# Ultra-sensitive LC-MRM analysis for trastuzumab-emtansine quantification in rat plasma

***Featuring the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready, powered by SCIEX OS Software***

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With the adoption of immunoaffinity based sample preparation, an ultra-low LLOQ of 1 ng/mL was achieved. The assay shows high precision, accuracy, and good linearity, demonstrating the robustness and performance of the developed method.

Quantification of protein therapeutics in biological matrices, play a critical role in the drug discovery and development process. LC-MS/MS has been routinely adopted for quantification of this molecular class in bioanalytical laboratories, serving as an alternative technology to the traditional ligand binding assays (LBAs). Although mass spectrometry offers higher specificity than LBA, there still remains a need for improved sensitivity for accurate quantification of low concentration analytes in complex matrices while still maintaining high analysis throughput, instrument easy-of-use, and robustness.

In this work, the SCIEX Triple Quad 7500 System coupled with an analytical flow HPLC system is employed to quantify trastuzumab-emtansine in rat plasma. Multiple hardware improvements on the ion source and the front end of the mass analyzer significantly boost the system sensitivity.[1]

## Key features of the peptide quantification workflow

- Immunoaffinity-LC-MRM workflow[2] offers solid quantification of trastuzumab-emtansine in rat plasma at 1 ng/mL, with high precision, accuracy, and linearity
- Hardware improvements on the SCIEX 7500 System provide significant gains in sensitivity for peptide quantification: the OptiFlow® Pro Ion Source with E Lens™ Technology provides improvements in ion generation and the D Jet™ Ion Guide improves ion sampling
- SCIEX OS Software—an easy to use, compliance ready, single platform for acquisition, processing and data management

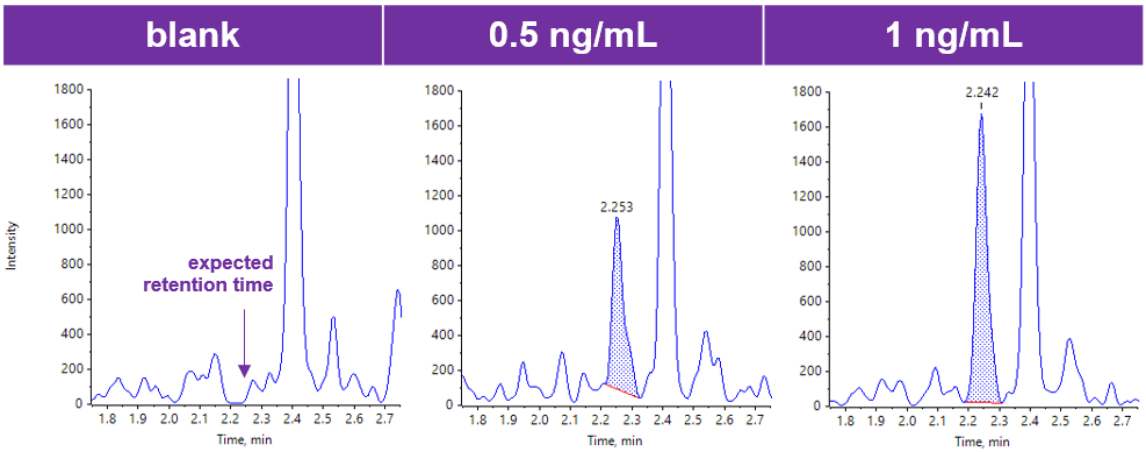


**Figure 1. Extracted ion chromatograms (XICs) of selected MRM for trastuzumab-emtansine in rat plasma.** From left to right are blank, 0.5 ng/mL (LOD) and 1 ng/mL (LLOQ).

## Methods

**Immunocapture:** A streptavidin coated immunoaffinity magnetic bead slurry was aliquoted and washed with PBS buffer (1x) three times. Biotinylated goat anti-human IgG antibody was added to the beads and incubated at room temperature for 1 hour with shaking. The conjugated beads were washed three times and re-suspended in PBS buffer. Calibration standard samples were prepared by spiking trastuzumab-emtansine and SILuMab (internal standard) into rat plasma and performing serial dilution. The concentrations of trastuzumab-emtansine in plasma ranged from 0.5 - 20,000 ng/mL. To each calibration standard sample, 250 µL of PBS Buffer (1×) and 50 µL conjugated bead slurry were added and the mixtures were incubated at room temperature for 1 hour with shaking. The beads were accumulated by magnetic stand and washed sequentially with PBS buffer and 10 mM ammonium bicarbonate. The target proteins were eluted by incubating the beads with 0.1% TFA in water and vortexing for 10 min.

**Protease digestion of immuno-enriched eluents:** The eluents were neutralized with 500 mM ammonium bicarbonate in water and incubated at 95 °C for 10 mins with shaking. The samples were cooled to room temperature and incubated with 1 µg of trypsin/Lys-C mix per sample overnight at 37 °C. The digestion was aborted by adding formic acid. The samples were centrifuged at 15,000×g for 5 min. The supernatants were collected and subjected to LC-MS/MS analysis.

***LC-MS/MS conditions:*** Samples were analyzed in triplicate by a SCIEX Triple Quad 7500 System, coupled with an ExionLC System. The method details are summarized in Tables 1, 2 and 3. The data were processed using the Analytics module in SCIEX OS Software 2.0.

**Table 1. Chromatographic conditions.**

| Parameter | Value |
|---|---|
| *Column* | *Phenomenex bioZen Peptide XB-C18 50×2.1 mm; 2.6 µm* |
| *Mobile phase A* | *Water with 0.1 % formic acid* |
| *Mobile phase B* | *Acetonitrile with 0.1 % formic acid* |
| *Flow rate* | *500 µL/min* |
| *Column temperature* | *40 °C* |
| *Injection volume* | *30 µL* |

**Table 2. Gradient information.**

| Time [min] | Mobile phase A [%] | Mobile phase B [%] |
|---|---|---|
| *Initial* | *95* | *5.0* |
| *0.7* | *95* | *5.0* |
| *0.8* | *90* | *10* |
| *3.5* | *75* | *25* |
| *4.0* | *60* | *40* |
| *4.5* | *10* | *90* |
| *6.0* | *10* | *90* |
| *6.1* | *95* | *5.0* |
| *7.7* | *95* | *5.0* |

**Table 2. Mass spectrometric conditions.**

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| *Curtain gas* | *40 psi* | *Source temperature* | *500 °C* |
| *Ion source gas 1* | *50 psi* | *Ion source gas 2* | *50 psi* |
| *CAD gas* | *11* | *Ion spray voltage* | *1700 V* |

| *Name* | *Q1* | *Q3* | *CE* | *CXP* |
|---|---|---|---|---|
| *IYPTNGYTR1* | *542.8* | *808.4* | *23* | *13* |
| *IYPTNGYTR2* | *542.8* | *405.1* | *23* | *13* |
| *FTISADTSK1** | *485.2* | *721.3* | *23* | *15* |
| *FTISADTSK2* | *485.2* | *608.2* | *22* | *15* |
| *GPSVFPLAPSSK1* | *593.8* | *699.4* | *31* | *15* |
| *GPSVFPLAPSSK2* | *593.8* | *846.5* | *28* | *15* |
| *FNWYVDGVEVHNAK[H]* | *562.9* | *713.3* | *23* | *15* |
| *GPSVFPLAPSSK[H]* | *597.8* | *707.4* | *28* | *15* |

** MRM transition for the best sensitivity*
*[H] MRM transitions of internal standard peptides from SILuMab*

## Quantification results

| Component ... | Actual Co... | Num. Values | Mean | Standard Deviation | Percent CV | Accuracy |
|---|---|---|---|---|---|---|
| FTISADTSK1 | 0.00100 | 3 of 3 | 9.852e-4 | 9.735e-5 | 9.88 | 98.52 |
| FTISADTSK1 | 0.00500 | 3 of 3 | 5.293e-3 | 2.785e-4 | 5.26 | 105.86 |
| FTISADTSK1 | 0.01000 | 3 of 3 | 1.024e-2 | 7.576e-4 | 7.40 | 102.44 |
| FTISADTSK1 | 0.05000 | 3 of 3 | 5.083e-2 | 4.477e-3 | 8.81 | 101.65 |
| FTISADTSK1 | 0.10000 | 3 of 3 | 1.020e-1 | 6.762e-3 | 6.63 | 101.99 |
| FTISADTSK1 | 0.50000 | 3 of 3 | 5.176e-1 | 1.101e-2 | 2.13 | 103.52 |
| FTISADTSK1 | 1.00000 | 3 of 3 | 1.060e0 | 9.045e-2 | 8.53 | 105.99 |
| FTISADTSK1 | 5.00000 | 3 of 3 | 4.989e0 | 3.620e-1 | 7.26 | 99.78 |
| FTISADTSK1 | 10.00000 | 3 of 3 | 9.388e0 | 9.304e-1 | 9.91 | 93.88 |
| FTISADTSK1 | 20.00000 | 3 of 3 | 1.728e1 | 4.775e-1 | 2.76 | 86.38 |

**Figure 2. Quantification result summary.**

The SCIEX Triple Quad 7500 System integrates innovations that provide improvements in both ion generation and ion sampling. The OptiFlow Pro Ion Source with E Lens Technology provides improvement in ion generation and the D Jet Ion Guide efficiently captures and transmits the ions in the high gas flow behind the orifice plate.[1]

With the optimized method, the presented immunocapture-LC-MRM assay demonstrated ultra-high sensitivity to quantify trastuzumab-emtansine in rat plasma, with the LLOQ at 1 ng/mL and the LOD at 0.5 ng/mL (Figure 1). As summarized in Figure 2, the assay accuracy is 86-106% and %CVs are below 10% for all tested samples. The calibration curve covered 4.5 orders of magnitude (1-20000 ng/mL) (Figure 3) and displayed a regression coefficient (r) of 0.996 using a weighting of $1/x^2$.

## Conclusions

- A highly sensitive immunoaffinity-LC-MRM workflow for quantifying trastuzumab emtansine in rat plasma was demonstrated
- By using the SCIEX Triple Quad 7500 System coupled with analytical flow HPLC, trastuzumab-emtansine was solidly quantified at 1 ng/mL level
- Good reproducibility, accuracy and wide linear dynamic range as 4.5 orders of magnitude were achieved at the same time

## References


1. Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.
2. Quantification of trastuzumab in rat plasma using an improved immunoaffinity-LC-MS/MS method. SCIEX technical note RUO-MKT-02-7597-A.


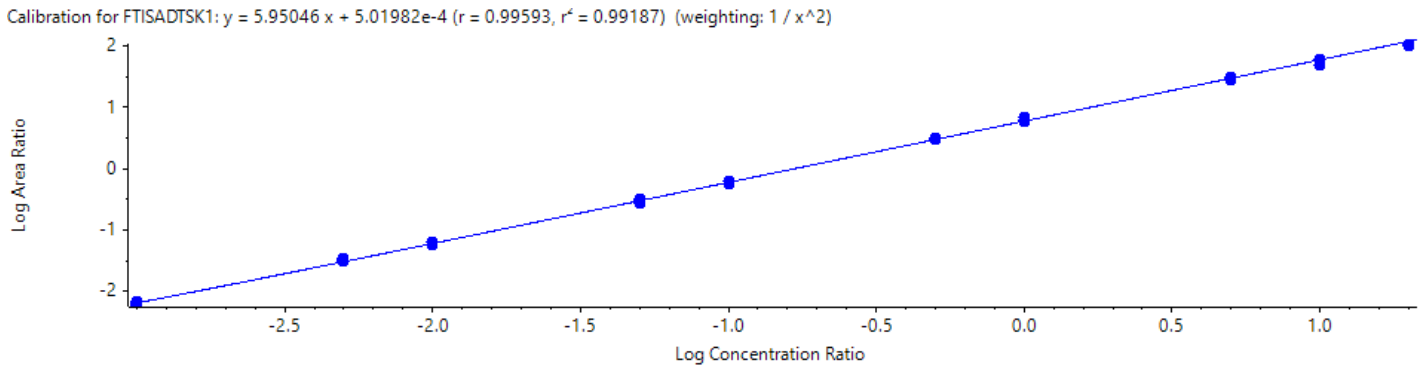


**Figure 3. Calibration curve for trastuzumab-emtansine in rat plasma displayed with log-log format.** Concentrations range from 1 ng/mL to 20,000 ng/mL.



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