

CATALYST Cue

VOLUME 02

ISSUE 01

AUGUST 2025

EXPLORING THE AGE OF INFORMED AUTOMATION



AUTONOMOUS WORKFLOW

ROBOTICS AND ML RESHAPE
CHEMICAL SYNTHESIS



CRYSTAL POWDER DOSE



NEW PARTNERSHIP

CENTRIFUGAL PARTITION CHROMATOGRAPHY
FOR SCALABLE, HIGH-PURITY SEPARATIONS





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Dear Readers,

With this issue, we begin Volume 2 of CATALYSTCue.

We open this edition with our cover story on **Self-Driving Laboratories**. AI is no longer just assisting with data—it is now designing experiments, running them, learning from the results, and improving outcomes with minimal human input. It is a shift that is already happening, and this story breaks down what that means for researchers.

Our features **explore how freeze-drying preserves structural integrity** and stability, along with a useful breakdown of what to expect in the **Indian Pharmacopoeia's updated Weighing Chapter for IP 2026**.

In the interviews section, Dr. Arunraj Chidambaram (Chemspeed) discusses how modular systems and smarter workflows are shaping autonomous labs, while Dr. Ravishankaran (GeNext Genomics) highlights the Implen NP80's role in precise, low-volume analysis.

The **Application Showcase** spans FTIR microanalysis for pharma troubleshooting, Kjeldahl-based nitrogen/protein testing in rice, nucleic acid quality checks, mRNA synthesis optimization, and ultrapure water's impact on cell culture.

We are introducing two new segments: **AI and Automation**, which explores Chemspeed's SWING and iSYNTH in action, including a case study where machine learning streamlined reaction optimization, and **Chromatography Edge** which features Semaglutide purification (RotaChrom CPC), carbohydrate separation (Puriflash + ELSD), anti-diabetic drug analysis (Shodex HPLC), and PFAS profiling (Waters Xevo TQ Absolute).

The Tech Corner simplifies an often-overlooked routine—**balance cleaning**. Our Product Highlight turns the spotlight on **BUCHI's Lyovapor freeze drying systems** and how they bring safety, control, and performance together in one package.

This issue also includes a special supplement—**Technologies Tailored to Fit Your Lab Process**—featuring the instruments and solutions showcased at **ANACON-Hyderabad**, organised in a way that mirrors how real labs work.

This is just the start of Volume 2. If something in this issue sparks a thought, question, or disagreement, I hope you will write in. CATALYSTCue is not just a magazine—it is a conversation. Thank you for being part of it.

Best regards,

A handwritten signature in black ink, appearing to read 'Arun Mathrubootham'.

Arun Mathrubootham
Director
Inkarp Instruments Pvt. Ltd.

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Editor
Arun Mathrubootham

Creative Team
Apoorva Nagarajan
Halim Baig
Raju Dasari

Marketing Team
Venkata Pavan Kumar
Sanghavi Suresh



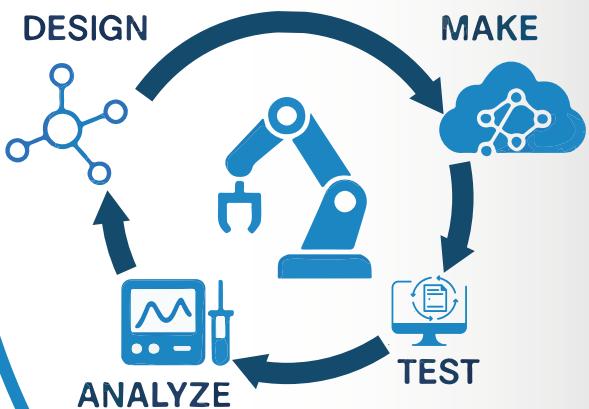
THE RISE OF SELF-DRIVING SCIENCE

Reimagining Research in the Age of AI

Fundamentally, science is an act of curiosity. However, the conventional bench-top lab is giving way to something far more intelligent as the questions become more complex and the need for speed, reproducibility, and scale increases. A silent revolution is taking place in both academic and industrial research environments, one in which cloud-connected systems, learning algorithms, and robotic arms work together in real time. This is not a work of science fiction. The new normal is this.

Welcome to the world of AI-guided automation, where every experiment produces not only answers but also new questions, and where manual labour is no longer a barrier to discovery.

The Automated DMTA Loop



Pipettes to Programmable Labs

Industrial labs and pharmaceutical behemoths have long been linked to automation in research. However, in academic and exploratory science today, it is presenting a strong argument. Research automation, which was previously hampered by strict procedures and exorbitant expenses, is now modular, more reasonably priced, and driven by intelligent software that can adjust to evolving workflows.

A revised Design–Make–Test–Analyze (DMTA) cycle is at the core of this change. A continuous, adaptive loop is taking

the place of what was formerly a laborious manual and linear process. Robotic systems carry out multi-step protocols, scientists input their theories, and AI evaluates results in real time, recommending what to try next. It is extremely quick, intelligent, and iterative.

THE DEVELOPMENT OF AUTONOMOUS RESEARCH FACILITIES

The 21st century is about platforms and prediction, whereas the 20th century was about pipettes and petri dishes. Once

controversial, the idea of a "robot scientist" now boosts productivity. Mobile robotic chemists and fully autonomous biology labs are examples of systems that can conduct hundreds of experiments per week without interruption, without human intervention, and with continuous learning.

AUTOMATION IN ACTION

These are decision-makers, not merely machines. Labs can investigate large chemical or biological spaces without human fatigue or guesswork thanks to closed-loop optimisation, which is fuelled by Bayesian algorithms and neural networks. They think in addition to performing.

- ▶ **"Adam," Robot Scientist:** Without human assistance, Adam independently developed and tested yeast genomics hypotheses to determine the functions of genes. It was among the first examples of a robot performing an entire scientific cycle.
- ▶ **Agile BioFoundry²:** During COVID-19² a automated biofoundry that was initially constructed for synthetic biology was modified to create COVID-19 diagnostics, speeding up the development of tests with the use of modular workflows and liquid-handling robots.
- ▶ **Robotic Mobile AI Chemist³:** A mobile robotic chemist equipped with AI navigated a lab autonomously, mixing reagents, performing experiments, and optimizing reaction conditions using Bayesian search. It worked 24/7 and completed 688 experiments in 8 days, demonstrating a dramatic acceleration of discovery.
- ▶ **Regenerative Medicine with AUTOSTEM⁴:** AUTOSTEM facilitated consistent quality production of cell therapy at the clinical scale by automating the culture and harvesting of mesenchymal stem cells.
- ▶ **Cloud Laboratories:** Automated cloud labs with remote access let researchers conduct experiments from any location. For startups and small teams, these "labs-as-a-service" lower the infrastructure barrier.

HUMAN IN THE LOOP

Automated systems aren't perfect, and they're definitely not

autonomous, even with their speed and accuracy. Collaboration, not replacement, is where this transformation's true power resides.

Today's scientists are interpreting machine-driven hypotheses, curating data, and designing workflows. Models of adaptive automation are being developed to recognise human workload, modify their degree of autonomy, and request user input as necessary. The goal is to elevate the role of the scientist, not to cede control.

Our approach to ethics, training, and UI design must change along with these systems. Because the success of the automated lab of the future will be determined by factors like trust, transparency, and usability.

WHY AUTOMATE?

The solutions are both pragmatic and philosophical:

Reproducibility: Reduced errors and variables.

Throughput: Tasks that used to take months can now be completed in a matter of days.

Safety: Machines are more adept than humans at handling dangerous chemicals.

Scalability: Automation greatly facilitates the transition from benchtop to pilot scale.

Understanding: Data analysis gains strength and predictiveness as each experiment is recorded and labelled.

Automation is not only quicker, but also more intelligent.

OBSTACLES AND FACTS

Friction is a part of every revolution. Not all labs are able to pay the initial outlay. Some researchers are concerned that the "art" of practical experimentation may be lost due to strict protocols. Others are concerned about restricting exploratory freedom and over-automating.

It is evident that a balance is necessary for successful adoption: systems need to be both reliable and adaptable,

data-rich and user-friendly. The goal is to create a lab that learns alongside the researcher, not to replace them.

HYBRID INTELLIGENCE

Future labs won't be robotic strongholds. It will be a hybrid environment where algorithmic rigour and human intuition coexist. Where failures become feedback loops rather than dead ends. Where systems and scientists collaborate to learn.

AI is the co-pilot in this vision, not the solution. Although the pipette may never completely vanish, its function is evolving. It is replaced by a new type of research that is much more collaborative, quicker, and smarter.

FINAL THOUGHTS

AI-guided automation enhances scientific thinking rather than replacing it. It enables researchers to go beyond the bounds of human speed, take on more challenging issues, and pose more ambitious questions.

From Operator to Orchestrator: Scientists are now spending more time creating intelligent workflows and less time on manual setup.

From Executor to Explorer: Researchers can concentrate on new hypotheses, imaginative experimental design, and interdisciplinary innovation when routine tasks are delegated.

From Expert to Strategist: The modern scientist's skill set is increasingly centred around data fluency, systems thinking, and AI literacy.

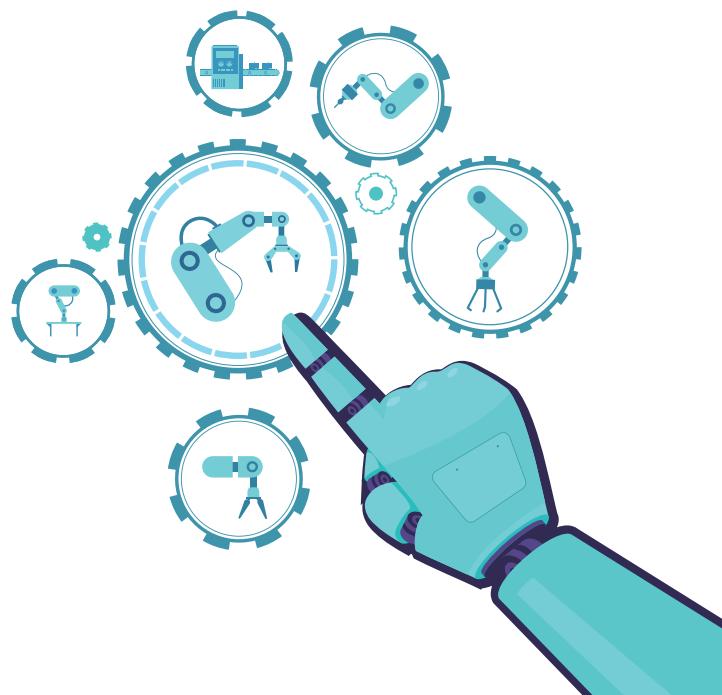
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Letting the machines assist is possibly the most human thing we can do in a world where discovery cannot wait!



UNDERSTANDING THE INDIAN PHARMACOPOEIA'S WEIGHING CHAPTER FOR IP 2026



A Practical Breakdown of the Revisions

When combined with automated small-scale reactor formats (from microlitre to millilitre scale) and intuitive software, overhead gravimetric dispensing of solids and liquids provides a potent method for speeding up, standardising, and digitising small-molecule reaction screening, from preparation and photochemistry to work-up and analysis.

In light of this, the Indian Pharmacopoeia Commission (IPC) has published a draft of a new general chapter, 2.1.7 Weights and Balances, which is intended to be included in the Indian Pharmacopoeia (IP) 2026. This draft introduces methodical approaches to performance verification, calibration, and minimum weight determination while aligning India's regulatory guidance on analytical weighing with international standards.

The Ministry of Health and Family Welfare's Indian Pharmacopoeia Commission (IPC) is in charge of establishing the country's pharmaceutical standards. The official list of quality requirements for pharmaceutical ingredients and goods is the Indian Pharmacopoeia (IP). It guarantees the safety, efficacy, and constant quality of medications made and sold in India. IPC's growing emphasis on enhancing analytical reliability and aligning procedures with global standards like the USP and EP is reflected in the introduction of general chapters like 2.1.7.

This article explores the update's salient features, real-world applications, and proactive ways that labs can comply with the updated standards.

BALANCE TYPES DESCRIBED IN THE IP 2026 DRAFT

In accordance with international metrology definitions, the draft groups balances according to their sensitivity and scale interval (d). These classifications aid in assessing appropriateness according to application and precision:

Balance Type	Typical Readability (d)	Sensitivity	Application Focus
Precision	$\geq 1\text{mg}$	Low	Raw material weighing, general prep
Analytical	0.1mg	Medium	Pharmacopoeial sample prep, dilutions
Semi-micro	0.01mg	High	Potent APIs, assay prep
Micro	1–10 μg	Very High	R&D, genotoxic impurities
Ultra-micro	$\leq 1\mu\text{g}$	Extremely High	Mass standards, trace contaminants

This draft only covers balances used in analytical workflows, not in manufacturing or formulation.

THE INTENT OF CHAPTER 2.1.7

Chapter 2.1.7's introduction is more than just a procedural change; it is part of IPC's larger strategic effort to align analytical practices in India with globally standardised standards. Among the main goals are:

- ▶ Creating consistent weighing procedures in all regulated labs
- ▶ By requiring scientific performance metrics (like Mmin), analytical variability can be decreased.
- ▶ Encouraging data integrity by documenting and tracing weighing operations
- ▶ Conforming to international pharmacopoeial standards, including EP 2.1.7, USP <41>, and <1251>
- ▶ Making certain that weighing is not viewed as a routine task but rather as a qualified analytical activity

This chapter presents a paradigm shift by highlighting the importance of precise weighing in relation to analytical compliance and product safety.

CHAPTER 2.1.7: WEIGHTS AND BALANCES: WHAT'S NEW?

Robust guidelines for the selection, calibration, verification, and use of electronic balances used in analytical procedures are outlined in the chapter. Interestingly, it doesn't apply to manufacturing balances or non-analytical contexts.

1. Classification and Scope

- ▶ Only balances used in analytical labs, especially for pharmacopoeial procedures, are subject to the guidelines. Balances used in general laboratory work or manufacturing are not included. Although it acknowledges both mechanical and electronic balances, because of their accuracy and extensive use, it primarily concentrates on electronic systems.

2. Equilibrium Setting up and the surroundings

- ▶ It is necessary to place balances on sturdy, vibration-free surfaces. It is advised to use dampening pads or anti-vibration tables.
- ▶ Installation spaces need to have regulated temperatures (typically 20–25 °C) and little fluctuation in humidity (40–60%).
- ▶ It is necessary to protect balances from environmental disruptions like sunlight, drafts, magnetic fields, and electrostatic charges. In some circumstances, ionising bars or enclosures may be required.
- ▶ Prior to each use, levelling indicators need to be examined. To avoid static accumulation, balances must also be correctly grounded.

3. Performance Qualification (PQ)

Prior to a balance being used regularly, PQ is required. It entails proving that, in real-world operating circumstances, the balance satisfies specified standards for accuracy and precision.

PQ consists of:

- ▶ Testing for repeatability across several measurements with standard weights.
- ▶ Sensitivity testing to confirm that the balance is linear and responsive to known weights.

4. Repeatability

- ▶ The same standard weight must be used for a minimum of ten weighings. The repeated measurements are used to calculate the standard deviation (SD), and for the balance to be deemed appropriate, the coefficient of variation (%RSD) cannot be greater than 0.10%.
- ▶ This test supports the M_{min} calculation and guarantees the accuracy of the instrument under real-world use conditions.

5. Sensitivity

Also referred to as the accuracy or deviation check, this test assesses the balance's capacity to provide an accurate value for a known test weight.

- ▶ The certified weight and the measured value cannot differ by more than $\pm 0.05\%$.
- ▶ This test is necessary to confirm that the balance is appropriately calibrated and appropriate for analytical work.

6. Determining the Minimum Weight (M_{min})

The smallest amount that can be weighed with respectable accuracy and repeatability is denoted by M_{min} .

- ▶ The formula is $M_{min} = (2 \times SD / 0.001) \times k$, where k is the coverage factor (usually 2 for 95% confidence).
- ▶ It is necessary to label or document balances with their verified M_{min} value. To maintain data integrity, analysts must refrain from weighing less than this threshold.

- ▶ This requirement is essential to guaranteeing measurement traceability and is in line with international best practices (USP 41>, EP 2.1.7).

7. Routine Verification

Depending on the laboratory's risk-based SOPs, balances in use must go through regular performance checks on a daily, weekly, or per-use basis. Among these checks are:

- ▶ A low or mid-range test weight to check for repeatability (precision).
- ▶ A certified weight traceable to national standards is used for the sensitivity (accuracy) check.
- ▶ Out-of-specification events must prompt corrective actions, and results must be documented.

8. Traceability and Calibration

Weights traceable to SI units via a national metrology institute (e.g., NABL in India) must be used for calibration, which must be done at least once a year or whenever there is a change in location, a suspected malfunction, or a service intervention.

The following must be included in calibration certificates:

- ▶ Measurement uncertainty
- ▶ Traceability statement
- ▶ Identification and status of test weights used
- ▶ Environmental conditions during calibration
- ▶ External calibration cannot be replaced by internal calibration with motorised weights, if applicable.

CONCLUSION

A significant step towards modernising and fortifying India's regulatory framework for analytical weighing has been taken with the addition of Chapter 2.1.7 to the Indian Pharmacopoeia (IP 2026). The IPC has brought national expectations into line with globally accepted standards like USP 41> and EP 2.1.7 by incorporating concepts like traceable calibration, routine verification, and minimum weight determination.

To guarantee preparedness, laboratories in research, regulatory testing, or quality control must take immediate action. This entails updating SOPs, figuring out Mmin for every balance, making sure test weights are traceable, and recording every performance check. More significantly, labs need to promote a culture of accuracy and responsibility in their measurement procedures.

In addition to protecting against regulatory scrutiny, early compliance will increase trust in the accuracy of analytical results. The message is clear for the pharmaceutical industry, where variations at the microgramme level can affect safety: every milligram matters when it comes to weighing.

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HOW DOES FREEZE DRYING PRESERVE STRUCTURE AND STABILITY?



Freeze drying is the gentlest and most effective way to preserve delicate materials that are prone to degradation and/or decomposition.

The principle of freeze drying is based on sublimation – direct conversion of solid to gaseous state. Although water is the most common solvent to be removed from samples, other solvents like ethanol have been used to a certain extent. Initially, the product is frozen and then dried by sublimation in an environment of reduced pressure. The low pressure enables direct changeover of the frozen solvent into vapor. Product stability is massively increased by reducing its water content due to the direct link between water presence and biological and chemical activity which are mainly responsible for product degradation. Compared to other dehydration methods which use high temperatures that affect the shape and colour of a product, freeze drying ensures less product damage, avoids shrinkage or agglomeration of the product. Freeze drying is highly suitable in cases of products that are heat sensitive

PRINCIPLE OF FREEZE DRYING: SUBLIMATION

The initial freezing process creates ice crystals within and on the surface of the product. By turning into ice, the individual

water molecules lock up into a well-defined grid. As the water molecules sublimate from the product, they leave little pores and gaps with the product and thus help maintain its shape and structure.

Rehydration of the product is therefore quick and simple—a particularly important requirement in pharmaceutical applications.

As shown in Figure 1, water goes through all physical states (solid, liquid and gaseous) as long as the prevalent pressure is above 6.11 mbar. At exactly 6.11 mbar, the three states coexist (triple point), and below 6.11 mbar water occurs only as ice or water vapor.

The product to be freeze dried is initially often a liquid. Considering the phase diagram, the freeze drying process leads via two phase changes, first to the solid and further to the gaseous phase.

For sublimation to take place once the product is completely frozen, the pressure must be below the triple point.

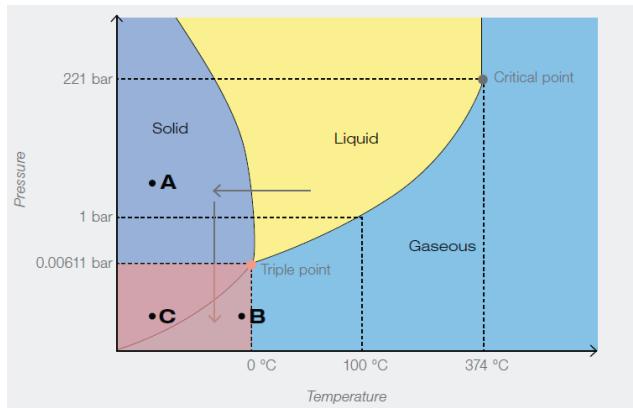


Figure 1. Phase diagram of water as a function of pressure and temperature. All states coexist at the triple point. Freeze drying occurs below the triple point and requires low pressures as well as temperatures.

KEY PARAMETERS: PRESSURE, TEMPERATURE AND CONTROL

The freeze drying process involves three stages-freezing, primary drying and secondary drying. Each of these steps has distinctive requirements in terms of pressure and temperature. For the primary drying stage, conditions for efficient sublimation need to be established. At the same time, it is essential to maintain product characteristics by ensuring the product's temperature remains below a distinct temperature- its critical temperature. Above this temperature the product structure collapses leading to shrinkages or cracks.

Ideally, freeze drying is performed at temperatures just below the critical temperature. The drying chamber pressure is decreased to activate the drying process. The prevailing pressure and temperature readings are now below the triple point (point B).

Sublimation creates water vapour in the drying chamber. If not removed from the system, the water vapour equilibrium and no further ice particles sublimate. The vapour particles are removed from the system by means of the ice condenser, a cooling device running at temperatures far below the critical product temperature (point C).

The vapour pressure resulting from the low temperature of the ice condenser is therefore lower than the vapour pressure of the product and naturally forces the water molecules to travel towards the ice condenser. Consequently, the water vapour, as well as other condensable gases, freeze on the ice collector, forming ice.

The rate of sublimation is basically defined by the difference in vapour pressures developed over the product and the ice condenser. Larger this difference, faster is the rate of sublimation. Also, the closer the product temperature is to the triple point, the larger is the pressure difference.

DRIVING FORCE HEAT

Sublimation is an endothermic process. The product undergoing sublimation gives off heat and would eventually cool down if the required heat is not supplied otherwise. An additional input of heat boosts the sublimation process and represents the real drive of freeze drying. Heat transfer methods include conduction, convection and radiation.

Shelf drying relies primarily on thermal conduction of the heated shelves, allowing precise control of the temperature.

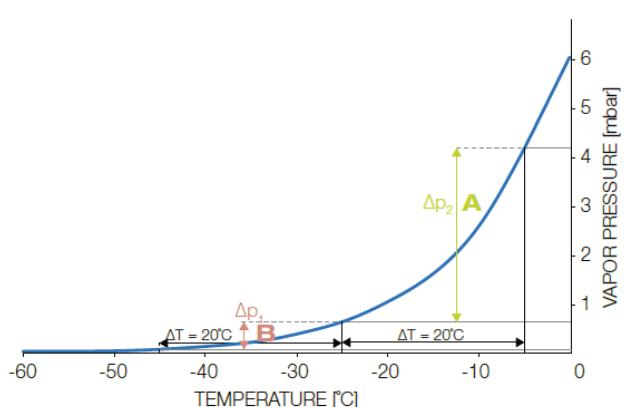
Manifold drying depends on the ambient heat transferred through convection and radiation, offering less control over heat input.

COMPONENTS OF A FREEZE DRYER

The main components of a freeze dryer are drying chamber, the vacuum pump and the ice condenser.

The sample to be freeze dried is either placed in a system of shelves inside the drying chamber or filled into single flasks that are attached to the manifold. The vacuum pump is connected to the drying chamber via ice condenser and is responsible for evacuation of the drying chamber. Additionally, the vacuum pump removes all non-condensable gases that did not aggregate at the ice condenser.

The main role of the ice condenser is to collect water vapour and all other condensable gases. Water molecules travel naturally towards the ice condenser due to the difference in vapour pressures. The temperature of the ice condenser must be significantly lower than the product temperature- at least 15 °C colder.



PROCESS STAGES: FREEZING, PRIMARY & SECONDARY DRYING

The main steps in freeze drying are freezing, primary and secondary drying.

Freezing takes place in a deep freezer under liquid nitrogen or via a low temperature bath. The freezing temperature should be chosen well below the critical point of the solvent to ensure the sample is fully frozen (Figure 2, point A). At the end of external freezing, the freeze dryer is prepared for operation by initialising the condenser cooling (Figure 2, point C). Once freezing is completed, the samples are placed in the drying chamber, or the vessels are attached to the manifold ports. The drying chamber is then evacuated, and the application specific vacuum settings are applied.

In case of heatable shelves, the shelf temperature is raised, increasing the temperature of the sample. Even so, the sample temperature must be maintained below the critical

(Figure 2, point B). The sublimation process is now activated. When liquid formulations freeze, they form ice crystals- rapid cooling produces small crystals while slower cooling produces larger, easier-to-remove crystals. Freezing behaviour depends on the composition of the sample.

Generally, formulations fall into two categories:

Eutectic Mixtures: These contain components that freeze at lower temperatures than water. While water may appear frozen, residual liquids can remain until the eutectic temperature (temperature where all components of the mixture are properly frozen) is reached. Also, applying vacuum too early can damage the sample.

Amorphous Mixtures: These do not crystallise but form a glass-like solid as they cool. Their stability is governed by the collapse temperature, slightly below the glass transition point. These mixtures are more difficult to freeze dry due to their complex behaviour.

In both cases, knowing the critical temperature is essential to prevent structural damage during drying.

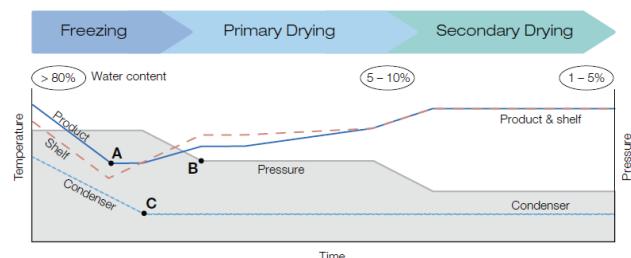


Figure 2. The freeze drying process includes three main stages: freezing, primary drying and secondary drying. The initial water content is usually reduced to more than 95%.

Primary Drying

This first drying phase removes the bulk of the water within the sample. To optimise efficiency, the sample is kept at the highest possible temperature below its critical point, maximising vapour pressure, without risking its collapse.

Heated shelves gradually raise the temperature at a controlled rate, while real-time temperature monitoring helps determine when drying is complete.

Secondary Drying

After primary drying, most ice is sublimed, but 5-10% moisture may remain, bound within the sample structure. Secondary drying removes this residual water through desorption requiring lower pressure and a higher shelf temperature-without compromising sample stability. This phase is shorter, aiming to reduce moisture to 1-5% for optimal long-term preservation.

END POINT DETECTION

Knowing the end point is crucial for sample stability. Several well-established methods are used to determine drying endpoints:

Temperature Difference Method

During primary drying, the sample temperature remains below the shelf temperature due to energy loss from sublimation. Once this ends, the sample and shelf temperatures converge, indicating completion.

Pressure Rise Test

When the chamber is temporarily isolated, any residual sublimation causes a rise in pressure. A stable pressure implies completion of drying.

Pressure Difference Test

This method compares readings from a Pirani gauge (affected by water vapour) and a capacitance gauge (unaffected by water vapour). Converging readings indicate the absence of water vapour and therefore, end of drying.

DRYING CHAMBER CONFIGURATIONS

Drying Type	Heat Source	Control Level	Use Case
Manifold	Ambient (convection/radiation)	Low	Multi-user, flexible
Shelf	Direct conduction	High	Precise drying, batch stoppering

POST-DRYING SEALING AND STORAGE

Freeze-dried products are hygroscopic and sensitive to moisture and oxygen. Proper sealing, ideally under vacuum or inert gas, is essential.

Stoppering systems, notched stoppers allow vapour to escape during drying and then pressed into vials under vacuum for airtight closure.

For products not suited to vacuum storage (e.g., products that are rehydrated via syringe), backfilling with inert gas like nitrogen is recommended before sealing.

CONCLUSION

Freeze drying is one of the most reliable and scientifically rigorous methods for preserving sensitive pharmaceutical, biotechnological and food products. By combining controlled freezing, precise pressure regulation and calculated heat application, this process ensures structural integrity, stability and long-term shelf life-without compromising the chemical or biological properties of the sample.

Understanding the principles of freeze drying process allows scientists to tailor their freeze drying workflows.

REFERENCE

www.buchi.com





Dr. Arunraj Chidambaram

Sales, EMEA Market, Chemspeed Technologies

Dr. Arunraj Chidambaram works as a sales manager for materials science market of EMEA in Chemspeed since 2021. He has a background in chemical engineering, materials chemistry, and automation chemistry with degrees from EPFL, TU Delft and Anna University. For his PhD thesis research work at EPFL, he was using the Chemspeed robotic system to accelerate the discovery of porous materials for energy applications. He combined automated synthesis, characterization driven by AI/ML algorithms to perform accelerated closed loop materials discovery research. In his current job as sales manager, he enables materials science researchers to introduce automation solutions of Chemspeed in their labs through consultative approaches.

In this exclusive interview, we speak with Dr. Arunraj Chidambaram who offers an insider's perspective on the evolving landscape of automated laboratories. From key trends like the Design-Make-Test-Learn paradigm, the rise of autonomous workflows, and the growing demand for modular systems, this conversation sheds light on how smart automation is not just a tool, but a catalyst for innovation.

Driving the Future of R&D and QC Workflows

Since the launch of Chemspeed's Automated Synthesis Workstation (ASW) product line in 1997, in what ways has the landscape of automated repetitive R&D workflows changed?

Over the past 28 years, the approach in general, towards automation and digitalization in R&D and QC have transformed significantly. Initially, automation was primarily adopted by pharmaceutical companies, which were pioneers in integrating high-throughput methodologies into research. In fact, Chemspeed itself was founded in response to this need—our former CEO, Dr. Rolf Gueller, developed the idea during his time at Roche, where he recognized the limitations of traditional workflows in productivity as well as quality and envisioned a more efficient, automated approach.

What began as a niche innovation in pharma has now expanded into a broad spectrum of scientific domains. Today, Chemspeed systems are deployed across Life Science, Materials Science and Consumer Goods labs in industries and academia. This shift reflects a growing awareness of the benefits automation brings via acceleration, standardization and digitalization and thus maximizing output / productivity related to quantity and quality.

In the past four to five years, the landscape has further evolved with the advent of self-driving laboratories—integrated environments that connect automation with AI / ML. These systems rely on vast, high-quality standardized datasets to drive predictive models and autonomous decision-making. Automation plays a critical role here, not only in ensuring around-the-clock operation but also in producing reliable, standardized data that fuels the evolution of AI models. As such, automation is no longer a luxury / pioneering tool—it's becoming essential to / fuels the next generation of intelligent research.

Chemspeed has a long history in lab automation. How have recent advancements in your product line focused on streamlining and simplifying workflows?

In recent years, Chemspeed has introduced several strategic innovations designed to lower the barrier to automation and simplify lab workflows, especially for those hesitant to adopt automated solutions.

One of the notable developments are the CRYSTAL Powder Dispensing Systems, launched in 2022. This benchtop unit is designed for gravimetric dispensing of powders from mg to mg—an operation that, surprisingly, is still carried out manually in many laboratories, even in regions with high labour costs such as Europe and USA. The goal behind CRYSTAL SWILE and CRYSTAL POWDERDOSE was to offer an accessible, entry-level automation solution that addresses a fundamental yet repetitive task: weighing out solids. Its compact form factor and focused functionality make it easy to integrate into existing lab environments, whether on a sturdy benchtop or inside a fume hood.

Importantly, CRYSTAL also plays a strategic role in addressing resistance to automation. Often, technicians and scientists perceive robots as a threat to their roles. With CRYSTAL, the message is clear: automation exists to augment human capability, not replace it. By automating tedious and error-prone tasks like manual weighing, scientists can redirect their focus to more value-added activities such as data analysis and experimental planning.

Another major advancement reflects Chemspeed's evolution under the Bruker Corporation, which acquired the company in 2024. This strategic alignment allows Chemspeed to contribute to a broader, end-to-end vision of the self-driving laboratory. Under Bruker, an emphasis is on the Design-Make-Test-Learn (DMTL) cycle—a comprehensive approach that combines molecule / materials design (using AI and ML), synthesis / formulation (Chemspeed platforms), characterization (e.g. Bruker instruments like NMR and XRD), and feedback-based learning.

To support this vision, Chemspeed launched integrated automation packages in collaboration with Bruker. For example, labs can now pair automated synthesis or sample preparation with benchtop analytical instruments such as NMR, XRD, IR—enabling seamless, high-throughput experimentation and characterization. This is particularly impactful in applications ranging from molecules and materials discovery to corresponding process research / development.

These recent advancements—from entry-level automation

like CRYSTAL to end-to-end integration through the Bruker ecosystem—demonstrate Chemspeed's commitment to simplifying workflows, increasing accessibility, and shaping the future of lab automation and digitalization in a data-driven world.

“ Today, Chemspeed systems are deployed across Life Science, Materials Science and Consumer Goods labs in industries and academia. This shift reflects a growing awareness of the benefits automation brings via acceleration, standardization and digitalization and thus maximizing output / productivity related to quantity and quality. ”

In your opinion, what are the key trends that are shaping the future in lab automation and digitization, and how is Chemspeed positioned to adapt/lead in this direction?

One of the most transformative trends shaping the future of lab automation is the Design-Make-Test-Learn (DMTL) framework, which lies at the heart of the self-driving laboratory concept. While initially pioneered by pharmaceutical companies to accelerate drug discovery, this paradigm is now expanding rapidly into materials science and consumer goods.

In essence, DMTL represents a closed-loop research approach where molecules or formulations are first computationally designed (using AI/ML), then automatically synthesized / formulated, tested, and analysed—feeding data back into the system to evolve with the next iteration. This iterative process enables faster innovation cycles, data-driven decision-making, and enhanced reproducibility.

Chemspeed is uniquely positioned to lead in this space. Through its integration into the Bruker ecosystem, the company now offers modular and scalable automation platforms that not only perform the Make but also connect seamlessly with analytical instruments like NMR and XRD. These integrated solutions support high-throughput experimentation and are designed to meet the specific needs of modern, data-centric laboratories.

An important aspect to consider is that the degree of automation varies significantly across organizations. Some institutions aim for fully autonomous labs—where robots manage everything from sample prep to characterization—while others prefer semi-automated setups that still rely on human oversight for certain tasks (human-in-the-loop), such as transferring samples or managing logistics. There is even growing interest in the use of mobile collaborative robots or hubots that transport samples between systems within a lab environment, offering a flexible alternative where full integration isn't feasible.

At Chemspeed, this diversity in automation maturity is met with a consultative and customizable, off-the-shelf modularity approach (LEGO concept). Rather than promoting a one-size-fits-all model, the company engages with clients to understand their workflows, constraints, safety requirements, and long-term visions. Based on this, tailored automation solutions are designed, delivered, and supported—from proof-of-concept to installation and training.

As the industry continues to shift toward digitalized, AI-driven R&D and QC, Chemspeed remains focused on enabling this transformation—not only by advancing automation hardware, but also by ensuring that the systems are data- and AI-ready, modular, and scalable for future integration with intelligent design and analytics tools.

Researchers have extremely specific needs. How are your systems customized and adaptable to such versatile research workflow and application requirements?

That's a crucial point—and one we often hear directly from our customers. No matter the size of the investment, whether it's a benchtop solution like the CRYSTAL or a larger, custom

configured FLEX or SWING platform up to a connected lab solution, researchers in R&D want modularity and adaptability at the core of any automation system they bring into their lab. This is because chemistry science evolves e.g. shifting to the increasing emphasis on green chemistry and circular economy principles.

At Chemspeed, we've made modularity and cross-discipline versatility a cornerstone of our design and offering. Our hardware platforms are built with interchangeable components and scalable modules, allowing researchers to customize their workflows based on evolving needs. For example, the same process reactor on one of our systems might be used by:

- ▶ A pharmaceutical company for chemical development of drug candidates,
- ▶ A polymer manufacturer for developing new polymers, or
- ▶ A battery R&D lab for producing electrode materials.

This flexibility allows customers to future-proof their investment. In fact, especially in European and North American universities, there is a growing trend to purchase an automation solution for use across an entire department. That means organic, inorganic, polymer, nano, and physical chemists all need to be able to use the same platform for different applications. If a system isn't adaptable to multiple research areas, it's often a deal-breaker.

To support this adaptability, we have a dedicated team of workflow architects who engage closely with our clients from day one. We ask them to share:

- ▶ Standard Operating Procedures (SOPs),
- ▶ Specific research workflows, or
- ▶ Experimental sections from published work.

Our team then translates those manual procedures into automated protocols, designing a solution that complies—and often enhances—their current process. This ensures that the automation we deliver fits seamlessly into the customer's research objectives rather than requiring

them to fit into a rigid automation box.

Ultimately, this approach ensures Chemspeed systems aren't just tools—they become research / researcher enablers, capable of evolving with the science itself.

Could you share some impactful R&D success stories with us?

Absolutely. In fact, there's a growing body of published work from our customers (<https://www.chemspeed.com/news/>) where Chemspeed's solutions have played a central role in breakthrough R&D efforts. If you visit our website, you'll find a comprehensive collection of scientific publications and case studies—all of them detailing real-world applications where our systems have enabled researchers to push boundaries.

These range across industries (Life Science, Materials Science, Consumer Goods). What's particularly rewarding is seeing how our platforms help customers accelerate their design-make-test-learn (DMTL) cycles. In many of these success stories, researchers were able to reduce experimentation timelines from months to weeks or even identify optimal formulations and reaction conditions with far fewer iterations than traditional methods.

We've worked with top universities, R&D centres, and leading companies globally, and many of them have published their results using Chemspeed platforms—something we're very proud of. These publications are not just testimonials; they're evidence of how automation is transforming scientific innovation.

Let me outline a fascinating case study— Autonomous Optimization of Discrete Reaction Parameters: Mono-Functionalization via a Suzuki–Miyaura Reaction

Researchers at the Institute for Chemical Reaction Design and Discovery (WPI-ICReDD), Hokkaido University, employed a Chemspeed automated synthesis platform to achieve selective mono-functionalization of a bifunctional substrate via the Suzuki–Miyaura reaction.

The team integrated a fully autonomous system combining robotic synthesis, supercritical fluid chromatography, and Bayesian optimization. This closed-loop setup explored 192

reaction conditions over 47 iterative cycles. The result? A fivefold increase in product yield — from 9.5% to 49% — with precise tuning of ligand, base, and solvent parameters.

This data-driven approach pinpointed eight critical descriptors of reactivity, offering a framework for future autonomous experiments. The generated mono-functionalized products have promising applications in organic photovoltaics, reflecting how AI-powered lab automation is redefining the landscape of reaction discovery.

How important is the Indian market to Chemspeed's overall global strategy?

India is undeniably a high-potential market, but at the moment, it still lags in the widespread implementation of lab automation. There are multiple factors at play here. One of the most cited is the availability of low-cost labour, which often makes traditional manual research workflows more economically viable—at least on the surface.

However, if you compare this with China, which has a similar population scale, we see a sharp contrast. China has invested heavily in automation. The difference lies in vision and long-term strategic investment. In India, while the potential is significant, the vision for adopting lab automation is still maturing.

Another major roadblock is funding. The kind of capital required to implement, scale, and standardize R&D through automation is decent to significant. In many European countries, USA, and Canada, you'll find a combination of state and federal funding mechanisms that actively support labs to modernize in view of the global mega trends. Unfortunately, such structured support isn't yet as prevalent in India. Many researchers still struggle to secure basic funding for PhD projects or essential laboratory infrastructure, let alone dream of self-driving labs or end-to-end automated platforms.

That said, we do see a very promising entry point in India—especially with solutions like our CRYSTAL Powder

Dispensing Systems—it's a practical first step for many Indian companies and institutions. Once they adopt a foundational automation tool and experience its benefits firsthand, the transition toward broader lab automation becomes much more natural. It changes how they perceive lab efficiency, reproducibility, and throughput. India is strategically important to Chemspeed's global outlook, but we also recognize that it's a long-game market. The interest is growing, especially among progressive research institutions and industries, and we're here to support that growth when the ecosystem—particularly funding and policy—begins to align with the potential we already see.

Lastly, from your perspective, what is the value of partnering with Inkarp in serving the research community in India?

In a country as vast and dynamic as India, it is clear that having a strong domestic partner is not just helpful — it is essential. Local presence means much more than just faster service; it means understanding the unique challenges, expectations, and ways of working that are part of everyday research life. A partner like Inkarp brings the ability to respond quickly and practically, with the right mix of technical support, application expertise, and business understanding.

From our side, it is important that researchers feel they are supported not only by a global company but also by a team that speaks their language, knows their environment, and can help solve problems in real time. With Inkarp's established experience and network across India, we can make sure that our technologies are not only delivered but truly integrated into the researchers' workflows — helping them move faster and achieve better outcomes.

In the end, it's about being closer to the community we serve, and Inkarp helps us do exactly that.



Dr. Ravishankaran R

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

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, Cry X, ASFV, CSFV.

His strategic leadership encompasses securing and executing BIRAC- and DBT-funded projects, establishing high-throughput immunotechnology laboratories, and commercializing diagnostic reagents for global corporations. Committed to translational innovation, Dr. Ravishankaran fosters industry-academia partnerships to drive next-generation antibody-based diagnostic solutions from bench to market.



Micro Volume, Macro Impact

Dr. Ravishankaran R shares how the Implen NanoPhotometer NP80 empowers GeNext Genomics Pvt. Ltd. with precise, reproducible results—even at low volumes—making it a trusted tool in quality control.

You work with microvolume nucleic acid samples — how critical is real-time pathlength control in ensuring consistency in your readings?

Based on our team's continual measurements, the Implen NanoPhotometer has shown to be quite reliable. For instance, measurements stay consistent when we separate nucleic acids from a sample, measure the concentration at one point, and then measure it again from the remaining section of the same sample.

We have compared the concentration of the same sample obtained on the first day with findings taken after 10 to 15 days, and the readings were almost the same. This is even more comforting. This indicates that the NanoPhotometer is highly stable over time in addition to being accurate in the near term. In our CRO and QC workflow, that degree of reproducibility is crucial, and it has continuously produced accurate and dependable readings.

How has microvolume analysis changed the way you prioritize or handle samples?

Microvolume approaches need 2 to 3 μL of sample. The NanoPhotometer provides accurate, dependable concentration readings even with such a tiny amount. Our approach to sample prioritisation has evolved as a result. We no longer feel the need to devote big volumes of rare or costly materials when we can receive high-quality data from such low volumes. It's a definite benefit that has improved the efficiency and reduced waste of our workflows.

Although recovering a portion of the material after measurement is occasionally feasible, there is a significant chance of losing roughly 50 μL . In addition, cuvettes must always be completely cleaned in between uses to prevent contamination. Over time, these minor but frequent dangers mount up.

When comparing it to other systems you've personally used, what difference did you observe in background correction or baseline drift?

I've had the chance to experiment with a variety of instruments for protein and nucleic acid measurement throughout the years. In that regard, the Implen

NanoPhotometer's exceptional accuracy and stability are what truly make it stand out. This NanoPhotometer has been continuously dependable in every test we've run, in contrast to some systems where you could have baseline drift or variable readings over repeated tests.

Additionally, it requires very little maintenance and is incredibly user-friendly. From the standpoint of an operator, that is a significant advantage. You don't have to worry about breakable parts or undertake regular recalibration. Regardless of whether you use different measurement channels or repeat the same test several times, the data is quite repeatable.

I favour using the NanoPhotometer in all of my workflows because of these benefits. It has continuously produced accurate results.

Are there particular challenges in quantifying low-concentration or partially degraded DNA/RNA that the NanoPhotometer helped you overcome?

Of course. Working with low-concentration or partially degraded DNA or RNA samples is often tough, especially when you're unsure about the purity or stability of the material. We haven't encountered these problems, though, thanks to the NanoPhotometer.

The device provides accurate concentration readings even with subpar samples, such as those with slightly degraded content or poorer purity. One feature that I really value is how the results section instantly displays both purity and concentration by values and via graphical representations. This allows us to assess not just how much material we have, but also how pure it is — which is critical when you're working with sensitive assays. We're currently using the NP80 model, which supports a very wide quantification range for DNA, RNA, and protein.

Have you ever tested the same sample on two systems? What stood out in terms of reproducibility or variability?

Yes, we frequently do this. Our workflows as CROs require strict quality control, and we routinely validate data across many platforms.

The same sample is usually tested with the NanoPhotometer,

and the results are cross-checked using a second technique, usually gel electrophoresis or SDS-PAGE. We can verify whether the same concentration is mirrored across platforms with the use of this two-pronged technique. This has been used in a number of projects, including internal research and commercial antigen and antibody programs. The consistency of the results from the NanoPhotometer is noteworthy. It has produced accurate and repeatable results even when working with samples that were obtained from outside sources, where there is always a chance that concentrations would be misrepresented. The data we get from it is now a routine component of our QC paperwork because it has gained our trust over time.

Has the system helped reduce experimental failures or variability in your workflows?

Yes, without question. Our quality control system now includes the NanoPhotometer as a crucial component. We use this technology to quantify all raw materials, including DNA, RNA, and protein, before we begin or finish any workflow. It serves as our initial checkpoint to make sure the inputs fulfil our requirements for quality.

To ensure repeatability over time, we also retest concentrations at various intervals in some investigations, particularly time-point studies. This enables us to keep an eye out for any possible deterioration or fluctuation under actual circumstances.

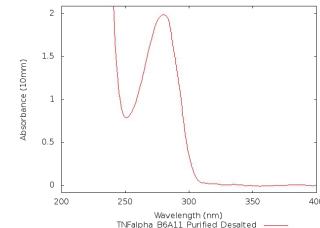
Because of this routine quantification, we've been able to maintain consistency in our input materials, which directly translates into lower variability and fewer failed trials. The technology gives us assurance that our baseline measurements are correct, which is extremely helpful in a CRO setting when client trust and consistency are crucial.

Implen NanoPhotometer®

Parameter		Wavelength (nm)		280	320 nm
Method	Protein UV	Background Correction		280	
Type	IgGMouse	Air Bubble Recognition		320 nm	
Mode	NanoVolume	On			
Protein Factor	0.714	Manual Dilution Factor		1.000	
Volume (uL)	1-2				

#	Name	Conc.	Units	A230	A260	A280	A320
1	Blank 1	0.0000	mg/mL	0.000	0.000	0.000	0.000
2	TNFalpha_B6A11_Purified_Desalted	1.4052	mg/mL	11.29	1.064	1.987	0.019

#	Name	A260/A280	Dilution
1	Blank 1	0.000	
2	TNFalpha_B6A11_Purified_Desalted	0.531	15

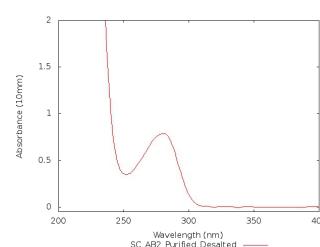


Implen NanoPhotometer®

Parameter		Wavelength (nm)		280	320 nm
Method	Protein UV	Background Correction		280	
Type	IgGMouse	Air Bubble Recognition		320 nm	
Mode	NanoVolume	On			
Protein Factor	0.714	Manual Dilution Factor		1.000	
Volume (uL)	1-2				

#	Name	Conc.	Units	A230	A260	A280	A320	A260/A280
1	Blank 1	0.0000	mg/mL	0.000	0.000	0.000	0.000	0.000
2	SC_AB2_Purified_Desalted	0.5605	mg/mL	4.371	0.442	0.785	-0.000	0.563

#	Name	Dilution
1	Blank 1	
2	SC_AB2_Purified_Desalted	15

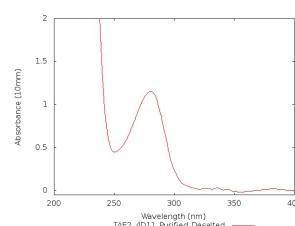


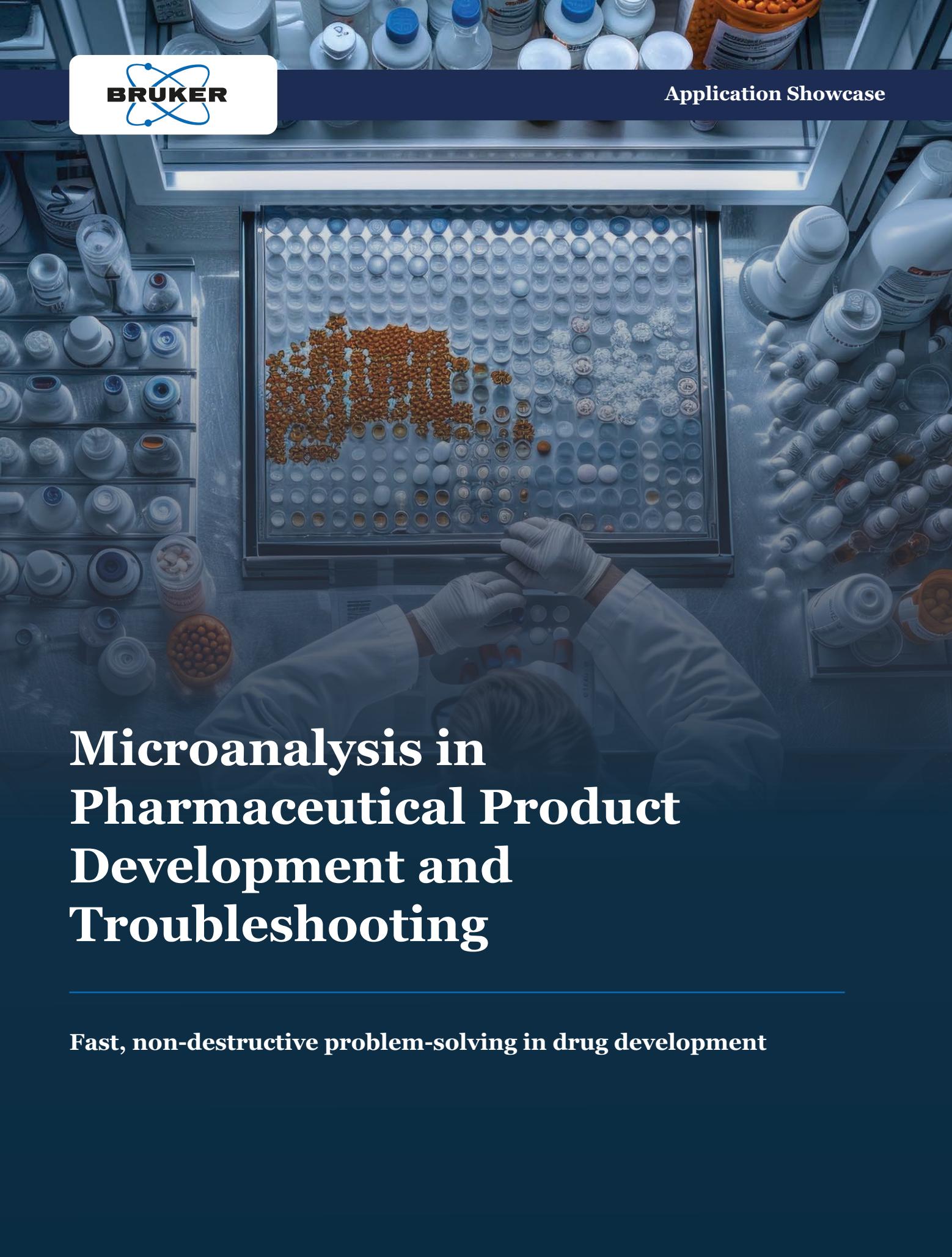
Implen NanoPhotometer®

Parameter		Wavelength (nm)		280	320 nm
Method	Protein UV	Background Correction		280	
Type	IgGMouse	Air Bubble Recognition		320 nm	
Mode	NanoVolume	On			
Protein Factor	0.714	Manual Dilution Factor		1.000	
Volume (uL)	1-2				

#	Name	Conc.	Units	A230	A260	A280	A320	A260/A280
1	Blank 1	0.0000	mg/mL	0.000	0.000	0.000	0.000	0.000
2	TAE2_4D11_Purified_Desalted	0.8132	mg/mL	6.449	0.587	1.155	0.016	0.501

#	Name	Dilution
1	Blank 1	
2	TAE2_4D11_Purified_Desalted	15



A photograph of a scientist in a white lab coat and blue gloves, working in a laboratory. The scientist is looking down at a large tray filled with numerous small, circular samples, possibly microanalysis samples. The background is filled with shelves of laboratory glassware and equipment, creating a professional and scientific atmosphere.

Microanalysis in Pharmaceutical Product Development and Troubleshooting

Fast, non-destructive problem-solving in drug development

Through the conversion of visual abnormalities into chemical insights, this application note investigates how spatially resolved infrared microanalysis deepens pharmaceutical problem-solving. Beyond simple identification verification, it illustrates how FTIR microscopy closes the gap between observation and action by facilitating data-driven, direct decision-making in situations involving contamination, formulation flaws, or unforeseen variability. Tablets, biopharmaceutical solutions, and excipient mapping are used as case studies to show how focused spectrum analysis is a useful tool in quality control and research and development settings since it not only identifies a material but also explains its presence.

Keywords or phrases:

Pharmaceutical products, FTIR microscopy, quality control, troubleshooting, particles and contaminants

INTRODUCTION

The demand on the quality of pharmaceutical products is very high. Raw and packaging materials as well as intermediate and final products must contain the correct components and need to be free from contaminations. In quality control, analytical methods like Mid- and Near-IR spectroscopy and others allow to verify an overall correct identity and composition of the sample.

However, contaminants like particles in liquid formulations or inclusions in tablets are often extremely small and cannot be detected selectively by such macroscopic methods. Therefore, further analytical methods are needed to determine the accurate chemical nature of the particle or inclusion – valuable information that allows to find the source of contamination.

This application note demonstrates how FTIR microanalysis using the Bruker LUMOS II supports both troubleshooting and pharmaceutical development, offering fast, automated, and spatially resolved insights across a variety of sample types.

IDENTIFICATION OF AN INCLUSION INSIDE A PHARMACEUTICAL TABLET

Pharmaceutical tablets have to be free from inclusions. If an inclusion is detected e.g. by visual inspection its chemical nature has to be determined to raise the chances finding its origin. Figure 1 shows a white tablet with a yellow spot inside.



Figure 1. Pharmaceutical tablet with contamination (yellow).

To discriminate the inclusion from the general matrix of the tablet a series of positions was measured on the yellow spot and next to it. Using the fully automated ATR (Attenuated Total Reflectance) mode of the LUMOS II the region of interest was analysed without any sample preparation; the tablet just had to be fixed in a micro vice on the microscopic stage. Guided by the software microscopic images of the region of interest were taken and measurement positions defined before the measurement was performed by the LUMOS II automatically. An area of $10 \times 10 \mu\text{m}$ per spot was measured with the spectrum acquisition taking 10 seconds, respectively. Figure 2 shows the visual microscopic image of the inclusion inside the tablet together with the measurement positions. The spectra are coloured according to their position.

It is obvious that the spectra measured on the inclusion (blue) have a much different signature than the ones on the general matrix of the tablet (red).

Result of IR-microscopic measurement

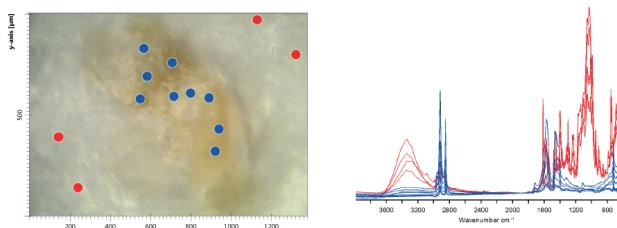


Figure 2. IR-microscopic mapping on pharmaceutical tablet with unwanted yellow inclusion. The spectra on the inclusion (blue) show a different signature compared to those from the tablet matrix (red)

The identification of the inclusion as magnesium stearate was achieved within a few seconds by searching the comprehensive spectral library ATR-COMPLETE which contains > 26.000 spectra of various substances. Figure 3 shows the high similarity of the library spectrum with the measured spectrum.

Similarity library spectrum / measured spectrum

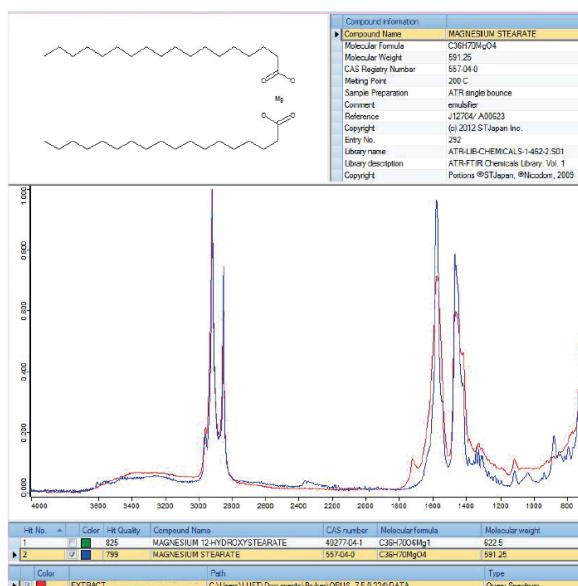


Figure 3. Clear identification of the inclusion as magnesium stearate by library search

DETERMINATION OF THE CHEMICAL IDENTITY OF A PARTICLE

Liquid sterile formulations must be free from all visible and even smaller particles. Unwanted particles might originate from the manufacturing equipment, the personnel or the used packaging material. Also unwanted precipitation of the active ingredient and excipient might result in particles.

Hence, the chemical composition of potential particles covers a wide range including plastic and rubber fragments, cellulose fibres, glass, metal or biological material like protein. For further analysis, particles are isolated from the liquid formulation using a gold filter (Figure 4) which can be used directly as substrate for the IR-microscopic measurement.



Figure 4. Gold filter for the isolation of particles from liquid formulations.

Figure 5 shows the microscopic image of several fibres that were filtered from the liquid formulation of a protein drug.

The spectra were measured without further sample preparation directly on the fiber bundle and on the filter substrate next to it with the LUMOS II by applying the ATR-technique. Whereas the spectra on the filter are typical for the protein drug (amide I at 1640 cm^{-1} and amide II 1550 cm^{-1} ; not shown) the fiber has a strong double peak around 1200 cm^{-1} .

Microscopic image and spectrum of particle

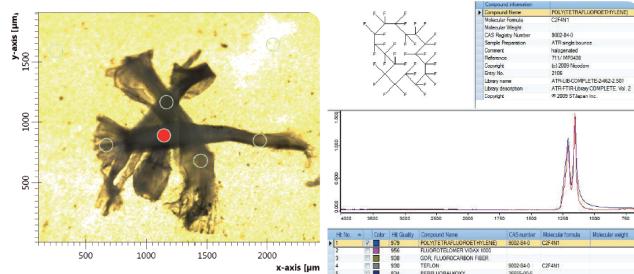


Figure 5. Left: Microscopic image of a bundle of fibres on a gold filter separated from a liquid biopharmaceutical. Right: Identification of the spectrum on the indicated measurement position as Poly(tetrafluoroethylene) (Teflon) by library search tablet covering an area of 500x325 μm . The spatial resolution of 25x25 μm (indicated by red rectangles) is defined by the operator using an automated physical aperture which is integrated in the LUMOS II.

ANALYSIS OF THE DISTRIBUTION OF API AND EXCIPIENTS IN A PHARMACEUTICAL TABLET

The quality of a pharmaceutical tablet is not only defined by its components but also by their distribution inside the tablet. As an example, a protective layer around the tablet as well as the homogeneity of the active pharmaceutical ingredient (API) distribution defines where and at which speed the API is released inside the body. IR-microscopic mapping measurements create chemical images of the API and the excipients distribution.

In this example, a fully automated ATR-mapping on the cross-section of a pharmaceutical tablet of the anti-inflammatory drug ibuprofen was performed using the LUMOS II. An area of 500x325 μm was measured with a spatial resolution of 25x25 μm (Figure 6).

Apart from the active pharmaceutical ingredient ibuprofen the excipients lactose, microcrystalline cellulose and sodium dodecyl sulphate are present in the tablet. To determine their distribution inside the tablet a linear combination of previously measured pure substance spectra was

performed to explain the composition of each individual mapping spectrum.

The calculation was done fully automatic using the OPUS spectroscopic software.

Rectangular mapping grid

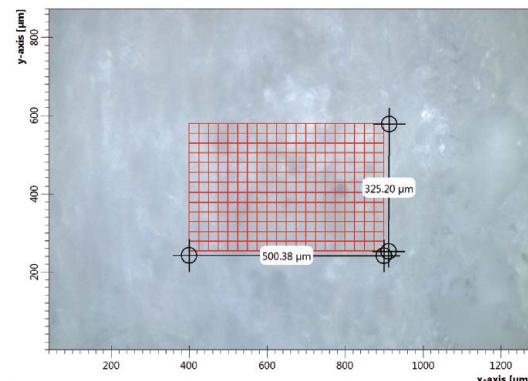


Figure 6. Rectangular map on the cross section of a pharmaceutical tablet covering an area of 500x325 μm . The spatial resolution of 25x25 μm (indicated by red rectangles) is defined by the operator using an automated physical aperture which is integrated in the LUMOS II.

The chemical images in Figure 7 visualize the composition of the tablet at each measurement spot using a colour scheme ranging from black (no contribution of component) to bright orange (high contribution of component).

Combination of the chemical images shows which part of the tablet is dominated by which component (Figure 8).

Chemical images

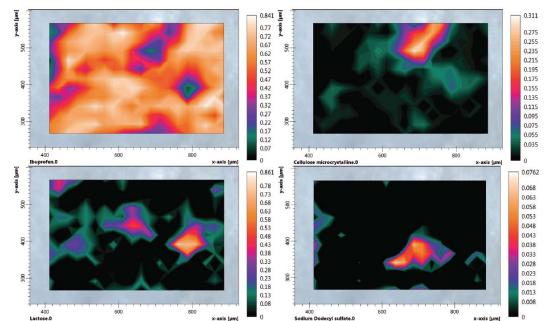


Figure 7. Chemical images of the distribution of different compounds in a pharmaceutical tablet: ibuprofen (upper left), microcrystalline cellulose (upper right), lactose (lower left) and sodium dodecyl sulphate (lower right).

CONCLUSION

IR-microanalysis is an extremely powerful and valuable technique to effectively track the source of product contaminations found in routine quality control. Furthermore, the spatially resolved analysis of pharmaceutical products like tablets or lyophilizates provides insight in their composition and homogeneity. This knowledge helps pharmaceutical research and development to optimize products.

Using the compact FTIR microscope LUMOS II even inexperienced users are able to perform an IR-microscopic analysis with minimal training. Its full automation and intuitive software interface result in a user comfort that will save valuable time.

Combination of the chemical images

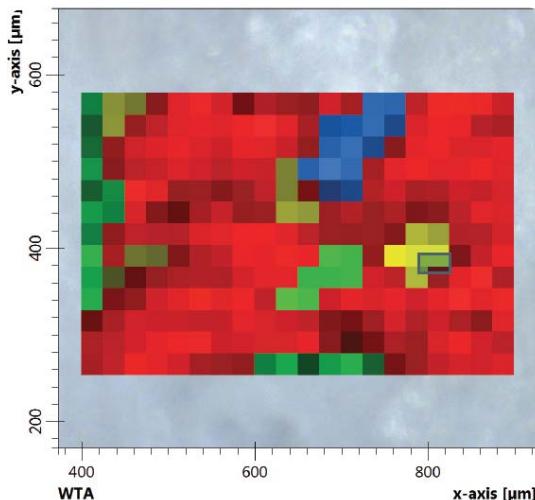


Figure 8. Combined chemical images of the distribution of different compounds in a pharmaceutical tablet: ibuprofen (red), microcrystalline cellulose (blue), lactose (yellow) and sodium dodecyl sulphate (green).



LUMOS II FTIR microscope



Handle with Precision Protect with Confidence

Applications:

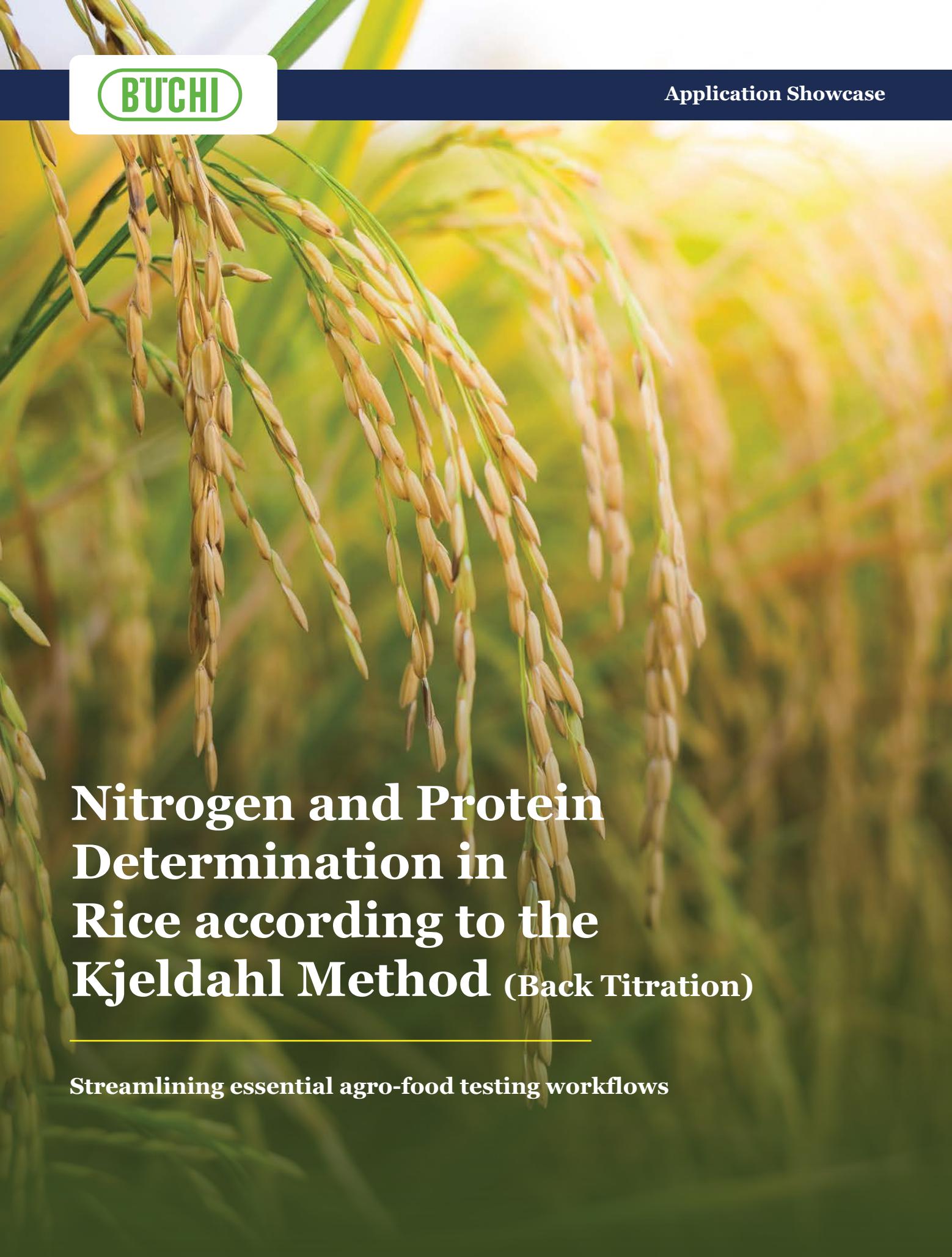
- **Pharmaceutical and Medical:** Drug synthesis, Toxic sample handling
- **Battery Research/Manufacturing:** Electrolyte handling, electrode preparation, battery assembly.
- **OLED/Display Manufacturing:** Handling of organic materials, deposition processes.
- **Semiconductor Industry:** Wafer handling, etching, and deposition processes.
- **Energy Research:** Battery development (lithium-ion, etc.), fuel cell research, and solar cell fabrication.
- **Nuclear Industry:** Handling of radioactive materials and waste.
- **Automotive Industry:** Research and development of advanced materials for vehicles.

Features:

- Closed loop recirculation
- Attainable oxygen and moisture level < 1 ppm
- Modular design for extension in future
- Smart Energy Mode feature
- Automatic control of antechamber
- Automatic box purging function
- Negative and/or positive pressure operation
- Foot pedal switch for pressure adjustment
- Large purification column to absorb 44 L of oxygen and 1850 g of moisture.

**Pure Protection for
Sensitive Workflows**

GLOVEBOX TECHNICAL DATA	
Box Structure	Modular Design
Material	Stainless Steel (US304, 1.4301)
Leak Rate	< 0.01 vol%/h
Window	Safety glass (thickness 10 mm) or Polycarbonate (thickness 10 mm)
Glove Ports	Black anodised Al or POM Polymer, port diameter 220 mm
Gloves	Butyl rubber (thickness 0.4 mm)
Light	LED Lamp
Feedthroughs	4xDN40KF Flange
Dust Filter	Inlet/Outlet, HEPA H13
Electrical Feedthrough	1
Shelves	6 on rear panel with adjustable height



Nitrogen and Protein Determination in Rice according to the Kjeldahl Method (Back Titration)

Streamlining essential agro-food testing workflows

The determination of protein in food is a routine procedure for quality assurance and labelling. A simple and fast procedure for protein determination in rice, as described in the AOAC 979.09, is introduced below. The sample is digested with sulfuric acid using the SpeedDigester K-436 or K-439, followed by distillation and back titration with the KjelFlex K-360. The determined protein contents correspond to the labelled values.

Keywords or phrases:

Kjeldahl method, protein determination in rice, nitrogen analysis, back titration, SpeedDigester K-436/K-439, KjelFlex K-360, AOAC 979.09

INTRODUCTION

Protein determination is one of the key analyses performed in the food industry. The samples require digestion with sulfuric acid to convert nitrogen into ammonium sulphate. After conversion to ammonia through the alkalinization with sodium hydroxide, the sample is distilled into a sulfuric acid receiver by steam distillation, followed by a back titration with sodium hydroxide solution. The nitrogen content is multiplied by a sample-specific factor (6.25 for rice) to obtain the protein content.

EXPERIMENTAL

Instrumentation: SpeedDigester K-436, K-439, KjelFlex K-360, external dosage device, 848 Titrino plus

Samples: Rice, protein content 7.36 g/100g

Chemicals and Materials: Sulphuric acid conc. 98 %, catalyst, Hg/Se-free, NaOH 32 %, H_2SO_4 0.25 mol/L, standard solution, NaOH 0.5 mol/L, standard solution, neutralization solution for the Scrubber: 600 g sodium carbonate, calcined,

technical, about 2 ml ethanol and a spatula tip of bromthymol blue, diluted to 3 L with distilled water, glycine (assay: 99.7 %)

PROCEDURE

The determination of nitrogen and protein in rice includes the following steps:

- ▶ Homogenization of the sample
- ▶ Digestion of the sample, using SpeedDigester K-436, K-439
- ▶ Distillation of the sample, using KjelFlex K-360
- ▶ Back titration with Metrohm 848 Titrino Plus

Digestion method - glycine (verification of the method)

- ▶ Place approx. 0.25 g glycine in a 300 ml sample tube
- ▶ Add 2 Kjeldahl tablets and a portion of 20 ml of sulfuric acid (98 %)
- ▶ Prepare additional blanks, chemicals without sample
- ▶ Carefully suspend the sample by gently swirling the tube
- ▶ Connect the Scrubber B-414 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- ▶ Insert the rack containing the samples into the preheated unit

Digest the samples according to the "cereal products" method (K-439) or the parameters listed in Table 1.

Digestion method samples

- ▶ Place approx. 2.5 g of the sample (depends on concentration of protein and organic matrix) in a 300 ml sample tube.
- ▶ Add 2 Kjeldahl tablets and a portion of 25 ml of sulfuric acid (98 %)
- ▶ Prepare additional blanks, chemicals without sample
- ▶ Carefully suspend the sample by gently swirling the tube

- ▶ Connect the Scrubber B-414 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- ▶ Insert the rack containing the samples into the preheated unit
- ▶ Digest the samples according to the “cereal products” method (K-439) or the parameters listed in Table 1.

	K-439		K-436	
Step	Temp. [°C]	Time [min]	Level	Time [min]
Preheat	480	-	8.5	10
1	480	10	8.5	10
2	550	10	9.5	15
3	490	65	8.5	75
Cooling	-	30	-	30

Table 1. Temperature profile for digestion with the K-436, K-439 K-439 K-436.

NOTE: When the samples are placed in the cooling position it takes approx. 30 min to cool them down; when they are left in the heating chamber it takes at least 60 min.

Distillation and Titration

Distill the samples according to the parameters listed in Table 2. The determination was carried out with a KjelFlex K-360 connected to an external dosage device and a Metrohm 848 Titrino plus.

KjelFlex K-360		Metrohm 848 Titrino plus	
Water	50 ml	Receiving solution ¹	20.0 mL H ₂ SO ₄ 0.25 mol/L
Sodium hydroxide	90 ml	Titration Solution	NaOH 0.5 mol/L
Reaction Time	5 s	Endpoint	pH 4.65
Steam Power	100%	Titration Rate	Optimal
Dist. Time	240 s	Stop Crit.	Drift
Titration Start	240 s	Stop Drift	20 µl/min
Titration Type	Back Titration	Stop Volume	40 ml
Stirrer Sp. Dist.	5	Stop Time	Off
Stirrer Sp. Titr.	7	Filling Rate	Max. mL/min

Table 2. Parameters for distillation with the KjelFlex K-360 and titration.

1) The external dosage device is connected to the port for the manual key button. The volume of the receiving solution must be entered at the external dosage device. For this purpose, refer to the Operation Manual of the dosage device.

Configuration K-360: Signal “ready”: No

Signal “active”: No

Signal “end”: 40 s

CALCULATION

The results are calculated as 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) are used to calculate the results.

$$W_N = \frac{(V_{\text{Blank}} - V_{\text{Sample}}) \cdot C \cdot f \cdot M_N}{m_{\text{Sample}} \cdot 1000} \quad (1)$$

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

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

W_N : weight fraction of nitrogen

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

V_{Sample} : amount of sodium hydroxide titrant for the sample [mL]

c : titrant concentration [mol/L]

f : factor of the titrant (for commercial solutions normally 1.000)

M_N : molecular weight of nitrogen (14.007 g/mol)

m_{Sample} : sample weight [g] (recovery: consider the assay of glycine)

1000 : conversion factor [mL/L]

%N : percentage of weight of nitrogen

%P : percentage of weight of protein

PF : sample-specific protein factor (6.25 for rice)

RESULTS

Digestion with SpeedDigester K-439

Recovery of glycine

The results of the nitrogen determination and recovery in glycine are presented in Table 3. The nominal value of glycine (assay: 99.7 %) is 18.60 % nitrogen. The recoveries are within the specification of 98 – 102 %².

The results of the nitrogen determination and recovery in glycine are presented in Table 3. The nominal value of glycine (assay: 99.7 %) is 18.60 % nitrogen. The recoveries are within the specification of 98 – 102 %².

Glycine	m _{Sample} [g]	V _{Sample} [mL]	%N	Recovery [%]
Sample 1	0.2607	13.028	18.62	100.1
Sample 2	0.2348	13.754	18.51	99.5
Sample 3	0.2339	13.764	18.55	99.7
Sample 4	0.2674	12.908	18.47	99.3
Average	-	-	18.54	99.6
Rsd [%]	-	-	0.35	0.35

Table 3. Results for the recovery of nitrogen in glycine with K-439. The mean blank volume for this sample was 19.959 mL (n = 4).

Protein determination in rice

The results of the determination of nitrogen in rice are presented in Table 4.

Rice	m _{Sample} [g]	V _{Sample} [mL]	%N	%P
Sample 1	2.5409	15.62	1.198	7.49
Sample 2	2.595	15.548	1.192	7.45
Sample 3	2.5755	15.59	1.19	7.44
Sample 4	2.5445	15.658	1.186	7.41
Average	-	-	1.192	7.45
Rsd [%]	-	-	0.43	0.43

Table 4. Results for the determination of nitrogen in rice with K-436 (literature protein content 7.36 %1).

The mean blank volume for this sample was 19.967 mL (n = 4).

Digestion with SpeedDigester K-436

Recovery of glycine

The results of the nitrogen determination and recovery in

glycine are presented in Table 5. The nominal value of glycine (assay: 99.7 %) is 18.60 % nitrogen. The recoveries are within the specification of 98 – 102 %².

Glycine	m _{Sample} [g]	V _{Sample} [mL]	%N	Recovery [%]
Sample 1	0.2529	13.266	18.54	99.6
Sample 2	0.2607	13.07	18.51	99.5
Sample 3	0.2559	13.164	18.6	100
Sample 4	0.266	12.922	18.53	99.6
Average	-	-	18.54	99.7
Rsd [%]	-	-	0.21	0.21

Table 5. Results for the recovery of nitrogen in glycine with K-436.

The mean blank volume for this sample was 19.959 mL (n = 4).

Protein determination in rice

The results of the determination of nitrogen in rice are presented in Table 6.

Rice	m _{Sample} [g]	V _{Sample} [mL]	%N	%P
Sample 1	2.5727	15.644	1.183	7.39
Sample 2	2.4674	15.832	1.18	7.37
Sample 3	2.702	15.424	1.183	7.39
Sample 4	2.5488	15.702	1.178	7.36
Average	-	-	1.181	7.38
Rsd [%]	-	-	0.21	0.21

Table 6. Results for the determination of nitrogen in rice with K-436 (literature protein content 7.36 %1).

The mean blank volume for this sample was 19.989 mL (n = 4).

COMPARISON TO STANDARD METHODS

This application note is based on the standard method AOAC 979.09.

	This application note	Standard method	Notes/Impact
Sample weight	2.5 g	0.7 – 2.2 g	In back titration higher contents of nitrogen are needed to obtain a reasonable difference in titrant consumption between blanks and samples.
Catalyst	10 g Tablets cont. - 47.7 % K ₂ SO ₄ - 47.7 % Na ₂ SO ₄ - 2.8 % TiO ₂ - 1.8 % CuSO ₄	15 g K ₂ SO ₄ + 0.7 g HgO	Easy to handle especially in routine analytics. The choice of catalyst does not influence the result. No toxic Hg.
Water	50 mL	200 mL	The K-360 generates steam in a separate vessel; therefore it is not necessary to add such a high amount of water to the digested sample as described in the standard method.

Table 7: Differentiation to the standard method

CONCLUSION

The determination of nitrogen and protein in rice using the SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results that correspond to the literature values with low relative standard deviations. There are no differences between the results obtained with the K-436 and the K-439 respectively.

The digestion time is very fast: 85 min for the K-439 and 100 min for the K-436. The recoveries with glycine were 99.6 % (K-439) and 99.7 % (K-436) respectively and are within the specification of 98 – 102 %.

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Operation manual of SpeedDigester K-439
Operation manual of Scrubber B-414
Operation manual of KjelFlex K-360

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KjelMaster K-375 & KjelSampler K-376 / K-377



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- › **Spectrum: 200–900 nm**



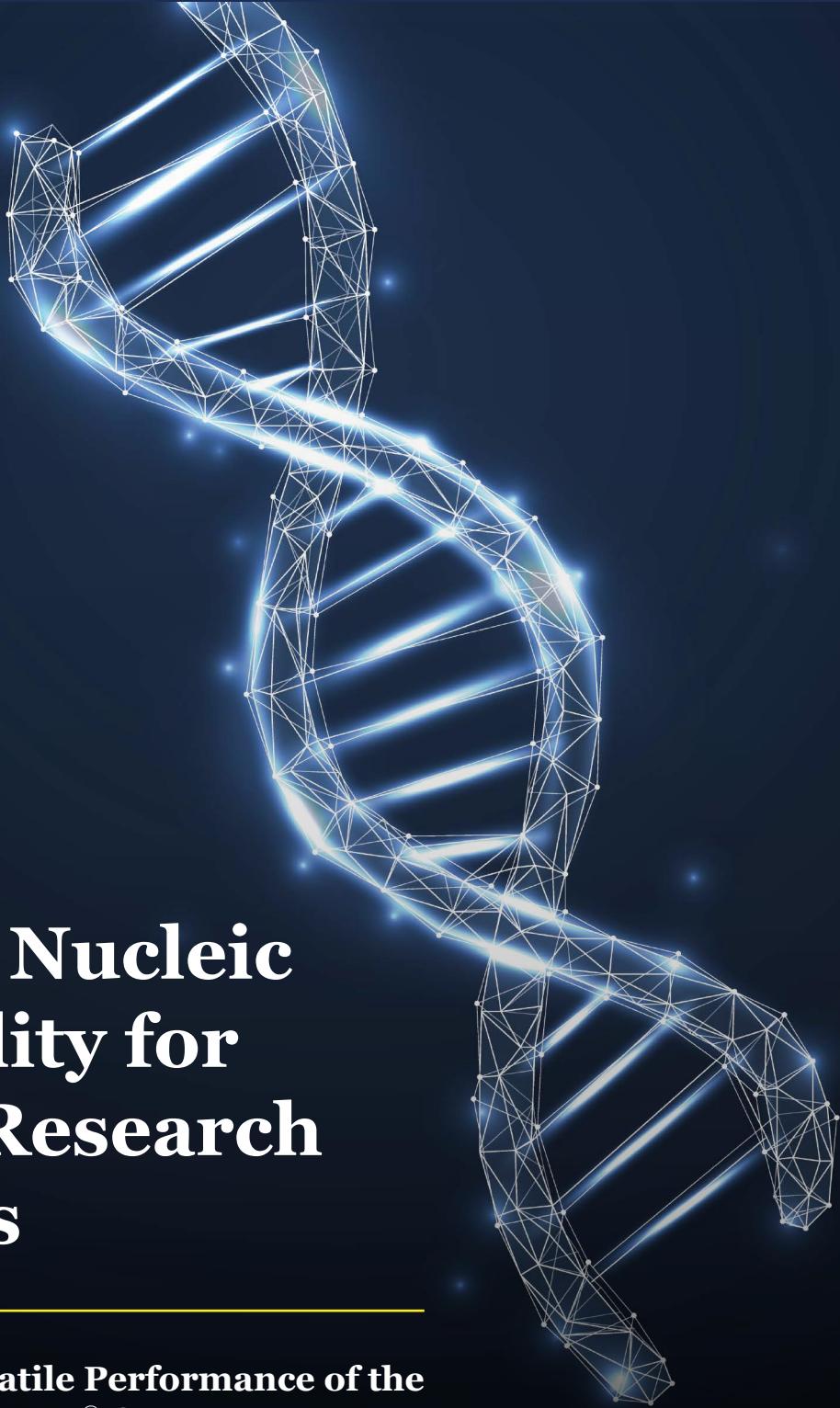
NanoPhotometer® C40 Cuvette Spectroscopy

Technical Specifications

Detection Range	dsDNA: 0.1–130 ng/µl BSA: 0.003–3.7 mg/ml
Wavelength Range	200–900 nm
Measurement Time	2.5 seconds
Photometric Range	0–2.6 OD (expandable to 26 OD with DiluCell™ 10)
Cuvette Types	Quartz, glass, plastic 12.5 x 12.5 mm, Z-height: 8.5 mm
Temperature Control	37°C ± 0.5°C
Absorbance Accuracy	< 1.75% @ 0.7 A @ 280 nm
Absorbance Reproducibility	< 0.002 A @ 0–0.3 A @ 280 nm CV < 1% @ 0.3–2.0 A @ 280 nm
Resolution	< 1.5 nm

Ensuring Nucleic Acid Quality for Reliable Research Outcomes

Demonstrating Versatile Performance of the
Implen NanoPhotometer® C40



The success of subsequent procedures like PCR, gene expression analysis, and metagenomic sequencing is directly impacted by accurate nucleic acid quantification, which is a crucial stage in molecular biology and environmental research. This paper reports two practical applications of the Implen NanoPhotometer® C40: siRNA-based gene silencing in breast cancer research and environmental microbiology, where DNA was tracked during petroleum biodegradation. The NanoPhotometer® C40 ensured data quality and reproducibility in both situations by enabling quick, accurate DNA and RNA quantification with small sample volumes. The instrument is a vital tool in a variety of scientific contexts due to its sturdy design, full-spectrum detection, and contamination alerts.

Keywords or phrases:

NanoPhotometer C40, nucleic acid quantification, UV-Vis Spectrophotometry, DNA purity, RNA quality control, environmental bioremediation, siRNA gene silencing, qRT-PCR validation, microbial community profiling, Implen spectrophotometer

INTRODUCTION

In workflows ranging from therapeutic gene expression studies to microbial community profiling, accurately and confidently quantifying nucleic acids is essential. Whole experiments may be jeopardised by inconsistent measurements or contaminants that are not yet detected. Even though they are accurate, traditional UV-Vis instruments frequently need larger sample volumes, cuvettes, and labour-intensive maintenance. In order to overcome these difficulties, the Implen NanoPhotometer® C40 provides full-spectrum, ultra-low volume quantification without the need for cuvettes or recalibration, as well as immediate contamination alerts.

This study demonstrates the use of the NanoPhotometer® C40 in two research settings:

(1) determining the integrity of DNA during hydrocarbon bioremediation, and (2) analysing the quality of RNA in a model of breast cancer cells for RNA interference investigations.

INSTRUMENT OVERVIEW

Protein and nucleic acid concentrations can be determined using the Implen NanoPhotometer® C40, a UV-Vis spectrophotometer, from volumes as small as 0.3 µL. It allows for high dynamic range analysis without sample dilution by supporting pathlengths of 0.67 mm and 0.07 mm. With integrated performance verification and no need for recalibration, the system offers automatic contamination detection and purity evaluation using absorbance ratios (A260/280 and A260/230).

Case Study 1: DNA Quantification in Bioremediation

The ability of enriched microbial communities to break down crude oil in marine sediment was examined in the study "Bioremediation of Petroleum Hydrocarbons Using Microbial Consortia." To improve the microbial consortia's capacity to degrade hydrocarbons, they were grown in oil-supplemented conditions during several enrichment cycles.

The MO BIO PowerSoil® DNA Isolation Kit was used to extract genomic DNA from culture samples after each enrichment. Before moving on to microbial profiling procedures like PCR-DGGE (Denaturing Gradient Gel Electrophoresis) and Illumina sequencing, the NanoPhotometer® C40 was used to evaluate the quantity and quality of DNA.

Experimental

DNA concentration was measured to guarantee uniformity among enriched consortia.

To verify sample purity and rule out the possibility of protein or salt contamination, the A260/280 and A260/230 ratios were employed.

Researchers were able to identify any extraction carryover

(such as leftover phenol or humic acids), which is typical in soil-based samples, thanks to real-time spectrum visualisation.

The C40's precise quantification made sure that only high-quality DNA moved on to sequencing, boosting the accuracy of analyses of microbial diversity and cross-enrichment generation comparisons. As a result, strong consortia dominated by Alcanivorax, Marinobacter, and other hydrocarbon-degrading taxa were identified.

Case Study 2:

RNA Quantification in Breast Cancer Research

In order to silence the gene CRIF1 in MCF-7 human breast cancer cells, researchers created a siRNA-loaded lipid nanoparticle (LNP) system, which was published in the International Journal of Molecular Sciences. The objective was to assess how CRIF1 knockdown affected p53, p21, and TIGAR-mediated tumor-suppressive pathways.

Following LNP transfection, TRIzol reagent was used to extract total RNA from both treated and control cells. Prior to reverse transcription and qRT-PCR, the extracted RNA was examined with the NanoPhotometer® C40.

Experimental

RNA concentrations were measured in order to standardise the inputs for cDNA synthesis across conditions. RNA purity appropriate for enzymatic reactions was confirmed by A260/280 ratios greater than 2.0.

Degradation or leftover solvents could be found using visual absorbance profiling, which is essential for maintaining the accuracy of subsequent gene expression analysis.

The researchers confidently carried out the qRT-PCR analysis, which demonstrated successful gene silencing and related downstream effects, because of the NanoPhotometer®'s accuracy.

RESULTS AND DISCUSSION

Because it provided quick, accurate nucleic acid quantification with small sample volumes and no consumables, the NanoPhotometer® C40 was essential to both investigations. The highest analytical standards were maintained in clinical and environmental research workflows thanks to its capacity to compute purity ratios and provide instant visual feedback.

It enabled the following in the bioremediation study:

- ▶ DNA input that is constant throughout DGGE and sequencing runs
- ▶ Dependable observation of the dynamics of microbial communities

It made it possible for the oncology study to:

- ▶ Normalisation of RNA input with confidence for qRT-PCR
- ▶ Reliable confirmation of the therapeutic effects of siRNA

CONCLUSION

In two different fields—microbial environmental monitoring and molecular oncology—the Implen NanoPhotometer® C40 showed great utility. It is a vital tool in contemporary labs because of its capacity to provide accurate nucleic acid quantification results with incredibly small sample volumes. The C40 guarantees that sample quality is never a bottleneck, whether it is for analysing RNA from cancer cells or sequencing bacterial DNA from sediments contaminated by oil.

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OPTIMISING IN VITRO TRANSCRIPTION FOR SCALABLE mRNA SYNTHESIS

**Improving Process Control and Reproducibility with
Radleys Reactors**

The development of high-quality biological therapeutics requires efficient and reliable in vitro transcription (IVT). Conventionally, in vitro transcription is performed in batch reactions in single-use setups, which have been shown to exhibit batch-to-batch variation due to fluctuating reaction conditions. Glass reactors are a promising method to address this batch variation issue, and with a leaning protocol in place, the reactors can be used repeatedly. This application note discusses the use of glass jacketed reactors in improving in vitro transcription's efficiency and reliability, particularly for mRNA synthesis.

Keywords or phrases:

mRNA synthesis, in vitro transcription (IVT), Radleys reactors, bioprocess scale-up, process control, RNA therapeutics

INTRODUCTION

In vitro transcription (IVT) is a widely used technique for synthesising ribonucleic acid (RNA) in the laboratory. It involves the use of a deoxyribonucleic acid (DNA) template, RNA polymerase enzyme, and nucleotides to produce RNA molecules of interest.

The reaction conditions in IVT are critical for the efficiency and yield of RNA synthesis. The conventional method of in vitro transcription is performed in batch reactions, which have been shown to exhibit batch-to-batch variation due to fluctuating reaction conditions.

One way to optimise these conditions is through the use of jacketed glass reactors. This application explores the advantages of using jacketed glass reactors in IVT reactions, specifically for mRNA synthesis.

THE ROLE OF mRNA IN THE PHARMACEUTICAL INDUSTRY

The pharmaceutical industry has witnessed a paradigm shift with the advent of mRNA technology. mRNA has emerged as a powerful tool for therapeutic interventions, enabling innovative approaches in personalised medicine and vaccine development. It became a global game-changer during the COVID-19 pandemic—delivering the world's first mRNA-based vaccines at unprecedented speed.

mRNA: A PLATFORM FOR PERSONALISED MEDICINE

Creating treatments that are tailored to each patient's distinct genetic profile holds the promise of personalised medicine, and mRNA is quickly emerging as one of the most flexible platforms for this purpose.

Synthetic mRNA can be modified to encode therapeutic proteins specific to a patient's disease biology through the use of in vitro transcription (IVT). These consist of tumour-specific antigens for cancer immunotherapy, enzymes, antibodies, and immune modulators. Since mRNA therapeutics can be easily modified to encode almost any protein of interest, they are not constrained by target specificity like traditional medications are.

When treating rare or genetically complex diseases, where one-size-fits-all treatments frequently fail, this flexibility is essential. Clinicians can use customised mRNA constructs to target molecular abnormalities by combining genomic profiling with IVT technology, enabling not only precise but also faster development and safer administration.

mRNA VACCINES: PANDEMIC RESPONSE TO BROAD-SPECTRUM PREVENTION

For mRNA technology, the COVID-19 pandemic marked a turning point. The creation, approval, and distribution of mRNA vaccines in less than a year revolutionised vaccine science and international public health policy.

mRNA vaccines provide genetic instructions that cause the body's own cells to produce a disease-specific antigen, in contrast to conventional vaccines that employ inactivated pathogens or protein subunits. Without integrating into the patient's genome or exposing them to live virus, this sets off a focused immune response.

Why mRNA vaccines are revolutionary

Speed: Vaccine candidates can be created in a matter of weeks after a viral sequence is known.

Adaptability: The mRNA sequence can be promptly updated to address new variants.

Safety: The mRNA doesn't change the host DNA and is only temporary.

mRNA-based vaccines against influenza, RSV, HIV, and emerging zoonotic diseases have become widely available as a result of the success of the Pfizer-BioNTech and Moderna COVID-19 vaccines, which are both based on this technology.

mRNA platforms are positioned to play a key role in vaccine development, not only for infectious diseases but also possibly for cancer and chronic conditions, as the focus of the world moves from crisis response to proactive prevention.

CHALLENGES IN mRNA SYNTHESIS

mRNA therapies still have a long way to go before they are widely used in clinical settings, despite their revolutionary potential. Making sure that mRNA reaches the appropriate cells and is effectively translated into useful protein is still a major challenge in targeted delivery.

- ▶ **Batch-to-batch variability** due to inconsistent temperature or mixing

- ▶ **Enzyme deactivation** from local overheating or poor pH stability
- ▶ **Incomplete transcription** leading to short or truncated RNA species
- ▶ **Residual DNA or impurities** interfering with downstream purification
- ▶ **Low reproducibility** when scaling up using single-use or open systems

IN VITRO TRANSCRIPTION

A laboratory technique called in vitro transcription (IVT) is used to create RNA molecules from a DNA template outside of living cells. By using RNA polymerase to create RNA strands that carry genetic instructions, it mimics the natural transcription process.

A DNA template with a promoter sequence that the polymerase recognises (typically T7, SP6, or T3) is used to start the process. This PCR or cloning-prepared template is incubated with:

- ▶ **RNA polymerase**
- ▶ **Ribonucleotides (ATP, GTP, CTP, and UTP)**
- ▶ **Essential cofactors and buffer**

Under regulated circumstances, RNA polymerase attaches itself to the promoter and adds ribonucleotides in a specific order to create a complementary RNA strand. Until a predetermined endpoint or termination signal is reached, the reaction keeps going.

IVT is a foundational step in the production of mRNA therapeutics, vaccines, guide RNAs (CRISPR), and RNA-based assays—offering precision, flexibility, and scalability for modern molecular biology.

REACTOR-BASED SOLUTIONS FOR IVT

Because of their scalability, temperature control, and chemical resistance, glass jacketed reactors

are being used more and more for mRNA *in vitro* transcription (IVT). These reactors, which are made of inert, corrosion-resistant glass, are perfect for the production of biopharmaceuticals, where process consistency and sterility are crucial. To guarantee even mixing, which is crucial for preserving enzyme activity and reaction homogeneity during RNA synthesis, they are outfitted with a variety of agitator types.

ADVANTAGES OF REACTOR SYSTEMS:

- ▶ Stable temperature control (typically ± 0.5 °C)
- ▶ Uniform mixing to avoid local substrate or enzyme gradients
- ▶ Real-time monitoring of pH, temperature, and process kinetics
- ▶ Efficient RNA recovery and sample handling
- ▶ Scalable volumes, from millilitres to tens of litres

RADLEYS SYSTEMS FOR CONTROLLED IVT

mRNA therapies still have a long way to go before they are widely used in clinical settings, despite their revolutionary potential. Making sure that mRNA reaches the appropriate cells and is effectively translated into useful protein is still a major challenge in targeted delivery.

Mya 4 Reaction Station

- ▶ Ideal for developing new methods
- ▶ 4 Reaction Station (2–400 mL)
- ▶ Data logging, heating/cooling, and stirring automation
- ▶ Allows for multiple parallel reactions to optimise the process.

Reactor-Ready & Reactor-Ready Pilot

- ▶ Glass jacketed vessels (100 mL to 35 L)
- ▶ Using circulators to precisely regulate the temperature
- ▶ Simple vessel swapping and tool-free assembly
- ▶ Facilitates sampling, filtration, and integration with inline probes.

KEY CONSIDERATIONS FOR REACTOR-BASED IVT

Parameters	Impact on IVT
Temperature	Affects RNA polymerase activity and product integrity
pH control	Stabilises enzyme function, reduces degradation
Mg ²⁺ and buffer systems	Support efficient NTP incorporation
Mixing	Prevents hot spots or localised gradients
Volume scalability	Allows same conditions to be maintained from lab to pilot scale
Cleanability & validation	Essential for GMP-readiness and multi-batch operation

CASE STUDY: The Scale-Up Success of Aldevron

Radleys' Reactor-Ready Pilot system was used by Aldevron, a top producer of therapeutic proteins, mRNA, and plasmid DNA, to support their expanding RNA production capacity.

They needed a platform that could:

- ▶ Allow for different batch sizes.
- ▶ During multi-hour IVT runs, maintain strict thermal control.
- ▶ Encourage the integration of quality systems and purification workflows.

Aldevron was able to transition from lab-scale to pilot production without sacrificing RNA integrity, yield, or reproducibility thanks to its robust stirring, tool-free operation, and scaling capability from 100 ml to 35 L.

CONCLUSION

Consistent, scalable, and strictly regulated in vitro transcription procedures are more important than ever as the need for high-quality mRNA in biopharma and vaccine development keeps growing. Precision and reproducibility are frequently lacking in traditional batch methods, particularly when scaling up.

Researchers and manufacturers can overcome important obstacles like temperature drift, mixing inefficiencies, and batch-to-batch variability by incorporating Radleys reactors into the IVT workflow. Radleys systems provide a stable, adaptable platform that connects laboratory research and development with pilot-scale manufacturing thanks to their accurate thermal control, real-time monitoring, and scalable volumes.

In the end, this method facilitates the dependable and GMP-compliant production of next-generation RNA therapies in addition to streamlining mRNA synthesis.

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REACTOR READY



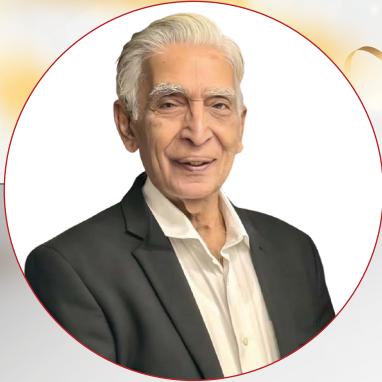


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Through evolving technologies and growing partnerships, the brand has remained committed to advancing science. *Today, we celebrate 40 years of unwavering innovation, integrity, and impact.*

• **ADVANCING DISCOVERY SINCE 1985** •



S. Balu

Chairman & Managing Director, Inkarp Group

A 40-Year Journey: Then, Now, and What Comes Next

On August 20th 2025, Inkarp turns 40. Coincidentally, it is also my birthday. I was born on August 20, 1942. Exactly four decades later, in 1985, we started this company with one goal in mind—to bring meaningful science and technology closer to Indian researchers. Not as middlemen, but as long-term partners. Partners who understand what is needed, who stay committed, and who do not make promises they cannot keep.

Back then, the world was different. No internet. No real-time tracking. No fancy dashboards. Business was personal. A delay meant you made a phone call or showed up at the customer's door. A service complaint was your responsibility, not someone else's. That mindset shaped everything we built at Inkarp.

We did not begin with funding or infrastructure. We began with belief. And slowly, we earned the trust of a few global principals. We built relationships one by one. We listened to what scientists needed, adapted to changing demands, and stayed the course—quietly, consistently. That is how Inkarp was built.

Of course, the journey has had its share of challenges. There were years of uncertainty, intense competition, and lessons learned the hard way. But what carried us through was a clear sense of purpose. We never ran after shortcuts or tried to be what we were not. We focused on being real and being reliable. That is what brought us here.

When I walk into a lab and see Inkarp's solutions are in use—or hear a customer speak about how our team supported them—I feel a quiet kind of pride. Not the kind that shouts, but the kind that says, yes, this has been worth it.

Today, the world is moving faster. Labs are more connected. Automation is everywhere. AI is rewriting the rules. It is a different game. But I believe the core does not change. Relationships still matter. Integrity still matters. And people still matter.

This next generation of leadership at Inkarp brings energy, speed, and new ideas. They speak a different language, but their values are rooted. That gives me confidence. They know where this company came from, and they know where it can go.

As we celebrate 40 years, I want to say thank you—to everyone who trusted us, supported us, and stayed with us. You are part of this story. Whether you are a principal, a customer, an employee, or a well-wisher—you helped shape Inkarp.

To our team, I will say this: Inkarp is not just a company. It reflects the people inside it. How you speak, how you follow up, how you show up for your customer—that is what builds trust. That is what keeps this legacy alive.

To our principals, thank you for choosing to partner with us when it was not the easy or obvious choice. You believed in us before the world looked at India seriously. That will never be forgotten.

To our customers, your work drives ours. We are proud to serve you. And we promise to stay committed, curious, and consistent in everything we do.

Inkarp's story is not just about the past. It is about what we do next. There is still more to build, more to solve, and more to give back. And as always, we will do it the way we know best—with integrity, with humility, and with people at the centre of it all.

Thank you for walking this journey with us.



MOMENTS THAT MADE US...



INCORP

1985

1991

Incorp

2002

Spincorp





40 YEARS & COUNTING...



Celebrating 40 years of excellence – and a partnership built on “**Research made easy**”

“

This year marks the 40th anniversary of our long-standing partner, Inkarp – and with it, a shared success story that spans nearly as many years.

From the very beginning, Inkarp has been more than a distributor: a dedicated ambassador of our mission, bringing “Research Made Easy” to life down to the last molecule. Who else would take our evaporator by train to the most remote provinces – just to personally introduce it with pride? That kind of commitment defines true partnership. Challenges have come and gone, but through open dialogue, trust, and shared values, we’ve always moved forward – stronger, together.

Congratulations to Inkarp on four decades of excellence. Here’s to the future – and many more years of outstanding collaboration and customer service.

”

Team Heidolph !!



Cheers to Inkarp !

“

We are honored to celebrate 40 years of Inkarp, a remarkable milestone that speaks to the dedication, integrity, and excellence that define your organization.

PolyScience partnership with Inkarp has been built on a foundation of trust, collaboration, and a shared commitment to delivering quality and innovation. Over the years, we’ve witnessed the strength of this relationship grow, bringing forward meaningful achievements and lasting connections. Each visit to your team and customers fills us with joy, not only because of the professionalism and enthusiasm we see, but also because of the warm and genuine welcome we always receive. Your hospitality and spirit make every visit a true pleasure and a testament to the values that make this partnership so special.

Congratulations to the entire Inkarp team on this significant anniversary. We are proud to be part of your journey and look forward to many more years of success together.

Team PolyScience !!

”



Congrats !

“

As Inkarp celebrates an incredible 40-year journey, we at GEA Homogenizer are honored to reflect on the strong and enduring partnership we’ve shared over the years. This milestone is a testament to Inkarp’s dedication, innovation, and consistent impact across the industry.

Our collaboration has always stood out—not only for its technical achievements and shared objectives, but also for the professionalism, reliability, and human connection we’ve experienced with the entire Inkarp team.

The mutual trust and respect built over time have made every initiative smooth and rewarding. The most memorable highlight of our collaboration has been our joint success in introducing innovative and customized homogenizer solutions to the Indian market—an achievement that truly reflects the strength and synergy of our partnership.

Here’s to celebrating the past and looking forward to many more shared successes ahead.

Team GEA !!

”



Cheers to 40 Amazing Years!

“ Congratulations on hitting such a huge milestone – 40 years! What an incredible journey it's been. Inkarp teams' dedication, hard work, and loyalty over the years have made a real difference, and it's something to truly celebrate. We, Hitachi High Tech Analytical India and Japan team, extend our heartfelt congratulations on reaching this incredible milestone of dedicated service and unwavering commitment.

Hitachi had an association with Inkarp since 2007 for TA Analyser products and since then your journey is a true testament to your passion, perseverance, and loyalty to us. In last few years, we are seeing a vertical growth journey for TA products business in India and many Hitachi TA users, mainly scientists and technocrats, have produced good quality of research across India and private customer experience for their high-quality products. This has made a good economic impact on our nation to a great extent. It has been an absolute pleasure having you as part of our associate all these years. Thank you for everything you've contributed. Here's to the memories, the impact you've made, and the many more good times to come. Congrats again!

Team Hitachi !!

”



Congratulations!

“ Kubota Corporation cherishes its long-standing relationship with Inkarp Instruments, a collaboration based on trust and a shared dedication to excellence. Kubota has been able to spread its legacy of precision centrifugation to a larger audience thanks to Inkarp's extensive presence in Indian labs. This partnership is essential to ensuring that researchers who rely on performance, dependability, and usability in every spin can access Kubota's cutting-edge lab technologies.

Team Kubota !!

”



From one innovator to another – congratulations!

“ Inkarp is a perfect partner in India because of their strong technical capabilities, dedication to scientific advancement, and thorough understanding of the Indian market. In order to enable Indian labs to innovate more quickly, operate more efficiently, and embrace the future of digital science, we are promoting the adoption of intelligent, modular automation for R&D and QC, & Inkarp is helping us achieve this.

Team Chemspeed !!



”



Cheers to 40 Amazing Years!

“

Inkarp Instruments has been our valued distributor in India for over a decade, and they have helped us evangelize benchtop NMR in that territory. Mr. Madhu and team have built a very successful company, and we look forward to working with them to continue to drive adoption of benchtop NMR in vertical markets such as pharma, food, polymers, energy and so on. Congratulations on your 40th anniversary Inkarp!!!

”

Team Nanalysis !!

Congratulations!

“

Heartfelt congratulations to Inkarp Instruments on completing 40 remarkable years of excellence in the scientific industry. It has been our privilege at Implen GmbH to be part of this journey since 2007. Over nearly two decades, we've witnessed Inkarp's unwavering commitment to delivering quality, innovation, and trusted service to customers across the Life Science and Pharma sectors. Your dedication has been instrumental in bringing our NanoPhotometers to labs across India with unmatched professionalism and care. Here's to many more years of impactful collaboration and shared success!

”

Team Implen !!



To every brand, every partner, and every friend – thank you for being part of our 40-year journey. Here's to building the future of science, together.

Team Inkarp!



HAPPY CUSTOMER...





LONG MILES, LOYAL VOICES...



D. Saritha - Manager, Commercials

...

Happy 40th Anniversary to Inkarp and my hearty Congratulations to Balu Sir and the leadership on this remarkable milestone!

“ An opportunity to work at Inkarp has made my career growth. I am thankful to Balu Sir for giving me an opportunity when requested by me to join Inkarp way back in 2001. It's been a wonderful experience working in Inkarp since 2001. Proper guidance and mentoring from Balu Sir and Natesh Sir have groomed me to the current position in Inkarp. Thank you for providing a workplace where we are given freedom to work and grow in our careers. It's truly valuable to work in an environment that encourages both individual contribution and professional development. Feeling incredibly grateful on the company's journey and the impact it has made not only to the industry that it has been serving but as well as on our lives who have been a part of it. Excited to see what the future holds! Looking forward to many more years of success and continued growth! ”



M. Srinivas - General Manager

...

Congratulations and best wishes to all fellow Inkarpians on the 40th anniversary of Inkarp!

“ I feel truly proud and happy to have witnessed 23+ years of Inkarp's remarkable journey and achievements.

Inkarp has given me numerous opportunities to grow in my career, and it continues to extend such opportunities to every Inkarpian. Many have embraced these opportunities and now hold influential, decision-making roles. I take this opportunity to sincerely thank the leadership team for their unwavering support and guidance. To the young Inkarp team- my message is to take ownership of your work, stay positive, remain practical, and stay committed, success will surely follow.

I wish Inkarp continued success and hope it remains a leader in this field for as long as research and innovation thrive. ”



I. Hari Krishna - Warehouse Incharge

...

Firstly Happy 40th Anniversary to Inkarp!

“ Big congratulations to Balu Sir and the whole leadership team on reaching this great achievement!

I'm thankful for the chance to be part of this amazing journey. Joining Inkarp in 1992 was a turning point in my career. I am especially thankful to Balu Sir for believing in me and offering me the opportunity to join the organization when I approached him.

I feel truly proud to be a part of Inkarp's journey and to see the positive difference it has made—not just in the industry, but also in the lives of everyone who are a part of this company.

As we celebrate 40 years of Inkarp, I'm excited about the future and look forward to many more years of growth, new ideas, and success together. ”

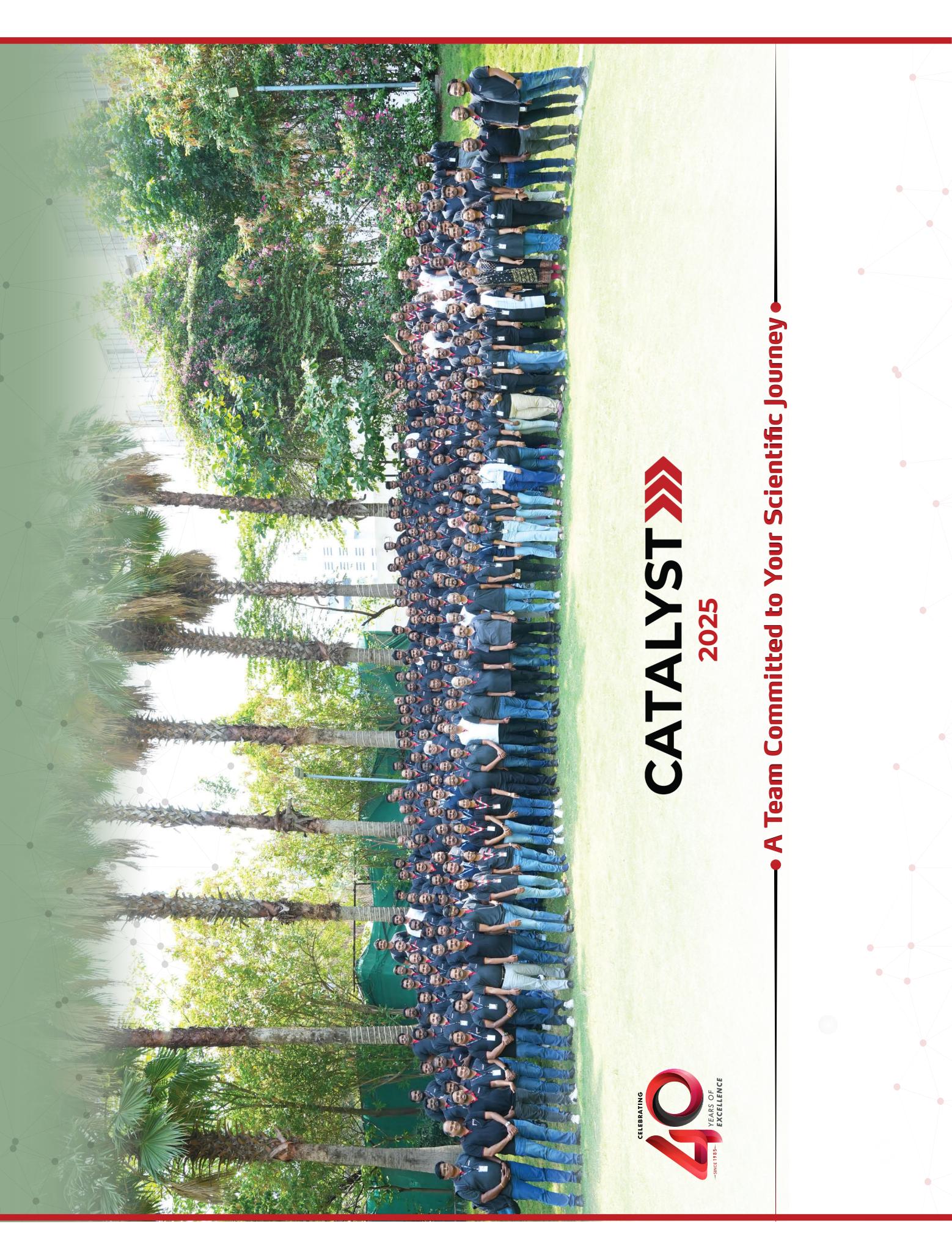


L. Vijaya Bhaskar - Service Manager

...

Congratulations!

“ As we celebrate 40 years of this incredible company, I feel truly grateful and proud to have spent 21 of those years as part of this journey. These years have given me not just professional growth, but lasting relationships and countless memories. I thank the company, my colleagues, and mentors for their trust, support, and inspiration along the way. It's been an honour to contribute to this legacy—and I look forward to many more milestones together. ”



CATALYST

2025

• A Team Committed to Your Scientific Journey •





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Water is Life: Ultrapure Water in Cell Cultivation

**Pure Water, Pure Outcome: Powering Cell Cultures with
Arium® Ultrapure Water**

Anil Kumar Rathod¹, Sheokant Diwakar¹, Dr. Ashok Mundrungi¹, Dr. Elmar Herbig²

¹Sartorius Stedim India Pvt. Ltd., Bangalore, India

²Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany

Water is a major component of all cell culture media and is, therefore, needed to prepare media, buffers, and additives, as well as to serve many ancillary functions, such as heating, cooling, cleaning and rinsing. Thus, water quality plays an important role in the outcome of cell culture experiments.

Keywords or phrases:

Ultrapure Water, Cell Cultivation, Media and Buffer Preparation, Recombinant Proteins, Monoclonal Antibodies (mAb Production), Manufacturing of Therapeutic Proteins, PER.C6 EpCAM Cells

INTRODUCTION

Contaminants in water used for cell cultures can occur in many forms, such as bacteria, yeasts, or moulds. These contaminants are usually visible to the eye or by optical microscopy. However, contamination from chemicals or other biological agents may also affect growth, morphology, or behaviour of cultured cells, yet be undetectable to the unaided eye. Water used in cell cultures must, therefore, be free of microorganisms and, in particular, of endotoxins, inorganic ions (heavy metals such as lead, zinc, etc.), and organic compounds (humic acids, tannins, pesticides, etc.). For more detailed information, please refer to the reference literature.^{1,2}

Examples of typical impurities in tap water and target values for cell culture work are shown in Table 1.

Parameter	Tap Water	Water for Cell Culture	% Reduction
Conductivity (µS/cm)	50 to 900	0.2	99.95
Calcium (mg/L)	20 to 150	< 0.01	> 99.99
Sodium (mg/L)	20 to 150	< 0.01	> 99.99
Iron (mg/L)	0.01 to 0.1	< 0.001	> 98
Bicarbonate (mg/L)	30 to 300	< 0.01	> 99.99
Chloride (mg/L)	10 to 150	< 0.01	> 99.99
Sulphate (mg/L)	1 to 100	< 0.01	> 99.98
TOC (mg/L)	0.2 to 5	0.1	96
Free chlorine (mg/L)	0.1 to 0.5	< 0.01	> 97
Bacteria (CFU/100 mL)	100 to 1000	< 10	> 98
Endotoxin (IU/mL)	1 to 10	< 0.1	> 98
Turbidity	0.1 to 2	< 0.01	99

Table 1: Typical tap water impurities and target values for cell culture work²

The objective of the present test series was to evaluate whether ultrapure water produced by Arium® Pro UF can be readily used for cell culture applications without entailing any problems. In this study, we cultivated PER.C6 EpCAM cells in ready-made CDM4PERMab (Hyclone) media employed as controls, as well as in CDM4PERMab (Hyclone) powder media prepared using ultrapure water obtained with Arium® Pro UF (UF water) and RO water, respectively, for test purposes. Data for RO water in this Application Note were obtained using the predecessor model (Arium® RO) of the current Arium® advance system. The results of each culture were then used to assess whether the water produced by Arium® Pro UF is suitable for use in the cultivation of PER.C6 EpCAM cells.

The PER.C6 cell line derived from human retinoblast cells, described and employed in our test series, is also used today for the expression of recombinant proteins and monoclonal antibodies (mAb production) and for the manufacture of therapeutic proteins and monoclonal antibodies.

Ultrapure Water System

The system is designed to produce ultrapure water from pre-treated water sources by removing trace levels of residual contaminants. Production of ultrapure water requires continuous recirculation and constant flow. This is carried out by a pump system that controls the pressure. The system measures the conductivity of the water at both the water feed inlet and the water outlet (downstream product).

The system works with two different cartridge kits. These cartridges are filled with a special active carbon adsorber and a special mixed-bed exchange resin designed to deliver

high-purity water with low extractables. A final microfilter at the outlet is usually installed to remove any particles or bacteria from the ultrapure water as it is dispensed. The general process described for water purification is depicted in Figure 1.

For the tests mentioned below the feed water for the Arium® Pro UF was prefiltered by an Arium® RO reverse osmosis system. This configuration is in accordance with Whitehead² for the water purification treatment for small scale cell cultivation in laboratories.

MATERIALS AND METHODS

PER.C6 EpCAM cells (eye catcher) were cultivated in T-75 flasks, with vented caps (Nunc) for gas exchange, in

(3.2 g/L, Merck) and pluronic acid F-68 (0.5 g/L, Sigma), and filtered through a final 0.2 µm sterilizing-grade filter using 1000 mL disposable filtration units (Sartolab®, Sartorius) under aseptic conditions. The cells were seeded at a seeding density of 0.3×10^6 cells/mL in T-75 flasks and 0.7×10^6 cells/mL in spinner flasks. The T-flasks and spinner flasks were incubated in a CO₂ incubator at 37°C, 5% CO₂ and 85% humidity. In the CO₂ incubator, the spinner flasks were incubated on a magnetic stirrer at 80 rpm with the side arm of each spinner flask loosely capped to facilitate gas exchange inside. Samples were taken every day except for weekends (Days 4 and 5) from the spinner flasks and every third day from the T-flasks to determine the viable cell density. The viable cell density was measured according to the Trypan Blue exclusion method using a haemocytometer. Comprehensive information about the basic techniques of cell cultivation is given in the reference literature.³

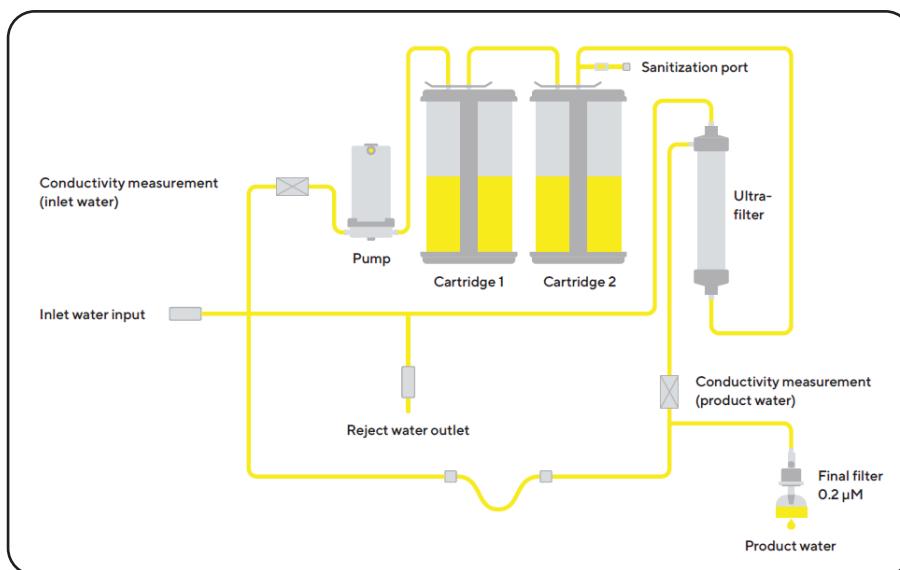


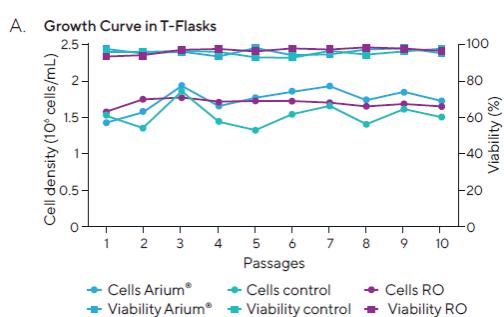
Figure 1. Schematic drawing of the working principle of the Arium® Pro UF ultrapure water system

duplicates (12 mL media in each flask) for 10 passages and in 125 mL spinner flasks (Wheaton, VWR) with 50 mL of media in duplicates.

The PER.C6 EpCAM cell line was cultivated in CDM4PERMab ready-made media (Hyclone) and in CDM4PERMab powder media (Hyclone). The CDM4PERMab powder media was reconstituted with either UF water or with RO water, along with 4 mM L-glutamine (Lonza), sodium bicarbonate

Results and Discussion

The average cell density yield in the control T-flasks (cells cultivated in ready-made media as controls) was 1.52×10^6 cells/mL and the average viability for these controls was 95.23% (Figure 2A). For cells in T-flasks cultivated in media reconstituted with UF water, the average cell density was 1.73×10^6 cells/mL, and an average viability of 95.7% was obtained. In comparison with these results, an average cell density yield of 1.68×10^6 cells/mL and a viability of 95.59% (Figure 2A) were obtained in the cells cultivated in media reconstituted in RO water.



B. Growth Curves in Spinner Flasks

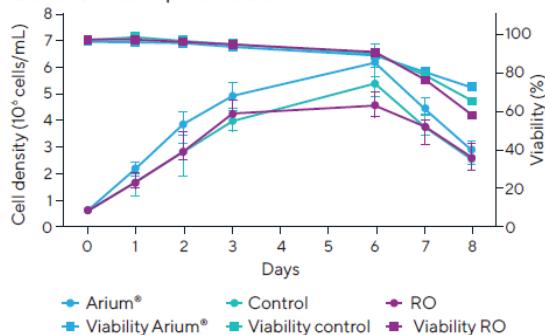


Figure 2. (A) Growth curves of PER.C6 EpCAM cell lines in T-flasks. (B) Growth curves of PER.C6 EpCAM cell lines in spinner flasks.

In another experiment, the PER.C6 EpCAM cell lines were cultivated in spinner flasks with ready-made media (control), media reconstituted using UF water and media reconstituted using RO water (Figure 2B). The maximum cell density obtained in control spinner flasks was 5.42×10^6 cells/mL with an 88.47% viability, 6.24×10^6 cells/mL with an 88.55% viability in UF water spinner flasks and 4.60×10^6 cells/mL with an 89.85% viability in RO water spinner flasks on Day 6 of cultivation (Figure 2B). Microscopic examination of the cells in the RO spinner flasks indicated that cells grown in media reconstituted with RO water look unhealthy compared with cells grown in the ready-made media and in media reconstituted with UF water. The viability of the cells grown in spinner flasks with media reconstituted in RO water decreased more rapidly compared with those cultivated in the ready-made media and media reconstituted with UF water. This decrease was not observed when the cells were cultivated in T-flasks. The rapid decline in viability inside the spinner flasks can be attributed to the presence of endotoxins and inorganic salts present in RO water which affect the growth and viability of the cells. However, these adverse effects of endotoxins or inorganic salts were not observed in static cultures (small-scale cultivation), such as in the T-flasks, because in the latter case, growth is limited by the O₂ concentration in the medium and not by the endotoxin or inorganic salt concentration (no typical growth curve can be observed in the T-flasks compared with the cultures in the spinner flasks).

The actual effects can be observed only in spinner flask

cultivation where higher cell densities occur and O₂ is not a limiting factor. In spinner flasks in which high cell densities can be achieved, the effect of higher concentrations of endotoxins and inorganic salts in media reconstituted with RO water result in a reduced growth rate (lower cell density and lower viability) compared to values obtained for the controls (ready-to-use media) or for the samples in medium reconstituted with UF water. These results are confirmed by the antibody production (mAb) experiments. MAb production in spinner flasks (Figure 3) of cells cultivated in media reconstituted with UF water was 0.84 mg/mL (example Day 8) and is thus higher than the values obtained for mAb production with the manufacturer's ready-made media as controls (0.71 mg/mL) or in the samples reconstituted with RO water (0.42 mg/mL). The productivity of cells, i.e., mAb production in T-flasks, was not measured because the amounts of antibodies were too low and a reliable comparison of such low values was not statistically

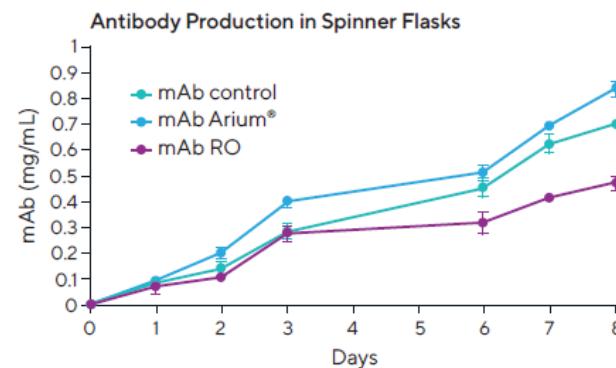


Figure 3. Antibody production in cells cultivated in media reconstituted with UF water (mAb Arium®), ready-made media (mAb Control) and RO water (mAb RO) in spinner flasks.

CONCLUSION

The results above clearly demonstrate that dehydrated media (CDM4PERMab media) that are reconstituted with UF water are suitable for use in cultivation of PER.C6 EpCAM cell lines instead of commercially available ready-to-use media. The growth characteristics of the PER.C6 EpCAM cell lines cultivated in media reconstituted with UF water are similar to those of the PER.C6 EpCAM cell lines cultivated in ready-to-use CDM4PERMab media used as controls. Moreover, there was an enhanced growth in cell line samples cultivated in media reconstituted with UF water compared with the samples cultured in media reconstituted with RO

SARTORIUS

water when the experiments were carried out in spinner flasks. In this case, higher cell densities normally occur and O₂ is not a limiting factor. It can, therefore, be concluded that the higher concentration of endotoxins or inorganic salts in RO water caused this decrease in growth.

These results were confirmed and reflected by the mAb production in the PER.C6 EpCAM cell line cultivated in spinner flasks. MAb production from PER.C6 cell lines showed the highest values in the samples reconstituted with Arium® Pro UF ultrapure water, followed by those of the controls (ready-to-use media). These values were unlike those in the RO water samples, where mAb production decreased.

Hence, it was concluded that water from the Arium® Pro UF system is excellently suited for PER.C6 EpCAM cell cultivation because this water system minimizes the content of impurities, such as inorganic ions, organic compounds and, in particular, it reduces endotoxins to exceptionally low levels, as was recently confirmed in newer experiments.⁴

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Acknowledgement

The authors acknowledge Dr. Alexander Tappe and Dr. Yvonne Martin of Sartorius-Stedim Biotech GmbH, Goettingen, Germany, for providing the photograph of the PER.C6 cell line and further technical discussions.

First published in G.I.T. Laboratory Journal Europe 9-10, Volume 16



Arium® Pro UF water system

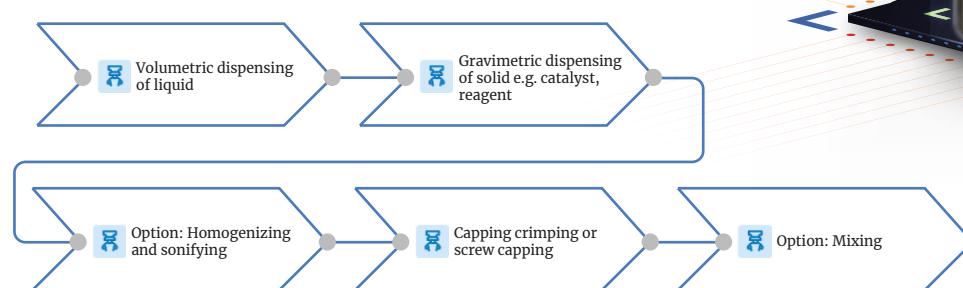
AUTOMATED SOLUTIONS FOR SOLID AND LIQUID DISPENSING/ DOSING

Workflows are blueprints for self-driving experimentation in the rapidly changing field of autonomous laboratories. To make AI-driven chemistry a reality, Chemspeed Technologies has developed a series of modular, automation-ready platforms. Chemspeed technologies' workflows, such as SWING RP and ReactScreen iSYNTH, are fundamental components in smart labs in both academia and industry, ranging from intelligent sample preparation to parallel reaction execution. Example workflows are overviewed in this article along with how each one supports hands-free, high-throughput discovery.

EXAMPLE WORKFLOW FOR HIGH THROUGHPUT, VERSATILE SOLID AND LIQUID REACTION PREPARATION

Automated solid and liquid reaction preparation for e.g. library synthesis, catalyst screening.

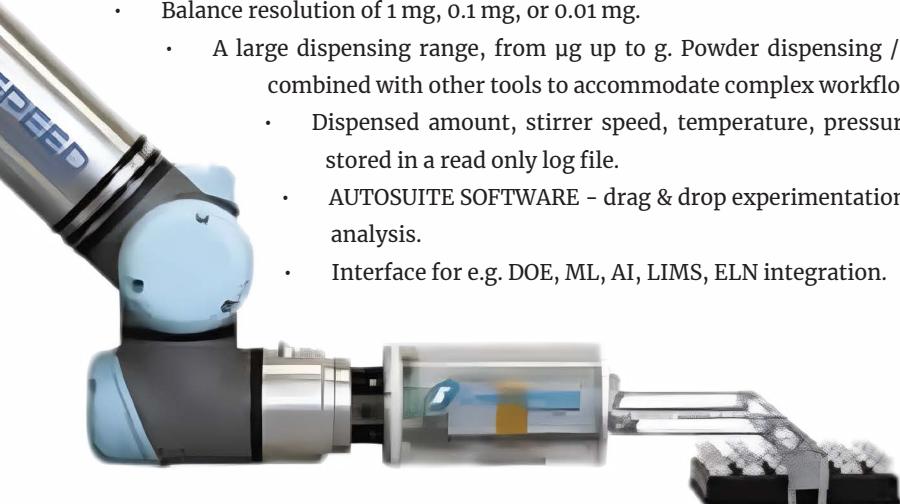
The smooth automation of powders, solids, extrudates, and liquids into a variety of vessel types is made possible by Chemspeed Solutions' patented precision solid and liquid dispensing technologies that are driven by overhead gravimetric systems and their user-friendly control software.



Automated solid and liquid reaction preparation for e.g. library synthesis, catalyst screening.

ADVANTAGES

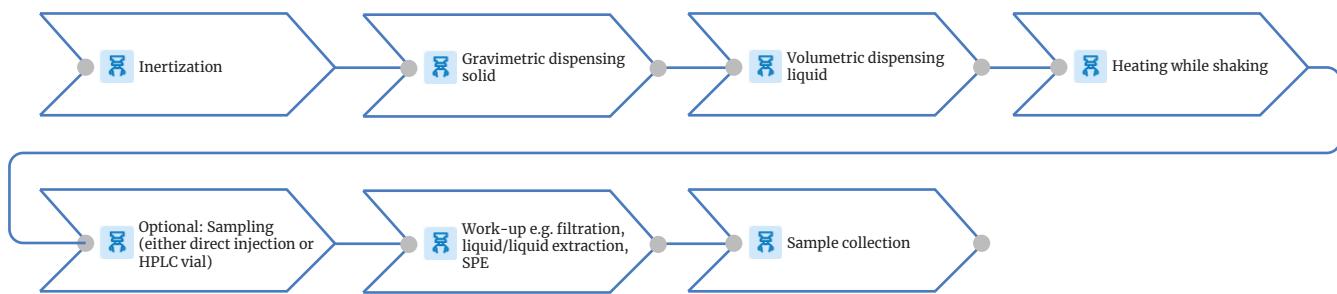
- Decrease in cost per sample up to 90 %.
- Increase in productivity by a factor of 10+.
- Gravimetric solid dispensing (low density, sticky, any particle size, spherical or granulates shapes, beads and tablets) into an unlimited choice of vial / plate / reactor formats.
- 4-Needle Head for volumetric liquid handling and sampling.
- The balance moves to the destination, leading to increased speed and efficiency.
- Balance resolution of 1 mg, 0.1 mg, or 0.01 mg.
 - A large dispensing range, from μg up to g. Powder dispensing / dosing robotic tool can be combined with other tools to accommodate complex workflows.
 - Dispensed amount, stirrer speed, temperature, pressure, time and other data are stored in a read only log file.
 - AUTOSUITE SOFTWARE - drag & drop experimentation with easy interface to data analysis.
 - Interface for e.g. DOE, ML, AI, LIMS, ELN integration.



EXAMPLE WORKFLOW FOR AUTOMATED REACTION SCREENING (μL TO mL SCALE)

Automated small-molecule reaction screening (μL to mL scale) in e.g. late-stage drug discovery

When combined with automated small-scale reactor formats (from microlitre to millilitre scale) and intuitive software, overhead gravimetric dispensing of solids and liquids provides a potent method for speeding up, standardising, and digitising small-molecule reaction screening, from preparation and photochemistry to work-up and analysis.



ADVANTAGES

- ▶ Fully automated, integrated reaction preparation, synthesis, work-up, analysis, output to storage vials for solid-phase and liquid-phase μL and mL reaction screening.
- ▶ E.g. easy-to-use SBS-plate based disposable multi-functional, high-precision glass reactor arrays with μL reaction scale, screwless and self-sealing opening / closing, mixing by shaking (up to 1'000 rpm), heating (up to 150°C) and cooling (-20°C).
- ▶ Work-up such as evaporation, L/L extraction, filtration, solid-phase extraction, crystallization and drying.
- ▶ Proprietary robotic tool exchange.
- ▶ Gravimetric solid handling from μg to g .
- ▶ Gravimetric liquid handling from mg to g / μL to mL .
- ▶ Chemistry proven liquid handling with e.g. pH, filtration, heated needle extension.
- ▶ Throughput, e.g. 24 / 48 / 96 / ... parallel reactions per run based.
- ▶ Sampling and various integrated analytics such as benchtop online NMR, HPLC (MS), UPLC (MS), GC (MS), UV-VIS, NIR / MIR / DLS measurement cell.
- ▶ Ventilated hood for safety and conditioned operation.
- ▶ AUTOSUITE SOFTWARE - drag & drop experimentation with easy interface (e.g. python custom device) to e.g. LIMS, ELN, data analysis, AI / ML closed loop.
- ▶ Optional integrated online benchtop NMR - including but not limited to 80 MHz Fourier RxnLite, NMR device, sample injection port, software with advanced chemical profiling and hardware integration, H / F / C / P / Si / B, 1D and 2D spectra, online data acquisition via flow cell or offline via standard 5 mm sample tubes, permanent magnet - no liquid nitrogen or helium required, 2 Gauss line inside NMR device housing.

ISYNTH REACTSCREEN FOR REACTION SCREENING



SWING RP



CASE STUDY

AI-GUIDED AUTOMATION OF THE SUZUKI-MIYaura REACTION FOR SELECTIVE MONO-FUNCTIONALIZATION

KEY FOCUS AREAS

- Autonomous reaction optimization
- AI-guided ligand/base/solvent screening
- Suzuki-Miyaura cross-coupling
- Mono-functionalization of bifunctional substrates
- Discrete reaction parameter control
- Chemspeed SWING RP + iSYNTH workflow
- Closed-loop experimentation
- Supercritical fluid chromatography (SFC)
- Bayesian optimization in synthesis
- Data-driven methodology development
- Organic electronics precursor synthesis
- High-throughput catalyst screening

In synthetic chemistry, selective mono-functionalization of symmetric bifunctional substrates such as dihalogenated arenes is an important but understudied. In these systems, achieving high selectivity frequently necessitates meticulous control over discrete parameters like the solvent, base, and ligand type.

Conventional approaches have a limited throughput, a heavy reliance on manual trial-and-error, and an inability to efficiently explore large chemical spaces.

By tackling this persistent synthetic problem with autonomous experimentation and AI-driven optimisation, researchers at WPI-ICReDD aimed to advance the field of organic synthesis. Creating a self-governing experimental loop that can optimise discrete reaction conditions for a Suzuki-Miyaura coupling reaction was their aim.

SOLUTION: FULLY INTEGRATED AUTONOMOUS OPTIMIZATION SYSTEM POWERED BY CHEMSPEED

In order to achieve this goal, the group built a closed-loop autonomous optimisation platform that included the following components¹:

- ▶ **Chemspeed SWING RP Robotic Platform:** Automated vial preparation, gravimetric solid/liquid handling, and reagent dispensing
 - ▶ **ReactScreen iSYNTH Module:** Accurate thermal and inert control for parallel synthesis across 48 reactors
 - ▶ **Supercritical Fluid Chromatography (SFC):** Fast, high-resolution analysis of complex product mixtures
 - ▶ **PHYSBO (Bayesian Optimization Engine):** Gaussian process regression to propose new experiments and optimize the objective function
 - ▶ **AutoSuite Software:** Central control of synthesis hardware and workflow orchestration.
- The components of the reaction loop were:
- Establishing reaction conditions using recommendations from algorithms
 - Using the Chemspeed iSYNTH reactors to run automated reactions
 - Automated sample injection and detection for online SFC analysis
 - Updates to the model and fresh experimental ideas

DESIGN OF EXPERIMENTS

Target Reaction: Mono-functionalization of 2,7-dibromo-9,9-dihexylfluorene with 4-methoxyphenylboronic acid via Suzuki-Miyaura coupling

Optimised Variables: 15 Ligands (such as triphenylphosphine, tris(4-methoxy-3,5-dimethylphenyl)phosphine, etc.)

- 10 bases, such as Cs_2CO_3 , NaHCO_3 , K_3PO_4 , and so forth
- 10 Solvents (such as 1,4-dioxane, 1-propanol, and acetonitrile, among others)
- 1500 distinct condition sets make up the search space

Cycle Time: approximately three hours for each cycle of an independent experiment

ML Modelling Descriptors:

- ▶ **Ligands:** ^{31}P NMR shift, Cone Angle, and Tolman Electronic Parameter (TEP)
- ▶ **Bases:** Ionic Radius, pK_a
- ▶ **Solvents:** Hansen Solubility Parameters (hydrogen bonding, polarity, and dispersion) Maximise $\log(\text{yield of desired mono-functionalized product})$ is the objective.

RESULTS

- ▶ 9.5% is the initial yield (Condition #1).
- ▶ Yield optimisation (Condition #102): 49.0%
- ▶ 192 conditions were screened in total (47 iterative cycles plus 4 initial trials).

Best Optimised Conditions:

- ▶ **Ligand:** L3 (tris(4-methoxy-3,5-dimethylphenyl) phosphine)
- ▶ **Base:** B8 (Cs_2CO_3)
- ▶ S3 (acetonitrile) is the solvent.
- ▶ **Performance of the Model:**
- ▶ **Training Set R₂:** 0.98
- ▶ **Test Set R₂:** 0.46
- ▶ TEP (ligand electronics) and δ_h (solvent hydrogen bonding ability) are the descriptors with the highest predictive power.
- ▶ Using SFC pressure stability and yield logic, 17 unsuccessful experiments were automatically eliminated through error detection.

IMPACT

A series of π -conjugated oligomers was also synthesised using the monofunctionalized product, showing:

- ▶ Weights of molecules $>1000 \text{ g/mol}$
- ▶ High quantum yields ($\Phi = 0.72\text{--}0.80$)
- ▶ Molar absorptivity $>105 \text{ L mol}^{-1} \text{ cm}^{-1}$
- ▶ These materials exhibit potential for use in photonic and organic electronic applications. More generally, the project confirms that it is feasible to solve discrete-variable problems in reaction chemistry—a field that has not received enough attention—by utilising Chemspeed's modular automation and AI optimisation.

CONCLUSION

This example shows how powerful it is to combine the Chemspeed SWING + ReactScreen iSYNTH + ML algorithms in a self-sufficient lab environment. It made it possible for researchers to find unexpectedly ideal conditions, navigate a vast discrete parameter space with little assistance from humans, and convert findings into useful materials. An actual advancement in autonomous chemical discovery.

REFERENCES

1. Akiyama, S.; Akitsu, H.; Tamura, R.; Matsuoka, W.; Maeda, S.; Tsuda, K.; Nagata, Y. Autonomous Optimization of Discrete Reaction Parameters: Mono-Functionalization of a Bifunctional Substrate via a Suzuki-Miyaura Reaction. 2024. <https://doi.org/10.26434/chemrxiv-2024-bnj6p-v2>.
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Noise level	< 60 dB	< 68 dB	< 68 dB
Minimum system vacuum (with vacuum pump / without samples)	0.03 mbar	0.03 mbar	0.03 mbar
Global Warming Potential (GWP)-Refrigerant	4,000	4,000	3,559
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Biotechnology	Peptides, proteins, enzymes, cells, bacteria, viruses
Chemicals	Organic/inorganic compounds, nanotech
Environmental	Sample prep, quality/pathology analysis
Food	Dairy, beverages, fruits, meat, smart food
Natural Extracts	Nutraceuticals, plant-based compounds



8 CRUCIAL STEPS TO CLEAN A BALANCE AND 5 SOLUTIONS TO MAINTAIN IT

A clean balance is a first step towards safe and accurate weighing and is the most important action to prolong instrument life. Furthermore – and even more important – cleaning increases user safety and reduces the risk of cross-contamination.

This guide imparts knowledge about cleaning methods and agents, provides instructions on how to clean a balance in 8 easy steps. In addition, 5 solutions are discussed on how to avoid making your balance dirty in the first place.

CLEANING IS ESSENTIAL

If you work in a laboratory, then you know that ensuring its cleanliness has a significant impact on both operator safety and cross-contamination risk.

Keeping a balance clean is important. It can:

- Minimize cross-contamination risk

MUST KNOW DEFINITIONS

- ▶ Enhance user safety
- ▶ Increase operating reliability, and
- ▶ Reduce equipment failure rates.

Each of these points can represent a significant source of expense for your lab, whether in rework, health costs, or equipment service and replacement costs.

Keeping balances clean is a great first step towards reducing these costs.

This guide covers all aspects of balance cleaning including:

- ▶ How to clean various balance components
- ▶ Which cleaning agents are appropriate
- ▶ How to avoid making your balance dirty
- ▶ How intelligent solutions eliminate spills altogether.

WHICH CLEANING AGENTS SHOULD I USE?

Depending on the laboratory, different risks and potential for contamination exist.

In chemical and pharmaceutical laboratories, the main risk is the potential for exposure to harmful chemicals or active substances, either by inhalation, ingestion, or direct skin contact. In biological laboratories, risks also include the potential for exposure to microorganisms that could result in infection and illness. The level of potential exposure to biological contaminants will in part determine the type of cleaning agent chosen. For example, an analytical laboratory primarily working with chemical agents might prefer 70% isopropanol, while a biochemical laboratory would use 70% ethanol to reduce contamination. Secondarily, the cleaning agent selected must be able to fully remove contamination yet not damage the equipment.

Again, balances should always be cleaned after weighing potentially toxic reagents.

IS IT TIME TO CLEAN MY BALANCE?

Laboratories differ on how often a balance should be cleaned, depending on industry, application and frequency of use. Typically, there are SOPs that specify the frequency for balance cleaning.

However, it is recommended as a general rule that the balance should be cleaned immediately after every use or change of weighing substance.

Cleaning: Physical removal of foreign material, e.g. dust, soil, organic material such as secretions, excretions and microorganisms.

Cleaning generally removes rather than kills microorganisms. It is accomplished with water, detergents and mechanical action.

Decontamination: Removal of microorganisms, radioactive substances or hazardous material to leave an item safe for further handling.

Disinfection: Inactivation of disease-producing microorganisms. Disinfection does not destroy bacterial spores. Disinfectants are used on inanimate objects in contrast to antiseptics, which are used on living tissue. Disinfection usually involves chemicals, heat or ultraviolet light. The nature of chemical disinfection varies with the type of product used.

Sterilization: Destruction of all forms of microbial life including bacteria, viruses, spores and fungi. Items should be cleaned thoroughly before effective sterilization can take place.

Every user expects that the previous user has left the instrument clean. However, control is always better than trust.

When handling potentially toxic substances, the balance must always be cleaned immediately after use. This avoids cross-contamination and exposure risk for the person who uses the instrument next.

BALANCE CLEANING PROCEDURE:

1. Get ready: Protect yourself and prepare instructions.

- a) Wear appropriate personnel protective equipment (coat, goggles and gloves).
- b) Consult the operating instructions on how to disassemble and reassemble the removable parts of your balance.
- c) Consult the operating instructions on which cleaning methods and agents are recommended to clean the balance parts.

2. Switch off the balance display.

Unplugging the power is recommended but not necessary. If possible, leave the balance in its normal operating position, do not tilt it sideways and don't unplug connected peripherals.

3. Clean the area around the balance.

Use a tissue to remove any dirt surrounding the unit to avoid further contamination.

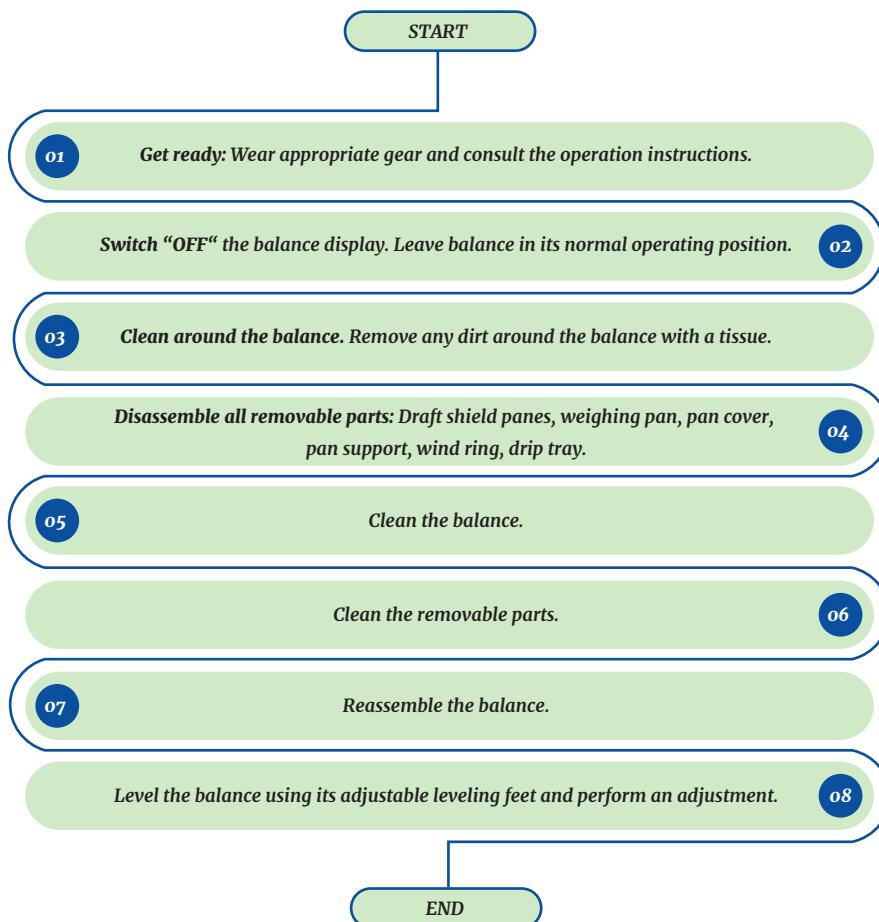
4. Disassemble all removable parts.

Depending on your balance:

- a) Unlock and gently remove the draft shield panes.
- b) Remove the inner draft shield.
- c) Remove weighing pan, pan cover, pan support, wind ring and drip tray.

5. Clean the balance itself.

Use a lint-free cloth moistened with a mild cleaning agent to clean and remove any spilt material on the surface of the balance.



First remove powder and dust, then sticky substances.

- a) For removal of powder or dust use a disposable tissue.
- b) Never blow; this could transport the dirt inside the balance or outside in your laboratory environment.
- c) For removal of sticky substances use a damp lint-free cloth and mild solvent (isopropanol or ethanol 70%); avoid abrasive materials.
- d) Wipe away from the hole in the middle of the balance. Do not allow any substance to enter the balance's internal parts and do not spray or pour liquids on the balance. This might lead to damage of the weighing cell or other essential balance components.

6. Clean the removable parts.

Use a damp cloth or tissue and clean with a mild cleaning agent containing ethanol (e.g. a commercial window cleaner) or simply place them in the dishwasher.

7. Reassemble the balance.

Make sure the parts are correctly positioned, switch the balance on and check that it is working correctly. If completely switched off: Ensure enough warm-up time before first use (check the operating instructions for manufacturer's recommendation).

8. Ensure the balance is ready.

- a) Ensure that the balance is leveled or take appropriate action to re-level if it is not.
- b) Calibrate with external weights or execute an internal adjustment such as FACT.
- c) Place a test weight on the weighing pan and perform a routine test to check accuracy.

AVOID MAKING YOUR BALANCE DIRTY

5 solutions from **METTLER TOLEDO** that will help you prevent your balance from getting dirty in the first place.



1. Use a balance designed to avoid dirt

When evaluating a new balance, keep an eye on gaps and shoulders in the design and check if the draft shield can be dismantled without tools. Better yet, determine if it can be removed in just a few steps and put in the dishwasher. This can help ensure easy clean-up when spills occur.

2. Avoid touch contamination or soiling

A balance that allows touchless operation – achieved through a built-in functionality, an optical hand-sensor, or addition of a foot switch – can help prevent soiling. This can be especially helpful when working with toxic substances. A balance that allows you to detach the balance terminal and move it away from the weighing platform can also help avoid contamination, as can the use of a secondary display or display stand. Finally, if possible, use fewer cables, as these offer an excellent way to trap undesirable dirt and dust around the weighing unit.





3. Protect the balance in-use

Replaceable protective covers and mats safeguard your balance in-use from soiling, prevent scratches and ensures a long instrument lifetime.

4. Safe dosing

Sample and standard preparation require substance transfer from the original container to the preparation vessel, such as volumetric flask, test tube, and other. All material transfers bear a risk of spills and potentially create a need for cleaning.

- a) Avoid scattering during transfer
- b) Safely dose directly into tare containers
- c) Secure dangerous powders



5. Protect the balance when it is not in use

They may be simple, but they work: Dust covers are excellent barriers against environmental contaminants like dust and moisture. They also protect against accidental soiling or spills.

 **Poor cleaning frequently goes unnoticed until it affects outcomes.**

The best investment you can make in the precision and longevity of your lab is preventive care!



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Applications

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- DC motor drive, for smooth and quiet operation
- Safe, durable and robust rotor with clamp locking design
- Compatible with 0.5/1.5/2mL×12 tubes+4×PCR8 strips, 0.2mL×44 PCR tubes;
- Screen buttons can adjust the start/stop of the motor
- supports both soft braking and instant stop modes
- Simple appearance and structure, only weight 1.5Kg



Specifications	D1012UA
Speed Range	2000-10000rpm, increment:1000rpm
Max.RCF	6708×g-1.5/2mL ; 6596-7490×g-PCR8 strips
Rotor Capacity	0.2mL×44 PCR tubes 0.5/1.5/2mL×12+ 0.2mLPCR 8 strips×4
Run Time	10s-20min
Display	OLED
Acceleration/Deceleration	1↑/2↓
Driving Motor	DC motor
Power	AC100-240V/50Hz/60Hz 40W
Noise Level	≤58dB
Dimension (D x W x H)	210×180×130mm
Weight	1.5kg



CENTRIFUGAL PARTITION CHROMATOGRAPHIC PURIFICATION OF SEMAGLUTIDE

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

The limitations of traditional multi-step HPLC workflows are addressed in this application by introducing a centrifugal partition chromatography (CPC) method for the effective purification of semaglutide. To improve selectivity and resolution, a benchtop CPC system that alternated between descending and ascending phases was used in a multiple dual-mode (MDM) configuration. To maintain peptide solubility and attain optimal partitioning behaviour, a quaternary organic-aqueous biphasic solvent system was refined. Diode array detection and subsequent HPLC analysis verified that the purification of 900 mg of crude semaglutide produced a clear separation of impurities. The technique provides a gentle, scalable, and economical downstream approach that is especially well-suited for resolving complex impurity profiles and structurally similar peptide variants.

Keywords or phrases:

Centrifugal Partition Chromatography (CPC), Semaglutide purification, Multiple Dual-Mode (MDM-CPC), peptide impurities, biphasic solvent system, downstream processing



CPC Modeler

INTRODUCTION

Semaglutide is currently one of the most promising antidiabetic drugs. Approved as a second-line treatment option for type 2 diabetes mellitus, it is highly effective in improving glycemic control and promoting weight loss.¹ It is also under investigation for anti-obesity applications.² Semaglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist available as monotherapy in both subcutaneous and oral dosage forms, with the oral formulation being the first approved oral GLP-1 receptor agonist. It has been optimized for an extended half-life in humans, approximately 7 days, facilitating once-weekly dosing. This prolonged half-life is achieved through structural modifications that enhance albumin binding and confer resistance to degradation by dipeptidyl peptidase-4 (DPP-4).^{1,3}

However, semaglutide production remains costly, and achieving high yield, low cost, and high purity continues to pose a significant challenge.

KEY PURIFICATION CHALLENGES

A multi-step purification process, typically involving multiple reversed-phase high-performance liquid chromatography (RP-HPLC) steps using different stationary phases.

The presence of racemized impurities and sequence variants (structurally similar to the target peptide) pose significant challenges for chromatographic separation and purification.

A complex impurity profile, including high molecular weight proteins (HMWPs) and a variety of hydrophilic and hydrophobic impurities.

The need for rigorous impurity control to ensure product safety and efficacy.

Difficulties during pH adjustment, particularly precipitation near the isoelectric point, along with various stability concerns⁴.

Considering the chromatographic complexities and the high financial burden associated with these purification steps, a more cost-effective and promising alternative could be centrifugal partition chromatography (CPC).

CENTRIFUGAL PARTITION CHROMATOGRAPHY

CPC is a liquid-liquid chromatographic technique, in which separation occurs between two immiscible liquid phases.

Both the mobile and stationary phases are liquids, and the liquid stationary phase is immobilized by a strong centrifugal force in the cells which are connected by a series of ducts attached to a rotor.

The solute's retention depends simply on the liquid-liquid partition coefficient (Kd). Practically, it is the ratio of the solute concentration in the stationary phase over the solute concentration in the mobile phase.

THE ADVANTAGES OF CPC IN THE DOWNSTREAM PROCESS

- ▶ No solid stationary phase required
- ▶ Gentle separation technique
- ▶ Wide compound compatibility
- ▶ Multiple operating modes available (ascending, descending, dual-mode)
- ▶ Flexible method development
- ▶ Stable on both acidic and basic pH values

MULTIPLE DUAL-MODE CPC (MDM-CPC)

Multiple dual-mode (MDM) separation in CPC consists of a sequence of dual-mode runs, i.e., repeated inversions of the stationary and mobile phases, enabled by the liquid-liquid nature of the system. Each cycle includes two steps: descending (DSC) and ascending (ASC) elution modes, which correspond to reversed and normal-phase operation, respectively.

By switching flow direction and phase roles, MDM enhances resolution and improves the purity of the target compound. It is especially effective for isolating closely eluting or intermediate components from complex mixtures, an advantage unique to liquid–liquid chromatography.

EXPERIMENTS AND RESULTS

To identify the most suitable solvent system, several biphasic solvent systems were evaluated using the shake-flask method to determine ideal partition coefficients for the target compounds. Ultimately, a quaternary organic–aqueous mixture was used, consisting of organic solvents and water. This combination formed a biphasic system with tuneable polarity, which made it suitable for peptide separation. The system provided sufficient stability for a scalable CPC run, and the solubility of semaglutide in this mixture was ideal (Figure 1).

The starting synthetic crude material contained 77–80% semaglutide by weight along with a significant number of impurities. A total of 900 mg of crude was injected into the Benchtop CPC system operating in multiple dual-mode (method parameters are shown in Table 1.). With this specific purification method, the system switches multiple times between descending and ascending modes.

As a result, the process consists of three consecutive segments: DSC, followed by ASC, and then DSC again, representing 1.5 cycles of the MDM elution steps. This

continuous switching between two operational modes enhances the selectivity between semaglutide and its impurities, as they migrate at different rates under each elution condition. This results in their separated elution at opposite ends of the rotor.

Parameter	Details
Instrument	Benchtop CPC purification system equipped with a 250 mL rotor
Solvent system	Organic–aqueous biphasic system
Mode	DSC-ASC-DSC
RPM	2500
Flowrate	10 mL/min
Sample loading	900 mg injected semaglutide crude dissolved in 10 mL upper phase
Detection	DAD (254 nm)
Runtime	80 minutes

Table 1. The parameters of the CPC method

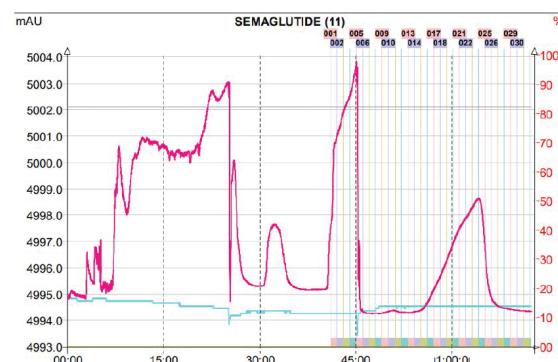


Figure 2. CPC chromatogram of semaglutide purification (detection: $\lambda=254$ nm). Semaglutide peak marked by rectangle.

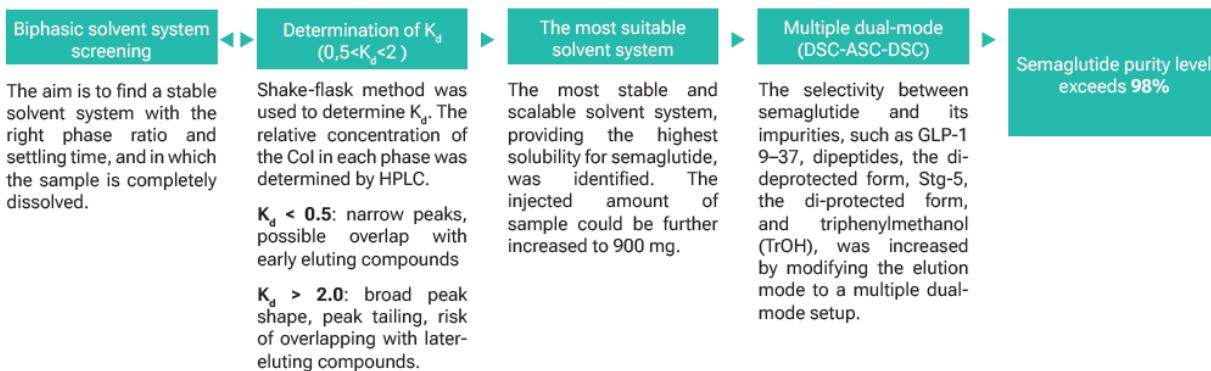


Figure 1. CPC method development for semaglutide purification

Parameter	Details
Column	HPLC C18 column (150 x 4.6 mm, 2.6 μ m)
Mode	gradient
Flowrate	0.7 mL/min
Detection wavelength	254 \pm 4 nm
Mobile phase	A: water +0.1% TFA; B: acetonitrile
Timetable	0.00–3.00 min – 35% B isocratic 3.00–20.00 min – 35–80% B gradient 20.00–22.00 – 80% B isocratic 22.01–25.00 – 35% B isocratic
Injection volume	10 μ L
Column temperature	40 °C
Diluent and Blank	MeOH

Table 2. The analytical parameters of the HPLC method

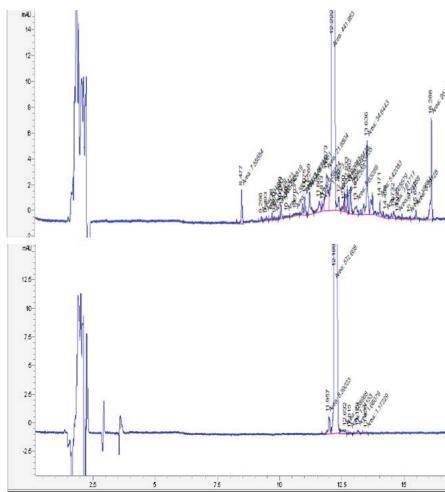


Figure 3. The chromatogram of the crude sample (top), and the chromatogram of the purified semaglutide fraction (bottom).

CONCLUSION

For the purification of semaglutide, centrifugal partition chromatography (CPC), especially in multiple dual-mode (MDM) operation, provides a reliable and scalable substitute for conventional reversed-phase HPLC techniques. This method successfully separates semaglutide from complex contaminants and structurally similar impurities by utilising alternating flow modes and a carefully tuned biphasic

solvent system. The procedure lessens dependency on pricey solid-phase materials while also streamlining downstream workflows. All things considered, MDM-CPC shows great promise as a productive, adaptable, and affordable peptide purification method for both manufacturing and research settings.

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FLASH PURIFICATION OF CARBOHYDRATES USING INTEGRATED ELSD DETECTION

Effortless Carbohydrate Purification Using puriFlash® 5.030 with Smart ELSD Detection

Authors: Applications Team, Advion Interchim Scientific

Carbohydrates are non-chromophore compounds which can often lead to flat signals when using UV detection only. In this application note, we show carbohydrate purification with ELSD and how such detection can help in purification.

Keywords or phrases:

Carbohydrate Purification, flash chromatography, ELSD detection, non-chromophoric compounds, evaporative light scattering detector, UV-invisible sugars, low-temperature detection

INTRODUCTION

Carbohydrates are non-chromophore compounds that often lead to flat signals when using UV detection only. In this application note, we show carbohydrate purification with ELSD and how such detection can help in purification.



puriFlash® 5.030

WHY USE ELSD FROM ADVION INTERCHIM SCIENTIFIC?

- ▶ Detect chromophotic and non chromophoric compounds as carbohydrates
- ▶ Low maintenance with Isopropanol make-up solvent for ELSD
- ▶ IPA push the sample to the ELSD
- ▶ System cleaning meanwhile processing to purification almost maintenance free
- ▶ Automatic refill
- ▶ Eliminates risk of signal saturation or non-detection
- ▶ Allow to detect and collect low concentration compounds
- ▶ Easy set up (no parameters to predict, easy to use)
- ▶ Automatic gain to clearly see all compounds at the same time (Figure 1)
- ▶ Low sample consumption 40µL/min
- ▶ ELSD has a destructive detection mode
- ▶ Low gas consumption (1 - 1.5L/min at 1-1.5bar)
- ▶ Low temperature technology

METHOD

Flash chromatography system: puriFlash® 5.030 with pack iELSD

Column puriFlash®: 50 µm NH2 F0025

Sample: D(-)Fructose 100 mg, Alpha (D)-Lactose 100 mg

Detector: ELSD 35°C

UV: 254nm

Time (min)	% ACN	% Water
0	90	10
5	90	10
15	70	30
20	70	30
30	0	100

RESULTS & DISCUSSION

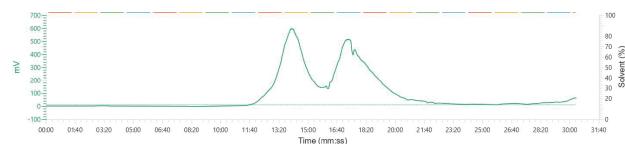
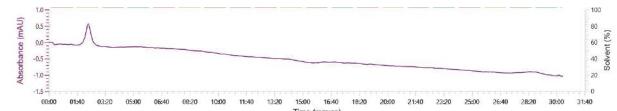


Figure 2: ELSD showing a good signal intensity and smoothing



Figures 3 & 4: Compounds are not visible with UV. Scan start to show low intensity for the first compounds, but not enough to get good collection

CONCLUSION

Since they are not chromophoric substances, carbohydrates are invisible to UV detectors. For their analysis, an Evaporative Light Scattering Detector (ELSD) is therefore necessary. The AIS-proposed ELSD system has several easy-to-use benefits. Most notably, it does away with the necessity of manually modifying gain settings according to sample loading. Additionally, a greater variety of compounds, including volatile and temperature-sensitive analytes, can be detected thanks to its low-temperature evaporation technology. Water served as the mobile phase in this application, and a temperature of 35 °C was adequate to suppress the water signal and produce a flat, stable baseline.

HPLC ANALYSIS OF ANTI-DIABETIC DRUGS

HILIC and SEC-Based Separation Using Shodex Columns

The structural diversity of antidiabetic drugs—including peptides, small molecules, and carbohydrates—presents significant analytical challenges in high-performance liquid chromatography (HPLC). This article showcases the application of various Shodex columns for the effective separation and quantification of three key antidiabetic drugs: metformin, insulin, and liraglutide.

Keywords or phrases:

Antidiabetic Drug Analysis, Shodex Columns, metformin HILIC, insulin, liraglutide, Size Exclusion Chromatography (SEC), High-Performance Liquid Chromatography (HPLC, MRM Quantification

INTRODUCTION

Drugs, including antidiabetic drugs, with different targets have various chemical structures. This makes it difficult for an HPLC method to simultaneously analyse all antidiabetic drugs which may contain peptides, sugars, and low-molecular weight compounds of different properties. Thus, analysis of each drug requires a specific method with an appropriate column and optimized analytical conditions. This technical article introduces application data for the analysis of four antidiabetic drugs using Shodex columns.



Asahipak NH2P series

1. ANALYSIS OF AN ORAL ANTIDIABETIC DRUG (METFORMIN)

Metformin is an oral antidiabetic drug. It suppresses glucose production to lower blood sugar level as well as to elongate life expectancy. To understand its various actions and utilize the obtained information to the treatment, it requires highly sensitive and selective analysis methods.

Since polarity of metformin is very high, it is not retained by reversed phase mode. Meanwhile, HILIC mode is often suitable for the analysis of high polarity compounds. We analysed metformin using a Shodex HILIC column and a triple quadrupole mass spectrometer, aimed for a highly sensitive detection.

Shodex Asahipak NH2P-40 2D is a column filled with polymer-based packing materials modified with amino groups. By using an eluent with a large acetonitrile concentration, we attempted to retain metformin under HILIC mode.

1.1. EXPERIMENTAL

Sample pretreatment

The test sample was prepared by mixing metformin standard and guinea pig serum following the below steps:

1. Add an equal volume of 0.05- μ M standard solution to the serum.
2. Add 1+4 volume of CH₃CN to the mixture to let the protein precipitate.
3. Centrifuge the mixture. Add an equal volume of CH₃CN to the obtained supernatant and filter the mixture with a 0.45- μ m membrane filter.

The final test sample contains 90% CH₃CN.

1.2. RESULTS

The obtained chromatograms show sufficient retention of metformin that cannot be achieved by an ODS column (Figure 1). The recovery rate was 103%. This indicates that the metformin peak was not affected by ion suppression even under the presence of guinea pig serum.

The test sample was quantified by standard addition method. A good linearity was obtained for the calibration curve with a coefficient of determination $R^2 \geq 0.99$. The MRM mode was used in this experiment. The selectivity and sensitivity of SIM is not as good as that of MRM, but it can also be used in place of a UV detector, by monitoring m/z 130(+).

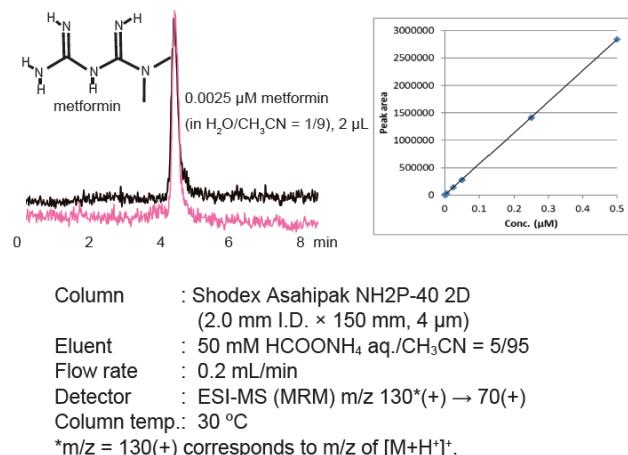


Figure 1. Chromatograms of (black) metformin standard and (pink) metformin standard with guinea pig serum.

1.3. CONCLUSION

Metformin was retained and analysed effectively using the Shodex Asahipak NH2P-40 2D. The use of MRM mode allows the quantification of metformin.

2. ANALYSIS OF INSULIN

Since its discovery in the early 20th century, insulin has been taking a major role in the treatment of diabetes. Insulin forms dimers and hexamers; however, unlike insulin monomers, they do not have blood sugar lowering effects. Thus, it is important to separate and quantify monomers and other aggregates of insulin in insulin drugs.

Dimers of proteins, including insulin, are often separated by size exclusion chromatography. The US Pharmacopoeia (USP) General Chapters mentions size exclusion chromatography separation in [<121.1> PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS, LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS](#). In this application, we examined a Shodex column's capability for fulfilling the system suitability.

2.1. EXPERIMENTAL

The USP method states to use a column filled with 5- to 10- μ m diameter, L20 column packing material. The Shodex PROTEIN KW-802.5 is an aqueous SEC column filled with 5- μ m modified silica gels, and this fulfills the requirement. The method also states to use an ambient temperature for the column temperature; however, to ensure obtaining the repeatable results, we set the column temperature at 25°C.

The “resolution solution” used for the system suitability test was prepared following the USP method. The test sample is required to contain more than 0.4% “high molecular proteins” (i.e., dimers and hexamers). We prepared 4-mg/mL insulin (bovine) sample in 0.01 N HCl (aq). The injection volume used was 100 μ L.

2.2. RESULTS AND DISCUSSION

Figure 2 shows the obtained chromatogram. The sum of peak areas 1 and 2 was 9.1% of the total peak area (sum of peak areas 1, 2, and 3). This fulfills the requirement stated in the “Resolution solution” (the sum peak area of peaks 1 and 2 to be $\geq 0.4\%$).

The system suitability requirements include the elution times of insulin aggregate, dimer, and monomer to be between 13 and 17 minutes, 17.5 minutes, and between 18 and 22 minutes, respectively. Also, the system suitability requires the ratio of dimer peak height and valley height (a valley between the dimer and the monomer peaks; peak-to-valley ratio) to be ≥ 2.0 .

The peaks 1, 2, and 3 in the figure 2 correspond to aggregate, dimer, and monomer, respectively. The observed retention times are very similar to the requirements. The obtained peak valley ratio was ≥ 10.0 . The results demonstrated that the HPLC system using the Shodex PROTEIN KW-802.5 to fulfil all system suitability requirements.

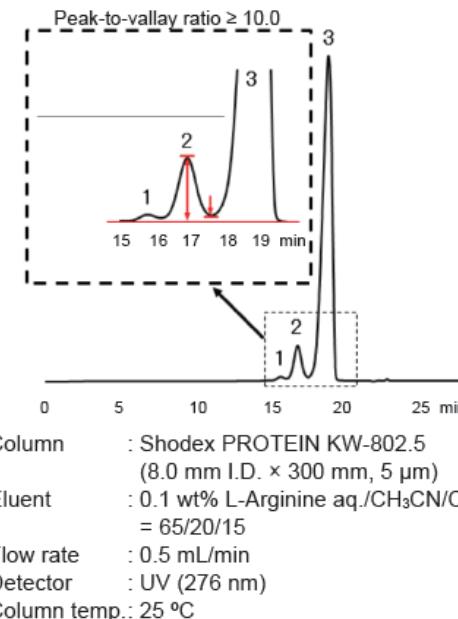


Figure 2. Chromatogram of insulin (bovine)

2.3. CONCLUSION

The HPLC method using Shodex PROTEIN KW-802.5 fulfilled the system suitability stated in the USP [<121.1> PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS, LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS](#).

3. ANALYSIS OF LIRAGLUTIDE

Liraglutide is a drug for treating type 2 diabetes. It promotes insulin secretion. It is an acylated peptide having a structure similar to GLP-1 secreted in a body. The molecular weight of liraglutide is 3,751 while that of GLP-1 is 4,170. Since size exclusion chromatography is known to effectively separate many peptide/protein drugs, including insulin and their dimers, liraglutide and its aggregates are also expected to be separated under size exclusion mode.

Therefore, we analysed liraglutide and its dimer and other aggregates using the Shodex PROTEIN KW-802.5.

3.1. EXPERIMENTAL

Shodex PROTEIN KW-802.5 is an aqueous SEC column filled with modified silica gel. The test sample was prepared by diluting the liraglutide five times with ultrapure water. We prepared another test sample by heating the liraglutide drug at 80 °C for 17 hours to purposely increase its dimer content. Prepared samples were used to test the method's capability for separating monomer and dimer. The heated sample was diluted five times with ultrapure water. The injection volume used was 5 μ L.

3.2. RESULTS AND DISCUSSION

Figure 3 shows the obtained chromatogram. The peaks 1 and 2 correspond to liraglutide monomer and phenol (an additive in the drug), respectively. The peaks eluted prior to liraglutide monomer are considered to be its dimer and trimer. The amount of those components increased after heating. The resolutions between the monomer and the dimer peaks in both heated and non-heated test samples were about 2.4. Each component was successfully separated for its quantification.

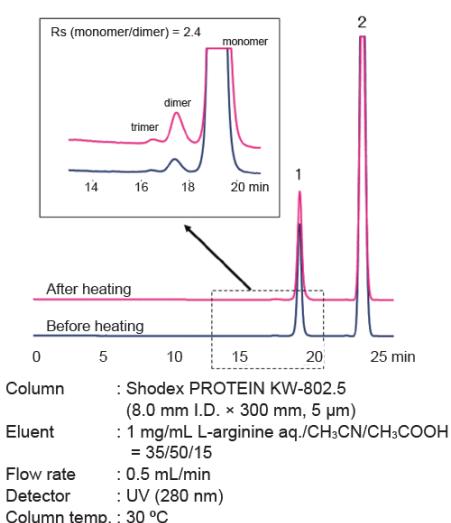


Figure 3. Chromatogram of Liraglutide

3.3. CONCLUSION

The results demonstrated Shodex PROTEIN KW-802.5's effectiveness in separating monomer and aggregates of liraglutide as well as phenol, an additive, in the drug.



ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN ACCORDANCE WITH EPA 1633 PART 3

Comprehensive PFAS Profiling in Soil and Tissue Using Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer

Kari L. Organtini, Kenneth J. Rosnack, Chelsea Plummer, Peter Hancock, Oliver Burt

Waters Corporation

US EPA Method 1633 has become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids, and tissues in the United States. The method consists of sample preparation using weak anion

exchange (WAX) solid phase extraction (SPE) with graphitized carbon black (GCB) clean up. This application note is the third in a series demonstrating a comprehensive solution for performing the EPA 1633 methodology.

The focus of this note is preparation and analysis of soil and fish tissue samples utilizing a bilayer dual-phase SPE cartridge and LC-MS/MS method on an ACQUITY™ Premier BSM FTN LC System coupled to a Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer.



Keywords or phrases:

PFAS analysis, EPA method 1633, Xevo TQ Absolute, Oasis GCB/WAX SPE, soil and tissue extraction, LC-MS/MS, *m/z* quantitation

Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer

INTRODUCTION

US EPA Method 1633 was first introduced in August 2021 to become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids, and tissues.¹ EPA 1633 was finalized in January 2024 and is multi-lab validated for each type of sample matrix included in the method.² The method covers 40 PFAS and utilizes isotope dilution calibration and quantitation. Required sample preparation differs slightly depending on sample type but all sample types utilize solid phase extraction (SPE) on a weak anion exchange (WAX) cartridge in combination with graphitized carbon black (GCB) clean up. EPA 1633 was created to support sample analysis for the Clean Water Act (CWA) and Department of Defense (DoD) monitoring and remediation, but it covers such a wide range of matrices and compounds that its applicability is expected to be widespread.

This is the third in a series of application notes addressing sample preparation, analysis, and method performance of EPA 1633 using a comprehensive workflow of Waters technologies.

This application will focus on the preparation of authentic soil and tissue samples with analysis utilizing the LC-MS/MS method established in Part 1 on an ACQUITY Premier BSM FTN UPLC System coupled to a Xevo TQ Absolute Mass Spectrometer.³ The use of a combined WAX and GCB sample extraction and cleanup workflow is demonstrated on soil and fish tissue.

EXPERIMENTAL

Sample Preparation

Samples discussed in this application include soil and fish tissue. Salmon was used as the fish tissue matrix studied and was sourced at a local market. The fish tissue was homogenized using a blender before subsampling. The soil was a custom soil reference material created by the ERA that is similar to the PFAS in Soil CRM currently offered

(Item number 603 <<https://www.eraqc.com/pfas-in-soil-soil-era001675?returnurl=%2fsearch%3fq%3d603%26analytessearchonly%3dfalse>>).

It contained the 40 EPA 1633 PFAS at a known concentration. Samples were frozen until sample analysis according to EPA 1633 guidelines and holding times.

Oasis GCB/WAX for PFAS dual-phase SPE cartridges containing both WAX and GCB sorbents required for sample cleanup were used instead of using dispersive GCB. For soil and tissue analysis, the GCB is packed on top of the WAX sorbent to replicate the EPA 1633 where the GCB is used to clean the sample prior to WAX SPE.

Full sample preparation details are listed in Figures 1 and 2 and are adapted directly from the EPA 1633 method.

Figure 1 details the two different extraction procedures used for soils/solids and tissues. Figure 2 details the SPE procedure used for all sample types. The dispersive GCB step was combined into the SPE cartridge, as described previously, providing the convenience of minimizing complications from using loose GCB material and reducing the number of steps during sample preparation without compromising the method.

Soil samples were spiked with 0.25–2 ng/g (sample concentration equivalent) of the required extracted internal standard (EIS) prior to extraction and 0.25–1.0 ng/g (sample concentration equivalent) of the required non-extracted internal standard (NIS) after extraction.

Tissue samples were spiked with 0.625–5 ng/g (sample concentration equivalent) of the required extracted internal standard (EIS) prior to extraction and 0.625–2.5 ng/g (sample concentration equivalent) of the required non-extracted internal standard (NIS) after extraction.

Individual concentrations vary dependent on the concentration of each component in the Wellington standard mixes.

The calibration curve range for each analyte (in vial equivalent) is listed in Appendix Table 2 and was determined from the data acquired and presented in Part 1 of this application note series.³ All standards were obtained as mixes from Wellington Laboratories.

Soils/Solids	Tissues
<ul style="list-style-type: none"> Weigh 5 g sample into 50 ml tube Spike with Extracted Internal Standard Mix (MPFAC-HIF-ES from Wellington) Add 10 ml 0.3% ammonium hydroxide in methanol Shake 30 mins, centrifuge, transfer supernatant to clean tube Add 15 ml 0.3% ammonium hydroxide in methanol Shake 30 mins, centrifuge, transfer supernatant (combine with previous step) Add 5 ml 0.3% ammonium hydroxide in methanol Shake 5 mins, centrifuge, transfer supernatant (combine with previous steps) Concentrate under nitrogen to 7 ml Reconstitute up to 50 ml with water Check pH and adjust to approximately pH 6 	<ul style="list-style-type: none"> Weigh 2 g sample into 15 ml tube Spike with Extracted Internal Standard Mix (MPFAC-HIF-ES from Wellington) Add 10 ml 0.05 M KOH in methanol Shake gently for 16 hours, centrifuge, transfer supernatant to clean tube Add 10 ml acetonitrile Sonicate 30 mins, centrifuge, transfer supernatant (combine with previous step) Add 5 ml 0.05 M KOH in methanol Shake 5 mins, centrifuge, transfer supernatant (combine with previous steps) Add 1 ml water Concentrate under nitrogen to 2.5 ml Reconstitute up to 50 ml with water Check pH and adjust to approximately pH 6

Figure 1. Full method details of the extraction procedure used for soil and tissue. Adapted from EPA Method 1633.

1

- Pack SPE cartridge with glass wool to half height of barrel
- Condition SPE cartridges
 - 15 ml 1% (v/v) ammonium hydroxide in methanol
 - 5 ml 0.3 M formic acid

2

- Load sample at 5 ml/min
- Wash cartridge with 10 ml of reagent water, ensuring to rinse reservoir with this solution
- Wash with 5 ml of 1:1 0.1 M formic acid:methanol, ensuring to rinse reservoir with this solution
- Dry cartridge for 15 seconds

3

- Place collection tubes in manifold
- Rinse bottle with 5 ml 1% (v/v) ammonium hydroxide in methanol. Transfer to cartridge and elute
- Add 25 μ L acetic acid to each sample
- Spike each sample with Non-Extracted Internal Standard (MPFAC-HIF-IS from Wellington)

Figure 2. Full method details of the SPE procedure used for soil and tissue. Adapted from EPA Method 1633.

LC CONDITIONS

LC system: ACQUITY Premier BSM with FTN

- Vials:** 700 μ L Polypropylene Screw Cap Vials
- Analytical column:** ACQUITY Premier BEH™ C18 2.1 x 50 mm, 1.7 μ m
- Isolator column:** Atlantis Premier BEH C18 AX 2.1 x 50 mm, 5.0 μ m
- Column temperature:** 35 °C
- Sample temperature:** 10 °C
- PFAS kit:** PFAS Install Kit with OASIS™ WAX 150 mg
- Injection volume:** 2 μ L
- Flow rate:** 0.3 mL/min
- Mobile phase A:** 2 mM ammonium acetate in water
- Mobile phase B:** 2 mM ammonium acetate in acetonitrile

Time (min)	%A	%B	Curve
0	95	5	initial
0.5	75	25	6
3	50	50	6
6.5	15	85	6
7	5	95	6
8.5	5	95	6
9	95	5	6
11	95	5	6

MS CONDITIONS

MS system: Xevo TQ Absolute Mass Spectrometer

- ▶ Ionization mode: ESI-Capillary voltage: 0.5 kV
- ▶ Source temperature: 100 °C
- ▶ Desolvation temperature: 350 °C
- ▶ Desolvation flow: 900 L/hr
- ▶ Cone flow: 150 L/hr

Data Management

- ▶ Software: waters_connect™ for Quantitation

RESULTS AND DISCUSSION

Recovery in Soil and Tissue Samples

EPA 1633 is a performance-based method that allows modifications as long as the performance criteria outlined in the method are all met. The only modification presented in this work was to use a bilayer dual-phase SPE cartridge that combines the otherwise dispersive GCB clean up step into the WAX SPE cartridge. GCB is difficult to work with and accurately measure, therefore utilizing a bilayer cartridge eliminates the untidy dispersive GCB step. More importantly, combining the GCB cleanup step into the SPE extraction saves valuable time in the laboratory during the sample preparation process. Additionally, less preparation steps allow for fewer opportunities for introduction of unintended PFAS sample contamination. For this work, a cartridge with the GCB stacked on top of the WAX was utilized to replicate the workflow of EPA 1633 where the GCB clean up step occurs before loading the sample onto the WAX cartridge.

One of the important performance criteria that must be established in order to prove equivalence of this approach is the target analyte (natives) and extracted internal standard (EIS) recovery acceptance limits (Table 7).¹ The individual recovery performance of the bilayer dual-phase SPE cartridge for soil and fish tissue are listed for each EIS in

Table 1. The data reported in Table 1 is the average recovery and %RSD for 3 replicate extractions of each matrix type. The mean recovery of all EIS among the soil and tissue samples extracted was 81% and 85%, respectively, with mean RSDs of 2.8% and 9.2% for soil and tissue, respectively.

Figure 3 directly compares the average recovery for the EIS in each sample type with the allowable recoveries in EPA 1633 Table 7. All PFAS analyzed in this study in both soil and fish tissue, were easily within the recovery acceptance limits for each compound, and in all cases were significantly above the minimum recovery level, demonstrating that the bilayer GCB/WAX SPE cartridge has equivalent performance as using dispersive GCB and is fit-for-purpose.

Compound	Soil	%RSD	Fish Tissue	%RSD
	Average Recovery %		Average Recovery %	
¹³ C ₆ -PFBA	93.9	16	79.8	13.6
¹³ C ₆ -PFPeA	914	2.3	83.3	11.4
¹³ C ₆ -PFHxA	95.1	18	82.6	9.3
¹³ C ₆ -PFHpA	92.2	18	812	12.2
¹³ C ₆ -PFOA	92.3	15	77.8	8.3
¹³ C ₆ -PFNA	92.4	13	81	8.7
¹³ C ₆ -PFDA	93.3	14	82.4	8.2
¹³ C ₁₀ -PFUnDA	914	18	82.3	6.3
¹³ C ₂ -PFDoDA	914	14	82.4	6.7
¹³ C ₃ -PFTrDA	84.8	2.3	813	6.2
¹³ C ₂ -PFTeDA	84.8	2.3	813	6.2
¹³ C ₆ -PFBS	81	2.7	86.2	8.2
¹³ C ₆ -PFHxS	89.7	2.1	88.1	7.7
¹³ C ₈ -PFOS	89	17	82.3	8.4
¹³ C ₈ -PFDS	915	13	84.9	6.4
¹³ C ₆ -PFHpS	89.7	2.3	813	8.2
¹³ C ₆ -PFNS	93.7	2	82.9	8.3
¹³ C ₆ -PFDs	93.3	16	82.3	8.5
d ₃ -NEtFOSAA	83.5	4.1	76.7	6.7
d ₅ -NMeFOSAA	83.6	4	76.7	6.7
d ₄ -NETFOSAA	65.4	4.6	76.7	6.7
d ₅ -NMeFOSA	65.4	4.6	76.7	6.7
d ₃ -PFOSA	84.9	2.3	83.2	6.2
d ₄ -NMeFOSAE	52.2	19	63.2	5
d ₅ -NETFOSAE	52.2	18	63.2	5

Table 1. Average recovery of the extracted internal standards (EIS) using the bilayer dual-phase SPE cartridge for soil and fish tissue (n=3).

ANALYSIS OF A CERTIFIED REFERENCE MATERIAL FOR SOIL

Accuracy of analysis is important for quantitating PFAS in customer samples. A custom certified reference material (CRM) from Waters ERA was processed to demonstrate workflow accuracy. The reference material analyzed contained all 40 EPA 1633 PFAS compounds in a representative soil matrix to evaluate method performance in a known sample free from PFAS. Figure 5 shows the average quantitative results for three replicate extraction and analyses of the soil CRM. The dotted and dashed red lines indicate $\pm 20\%$ of the designated concentration of the CRM (solid blue line) and the solid grey line represents the average experimental quantitated value determined during sample analysis.

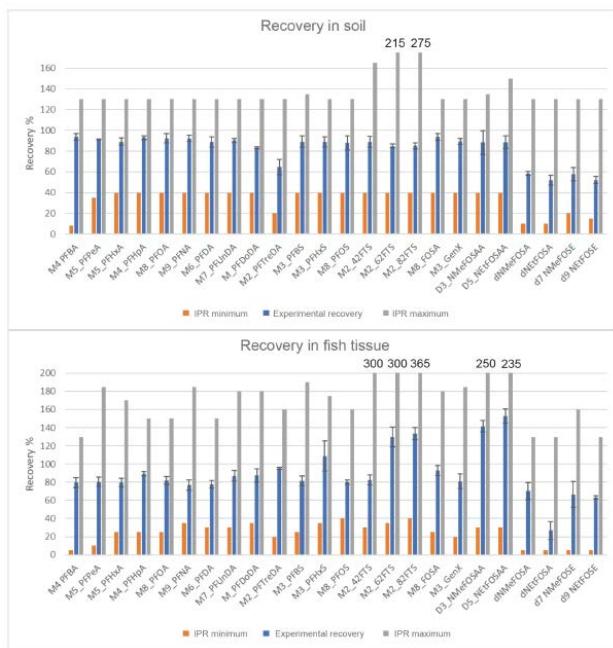


Figure 3. Average recovery of the extracted internal standards (EIS) in soil (top) and fish tissue (bottom). Experimental values (blue) are compared to the minimum (orange) and maximum (grey) percent recoveries allowed in the EPA 1633 method. n=3 replicates for each sample matrix. Bars are labelled that go off the scale of the graph.

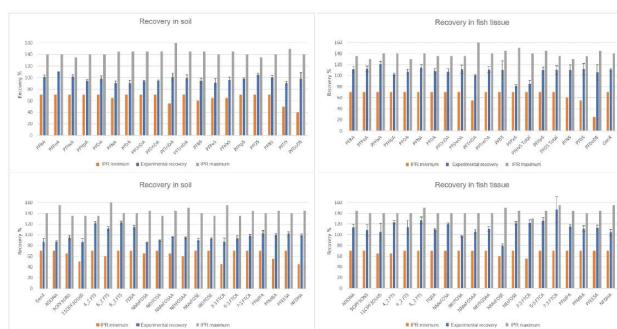


Figure 4. Average recovery of the target PFAS analytes in soil (left) and fish tissue (right). Experimental values (blue) are compared to the minimum (orange) and maximum (grey) percent recoveries allowed in the EPA 1633 method. n=3 replicates for each sample matrix. Bars are labelled that go off the scale of the graph.

All 40 target PFAS in EPA 1633 were quantified within $\pm 20\%$ of the concentration range with a mean trueness of 97% and trueness range of 85–120%

This demonstrates confidence in accuracy of the sample preparation, analysis, and data processing workflow using Waters solutions.

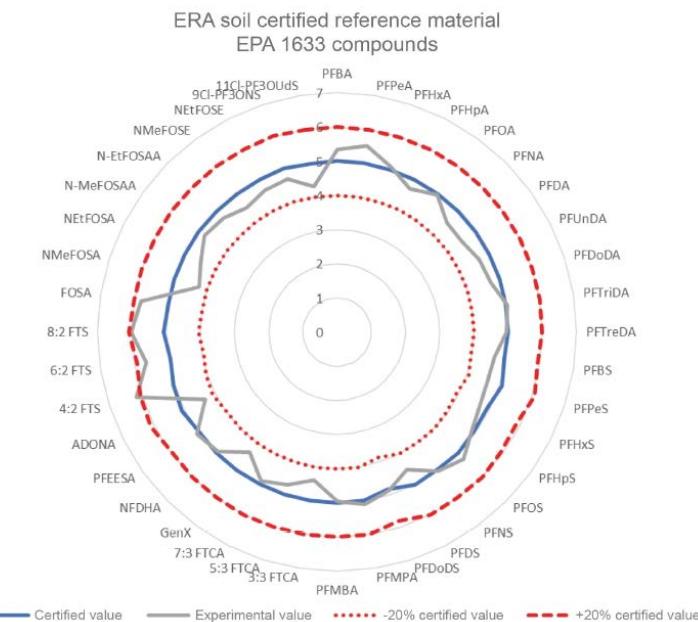


Figure 5. Quantified values of all 40 EPA 1633 target analytes in a custom Waters ERA PFAS in Soil CRM. Red lines represent $\pm 20\%$ of the certified value range of the CRM. The blue line represents the certified value. The solid grey line represents the average experimental quantitated value ($n=3$).

CONCLUSION

Sample preparation and analysis was performed for soil and fish tissue samples using EPA 1633 procedures. Oasis GCB/WAX bilayer SPE cartridges containing both WAX and GCB were utilized for the sample extraction and clean up in place of performing the extraction and clean up in two separate steps with dispersive GCB. This cartridge provides a better user experience and reduces time spent in sample preparation. All recoveries were within the acceptance criteria ranges with the mean EIS recovery of 81% and 85% for soil and fish tissue respectively. Mean RSDs were 2.8% and 9.2% for soil and tissue, respectively. This demonstrates the equivalence of the bilayer dual-phase SPE cartridge as a suitable single step replacement for the multi-step extraction and clean up presented in EPA 1633.

Additionally, a Waters ERA soil reference material processed and analyzed using the same method was easily within the certified reference value range, giving high confidence in

method accuracy. The data presented demonstrates that the Oasis GCB/WAX for PFAS SPE cartridge in combination with the LC-MS/MS system easily fulfills all requirements for analysis of solids and tissues for EPA 1633.

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3. 720008230, February 2024, Waters Corporation.

FUN LAB TIPS

Pipetting tips: Avoid Dripping and Backflow

- **Pre-wet the tip:** Aspirate and dispense the liquid 2–3 times to condition the tip.
- **Use correct immersion depth:** 2–3 mm for small volumes; don't submerge too deep.
- **Hold the pipette vertically while aspirating** to avoid drawing air or overfilling.
- **Keep the pipette at a 10–20° angle** while holding a loaded tip to prevent liquid from flowing back into the shaft (especially important with volatile or viscous samples).
- **Use smooth, consistent thumb control** to avoid pressure surges or air bubbles.
- **Pause briefly after aspiration** before lifting the pipette to prevent drips.
- **Dispense against the side wall** of the receiving vessel to control flow and reduce splashes.
- **Withdraw the tip slowly and steadily** to avoid trailing drops.

Don't Let a Drop Ruin Your Data!



Hipette

Updates

Recent Novel FDA Approved Drugs



1. Sunvozertinib (Zegfrovy)

— Recently greenlit by the FDA on July 2, 2025 for metastatic NSCLC with EGFR exon 20 insertion mutations, following progression after platinum-based chemotherapy.

Why it matters: Targets a rare lung cancer subtype with limited treatment options, offering new hope post-chemotherapy.

2. Linvoseltamab-gcpt (Lynzyfic)

— Received FDA clearance on July 2, 2025 for relapsed or refractory multiple myeloma in adults who have failed four or more lines of therapy.

Why it matters: A novel BCMA×CD3 bispecific antibody that re-engages T cells for hard-to-treat myeloma cases.

3. Datopotamab deruxtecan-dlnk (Datroway)

— Granted FDA marketing authorization on June 23, 2025 for EGFR-mutant NSCLC after prior targeted and platinum-based therapies.

Why it matters: Combines antibody precision with cytotoxic delivery, tackling resistance in advanced lung cancer.

4. Sephience (Sepiapterin)

— Approved by the FDA on July 28, 2025 for the treatment of phenylketonuria (PKU), a rare metabolic disorder.

Why it matters: Provides a much-needed oral option beyond dietary restrictions, improving quality of life for PKU patients.



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