

***JBF activities for protein analysis  
by LC-MS  
Past and future perspective***



**Japan Bioanalysis Forum (JBF)  
steering committee**

*Please note the presentation came from the discussions in JBF but it should not be construed to represent JBF's official views or policies*

# JBF activities for large molecule analysis by LC-MS



- JBF Task Force for large molecule MS  
2014-2018

>LC-MS for **large molecule therapeutics** (mainly **therapeutic antibody**)

- JBF Discussion Group  
2016-25 and 2017-31

“Quantitative analysis of endogenous large molecule substance by LC-MS”

>LC-MS for **protein biomarkers**

***protein analysis by LC-MS***

- JBF Discussion Group  
2018-36

“ Quantitative analysis of oligonucleotide therapeutics by LC-MS”

> LC-MS for **oligonucleotides**

Link to each presentation material are listed in the end of slide  
(supplemental materials)

<http://bioanalysisforum.jp/>

# JBF task force for large molecule MS



- **Scope**

LC-MS quantification of therapeutic antibody utilizing enzymatic digestion (endogenous substance, i.e. biomarker, is out of scope)

- **Outcomes**

- ✓ **Deliver Q&A** for BMV of large molecule LC/MS (by TF team)

[http://bioanalysisforum.jp/images/2015\\_6thJBFS/62\\_Outcomes%20from%20large%20molecule%20MS%20task%20force\\_goda.pdf](http://bioanalysisforum.jp/images/2015_6thJBFS/62_Outcomes%20from%20large%20molecule%20MS%20task%20force_goda.pdf)

- ✓ **Deliver Review paper** (Chromatography 2018, 39, 7-9)

(as a outcome of large molecule LC/MS Working Group supported by AMED programs\*)

<https://doi.org/10.15583/jpchrom.2017.018>

\*1 Studies on the acceleration of global harmonization for regulating safety and quality assurance of pharmaceuticals

\*2 Establishment and standardization of novel safety evaluation methods for accelerating innovative medicine development and data acquisition using these methods

# Key discussion points (technical)



**Large molecule (LM) MS TF teams discussed and addressed the point to be considered for LM-LCMS compared with small molecule LC-MS**

- **Selection of surrogate peptides**

Specificity of surrogate peptides in sample matrices

CDR peptide is considered as the most specific peptide

For non-clinical application, IgG-Fc peptides can be applicable as a generic peptides

- **Sample preparation for LC-MS**

Immunoaffinity purification

Denaturing

Reductive alkylation

Digestion

Peptide purification

Reproducibility of each step is important

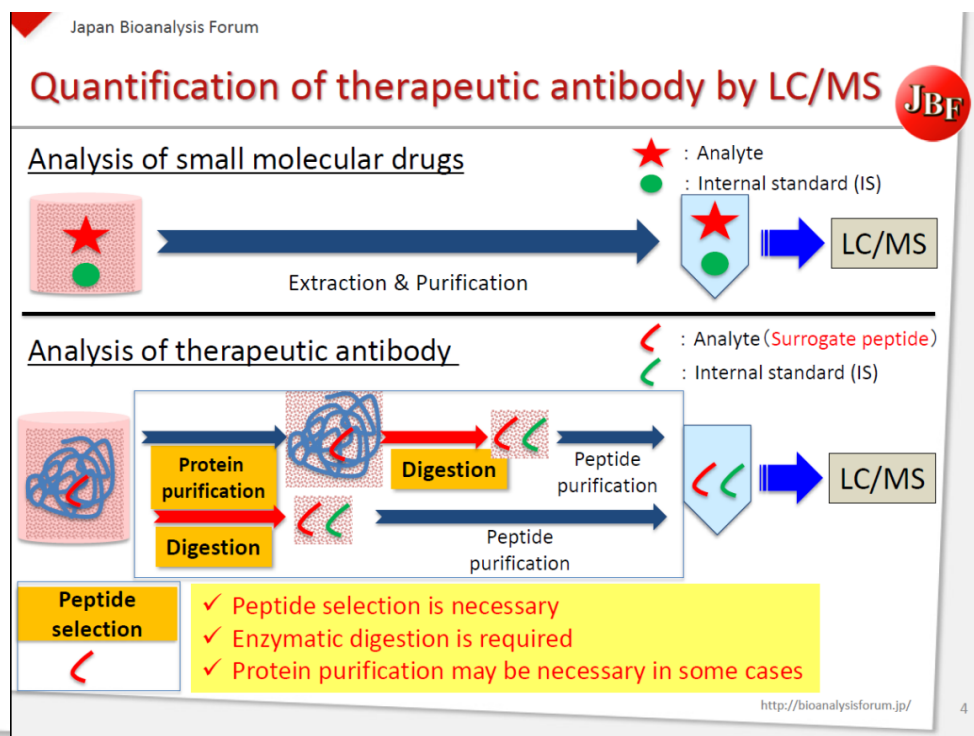
- **Selection of internal standard (IS)**

SIL peptide

SIL protein

Extended SIL-peptide

Timing for addition of IS was discussed



# Key discussion points (validation)



- It was recommended to refer the LC guidelines for sample preparation procedures and validation items since the assay platform is LC-MS.  
(Summary of proposed BMV parameters by JBF TF are attached at the end of the slide, supplemental materials)
- It would be appropriate to refer the LBA guidelines for acceptance criteria, since therapeutic antibody have been analyzed by LBA method.

Proposed assay criteria for accuracy and precision;

≥4 conc. Levels (LLOQ, low, middle high)

N ≥ 5 in a run, ≥3 runs

Accuracy (mean): ≤± 25% at LLOQ, ≤± 20% at others

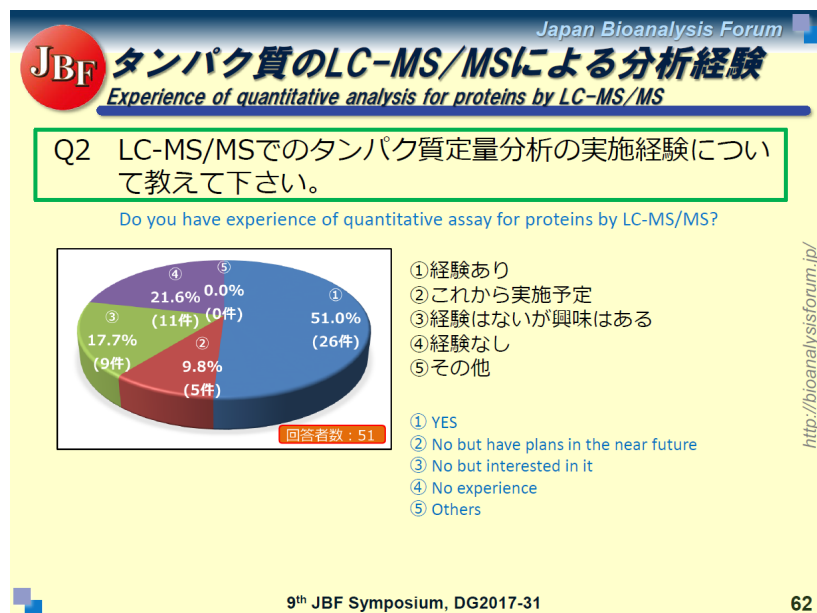
Precision: ≤25% at LLOQ, ≤20% at others

## JBF Discussion Group 2016-25 and 2017-31

## “Quantitative analysis of endogenous large molecule substance by LC-MS”



- JBF DG actively discussed **technical aspect** of protein LC-MS such as pretreatment procedures for large molecules, and utility of databases and software to searching the appropriate amino acid sequence for quantification, etc.
- DG also took a questionnaire in DG supporter and JBF partners to summarize the current situation of the technologies in Japan



51% of respondent (include Pharma, CRO, and others) has an experience of protein LC-MS.

Antibody drugs and endogenous substances are main targets for LC-MS (as of 2018).

[http://bioanalysisforum.jp/images/2017\\_8th/BFS/P6\\_DG2016-25\\_HP.pdf](http://bioanalysisforum.jp/images/2017_8th/BFS/P6_DG2016-25_HP.pdf)

[http://bioanalysisforum.jp/images/2018\\_9th/BFS/P4\\_DG2017-31.pdf](http://bioanalysisforum.jp/images/2018_9th/BFS/P4_DG2017-31.pdf)

# Summary



- Protein LC-MS is not clearly included as a scope of the existing bioanalytical method validation (BMV) guidelines in Japan
- Protein LC-MS analyses are becoming increasingly important in pharmaceutical development.

## **Future perspective**

- JBF would like to continue to facilitate discussion on Protein LC-MS in Japan.

# Announcement of next JBF Symposium



## 12th JBF Symposium For the Next Generation

March 9-11, 2021

Tower Hall Funabori, Tokyo, Japan

Chair : Hitoshi Uchiyama (Towa Pharmaceutical Co., Ltd.)  
Vice Chair : Takeru Yamaguchi (Sumitomo Chemical Co., Ltd.)

**12<sup>th</sup> JBF Symposium will be held from March 9 to 11, 2021  
at Tower Hall Funabori (Tokyo) and Online**

**[http://bioanalysisforum.jp/en/topics/12th\\_JBFsympo\\_info.html](http://bioanalysisforum.jp/en/topics/12th_JBFsympo_info.html)**





## *Supplemental materials*

# Link to presentation materials



- **JBF Task Force for large molecule MS, presented at JBF symposium in 2015-2018**

[http://bioanalysisforum.jp/images/2015\\_6thJBFS/62\\_Outcomes%20from%20large%20molecule%20MS%20task%20force\\_goda.pdf](http://bioanalysisforum.jp/images/2015_6thJBFS/62_Outcomes%20from%20large%20molecule%20MS%20task%20force_goda.pdf)

[http://bioanalysisforum.jp/images/2015\\_6thJBFS/52\\_Large%20BMVmolecule%20MS%20task%20force.pdf](http://bioanalysisforum.jp/images/2015_6thJBFS/52_Large%20BMVmolecule%20MS%20task%20force.pdf)

[http://bioanalysisforum.jp/images/2018\\_9thJBFS/5\\_3\\_Nozomu%20Kato.pdf](http://bioanalysisforum.jp/images/2018_9thJBFS/5_3_Nozomu%20Kato.pdf)

<https://doi.org/10.15583/jpchrom.2017.018> (Review paper form large molecules LCMS WG in the BMV study Group)

- **JBF Discussion Group 2016-25 and 2017-31  
“Quantitative analysis of endogenous large molecule substance by LC-MS”,  
presented at JBF symposium in 2017 and 2018**

[http://bioanalysisforum.jp/images/2017\\_8thJBFS/P6\\_DG2016-25\\_HP.pdf](http://bioanalysisforum.jp/images/2017_8thJBFS/P6_DG2016-25_HP.pdf)

[http://bioanalysisforum.jp/images/2018\\_9thJBFS/P4\\_DG2017-31.pdf](http://bioanalysisforum.jp/images/2018_9thJBFS/P4_DG2017-31.pdf)

- **JBF Discussion Group 2018-36 “ Quantitative analysis of oligonucleotide therapeutics by LC-MS”, presented at JBF symposium in 2019**

[http://bioanalysisforum.jp/images/2019\\_10thJBFS/DG2018-36.pdf](http://bioanalysisforum.jp/images/2019_10thJBFS/DG2018-36.pdf)

# TF Proposal: Comparison with Guidelines (1)



	LC Guideline (2013)	LBA Guideline (2014)	JBF Task Force
Scope	Chromatography (LC/MS)	LBA	LC/MS
	Low-molecular-weight drugs	Peptides, Proteins, Low-molecular-weight drugs	Therapeutic Antibodies
Selectivity	<ul style="list-style-type: none"> <li>➤ At least 6 individual sources</li> <li>➤ Interfering response: <math>\leq 20\%</math> of analyte &lt;LLOQ&gt; <math>\leq 5\%</math> of IS</li> </ul>	<ul style="list-style-type: none"> <li>➤ At least 10 individual sources</li> <li>➤ Blank samples, <math>\geq 80\%</math>: below LLOQ</li> <li>➤ Accuracy: <math>\geq 80\%</math> of samples <math>\leq \pm 20\%</math> at near-LLOQ (<math>\leq \pm 25\%</math> at LLOQ)</li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>6-10 individual sources</b></li> <li>➤ <b>Interfering response: <math>\leq 20\%</math> of analyte &lt;LLOQ&gt; <math>\leq 5\%</math> of IS</b></li> </ul>
Specificity	<ul style="list-style-type: none"> <li>➤ N/A</li> </ul>	<ul style="list-style-type: none"> <li>➤ Evaluate: blank samples and blank samples spiked with related substance</li> </ul>	<ul style="list-style-type: none"> <li>➤ N/A</li> </ul>
LLOQ	<ul style="list-style-type: none"> <li>➤ Interfering response: <math>\geq 5</math> times of blank sample response</li> <li>➤ Accuracy (mean): <math>\leq \pm 20\%</math></li> <li>Precision: <math>\leq 20\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ Accuracy (mean): <math>\leq \pm 25\%</math></li> <li>Precision: <math>\leq 25\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>Interfering response: <math>\geq 5</math> times of blank sample response</b></li> <li>➤ <b>Accuracy (mean): <math>\leq \pm 25\%</math></b></li> <li><b>Precision: <math>\leq 25\%</math></b></li> </ul>

# TF Proposal: Comparison with Guidelines (2)



	LC Guideline (2013)	LBA Guideline (2014)	JBF Task Force
Calibration Curve	<ul style="list-style-type: none"> <li>➤ ≥6 conc. levels</li> <li>➤ Accuracy: ≤±20% at LLOQ ≤±15% at others</li> <li>➤ Meet criteria (accuracy) ≥75% of standards ≥6 conc. levels including LLOQ &amp; ULOQ</li> </ul>	<ul style="list-style-type: none"> <li>➤ ≥6 conc. levels</li> <li>➤ Accuracy: ≤±25% at LLOQ &amp; ULOQ ≤±20% at others</li> <li>➤ Meet criteria (accuracy) ≥75% of standards ≥6 conc. levels including LLOQ &amp; ULOQ</li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>≥6 conc. levels</b></li> <li>➤ <b>Accuracy:</b> <b>≤±25% at LLOQ</b> <b>≤±20% at others</b></li> <li>➤ <b>Meet criteria (accuracy)</b> <b>≥75% of standards</b> <b>≥6 conc. levels</b> <b>including LLOQ &amp; ULOQ</b></li> </ul>
-Accuracy -Precision	<ul style="list-style-type: none"> <li>➤ ≥4 conc. levels (LLOQ, low, middle, high)</li> <li>➤ N≥5 in a run</li> <li>➤ ≥ 3 runs</li> <li>➤ Accuracy (mean): ≤±20% at LLOQ ≤±15% at others</li> <li>➤ Precision: ≤20% at LLOQ ≤15% at others</li> </ul>	<ul style="list-style-type: none"> <li>➤ ≥5 conc. levels (LLOQ, low, middle, high, ULOQ)</li> <li>➤ ≥ 6 runs</li> <li>➤ Accuracy (mean): ≤±25% at LLOQ &amp; ULOQ ≤±20% at others</li> <li>➤ Precision: ≤25% at LLOQ &amp; ULOQ ≤20% at others</li> <li>➤ Total error: ≤40% at LLOQ &amp; ULOQ ≤30% at others</li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>≥4 conc. levels (LLOQ, low, middle, high)</b></li> <li>➤ <b>N≥5 in a run</b></li> <li>➤ <b>≥ 3 runs</b></li> <li>➤ <b>Accuracy (mean):</b> <b>≤±25% at LLOQ</b> <b>≤±20% at others</b></li> <li>➤ <b>Precision:</b> <b>≤25% at LLOQ</b> <b>≤20% at others</b></li> </ul>

# TF Proposal: Comparison with Guidelines (3)



	LC Guideline (2013)	LBA Guideline (2014)	JBF Task Force
<b>Matrix Effect</b>	<ul style="list-style-type: none"> <li>➤ At least 6 individual sources</li> <li>➤ MF Precision: <math>\leq 15\%</math></li> <li>or</li> <li>➤ Determined conc. (spiked sample) Precision: <math>\leq 15\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ N/A</li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>6-10 individual sources</b></li> <li>➤ <b>MF Precision: <math>\leq 20\%</math></b></li> <li>or</li> <li>➤ <b>Determined conc. (spiked sample) Precision: <math>\leq 20\%</math></b></li> </ul>
<b>Carry-Over</b>	<ul style="list-style-type: none"> <li>➤ Blank sample response after ULOQ sample: <math>\leq 20\%</math> of analyte &lt;LLOQ&gt; <math>\leq 5\%</math> of IS</li> </ul>	<ul style="list-style-type: none"> <li>➤ N/A</li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>Blank sample response after ULOQ sample: <math>\leq 20\%</math> of analyte &lt;LLOQ&gt; <math>\leq 5\%</math> of IS</b></li> </ul>
<b>-Dilution Integrity -Dilution Linearity</b>	<ul style="list-style-type: none"> <li>➤ Dilution integrity</li> <li>➤ <math>N \geq 5</math></li> <li>➤ Accuracy (mean): <math>\leq \pm 15\%</math></li> <li>➤ Precision: <math>\leq 15\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ Dilutional linearity</li> <li>➤ Accuracy (mean): <math>\leq \pm 20\%</math></li> <li>➤ Precision: <math>\leq 20\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ <b>Dilution integrity</b></li> <li>➤ <b><math>N \geq 5</math></b></li> <li>➤ <b>Accuracy (mean): <math>\leq \pm 20\%</math></b></li> <li>➤ <b>Precision: <math>\leq 20\%</math></b></li> </ul>
<b>Stability In Matrix</b>	<ul style="list-style-type: none"> <li>➤ <math>N \geq 3</math> at Low &amp; High conc. levels</li> <li>➤ Accuracy (mean): <math>\leq \pm 15\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ <math>N \geq 3</math> at Low &amp; High conc. levels</li> <li>➤ Accuracy (mean): <math>\leq \pm 20\%</math></li> </ul>	<ul style="list-style-type: none"> <li>➤ <b><math>N \geq 3</math> at Low &amp; High conc. levels</b></li> <li>➤ <b>Accuracy (mean): <math>\leq \pm 20\%</math></b></li> </ul>