M1530-02-09

Development of a High Throughput LC-MS/MS 1.5 Plex Assay for Quantitation of Total Antibody (TAb) and Intact Antibody Drug Conjugate (ADC) using Immunoprecipitation by Specific Receptor Capture

Ryan Darling, Amrit Chakravarty, Dawn R. Dufield, Jared Stark, Rathna J. Veeramachaneni

KCAS Bio

CONTACT INFORMATION: Ryan.Darling@kcasbio.com

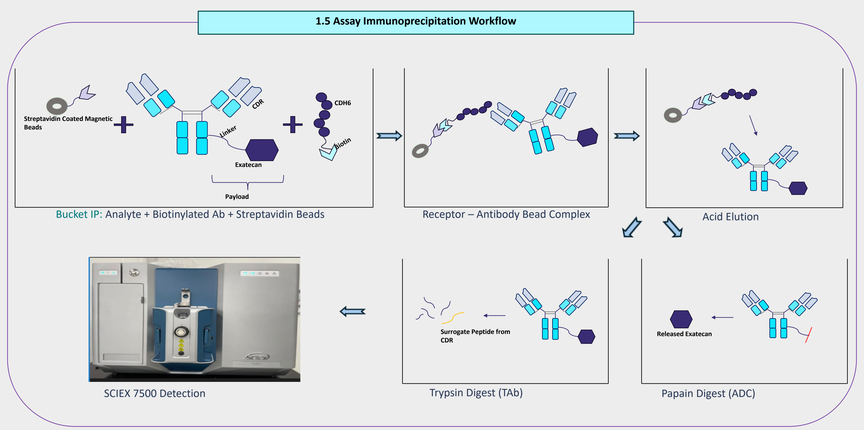
aaps National Biotechnology CONFERENCE

PURPOSE

Historically, intact ADC and total antibody determinations in plasma are performed using two separate immunoassays differing by either a protein-specific or conjugate-specific antibody capture. Due to requiring two separate assays, samples will undergo multiple freeze-thaws along with increased processing time. More recently, there has been a trend toward supporting these assays by Hybrid LC-MS/MS. Development of an innovative high-throughput 1.5 plex Hybrid LC-MS/MS method with minimal sample handling to generate total antibody and intact ADC pharmacokinetic data from a single extraction would benefit studies with limited sample volume, analyte stability issues, and expedited data deliverables as well as provide consistency with both sets of data coming from a single aliquot.

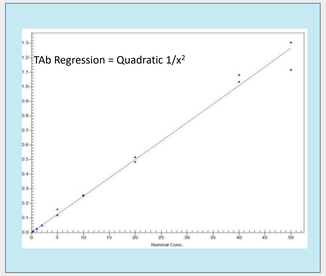
METHOD(S)

This novel approach utilizes a single immunoaffinity step coupled with a split enzymatic digestion to yield both total antibody and intact ADC quantitation. This approach works for most ADCs with cleavable linkers. Many hybrid assays have traditionally used antibodies for the capture step. In cases where there is not a viable Ab available, we have adopted an approach to using a target or receptor for the capture step. We biotinylated the targeted receptor (cadherin-6) and utilized its affinity to capture and isolate the ADC from human plasma. An MRD of 10 is performed to mitigate IP capture limitations during the IP process due to the range and ULOQ requirements. Magnetic streptavidin beads were utilized to bind to the biotinylated receptor to facilitate the IP extraction from matrix followed by wash and elution steps performed using a KingFisher Flex system. The isolated analyte is then split into two parts. One part is subjected to reduction and alkylation followed by trypsin digestion to generate surrogate peptides from the complementarity-determining region (CDR). The CDR peptide is monitored as an indicator for TAb. The second part is digested with papain enzyme to release the payload (Exatecan). The payload is monitored as an indicator for the intact ADC. This would be considered a measurement of the ADC by drug conjugate and does give some information about average DAR. Both TAb and intact ADC final extracts were analyzed on an Applied Biosystems Sciex API 7500 LC-MS/MS using an Ascentis Peptide Express ES-C18, 75 x 2.1 mm, 2.7 µm column for chromatographic separation.

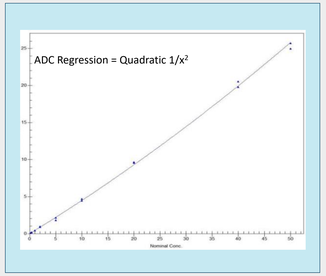


RESULT(S)

The method is developed and fully validated for a 0.200 – 50.0 µg/mL curve range in human plasma meeting acceptance criteria of ± 20% from nominal (± 25% at LLOQ). Inter-assay Accuracy of TAb ranged from 3.6 to 10.7% of nominal and 3.4 to 8.0% for intact ADC. Inter-assay Precision of TAb ranged from 6.4 to 12.2% and 3.9 to 9.3% for intact ADC. The method was shown to be selective by assessing six individual human plasma lots with no observed interferences at analyte and IS retention time for both TAb and intact ADC assays. Matrix Effect assessment of six individual lots at low and high quality control levels (n=4) show acceptable accuracy and precision (± 20% from nominal) for both TAb and intact ADC.



Total Antibody Regression-Surrogate Peptide Quantitation



Intact ADC Regression-Exatecan Quantitation

0.6 µg/mL	ME Low QC Lot-1	ME Low QC Lot-2	ME Low QC Lot-3	ME Low QC Lot-4	ME Low QC Lot-5	ME Low QC Lot-6
Mean	0.549	0.592	0.673	0.645	0.619	0.581
%CV	3.4	5.8	6.4	5.3	4.4	6
%Bias	-8.5	-1.3	12.2	7.5	3.2	-3.2
n	4	4	4	4	4	4
37.5 µg/mL	**ME Low QC Lot-1**	**ME Low QC Lot-2**	**ME Low QC Lot-3**	**ME Low QC Lot-4**	**ME Low QC Lot-5**	**ME Low QC Lot-6**
Mean	37	39.9	43.5	42.1	38.8	37.4
%CV	3.4	5	10	6.4	4	4.4
%Bias	-1.3	6.4	16	12.3	3.5	-0.3
n	4	4	4	4	4	4

Total Antibody Matrix Effect

0.6 µg/mL	ME Low QC Lot-1	ME Low QC Lot-2	ME Low QC Lot-3	ME Low QC Lot-4	ME Low QC Lot-5	ME Low QC Lot-6
Mean	0.584	0.645	0.618	0.599	0.641	0.585
%CV	4.4	7.9	7.2	8.9	3.6	7.2
%Bias	-2.7	7.5	3	-0.2	6.8	-2.5
n	4	4	4	4	4	4
37.5 µg/mL	**ME Low QC Lot-1**	**ME Low QC Lot-2**	**ME Low QC Lot-3**	**ME Low QC Lot-4**	**ME Low QC Lot-5**	**ME Low QC Lot-6**
Mean	37.7	39.5	39.7	39.1	39.4	38.8
%CV	0.8	4.4	2.5	3	1.4	1.8
%Bias	0.5	5.3	5.9	4.3	5.1	3.5
n	4	4	4	4	4	4

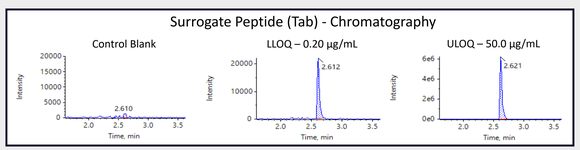
Intact ADC Matrix Effect

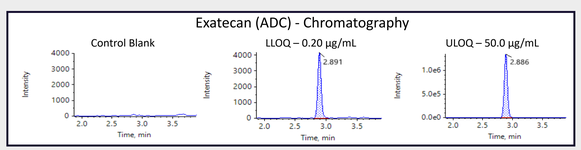
QC Level	0.200 µg/mL	0.600 µg/mL	15.0 µg/mL	37.5 µg/mL
Mean Concentration Found (µg/mL)	0.228	0.673	16.4	39.9
Inter-run %CV	9.6	10.7	3.6	4.4
Inter-run %Bias	14	12.2	9.3	6.4
n	18	18	18	18

Total Antibody Inter-Assay – 3 separate batches

QC Level	0.200 µg/mL	0.600 µg/mL	15.0 µg/mL	37.5 µg/mL
Mean Concentration Found (µg/mL)	0.215	0.623	16.4	39.7
Inter-run %CV	3.8	8	4.3	3.4
Inter-run %Bias	7.5	3.8	9.3	5.9
n	18	18	18	18

Intact ADC Inter-Assay – 3 separate batches





CONCLUSION(S)

We have successfully developed and validated a single immunoprecipitation extraction for TAb and Intact ADC assessment, which we have named a 1.5 plex assay, with acceptable accuracy, precision, selectivity, and specificity in human plasma for PK analysis in ADC clinical trials. This methodology allows for a rapid and economical bioanalytical workflow for sample analysis while minimizing sample handling and stability issues in complex biologics.

The prevalence of protease cleavable linkers in ADCs allow for the 1.5 methodology to be applied to a variety of therapeutics. This approach has been applied and successfully validated for multiple analytes for total and intact ADC at KCAS Bio.

Kcas bio SCIENCE ACCELERATED