Impact of Bioanalytical Methodology on ADC Pharmacokinetics and Clinical Interpretation

Complete 20-Minute Presentation Guide for Clinical Pharmacology Audience

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DETAILED PRESENTATION OUTLINE (12-15 Slides with Time Allocation)

SLIDE 1: Title Slide (1 minute)

Title: "Why Bioanalytical Methods Matter: Impact on ADC Pharmacokinetics and Clinical Decisions"

Subtitle: Understanding How We Measure ADCs Changes What We See

Content:

- Your name, affiliation
- Date and venue
- Brief background: Clinical pharmacology perspective on ADC bioanalysis

Opening Statement (Memorize): "Have you ever seen PK data from two different labs for the same ADC that don't match? Or wondered why exposure-response relationships seem inconsistent across studies? The answer often lies in which bioanalytical method was used. Today I'll show you how the choice of analytical method fundamentally changes PK interpretation for antibody-drug conjugates, and more importantly, how this impacts clinical decisions for patients."

SLIDE 2: The Clinical Challenge (2 minutes)

Title: "ADCs are falling apart in patients - and we need to measure the pieces"

Visual: Simple animation showing intact ADC breaking down into three components

- Intact ADC (conjugated antibody) in green
- Naked antibody (unconjugated) in blue
- Free payload in red
- Arrow showing deconjugation process over time

Content (Bullets):

- ADCs undergo continuous deconjugation in circulation PubMed ACS Publications

- Creates multiple analytes with different PK profiles ACS Publications
- Each analyte correlates differently with efficacy and toxicity
- **Key point:** "What we measure determines what we see"

Talking Points:

- "Think of an ADC as a smart bomb - antibody is the GPS, payload is the explosive"
- "But in patients, this 'smart bomb' gradually disassembles. By week 2, only 40% of circulating antibody may still have drug attached"
- "This means if we measure 'total antibody,' we're counting both armed and disarmed missiles - very different from measuring only the intact weapon"
- "The clinical question is: which measurement tells us what we need to know for dosing and safety monitoring?"

SLIDE 3: ADC Structure and PK Complexity (2 minutes)

Title: "One drug, multiple analytes: the ADC pharmacokinetic challenge"

Visual: Left side - ADC schematic with components labeled

- Antibody (150 kDa, half-life 7-21 days)
- Linker (cleavable vs non-cleavable)
- Payload (cytotoxic drug)
- DAR label (Drug-to-Antibody Ratio: typically 3-4)

Right side - Table of PK parameters showing differences:

Analyte	Half-Life	What It Tells Us
Total Antibody	7-21 days	Overall drug-related exposure
Conjugated ADC	3-14 days	Active drug delivery
Free Payload	Hours-days	Systemic toxicity

Talking Points:

- "An ADC isn't one drug - it's a complex mixture that changes over time" PubMed Central

- "Total antibody includes both conjugated and naked antibody (ScienceDirect) - can overestimate active drug by 2-3 fold"
- "Conjugated ADC represents the intact therapeutic - best correlates with tumor killing"
- "Free payload drives off-target toxicity like neuropathy and cytopenias"
- "Different analytes = different half-lives = different exposure patterns"
- **Clinical pearl:** "For efficacy, measure conjugated drug. For safety, measure free payload. For complete understanding, measure all three."

SECTION 2: BIOANALYTICAL METHODS (4 minutes total - Slides 4-5)

SLIDE 4: Overview of Bioanalytical Methods (2 minutes)

Title: "Three platforms, three perspectives: LBA, LC-MS/MS, and hybrid approaches"

Visual: Side-by-side comparison flowchart (simplified, 3-4 steps each)

LBA (Left):

1. Capture with antibody
2. Detect with labeled antibody
3. Signal proportional to ADC

- ✓ High sensitivity (pg/mL)
- ✓ High throughput
- ✗ Can't measure DAR
- ✗ Can't distinguish structures

LC-MS/MS (Middle):

1. Extract from plasma
2. Chromatographic separation
3. Mass spectrometry detection

- ✓ High specificity
- ✓ Measures free payload
- ✗ Lower sensitivity

- ✗ Can't directly measure intact ADC

Hybrid (Right):

1. Immunocapture ADC
2. Release payload/digest antibody
3. LC-MS/MS quantification

- ✓ Multiple analytes
- ✓ DAR information
- ✗ Complex, low throughput
- ✗ Specialized expertise

Talking Points:

- "No single method can measure everything - each has trade-offs" (nih)
- "LBA is like using a lock-and-key system - highly sensitive but can't tell us structural details"
- "LC-MS is like sorting mail by address AND weight - very specific but less sensitive"
- "Hybrid methods combine the best of both but are more complex and time-consuming" (ACS Publications) (nih)
- **Key message:** "The method you choose determines which analyte you measure, which determines the PK profile you see"

SLIDE 5: How Method Choice Impacts PK Parameters (2 minutes)

Title: "Same drug, different methods = different PK parameters"

Visual: Two-panel PK curve comparison

- **Left panel:** Concentration vs Time curves (semi-log scale)
  - Green solid line: Total antibody (highest, slowest decline)
  - Blue dashed line: Conjugated ADC (lower, faster decline)
  - Red dotted line: Free payload (lowest, much faster)
  - Annotate: "2-3 fold difference in AUC"
- **Right panel:** Bar graph comparing PK parameters
  - Clearance (L/day): Total Ab = 0.2, Conjugated = 0.6, Payload = 2.0

- Half-life (days): Total Ab = 14, Conjugated = 7, Payload = 2
- Bold text: "3-fold difference!"

Talking Points:

- "This is the same drug in the same patient - just measured differently"
- "Total antibody overestimates active drug exposure because it includes naked antibody"
- "Conjugated ADC clears 3x faster than total antibody due to deconjugation"
- "Free payload has completely different kinetics - formation-limited, cleared by liver"
- **Clinical implication:** "If you're using total antibody for exposure-response analysis, you might conclude there's no PK-efficacy relationship when in fact you're not measuring the right analyte"
- **Key message:** "Method choice isn't just a technical detail - it fundamentally changes our understanding of drug disposition"

SECTION 3: REAL-WORLD CASE STUDIES (8 minutes total - Slides 6-10)

SLIDE 6: Case Study 1 - Kadcyla (T-DM1): The Deconjugation Discovery (2 minutes)

Title: "Kadcyla revealed that ADCs fall apart faster than we thought"

Visual:

- Left: Chemical structure showing non-cleavable thioether linker
- Right: Graph showing DAR distribution shift over time
  - Day 0: Peak at DAR 3-4
  - Day 7: Peak shifting to DAR 1-2
  - Day 14: Only 40% remains as conjugated ADC (rat data)

Key Data Box:

- Conjugate clearance: 0.676 L/day (nih)
- Total antibody clearance: ~0.2 L/day (**3.4-fold difference**)
- Conjugate t½: 3.9 days
- Total antibody t½: ~10 days (**2.5-fold difference**)

Talking Points:

- "T-DM1 was approved in 2013 for HER2+ breast cancer - first major ADC success" PubMed Central
- "Early PK data showed a puzzle: why was drug clearing faster than expected for a typical antibody?"
- "Answer came from measuring conjugated antibody separately: massive deconjugation via maleimide exchange" nih nih
- "By day 14, only 40% of antibody in circulation still had drug attached" ACS Publications
- **Three biotransformation pathways identified:** maleimide exchange (predominant), ester hydrolysis, linker-drug hydrolysis NCBI nih
- "This was a wake-up call for the field: we can't assume ADCs stay intact"
- **Clinical outcome:** Despite PK complexity, flat exposure-response curve meant method choice less critical for dose selection - but essential for understanding mechanism

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SLIDE 7: Case Study 2 - Enhertu (T-DXd): Why Three Analytes Are Essential (2 minutes)

Title: "Enhertu teaches us that payload measurement is critical for cleavable linker ADCs"

Visual: Three-panel comparison

- **Panel 1:** Structure comparison (T-DM1 vs T-DXd)
  - T-DM1: Non-cleavable, DAR ~3.5
  - T-DXd: Cleavable tetrapeptide, DAR ~8 Biopharma PEG
- **Panel 2:** Table of analytes measured

Analyte	T-DM1 Role	T-DXd Role
Total Ab	Moderate importance	Important
Conjugated ADC	High importance	Critical
Free payload	Low (minimal release)	**Critical for ILD risk**

- **Panel 3:** Key safety finding
  - Free DXd levels correlate with interstitial lung disease risk
  - Renal impairment → altered payload clearance → higher ILD incidence
  - Box: "13.6% ILD rate overall; higher in renal impairment"

Talking Points:

- "Enhertu is structurally different from Kadcyla - cleavable linker, higher DAR"

- "With cleavable linkers, payload release is the intended mechanism - drug gets released inside tumor cells"
- "But this means free payload circulates and can cause off-target toxicity"
- "Most notable: interstitial lung disease (ILD/pneumonitis) - can be fatal" (American Society of Clinical O…)
- **Critical finding:** "Patients with moderate renal impairment showed higher ILD rates - likely due to impaired free payload clearance" (FDA)
- "Measuring only total or conjugated antibody would have completely missed this mechanistic link"
- **Clinical impact:** This led to enhanced monitoring recommendations for renally impaired patients, though not dose adjustment
- **Key lesson:** "For cleavable linker ADCs, measuring released payload isn't optional - it's essential for understanding toxicity" (nih)

SLIDE 8: Case Study 3 - Blenrep: The Definitive Example (2.5 minutes)

Title: "Blenrep shows different analytes predict different toxicities - and drive different clinical decisions"

Visual: Two-panel exposure-response graph

- **Left panel:** "ADC Exposure vs Corneal Toxicity"
  - Scatter plot with fitted curve
  - Steep positive relationship
  - Annotate: OR = 2.58 per unit increase ($p<0.001$)
- **Right panel:** "Free Payload vs Thrombocytopenia"
  - Scatter plot with fitted curve
  - Positive relationship
  - Annotate: OR = 1.61 per log unit ($p<0.001$)

Key Data Box:

- 2.5 mg/kg: 44% Grade 3-4 keratopathy
- 3.4 mg/kg: Higher severe keratopathy rates
- **Critical finding:** Safety increased without efficacy improvement at higher dose
- **Decision:** 2.5 mg/kg selected; REMS program mandated

Talking Points:

- "Blenrep (belantamab mafodotin) for multiple myeloma provides the clearest example of why measuring multiple analytes matters clinically" PubMed Central
- "Three analytes measured: intact ADC, total mAb, free cys-mcMMAF payload" nih PubMed Central
- **Key finding 1:** "ADC exposure strongly predicted corneal toxicity - the dose-limiting toxicity. Odds ratio of 2.58 means corneal events more than double with each unit exposure increase" nih NCBI
- **Key finding 2:** "But free payload exposure predicted thrombocytopenia, not ADC exposure. Different analyte, different toxicity" nih
- "Even more interesting: disease burden (sBCMA, IgG, albumin) confounded exposure-efficacy relationships but NOT exposure-safety relationships" nih NCBI
- **The clinical decision:** "At 3.4 mg/kg, Grade 3+ corneal events increased 20% more than efficacy improved - safety E-R slope steeper than efficacy" nih
- "Result: 2.5 mg/kg selected as recommended dose, REMS program with mandatory ophthalmologic monitoring" nih NCBI
- **Key lesson:** "If they'd measured only one analyte, they couldn't have made this nuanced safety-driven dose selection. This is precision pharmacology in action"

SLIDE 9: Case Study 4 - Telisotuzumab Vedotin: When Data Gaps Exist (1.5 minutes)

Title: "Not all ADCs have published exposure-response data - but bioanalytical strategy still matters"

Visual: Timeline showing development

- Phase 1: Multiple analytes measured (ADC, MMAE, total Ab)
- Dose/schedule selection: 1.9 mg/kg Q2W vs 2.7 mg/kg Q3W
- FDA approval May 2025: E-R analysis required as PMR
- Text box: "Limited published E-R data demonstrates ongoing need for comprehensive bioanalysis"

Key Points Box:

- ADC t½: 3-4 days PubMed (shorter than Blenrep)
- Schedule-dependent neuropathy (cumulative MMAE exposure) PubMed
- c-Met biomarker-selected population
- **FDA PMR:** Conduct exposure-response analyses using popPK methods FDA

Talking Points:

- "Not every ADC has extensive published E-R data like Blenrep"
- "Telisotuzumab vedotin approved in 2025 for c-Met+ NSCLC" Lung Cancer Today +2
- "Measured ADC, total antibody, and free MMAE - but detailed E-R analyses not publicly available" PubMed
- "What we know: schedule mattered for neuropathy, likely driven by cumulative MMAE exposure" PubMed PubMed Central
- "FDA required post-marketing E-R analysis as a PMR - tells us regulators view this as essential even when not completed pre-approval"
- **Key lesson:** "Even with accelerated approvals, comprehensive bioanalytical characterization remains necessary for understanding benefit-risk"

SLIDE 10: Comparative Summary - Key Lessons from Four ADCs (2 minutes)

Title: "Four ADCs, four lessons: What bioanalytical methods revealed"

Visual: Comparative table

ADC	Linker	Key Method Discovery	Clinical Impact
Kadcyla	Non-cleavable	2-3x faster clearance of conjugated vs total Ab; PubMed Central 40% deconjugation by Day 14	Mechanistic understanding; flat E-R made method choice less critical for dosing
Enhertu	Cleavable	Free payload essential; correlates with ILD risk in renal impairment FDA	Enhanced monitoring for renal impairment; all 3 analytes required
Blenrep	Non-cleavable	**ADC → corneal toxicity; Free payload → thrombocytopenia**	**2.5 vs 3.4 mg/kg dose selection; REMS program**
Teliso-V	Cleavable	Schedule-dependent neuropathy (cumulative MMAE)	Q2W vs Q3W schedule selection; FDA PMR for E-R

Key Insights Box (Bold):

- **Non-cleavable linkers:** Still undergo deconjugation (maleimide exchange)
- **Cleavable linkers:** Free payload measurement critical for toxicity
- **No single analyte tells complete story**
- **Method choice directly impacts clinical decisions**

Talking Points:

- "Let's step back and see patterns across these four ADCs"
- "Even 'non-cleavable' linkers aren't truly stable - Kadcyla and Blenrep both show deconjugation"
- "Cleavable linkers like Enhertu and Teliso-V require free payload measurement for toxicity prediction"
- "Blenrep is the gold standard example: measuring multiple analytes revealed different analytes drive different toxicities"
- **Key insight:** "Disease characteristics can confound exposure-efficacy but exposure-safety relationships are often clearer - as seen with Blenrep" (nih)
- "Method choice isn't academic - it drove dose selection for Blenrep, enhanced monitoring for Enhertu, and schedule selection for Teliso-V"
- **Transition to next section:** "So what do regulators say about all this?"

SECTION 4: REGULATORY PERSPECTIVE (2 minutes - Slide 11)

SLIDE 11: FDA Guidance - What Regulators Expect (2 minutes)

Title: "FDA now requires comprehensive bioanalytical strategies for ADCs"

Visual: Timeline showing regulatory evolution

- Pre-2013: Single LBA often sufficient
- 2013-2023: Growing recognition of complexity
- **March 2024: FDA issues ADC-specific guidance** ← Highlight this

Key Requirements Box: FDA Guidance (March 2024): Core Expectations ✓ Measure ADC and constituent parts from first-in-human (ScienceDirect) ✓ Core analytes: ADC (DAR≥1), total antibody, unconjugated antibody, free payload, active metabolites ✓ Conduct exposure-response for BOTH safety and efficacy (ScienceDirect) ✓ All methods validated per ICH M10 (November 2022) ✓ May exclude certain analytes in later development with justification ✓ Consider shed target interference

Visual: Checkmark/X graphic showing evolution

- ❌ Old approach: Single ELISA
- ✓ New standard: Multiple analytes + E-R analysis

Talking Points:

- "In March 2024, FDA issued first comprehensive guidance specifically for ADC clinical pharmacology"

- "Key message: measuring multiple analytes isn't optional anymore - it's expected from first-in-human studies"
- "You must measure ADC with at least one drug attached, total antibody, and unconjugated payload"
- "FDA explicitly states: conduct exposure-response analysis for both safety AND efficacy using multiple constituents"
- "There's some flexibility: you can justify excluding certain analytes in Phase 2/3 if Phase 1 data shows they're not informative"
- "All methods must meet ICH M10 validation standards - harmonized global guidance from November 2022" (nih)
- **Practical implication:** "If you're designing an ADC trial now, plan for multiple validated assays from the start. Don't wait until Phase 2 to discover you measured the wrong analyte"
- **Key message:** "Regulatory expectations have evolved dramatically - comprehensive bioanalytical strategies are now the standard"

SECTION 5: FUTURE DIRECTIONS (2 minutes - Slide 12)

SLIDE 12: Emerging Technologies and Future Directions (2 minutes)

Title: "Next-generation methods for next-generation ADCs"

Visual: Four quadrants showing emerging technologies

Quadrant 1: Intact LC-HRMS

- Icon: Mass spectrum showing intact ADC
- Real-time DAR distribution in vivo (ACS Publications +2)
- Biotransformation characterization (ACS Publications)
- No digestion artifacts

Quadrant 2: Native MS

- Icon: Intact protein complex
- Direct measurement of all DAR species (ResearchGate)
- Comprehensive single analysis
- Higher complexity

Quadrant 3: Automated Hybrid Platforms

- Icon: Robotic system
- High-throughput immunocapture-LC-MS ScienceDirect
- Multiple analytes simultaneously
- Reduced manual labor

Quadrant 4: Advanced PK Modeling

- Icon: Computer with equations
- Mechanistic PK/PD models PubMed +2
- PBPK for ADCs PubMed
- QSP approaches integrating tumor biology PubMed

Future Challenges Box:

- Site-specific conjugation → simplified PK
- Bispecific ADCs → even more complex analytics
- Dual-payload ADCs → measure both payloads Nature
- Tumor tissue measurements for precision dosing

Talking Points:

- "Technology is rapidly evolving to meet ADC complexity" Springer
- "Intact LC-HRMS allows real-time DAR monitoring without digestion - you can watch the ADC change in patients" Springer PubMed
- "Native MS can measure all DAR species simultaneously in a single run - comprehensive but technically challenging" NCBI ResearchGate
- "Automated hybrid platforms are bringing the precision of LC-MS with the throughput needed for clinical trials"
- "On the modeling front: mechanistic PK/PD models can predict drug exposure at the tumor site, not just plasma"
- **Next-generation challenges:** "Site-specific conjugation simplifies some PK complexity but doesn't eliminate it"
- "Bispecific and dual-payload ADCs are coming - analytics will be even more complex"

- **Vision for future:** "Ultimately, we want to measure ADC exposure in tumors, not just blood, for true precision dosing"
- **Key message:** "The future is moving toward comprehensive characterization enabling precision ADC dosing based on individual patient PK and tumor characteristics" Springer

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SECTION 6: CONCLUSIONS AND RECOMMENDATIONS (3 minutes - Slides 13-14)

SLIDE 13: Key Takeaways for Clinical Pharmacologists (1.5 minutes)

Title: "What you need to remember when interpreting ADC pharmacokinetics"

Visual: Numbered list with icons

1. ADCs are not simple drugs 🧪

- Multiple analytes with different PK profiles PubMed ACS Publications
- In vivo deconjugation is universal
- What you measure determines what you see

2. Method choice has clinical consequences ⚕️

- Drove dose selection (Blenrep: 2.5 vs 3.4 mg/kg)
- Enabled safety monitoring (Enhertu: ILD in renal impairment)
- Guided schedule optimization (Teliso-V: Q2W vs Q3W)

3. Different analytes predict different outcomes 📊

- **Efficacy:** Conjugated antibody or conjugated payload
- **Toxicity:** Often free payload (neuropathy, ILD)
- **On-target toxicity:** May be ADC-driven (Blenrep corneal events)

4. Regulatory expectations are clear 📋

- FDA March 2024 guidance mandates multi-analyte approach
- ICH M10 validation standards apply
- Exposure-response required for safety AND efficacy

5. Always ask: which analyte was measured? 🔍

- When reviewing PK data from trials

- When interpreting exposure-response relationships
- When comparing across studies or labs

Talking Points:

- "Let me give you five practical takeaways for your clinical work"
- "First: Never assume an ADC is a simple drug. It's a dynamic mixture that changes in patients"
- "Second: Method choice isn't just technical - Blenrep's dose selection, Enhertu's monitoring strategy, Teliso-V's schedule - all driven by measuring the right analytes"
- "Third: There's no universal 'best analyte.' Conjugated drug usually predicts efficacy, free payload often predicts systemic toxicity, but exceptions exist"
- "Fourth: Regulatory expectations are now clear - FDA guidance says measure multiple analytes and do E-R analysis for both safety and efficacy"
- "Fifth and most practical: **When reviewing any ADC PK data, always ask 'which analyte was measured?'** Total antibody? Conjugated ADC? Free payload? That context is essential for interpretation"

SLIDE 14: Recommendations for Practice (1.5 minutes)

Title: "Practical recommendations when working with ADCs"

Visual: Three columns for different stakeholder groups

For Clinical Trial Design:

- Plan multiple assays from Phase 1
- Budget for LBA + LC-MS + hybrid
- Collect samples for retrospective analysis
- Pre-specify E-R analysis plan

For Data Interpretation:

- Confirm which analyte was measured
- Consider method limitations
- Don't compare across different methods directly
- Evaluate disease burden as confounder

For Clinical Practice:

- Understand which analyte correlates with toxicity
- Know what your lab measures
- Therapeutic drug monitoring may use different methods than trials
- When in doubt, consult pharmacology team

Resources Box:

- FDA Guidance (March 2024): Clinical Pharmacology Considerations for ADCs
- ICH M10 (November 2022): Bioanalytical Method Validation
- AAPS Position Papers (2013-2024): Multiple white papers on ADC bioanalysis

Talking Points:

- "Here are practical recommendations for different scenarios"
- **For trial design:** "Don't wait until Phase 2 to figure out which analyte matters. Measure multiple analytes from Phase 1, even if it's expensive. It's much more expensive to dose wrong"
- "Budget for comprehensive bioanalysis upfront - LBA for throughput, LC-MS for specificity, hybrid for mechanistic insights" (nih)
- **For data interpretation:** "Always confirm which analyte was measured before interpreting PK data"
- "Don't directly compare PK parameters from different methods - a 50% difference might just be method-dependent"
- "Watch for disease burden confounding E-R relationships - Blenrep showed us this clearly" (nih)
- **For clinical practice:** "If you're involved in ADC therapeutic drug monitoring, know what your clinical lab measures - it may differ from what was measured in pivotal trials"
- "When in doubt about ADC PK interpretation, loop in your clinical pharmacology team"
- **Resources:** "I've listed key guidance documents - FDA March 2024 guidance and ICH M10 are essential reading"

SLIDE 15: Final Summary Slide

Title: "Bioanalytical methodology fundamentally shapes our understanding of ADC pharmacokinetics and clinical decisions"

Visual: Central diagram with ADC structure connected to three outcomes

Central Image: ADC breaking down into components (repeat of Slide 2 visual for bookending)

Three Outcomes Connected by Arrows:

1. Different PK Profiles

- 2-3 fold differences in CL, t½, AUC PubMed Central
- Method-dependent parameters

2. Different Clinical Interpretations

- What predicts efficacy?
- What predicts toxicity?
- Which patients need monitoring?

3. Different Clinical Decisions

- Dose selection
- Schedule optimization
- Safety monitoring strategies
- Regulatory actions

Bold Bottom Text: "When interpreting ADC pharmacokinetics, always ask: Which analyte was measured?"

Closing Statement (Memorize):

- "To bring this full circle: we started by asking why PK data might not match across labs or studies. The answer is that ADCs are uniquely complex drugs where method choice fundamentally determines what you see"
- "We've seen how measuring conjugated antibody versus total antibody can show 2-3 fold PK differences" PubMed Central
- "More importantly, we've seen real clinical consequences: Blenrep's dose selection, Enhertu's monitoring strategy, regulatory REMS programs - all driven by measuring the right analytes"
- "The field has matured enormously - regulatory expectations now mandate comprehensive strategies from first-in-human"

- "My final message: **Whenever you review ADC PK data, interpreting exposure-response, or designing a trial, always ask which analyte was measured. That single question can prevent major misinterpretations**"
- "Thank you. I'm happy to take questions."

BACKUP SLIDES (For Q&A - Not Presented Unless Asked)

BACKUP SLIDE 1: Technical Details of Hybrid LBA-LC-MS/MS

Detailed workflow, validation parameters, typical sensitivity ranges

BACKUP SLIDE 2: DAR Measurement Methods Comparison

HIC, RP-HPLC, UV/Vis, SEC-MALS, intact MS - advantages/limitations Nature Creative Biolabs

BACKUP SLIDE 3: Immunogenicity Impact on PK Assays

ADA interference, need for ADA-tolerant methods, bridging assay approach

BACKUP SLIDE 4: Population PK Models for ADCs

Covariate effects, time-varying clearance, target-mediated disposition PubMed Central

BACKUP SLIDE 5: Regulatory Inspection Common Findings

Validation deficiencies, ISR failures, stability issues, documentation gaps

BACKUP SLIDE 6: Cost and Timeline Considerations

Method development timelines, cost per sample, throughput comparison

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VISUAL DESIGN RECOMMENDATIONS

Consistent Color Scheme Throughout Presentation

- **Intact ADC/Conjugated antibody:** Green (#2E7D32)
- **Total/naked antibody:** Blue (#1976D2)
- **Free payload:** Red (#C62828)
- **Neutral elements:** Gray (#616161)
- **Emphasis/highlights:** Gold/amber (#F57C00)

- **Background:** White for small rooms, consider dark for large auditoriums

Key Diagrams to Include

1. ADC Structure Diagram (Slides 2, 3, 15):

- Use consistent molecular cartoon style
- Color-code antibody (blue Y-shape), linker (black line), payload (red circle)
- Label DAR clearly
- Show progression: Intact → Partially deconjugated → Fully deconjugated

2. Method Workflow Comparisons (Slide 4):

- Side-by-side flowcharts
- Use icons: test tube (sample), magnet (immunocapture), vial (separation), graph (detection)
- Keep to 3-4 major steps per method
- Color-code what each method measures

3. PK Curves (Slide 5):

- Semi-log plot: Time (0-21 days) on x-axis, Concentration (ng/mL, log scale) on y-axis
- Three curves with different line styles AND colors
- Legend in top-right corner
- Annotate key features: "Curves diverge at Day 3", "2-3 fold AUC difference"
- Include error bands if space permits

4. Exposure-Response Plots (Slide 8 for Blenrep):

- Scatter plots with fitted logistic curves
- X-axis: ADC Ctau (μg/mL)
- Y-axis: Probability of event (0-100%)
- Individual patient data points as semi-transparent dots
- Confidence intervals as shaded regions
- Key statistics in text box: OR, 95% CI, p-value

5. Comparative Tables (Slides 10, 13):

- Clear row/column headers
- Alternate row shading for readability
- Bold key findings
- Use checkmarks (✓) and X marks (✗) for quick scanning
- Limit to 4-5 columns maximum

Typography Guidelines

- **Slide titles:** 36-40pt, Bold, Sans-serif (Arial/Calibri)
- **Body text:** 24-28pt, Regular, Sans-serif
- **Figure labels:** 20-22pt
- **Citations:** 14pt at bottom (Author, Journal, Year format)
- **Emphasis:** Bold or color, NOT underline or all-caps

Animation Strategy

- Use sparingly and purposefully
- Progressive reveal for complex diagrams (build ADC structure piece by piece)
- Step-through for PK curves (show each curve sequentially, then together)
- Fade-in for bullet points (one at a time) only if it supports the narrative
- Avoid gratuitous animations (spinning, bouncing, etc.)

STUDY MATERIALS COMPILATION

PRIMARY REGULATORY DOCUMENTS

1. FDA Clinical Pharmacology Considerations for Antibody-Drug Conjugates (March 2024) Link: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-pharmacology-considerations-antibody-drug-conjugates-guidance-industry Key sections: Bioanalytical methods (Section 4), Exposure-response (Section 6), Organ impairment (Section 8)

2. ICH M10 Bioanalytical Method Validation (November 2022) Link: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m10-bioanalytical-method-validation-and-study-sample-analysis Key sections: LBA validation (Section 8), Cross-validation (Section 9)

3. EMA Guideline on Monoclonal Antibodies and Related Products (2016) Link: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-production-characterisation-specification-monoclonal-antibodies-related_en.pdf Note: ADCs covered under "conjugated mAbs" section

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ESSENTIAL REVIEW ARTICLES

4. Kaur S., et al. "Bioanalytical assay strategies for the development of antibody-drug conjugate biotherapeutics." Bioanalysis, 5(2):201-226, 2013. PMID: 23330562 Why essential: Foundational paper establishing multi-analyte strategy; includes T-DM1 case studies

5. Gorovits B., et al. "Bioanalysis of antibody-drug conjugates: AAPS ADC Working Group position paper." Bioanalysis, 5(9):997-1006, 2013. PMID: 23641692 Why essential: Industry consensus on best practices; regulatory perspective

6. Wang J., et al. "Antibody-drug conjugate bioanalysis using LB-LC-MS/MS hybrid assays." Bioanalysis, 8(13):1383-1401, 2016. PMID: 27277879 Why essential: Detailed comparison of platforms; when to use each method

7. Qian Q., Gong L. "Current Analytical Strategies for Antibody-Drug Conjugates in Biomatrices." Molecules, 27(19):6299, 2022. PMC: PMC9572530 Why essential: Most recent comprehensive review covering all platforms

8. Recent Advances in Bioanalytical Methods for Quantification and Pharmacokinetic Analyses of Antibody-Drug Conjugates. AAPS Journal, 2025. DOI: 10.1208/s12248-025-01115-9 Why essential: Cutting-edge technologies; future directions

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KEY METHODOLOGY PAPERS

9. Huang Y., et al. "Characterization of ADC Pharmacokinetics and in Vivo Biotransformation Using Quantitative Intact LC-HRMS." Analytical Chemistry, 93(15):6135-6144, 2021. PMID: 33835773 Why important: State-of-the-art intact mass approach; DAR profiling

10. He J., et al. "Characterization of in vivo biotransformations for trastuzumab emtansine by high-resolution mass spectrometry." mAbs, 10(7):960-967, 2018. PMID: 29958059 Why important: T-DM1 biotransformation; mechanism of deconjugation

11. Dere R., et al. "Evaluation of a sensitive and specific LC-MS/MS method for quantitation of in vivo dethiolated antibody-drug conjugate." Bioanalysis, 5(9):1025-1040, 2013. PMID: 23641694 Why important: First hybrid immunocapture-LC-MS/MS for conjugated payload

CLINICAL PHARMACOLOGY / EXPOSURE-RESPONSE

12. Ferron-Brady G., et al. "Exposure-Response Analyses for Therapeutic Dose Selection of Belantamab Mafodotin." Clinical Pharmacology & Therapeutics, 110(5):1282-1292, 2021. DOI: 10.1002/cpt.2372 Why critical: Definitive Blenrep E-R analysis; differential analyte-toxicity relationships

13. Rathi C., et al. "Population pharmacokinetics of belantamab mafodotin in patients with relapsed/refractory multiple myeloma." CPT Pharmacometrics & Systems Pharmacology, 10(8):851-863, 2021. DOI: 10.1002/psp4.12660 Why critical: PopPK with disease covariates; sBCMA effect on PK

14. Lu D., et al. "Population pharmacokinetics of trastuzumab emtansine (T-DM1), a HER2-targeted antibody-drug conjugate, in patients with HER2-positive metastatic breast cancer." Cancer Chemotherapy and Pharmacology, 74(2):399-410, 2014. PMID: 24942146 Why important: First comprehensive ADC popPK; established modeling framework

15. Girish S., et al. "Clinical pharmacology of trastuzumab emtansine (T-DM1): an antibody-drug conjugate in development for the treatment of HER2-positive cancer." Cancer Chemotherapy and Pharmacology, 69(5):1229-1240, 2012. PMID: 22271209 Why important: Early T-DM1 clinical pharmacology; established PK-PD principles

16. Vasalou C., et al. "Quantitative evaluation of trastuzumab deruxtecan distribution in HER2+ breast cancer brain metastases." CPT Pharmacometrics & Systems Pharmacology, 13(3):522-535, 2024. PMID: 38287477 Why important: T-DXd tissue distribution; tumor vs plasma exposure

17. Yang A., et al. "Review of dose justifications for antibody-drug conjugate approvals from clinical pharmacology perspective." Journal of Pharmacokinetics and Pharmacodynamics, 2024. PMID: 39374692 Why essential: Comprehensive review of all FDA-approved ADCs; E-R patterns

18. Masters J.C., et al. "Clinical toxicity of antibody drug conjugates: a meta-analysis of payloads." Investigational New Drugs, 36(1):121-135, 2018. PMID: 29027591 Why important: Payload-specific toxicity patterns; free drug correlations

19. Wang C., et al. "Meta-Analysis of Exposure-Adverse Event Relationships for Antibody-Drug Conjugates." Journal of Clinical Pharmacology, 65(4):486-498, 2025. PMID: 39539040 Why important: Recent comprehensive E-R meta-analysis; which analytes matter for which AEs

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SPECIFIC ADC CASE STUDY DOCUMENTS

20. FDA Clinical Pharmacology Review: Kadcyla (T-DM1) - NDA 125427 (2013) Available: FDA Drugs@FDA website Sections to review: Clinical Pharmacology, PopPK, E-R analysis

21. EMA Assessment Report: Kadcyla (T-DM1) - EMEA/H/C/002389/0000 (2013) Available: EMA website Why important: European perspective; comprehensive bioanalytical discussion

22. FDA Multi-Disciplinary Review: Enhertu (T-DXd) - BLA 761139 (2019) Available: FDA Drugs@FDA website Sections to review: Clinical Pharmacology (Section 4.5), Renal impairment substudy

23. FDA BLA Review: Blenrep (belantamab mafodotin) - BLA 761158 (2020) Available: FDA Drugs@FDA website - particularly Appendix 20.4.1 Why critical: Most detailed E-R documentation; disease covariate discussion

24. Richardson PG., et al. "Belantamab mafodotin for relapsed or refractory multiple myeloma: Phase 1 study." Blood Cancer Journal, 10(10):106, 2020. PMID: 33060564 Why important: Clinical context for Blenrep dose selection

25. Strickler JH., et al. "First-in-Human Phase I Study of Telisotuzumab Vedotin, an Antibody-Drug Conjugate Targeting c-Met, in Patients With Advanced Solid Tumors." Journal of Clinical Oncology, 36(33):3298-3306, 2018. PMID: 30265611 Why important: Teliso-V clinical pharmacology; schedule selection rationale

26. Camidge DR., et al. "Phase I Study of 2- or 3-Week Dosing of Telisotuzumab Vedotin, an Antibody-Drug Conjugate, in Patients With c-Met Protein–Overexpressing Advanced Non-Small Cell Lung Cancer." Clinical Cancer Research, 28(15):3248-3259, 2022. PMID: 35560205 Why important: Schedule-dependent toxicity; cumulative exposure effects

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ADDITIONAL SEARCH QUERIES FOR ONGOING LEARNING

PubMed Search Strategies:

1. "antibody drug conjugate" AND "bioanalysis" AND "pharmacokinetics" [Filter: Review, last 5 years]
2. "ADC" AND "exposure response" AND "clinical pharmacology" [Filter: Clinical Trial]
3. ("trastuzumab emtansine" OR "trastuzumab deruxtecan" OR "belantamab mafodotin") AND "bioanalytical"
4. "hybrid immunocapture" AND "LC-MS" AND "antibody drug conjugate"
5. "intact mass spectrometry" AND "ADC" [Filter: last 3 years]

Google Scholar Searches:

1. site:fda.gov "antibody drug conjugate" pharmacology review
2. "ADC bioanalysis" filetype:pdf review
3. "AAPS" "antibody drug conjugate" "white paper"

Journal Monitoring:

- Set up alerts in Bioanalysis, AAPS Journal, mAbs, Clinical Pharmacology & Therapeutics
- Keywords: "antibody drug conjugate", "ADC", "bioanalysis", "exposure response"

BEST PRACTICES FOR EXPLAINING TO CLINICAL PHARMACOLOGY AUDIENCE

Key Principles

1. Start with Clinical Context, Not Methodology

- ❌ Don't say: "LBA uses sandwich ELISA with capture and detection antibodies..."
- ✓ Do say: "We need a sensitive method to detect low ADC concentrations in patient blood. LBA is like a lock-and-key system that can find one specific molecule in a sea of proteins."

2. Use the "Why-What-How" Framework

- **Why:** Why does this matter for patients/clinicians?
- **What:** What did we find/measure?
- **How:** How did we do it? (briefest part)

3. Anchor Complex Concepts to Familiar Ideas

- ADC structure → "Smart bomb with GPS guidance"
- LBA → "Lock and key" or "How we measure cholesterol"
- LC-MS → "Sorting mail by address AND weight"
- DAR → "How many bombs are attached to each GPS unit"
- Deconjugation → "The smart bombs are falling off the GPS system"

4. Quantify Impact Whenever Possible

- Instead of: "Different methods give different results"
- Say: "Measuring total antibody overestimates active drug by 2-3 fold compared to measuring conjugated ADC"
- Numbers make abstract concepts concrete

5. Connect to Clinical Decisions

- After every major point, add: "Why does this matter clinically?"
- Example: "This 3-fold PK difference meant Blenrep's dose was selected at 2.5 mg/kg instead of 3.4 mg/kg - preventing unnecessary toxicity in thousands of patients"

Common Pitfalls to Avoid

Jargon Without Definition:

- Never use: MRM, UPLC, ECLA, HIC, SPE without explaining
- Instead: "We use triple quadrupole mass spectrometry - think of it as a highly specific detector that can identify molecules by their exact weight"

Assuming Method Knowledge:

- Don't assume audience knows how mass spectrometry works
- Brief analogy is better than no explanation

Method Details Over Clinical Impact:

- Limit method details to 20% of any given slide
- Spend 80% on what it tells us and why it matters

Information Overload:

- One major message per slide
- If explaining three analytes, use three slides or progressive reveal
- Don't show a table with 10 rows and 8 columns

Tables in Presentations:

- Minimize tables - they're hard to digest during presentations
- If you must use a table, limit to 4 rows x 4 columns maximum
- Convert data to graphs whenever possible

Engagement Techniques

Rhetorical Questions:

- "Have you ever wondered why PK data from two labs don't match?"
- "What would happen if we dosed patients based on total antibody when only half is actually active drug?"

- Pause briefly for mental processing

Brief Anecdotes:

- "When Blenrep was being developed, they noticed patients with lower disease burden had better responses. Was this because the drug worked better? Or because these patients had different PK? Measuring multiple analytes revealed..."

Previewing/Signposting:

- "I'm going to show you three case studies. The first, Kadcyla, taught us ADCs fall apart. The second, Enhertu, showed us we must measure released payload. The third, Blenrep, is the gold standard for how different analytes predict different toxicities."

Repetition of Key Messages:

- Repeat your core message ~7 times throughout the talk
- Rephrase each time: "Method choice matters" → "What we measure determines what we see" → "Different assays tell different stories" → "The analytical method fundamentally shapes PK interpretation"

Handling Technical Questions During Q&A

If Asked About Detailed Methods:

- "That's a great technical question. Let me give you the high-level answer now, and I'm happy to discuss details after the session."
- Have backup slides with technical details
- Offer to share protocols or references

If Asked to Compare Methods Quantitatively:

- "For sensitivity, LBA typically achieves 10-100 pg/mL while LC-MS/MS is in the 1-10 ng/mL range. For throughput, LBA handles 96-384 samples per day while hybrid methods are more like 20-40. The trade-off is specificity and structural information."

If Asked About Cost:

- "LBA is generally $50-150 per sample for routine analysis. LC-MS/MS is $100-300. Hybrid methods can be $300-500. But the real cost is measuring the wrong analyte and making incorrect clinical decisions."

If Asked About Regulatory Preference:

- "FDA's March 2024 guidance is method-agnostic - they care that you measure the right analytes and validate properly per ICH M10. LBA remains most common for clinical trials due to throughput, but LC-

MS is increasingly accepted especially for payloads."

FINAL PREPARATION CHECKLIST

Content Preparation

- ✓ Presentation timed to 17-18 minutes (leaving 2-3 min for Q&A)
- ✓ Every slide has clear declarative title stating the main message
- ✓ Key message repeated approximately 7 times throughout
- ✓ All acronyms defined on first use
- ✓ All figures have clear axis labels and legends
- ✓ Citations included at bottom of slides (Author, Journal, Year format)
- ✓ Backup slides prepared for technical questions
- ✓ Opening statement memorized
- ✓ Closing statement memorized
- ✓ Transitions between sections smooth and logical

Visual Design

- ✓ Consistent color scheme throughout (green=ADC, blue=antibody, red=payload)
- ✓ Font sizes: Titles 36-40pt, Body 24-28pt, Citations 14pt
- ✓ Sans-serif fonts used throughout
- ✓ High contrast colors (avoid red-green combinations)
- ✓ Lots of white space - not crowded
- ✓ Tables limited to 4x4 or converted to graphs
- ✓ Animations used sparingly and purposefully
- ✓ All graphs have clear labels and legends

Technical Aspects

- ✓ Presentation tested on presentation computer
- ✓ Backup copy on USB drive
- ✓ PDF version prepared as backup

- ✓ Videos/animations work properly (if used)
- ✓ Hyperlinks functional (if any)
- ✓ Slide numbers included
- ✓ File size manageable for email if needed

Delivery Preparation

- ✓ Presentation practiced 3+ times
- ✓ Practiced with timer to ensure proper pacing
- ✓ Key sections practiced out loud (not just mentally)
- ✓ Complex slides practiced for smooth explanation
- ✓ Case study narratives rehearsed
- ✓ Anticipated questions brainstormed
- ✓ Backup slides reviewed
- ✓ Handout prepared with references (optional)

Day-of Checklist

- ✓ Arrive early to test equipment
- ✓ Check audio/visual setup
- ✓ Have water available
- ✓ Remote clicker tested (if using)
- ✓ Presenter notes available (but don't read from them)
- ✓ Contact information on title slide or final slide
- ✓ Business cards available for post-talk discussions

ADDITIONAL RESOURCES

Recommended Background Reading Before Presenting

For Comprehensive Understanding:

1. Read FDA March 2024 Guidance in full (30 pages)
2. Review Blenrep E-R paper (Ferron-Brady 2021) - best case example

3. Skim T-DM1 popPK papers (Lu 2014, Girish 2012) for historical context

For Technical Depth:

1. Kaur 2013 review (foundational bioanalytical strategies)
2. Wang 2016 review (hybrid methods)
3. Recent AAPS Journal 2025 review (emerging technologies)

For Clinical Context:

1. Masters 2018 (payload-specific toxicities)
2. Yang 2024 (dose justifications for all approved ADCs)
3. Wang 2025 meta-analysis (E-R relationships)

Professional Development Opportunities

Conferences Where ADC Bioanalysis Featured:

- AAPS Annual Meeting (November each year)
- WCBP (World Conference on Bioanalysis and Pharma)
- ACoP (American Conference on Pharmacometrics)
- AACR (American Association for Cancer Research) - clinical focus

Online Resources:

- Bioanalysis Zone (https://www.bioanalysis-zone.com/) - news and articles
- AAPS website - white papers and position statements
- FDA website - all regulatory guidance documents

Training Opportunities:

- AAPS short courses on ADC bioanalysis
- Vendor-sponsored LC-MS workshops (Thermo, Agilent, Waters)
- Academic courses: UW-Madison, UMD, USC offer bioanalysis programs

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CONCLUSION

This comprehensive presentation guide provides everything needed to deliver an impactful 20-minute talk on

ADC bioanalytical methodology for a clinical pharmacology audience. The structure balances technical rigor with clinical relevance, uses real-world case studies to illustrate key principles, and emphasizes actionable takeaways.

The Core Message: Bioanalytical method choice isn't just a technical detail - it fundamentally shapes PK interpretation and drives clinical decisions. From Blenrep's dose selection to Enhertu's safety monitoring, measuring the right analytes has real consequences for patients.

Success Metrics for Your Presentation:

- Audience can explain why measuring total antibody alone is insufficient
- Audience understands that different analytes predict different outcomes
- Audience knows to ask "which analyte was measured?" when reviewing PK data
- Audience appreciates that this has regulatory and clinical implications
- Audience feels equipped to apply these principles in their work

With thorough preparation using this guide, you'll deliver a presentation that educates, engages, and equips your clinical pharmacology audience to better understand and interpret ADC pharmacokinetics.

Good luck with your presentation!