Quantification of antibody-Conjugated Drug in Cleavable ADCs: A Universal, Automated, and Cost-Effective LC-MS/MS Platform

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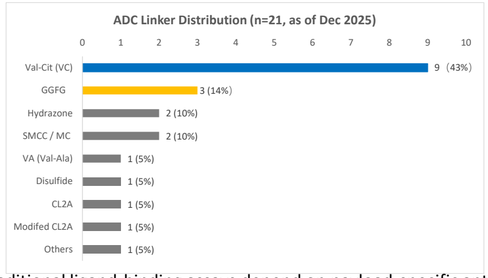
Background

Antibody-drug conjugates (ADCs) are complex biotherapeutics requiring quantification of antibody-conjugated drugs—the pharmacologically most relevant species.
Most approved ADCs use cleavable peptide linkers. Among 21 FDA-approved ADCs (as of Dec 2025), Val-Cit (representing 43%) and GGFG (14%) are the two most widely used cleavable linkers.



However, traditional ligand-binding assays depend on payload-specific antibodies (often unavailable in early discovery). Existing methods are typically developed on a case-by-case basis for specific linker–payload combinations.

There is an unmet need for a universal, systematic, and automated workflow that can be readily adapted to multiple cleavable linkers without case-by-case development.

- ☑Systematic workflow – Linker understanding → cleavage optimization → sample prep → analysis → ICH M10 validation
- ☑Model validation – Val-Cit and GGFG as two representative linker classes
- ☑Platform features – Robust, automated, cost-effective LC-MS/MS
- ☑The systematic workflow is adaptable to other cleavable linkers (e.g., disulfide, β-glucuronidase) by re-optimizing the cleavage step accordingly

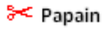
Automated Workflow ADC Quantification

Step 1: Capture 

Plasma + Anti-human Fc Dynabeads magnetic beads(Generic capture of all IgG based ADCs)

Step 2: Isolation 

KingFisher system · Automated positive isolation

Step 3: Cleavage 

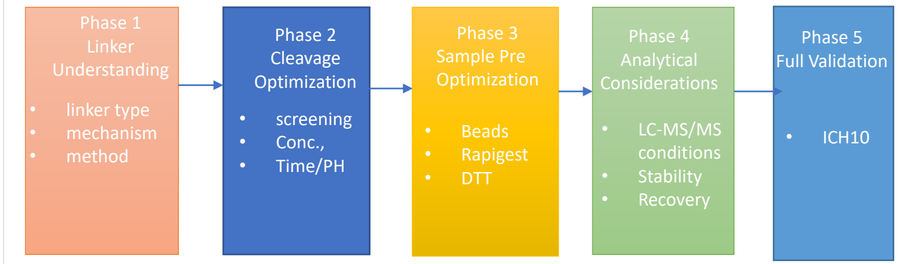
Papain digestion · 2h (Val-Cit / GGFG)

Step 4: Analysis 

LC-MS/MS · SIL-IS · MRM quantitation of released payloads

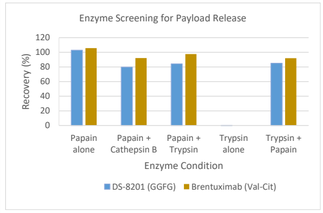
Key point: A single unified workflow was successfully applied to both Val-Cit and GGFG linker classes.

Systematic Method Development Workflow for Cleavable ADCs



Method Development and Optimization (Using Brentuximab and DS-8201 as Model Compounds)

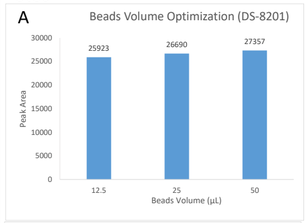
1. Enzyme Screening

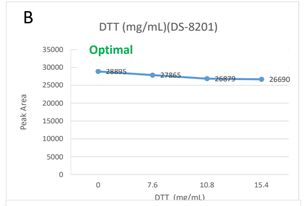


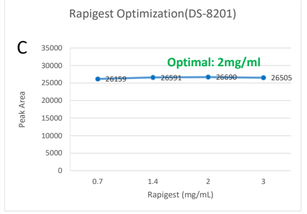
Mechanism-based enzyme selection:
• Both Val-Cit and GGFG linkers are substrates for lysosomal cathepsins (including cathepsin B), which belong to the cysteine protease family .
•.Papain has been successfully used for Val-Cit [Sanderson et al., 2016] and GGFG.
•This work, Integrated papain-based cleavage into a unified, automated LC-MS/MS workflow for both linker classes.
Experimental validation (this work):
☑Papain alone: efficient release for both linkers
☒Trypsin (serine protease, different mechanism) → negligible release.
☒Adding cathepsin B or trypsin → no additional benefit.
Conclusion: A single protease (papain) is sufficient for both linker classes. The same mechanism-driven strategy can be applied to other cleavable linkers.

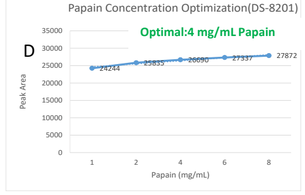
2. Multi-Parameter Optimization

➢ Optimization results shown for DS-8201 (GGFG). Similar trends observed for Brentuximab vedotin (Val-Cit) unless noted.









➢ **Key Findings**:
A: Beads Volume:
Optimal: 25 μL (sufficient and cost-effective)

B: DTT Optimization:
no effect on Val-Cit; slight decrease for GGFG in high concentrations.

➢ Key Findings:
C: Rapigest Optimization

Optimal: 2.0 mg/mL
- Payload release: no significant difference
- But total antibody detection is sensitive to Rapigest denaturation

D: Papain
Optimal: 4 mg/mL
4 mg/mL selected for cost efficiency

Recovery Assessment

ADC	IC Recovery	Overall Recovery (IC + Cleavage + Processing)
Brentuximab vedotin	103.5% (RSD 5.48%)	98.8% (RSD 6.0%)
DS-8201	90.5% (RSD 5.49%)	89.8% (RSD 7.31%)

•IC Recovery: immunocapture efficiency.

•Overall Recovery: total process recovery (capture → cleavage → analysis). Both ADCs met acceptance criteria (80–120%) with RSD <8%.

Validation results

Parameter	Brentuximab vedotin (Val-Cit)	DS-8201 (GGFG)
Plasma Volume	12.5 ul	12.5 ul
Linear range	0.025-50 ug/ml	0.2-100 ug/ml
LLOQ	0.025 ug/ml	0.2 ug/ml
Intra-run RE(QC)	-4.86% to 4.29%	-5.8% to 5.27%
Intra-run RSD(QC)	7.45%	≤7.89%
Inter-run RE	-1.39% to 3.49%	-2.91% to 2.97%
Inter-run RSD	≤10.0%	≤14.7%

Selectivity, stability, and matrix effect were also evaluated and met ICH M10 acceptance criteria (data not shown).

CONCLUSIONS

- ☑ Systematic workflow established – Linker understanding → cleavage optimization → sample prep → analytical considerations → ICH M10 validation.
- ☑ Universal applicability – Successfully validated on two representative linker classes: Val-Cit and GGFG
- ☑ Optimized conditions
- ☑ Performance – – LLOQ: 0.025 μg/mL (Brentuximab) / 0.2 μg/mL (DS-8201); recoveries 89.8–98.8%; intra-run RSD ≤7.9%; inter-run RSD ≤14.7%.
- ☑ Compliant & automated – ICH M10 full validation; KingFisher automated workflow.
- ☑ Cost-effective – ~10× lower enzyme cost vs. cathepsin B.
- ☑ This workflow is designed based on linker cleavage mechanism, not on specific ADC. The same logic can be applied to any cathepsin B-sensitive cleavable linker.
- ☑ We have developed a systematic and adaptable LC-MS/MS workflow for Val-Cit and GGFG linker classes. While payload-specific MS optimization is still required, the entire upstream sample preparation and digestion process is unified and automated

REFERENCES

1. Klussman, K., et al. Bioconjugate Chem. 2004, 15, 765-773.
2. Sanderson RJ, et al. Bioanalysis. 2016;8:55-63.
3. Li Y, et al. mAbs. 2016;8:698-705.
4. Yang L, et al. J Med Chem. 2025;68:18099–18113.
5. Balamkundu S, Liu C-F. Biomedicines. 2023;11(11):3080.