

Quantification of therapeutic proteins in biological samples by liquid chromatography/mass spectrometry

Bioanalysis of biopharmaceuticals by LC/MS

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Outline

Bioanalysis of biopharmaceuticals by LC/MS

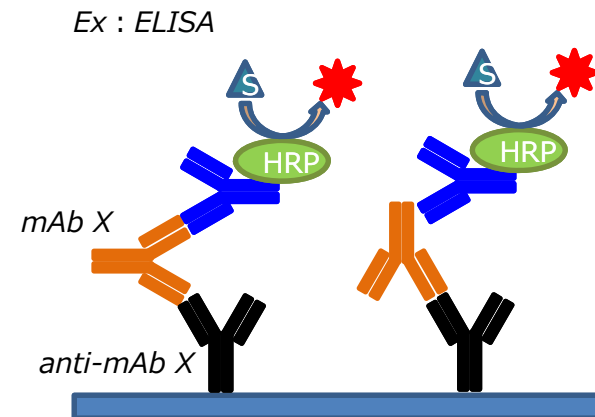
- ✓ Current status and issues
- ✓ Our efforts
 - Development of quantification method for therapeutic monoclonal antibody in human serum by LC/MS

Current bioanalytical approach of biopharmaceuticals

Ligand binding assays (LBAs)

Issues

- Selectivity/Specificity
 - Interference by ADA
 - Cross reactivity
- Narrow dynamic range
- Long method development time
 - Reagent development needed prior to starting method development



Current bioanalytical approach of biopharmaceuticals

Liquid chromatography/mass spectrometry (LC/MS)

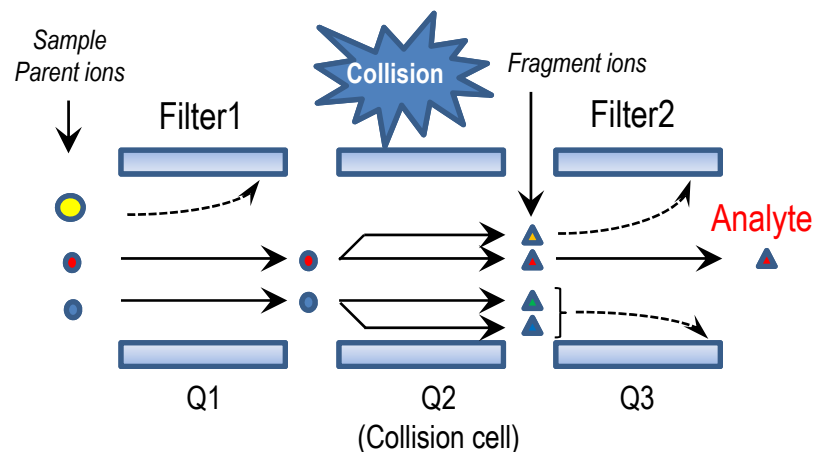
Advantage of LC/MS

- ✓ High selectivity/specificity
- ✓ Analysis of multiple peptides
 - *Monitoring of post-translational modifications*
 - *Multi-domain detection (ex. Fab and Fc domains)*
- ✓ Fast method development
- ✓ Broad linearity range (3-5 orders)
- ✓ Avoid interference by serum factors
(e.g. endogenous ligand molecule, ADA)

LC/MS used to complement LBA

Current LC/MS methods

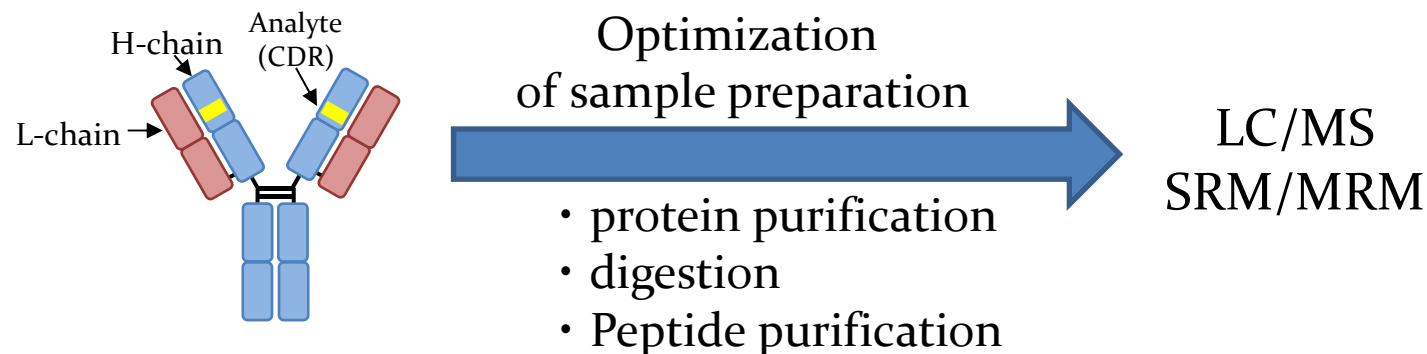
- **Triple quadrupole tandem mass spectrometer**
Selected reaction monitoring/ Multiple reaction monitoring (SRM/MRM)



- **High resolution mass spectrometer (HRMS)**
Selected-ion monitoring (SIM)
Parallel-reaction monitoring (PRM)

Development of quantification method for therapeutic monoclonal antibody by LC/MS

Collaborators: Ajinomoto Co. Inc., Ig-M Co., Ltd., CMIC Pharma Science Co., Ltd., LSI Medience Corporation, Takeda Pharmaceutical Company Limited, and Towa Pharmaceutical Co. Ltd.



Common materials : Trastuzumab (IgG₁), Trypsin, SIL-IS, human serum

Optimization of sample preparation: mAb-purification, enzymatic digestion, and peptide clean-up by collaborative study

Target LLOQ: 0.1 µg/ml

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Issues regarding bioanalysis of biopharmaceuticals by mass spectrometric method

- ✓ Improvement of sensitivity compared with LBA method
- ✓ Sample preparation (Protein purification)
- ✓ Automation of sample preparation
- ✓ LC/MS bioanalytical method for microsampling sample

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Thank you for your attention !