2018/2/7

抗体医薬品のLC/MS分析の解説＆提案

Commentaries and Proposals on Bioanalytical Quantification of Therapeutic Antibodies by LC/MS

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Overview

- Current status
- Purpose of activity
- Peptide selection
- Sample preparation
- Analytical method validation
- Summary

Current status

- Liquid chromatography/mass spectrometry (LC/MS) method is becoming an important approach for therapeutic antibody assays.

  （There are several advantages compared to the LBA method）

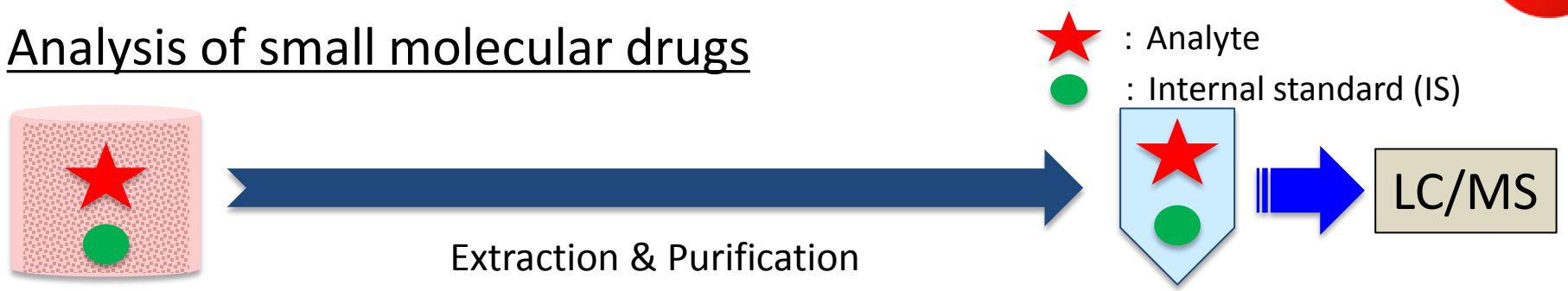
  ○High selectivity/specificity
  ○Broad linearity range (3-5 orders)
  ○Fast method development
  ○Analysis of multiple peptides
    - Monitoring of post-translational modifications
    - Multi-domain detection (ex. Fab and Fc domains)

- On the other hand, the following processes are complicated.

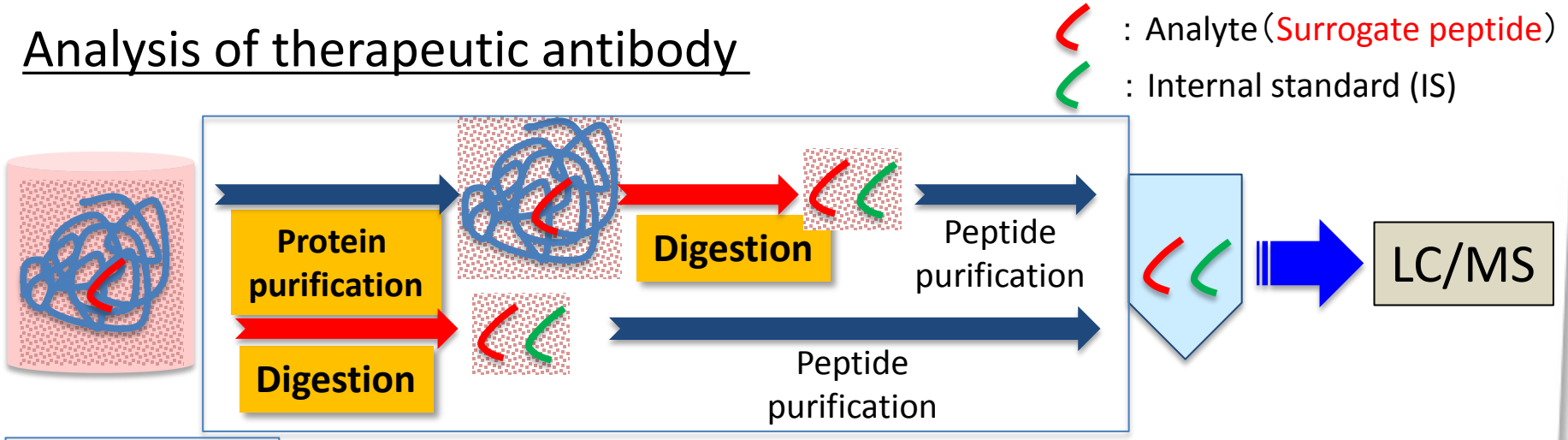
  ○Enzymatic digestion
  ○Denaturation, Reduction and Alkylation
  ○Protein purification（Affinity purification et al）

Quantification of therapeutic antibody by LC/MS

Analysis of small molecular drugs



Analysis of therapeutic antibody



Peptide selection

- ✓ Peptide selection is necessary
- ✓ Enzymatic digestion is required
- ✓ Protein purification may be necessary in some cases

Purpose of activity

- The large molecules LC / MS working group have discussed general requirements for developing bioanalytical LC/MS methods for therapeutic antibodies.

- This presentation will introduce commentaries and proposals from the review (Chromatography 2018*) made by large molecules LC / MS WG in the BMV study Group.

- Please note that this presentation is a summary of the discussion of large molecules LC / MS WG and dose not necessarily represent the views, opinions or practices of companies which members belong.

*Chromatography 2018, 39, 7-9
「Bioanalytical Quantification of Therapeutic Antibodies by Liquid Chromatography/mass Spectrometry」 https://doi.org/10.15583/jpchrom.2017.018

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分担研究：バイオアナリシスに関する研究
（研究分担者：国立衛研　香取典子先生）
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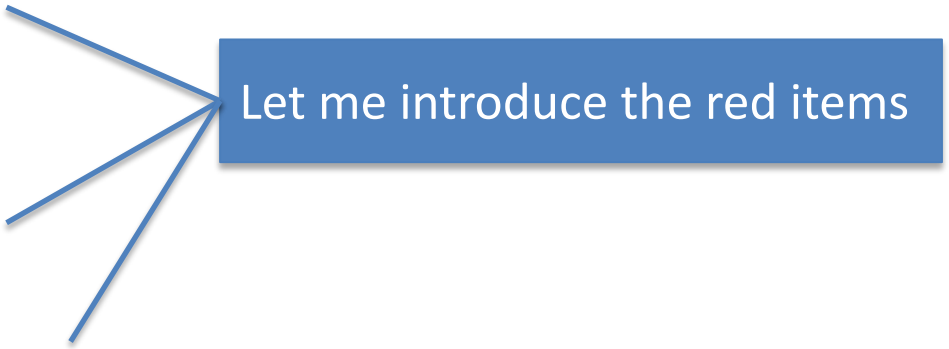
（研究代表者：国立衛研　斎藤嘉朗先生）

The discussion by large molecules LC / MS WG

（Contents）

- Peptide selection
- IS selection
- Sample preparation
- Validation



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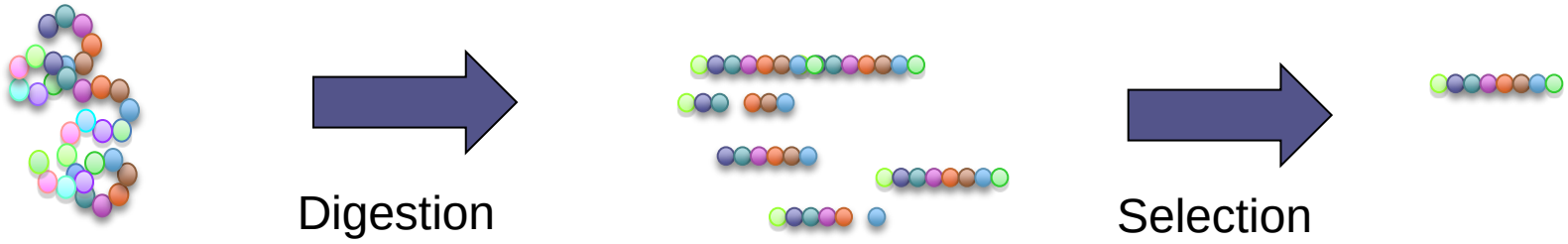
Importance of peptide selection

LC / MS can not quantify the protein itself. Therefore, quantification of the protein is carried out by measuring the surrogate peptide after enzyme digestion.



Target Protein
（low sensitivity）

Enzymatic peptide

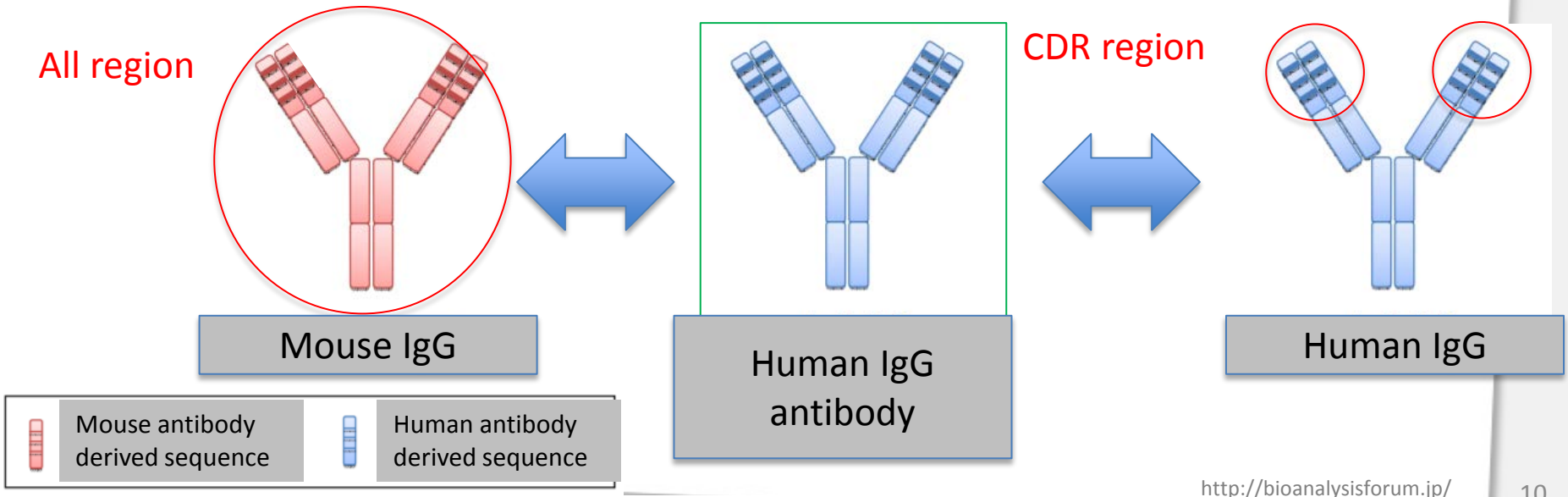
Surrogate peptide
（high sensitivity）

Points to be considered selecting of surrogate peptides

- **Specificity：**
  Derived from therapeutic antibody , Depend on the enzyme species, Distinguishable from other peptides
- **Sensitivity：**
  High peak intensity, Exclude unexpected modification, Appropriate length, Simple fragmentation
- **Stability：**
  Stability during pretreatment, High recovery rate

Peptide selection: differences between a clinical and a non-clinical sample for therapeutic antibodies

Research stage	Non-clinical	Clinical
Requirements	Differentiation between human IgG-derived antibody and animal IgG is necessary	Differentiation between human IgG-derived antibody and human IgG is necessary
Sequences	・CDR * and human IgG constant region. When selected from the constant region, it can be used as a universal.	・CDR region



*Complementarity-determining regions (CDRs) are part of the variable chains in immunoglobulins (antibodies)

Peptide selection flow in therapeutic antibodies

Confirmation of CDR region based on base sequence information (using IGBLAST)

Confirmation of CDR region based on base sequence information (using Multiple alignment)

Unique sequence

In silico digestion (ProteinProspector et al)

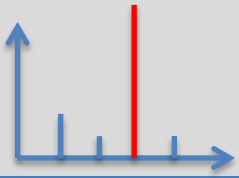
Peptide length and modification, which obtained by enzyme digestion

Homology search (Protein Blast search)

Specificity

Peptide mapping by LC/MS

- High peak intensity
- Exclude unexpected modification
- Appropriate length
- Simple fragmentation



Sensitivity and modification by actual measurement

Analysis in matrix

- Not detected from matrix

Selectivity

Surrogate peptide

IGBLAST, https://www.ncbi.nlm.nih.gov/igblast;
ClustalW, http://www.genome.jp/tools/clustalw;
ProteinProspector, http://prospector.ucsf.edu/prospector/mshome.htm;
Protein BLAST, https://blast.ncbi.nlm.nih.gov.

Confirmation of CDR region based on the sequence information (using Multiple alignment)

As a result of performing multiple alignment analysis using amino acid sequences of some approved for therapeutic antibodies, the CDR regions of both H chain and L chain are approximately in the vicinity of the amino acid sequences at positions 20 to 30, 50 to 60, and 100 to 110.

Heavy chain

Light chain

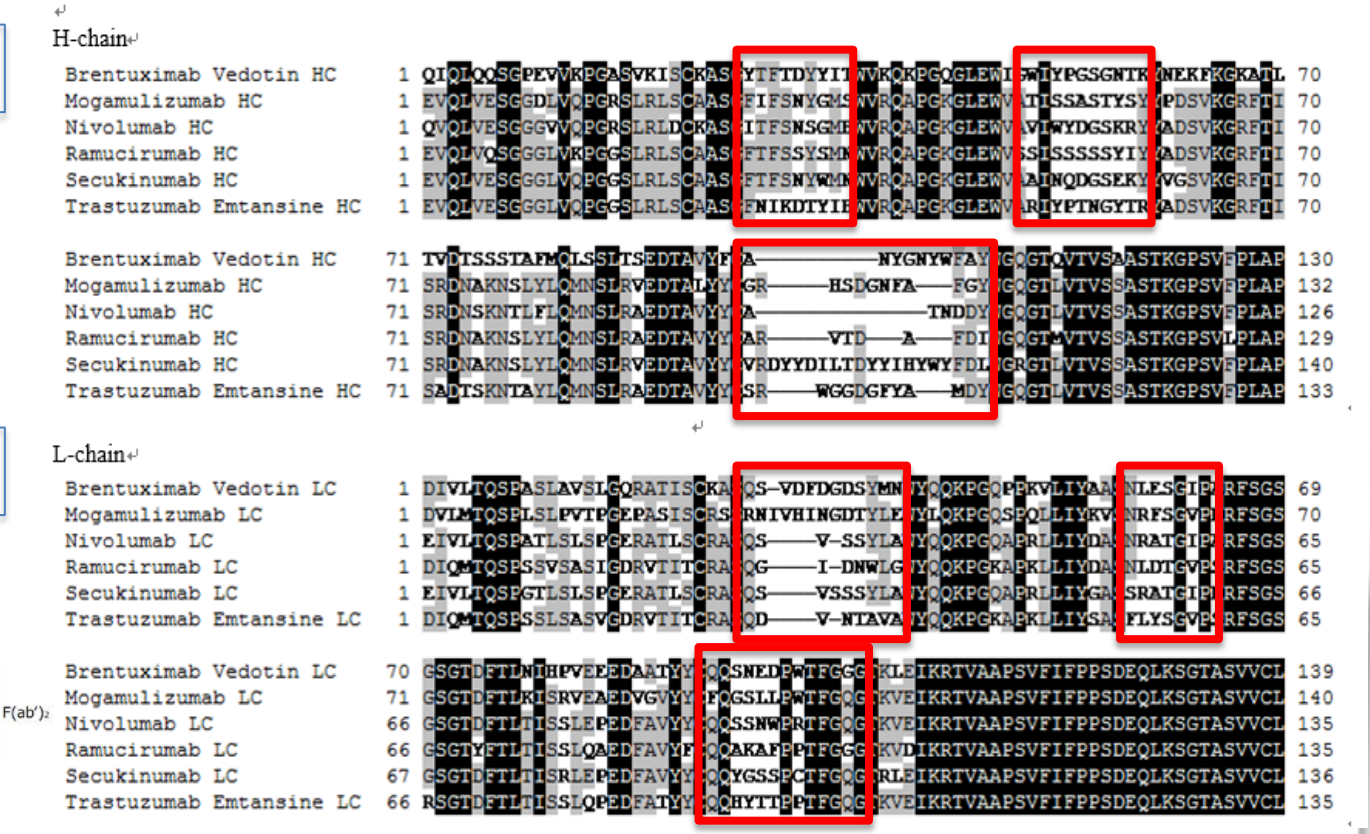
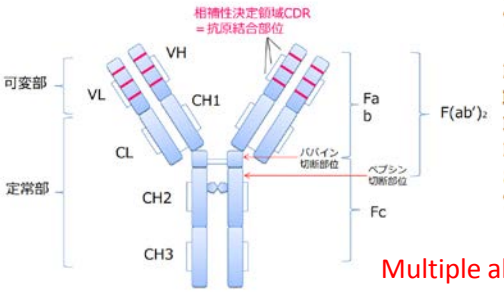


図1　ヒトIgG1の構造模式図



Multiple alignment analysis was performed using GENETYX Ver.10 software.

Sequence information : WHO International Nonproprietary Names (INNs) list.

Peptide selection-other conditions

To reduce potential sources of variability

Amino acids that should be removed (Exclude as much as possible)	Other notes (According to need)
• Methionine (Met) ⇒ Oxidation • Asparagine (Asn) ⇒ Deamidation • Histidine (His) ⇒ Decrease sensitivity • Asn-X-Ser or Asn-X-Thr (X excludes Pro) ⇒Glycosylation • Cysteine (Cys) ⇒Alkylation • N-terminal glutamine (Gln) & Glutamic acid (Glu) ⇒ Pyroglutamate	• Contents of hydrophobic amino acids ⇒ Adsorption

Huang, L. Anal Chem 2005, 77, 1432-1439.
Furlong, M.T. Biomed Chromatogr 2012, 26, 1024-1032.
Chelius, D. Anal Chem 2005, 77, 6004-6011.
Kamiie, J. Pharm Res 2008, 25, 1469-1483.

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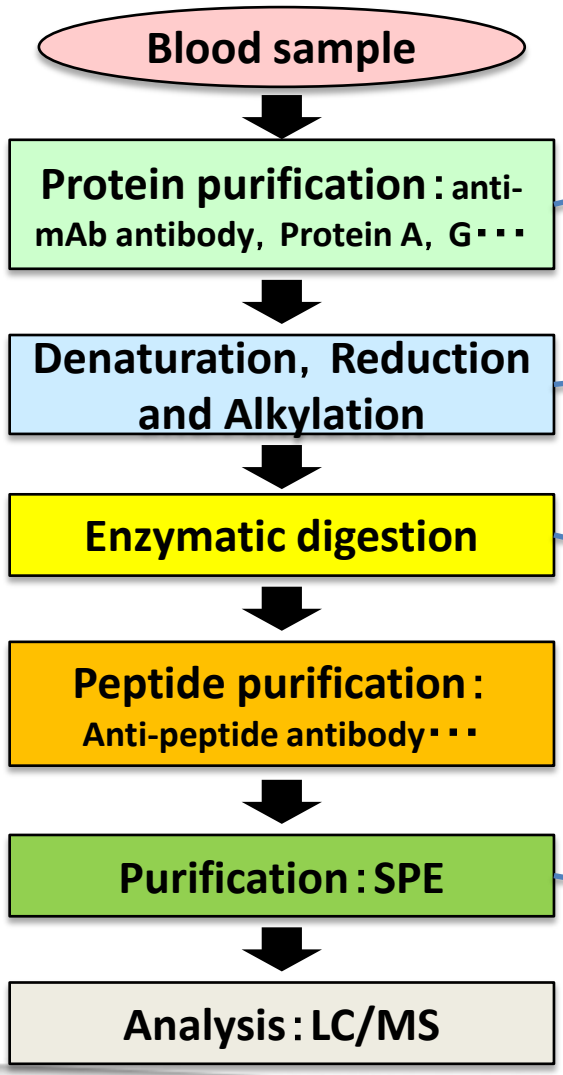
Outline of sample preparation (Points to be noted)



It contributes most to improving sample complexity. There are various methods. The degree of difficulty of optimization is high.

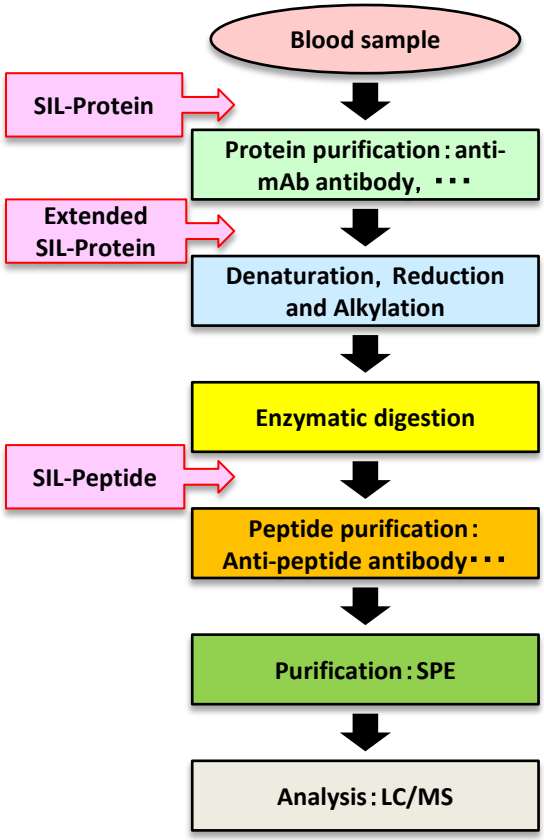
It contributes to the efficiency of enzyme digestion. Pay attention to pH change by reagent.

It depends on the performance of the enzyme selected. Attention should be paid to the optimum pH of the enzyme. Confirmation of reproducibility & concentration dependence of enzyme reaction is necessary.

Mainly remove reagents and salts in the sample. Easy to optimize. However, it does not lead to improvement of complex samples.

Concept of Workflow Selection

General method reported in some reference



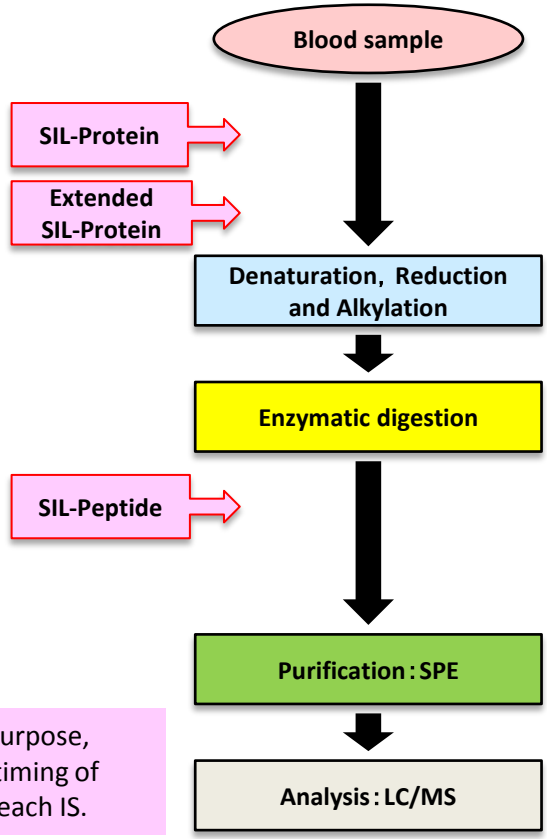
Pros :
- **High selectivity**
- **High sensitivity**

Cons :
- Complicated sample preparation
- High optimization difficulty
- Long development time

IS was selected according to purpose, synthesis cost and time. The timing of addition varies depending on each IS.

Suitable for low concentration samples such as clinical trials

Focus on speed

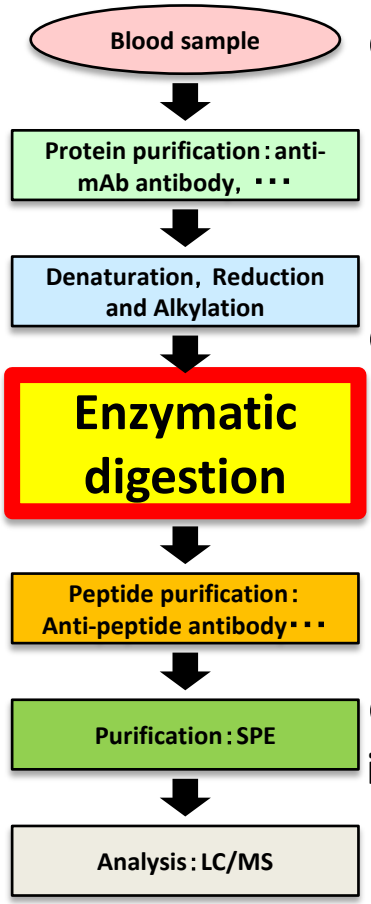


Pros :
- **Simple sample preparation**
- **Low optimization difficulty**
- **Short development time**

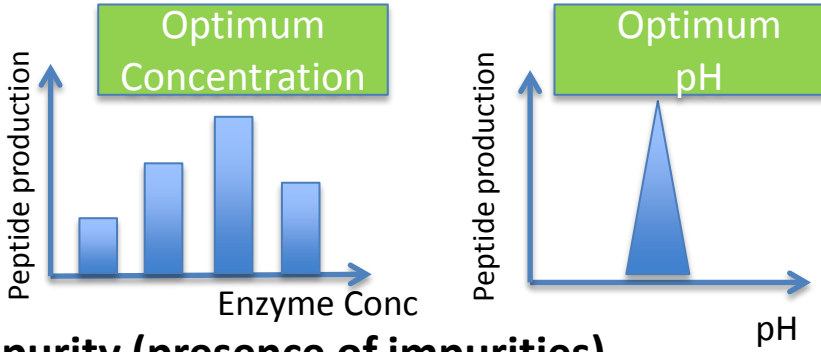
Cons :
- Low selectivity
- Low sensitivity

Suitable for high concentration samples such as non-clinical studies

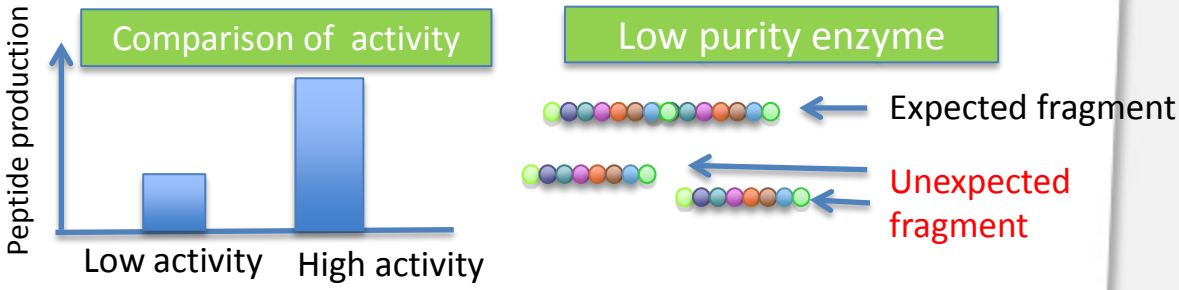
Example) Optimization of enzyme digestion



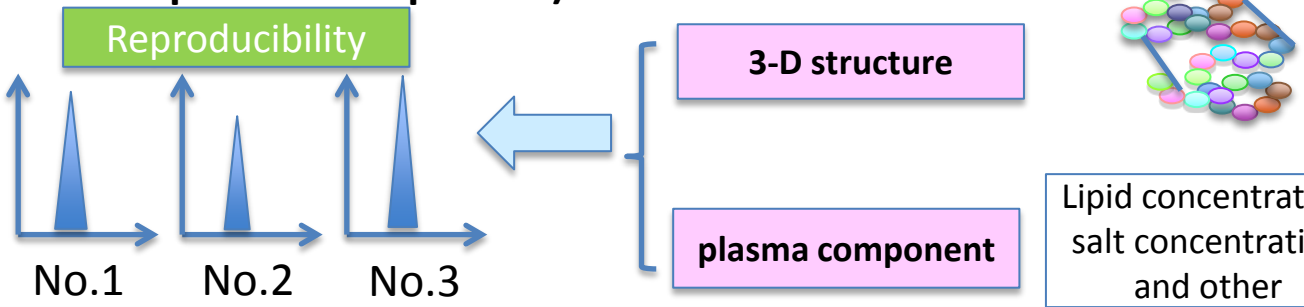
○Optimum concentration, Optimum pH



○ Enzyme activity, enzyme purity (presence of impurities)

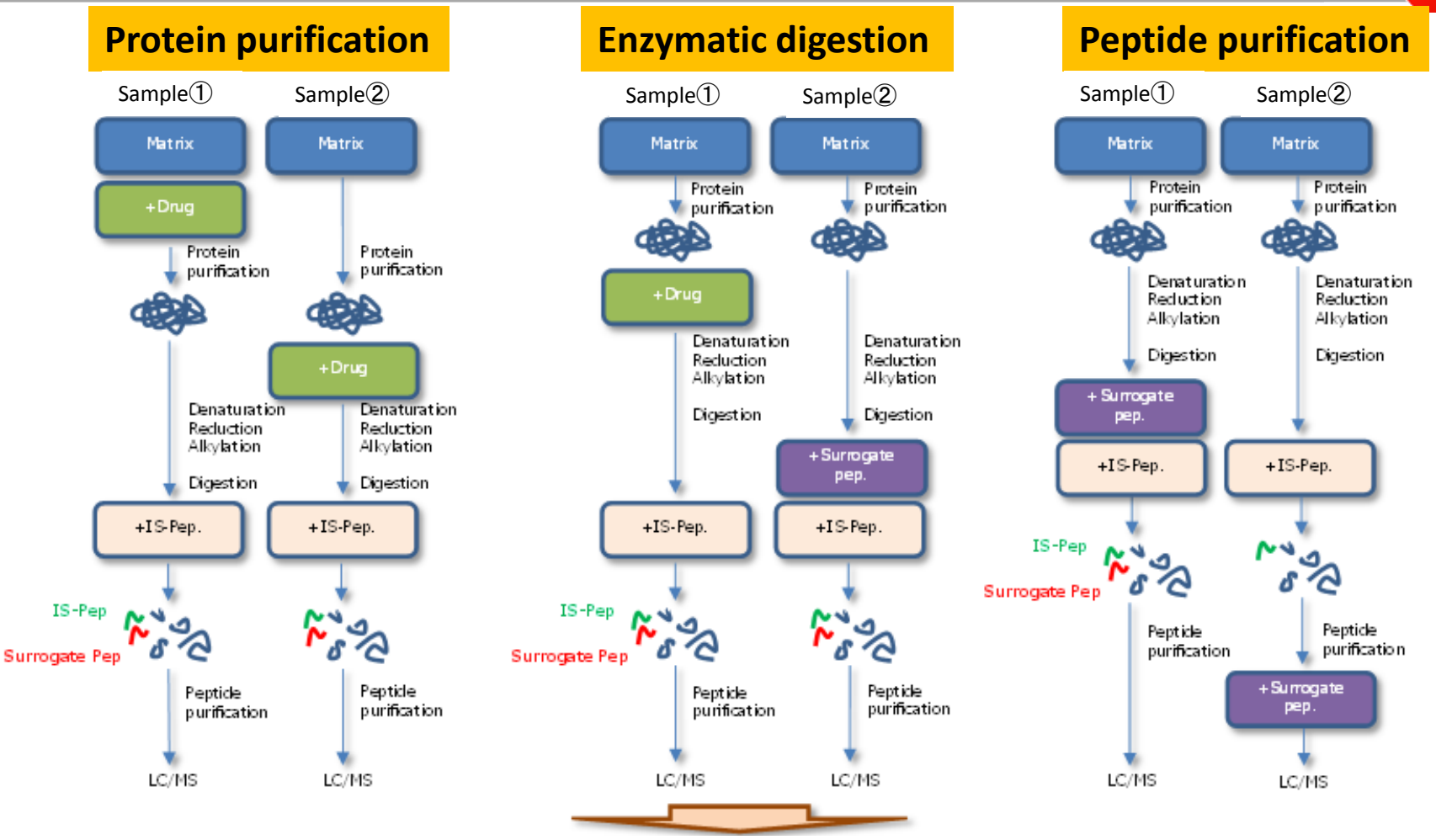


○Reproducibility of enzyme digestion (3-D structure of protein, influence of plasma component)



There are multiple factors for optimization

The evaluation of the Recovery rate for each process



Recovery rate = sample ① / sample ②

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Bioanalytical method validation

- We would propose the validation items and criteria for quantification of therapeutic antibody in plasma/serum/blood using LC/MS.
- It is recommended to refer to the LC guidelines for preparation procedures and evaluation items, since the platform is LC/MS.
- It would be appropriate to refer to the LBA guidelines for acceptance criteria (accuracy and precision), since therapeutic antibody have been analyzed by LBA method.

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	➢ At least 6 individual sources ➢ Interfering response: ≤20% of analyte <LLOQ> ≤5% of IS	➢ At least 10 individual sources ➢ Blank samples, ≥80%: below LLOQ ➢ Accuracy: ≥80% of samples ≤±20% at near-LLOQ (≤±25% at LLOQ)	➢ **6-10 individual sources** ➢ **Interfering response: ≤20% of analyte <LLOQ> ≤5% of IS**
Specificity	➢ N/A	➢ Evaluate: blank samples and blank samples spiked with related substance	➢ **N/A**
LLOQ	➢ Interfering response: ≥5 times of blank sample response ➢ Accuracy (mean): ≤±20% Precision: ≤20%	➢ Accuracy (mean): ≤±25% Precision: ≤25%	➢ **Interfering response: ≥5 times of blank sample response** ➢ **Accuracy (mean): ≤±25% Precision: ≤25%**

TF Proposal: Comparison with Guidelines（2）

	LC Guideline（2013）	LBA Guideline（2014）	JBF Task Force
Calibration Curve	➢ ≥6 conc. levels ➢ Accuracy: ≤±20% at LLOQ ≤±15% at others ➢ Meet criteria (accuracy) ≥75% of standards ≥6 conc. levels including LLOQ & ULOQ	➢ ≥6 conc. levels ➢ Accuracy: ≤±25% at LLOQ & ULOQ ≤±20% at others ➢ Meet criteria (accuracy) ≥75% of standards ≥6 conc. levels including LLOQ & ULOQ	➢ **≥6 conc. levels** ➢ **Accuracy:** **≤±25% at LLOQ** **≤±20% at others** ➢ **Meet criteria (accuracy)** **≥75% of standards** **≥6 conc. levels** **including LLOQ & ULOQ**
-Accuracy -Precision	➢ ≥4 conc. levels (LLOQ, low, middle, high) ➢ N≥5 in a run ➢ ≥ 3 runs ➢ Accuracy (mean): ≤±20% at LLOQ ≤±15% at others ➢ Precision: ≤20% at LLOQ ≤15% at others	➢ ≥5 conc. levels (LLOQ, low, middle, high, ULOQ) ➢ ≥ 6 runs ➢ Accuracy (mean): ≤±25% at LLOQ & ULOQ ≤±20% at others ➢ Precision: ≤25% at LLOQ & ULOQ ≤20% at others ➢ Total error: ≤40% at LLOQ & ULOQ ≤30% at others	➢ **≥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**

TF Proposal: Comparison with Guidelines（3）

	LC Guideline（2013）	LBA Guideline（2014）	JBF Task Force
Matrix Effect	➢ At least 6 individual sources ➢ MF Precision: ≤15% or ➢ Determined conc. (spiked sample) Precision: ≤15%	➢ N/A	➢ **6-10 individual sources** ➢ **MF Precision: ≤20%** **or** ➢ **Determined conc. (spiked sample) Precision: ≤20%**
Carry-Over	➢ Blank sample response after ULOQ sample: ≤20% of analyte <LLOQ> ≤5% of IS	➢ N/A	➢ **Blank sample response after ULOQ sample:** **≤20% of analyte <LLOQ>** **≤5% of IS**
-Dilution Integrity -Dilution Linearity	➢ Dilution integrity ➢ N≥5 ➢ Accuracy (mean): ≤±15% ➢ Precision: ≤15%	➢ Dilutional linearity ➢ Accuracy (mean): ≤±20% ➢ Precision: ≤20%	➢ **Dilution integrity** ➢ **N≥5** ➢ **Accuracy (mean): ≤±20%** ➢ **Precision: ≤20%**
Stability In Matrix	➢ N≥3 at Low & High conc. levels ➢ Accuracy (mean): ≤±15%	➢ N≥3 at Low & High conc. levels ➢ Accuracy (mean): ≤±20%	➢ **N≥3 at Low & High conc. levels** ➢ **Accuracy (mean): ≤±20%**

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Summary

- We have shown points to be considered regarding selection of surrogate peptides and sample preparation in by large molecules(LM) LC/MS method.
- Furthermore, we have proposed criteria for validation of by LM-LC/MS method.
- We hope the review article* will aid in the development of LC/MS methods for therapeutic antibodies.

（*Review article）

Chromatography 2018, 39, 7-9

“Bioanalytical Quantification of Therapeutic Antibodies by Liquid Chromatography/mass Spectrometry”
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Acknowledgments

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