

Challenges and Solutions Associated with the Bioanalysis of Antibody Drug Conjugates in Support of Clinical Studies – A Case Study on Kadcyła®

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Introduction:

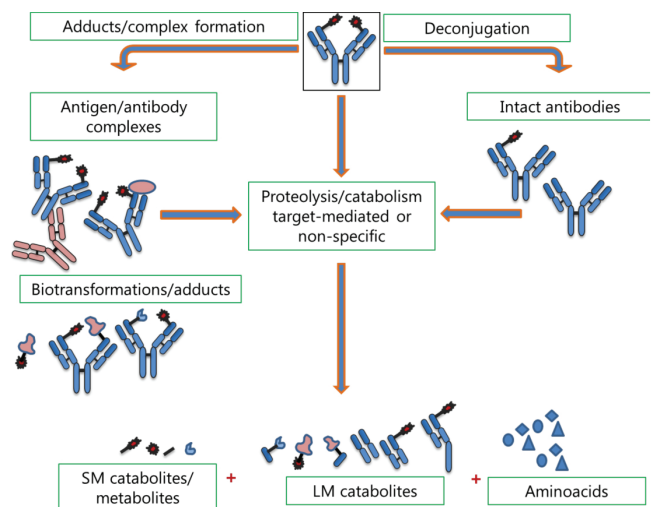
Antibody-drug Conjugates (ADCs) are one of the most innovative, and fast growing classes of potent bio-therapeutic drugs predominantly designed for targeted cancer therapy. ADCs are composed of a potent cytotoxic drug attached to a monoclonal antibody (mAb) via a linker. The chosen mAb either binds to a receptor or a cell-surface protein that is over-expressed in cancer cells. ADCs are thus capable of discriminating between healthy and cancerous cells, and therefore target their cytotoxicity on the cancerous cells.

Variability between ADCs can arise from the mAb (chimeric, humanized), the conjugation site of the linker to the mAb - non-specific (lysine or inter-chain disulfide) or site-specific (engineered cysteines); the type of linker used (peptide/ maleimide/ disulfide) and the type of bioactive drug (tubulin inhibitors or DNA alkylators).

Challenges:

The biological presence of the ADCs is a heterogeneous mixture due to its complex composition. ADC heterogeneity can arise from stoichiometry of the drug to Ab ratio, the specific amino acid sites of attachment on the mAb, and the stability or reversibility of the conjugation chemistry. Figure 1 illustrates the theoretical metabolism of an ADC and the potential analytical targets.

Figure 1. Flow chart showing the various theoretical catabolic pathways of ADCs [1].

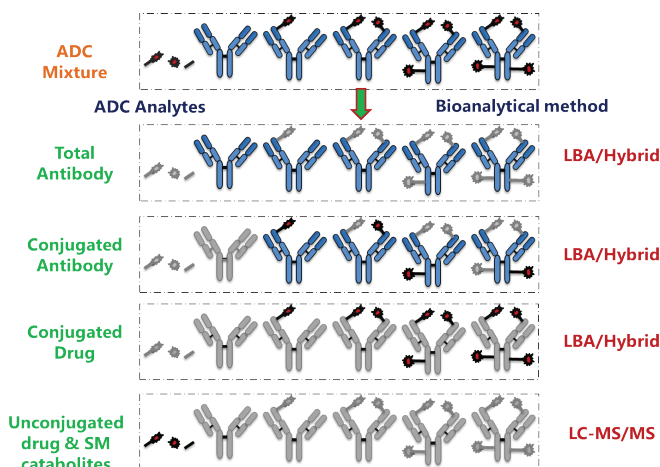


1. Loss of drug through de-conjugation
2. Complex/adduct formation
3. Lysosomal degradation and proteolysis after internalization

While ligand binding assays (LBAs) offer sensitivity with minimal sample volume in a high throughput setting, in comparison, they are not selective, prone to greater imprecision and are limited to a narrow dynamic range than LC-MS/MS.

The various possible ADC analytes pose a challenge for the accurate pharmacokinetic (PK) measurement of ADCs in a biological matrix. Multiple PK assay strategies are essential to understand the fate of these ADC analytes. Figure 2 shows the possible ADC analytes in a heterogeneous mixture and the current bioanalytical assays adapted for the measurement of the respective ADC analytes.

Figure 2. Figure showing the various ADC analytes (green) {total antibody (TAb) (conjugated and unconjugated); conjugated antibody (antibody with at least one drug present; conjugated drug (one or more drug(s) that is attached to the antibody) and unconjugated drug (drug that is not attached to any antibody)}. The multiple assays (red) that are currently used for the measurement of these analytes are also listed [2].

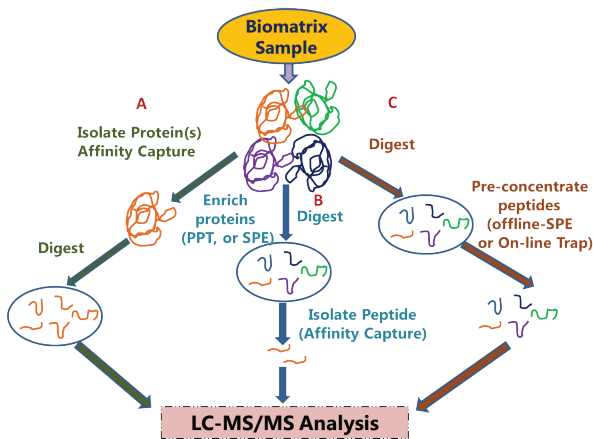


Solutions:

There is a need for novel bioanalytical hybrid approaches and strategies for the bioanalysis of ADCs including a combination of immunoaffinity (IA) and LC-MS-based platforms. This will allow for combining the high sensitivity of LBA assays with the greater selectivity, broad dynamic range and improved precision of LC-MS assays. IA-LC-MS technique can be orthogonal and complementary to traditional LBA assays.

Typical IA-LC-MS/MS approaches can be described as follows in figure 3.

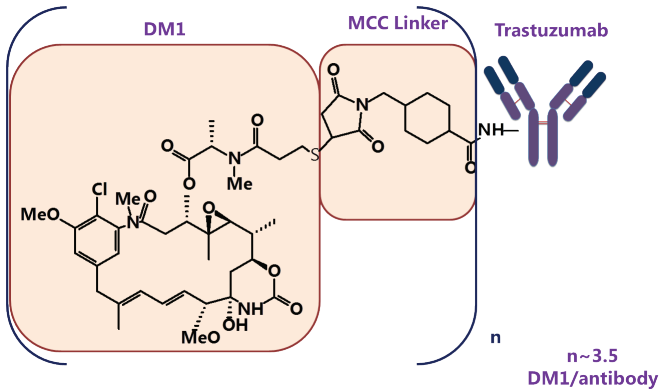
Figure 3. Figure showing the various workflows that can be adapted based on the requirement for an IA-LC-MS/MS methodology. A. Protein level affinity capture B. Peptide level affinity capture and C. affinity reagent-free procedure [3].



Case Study:

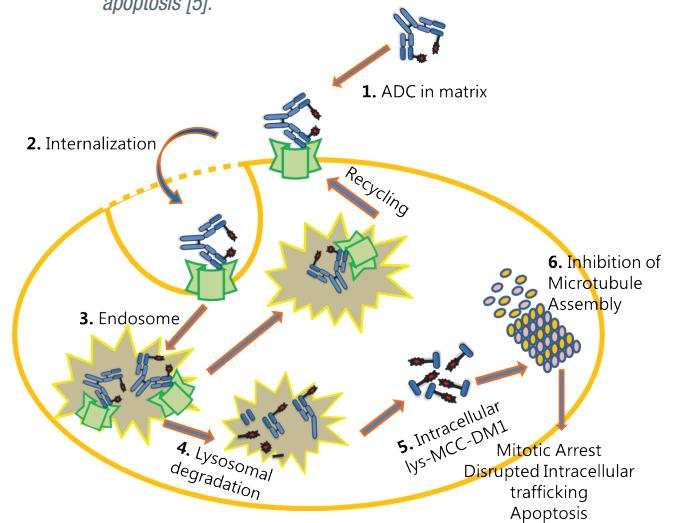
Kadcyla® (ado-trastuzumab emtansine) is a humanized mAb (IgG1) Herceptin® against HER2 receptor that is chemically conjugated to non-specific lysine via a heterobifunctional cross linker to a cytotoxic maytansinoid derivative, Emtansine (DM1) as shown in figure 4.

Figure 4. Structure of Kadcyla®. The average drug to antibody ratio (DAR) for Kadcyla® derived from studies is 3.5 [6].



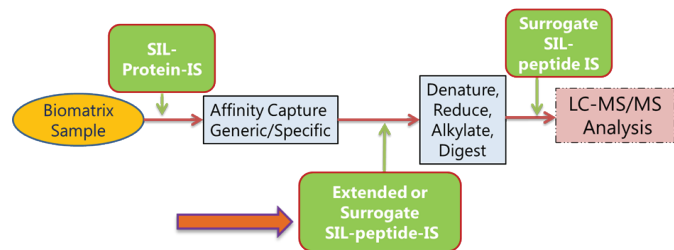
Kadcyla® is prescribed for the treatment of human epidermal growth factor (HER2) positive breast cancer. The proposed mechanism of how Kadcyla® acts is described in figure 5 as follows.

Figure 5. Kadcyla® binds to HER2 receptor on the membrane and enters into the cell via receptor-mediated endocytosis. Internalized endocytic vesicles either form early endosomes or recycled back to the cell membrane. The early endosome can mature to a lysosome and releases DM1 via proteolytic degradation. Intracellular lysine (lys)-MCC-DM1 inhibits microtubule assembly, causing mitotic arrest and apoptosis [5].



Total antibody (Tab) analysis of Kadcyla® in human plasma/serum using IA-LC-MS/MS is carried out by affinity capture at the protein level with biotinylated-HER2 receptor immobilized on streptavidin-paramagnetic beads, and the addition of stable isotopically labelled (SIL) extended peptide as internal standard that includes the exact amino acid sequence of the signature peptide monitored for quantification. The strategy is executed as follows in figure 6.

Figure 6. Workflow of protein level affinity capture with the addition of internal standard (with flanking ends) after the capture and before the hydrolysis step and subjecting the sample to LC-MS analysis post digestion [3].



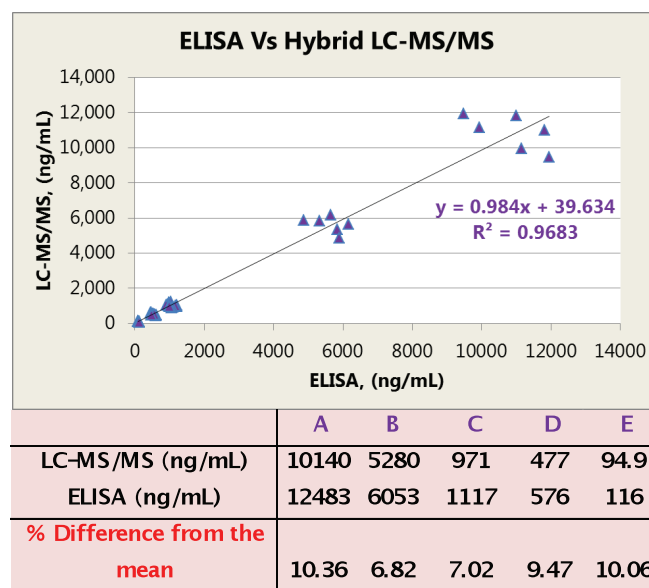
The assay parameters and the data obtained from the 3 precision accuracy (PA) batches of the IA-LC-MS/MS analysis is outlined in table 1:

Table 1. Assay parameters and the data obtained from the PA batches of IA-LC-MS/MS analysis of Kadcyła®.

Assay parameters	Data
Analyte	Kadcyła (Ado-Trastuzumab Emtansine (IYPTNGYTR signature peptide))
Internal Standard (IS)	WVARIY[¹³ C ₅ ; ¹⁵ N]PTN[¹³ C ₂ ; ¹⁵ N]GYTRYANS
Method Description	Immunoaffinity coupled with bottom-up LC-MS/MS
Limit of Quantitation (ng/mL)	10000 ng/mL
Average Recovery of Drug (% Mean)	116% at 75.0 ng/mL 102% at 8000 ng/mL
Standard Curve Concentrations (ng/mL)	50.0, 100, 250, 500, 1000, 5000, 8500, and 10000 ng/mL
QC Concentrations (ng/mL)	50.0, 150, 700 and 8000 ng/mL
QC Intra-Batch Precision Range (% CV)	8.2 to 22.2%
QC Intra-Batch Accuracy Range (% Bias)	-19.4 to 21.4%
QC Inter-Batch Precision Range (% CV)	13.5 to 19.4%
QC Inter-Batch Accuracy Range (% Bias)	-7.0 to 12.9%
Bench-Top Stability (Hrs)	Short-Term Stability: 8 hours in polypropylene tubes at ambient temperature under white light (QCs stored at -20°C)
Freeze-Thaw Stability (Cycles)	3 freeze (-20°C)-thaw (ambient temperature) cycles in polypropylene tubes under UV-shielded light
Dilution Integrity	Up to 25,000 ng/mL, diluted 10-fold
Selectivity	No significant interference at the retention time and mass transition of Kadcyła was observed from endogenous components in any of the 10 human plasma (EDTA) lots screened or of IS in any of the 10 human plasma (EDTA) lots screened
Matrix	Human Plasma
Anticoagulant	K ₂ EDTA
Detector	AB SCIEX API 6500™
Assay Volume Required	0.100 mL
Regression Type	Weighted linear (1/concentration ²)
Quantitation Method	Peak area ratio
Batch Size	96

Comparison of data generated for TAb analysis of Kadcyła® using a traditional enzyme linked immune sorbent assay (ELISA) and IA-LC-MS/MS assay as shown in figure 7. Samples with various concentrations of Kadcyła® were analyzed using Celerion's IA-LC-MS approach and the same samples was also analyzed by Somru BioScience using their Kadcyła® PK ELISA kit.

Figure 7. Comparable and consistent results obtained from an ELISA assay and the IA-LC-MS/MS assay.



Conclusion:

- Both ligand-binding and tandem mass- spectrometric methods are useful in understanding the pharmacokinetic and metabolic fate of heterogeneous ADCs due to their dynamically changing and complex nature.
- While ELISA based assays have traditionally been used for bioanalysis of ADCs, IA-LC-MS/MS analysis is emerging as a complementary method, providing more reliable measurements for ADC species by combining the structural sensitivity of IAs with the mass selectivity and, broad dynamic range of LC-MS/MS.
- Kadcyła® is used as a case study to demonstrate the bioanalysis of ADCs using IA-LC-MS/MS strategy. Comparable results were obtained for the Total antibody (TAbs) (conjugated and unconjugated) quantification of Kadcyła® using ELISA and IA-LC-MS/MS analysis.

Acknowledgement:

We would like to thank Somru BioScience Inc. for analyzing samples in using ELISA method and for sharing providing the data analysis using the ELISA method.

References

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