

QUANTIFICATION OF THE ANTIBODY DRUG CONJUGATE, TRASTUZUMAB EMTANSINE, AND THE MONOCLONAL ANTIBODY, TRASTUZUMAB, IN PLASMA USING A GENERIC KIT-BASED APPROACH

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INTRODUCTION

As drug development efforts focus on large molecules such as mAbs and ADCs, the desire to employ LC/MS in bioanalysis is also increasing. However, LC/MS protein quantification presents many challenges. There is no single workflow and the various workflow options can be complex and laborious, making it difficult for a scientist to achieve success. Additionally, due to their complex and heterogeneous nature, ADCs often require multiple bioanalytical assays to determine efficacy, toxicity, and PK/PD response during drug development.

This work describes the total mAb quantification of the ADC, ado-trastuzumab emtansine (T-DM1), and mAb, trastuzumab, from plasma using a kit-based approach and generic protocol which can be applied equally, not only to ADCs and mAbs, but to small proteins as well.

METHODS

Sample Preparation

To prepare standards and quality control samples (QC), trastuzumab and T-DM1 were spiked separately into rat plasma at various concentrations (0.1–500 µg/mL). An intact murine monoclonal antibody standard (Waters) was used as a generic internal standard. Plasma samples (35 µL) were directly digested and prepared for LC/MS analysis using the Waters ProteinWorks eXpress Digest Kits digestion kit and protocol.

LC/MS Conditions

LC-MS/MS quantification was performed using a Waters Xevo TQ-S triple quadrupole MS (ESI+). Chromatographic separation was achieved using an ACQUITY UPLC system with an ACQUITY UPLC Peptide BEH C18, 300A, 1.7 µm, 2.1 mm x 150 mm column, at a flow rate of 0.3 mL/min using a linear gradient with 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Three signature tryptic peptides of trastuzumab were used for quantification: IYPTNGYTR, FTISADTSK, and GPSVFPLAPSSK. Final injection volume was 5 µL.

Protein	Peptide	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
T-DM1/Trastuzumab	IYPTNGYTR	542.77>249.16	36	16
		542.77>808.40	12	16
Trastuzumab	FTISADTSK	485.20>721.40	28	22
		485.20>608.30	28	22
Trastuzumab	DTYIHWWR	543.30>597.30	28	24
		545.30>710.40	28	28
Trastuzumab	GPSVFPLAPSSK *	593.83>699.40	31	21
		1073.17>547.20	35	38
T-DM1 miscleavage with small molecule drug attached	FTISADTSKNTAYLQMNSLR	1073.17>547.20	35	38
		1073.17>485.22	35	38
Murine mAb (IS)	GPSVFPLAPSSKSTSGTAALGCLVK	1149.23>547.20	35	38
	SVSELPIMHQDWLNGK	618.64>834.41	16	12
	VNSAAFPAPIEK	622.30>654.44	28	16

*Generic IgG peptide

Table 1. MRM conditions for trastuzumab and trastuzumab emtansine (T-DM1).

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RESULTS

I. Quantification of Trastuzumab and T-DM1 Through Direct Digestion

Peptide	Std. curve range (µg/ml)	Weighting	Linear fit (r^2)	Mean % accuracy
IYPTNGYTR	0.5 - 500	1/x ²	0.995	100.01
FTISADTSK	1.0 - 500	1/x	0.999	100.01
GPSVFPLAPSSK*	0.5 - 500	1/x ²	0.990	100.00

Table 2. Linear dynamic range, weighting, and average accuracy for standard curves for trastuzumab in plasma, digested and extracted using a protein quantification direct digestion kit.

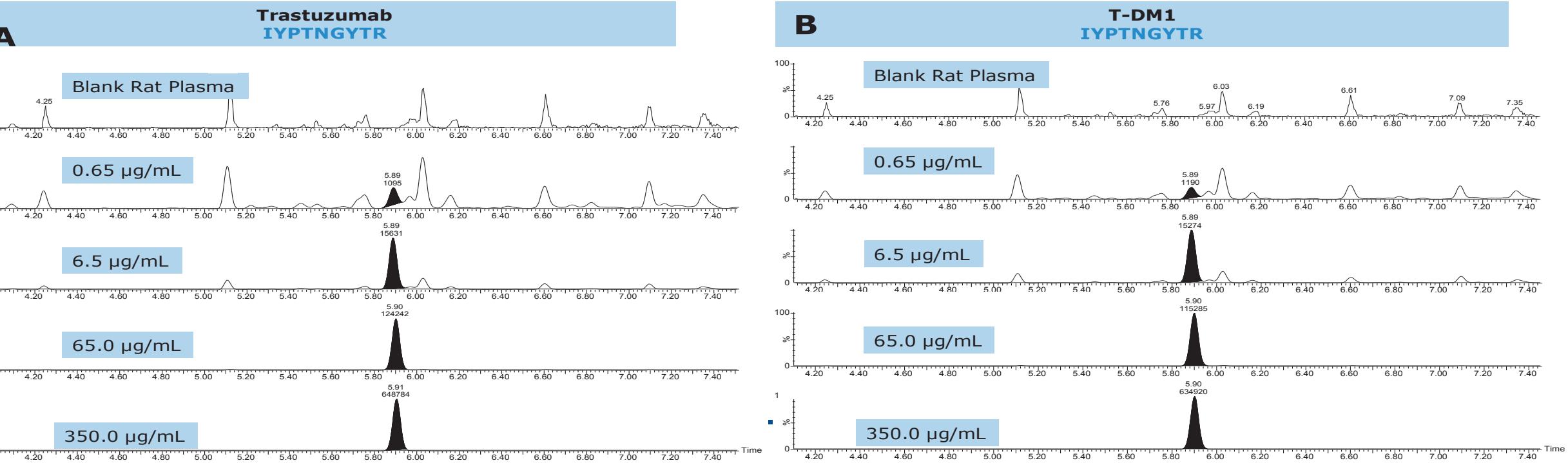


Figure 1. QC chromatograms of trastuzumab (A) and T-DM1 (B) for the IYPTNGYTR unique signature

II. Detection and Confirmation of T-DM1 Miscleavage Signature Peptides FTISADTSKNTAYLQMNSLR and GPSVFPLAPSSKGGTAALGCLVK

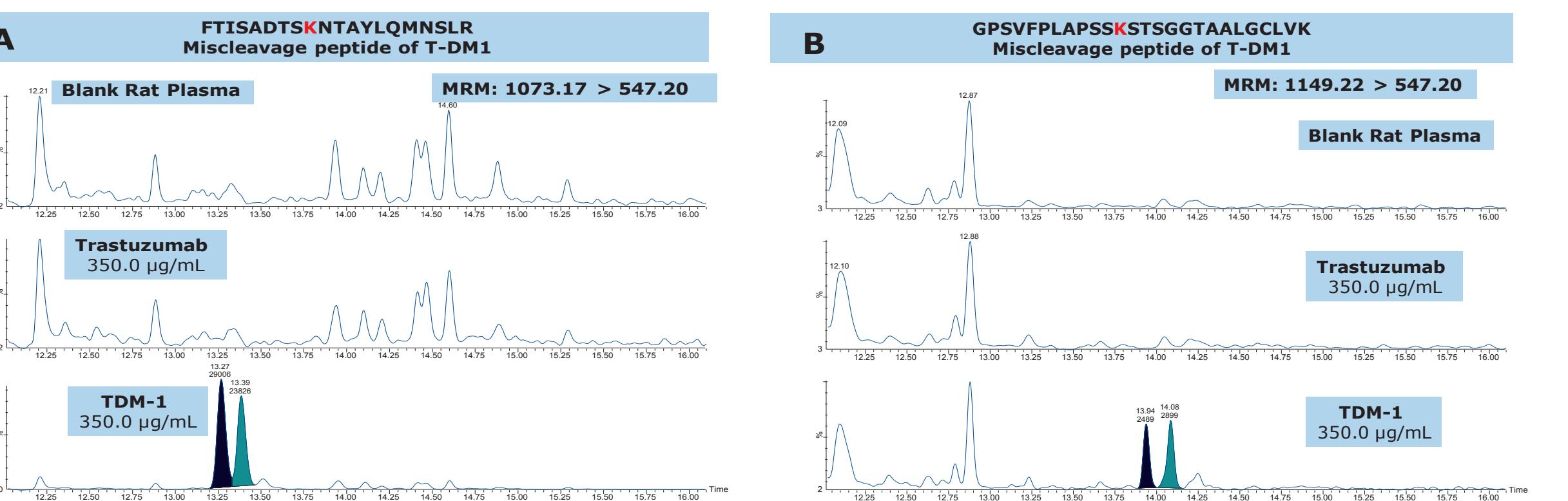


Figure 2. Chromatograms demonstrating the presence of the miscleavage peptides in T-DM1 samples (350 µg/mL), as compared to trastuzumab (350 µg/mL), and blank rat plasma.

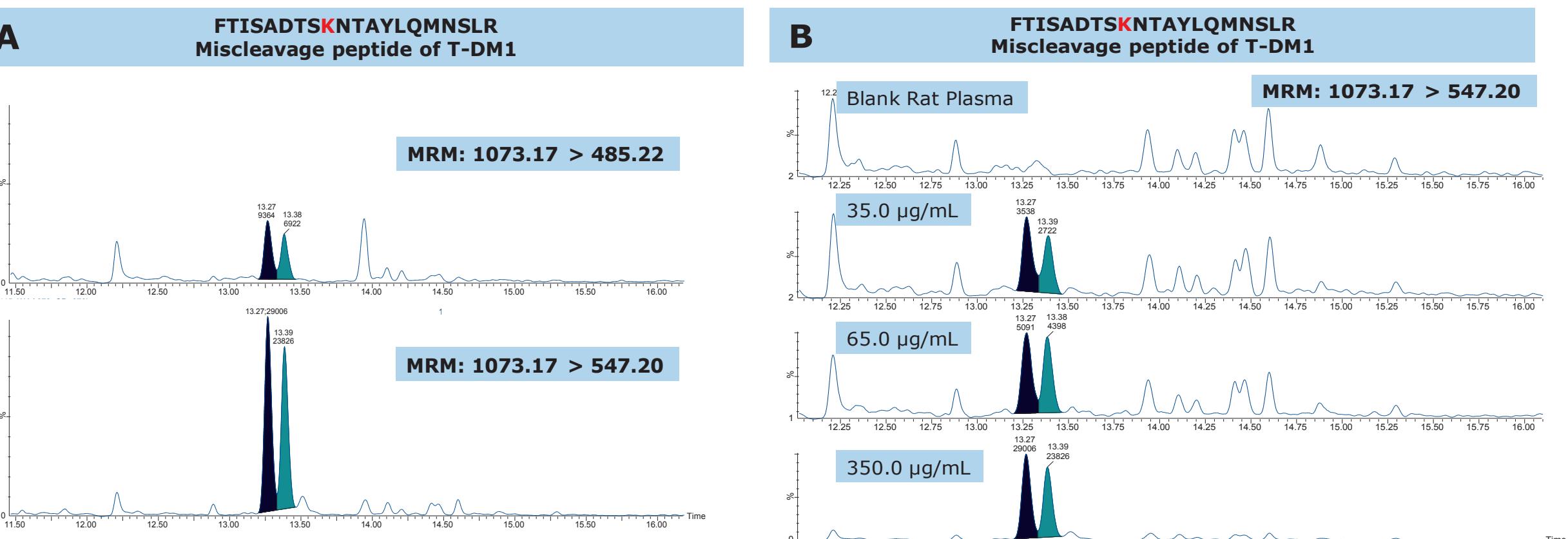


Figure 3. Chromatograms confirming the presence of the FTISADTSKNTAYLQMNSLR conjugated peptide by multiple MRM transitions (A) and the increase of signal for the conjugated peptides of T-DM1 with increasing concentration in plasma (B).

DISCUSSION

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate (ADC) and is used as a treatment for patients with advanced breast cancer.^{1,2} Using a commercially available protein digestion kit and protocol, successful total antibody quantification of T-DM1 and trastuzumab was achieved.³

- Through direct digestion (2 hours) of 35 µL of plasma, quantification limits of 0.5–1 µg/mL were achieved for the three signature tryptic peptides of trastuzumab (Table 2).
- Sensitivity, linearity, accuracy and precision data met typical method validation requirements.⁴ Standard curves were linear over 3.5 orders of magnitude with the average accuracies of 100% for all points (Table 2).
- The accuracy and precision for trastuzumab and T-DM1 QC samples, quantified using the trastuzumab standard curve, were excellent with accuracies ranging from 87–110% and % CVs <8 (Table 3). Representative QC chromatograms are illustrated in Figure 1, Panels A and B.
- Following tryptic digestion of T-DM1 (a lysine-conjugated ADC), there is potential for miscleavage on the lysine residue when it is conjugated with the payload. For the two lysine containing peptides, GPSVFPLAPSSK and FTISADTSK, "mislceavage peptides" were detected in TDM-1 samples (Figure 2, Panels A and B).
- Presence of the T-DM1 conjugated peptides were confirmed based on their: late elution in the chromatographic run (due to hydrophobicity of the small molecule cytotoxic drug) as isomeric pairs (isomers from the conjugation), the common CID MS fragment 547.2 m/z (from the small molecule drug payload), and increase in signal with increasing T-DM1 concentration (Figure 3, Panels A and B).

CONCLUSION

Using a generic kit-based approach, total antibody quantification of a mAb therapeutic (Trastuzumab) and ADC (Trastuzumab Emtansine) was achieved with successful detection and identification of miscleavage peptides. Quantification limits of 0.5–1 µg/mL were achieved, while maintaining excellent linearity, precision and accuracy. The universal, kit-based approach allows scientists to achieve high sensitivity with a simple step-wise protocol and standardized, pre-measured reagents, ensuring both the sensitivity and reproducibility required in discovery studies to make time sensitive and critical project decisions.

REFERENCES

1. Peddi PF, Hurvitz SA. Trastuzumab emtansine: the first targeted chemotherapy for treatment of breast cancer. Future oncology (London, England). 2013;9(3):10.2217/fon.13.7. doi:10.2217/fon.13.7.
2. BarokM, JoensuuH, IsolaJ. Trastuzumab emtansine: mechanisms of action and drug resistance. Breast Cancer Res. 2014 Mar;16(2):209. doi:10.1186/bcr3621
3. Application Note: 720005619EN, Quantification of the Antibody, Trastuzumab Emtansine and Monoclonal Antibody using a Generic Kit-Based Approach.
4. FDA Guidance for Industry for Bioanalytical Method Validation, CDER