why use the SCIEX 7500+ system

The SCIEX 7500+ system enables quantitative, high-sensitivity LC-MS workflows that support confident measurement of critical ADC components across development.

By combining sensitive detection with reproducible performance, it enables accurate quantitation of DAR and ADC components in biological samples and the detection of low-abundance variants that may impact product quality. These workflows are designed to support high-throughput analysis and consistent tracking across large sample sets, enabling more efficient evaluation of ADC candidates and process conditions.

Comprehensive LC MS workflows for ADC bioanalysis

Ebru Selen
SCIEX

Q1. What challenge does ADC bioanalysis need to address?

ADC bioanalysis requires measuring multiple components that behave differently in biological systems.

Because ADCs consist of an antibody, linker, and cytotoxic payload, no single measurement captures the full picture. Instead, multiple assays are required to understand how the ADC distributes, degrades, and releases payload, and how these factors impact safety and efficacy.

We demonstrate multiple LC MS assays, including free payload quantitation, conjugated payload quantitation, total antibody quantitation, and payload–based measurement strategies. Together, these assays provide insight into ADC composition and behavior, enabling a more complete pharmacokinetics (PK) and pharmacodynamics (PD) evaluation.

Q2. Why is quantifying free payload and conjugated payload important?

Quantifying both free and conjugated payload is essential because each provides distinct and complementary insight into ADC performance. Free payload from biological samples reflects payload release, which is directly linked to potential toxicity and off-target effects. Conjugated payload (ADC) reflects drug still attached to the antibody, providing insight into stability and in vivo behavior.

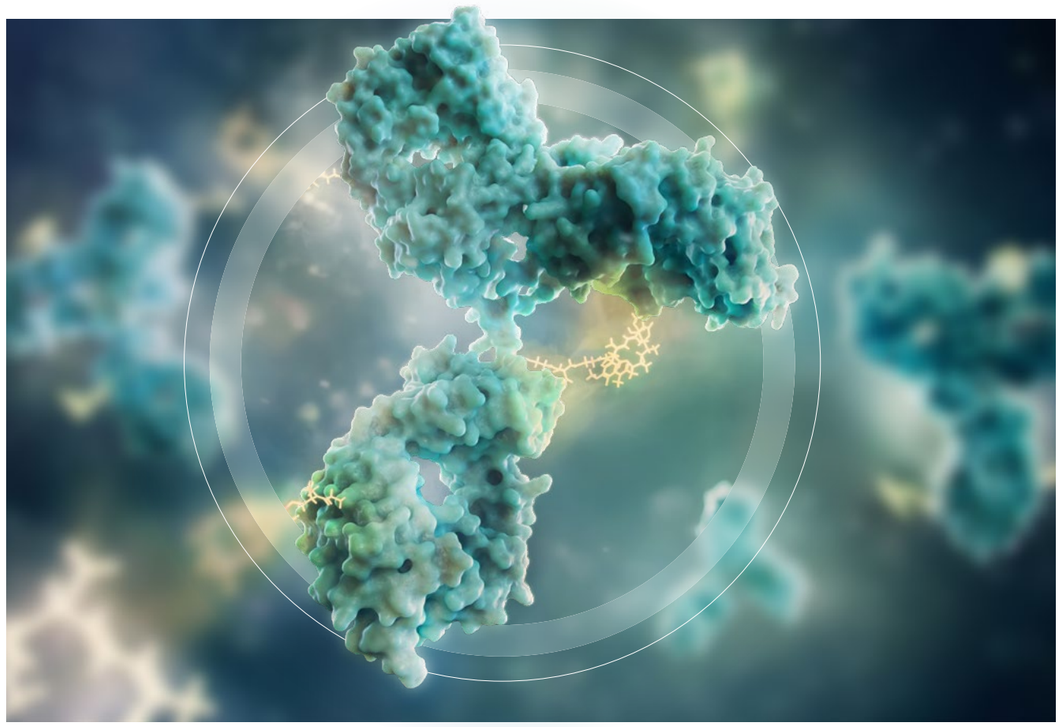
The workflows demonstrated in this technical note include sensitive assays for both, achieving lower limits of quantitation (LLOQ) of 0.005 ng/mL for free payload and 0.005 µg/mL for conjugated payload and ADC measurements.

Together, these measurements enable a more complete understanding of drug exposure, stability, and therapeutic window.

Q3. How do these workflows support lifecycle-ready ADC analysis?

Workflows become more scalable when they combine sensitivity, reproducibility, and comprehensive assay coverage.

The study demonstrates that integrating multiple LC-MS assays, such as free payload, total antibody, and ADC quantitation, enables consistent, quantitative assessment across components, supporting PK/PD evaluation. These workflows provide the sensitivity and reproducibility required to generate accurate data across a broad dynamic range, enabling reliable analysis as programs progress.



Comprehensive LC-MS workflows for antibody drug conjugate (ADC) analysis

This technical note demonstrates a sensitive workflow for 4 key quantitative pharmacokinetic/pharmacodynamic (PK/PD) assays using trastuzumab deruxtecan (TDX) in rat plasma on a nominal mass spectrometer. A lower limit of quantitation (LLOQ) of 0.005 ng/mL was achieved for the free payload (deruxtecan; Dxd) and 0.005 ug/mL was achieved for the conjugated payload, total antibody and ADC quantitation

Read technical note

SCIEX 7500+ system

The SCIEX 7500+ system is a triple quadrupole engineered to maintain our highest sensitivity for up to twice as long in complex matrices.

Learn more

Mass Guard technology

The innovative ion filtering technology that reduces the risk and frequency of instrument contamination, maintaining our highest sensitivity for up to twice as long.

Speeds of up to 800 MRM per second

The fastest SCIEX Triple Quad yet increases the capacity for large quantitation panels that incorporate new compounds of concern.

Ionization Source

The OptiFlow Pro ion source with E Lens incorporates the reliability and efficiency of the legendary Turbo V ion source while providing flexibility for quickly switching flow rates.

Newly designed removable DJet+ assembly

The removable DJet+ assembly allows users to clean and maintain it themselves, making it easier to schedule front-end cleaning.

More energy efficient

The SCIEX 7500+ system is compatible with dry roughing pumps, which can reduce electricity consumption by up to 24% relative to oil-sealed pumps.

24%

reduction in electricity consumption

QTRAP system

Exclusive to SCIEX, QTRAP functionality like enhanced product ion scans enable improved confidence, while MRM3 workflows push quantitation levels through matrix interferences.

Deliver on important timelines

SCIEX OS software automated decision making means your system spends more time generating the results you need to hit your project deadlines.

Detection

Precise and robust engineering of the ion rail delivers consistent and reproducible analysis time after time, by focusing and transmitting the crucial ions you need for your study.

High-resolution insight—from development to lot release

The role of capillary electrophoresis in ADC development

Build on a strong backbone. The structural complexity of ADCs requires analytical methods capable of resolving subtle differences in structural integrity and charge.

While it is important to understand the complexity of the ADC drug substance, the unconjugated mAb is fundamental to the success of the final drug product and requires intensive characterization.

The challenge

Balancing fast turnaround time with routine-ready data quality while keeping data continuity as programs progress.

Analytical strategies must address:

- Structural variability impacting purity and integrity
- Charge heterogeneity driven by conjugation and PTMs
- Need for consistent, transferable methods across development and manufacturing

Separation is essential, but it must also be robust, reproducible, scalable, and transferable to routine use.

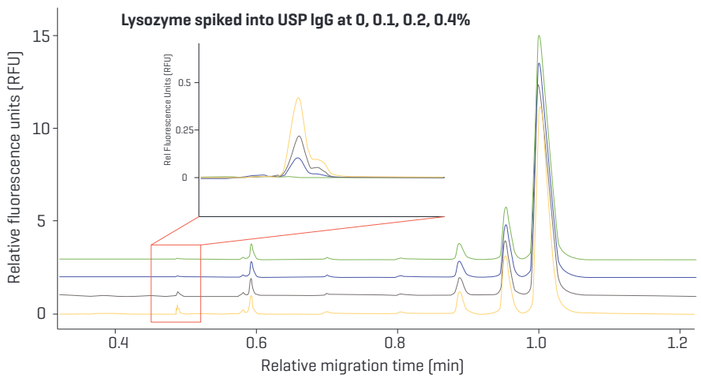
How capillary electrophoresis (CE) addresses these challenges

CE delivers:

- High-resolution size and purity analysis to assess structural integrity with capillary electrophoresis sodium dodecyl sulfate (CE-SDS)
- Flexible charge separation to profile conjugation-driven differences with capillary isoelectric focusing (cIEF) and capillary zone electrophoresis (CZE)
- Highly sensitive separation of closely related species for consistent monitoring with different detection modes including ultraviolet (UV), laser-induced fluorescence (LIF), native fluorescence (NF)
- CE methods can be applied from early characterization through comparability and QC, supporting consistent monitoring of ADC quality attributes

Together, these capabilities support consistent, high-confidence monitoring of purity, impurities, and charge heterogeneity, enabling reliable characterization, comparability studies, and lot release testing for complex ADCs.

Confident detection and quantitation of low abundant species with CE-SDS analysis



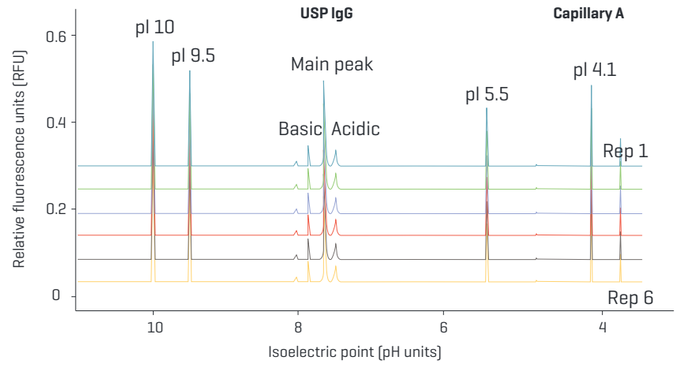
Quantitation of 0.01% spiked in lysozyme. CE-SDS of non-reduced USP IgG at 1 mg/mL with spiked in lysozyme (lysozyme spiked into USP IgG at 0, 0.1, 0.2, 0.4%) run on BioPhase 8800 system with NFD.

SCIEX CE solutions for ADC development

SCIEX CE platforms deliver reproducible, high-resolution workflows for ADC and intermediate analyses across the product lifecycle. Proven method transfer supports continuity from development through lot release, while automation-ready workflows help increase turnaround time and consistency.

- **BioPhase 8800 system:** 8-parallel capillaries enable higher-throughput analysis while maintaining resolution, supporting both development and routine testing.
- **PA 800 Plus system:** Established single-capillary platform, supporting workflows for routine use and lot release in regulated environments.
- **Validated kit-based assays:** CE SDS, CZE, and cIEF kits provide consistent reagents and protocols, enabling reliable method performance and easier transfer across laboratories

High consistency in peak shape, migration time, and baseline stability for cIEF separations



cIEF-NFD separations of USP-IgG

BioPhase 8800 system

The BioPhase 8800 system is a multi-capillary electrophoresis system that enables biopharma scientists to run multiple samples in parallel, accelerating the development and execution of sensitive, high-throughput analytical methods.

Learn more

Parallel processing

Accelerate analysis without compromising consistency. 8-capillary parallel processing enables faster method development while maintaining uniform separation conditions across channels. Factory-built cartridges and push-in loading simplify setup and reduce user variability.

Multiple detectors

Adapt detection to your workflow needs. UV detection supports legacy method transfer, while NF simplifies identification and quantitation. LIF enables high-sensitivity detection for labeled proteins and oligonucleotides.

Software

Simplify method development and streamline data analysis. Intuitive drag-and-drop programming reduces setup time, while remote access supports efficient workflows. Integration with Empower™ chromatography data system ensures compliance and data integrity.

Temperature control

Maintain reproducibility and sample stability. Individual capillary temperature control, combined with uniform plate cooling, ensures consistent separation conditions, while rapid temperature adjustment enables flexibility across a wide range of workflows.

Injection modes

Ensure precise and consistent sample introduction. Pressure-based injections enable uniform capillary filling, while electrokinetic injections support high-resolution separations, providing flexibility to match the injection mode to your analytical requirements.

Up to

5x

increase in sensitivity for CE-SDS, CZE, and cIEF

PA 800 Plus system

Capillary electrophoresis (CE) on the PA 800 Plus system enables ultra-high quality separation day in and day out for the development of proteins and next generation therapeutics.

Learn more

Liquid temperature control

Maintain precise separation conditions for consistent results. A recirculating liquid coolant system provides accurate temperature control in the capillary, minimizing variability and ensuring reproducible performance across applications.

Multiple injection modes

Achieve consistent, high-quality separations with flexible injection options. Electrokinetic injection supports high resolution, hydrodynamic injection addresses varying sample conditions, and field-amplified stacking enhances sensitivity.

Separation voltage

Optimize performance for your application. Adjustable separation voltage up to 30 kV enables you to balance resolution and analysis time, tailoring methods to different sample types and analytical requirements.

Detection wavelength

Improve sensitivity and accuracy across analytes. A wide range of detection wavelengths allows you to optimize signal-to-noise ratios and ensure reliable, reproducible results for diverse applications.

Detector type

Adapt detection to your sensitivity and application needs. Switch between UV and LIF detectors to support routine workflows or enable high-sensitivity detection of low-concentration analytes.

Up to

30kV

separation voltage

Validate kit based assays

Built on proven CE workflows and designed to evolve with new modalities, the SCIEX portfolio of validated kits gives you the tools to address distinct analytical questions without disrupting what already works.

Learn more

Protein CE-SDS Purity Analysis kit

Rapid determination of protein purity using capillary electrophoresis with sodium dodecyl sulfate.

CZE rapid charge variant analysis kit

Rapid charge variant analysis

- Simplify analysis and maximize lab time, with fast, powerful separations based on sample mobility.
- Resolve a native-state molecule's charge variants with greater resolution than icIEF or ion-exchange chromatography.
- Quantify charge variants with a buffer that serves as both a separation matrix and dynamic coating with a bare fused silica capillary.

BioPhase cIEF kit

Charge profile monitoring

- Capillary isoelectric focusing (cIEF) is used for rapid monitoring of charge heterogeneity in the development of therapeutic proteins.
- High-resolution separation and high reproducibility enable rapid and quantitative information to be gathered to establish the identity and stability of the molecule.
- Optimize protein charge heterogeneity screening using validated kits and workflows across the PA 800 Plus and BioPhase 8800 systems.

The power of precision

SCIEX Now support network

SCIEX Now

- Manage your instruments.
- Submit and manage support cases, track status and view history.
- Access online training courses and articles.
- Manage software licenses linked to your registered instruments.
- View and report critical instrument statistics when connected to StatusScope remote monitoring service.
- Be a part of the SCIEX community by submitting questions and comments.
- Receive notifications from SCIEX with content based on your preferences.

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SCIEX Now learning hub

- SCIEX Now learning hub success programs provide LC-MS and CE training customized to meet your exact needs.
- With a selection of training methods and certifications available, you can build a mass spectrometry program that is most suited to your lab and users.
- Starting with a clear understanding of your desired learning outcomes, we aim to help you improve lab productivity and consistency by designing and delivering a program that is focused on knowledge advancement and retention.

Find out more

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