

The Application Book 2021

Summarizing recent application notes across
a range of exciting topics

Please click
the circles
to navigate

High Resolution
Multi-Reflecting
Time-of-Flight
Mass Analyzer

Removing User
Bias from Structure
Verification by NMR

A Novel Method
for Fast, Sensitive,
and Simple Analysis
of Total Fluorine in
Wastewater

Food Analysis
with Confocal Raman
Microscopy

The Revolutionary
and Novel Properties
of Meta-Materials

Detection
of Regulated
Nitrosamine Impurities
from the Drug
Manufacturing
Process

MAb Aggregation
Study Using SEC
With Multi-Angle
Light Scattering

Direct Trace-Element
Analysis in Cell Culture
Media and Raw
Materials with ICP-MS

Multi-Element
Analysis of Drinking
Water Using ICP-MS
Following ISO 17294-2
and (EU) 2020/2184



HIGH RESOLUTION
MULTI-REFLECTING TIME-
OF-FLIGHT MASS ANALYZER

REMOVING USER BIAS
FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

High Resolution Multi-Reflecting Time-of-Flight Mass Analyzer with Folded Flight Path®

Celebrating Ten Years of High Resolution

By Viatcheslav Artaev

LECO's Folded Flight Path® (FFP®) technology revolutionized high-resolution Time-of-Flight Mass Spectrometry. Using planar gridless electrostatic mirrors and an array of Einzel focusing electrostatic lenses, the FFP can create a flight path of 40m in a sensible footprint, enabling resolving powers beyond 50,000.

The first Time-of-Flight (TOF) mass spectrometer was proposed in 1946, and the design has been refined and iterated upon ever since. With an unlimited mass range visible in each spectrum and a high spectral generation speed, the advantages of TOF mass spectrometry were immediately clear. Though the instruments improved, they did eventually hit physical limitations that restricted the resolving power. More work had to be done.

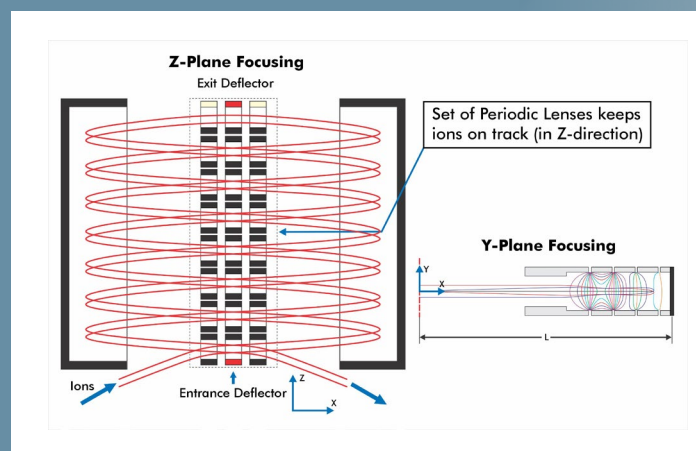
In 1973, TOF analyzers took a huge step forward with the introduction of the reflectron, an electrostatic ion mirror. Reflectrons provided time-energy focusing and improved the resolving power from several hundred to several thousand or more. To increase resolving power beyond 15,000, though, the height of the analyzer's flight tubes had to increase significantly.

The next major iteration in TOF resolution came with multi-reflecting reflectrons. Ion mirrors can bounce the ions once, twice,

or three times through grids defining electrical fields before they arrive at the detector, increasing the flight time significantly. However, these grids also cause significant ion losses, limiting the usefulness of too many reflections.

In 1989, a planar gridless electrostatic mirror was proposed. Without the grids, ion loss from the reflections was minimized. Unfortunately, this design could not mitigate the ion trajectories' divergence in the Z-direction, and there was still a significant loss of ions, resulting in a lack of practical improvement over the previous reflectron designs.

In 2011, LECO engineers took that planar gridless electrostatic mirror and worked in an array of Einzel focusing electrostatic lenses. This allowed for both Y-direction and Z-direction focusing, minimizing ion losses. With this Folded Flight Path (FFP®) design, resolving power for TOF mass spectrometry took a huge leap forward, easily reaching 25,000 within a sensible instrument footprint. An additional reflection within the FFP can send the ions through the array in another pass, doubling resolving power to 50,000.



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Separation Science
Mass Spectrometry

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OF-FLIGHT MASS ANALYZER

REMOVING USER BIAS
FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-MS

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

Removing User Bias from Structure Verification by NMR

Establishing a More Accurate Structure Verification with NMR Workbook

By Dimitris Argyropoulos, Sergey Golotvin, Rostislav Pol, Vladimir Mikhailenko & Yalda Liaghati Mobarhan

ACD/NMR Workbook Suite offers three levels of automated structure verification. The most rigorous of these is Unbiased Structure Verification (UBV), which is shown here to have identified the correct structure based on the NMR data of two commercially available substances, despite the incorrect structure having been proposed by the user.

Once you have determined a plausible chemical structure based on NMR data, how certain can you be that it is the correct one? Verifying a proposed structure based on NMR data is one of the most fundamental, and yet challenging practices in synthetic chemistry.

Automated structure verification using NMR data has quickly become an invaluable tool for timely and unambiguous characterization of chemical structures. Here we present the ACD/NMR Workbook suite, the only commercially available software that performs structural verification on three levels of increasing rigor to give chemists the highest levels of confidence in their proposed chemical structures.

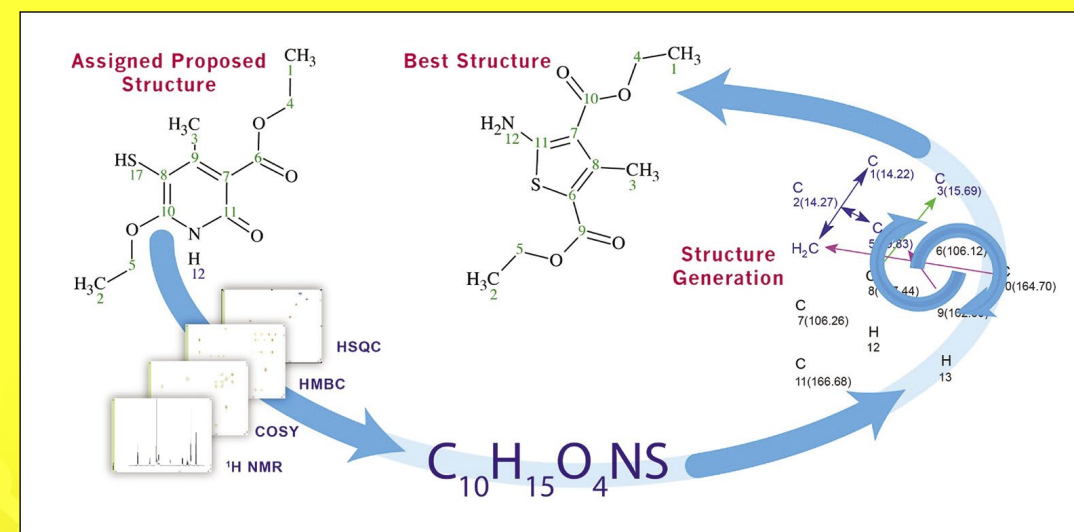
The simplest form of automated structure verification is Single Structure Verification (SSV). While this method can confidently identify when a proposed structure fails to match the provided NMR data, without alternative structures to consider, there is a significant chance of false positives.

The next level of verification, Combined and Concurrent Verification (CCV), reduces the false positive rate by considering the proposed structure as a defined number of generated isomers. However, since this proposed structure is provided by the user, the user's initial bias may still affect the result.

The Unbiased Structure Verification (UBV) workflow provides the highest level of confidence, provided minimum requirements are met. UBV is based on the most advanced structure generators and best ranking system available on the market. With this method, all possible structures compatible with the provided NMR data and molecular formula (derived from the proposed structure) are generated and ranked to determine the best possible structure independent from user bias.

We put the UBV method to the test in two examples presented here by submitting NMR datasets of commercially available compounds with intentionally incorrect proposed structures. In both cases, SSV and CCV returned false positive results. UBV on the other hand identified the correct structure in both cases.

UBV is especially beneficial to synthetic chemists – providing an ultimate level of confidence in structure verification free from false positive errors. Furthermore, the improved productivity, high reliability, and reduced chemist supervision involved in this structure verification workflow enables a greater return on investment for analysts and NMR labs.



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Unbiased Structure Verification

Single Structure Verification

Combined & Concurrent Verification

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NMR Software
for Data Analysis and
Structure Verification

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OF-FLIGHT MASS ANALYZER

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FROM STRUCTURE
VERIFICATION BY NMR

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FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

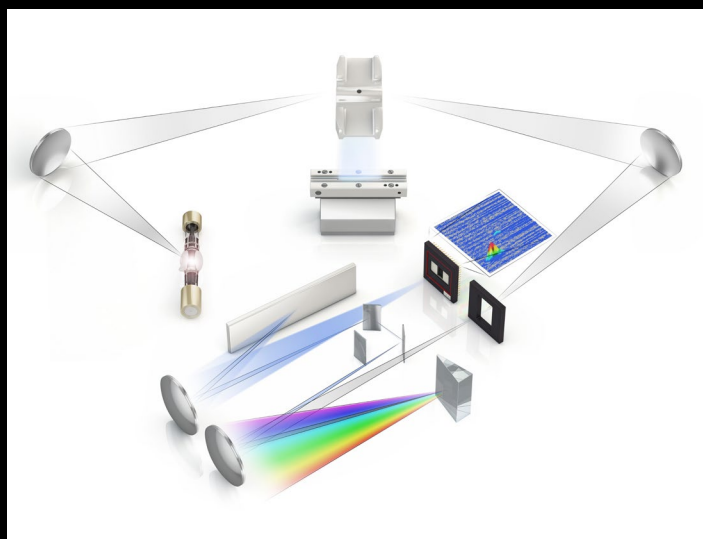
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OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

A Novel Method for Fast, Sensitive, and Simple Analysis of Total Fluorine in Wastewater

Presenting a TOF detection method for environment pollution monitoring and control based on a combination of solid-phase extraction (SPE) and high-resolution-continuum source graphite furnace molecular absorption spectrometry (HR-CS GF MAS)

By Siqi Sun, PhD

We describe a simple, fast, and sensitive method to detect total fluorine in wastewater. In order to determine a sum parameter of fluorinated compounds, a species-unspecific response of high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS GF MAS) was developed.



The introduction of carbon-fluorine bonds into organic compounds is known to profoundly influence their chemical and physical properties when compared to their non-fluorinated analogues. Many of the most important products used in the chemical and life-science industries – from pharmaceuticals, to fine and specialty chemicals, to polymers – rely on organic fluorine chemistry for their useful properties. The accelerating production volumes of fluorinated organic substances is expected to lead to increased release into the environment. Some of these substances are already listed as persistent organic pollutants by the Stockholm Convention (Annex B -restriction) and the European water framework directive (WFD).

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals that present numerous analytical challenges, including their widespread presence in a variety of environmental samples. In the US, PFAS may contaminate public drinking-water systems that serve an estimated 19 million people. The US Environmental Protection Agency (EPA) is continuing to aggressively implement their PFAS Action Plan – the most comprehensive cross-agency plan ever to address an emerging chemical of concern. The EPA has established a non-enforceable health advisory level of 70 parts per trillion (ppt) for the sum of pentadecafluorooctanoic acid (PFOA) and heptadecafluorooctanesulfonic acid (PFOS) for drinking water. The EPA is developing a rapid screening tool to identify total PFAS presence and absence in 2021. This eventual standard operating procedure will be used to quantify Total Organic Fluorine (TOF).

In this study, a fast, easy, and sensitive complementary method for total fluorine analysis in wastewater is presented. This study shows that the MAS method can be successfully applied using the contrAA 800 G for measurements. The optimized furnace program and calibration strategy provide high sample throughput, sensitivity, and accuracy. Only 3.5 minutes/replicate is needed for each sample. And 100 percent recovery rate of QC sample is successfully achieved with an inorganic and organic mixture calibration strategy. The lowest LOD of 4 ppb is also achieved with the inorganic and organic mixture calibration strategy. The results of fluorine determination by HR-CS-GF MAS show good reproducibility and long-term stability. Sample dilution and spiking can be easily achieved with an AS-GF autosampler. No additional cleaning steps are required for this method.

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FROM STRUCTURE
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A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

Food Analysis with Confocal Raman Microscopy

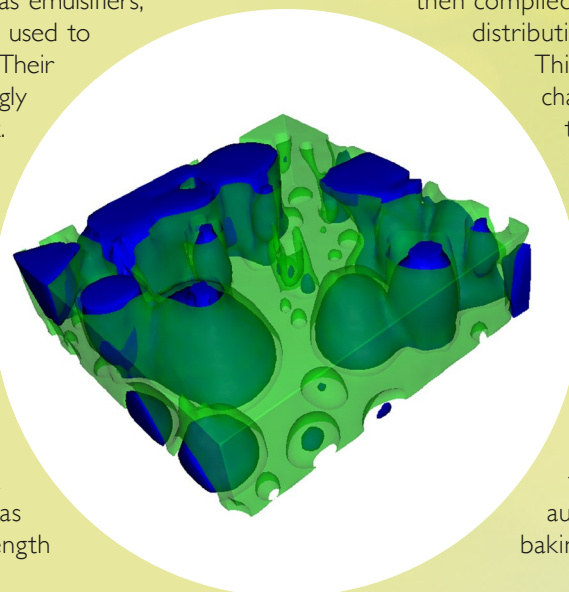
High-resolution chemical characterization reveals how the distribution and structure of ingredients and additives affect food properties

By WITec GmbH

This application note shows how confocal Raman imaging, topographic Raman imaging and Raman-based particle analysis can characterize components in food products. Samples measured include: fat spreads, conventional and spreadable butter, white chocolate, honey, sugar, banana pulp, particulate baking ingredients and more.

Various ingredients and additives such as emulsifiers, stabilizers or thickeners are commonly used to optimize the texture or flavor of food. Their distribution and microstructure strongly influence the properties of the final product. Therefore, food industry research and development, as well as quality control, require powerful analytical methods for studying the distribution of compounds in high resolution.

Confocal Raman microscopy is a versatile tool for analyzing the chemical composition of samples on the sub-micrometer scale and is well suited to analyses in food science. The physical phenomenon that underlies Raman microscopy, known as the Raman effect, is the slight wavelength



shift in light that has been inelastically scattered by molecules of gaseous, liquid or solid materials. The incident photons from a monochromatic excitation source cause vibrations of the molecule's chemical bonds, leading to a specific change in energy that is visible in a Raman spectrum. Every chemical compound produces a unique Raman spectrum when excited and can be quickly identified by it. Raman imaging microscopy acquires a Raman spectrum at each image pixel over a sample area. This information is then compiled in an image that visualizes the distribution of its chemical components.

This survey shows how Raman imaging can characterize food samples to help understand the products and production processes with confocal measurements, scans guided by an integrated profilometer, and investigations that employ a Raman spectral database. It describes experiments on white chocolate, fat spreads, a sugar bar, a squashed banana pulp sample and a honey pollen grain. It also features 3D Raman imaging of conventional and spreadable butter, topographic Raman imaging of frosted gingerbread, and Raman-based automated particle analysis of a mixture of baking ingredients.

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OF-FLIGHT MASS ANALYZER

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FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

The Revolutionary and Novel Properties of Meta-Materials

One key feature of meta-materials is that they can be designed with different optical responses at certain frequency ranges. Different sub-wavelength structures produce various optical effects appearing at different ranges: from several THz (far-infrared) to several eV (visible and UV). Therefore, a flexible spectrometer covering wide spectral range is very necessary for meta-material research.

Meta-materials have been defined as artificial materials engineered to have properties that may not be found in nature. They are assemblies of multiple individual elements fashioned from conventional microscopic materials such as metals or plastics, but usually arranged in pre-designed periodic patterns.

During the last two decades, many scientists in different fields have worked hard to tailor the novel properties of meta-materials – optical ones in particular. And meta-materials have been designed with different optical responses at certain frequency ranges, for example. Different sub-wavelength structures produce various optical effects appearing at different ranges: from several THz (far-infrared) to several eV (visible and UV). Therefore, a flexible spectrometer covering wide spectral range is very necessary for meta-material research.



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OF-FLIGHT MASS ANALYZER

REMOVING USER BIAS
FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

Detection of Regulated Nitrosamine Impurities from the Drug Manufacturing Process

Nitrosamines are classified as probable human carcinogens and are believed to be introduced into finished medicines as byproducts during manufacturing

By Chander Mani, Kartheek Srinivas Chidella, Saikat Banerjee, and Samir Vyas

In this application note, we have developed a highly sensitive, triple quadrupole-based, liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of eight nitrosamine impurities in metformin drug substance and drug products.

Nitrosamine impurities have recently become a focus for major regulatory bodies such as the U.S. Food & Drug Administration

and European Medicines Agency. For example, a major recall was announced for angiotensin II receptor blocker drug products due to the potential for these products to contain N-nitroso-dimethylamine (NDMA). Nitrosamine impurities pose a significant risk even in trace amounts, as they may interact with DNA, potentially causing harm to the patient. Because of the of potential mutagenic and genotoxic nature of these types of contaminants, there is now widespread regulatory scrutiny and governance.

Nitrosamine impurities are often an unintentional byproduct of the drug manufacturing process and are present in trace-level quantities. This makes analysis extremely difficult because the drug product and other excipients are present in quantities that are magnitudes greater than the impurities themselves. For this reason, the sensitivity and selectivity provided by LC/MS/MS-based methods have served as the analysis platform of choice.

To demonstrate the utility of LC/MS/MS analysis for the safeguarding of pharmaceutical drug products, the methods described in this application note provide a comprehensive analysis of eight nitrosamine impurities at very low detection limits with two different sample preparation and column chemistries. This workflow demonstrates that the Agilent 6470 triple quadrupole LC/MS system is fit to meet the detectability requirements demanded by today's regulatory bodies.

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Triple Quadrupole LC/MS
6470B Triple quadrupole LC/MS

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FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
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SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-MS

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

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MAB Aggregation Study Using SEC With Multi-Angle Light Scattering

**Characterization of antibody aggregates and fragments
on one high-resolution column with advanced detection**

By Miriam Lossa and Jasmin Preis

SEC, equipped with UV, RI, and static light scattering detection, is a powerful analytical tool to determine the content and size of mAb aggregates and fragments. Modern high-resolution SEC columns with an optimized separation range unlock the method's full potential.

Size exclusion chromatography (SEC) with multiple detection techniques – including UV, RI, and light scattering (LS) – is a powerful analytical tool for characterization of (monoclonal) antibody aggregates and fragments.

The following information can be obtained from data using a multi detection setup:

- absolute molar mass and radius of gyration (R_g), of monomer, dimer, trimer (requires light scattering detector; e.g. PSS SECcurity² SLD2020 light scattering detector)

- molar mass of fragments by relative method (calibration, requires concentration detectors, e.g. PSS SECcurity² I 260 MWD/RI)
- purity: quantity of high aggregates, associates, monomer, and fragments (requires concentration detectors, e.g. PSS SECcurity² I 260 MWD/RI).

The applied PSS MAB SEC column is well-designed for protein and monoclonal antibody (mAb) applications. Covering the complete separation range required for mAb monomers, associate, aggregate, and fragment monitoring, the PSS MAB column also provides a long-term performance with high resolution for the determination of the CQAs of mAbs. Moreover, the PSS MAB column is pre-equilibrated for hyphenation with light scattering.

Figure 1 shows an overlay of chromatograms obtained for a native antibody with associates and for a fragmented antibody sample by reduction. The half-body mAb is clearly visible in the fragmented sample and more fragments are detected.

Figure 2 shows all three detector signals for the analysis of monoclonal antibodies. The light scattering signal shows enhanced sensitivity for aggregates with high molar masses. The latter does not mean that light scattering is used for quantitation, but that monitoring of CQAs and the investigation of mAb aggregation processes is simplified. However, the light scattering signals are used to determine absolute molar masses and sizes.

The PSS SECcurity² GPC/SEC system, the PSS SLD2020, and the PSS MAB column are also available in bioinert fabrication to avoid contact with stainless steel during analysis.

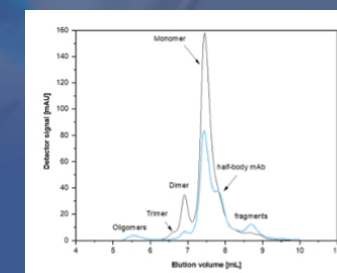


Figure 1: The black curve shows the UV signal of a native antibody and its associates plotted against the elution volume. The blue curve is the chromatogram of fragmented antibody sample by reduction.

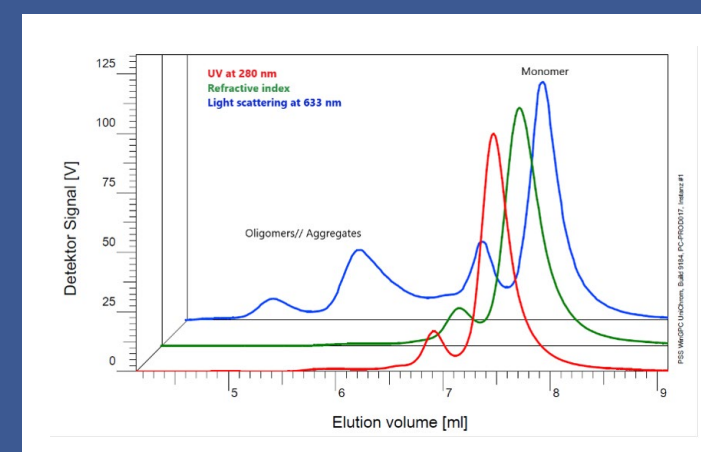


Figure 2: Sensitive analysis of antibody aggregates. The light scattering signal for the antibody associates and higher aggregates is relatively high compared to monomer signal due to molar mass dependency and provides enhanced sensitivity for the detection of high aggregates at very low concentrations.

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VERIFICATION BY NMR

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SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

Direct Trace-Element Analysis in Cell Culture Media and Raw Materials with ICP-MS

Interference-free trace elemental analysis and low detection limits for cell culture media samples with PerkinElmer NexION 5000 Multi-Quadrupole ICP-MS

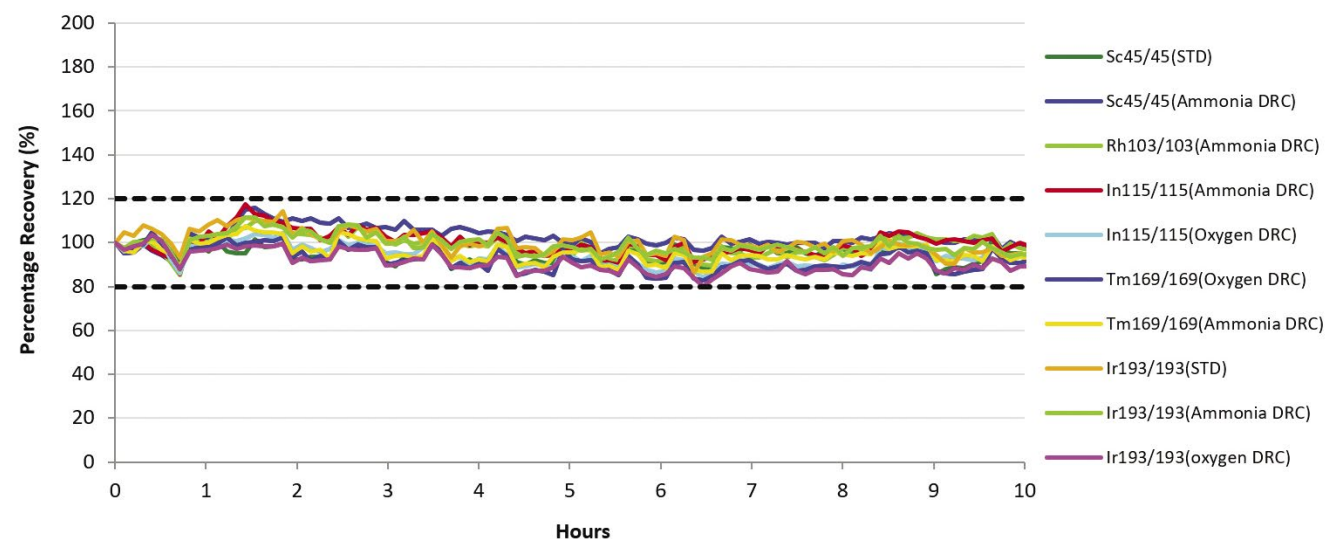
By Liyan Xing, Chady Stephen, Aaron Hineman, and Sherri Naughton

In this application note, a wide spectrum of elements in cell culture media samples were analyzed with ICP-MS. Culture media is a complex mixture which can generate interferences that can compromise analytical accuracy. The PerkinElmer NexION® 5000 ICP-MS, equipped with Extended Dynamic Range (EDR), allowed for the interference-free analysis of both low and high concentrations of elements within a single analytical run which, coupled with HTS, dramatically contributed to the overall accuracy, stability, and improved productivity of the system.

Raw material preparation and formulation is a known source of elemental content and variation in cell culture media. Other sources of trace metal concentrations and variability include the leaching of trace metals from bioreactors, preparation vessels and storage containers. Quality control in cell culture media is essential for reducing variability and ensuring improved production yields.

In this application note, a wide spectrum of elements in cell culture media samples were analyzed with ICP-MS – a powerful tool for elemental analysis with multi-element analysis capability, high sensitivity, low detection limits, wide linear dynamic range, and easy automation. However, as with all other analytical techniques, ICP-MS analysis is subject to interferences. Culture media is a complex mixture containing a substantial amount of inorganic salts and organic compounds, which can generate polyatomic and other interferences that increase the spectral background and can compromise analytical accuracy.

The PerkinElmer NexION 5000 ICP-MS, equipped with Extended Dynamic Range (EDR), allowed for the analysis of both low and high concentrations of elements within a single analytical run which, coupled with HTS, dramatically contributed to the overall accuracy, stability, and improved productivity of the system.



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FROM STRUCTURE
VERIFICATION BY NMR

A NOVEL METHOD FOR
FAST, SENSITIVE, AND
SIMPLE ANALYSIS OF TOTAL
FLUORINE IN WASTEWATER

FOOD ANALYSIS
WITH CONFOCAL
RAMAN MICROSCOPY

THE REVOLUTIONARY
AND NOVEL PROPERTIES
OF META-MATERIALS

DETECTION OF REGULATED
NITROSAMINE IMPURITIES
FROM THE DRUG
MANUFACTURING PROCESS

MAB AGGREGATION STUDY
USING SEC WITH MULTI-
ANGLE LIGHT SCATTERING

DIRECT TRACE-ELEMENT
ANALYSIS IN CELL CULTURE
MEDIA AND RAW MATERIALS
WITH ICP-M

MULTI-ELEMENT ANALYSIS
OF DRINKING WATER USING
ICP-MS FOLLOWING ISO
17294-2 AND (EU) 2020/2184

Multi-Element Analysis of Drinking Water Using ICP-MS Following ISO 17294-2 and (EU) 2020/2184

Meeting and/or exceeding the specifications of ISO
and EU directives for drinking water analysis with the
PerkinElmer NexION 1000 ICP-MS

By Liyan Xing Sr. Application Specialist

Elemental content is critical to the quality of drinking water. The international standard method ISO 17294 outlines the analysis of elements in water samples (for example, drinking water, surface water, groundwater, wastewater, and eluates) using ICP-MS. This application note presents the performance of the PerkinElmer NexION® 1000 ICP-MS for the compliance with ISO 17294-2 and (EU) 2020/2184.

ISO 17294-1:2004 provides the general guidelines for using the ICP-MS technique, and ISO 17294-2:2016 describes the determination of selected elements, including uranium isotopes in drinking water and relatively unpolluted waters. This directive aims to protect human health from the adverse effects of any contamination of water intended for human consumption.

Compared to other analytical techniques, ICP-MS has the advantages of multi-element detection capability, low detection limits, high-speed of analysis, wide dynamic range, and so on. However, it is affected by plasma, as well as matrix-based polyatomic interferences and doubly charged species, which need to be accounted for by applying mathematical corrections and/or collision/reaction mechanisms.

The NexION 1000 ICP-MS has demonstrated the ability to meet and/or exceed the specifications contained within the ISO and EU directives for the analysis of drinking water. In addition, accuracy and robustness have been validated through the analysis of certified reference materials, the matrix spike recovery test, and the stability of ISTD and CCV.

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