NIM Newsletter

ISSUE 02
DEC 2020

Table of Contents

Foreword

The year of 2020 is a truly turbulent year. The COVID-19 pandemic has made the world realize the most precious assets in our lives. It has also driven us as metrologists to think about how we could do our part to safeguard our life and health.

This issue focuses on NIM's activities in metrology for therapeutics and diagnostics. We have selected six articles to introduce our research on reference materials and reference methods supporting therapeutics and diagnostics.

Another event worth to mention is the International Workshop on Therapeutics and Diagnostics: Measurements, Standards, Quality and Safety (TD-MSQS) jointly organized by NIM and the BIPM from 10 to 12 November 2020 in Nanjing of China, which has provided a forum for metrologists and therapeutic and diagnostic industry stakeholders to exchange information that may forge further cooperation.

In this issue you will also see a brief update of the NIM Fellow policy and the appointment of six new NIM Fellows. An improvement was made to this mechanism to better foster and leverage high quality experts.

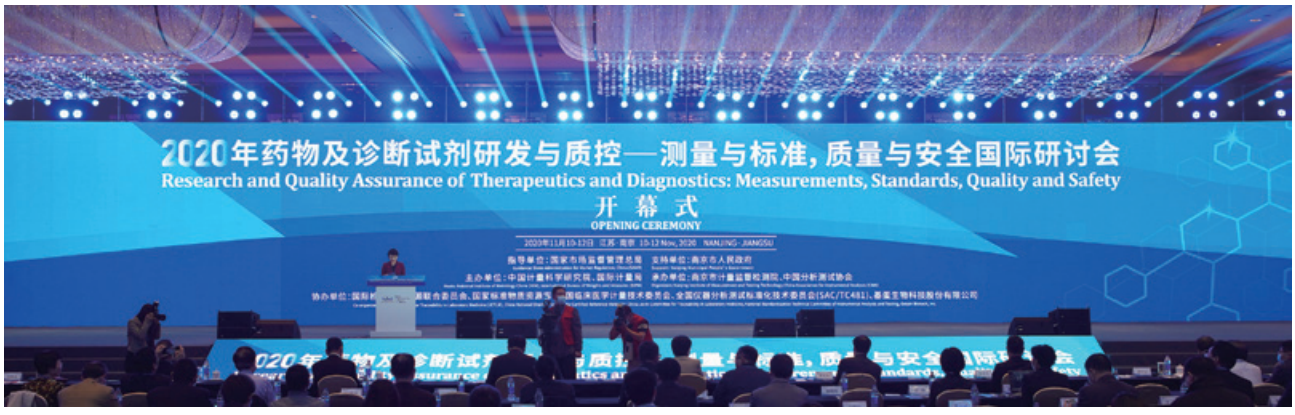
2020 makes us understand the strength of cooperation more than ever before. As 2020 draws to a close, we take this opportunity to thank our colleagues and partners around the world for your friendship and cooperation especially during this very challenging year. We wish you a healthy, happy and prosperous New Year!

International Workshop on Therapeutics and Diagnostics: Measurements, Standards, Quality and Safety (TD-MSQS)

Validity evaluation and quality control of drugs and diagnostic reagents is a major challenge facing global biopharmaceutical and diagnostic industries. It is a research priority for biological enterprises. It is also a prerequisite for standardization of process and development of commercial products. To promote the communication and collaboration between metrology community and stakeholders in therapeutic and diagnostic fields, NIM and the BIPM jointly organized an International Workshop on Therapeutics and Diagnostics: Measurements, Standards, Quality and Safety (TD-MSQS) from 10 to 12 November 2020 in Nanjing, China. On similar topics, NIM and the BIPM jointly held two International Workshops on Protein and Peptide Therapeutics and Diagnostics (PPTD) in Chengdu, China in 2016 and 2018, respectively.

Under the theme of "Measurements, Standards, Quality and Safety" and with measurement technology as the core content, this Workshop aims at bridging the gap between scientific research in drug and diagnostic reagents and their applications in industries and sectors, and promoting open and closer collaboration among key players in this industrial chain, from research, to quality assurance, through to application. Reports in the plenary session and topics of the three sub-sessions are shown on the next page.

In the opening ceremony, a "China Therapeutics and Diagnostics Industrial Measurement Consortium", coordinated by NIM, was launched. The Consortium is open to universities, institutes, enterprises, and testing laboratories that engage in scientific research, technology development, manufacturing, and application of drug and diagnostic reagents. The first members include thirty organizations who had volunteered to join the Consortium. As a link between Chinese metrology community and industry, the Consortium will be committed to promoting technical progress and the development of therapeutic and diagnostic industries in China.



Plenary Session

Topic	Speaker	Organization
Molecular classification of disease	Prof. Weihong Tan	Hunan University, China
Impact of measurements and standards on the characterization of biopharmaceuticals	Prof. Fouad Atouf	United States Pharmacopeia (USP), USA
Proteome analysis for precision medicine	Prof. Yukui Zhang	Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), China
CDC's clinical standardization programs to ensure quality of in vitro diagnostics (IVD)	Prof. Hubert Vesper	Centers for Disease Control and Prevention (CDC), USA
Hybridizing qNMR and LC-UV data for enhanced quantitative characterization of pure substances. Examples of synthetic pharmaceuticals	Prof. Cees-Jan Nap	European Directorate for the Quality of Medicines (EDQM)
Standardization by the IFCC Scientific Division: challenging the status quo!?	Prof. Christa Cobbaert	Leiden University Medical Centre (LUMC), Netherlands/International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
The current status and demand of IVD standardization for cardiovascular and cerebrovascular disease diagnosis in China	Prof. Hongmei Li	National Institute of Metrology (NIM), China
Green Bio-manufacturing	Prof. Tianwei Tan	Beijing University of Chemical Technology (BUCT), China
Impact of measurement accuracy on clinical decision making	Prof. Ian Young	Joint Committee for Traceability in Laboratory Medicine (JCTLM)

Sub-sessions

1. Drug characterization and quality assurance
2. Research and quality control for IVD
3. Reference standards, regulation and metrology

For more information about the Workshop, please contact Ms. JIAO Hui at jiaohui@nim.ac.cn.

Development of Amyloid-beta Certified Reference Materials for Diagnosis of Alzheimer's Disease

Due to the trend in population aging, the impact of neurodegenerative disorders, predominantly Alzheimer's disease (AD), is increasing rapidly. And the pathogenesis and early diagnosis of AD have attracted widespread attention. Numerous clinical studies have shown that abnormal levels of amyloid-beta (Aβ) in cerebrospinal fluid (CSF), blood and brain tissue are closely related to the progression of AD and hence Aβ has become one of the most important biomarkers for studying AD. Therefore, accurate measurement of Aβ can improve the accuracy of early diagnosis of AD. However, the lack of Aβ certified reference materials (CRMs) and reference measurement procedures led to variances in measurement results from different laboratories, resulting in low clinical utility of Aβ markers.

To solve this problem, Aβ40 (GBW09874) and Aβ42 (GBW09875) solution CRMs were developed by NIM, China with the certified values and uncertainties of (7.58 ± 0.30) μg g-1 and (7.62 ± 0.30) μg g-1 respectively. After identification and qualitative analysis of the candidates for raw materials, amino acids-based and online sulfur-based isotope dilution strategies were employed together for the first time to certify the Aβ CRMs. In the amino acids-based approach, the AA hydrolysis conditions were optimized. In the online sulfur-based isotope dilution approach, the separation conditions of Aβ suitable for ICP-MS and the influence of species-unspecific 34S spike on 32S/34S ratio were investigated. The two approaches achieved consistent results and the mean value of the results was used as the certified value.

The two Aβ CRMs, which were developed for the first time ever, are at the top of the ISO17511 traceability chain. They can be used as primary calibrators for value assignment for secondary calibrators or secondary RMs with clinical matrix, which are helpful for early diagnosis of AD. Moreover, the certification strategy of this study can provide a new idea for certification of high-purity protein reference materials.

Reference

Feng L., Huo Z., Xiong J., Li H. (2020). *Analytical Chemistry*, 92(19), 13229-13237.

Contact

LI Hongmei, lihm@nim.ac.cn

FENG Liuxing, fenglx@nim.ac.cn

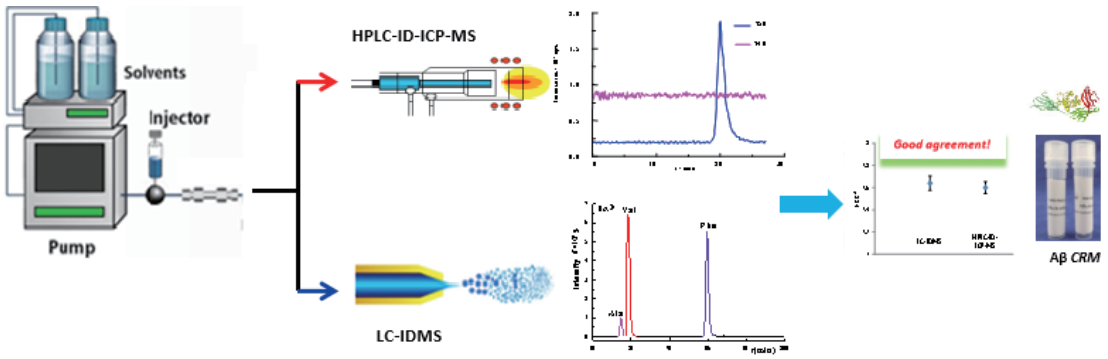


Fig.1 Certification strategy of Aβ CRMs by LC-IDMS and HPLC-ID-ICP-MS

In-depth Structural Analysis of Charge Isomers of the NIMCmAb Monoclonal Antibody Reference Material

Biologics are life-saving medications for cancers, autoimmune conditions, and heart diseases. The largest class of biologic drugs is protein therapeutics such as monoclonal antibodies (mAbs). Large, complex biologic drugs are heterogeneous and a shift or change in their quality attributes may occur over their lifecycle, which makes it difficult to define their quality attributes by measurement. To assist quality assessment of biopharmaceutical products, NIM has developed a monoclonal antibody reference material (NIMCmAb), a kind of humanized IgG1 κ reference material, to provide a common control material and representative test molecule for development of new techniques for biopharmaceutical manufacturers.

For quantitatively characterizing the posttranslational modification status of charge isomers (CIs) of NIMCmAb and exploring the impact of posttranslational modifications on its charge heterogeneity, the CIs of NIMCmAb were fractionated by strong cation exchange chromatography and verified by capillary isoelectric focusing-whole column imaging detection, followed by stepwise structural characterization at three levels (Fig. 2).

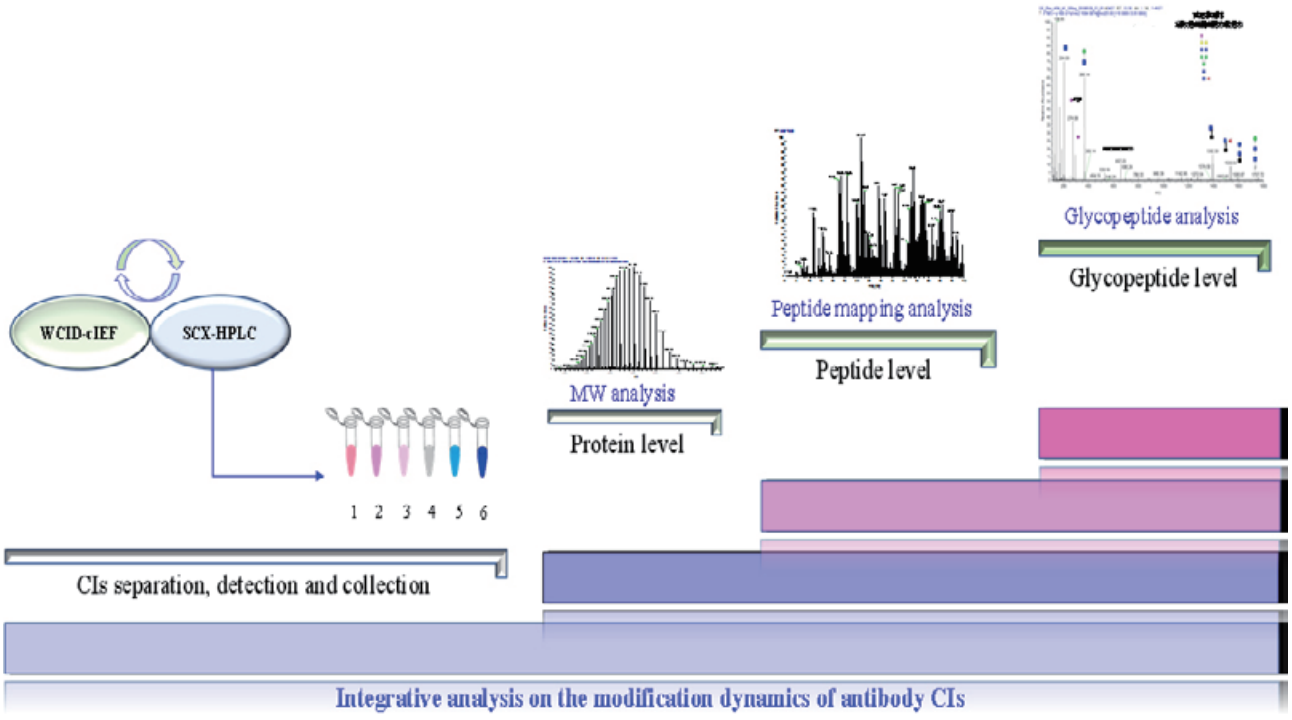


Fig. 2 Experimental design for in-depth structural analysis of charge isomers of NIMCmAb, consisting of SCX-HPLC, strong cation exchange chromatography, cIEF-WCID, and capillary isoelectric focusing-whole column imaging detection.

Posttranslational modifications of antibody products affect their stability, charge distribution, and drug activity and thus are their critical quality attributes. The comprehensive mapping of antibody modifications and different CIs are of the utmost importance. In this study, based on proteomics technology, the effects of modifications on CIs were comprehensively characterized. The results suggested that the sialic acid and deamidation modifications were the most critical factors affecting the charge heterogeneity of NIMCmAb.

In this study, global characterization of modifications to the CIs of NIMCmAb was performed, providing a stepwise and in-depth approach to studying the influence of CIs on therapeutic proteins. It can be applied to the quality control of pharmaceutical production by antibody drug manufactures for drug registration and batch release, which could improve safety and efficacy of antibody products. Additionally, as shown in this study, the NIMCmAb reference material can be used for method qualification, development of innovative approaches to identity, purity and stability testing, and evaluation and harmonization of best practices.

Reference

Cui X., Mi W., Hu Z., Ying W. (2021). *Journal of Pharmaceutical Analysis*. (Accepted)
DOI: 10.1016/j.jpha.2020.11.006

Contact

HU Zhishang, huz@nim.ac.cn

Traceability System for Accurate Measurement of C-Reactive Protein

C-reactive protein (CRP) is one of the most well-known biomarkers for systemic inflammation and also a good diagnostic marker for cardiovascular and cerebrovascular diseases. CRP is a pentameric protein, consisting of five noncovalently bound, identical monomers. The molecular weight of each monomer is 23 kDa, and the molecular weight of the pentamer has reached 115 kDa. The valuation of CRP has always been a popular challenge for National Metrology Institutes (NMIs).

Before this study, there were only two primary reference materials of CRP, i.e. SRM 2924, developed by the National Institute of Standards and Technology (NIST) of the US, and CRM 6201-b, developed by the National Metrology Institute of Japan (NMIJ). These two reference materials are based on the recombinant expression of Escherichia coli and exist in the form of monomers, which are different from natural CRP. Due to the complexity of serum matrix, it is difficult to develop secondary reference materials of serum matrix. Only ERM-DA474 developed by the Institute of Reference Materials and Measurement (IRMM) of the European Union and 85/506 developed by the National Institute for Biological Standards and Control (NIBSC) of the UK are reference materials of serum matrix available in the world at present. These two reference materials adopt multiple kits' values and have only one concentration, which cannot meet the clinical demand for reference materials with a series of concentration gradients. Clinically, concentrations of CRP range from 0 to 100 mg/L or even higher, so the accurate measurement of the linear range of different concentrations requires multiple reference standards with corresponding concentrations.

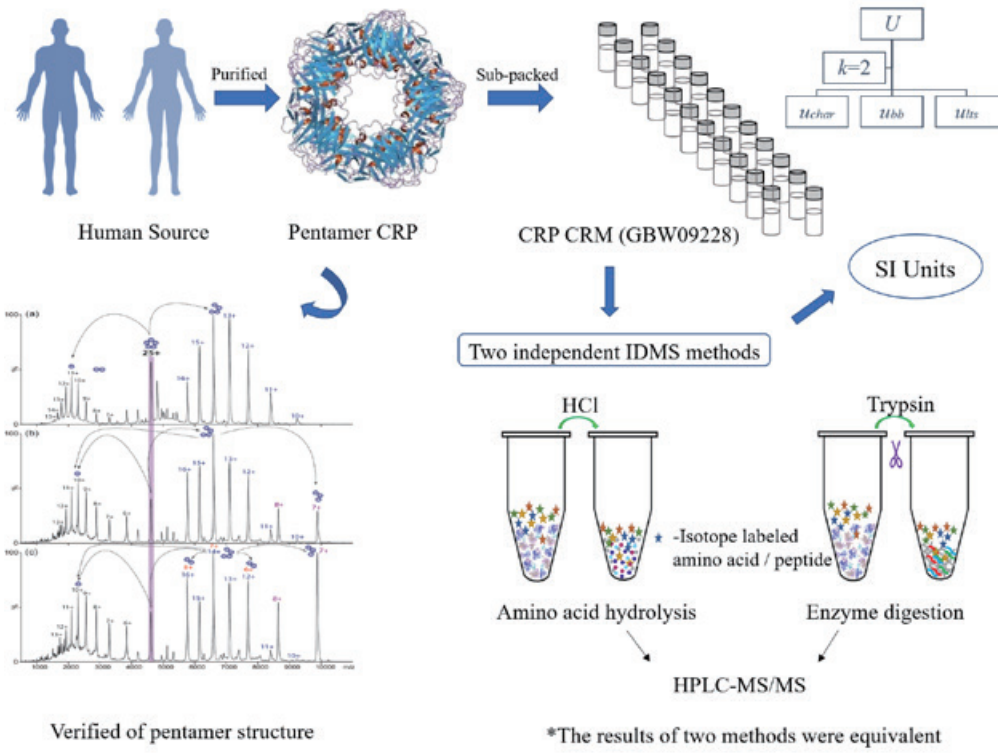


Fig.3 Development process of the C-reactive protein certified reference material GBW09228

A primary reference material of CRP (GBW09228) has been developed at NIM. It is the first pentamer structure CRP certified reference material (CRM) extracted from the human body. This reference material was certified using two primary isotopic dilution mass spectrometry methods, including amino acid hydrolysis isotope dilution mass spectrometry (IDMS) and signature peptide IDMS. The result is traceable to SI, with an uncertainty of 5.1%. Based on this primary reference material, NIM developed a serum CRP certified reference material (GBW09865-09868) with four levels of concentration, consisting of 1.5±0.1 mg/L, 12.3±0.9 mg/L, 51.6±4.1 mg/L, and 81.5±6.5 mg/L, in 2020. The certification method used to determine the CRP in serum is immunomagnetic bead enrichment combined with signature peptide isotope digestion isotope dilution mass spectrometry. The verification result showed that this secondary reference material had good commutability with 8 mainstream measurement systems from companies such as Roche, Abbott, and Siemens.

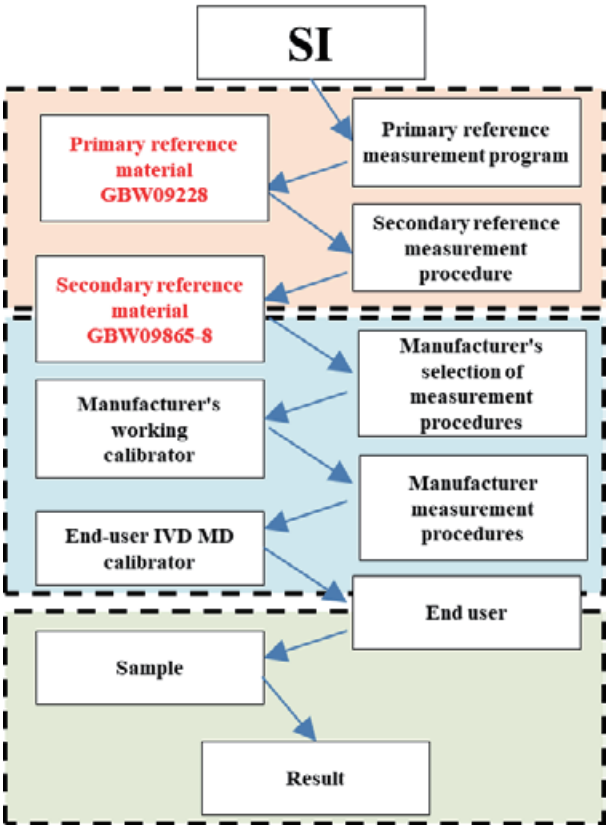


Fig.4 The clinical traceability chain of C-reactive protein

The primary reference material of CRP, the serum matrix secondary reference material of CRP and the accurate quantification method of CRP in serum developed by NIM can form a complete traceability chain from pure products to serum matrix reference materials, which provides a sound basis for accurate measurements for users such as CRP diagnostic reagent manufacturers and CRP clinical testing laboratories. At present, the series of CRP certified reference materials developed by NIM have been used to provide metrological traceability for external quality evaluation of C-reactive protein in 91 hospitals in China, supporting more than 200,000 measurements per year, and provide traceability services for more than 100 IVD companies. This accurate determination technique of CRP is also useful for development of reference materials and related reference methods for other protein diagnostic markers.

Reference

Song D., Dong X., Xu B., Wu Q. (2014). *Journal of Chinese Mass Spectrometry Society*, 35(5), 462–466.

Contact

SONG Dewei, songdw@nim.ac.cn

Absolute Measurement of Active Protein Concentrations Improving Accuracy of Diagnosis

As a kind of immuno-diagnostic biomarker of critical diseases, such as tumors, cardiovascular and cerebrovascular diseases, proteins are widely quantified and the accuracy of the results has become the basis for treating the diseases effectively. However, immuno-assay results often differ by several times or even dozens of times, and the results from different hospitals are not comparable, which hampers the mutual recognition of clinical trial results. One reason is the lack of an absolute measurement method of active protein concentrations, which makes it difficult to define whether the "measurand" in the traceability chain is the same or not. Therefore, a potential broken traceability chain leads to comparability issues and inaccuracy of immuno-diagnosis results.

Regarding the issue of absolute measurement of active protein concentrations, NIM and Beijing University of Chemical Technology cooperated to quantify the concentrations by measuring the diffusion rate under the condition of mass transfer limitation using surface plasmon resonance (SPR). Regarding the measurement of active protein concentrations in the sandwich immunoassay mode, a digital enzyme linked immunosorbent assay (ELISA) was established by labeling antibodies with oligonucleotide. By relying on the proximity ligation assay (PLA) and digital polymerase chain reaction (dPCR), the active concentration of the target protein in the sample was measured directly. By comparing with the physicochemical concentration, the consistency of "measurands" can be determined effectively.

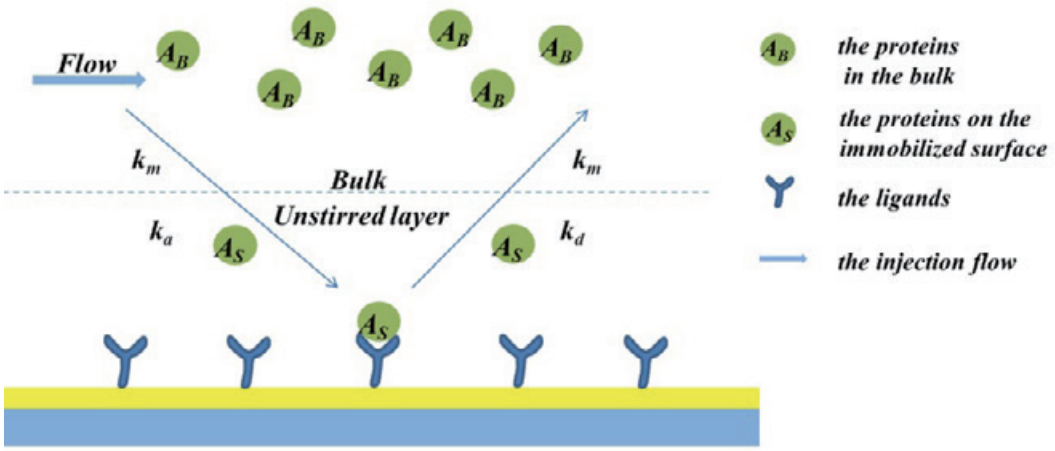


Fig.5 Schematic diagram of the measurement of protein immuno-activity concentrations by surface plasmon resonance

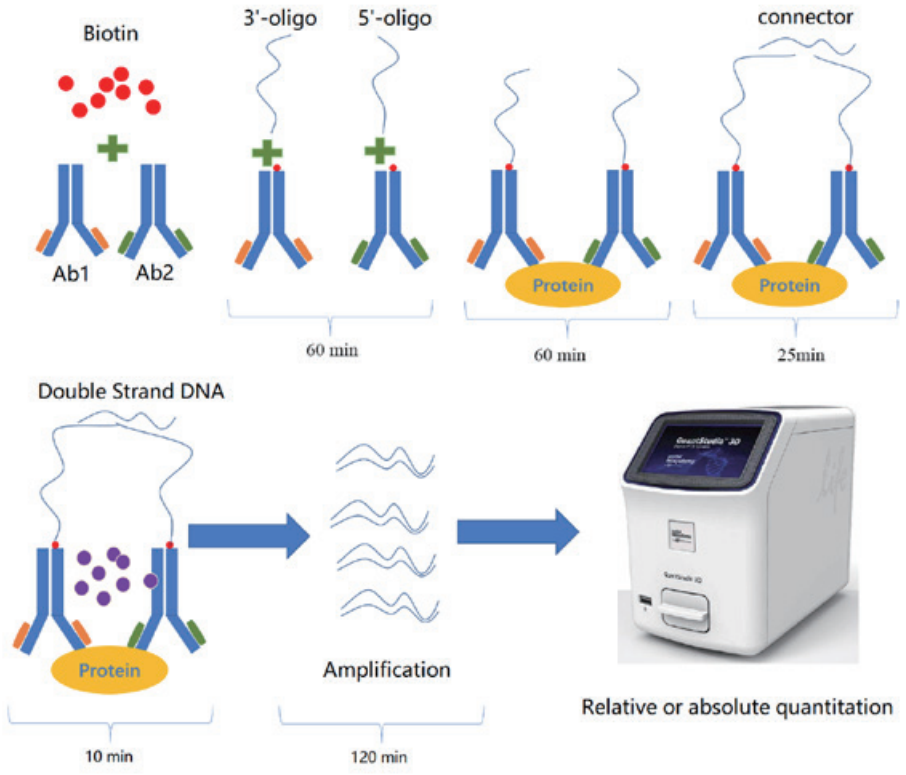


Fig.6 Schematic diagram of the measurement of protein immuno-activity concentrations by digital ELISA

The results show that the immuno-activity concentration of the protein is usually lower than the physicochemical concentration, indicating that the "measurands" are inconsistent. Therefore, an effective calibration of the measurement system cannot be realized. However, when using an antibody recognizing the external linear epitope of the protein, the immuno-activity concentration is consistent with the physicochemical concentration,indicating that the "measurands" are consistent. Therefore, this measurement system can be calibrated effectively. The accuracy and comparability of immuno-diagnosis results can be significantly improved.

The absolute quantification of immuno-activity concentrations has been used for the validation of commutability and an unbroken traceability chain. The related technique has been applied for a patent and used in the development of IVD reagents for cardiovascular and cerebrovascular disease biomarkers on trial.

Measuring platforms with the same "measurand" can be well calibrated with protein reference materials, and the coefficient of variation between different platforms is significantly reduced after calibrations. It is anticipated that both the quality of immuno-diagnosis products and the comparability of immuno-diagnosis results will be greatly improved after wide application of this technique. It can reduce economic loss and waste of resources caused by invalid calibrations and repeated testing and make the diagnosis of critical diseases more accurate.

Reference

1. Hu T., Wu L., Sun X., Su P., Yang Y. (2020). *Analytical and Bioanalytical Chemistry*, 412(12), 2777–2784.

2. Su P., He Z., Wu L., Li L., Yang Y. (2020). *Talanta*, 178, 78–84.

3. Hu T., Zheng K., Su P., Yang Y., Li L., Meng Z., Wu L. (2020). *Microchemical Journal*, 157, 104954.

Contact

WU Liqing, wulq@nim.ac.cn

An Accurate Characterization Technique for Structurally Related Impurities in Peptide Drugs

Peptides are important drugs and biomarkers in clinical diagnosis and treatments. It is classified as a separate drug category, positioned between small organic molecules and large biomolecules such as proteins. Peptide drugs are easier to be metabolized in human bodies, compared with small molecule drugs, and more stable than large protein drugs. At present, peptide drugs have been widely used in the treatment of tumors, diabetes, AIDS and other diseases. In the process of peptide synthesis, storage and transportation, its amino acid active side chain groups are prone to side reactions such as modification reactions, which will produce structurally related impurities that are similar to the main component peptide sequences. These impurities may have potential toxicity, which could induce adverse reactions and affect the quality and safety of peptide drugs. Therefore, it is necessary to make comprehensive and accurate characterization of these structurally related impurities in the corresponding peptide drugs. The current edition of the European Pharmacopoeia puts forward related qualitative and quantitative requirements on an account of the mass fraction levels of the structurally related impurities in peptide APIs. In particular, impurities with mass fraction above 0.1% need to be reported to clarify their existence.

However, the longer the peptide sequences are, the more structurally related peptide impurities are expected to be formed due to the more active side chain groups contained in peptides. So it is extremely difficult to achieve a comprehensive identification and accurate quantification of these structurally related peptide impurities, especially for the impurities with mass fraction at about 0.1% and the impurities that are hard to be separated from the main component peptide such as deamidated impurities.

In a recent study, the pharmaceutical and clinical chemistry group at the Division of Chemical Metrology and Analytical Science of NIM has developed, based on the HPLC-HRMS technique, an approach that is capable of improving the detection of impurities with mass fraction of 0.1% or lower. The detection of impurities with mass fraction of as low as 0.02% can be realized. Thus, impurities at various mass levels can be fully characterized through the main component cutting off technique and data-dependent information acquisition in HPLC-HRMS. In addition, different chromatographic separation modes and chromatographic elution gradient programs were adopted to realize the analysis of impurities of different types, such as deamidation impurities. In 2020, 23 impurities in the calcitonin salmon USP reference material, of which 21 are newly found impurities that are not recorded in the US Pharmacopoeia, were successfully characterized using this method.

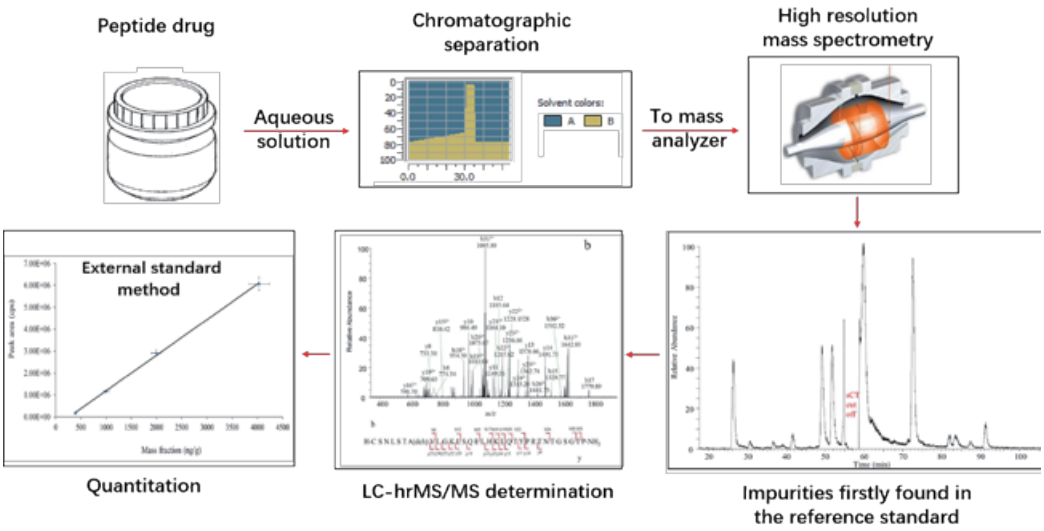


Fig.7 Process of the method in this study

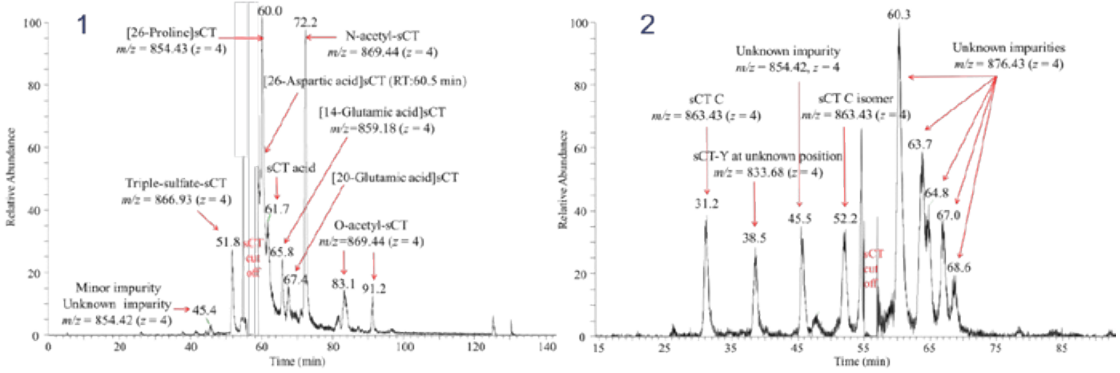


Fig.8 Structurally related impurities in calcitonin salmon USP reference material (the left picture is an overlayed EIC of impurities with mass fraction above 0.5%, and the right picture is an overlayed EIC of impurities with mass fraction less than 0.1%)

The above-mentioned method can be used to evaluate the efficacy and safety of peptide drugs and then establish a quality control system based on impurity profiles of peptide drugs. At present, this technique has been applied to the analysis of impurities in C peptide, calcitonin salmon and oxytocin. Moreover, this technique can be adopted to develop peptide reference materials, providing basis for the validity, consistency, and accuracy of measurement results of peptide purity assignment from different laboratories in a country or across the globe. NIM has developed an oxytocin purity reference material based on this technique.

Reference

1. Wu P., Li M., Kan Y., Wu X., Li H. (2020). *Journal of Pharmaceutical and Biomedical Analysis, 186*, 113271.

2. Li M., Josephs R. D., Daireaux A., Choteau T., Westwood S., Wielgosz R. I., Li H. (2018). *Analytical and Bioanalytical Chemistry, 410*(20), 5059–5070.

Contact

WU Peize, wupz@nim.ac.cn

LI Ming, liming@nim.ac.cn

Deciphering the Molecular Structures of Heparin and Low-molecular-weight Heparin

Heparin is one of the oldest biological drugs in the field of thrombosis and haemostasis. Following its discovery in 1916 and early clinical trials in the 1930s and 1940s, it became the mainstay of treatments for preventing blood clots and treating deep vein thrombosis, pulmonary embolism and myocardial infarction. In the 1980s, the development of low-molecular-weight heparin (LMWH) improved the effectiveness of this class of drugs, and for many indications, LMWH has replaced its parent compound. Ever since the heparin crisis in 2007 and 2008, which resulted in nearly 100 deaths in the United States, drug administrations worldwide have implemented increasingly stringent regulations and supervision. Particularly, they require extensive structural characterization of heparin for drug efficacy and safety. Despite numerous efforts to characterize the molecular structures of heparin and its derivatives, technical barriers still exist due to the complexity and heterogenity of drugs, especially in characterizing epimer configurations.

In a recent study, the pharmaceutical and clinical chemistry group at the Division of Chemical Metrology and Analytical Science of NIM has established an approach capable of evaluating both the epimerization and composition of heparin and dalteparin (a species of LMWHs) through a UHPLC-HILIC/WAX-MS approach. Nitrous acid treatment at pH 1.5 selectively cleaved the glycosidic bonds between N-sulfated glucosamine and hexuronic acid and resulted in building blocks mainly consisting of disaccharides, as well as tetra-, tri-, and mono-saccharides, all of which maintained the epimeric conformations of the hexuronic acid. Resulted oligosaccharides were separated and analyzed using the UHPLC-HILIC/WAX-MS method, with successful structural characterization of 19 tetrasaccharides, 19 trisaccharides, 10 disaccarides and 4 monosaccharides species. Isomer identifications were achieved through high-resolution tandem mass spectrometry analysis with reference to elaborately prepared standards. The method was successfully applied to the sameness study of generic dalteparins in combination with an isotopic labeling procedure. A graphical representation of the technique of this study is shown in Fig.9.

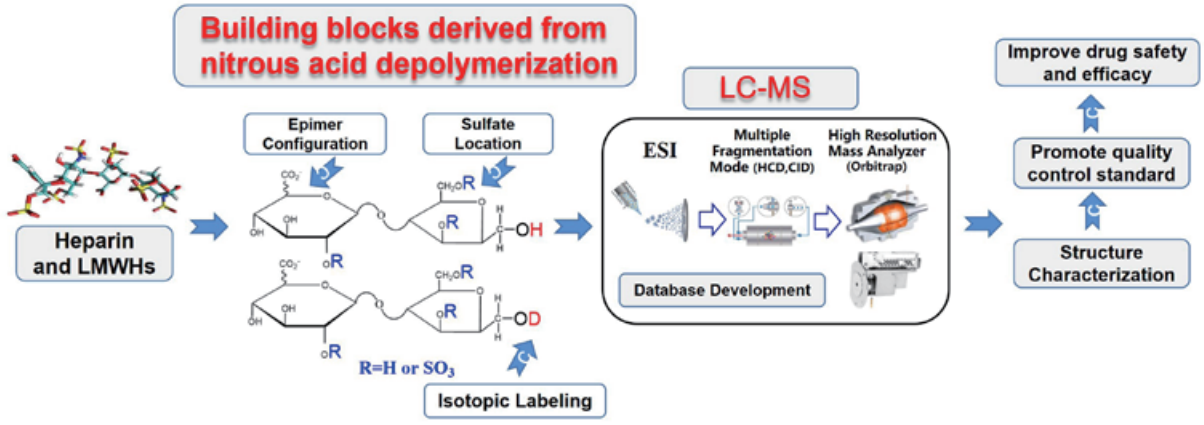


Fig.9 A graphical representation of the technique of this study

The above mentioned technique has been used successfully in the quality control process of pharmaceutical production of heparin and LMWHs by several Chinese manufacturers for drug registration. We would like to recommend this new technique to drug authorities in combination with corresponding reference materials, for improving efficacy and safety of heparin drugs.

Reference

1. Zhang T., Liu X., Li H., Wang Z., Chi L., Li J. P., Tan T. (2019). *Carbohydrate Polymers, 203*, 87–94.

2. Zhang T., Xie S., Wang Z., Zhang R., Sun Q., Liu X., Tan T. (2020). *Carbohydrate Polymers, 231*, 115695.

Contact

ZHANG Tianji, zhangtianji@nim.ac.cn

LI Hongmei, lihm@nim.ac.cn

Improvement of the NIM Fellow Mechanism

NIM Fellow is an internal expert management mechanism to motivate and recognize scientists and technicians with outstanding scientific and engineering achievements.

In a recent improvement to this mechanism, two types of titles are defined and granted to NIM Fellows, for experts majoring in scientific research and in technology development and engineering, respectively: Principal Researcher and Principal Metrologist.

NIM Fellows have the responsibility:

- to advise NIM on development strategies for major metrological fields following the developing trends of measurement science; to advise NIM on metrological engineering and application directions and initiatives that will support the country's industries and grand projects;
- to propose research directions and programs in both fundamental and applied metrology fields; to lead the study and discussion of major metrological issues relating to engineering and measurement application;
- to lead teams to push back the frontiers of research and explore new and promising research areas; to advise NIM on commercialization of scientific and research findings, knowledge transfer, and the operation and optimization of quality system;
- to advise NIM on human resource development, especially the cultivation of high-level scientists and technical staff.

The updated NIM Fellow policy was launched in November 2019. Together with it, six new Fellows were appointed, including two Principal Researchers and four Principal Metrologists.

New NIM Fellows

Principal Researcher: ZHANG Jintao

Main research interests: precision measurements of thermophysical quantities

Awardee of the First Prize of the State Scientific and Technological Progress Award in 2018

E-mail: zhangjint@nim.ac.cn

Principal Researcher: WANG Jun

Main research interests: metrology in chemistry, inorganic mass spectrometry and isotope analysis technologies

Awardee of the Second Prize of the State Natural Science Award in 1997
Awardee of the Second Prize of the State Scientific and Technological Progress Award in 2011

E-mail: wangjun@nim.ac.cn

Principal Metrologist: YUAN Zundong

Main research interests: radiation thermometry

Awardee of the Second Prize of the State Scientific and Technological Progress Award in 2002

E-mail: yuanzd@nim.ac.cn

Principal Metrologist: HE Qing

Main research interests: quantum electrical primary standards, Joule Balance for a quantum primary mass standard

Awardee of the First Prize of the State Scientific and Technological Progress Award in 2007

E-mail: heqing@nim.ac.cn

Principal Metrologist: GAO Sitian

Main research interests: nanometrology and dimensional metrology

Awardee of the First Prize of Science and Technology Excellence Award of the General Administration of Quality Supervision, Inspection and Quarantine(AQSIQ) in 2015

E-mail: gaost@nim.ac.cn

Principal Metrologist: FANG Zhanjun

Main research interests: optical frequency standards (optical clocks), optical frequency combs and their applications

Awardee of the Second Prize of the State Scientific and Technological Progress Award in 2002 and 2009

E-mail: zfang@nim.ac.cn

Special thanks to Ms. JIAO Hui, Ms. SONG Dan and Ms. KAN Ying for their kind assistance in editing this issue.
Special thanks to Mr. CHEN Haoyang for the cover design .

Edited and issued by the Department of International Cooperation, National Institute of Metrology (NIM), China.
No.18, Bei San Huan Dong Lu, Beijing 100029, China
For more information, please contact:
E-mail: lijinyuan@nim.ac.cn yanwen@nim.ac.cn
Phone: +86 10 6421 8565 Fax: +86 10 6421 8703
Website: http://en.nim.ac.cn