

ENHANCING LABORATORY EFFICIENCY

WATER QUALITY MATTERS

REDEFINING PURE WATER STORAGE FOR LABS- IN CONVERSATION WITH
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From the editor

Dear Readers,

It is with great excitement that I welcome you to the third edition of CATALYSTCue, where we continue our journey of exploring innovations that transform laboratories into hubs of efficiency and precision. This issue, themed **Enhancing Laboratory Efficiency**, brings together a diverse range of applications and methodologies to inspire scientists, researchers, and innovators alike.

In today's era, sustainability in research is crucial in minimizing negative environmental impact.

Our Cover Story sheds light on the Critical Importance of Sustainability in the Laboratory Sector, a BWB's Commitment to a Greener Future.

As part of our in-depth applications, we have incorporated Complement Flow Synthesis with In-Line Purification, a game-changing process enabled by flash chromatography from Advion Interchim. This innovation not only accelerates workflows but also ensures accuracy in compound purification. For those working with liposomes, we present an in-depth study on High-Pressure Homogenization using the GEA PandaPLUS 2000, highlighting its ability to deliver consistent results in lipid-based research.

Furthering the focus on efficiency, we dive into batch ethanol recovery from winterized botanical extracts using Heidolph Hei-Vap Industrial, which streamlines processes in botanical and natural product research. For thermal analysis enthusiasts, Hitachi's Temperature-Modulated DSC with the NEXTA® DSC200 offers an unparalleled level of precision in characterizing materials.

This issue also covers advanced analytical techniques like purity and content uniformity testing of tadalafil using the compact and powerful Nanalysis 60 PRO benchtop NMR, which simplifies pharmaceutical analysis. Additionally, eliminating background signals in chromatography and mass spectrometry becomes possible with the Sartorius Arium® Mini Plus, offering a clean slate for accurate and reliable results.

The applications section concludes with innovations in synthetic chemistry and material testing: The performance verification of Sorbitan Monooleate using Gel Permeation Chromatography (GPC) with RI detection on the Waters Arc™ HPLC System, and the Efficient Dissolution of Be from a Pu/Be mixture in glove box conditions.

In our Features Section, we delve into the nuances of determining moisture, water content, and loss on drying with a comprehensive guide by Mettler Toledo. Sustainability takes the spotlight as Radleys shares a comparative study on greener, more sustainable alternatives to traditional oil baths, a step toward eco-conscious lab practices.

Also, this issue brings you expert perspectives: insightful interviews on the use of CO₂ incubators and ULT freezers, and a candid discussion with Sartorius about their role in advancing laboratory water systems. In our Tech Corner, we present an A-to-Z guide on lab water applications, emphasizing the transformative capabilities of the Sartorius Arium family.

To round it all off, the Product Highlight section features the Implen OD600, a practical tool that simplifies bacterial density measurements, enhancing workflow efficiency in microbiology labs.

As always, CATALYSTCue remains dedicated to delivering insights that resonate with the scientific community. I hope this edition inspires new ideas and solutions for your research and laboratory needs. We value your feedback and look forward to hearing your thoughts on how we can continue to serve and support your goals.

Best regards,

Arun Mathrubootham
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JANUARY 2025



Hozan Edwards

Managing Director

BWB Technologies Ltd



The Critical Importance of Sustainability in the Laboratory Sector: BWB's Commitment to a Greener Future

As the world continues to grapple with pressing environmental challenges, the call for sustainability resonates louder than ever before. In laboratories around the globe, the importance of adopting sustainable practices cannot be overstated. Laboratories, by their very nature, consume large amounts of energy, water, and raw materials, generating significant amounts of waste. Transitioning towards greener practices is not just a regulatory obligation but a moral imperative. At BWB, we are proud to be at the forefront of this movement, taking active strides in reducing our environmental footprint while maintaining the highest standards of precision and efficiency.

Why Sustainability in Laboratories is Critical

The scientific sector plays a crucial role in advancing knowledge, innovation, and medical breakthroughs. However, these accomplishments often come with unintended environmental costs. According to *My Green Lab*, a non-profit organization promoting sustainability in laboratories, the global laboratory sector accounts for a staggering 2% of global plastic waste, while energy consumption in laboratories can be three to five times higher than in office spaces. Moreover, with the increase in research and development, the pressure on natural resources continues to escalate.

Sustainability is not merely about reducing waste or energy consumption—it's about rethinking how we design, operate, and manage laboratories. From sourcing eco-friendly materials to optimizing energy usage, the goal is to minimize the negative environmental impacts while still pushing the boundaries of scientific discovery. Initiatives such as the My Green Lab Certification offer guidelines for creating more sustainable laboratory environments, highlighting areas like responsible resource management, energy-efficient equipment, and environmentally conscious chemical use.

BWB's Sustainable Innovations: Leading by Example

At BWB, we have made sustainability a core pillar of our mission. Understanding the impact of laboratory practices on the environment, we have worked tirelessly to innovate and integrate greener solutions into our products and processes. Here are some of the key ways BWB is contributing to a more sustainable future:

» Energy Efficiency in Instrument Design

Laboratories rely heavily on analytical instruments, many of which consume large amounts of energy. BWB has re-engineered its flame photometers to be more energy-efficient. Our modern designs incorporate state-of-the-art technology that minimizes power consumption without compromising on performance. This not only reduces operational costs but also cuts down on greenhouse gas emissions associated with energy use.

» **Minimizing Waste through Product Lifecycle Management**

Sustainability in laboratories extends beyond energy use; it also involves careful consideration of the lifecycle of products and materials. BWB is committed to reducing waste by designing products with longevity and recyclability in mind. Our devices are built to last, and we offer extensive support to ensure that they remain in operation for as long as possible. When their lifecycle comes to an end, many components can be recycled, thereby reducing the burden on landfills.

» **Sourcing Sustainable Materials**

The materials we choose matter. BWB is taking active steps to source materials responsibly, opting for eco-friendly alternatives where possible. We prioritize suppliers that adhere to sustainable practices and continuously assess our supply chain to ensure we meet the highest environmental standards.

» **Sustainable Practices in Manufacturing and the Workplace**

Beyond our products, BWB is also focused on implementing sustainability initiatives within our own operations and manufacturing processes. In our manufacturing facilities, we've taken significant steps to reduce waste and energy consumption by optimizing production lines and introducing energy-efficient lighting and equipment. Furthermore, we have established robust recycling programs for materials used in both manufacturing and office environments, reducing our overall waste output. We also encourage green commuting options for our employees and have implemented a cycle to work scheme where possible to minimize the carbon footprint associated with travel. These actions are a testament to our belief that sustainability must be embraced at every level of the organization, from product design to daily operations.

» **Empowering Sustainable Lab Practices Globally**

Sustainability is not just an internal effort. We collaborate with partners across the globe, including The SME Climate Hub initiative and GAMBICA's Environmental Impact Programme —a UK-based association representing the instrumentation industry—to drive sustainable practices throughout the sector. Together, we aim to inspire the wider scientific community to embrace eco-conscious practices, not only in the laboratory but in the manufacturing and distribution of laboratory equipment.

The Path Forward: Research, Innovation, and Collaboration

The laboratory sector stands at a pivotal point in the sustainability movement. With the rising awareness of environmental issues, the time to act is now. Future research and innovation will play a critical role in driving the transition to greener laboratories. Organizations like My Green Lab and The SME Climate Hub provide the tools and networks to help laboratories and manufacturers integrate sustainable practices at all levels.

At BWB, we remain dedicated to exploring new frontiers in sustainability. Our research efforts focus on reducing resource usage, enhancing the recyclability of materials, and developing cutting-edge technologies that push the boundaries of energy efficiency. By working closely with the global scientific community and regulatory bodies, we aim to lead the way in setting new standards for sustainability in laboratory environments.

In conclusion, sustainability in the laboratory sector is not a passing trend—it's the future. BWB is proud to be part of this journey, and we invite others in the industry to join us in making meaningful changes that will benefit not only the environment but also future generations of scientists and researchers.



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flame photometer with
built-in compressor and
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to your requirements.**



Moisture content, Water content, Loss on drying

What exactly is meant and how are these quantities determined?

The presence of water in polymers, pharmaceutical substances or foodstuffs is often undesirable. A water content that is too high can reduce the shelf life of rice, make the processability of pharmaceutical products more difficult (for example tabletting), or lead to quality problems with plastic products. The determination of the water content in different materials is therefore very important. Besides "water content", the terms "moisture content" or "loss on drying" are also often used.

In this article, we explain what the terms mean and discuss some of the techniques used to determine these quantities.

Introduction

In everyday English, by "moisture" we usually mean the presence of small amounts of water on the surface of a solid or weakly bound within a solid. Water present in the atmosphere is referred to as humidity. If "moisture content" refers explicitly to water and not to other substances, then the term "water content" should be used. The "water content" is then the quantity of water in the sample mass.

"Moisture content" can however also refer to any other volatile substances (volatiles) including water in materials. In the context of techniques and methods for moisture content determination, the term "loss on drying" (LOD) is synonymous with "moisture content".

As shown in Figure 1, moisture content or water content can be determined by gravimetric, chemical, or physical methods.

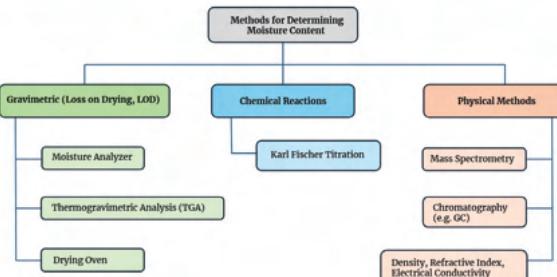


Figure 1. An overview of some of the methods used to determine moisture or water content.

Gravimetric methods measure changes in mass of a sample after or during a defined temperature program using a balance. The total loss of mass is referred to as "loss on drying". Whether this is exclusively water or whether other volatile substances or even decomposition products are released cannot be determined without further analysis. Gravimetrically determined moisture contents depend on the temperature program used and (to a lesser extent) on the relative humidity and pressure in the drying oven. So-called LOD analyses are always carried out using gravimetric methods; these methods always determine the total volatile content.

Chemical methods include methods in which a chemical reaction takes place between water and a reagent. The amount of water converted during the reaction can be determined from the amount of reagent consumed.

The most well-known method based on this principle is the Karl Fischer titration (KFT). The advantage of this method is its selectivity – the Karl Fischer titration is specific for water.

Physical techniques (for example mass spectrometry, chromatography) are more time-consuming and expensive but are also selective and ideal for determining low water contents even in very small samples. Other physical methods are based on the measurement of density, refractive index or electrical conductivity. Herein, we discuss gravimetric methods and the Karl Fischer titration.

Gravimetric methods

In a gravimetric method, a sample is first weighed and then dried using a suitable temperature program until the sample mass reaches a “constant” value. At first sight, this measurement principle appears simple and reliable. However, several fundamental difficulties arise:

The loss of mass can be caused both by the release of water and other volatiles (gravimetric methods are not specific with respect to water). Besides this, the material can also decompose during drying and thereby release decomposition products. Gravimetric methods are therefore only suitable for the measurement of water content if one can assume that decomposition processes do not take place and that other volatiles are not present. Gravimetric methods can therefore only determine the loss on drying (content of moisture and volatiles) and then only when the sample does not decompose during drying.

The drying conditions (temperature, ambient pressure, ambient humidity) influence the drying process. In principle, complete drying of a material takes a long

time. In practice (for example with the halogen moisture analysers, see below) a drying criterion is therefore defined. This is done by setting a maximum value for the mass loss, Δm_{\max} that occurs in a certain time interval, Δt . If the mass loss rate

$\frac{\Delta m}{\Delta t}$ is higher than $\frac{\Delta m_{\max}}{\Delta t}$ after a certain time,

the material is not dry, below this value it is considered to be “dry” (see Figure 1).

Moisture contents or water contents are usually given in percent of the sample mass. The question arises concerning the reference mass: Is the reference mass the mass of the sample before the drying process or the mass of the dried product? In the latter case, one refers to the “dry mass” as the reference mass. But is the sample really “dry” after drying? For practical reasons, the mass of the sample before drying is often used as the reference mass. This assumes that the moisture content of the sample does not change during sample preparation, for example through evaporation or adsorption of water – which would then have consequences for the sample preparation.

The classical method for the determination of the moisture content (i.e. loss on drying) is to use a drying oven. One (or more) samples are dried in an oven at a certain temperature by means of hot (and ideally dry) air (sometimes also under reduced pressure). The moisture content is calculated after drying from the difference between the starting mass and the final mass. The procedure is described in detail in USP 40 <731>¹. The determination of the moisture content using a drying oven takes a long time (typically 2 to 3 hours) and involves a lot of work (the weighing process is done manually). The use of drying ovens is however still the most important reference method for the determination of moisture content or loss on drying.

If halogen moisture analysers are used, the moisture content (i.e. loss on drying) of a material can be determined in 5 to 15 minutes. In a halogen moisture analyser such as the METTLER TOLEDO HX204, the sample is heated using halogen heating technology to

the drying temperature (typically 105 °C). During the heating phase and the following isothermal phase, the mass of the sample is continuously measured and the drying curve of the sample displayed. When the drying criterion defined in the measurement method is reached, the corresponding moisture content is calculated.

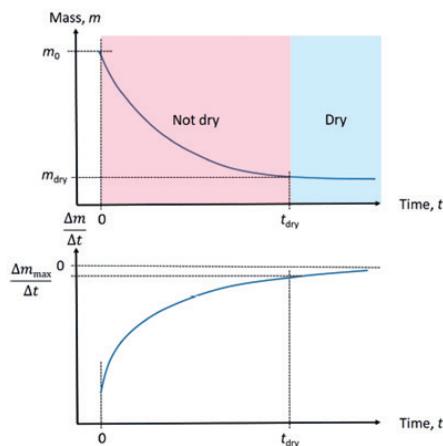


Figure 2. Characterization of drying and the drying criterion by the critical mass loss rate. The drying process shown was performed at constant temperature (isothermally).

In fact, the actual sample temperature can deviate by a few degrees from the drying temperature set. This is due to the different absorption properties of the materials investigated – the samples are heated by the absorption of the radiation emitted from the halogen lamp, which is mainly in the IR region.

The sample is heated with the halogen lamp to the drying temperature and then dried typically for 10 minutes. The sample mass is continuously measured. The moisture content is determined according to the drying criterion set by the user.

Moisture contents can also be measured by thermogravimetric analysis (TGA). In contrast to drying ovens and halogen moisture analysers, TGA measurements can be performed with much smaller samples. Besides this, there is also the possibility of performing measurements under reduced pressure.

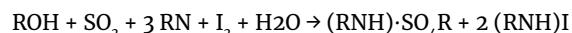
If the TGA is coupled to an evolved gas analysis system

such as a mass spectrometer MS or a Micro GC/MS), water can be distinguished from other volatile substances or from decomposition products. In this case, the TGA method is then specific for water.

Karl Fischer Titration

Titration is an analytical method by which the concentration of a particular substance in a solution (the sample solution) is measured. The substance concerned in the sample solution is titrated in a chemical reaction with a standard solution (the titrant) of known composition.

The volume of the standard solution consumed is measured and the unknown concentration of the substance in the sample solution is calculated based on the stoichiometry of the reaction with the standard solution. Water is detected using the so-called Karl Fischer titration (KFT) according to the reaction equation:



where ROH is an alcohol (usually methanol) and RN a base (usually imidazole)². If the sample contains no other substances besides water that directly or indirectly react with the Karl Fischer reagent, then the Karl Fisher titration can be used as a method to selectively determine water content.

Modern titrators enable the titration to be performed automatically. The course of the titration is monitored by means of a potentiometric electrode whose potential is continuously measured throughout the automated addition of the titrant. In the Karl Fischer titration, the potential depends on the amount of water in the sample: the larger the water content, the higher the potential. During the titration, the water molecules are titrated according to the reaction equation and the measured potential decreases. This is continued to the endpoint of the titration, that is, until no more water is left to titrate. The endpoint has a defined potential (100 mV). As soon as this endpoint is reached the titration process is terminated.

Two different measurement techniques are used to perform the Karl Fischer titration depending on the water content expected in the sample:

For samples with a water content of 100 ppm to 100%, a methanolic solution of iodine is dosed from a burette into the titration cell (volumetric KFT).

For water contents between 1 ppm to 5%, iodine is generated electrochemically in the titration cell (coulometric KFT). The amount of iodine produced and hence the amount of water that has reacted with the iodine can then be determined by measuring the total amount of current used for iodine production (in coulombs).

To successfully carry out a Karl Fischer titration, the water molecules in the KF solution in the titration cell must be freely available. Depending on the sample, this can be achieved by means of a suitable sample preparation technique before the titration. Sample preparation methods frequently used are dissolution at slightly higher temperatures (e.g. the determination of the water content of honey), external extraction (e.g. the determination of surface water in crystalline sugar) or gas phase extraction (heating the sample, see details below).

Detailed information about the Karl Fischer titration can be found in reference 3.

Conclusion

Gravimetric measurements are always LOD methods and yield the total volatile content (including water) of samples. In contrast KFT or physical methods are specific for water and determine the water content of samples. Gravimetric methods tend to be more suitable for materials in which moisture is only weakly bound to the material. In honey for example, water is strongly bound to sugar molecules by hydrogen bonding. As a result, the gravimetric determination of the moisture content takes a long time, whereas the determination of the water content in honey can be performed by KFT in just a few minutes⁴.

The result of a determination of the moisture content (or water content) depends on the technique used and the measurement and evaluation parameters used (temperature program, drying criterion, etc.). Besides this, the release of moisture and decomposition often overlap, which makes the determination of the actual moisture content more difficult. The apparently simple question "what is the moisture content" then becomes anything other than a simple analytical task. This means that uncertainties must be expected that are significantly greater than the standard deviations obtained after several repeat determinations using a particular technique and a particular measurement method.

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METTLER TOLEDO
HX204 Halogen Moisture Analyzer

Heat-On™: The Safer, Faster, and More Efficient Alternative to Oil Baths



Introduction

In chemistry labs, heating solutions in round-bottom flasks has traditionally relied on oil baths and heating mantles. However, due to safety risks, inefficiency, and maintenance costs, these conventional methods are increasingly being replaced by innovative heating solutions. Heat-On™, a specially designed aluminium block system, offers chemists a safer, cleaner, and faster alternative to oil baths while delivering significant energy and cost savings.

The Challenges of Oil Baths and Heating Mantles

Oil baths and heating mantles pose a series of safety and operational concerns:

- » **Safety Risks:** Hot oil spills, oil fires, and water contamination risks pose serious hazards in laboratories.
- » **Maintenance Costs:** Thermal aging, contamination, and oil disposal contribute to recurring expenses.
- » **Operational Inefficiency:** Oil baths tend to overshoot target temperatures and require continuous monitoring.
- » **Cleanliness Issues:** Oil is messy, difficult to clean, and often degrades over time.

Given these challenges, the adoption of Heat-On™ blocks offers a transformative improvement for heating round-bottom flasks in laboratories.

What Makes Heat-On™ Superior?

Heat-On™ blocks are meticulously designed for efficiency, safety, and ease of use. Key features include:

- » **Enhanced Chemical Resistance:** Available with a fluoropolymer coating for superior chemical resistance compared to anodized blocks.
- » **Faster Heating and Cooling:** Heat-On™ blocks have a lower thermal mass, reducing heat-up and cool-down times significantly.
- » **Innovative Well Design:** Proprietary well designs eliminate flask sticking and cracking while maximizing the heated surface area for efficient heat transfer.
- » **Energy Efficiency:** Heat-On™ uses up to 30% less energy than comparable block systems, reducing both costs and environmental impact.
- » **Safe Handling:** Optional detachable safety lifting handles make operation safer without compromising design integrity.

Performance: Heat-On™ vs Traditional Systems Energy Savings

In controlled experiments comparing Heat-On™ with another leading brand (Brand A), Heat-On™ demonstrated up to **30% lower energy consumption**:

- » **500ml Flask:** 7 hours at 100 °C
 - Heat-On™: 0.87 kWh | Brand A: 1.24 kWh
- » **2000ml Flask:** 7 hours at 100 °C
 - Heat-On™: 1.64 kWh | Brand A: 1.84 kWh

Faster Heat-Up Times

In heat-up comparisons:

- » **2000ml Flask (1000ml water, 140 °C):** 7 hours at 100°C
 - Heat-On™: Reached boiling in 53 minutes.
 - Brand A: Failed to boil after 120 minutes.
- » **500ml Flask (200ml water, 130 °C):**
 - Heat-On™: 45 minutes to boil vs. Brand A's 55 minutes (18% faster).
- » **100ml Flask (50ml water, 130 °C):**
 - Heat-On™: 23 minutes to boil vs. Brand A's 35 minutes (34% faster).

Cost Comparison: Heat-On™ vs. Oil Baths

Over a three-year period, Heat-On™ systems demonstrate significant cost savings compared to oil baths:

Flask Size	Heat-On™ Cost (INR)	Oil Bath Cost (3 Years) (INR)	Savings (INR)
500ml	26910	₹ 95,310	₹ 68,400
1 Litre	28710	₹ 1,76,850	₹ 1,17,900
2 Litre	39330	₹ 1,84,950	₹ 1,23,300

Note: Costs include oil purchases and disposal fees.

Sustainability: Reducing Water and Energy Usage

Collaborative experiments with the University of Oxford demonstrate the environmental benefits of replacing oil baths and water-cooled condensers with Heat-On™ blocks and air-cooled condensers (Findenser):

Results of Comparative Analysis

System	Energy Consumption	Water Consumption	Oil Costs/Year (INR)
Oil Bath with Water Condenser	1.12 kWh	757.44 Litres	₹ 16,200
Heat-On™ Block with Findenser	0.61 kWh	0 Litres	₹ 0

Key Findings:

- » **84% Energy Savings:** Heat-On™ consumes significantly less energy.
- » **Water Savings:** Eliminates water consumption entirely.
- » **Zero Oil Costs:** Removes recurring oil replacement expenses.

Payback Periods

Runs Per Year	Water Saved	Cost Savings (INR)	Payback (Full System) (INR)	Payback (Excl. Hotplate) (INR)
4 (Quarterly)	15.1 m³	24790	₹ 1,20,330	59760
12 (Monthly)	45.4 m³	38371	₹ 1,33,710	52960
24 (Twice Monthly)	90.9 m³	58742	₹ 1,30,830	₹ 45,650

Key Advantages of Heat-On™ Systems

Dr. Antonio Martinez-Martinez's studies highlight the practical benefits of Heat-On™ systems:

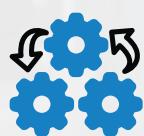
- » **Safety:** No risks of hot oil spills, lab flooding, or hazardous overshooting.
- » **Temperature Control:** Greater stability with no temperature overshoot.
- » **Energy Efficiency:** Reduced heat loss due to superior insulation.
- » **Operational Ease:** Stable flask support and faster cool-down times.

Conclusion

Heat-On™ blocks are redefining laboratory heating practices by offering safer, more efficient, and sustainable alternatives to oil baths and heating mantles. By reducing energy consumption, eliminating oil usage, and improving safety, Heat-On™ systems deliver measurable savings and a safer working environment. With a lifespan of at least 10 years, these systems provide long-term benefits for researchers and laboratory budgets alike.

Heat-On™ – The Safer, Faster, and Smarter Choice for Modern Chemistry Labs.

For more information, visit www.inkarp.co.in or contact sales@inkarp.co.in.



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- Work-up
- Purification
- Analysis and Product transfer to storage



Water Quality Matters

Using high-grade water significantly improves research output. To delve deeper into this critical aspect, CATALYSTCue interviewed a leading expert in water purification systems at Sartorius. In this insightful conversation, we explore the growing demand for high-quality water in Indian research laboratories, the challenges and how Sartorius is addressing these needs.



Mr. Abhijit Ghosh

Head of Sales Lab Products and Solutions, India & SAARC region



Abhijit Ghosh, the Head of Sales for Lab Essentials in India and the SAARC region, has been with the organization for over 25 years and has played a pivotal role in expanding the business in India and SAARC including Bangladesh, Nepal, Sri Lanka, and Pakistan. He is recognized for his expertise in driving change management, tackling complex challenges, and transforming them into sustainable and profitable business models. Abhijit is known for his exceptional communication skills and extensive experience in building high-performance teams, fostering operational excellence and maintaining a strong passion for both the company and its customers.

Scientific Developments

1. What are your perspectives on the water purification systems existing in the research labs, especially in India?

In India the demand for water systems is high due to the various seasons which alters the ground water quality which hinders the customers to get the desired laboratory grade water for research. Lab water systems provide the labs with consistent quality of pure or ultrapure water irrespective of changing the quality of ground water.

2. How has the demand for high quality water evolved in research?

Post-covid, we have observed research scaling up and new research areas have been derived due to the demands in the Life science research for new drug developmental studies etc. Analytical chemistry and Cell culture analysis are the core areas of research in India which demands for High quality water to get accurate results in Research and Development & Production phase.

3. How does Sartorius ensure the highest quality water for sensitive applications?

The main challenge is to remove microbial load which is controlled by our Arium® Patented Bagtank technology. The pure water stored in the closed loop Bagtank ensures quality and requires no cleaning and laborious sanitizations costs. The sensitive applications would require Ultrapure water of TOC to be controlled for high analytical applications, which are achieved by Bagtank technology with no extra costs.

Associating with researchers

4. Can you share success stories/ collaborations that made a significant impact on research outcomes?

Certainly. We recently installed the Arium® Comfort II Lab Water System at Lotus Pharma in Hyderabad, with a project value estimated at €20,000, aimed at upgrading their analytical QC lab. The primary challenge faced by the customer was the high recurring costs of consumables and spare parts from their existing supplier, Merck, which indirectly increased their research overhead. We proposed switching to our Arium® Comfort II Lab Water System, which offers a more affordable maintenance cost and a combined consumables package. This transition has significantly optimized operational costs in the lab, allowing more funds to be allocated to research workflows.

5. There are diverse research applications which utilise ultra-pure water. What are the customisations that Sartorius provides to cater to such needs?

Sartorius Arium® Lab Water System is customized to the application requirement of the customer. The Arium® Ultrapure Water Systems come in different application specific models catering to either Analytical or Biological and also Analytical and Biological combined in a single system. The customizations are application specific based keeping the cost at control.



Scan the QR code to learn more about Arium® Lab Water System and discover how we can support your laboratory water system requirements.

6. How does Sartorius engage with the scientific community to develop knowledge sharing?

Under the leadership of Abhijit Ghosh, Head of Sales LPS India & SAARC, and Sankar Paruk, Manager of Field Account Management LE, Sartorius India actively engages with various scientific communities to foster knowledge sharing through both in-house and customer-centric programs. Our in-house programs are conducted at our state-of-the-art Application Centre at the Bangalore Headquarters. Meanwhile, customer-centric initiatives, such as Lab Water seminars, Arium® Health Checks, and roadshows, are tailored to meet specific customer requests, ensuring a collaborative and responsive approach to knowledge development.

7. From your experience, what parameters should researchers look for while choosing a water purification system for their applications?

The first and foremost parameter is ensuring the quality parameters of the feed water to the water system. The water systems are designed around the input feed water quality. Arium® Lab Water System offers various models to the feed water provided at the facility which can deliver the desired quality of water at economically recurring costs.

Industry Insights

8. As water purification systems continue to evolve, how does Sartorius safeguard its relevance?

Arium® Lab Water System offers the patented Bagtank Technology which safeguards microbial contamination without any chemical cleaning sanitization methods. The system provides water recovery functionality programme as a part of sustainable Go Green practices. The system is also provided with a customized specific Prefilter to ensure an economical reoccurring cost.

Interview

9. How do you foresee the role of water purification systems evolving? What would be your key technological focus areas in future?

The key areas we are working on Arium® Lab Water Systems are :

- Dispense functionality features.
- Data integrity in 21 CFR compliance perspective
- Data Transfer interface platform

10. How does the design of Arium Bagtank contribute to their functionality and durability?

Arium® Patented Bagtank technology safeguards microbial contamination without any chemical cleaning sanitization methods delivering consistent quality of Pure water. The design is a closed loop to protect from any external contamination.

The Bagtanks are highly durable with a transparent design made of EVA material. The water can be dispensed out without any dead leg space which can cause microbial contamination.



Scan the QR code to discover more about the unique feature of **Arium® Bagtank technology** and find the right Bagtank for your laboratory needs.

Arium® Comfort II & Advance EDI with 100L Bagtank installation at Lotus Pharma QC Lab.



Arium® Comfort II & Advance EDI with 100L Bagtank installation at Lotus Pharma QC Lab.

Interview

In the complex and ever fast pacing field of immunology, having the right laboratory equipment is essential for conducting successful research. In a sparkling interview with CATALYSTCue, a researcher from a premier institute in India, talks about how incorporating a -86 °C Ultra Low Temperature Freezer and CO₂ incubator has been an indispensable part of their research. Below is a glimpse of the interview, capturing the core of the discussion.



HAPPY CUSTOMER

being Laboratory
Instrument
Manufacturer

-86 °C ULTRA LOW TEMPERATURE FREEZER



1. Could you briefly describe your area of research?

I work in the field of immunology, with particular interest in immune signaling, vascular drug targets and inflammation.

2. What type of biological samples do you store ? And what is the usual sample size?

We store a variety of biological samples required for our research, like cellular extracts, cell cultures, tissue samples and biological reagents. The sample sizes vary but commonly range from 10 µL to 1000 µL, depending on the experimental requirements.

3. Can you elaborate on how the ULT helps maintain the integrity of your samples?

Most of the samples require constant freezing at very low temperatures and the ULT is therefore crucial in maintaining this. It ensures that the samples remain viable without undergoing any degradation or compromising their quality.

4. Do you follow any specific sample preservation protocols?

Yes, we follow very specific and meticulous protocols when it comes to preserving sample integrity. All samples are stored in high quality tubes and vials which are air sealed. A very crucial step here is to maintain sample viability, and of course, special care is also taken to avoid leaks, spillage and contaminations of any kind. There are also standardized procedures for sample preparation and handling, and we strictly adhere to them.

5. Are there any sample organisation methods you practice ensuring easy sample retrieval without compromising on temperature fluctuations?

Having a well-structured sample organisation method greatly eases sample retrieval. Firstly, we arrange samples into labelled racks that are carefully arranged and allow us easy sample accessibility. These racks are in turn organized according to sample types or experimental categories. The labels on the racks carry clear information such as sample code, date of storage

Interview

etc. These practices reduce the risk of temperature fluctuations.

6. Is there a maintenance routine that you adhere to?

We have regular maintenance checkups to ensure optimal performance of our ULT. These checkups include temperature monitoring, checking for potential leaks, ensuring seals are intact, etc., to make sure we have smooth running of the ULT at the desired temperature. Apart from standard maintenance, we are required to follow biosafety regulations to maintain a sterile environment. We also sterilize the ULT periodically with surface disinfectants that do not interfere with the sample in any way.

CO₂ INCUBATOR

7. How would you handle the risk of temperature fluctuations, given the importance of sample preservation?

We have electrical backup systems so seldom face issues with fluctuations or electricity failures. However in rare cases, under such conditions, we restrain from opening the freezer until the systems are back and running and the freezer regains the set temperature.



1. What kind of cell types/samples do you usually grow in the CO₂ incubator?

Depending on the experimental models, we deal with various types of cell cultures.

2. What are some of the applications that require the use of CO₂ incubator?

My research revolves around how various diseases interact with different drugs, especially immune signaling.

3. Do you perform media changes or culture manipulation inside the incubator?

We do not perform any sort of manipulation inside the incubator. All media changes are performed in a sterile environment, inside a biosafety cabinet typically, and

then placed inside the incubator for growing.

4. In case of multiple sample storage, how do you ensure uniform conditions?

Incubator conditions are standard for all samples we deal with; a temperature of ~35°C and 85–95% humidity, 5 % CO₂ and 21% O₂. These are the standard conditions one would look for in an incubator. So, the multiple cell types we put in the incubator need these conditions to grow.

5. How would you handle contamination risks when placing multiple samples?

First of all, the incubator itself has an inbuilt sterilization program. We have UV sterilization in our

Interview

incubator, which we turn on before placing any cell culture in it. Apart from UV sterilization, we also use water mixed with a disinfectant to maintain a sterile environment. These disinfectants are chosen carefully to avoid harming the cells and equipment. The concentration of the disinfectants after vaporization is so minuscule, so they are non-threatening. Additionally, because we work with animal cell cultures, we are required to adhere to strict sterility codes.

6. How frequently do you check the condition of samples during incubation?

It is absolutely necessary to check the condition of samples, we check them every day without fail.

7. How do you ensure long-term viability of samples inside the incubator?

There are criteria to keep the cells viable. Long-term viability of the cells inside the incubator requires maintaining a highly controlled environment that mimics the physiological conditions the cells would experience in their natural conditions. Monitoring temperature, CO₂ and O₂ levels, humidity, and proper feeding are critical factors. The incubator has inbuilt sensors for monitoring these conditions. And of course,

proper sterilization, preventing contamination and minimizing disturbances—such as frequently opening the door—are some essential practices to preserve the viability of the samples.



8. Do you think automated monitoring and tracking of samples would increase your efficiency?

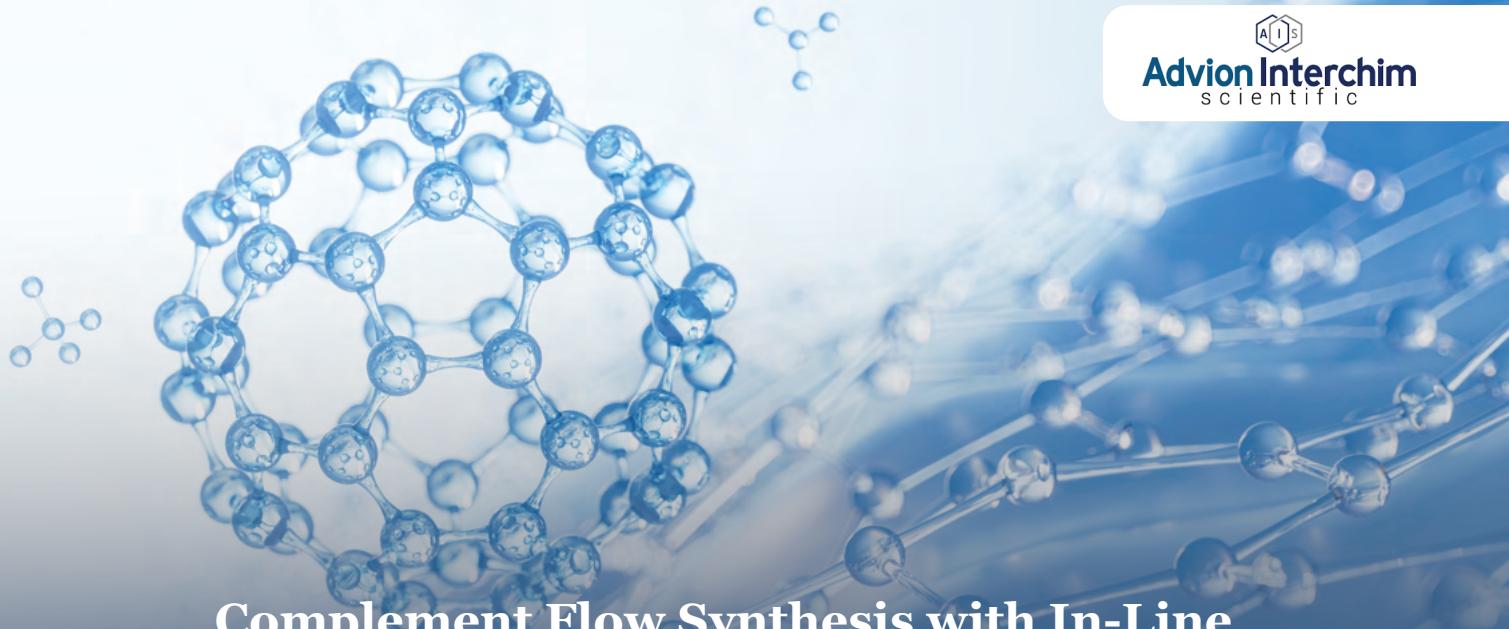
Automated monitoring will definitely increase efficiency in the lab. In fact, there are large-scale automated incubators that are becoming increasingly common in research settings.

9. Do you keep track of incubation conditions to ensure repeatability and quality control?

We meticulously record all the data related to incubation conditions. Every step, from temperature setting to the exact volume of media added, is thoroughly documented in our lab manual and protocol book. This detailing of data allows us to maintain experimental consistency, ensuring repeatability and quality control.

**Your satisfaction
is our top priority**





Complement Flow Synthesis with In-Line Purification — Using Flash Chromatography

A smooth approach for continuous isolation of flow synthesis products with the Advion Interchim Scientific puriFlash® 5.250 prep LC System

A key problem in flow chemistry is effectively isolating the desired products from residual starting materials, catalysts and by-products. This complicates the purity and yield of the desired product. Isolation methods involve manual intervention, at large, requiring multiple steps. This process is tedious and quite unproductive. Recently, advancements in preparative liquid chromatography have significantly improved the isolation of flow synthesis products. This application note describes a novel method to perform the continuous isolation of flow synthesis products from residual starting materials, catalysts or by-products to expedite chemical discovery. A promising new approach is highlighted here, featuring the Advion Interchim Scientific puriFlash® 5.250 preparative LC system.

The first results of our cooperation with the VilelaLAB and Continuum Flow Lab at Heriot-Watt University, Edinburgh, on this topic are outlined here.

Keywords: Isolation, Flow Synthesis, Preparative LC, Liquid Chromatography, Flash Chromatography, Advion, puriFlash® 5.250

Introduction

The continuous isolation of flow synthesis products from residual starting materials, catalysts or by-products is desirable to expedite chemical discovery. A promising new approach to achieve this goal is to distribute the effluent from an optimized flow reactor setup into two alternating flash chromatography paths, perform the purification in a staggered and continuous manner, automatically collecting the desired fractions.

Interchim's flash/prep LC chromatography system puriFlash® 5.250 is ideally suited for this purpose.

Application Showcase

Method

Instrumental Set-up

A flow reactor system is used to perform synthesis and deliver the crude reaction mixture directly or via in-line extractors to the injection port of Advion Interchim Scientific's puriFlash® 5.250 chromatograph. The latter contains a 10-port valve that directs the flow of crude material alternately into one of two sample loops.

A repeating sequence, in which one of two identical flash columns is loaded with the reaction mixture while a chromatography run plus re-equilibration is performed on the other column, ensures that the contents of the sample loops are processed without substance loss. The separation is monitored using UV + ELSD detection and the fractions of interest are collected accordingly. (Figure 1)

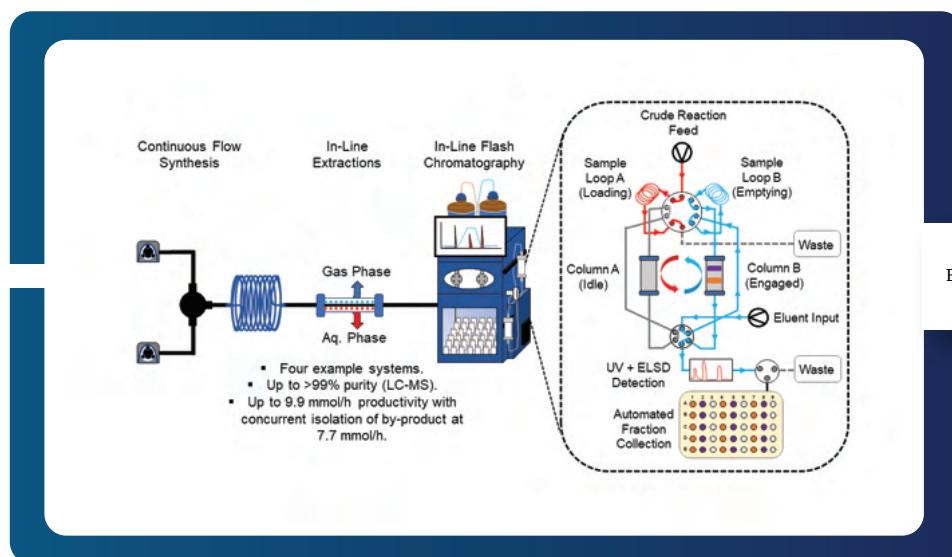
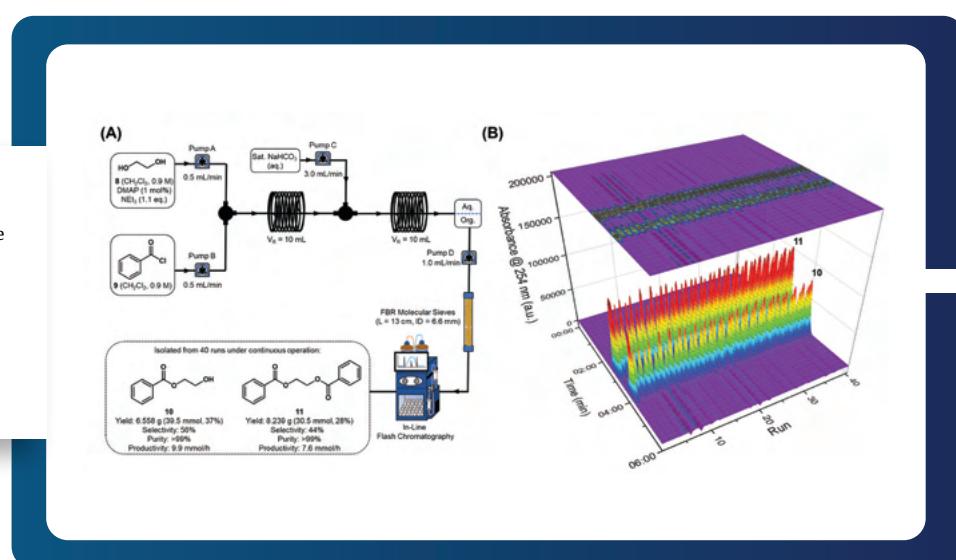


Figure 2: (A) Schematic flow diagram for the continuous flow esterification of ethylene glycol and benzoyl chloride with in-line normal-phase flash chromatography.

DMAP: 4-(Dimethylamino) pyridine, FBR: Fixed Bed Reactor. (B) Stacked plot of 40 processed chromatograms from the continuous isolation of the ester products.



Application Showcase

Principle of Method Development

The following systematic approach to achieve continuous purification is followed:

- » Optimization of flow reactor setup to obtain the maximum yield of products.
- » Minimizing the time required for flash chromatography-based purification of the crude reaction mixture in offline experiments.
- » Synchronizing the duration of the chromatography method with a suitable feed flow rate from the flow reactor to achieve a successful coupling.

Results

A proof-of-principle study using a test mixture (4-methoxyphenol and 2,5-dibromo-p-xylene in n-hexane/ethyl acetate) was successfully performed, proving the feasibility of flow chemistry - puriFlash® 5.250 integration and confirming the durability of Advion Interchim Scientific's flash column cartridges.

Encouraged by the success of the methodology, three different flow chemistry syntheses were successfully complemented with in-line normal or reversed-phase flash chromatography, resulting in the continuous isolation of products with 97–99% purity. It was realized that the flash chromatograph could also serve important secondary functions:

- » The possibility of cost-saving inline recovery of homogeneous (photo)catalysts was demonstrated using the example of the continuous flow oxidation of Fmoc-L-methionine with air to the corresponding sulfoxide via 4,7-diphenyl-2,1,3-benzothiadiazole as a homogeneous photosensitizer.
- » In-line process monitoring to ensure process consistency of a flow chemistry system could be conveniently performed using onboard flash chromatography. As an example, the quality control of continuous heterogeneous catalytic systems was

realized by inline reversed-phase flash chromatography for a flow coupling of thiophenol to diphenyl disulfide via heterogeneous photo-redox catalysis by TiO₂ nanoparticles.

- » The potential of the flow-integrated puriFlash® 5.250 to simultaneously isolate two products in grams per hour with >99% purity from a crude flow chemistry reaction mixture with polyfunctional substrates is described. In the continuous flow esterification of ethylene glycol and benzoyl chloride, productivity values of 9.9 and 7.6 mmol/h were achieved for the two desired esters.

Conclusion

Integrating our off-the-shelf flash chromatography system into your custom flow chemistry has no technological barrier to entry. The coupling easily adapts to the needs of the chemist and will help to intensify and accelerate chemical discovery. Automating routine flow synthesis of key intermediates required for compound library generation will particularly profit.

Reference

1. Thomson, C.; Banks, C.; Allen, M. G.; Barker, G.; Coxon, C. R.; Lee, A.-L.; Vilela, F. Expanding the Tool Kit of Automated Flow Synthesis: Development of In-Line Flash Chromatography Purification. *Journal of Organic Chemistry* 2021, 86 (20), 14079–14094. DOI: 10.1021/acs.joc.1c01151.





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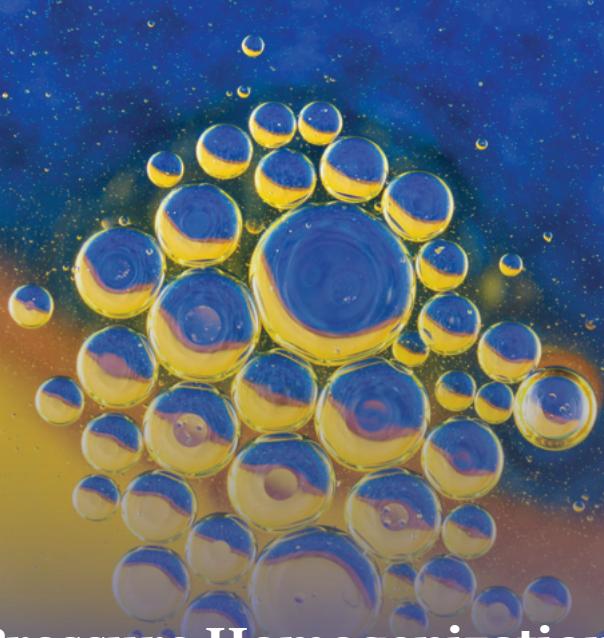


Applications:

- Degreasing Machine Parts
- Degassing Solvents, Media for Cell Culture, Coatings and Films, Etc
- Instrument Sterilization

Technical Specifications:

Model	Volume (L)	Power (W)	Frequency (KHz)	Heating Range (°C)
INK-140DM-4.3L	4.3	140	40/80	Amb-100
INK-180DM-6.5L	6.5	180	40/80	Amb-100
INK-240DM-10L	10	240	40/80	Amb-100
INK-600DM-22L	22	600	40/80	Amb-100



High-Pressure Homogenization of Liposomes

A Study Using the GEA PandaPLUS 2000

Homogenization plays a critical role in numerous industries. By reducing the particle size and creating a more uniform distribution, the efficiency of particles multiplies. In pharmaceutical industry, homogenization facilitates drug delivery. The reduction in particle size aids in increased bioavailability of the drug.

This study demonstrates the effective use of a GEA PandaPLUS 2000 homogenizer for reducing the particle size and providing uniform distribution of liposomes.

Keywords or phrases : Homogenization, particle size, uniform distribution, liposome, GEA PandaPLUS 2000, homogenizer.

Introduction

Liposomes have wide applications in drug delivery, cosmetics and other biomedical applications. Achieving a uniform and consistent liposome distribution is necessary for their efficiency.

Structure

Liposomes are closed, spherical shells containing aqueous solution; the shell is made by one or more lipid bilayers, and a lipid is any type of molecule soluble in no polar, organic solvents such as chloroform and ether. The type of lipid associated with liposomes is phospholipid.

The phospholipid molecule has a water-soluble end and an oil-soluble end, and an example of a phospholipid is lecithin in egg yolk.

Application Showcase

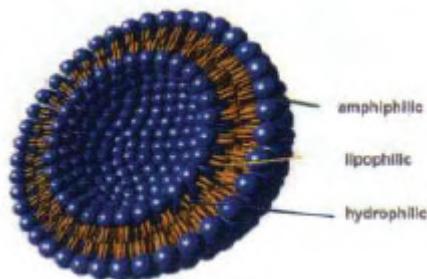


Figure 1: Structure of a liposome

The double layer is composed of two monolayers of phospholipid with the non-polar (oil-soluble) molecular ends facing inward and with the polar ends of both monolayers facing out. The liposome vesicle can be made up of a single bilayer or multiple bilayers. They are described with respect to size and number of layers (lamella):

- a) 0.02 to 0.05 μm - small unilamellar vesicles
- b) 0.10 to 1.00 μm - large unilamellar vesicles
- c) 0.10 to 5.00 μm - multilamellar vesicles

The importance of liposomes lies in the fact that they can be used as carriers for drugs, enzymes and other biologically active molecules. These active ingredients are encapsulated in the liposome and are transported by these vesicles to different organs in the body.

Liposome vesicles can be prepared with different methods:

- » Homogenisation
- » French press
- » Sonication by probe or cleaning bath
- » Detergent dilution and dialysis
- » Injection of ethanol solution into an aqueous solution

Even though liposomes are expensive to prepare, usually because of the high cost of ingredients and the small sample size (2 ml), their potential importance and usefulness have led researchers into the development of larger batches.

The Homogenizing Process

Product: Liposomes Emulsion

Composition: Water, Sodium Salicylate, Phospholipids

Application: Cosmetic and Pharmaceutical

Advantages of Homogenization: Before the treatment, the product was separated in two phases- fat and water. After the high-pressure homogenization, it was possible to obtain a stable emulsion, reducing the mean size of the fat droplets.

Homogenizer: PandaPLUS 2000

nºStage: 1

Valve 1st: "R"

Suggested Pressure: 1000 bar X2 pass

Suggested Temperature: 65–70 °C

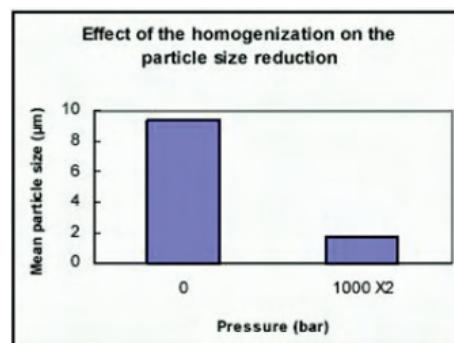


Figure 2. Effect of homogenization on particle size reduction.

In order to prepare liposomes of smaller diameter (about 110 nm), the suitable homogenising pressure is very high, about 1000–1500 bar and normally needs multiple passes (about 3) to obtain a narrow size distribution.

The results obtained can be checked utilizing different analysis technologies : electron microscopy, light scattering and dynamic light scattering such as performed with a Coulter analyser.

The homogenizer used for the treatment is a machine designed for 1200 bar for

Application Showcase

- » Very high pressure (VHP)
- » High abrasion
- » Pharma execution

Conclusion

This study demonstrates the successful utilisation of the GEA PandaPLUS 2000 high-pressure homogenizer for the efficient and controlled reduction of liposome size. By systematically varying processing parameters, such as pressure, number of passes, and temperature, a significant decrease in liposome diameter and narrowing of liposome diameter were observed.

The Niro Soavi homogenisers suitable for very small production, can be used successfully for bigger scale preparation of liposome vesicles larger batches.

Ensuring Sterile Homogenization

Maintaining sterility is imperative to ensure the aseptic processing of materials. For this purpose, the homogenizers can be integrated with Steam Jet Sterilizers.

Unlike traditional methods that require removing parts and placing them in an autoclave, Steam Jet Sterilizers utilize high-pressure jet steam to sterilize the entire homogenizer in place.

Because of this major advantage, they are termed as SIP – Sterilization in Place.

In-place sterilization is a process that sterilizes production equipment without the need to disassemble it first, saving time.

This is typically achieved using superheated steam. Sterilization in Place systems is employed in environments where microbial contamination control is critical.

One of the main applications of SJS technology is the sterilization of homogenizers.

The optimum conditions are 121 °C, 2-3 bar for 20 mins. (conditions vary by application).

By incorporating a steam jet sterilizer into the homogenization process, it is possible to achieve both particle size reduction and complete sterility, whilst ensuring the safety of the final product.



GEA PandaPLUS 2000 in conjunction with Inkarp Steam Jet Sterilizer





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Batch ethanol recovery from winterized botanical extract with AUTOaccurate

Increased Efficiency in Solvent Recovery using the Heidolph Hei-VAP Industrial Package

Ethanol is one of the most preferred solvents for extraction and winterization processes. The main reasons are the low toxicity, and the easy handling compared to other methods and solvents. Since it is, mostly due to taxes, one of the more expensive solvents, recycling and re-use of the ethanol is very important to minimize production costs and do have a positive effect on the environmental footprint of companies. Many manufacturers also do not want to deal with finding the perfect vacuum settings and therefore prefer automatisms that guarantee a high-performance distillation process for solvent recovery. This whitepaper shows how the ethanol of a batch of winterized botanical extract was effectively recovered with Heidolph's automatic boiling point detection AUTOaccurate.

Keywords: Solvent recovery, ethanol, automatic, distillation, Hei-VAP, industrial rotary, AUTOaccurate.

Introduction

Ethanol that has been recovered from a winterized batch of botanical extract can be re-used for the extraction and winterization processes for several times. Since batch evaporation is a dynamic process, a performant system requires a frequent adjustment of the vacuum values during the separation, to ensure a highly productive workflow and a high-quality result with no residual ethanol present in the botanical extract. For unexperienced users it can be a challenge to find and to maintain the right parameter settings of their evaporator that on the one hand guarantee for a good separation and on the other hand work in an efficient way.

New options due to automated process control

Many manufacturers try to meet this challenge by hiring one or more person just to have an eye on the evaporator

Application Showcase

and to adjust the vacuum values when necessary. Those technicians often are not experienced in working with evaporators and run the machine with low efficiency, decreasing the daily output remarkably.

With the Hei-VAP Industrial packages, Heidolph offers a system solution that can increase efficiency, costs and product quality. With the AUTOaccurate function, the perfect vacuum setting is found, held and adjusted to the changes during the whole evaporation process. It omits the necessity to constantly check, if the process runs performant, because the AUTOaccurate sensor ensures optimal efficiency of the condenser's capacity. After the evaporation of the solvent, the vacuum is further lowered to a preset final pressure, to ensure that all solvent has been recovered. After the process, the evaporator automatically goes into a safe condition by switching off and lowering the heating bath, stopping the rotation and aerating the system.

Method: Successful batch recovery of ethanol from winterized botanical extract using AUTOaccurate

The Heidolph Hei-VAP Industrial package was used to recover the ethanol from a batch of winterized botanical extract using the AUTOaccurate function. Therefore, the recirculating chiller was set to 5 °C, the heating bath was pre-heated to 60 °C and the rotation speed set to 130 rpm. In the AUTOaccurate settings a Delta T of 5 °C was chosen and a final pressure of 30 mbar. To facilitate the filling of the evaporation flask, and omit the risk of spilling, the replenishment valve was used instead of detaching the flask and fill it manually. With a vacuum setting of 200 mbar in the Set Pressure mode a batch size of 8 l was introduced into the evaporation flask via opening the replenishment valve. After changing to the AUTOaccurate mode, the process was started. From there on the AUTOaccurate function takes over the regulation of the vacuum setting for optimal conditions until the final pressure is reached. The result is a clear and colourless ethanol distillate, that is ready to be reused for extraction and winterization and a clear, golden coloured botanical extract with no solvent residue.

Conclusion:

Heidolph meets the challenge

This example shows that Heidolph products optimize and facilitate batch ethanol recovery from winterized botanical extracts for the re-use in extraction and winterization. In comparison to manual evaporation working with AUTOaccurate ensures that the process runs performant and with optimal conditions, saving time and resulting in a high-quality product with no residual solvent.



Highlighting Cutting-Edge Measurements in Thermal Analysis

Introducing the Temperature Modulated DSC with Hitachi's NEXTA® DSC200

Temperature modulated differential scanning calorimetry (TM-DSC) is a powerful thermal analysis technique that enables the study of materials' thermal degradation with high precision. TM-DSC can separate and quantify complex thermal transitions such as heat capacity, glass transitions, crystallisation, melting etc., for allowing a deeper understanding of material properties. This application note outlines various measurements examples of TM-DSC using the Hitachi NEXTA® DSC200.

Keywords: Temperature, modulated, DSC, heat, flow, transitions, Hitachi, NEXTA DSC200.

Introduction

The conventional DSC method, we measure heat flow as well as temperature, such as glass transition, crystallization, or melting, by detecting differences in heat flow to a sample and reference material by heating or cooling at a constant rate. AC calorimetry, a technique that measures the specific heat capacity of a sample by measuring the oscillated temperature of that sample while periodically-heated it, has been used extensively as a method for measuring specific heat capacity. Temperature Modulated DSC (TM-DSC) is a technique that combines periodic temperature control performed by AC calorimetry and constant rate temperature control performed by standard DSC measurement. As with AC calorimetry, this method can simultaneously obtain heat capacity component (reversing heat flow) data that corresponds to the specific heat capacity, besides information obtained from conventional DSC measurements, while heating a sample at a constant

Application Showcase

rate by repeating sinusoidal temperature control. The data equivalent to DSC curve provided from conventional DSC measurement is called the Total Heat Flow. Data of components not involved in specific heat capacity can be obtained by subtracting the Heat Capacity Component from the Total Heat Flow. This component data is called the Kinetic Component (non-reversing heat flow). Here, we will introduce principles of TM-DSC and applications using this technique.

Temperature Modulated DSC

1.1 Principle

DSC measures temperature differences in the heat flow path to the sample and reference material by constant rate heating and detects differences in heat flow (ΔQ) that flows to both the sample and reference material. TM-DSC heats samples by a temperature program that adds sinusoidal temperature control to linearly heating at constant rate. Sample temperature is raised on average at a constant rate by repeated heating and cooling of the temperature in short time period (Figure 1).

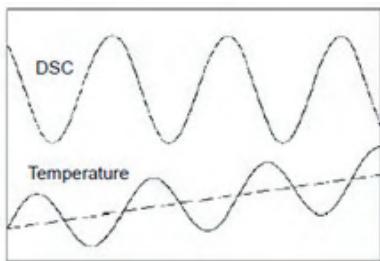


Figure 1: Temperature curve and DSC signal for TM-DSC

Generally, the DSC signal can be expressed by the following equation.

$$dQ/dt = -C_p \cdot (dT/dt) + K(T, t) \dots (1)$$

dQ/dt : DSC Total Heat Flow

dT/dt : Heating rate

C_p : Specific Heat Capacity of Sample

t : Time

T : Temperature

$K(T, t)$: Heat flow change with behaviour

Equation (1) shows that the Total Heat Flow is composed of two different components, heat capacity dependent component and other phenomenon (kinetic) dependent component.

Heat flow changes that accompany the reaction mostly depend on temperature and assume that the time dependence is small. Terms of heat flow change that go with this phenomenon can be expressed as:

$$K(T, t) = K(T) \dots (2)$$

If the sign of time is reversed here, the (1) equation becomes:

$$dQ/dt = -C_p \cdot (dT/dt) - K(T) \dots (3)$$

When comparing equation (3) and (1), terms that depend on heat capacity have the same symbol; whereas terms that depend on the phenomenon see the signs change. Thus, the first term on the right-hand of equation (3) expresses a reversing component for the heating and cooling from the time-reversal invariance, and the second term expresses a non-reversing component.

Measurement example by TM-DSC is shown in Figure 2. The DSC curve in raw data is oscillated shape as shown in Figure 2a. This raw data is separated into each component data by the following procedure.

First, the Total Heat Flow in Figure 2b can be obtained by performing contour integration on raw data (Figure 2a). This data is equivalent to the DSC curve obtained by the conventional DSC measurement at constant rate heating (Figure 2e).

Next, the heat capacity component of Figure 2c can be obtained by performing amplitude ratio calculations of the DSC signal and temperature signal using the Fourier transform same as AC calorimetry.

Application Showcase

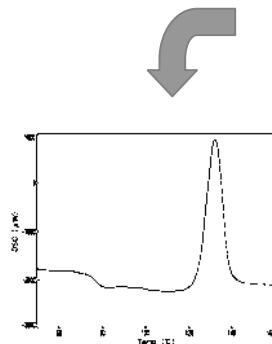
Heat capacity component

$$\begin{aligned}
 &= -B \cdot |Cp^*(\omega)| \\
 &= \frac{-B}{\omega} \cdot \left| \frac{\frac{dQ}{dt}(\omega)}{\tilde{T}(\omega)} \right| \quad \dots \dots \quad (4)
 \end{aligned}$$

Next, a kinetic component other than the heat capacity component in Figure 2d can be obtained by subtracting heat capacity component (Figure 2c) data from the Total Heat Flow (Figure 2b).

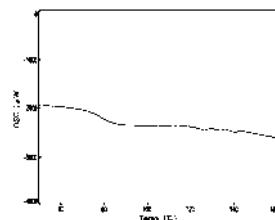
Thus, by TM-DSC measurements, the heat capacity component and kinetic component can be obtained by a single measurement, in addition to Total Heat Flow equivalent to the DSC curve from conventional measurements at constant rate heating.

Contour integration

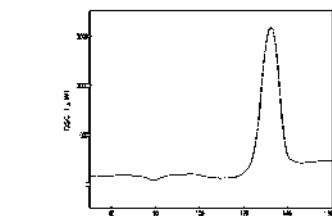


b. Total Heat Flow data

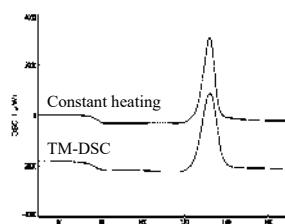
Fourier transform



c. Heat Capacity Component data



d. Kinetic Component (d.=b. -c.)



e. DSC curves for constant rate heating and TM-DSC

Figure 2: DSC data of Temperature Modulated measurement

Application Showcase

1.2 Features

Because the glass transition phenomenon changes the specific heat capacity of a material before and after, the DSC signal that corresponds to that change appears in the heat capacity component. On the other hand, phenomenon (irreversible phenomenon) observed as peaks, which include enthalpy change such as thermal curing reaction, cold crystallization, vaporization, or volatilization appear in the kinetic component (Table 1). TM-DSC can retrieve by a single measurement data not obtained by conventional methods separated into a heat capacity component and kinetic component. These can be separated when the glass transition and other irreversible phenomenon overlap. Also, when two or more measurements were required in the past because of reasons such as water evaporation or elimination of thermal history, measurement efficiency has improved because data can be obtained with a single measurement.

Specific Heat Component	Kinetic Component
Glass Transition	Evaporation Crystallization Curing Decomposition, etc.

Table 1: Classification of DSC Measurement Phenomena

A summary of the advantages of TM-DSC are listed below-

- » Improved separation of proximities or overlapped transitions
- » Improved detection of small transitions and secondary transitions
- » Separation into a heat capacity component and kinetic component
- » Specific heat capacity measurement (In particular, specific heat capacity measurement under the low heating rate.)

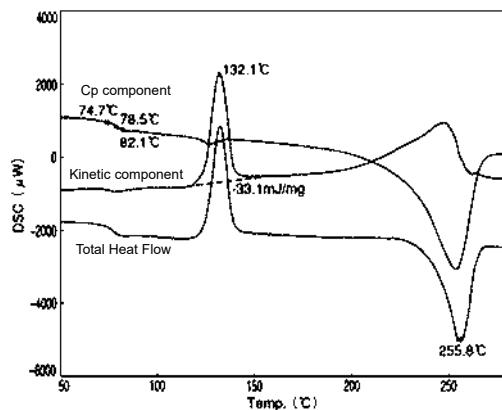


Figure 3: TM-DSC measurement of amorphous PET

Temperature Modulated DSC

2.1 Amorphous polyethylene terephthalate

Figure 3 shows a measurement example of a polyethylene terephthalate (PET) of amorphous by TM-DSC. Shifting due to glass transition at 75 °C to 82 °C, exothermic peak from cold crystallization at 132 °C, and endothermic peak from melting at 256 °C are observed in the Total Heat Flow. Glass transition and melting are observed in the heat capacity component; and cold crystallization and melting are observed in the kinetic component. Accordingly, phenomena measured by DSC are divided into a heat capacity component and kinetic component. Melting phenomena are shown to contain both components.

2.2 Glass transition with enthalpy relaxation

Figure 4 shows the measurement result of an epoxy resin (cured sample) as an example of glass transition with enthalpy relaxation using TM-DSC. Polymer glass transition may be included with the endothermic peak because of enthalpy relaxation due to the thermal history of the sample. The endothermic peak in the example in Figure 4 is large, resulting in the possibility of it being mistaken for a melting peak. The existence of the endothermic peak makes finding an accurate glass transition temperature difficult. Nonetheless, shifting of the DSC curve by glass transition and endothermic peak by enthalpy

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relaxation, as seen in Figure 4, are separate in TM-DSC measurements; and therefore, shifting by glass transition can be obtain clearly. Further, because the quantity of heat can be found from the area of the enthalpy relaxation peak separated as the kinetic component, the size of the relaxation phenomenon can be estimated.

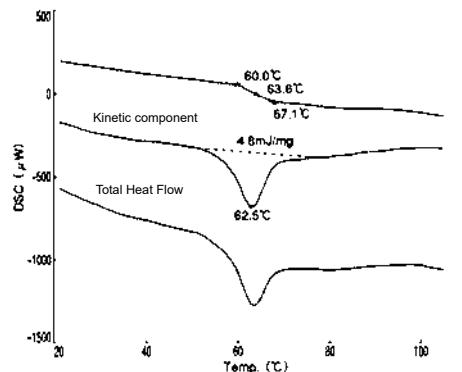


Figure 4: TM-DSC measurement of glass transition with enthalpy relaxation

2.3 Curing reaction of Thermoset polymer

Figure 5 shows the measurement result of an epoxy resin (uncured sample), as an example of curing reaction of a thermoset resin. With Total Heat Flow, glass transition with enthalpy relaxation appears around 70 °C after which immediately the exothermic peak from thermal curing reaction across a wide temperature range near 180°C is observed. The curing peak appears only in the kinetic component when separated into each component, because the thermal curing reaction of the thermoset polymer is enthalpy change. This makes possible accurate reading of the end temperature of the glass transition and the start temperature of curing reaction.

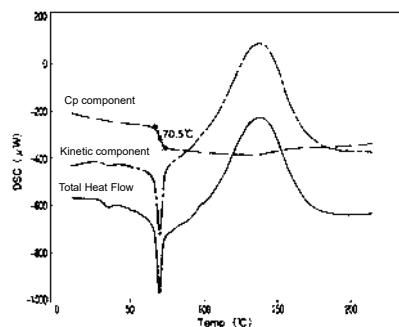


Figure 5: TM-DSC measurement of epoxy resin (uncured sample)

2.4 Immiscible polymer blend

Figure 6 shows measurement result of a PET and PC (polycarbonate) polymer blend as an example of measuring an immiscible polymer blend. Around 110 to 130 °C of Total Heat Flow, cold crystallization of PET and PC glass transition overlap. In such cases, accurate analysis of both PET cold crystallization and PC glass transition is difficult. However, separating data into each component shows the glass transition of PC into the heat capacity component and PET cold crystallization into the kinetic component. Thus, it is possible to be read the glass transition of PC at 114 to 123 °C.

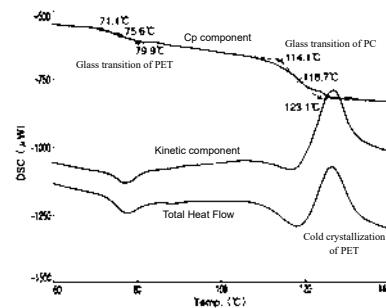


Figure 6: TM-DSC measurement of PET and PC polymer blend

2.5 Vaporization with volatilization system

Figure 7 shows measurement results of nylon fibre (nylon 6) as an example of measuring phenomenon with vaporization. Nylon easily absorbs moisture because the Amide Group, which is a Hydrophilic Function Group, is included in the molecule. During heating up nylon, vaporization occurs in the measurement. An endothermic peak from vaporization over a wide temperature range is observed by the Total Heat Flow in Figure 7. Since the glass transition of nylon overlaps this endothermic peak, the glass transition from this data cannot be observed.

However, the glass transition can be clearly observed in the separated Heat Capacity Component from around 0 to 40 °C. Vaporization is a kinetic phenomenon; therefore, the endothermic peak from this does not appear in the Heat Capacity Component.

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Thus, measurement of the glass transition of sample that includes water, or solvent can be measured by TM-DSC.

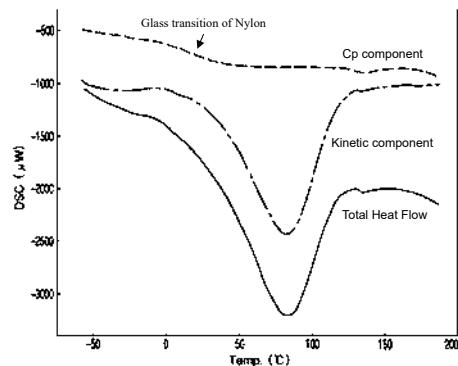


Figure 7: TM-DSC measurement of nylon fibre

2.6 Crystal Polymorphism

Due to the existence of a metastable phase of crystals in pharmaceuticals, there are many materials in which crystal polymorphism is observed by DSC. Figure 8 shows a measurement example of a pharmaceutical having crystal polymorphism. A broad endothermic peak from 40 to 80 °C is observed in Total Heat Flow. This peak cannot be observed in the Heat Capacity component; therefore, from this, vaporization of trace moisture or volatilization of residual solvent is estimated. Further, endothermic and exothermic peaks at around 130 °C and endothermic peak at 167 °C, are observed. Endothermic and exothermic action near 130 °C express the fusion of the metastable phase continuing transitions to stable crystals, and the endothermic peak at 167 °C is considered as a fusion of stable crystals. The endothermic peak is observed, and exothermic peak is not observed by the Heat Capacity component; therefore, the endothermic phenomenon that can be seen in the Total Heat Flow is melting, and the exothermic phenomenon is thought to capture crystallization, which corroborates investigations mentioned earlier.

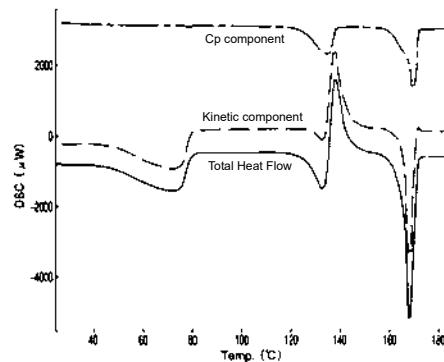


Figure 8: TM-DSC measurement of crystal polymorphism

Conclusion

Temperature modulated DSC offers significant advantages over conventional method as it provides detailed information by splitting the thermal data into heat capacity and kinetic components which has been shown to improve the analysis of parameters such as glass transition, crystallization, melting amongst others. Furthermore, TM-DSC differentiates between processes which are reversible and non-reversible, which in turn enables the understanding of the material's properties and behaviour. The application of TM-DSC in the analysis of polymers, pharmaceuticals and blends shows that this technology is powerful and can be employed to characterize materials efficiently and accurately.

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Assay of Tadalafil in formulated tablets via benchtop qNMR

Purity and content uniformity testing of tadalafil using Nanalysis 60 PRO benchtop NMR

A decade of developments has culminated in compact, cost-effective, and user-friendly benchtop NMR spectrometers as viable alternatives to conventional NMR spectrometers. These spectrometers have gained immense popularity due to their ease of use, compactness, and operating on a permanent magnet instead of cryogenic magnets unlike in their conventional counterparts.

Because of these advantages, these compact spectrometers have wide applications in the field of pharmaceutical analysis. Quantitative nature of NMR has been explored in many extensive ways. The pharmaceutical industry exploits this technique for various purposes such as for drugs, vaccines, natural products, excipients, peptides, etc. Here, the potential of Nanalysis 60PRO benchtop NMR has been explored as an analytical tool for the precise quantification of Tadalafil, critical selective phosphodiesterase-5 inhibitor.

Keywords: Benchtop NMR, Nanalysis 60PRO, quantitative NMR (qNMR), tadalafil, purity.

Introduction

Nuclear Magnetic Resonance (NMR) is an extremely powerful characterization technique that is frequently used for pharmaceutical research to identify new compounds, assess purity and characterise and optimize chemical reactions, either online (with a flow cell), off-line or at-line (for NMR tube reactions or aliquot analysis). Although less common, it has also been established as a useful technique in pharma for routine quantitative analysis.¹⁻⁴ Benchtop NMR has emerged as an accessible technology to extend the use of NMR Spectroscopy to start-ups and SME pharmaceutical

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companies, as well as provide an easy-to-use, compact and automatable alternative to allow qNMR to be incorporated directly in quality control labs and used by technicians.

In this context, this application describes the use of benchtop NMR for structure confirmation of an active drug ingredient; evaluation of degradation products and/or impurities, towards assessing the efficacy and performance of this technique for assay and content uniformity testing.

NMR has many advantages over commonly used methods, such as titration, infrared (IR) spectroscopy, Raman spectroscopy, and chromatography. It is chemically specific, non-targeted, non-destructive, inherently qualitative without calibration and requires very simple sample preparation. It does not require the preparation of dilution series, and does not consume large amounts of solvent, nor does it require an authentic standard substance⁵ for analysis.

This application note describes the evaluation of the purity of tadalafil bought as a reference standard (Figure 1A) using ¹H NMR and the assay test using two commercially available tadalafil-containing drugs. These drugs are sold under the brand name Cialis and generic name Apo-Tadalafil. These drugs are used to treat benign prostatic hyperplasia,⁶ erectile dysfunction,⁷ and treat pulmonary arterial hypertension⁸. Dimethyl terephthalate (Figure 1B) was used as the internal calibrant in this quantitative NMR analysis. It was chosen because at least one of its ¹H resonances do not overlap with those resonances of the target compound, and its T_1 values are shorter than other possible qNMR internal standards.

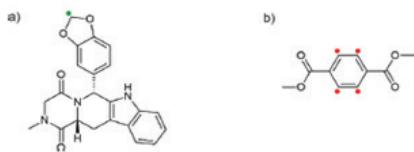


Figure 1A: Structure of tadalafil, and B: structure of dimethyl terephthalate (DMT). The green and red circles represent the protons under evaluation in this study.

Experimental: Tadalafil reference standard purity determination

The reference standard compound (tadalafil, 11.78 mg Lot#: LRAB8867 and a certified internal calibrant (dimethyl terephthalate (DMT), 2.73 mg Lot#: BCBT9974, were accurately weighed into a vial, and 600 μ L of DMSO-d6 was added. The solution was mixed using a vortex mixer until the sample and the internal standard were fully dissolved. The resultant clear solution was transferred to a 5 mm NMR tube, and the ¹H NMR spectrum was obtained after the T_1 values were determined. The T_1 value for the DMT signal at 8.08 ppm is 2.82 s, and the value for the tadalafil signal at 5.92 ppm is 0.53 s. All compounds were purchased from Sigma - Aldrich and used without further purification. The spectra were obtained at 32 °C using a Nanalysis 60PRO benchtop NMR spectrometer at a 60 MHz proton frequency (1.418 tesla). The experiments were performed with the following acquisition parameters: number of complex points, 8192; spectra width, 24 ppm; number of scans, 32; scan delay, 20 s; spectral center, 8.03 ppm; acquisition time, 5.57 s; 90° pulse duration, 15.1 μ s. Each spectrum was processed applying zero filling, number of points with zero filling equal 65536, phase and baseline correction were performed, Lorentz-to-Gauss window multiplication was applied to the FID (exponential line broadening (lb) and Gaussian line broadening (gb) were set to -0.1 and 0.3 Hz, respectively).⁹ The spectrum acquisition was performed in quintuplicate.

Results and discussion: Tadalafil reference standard purity determination

The purity of the analyte Px was calculated using the following Equation 1:

$$Px = \frac{Ax}{ADMT} \times \frac{NDMT}{Nx} \times \frac{Mx}{MDMT} \times \frac{Mx}{mDMT} \times PDMT$$

Where Ax is the integral value of the signal at 5.92 ppm which belongs to tadalafil; ADMT is the integral value of the signal at 8.08 ppm that belongs to DMT; NDMT and Nx correspond to the number of spins of DMT and

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tadalafil, respectively; M_x and M_{DMT} are the molecular weight of tadalafil and DMT, respectively; m_x is the weighted mass of tadalafil; and m_{DMT} and P_{DMT} are the weighted mass and the reported purity of DMT¹⁰, respectively.

A _x	A _{DMT}	N _{DMT}	N _x	M _x (g mol ⁻¹)	M _{DMT} (g mol ⁻¹)	m _x (mg)	m _{DMT} (mg)	P _{DMT} %	P _x %
5228386.44	4884533.93	4	2	389.4	194.186	2.73	11.78	99.95	99.4
5224447.26	4858522.55	4	2	389.4	194.186	2.73	11.78	99.95	99.9
5231152.01	4866928.42	4	2	389.4	194.186	2.73	11.78	99.95	99.9
5245944.95	4870028.1	4	2	389.4	194.186	2.73	11.78	99.95	100
5215474.05	4883227.2	4	2	389.4	194.186	2.73	11.78	99.95	99.2

Table 1: Determination of tadalafil (X) reference compound purity using dimethyl terephthalate (DMT) as an internal calibrant.

The pre-set integration region applied to each signal in Figure 2 was: 8.2791 to 7.9320 ppm for DMT and 6.0283 to 5.7700 ppm for compound X. Replacing the variable values of Equation 1 by the values reported in Table 1, a P_x purity value of 99.7 ± 0.4 is achieved. The calculated value agrees with the value range reported by the supplier company¹¹ (99.7 ± 0.1).

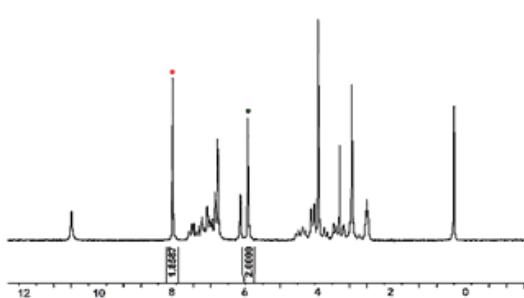


Figure 2: ¹H NMR spectrum of a mixture of tadalafil and DMT in DMSO-d6 acquired using a Nanalysis benchtop 60PRO. The green and red circles represent the protons under evaluation of tadalafil and DMT, respectively.

Experimental procedure: Cialis and Apo-Tadalafil assay test

The assay tests were performed by weighing and powdering five tablets of each drug. The drug boxes indicate that each tablet contains 20 mg of tadalafil. A portion, representing approximately 20 mg of the active compound of each well-mixed powder (210.91 mg for Cialis and 239.07 mg for Apo-Tadalafil) and the calibrant compound, DMT (2.25 mg for Cialis and 3.6 mg for Apo-Tadalafil), were weighed on an analytical balance (Mettler-Toledo MS105DU), followed by the addition of DMSO-d6 (950 µL). The two vials containing the mixtures were vortexed for 5 minutes, followed by

sonication for an extra 20 minutes. After centrifugation, the supernatants were filtered and added to a 5 mm standard NMR tube.⁵ The acquisition and processing parameters used in each ¹H NMR spectrum were the same as described in the procedure for purity determination.

Results and discussion: Cialis and Apo-Tadalafil Assay test

To determine the mean mass of tadalafil in each tablet, equation 2 was used

$$m_x = \frac{A_x}{ADM} \times \frac{NDMT}{Nx} \times \frac{M_x}{MDMT} \times \frac{mDMT}{mpowder} \times PDMT \times T$$

Where mpowder is the weighted mass of tablet powder sample taken for the assay test, and T is the average tablet weight. The meaning of any other parameters is identical as described in Equation 1.

A _x	A _{DMT}	N _{DMT}	N _x	M _x (g mol ⁻¹)	M _{DMT} (g mol ⁻¹)	m _{DMT} (mg)	m _{powder} (mg)	P _{DMT} %	T (g)	m _x (mg)
1278193.95	217851.56	4	2	389.4	194.18	2.25	210.91	0.9995	0.3619	19.8
277063.35	212686.4	4	2	389.4	194.18	2.25	210.91	0.9995	0.3619	20.2
273939.14	218779.6	4	2	389.4	194.18	2.25	210.91	0.9995	0.3619	19.4
277771.38	216568.5	4	2	389.4	194.18	2.25	210.91	0.9995	0.3619	19.9
276389.04	213979.2	4	2	389.4	194.18	2.25	210.91	0.9995	0.3619	20

Table 2: Determination of tadalafil average mass in Cialis tablets using dimethyl terephthalate (DMT) as a calibration compound.

A _x	A _{DMT}	N _{DMT}	N _x	M _x (g mol ⁻¹)	M _{DMT} (g mol ⁻¹)	m _{DMT} (mg)	m _{powder} (mg)	P _{DMT} %	T (g)	m _x (mg)
1278194	217851.59	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.8
277063.4	212686.38	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	20.2
273939.1	218779.56	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.4
277771.4	216568.53	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	19.9
276389	213979.22	4	2	389.4	194.18	2.25	210.91	0.9995	0.36191	20

Table 3: Determination of tadalafil average mass in Apo-Tadalafil tablets using dimethyl terephthalate (DMT) as a calibration compound.

The relative area of the chosen signals was determined by applying a pre-set integration region from 8.1964 to 7.9930 ppm for the DMT signal and from 5.9943 to 5.8340 ppm for the tadalafil signal (Figure 2). Replacing the values reported in Tables 2 and 3 for its respective variables in Equation 2, an average mass of 19.8 ± 0.3 mg per tablet for Cialis and 20.3 ± 0.2 mg for each Apo-Tadalafil tablet was obtained. Both values are in the range accepted by the USP monograph for tadalafil, reported as being 20 ± 2 mg, as well as with the high field data, which was determined as being 20.0 mg and 20.9 mg, respectively for Cialis and Apo-Tadalafil.

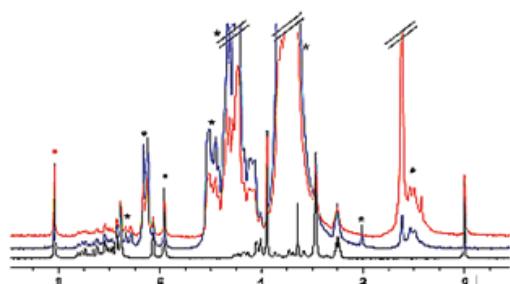


Figure 3: ^1H NMR spectrum of the tadalafil reference standard in DMSO-d6 (black); ^1H NMR spectrum of the extraction of Cialis tablets with DMT in DMSO-d6 (blue); ^1H NMR spectrum of the extraction of Apo-Tadalafil tablets with DMT in DMSO-d6 (red). All spectra were acquired in a Nanalysis 60PRO.

In addition to the quantitative information obtained in the assay tests, a fingerprint of the excipients can be observed and used for quality control when using NMR analysis (Figure 3). For the studied case, the following excipients were reported by the suppliers: croscarmellose sodium, hydroxypropylcellulose, hydroxypropylmethylcellulose, iron oxide, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium lauryl sulfate, talc, titanium dioxide and triacetin for Cialis and croscarmellose sodium, hydroxypropylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium lauryl sulfate, titanium dioxide, ferric oxide red, ferric oxide yellow, hypromellose, poloxamer, and polyethylene glycol for Apo-Tadalafil.

Conclusion

Nanalysis 60PRO benchtop NMR was successfully used to evaluate the purity of tadalafil and perform the assay test of two different tablets containing tadalafil as the active ingredient. Like other methods, a sample preparation step is required before the data acquisition and evaluation. However, with NMR, the data can be acquired in minutes. In addition, the data evaluation is straightforward since the peak area in NMR is directly proportional to the number of protons present in the molecule. The internal calibrant, which has a known concentration and structure, has the role of providing one or more signals in areas that can be used for the quantification of the target analyte. This study indicates how powerful benchtop NMR can be in quality control analysis and how it can be used as a complementary

method in many steps of drug evaluation. Once the method is developed and validated, the results can be obtained quickly, with a low volume of waste solvent, no requirement for a specialist to run the sample or to evaluate the data, without mentioning the wealth of information obtained.

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Arium® Ultrapure Water for Trace Analysis

Eliminating background signals in chromatography and mass spectrometry with the Sartorius Arium® Mini Plus

The purity of the solvents used, primarily that of water, is a decisive criterion for interference-free and reproducible analysis by liquid chromatography and for the sensitivity of this method, especially for applications in trace analysis. In a study, different sources of ultrapure water used as eluents were compared in high-performance liquid chromatography with diode-array detectors (HPLC-DAD) and mass spectrometry (MS) systems in various experiments.

Keywords: Arium® Mini Plus, ultrapure water, HPLC-DAD, LC-TOF-MS, liquid chromatography, flavour and fragrance industry

Introduction

In the flavour and fragrance industry, many products are based on natural raw materials, such as vanilla beans, citrus fruits, blossoms and other materials of plant origin. HPLC systems coupled to various detectors (e.g., mass spectrometers, DAD, or refractive index (RI) detectors), are used for quality control of such raw materials in incoming goods inspection and final quality control of outgoing products, as well as in research and development of new products.

A routine analysis performed both in research and in quality control is, for instance, the quantification of vanillin in various samples (e.g., vanilla beans, vanilla extract, vanillin sugar, chocolate, beverages and flavourings) by HPLC-DAD.

Besides the quantification of certain analytes, screening methods for identification of partially unknown

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substances contained in raw extracts and natural products, among others, play a significant role in research. Liquid chromatography (LC) systems are predominately used in such methods and are coupled to high-resolution (TOF)-MS instruments.

Avoiding High Background Noise

The purity of the solvents used, primarily that of water, is a decisive criterion for interference-free and reproducible analysis by liquid chromatography and for the sensitivity of this method, especially for applications in trace analysis.

Contaminants in the eluent can result in relatively high baseline noise originating from the detector and thus to a poorer signal-to-noise ratio (S/N) of a peak. In DAD, such contaminants are, for example, organic compounds that absorb light in the UV/VIS range. In LC-MS applications, the concentration of ions (Na^+ , K^+) should be kept as low as possible to prevent the formation of adducts with analytes during ionization. Water contaminants that are enriched in the stationary phase can also be eluted with a higher percentage of organic solvent and occur as potentially co-eluting peaks in the chromatogram.

For these reasons, a number of specially treated and filtered types of ultrapure water are commercially available in different grades (HPLC and LC-MS grades). An alternative to these grades of water that are usually filled in 1- or 2.5 - litre bottles is to use water purified by ultrapure water systems such as the Arium® Mini Plus ultrapure water system (Figure 1). Use of ultrapure water freshly produced by such a system to prepare an eluent for HPLC-DAD and MS systems was compared in various experiments with two commercially available brands of bottled water of LC-MS grade.

For this purpose, the background signal in the chromatogram – usually detectable as a baseline – was examined after a relatively long accumulation phase for each particular water sample used in the chromatographic system and subsequent gradient elution performed on two different detectors (DAD- and

TOF-MS). In addition, representative routine analyses, such as the analysis of vanillin by HPLC-DAD and screening of a natural product by LC-MS, were run with three different sources of ultrapure water as part of the mobile phase and compared.

Production of Ultrapure Water Using Arium® Mini Plus

To produce ultrapure water, Arium® Mini Plus (Figure 1) is directly connected to the tap water feed to purify this water in a two-stage process. In the first stage, this compact system produces pure water, reverse osmosis water (RO water), and in the second stage, ultrapure water. As lower flow rates are reached during RO purification and this stage therefore has a limiting effect on such rates, an Arium® bag is connected as a reservoir between the two stages (flow diagram in Figure 1).

In the first stage of the Arium® Mini Plus system, feed water is passed from the system inlet through a pretreatment cartridge, an RO module, by using a diaphragm pump. The RO module has two outlets, one for the permeate flow and the other for the concentrate flow. The latter flow path is connected to the system's outlet to drain off the water removed from the RO purification stream, "rejected water." The permeate flow is purified RO water (i.e., pure water) that fills the bag and is monitored in the process by a conductivity cell.

In the second downstream stage, the pure water obtained is transported by a further pump out of the bag to the actual purification cartridge for generating ultrapure water.

Here, pure water is transformed into ultrapure water using an optional UV lamp (has an oxidizing at wavelengths of 185 nm and 254 nm, respectively) and by passing through a cartridge filled with active carbon and ion exchange resin. During purification, the quality of ultrapure water is continuously monitored by a second conductivity cell to maintain a conductivity of $0.055 \mu \text{S}/\text{cm}$ (corresponds to a resistivity of $18.2 \text{ M}\Omega \times \text{cm}$),

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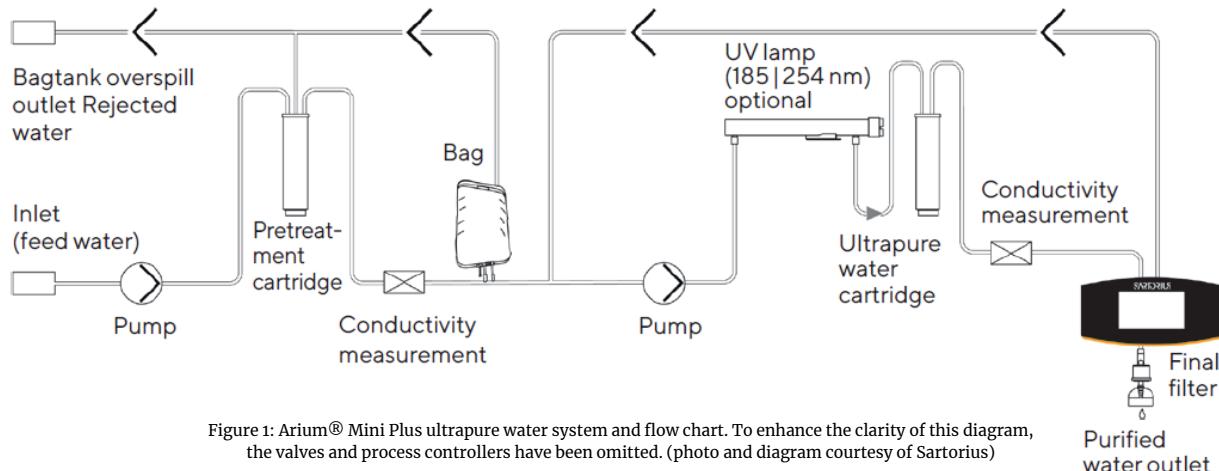


Figure 1: Arium® Mini Plus ultrapure water system and flow chart. To enhance the clarity of this diagram, the valves and process controllers have been omitted. (photo and diagram courtesy of Sartorius)

compensated to 25 °C. Then in the last purification step, purified water is dispensed via a final sterilizing-grade filter.

This process is shown as a schematic diagram in Figure 1.

Materials and Methods

The water sources tested included two commercially available, certified water grades for LC-MS applications (LC-MS grade water A and B) besides ultrapure water freshly produced by the Arium® Mini Plus system. To examine the background signal in the HPLC-DAD and LC-MS chromatograms, the different water sources were each passed through an RP-C18 column, under standard flow conditions and without the addition of modifiers (e.g., formic acid or buffer), for a period of 40 min. for the HPLC-DAD method and 16 min. for the LC-MS method, respectively, to concentrate any contaminants present in each water source (accumulation phase).

Then the potential contaminants were eluted by running a gradient of water used as the solvent to 100% acetonitrile. At the end of each run, the column was reconditioned with the respective water source. This was carried out on two different systems: HPLC-DAD (system 1 supplied by Agilent based in Waldbronn, Germany) and LC-TOF-MS (system 2 supplied by

Waters, Eschborn and Bruker based in Karlsruhe, Germany). The device parameters are listed in Table 1.

In routine analysis, system 1 is used, for example, to quantify vanillin, whereas system 2 is mainly employed for screening of compounds, such as those in natural products. For these two applications, trial runs were performed using the different water sources.

Results

Background Signal in UV and MS Detection

The resulting chromatograms of the trial runs conducted on the HPLC-DAD system (system 1) are shown in Figure 2.

In the top graph (Figure 2A), the chromatograms of the three samples of ultrapure water are overlaid, and the gradient profile is marked.

On the chromatograms, both the commercially available bottled water grades and ultrapure water freshly produced by the Arium® Mini Plus system show similar contaminants that were accumulated in the separation column. At approx. 45 minutes, a broad peak was observed, which exhibits a substantially higher peak area for accumulation using Arium® Mini Plus water and, due to its asymmetry, indicates overlay of several contaminants.

Application Showcase

	System 1	System 2
Device	Agilent 1290 with DAD	Waters Acuity UPLC, Bruker microTOF II
Separation Column	Grom Sapphire C18, 150 x 2.1 mm, 5 µm (Grom analytical + HPLC, Herrenberg, Germany)	Kinetex RP-C18, 100 x 2.1 mm, 1.7 mm (Phenomenex, Aschaffenburg, Germany)
Mobile phase	A: Water B: Acetonitrile	
Gradient	0 min. 100% A 0% B	0 min. 100% A 0% B
	40 min. 100% A 0% B	16 min. 100% A 0% B
	50 min. 0% A 100% B	19 min. 5% A 95% B
	60 min. 0% A 100% B	23 min. 0% A 100% B
	70 min. 100% A 0% B	26 min. 100% A 0% B
		30 min. 100% A 0% B
Temperature	40 °C	50 °C
Flow rate	1 mL/min.	0.55 mL/min.
Detection	200 nm	50-1600 Da (ESI- and ESI+)

Table 1: Device Parameters of Systems 1 and 2 for Analysis of Contaminants Present in the Different Water Sources

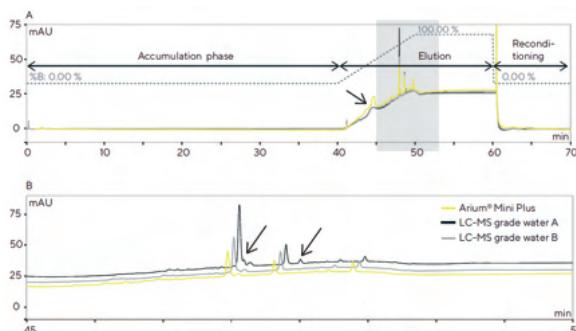


Figure 2: (A) HPLC-DAD chromatograms (detection: 200 nm) after a 40-min. accumulation phase for each of the three water sources tested in the column and subsequent elution of the contaminants performed with acetonitrile. (B) Magnified view of the coloured section in A; differences in the peak profile are identified by arrows.

In the coloured section (45 – 53 min.; magnified view in Figure 2B), there are slight differences in the peak profile.

Here, the profiles for Arium® Mini Plus water and water B are comparable, whereas in water A, contaminants that cannot be observed in the other chromatograms are detected. This accumulation experiment delivered reproducible results (n = 5).

This trial conducted to examine the background signal after accumulation of the contaminants for the various samples of ultrapure water on an RP-C18 separation column was additionally performed with a

high-resolution TOF-MS system (system 2, Figure 3). After electrospray ionization in positive mode (ESI+), hardly any differences can be seen between the peak profiles (data not shown). By contrast, differences can be seen in the peak profiles obtained in the ESI- mode (Figure 4).

Thus, the chromatogram for Arium® Mini Plus water in the range of 22 – 25 min. shows fewer peaks of contaminants in comparison to those obtained for the commercially available brands of bottled water.



Figure 3: Waters Acuity UPLC with Bruker microTOF II (location and photo: Symrise)

Application Showcase

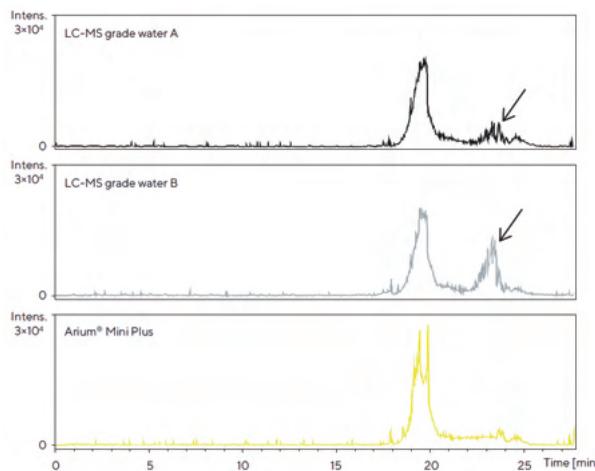


Figure 4: LC-TOF-MS chromatograms after the accumulation phase for each of the three water sources tested in the column and subsequent

In addition, the particular water samples were injected by a syringe pump directly into the TOF-MS system (Bruker microTOF II).

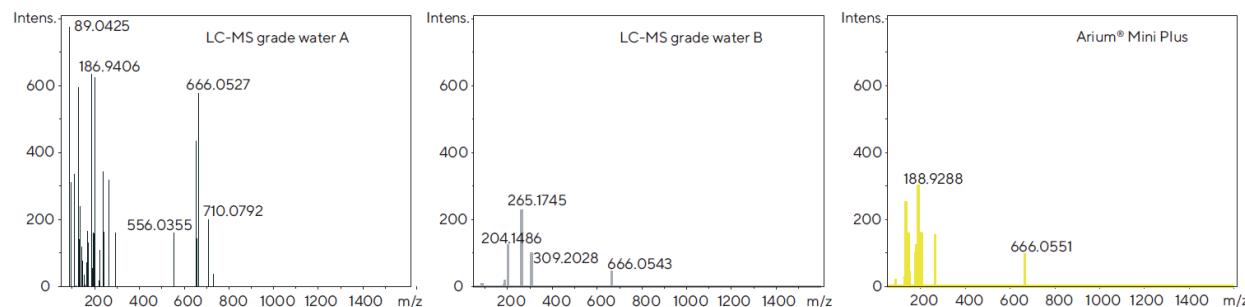


Figure 5: Mass spectra of each of the tested water sources, obtained after direct injection by syringe pump following electrospray ionization in the negative mode (ESI-).

Comparison of Water Sources in Routine Analyses

To test the usability of the different ultrapure water sources in routine analyses, these sources were each employed as solvents in sample chromatographic runs.

Quantification of Vanillin in Vanilla Extract

After diluting with methanol (approx. 1:1,000), vanilla extract was analysed using each of the three different water sources as a component of the respective eluents run through system 1.

The vanillin concentration of each injected solution corresponded in this case to approx. 4 µg/mL.

As special experiments are carried out by direct injection just as is generation of reference spectra both in the scan and MS/MS modes, it is also important in these cases that the quantity of interfering ions produced from the solvents used be kept as low as possible. The spectra recorded were averaged by software over a time span of 1 min.

Figure 5 shows examples of the spectra obtained in the ESI mode. By comparison, LC-MS grade water B and water from the Arium® Mini Plus system show fewer signals of potentially interfering ions, whereas LC-MS grade water A generates considerably more signals. This can also be observed in the ESI+ mode (data not shown) and supports the observations made in assessing the chromatograms depicted in Figure 4.

The resulting chromatograms (Figure 6A) are nearly congruent, and the peak areas of vanillin do not differ at all.

However, if the vanillin concentration is within the range of the detection limit (9 ng/mL), the background signal, as a result of the water purity, does play a role, and substantial differences can be seen (Figure 6B). In view of the baseline curve and signal-to-noise ratio (S/N) of the vanillin peak, Arium® Mini Plus water and LC-MS grade water B are comparable, whereas the chromatogram for LC-MS grade water A shows a higher baseline and more potentially interfering peaks.

Application Showcase

This confirms the observations made in the experiments described above with regard to the background signals in the HPLC-DAD system and after direct injection into the TOF-MS system.

Screening of Orange Oil Using LC-TOF-MS

Furthermore, the three water sources were tested in a qualitative screening method by high-resolution LC-TOF-MS (system 2) to identify individual compounds in mixtures.

A specific type of orange oil was used as the sample material. The chromatograms are shown in Figure 7. Comparable performance regarding the peak height, peak area, retention time and separation was observed. Likewise, in view of the baseline curve and signal-to-noise ratio, hardly any differences are seen (Figure 7, magnified view).

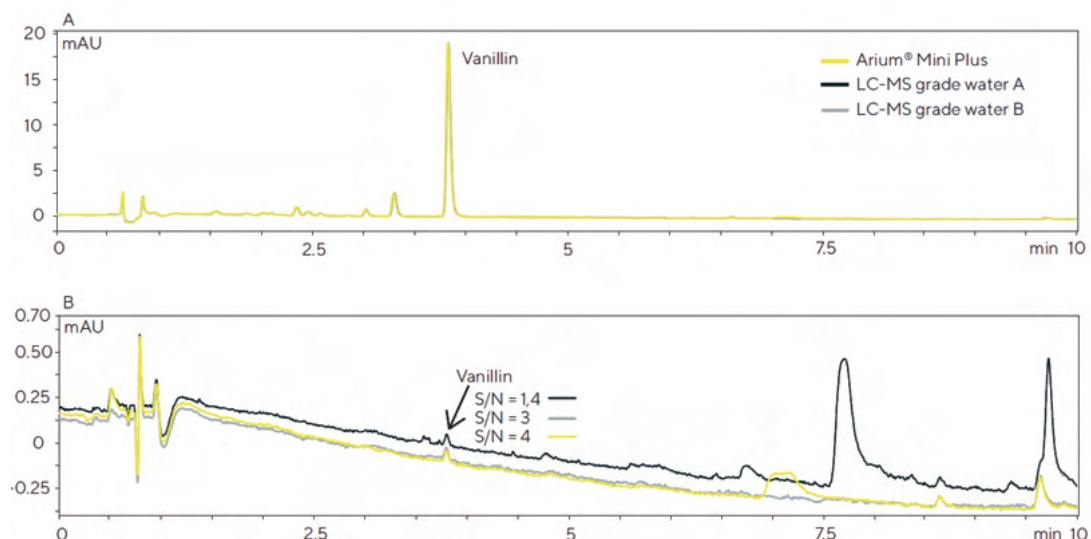


Figure 6: HPLC-DAD chromatograms of a vanilla extract (A) and an aqueous vanillin solution (B, 9 ng/mL) obtained with the different water sources used in the mobile phase (detection: 280 nm; column: Poroshell 120 SB-C18, 2.7 μ m, 100 \times 2.1 mm; eluents: acetonitrile and water with 0.1% formic acid in the gradient mode; flow rate: 0.4 mL/min.).

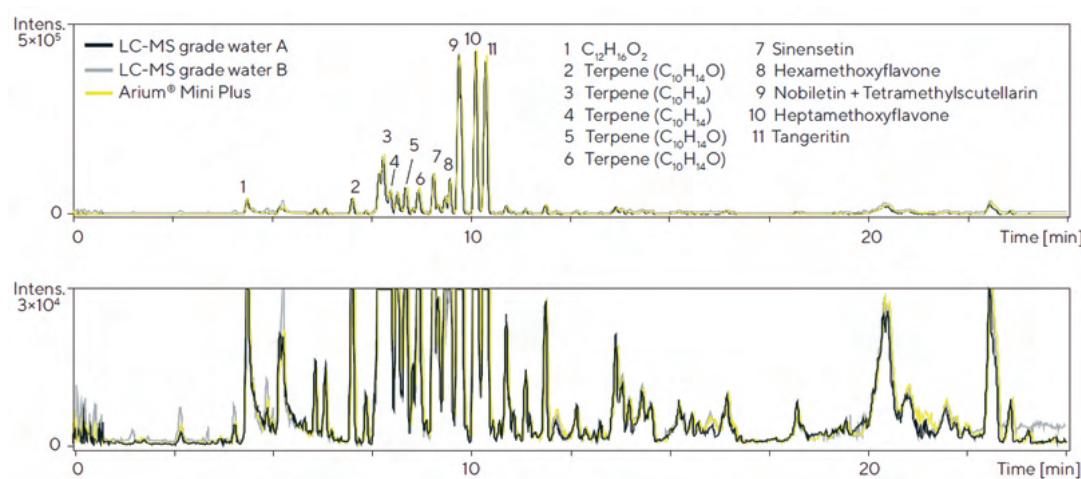


Figure 7: LC-TOF-MS chromatograms (BPC, base peak chromatogram; 70 – 1,600 Da) of an orange oil (diluted 1:20) obtained with the respective water sources in the mobile phase (detection: 50 – 1,600 Da (ESI+); column: Kinetex RP-C18, 1.7 μ m, 100 \times 2.1 mm; eluents: acetonitrile and water, each with 0.1% formic acid in the gradient mode; flow rate: 0.55 mL/min.; lower chromatogram: magnified view).

Application Showcase

Discussion

Based on the experiments conducted, it could be shown that Arium® Mini Plus ultrapure water is excellently suited for use in chromatography and mass spectrometry (MS). In view of the potentially co-eluting peaks and the back-Ground signal in UV and MS detection, it was observed that Arium® Mini Plus water is comparable with the tested quality grades of commercially available bottled water.

The background signal, which primarily depends on the purity of the solvent used in chromatographic analysis, must be as low as possible as this signal is highly significant for the sensitivity of the analytical method and for reliable quantification. Besides LC-MS grade water B, Arium® Mini Plus water with a higher S/N excels especially in trace analysis requiring high sensitivity, as shown in the example of the vanillin peak obtained on the HPLC-DAD chromatogram.

Unlike commercially available, bottled ultrapure water, an ultrapure water system offers the considerable advantage of being able to freshly purify water in any quantity on demand. From an economic point of view, this feature is thus a good alternative to purchased ultrapure bottled water. Fresh purification also prevents water from standing in opened bottles for long periods because such water stored in opened bottles can be contaminated by absorption from the laboratory atmosphere^{1,2} and dissolve CO₂ from air, among other contaminants. Organic contaminants in water are detectable by an increase in the TOC level (total organic carbon). At high TOC levels, identification and quantification of trace components can be compromised¹, for instance, by shifts in the baseline^{1,2} or by the occurrence of ghost peaks³.

In addition, if bottled water is stored for relatively long periods, Na-cations, for example, can leach from the glass bottles, which, in turn, can lead to increased formation of adducts during ionization in LC-MS systems. An accordingly lower yield of ions used for

evaluation (usually [M+H]⁺ or [M-H]⁻) ions can have a negative impact on the sensitivity of the method⁴.

The high suitability of fresh ultrapure water, produced by Arium® Pro systems, in different chromatography techniques and the increasing use of these technologies in the most diverse applications will very likely contribute to the growing acceptance and pervasiveness of laboratory water purification systems.

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Performance verification of a proposed USP monograph for Sorbitan Monooleate using a Gel Permeation Chromatography (GPC) Method with Refractive Index (RI) Detection

Exemplifying High Performance on the Waters Arc™ HPLC System

The United States Pharmacopeia (USP) proposed revisions to the monograph for sorbitan monooleate to update the procedure for assay and add a limit for organic impurities test, employing a GPC method (USP-PF 50(2)). In this work, the proposed GPC method was run on an Arc™ HPLC System with a strong solvent compatibility kit and RI detector. The GPC results met the USP system suitability requirements and acceptance criteria for both the assay and organic impurities analysis in sorbitan monooleate.

Keywords: Sorbitan monooleate, Gel Permeation Chromatography, USP, HPLC, Waters Arc HPLC

Introduction

Sorbitan monooleate, also known as Span 80, is a non-ionic surfactant used as a stabilizer and emulsifier in cosmetic, pharmaceutical, and food products¹. It helps to stabilize formulations by creating a stable mixture between immiscible ingredients, such as oil and water. Additionally, sorbitan monooleate increases stability of food products and helps to elevate their texture and consistency¹.

The USP is updating monographs for Chemical Medicines and excipients across the compendia with new methodologies and technologies. As part of the modernization efforts, the USP proposed revisions to the monograph for sorbitan monooleate². The proposed USP monograph updates an assay procedure with a GPC method designed for analysis of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters.

Application Showcase

Additionally, the USP proposes addition of a limit test for organic impurities utilizing the same GPC method conditions as that used for the assay.

In this work, the proposed USP monograph for sorbitan monooleate was run on a GPC system composed of an Arc HPLC System with a strong solvent compatibility kit and RI detector. The experimental work, chromatographic separation, and calculations were performed according to the proposed USP monograph assay and limit of organic impurities procedures.

The success of the analysis was measured by comparing GPC results against the USP requirements and acceptance criteria for assay and organic impurities analysis. Empower™ Software was used for data acquisition and analysis.

Introduction

- » Excellent performance of a proposed USP monograph for sorbitan monooleate by meeting the requirements for the assay and limit of organic impurities analysis.
- » Reliable GPC analysis using the Arc HPLC System with a strong solvent compatibility kit and RI detector.

Experimental

Solutions preparation and experimental conditions proceeded as described in the proposed USP monograph for sorbitan monooleate².

Materials

Tetrahydrofuran (THF) HPLC grade, no preservatives, purchased from Fisher Chemicals.

Isopropyl alcohol (IPA) purchased from Honeywell. Sorbitan monooleate purchased from Sigma-Aldrich.

Sample Description

Standard Solutions

Standard solution for the assay was prepared by dissolving each of oleic acid, 1,4-sorbitan, and

isosorbide in THF at 1.0 mg/mL.

For organic impurities, preparation of the standard solution proceeded as described for the assay.

Sample Solutions

Sample solution for the assay testing was prepared by dissolving sorbitan monooleate in THF at 1.0 mg/mL. For organic impurities, preparation of the sample solution proceeded as described for the assay.

Method Conditions

- » **System:** Arc HPLC System with quaternary solvent manager (QSM), flow through needle (FTN) sample manager, and strong solvent compatibility kit (p/n: 205002572).
Column heater/cooler (p/n: 186179100)
- » **Detector:** Refractive Index (RI)
Flow cell temperature: 30 °C
Sampling rate: 10 pts/sec
Polarity: positive
- » **Mobile phase:** Tetrahydrofuran
- » **Separation:** Isocratic
- » **Columns:** Columns with 7.8 x 300 mm with 5 µm, connected in series using a joining tube (p/n: WAT084080) supplied with columns.
 - Styragel™ HR 1, 100 Å, molecular weight range: 100–5,000 (p/n: WAT044234)
 - Styragel HR 0.5, 50 Å, molecular weight range: 0–1,000 (p/n: WAT044231)
- » **Column temperature:** 30 °C
- » **Sample temperature:** 25 °C
- » **Flow rate:** 0.9 mL/min
- » **Injection volume:** 20 µL

Application Showcase

- » Run time: 30 minutes
- » Vials: LCMS Maximum Recovery 2 mL volume (p/n: 600000670CV)
- » Wash solvents: Sample manager/purge wash: tetrahydrofuran
- » Seal wash: Isopropyl alcohol

Data Management

Chromatography software: Empower 3 Feature Release 5 Service Release 5 (FR5 SR3) for data acquisition and analysis.

Results and Discussion

The proposed USP monograph for sorbitan monooleate describes a revised assay procedure and adds a limit for organic impurities testing, both utilizing a GPC method². The procedure for organic impurities employs the same standard solution, sample solution and chromatographic conditions as that for the assay.

Waters Styragel HR 0.5 and HR 1 columns are recommended for the GPC separation with THF as a mobile phase.

In this work, the GPC analysis was performed following the updated procedures in proposed USP monograph for sorbitan monooleate. Columns were connected in series with the larger pore size column first to reduce back pressure. Calculations were performed using Empower Software.

Peak Assignment

The USP lists relative retention times (RRT) to aid in peak assignment and identification of components for the assay and organic impurities testing (Table 1). These values were used to identify peaks in the chromatographic separation of standard and sample solutions (Figure 1).

Procedure	Peak	RRT
Assay	Sorbitan tri-/higher esters	0.73
	Sorbitan diesters	0.75
	Sorbitan monoesters	0.8
	Oleic acid	0.86
	1,4-Sorbitan	0.91
	Isosorbide	1.0
Organic Impurities	Isosorbide monoesters	0.83
	Fatty acid (oleic acid)	0.86
	1,4-Sorbitan	0.91
	Isosorbide	1.0

Table 1. Relative retention time (RRT) to aid in peak assignment for assay and limit of organic impurities testing according to the proposed USP monograph for sorbitan monooleate².

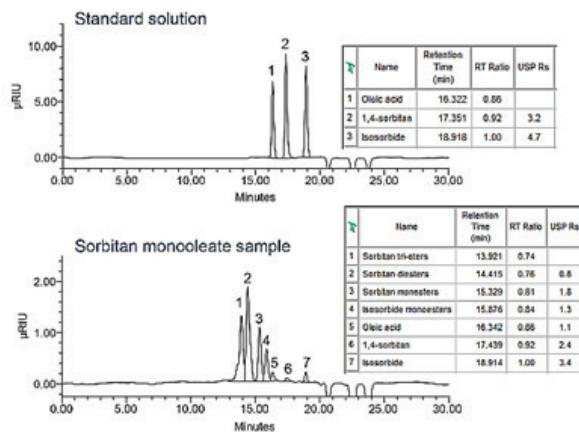


Figure 1. GPC separation of the standard solution and sorbitan monooleate sample using an Arc HPLC System with a strong solvent compatibility kit and RI detector.

Empower RT ratio: relative retention time (RRT).

System Suitability

System suitability was measured using the standard and sample solutions as indicated in the proposed USP monograph for sorbitan monooleate². The results for six replicate injections of the standards solution, showed excellent relative standard deviations (RSD) for peak areas and retention times of $\leq 0.68\%$ and $\leq 0.01\%$, respectively (Figure 2). A summary of the USP system suitability requirements and results generated by the GPC method for assay and limit of organic impurities are

Application Showcase

shown in Table 2. The GPC method was run on an Arc HPLC System with strong solvent compatibility kit met all the USP acceptance criteria for resolution and RSD of six replicate injections of standard solution.

Procedure	Parameter	USP Requirement ²	GPC Results
Assay	Resolution: Between the sorbitan diesters and sorbitan monoester peaks (sample solution)	Not less than (NLT) 1.0	1.8
Assay and organic impurities	Relative standard deviation: for the oleic acid, 1,4-sorbitan, and isosorbide peaks (six replicate injections of standard solution)	<5.0%	Oleic acid peak: -RSD of areas: 0.58%; RSD of RT: 0.01% 1,4-sorbitan peak: -RSD of areas: 0.38%; RSD of RT: 0.01% Isosorbide peak: -RSD of areas: 0.68%; RSD of RT: 0.01%
Organic Impurities	Resolution: Between the 1,4-sorbitan and isosorbide peaks (standard solution)	NLT 1.5	4.7

Table 2. System suitability for assay and limit of organic impurities in sorbitan monooleate. USP requirements and results generated by the GPC method.²

Assay: Analysis of Sorbitan Tri-/Higher Esters, Sorbitan Diesters, and Sorbitan

Monoesters

The percentage (%) of each sorbitan ester component in the sorbitan monooleate sample was calculated by area normalization as instructed by the USP². The peak area of individual peak was divided by the sum of the relevant peak areas and multiplied by 100.

The results generated by the GPC method for the sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters met the USP acceptance criteria ranges (Table 3).

Sorbitan ester	USP acceptance criteria: range (%) ²	GPC results (%)
Sorbitan tri-/higher esters	25.0-40.0	27.4
Sorbitan diesters	30.0-40.0	37.6
Sorbitan monoesters	15.0-20.0	17.6

Table 3. GPC results for the assay of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters in sorbitan monooleate sample (n=6).

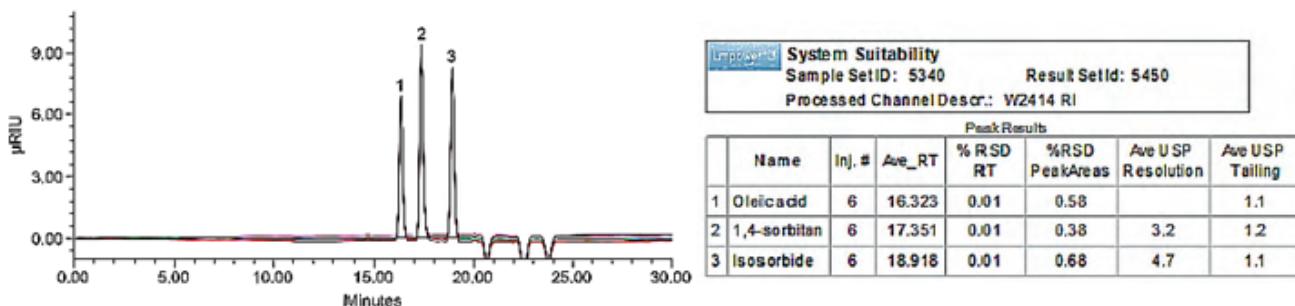


Figure 2. Results for six replicate injections of standard solution. RT: retention time; RSD: relative standard deviation.

Application Showcase

Limit of Organic Impurities

The percentage of each impurity peak in the sorbitan monooleate sample was determined by comparing the area of each peak to the sum of the relevant peaks. The GPC results were within the USP limits for organic impurities content (Table 4).

Impurity	USP acceptance criteria: limit, NMT (%)	GPC results (%)
Isosorbide monoesters	15.0	10.6
Oleic acid	5.0	2.7
1,4- sorbitan	2.5	1.8
Isosorbide monoesters	3.0	2.4

Table 4. GPC results for limit of organic impurities in sorbitan monooleate sample (n=6).

Conclusion

The GPC method described in the proposed USP monograph for sorbitan monooleate was successfully run on the Arc HPLC System with a strong solvent compatibility kit and refractive index detector, meeting all the requirements for system suitability, assay, and limit of organic impurities testing.

The GPC method demonstrated excellent relative standard deviations (RSD) for peak areas and retention times of $\leq 0.68\%$ and $\leq 0.01\%$, well below the required acceptance criteria of 5.0%. The USP criteria for resolution were also met.

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Dissolution of Be from Pu/Be metal mixtures using the NaOH Process

Highlighting Glovebox Procedure in Eliminating Boil Over Problems

Dissolution processes are highly exothermic, and produce excessive amounts of hydrogen gas, which can lead to violent boiling, and pose safety risks. This application note emphasises on the significance of a glove box in mitigating these risks. Key safety concerns include addition of NaOH, stirring, temperature monitoring and use of anti-foaming agents in the dissolution process.

Keywords or phrases: Glovebox, Pu/Be, Beryllium Dissolution, NaOH, Boil-Over, Safety, Nuclear Materials

Introduction

The dissolution of beryllium (Be) from plutonium-beryllium (Pu/Be) metal mixtures is challenging due to the exothermic nature of the reaction and the potential for hazardous byproducts. This process, often involving the use of strong bases like sodium hydroxide, can lead to violent boiling, known as "boil-over," posing serious safety risks. This application note delves into the critical role of glove boxes in mitigating these risks and ensuring the safe and efficient processing of Pu/Be materials¹.

Experimental

This application note exemplifies the use of a glove box in the dissolution of Be from Pu/Be metal materials¹.

This procedure aims to control the reaction rate to prevent boil-over and minimize H₂ release. It is designed for 100 gram samples in 1 litre of water.

Application Showcase

Due to pyrophoricity of the Pu/Be mixture, they are stored in Ar-filled glove boxes until the plutonium is calcined to PuO_2 for safe and long-term storage. Calcining Pu/Be directly results in a mixture of oxides with a high neutron dose rate. Thus, Be has to be removed from Pu/Be mixture by dissolution in conc. NaOH , preventing the high neutron dose rate problem.

However, this exothermic reaction can lead to violent boiling ("boil-over") for larger samples (>10 grams).

This document outlines a procedure to safely dissolve ~100 grams of Pu/Be in NaOH by gradually increasing the NaOH concentration, in a glove box.

The reaction: $\text{Be} + 2\text{OH} \rightarrow \text{BeO}_2 + \text{H}_2(\text{g})$ generates heat (8.8 kcal/g Be) and hydrogen gas. While Pu is insoluble in NaOH , rapid Be dissolution can cause uncontrolled heat release and H_2 evolution, leading to boil-over.

Challenges of Beryllium Dissolution

Exothermic Reactions:

The dissolution of Be in NaOH is a highly exothermic reaction, producing H_2 gas as a by-product. This gas has to be vented properly to avoid its accumulation within the reaction vessel to reduce the internal pressure.

The unwanted pressure build-up can exacerbate the risk of localised superheating and eventually, a violent 'boil-over', causing explosive eruptions.

Pyrophoricity of Pu/Be Mixtures:

Pu/Be mixtures are highly pyrophoric. Handling these materials outside a controlled environment significantly increases the risk of fire and explosion. Furthermore, the presence of finely divided Pu/Be particles can increase the reactivity and the likelihood of uncontrolled reactions.

Potential for Chemical Splashes and Inhalation Hazards:

Boil-over events can result in the forceful eruptions of hot, caustic solutions and potentially hazardous fumes. This poses a significant risk of chemical splashes to the handler, causing severe burns and other injuries. Inhalation of these fumes can also have serious health consequences.

The Critical Role of Glove Boxes

Glove boxes provide a crucial safeguard against the hazards associated with dissolutions from its mixture. By acting as a physical barrier, they create a controlled and isolated environment, they significantly reduce the risks mentioned above and enhance overall laboratory safety.

Inert Atmosphere Control and Controlled Handling of Materials:

Under conditions of handling highly reactive materials/compounds, filling the glove box with an inert gas (Ar or N_2) will prevent the ignition of H_2 gas due to atmospheric exposure. An inert environment will also control the unnecessary oxidation of reactive compounds, maintaining the stability of the reaction.

Controlled Addition of Reagents:

- » The gradual and controlled addition of NaOH pellets is critical to regulate the reaction rate and minimize the risk of sudden temperature increases.
- » The use of a controlled feed mechanism or the manual addition of small aliquots of NaOH can help maintain a steady reaction rate.

Temperature Control and Monitoring:

- » Before transferring the Pu/Be sample into the glove box, the background gamma and neutron radiation were measured through the glove box window. The dissolving equipment was set up and the sample was loaded into the beaker using proper glove box techniques.

Application Showcase

- » Continuous monitoring of the solution temperature is essential to prevent excessive heating and potential boil-over events.
- » The use of thermocouple probes and controllers allow for precise control of the reaction temperature.

Stirring and Mixing:

- » NaOH pellets were added slowly in a controlled way to the solution at 100rpm. The main goal of controlled addition is to prevent boil-over. This is also done to maintain a low reaction concentration of NaOH, until the dissolution of Be starts, and to maintain a moderate reaction rate.
- » Effective stirring is crucial to ensure even heat distribution and prevent the formation of localized hot spots.

Anti-foaming Agents:

- » The addition of anti-foaming agents, e.g., n-octanol, can help to suppress the formation of foam, which can contribute to boil-over and hinder efficient gas evolution.

Conclusion

Ten 100 gram batches of Pu/Be samples were processed within a glove box environment for the first phase of development. There were two incidents, one boil-over and another near-boil-over. The neutron dose rates after calcination were erratic, with seven samples less than 2.5 mR/h and three samples having higher rates, which included 12 mR/h, 7 mR/h, and 5 mR/h.

The process was then refined and again carried out inside the glove box, with eight 100-gram batches being processed. No boil-over events occurred during this stage. Neutron dose rates after calcination were consistently below 2 mR/h for all samples, typically about 1 mR/h.

These results demonstrate the effectiveness of the glove box environment in mitigating boil-over risks and achieving more consistent and lower neutron dose rates

following calcination, indicating improved process control and enhanced safety.

Reference

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An Essential Guide to Laboratory Water Applications

Finding the Right Arium® System- Supporting Research Across Disciplines

In the daily work of laboratories, laboratory water is the most used solvent. Used in almost all laboratories, it is an important component of a wide range of applications such as preparation of buffers, samples, media and other solutions, and as feed water for laboratory devices. With a laboratory water purification system, you have access to on-demand, consistent, reliable and high-quality water. Depending on your application, you will need different types of water to have consistent and reliable results.

This guide helps you to determine what type of water is needed for different applications.

Lab water is the most common solvent used in laboratories and is used in a wide range of applications such as preparing blanks, buffers, samples, media and other solutions, as well as feed water for equipment such as washing machines, autoclaves and ice machines. Sartorius AG manufactures its own water purification systems (Arium®). The Arium® family has several variations and can effectively accommodate your laboratory's requirements and provide you with high quality, on-demand laboratory water.

This guide describes common applications as well as providing recommendations for which Arium® system would be suitable.

If you have several applications which require different quality water, make sure to have a system that produces the needed quality for the more critical application, which has higher requirements. This is because, generally, the higher quality water will also be suitable for applications with lower requirements, while the same cannot be said the other way around.

The Sartorius combined water purification systems (Comfort I & II or Mini Plus) that can produce two water qualities with the same system using tap water as its feed source.

For the effectiveness of this guide, water applications have been categorised into Non-critical, Critical Life Science and Critical Analytical Applications. For Non-critical Applications, there are no other specific concerns but the water type (quality) itself – so if RO, pure or ultrapure water is used. Some instances when these applications are used, they can also be critical and therefore may have higher requirements on the water quality, this depends on your specific case. In Critical Life Science, in addition to ultrapure water, endotoxins, nucleic acids, nucleases etc. should also be considered. Similarly in Critical Analytic Applications the TOC level, in addition to being ultrapure water, should be considered.

Feed Water Applications

There is likely a variety of equipment in your laboratory in need of a reliable source of purified water as feed water. Tap water is a typical source for feed water and is what has been considered in this guide. However, tap water generally needs some sort of pre-treatment before used as feed water to minimize risk for calcification, deposits or other buildups, corrosion etc.

Sartorius provides solutions within the Arium® family for both tap water purification and intermediate storage (Arium® Bagtank) of purified water for later use as feed water. It is important to recognize the connections, needed pressure, volume and flow rate to make sure the feed water source is compatible with the equipment. It is also important to remember that some equipment already has internal pumps of their own, so an additional pump in the Arium® Bagtank is not needed and can harm the equipment if the pressure is too high. In such a case, a Bagtank version without the pump can be selected.

Some equipment might not have a connection for a direct feed source and require manual filling. The Bagtank accommodates these needs as well by having a Smart Station connected to the Bagtank that allows water to be dispensed directly from the Bagtank to any vessel.

Feed Distilled Systems

Stills produce purified water by evaporating feed water, leaving impurities behind and condensing the steam. Extra stages are required to minimize carry-over and collection of volatiles.

To reduce excessive build-up of deposits which would require frequent cleaning, and minimize carry-over, pretreated water is needed. Reverse osmosis permeate or deionized water are generally used and sufficient.

Feed Ultrapure Water Systems

Type 1 ultrapure water is widely used in laboratories. It is often prepared in two stages: a source of pre-purified water followed by a “polisher” to achieve ultrapure water quality.

Pre-purified water is produced by reverse osmosis (possibly with ion-exchange or electrode ionization) and stored in an intermediate water storage unit before polishing. The quality of the pre-treated water affects the performance of the system and cartridge life. Type 3 RO or Type 2 pure water is sufficient.

Feed Water for Laboratory Equipment (Autoclaves | Washing Machine | Ice Machine etc.)

Plenty of laboratory equipment needs reliable and constant feed of pretreated water to reduce build-up, calcification, residues and other problems that could arise if using water with too low quality.

Therefore, a reliable and constant source of pre-treated feed water is needed in various volumes, depending on the equipment. High purity is not needed, and general Type 2 pure water or Type 3 RO water would be satisfactory.

Manual Feed for Laboratory Equipment (Water Bath etc.)

Some equipment in the laboratory needs purified water but does not have an inlet for automatic feed water. One example is water baths, which can keep water at a constant temperature over a long period of time. They are used for practices like

incubating samples in water and provide a reliable surrounding temperature. This equipment needs manual filling either directly from a water tank or via another vessel. Either way, easy and flexible filling of water to any vessel or basin is required.

Water baths usually use purified water to avoid corrosion and build-ups in the basin.

Non-Critical Applications

During an average day of laboratory work, there are numerous routines and tasks that require purified water. Depending on the application – and its criticality and sensitivity – different quality water may be needed, sometimes with specific parameters in mind. Generally, Type 2 (pure) water is sufficient when it comes to general laboratory tasks. Both classical methods and instrumental methods are included in this segment, which will determine some degree of sensitivity by itself.

AAS (Atomic Absorption Spectroscopy)

AAS is a technique for determination of the total concentrations of specific elements in a sample. It is used for trace analysis at ppm (mg/L) or ppb (µg/L) levels. Analysis by AAS requires the use of high-purity reagents and solvents during sample preparation and calibration to ensure the accuracy and precision of measurements. Type 1 ultrapure water is required for high-sensitivity, while Type 2 pure water can be used for general AAS.

Buffer, Media and pH Solutions

Buffers are used in several fields to create an environment that is more stable and resistant to pH level changes. Media on the other hand creates an environment to allow cells to grow by introducing important nutrients. Finally, pH solutions are needed for pH measurements, which is a quick and easy way to determine the acidity or basicity of a solution, which is important in many applications.

The water used to prepare buffer, media and pH solutions should be devoid of significant concentrations of acids or bases and free of other substances that can modify pH. Generally, Type 2 pure water is sufficient, but depending on criticality and application, higher quality water might be needed. It is also important to understand how TOC or endotoxins may play a role as well!

ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is a technique to detect the presence of antigens, antibodies or other analytes in a sample. It can be used as qualitative or quantitative method. It is a highly sensitive technique for determining infections and diseases as well as food allergens. It can also be used in toxicology.

Washing is needed in several steps of the analysis. For this, Type 2 pure water is generally sufficient for preparing the washing solution. For more critical applications, Type 1 ultrapure water might be needed.

Electrophoresis

Electrophoresis is used to separate molecules based on charge, size and affinity with the help of an applied electrical field and an adequate buffer solution. The molecules typically migrate through a gel medium (such as SDS-Polyamid or Agarose) with a specific velocity and distance and creates a pattern that can be analysed. The technique is widely used, especially to separate and analyse biomolecules such as DNA, RNA and proteins. There are different sub-techniques that use electrophoresis depending on application and sensitivity.

Tech Corner

Arium® Lab Water System

Lab Water Quality by System	Advanced RO	Advanced EDI	Mini	Mini UV	Mini Essential
Type 1 Water			■	■	■
Type 2 Water		■			
Type 3 Water	■				
Daily Water Consumption by System¹					
Type 1 ultrapure water up to approx. 10 Liter/day			■	■	■
Type 1 ultrapure water approx. 10–40 Liter/day					
Type 1 ultrapure water approx. 40–100 Liter/day					
Type 2 pure water up to approx. 120 Liter/day (5 L/h)	■				
Type 2 pure water up to approx. 150 Liter/day (10 L/h)		■			
Type 3 pure water up to approx. 140 Liter/day (8 L/h)		■			
Type 3 pure water up to approx. 200 Liter/day (16 L/h)		■			
Type 3 pure water up to approx. 270 Liter/day (24 L/h)		■			

Lab Water Application Overview | System Requirements by Application

Feed Applications					
Feed ultrapure water systems	■	■			
Feed distilled systems	■	■			
Water for Laboratory devices (Autoclaves Washing Machine Ice Machine etc.)	■	■			
Manual Feed for Laboratory Equipment (Water Bath etc.)	■	■			
Non-critical Applications					
Buffer, media, and pH solutions	■	■	■	■	■
Rinsing	■	■	■	■	■
AAS (Atomic Absorption Spectroscopy)	■	■	■	■	■
ELISA (Enzyme-Linked Immunosorbent Assay)	■	■	■	■	■
Electrophoresis	■	■	■	■	■
Histology	■	■	■	■	■
Photometry	■	■	■	■	■
Preparation of reagents, blank samples, etc.	■	■	■	■	■
Solutions for chemical analysis and synthesis	■	■	■	■	■
Critical Life Science Applications					
DNA Sequencing		■ ²		■ ²	
Endotoxin analysis		■ ²		■ ²	
Immunocytochemistry		■ ²		■ ²	
Northern Blot		■ ²		■ ²	
Nutrient media for cell culture (Mammalia & plant)		■ ²		■ ²	
PCR (Polymerase Chain Reaction)		■ ²		■ ²	
Production of monoclonal antibodies		■ ²		■ ²	
Southern Blot		■ ²		■ ²	
Western Blot		■ ²		■ ²	
Critical Analytical Applications					
GC-MS (Gas Chromatography-Mass Spectrometry)		■			
GF-AAS (Graphite Furnace Atomic Absorption Spectrometry)		■			
HPLC (High-Performance Liquid Chromatography)		■			
IC (Ion chromatography)		■			
ICP-MS (Inductively Coupled Plasma Mass Spectrometry)		■			
SPE (Solid phase extraction)		■			
TOC analysis		■			
Trace metal analysis		■			

All displayed applicable systems starting with the minimal requested water quality criteria: ¹Average indication depending on incoming feed water, ²Only in combination with an Arium® Cell Plus ultrafilter.

Tech Corner

Histology

Histology is the study of the microscopic anatomy of cells and tissues of plants and animals, performed by examining a thin slice (section) of tissue under an electron or light microscope. Water is used for rinsing, preparation of buffered solutions, etc. Histopathology, the microscopic study of diseased tissue, is an important tool in the diagnosis of cancer and other diseases.

Photometry

Photometry studies measurement of visible light (around 360 nm to 830 nm). In chemistry, it is used for example to determine substances and their concentrations (ppm level) by colour reaction and light absorbance. Every substance has a specific chemical property at a specific wavelength. The measurements are done by a photometer, of which there are different kinds depending on application.

Water is used in photometry to prepare samples. Impurities in the water may affect the results, but in general Type 2 pure water is sufficient. For more critical applications, Type 1 ultrapure water with low level of TOC might be needed.

Preparation of Reagents, Blank Samples, etc.

The water required for diluting samples, blanks, reagents and standards must be of sufficient purity that subsequent analyses are not affected. These can range from ppb to ppt levels. Minimum Type 2 pure water is recommended, but for more critical applications higher quality might be needed with specific focus on either TOC or endotoxins, pyrogens or nucleic acids.

Rinsing

Rinsing is a common laboratory practice. Washing glass or plastic ware between experiments or washing equipment are two examples of when it might be necessary to rinse off chemical or particle residues.

Purified water is usually used to avoid deposits and impurities from tap water. RO or pure water is usually sufficient for rinsing, but for some applications higher quality water might be needed.

Solutions for Chemical Analysis and Synthesis

Chemical analysis includes classical and instrumental methods. Both methods can be used for identification, separation and quantity measurements with various sensitivity. Chemical synthesis is used to produce new products with the help of reagents. Water is often used to produce the solution in which the reagents can react, after which a purification process might be needed to isolate the final product.

The water used in producing the solutions should be free of impurities that could inhibit reactions or falsify results. Generally, Type 2 pure water is sufficient but, depending on criticality and method, ultrapure water with low TOC might be needed.

Critical Life Science Applications

When it comes to Life Science Applications, water is commonly used to prepare buffers or other solutions and samples, or for washing the samples. Therefore, it is important to have water with a low level of endotoxins and other pyrogens, nucleic acids, nucleases or microorganisms to ensure reliable and reproducible results. This generally means ultrapure water is needed with special attention to these impurities.

DNA Sequencing

DNA sequencing refers to sequencing methods for determining the order of nucleotide bases in a molecule of DNA. The generation of DNA fragments – generally by a PCR-based technique – is followed by separation of the fragments by capillary or regular agarose gel electrophoresis. Endotoxin and pyrogen free Type 1 ultrapure water is needed.

Endotoxin Analysis

Bacterial endotoxins are generally lipopolysaccharides (LPS) which have pyrogenic effects and are therefore very undesired in some applications. Endotoxin analysis is performed by three technologies with the help of a microplate reader:

Gel-Clot, Endpoint Analysis and Kinetic Assays. All techniques are based on the LAL-test (limulus amoebocyte lysate), which is a sensitive reagent for detecting bacterial endotoxins.

Water is used to prepare buffers and sample dilutions. Endotoxin and pyrogen-free water is essential.

Immunocytochemistry

Immunocytochemistry (ICC) – also known as immunofluorescence (IF) – is used to visualize/label proteins or antigens, typically with the help of an antibody (primary or secondary) with an attached fluorophore. However, magnetic or radioactive labelling is possible. This determines if a specific protein or antigen is present in a sample and in that case where it is present.

Immunocytochemistry is a sensitive technique that requires ultrapure water to avoid interferences.

Northern and Southern Blot

The Southern blot is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.

The Northern blot is a technique used to study gene expression by detection of RNA in a sample.

Pyrogen and nuclease-free Type 1 ultrapure water is used for many steps of blotting: sample, buffer, gels and preparation of rinsing solutions. Prior to blotting itself – for Southern or Northern blotting – sample preparation requires the highest purity of water to protect the raw material DNA or RNA.

Nutrient Media for Cell Culture (Mammalian and Plant)

To support the growth of cells in cell culture, a nutrient or growth media is needed. Depending on the cells cultivated, different components or ratios of nutrients might be needed in the media.

Nutrient/growth media can be produced by mixing water with the necessary nutrients. It is recommended to use ultrapure water that is low in nucleic acids, nucleases and pyrogens | endotoxins.

PCR (Polymerase Chain Reaction)

PCR is a technique used in molecular biology to amplify a single piece of DNA (or a few copies) by several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

PCR and related PCR-based techniques, including quantitative PCR and reverse transcriptase PCR, require nuclease-free and endotoxin-free water to avoid degradation of the nucleic acid. Water should also be free of specific ions, organics and bacteria. Pyrogen and nuclease-free Type 1 ultrapure water is essential.

Production of Monoclonal Antibodies

Monoclonal antibodies are antibodies that can bind to a single epitope of an antigen. They are widely used in biochemistry, molecular biology and medicine where they can help determine the presence of a specific antigen in a sample. Monoclonal antibodies are also used to diagnose and treat several diseases.

To produce monoclonal antibodies, you will need ultrapure water with low level of pyrogens, endotoxins, nucleases or nucleic acids.

Western Blot

The western blot is an analytical technique used to detect specific proteins in a sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native | non-denaturing conditions).

In Western blotting, water is used to prepare the transfer buffer, buffers used to prepare the blocking solution, primary and secondary antibodies and the wash solutions. It is critical to use water free of impurities that are biologically active and could interfere. Pyrogen free Type 1 ultrapure water is essential.

Critical Analytical Applications

When sensitivity or accuracy is needed, it also brings higher requirements on the quality and purity of the water used. Critical Analytical Applications refer to applications where analyses are performed with highly sensitive instruments in a wide range of areas. At this stage, TOC (total organic carbon) is usually a concern. Therefore, water for preparing blanks and samples needs to be low in particles and other impurities that can interfere with the analysis or create blockage in the instrument itself.

GC-MS (Gas Chromatography–Mass Spectrometry)

GC and GC-MS are the most sensitive and widely used techniques for separation and determination of the components of mixtures of volatile or semi-volatile compounds. Samples are volatilized and separated on a column. The technique is especially powerful when a mass spectrometer is used as a detector, as individual components can then be characterized and identified directly.

Type I ultrapure water is typically used in sample preparation. Low TOC is essential.

GF-AAS (Graphite Furnace Atomic Absorption Spectrometry)

GF-AAS is a variant of AAS which uses a small carbon tube rather than a flame to atomize the sample. It is a more sensitive method than AAS, and suitable for measurements of ultra-trace levels of elements. The detection limits for many elements are well below 1 ppb.

Water is used to prepare blanks, standards and sample preparation and needs to be extremely low in relevant impurities. Type 1 ultrapure water is required.

HPLC (High-Performance Liquid Chromatography)

HPLC can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. It is one of the most powerful tools in analytical chemistry. With suitable choice of methodology, HPLC can separate, identify and quantify most compounds present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion may be identified with the appropriate detector.

Type 1 ultrapure water with typical TOC less than ≤ 2 ppb is needed for the more demanding applications.

IC (Ion Chromatography)

IC is a chromatographic technique that allows the separation of ions and polar molecules based on their interaction with a column of ion-exchange media. It can be used for almost any charged molecule including large proteins, small nucleotides and amino acids. It is widely used for determining inorganic and organic cations and especially anions. With pre-concentration, low-ppt concentrations may be measured. Type 1 ultrapure water is needed for blanks, standards, sample preparation and eluents.

ICP-MS (Inductively Coupled Plasma Mass Spectrometry)

ICP-MS uses a high temperature source and a mass spectrometer to provide the most sensitive method for general analysis of elements in solution with detection limits below 1 ppt for many elements. Clean room conditions are preferred.

Water is extensively used for sample preparation, standards and blanks. Type 1 ultrapure water, free from elemental impurities, is essential. Low levels of organics are also desirable.

SPE (Solid Phase Extraction)

SPE is a semi-automated separation and concentration process by which compounds that are dissolved or suspended in a liquid mixture are taken up on a solid substrate and subsequently eluted before analysis.

For trace analysis, water of the highest organic purity is needed to prepare blanks and standards and to rinse the solid phase. Type 1 ultrapure water with a TOC of less than ≤ 2 ppb is needed.

TOC Analysis

TOC is the amount of carbon bound in organic compounds, (i.e., in an aqueous solution). It is often used as a non-specific indicator of the level of organic impurities in water or cleanliness of pharmaceutical manufacturing equipment.

Water is used to prepare the standards necessary for instrument calibration, run the blanks and to clean or rinse the instrument. To optimize the performances of TOC analysis – in particular the concentration range below 1 ppm – it is important to have water with very low TOC.

Trace Metal Analysis

Trace metal detection is carried out by a variety of methods. Due to its extremely high sensitivity and specificity, ICP-MS has become very popular. ICP- AES is still widely used for multi-element analysis, and AAS provides a cheaper alternative when fewer elements are being determined.

Spectrophotometric and electrochemical detection have advantages in specific cases. Water purity requirements depend on the sensitivity needed. Type 1 ultrapure water is generally recommended.

Implen OD600®: Revolutionizing Microbial Growth Analysis

The Implen OD600® is a user-friendly device for measuring sample optical density at ~600 nm, ideal for various applications including cell growth rates and McFarland turbidity measurements.

Key Features

- ◆ Higher absorbance range, accuracy and reproducibility
- ◆ Utmost compatibility (DiluCell, Cuvette, Tubes) without adapters
- ◆ No maintenance or recalibration needed ever
- ◆ Pre-programmed with Intuitive OD600 & McFarland Applications
- ◆ Glove-Compatible 3.5" Touchscreen Display
- ◆ High Precision Versatile Measurements, up to 4 A
- ◆ Extremely Lightweight, Compact and Portable

The new Implen
OD600®



Product Highlight



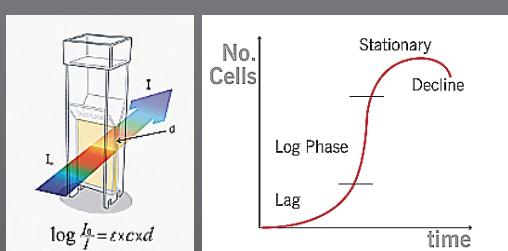
The Implen OD600® is highly adaptable and versatile, supporting various cuvettes and test tubes without the need for adapters, including DiluCells, 10 mm macro and semi-micro cuvettes, and 10-, 12-, 16-, and 18-mm glass/falcon tubes.



Built-in Applications to Measure Bacterial Growth

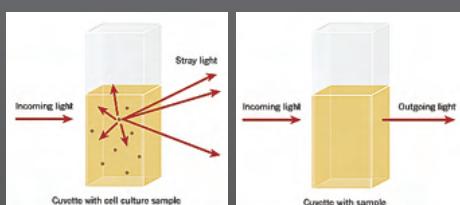
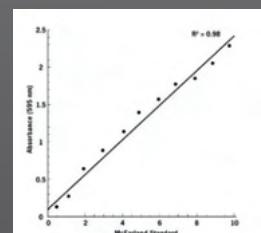
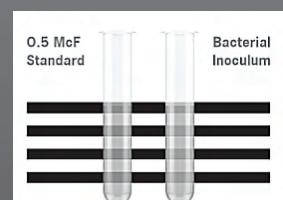
OD600 Application

- ◆ Cell Density, Bacterial Growth, Yeast Growth
- ◆ Beer-Lambert Law



McFarland Application

- ◆ Cell Suspension Density, Turbidity Solutions
- ◆ The McFarland method



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Air jacket CO_2 incubator

Touch Screen Controller (With timing function)

- Precision temperature control (37%)
- stable CO_2 concentration control(5%)
- Saturated humidity environment(≥95%)
- Effective microbial contamination prevention

Applications:



Basic research



Cell Biology



Biotechnology



Clinical University Hospital



Organizational engineering



IVF

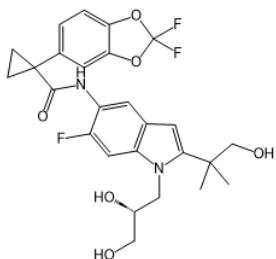
Features:

- LCD screen controller
- CO_2 concentration sensor
- Temperature control and monitoring system
- Pollution proof control
- Cycle fan speed adjustable
- CO_2 inlet control system
- Safety Functions
- Documentation and failure diagnostic display (Option)
- automatically



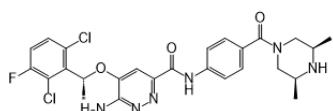


Recent novel FDA approved drugs



Tezacaftor

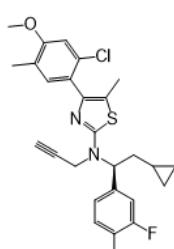
Alyftrek Containing active ingredients vanzacaftor, tezacaftor, and deutivacaftor, it is used for the treatment of cystic fibrosis (CF) in people aged 6 years and older who have at least one F508del mutation or another responsive mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.



Ensartinib

Ensacove Contains active ingredient ensartinib, used to treat adults with non-small cell lung cancer (NSCLC) that has spread and is caused by an abnormal ALK gene.

Ensacove works by blocking a specific protein in cancer cells called anaplastic lymphoma kinase (ALK). ALK is a protein that helps cancer cells grow and spread. By blocking ALK, Ensacove can help to stop cancer cells from growing and spreading.



Crenessity

Crenessity is used to treat adults and children 4 years of age and older with classic congenital adrenal hyperplasia (CAH).

CAH is a genetic disorder that affects the adrenal glands, which are located on top of the kidneys. The adrenal glands produce hormones that help regulate the body's metabolism, blood pressure, and immune system. People with CAH have a deficiency in an enzyme that is needed to produce certain hormones. This can lead to an overproduction of other hormones, such as androgens.

Crenessity works by blocking a protein called corticotropin-releasing factor type 1 receptor (CRF1). CRF1 is involved in the production of androgens. By blocking CRF1, Crenessity can help to reduce the production of androgens.

Industry Buzz

CPHI & PMEC
India Expo
Greater Noida, 2024

PHARMLABCHEM EXPO

Indore, 2025



A sneak-peak at our events

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**Hei-TORQUE
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Light weight, stirs powerfully up to 40 Ncm



**Hei-TORQUE
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Powerful stirring up to 400 Ncm



**Hei-TORQUE
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Powerful stirring up to 400 Ncm with full control

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- Easy to change the stirrer - without the need for extra tools
- Achieve perfect homogeneity with a complete system solution



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