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Immunoaffinity Coupled with Mass Spectrometry (IA-LC-MS/MS) as a Quantitative Tool for Bioanalysis of Ado-Trastuzumab Emtansine in Human Plasma

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Overview

- Development and optimization of a signature-peptide LC-MS/MS method for Kadcyla®, a human IgG antibody drug conjugate (ADC) using HER2 receptor for the specific immunoaffinity (IA) extraction from human plasma
- Optimization of reduction, alkylation and trypsin digestion steps
- Utilization of a flanked, stable isotope-labeled internal standard (FSLIS) corresponding to the signature peptide monitored for the quantitation of Kadcyla®
- Application of the optimized IA-LC-MS/MS method to pre-validate precision and accuracy batches that included stability, matrix effect, hemolyzed and lipemic samples

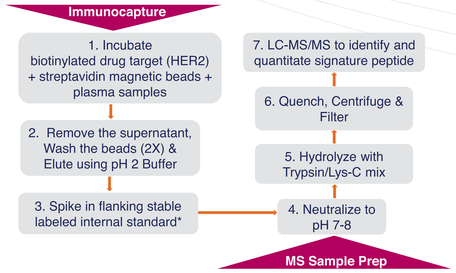
Introduction

Kadcyla® (ado-trastuzumab emtansine) is an ADC that specifically targets the Human Epidermal growth factor Receptor 2 (HER2) that is overexpressed in some types of breast cancer cells. The targeted specificity of ADCs increases potency and reduces toxicity as they are comprised of a cytotoxic drug covalently attached to a highly specific monoclonal antibody. The number of cytotoxic drug molecules per antibody, typically varies with the chemistry of conjugation and the accessibility of linking sites, increasing the heterogeneity of ADCs and making their analysis challenging. Combining the selectivity of immunoaffinity (IA) enrichment and the sensitivity and selectivity of tandem mass spectrometry offers a method with a broad dynamic range that requires a low sample volume for reliable and reproducible analysis of ADCs including Kadcyla®.

In the current study, immunoaffinity extraction of Kadcyla® from 0.100 mL of human plasma was carried out using biotinylated HER2, and streptavidin-coated magnetic beads. The immunoaffinity extracted samples were fortified with FSLIS and subjected to trypsin hydrolysis, to generate peptides of suitable mass for LC-MS/MS. The fragmentation of a signature peptide specific to Kadcyla® was monitored and used for quantitation. All PA batches were 96 samples in length and all steps were carried out manually for the current study.

Methods

Figure 1. Assay Workflow



*A stable labeled flanking peptide (WVARIY[$^{13}C_6$;^{15}N]PTN[$^{13}C_5$;^{15}N]GYTRYANS) corresponding to signature peptide (IYPTNGYTR) is used for the quantitation of Kadcyla®
^Reduction and alkylation steps are eliminated from the workflow after optimization

The flow chart above outlines the steps followed for the extraction and analysis of Kadcyla® in human plasma. BioBA sample prep kits (SCIEX) are used in the development and optimization of the current method. Briefly, 0.100 mL of plasma containing Kadcyla® was incubated for 1 hour with 1:8 molar ratio of HER2 receptor and 25 µL of streptavidin-coated magnetic beads. The unbound plasma components were removed by two buffer washes and the bound Kadcyla® was eluted from the HER2 receptor using 0.100 mL of pH 2 reagent. The eluted sample was fortified with FSLIS then neutralized and hydrolyzed using Trp/Lys-C mix for 2 hours. The reaction was quenched using 0.1% formic acid and was subjected to LC-MS/MS analysis. A universal IS named SILuMAb is used as IS for this study earlier in the method development because it required reduction and alkylation steps. But optimization of the method with FSLIS gave similar results with and without reduction and alkylation steps as the FSLIS did not contain any sulfhydryl groups.

Figure 2. MS Analysis Parameters

System	Waters ACQUITY UPLC
Column	Phenomenex, Kinetex C18 Column, 50 X 3 mm, 2.6 µm
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Flow rate	350 µL
Column Temperature	40°C
Injection Volume	10 µL

CUR	45
CAD	10
IS	5500
TEM	300
GS1	50
GS2	60

Time (min)	% B
0.70	5
0.80	10
3.50	25
5.00	50
6.00	5
7.00	50
7.50	5

Name	Q1	Q3	Dwell	DP	CE	CXP
IYPTNGYTR	542.8	808.4	80	60	24	11
IYPTNGYTR (IS)	547.5	817.4	80	120	22	14

The tables in the figure above outline the MS parameters and mass transitions for the analysis of signature peptide IYPTNGYTR and its stable labeled flanking peptide.

Results

Figure 3. Chromatogram Showing Comparison of IYPTNGYTR Peak at LLOQ with Control Blank

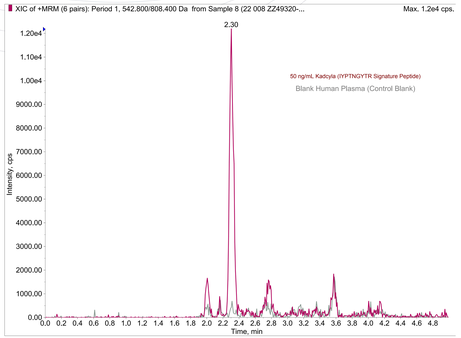


Figure 3 shows the chromatogram highlighting the retention time (2.30 min) and peak height of signature peptide (IYPTNGYTR) monitored at a lower limit of quantitation (50 ng/mL). The chromatogram of the signature peptide (burgundy) is overlaid with control blank human plasma sample (gray).

Figure 4. Standard Curve for the Quantitation of IYPTNGYTR Signature Peptide

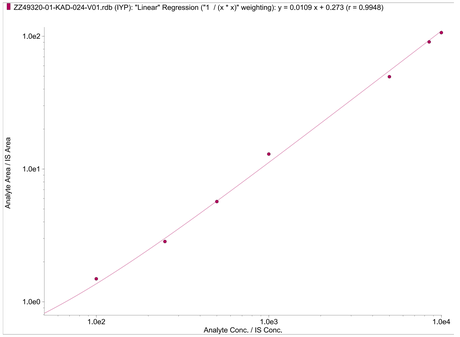


Figure 4 shows the log scale standard curve for Kadcyla® in human plasma from 50-10,000 ng/mL range. A regression of linear with 1/x2 weighing is applied to the curve. Ratio of Kadcyla® to flanking peptide corresponding to signature peptide is used in the calculation of the concentrations of the standards.

Table 1. Intra- and Inter- Batch Precision and Accuracy Table

Intra- and Inter- Batch Precision and Accuracy

Batch		50.0 ng/mL	150 ng/mL	700 ng/mL	8000 ng/mL
PA Batch 1	Intra-batch Mean	50.1	156	735	8700
	Intra-batch % CV	19.7	18.7	20.5	19.4
	Intra-batch % Bias	0.2	4.0	5.0	8.7
	n	6	6	6	6
PA Batch 2	Intra-batch Mean	49.1	146	785	9130
	Intra-batch % CV	11.2	9.3	8.2	18.3
	Intra-batch % Bias	-1.8	-2.7	12.1	14.1
	n	6	6	6	6
PA Batch 3	Intra-batch Mean	40.3	155	850	8540
	Intra-batch % CV	22.2	11.5	9.9	8.7
	Intra-batch % Bias	-19.4	3.3	21.4	6.7
	n	6	6	6	6
Inter-batch	Inter-batch Mean	46.5	152	790	8790
	Inter-batch % CV	19.4	13.5	14.1	15.6
	Inter-batch % Bias	-7.0	1.3	12.9	9.9
	n	18	18	18	18

Table 1 shows the intra (3 PA batches) and inter-batch precision, and accuracy data for quality control (QC) samples of Kadcyla® in human plasma.

Table 2: Data for Kadcyla® Matrix Effect, Hemolyzed and Lipemic Test Samples

Matrix Effect (Matt Eff), 2% Hemolyzed (2% HEM) and Lipemic (LPM)

	Lot#	Low QC 150 ng/mL	% Dev.	High QC 8000 ng/mL	% Dev.
Matt Eff	1	125	-16.7	6860	-14.3
	2	171	+14.0	6920	-13.5
	3	73.8	-50.8	9420	+17.8
	4	178	+18.7	8450	+5.6
	5	141	-6.0	6550	-18.1
	6	173	+15.3	6630	-17.1
	7	175	+16.7	9190	+14.9
	8	158	+5.3	9110	+13.9
	9	171	+14.0	7220	-9.8
	10	196	+30.7	7150	-10.6
Mean		156		7750	
% CV		22.5		14.9	
% Theoretical		104.0		96.9	
n		10		10	
2% HEM	1	120	-20.0	9410	+17.6
	2	148	-1.3	10100	+26.3
	3	167	+11.3	9390	+17.4
Mean		145		9630	
% CV		16.3		4.2	
% Theoretical		96.7		120.4	
n		3		3	
LPM	1	170	+13.3	8020	+0.3
	2	165	+10.0	9750	+21.9
	3	127	-15.3	8040	+0.5
Mean		154		8600	
% CV		15.3		11.5	
% Theoretical		102.7		107.5	
n		3		3	

Table 2 shows the data obtained from matrix effect, 2% hemolyzed and lipemic samples tested at low and high QC levels of Kadcyla®. 10 different human plasma lots were used to test matrix effect. The percent deviations from the theoretical concentration are also noted for matrix effect, 2% hemolyzed and lipemic samples. The percent deviation is within 20% for 8 out of 10 plasma lots at low QC level, while all the 10 lots of plasma quantified with <20% deviation from theoretical at high QC level.

Table 3. Stability Data for Kadcyla

Freeze Thaw (FT) and Short-Term Stability (STS)

	Low QC 75.0 ng/mL	High QC 8000 ng/mL
STS	73.5	9460
	72.8	7400
	79.1	8510
	48.0*	7470
Mean	75.1	8210
% CV	4.6	11.9
% Theoretical	100.1	102.6
n	3	4
FT	66.8	9180
	71.3	9060
	62.0	9800
		7780
Mean	66.7	8960
% CV	7.0	9.5
% Theoretical	88.9	112.0
n	3	4

*Eliminated from the regression analysis

Short term stability (STS) and freeze-thaw (FT) stability data of Kadcyla® in human plasma tested at a low and high QC level is shown in the table above. STS is tested after 8 hours under UV-shielded light at ambient temperature. Kadcyla® in human plasma is shown to be stable over 8 hours at ambient temperature under UV-shielded light. FT stability is tested after 3 FT cycles and found to be stable.

Conclusions

The amount of Kadcyla® (drug) to HER2 receptor (specific capture) was optimized to 1:8 molar ratio. The incubation time of streptavidin magnetic beads, biotinylated HER2 receptor and Kadcyla® together for affinity capture has been optimized to one hour. Reduction and alkylation steps were eliminated from the assay workflow and trypsin digestion time was optimized to 2 hours for optimal quantitation. Three PA batches were run with 50 - 10,000 ng/mL range standard curve. Kadcyla® has shown 8 hours of short term stability (STS) and 3 freeze thaw (FT) cycles. IA-LC-MS/MS has proven to be a feasible method to quantitate low ng levels of ADCs in human plasma with acceptable accuracy- and precision using manual handling. Automation of the steps in the workflow is expected to improve the accuracy and precision significantly.

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