MEDICILON

Abstract Presentation No. 7670

Integrated Mass Spectrometric Workflows Enabling Next-Generation Conjugate Therapeutics in Cancer Research

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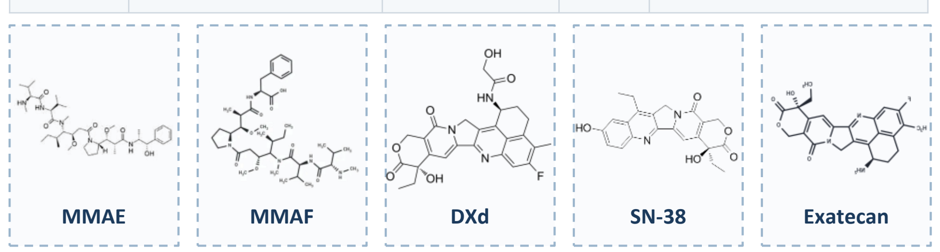
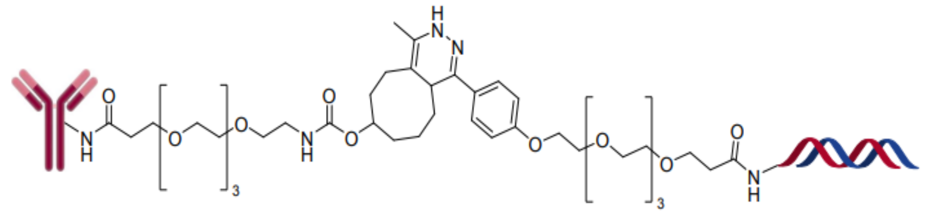
ABSTRACT

Antibody–drug conjugates (ADCs) and related Antibody-X Conjugates (AXCs), including Antibody–Oligonucleotide Conjugates (AOCs), represent rapidly expanding therapeutic modalities in oncology. Their growing clinical significance demands precise bioanalytical methods capable of quantifying conjugated species, released payloads, and complex biotransformation pathways. Similarly, click-chemistry–enabled drug systems designed for tumor-selective activation present analogous analytical challenges requiring high sensitivity and specificity. We developed an integrated LC-MS/MS platform to support these next-generation cancer therapeutics. For ADC, AXC, and AOC programs, optimized controlled-reduction and selective-extraction workflows enabled accurate quantification of diverse payload classes—including auristatins (MMAE/MMAF) and topoisomerase-I inhibitors (DXd, SN-38, exatecan)—while minimizing artificial payload release. The platform demonstrated high sensitivity, linearity, and matrix robustness across plasma, tumor tissue, and cell-based systems, supporting comprehensive in vitro, ex vivo, and in vivo pharmacokinetic studies. In parallel, we established complementary bioorthogonal click-chemistry workflows using azide–alkyne and TCO–tetrazine reactions to selectively tag precursor and product species, enabling quantitative assessment of conversion efficiency and activation kinetics under biologically relevant conditions. Together, these LC-MS/MS–based approaches provide a unified analytical solution for ADCs, AOCs, AXCs, and click-activated therapeutic platforms. By enabling rigorous characterization of payload release, linker stability, and tumor-selective activation, this integrated toolkit accelerates the discovery and preclinical development of emerging oncology therapeutics.

COMMON CYTOTOXIC PAYLOADS WITH ADC AND AOC

Payload	Full Name	MOA	MW	Key ADC
MMAE	Monomethyl Auristatin E	Microtubule inhibitor	718.0	Brentuximab vedotin (Adcetris)
MMAF	Monomethyl Auristatin F	Microtubule inhibitor	731.5	Belantamab mafodotin
DXd	Deruxtecan (exatecan deriv.)	Topo-I inhibitor	493.5	Trastuzumab deruxtecan (Enhertu)
SN-38	7-Ethyl-10-hydroxycamptothecin	Topo-I inhibitor	392.4	Sacituzumab govitecan (Trodelvy)
Exatecan	Exatecan mesylate	Topo-I inhibitor	436.5	Next-gen ADC programs

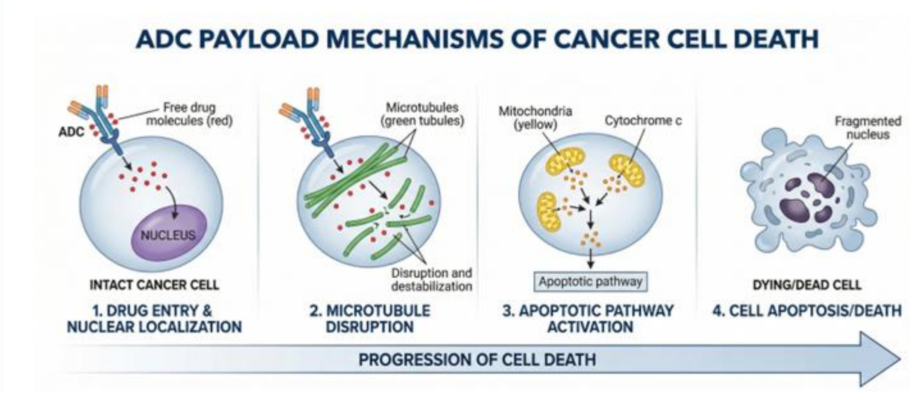




AOC

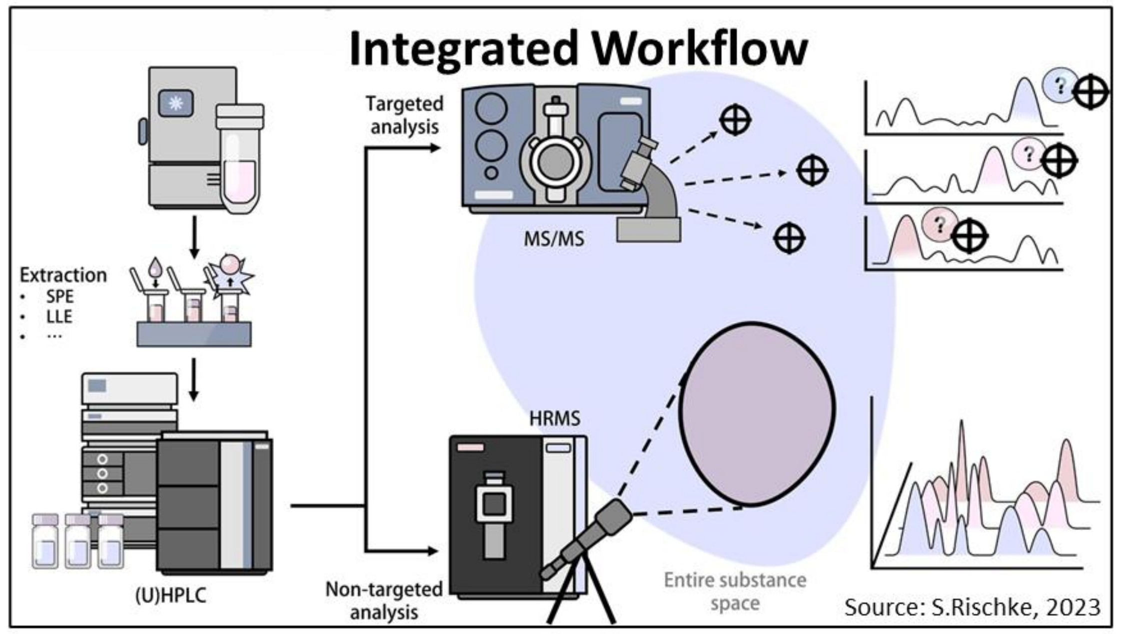
Source: M.Cochran, 2024

PAYLOAD RELEASE MECHANISMS AND CLICK CHEMISTRY IN ADCS



Application of click chemistry in ADC

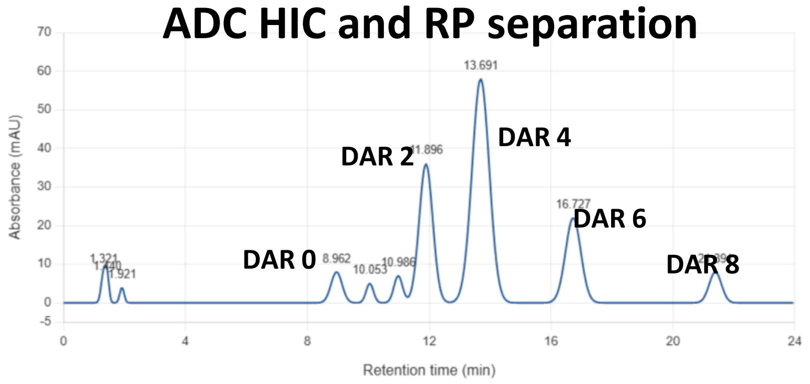
HRMS: High-resolution mass spectrometry (HRMS) enables non-targeted profiling of ADCs and supports accurate drug-to-antibody ratio (DAR) determination.

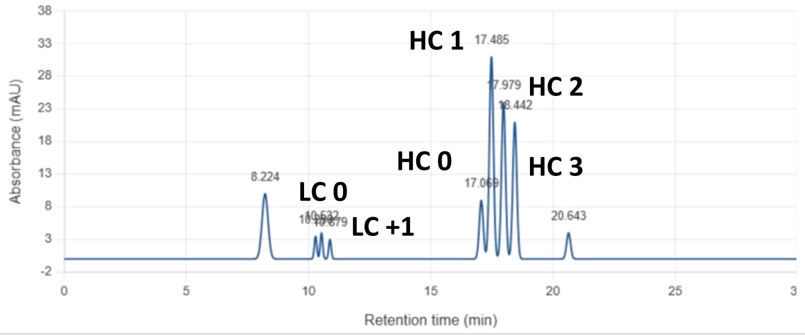


Triple quadrupole MS/MS: provides sensitive and selective targeted quantification of predefined ADC analytes using optimized MRM transitions.

HPLC & HRMS ANALYSIS OF DRUG-TO-ANTIBODY RATIO (DAR) IN ADCS

ADC HIC and RP separation



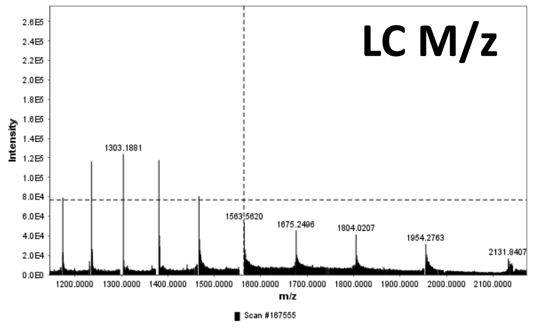


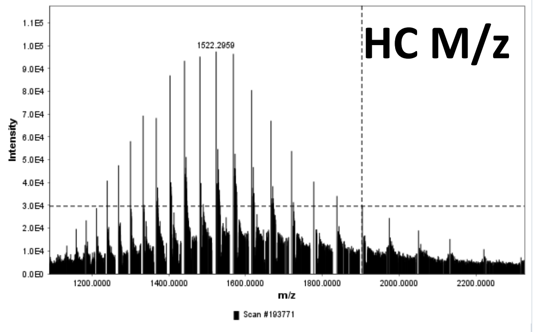
LC intact mass analysis

Component	Calculated Mass (Da)
LC	23439
LC+1	24756

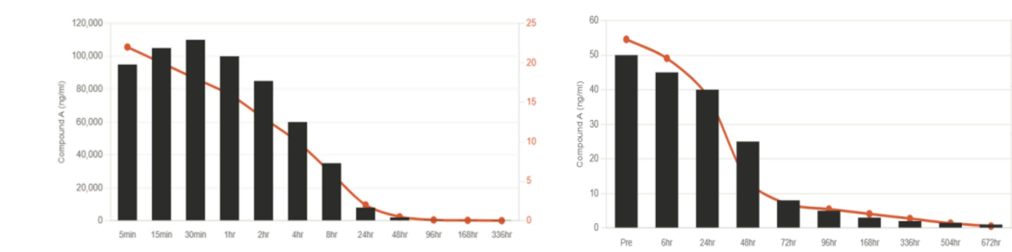
HC intact mass analysis

Component	Calculated Mass (Da)
HC	51911
HC+1	53228
HC+2	54544
HC+3	55861

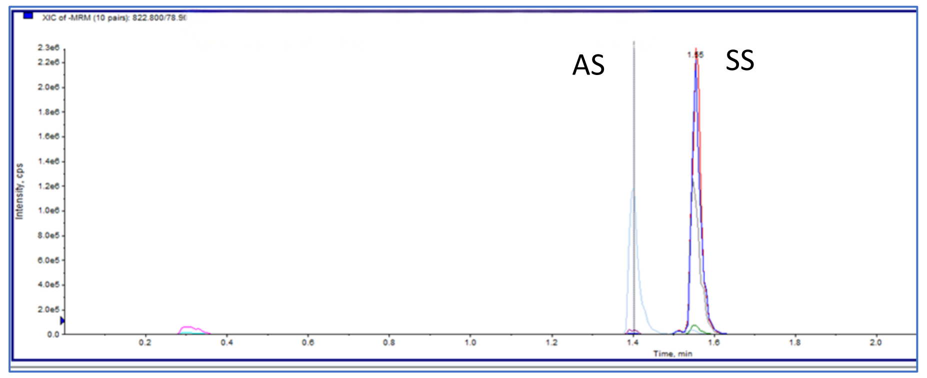




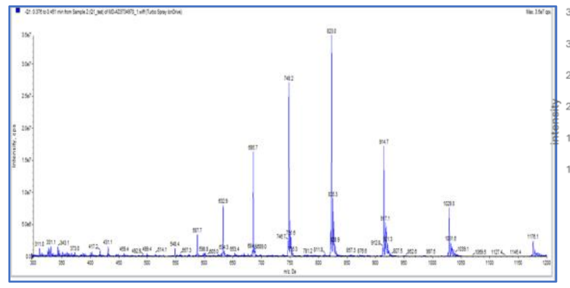
QQQ MASS SPEC ANALYSIS OF THE LINKER-PAYLOAD FROM IN VIVO STUDY



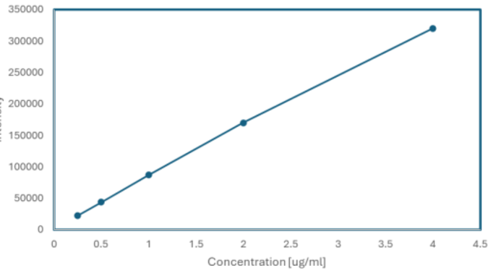
Analytes: Compound **A** and the payload quantified in plasma and tumor
Activation: TCO-tetrazine bioorthogonal reaction for selective tagging
MS benefit: Enhanced detectability; quantitative conversion & kinetics assessment
Matrices: Mouse plasma and tumor tissue; good reproducibility CV < 10%



Extracted ion chromatogram (XIC) from MRM analysis of the linker payload of an AOC



Calculated mass: Sense Strand



Calibration curve of SS

KEY PLATFORM CAPABILITIES

- **Multi-matrix Support:** Plasma, tumor tissue, liver, cell-based systems
- **Broad Payload Coverage:** Auristatins (MMAE/MMAF), Topo-I inhibitors (DXd, SN-38, exatecan), siRNA, peptides, LNP components
- **High Sensitivity:** LLOQ 1-10 ng/mL; CV < 10%; excellent linearity
- **Comprehensive PK Design:** In vitro, ex vivo, in vivo; robust PK/PD correlation and exposure profiling
- **Click Chemistry Toolkit:** Chemical compound synthesis; CuAAC and TCO-Tz workflows; conversion efficiency, reaction kinetics, activation profiling
- **ADC/AXC/AOC Support:** Controlled-reduction extraction, linker-drug quantification, biotransformation assessment

We developed an integrated LC-MS/MS platform supporting next-generation oncology therapeutics including ADCs, AOCs, AXCs, and click-activated systems. Through optimized sample preparation and bioorthogonal workflows, the platform enables sensitive and reliable quantification of conjugates, payloads, and biotransformation products across diverse biological matrices. This unified, scalable analytical toolkit supports comprehensive PK and biotransformation studies, accelerating preclinical development of emerging therapeutic modalities.

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