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Application News

No. LC-31-ADI-54

LC/MS
nSMOL and Liquid Chromatography Mass Spectrometry

Bioanalytical assessment of Cetuximab in Wistar rat plasma using nSMOL and LCMS-8060

Introduction

Cetuximab is a human monoclonal antibody (mAb) for the treatment of metastatic colorectal carcinoma, head and neck cancer. Cetuximab was first approved by U.S. FDA in 2004.

Generally bioanalysis of mAB drugs is based on Ligand Binding Assay (LBA). However LBA tends to show larger variability and development time for assay can be longer. Hence in this work, we present bioanalysis of Cetuximab in Wistar rat plasma using nSMOL™ (nano surface molecular orientation limited proteolysis) reagent kit and LCMS-8060, triple quadrupole from Shimadzu Corporation.

nSMOL™ Antibody BA kit features

nSMOL is Shimadzu's completely new and breakthrough technology that enables selective proteolysis of the Fab region of monoclonal antibodies. This technique facilitates method development independent of a variety of antibody drugs and achieves a paradigm shift in the bioanalysis of antibody drugs.

Furthermore, numerous bioanalytical method validation have been reported using nSMOL technique as per the criteria of "Guideline on Bioanalytical Method Validation in Pharmaceutical Development" for low MW drug compounds issued by the Japanese Ministry of Health, Labour and Welfare. Shimadzu also offers optimization methods and protocols, and nSMOL can be applied to clinical research at various institutions.

nSMOL principle

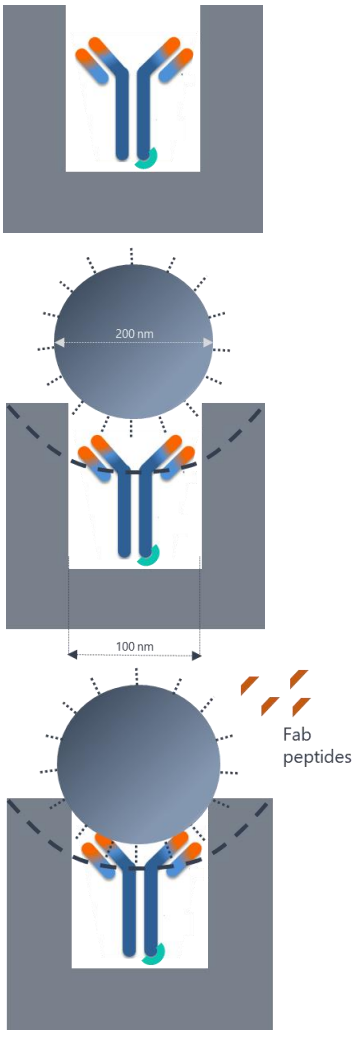
nSMOL works on selective proteolysis of Fab by making use of the difference in size of the protease nanoparticle diameter (200 nm) and the antibody resin pore size (100 nm). With the use of nSMOL one can maintain the specificity of the antibody sequences while minimizing the sample complexity as well as the elimination of extra protease. This approach leads to the shortening of analytical time, LCMS robustness, wide dynamic range, and considerable improvement in sensitivity using LCMS-8060.

Sample Preparation

The standard samples of Cetuximab with concentration between 0.295 to 150 ug/mL in rat plasma were prepared for calibration curve and QC samples with concentration of 0.295 and 0.59 ug/mL were prepared to validate the analysis results. Standards and QC samples were processed based on nSMOL, which involves selective cleavage of Fab region from mAb using trypsin nano patricles. P14 was used as internal standard.

Selection of signature peptide and MRM optimization

Unique peptide for Cetuximab was selected by sequence alignment using skyline. 'GPSVFPLAPSSK' peptide selectively represents Cetuximab from rat plasma. However, this peptide is generic signature peptide. Therefore, this method can be selectively used for only assessment of a singular chimeric mAb in rat plasma. For analysis of Cetuximab from human plasma, peptide sequence of 'SQVFFK' should be used. MRM was optimized using Skyline and MRM optimization tool from Labsolutions.



| Capture

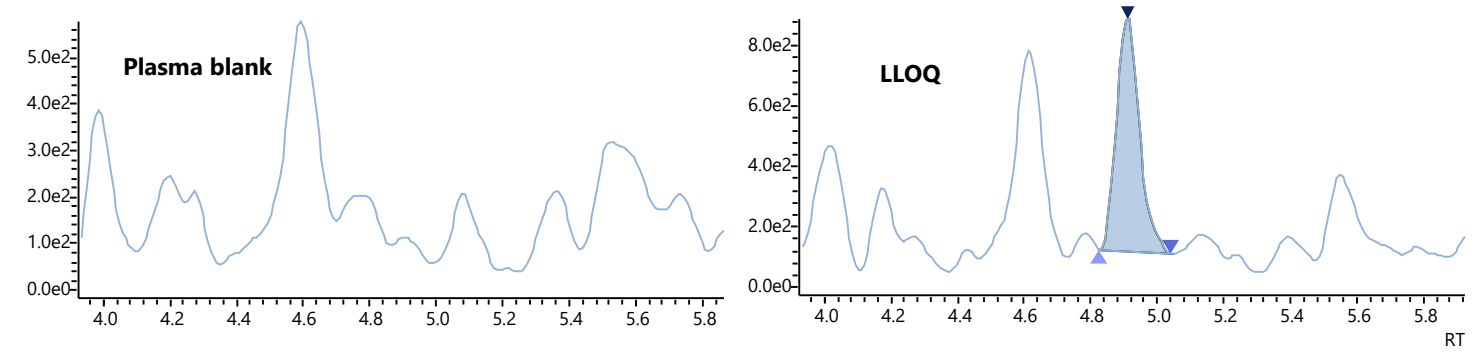
Fab region is captured in collection resin and oriented towards solution

| Digestion

Limited proteolysis by trypsin immobilized on nano particle

| Analysis

Fab derived peptides are harvested and injected in LCMS-8060



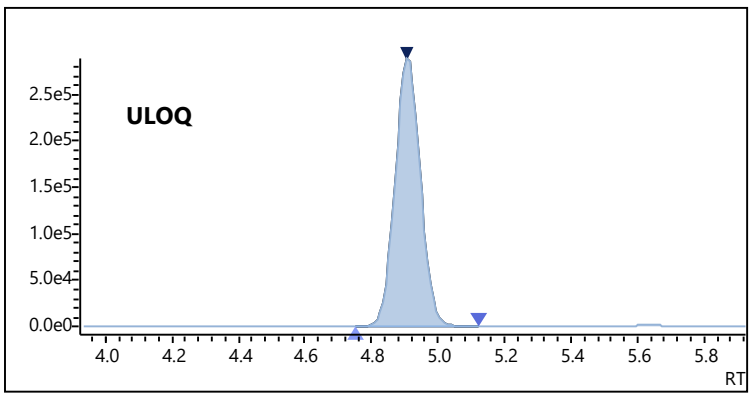


Fig. 1: MRM chromatograms of blank rat plasma, LLOQ level (0.295 ug/mL) and ULOQ (150 ug/mL) in rat plasma for Cetuximab

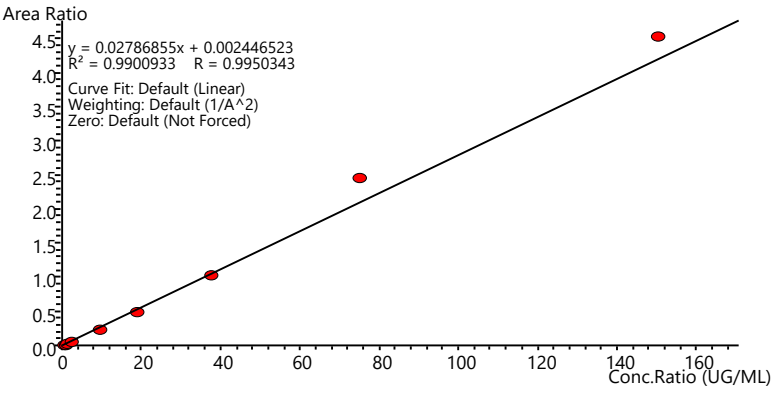


Fig. 2: Calibration curve for Cetuximab in rat plasma from 0.295 to 150 ug/mL

Results and Discussions

Chromatograms for blank rat plasma, LLOQ and ULOQ level of Cetuximab in rat plasma are shown in **Fig. 1** and the calibration curve is shown in **Fig. 2**. Good linearity was obtained over the concentration range of 0.295 to 150 ug/mL of the Cetuximab in rat plasma with a correlation coefficient of 0.9900. The accuracy of linearity and QC samples for Cetuximab in rat plasma is shown in **Table 3**. For all the linearity and QC samples accuracy was within acceptance criteria.

Table 3: Results of linearity and QC samples for analysis of Cetuximab from rat plasma

Sample Name	Conc. (ug/mL)	Accuracy
BLK	-----	-----
CS 01	0.295	95.73
CS 02	0.59	111.82
CS 03	1.17	99.99
CS 04	2.34	96.09
CS 05	4.69	80.09
CS 06	9.35	88.53
CS 07	18.75	95.41
CS 08	37.5	98.67
CS 09	75	117.84
CS 10	150	108.39
QC 1	0.295	98.69
QC 2	0.59	99.79

References

[1] EMA Guideline on Bioanalytical Method Validation. EMEA/CHMP/EWP/192217/2009 Rev.1 Corr. 2**

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[3] Iwamoto N, et al., Analyst, Volume 139, Issue 3, (2014), 576-580.

[4] Iwamoto N, et al., Analytical methods, Volume 7, Issue 21 (2015), 9177-9183.

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