

COMPLIANCE BY DESIGN

A DEEP LOOK AT THE PFAS LANDSCAPE

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NANALYSIS' VISION FOR PORTABLE, AUTOMATED SPECTROSCOPY

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NON-DESTRUCTIVE INSIGHT INTO FORMULATION QC AND EFFICACY

ZEISS Research Microscopy Solutions



FLEX EP

AUTOMATED PRECISION FOR PFAS SAMPLE PREPARATION

THE NEXT FRONTIER OF RAPID ANIMAL DIAGNOSTICS

Dr. Ravishankaran R
GeNext Genomics Pvt. Ltd.

TURNING CHEMISTRY INTO THRUST

Ms. Sivakami S & Mr. Henry Sam
Manastu Space





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of Pioneering the Scientific Frontier

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Dear Readers,

Compliance usually comes at the end. The test is done, the form is filled, the report is archived. That mindset is what many labs have followed for years. But it is changing. What we are seeing now is a shift from reactive checks to proactive design. Systems that are built to do the right thing by default, not just to catch mistakes after they happen.

This issue starts with that idea. The cover story looks at how quality is no longer inspection-driven. As labs digitise their infrastructure, the focus is moving toward real-time control. Automation is not just speeding things up. It is enforcing standards with logic and repeatability. And that is where reliability actually begins.

We also go deep into the PFAS challenge. From shifting regulations to the analytical methods that need to catch up fast, this article gives clarity. The focus is not just on awareness. It is on the tools that are helping labs get reliable results and stay compliant, especially when the rules keep changing.

The interviews in this issue bring different angles. Ms. Susanne Riegel from Nanalysis talks about pushing NMR into smaller labs without losing scientific depth. Dr. Ravishankaran from GeNext Genomics explains how speed and accuracy are reshaping animal diagnostics. And the R&D team from Manastu Space shares what it really takes to develop green propellants from scratch—not in theory, but inside a working lab.

In the application section, you will find practical examples that make a point quickly. Quality checks using Bruker FTIR, cost control in nitrogen analysis with BUCHI, moisture analysis that follows lean principles with Mettler Toledo, water system design for better HPLC results from Sartorius, and how ZEISS uses X-ray imaging and AI to explore formulations without destroying the sample.

There is also a closer look at Flex EP, a workstation that fits straight into your lab data system. Built to support compliance and business continuity without slowing down the workflow.

The tech corner this time is short and sharp. It covers protein and antibody quantification using tools from Implen, and focuses on what not to miss when you need both speed and accuracy.

Our product highlight features benchtop NMR by Nanalysis, not as a stripped-down version of traditional NMR, but as a reliable everyday solution.

The final section focuses on chromatography. There is a range of methods here—oligonucleotide analysis using Advion, high purity separation of remdesivir with RotaChrom, protein and biopolymer work with Shodex SEC columns, and stress-induced impurity profiling using the BioAccord system from Waters.

If there is one theme that runs through this issue, it is this – Real compliance does not come from supervision. It comes from design. From choosing the right workflows, the right instruments, and the right mindset from the beginning.

Best regards,



Arun Mathrubootham

Director

Inkarp Instruments Pvt. Ltd.

Cover Story

01

01 - When quality is engineered, not designed

Features

03

03 - Influence of a Built-In Crossflow Ultrafiltration Module on the TOC Level in Ultrapure Water

06 - PFAS Landscape: Global Regulations, India's Framework, Analytical challenges and Waters' Solutions

Mr. Jagadeesh Banda

Product Manager, Chemistry & Lab Automation, Waters India Pvt. Ltd.

Interviews

12

12 - From High-Field Exclusivity to Everyday Insight, Nanalysis' Vision for Portable, Automated Spectroscopy

Ms. Susanne Riegel

VP Marketing and NMR Product Manager, Nanalysis

16 - Turning Chemistry into Thrust

Ms. Sivakami S & Mr. Henry Sam

R&D team, Manastu Space

20 - The Next Frontier of Rapid Animal Diagnostics: Inside GeNext Genomics' ASFV Revolution

Dr. Ravishankaran R

Team Lead- Diagnostics Division, GeNext Genomics Pvt. Ltd.

Application Showcase

25

25 - Quality Control with Bruker's ALPHA II FTIR

29 - Reducing cost per analysis for Kjeldahl Nitrogen Determination Using BUCHI's Reaction Detection Sensor® Technology

42 - Boosting Food Production Efficiency with Mettler Toledo's Lean Principles in Moisture Determination

47 - The Role of Ultrapure Water for HPLC Analysis enabled by the Sartorius Arium® Water Purification System

52 - Non-destructive insight into formulation quality and efficacy enabled by ZEISS X-ray microscopy and AI

AI and Automation

NEW

60

- 60 - Chemspeed Technologies' Flex EP – Compliance ready workstation with assured business continuity, seamlessly integrated into your lab information management system.

Product Highlight

62

- 62 - Portable, Automated NMR Spectroscopy - Nanalysis

Tech Corner

64

- 64 - Best Practices for Protein/Antibody Quantifications

Advances in Precision Chromatography

NEW

67

- 67 - Qualitative Analysis of Oligonucleotides Using the Advion Interchim Scientific HPLC-UV/MS System

- 71 - Separation of High-Purity Remdesivir using RotaChrom's Continuous Centrifugal Partition Chromatography Platform

- 74 - Shodex KW400 Series: High-Efficiency Semi-Micro SEC Columns for Advanced Protein and Biopolymer Analysis

- 79 - Streamlined LC-MS Analysis of Stress Induced Impurities of a Synthetic Peptide using the BioAccord™ System

Industry Buzz

86

- 86 - Pharma updates: Recent novel FDA approved drugs

- 87 - A sneak-peak at our events



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WHEN QUALITY IS ENGINEERED, NOT DESIGNED

For decades, “quality” meant inspection. Lines of analysts, painstakingly verifying data, checking tolerances and approving results. This was a human safety net built on diligence and repetition. It worked, but the more complex the processes became, the harder it was to keep up.

Slowly the cracks started showing audit findings, data integrity failures and rising compliance costs that checked innovation. At this moment, quality becomes everyone’s problem. Did the sample degrade before analysis? Was the correct reagent lot used? Was the mobile phase freshly prepared? Was the temperature the same as last time? For decades, these questions were asked after the work was done. Quality was something you checked, verified or even defended, and often under pressure.

The turning point came with digital transformation. Suddenly, data was everywhere; not confined to paper records or individual workstations, but flowing through integrated LIMS, ELNs and cloud-based analysis hubs.

However, data alone isn’t the resolution. The real breakthrough came when labs began to trust automation—when inspection turned proactive and instruments became extensions of the quality system itself.

The term “Quality by Design” isn’t new. Regulatory bodies like the FDA and EMA have promoted them for years. What’s changed is how it’s now being realized.

Modern labs are embedding quality directly into their infrastructure, through connected instruments, secure data ecosystems, and AI-driven process control.



Compliance conversations are asking a different question: “What if quality wasn’t something we verified later, but something that the system enforced by default?”

Think of it as the lab going from reactive compliance to engineered assurance.

Instead of relying on corrective actions, modern labs are

- ▶ Standardizing workflows
- ▶ Automating repetitive steps
- ▶ Digitizing records at the source
- ▶ Embedding compliance logic into instruments and software

One of the most powerful aspects of engineered quality is that it often looks so ordinary. It doesn't look rigid. It doesn't look bureaucratic.

Automated data integrity: Systems now cross-check measurements, timestamps and calibration histories faster than ever.

Predictive maintenance: Equipment "learns" when it's about to drift out of specification, preventing downtime or invalid runs.

Procedure standardization: Intelligent workflows guide every technician identically, ensuring reproducibility becomes effortless.

INTEGRATION OF CULTURE AND CODE

Technology alone doesn't build a compliant lab. The biggest transformation is cultural. How teams think about quality itself matters greatly. In older setups, quality itself was a final hurdle, a box to tick before release. Today's labs design processes collaboratively, where R&D, production and QA teams all speak the same data-driven language.

WHAT DOES THE DESIGN LANGUAGE OF COMPLIANCE LOOK LIKE?

This is what confidence in a QbD lab looks like:

Transparency by design: Every change, result and decision is instantly traceable. No hidden folders or manual reconciliations.

Contextual intelligence: Instruments talk. Data carries meaning. Quality isn't just recorded; it is interpreted in real time.

Continuous readiness: Regulatory audits no longer cause panic. The lab is always "inspection-ready".

**Compliance isn't an event
-it's a state of being**

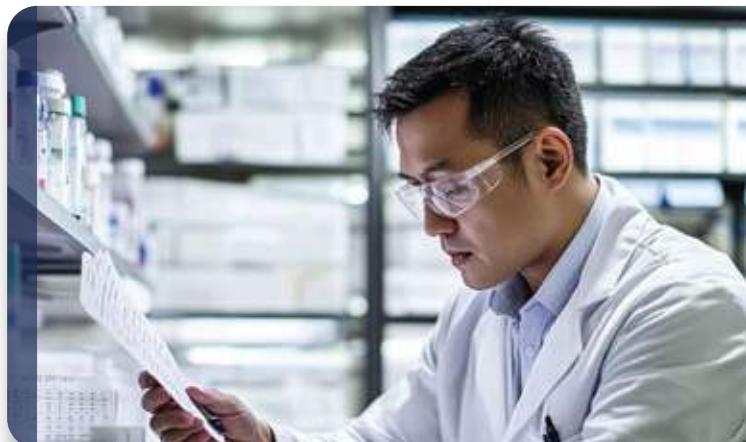
THE FUTURE

Tomorrow's labs will be less about departments and more about digital ecosystems.

Integrated sensors, AI validation engines, blockchain-secure records, and self-calibrating workflows will blur the line between R&D, QC and QA.

We might soon drop the word "compliance" altogether. Instead, we will speak of "Operational Integrity", where systems are so harmonized that trust and transparency are built in from the molecular level up.

One truth stands tall though; inspection may have built the last century of quality, but only engineering will build the next.



INFLUENCE OF A BUILT-IN CROSSFLOW ULTRAFILTRATION MODULE ON THE TOC LEVEL IN ULTRAPURE WATER

Dr. Herbert Bendlin

INTRODUCTION

The basis for successful and reliable analyses using analytical technologies such as HPLC, GC-MS, and ICP-MS among others is ultrapure water. As these methods become more sensitive, they also become more susceptible to interference due to trace levels of contaminants in the buffers and solutions used. As a result, the quality requirements for the various system components and consumables also increase.

The following water purification technologies have proven successful over the past few years in providing the high-quality ultrapure water required for these applications.

For applications requiring particularly low levels of total organic carbon (TOC) in the product water, initial water purification is normally performed using activated carbon and particulate filters, followed by reverse osmosis technology (with an EDI stack as an option). These processes typically remove 95–98% of the contaminating substances found in water (e.g., salts, particles, and organic compounds). The final purification steps are performed by an Ultrapure Water System producing product water of a quality to meet and exceed the ASTM Type I water standard.

This is enabled by utilizing the following technologies:

- ▶ Pre-treatment purification. Cartridge 1, containing a specifically tailored mixture of activated carbon and deionization resins for different feedwater conditions, removing most of the remaining ions in the water as well as some of the remaining TOC molecules.
- ▶ UV irradiation with 185/254 nm wavelengths, reducing TOC levels through oxidation.
- ▶ Polishing Cartridge 2, specifically tailored for low TOC applications, contains activated carbon to absorb TOC breakdown products and deionisation resins to polish the water to a resistivity of 18.2 MΩ.
- ▶ Built-in Ultrafiltration (UF) in crossflow system.
- ▶ A 0.2 µm sterilizing grade final filter retains particles and bacteria, providing particle-free and sterile water at the point of use.

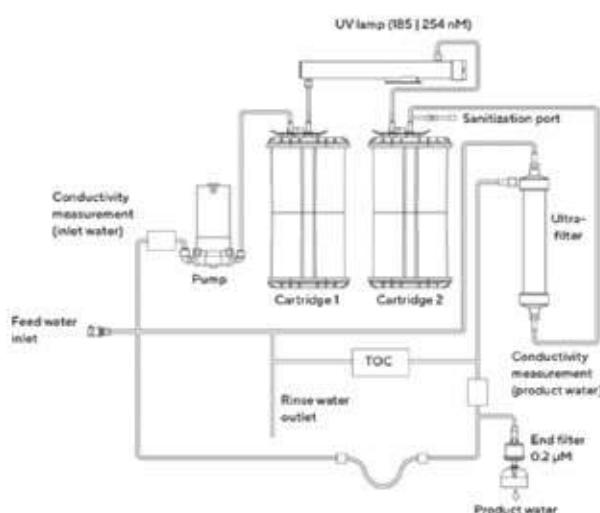


Figure 1: Arium flowchart

THE IMPORTANCE OF MOLECULAR WEIGHT CUT OFF (MWCO)

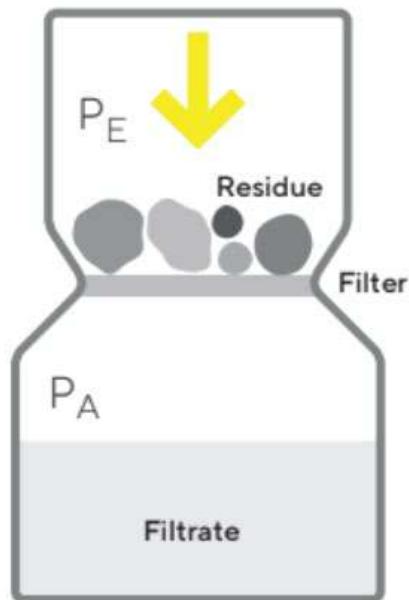
Organic residues (Total Organic Carbon (TOC)) can have a significant impact on chromatographic applications such as HPLC, ICP-MS, etc. Because of this, they need to be reduced to the lowest possible levels in the ultrapure water used in these analyses using the methods described above. As well as the standard methods used to remove TOC (e.g. activated carbon or UV oxidation), it may be recommended to also use an ultrafilter for final purification, depending on the size of the solved organic components.

Built-in UF using crossflow (or tangential flow) filter systems have been extensively used to remove any remaining functional enzymes (especially nucleases) and any nucleic acids from the product water for use in biological applications such as cell culture. An ultrafiltration stage could also be used to reduce a variety of particularly non-ionic TOC compounds that are not removed by absorption.

UF is a common technique used when organic matter must be removed from or concentrated in a solution and can be

implemented for a wide range of molecule sizes and types by varying the membrane material and MWCO specifications. Molecules which are larger than the corresponding MWCO of the membrane are retained within the concentrate, while smaller molecules pass through the membrane with the permeate. Ionic charge or biological activity have little effect on the efficiency of this technique. Separation is based on the size and conformation of the molecule in question, and it does not matter whether these are biologically active molecules or simple humic substances from the water supply. Using UF membranes as part of the purification process for ultrapure water will lead to a reduction in product water TOC values compared to using devices without UF technology.

Importantly, the integrity of the UF membrane can also be verified. This is a considerable advantage because it means that only intact membranes are used, thus ensuring a constant and efficient retention of molecules larger than the specified MWCO.



$$\Delta P = P_E - P_A$$

The built-in Sartorius UF membrane within the Arium® Pro Ultrapure Water Systems has the additional advantage of using a crossflow (or tangential flow) filtration technology (Figure 2.2), rather than a point of use device which uses dead-end filtration (Figure 2.1). The flow of water across the membrane surface prevents the deposition of molecules on the membrane, significantly reducing fouling and blockage of the UF membrane. Rinsing of the filter prior to dispensing water, which is required with a point of use filter, is not necessary, leading to a reduction in the use of environmental resources. An optional cleaning procedure for the built-in UF module also increases the service life of the filter, reducing the total cost of ownership.

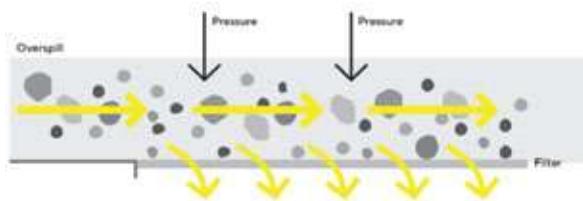


Figure 2.2: Schematic diagram of crossflow filtration. The varied distribution along the filter surface and the impact on performance becomes quickly apparent.

SUMMARY

Generally, reductions of the TOC level are successfully made using adsorption by upstream activated carbon filters and via oxidation using a UV lamp built into the ultrapure water system. A built-in ultrafiltration module can be used for the removal of residual TOC which cannot be reduced using standard technologies. This plays an important role in life science applications where the removal of nucleic acids, nucleases, and proteases can be critical, and in analytical applications for the further reduction of TOC moieties in general. As a result, uncharged organic components that have not been completely removed by previous purification steps can be significantly reduced with help of the built-in ultrafiltration module.

SARTORIUS



Arium® Laboratory-Grade Water Purification Systems

PFAS LANDSCAPE: GLOBAL REGULATIONS, INDIA'S FRAMEWORK, ANALYTICAL CHALLENGES AND WATERS' SOLUTIONS



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Per- and polyfluoroalkyl substances (PFAS) – often called “forever chemicals”—are now routinely found in water, soil, food packaging, biosolids and a wide range of consumer products. Their extreme persistence, bioaccumulation potential and associated health risks have made PFAS a global regulatory priority.

In India, FSSAI, CPCB and BIS are fast aligning with Stockholm Convention, EU REACH and U.S. EPA/USDA standards — with FSSAI's 2025 draft ban on PFAS in food-contact materials leading the charge.

This transition presents both challenges and opportunities for industries and analytical laboratories. As regulatory requirements evolve, laboratories must significantly strengthen ultra-trace analytical capabilities to monitor and mitigate PFAS across complex food, environmental and diverse matrices to support national environmental objectives, public health protection and global trade compliance.

This article outlines the evolving PFAS regulatory landscape, highlights India's accelerating efforts and shows how Waters end-to-end total workflow solutions – enable precise, reliable compliance. Featuring our collaboration with CSIR-IITR, it demonstrates how these solutions are strengthening India's PFAS testing ecosystem and supporting safer, sustainable practices.



GLOBAL PFAS REGULATIONS 2025: KEY IMPERATIVES

In 2025, PFAS regulations intensified globally due to health and environmental risks, enforcing ppt-level limits and bans—requiring laboratories to adopt ultra-sensitive, contamination-controlled PFAS workflows for compliance.

EUROPEAN UNION: PHASED RESTRICTIONS

- ▶ **Drinking Water Directive (EU) 2020/2184:** Limits of $\leq 0.1 \mu\text{g/L}$ (sum of 20 PFAS) and $\leq 0.5 \mu\text{g/L}$ total PFAS apply by 2026.
- ▶ **Firefighting Foams Ban (Reg. (EU) 2025/1988):** PFAS-based AFFF phased out by 2030 ($\geq 1 \text{ mg/L}$ sum threshold). Adopted & formalized in Oct 2025.
- ▶ **ECHA Proposal:** Covers 10,000+ PFAS, with 18-month transition periods and essential-use exemptions. (e.g., medical)
- ▶ **Packaging Reg. (EU) 2025/40:** Bans PFAS in food packaging from Aug 2026 (25 ppb individual, 250 ppb sum, 50 ppm total).

United States: Enforceable Limits & Standards

- ▶ **EPA NPDWR (2024/2025):** MCL: 4 ppt (PFOA/PFOS), 10 ppt (PFNA/PFHxS/GenX); compliance through 2031.

- ▶ **Method 1633A (Dec 2024):** Definitive LC-MS/MS method for 40 PFAS in multi-matrices across water, soil, biosolids.
- ▶ **USDA FSIS (2025):** PFAS Validation for meat/poultry/eggs started January 2025; post-FDA phase-out of grease-proofing agents.

International Benchmarks & Standards Bodies

- ▶ **Stockholm Convention (2025 Update):** Long-chain PFCAs listed Annex A (effective 2026); prior controls: PFOS (2009), PFOA (2019), PFHxS (2022).
- ▶ **EFSA TWI:** 4.4 ng/kg bw/week for four key PFAS (PFOS, PFOA, PFHxS, PFNA)—guiding 2025 assessments.
- ▶ **WHO / Codex** — WHO guidance on drinking-water PFAS risk management and Codex deliberations informing international food limits and traceability.
- ▶ **AOAC SMPR® 2025.001:** AOAC — SMPR development and method calls for PFAS in food/packaging to establish consensus analytical performance standards.
- ▶ **AOAC SMPR® 2023.003:** Defines performance requirements for PFAS analysis in food matrices such as produce, beverages, dairy, eggs, seafood, meat, and feed.

INDIA'S EMERGING PFAS FRAMEWORK: MOMENTUM MEETS OPPORTUNITY

India's PFAS regulatory landscape is accelerating under global trade requirements and health concerns. As of December 2025, no enforceable national limits exist for water, food or environment, but data generation surveillance and global alignment are prioritizing standardization.

KEY REGULATORY INITIATIVES

- ▶ **FSSAI Packaging Reforms:** Draft amendment to Food Safety and Standards (Packaging) Regulations, 2018, issued October 6, 2025, proposes outright ban on PFAS and BPA in food-contact materials (e.g., polycarbonate, epoxy resins etc;). 60-day comment period ended December 2025; aligns with Stockholm Convention to curb migration risks.
- ▶ **CPCB & BIS:** Monitoring and Standards Development: CPCB, with CSIR labs, has intensified PFAS surveillance across major rivers (including Ganges, Yamuna); IIT-Madras study (2024) detected 23–136 ng/L in urban waters, reinforcing the need for standardization. BIS drafts sampling/analysis protocols for support future standards.
- ▶ **Trade & Research Synergies:** Export Inspection Agencies (EIAs) enforce EU/U.S.-aligned PFAS compliance for dairy, seafood, food products and textiles. CSIR baseline studies (2025) assess PFAS in milk, spices, packaged foods and drinking water, informing policy and method uniformity.

ANALYTICAL CHALLENGES IN PFAS DETECTION: FROM HURDLES TO HEROICS

PFAS testing is among the most demanding trace analyses today — ultra-low concentrations (ppt or lower), complex

matrices (fatty seafood, spices, textiles, sediments, packaged foods etc;), Achieving reliable results requires exceptional sensitivity, precision and contamination control across the workflow.

- ▶ **Ultra-trace Sensitivity & Matrix Interference:** Sub-ppt detection is mandatory. Demands matrix extraction SPE strategies (e.g., Oasis WAX + GCB cleanup) is required to achieve 90–115% recoveries in high-fat or pigmented samples.
- ▶ **Contamination Risk:** PFAS routinely leach from common labware, PTFE tubing and even HPLC seals, creating false positives. CSIR-IITR reported blank levels dropped dramatically after switching to certified PFAS-free consumables and inert PEEK flow paths and isolator kits.
- ▶ **Lack of Standardised Methods:** EPA 533/537.1/1633 cover water and biosolids well, but spices, marine products, textiles and packaged food etc; often require modified cleanups (Dual phase mixed-mode SPE) to remove lipids, colorants and particulates
- ▶ **Quality Assurance for Audit-Ready Data:** CRMs, isotopically labeled standards, and ISO/IEC 17043-compliant PT programs (ERA) ensure linearity, precision, and export audits.

GLOBAL PFAS COMPLIANCE: OPPORTUNITY FOR INDIAN LABORATORIES

With growing global regulations—EU REACH restrictions, US EPA MCLs (4 ppt for PFOA/PFOS) and FSSAI's 2025 draft ban on PFAS/BPA—ultra-trace PFAS testing is now essential for India's export-focused food, textile and packaging sectors. Indian labs must adopt PFAS-free workflows, matrix-specific methods (e.g., EPA 1633), and strong QA to deliver defensible ppt-level data. Building these capabilities enhances compliance, boosts export readiness, and positions India to lead in safer food, water and sustainable trade.

WATERS COMPREHENSIVE PFAS WORKFLOW SOLUTIONS FOR GLOBAL COMPLIANCE

Waters offers integrated workflow solutions designed to minimize contamination, streamline sample preparation and deliver ultra-trace PFAS detection. Our portfolio aligns with global standards like EPA Method 1633 and EU Drinking Water Directive, ensuring compliance and accuracy.

Key Benefits: Eliminates background contamination, enhancing detection sensitivity for complex matrices.

► Certified PFAS-Free Consumables

Oasis WAX SPE Cartridges: High-purity, pre-screened for 32 PFAS, ideal for ultra-trace cleanup in aqueous, soil and food matrices. **Oasis WAX/GCB & GCB/WAX SPE:** For water and biosolid testing, compliant with EPA Method 1633, ensuring robust sample preparation.

► Advanced Analytical Platforms

ACQUITY UPLC + Tandem MS: Combines ACQUITY UPLC with Xevo TQ-S micro, TQ-XS or TQ-Absolute XR for high-sensitivity PFAS detection at parts-per-trillion (ppt) levels. **Automated SPE + Andrew+ Pipetting:** Integrates automated sample preparation for consistent, high-throughput workflows, reducing manual errors.

► Key Benefits: Delivers reproducible results for diverse matrices like drinking water, seafood and textiles etc.

Accessories for Contamination Control

► PFAS Accessories Kit: Includes tubing assemblies, isolator columns and solvent inlet filters to minimize system-related PFAS contamination in UPLC setups.

► **ACQUITY/Alliance Bottle Accessory Kit:** Features PEEK mobile phase solvent lines to reduce contamination risks in solvent delivery systems.

► **Isolator Column:** Enhances sample integrity by separating PFAS contaminants, improving analytical sensitivity.

► **Key Benefits:** Ensures clean, reliable results critical for meeting ultra-low regulatory thresholds.

► Proficiency Testing & Certified Reference Materials

Waters ERA portfolio offers ISO/IEC 17043-accredited PT programs aligned with EPA 1633, 533 and 537 for water, wastewater, soil, biosolids and tissue, enabling method validation and regulatory benchmarking. Trace-spiked PFAS CRMs support calibration, LOQ checks and routine QA/QC, ensuring independent accuracy verification and audit-ready data.

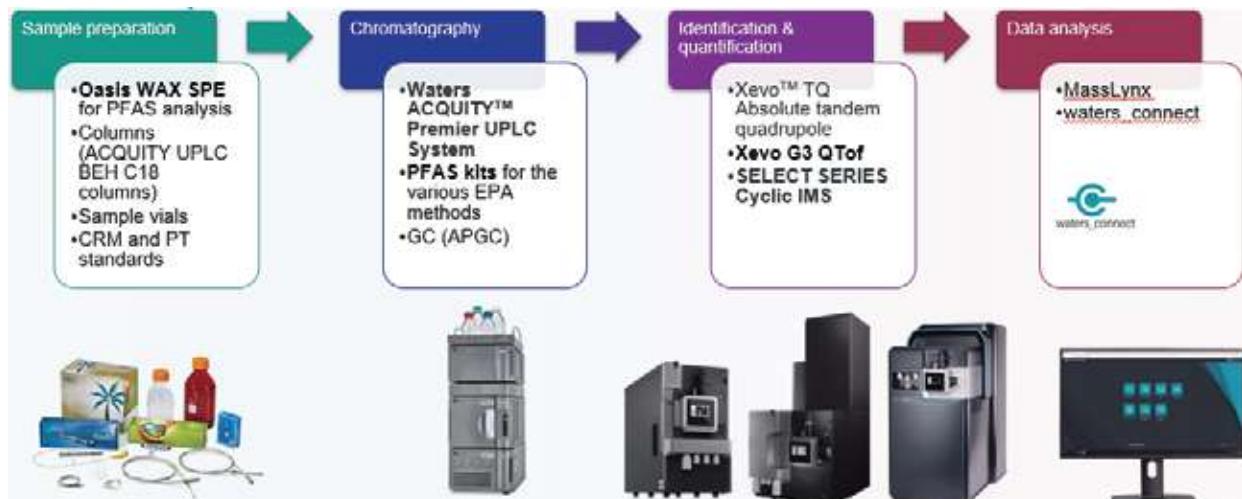
Reference: ERA PFAS Portfolio (eraqc.com).

► **Local Expertise & Implementation Support Waters India Application Labs:** Offer hands-on method development support, troubleshooting and workflow optimization specific to PFAS analysis.

► **Training & Adoption Support:** On-site and virtual training modules help labs rapidly implement ppt-level PFAS workflows aligned with EPA 1633 and global requirements.

Key Benefits: Enables laboratories to scale PFAS testing capability efficiently while maintaining accuracy, compliance and operational readiness. Waters' end-to-end solutions empower Indian laboratories to achieve global PFAS compliance, deliver defensible data and support sustainable, export-ready environmental and food testing.

PFAS ANALYSIS WORKFLOW



SPOTLIGHT: CSIR–IITR PARTNERSHIP – BUILDING INDIA’S PFAS-READY FUTURE

In June 2025, CSIR–IITR, Lucknow, inaugurated a national PFAS testing laboratory equipped with Waters’ PFAS workflows, including ACQUITY UPLC, Xevo TQ MS and PFAS-free sample prep. As noted, the lab achieves ppt-level detection in water and food using Waters certified LC–MS/MS kit. Together with Waters scientists, IITR developed a 10-minute method for 30 PFAS, now guiding national readiness. The center is training 50+ labs and contributing critical data to FSSAI, BIS and CPCB. IIT–Madras’ riverine PFAS mapping further reinforces India’s need for ultra-trace capability.

Sources: SelectScience (2025); Environmental Sciences Europe (2024).

OUTLOOK: A PFAS-RESILIENT INDIA

As India prepares for stricter PFAS controls—including the anticipated FSSAI 2025 packaging restrictions—robust, contamination-free workflows are essential. Waters’ integrated ecosystem—Oasis SPE, ACQUITY UPLC, Xevo TQ

MS, and ERA PT/CRMs—provides Indian laboratories with a scalable, compliant, ppt-ready pathway to meet global standards and protect public health.

2026 will be a big year – FSSAI ban likely to be final, export rules getting tighter and more labs starting routine PFAS monitoring. The labs that prepare today with reliable, certified, PFAS-free workflows will lead tomorrow.

**TOGETHER, LET'S MAKE
WATER, FOOD AND
ENVIRONMENT SAFER— ONE
ACCURATE RESULT AT A
TIME!**

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in separation
science is here**



Waters™

**PFAS Analysis
Solutions**



Ms. Susanne Riegel

VP of Marketing and Product Manager of Nanalysis Corp

FROM HIGH-FIELD EXCLUSIVITY TO EVERYDAY INSIGHT, NANALYSIS' VISION FOR PORTABLE, AUTOMATED SPECTROSCOPY

Ms. Susanne Riegel has been the VP of Marketing and Product Manager of Nanalysis Corp. for over 13 years. Over that time Nanalysis Corp has grown from a small technology start-up to a world leader in the development, manufacture, and sale of portable benchtop Nuclear Magnetic Resonance (NMR) spectrometers.

She has a background in chemistry, completing a M.Sc. in main group catena-phosphorus compounds at Dalhousie University in Halifax, Nova Scotia and a Ph.D. in Organometallic Chemistry at the University of Calgary. Throughout her time at Nanalysis she has grown a group focused on the development or validation of analytical methods to proliferate the use of NMR Spectroscopy into targeted markets in a variety of industrial applications.

 nanalysis

Accessible. Affordable. Automatable.

Could you brief on Nanalysis' journey in the space of benchtop NMR spectrometers?

Nanalysis began in 2008 with some licensed technology from Caltech looking at MEMS chips for force detected NMR Spectroscopy, a few months into this investigation, the company pivoted to make a more commercially relevant product – ultimately the benchtop NMR. Built from the ground up, Nanalysis' initial focus was to make the smallest, most homogenous permanent magnet they could and build the infrastructure around this product to make it easy-to-site and easy-to-use even for non-NMR experts. The first product was launched commercially in 2013 – a proton-only 60 MHz benchtop NMR spectrometer, which was joined by multi-nuclear offerings in 2015, and by the highest benchtop NMR field strength on the market – 100 MHz, in 2019. The focus has been providing NMR solutions to people who could benefit from the technique but have had insufficient access to it.

In what ways does Nanalysis' commitment to their brand statement "Accessible. Affordable. Automatable", guide innovation and product development strategies?

Nanalysis' vision is to democratize the use of NMR spectroscopy. This is an extremely powerful technique that has been mostly limited to large institutions who have the financial and human resources required to upkeep the expensive and complex instrumentation associated with high-field NMR spectroscopy.

The Nanalysis benchtop NMR product line has been focused on compact, portable, affordable and easy-to-use instrumentation for both data acquisition and processing.

The idea is to make NMR spectrometers more readily available regardless of the location and expertise.

Benchtop NMR technology has evolved over the years. What key advancements has Nanalysis incorporated into its systems?

Starting with proton-only 60 MHz (1.4 T), we evolved the observable nuclei to include heteronuclear options like carbon, phosphorus, fluorine, lithium, boron, as well as the number of available experiments, which include 2D homo- and hetero-nuclear datasets, as well as phase

sensitive experiments. With the release of our higher field instrument, the 100 MHz (2.35 T), we also looked to further expand these capabilities. The focus was on improving our temperature sensitivity to allow the instrument to be used reliably in a wider range of room temperatures, adding more sophisticated options such as pulsed-field gradients to allow 2D data to be acquired more rapidly, and accessories such as flow cells and an autosampler to expand the reach of the product. By increasing the flexibility of the interface and the optional access to pulse programming but keeping a simple, automatic 'black box' type interface, we have really made the instrument appealing as both a research tool, and for increased QA/QC.

Benchtop NMR systems are known for their compact size. How does this offer an advantage over traditional high-field systems?

Traditional high-field NMRs are extremely powerful instruments, and we are not looking to replace them or their use in research, but rather, we are trying to address some of the limitations of the nature of superconducting magnets that have limited the widespread usage of NMR spectroscopy. By miniaturizing the magnet, the benchtop NMR can start to allow this powerful technique to be incorporated into new applications – including mobile laboratory vehicles, or QC testing facilities.

What type of research fields would benefit the most from benchtop NMRs? Could you share some success stories of how your spectrometer(s) were used to achieve a breakthrough in research?

Our benchtop NMR spectrometers are aimed at providing access to underserved users of high-field NMR spectrometers, including academic teaching, and also for academic, industrial or government research. However, we view the benchtop NMR less as a tool to facilitate high-end research, and more as an added layer within the NMR workflow to provide the foundation for process analytical technology (PAT) and reaction monitoring, or as a valuable quality control/quality assurance (QC/QA) tool. Additionally, we have seen many exciting research work done within the fields of agrochemicals, pharmaceuticals/biochemistry, lithium mining, and oil and gas – including a life cycle analysis of H2S scavengers to determine the route of

deposition/precipitation to optimize the use of these important catalysts.

How can the portability of your systems be taken advantage of in real-time reaction monitoring and product analysis?

'Accessible. Affordable. Automatable.' is our benchtop NMR motto. NMR Spectroscopy is one of the most information rich techniques that exists, and it has been excluded from many applications due to the size, weight, cost, and maintenance requirements of high-field, super-conducting NMR magnet technology.

By using a permanent magnet-based product we have made products as small as a toaster oven that can be put directly on a process chemistry line, in a QA/QC environment, or even in a mobile lab or van.

What are some of the common concerns from researchers regarding benchtop NMR spectrometers? How do you address them?

Benchtop NMRs addresses some of the economic limitations of superconducting NMR technology, but, unfortunately, the permanent magnets are not as powerful. While this means the instruments are more robust and easier to site, it also means that they have inherently less sensitivity and peak dispersion. This means that sometimes samples must be run at a higher concentration, or additional software layers – e.g., databases, quantum mechanical modelling, CRAFT, etc. – must be included to ensure that the information can be extracted even if it is slightly more complicated to the human eye.

Pharma and biotech industries are often data driven. How does Nanalysis see its benchtop NMR technology evolving to meet these industry requirements?

Accurate and reliable data taken from analytical instrumentation is the cornerstone of data driven sciences. Synthetic chemistry is labour intensive and can limit the speed with which discovery is made. To address this limitation, there has been increased focus on high-throughput screening (HTS) and self-driving laboratories (SLDs). Since its conception, the benchtop NMR was designed with connectivity in mind – with WiFi, ethernet, USB, and

intra-laboratory network options, the data is easily incorporated with existing tools within the lab. The data itself is saved in a standard jcAMP-dx file format that can be automatically processed with reliability and connected to the laboratory workflow as required. As we learn more about these requirements, we make the modifications necessary to allow benchtop NMR to provide high quality, precise and reliable data to streamline pharmaceutical and biotechnology research.

In a field where innovation often races ahead of regulation, how does Nanalysis strike a balance between pushing analytical boundaries and ensuring regulatory validation?

As NMR spectroscopy is inherently quantitative, Nanalysis' benchtop NMR spectrometers are being validated in quality control environments even though NMR spectroscopy has largely been overlooked in this regard. For internal institutional assays, these can be developed and validated to give reliable answers quite rapidly. However, it is also important to work with regulatory organizations to ensure that key parameters and characteristics of products and materials are verifiable with a gold standard method.

Nanalysis is developing and validating methods and working with appropriate bodies to get these NMR-based methods certified as gold standard methods.

Instrument qualification and software validation often pose major barriers to regulatory adoption. Could you throw light on what steps Nanalysis has taken to make these processes more transparent and user-friendly for regulated labs?

To ensure that data acquired on the benchtop NMR is compliant, it is important to adhere to regulatory guidelines such as GxP. Instruments should be qualified with assay-appropriate assessments through the design qualification, installation qualification, operational qualification, and performance qualification stages. These options have been available on our benchtop NMR products for quite a while. Additionally, we are working to release software validation following guidelines outlined from FDA 21 CFR Part 11 to ensure that the data is traceable, and its integrity is maintained and can be audited throughout the analytical process and workflow.

How do you view the growth of Nanalysis in the Indian market? Could you comment on the value of Nanalysis' partnership with Inkarp Instruments in helping Indian researchers gain access to this technology?

Nanalysis has had the pleasure of working with Inkarp Instruments for over a decade. They are one of our longest running distributors and have been instrumental to understand the landscape of the Indian market in both research and industry. With the amount of chemical production occurring in India – particularly in the agrochemical and pharmaceutical industries, we think that the incorporation of benchtop NMR could be used to improve yields and assure the highest quality products without drastically increasing the price of the analysis.

Lastly, are there any exciting developments in Nanalysis that you would like our research customers to know about?

Nanalysis just re-released our 60 MHz on the same platform as our 100 MHz instrument. This has allowed us to increase the number of more sophisticated features that we can offer our customers, including improved performance, stability, gradients, a flow cell, and an autosampler. Additionally, on the new platform, we can offer software modules that help improve data integrity working towards GxP compliance, can analyse data automatically, allow people to build their own custom pulse sequences, etc.

The Nanalysis product line is growing to accommodate all types of users, and for anyone wondering if benchtop NMR can work for them, we encourage you to reach out to Inkarp Instruments and our application chemistry team to see if benchtop NMR can assist your program.





Ms. Sivakami Senthil
Head of Propellant Department



Mr. Henry Sam
Head of Catalyst Department

PROPELLING SUSTAINABILITY: MANASTU SPACE'S MS289 THRUSTS INTO GREEN SPACE PROPULSION

Ms. Sivakami Senthil — Head of Propellant Department

Sivakami hails from Tamil Nadu and earned a B. Tech in Nanoscience and Nanotechnology from KSR College of Tech, Namakkal. She joined Manastu Space in 2019 as a final-year intern, working on catalyst development with nanoparticles and nanomaterials. After completing her internship, she continued with Manastu and now leads the propellant team, developing green, high-performance fuels for sustainable space propulsion systems.

Mr. Henry Sam — Head of Catalyst Department

Henry, coming from a village Tirunelveli district Tamil Nadu, was Sivakami's junior at KSR College of Tech, where he completed his B. Tech in Nanoscience and Nanotechnology. Fascinated by the ability to tailor material properties at the nanoscale, he joined Manastu Space in the catalyst division after graduation. He now leads the catalyst team, developing innovative materials that power Manastu's green propulsion systems.



Turning Chemistry into Thrust

Starting with Sivakami — which comes first in development: the catalyst or the propellant?

Ms. Sivakami: You can say they go hand in hand. If you want any engine to work — take a car for example — the car needs petrol and it needs its engine. And not just that: when petrol goes into the engine, it will not start without an igniter or spark plug.

So, the propellant is like the petrol, the catalyst is like the spark plug or igniter, and the thruster is the engine. Without any of this, none of the system components will work.

Can you just tell us about MS-289 and what problem it's solving?

Ms. Sivakami: Yes. MS-289 is our proprietary green monopropellant, designed as a safer and high-performance alternative to toxic hydrazine. It addresses toxicity — hydrazine is highly toxic.

Hydrazine is widely used commercially, and the key problem MS-289 solves is reducing environmental and handling risk; it's an alternative to toxic hydrazine.

Hydrazine has been used for a long time — what led you to hydrogen peroxide as a basis for MS-289?

Ms. Sivakami: As I mentioned, MS-289 is a blend of hydrogen peroxide with additives and alcohols. Hydrogen peroxide has a long history in space applications — high-test peroxide was used as rocket fuel.

Peroxide's performance is lower than hydrazine, which is why hydrazine became dominant: better specific impulse and mass efficiency. But hydrazine's toxicity requires heavy protective measures and raises operational cost.



Alternatives are being pursued worldwide — ionic propellants like ADN and HAN, and NASA is testing peroxide mixtures too. Peroxide decomposes to oxygen and water and is less toxic, but performance and storability need work. We added alcohols and additives to improve performance and storage life, and after many combinations we arrived at our specific blend.

When you say performance, what metrics do you mean — specific impulse, thrust, chamber temperature?

Ms. Sivakami: Yes. Specific impulse is like mileage for rockets, that is thrust per kg of propellant. The mixture we're discussing gives about 15–20% better performance than pure hydrogen peroxide.

That must have taken a lot of trial and error.

Ms. Sivakami: Haha, yes. We've been at it for six years and are still improving. So far, we're achieving about three years shelf life; we're working toward five to ten years to meet satellite manufacturers' expectations.



Henry, what are the ideal properties you look for when designing a catalyst?

Mr. Henry: A catalyst participates in the reaction without being consumed. You need specific sites where the reaction occurs — that's selectivity. Second is structural integrity: in our engines the catalyst is often in pellet form and must withstand the load of liquid fuel expanding into gas. Third is mechanical strength: the pellet bed must transfer stress smoothly. So: chemical activity (selectivity), structural integrity and mechanical strength.

How do you balance performance with longevity under harsh conditions?

Mr. Henry: Catalysts don't like high temperature and pressure. We look at a material's characteristic velocity to know the reaction rate it can handle. If the system overloads the catalyst it degrades, so we design the engine to limit catalyst load and thus increase longevity.

Do you develop propellant and catalyst together? How do you handle mismatches?

Mr. Henry: We test components in the final system — thruster firings show how propellant and catalyst interact. Individually, we qualify the catalyst; in the system we assess interaction with the oxidizer. Oxidizers can corrode metals and form oxides that block active surfaces; if surfaces stay blocked, reactions can't occur. We constantly assess whether our combinations affect system performance.



What unforeseen failures did you encounter in development?

Mr. Henry: For any material there's a temperature — roughly 70–80% of its melting point — where internal structure

starts to rearrange and atoms interact with each other. We don't want the catalyst material to react with itself; we want it to react with the fuel. In our system temperature and pressure can spike within seconds, so designing for those transient extremes is a key challenge.

What simulations do you run, and how do you validate them?

Mr. Henry: We qualify components individually, then use MATLAB and other models to predict reactions in different zones of the catalyst bed. Simulations guide us, but system tests validate the models — simulations don't capture every interaction.



How central is QA and compliance?

Ms. Sivakami: Critical. Hydrogen peroxide for space must meet ASTM / military / Indian standards. We're talking ppb-level impurities (anions, cations, metal ions). Even tiny metal impurities can trigger decomposition. Measuring and controlling these impurities is difficult; if a batch fails spec, we reject it. So, QA determines flight readiness..

What instruments do you use in QA?

Ms. Sivakami: Ion chromatography (anions/cations), ICP (metal ions), TOC, pH, auto titrators, and structural/molecular checks with FTIR, NMR, and SEM. These tests are for MS-289 specifically, not the catalyst.

What is HAZOP and why does it matter?

Ms. Sivakami: HAZOP – Hazard and Operability Analysis – is a systematic pre-operation review of what could go wrong. For example, purifying industrial-grade peroxide to space grade involves high temperatures and vacuum: HAZOP asks, what if the temperature spikes? if glassware breaks? if there's overpressure? We set safety parameters and train staff on responses, so operations are controlled and safe

Now that you've combined components into a full system, what unexpected failures did you encounter at scale?

Mr. Henry: We studied additive combinations because additives can interact with catalysts and shorten life. Literature suggested fouling; testing showed ~80% of additives had little adverse effect — that was a big relief. That outcome couldn't be predicted in MATLAB; we had to experiment.

Ms. Sivakami: Also, lab tests use inert glassware, but the final system uses metal tanks. Hydrogen peroxide reacts with incompatible metals and impurities, reducing durability. Scaling up requires compatible materials, tank treatments



and a lot of extra work — new issues appear when you move from lab to system.

As the discussion ended, both reflected on surprises and learning moments that defined their R&D journey. From unexpected experimental outcomes to simulations playing out differently in real systems, those moments were key learning experiences. Trial, testing and observation, they said, shape the development of propellants and catalysts – and each challenge ultimately contributes to improved performance and longevity.

As our conversation wrapped up, Sivakami and Henry reflected on the unpredictable nature of R&D — those moments when experiments challenge assumptions and lead to new insights.

Their accounts emphasize how trial, testing, and observation shape the development of propellants and catalysts — and how each challenge ultimately contributes to improved performance and longevity.





Dr. Ravishankaran R

R&D Head, Diagnostics Division
GeNext Genomics Pvt. Ltd.

THE NEXT FRONTIER OF RAPID ANIMAL DIAGNOSTICS: INSIDE GENEXT GENOMICS' ASFV REVOLUTION

Dr. Ravishankaran R is a distinguished immune biotechnologist with 18+ years of expertise in monoclonal and polyclonal antibody development, specializing in infectious diseases, viral diagnostics, and insect toxin detection for human health, veterinary medicine, and agricultural applications.

A trailblazer in hybridoma technology, scFv engineering, and immunoassay development, he has successfully designed functional antibodies and immunoassays against high-impact targets, including: **Human Diseases: Malaria, Dengue, Filarial antigen, HIV, HCV, TNF α , ER/PR receptors, Toxins & Veterinary Diagnostics: Aflatoxin, 1EC, CryX, ASFV, CSFV.**



RAPID DIAGNOSIS OF ASF VIRUS INFECTION

In collaboration with



WHAT IS ASFV?

African Swine Fever (ASF) is a highly contagious and often lethal **viral disease** that affects both domestic and wild pigs. The disease has no available vaccine or treatment, making early detection and rapid containment critical for controlling outbreaks. Since 2007, ASFV has spread **widely across Africa, Europe, and Asia**. In recent years, it has become a **major threat in Asia, particularly in countries such as India, China and Vietnam**, severely impacting the pig farming sector and causing substantial economic losses.

NEED FOR EARLY DIAGNOSIS

ASFV outbreaks spread quickly and can devastate pig populations. Conventional diagnostic methods, while accurate, often require specialized laboratories, equipment, and trained personnel, leading to delays in detection. A rapid, field-deployable, and easy-to-use test is therefore essential for on-site disease monitoring, immediate decision-making, and effective control strategies.

Note: This SWIRUS diagnostic kit is free from any infectious components and poses no biological hazard to humans or animals.

WHAT IS SWIRUS?

SWIRUS is a **rapid lateral-flow antigen test** designed to detect African Swine Fever Virus (ASFV) in **whole blood, serum, or plasma** of pigs.

- ▶ Based on highly specific monoclonal antibodies targeting the ASFV p30 protein
- ▶ Detects **active infection in Early & Asymptomatic Stages**
- ▶ Suitable for **field, farm, and border-level screening**
- ▶ Delivers results in **10–15 minutes**
- ▶ Ideal for **Veterinary officers, DIOs, government recurring farms, breeding farms, small to large commercial farms, and emergency outbreak response units.**
- ▶ **Zero Lab Infrastructure Needed** – No PCR machine, no electricity dependency – works **anywhere**, even in remote farms.

SWIRUS

AFRICAN SWINE FEVER VIRUS

ANTIGEN DETECTION RAPID TEST KIT

SWIRUS, India's first indigenous ASFV Rapid **antigen** Detection Kit, delivers **accurate results in just 10 -15 minutes**, enabling on-site decision-making when every minute matters. Developed jointly by Assam **Agricultural University (AAU)** and **GeNext Genomics Pvt. Ltd.**, SWIRUS is optimized for field conditions, requiring **no lab setup or specialized training**. Designed for veterinarians, surveillance teams, and government agencies, it is a critical tool for strengthening national biosecurity and outbreak preparedness. African Swine Fever Virus (ASFV) point of care diagnostic test which detects antigen (active infection) in swine serum, plasma, and whole blood.



HYBRIDOMA TECHNOLOGY

Hybridoma-driven monoclonal antibody generation forms the backbone of your diagnostic platforms. How do you optimize clone screening and selection to ensure high-affinity antibodies that can perform reliably in rapid, field-ready ASFV detection kits?

First, I would like to thank **Dr. Ashwin Kashikar and Supriya Kashikar** for providing me with the opportunity to carry out this work and for their continuous support, valuable project insights, and access to advanced instrumentation. I also acknowledge the dedicated IVD-Hybridoma, Analytical, and Marketing teams at GeNext Genomics Pvt. Ltd.

I am grateful to my guide, **Prof. Anjali A. Karande (IISc)**, for her training and emphasis on rigorous experimental design.

From selecting native or near-native immunogens to planning effective immunization schedules, these foundational steps were essential for generating strong immune responses. Hybridoma development, particularly the fusion and early screening stages, demands precision and experience.

With over 20 years in this field, I have learned how critical it is to execute these steps efficiently and economically, as fusion and primary screening alone can take nearly three months. Strong fundamentals greatly increase the likelihood of obtaining high-affinity, antigen-specific clones.

Using the selected antibodies, we performed BLI (Octet) analysis to identify optimal capture-detector pairs, followed by extensive LFA-format pairing studies to determine the best-performing combinations. This systematic workflow enabled us to develop a highly sensitive and specific ASFV rapid detection kit within 10 months.

The kit has since been evaluated by multiple government agencies, all of which reported that it is among the most sensitive and specific ASFV rapid tests available globally.

Maintaining specificity while reducing background noise is critical for lateral-flow assay performance. What refinements has GeNext Genomics introduced in its hybridoma workflow to minimize cross-reactivity and enhance analytical sensitivity?

GeNext Genomics refines its hybridoma workflow through a series of targeted improvements that directly reduce cross-reactivity and boost analytical sensitivity:

- ▶ **Smart immunogen design** — use of native/near-native and epitope-focused antigens to steer the immune system.
- ▶ **Early negative selection** — parallel screening against related pathogens, host proteins, and common sample matrices to eliminate cross-reactive clones at the earliest stage.
- ▶ **High-resolution binding analytics (Octet-BLI)** — kinetic profiling (kon/koff/KD) and epitope binning ensure only high-affinity, non-competing clones are advanced, strengthening assay sensitivity.
- ▶ **Function-forward screening** — testing hybridoma supernatants directly in the intended assay matrices (blood/serum/plasma) and after nanoparticle/latex conjugation to detect matrix interference early.
- ▶ **Pair optimization for sandwich assays** — systematic evaluation of multiple capture-detector combinations to identify pairs with highest signal-to-noise and lowest LOD.
- ▶ **Stability & manufacturability gating** — selection of clones with strong thermal stability, low aggregation, and consistent expression, ensuring high sensitivity is retained after scale-up and storage.
- ▶ **Iterative subcloning & refinement** — confirmed single-cell cloning and re-validation to secure monoclonality and robustness, reducing the risk of drift and off-target binding.

Result: a streamlined hybridoma-to-LFA pipeline that yields highly specific, low-cross-reactive, and high-affinity monoclonal antibodies ideal for sensitive, field-ready

standards — is the most effective way to ensure consistent, high-quality monoclonal antibody supply for large-scale veterinary surveillance kits.

ASFV DIAGNOSTICS

ASFV's early infection stages present low viral loads and unpredictable field conditions. What scientific and technical hurdles did you encounter while designing a rapid ASFV test that remains accurate even in early-stage infections?

Thank you for the question. One of the major scientific challenges we encountered was the selection of an appropriate antigen target, particularly because African swine fever virus (ASFV) exhibits very low viral loads during the early stages of infection and field conditions are often unpredictable. Among the reported antigen candidates, p30 and p72 are the most commonly used, with p30 being expressed during early infection and p72 being associated with later, more established stages of disease. With valuable inputs and guidance from our collaborators, **Dr. Nagendra Nath Barman and Dr. Lukumoni Buragohain from Assam Agricultural University**, we carefully evaluated these targets for assay development.

To ensure early-stage detection, we focused on P30, even though most PCR-based molecular kits still rely on P72 primers. Identifying and validating P30 as the target was critical because it required confirming that the antigen is consistently detectable at low viral concentrations across varied field samples.

Once the antigen target was finalized, the next technical hurdle was developing highly sensitive and specific monoclonal antibody pairs. Leveraging our experience in hybridoma development, we systematically optimized the screening and pairing strategy to obtain antibodies capable of reliably capturing P30 under field-level variability. This allowed us to achieve the sensitivity and specificity required to build a robust LFA suitable for early ASFV detection.

Sensitivity is a major differentiator for point-of-care tests. What validation data or internal benchmarks support the high sensitivity claims of your SWIRUS® ASFV detection kit?

Our sensitivity claims for the SWIRUS® ASFV detection kit are supported by comprehensive internal validation studies using recombinant P30 antigen and ASFV-positive field samples. The assay consistently detected antigen concentrations as low as 1–5 ng/mL and demonstrated a **100% correlation** with PCR-confirmed positive samples in our pilot panel. Batch-to-batch evaluations further confirmed stable performance with no loss of sensitivity, underscoring the robustness of the antibody pair and overall assay design.

In addition to internal testing, the kit has been independently external evaluated by government agencies, including Indian Council of Agricultural Research – National Institute of Veterinary Epidemiology and Disease Informatics. Indian Council of Agricultural Research – National Institute of Veterinary Epidemiology and Disease Informatics (ICAR–NIVEDI), Bengaluru and the College of Veterinary Science, Assam Agricultural University (AAU), Guwahati. Both institutions validated the kit and reported **99.9% sensitivity and specificity**, further reinforcing its reliability for ASFV detection.

Differentiating ASFV from other swine pathogens is essential for outbreak management. How did you ensure high specificity in antigen/antibody pairing and avoid cross-reactivity with viruses like CSFV or PRRSV?

We ensured high specificity by rigorously screening monoclonal antibody pairs against a wide antigen panel, including CSFV and PRRSV. Only clones that showed strong binding to ASFV P30 and zero cross-reactivity with non-target swine pathogens were selected. Further confirmatory testing with field samples and recombinant proteins validated that the final antibody pair remained highly specific under diverse conditions.

Usability in rural or non-laboratory environments is crucial for real adoption. What design choices helped translate your lab-based development into a rugged, user-friendly kit deployable directly on farms and government surveillance sites?

LFA-based rapid diagnostic tests are inherently user-friendly and well-suited for mass surveillance programs, especially in rural or non-laboratory settings. Our primary focus was to develop a kit that can be operated by non-technical

diagnostic kits.

Scaling antibody production without compromising purity is a known bottleneck in commercial diagnostics. How do you maintain batch-to-batch consistency in monoclonal antibody manufacturing for high-volume veterinary surveillance kits?

Implementing these controls as an integrated system — cell banking + platform process + Real time In-process monitoring (PAT) + rigorous QC + change control + reference personnel and deliver clear, reliable results within minutes. The main scientific challenge was identifying highly sensitive and specific antibody pairs; once this was optimized, the rest of the design translated smoothly into a rugged and practical field-ready format.

By prioritizing stable reagents, simple test workflows, and easy-to-interpret visual readouts, we ensured that the kit could be confidently deployed directly on farms and government surveillance sites without the need for specialized equipment or trained laboratory staff.

IMPLEN NANOPHOTOMETER

Accurate quantification of recombinant antigens and hybridoma-derived antibodies is key to QC reliability. Where does the Implen NanoPhotometer fit into your quality-control workflow, and which performance parameters (A260/280, concentration linearity, low-volume accuracy) have been most valuable for your diagnostic production?

The Implen NanoPhotometer plays a central role in our QC workflow—not only for recombinant antigens and antibodies, but for all high-value reagents used in our diagnostic development. It provides precise quantification, purity assessment, and consistent low-volume measurements, which are critical for batch-to-batch standardization.

Key performance parameters such as A260/280 purity ratios, concentration linearity, and high accuracy with minimal sample volumes have been particularly valuable. These capabilities ensure that every antigen and antibody lot meets stringent quality requirements, supporting the overall

reliability and reproducibility of our diagnostic production.

Rapid QC cycles are essential when scaling diagnostic kits. What advantages have you observed with the NanoPhotometer compared to traditional spectrophotometry in terms of speed, sample economy, and reproducibility?

The NanoPhotometer has significantly accelerated our QC workflow compared to traditional spectrophotometers. Its rapid measurement time, low sample volume requirement, and high reproducibility allow us to complete more QC cycles in less time while conserving valuable reagents. The sealed microvolume design eliminates cuvette cleaning and handling errors, improving consistency across operators. Overall, it has enabled faster turnaround, better sample economy, and highly reliable batch-to-batch documentation during kit scale-up.

Looking ahead, veterinary diagnostics is shifting quickly toward faster, field-ready, and increasingly molecular platforms. What future innovations or technology directions is GeNext Genomics most excited about as you continue expanding India's diagnostic landscape for both animal and human health?

GeNext Genomics is driving innovation across Agriculture, Veterinary, and Human diagnostics with strong in-house capabilities in antigen cloning, expression, and purification, as well as hybridoma, scFv, and recombinant antibody development in gram-scale. Our collaborations with global industry and academic partners reinforce our role as one of India's few end-to-end antigen and antibody manufacturers. Looking ahead, we are particularly focused on advancing rapid field-ready diagnostics, molecular point-of-care platforms, multiplex assays, and next-generation recombinant antibody technologies. These efforts support India's diagnostic self-reliance and align with the “Hon’ble Prime Minister Shri Narendra Modi Ji’s” vision of “Aatmanirbharta” by strengthening indigenous solutions for both diagnostic and therapeutic needs.

A large, white, crystalline mound of material, possibly pharmaceutical ingredients, is in the foreground. In the background, several blurred containers are visible, suggesting a laboratory or industrial setting.

Quality Control with Infrared Spectroscopy

Bruker ALPHA II FTIR for Rapid, Reliable Material Verification in Modern QC Labs

Infrared spectroscopy is an essential tool for fast, non-destructive material analysis across industrial QC workflows. This application note demonstrates how the Bruker ALPHA II FTIR system streamlines identity testing, contamination detection and polymer characterization using intuitive ATR sampling and guided touchscreen operation.

This note demonstrates practical use case examples in raw material verification, and surface cleanliness assessment. The ALPHA II shows high-quality spectra, helping QC teams improve speed, consistency and decision-making without complex sample preparation or extensive expertise.

Keywords or phrases:

FTIR, Bruker Alpha II, Attenuated Total Reflection, incoming goods inspection, raw material verification, quality control, non-destructive analysis, rapid screening.

INTRODUCTION

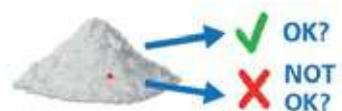
When it comes to up-to-date testing of the chemical nature of materials, Fourier Transform Infrared Spectroscopy (FTIR) has established itself as one of the most important analytical methods. Its speed and simplicity make it the method of choice in many applications. The technique is equally suitable for incoming goods inspection, failure analysis, competitive analysis, process- and quality control. The measurement can be performed within a short time and without any sample preparation or the use of consumables.

Many industrial sectors now rely on FTIR spectroscopy and over the years it has completely replaced a number of complex physical and wet-chemical methods of analysis.

It is widely used in the field of plastics processing, pharmaceuticals, chemical and petrochemical industries, as well as in automotive and electronics sectors. Moreover, new

and exciting fields of application are constantly being defined.

The focus of incoming goods inspection is the identity check of materials and to test if they are compliant to reference standards or whether they show deviations (for example due to contaminations). Multi-component products are tested for their composition to ensure that the formulation is within defined limits. All these tasks are conveniently performed by failsafe FTIR spectroscopy in just a few seconds.



MEASUREMENT PRINCIPLE

Infrared spectroscopy makes use of the invisible thermal radiation's property to stimulate molecular or lattice vibrations in the irradiated matter. This vibrational excitation results in the absorption of the incident light at certain wavelengths that are dependent from the absorbing medium. The FTIR spectrometer measures the absorbed energy as a function of the wavelength and provides a spectrum in which the absorption intensities are plotted against the wavelength. Since the number, relative intensity and location of these absorptions (bands) are substance-specific, this spectrum can be used for identification or for answering quantitative questions. Modern IR spectrometers make it possible to record and evaluate a spectrum in a matter of seconds.

INSTRUMENTATION

Since it is much more comfortable, the Attenuated Total Reflection (ATR) has almost completely superseded the traditional transmission measurement in incoming goods inspection and quality control. All types of samples (e.g. powders, pastes, fibres, components, liquids, solutions) are just put on the ATR unit and then measured directly.

As a chemically inert and above all robust material, the diamond used as an ATR measuring element allows the analysis of a wide range of samples.

Due to the modular principle of the ALPHA II FTIR spectrometer, only a few steps are needed to replace a measuring module and optimally adapt the spectrometer to almost any requirement. In addition to the ATR configuration, for example, modules for the measurement of reflection or transmission are available. These are typically used, when a contactless analysis of surfaces is required or when low-concentration components of a solution have to be quantified.

The ALPHA II is the next generation of its extremely successful predecessor. In addition to a higher measuring sensitivity, a temperature-stabilized detector and the electronically controlled CenterGlow™ source, the ALPHA II is characterized above all by the integrated touch panel. Due to the complete integration of spectrometer and user interface, the ALPHA II is particularly compact and self-explanatory in its operation. Tailored to the integrated touchscreen, the OPUS-TOUCH software guides the user intuitively through the measurement and evaluation process. It allows spectra to be easily measured and subsequently evaluated via a library search, a fast spectra comparison or a quantification method.

The ergonomic one-finger pressure mechanism of the Platinum ATR module simplifies the fixation of solid samples on the crystal. The freely rotatable pressure stamp provides the user unrestricted access to the measurement area, in order to comfortably place samples on the crystal and thoroughly clean it after the measurement.

INCOMING GOODS INSPECTION

Xanthan is a naturally occurring polysaccharide which is used, for example, as a thickening agent in the food and cosmetics industry. The incoming raw material needs to be thoroughly checked for any mixing or contamination with other white powders, while its identity must be unambiguously confirmed. The central question is whether a sample has the expected identity and composition. The quick spectra comparison of OPUS-TOUCH has two different comparison models available:

- ▶ **Verification:** Check whether the sample spectrum corresponds to a selected reference spectrum of the spectral comparison method.
- ▶ **Identification:** Compare the sample spectrum against all spectra inside the selected method.

For measurement, a background spectrum is first taken with a clean ATR crystal. Then a small amount of the powder is placed on the ATR crystal and pressed with the pressure mechanism onto the ATR crystal to ensure good contact between sample and crystal.

A preview spectrum indicates whether the crystal is clean and the sample is in good contact with the crystal.



After the measurement the complete spectrum is shown together with the evaluation possibilities for spectra comparison and identification.

A short touch shows the evaluation result: The Xanthan is well within specifications and can be accepted for further processing.

With “Next Sample” the measurement for the next sample can be started.



POLYMER ANALYSIS

The FTIR spectroscopy is an established method for the analysis of polymers and plastics. It can be used for the identification of different polymer types, additives and fillers and even allows to differentiate between different polymer subtypes (like HD- and LDPE or PA6, PA6.6 and PA12). Furthermore, it is possible to quantify parameters like

density, crystallinity or additive- and filler-content. In figure 2 the ATR spectra of three colourless plastic pellets from unlabelled containers are shown. The measuring process is very simple. Although the plastics look very similar from the outside, the different types of polymers are clearly distinguishable by their IR spectra, even for the untrained eye. Thanks to very extensive and dedicated spectral libraries, unknown substances are automatically identified within seconds.

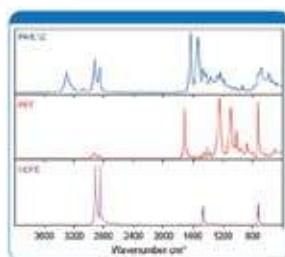


Figure 2: ATR spectra of different polymers



Figure 3: Identification result in OPUS-TOUCH after a search in spectral libraries.

(polymethylhydrosiloxane, PMHS). Obviously, the metal surface was contaminated with a very thin layer of silicon oil that was invisible to the naked eye.

SUMMARY

FTIR spectroscopy can be used in many ways in the field of materials testing. It quickly and easily provides both qualitative and quantitative results for a wide variety of sample types. Due to user friendly touch operation in the most modern spectrometers, it is now possible for untrained users to perform a measurement and evaluation without training.

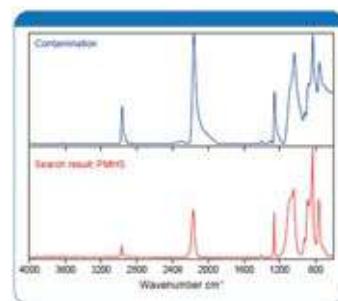


Figure 4: Spectrum of the contamination (blue) measured with the front reflection module and reference spectrum (red) found via a library search.



ALPHA II FTIR SPECTROMETER

with touch panel PC and Platinum ATR-module

Figure 4 shows the spectrum of the contamination (blue) and the reference spectrum found by spectra search

Reducing cost per analysis for Kjeldahl

A Resource-Efficient Approach to Kjeldahl Nitrogen Determination
Using BUCHI's Reaction Detection Sensor® Technology

The Kjeldahl method is enhanced by the Reaction Detection Sensor (RDS) technology, which significantly reduces sodium hydroxide usage and promoting cost efficiency and environmental sustainability. Traditional methods often use an excess of sodium hydroxide for alkalization, while the RDS technology optimizes this by accurately detecting the alkalization endpoint for a more environmentally friendly analysis.

Keywords or phrases:

Reaction Detection Sensor (RDS)-assisted Kjeldahl analysis, reduction of sodium hydroxide consumption, cost-per-analysis optimization in Kjeldahl distillation, environmentally sustainable nitrogen determination, automated alkalization endpoint detection, high-throughput Kjeldahl protein analysis, resource-efficient Kjeldahl distillation and titration

INTRODUCTION

The Kjeldahl method is an official worldwide standard that has been approved by various scientific associations including AOAC International, AACC International, AOCS, DIN, EPA, ISO, JAS, and USDA. It is mainly applied in the food and beverage industry but also used in environmental analysis, feed, pharma and the agricultural industry for the determination of nitrogen, protein, nitrates and ammonium. Due to the popularity of this method and its wide range of applications, there is a great demand to make the analysis more cost-effective.

In this Application Note, several Kjeldahl measurements are presented to show the potential of the Reaction Detection Sensor (RDS) technology in saving sodium hydroxide and therefore, lowering the costs of the analysis and making it more environmentally friendly.

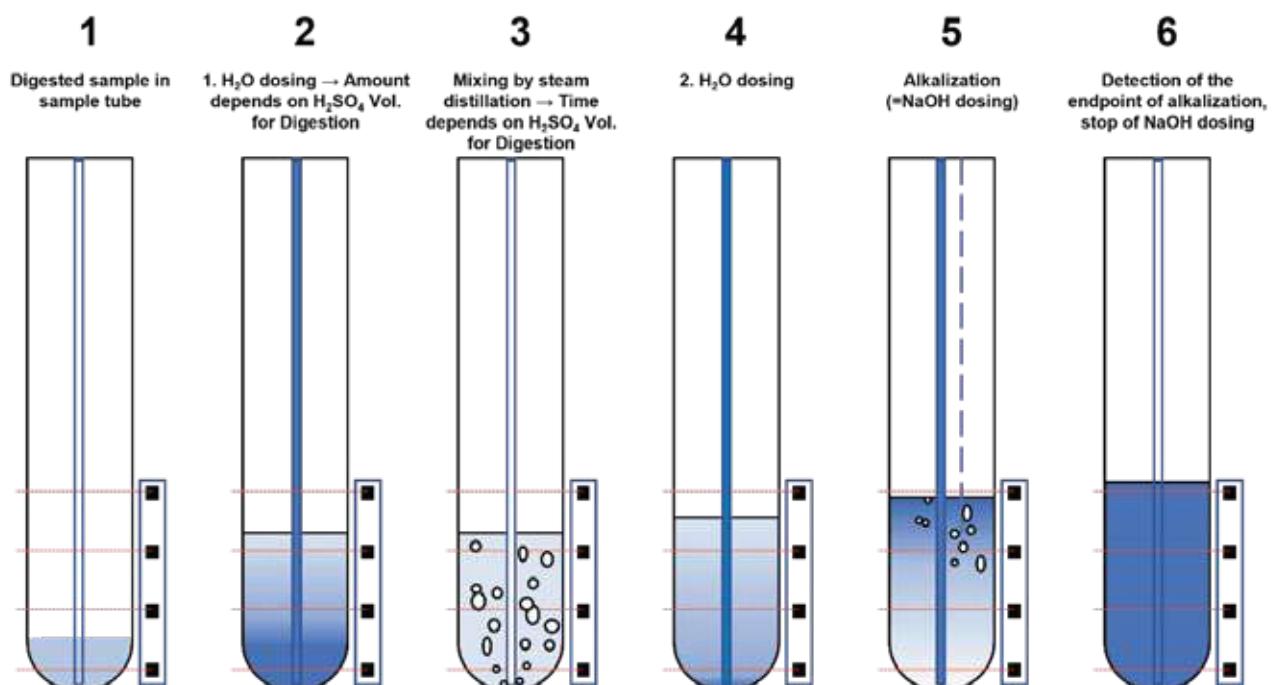


Figure 1: Functional principle of the Reaction Detection Sensor technology.

The Kjeldahl method starts with a catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salts. During this step, the organic nitrogen is converted into ammonium sulfate. In the next step, the solution is alkalized to liberate the ammonia which is further steam-distilled and determined by titration.^[3] Mineralization of the sample consumes sulfuric acid to form carbon dioxide, water and sulfur dioxide. The more organic matter in the sample, the more sulfuric acid is consumed, and the less acid remains in the sample tube.

In a classical Kjeldahl distillation, the dosed volume of sodium hydroxide solution is always the same and is based on the maximum possible amount of sulfuric acid after mineralization. It is generally dosed in excess to ensure completion of alkalization, which is crucial to release the ammonia and thereby quantify the protein content accurately. If a Kjeldahl distillation is performed with the RDS, the end of the alkalization is detected, and dosage of sodium hydroxide is stopped immediately. Therefore, less sodium hydroxide is dosed to samples that contain less sulfuric acid.

Figure 1 shows the functional principle of the RDS technology.

EQUIPMENT

- ▶ KjelDigester K-449 (1154492000).
- ▶ Scrubber K-415 TripleScrub ECO (114152331).
- ▶ MultiDist coupled with Metrohm EcoTitrator and Recirculating Chiller F-314 (11K36532211).
- ▶ Reaction Detection Sensor (11072666).
- ▶ Sample Tubes 300 mL (037377).
- ▶ BUCHI Mixer B-400 (034220).
- ▶ Analytical balance (accuracy ± 0.1 mg).

CHEMICALS AND MATERIALS

Chemicals:

- ▶ Sulfuric acid conc. 96%, VWR
- ▶ BUCHI Kjeldahl Tablets Titanium, Missouri, ECO & Antifoam.

- ▶ Sodium hydroxide 32%, VWR.
- ▶ BUCHI ready to use 4% boric acid pH 4.65 with Sher indicator.
- ▶ Sulfuric acid 0.1 mol/L, VWR.
- ▶ Neutralization solution for the Scrubber: 600 g sodium carbonate, calcined, technical, Synopharm and a spatula tip of bromthymol blue, Fluka (18460) diluted to 3 L with distilled water.
- ▶ Glycine, assay 99.7%, Sigma aldrich.
- ▶ BUCHI Weighing boats.

For safe handling please refer to all corresponding MSDS.

Sample name	Sample details	Declared / labeled protein content (g/100g)
Milk powder	Reference material from LVU (Nr. 18-4a)	20.38 \pm 0.37
Emmentaler mild	From Supermarket	29
Boiled sausage	Reference material from LVU (Nr. 20-01ab)	16.19 \pm 0.20
Milk chocolate	From Supermarket	7.7

Table 1: Sample details.

PROCEDURE

The determination of nitrogen and protein in various food samples includes the following steps:

- ▶ Sample homogenization
- ▶ Digestion of the sample
- ▶ Distillation and titration

SAMPLE HOMOGENIZATION

The milk powder sample was used directly for analysis. The boiled sausage sample was ground to a fine paste using a mixer. The Emmentaler cheese sample as well as the milk chocolate sample were cut into small pieces (\varnothing 2–3 mm).

DIGESTION OF THE SAMPLE

- ▶ After sample homogenization, digestion was carried out as follows:
- ▶ Samples were weighed in BUCHI weighing boats with nitrogen free paper. Each weighed portion of sample was placed in a 300 mL Sample Tube.
- ▶ The Kjeldahl/Antifoam Tablets and the corresponding volume of sulfuric acid (conc. 96%) were added.

- ▶ Additional blanks (chemicals without the sample) as well as reference samples (Glycine) were prepared.
- ▶ The racks containing the blanks and the samples were inserted into the Digestion Unit (KjelDigester K-449) and the suction module was closed.
- ▶ The digestion was started.

Table 2, 3 and 4 show an overview of the samples that were analyzed in the course of this study. The corresponding digestion methods are shown in Table 5, 6 and 7.

No.	Type	Kjeldahl Tablets type	Kjeldahl Tablets [pcs.]	Antifoam Tablets [pcs.]	Conc. H ₂ SO ₄ [mL]	Sample weight [g]
1–5	Blank	Titanium	1	8	-	-
6–10	Reference (Glycine, 99.7% purity)					0.09–0.10 g
11–15	No. 1: Milk powder					0.4–0.5 g
15–20	No. 2: Emmentaler					0.8–1.0 g
21–25	No. 3: Boiled sausage					1.6–1.8 g
26–30	No. 4: Milk chocolate		2	15	0.6–0.7 g	0.6–0.7 g
31–35	Blank					-
36–40	Reference (Glycine, 99.7% purity)					0.09–0.10 g
41–45	No. 1: Milk powder					0.4–0.5 g
46–50	No. 2: Emmentaler					0.9–1.0 g
51–55	No. 3: Boiled sausage					1.8–2.0 g
56–60	No. 4: Milk chocolate					1.0–1.1 g

Table 2: Overview of the samples that were digested using Kjeldahl Tablets Titanium.

No.	Type	Kjeldahl Tablets Type	Kjeldahl Tablets [pcs.]	Antifoam Tablets [pcs.]	Conc. H ₂ SO ₄ [mL]	Sample weight [g]
61–65	Blank	Missouri	1	10	-	-
66–70	Reference (Glycine, 99.7% purity)					0.09–0.10 g
71–75	No. 1: Milk powder					0.4–0.5 g
76–80	No. 2: Emmentaler					0.8–1.0 g
81–85	No. 3: Boiled sausage					1.6–1.8 g
86–90	No. 4: Milk chocolate		2	20	0.6–0.7 g	0.6–0.7 g
91–95	Blank					-
96–100	Reference (Glycine, 99.7% purity)					0.09–0.10 g
101–105	No. 1: Milk powder					1.0–1.2 g
106–110	No. 2: Emmentaler					0.9–1.0 g
111–115	No. 3: Boiled sausage					1.8–2.0 g
116–120	No. 4: Milk chocolate					1.2–1.4 g

Table 3: Overview of the samples that were digested using Kjeldahl Tablets Missouri.

No.	Type	Kjeldahl Tablets Type	Kjeldahl Tablets [pcs.]	Antifoam Tablets [pcs.]	Conc. H ₂ SO ₄ [mL]	Sample weight [g]
121–125	Blank	ECO	1	8	-	-
126–130	Reference (Glycine, 99.7% purity)					0.09–0.10 g
131–135	No. 1: Milk powder					0.4–0.5 g
136–140	No. 2: Emmentaler					0.8–1.0 g
141–145	No. 3: Boiled sausage					1.6–1.8 g
146–150	No. 4: Milk chocolate		2	16	0.6–0.7 g	0.6–0.7 g
151–155	Blank					-
156–160	Reference (Glycine, 99.7% purity)					0.09–0.10 g
161–165	No. 1: Milk powder					1.0–1.2 g
166–170	No. 2: Emmentaler					0.9–1.0 g
171–175	No. 3: Boiled sausage					1.8–2.0 g
176–180	No. 4: Milk chocolate					1.2–1.4 g

Table 4: Overview of the samples that were digested using Kjeldahl Tablets ECO.

Step	Temperature [°C]	Time [min]
1	250	0
2	280	20
3	380	30
4	420	80
Cooling	-	35

Table 5: Digestion parameters for samples no. 1–60 (Kjeldahl Tablets Titanium, 8 & 15 mL of conc. H₂SO₄).

Step	Temperature [°C]	Time [min]
1	250	0
2	280	20
3	380	30
4	420	90
Cooling	-	35

Table 6: Digestion parameters for samples no. 61–120 (Kjeldahl Tablet Missouri, 10 & 20 mL of conc. H₂SO₄).

Step	Temperature [°C]	Time [min]
1	250	0
2	280	20
3	380	30
4	420	100
Cooling	-	35

Table 7: Digestion parameters for samples no. 121–180 (Kjeldahl Tablet ECO, 8 & 16 mL of conc. H₂SO₄).

NOTE: As a first indication of successful digestion, the digested sample should be clear and blue green, with acceptable recoveries of a reference substance.

DISTILLATION AND TITRATION

After digestion, the samples were analyzed as follows:

- ▶ Alkalization of the sample to free up the nitrogen (ammonium).
- ▶ Steam distillation of the ammonia into boric acid receiver.
- ▶ Determination of the nitrogen content by boric acid titration.

Boric acid titration was carried out using sulfuric acid as titrant. An excess of boric acid guaranteed that all ammonia was bound in a complex. Table 8 shows the distillation and titration parameters. Before sample distillation, a priming was performed with the method used for sample analysis.

Method parameters MultiDist		Instrument Settings	
Reaction Detection	On	MaxAccuracy mode	On
H ₂ SO ₄ Vol. for digestion	* mL	Chiller / Tap water	Chiller F-314
Steam steps	No	Chiller temperature	15 °C
Steam power	1	AutoDist mode	On
Level detection	Off	Titrator model	Metrohm Eco Titrator
Distillation time	180 s		
Stirrer speed distillation	5		
Titration type	Boric Acid Titration		
H ₃ BO ₃ Volume	60 mL (4%)		
Stirrer speed titration	8		
Titration start time	160 s		
Sample tube aspiration	30 s		
Receiver aspiration	25 s		

Table 8: Parameters for distillation and titration with MultiDist (samples no. 1–180).

*Sample No. 1–30: 8 mL

Sample No. 31–60: 15 mL

Sample No. 61–90: 10 mL

Sample No. 91–120: 20 mL

Sample No. 121–150: 8 mL

Sample No. 151–180: 16 mL

Automated Titration with Eco Titrator

Method Eco Titrator	BUCHI blank; BUCHI sample
Titrant	H SO 0.1 mol/L
Sensor type	Potentiometric (pH)
Endpoint pH	4.65

wN: Weight fraction of nitrogen.

V_{Sample}: Amount of titrant for the sample [mL].

V_{Blank}: Mean amount of titrant for the blank [mL].

z: Molar valence factor (1 for HCl, 2 for H₂SO₄).

c: Titrant concentration [mol/L].

f: Titre value (for commercial solutions normally 1.000; refer to the product certificate).

MN: Molecular weight of nitrogen (14.007 g/mol).

m_{Sample}: Sample weight [g].

1000: Conversion factor [mL to L].

%N: Percentage weight of nitrogen.

%N_{Gly}: Percentage weight of nitrogen corrected for the purity of reference substance glycine [%].

%P: Percentage weight of protein.

P: Purity of the reference substance Glycine [%] as declared by the manufacturer.

PF: Sample-specific protein factor (sample type no. 1 & 2 / milk powder & Emmentaler: 6.38; sample type no. 3 & 4 / boiled sausage & milk chocolate: 6.25).

CALCULATION

The results were calculated as a percentage of nitrogen. In order to calculate the protein content of the sample, the nitrogen content is multiplied with a sample-specific protein factor. The following equations (1), (2), and (3) were used to calculate the results. For the reference substance, the purity of the Glycine is considered in equation (4).

$$wN = \frac{(V_{\text{Sample}} - V_{\text{Blank}}) \cdot z \cdot c \cdot f \cdot MN}{m_{\text{Sample}} \cdot 1000} \quad (1)$$

$$\%N = wN \cdot 100\% \quad (2)$$

$$\%P = wN \cdot PF \cdot 100\% \quad (3)$$

$$\%N_{\text{Gly}} = \frac{\%N \cdot 100}{P} \quad (4)$$

RESULTS

Measurements performed using Kjeldahl Tablets Titanium & Antifoam Tablets

Table 9 and 10 show the results of the samples that were digested using Kjeldahl Tablets Titanium & Antifoam Tablets. The relative standard deviations (RSD) of the blanks (required: < 5%) and the samples (required: < 1%) are fine. Besides that, the recovery rates of the reference samples are

within the required range (98.0–102.0%) and the determined protein contents are very close to the expected values.

Compared to the standard method (KjelOptimizer App), up to Ø 43.0% of NaOH per sample was saved for the first measurement series due to the RDS technology (Table 9). In total, 35.3% (=402 mL) of NaOH was saved. For the second measurement series, up to Ø 34.3% of NaOH per sample and 19.0% (= 359 mL) for the whole measurement series was saved (Table 10, p. 9).

Type		Titrated volume [mL]		N [%]	Ø Protein [%]		NaOH Volume [mL]		NaOH Vol. saved vs standard method [%]					
Blank	-	0.313	0.318	-	-	-	28	29.2 ± 2.4	23.2					
		0.320					30							
		0.321					27							
		0.320					28							
		0.315					33							
Ref (Gly)	-	0.096	18.81	-	-	101.120	29	29.4 ± 0.9	22.6					
		0.094					100.500							
		0.091					100.460							
		0.096					100.640							
		0.099					100.460							
Sample No. 1	-	0.460	3.24	20.70 ± 0.04	-	-	22	22.0 ± 0.7	42.1					
		0.458					21							
		0.479					23							
		0.487					22							
		0.487					22							
Sample No. 2	-	0.835	4.91	31.32 ± 0.17	-	-	25	22.6 ± 2.8	40.5					
		0.819					18							
		0.894					22							
		0.891					24							
		0.900					24							
Sample No. 3	-	1.693	2.63	16.41 ± 0.07	-	-	22	22.8 ± 0.8	40.0					
		1.634					22							
		1.641					23							
		1.672					24							
		1.705					23							
Sample No. 4	-	0.607	1.27	7.91 ± 0.01	-	-	20	21.6 ± 1.5	43.2					
		0.608					22							
		0.613					21							
		0.609					21							
		0.640					24							
Total NaOH Volume [mL]							738							
Expected NaOH Volume without RDS (38 mL/sample) [mL]							1140							
NaOH Volume saved [mL]							402							
NaOH saved [%]							35							

Table 9: Results of the measurements performed using 8 mL of conc. H₂SO₄, Kjeldahl Tablets Titanium & Antifoam Tablets (No. 1–30; RSD: blank = 1.00%; ref = 0.25%; S 1 = 0.17%; S 2 = 0.55%; S 3 = 0.43%; S 4 = 0.17%).

Type		Titrated volume [mL]		N [%]	Ø Protein [%]	NaOH Volume [mL]		NaOH Vol. saved vs standard method [%]				
Blank	-	0.2640	0.261	-			60	5.4				
		0.2600		-			60					
		0.2600		-			59	5.4				
		0.2610		-			59					
		0.2620		-			60					
Ref (Gly)	0.0945	6.5690	18.69	-	-	100.71	58	7.6				
	0.0930	6.4560				100.50	57					
	0.0933	6.4900				100.72	59					
	0.0996	6.8620				99.99	59					
	0.0959	6.6420				100.39	58					
Sample No. 1	0.4262	5.1570	3.24	20.69	20.58 ± 0.02	-	51	15.2				
	0.4761	5.7370					52					
	0.4578	5.5230					53					
	0.4164	5.0510					61					
	0.4230	5.1160					50					
Sample No. 2	0.9179	16.3960	4.91	31.36	31.11 ± 0.26	-	47	26.3				
	0.9740	16.9710					45					
	0.9595	16.9120					45					
	0.9436	16.5770					48					
	0.9478	16.8280					47					
Sample No. 3	1.9265	17.9830	2.63	16.43	16.15 ± 0.03	-	44	25.1				
	1.8788	17.5930					48					
	1.8711	17.4730					50					
	1.9203	17.9590					48					
	1.8676	17.4080					46					
Sample No. 4	1.0941	5.1700	1.27	7.93	7.86 ± 0.01	-	41	34.3				
	1.0887	5.1350					40					
	1.0291	4.8720					44					
	1.0772	5.0840					42					
	1.0954	5.1640					40					
Total NaOH Volume [mL]							1531					
Expected NaOH Volume without RDS (38 mL/sample) [mL]							1890					
NaOH Volume saved [mL]							359					
NaOH saved [%]							19.0					

Table 10: Results of the measurements performed using 15 mL of conc. H₂SO₄, Kjeldahl Tablets Titanium & Antifoam Tablets (No. 31–60; RSD: blank = 0.57%; ref = 0.27%; S 1 = 0.08%; S 2 = 0.84%; S 3 = 0.17%; S 4 = 0.09%).

Type		Titrated volume [mL]	N [%]	Ø Protein [%]	NaOH Vol. [mL]	Ø NaOH Vol. saved vs standard [%]	
Blank	-	0.296	-	-	40	39.2 ± 2.9	
		0.298			44		
		0.294			38		
		0.295			37		
		0.293			37		
		18.69			100.44		
Ref (Gly)	-	18.48	-	-	99.32	40.2 ± 0.4	
		18.55			99.7		
		18.63			100.12		
		18.61			100.05		
		0.0913	6.373	100.44	40		
Sample No. 1	-	0.0924	6.378	99.32	40	40.2 ± 0.4	
		0.0973	6.725	99.7	40		
		0.0962	6.679	100.12	41		
		0.0919	6.389	100.05	40		
		0.4599	5.604	32	32	31.8 ± 0.8	
Sample No. 1	-	0.464	5.649	31	31		
		0.4605	5.615	33	33		
		0.4847	5.897	31	31		
		0.4882	5.948	32	32		
		3.24	20.67	32	32		
Sample No. 2	-	3.24	20.66	31	31	31.8 ± 0.8	
		3.24	20.69	33	33		
		3.24	20.7	31	31		
		3.25	20.74	32	32		
		4.77	30.41	31	31		
Sample No. 2	-	4.81	30.71	29	29	31.8 ± 0.8	
		4.82	30.72	31	31		
		4.82	30.73	33	33		
		4.79	30.59	27	27		
		4.77	30.41	31	31		
Sample No. 3	-	1.729	16.489	35	35	30.2 ± 2.3	
		1.6854	15.907	24	24		
		1.6772	15.871	29	29		
		1.6708	15.982	22	22		
		1.7831	16.754	35	35		
Sample No. 4	-	2.63	16.43	35	35	30.2 ± 2.3	
		2.6	16.25	24	24		
		2.61	16.29	29	29		
		2.64	16.47	22	22		
		2.59	16.19	35	35		
Sample No. 4	-	1.26	7.88	29	29	30.2 ± 2.3	
		1.26	7.9	25	25		
		1.26	7.88	31	31		
		1.26	7.88	36	36		
		1.26	7.88	23	23		
Total NaOH Volume [mL]					996		
Expected NaOH Volume without RDS (38 mL/sample) [mL]					1350		
NaOH Volume saved [mL]					354		
NaOH saved [%]					26		

Table 11: Results of the measurements performed using 10 mL of conc. H₂SO₄, Kjeldahl Tablets Missouri & Antifoam Tablets (No. 61–90; RSD: blank = 0.58%; ref = 0.38%; S 1 = 0.12%; S 2 = 0.40%; S 3 = 0.65%; S 4 = 0.10%).

Measurements performed using Kjeldahl Tablets ECO & Antifoam Tablets

Table 13 and 14 show the results of the samples that were digested using Kjeldahl Tablets ECO & Antifoam Tablets. The

relative standard deviations (RSD) of the blanks and the samples are fine. Moreover, the recovery rates of the reference samples are within the required range (98.0–102.0%) and the determined protein contents are very close to the expected values.

Compared to the standard method (KjelOptimizer App), up to an average of 39.5% NaOH per sample was saved for the first measurement series (Table 13). In total, the NaOH consumption was reduced by 28.1% (= 320 mL). For the second measurement series (Table 14, p. 13), up to 17.3% of

NaOH per sample was saved. In total, 9.4% (= 188 mL) of the NaOH was saved. However, up to 2.7% more NaOH in average was used for samples containing high volumes of conc. sulfuric acid after digestion (blanks & references).

Type		Titrated volume [mL]		N [%]		Ø Protein [%]		NaOH Vol. [mL]		Ø NaOH Vol. saved vs standard [%]						
Blank	-	0.302	0.3062	-	-	-	-	101	84.8 ± 13.5	-4.7						
		0.304						74								
		0.307						74								
		0.311						77								
		0.307						98								
		0.0959						99.96								
Ref (Gly)		6.679	18.65	-	-	-	-	104	92.0 ± 10.3	-13.6						
		6.557						100.14								
		6.885						92								
		6.75						100.16								
		6.761						85								
Sample No. 1		12.711	20.65 ± 0.01	20.65	-	-	-	100.24	63.2 ± 12.8	22						
		13.072						79								
		12.198						100								
		13.146						100								
		13.059						100								
Sample No. 2		17.422	31.88 ± 0.11	31.88	-	-	-	57	74.0 ± 13.7	8.6						
		16.532						64								
		17.25						85								
		17.629						54								
		16.964						56								
Sample No. 3		17.89	16.17 ± 0.02	16.17	-	-	-	63	74.6 ± 10.7	7.9						
		18.621						74								
		18.348						66								
		18.476						81								
		18.697						89								
Sample No. 4		6.005	7.89 ± 0.01	7.89	-	-	-	55	56.2 ± 3.8	30.6						
		6.099						53								
		6.319						53								
		6.423						58								
		6.523						62								
Total NaOH Volume [mL]								2224								
Expected NaOH Volume without RDS (81 mL/sample) [mL]								2430								
NaOH Volume saved [mL]								206.0								
NaOH saved [%]								8.5								

Table 12: Results of the measurements performed using 20 mL of conc. H₂SO₄, Kjeldahl Tablets Missouri & Antifoam Tablets (No. 91-120; RSD: blank=1.00 %; ref=0.10 %; s 1=0.05 %; s 2=0.34 %; s 3=0.14 %; s 4=0.12 %).

Measurements performed using Kjeldahl Tablets ECO & Antifoam Tablets

Table 13 and 14 show the results of the samples that were digested using Kjeldahl Tablets ECO & Antifoam Tablets. The

relative standard deviations (RSD) of the blanks and the samples are fine. Moreover, the recovery rates of the reference samples are within the required range (98.0–102.0%) and the determined protein contents are very close to the expected values.

Type		Titrated volume [mL]		N [%]		Ø Protein [%]		NaOH Vol. [mL]		Ø NaOH Vol. saved vs standard [%]				
Blank	-	0.322	0.3198	-	-	-	-	27	31.4 ± 3.6	17.4				
		0.315						36						
		0.321						30						
		0.323						34						
		0.318						30						
Ref (Gly)	-	0.0981	6.814	-	18.58	-	99.88	31	28.8 ± 1.8	24.2				
		0.0935	6.547		18.69		100.49	29						
		0.0993	6.941		18.72		100.6	27						
		0.0923	6.467		18.69		100.49	27						
		0.0953	6.613		18.54		99.63	30						
Sample No. 1	-	0.4329	5.328	0.3198	3.25	20.72	20.69 ± 0.02	30	26.6 ± 2.3	30				
		0.404	4.982		3.24	20.67		27						
		0.4838	5.908		3.24	20.69		24						
		0.4496	5.515		3.24	20.69		25						
		0.4816	5.878		3.24	20.67		27						
Sample No. 2	-	0.9956	17.203	-	4.76	30.37	30.64 ± 0.20	17	23.0 ± 6.0	39.5				
		0.9698	16.941		4.81	30.69		21						
		0.9527	16.697		4.83	30.78		22						
		0.944	16.383		4.78	30.47		22						
		0.9845	17.309		4.84	30.9		33						
Sample No. 3	-	1.6239	15.628	-	2.65	16.54	16.45 ± 0.06	23	27.2 ± 5.7	28.4				
		1.6496	15.803		2.63	16.47		27						
		1.6907	16.082		2.62	16.36		37						
		1.7391	16.585		2.63	16.41		24						
		1.6514	15.833		2.64	16.48		25						
Sample No. 4	-	0.6258	3.137	-	1.26	7.9	7.89 ± 0.02	26	27.0 ± 1.6	28.9				
		0.6226	3.108		1.26	7.86		27						
		0.697	3.461		1.27	7.91		28						
		0.6303	3.158		1.26	7.9		25						
		0.64	3.2		1.26	7.9		29						
Total NaOH Volume [mL]								820						
Expected NaOH Volume without RDS (38 mL/sample) [mL]								1140						
NaOH Volume saved [mL]								320						
NaOH saved [%]								28.1						

Table 13: Results of the measurements performed using 8 mL of conc. H₂SO₄, Kjeldahl Tablets ECO & Antifoam Tablets (No. 121–150; RSD: blank = 0.91%; ref = 0.39%; s 1 = 0.09%; s 2 = 0.64%; s 3 = 0.38%; s 4 = 0.22%).

Compared to the standard method (KjelOptimizer App), up to an average of 39.5% NaOH per sample was saved for the first measurement series (Table 13). In total, the NaOH consumption was reduced by 28.1% (= 320 mL). For the second measurement series (Table 14, p. 13), up to 17.3% of

NaOH per sample was saved. In total, 9.4% (= 188 mL) of the NaOH was saved. However, up to 2.7% more NaOH in average was used for samples containing high volumes of conc. sulfuric acid after digestion (blanks & references).

Type		Titrated volume [mL]		N [%]		Ø Protein [%]		NaOH Vol. [mL]		Ø NaOH Vol. saved vs standard [%]						
Blank	-	0.3130	0.3090	-	-	-	-	76	68.8 ± 5.0	-2.7						
		0.3080						66								
		0.3100						72								
		0.3050						66								
		0.3090						64								
Ref (Gly)	-	0.095	6.5940	18.57	-	-	-	99.88	66.6 ± 3.7	0.6						
		0.0949	6.5780					100.49								
		0.0967	6.7100					100.6								
		0.0963	6.6960					100.49								
		0.0908	6.3300					99.63								
Sample No. 1	-	0.4732	5.7540	3.23	20.61	20.62 ± 0.01	-	57	66.6 ± 3.7	16.1						
		0.4635	5.6480					55								
		0.4484	5.4690					54								
		0.4614	5.6280					56								
		0.4789	5.8220					59								
Sample No. 2	-	0.9418	16.3330	4.78	30.47	30.77 ± 0.21	-	64	56.2 ± 1.9	10.7						
		0.9143	16.1590					56								
		0.9202	16.1990					51								
		0.913	15.9130					66								
		0.9675	16.9450					62								
Sample No. 3	-	1.9754	18.4720	2.58	16.13	16.14 ± 0.03	-	65	59.8 ± 6.2	17.3						
		1.9465	18.2240					50								
		1.9767	18.4430					53								
		1.961	18.3400					60								
		1.9851	18.6100					49								
Sample No. 4	-	1.0445	5.0110	1.26	7.90	7.90 ± 0.01	-	64	57.6 ± 10.3	14.0						
		1.059	5.0760					72								
		1.022	4.9110					50								
		1.1098	5.3090					47								
		1.0452	5.0080					55								
Total NaOH Volume [mL]								1822								
Expected NaOH Volume without RDS (38 mL/sample) [mL]								2010								
NaOH Volume saved [mL]								188								
NaOH saved [%]								9.4								

Table 14: Results of the measurements performed using 16 mL of conc. H₂SO₄, Kjeldahl Tablets ECO & Antifoam Tablets (No. 151–180; RSD: blank = 0.84%; ref = 0.15%; s 1 = 0.07%; s 2 = 0.68%; s 3 = 0.16%; s 4 = 0.07%).

Comparison Kjeldahl Distillation with & without Reaction Detection Sensor

Table 15 shows an overview of the distillation parameters/steps for a Kjeldahl distillation with and without RDS. Instead of three parameters (H₂O Volume, NaOH Volume & Reaction Time), only one parameter (H₂SO₄ Vol. for Digestion) needs to be defined in the method if the RDS is used. Based on the entered value, the system automatically performs a sample pre-conditioning and a water dosage. Furthermore, the end of the alkalization is detected and the dosing of NaOH is stopped automatically.

Distillation parameter/step	Without RDS	With RDS
Sample pre-conditioning	Not performed	Performed
H ₂ SO ₄ Vol. for digestion	Not required	Required (8–30 mL)
H ₂ O Volume	Required (xx mL)	Not required (automatically closed)
NaOH Volume	Required (xx mL)	Not required (dosage stops automatically after detection)
Reaction Time	Required (xx s)	Not required

Table 15: Comparison Kjeldahl method programming with and without RDS.

CONCLUSION

The determination of nitrogen/protein in milk powder, Emmentaler cheese, boiled sausage and milk chocolate using the KjelDigester K-449 and KjelLine systems with the Reaction Detection Sensor provides reliable and reproducible results. All results correspond well to the labelled values with low relative standard deviations. Furthermore, all recovery rates for Glycine are within the required range (98.0–102.0%).

Due to the RDS technology, sodium hydroxide solution was saved in all measurement series. The RDS shows the best performance for samples digested with Kjeldahl Tablets Titanium. In those cases, an average NaOH saving of up to

43.2% per sample was reached with a very reproducible detection of the alkalization endpoint. For samples containing Kjeldahl Tablets Missouri or ECO, the performance is less good. Compared to a Kjeldahl distillation without RDS, even more sodium hydroxide is dosed in some cases, especially if a high volume of sulfuric acid remains in the sample tube after digestion (e.g. blank, reference or sample with little organic matter). Nevertheless, the RDS can also be used with these Kjeldahl Tablets to significantly reduce sodium hydroxide consumption. At low sulfuric acid volumes for digestion (e.g. 8 or 10 mL), sodium hydroxide was saved in all measurements. In addition, the sodium hydroxide consumption is significantly reduced for samples containing a high amount of organic matter and therefore, consumed a lot of sulfuric acid during digestion.

REFERENCES

- [1] Technical Note No. 772: Reaction Detection Sensor
- [2] Kjeldahl Optimizer App
- [3] Kjeldahl Knowledge Base
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- [5] Operation Manual of Scrubber K-415
- [6] Operation Manual of Kjel Line
- [7] Operation Manual of Dist Line
- [8] Application Note – N°. 777/2021



Kjel Line

Meeting the highest demands in nitrogen determination





Boosting Food Production Efficiency

How Mettler Toledo Halogen Moisture Analysis Enables Faster QC, Lean Changeovers, and Quicker Batch Release

Moisture content affects the quality of many food products, which must retain their properties up until the time they are consumed. For example, in snack production, moisture content impacts product texture, taste and shelf life, which means it helps to determine optimum storage conditions. However, moisture content determination has historically been a time-consuming process.

This application note describes how considering the lean method of Single-Minute Exchange of Die (SMED), along with other lean concepts in the moisture analysis process, can contribute to more efficient QC testing and faster batch release to help optimize production.

Keywords or phrases:

Halogen Moisture Analysis (HMA), Single-Minute Exchange of Die (SMED), food quality control, loss-on-drying (LOD), lean manufacturing, batch release optimization, rapid changeover strategies in QC testing, accelerating batch approval with halogen moisture analysis

INTRODUCTION

Generally, as a part of a continual improvement process (CIP1 or CI), SMED incorporates efforts to reduce the time it takes to complete equipment setup or changeover. The concept is to “externalize” as many changeover steps as possible — that is, make it possible for them to be performed while equipment is running.

This serves to:

- ▶ Reduce downtime
- ▶ Simplify changeover processes
- ▶ Optimize productivity

The original goal of SMED was to reduce changeover time to single digits, or less than 10 minutes. After “lean” principles such as CIP and SMED had been applied for the first time, tool

changeover times went from hours in the 1950s to minutes in the 1990s.

Today, “zero changeover (a kind of super SMED)” is even more dynamic, with changeover times reduced to less than three minutes. In food processing, this kind of rapid changeover has proven key to reducing the time it takes to approve production batches and therefore improve throughput.

For moisture analysis, loss-on-drying (LOD) in a drying oven is the typical reference method noted in food regulations. However, a halogen moisture analyser such as the HX204 from METTLER TOLEDO — which uses halogen moisture analysis (HMA) and is based on the LOD principle — can provide much faster and cost-effective moisture determination. In fact, results are typically delivered in minutes instead of hours.

Using HMA, the thermogravimetric principle is applied: a sample is heated by absorption of infrared radiation from halogen lamp. While the heat source is different, the principle is similar. Hence, the results from the moisture analyser and the drying oven are comparable.

However, HMA, can match the drying-oven reference method in minutes.

Depending on the sample, a moisture analyser requires just 3 to 15 minutes. Moisture determination using a halogen moisture analyser can be even faster with a unique and innovative function known as QuickPredict.

In just two minutes, METTLER TOLEDO QuickPredict predicts the expected measurement result. This allows the laboratory or production manager to decide at a much earlier stage what further steps need to be taken if an OOE (Out-of-Expectation) result is predicted.

When applied to moisture analysis, a faster setup and changeover of samples significantly reduce time to result. Overall, fast moisture analysis speeds up sample processing, which in turn reduces production loss and output variability.

IMPLEMENTING SMED IN FOOD PRODUCTION AND QUALITY CONTROL

Reducing changeover with SMED in any production process requires thorough analysis of the given process.

Generally, this will involve the following actions:

- ▶ Observe the current process.
- ▶ Separate the external and internal production steps.
- ▶ Internal activities are those performed when the process is stopped. External activities can be done while production is ongoing.
- ▶ Convert as many internal processes as possible to external processes.
- ▶ Simplify remaining internal activities.
- ▶ Document the new process.
- ▶ Review new method results to see if productivity gains have been made.

SUPPORTING SMED USING HMA

One snack-food company sought faster, higher-quality moisture content results in batch and sample control at the line based on thermogravimetric measurement.

The analyser would be used by shift workers instead of trained lab technicians. This required equipment that was robust and easy-to-use.

Could the HX204 provide the required control in much less time? And could the sampling process and cleanup be reduced to single digits (less than 9 minutes 59 seconds)?

It turns out, the HX204 could reduce the sampling process to meet SMED standards, and even reduced sample changeover to super-SMED status, as described in the following section.

ANALYSING SMED IN-PROCESS

Using the HX204, the production-line sampling process at the company in question involved the following seven steps:

- ▶ The user takes the sample (e.g. potato chips).
- ▶ The sample is prepared (crunched, homogenized).
- ▶ The user logs into the operator's profile. Security levels can also be set, so the operator is only able to change the settings required for his or her profile.



- ▶ The method is started with a pre-defined shortcut. This company uses A and B buttons for method saving. Behind each letter is a pre-defined method e.g. for crisp and cereals.
- ▶ The sample is applied onto the pan and weighed. It is important to always use the same sample weight (e.g. 5 g $\pm 10\%$). HX204 offers a weighing aid feature that, when activated, ensures that the operator cannot start the measurement if the sample weight is out of tolerance. Furthermore, with HX204, this is an ergonomic process because of the moisture analyzer stand. (Operators tend to suffer from problems caused by poorly designed devices. Providing comfort during work is an aspect of CIP.)
- ▶ The measurement is in progress.
- ▶ The result is displayed. Depending on the method and sample characteristics, results will be showed on the terminal in less than 10 minutes. Result documentation can be done via network printer or stored directly as a PDF-A file fulfilling the highest security requirements, such as those of the FDA².



The measured sample and pan are removed, and a new pan is added, making the equipment ready for the next sample almost immediately. In case of spills, they can be quickly cleaned/wiped off the flat stainless-steel surfaces. The unit's construction reduces sample changeover time to less than 30 seconds (well under the required 9:59 for SMED and exceeding zero changeover).

The results of the super-SMED process can be presented in terms of ROI using the following calculation:

Before, SMED (as found) used to take 11 minutes, 3 times per day (3 shifts 6 days per week), 200 working days per year. In total non-added value time: 6,600 minutes per year or 110 hours were lost to the process.

SMED (as left) takes 30 seconds \times 3 shifts, 6 days per week or 200 working days per year. The zero-changeover process required just 300 minutes or 5 hours over the course of the year.

The benefits of the changes over a year are as follows:

- ▶ 105 hours saved
- ▶ Fewer non-added value steps thanks to implementing KPI (Key Performance Indicators) and SMED to reduce sample setup and changeover times for each device.
- ▶ A process (SMED) that can be applied to all the other devices in their manufacturing plants and laboratories to create additional time, effort, and cost savings.

The device can also be tested for accuracy. With the in-built test procedure and SmartCal, a Moisture Analyzer Test Substance, an operator can easily test the device in 10 minutes.

SmartCal adheres to principles of Good Manufacturing Practice (GMP), Good Laboratory Practices (GLP) and ISO as a test substance certified in Bundesanstalt für Materialforschung und -prüfung (BAM), a Germany-based Testing Laboratory.



Moreover, EasyDirect™ Moisture Software enables the simplification and optimization of moisture analyzer data management by collecting data automatically, creating reports, visualizing results and more (www.mt.com/moisture-software).

ADVANTAGES OF SMED IMPLEMENTATION

Applying SMED to processes in your food production facility such as moisture determination, when combined with the latest technology, can bring the following benefits:

- ▶ **Accurate results.** In this instance, advanced halogen technology proved key to fast heating and precise temperature control.
- ▶ **Better use of bench space.** The device is small, barely the size of a piece of paper, hence optimizing lab space (another aspect of lean processing). The stand reduces the footprint further and enables ergonomic operation.
- ▶ **Shorter production time.** The reduction of moisture determination from hours to minutes, as well as a 30-second sample changeover and cleaning procedure,

makes moisture determination an easy, repeatable part of batch release.

- ▶ **Fast initiation.** The graphical One Click™ user interface significantly reduces instrument training time and also allows methods to be started with one motion.
- ▶ **Better process control.** Smart graphics including a real-time drying curve visualize the ongoing measurement on the display. The user can control charts and trend moisture content over time.
- ▶ **Improved safety and quality.** With password protected user profiles, safety is ensured for fully regulated operations. HX204 offers simpler setup, easier method-initiation.
- ▶ **User-friendly operation.** An ergonomic design helps to ensure operator safety and eliminate repetitive stress injuries.

▶ **Fast cleaning.** The flat surface of the weighing pan is completely smooth and sealed. Cleaning a moisture analyzer has never been as easy and fast.

Spills are contained in the draft shield and can be removed before the next measurement. The position of the display on the stand also helps to reduce the risk of getting the terminal dirty and protects it from damage caused by heavy-duty use.

▶ **Regulatory compliance.** Password-protected profiles, secure database management and more help to be compliant with regulations.

▶ **Data-collection ease.** Software featuring the EasyDirect functionality allows the connection of up to five devices to collect results in a central database.

It also provides excellent handling for shift workers and experienced lab technicians alike.



Halogen Moisture Analyzer HX204

The Role of Ultrapure Water for HPLC Analysis

Baseline integrity and contaminant-free eluants enabled by Sartorius Arium® ultrapure water



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The reliability, sensitivity and reproducibility of high-performance liquid chromatography greatly depends on the quality of water used as the mobile phase. Trace inorganic ions, organic contaminants, microorganisms, or particulates present in inadequately purified water can lead to baseline noise, retention time shifts, column fouling and the appearance of ghost or phantom peaks, particularly during gradient elution. This application note evaluates the suitability of ultrapure water produced by the Sartorius Arium® Pro VF system for use as an eluant in HPLC of water-soluble saccharides.

Keywords or phrases:

Ultrapure Water for HPLC Analysis, No ghost peaks – for optimal results

INTRODUCTION

HPLC is an analytical procedure for separation, identification and quantification of substances using liquid chromatography.

The beginnings of HPLC – High Pressure Liquid Chromatography – go back to the 60's. Thanks to improved column materials and equipment, it has come to be known as High Performance Liquid Chromatography since the end of the 70's.¹

In HPLC, the mixture to be separated is transferred to a column with a solvent (eluting agent) or with a solvent mixture (eluant | mobile phase), by an injector and a pump.

The column is a tube, in most cases of stainless steel, filled with the so- called stationary phase. The stationary phase usually consists of porous silica gel or polymer particles with chemical ligands bound on their surface. These ligands are responsible for the selective interactions between the analytes and the stationary phase, which are necessary for effective chromatographic separation. Depending on the sample and stationary phase, the separation mechanisms involved are, for example, adsorption by Van der Waals forces, ion exchange, ion exclusion, etc.

The substances of a sample are retained on the column packing material for different lengths of time and therefore exit the column after different retention times. The individual components of the sample are then registered by a detector and evaluated by a computer. The result is a chromatogram. The number of peaks corresponds to the number of separated components in the sample, and the area is proportional to the concentration of these separated components (according to Kromidas 20001).

Among the typical applications for HPLC is sugar analysis. This was performed within the scope of various tests conducted in order to characterize the quality of membranes. On the one hand, membranes were tested for their ability to remove sugar molecules, and, on the other hand, the activity of enzyme-immobilized membranes was determined.

For this purpose, sugars, such as raffinose, glucose and fructose, were assayed. These types of sugar can be specifically detected by using enzymatic methods like the GOD | POD assay for glucose² or spectroscopic methods, such as determination of fructose according to Dische & Borenfreund.³

In advanced analysis, sugar is now frequently assayed by thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC). These methods are used especially when mixtures containing several types of sugar must be separated.⁵

In HPLC as described here, the eluant must have especially high physical and chemical purity, and may not contain suspended mechanical particles or any dissolved substances

that can be released by the column at a delayed time and thus generate a signal. The quality of a solvent is often decisive for the reliability of an HPLC analytical run. The presence of trace contaminants during gradient elution can result in “ghost or phantom peaks”. Such trace substances accumulate in the column during an analytical run and are increasingly released when the eluting agent is subsequently changed. Water used as an eluant must be free of microorganisms. For this purpose, substances that prevent the growth of microbes and algae in the solvent mixture, such as copper salts or sodium azide, can be added.⁵ In doing so, the column manufacturer’s recommendations need to be followed as the use of incorrect additives can result in irreversible damage to the column.

Deionized or distilled water still contains considerable quantities of organic substances, which can cause ghost peaks.⁵ Contaminated solvents can lead to the buildup of deposits on the stationary phase and thus result in blockage of the column, which would be manifested by an increase in pressure and a shift in the running time for the samples.

EMPLOYING ARIUM® PRO VF TO PURIFY WATER FOR USE AS AN ELUANT

Water of the special quality required for HPLC can be purchased from various manufacturers or be produced directly on site for use on demand by employing a water purification system, such as the Arium® Pro VF system.

The following describes the tests performed for separation of sugar mixtures in which ultrapure water produced by Arium® Pro VF was used as the mobile phase (eluant).

DESCRIPTION OF THE ARIUM® PRO VF ULTRAPURE WATER SYSTEM

The Arium® Pro VF system has been designed to produce ultrapure water from pretreated drinking water by removing contaminants that are still present in this potable water feed. Production of ultrapure water requires continuous

recirculation and a constant water flow rate, which is achieved using a built-in pump system with controlled pressure.

The conductivity of the water is measured at the feed water inlet and directly at the downstream port (product water outlet). The Arium® Pro VF system used in the studies described in this paper (a predecessor model with the same technical specifications as the currently redesigned system shown next page) works with two different cartridges. These are filled with a special active carbon adsorber and mixed-bed ion exchange resins to deliver ultrapure water with a low TOC content.

In addition, the system has an integrated UV lamp that has an oxidizing effect at wavelengths of 185 | 254 nm.

Moreover, the Arium® Pro VF ultrapure water system has a built-in ultrafilter module used as a crossflow filter. The ultrafilter membrane incorporated in this filter retains colloids, microorganisms, endotoxins, RNA and DNA. A 0.2 μ m final filter installed at the water outlet serves to remove particulates and bacteria during dispensing of the ultrapure water produced. The process that the unit employs to purify water is depicted in Figure 1 (flow diagram of Arium® Pro VF).

MATERIALS AND METHOD

The samples were analyzed using an HPLC Agilent 1200 Series system with a Rezex RNM Carbohydrate Na+ 8 % HPLC column.

This column is filled with a cross-linked polystyrene-divinylbenzene- copolymer modified by sodium sulfonate groups and uses an ion exclusion mechanism. This means that analytes are separated based on different ionic interactions.

Because of the sulfonate groups on the surface of this column packing material, the pores have a negative charge. As a result, negatively charged molecules cannot penetrate into the pores of the material, which causes them to elute earlier. This ion exclusion mechanism is based on Gibbs-Donnan equilibrium that governs the behaviour of ions near a membrane. Analytes that are able to penetrate the

membrane's pores are subsequently separate based on steric differences as well as on hydrophobic and polar interactions with the functional groups on the surface of the stationary phase.

The retention times for various types of sugar are determined by the absorbance of the refractive index (RI) signal.

This RI signal is expressed as a dimensionless number in nano Refractive Index Units (nRIU) and indicates the difference between the refractive index of the sample in the sample cell and the mobile phase in the reference cell.

Ultrapure water produced by the Arium® Pro VF system was used as the mobile phase. For degassing the eluent in the HPLC system, this ultrapure water was filtered by vacuum through a Sartolab BT 500 Bottle Top disposable unit equipped with a 0.2 µm membrane (Sartorius Sartolab BT 180C5).

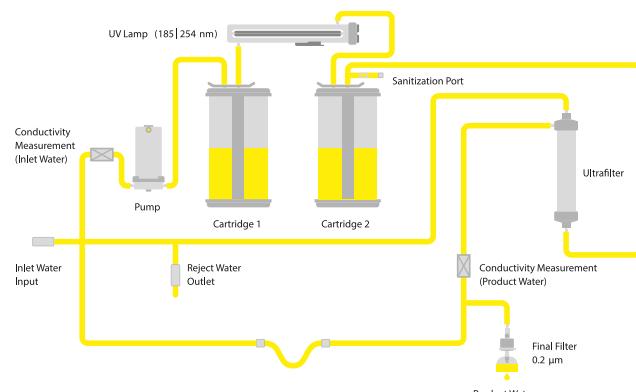


Figure 1: Schematic flow diagram of the Arium® Pro VF ultrapure water system (the valves and their controllers are omitted for better clarity).

PROCEDURE FOR HPLC ANALYSIS

To prepare for the analytical runs, the Rezex column was heated to 75 °C in the column compartment (heater) and flushed overnight with Arium® Pro VF ultrapure water at 0.6 mL/min. The optical unit of the RI detector was heated to 35 °C. Samples to be analyzed were prepared using Arium® Pro VF ultrapure water and prefiltered through a 0.2 µm syringe

filter unit (Sartorius Minisart® RC4, no. 17822). The samples were analyzed using HPLC according to the parameters defined by an HPLC method6 (Table 1).

Flow rate [mL/min]	0.6
Time [min]	25
Maximum pressure [bar]	70
Temperature of the Column Compartment [°C]	75
Temperature of the RI detector [°C]	35
Injection volume [µL]	2

Table 1: HPLC method

Sugar	Retention Time [min]	
Raffinose	Fluka 83400	8.96
Maltose	SIGMA M5885	10.3
Glucose	ROTH 6887.0	12.53
Fructose	SIGMA F0127	13.62

Table 2: Retention Times for Sugar Samples Passed through a Rezex RNM Carbohydrate Na⁺ 8 % Column

Concentration [mg/mL]	Retention time [min]	Peak aura [nRIU*s]
0	-	-
0.015	8.96	603
0.03	8.96	1088
0.06	8.96	2327
0.125	8.96	4178
0.25	8.96	7607
0.5	8.96	15097
1	8.96	30495

Table 3: Retention Times for Sugar Samples Passed through a Rezex RNM Carbohydrate Na⁺ 8 % Column

RESULTS

To determine the retention times of the individual types of sugar (Table 3), these were prepared and injected individually. As different sugars interact with the stationary phase to a varying degree, specific retention times are recorded by the RI detector once each sugar has moved through the column. After the individual types of sugar had been determined, a sugar mixture was prepared and separated. The individual sugar components were separated from one another. The peaks for the different retention times could be allocated to the individual sugar samples assayed. The effect of contaminants, or the influence of salts, was simulated by injecting potassium phosphate buffer and tap water.

Injection of tap water with a conductivity of 265 µS/cm and of potassium phosphate buffer with a conductivity of 1,700 µS/cm showed clear signals and can therefore be distinctly identified as contamination.

Multiply charged ions are especially prone to binding with sulfonate groups. This alters the dissociation equilibrium and can affect the retention time for a particular sugar.

For this reason, the mobile phase must be free of salts and other contaminants in order to perform reliable HPLC analysis with stable retention times and to avoid ghost peaks. The Arium® ultrapure water used in this analysis has a conductivity of 0.055 µS/cm and is largely free of interfering contaminants, which is expressed as a flat baseline without peaks (see green baseline in Figure 4). The column pressure during the analytical runs consistently remained at 23 bar (~334 psi).

This shows that no deposits had built up in the column. Blank runs at the beginning and the end did not show any change, i.e., there were no contaminants in the mobile phase. Standard series with different concentrations were analyzed to determine the reproducibility and detection limit. Raffinose is given as an example of these series performed. The retention times and peak areas were recorded and are listed in the Table 4. The consistent retention times repeatedly obtained show excellent reproducibility. The raffinose standard series shows a linear curve up to a concentration of 0.015 mg/mL (Figure 5). By generating a standard straight line based on the peak areas a quantification of the sample, in this case raffinose is possible.

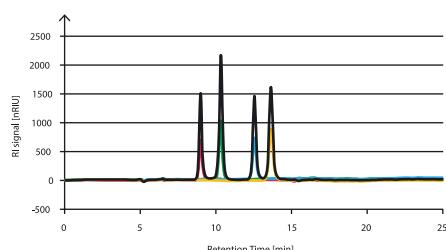


Figure 3: Separation of individually injected sugar samples and of a sugar mixture using a Rezex RNM Carbohydrate Na+ 8 % column with ultrapure water.
Black curve: sugar mixture

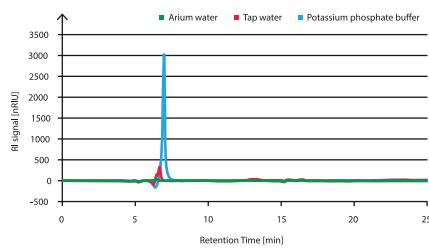


Figure 4: Chromatograms of 2 µL of 10 mM of postpotassium phosphate buffer, 2 µL of tap water and 2 µL of Arium® ultrapure water

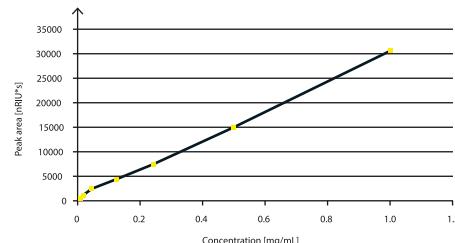


Figure 5: Standard series for raffinose (values from Table 4) passed through a Rezex RNM Carbohydrate Na+ 8 % column with Arium® ultrapure water

CONCLUSION

The results show that ultrapure water produced by Arium® Pro VF can be readily used as the mobile phase for HPLC analysis of the water-soluble saccharides described in this paper. The interactions of the sample with the stationary phase are not affected by the mobile phase as the ultrapure water that is produced with a conductivity of 0.055 µS/cm can be considered virtually free of contaminants. As a result, there are no salts present that would otherwise cause ghost or phantom peaks.⁵

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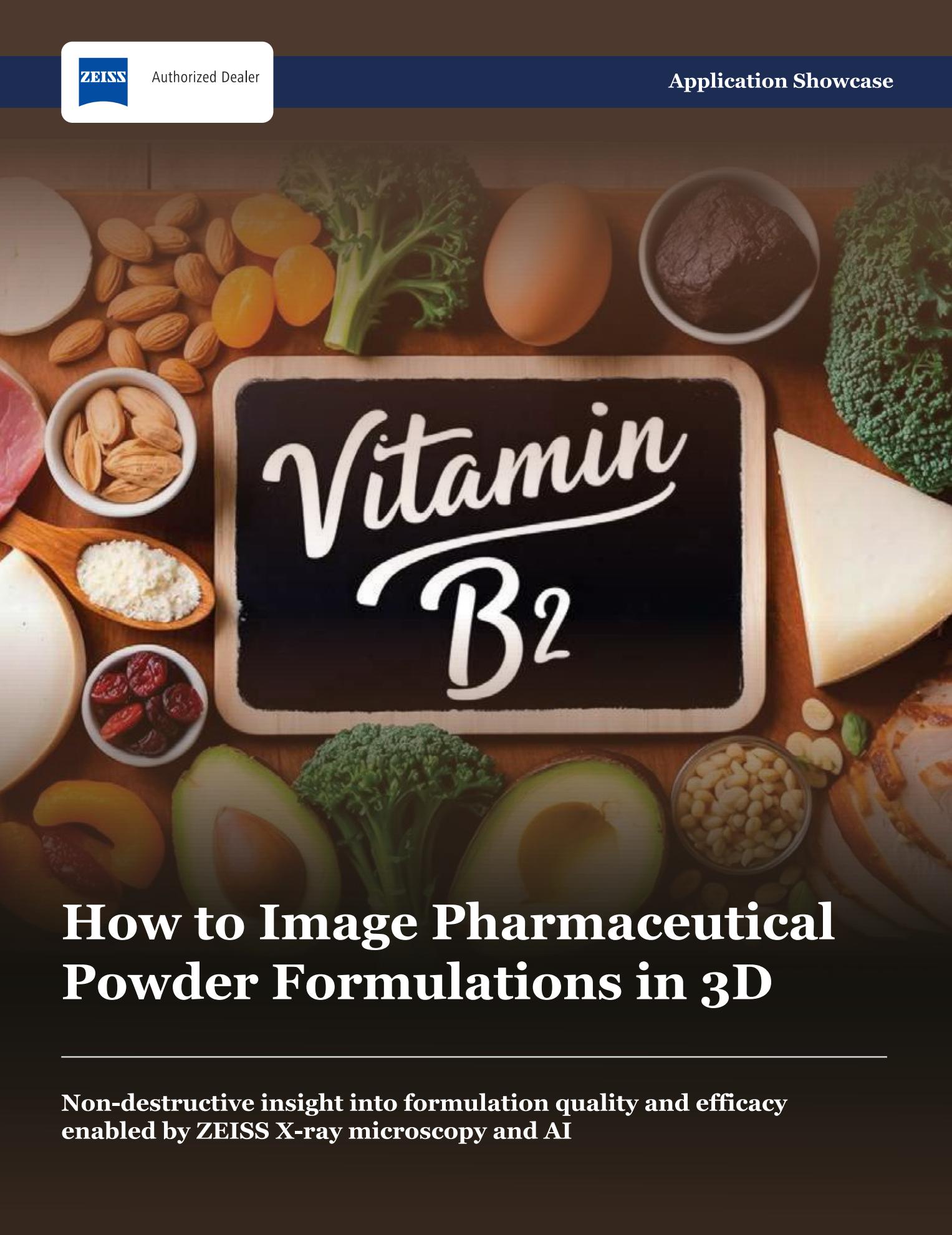


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How to Image Pharmaceutical Powder Formulations in 3D

Non-destructive insight into formulation quality and efficacy
enabled by ZEISS X-ray microscopy and AI

Dr Ria L. Mitchell, Andy Holwell

ZEISS Research Microscopy Solutions, Cambourne, UK

Consistent quality and efficacy are essential in drug formulations for human health. Therefore, easy-to-use, fast, and reliable quantitative characterization methods are needed to ensure pharmaceutical formulations contain the correct materials in the correct doses. This could include ensuring the correct particle sizes, verifying the proportion of active materials and excipients, and their production to specification. Here, a novel 3D imaging and phase identification-based workflow is introduced. 3D X-ray Microscopy (XRM) is used to non-destructively image riboflavin (vitamin B2) capsule powders at high resolution. Following this, the 3D data is reconstructed using two novel AI-based methods for de-noising, contrast improvement, phase enhancement, and phase artefact removal.

Finally, a phase classification, segmentation, and quantification method is used to understand the formulations in 3D. Further, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (SEM-EDS) are used as a ground truth and to provide additional chemical information. These approaches form a unique workflow for the deeper understanding of pharmaceutical formulations and to ensure efficacy and QA/QC in the industry.

Keywords or phrases:

3D X-ray Microscopy (XRM), non-destructive pharmaceutical characterization, AI-driven image reconstruction and segmentation, pharmaceutical powder formulation analysis, quality assurance and efficacy assessment, phase identification and particle morphology

INTRODUCTION

1. Pharmaceutical powder formulations and riboflavin background

Pharmaceutical powders are dry particulate formulation mixtures of active pharmaceutical ingredients (APIs) and

excipients. Powders can refer to an individual type of dosage form (e.g., orally administrated powders such as inhalation/nasal delivery blends, powders within capsules) or the raw materials that are later manufactured into other dosage forms (e.g., pressed tablets). Powder characterization at an early stage of the production process is important to understand the structure of the raw materials that will eventually become manufactured products, through to ensuring quality assurance and quality control (QA/QC) of the finished product for efficacy reasons.

For example, while APIs generally have a smaller particle size compared with excipients¹, API and excipient powder particle size, shape and surface features influence flow during production², and at the other end of the pipeline, a large particle size distribution will affect dissolution and ultimately how well the drug is taken up by the body³. Additionally, the quantities and balance of various excipients is important. Therefore, the right characterization methods should be used before, during, and after the powder production process. Here, an example of 3D capsule powder characterization within a riboflavin (vitamin B2) migraine relief supplement is shown.

Riboflavin naturally occurs in food groups such as dairy products, meat, and nuts. It is a water-soluble micronutrient that is essential for the reproduction of cells and growth, and the prevention of inflammation, cancer, and sepsis, whilst also acting as an antioxidant^{4,5}. It has also been found to contribute towards the alleviation of migraine symptoms⁴ by the reduction of oxidative stress, mitochondrial dysfunction, and neuroinflammation⁴⁻⁸, and is therefore being increasingly administered to patients because of its preventative effects⁴.

While it is common in multi-vitamins and dietary supplements, numerous manufacturers now produce riboflavin specifically as a migraine relief product, which is facilitated by a cost-effective single fermentation production step⁹. As well as the active riboflavin particles, variable excipients are also present; these include anti-caking (or flow) agents, designed to act as lubricants and facilitate a homogeneous mix, and binders, fillers, or diluents, which add cohesivity. Because of the various ways in which

riboflavin is manufactured between manufacturers, and the variety of excipient additives incorporated, characterization and quantification is needed to understand their structure, distribution, and effectiveness¹⁰.

In this work, the aim is to quantify and characterize powders using a simple 3D workflow to understand particle structure, morphology, and distribution. Two capsules from two different manufacturers are used; capsule 1, which reportedly was ineffective, and capsule 2, of good-reported efficacy. Firstly, X-ray Microscopy (XRM) is undertaken to image the powders non-destructively in 3D. Secondly, various advanced deep learning and AI based reconstruction approaches, namely ZEISS DeepRecon Pro and ZEISS PhaseEvolve, are applied to the 3D data to enhance data quality. Finally, an automated phase classification method (ZEISS Phase Identifier 3D) is applied to understand the chemical and phase distributions of the different components in 3D. This approach highlights the benefits of using 3D imaging, deep-learning and AI to enhance 3D data, and automated phase classification for segmentation and quantification of pharmaceuticals. This workflow lends itself to powder, capsule, and pressed tablet characterization.

2. Materials and methods

The first step of the workflow is the collection of a series of non-destructive multi-scale X-ray Microscopy (XRM) scans using a ZEISS VersaXRM (summary in Figure 1). Powders were decanted into polyimide tubing for ease of scanning and thermal stability and were packed to prevent static movement during scanning. Two samples were scanned from two different manufacturers: capsule 1 and capsule 2, both of which reportedly contained riboflavin (API), and Mg stearate (likely as a lubricant). At this point, there was no additional information regarding how each of the powders were manufactured.

Multi-scale scans were taken at 4.23, 1.50, and 0.72 μm voxel sizes, where 801 projections were collected per scan at bin 2 (Figure 2). Further higher resolution tomographies were obtained for capsule 2; these were collected using the 20 \times objective at 607 nm voxel size.

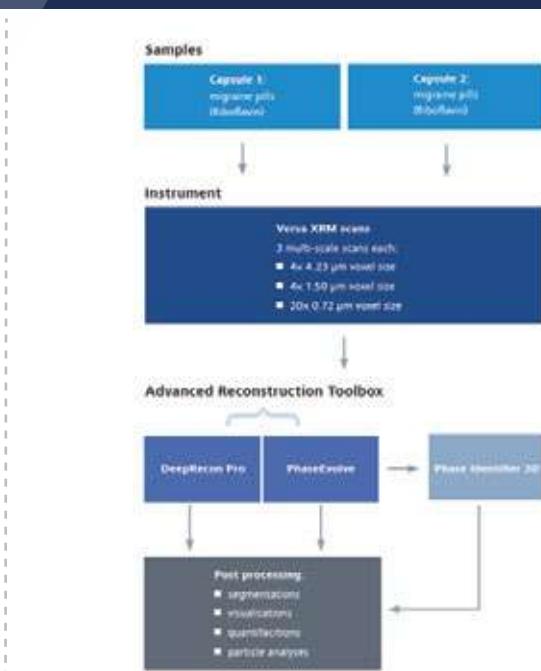


Figure 1: Summary of the workflow used in this study.

Initial qualitative investigation of the XRM data reveals that each set of powders appear to have very different morphological characteristics; capsule 1's powders being crystalline, porous and angular, and capsule 2's powders having a larger range of sizes, non-porous and rounded (Figure 2). This difference may be the result of different manufacturing processes, capsule 1 likely being formed by spray drying, and capsule 2 likely being formed by granulation.

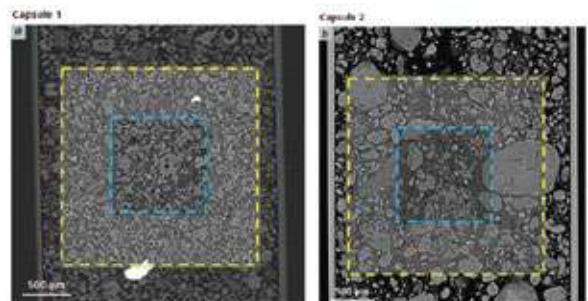


Figure 2: 2D slice view of the position of the three successively higher resolution multi-scale scans. Low resolution, full field of view scan is 4.23 μm voxel size, yellow outlined scan is 1.50 μm , and blue outlined high-resolution scan is 0.72 μm .

Further 2D imaging and analysis was undertaken using a ZEISS Sigma 560 VP field emission scanning electron microscope (SEM), equipped with an Oxford Instruments Ultim Max 170 EDS detector.

3. AI and deep-leaning reconstruction for de-noising, contrast improvement, and low-z phase enhancement

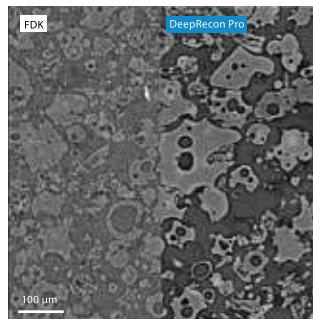


Figure 3: 2D slice comparison of FDK and ZEISS DeepRecon Pro reconstructed data for capsule 1. This high-resolution dataset was collected at 720 nm voxel size. Adapted from [10].

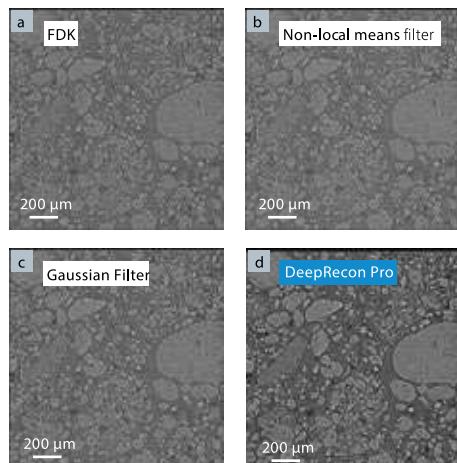


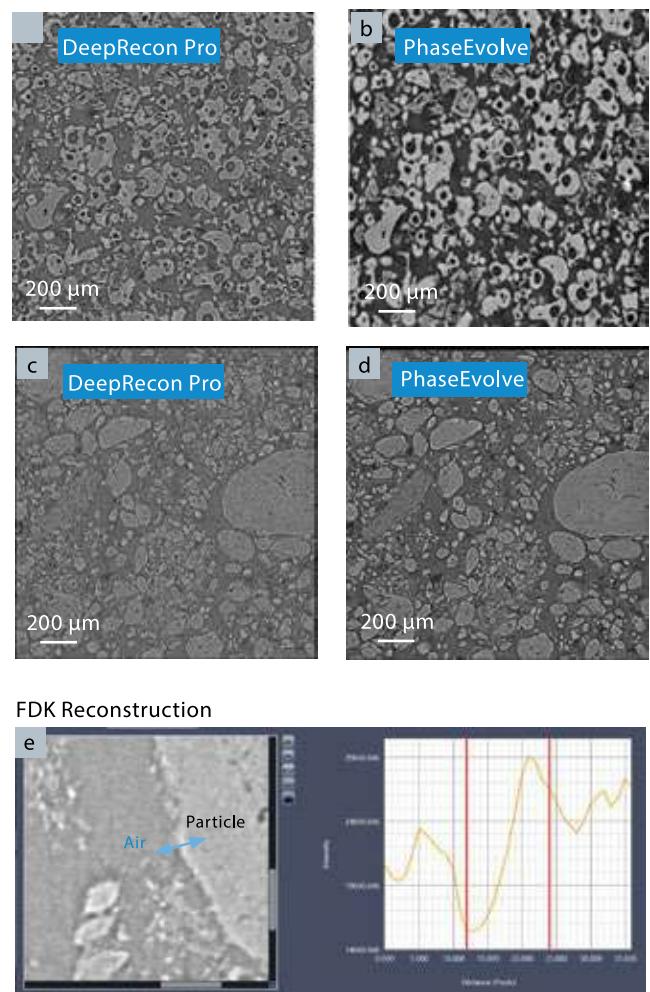
Figure 4: Comparison of FDK (a), two generic post-processing filter types (Nonlocal means and gaussian) (b, c), and the DeepRecon Pro reconstruction (d) results for capsule 2 powders.

Additionally, ZEISS PhaseEvolve is used to complement ZEISS DeepRecon Pro reconstructed data to a) improve contrast between low-density particles, b) remove unwanted phase fringe artefacts (“Fresnel Fringes”) from the outside of particles, and c) reveal differences in absorption at low kV which can be related to subtle chemical compositional differences^{12, 13}.

It is particularly important to remove the unwanted phase fringe artefacts which can contribute towards erroneous segmentations downstream. It works by applying an AI

algorithm to data to enhance the contrast enough that it exposes different material contrasts uniquely revealed by XRM and removes phase effects using an analytical description of the associated artefact¹². In the data presented here, it not only removes the unwanted phase fringe artefacts, but also reveals an additional particulate phase in capsule 2 (Figure 5, 6) and leads to more accurate segmentations of the particulate phase compared with FDK (Figure 6).

Also shown is how PhaseEvolve smooths greyscale fluctuations and noise at particle boundaries to eliminate the Fresnel Fringes (Figure 5e-f). DeepRecon Pro and PhaseEvolve are complementary, resulting in more accurate data for segmentation and quantification.



PhaseEvolve Reconstruction

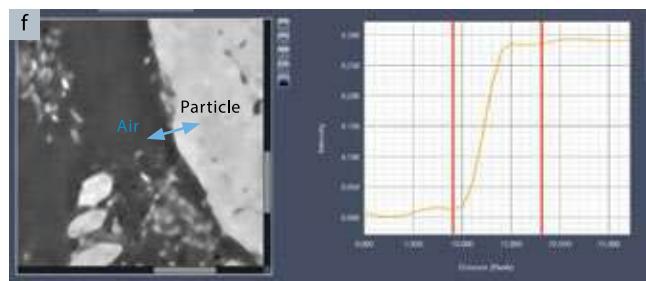


Figure 5: 2D slice comparisons of DeepRecon Pro and PhaseEvolve for capsule 1 (a, b) and capsule 2 (c, d). Phase fringes are eliminated, and a new phase previously unseen is revealed for capsule 2 using PhaseEvolve (d). Also shown are line plots of greyscale variations in FDK (e) and PhaseEvolve (f) reconstructed data across a particle – air boundary.

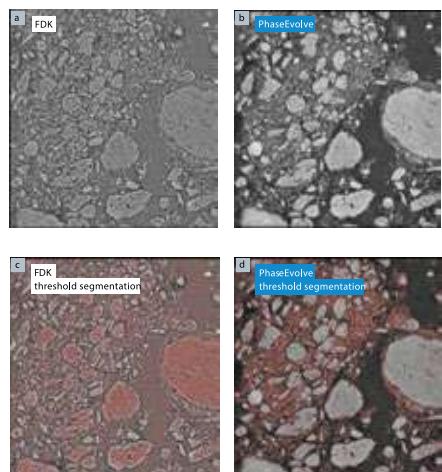


Figure 6: Comparison of (a) FDK, (b) PhaseEvolve, (c) FDK threshold segmentation, and (d) PhaseEvolve threshold segmentation in a highresolution scan of capsule 2. Data collected using the 20x objective at 607 nm voxel size. Images show the segmentation of needle shaped particulates is more successful in the PhaseEvolve enhanced data.

4. 3D phase classification, segmentation and quantification of pharmaceutical materials

ZEISS Phase Identifier 3D software allows for the automated phase identification, classification, segmentation, and quantification of pharmaceutical materials, as well as others¹⁴⁻¹⁶. It works by first utilizing DeepRecon Pro to enhance contrast-to-noise for phase discrimination. Following the creation of a bespoke phase library, the software then uses AIbased methods to identify the unique phase locations on peaks on the data histogram, based on

linear attenuation values¹⁵ (Figure 7). These phases are then segmented and quantified, and accurate phase-based particle analysis can be completed.

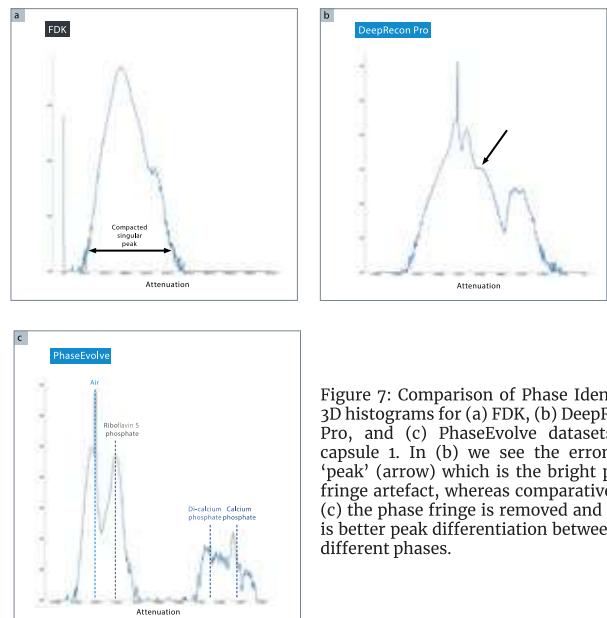


Figure 7: Comparison of Phase Identifier 3D histograms for (a) FDK, (b) DeepRecon Pro, and (c) PhaseEvolve datasets for capsule 1. In (b) we see the erroneous 'peak' (arrow) which is the bright phase fringe artefact, whereas comparatively in (c) the phase fringe is removed and there is better peak differentiation between the different phases.

The PhaseEvolve dataset was used in Phase Identifier 3D for segmentation of capsule 1 because it clearly identifies riboflavin as the lowest density phase (besides air; Figure 7) but also reveals three other findings. Firstly, nothing on the ingredients list matched with the highest density/attenuating phase (i.e., the peak furthest to the right), suggesting something is in the sample that wasn't listed on the ingredients. Subsequent SEM imaging and SEM-EDS analysis was undertaken to help identify the higher density phase (Figure 8a-c), which was identified as calcium phosphate, commonly used as a filler. This phase was then added to the Phase Identifier 3D phase library for classification and resulted in a peak match (Figure 7c). So, the combination of SEM and SEM-EDS analysis, and Phase Identifier 3D classification, identified the presence of a phase which is not listed on the ingredients in this specific supplement. Secondly, segmentation of the DeepRecon Pro data revealed that the minor peak (arrow in Figure 7b) was in fact the phase (Fresnel) fringe artefact surrounding the particles, and not 'real' data at all. In the enhanced PhaseEvolve dataset, this minor peak has been eliminated, leaving only the correct phase retrievable data for

segmentation, highlighting the need to use artefact free data. Finally, magnesium stearate, which was listed on the ingredients, is not identifiable from Phase Identifier 3D, indicating it is not present in at least this capsule (or it could be present but in proportions beyond the resolution of this dataset). Magnesium stearate is generally used as a lubricant for flow and/or as an anti-caking agent, which could indicate why the capsules were ineffective (i.e., limited flowability, lack of homogenisation). These results exhibit why it is important to enhance pharmaceutical data using PhaseEvolve before segmentation and quantification.

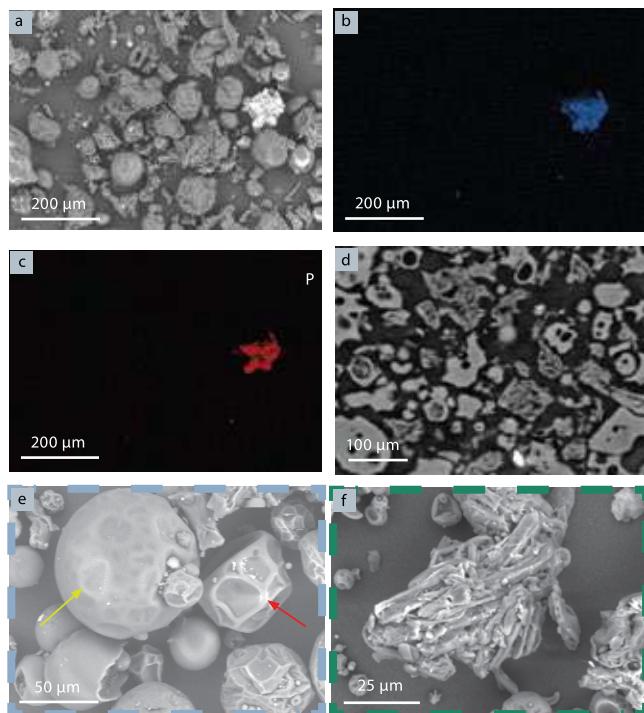


Figure 8: Complimentary SEM imaging and SEM-EDS analysis to establish the composition of the unknown, high-density phase in capsule 1 (yellow circle in (a)). (a) SEM image of capsule 1 powder, (b, c) SEM-EDS reveals the composition of this high-density grain as calcium phosphate, (d) shows a 2D slice through the XRM data highlighting the two different particle types, flakey (green) and rounded (blue), and (e, f) show SEM comparative ground truth images of the particles to validate the presence of the variable particle types. Arrows in e: yellow = 'intact' particle, red = collapsed particle examples.

5. Particle analysis of pharmaceutical powders following AI-based segmentation

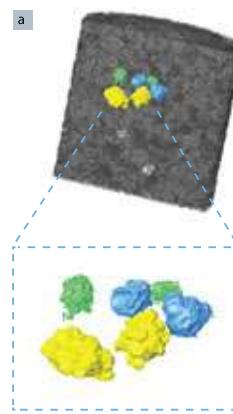
The XRM data reveals that while some of the powders in capsule 1 are intact and are near spherical, some powders are

collapsed with shrinkage (Figure 8, 9). This variation is likely due to different drying rates during the manufacturing process, likely at a lower-than-normal temperature for the collapsed powders, and variation in the characteristics of the powder crusts (skin) 200 μm 200 μm P 200 μm Ca 100 μm 50 μm 25 μm a b c d e¹⁷ leading to a range of morphologies and rates of collapse.

Many of the particles in capsule 1 are also porous and have hollow interiors; this increases surface area and may beneficially allow for greater solubility and dissolution. This porosity would not be identifiable from using SEM alone, and benefits from a non-destructive 3D XRM analysis.

Direct comparison of the segmented particle characteristics in the two capsules (Figure 10) highlights the morphological differences resulting from the different production methods employed by each manufacturer. This highlights that there can be considerable variation in the powders used in vitamin supplements, which ultimately can affect how effective the APIs are.

	Capsule 1	Capsule 2
Voxel Size	1.50 μm	1.50 μm
Field of View	1.5 x 1.5 mm	1.5 x 1.5 mm
Spatial resolution (4x objective)	1.9 μm	1.9 μm
Total Active Particles (Riboflavin)	3,40,647	2,04,229
Total Particles >4.5 μm volume	77,247	1,60,671
mean particle volume	164,301 μm^3	6672 μm^3
range particle volume	10,116,343 μm^3	52390178 μm^3
Average particle aspect ratio	0.43	0.36
Average feret diameter	20.42	12.78



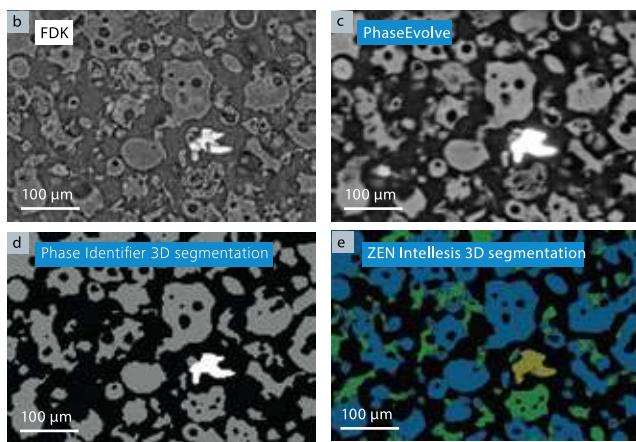


Figure 9: Example of how a combination of AI-based processes (PhaseEvolve, Phase Identifier 3D, and ZEN Intellesis) in a correlative arrangement can provide accurate segmentations of the different particle types in pharmaceutical powders. Blue = rounded particles, green = flaky particles, yellow = calcium phosphate. Width of a) is 2 mm.

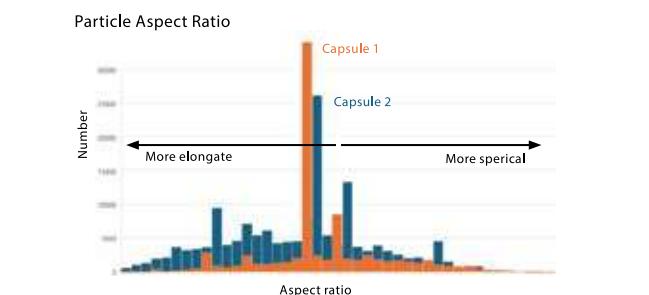
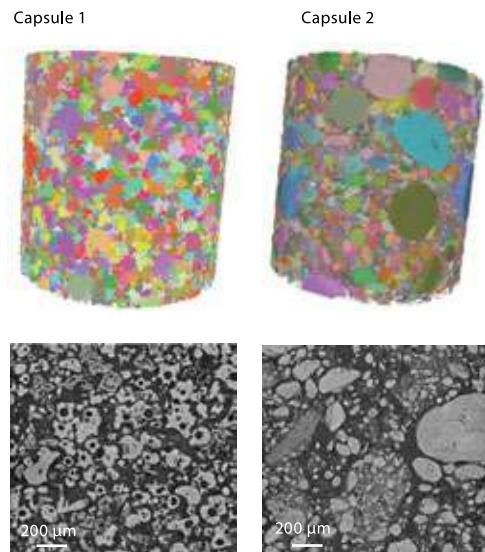


Figure 10: Particle analysis comparison of capsules 1 and 2, highlighting the different particle sizes, structures, and shapes between the two different manufacturers. Only particles 3x the voxel size ($>4.5 \mu\text{m}$) included.

Phases from Phase Identifier 3D segmentation	Contents (%)
Riboflavin	99.16
Ca Phosphate	0.84

Phases from further Intellesis segmentation	Contents (%)
Riboflavin flakey (less Na)	26.43
Riboflavin rounded (more Na)	72.95
Ca Phosphate	0.62

Table 1: 3D composition of capsule 1 based on Phase Identifier 3D and Intellesis segmentations.

SUMMARY

Direct comparison of the segmented particle characteristics in the two capsules (Figure 10) highlights the morphological differences resulting from the different production methods employed by each manufacturer. This highlights that there can be considerable variation in the powders used in vitamin supplements, which ultimately can affect how effective the APIs are.

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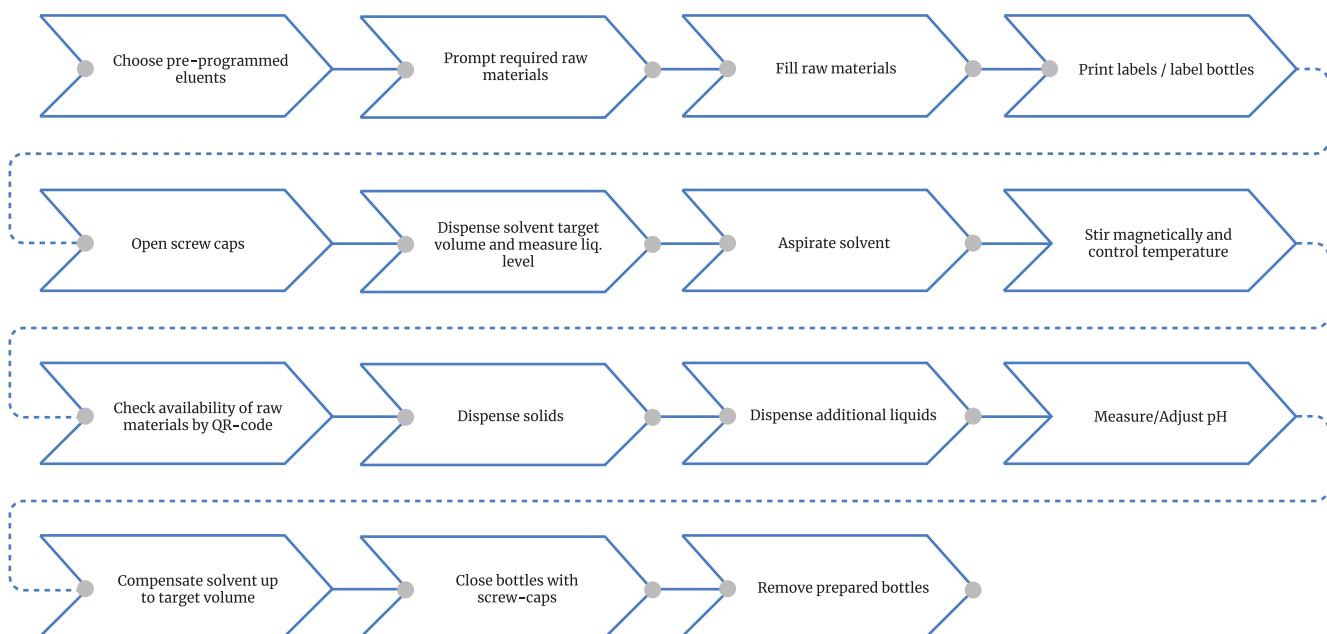
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INK-180DM-6.5L	6.5	180	40/80	Amb-100
INK-240DM-10L	10	240	40/80	Amb-100
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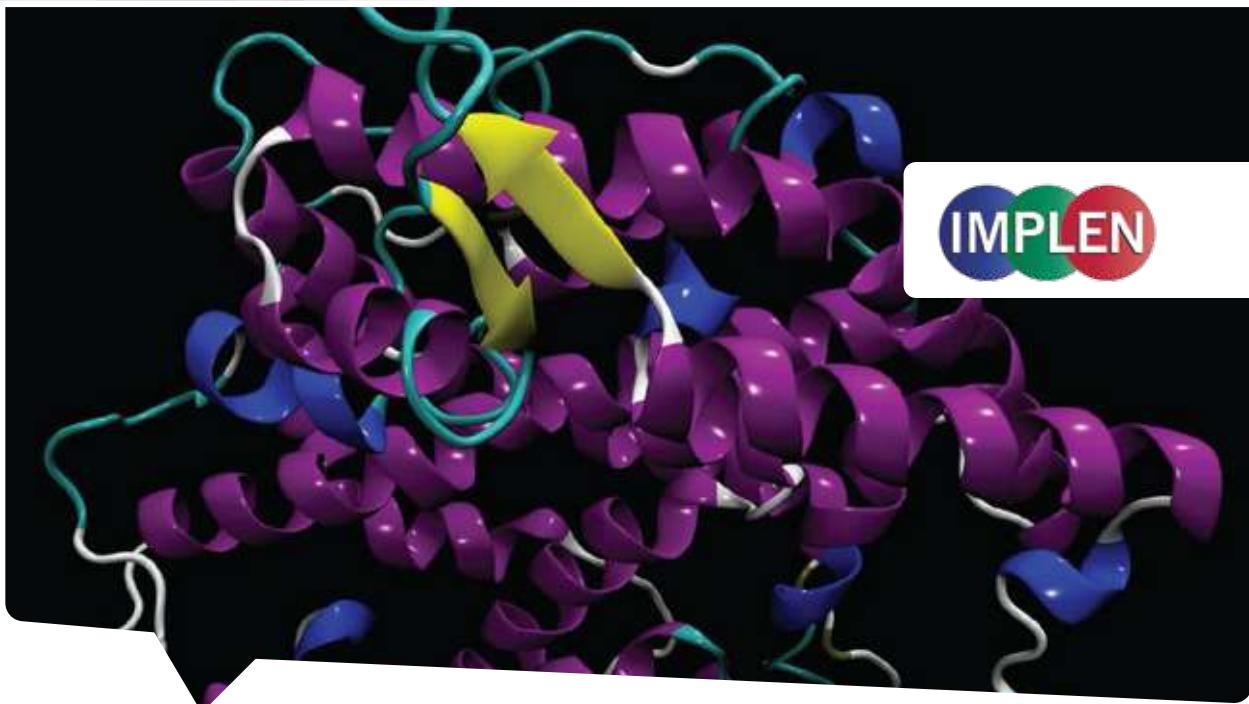
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Resolution LW (0.55%)	< 10 Hz (< 0.17 ppm)	< 10 Hz (< 0.010 ppm)
Sensitivity (1% Ethylbenzene, 1 scan)	>180:1 single; >130:1 dual	>250:1 single; >220:1 dual
Dimensions (W×H×D)	13×12.25×23.75 in / 33.02×31.1×60.3 cm	17×15.25×32 in / 43.2×38.74×81.28 cm
Screen size & resolution	13.3", 16:9, 1920×1080	15.6", 16:9, 1920×1080
Weight	96.5 lbs / 43.8 kg	243 lbs / 110 kg
Magnet	Permanent, no cryogens	Permanent, no cryogens
User Interface	Built-in touchscreen; optional remote	Built-in touchscreen; optional remote
Nuclei	¹ H/ ¹ F; ¹³ C/ ⁷ Li/ ³¹ P options	Same as Nanalysis-60
Lock	Internal ¹ H and ² H options	Internal ¹ H and ² H options
Sample	Standard 5 mm tubes; optional flow cell	Standard 5 mm tubes; optional flow cell
Compatibility	JCAMP-DX, CSV; Mnova, ACD/Labs, TopSpin, etc.	Same as Nanalysis-60
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Connectivity	Ethernet/WiFi, USB, Serial, HDMI	Ethernet/WiFi, USB, Serial, HDMI

Advantages of Nanalysis benchtop NMR spectrometers

- **Accessible** – with a compact footprint, affordable price tag, and minimal to no maintenance required, these instruments can be placed directly at the point of need.
- **Non-targeted** – you do not need to compare an unknown to known standards for identification.
- **Non-destructive** – you can reclaim your sample after analysis.
- **Inherently quantitative** – linear data can be used to extract qualitative and quantitative information.
- **Versatile** – can look at a variety of molecules with a variety of different nuclei.
- **Automatable** – adding software layers can turn what is often considered to be an expert technique into a yes/no, green light/red light analyzer.





Best Practices for Protein/Antibody Quantifications

Protein concentrations are frequently used in life science research. Although there are several assays available, care should be taken to select the optimum assay or method for the sample type. The decision on which assay or method to use is typically based on convenience, quantity and purity of protein available.

This technical guide provides best practice advice for using the NanoPhotometer® to perform small volume protein quantification at 280 nm (Protein UV280) and point to protein assays including Bradford, BCA, etc. for the indirect determination of the protein concentrations via a chemical binding reaction.

Proteins have a characteristic absorption spectrum peak at 280 nm, predominantly from the aromatic amino acids phenylalanine, tyrosine, and tryptophan. The Beer-Lambert law can be applied to determine the protein concentration of a sample. This UV based approach depends strongly on the purity and primary sequence of a protein. Some components which are commonly present in protein samples show strong UV absorbance and should be corrected for or avoided altogether. Since the NanoPhotometer® is scanning every sample from 200 – 900 nm, dye labels absorbing in the visible range can be detected simultaneously to calculate the dye concentration as well as the degree of labelling.

The NanoPhotometer® has a unique Blank Control™ feature that will automatically warn the user if high background is present in a blank from buffers or contaminants.

For protein lysates containing agents blocking the entire UV range, e.g. RIPA buffer, protein UV280 is not available. If these buffers cannot be replaced with non-interfering agents, indirect determination utilizing a protein assay is mandatory. If these buffers cannot be replaced with non-interfering agents, indirect determination utilizing a protein assay is mandatory.



Figure 1: The pedestal and mirror are being cleaned

To obtain optimal readings, the following steps are highly recommended:

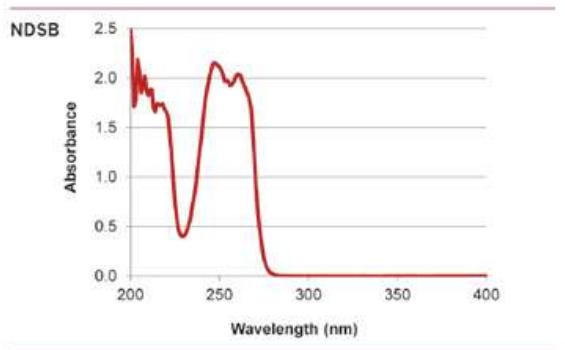
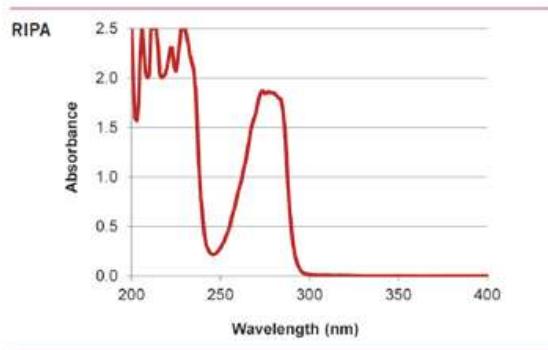
- ▶ Protein samples tend to stick. Clean the measurement head (both bottom and top/mirror) using a water dampened, lint free tissue (avoid vigorous polishing to prevent lint deposits on the quartz window) and apply a drying step with a fresh tissue after a wet wipe.
- ▶ If the pedestal is severely dirty, apply 5 μ l of water onto the pedestal to soak the spot for at least one minute and wipe the pedestal and mirror thoroughly afterwards.
- ▶ Blank with the same buffer solution used to elute/dissolve the sample.
- ▶ Avoid using the following buffers or reagents that show similar characteristics like the following:



Figure 2: Bad Blank warning

If you see peaks in interest or even a concentration displayed, replace the components with alternatives not showing the interference.

- ▶ Control the pH value and ionic strength of your sample. Changes of the pH and/or ionic strength can lead to a change in the absorbance spectrum of your sample and to incorrect concentration and purity calculations.
- ▶ Use low-binding tubes for samples with low concentrations.
- ▶ Measure the blanking solution as a sample to identify a bad blank.
- ▶ Homogenize the sample using the integrated vortex or by pipetting up/down.
- ▶ Use illuminated pedestal to identify if sample is well positioned and no bubbles are visible.



To check on whether a buffer is interfering, run a water blank and then check your intended buffer and/or reagents as a sample using a full wavelength scan.

- ▶ Activate “Air Bubble Recognition” if using buffers containing detergents like Tween or Triton.

- ▶ Use the reverse pipetting technique when applying the sample onto the measurement head to avoid air bubbles.
- ▶ Measure replicates if sample concentration is <0.1 mg/ml, expect greater % errors.
- ▶ Check the 260/280 ratio and graph data for contaminants. Sample Control will flag bad samples.
- ▶ Properly clean pedestal after each use.

Message Text	Explanation/Solution
Air bubble, lint residue or bad sample. Please reapply sample.	Air bubble recognition is on and has detected either an air bubble, lint residue or a bad sample like e.g. a turbid sample. Check sample, clean the sample window on pedestal and mirror in the lid arm thoroughly and reapply sample carefully. To avoid air bubbles apply sample by reverse pipetting.
High absorbance at 250 - 280 nm, 280 - 340 nm, 340 - 400 nm, 400 - 475 nm, 475 - 550 nm, 550 - 625 nm, 625 - 700 nm. Bad Blank or insufficient cleaning.	The warning message is shown when a blank measurement (NanoVolume) detects a significant absorbance a potential area of interest. The warning message shows the wavelength range where the absorbance is appearing. Two things can cause high absorbance in a blank measurement: either the blank solution/buffer has an absorbance in this wavelength range or the sample window on pedestal and the mirror in the lid arm was not cleaned properly after the last reading. Clean the sample window on pedestal and the mirror in the lid arm thoroughly and check the blank solution for absorbance (Use water for blank and measure the blank buffer as sample).
Lint residue or bad sample. Please reapply sample.	Displayed when Air Bubble Recognition is off. Software has detected either a lint residue or a bad sample like e.g. a turbid sample. Check sample, clean the sample window on pedestal and mirror in the lid arm thoroughly and reapply sample carefully.
Maximum absorbance level at specified wavelength reached. Calculations may lead to low/wrong results.	The concentration of the sample used is too high and exceeds the specified absorbance range. Maximum absorbance is 330 A (10 mm path) for NanoVolume and 2.65 A for cuvette measurements. Dilute sample and measure again.
Maximum level exceeded.	This message is shown when for example, too many dyes or wavelength are added with the Add + button. It is possible to add up to 20 dyes or wavelengths.
Sample concentration too low for 0.3 µl - change volume and parameter settings to 1 µl	The measurement parameters for 0.3 µl NanoVolume samples read only the 0.07 mm path length (dilution 1:40). The utilized sample concentration is too low. Please use 1 µl of the sample and change the volume setting to 1 - 2 µl. Minimum concentrations for the 0.07 mm path length for dsDNA 420 ng/µl and for BSA 12.6 mg/ml

Qualitative Analysis of Oligonucleotides Using the Advion Interchim Scientific HPLC-UV/MS System

Changtong Hao,
Advion Interchim Scientific

This application note aims to demonstrate the HPLC/UV analysis of multiple oligo samples and utilize HPLC/CMS analysis to determine their molecular weight.

INTRODUCTION

Oligonucleotides have gained significant attention in biopharmaceutical development due to their ability to modulate gene or protein expression. Their clinical success is evident with the approval of several oligonucleotide-based drugs or their advancement into clinical trials.¹

Keywords or phrases:

Oligonucleotide characterization, ion-pair reversed-phase HPLC, HPLC-UV analysis, HPLC-MS molecular weight determination, TEAA / HFIP-TEA ion-pairing reagents, Advion Interchim Scientific HPLC-UV/CMS system



AVANT™ (U)HPLC

These drugs include antisense oligonucleotides, small interfering RNA (siRNA) therapeutics, and mRNA-based vaccines, exemplified by the successful development of COVID-19 vaccines.

Such accomplishments have spurred further interest and investment in oligonucleotide research and development.

Solid-phase synthesis is a commonly used method to produce oligonucleotide sequences. The raw material is typically purified through various techniques, such as desalting, ultrafiltration, solid-phase extraction (SPE), high-performance liquid chromatography (HPLC), or preparative liquid chromatography (prepLC), depending on the desired purity level. Ion-pairing HPLC or prepLC methods are often preferred as they offer higher purity compared to other techniques.

This application note aims to demonstrate the HPLC/UV analysis of multiple oligo samples and utilize HPLC/CMS analysis to determine their molecular weight.

METHOD

HPLC-UV/CMS System

With a quaternary pump and column selection valve, the process of switching between different buffers and columns for various analyses becomes remarkably straightforward, eliminating the need for manually removing the column and changing the solvent. This automation greatly enhances efficiency and convenience in the analytical process.

With a quaternary pump and column selection valve, the process of switching between different buffers and for manually removing the column and changing the solvent. This automation greatly enhances efficiency and convenience in the analytical process. columns for manually removing the column and changing the solvent. This automation greatly enhances efficiency and convenience in the analytical process.

HPLC/MS	
A-2155	AVANT Autosampler, 18,850 psi, with cooling
A-4112	AVANT HPLC Quaternary Gradient Pumping System
A-2031	Column Oven (column auto selection feature is optional)
A-2046	UV/Vis-DAD diode array detector
MS	Advion expression-L Compact mass Spectrometer with ESI Source

Table 1: Item Instrument List

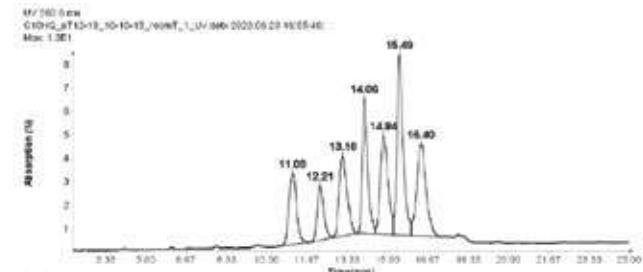


Figure 1: HPLC/UV analysis of Oligo(dT)12-18

RNA Standard with 4 components

The RNA oligonucleotides mixture (Agilent Technologies, CA) was prepared by diluting it 10 times with DI water before HPLC analysis. The sequences of four RNA standards are as follows: 14 mer (CACUGAAUACCAAU), 17 mer (UCA-CACUGAAUACCAAU), 20 mer (UCAUCACACUGAAUACCAAU), and 21 mer (GUCUCAUCACACUGAAUACCAAU).

The separation of these RNA samples was conducted using a similar HPLC method as that for oligo(dT)12-18 primers, with slight modifications.

The HPLC analysis proceeded as follows: After injection of the sample, mobile phase B was set at 9% for 1 minute. It was then linearly increased to 10% over 24 minutes. At 25.1 minutes, it increased to 95% and kept at this level for 2.4 minutes to clean the column. Subsequently, at 27.6 minutes, it reduced to 9% and maintained for 2.4 minutes for column equilibration.

Figure 2 illustrates that the HPLC method effectively separates the four RNA samples, even with a 1-mer difference between the 20-mer and 21-mer RNA samples. This baseline separation for the 20-mer and 21-mer is crucial for analyzing synthetic oligonucleotides, as most impurities during synthesis are typically N=1 mer or N+1 mer.²

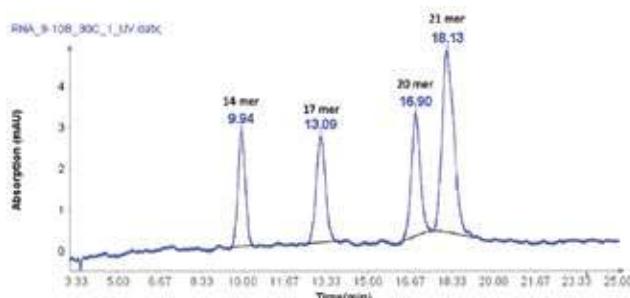


Figure 2: HPLC/UV analysis of four RNA samples

ssDNA Samples

Three single-strand DNA samples (ssDNA) with 17 mer (GTCAGCAAGGACATCGT), 18 mer (CATTTGAGTAGCCAAC-GC), and 19 mer (GGACACTTTCATGCGAGTT) were also tested using the modified HPLC method from that used for RNA samples.

The concentration of each ssDNA was 30 μ M, and 10 μ L aliquots were loaded onto the column for analysis.

The HPLC analysis was performed using the following gradient:

After the sample injection, mobile phase B (MPB) was set at 9% for 1 minute, then linearly increased to 15% over 24 minutes. At 25.1 minutes, it increased to 95% and maintained at this level for 2.4 minutes to clean the column. At 27.6 minutes, the MPB was reduced to 9%, and this level was maintained for 2.4 minutes for column equilibration. The flow rate for the analysis was set to 1.5 ml/min. Despite the greater change in solvent B per minute compared to the RNA samples, Figure 3 demonstrates that the method effectively separates the three ssDNA samples with good baseline resolution.

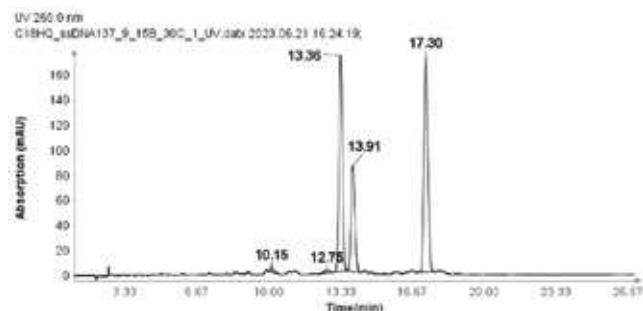


Figure 3: HPLC/UV analysis of three ssDNA samples 1-3, three single strand DNA samples with ssDNA-1: 17 mer (GTC AGC AAG GAC ATC GT); ssDNA-2: 18 mer (CAT TTG AGT AGC CAA CGC) and ssDNA-3: 19 mer (GGA CAC TTT CAT GCG AGT T).

Purity Analysis of a ssDNA Sample

With a same method used for ssDNA samples shown in figure 3, it was also employed for the purity analysis of an ssDNA sample: 19 mer (5'-TGGCGGGCGTACCTGGACT-3').

Figure 4 reveals that the 19-mer ssDNA 4 has a UV purity of 74.3% at 260 nm that was determined using Advion Data Express software.

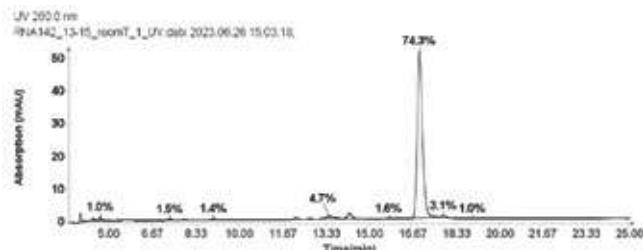


Figure 4: HPLC/UV analysis of ssDNA-4 (5'-TGG CGG GCG TAC CTG GAC T-3')

MS ANALYSIS OF ssDNA SAMPLE USED IN PURITY ANALYSIS

The HPLC/MS analysis of oligonucleotides was performed using an Advion AVANT HPLC system coupled with an Advion Expression CMS-L. For MS analysis, the Interchim Uptisphere Strategy 2.6 μ m C18-HQ column with dimensions 50 x 2.1 mm was used, with a flow rate of 0.2 ml/min. The column temperature was set to 55°C.

Compared to the use of TEAA for the mass analysis of oligonucleotides, the ion pair reagents combining TEA and HFIP offer significantly improved performance.

Therefore, this application note will focus on employing TEA and HFIP ion pair reagents for the HPLC/MS analysis of oligonucleotides.

The mobile phase consisted of 15 mM TEA and 10 mM HFIP in water as mobile phase A, and methanol as mobile phase B. The total HPLC run time was 25 minutes, starting with 5% of solvent B for 1 minute.

The percentage of B was then increased to 6% over 14 minutes, followed by an increase to 95% at 15.1 minutes that was kept for 2.9 minutes to elute the compounds of interest. Subsequently, the % B was reduced to 5% and kept at this level for 6.9 minutes to equilibrate the column before the next analysis.

The MS analysis was conducted in negative ESI mode with the MS scan range set from 500 to 2000 Da. Figure 5b shows the MS spectra of ssDNA-4, displaying a charged envelope with peaks at m/z 1463.9 (4-), 1170.8 (5-), (975.8 (6-), 936.0 (7-), 731.8 (8-), and 650.2 (9-). Through charge deconvolution in Data Express, the uncharged mass for the ssDNA sample was determined to be 5861.8 Da, which closely matches the theoretical value of 5860.8 Da.

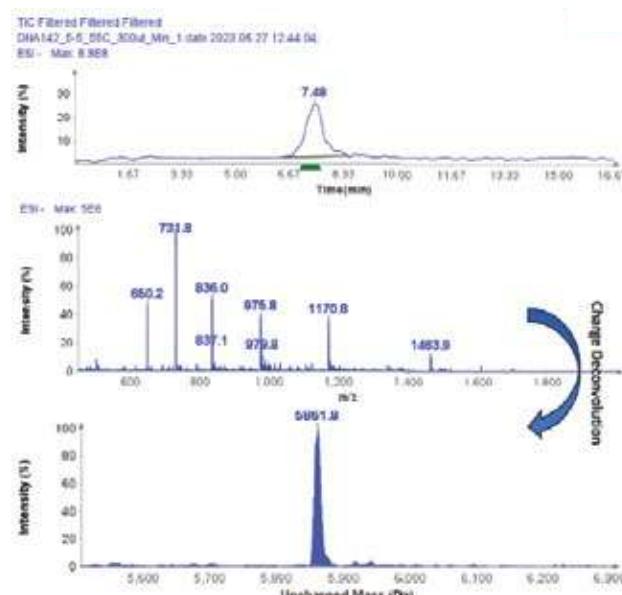


Figure 5: HPLC/MS analysis of ssDNA-4 (5-TGG CGG GCG TAC CTG GAC T-3), MW 5860.8 Da

The MS spectra of ssDNA-5 ($5'$ -GGG-TGG-CAT-TATGCT-GAG-T-3') are depicted in Figure 6, showing a charged envelope with peaks at m/z 1477.8(4-), 1182.1(5-), 984.7(6-), 843.8(7-), and 738.2(8-). By charge deconvolution the uncharged mass for the ssDNA-5 was determined to be 5913.9, which is in close agreement with the theoretical value of 5914.9.

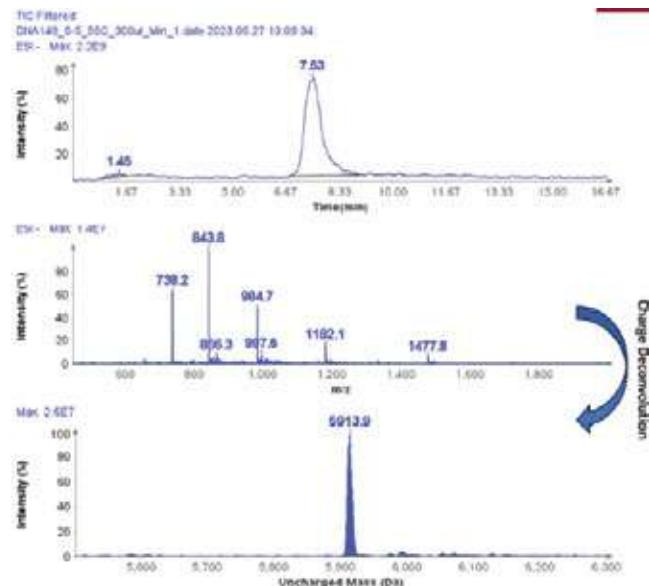


Figure 6: HPLC/MS analysis of ssDNA-5 ($5'$ -GGGTGG-

CONCLUSION

Using a 5 μ m particle size C18HQ column coupled with TEAA (Triethylammonium acetate) ion-pairing reagent has been demonstrated to be suitable solution for oligonucleotide HPLC analysis.

For MS analysis of Oligonucleotides, the same C18HQ column can be employed with HFIP (hexafluoroisopropanol) and TEA (triethylamine) as the ion-pairing reagent in conjunction with an AVANT[®] HPLC-UV/CMS system. This method has been proven to yield additional accurate mass measurements of Oligonucleotides.

Overall, utilizing the Interchim C18HQ column and the appropriate ion-pairing reagent in combination with the AVANT[®] HPLC-UV and -CMS system can provide a reliable solution for the purity analysis and characterization of oligonucleotides.

Separation of High-Purity Remdesivir using RotaChrom's Continuous Centrifugal Partition Chromatography Platform

Overcome Complex Purification Challenges with RotaChrom's High-Resolution MDM-CPC Technology

Efficient downstream purification remains a critical determinant of yield, throughput, and process economics for complex antiviral drug substances. This work evaluates a liquid-liquid chromatographic strategy that leverages centrifugal partition chromatography (CPC) to resolve closely related stereochemical variants under fully achiral conditions. Emphasis is placed on process design, operational flexibility, and performance across different CPC modes rather than on molecular or regulatory background.

Advanced CPC configurations enabled both rapid pilot-scale processing and selective enrichment of minor components without reliance on solid stationary phases. Multiple dual-mode and trapping workflows demonstrated distinct

advantages in productivity, solvent utilization, and purity control, supported by orthogonal analytical verification. The study illustrates how continuous CPC operation can be tuned to balance resolution, yield, and processing time, providing a scalable purification framework suitable for industrial implementation in high-value pharmaceutical manufacturing.



The Continuous CPC

In this application note two scenarios are presented:

- ▶ The successful isolation of both isomers at 95% purity level at pilot-scale (2 g of SP/RP 56/43 diastereomer mixture was processed in 52 min) with 66% and 58% yield by dual-rotor MDM-CPC (2x2.1 L rotor volume) (Figures 2. A, B).
- ▶ The analytical results of a high-purity (>99%) isolation of the rare RP isomer by trapping MDM CPC (2x90 mL rotor volume) is highlighted (38.6% yield, 510 mg of SP/RP 75/24 diastereomer mixture was processed in 180 min) (Figures 3. A, B).

Keywords or phrases:

Centrifugal partition chromatography (CPC), achiral liquid–liquid separation, diastereomer purification, multiple dual-mode (MDM) operation, trapping-MDM CPC, scalable pharmaceutical purification

INTRODUCTION

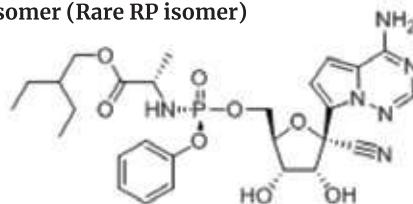
Remdesivir is a phosphoramidate nucleotide prodrug approved for the treatment of severe COVID-19 infection in 2020. It contains a chiral phosphorous centre with the current clinical compound being the SP-diastereomer (GS-5734). However, the pharmacological relevance of its RP-diastereomer, is also significant (Fig. 1.).

The chiral stationary phase utilizing a preparative HPLC method, which was initially reported by an originator, is an expensive and hardly scalable step in the total synthesis of remdesivir. We developed an achiral liquid-liquid chromatographic approach for the efficient and scalable separation of remdesivir P-diastereomers.

As a first step, we screened more than 200 binary, ternary, and quaternary solvent systems to find proper solubility, partition, and selectivity for the diastereomer pair. It was recognized that by careful selection of apolar organic solvents, cosolvents, solubilizing components, and pH selectivity, values as high as in chiral environments (1.45) can be achieved.

In addition, the efficiency-enhancing operation modes of the Continuous CPC, such as multiple dual-mode (MDM) and trapping- MDM modes successfully resolved the diastereomers.

S RP isomer (Rare RP isomer)



P isomer (Remdesivir)

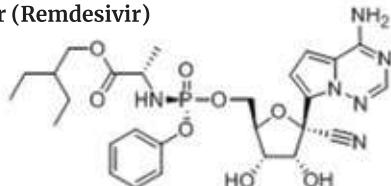


Figure 1. Chemical structures of the P-diastereomers of the antiviral remdesivir. Note that the SP isomer is utilized in coronavirus treatment.



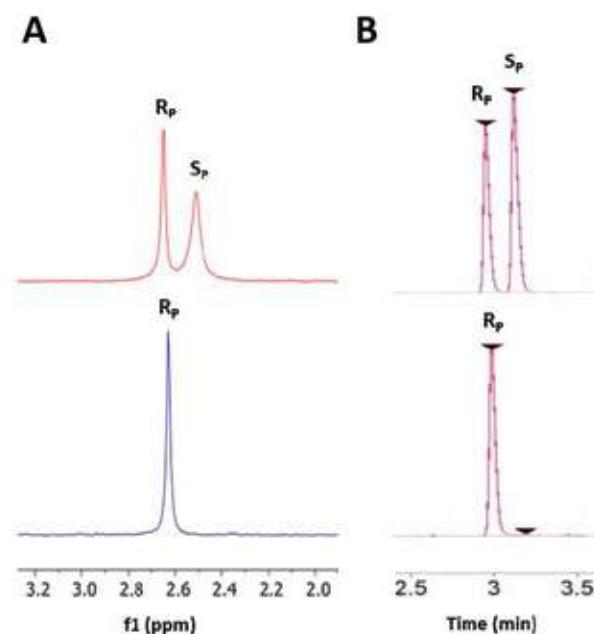
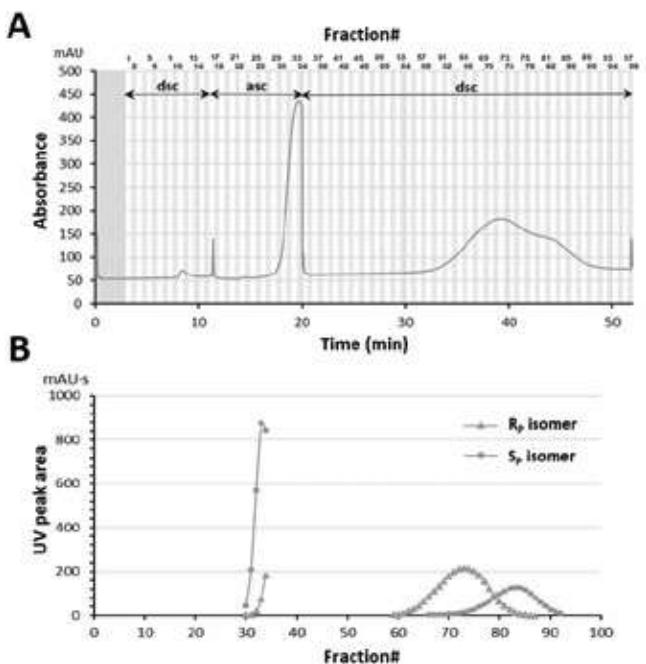
Continuous CPC

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[2] Andrew N. Bigley, Tamari Narindoshvili, and Frank M. Raushel, A Chemoenzymatic Synthesis of the (RP)-Isomer of the Antiviral Prodrug Remdesivir. *Biochemistry*, 2020, 59, 3038–3043.

[3] US10251898B2 patent.



Shodex KW400 Series: High-Efficiency Semi-Micro SEC Columns for Advanced Protein and Biopolymer Analysis

High-performance SEC semi-micro columns

High-resolution size-based analysis of proteins increasingly demands columns that deliver improved efficiency, sensitivity, and recovery while operating at reduced solvent consumption. This application note evaluates the performance of the Shodex KW400 series semi-micro size exclusion columns across a range of protein and peptide analyses. Comparative data demonstrate enhanced theoretical plate numbers and significantly improved detection sensitivity relative to conventional analytical SEC formats, alongside consistently high protein recovery indicative of minimal non-specific adsorption. The influence of flow rate on separation efficiency, peak resolution, and signal intensity is systematically examined to identify optimal operating conditions for routine and high-sensitivity analyses. Practical applicability of the KW400 series is further illustrated through real-sample separations, including control serum, dairy

matrices, lectins, and low-molecular-weight peptides, highlighting the series' versatility across diverse biopolymer workflows. Together, these results position the KW400 series as a robust SEC solution for laboratories seeking high-performance protein characterization using semi-micro HPLC systems.



Shodex KW400 series

Keywords or phrases:

Protein molecular weight profiling, semi-micro-SEC columns, high-efficiency silica packing, detection sensitivity enhancement, protein recovery performance, flow rate optimization

INTRODUCTION

Size exclusion chromatographic (SEC) columns are suitable to identify molecular weight of biopolymers, such as proteins. Shodex has developed a brand-new KW400 series of high-performance SEC semi-micro columns. This series is a downsized and higher performance version of PROTEIN KW-800, specialized for protein analyses.

Both series are filled with silica gel. This note introduces the features of KW-400 series.

Specifications

Product Code	Product Name	Exclusion Limit (Pullulan)	Exclusion Limit (Protein)	Plate	Particle Size (μm)	ID x Length (mm)
F6989201	KW402.5-4F	60000	150000	≥ 35,000	3	4.6 x 300
F6989202	KW403-4F	150000	600000	≥ 35,000	3	4.6 x 300
F6989203	KW404-4F	500000	1000000	≥ 25,000	5	4.6 x 300
F6989204	KW405-4F	1300000	20000000	≥ 25,000	5	4.6 x 300
F6700132	KW400G-4A	Guard column	Guard column	—	5	4.6 x 10

Table 1: Specification of KW400 Series Columns

For all Columns

Packing Material: Silica Gel with Hydrophilic Polymer

Coating

Housings: Stainless Steel

Recommended Flow Rate: ≤ 0.35mL/min

Maximum Flow Rate: 0.5mL/min

Maximum Pressure: 10 MPa (KW402.5-4F, KW403-4F, KW404-4F), 7MPa (KW405-4F)

Temperature: 5 - 45 °C

pH: 3.0 - 7.5

Organic Solvent: Up to 100% of Methanol, Ethanol or Acetonitrile

The KW400 series is a semi-micro type of a SEC column. It is recommended to use it with a semi-micro type HPLC system.

ADVANTAGES OF KW400 SERIES

Separation Performance

The KW400 series is a high-performance version of the conventional PROTEIN KW-800 series. Its finer packing material enabled a downsized column. Figure 3-1 shows chromatograms using the KW400 series and KW-800 columns for a protein mixture. A semi-micro type HPLC was used in this datum. The theoretical plate number of KW402.5-4F is 1.5 times better than that of KW-802.5.

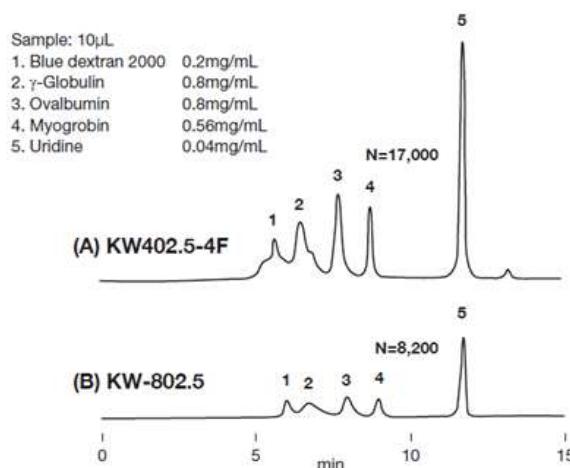


Figure 1: Comparison of KW402.5-4F and KW[AN1.1]-802.5

Recovery of Proteins

Recovery data of seven kinds of proteins are shown in Table 2. Both of KW402.5-4F and KW403-4F columns achieve a high recovery by the very low-level adsorption of proteins onto the packing materials.

Protein	KW402.5-4F Recovery (%)	KW403-4F Recovery (%)
γ -Globulin	98	96
Bovine serum albumin	89	96
Ovalbumin	89	97
Myoglobin	90	89
Cytochrome c	92	92
Lysozyme	87	98
α -Chymotrypsinogen	95	94

Influence of Flow Rate

Figure 2A shows the relationship between the theoretical plate number and the flow rate. It describes chromatograms of proteins at different flow rates. Table 3 shows the relationship between the flow rate and the peak separation. As shown in Figure 2A and Table 3, a lower flow rate contributes to a higher theoretical plate number and a better peak separation. Figure 2B indicates that a slower flow rate leads to a higher peak height.

The analysis is normally performed around 0.3 mL/min. For a better resolution and a higher sensitivity, the flow rate is recommended to be 0.2 mL/min or less. Please note that longer analysis time is needed in this case.

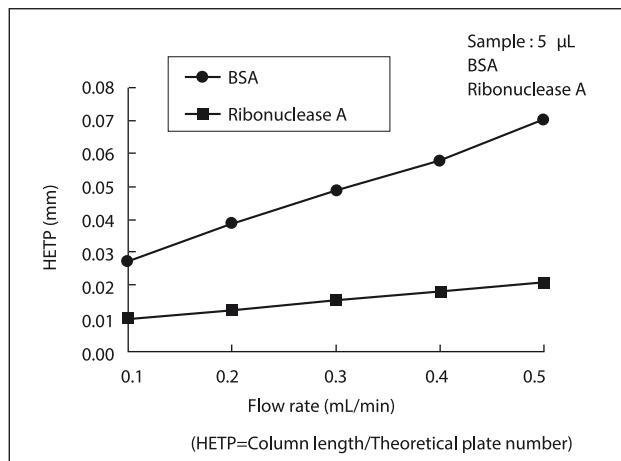


Figure 2A: Relationship of TPN and flow rate of KW402.5-4F

Column: Shodex KW402.5-4F (4.6mmID x 300mm)
 Eluent: 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Detector: UV (280nm)
 Column temp.: 25°C

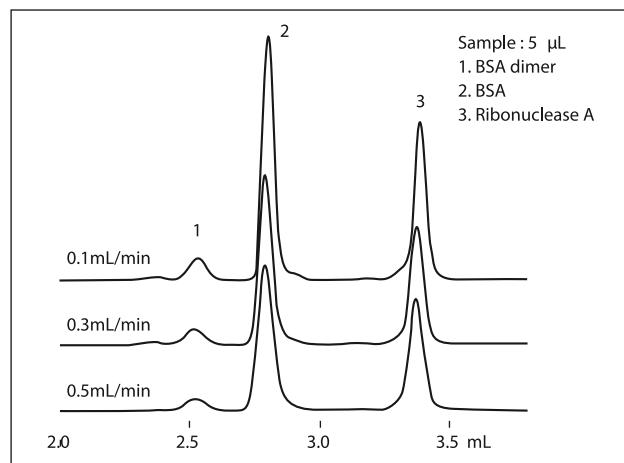


Figure 2B: Chromatograms of proteins at different flow rates

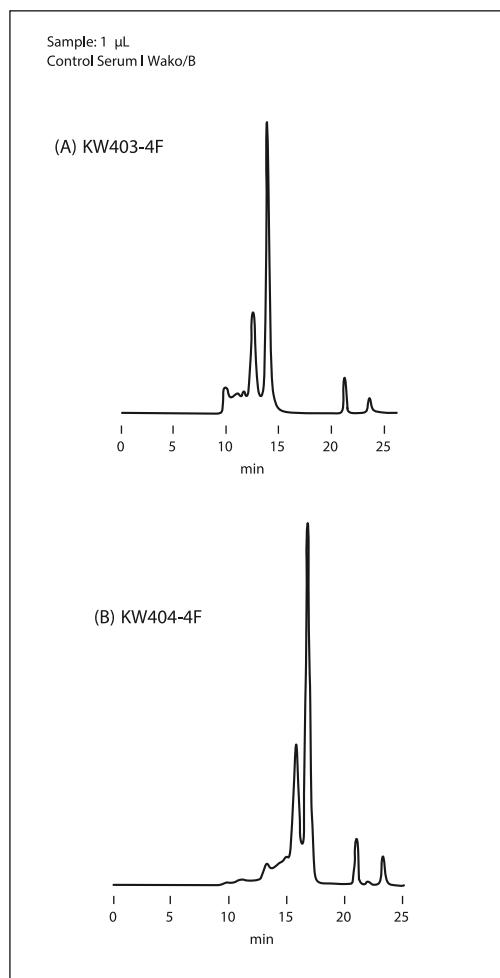
Column: Shodex KW402.5-4F (4.6mmID x 300mm)
 Eluent: 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
 Detector: UV (280nm)
 Column temp.: 25°C

KW402.5-4F		Flow Rate (mL/min)		
		0.5	0.3	0.1
Resolution	BSA dimer/BSA	1.49	1.68	2.04
	BSA/ Ribonuclease A	5.28	6.17	7.88

APPLICATIONS

1. Control Serum

Figure 6-1 shows chromatograms using KW403-4F and KW405-4F for a control serum. Since the pore size of the packing material of KW404-4F is larger than that of KW403-4F, KW404-4F can analyse large substances whose molecular weight is above the exclusion limit of KW403-4F.



2. Whey

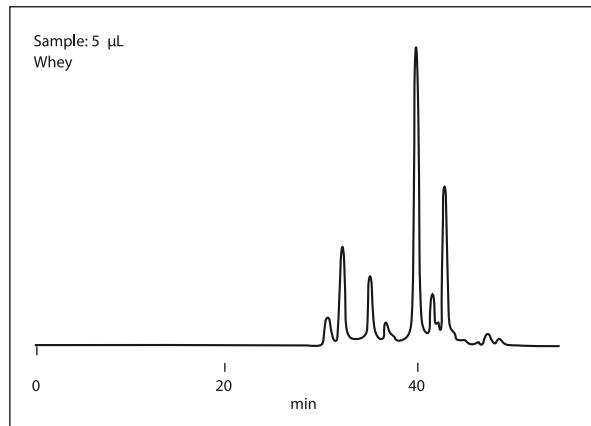


Figure: Whey in Yoghurt

Columns: Shodex KW402.5-4F + KW403-4F
(4.6mmID x 300mm each)

Eluent: 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)

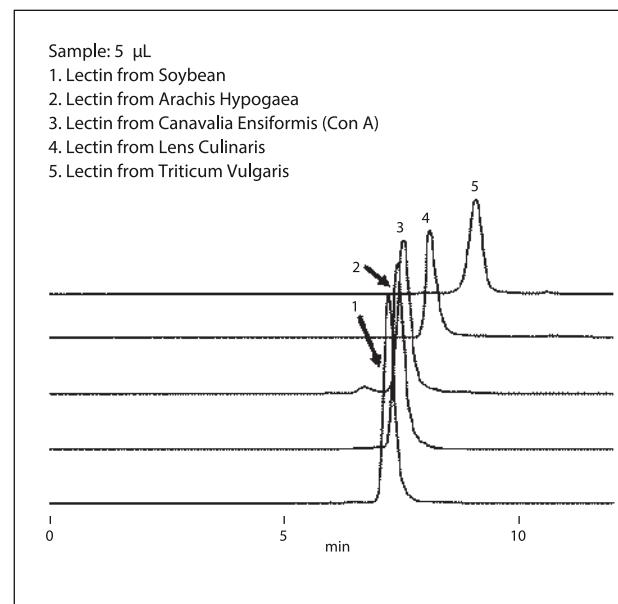
Flow Rate: 0.20mL/min

Detector: UV (280nm)

Column Temp.: 30°C

3. Lectins

The figure below shows chromatograms of lectins. Lectins are proteins with a special affinity to specific kinds of sugar, and their origins are diverse, such as glycoproteins and metal-containing.



Column: Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent: 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate: 0.33mL/min
Detector: UV (220nm)
Column Temp.: 30°C

4. Peptides

Figure below indicates chromatograms for peptides with a molecular weight of 269 to 1734. Because characteristics of side chains of amino acids influence the separation in peptide analyses, not like protein analyses, peptides containing lots of hydrophobic amino or basic acids might cause interactions besides size exclusion.

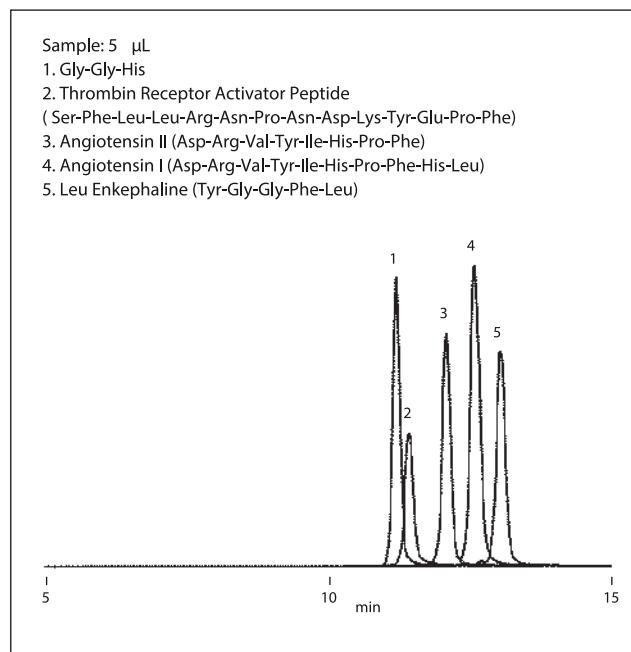
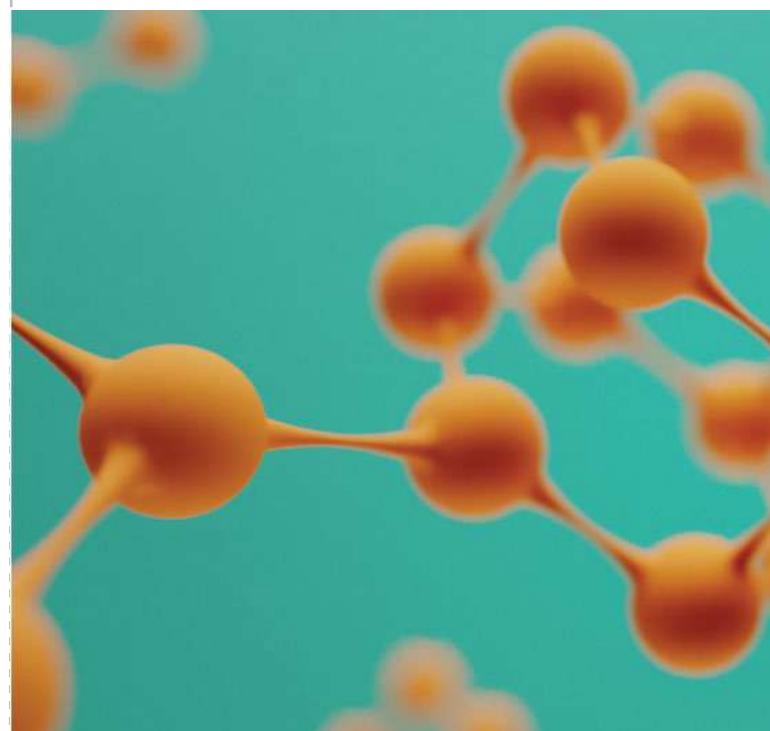


Figure: Peptides with a molecular weight of 269-1734
Column: Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent: 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH 7.0)
Flow Rate: 0.33mL/min
Detector: UV (220nm)
Column Temp.: 30°C

SUMMARY

This application note demonstrates how the Shodex KW400 series semi-micro-SEC columns address the growing need for high-efficiency, high-sensitivity protein and biopolymer analysis while reducing solvent consumption. By combining finer silica packing with a downsized column format, the KW400 series delivers markedly higher theoretical plate numbers and enhanced detection sensitivity compared with conventional SEC columns, without compromising protein recovery.

Systematic evaluation of flow rate effects highlights practical operating windows that balance resolution, sensitivity, and analysis time. The versatility of the column is further validated through real-world applications spanning control serum, dairy samples, lectins and low molecular-weight peptides. Overall, the results establish the KW400 series as a reliable and adaptable SEC platform for advanced protein characterisation in semi-micro HPLC workflows, supporting both routine QC and high-sensitivity research applications.



Streamlined LC-MS Analysis of Stress Induced Impurities of a Synthetic Peptide using the BioAccord™ System and the waters_connect Intact Mass™ Application

Nilini Ranbaduge, Henry Shion, Ying Qing Yu

Waters Corporation

This application note demonstrates the use of a compliance ready, automated workflow for the analysis of synthetic peptides and their impurities. The Intact Mass Application within the waters_connect informatics platform enables both detection and relative quantitation of the API (Active Product Ingredient) and its' impurities. A noticeable benefit of the streamlined workflow is that both targeted and untargeted data processing can be achieved by adding or removing target masses within the processing method.

The Intact Mass Application allows the scientist to obtain mass information, confirming the proper peptide was produced, and the relative quantitation of the detected impurities.

Keywords or phrases:

Keywords/Phrases: Synthetic peptide impurity profiling, LC-MS intact mass analysis, stress-induced degradation analysis, BioAccord™ LC-MS system, waters_connect™ Intact Mass™ application, targeted and untargeted impurity quantification



BioAccord LC-MS System

Benefits

- ▶ Streamlined LC-MS workflow for the mass confirmation and relative quantification of synthetic peptides and their impurities using the waters_connect™ Intact Mass Application
- ▶ Capable of targeted or untargeted impurity quantification
- ▶ Built-in thresholding and pass/fail criteria ensure high data quality and the confidence to make quick assessments of the safety and efficacy of the synthetic peptide

INTRODUCTION

Peptide therapeutics are amino acid polymers containing less than one hundred amino acids.¹ These are a popular class of biotherapeutics on the market, due to their relatively low toxicity, high biological selectivity, and the potential for treating a variety of diseases.²⁻³ Similar to other biopharmaceuticals subject to FDA approval, synthetic peptide regulatory filings require sufficient data to support its consistent manufacture, efficacy, potency, and safety. Impurities can be generated during the manufacturing process and storage.

Liquid chromatography with UV detection methods is commonly used to assess peptide impurity profiles, after those impurities are identified and risk assessed. LC-MS instrumentation and associated data processing software enables scientists to identify and quantify both the API and its impurities using increasingly simple and automated analytical workflows. This application note focuses on the capability of the recently developed waters_connect Intact Mass Application to streamline synthetic peptide analysis. The data processing workflow enables automated assignment of biomolecules based on their deconvoluted accurate mass and quantification of the components using integrated peak area from either the optical or MS data channels. This application can perform both targeted (pre-defined by mass) and untargeted analyses of analytes, enabling identity confirmation, and impurity monitoring.

To demonstrate the capabilities of the application software, we selected Exenatide as a test molecule. Exenatide is a glucagon-like-peptide-1 (GLP-1) analog, containing 39 amino acid residues. It is synthetically produced and is used in treating type II diabetes. The data shown in the application note represents a stressed Exenatide sample, a technique used in both routine and accelerated stability testing. The data were collected using the BioAccord™ LC-MS system, also operated by waters_connect operating system. This allows the full workflow automation from data acquisition to reporting of results to happen within a single compliant-ready and network scalable informatics platform. These results highlight the capability and functionality of waters_connect and the Intact Mass Application that can be used for synthetic peptide analysis across the full product life cycle of a synthetic peptide.

EXPERIMENTAL

Sample Description

Stress sample preparation: Exenatide peptide was purchased from the USP (The United States Pharmacopeial Convention, Rockville, MD). The sample was dissolved in 3.0 mL of milli-Q water to make a 0.9 mg/mL solution.

The concentration was adjusted to 0.5 mg/mL with pH 7.5 100 mM Tris buffer to obtain six aliquots of the diluted sample. Three aliquoted samples were covered to prevent exposure to light and incubated at 37 °C for two to four days. The second set of aliquots were incubated at room temperature, shielded from ambient light, for the same amount of time. At the end of the incubation period both samples were diluted in 0.1% formic acid to a final concentration of 0.1 mg/mL for LC-MS analysis. The on-column injection volume was 1 µL.

Exenatide Sequence Information

HGEGLFTSDL SKQMEEEAVR LFIEWLKNNGG PSSGAPPPS-NH₂

- ▶ **Monoisotopic mass:** 4184.02731 Da
- ▶ **Average mass:** 4186.57188 Da
- ▶ **LC-MS system suitability analysis:** MassPREP™ Peptide mixture was dissolved in 100.0 µL of 0.1% formic acid. Injection volume was 1 µL.

The peptides selected for monitoring and the neutral mass information are listed below.

- ▶ **Peptide name:** Bradykinin, Angiotensin II, angiotensin I, Renin substrate, Enolase T35
- ▶ **Molecular weight:** 1059.5613, 1045.5345, 1295.6775, 1757.9253, 1871.9604 Da
- ▶ **Relative quantification:** Peak area/total response/ exclude rawLC minimum peak area: 5%
- ▶ **Pass/warning/fail thresholding for impurities:** 5%/2%/1%
- ▶ **Intensity threshold (minimum):** 10% (of the largest peak)

Method Conditions

LC Conditions

LC system: ACQUITY™ Premier System

- ▶ **Detection:** TUV (214 nm), MS
- ▶ **Vials:** QuanRecovery with MaxPeak™ HPS (p/n: 186009186)
- ▶ **Column(s):** ACQUITY Premier Peptide CSH C18 Column 1.7 μ m, 130 \AA , 2.1 x 100 mm (p/n: 186009488)
- ▶ **Column temp:** 60 °C
- ▶ **Sample temp:** 6 °C
- ▶ **Injection volume:** 5 μ L
- ▶ **Flow rate:** 0.25 mL/min
- ▶ **Mobile phase A:** 0.1% formic acid in H₂O
- ▶ **Mobile phase B:** 0.1% formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.25	90	10	6.00
2.00	0.25	90	10	6.00
15.00	0.25	43	57	6.00
15.00	0.25	15	85	6.00
18.50	0.25	15	85	6.00
19.00	0.25	90	10	6.00
25.00	0.25	90	10	6.00

MS Conditions

MS system: BioAccord System with ACQUITY Premier

- ▶ **Ionization mode:** ESI+ (MS with fragmentation)
- ▶ **Acquisition range:** m/z 50–2000
- ▶ **Capillary voltage:** 1.2 kV
- ▶ **Collision energy:** 60–120 V
- ▶ **Cone voltage:** 20 V

Informatics Tools

- ▶ **Data processing application:** Intact Mass App v1.2
- ▶ **Informatics platform:** waters_connect v2.0

Selected Intact Mass App Processing Parameters

- ▶ **Deconvolution method:** Auto
- ▶ **Maximum number of peaks to deconvolute:** 10
- ▶ **LC minimum peak area:** 1
- ▶ **1–15 KDa algorithm:** BayesSpray
- ▶ **Output mass:** Monoisotopic
- ▶ **Type of biomolecule:** Protein
- ▶ **Relative quantitation:** Area, largest response, exclude raw
- ▶ **Specify intensity threshold (minimum):** 0.1% most intense peak

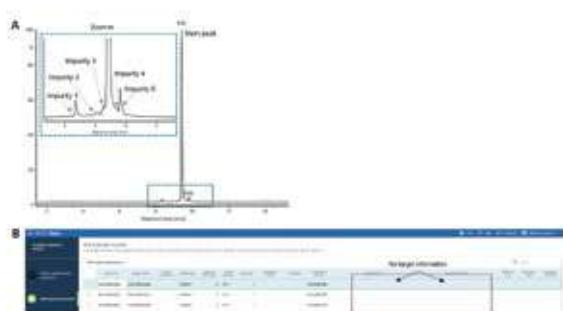


Figure 1. A) The TIC shows the main peak and impurity peaks found in the xenatide stressed sample. The most abundance peak is labelled as main peak and chromatographically separated peaks are labelled as impurities from 1–5. B) Untargeted analysis of exenatide stressed samples was performed to get accurate mass information for all components.

RESULTS AND DISCUSSION

LC-MS data was acquired with reversed-phase separation prior to MS acquisition using the BioAccord system (ACQUITY UPLC-ToF MS). Both the UPLC and the columns employ MaxPeak High Performance Surfaces (HPS) technology to minimize non-specific interactions between peptides and the surface of the fluidic path. ACQUITY Premier Reverse Phase Columns have shown to improve acidic peptide recovery resulting in the reduced need for column pre-conditioning and low variability between replicate measurements.^{4,5} The samples were separated using a 15-minute gradient on an ACQUITY Premier Peptide CSH C18 Column.

It was reported that Exenatide is susceptible to chemical degradation by pH variance, heat treatment, and light exposure.⁶ To generate a sample containing known impurities associated with thermal degradation process, half the aliquots of the Exenatide sample were incubated at elevated temperature for two to four days at pH 7.5. At the end of incubation period the samples were subjected to LC-MS analysis. The total ion chromatogram (TIC) of the heat stressed sample is shown as Figure 1A. The extracted region of the chromatogram, on the top left, shows the main peak of Exenatide and several potential degradation products (1–5).

An untargeted analysis (with no searched components based on mass) was performed on the initial Exenatide LC-MS data to assign the peaks for the API and its impurities, as shown in Figure 1B. In the analysis method, the expected masses were assigned to each injection to establish individual target masses for subsequent analyses.

Each summed mass spectra for peaks present above 0.5% peak area in the TIC (calculated relative to the total peak area of all the peaks) was charge deconvoluted to obtain neutral mass information. The Intact Mass Application can automatically adjust charge deconvolute settings based on the m/z profiles of analytes (e.g., isotopically resolved, and unresolved regions of spectra). The charge deconvolution settings used for Exenatide are listed under “biomolecule” in the “Define Deconvolution Parameters” section of the method (Figure 2A) using a refined isotopic model for

“Protein” molecules. The BayesSpray algorithm was selected to provide monoisotopic deconvolution results for molecules with mass between 1–15,000 Da, since the synthetic Exenatide has a MW at 4184.0273 Da.

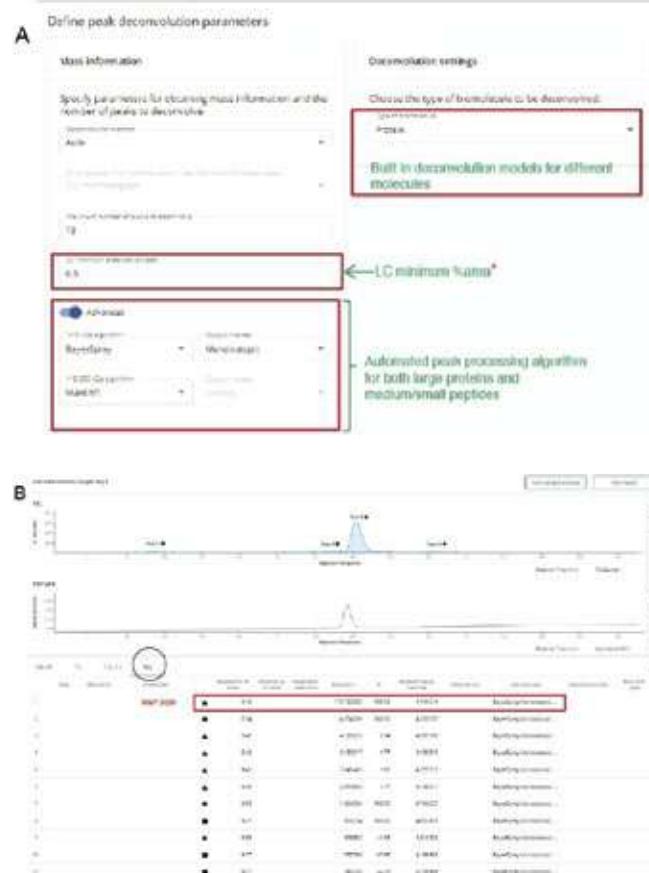


Figure 2. A) Intact Mass App processes data using built-in peak deconvolution algorithms. The BayesSpray algorithm was used with deconvolution settings for proteins for Exenatide. B) The untargeted analysis shows the neutral masses of compounds calculated based on the MS data. Each MS data point is marked by a symbol that refers to a chromatographically separated peak shown on the TIC above. Each mass shows a retention time that facilitates the identification of the chromatographic peak. The main peak 5 with 4184.018 Da is the most abundant component (API).

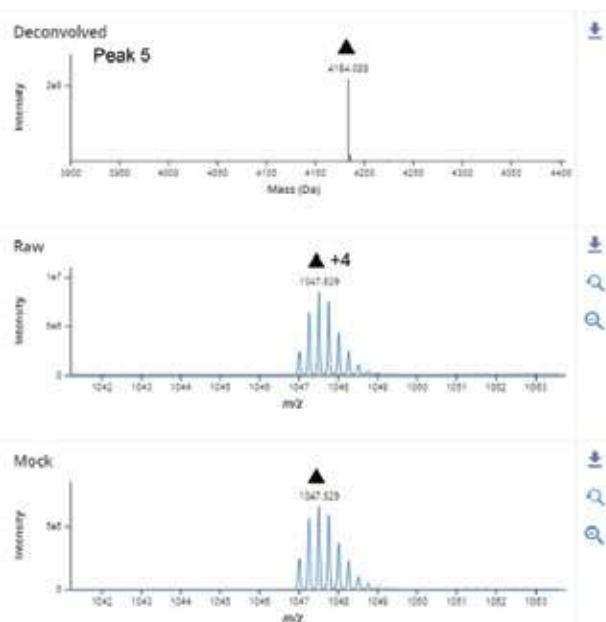


Figure 3. The figure shows XIC, charge deconvoluted, raw and mock spectra for Exenatide main peak. The raw data shows the zoomed-in +4 charge state.



Figure 4. A) The TIC shows the main peak and impurity peaks found in the Exenatide stressed sample. The highest abundance peak is labelled as main peak and other chromatographically separated peaks are labelled as impurities from 1–5.

B) Each injection is set up as untargeted analysis.

API PURITY AND IMPURITY QUANTIFICATIONS

API Impurity assignment is often followed by purity assessment of the Drug Product (DP). The peptide purity level was determined by the following equation: percent peptide purity = $[(\text{Area UV 220nm DP}) / (\text{Area UV 220nm all peaks})] * 100$; where: Area UV 220 UV nm DP is the peak area for the desired peptide in the LC chromatogram monitored at 220 nm, and Area UV 220 nm all peaks is the sum of the areas for

all peaks. Following the same formula for optical detection, the example data was used for purity calculation using MS response (TIC) as well.

Figure 5 shows the percent purity of the stressed sample (four days at elevated temperature). In the purity calculation, the C-terminal amidation was grouped together with native Exenatide MS response to obtain the total percent purity of the drug product. The C-terminal amidation is a common modification observed in synthetic peptides that improves the stability of the peptide

Like API purity, the Intact Mass Application calculates relative quantification for impurities using the peak area from TIC or UV trace. Further, the percent %abundance of an impurity can be calculated based on peak area or peak height relative to the total response of all the peaks or the most abundant peak. The Day 4 stressed impurities were calculated using the peak area of impurity relative to the peak area of the most abundant peak. A pass/fail criterion was implemented to demonstrate a built-in quality control feature. We reported the quantitation value using TIC due to low UV signal under the formic acid mobile phase conditions. Using lower levels of TFA as the ion pairing reagent would be beneficial for obtaining sensitive UV signals, should the quantitation using UV trace is preferred, but would require higher sample loadings to overcome TFA induced electrospray signal suppression.

The most recent guidelines published by FDA for synthetic peptide drugs require the relative quantification of impurities that are more than 0.5% abundance of the drug substance/API.7 Therefore, in this case study, 0.5% was selected as the minimum quantification threshold. In the example data, only peaks at or more than 0.5% abundance were reported. The most abundant impurity was the aggregate (dimer) of Exenatide which was measured at 16.8% abundance (day 2 stressed sample), and completely absent in the reference sample (day 0) confirming the susceptibility of Exenatide to elevated pH and heat effects. Other major impurities were oxidation at 4.7%, loss of methionine (desMet) at 1.4% and asparagine succinimide at 5.9% relative to the main peak.

The result table in Figure 5, displays a variety of other measurements: such as product purity, modifier assigned,

peak retention time, MW, mass error, quantitation, and pass/fail status. The purity of the Exenatide stressed sample was flagged due to the API purity which was below the expected value set at 95%. The lowest reported impurity (0.8%) identified in the Day 4 stressed sample had a double oxidation peak of Exenatide with 6.5 ppm mass accuracy (Figure 6). The abundance of this peak relative to the total MS response of all the identified peaks using deconvoluted MS spectra was ~0.6%, showing high quality results for both major and minor detected impurities.

CONCLUSION

- ▶ An automated LC-MS workflow for synthetic peptide mass confirmation and impurity profiling was accomplished using the BioAccord LC-MS system within the waters_connect informatics platform
- ▶ Stressed Exenatide peptide was used to show the process of API identity confirmation, purity assessment, and impurity definition and quantitation. Quality features such as System Suitability Test, selectable thresholding, and pass/ fail criteria were illustrated
- ▶ The utility to perform an initial untargeted impurity characterization for initial peak assignments and library generation, followed by subsequent targeted monitoring was shown to offer the flexibility and usability for analysts to evaluate the product quality attributes, and rapidly evolve methods with new product knowledge



Figure 5. The result table presents data for the Exenatide impurity analysis for day 2 stressed sample. The data shows %main peak and impurity levels calculated using TIC chromatogram. The purity threshold is set at 95%. The purity is flagged (Warning sign labelled in orange) due to low %Exenatide main peak level at 94.2% based on TIC.

all peaks. Following the same formula for optical detection, the example data was used for purity calculation using MS response (TIC) as well.

Day 4 Stressed

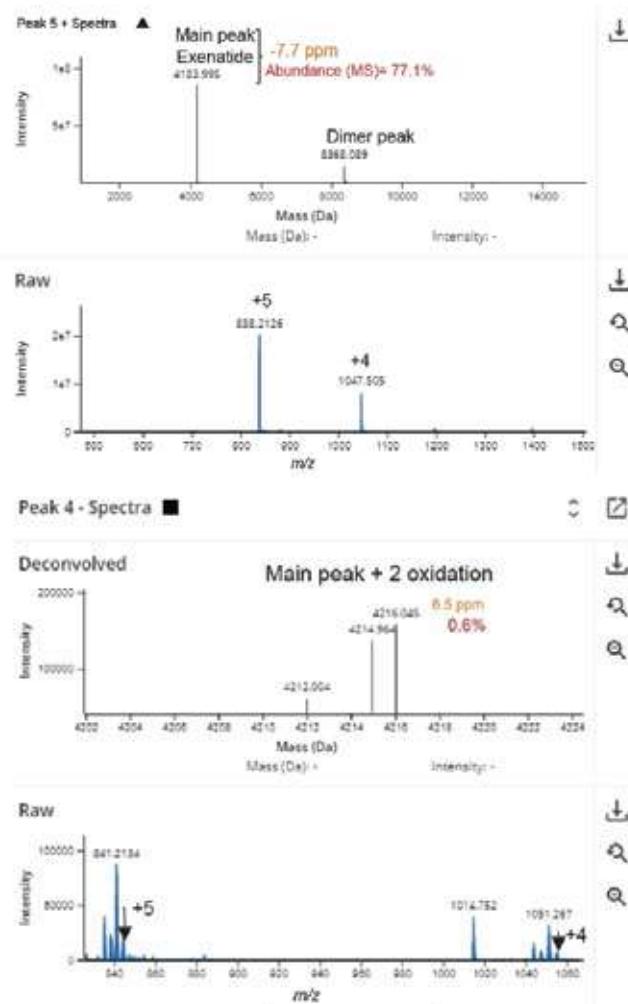


Figure 6. The Intact Mass App data shows high accuracy in data processing. The two peaks shown here are the main peak of Exenatide (left) and the one of the lowest impurity peaks (right) detected by the intact mass app.

Both peaks used the automated BayesSpray charge deconvolution processing parameters to detect the peaks with less than 10 ppm mass accuracy. The abundance of the peaks is 77.1% and 0.6% respectively for the main peak and the double oxidation peak based on the MS spectra.

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Ensure the instrument has completed warm-up and equilibration, pressures are stable, baselines are flat, no unresolved system alerts are present.



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Run a Quick Control or Blank

A blank or control injection before sample analysis helps identify carryover, contamination, or system noise early—before valuable samples are consumed or compromised.

Most analytical delays are not instrument failures—they are preventable realizations discovered too late.



Updates

Recent Novel FDA Approved Drugs

Myqorzo (aficamten)

Myqorzo, with the generic name aficamten, received FDA approval on December 19, 2025, as an oral therapy for adults with symptomatic obstructive hypertrophic cardiomyopathy (oHCM). It marks the first FDA-approved product from Cytokinetics and only the second cardiac myosin inhibitor in its class.

Mechanism of Action

Myqorzo is a **selective, reversible cardiac myosin inhibitor**. It works by reducing excessive myosin–actin cross-bridge formation in cardiac muscle, thereby **lowering hypercontractility** of the heart. In patients with obstructive hypertrophic cardiomyopathy (oHCM), this leads to a **reduction in left ventricular outflow tract (LVOT) obstruction**, improved ventricular filling, and relief of symptoms such as dyspnea and exercise intolerance.

By directly modulating sarcomere function—rather than heart rate or blood pressure—aficamten targets the **underlying pathophysiology of oHCM at the molecular level**.

Exdensur

Exdensur has received FDA approval for the **treatment of severe asthma characterized by an eosinophilic phenotype**, where it is used as an **add-on maintenance therapy**. The approval addresses a subset of asthma patients whose disease remains uncontrolled despite standard inhaled corticosteroids and long-acting bronchodilators.

By specifically targeting eosinophil-driven inflammation, Exdensur offers a precision-medicine approach for reducing exacerbations, improving asthma control, and lowering reliance on systemic corticosteroids in severe disease.

Mechanism of Action

Exdensur works by selectively targeting the eosinophilic inflammatory pathway central to severe asthma. It reduces eosinophil levels by interfering with signaling mechanisms essential for eosinophil survival and activity, leading to decreased airway inflammation and improved respiratory function.

This targeted biologic approach directly addresses the underlying immunologic driver of eosinophilic asthma rather than providing symptomatic relief alone.



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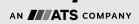
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