

CATALYST Cue

VOLUME 01

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The Rise of PFAS:
Are We All Contaminated?

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in PFAS Detection

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From the editor

Dear Readers,

Sometimes the smallest compounds leave the biggest impact. This issue of CATALYSTCue begins with one such story—the complex world of PFAS, often called the “forever chemicals.” Our cover story takes you through the threats PFAS pose to our health and why there is a global need to reduce these chemicals.

This issue brings together smart, efficient, and future-focused solutions across analytical chemistry, spectroscopy, chromatography, and materials science. From streamlining liquid chromatography workflows to automating UV/VIS calibration, we explore tools that help researchers scale faster, work smarter, and reduce human error.

You'll also find insights on:

- Accelerated drug discovery using combined TLC and mass spectrometry
- Improved freeze-drying techniques for sensitive pharmaceutical ingredients
- Thermal analysis for polymers and what structural changes reveal
- Simplified nitrogen and protein detection in everyday food samples
- Cleaner lab water workflows for improved compliance
- Flow chemistry systems that bring consistency to hydrogenation
- And a deep-dive into how labs are approaching PFAS analysis as per latest guidelines

Our Tech Corner simplifies weighing terminology for smoother lab routines, and the Product Highlight focuses on how automation and digital connectivity are reshaping both R&D and QC environments.

This edition also includes a special Core Lab Solutions supplement—a toolkit of ideas and workflows that support the full lab lifecycle, from synthesis and scale-up to stability and structure.

We hope this issue adds value to your work and inspires new directions in how you approach research, lab efficiency, and compliance.

Best regards,

Arun Mathrubootham
Director
Inkarp Instruments Pvt. Ltd.

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The Rise of PFAS

Are We All Contaminated?



Per- and polyfluoroalkyl substances (PFAS) are a group of over 4,000 synthetic chemicals used in a wide array of industrial and consumer products. Known for their water- and oil-resistant properties, PFAS have been the go-to solution for everything from nonstick cookware to waterproof clothing and fire-fighting foam.

The Rise of PFAS: A Miracle of Modern Chemistry

Developed in the mid-20th century, PFAS became prized for their unique properties. Their resistance to heat, water, and oil made them invaluable in a wide range of consumer products and industrial applications. Non-stick cookware, waterproof fabrics, food packaging, firefighting foam—PFAS were found in everything from the clothes we wear to the products we use in our kitchens.

Once hailed as a marvel of modern chemistry, PFAS are now the subject of growing scrutiny due to their widespread use and their potential long-term health impacts. But as we continue to learn more about these "forever chemicals," one question looms large: *Are we all contaminated?*

Why Are PFAS So Toxic?

The reason PFAS are so dangerous to both the environment and human health lies in their molecular structure. Composed of carbon-fluorine bonds, which are among the strongest in nature, PFAS are extraordinarily stable. These carbon-fluorine bonds make PFAS resistant to heat, light, and chemical reactions, meaning they do not degrade easily through natural processes like biodegradation or photodegradation. This earned them the nickname "*forever chemicals.*"

Their chemical stability, while beneficial for industrial uses, makes them a persistent environmental threat. Unlike other chemicals, they don't break down easily in the environment or in our bodies. The chemicals accumulate in the tissues of living organisms over time, a process known as bioaccumulation. Because of this, PFAS can move up the food chain, with concentrations rising as they pass from lower organisms to predators, including humans. This is particularly concerning for areas such as aquatic ecosystems, where PFAS have been found in everything from fish to aquatic plants, to humans who consume contaminated water or seafood.

Widespread Contamination

The issue with PFAS contamination isn't just limited to certain products or industries. It's a global problem. Studies have shown that PFAS can be found in water, air, and soil across the globe. Contaminated drinking water supplies are among the most significant sources of exposure. It's not just remote areas or industrial zones that are affected—PFAS contamination have been found in rural towns, urban centres, and even in the most pristine environments, far from any industrial activity.

Unique Health Concerns: The Toxicity of PFAS

Research into the health effects of PFAS exposure is still ongoing, but there is already compelling evidence linking PFAS to a wide range of serious health conditions. Studies have shown that certain PFAS chemicals are carcinogenic, increasing the risk of kidney and testicular cancers. Additionally, PFAS exposure has been associated with liver damage, thyroid disease, immune system suppression, developmental delays in children, and increased cholesterol levels. Given their widespread presence in everyday items, even people who are unaware of exposure may have PFAS in their bodies, heightening public health concerns.

Moreover, PFAS have been linked to reproductive issues, particularly in women. Some studies suggest that exposure to PFAS can reduce fertility and lead to complications during pregnancy, including preterm birth and low birth weight. These findings add to concerns about the long-term effects on future generations, as PFAS can be passed from mother to child through the placenta and breast milk.

High levels of PFOS and PFOA were found in infants; specifically, PFOS was detected in 297 out of 299 infants, and PFOA was found in all 299¹!

So, as we consider all this, we return to the fundamental question: Are we all contaminated? The answer, based on current scientific understanding, unfortunately leans towards a disturbing "YES"

What's Being Done

Recognizing the risks of PFAS contamination, governments and organizations around the world are acting. The U.S. Environmental Protection Agency (EPA) has started to set new guidelines for PFAS levels in drinking water and has pledged to investigate new methods for detecting and removing these chemicals from water supplies.

In addition, researchers are racing to develop better ways of detecting PFAS in the environment and in human tissue. Some companies are working on technologies to filter PFAS out of water supplies, and new techniques to break down PFAS in contaminated soils are also being tested.

However, the scale of the problem presents significant challenges. Clean-up efforts are slow, expensive, and complicated. The chemicals' persistence in the environment means that remediation projects will take years, if not decades, to fully address.

1. Apelberg, B. J.; Goldman, L. R.; Calafat, A. M.; Herbstman, J. B.; Kuklenyik, Z.; Heidler, J.; Needham, L. L.; Halden, R. U.; Witter, F. R. Determinants of Fetal Exposure to Polyfluoroalkyl Compounds in Baltimore, Maryland. *Environmental Science & Technology* 2007, 41 (11), 3891–3897. <https://doi.org/10.1021/es0700911>

Automated Performance Verification Advances in UV/VIS Spectroscopy

In UV/VIS spectroscopy, regular performance verification is essential to ensure accurate and reliable instrument performance. Widely accepted guidelines for performance verification of spectrophotometers are described in the US Pharmacopeia (USP). The recommended tests include the check of photometric accuracy and repeatability, wavelength accuracy and repeatability, instrument resolution as well as stray light measurement. The USP recently introduced a new chapter on ultra-violet visible spectroscopy and adapted the test for stray light. Here, the methods for measuring stray light have been compared, according to the current and previous version of the USP. Also, advantages of the new test have been assessed and automated optical performance verification has been introduced.

Performance verification in UV/VIS Spectroscopy

A. CertiRef™ – Automated and Pharmacopoeia-compliant Performance Verification

Regulated environments require performance verification of UV/VIS spectrophotometers on a regular basis. Instrument performance is the main factor directly affecting the accuracy and repeatability of measurements. It is therefore important that it be

monitored regularly, and documentary evidence is provided. As the procedure for performance verification is intricate and time consuming there are great benefits in integrating it in the analytical workflow. For this reason METTLER TOLEDO has developed an accessory, the CertiRef, for automatic calibration and performance tests of the UV/VIS Excellence spectrophotometer instrument line.

The CertiRef module offers a full US Pharmacopoeia compliant solution for the automated performance verification of the UV/VIS Excellence spectrophotometers UV7, UV5 and UV5Bio.

1. How it works

The quality of UV/VIS spectrophotometry measurements is quantified in its spectrum. Performance tests have to verify:

- That the wavelength positions (x axis) are correct (wavelength accuracy) and stable (wavelength repeatability),
- That the intensities, absorbances or transmittances (y axis) are correctly measured (photometric accuracy) and stable (photometric repeatability),

- That the measured shape of the spectrum is correct and not distorted (resolution toluene, stray light).

Certified reference materials (CRMs) manufactured and certified by Starna Scientific Ltd (England), are used to carry out the tests. The CRMs are contained in heat sealed, quartz cuvettes and accommodated in the CertiRef module which protects the cuvettes against light and damage, allowing long-term use. The fully automated system (Figure 1) runs the pharmacopoeia performance calibration tests in less than 10 minutes, displaying detailed results on the terminal screen once completed. As the results are also stored in the instrument, it is possible to call up the control charts of up to 100 past calibrations. The results of each test can be printed or stored as a PDF file.

When mounted, the compact design of the CertiRef module makes it an integral feature of the UV/VIS Excellence spectrophotometer. It can remain mounted in place at all times; the CertiRef does not interfere with any manual or automated measurements with the eight position CuvetteChanger or FillPalMini pump. Instruments increasingly have to be accredited to internationally recognized standards of Good Laboratory Practice, ISO/IEC 17025 or ISO 9001. This brings with it the need to provide evidence of control of the instrument performance and is only possible if the certified reference materials used adhere to the concept of traceability. The concept of traceability is defined in the "International Vocabulary of Basic and General Terms in Metrology (ISO, 1993)" It states that all Certified Reference Materials must have a defined traceable path to recognized primary materials. This ensures that compatible measurements are made across national borders, resulting in unambiguous and reliable communication of specifications.

Two types of CertiRef modules are available, namely a European Pharmacopoeia (EP) and a United States Pharmacopoeia (USP) compliant module, respectively. All CRMs are traceable to the USA National Institute of Standards and Technology (NIST) primary standards and are manufactured in an environment that is

accredited by UKAS under ISO Guide 34 (4001) and ISO 17025 (0659). The module can also be re-certified and the certificate is valid for a maximum period of two years from the date of issue – or sooner if specified by the user's own protocols. Table 1 shows the optical parameters, which can be automatically measured with the CertiRef module. Stray light, in particular, is one of the most important factors influencing the photometric accuracy and precision. The CertiRef USP configuration complies with the UV/VIS spectroscopy chapter <857> of the USP 40 and is described in more detail.



Figure 1: The CertiRef module mounted on top of a METTLER TOLEDO UV7 spectrophotometer.

| CertiRef Test | Reference Material | EP | USP |
|---|--|----|-----|
| Resolution | Hexane blank | ● | ● |
| | Toluene in hexane | ● | ● |
| Wavelength accuracy Wavelength repeatability | Holium oxide | ● | ● |
| | Perchloric acid blank | ● | ● |
| Photometric accuracy Photometric repeatability | Potassium dichromate | ● | ● |
| | Water blank | ● | — |
| Stray Light EP | Potassium chloride, 1.2% aqueous solution | ● | — |
| | Potassium chloride, 1.2% aqueous solution, 0.5cm | — | ● |
| Stray Light USP | Potassium chloride, 1.2% aqueous solution, 1 cm pathlength | — | ● |

Table 1: Optical parameters that can be automatically measured with the CertiRef module either in the European or United States Pharmacopoeia (EP, USP) compliant version.

2. What are the benefits of CertiRef?

In addition to traceable regulatory compliance, the CertiRef module offers the benefit of fully automating the tedious and error-prone manual workflow of repetitive measurements of the certified reference materials (CRM) in single sealed cuvettes, which include the following steps:

Preparatory work: Instrument warm-up and preparing CRM cuvettes

Running of tests: Changing CRM and blank cuvettes

After run handling: Cleaning and storing of CRM cuvettes

Calculation: Calculation and validation of results

Reporting: Compiling data and creating formal report

Important optical parameters such as resolution, wavelength accuracy/repeatability photometric accuracy/repeatability and straylight can be measured within 10 minutes, fully unattended. With CertiRef, the time to result, including traceable documentation and secure validation, is approximately 3 times faster than manually executed performance measurements. Workflow execution and performance data transcription errors can be excluded avoiding test repetition. In total, precious time is saved for other productive analytical tasks. Comparing the regular automated, mainly monthly, performance check of the instrument with manual checks using single CRMs in sealed cuvettes, the CertiRef investment pays back within a reasonable time frame of 1.5–2 years. This is dependent on the number of performance tests per individual run. As the unit can reside on the UV/VIS Spectrophotometer without interfering with sample measurements, performance checks prior to each sample series measurements can be considered. The execution is controlled simply with a One Click shortcut on the instrument's terminal. A further level of automation is possible when connecting the instrument to a PC with LabX software. The automated performance check with CertiRef can be scheduled as an automatic task by the LabX task

scheduler before the actual measurements takes place. This ensures that the instrument is always in the condition required for accurate spectroscopic measurements.

B. Stray Light – A Fundamental Parameter

Stray light, or stray radiant energy, is a common confounding factor in spectrophotometric measurements. It is defined as light from a source other than the instrument's light source, which does not follow the optical path. Although the measurement of absorbance or transmittance is, in theory, independent of monochromatic source intensity (it is a ratio measurement of intensities); in practice it affects linearity. When stray light reaches the detector it causes the negative deviation of the linear relationship between concentration and absorbance (Beer-Lambert law), especially at high concentrations (Figure 2). The resulting light intensity is measured as higher than it should be. Conversely, the absorbance of the sample appears lower. The results of sample measurements affected by stray light include a systematic error that can easily go unnoticed. Furthermore, the adverse effects of stray light tend to increase over time with aging of optical components and lamps in the spectrophotometer. The stringent requirements of the US and EU Pharmacopoeias require that stray light be smaller than 1% transmittance. Due to its significant effect on practical measurements, stray light is detected at a given wavelength with a suitable liquid filters (CRMs), as part of regular instrument performance verification.

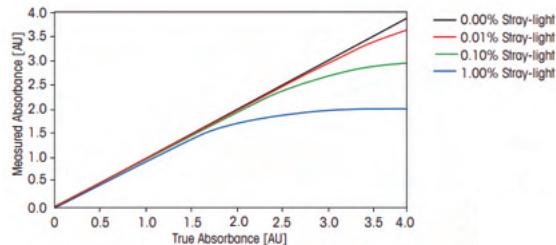


Figure 2: Effects of stray light on the linearity of optical spectrophotometer.

1. Compliance to the Latest USP Stray Light Regulations

In accordance with the pharmacopoeias, the CertiRef EUP and CertiRef USP apply ASTM E387-04 standard methods to test for stray light. The limits specified in both the European Pharmacopoeia (EP 8, chapter 2.2.25) and US Pharmacopeias (USP version 38 and 39,^{3,4}) are tested with the two methods described below:

1. Specific Wavelength Method (SWM) The specific wavelength method is well established and complies with the current EP 8, chapter 2.2.25 and the older USP 38. It was regarded as the preferred method for stray light testing by the US Pharmacopeia for many years. The method is based on measuring a material with a sharp cut-off wavelength against water as compensation liquid (blank). In theory, measurement of a cut-off filter would lead to infinite absorbance below the cut-off wavelength (all light is absorbed). In practice, the light that is detected results from stray light (Figure 2). The METTLER TOLEDO CertiRef EUP applies the specific wavelength method.

| | |
|-------------------|---|
| Definition | “...Stray light may be detected at a given wavelength with suitable filters or solutions: for example the absorbance of a 12 g/L solution of potassium chloride in a 1 cm cell should be greater than two at 198 nm when compared with water as the compensation liquid.” |
| Limits | >2.0 absorbance at 198 nm (KCl) |
| Test | SWM using KCl in a 1 cm cuvette against water as a blank in a 1 cm cuvette. |

Table 2: CertiRef EUP stray light test as described in the European Pharmacopoeia. (EP 8, chapter 2.2.25)

| | |
|--|---|
| Definition | “...Stray light can be detected at a given wavelength with a suitable liquid filter. These solutions are available as CRMs...This procedure simply requires the 10 mm cell measurement to be referenced against the 5-mm cell (filled with the same filter)...” with the same filter)...” |
| Spectral Range of Selected Material for Monitoring Stray Light | |
| Spectral Range (nm) | Liquid or Solution |
| 190 – 205 | Aqueous potassium chloride (12 g/L) |
| Limits | ≥0.7 absorbance at the peak maximum (KCl) |
| Test | SFRM using KCl in a 1 cm cuvette against KCl blank in a 0.5 cm cuvette |

Table 3: CertiRef USP stray light test as described in the US Pharmacopoeia. (USP 39 chapter 857)

Table 3: CertiRef USP stray light test as described in the US Pharmacopoeia. (USP 39 chapter 857)

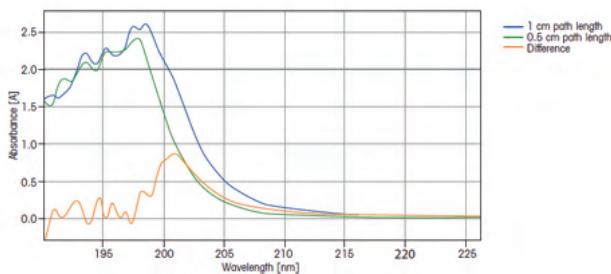


Figure 3: Comparison of the specific wavelength method (SWM) and the solution filter ratio method (SFRM). The blue line shows the measurement of KCl in a 1 cm cuvette against water as blank (SWM), the orange line shows the measurement of KCl in a 1 cm cuvette against a 0.5 cm cuvette as blank (SFRM).

2. Good UV/VIS Practice

Good UV/VIS Practice (GUVP™) improves measurement quality by minimizing risks through a 5-step lifecycle program. It provides professional evaluation & selection tools, comprehensive installation & qualification services and training and maintenance programs. Each step is thoroughly documented to support traceability and regulatory compliance.

Step 1 & 2 – Evaluation and Selection

The GUVP design qualification tool DQmate helps define current and future requirements.

Step 3 – Installation and Qualification

In order to fulfill regulatory requirements, qualification of the instrument is required at installation with easy to understand and traceable documentation.

Step 4 – Training

Correctly trained users make fewer errors. Guided by a specialist, the EduPac training package explains efficient and safe operation with practical exercises.

Step 5 – Routine Operation

Keeping the instruments in good working order is an important component of routine operation. For routine testing by the user, the CertiRef™ module provides automated USP- or EP-compliant performance verification based on NIST-traceable certified reference materials.

3. Instrument Qualification

EQPac

The METTLER TOLEDO EQPac provides a comprehensive installation and fully documented qualification solution for UV/VIS spectrophotometers. It complies with the strictest regulatory requirements regarding installation, operation, and performance qualification. The UV/VIS EQPac is the tool of choice for regulated environments:

IQ: Installation Qualification – Verification of complete delivery and correct installation in suitable environmental conditions.

OQ: Operational Qualification – Functional, operational and performance verification tests to confirm the accurate performance of the instrument in the installed location.

Initial Calibration – Traceable calibration certificate performed using the Pharmacopoeia-compliant CertiRef module to meet quality, industry & regulatory requirements.

User familiarization – Educational overview to operators and maintenance personnel to ensure correct operation of the instrument and accessories.

Lifecycle Support – Comprehensive testing and documentation supporting PQ & MQ for regulated environments.

Comprehensive documentation – Detailed documentary evidence of equipment qualification, suitable for highly-regulated industries.

Compliance with: GMDP/GLP; ISO; FDA; HACCP; USP; EP

StarterPac

If comprehensive fully documented qualification is not required, the StarterPac provides an easy and quickly

executed solution to document complete shipment and correct installation and functioning of the UV/VIS instrument. Users receive a basic introduction to the operation and care of the instrument. The UV/VIS StarterPac is the ideal choice for internal quality management system.

4. Routine Operation

Preventive maintenance

Preventive maintenance supports the requirement of accurate and reliable results. When performed by factory trained and certified technicians, it reduces the risk of breakdown by regularly monitoring equipment deterioration and restoring equipment to proper condition. Regular preventive maintenance will help to extend the lifetime of equipment.

Preventive maintenance includes:

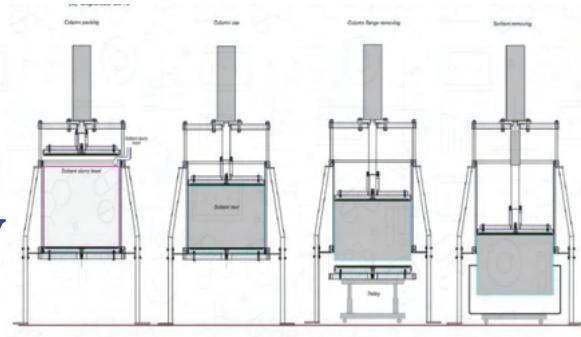
- Inspection and cleaning
- Functional and operational checks
- Documentary evidence of service, including equipment condition

Calibration and certification

With regular instrument calibration and adjustment using certified reference materials, you can trust your measurements, be compliant and avoid the costs of repeat analysis due to inaccurate results.



Process Development Optimisation and Scale-up in Liquid Chromatography



In industries like pharmaceuticals and biotechnology, efficient LC processes are much required for product purity and yield. Optimization reduces solvent consumption, analysis time and improves resolution. Scale-up on the other hand, poses challenges in maintaining performance and consistency.

This article explores the key aspects of process development, optimisation and scale-up in liquid chromatography, ensuring successful translation from lab-scale to industrial-scale production.

Initial stages of process development starts by identifying the objectives of separation, the critical quality attributes (CQAs) and selecting the appropriate chromatographic conditions like selecting the column type, mobile phase, detector. At this stage, considering factors such as, the complexity of the sample, analyte concentrations and separation specificity are extremely crucial.

Process Development and Optimisation

Careful optimisation of the separation conditions ensures highest efficiency and thus requires special attention. The objective is to maximise column load and minimise mobile phase wastage, hence allowing purification of larger sample quantity in minimum space.

While there are numerous techniques to achieve this, some of the key focus areas include:

A. Mobile phase:

- **Solvent selection:** The polarity of the solvents is based on the solubility properties of the analytes
- **pH Control:** Controlling the pH of the mobile phase significantly impacts the retention and selectivity, especially of the ionisable analytes.
- **Gradient optimisation:** In cases of a mixtures of samples, a solvent gradient is used to improve resolution. This gradual increase in the solvent strength helps in separating the analytes with varying polarities and affinities for the stationary phase.

B. Stationary phase:

- **Column selection and packing:** The column material like silica, polymer or hybrid materials is also chosen based on the nature of the analytes. A very important factor is the particle size of the column material. These factors ensure reproducibility, column longevity and separation efficiency.

- **Temperature and Pressure:** The temperature of the column and the pressure of the mobile phase affect the diffusion rates. These parameters are greatly useful in fine-tuning the separation conditions to improve speed and resolution.

Scale-up in LC

Scaling-up is essential for ensuring that processes and products are produced at the necessary quantities and meet commercial and regulatory standards.

Why is it necessary

- **Increased Production Needs:** As a drug candidate moves from discovery to commercialization, the required production quantities increase significantly.
- **Efficiency and Cost:** Scaling up LC methods allows for the efficient production of larger quantities of purified compounds, reducing costs and time.
- **Consistent Quality:** Maintain the quality and purity of the product at a larger scale.

Key Considerations for Scale-Up

- **Linear Scale-Up:** Maintaining the same column length and particle size (or adjusting L/dp ratio) during scale-up is crucial for maintaining separation efficiency and selectivity.
- **Non-Linear Scale-Up:** This may be necessary for bringing about significant changes in production volume.
- **Column Selection:** Using columns of identical chemistry, length, and particle size (or maintaining the L/dp ratio) simplifies the scale-up process.
- **Mobile Phase:** Using the same mobile phase composition and flow rate at both small and large scale is essential.
- **Sample Preparation:** Preparing samples at the same concentration and in the same diluent is critical.
- **Loadability:** Increasing the column diameter proportionally increases the loadability (the amount of sample that can be loaded onto the column).

Steps in LC Scale-Up

- **Method Development:** Develop a robust and efficient LC method on a small scale.
- **Scale-Up Calculations:** Perform accurate calculations to determine the necessary column dimensions and flow rates for the larger scale.
- **Pilot Plant Operations:** Test the scaled-up method in a pilot plant to validate its performance and identify any potential issues.
- **Optimization:** Optimize the scaled-up method to achieve the desired separation efficiency and throughput.

Column Selection

A DAC (Dynamic Axial Compression) column is a preparative chromatography device that uses dynamic axial compression technology for efficient and reproducible packing of chromatography columns, especially for pilot or production scale separations.

DAC columns are designed for preparative chromatography, a technique used to isolate and purify compounds in larger quantities than analytical chromatography.

Feature

Working

The DAC system uses a cylinder to apply pressure to the column, creating a uniform, high-density packed bed, which is crucial for efficient and stable separation.

Key Features

- **Axial Compression Technology (ACT):** This technology ensures a uniform and stable packing of the column material.
- **Optional Slurry Container:** Some DAC systems include a slurry container for automated packing procedures.
- **Cost-effective, Durable, and Reproducible:** DAC columns are designed to be practical and reliable for various separation scales.

Benefits

DAC columns offer a simple, user-friendly method for packing preparative scale chromatography columns, allowing for fast column packing of virtually any kind of packing material.

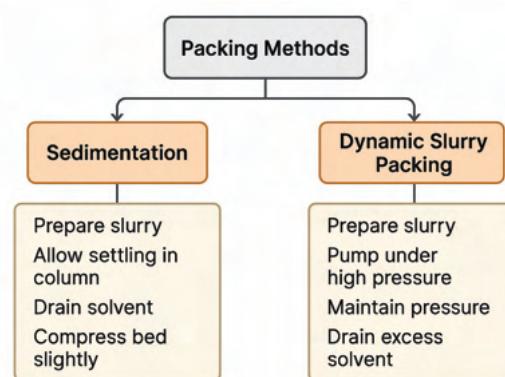
Column Packing Methods

Column packing is critical for obtaining high efficiency and reproducibility in HPLC, especially in prep columns. The two most common methods of packing are sedimentation packing and dynamic slurry packing.

Sedimentation packing relies on gravitational settling of the stationary phase. The slurry is then allowed to settle in the column. After the solvent is drained, the bed is dried and compressed.

Dynamic or High-Pressure Slurry Packing utilises high-pressure to pack the stationary phase in the column. The slurry is then introduced into the column under constant flow or pressure, ensuring dense and uniform packing.

| Feature | Sedimentation Packing | Dynamic Slurry Packing |
|-----------------------|-----------------------------|-----------------------------------|
| Packing Mechanism | Gravity/low vacuum settling | High-pressure flow |
| Equipment Required | Simple (beaker, vacuum) | High-pressure pump |
| Packing Quality | Can be uneven | Highly uniform |
| Column Efficiency | Lower | Higher |
| Best For | Low-pressure applications | High-performance preparative HPLC |
| Risk of Bed Shrinkage | High | Low |



Comparison: Sedimentation vs. Dynamic Slurry Packing

Scale-up Factors

While transitioning from analytical to prep HPLC, a scale-up factor is used to maintain linear velocity, resolution and efficiency.

- **Column diameter scaling/flow rate scaling:** A fundamental aspect of scale-up to increase the amount of sample load while maintain the same linear velocity.

$$\text{Flow rate}_{\text{prep}} = \text{Flow rate}_{\text{analytical}} \times (\text{Diameter}_{\text{prep}}/\text{Diameter}_{\text{analytical}})^2$$

Where units of flow rate is mL/min and diameter is mm.

- **Injection Volume:** The injection volume needs to be suitably scaled to maintain peak shape and loading capacity.

$$\text{Volume}_{\text{prep}} = \text{Volume} \times (\text{Diameter}_{\text{prep}}/\text{Diameter}_{\text{analytical}})^2$$

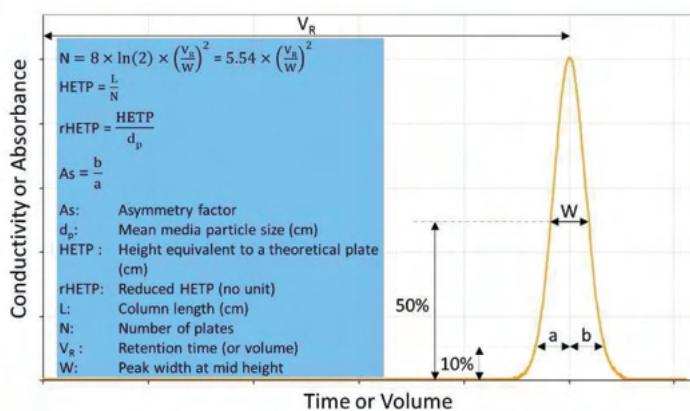
Where units of volume is μL and diameter is mm.

Packing Efficiency

One of the key factors in deciding the efficiency of chromatography columns is packing efficiency. As the name itself suggests, this describes how well a column is packed with the stationary phase. A well-packed column will give narrow and symmetrical peaks with good retention, whereas broad, distorted peaks with poor separation are seen in case of defectively packed columns.

Factor monitoring packing efficiency include

- **Theoretical plates (N):** It represents the number of hypothetical equilibrium stages within the column. Higher number denote increased column efficiency.
- **Height Equivalent to TP (HETP):** This is the length of column packing required to achieve one theoretical plate. It gives a measure of broadening of band within the column. A lower HETP value is better.
- **Reduced HETP (rHETP):** This factor is a dimensionless parameter, which allows comparisons of column efficiency, irrespective of the particle size. A lower rHETP value is better.



Conclusion

Process development, optimisation and scale-up from analytical to prep HPLC can be successfully carried out with a carefully calculated and systematic approach.

Parameters such as column dimensions, flow rates, sample load method significantly affect the output. By applying appropriate scale-up factor, it is possible to achieve high chromatographic performance while effectively increasing throughput.

TOP-QUALITY PREPARATIVE COLUMNS



DAC PC01-HPLC Columns
with Axial Compression



DAC PC02-MPLC (Medium-pressure)
Columns for Prep Chromatography



PC04-Non-metal Columns for
Prep Chromatography

Main Features And Benefits :

- › High-performance HPLC industrial-scale columns
- › For pharma and food industry uses, etc.
- › Highly polished inner surfaces and FDA-approved construction materials
- › Configurations for ATEX environment possible
- › Customer modifications available compared to a number of other column manufacturers
- › Excellent price/performance ratio



Expert Insights on Thermal Analysis

Dr. Premchand S. Jain

General Manager, Hitachi High Tech India Pvt Ltd.
Thermal Analysis Division for India and Southeast Asia.

Dr. Jain has dedicated over four decades to the field of analytical instrumentation, holding key leadership roles at companies like PerkinElmer and Labindia Instruments.

Currently, he is the General Manager, Thermal Analysis Division at Hitachi High Tech India. His expertise spans material characterization, inorganic spectroscopy, with special focus on thermal and hyphenated techniques.

Q. What are the key trends you see in thermal analysis currently, particularly in India?

As far as thermal analysis is concerned, majority of the applications in various industries are using DSC and TGA instrumentation to a maximum extent and sometimes, for critical applications related with mechanical properties, where regulatory methods have to be made, there is a need of TMA or DMA. This requirement also arises in cases where some scientists want to develop some new polymeric studies. DMA is surely an additional need in such cases of polymeric development processes.

Q. What are some of the major challenges Indian researchers face in thermal analysis?

In any analytical instrument, challenges always relate with the sensitivity, accuracy, precision, resolution, repeatability etc, of instrument. Concerns also arise regarding the software; whether the manufacturer is offering advanced or new software which suit the researcher's applications. While it is difficult to meet the

challenges of every researcher, we are always trying to optimise our product line in terms of excellent Baseline performance, Sensitivity, Accuracy, Precision, Furnace design, Software capabilities etc. These cumulative features shall offer the best results in any Thermal Analysis equipments.

Q. Could you provide an overview of the range of thermal analysis instruments Hitachi offers? What sets your instruments and services apart?

Under the banner of thermal analysis, Hitachi offers a wide array of high-sensitivity thermal analysis systems which fall into four categories-DSC, TGA/STA, DMA and TMA. After considering the thermal analysis requirements of various customers, we have incorporated important specifications/features in our instruments that have made significant impact on data quality and gained us more popularity and recognition in the Indian market.

Interview

Q. What are the specific characteristics (accuracy, precision, sensitivity, resolution) of Hitachi's instruments?

As I already mentioned, Hitachi has added some additional features in the current thermal analysis product line, which were missing in the predecessor models. We have improved our Product line in terms of the four main instrument requirements- Baseline parameters, sensitivity, accuracy, precision and resolution for DSC and TGA unit. This has put us in the competitive landscape and is one of the reasons we see there is a growth of Hitachi business in India.

Q. Could you provide an overview of Real View technology and its key features within the NEXTA series? What are the primary benefits of using RealView for thermal analysis experiments?

As the word indicates, we see the view/ image of the sample in real time. Earlier, when the samples were heated in DSC, TGA or DMA, we could not see the physical changes happening in the samples. But now, using camera attachment, called the Real View attachment, where we can observe/ collect the physical changes in the sample at various temperatures. It is possible to view if the sample is shrinking, elongating, converting into liquid form, polymorphic changes, change in colour and so on. So, all physical characteristics of the sample with respect to changes in temperature can be captured using RealView Technology.

This gives the researcher additional useful information about the sample, helping them streamline their research studies.

Q. Can you discuss the impact of experimental parameters (heating rate, atmosphere, sample preparation) on measurement accuracy?

For every Thermal instrument, certain experimental parameters are preset, according to which calibrations are carried out. Of course, now if the heating rate is changed, the output changes too. So, the way in which calibrations are done dictates the experimental parameters to get the desired results. As far as sample

preparation is concerned, there is no major issue in thermal analysis. Irrespective of the sample type, be it a powder, granule, fibre or pellet form, thermal analysis can be carried out with ease. In DSC, care has to be taken to ensure that the sample at the bottom is flat and in good contact with sensor. In TMA or DMA, it is anyway mandatory to have samples suitable to match measuring systems used.

Q. What advice would you give to researchers who are selecting a thermal analysis instrument for their specific needs? What are the key factors they should consider?

Any researcher would first look into their application, that goes without saying. They should look into the number of samples per day to be addressed to get desired productivity, regulatory compliance for quality control, meeting ASTM standards, critical high sensitive analysis, use of small sample size, etc. So, a researcher's requirement for selection of thermal analysis depends on above aspects and select most suitable unit or configuration to meet his critical studies/applications.

Q. How do you view the competitive landscape for thermal analysis in India?

The potential of thermal analysis is itself very huge in India and this gives us tremendous opportunity to grow much further. As India is growing market due to its economy, we are sure business of TA will improve sure in the years to come.

“

We are quite successful in the Indian market, since last 4 years and we are on the rise in the market at this stage.*

”



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Waters™

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Accordance With EPA 1633 Part 2

Analysis of Aqueous Matrices using the Waters Xevo™ TQ MS

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

Waters Corporation

US EPA Method 1633 has become the foundation method for analysis of PFAS in non-potable water matrices, soils, biosolids and tissues in the United States. The method consists of sample preparation using weak anion exchange (WAX) solid phase extraction (SPE) with graphitized carbon black (GCB) clean up. This application note is the second in a series demonstrating a comprehensive solution for performing the EPA 1633 methodology. The focus of this note is preparation and analysis of authentic water samples utilizing a prototype bilayer dual-phase SPE cartridge and LC-MS/MS method on an ACQUITY™ Premier BSM FTN LC System coupled to a Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer.

Keywords: PFAS (Per- and Polyfluoroalkyl Substances), EPA Method 1633, LC-MS/MS (Liquid Chromatography-Tandem MassSpectrometry), Solid phase extraction (SPE), wastewater analysis, Waters

Introduction

US EPA Method 1633 was first introduced in August 2021 to become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids, and tissues.¹ At the time of writing this document, Method 1633 is in the 4th Draft Phase with the final version expected to be released at the end of 2023.² By the final release of EPA 1633, it will have been multi-lab validated for each type of sample matrix included in the method. The method covers 40 PFAS and utilizes isotope dilution calibration and quantitation. Required sample preparation differs slightly depending on sample type

Application Showcase

but all sample types utilize SPE on a WAX cartridge in combination with GCB clean up. EPA 1633 was created to support sample analysis for the Clean Water Act (CWA) and Department of Defense (DoD) monitoring and remediation, but it covers such a wide range of matrices and compounds that its applicability is expected to be widespread.

This is the second in a series of application notes addressing sample preparation, analysis and method performance of EPA 1633 using a comprehensive workflow of Waters technologies. This application note will focus on the preparation of authentic water samples with analysis utilizing the LC-MS/MS method established in Part 1 on an ACQUITY Premier BSM FTN UPLC System coupled to a Xevo TQ Absolute Mass Spectrometer.³ The use of a combined WAX and GCB sample extraction and clean-up workflow is demonstrated on ground water, surface water, and wastewater (influent and effluent).

Experimental

Sample Preparation

Samples discussed in this application note include ground water and surface water that were collected locally, and influent and effluent wastewater that were kindly provided by a municipal wastewater treatment facility in the Midwest United States. All water samples were collected using grab sampling directly into 250 mL high density polypropylene bottles. Samples were frozen until sample analysis according to EPA 1633 guidelines and holding times. Sample bottles were weighed prior to sample preparation (full) and after sample preparation (empty) to determine the exact volume collected in each bottle. In addition to authentic samples, the Waters ERA PFAS in Wastewater certified reference material (CRM) was processed with the samples.

A prototype bilayer dual-phase SPE cartridge containing both Weak Anion Exchange (WAX) and Graphitized Carbon Black (GCB) sorbents was used for sample preparation instead of adding dispersive GCB into the

sample for cleanup. Full sample preparation details are listed in Figure 1 and are adapted directly from the EPA 1633 method. Two changes to the method were made including a sample volume change and combining the dispersive GCB step into the SPE cartridge, as described previously. Sample volume was reduced to 250 mL from the suggested 500 mL sample in the method due to instrument sensitivity of the Xevo TQ Absolute Mass Spectrometer. Reducing sample volume reduces sample shipping and storage costs, as well as allowing for faster sample preparation when loading half the volume of sample onto an SPE cartridge. Combining the GCB and WAX into the same cartridge provides the convenience of minimizing complications from using loose material and reducing the number of steps during sample preparation without compromising the method.

1. • Spike 250 mL water sample with Extracted Internal Standard Mix (MPFAC-HIF-ES from Wellington)
• Check pH and adjust to approximately 6 if necessary
2. • Pack SPE cartridge with glass wool to half height of barrel
• Condition SPE cartridges
• 15 mL 1% (v/v) ammonium hydroxide in methanol
• 5 mL 0.3 M formic acid
3. • Load sample at 5 mL/min
• Wash cartridge with 10 mL of reagent water, ensuring to rinse reservoir with this solution
• Wash with 5 mL of 1:1 0.1M formic acid:methanol, ensuring to rinse reservoir with this solution
• Dry cartridge for 15 seconds
4. • Place collection tubes in manifold
• Rinse bottle with 5 mL 1% (v/v) ammonium hydroxide in methanol. Transfer to cartridge and elute
• Add 25 μ L acetic acid to each sample
• Spike each sample with Non Extracted Internal Standard (MPFAC-HIF-IS from Wellington)

Figure 1. Full method details of the sample preparation process used for all water samples. Adapted from EPA Method 1633.

All samples were spiked with 5 ng/L (sample concentration equivalent) of the required extracted internal standard (EIS) prior to extraction and 5 ng/L (sample concentration equivalent) of the required non-extracted internal standard (NIS) after extraction. The calibration curve range for each analyte is listed in Appendix Table 2 and was determined from the data acquired and presented in Part 1 of this application note series.³ All standards were obtained as mixes from Wellington Laboratories.

Application Showcase

LC Conditions

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

Gradient Table

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

MS Conditions

| | |
|-------------------------|--|
| MS system | Xevo TQ Absolute |
| Ionization mode | ESICapillary |
| Voltage | 0.5 kV |
| Source temperature | 100 °C |
| Desolvation temperature | 350 °C |
| Desolvation flow | 900 L/hr |
| Cone flow | 150 L/hr |
| MRM method | See Appendix for Full MRM Method details |

Data Management

| | |
|----------|----------------------------------|
| Software | waters_connect™ for Quantitation |
|----------|----------------------------------|

Application Showcase

Results and Discussion

Recovery in Water Samples

EPA 1633 is a performance-based method that allows modifications as long as the performance criteria outlined in the method are all met. One major modification presented in this work is to use a bilayer dual-phase SPE cartridge that combines the otherwise dispersive GCB clean up step into the WAX SPE cartridge. GCB is difficult to work with and accurately measure, therefore utilizing a bilayer cartridge eliminates the untidy dispersive step.

More importantly, combining the GCB cleanup step into the SPE extraction saves valuable time in the laboratory during the sample preparation process. Additionally, less preparation steps allow for fewer opportunities for introduction of unintended PFAS sample contamination.

One of the important performance criteria that must be established in order to prove equivalence of this approach is the extracted internal standard (EIS) and non-extracted internal standard (NIS) recovery acceptance limits in the 4th Draft Method 1633 (See Table 6 within that document).¹ The individual recovery performance of the bilayer dual-phase SPE cartridge for ground water, surface water (with high organic matter content), influent wastewater (settled only), and effluent wastewater (fully treated discharge water) are listed for each EIS and NIS in Table 1.

The data reported in Table 1 is the average recovery and %RSD for five replicate extractions of each matrix type. The mean recovery of all EIS among the 20 samples extracted was 91.2% with a mean RSD of 9.2%.

| Compound | Ground water | | Surface water | | Influent water | | Effluent water | |
|---------------------------------------|----------------------|------|----------------------|------|----------------------|------|----------------------|------|
| | Average recovery (%) | %RSD |
| ¹³ C ₄ -PFBA | 100.0 | 2.7 | 111.9 | 8.1 | 85.8 | 9.2 | 86.6 | 13.8 |
| ¹³ C ₅ -PFPeA | 98.6 | 4.2 | 110.1 | 8.5 | 101.6 | 5.7 | 100.3 | 15.1 |
| ¹³ C ₅ -PFHxA | 97.2 | 3.1 | 111.2 | 8.0 | 111.0 | 8.4 | 102.5 | 14.3 |
| ¹³ C ₄ -PFHpA | 97.3 | 4.5 | 108.8 | 9.2 | 111.0 | 8.5 | 99.7 | 14.8 |
| ¹³ C ₈ -PFOA | 98.8 | 2.5 | 110.6 | 9.0 | 113.0 | 13.3 | 100.7 | 16.5 |
| ¹³ C ₉ -PFNA | 96.6 | 5.2 | 110.2 | 11.3 | 112.0 | 18.0 | 101.1 | 17.5 |
| ¹³ C ₆ -PFDA | 92.1 | 2.9 | 108.1 | 9.5 | 103.8 | 18.1 | 97.0 | 17.5 |
| ¹³ C ₇ -PFUnDA | 88.5 | 2.9 | 102.0 | 4.8 | 93.0 | 18.7 | 91.6 | 15.0 |
| ¹³ C-PFDoDA | 83.1 | 2.8 | 89.8 | 8.5 | 63.8 | 18.2 | 82.5 | 14.0 |
| ¹³ C ₂ -PFTreDA | 72.5 | 5.0 | 56.7 | 10.1 | 32.3 | 17.6 | 52.5 | 13.4 |
| ¹³ C ₃ -PFBS | 97.8 | 2.3 | 110.9 | 6.5 | 116.8 | 12.3 | 102.6 | 13.5 |
| ¹³ C ₂ -PFHxS | 97.0 | 5.0 | 113.1 | 6.9 | 112.5 | 8.3 | 104.8 | 14.5 |
| ¹³ C ₈ -PFOS | 93.2 | 1.8 | 108.7 | 7.8 | 108.5 | 13.9 | 97.8 | 17.3 |
| ¹³ C ₂ -4:2 FTS | 82.8 | 6.9 | 92.1 | 5.1 | 179.8 | 9.1 | 102.8 | 21.4 |
| ¹³ C ₂ -6:2 FTS | 94.1 | 2.8 | 95.0 | 5.0 | 197.8 | 11.0 | 101.9 | 19.1 |
| ¹³ C ₂ -8:2 FTS | 91.5 | 4.2 | 91.8 | 7.5 | 149.7 | 16.6 | 90.4 | 17.9 |
| ¹³ C ₈ -FOSA | 92.3 | 3.6 | 99.4 | 5.8 | 101.3 | 19.8 | 96.7 | 18.7 |
| ¹³ C ₃ -GenX | 98.0 | 3.5 | 105.9 | 6.6 | 85.2 | 7.5 | 98.6 | 14.8 |
| D ₅ -N-EtFOSSA | 91.3 | 3.4 | 91.9 | 6.0 | 127.0 | 15.4 | 93.3 | 18.3 |
| D ₃ -N-MeFOSSA | 89.0 | 4.8 | 90.3 | 5.5 | 137.4 | 16.2 | 91.6 | 20.3 |
| d ₅ NMeFOSSA | 63.5 | 5.9 | 64.0 | 13.2 | 52.8 | 24.3 | 82.3 | 19.2 |
| d ₅ NEtFOSSA | 61.1 | 7.0 | 61.9 | 13.5 | 38.9 | 23.8 | 78.5 | 19.6 |
| d ₇ -NMeFOSE | 70.0 | 5.7 | 74.0 | 11.8 | 56.9 | 20.3 | 74.5 | 17.7 |
| d ₉ -NEtFOSE | 67.2 | 5.9 | 71.1 | 12.3 | 52.9 | 20.2 | 71.1 | 18.3 |
| ¹³ C ₃ -PFBA | 134.8 | 20.0 | 112.2 | 6.5 | 89.6 | 6.6 | 114.1 | 7.1 |
| ¹³ C ₈ -PFHxA | 133.9 | 19.4 | 117.4 | 5.7 | 120.8 | 7.2 | 124.7 | 5.8 |
| ¹³ C ₄ -PFOA | 127.9 | 22.7 | 116.3 | 7.8 | 132.2 | 8.1 | 123.5 | 8.0 |
| ¹³ C ₅ -PFNA | 133.3 | 21.3 | 115.1 | 9.1 | 132.8 | 4.5 | 122.3 | 7.5 |
| ¹³ C ₂ -PFDA | 136.8 | 20.3 | 115.8 | 6.3 | 155.9 | 4.0 | 124.4 | 7.7 |
| ¹⁸ O ₂ -PFHxS | 133.6 | 21.4 | 115.7 | 7.4 | 112.2 | 7.0 | 119.8 | 6.6 |
| ¹³ C ₄ -PFOS | 132.2 | 22.7 | 116.1 | 6.6 | 118.9 | 7.2 | 121.6 | 8.2 |

Table 1. Average recovery of the extracted internal standards (EIS) and non-extracted internal standards (NIS) using the bilayer dual-phase SPE cartridge for each water sample type evaluated (n=5).

Application Showcase

Figure 2 directly compares the average recovery across all water sample types with the allowable recoveries in EPA 1633 Table 6 (Draft 4). The recoveries in water samples were easily within the recovery acceptance limits for each compound, and in all cases were significantly above the minimum recovery level. This demonstrates that even in the more complex water matrices, the cartridge has equivalent performance as using dispersive GCB and is fit-for-purpose.

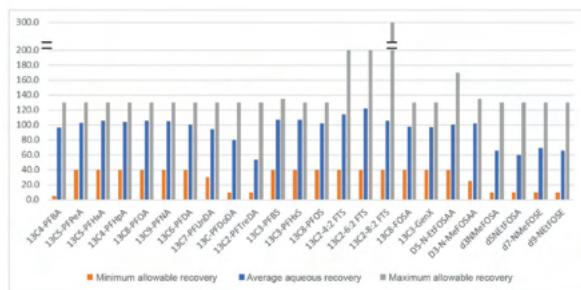


Figure 2. Average recovery of the extracted internal standards (EIS) in all four aqueous sample types (blue) compared to the minimum (orange) and maximum (gray) percent recoveries allowed in the EPA 1633 method ($n=20$). Note the split axis to accommodate the maximum recovery value for 13C2-8:2 FTS.

Analysis of a Certified Reference Material

Accuracy of analysis is important for quantitating PFAS in customer samples. A certified reference material (CRM) from Waters ERA was processed with the authentic samples as a benchmark for workflow accuracy. The PFAS in Wastewater CRM is certified for all EPA 1633 analytes, giving a representative reference material for method performance without having to spike unknown matrix samples which can become complicated without a sample free from PFAS.

Figure 3 shows the average quantitative results for three replicate extraction and analyses of the Wastewater CRM. The dotted and dashed red lines indicate the minimum and maximum certified value range of the CRM. The solid blue line represents the certified value. The solid gray line represents the average experimental quantitated value determined during sample analysis. All 40 target PFAS in EPA 1633 were quantified within the allowable minimum and maximum concentration

range with a mean trueness of 92% and trueness range of 73–112%. This demonstrates confidence in accuracy of the sample preparation, analysis and data processing workflow using Waters solutions.

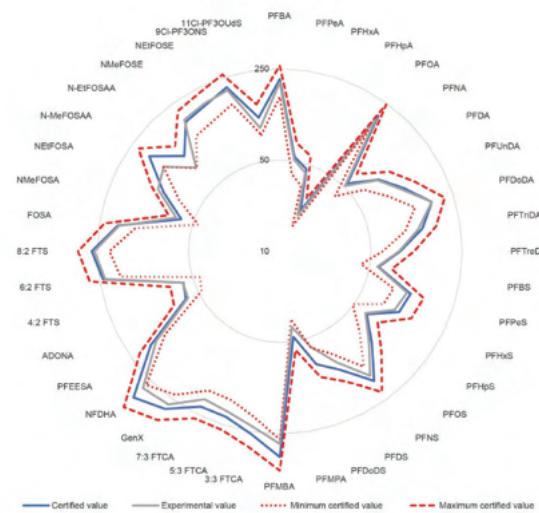


Figure 3. Quantified values of all 40 EPA 1633 target analytes in Waters ERA PFAS in Wastewater CRM. Red lines represent the minimum (dotted) and maximum (dash) certified value range of the CRM. The blue line represents the certified value. The solid gray line represents the average experimental quantitated value ($n=3$). Note the axis is represented using a log scale.

Analysis of PFAS in Authentic Water Samples

The presented workflow was utilized to detect and quantify the 40 target PFAS analytes in ground water, surface water, influent water, and effluent water.

The LC gradient method was designed to provide a minimum of 1 minute separation between potential cholic acid interferences and PFOS.² Because cholic acids are produced to aid digestion, they can be present in large amounts in wastewater samples.

Figure 3 demonstrates the presence of cholic acids in the influent and effluent wastewater samples and the successful separation of them from interference with PFOS. PFAS were detected in all samples, as highlighted in Table 2.

Application Showcase

| Analyte name | Ground water | | Surface water | | Influent wastewater | | Effluent wastewater | |
|--------------|----------------------|------|----------------------|------|----------------------|------|----------------------|------|
| | Concentration (ng/L) | %RSD |
| PFBA | 7.03 | 3.0 | 15.36 | 8.4 | 21.93 | 18.3 | 21.15 | 7.7 |
| PFPeA | 2.47 | 2.5 | 4.30 | 3.7 | 11.55 | 15.5 | 12.12 | 3.7 |
| PFHxA | 1.67 | 3.0 | 3.53 | 2.7 | 15.98 | 17.4 | 23.81 | 1.7 |
| PFHpA | 1.01 | 8.0 | 2.12 | 5.9 | 3.33 | 13.4 | 3.33 | 1.6 |
| PFOA | 0.69 | 10.0 | 4.86 | 6.7 | 27.02 | 14.5 | 14.89 | 8.5 |
| PFNA | N.D. | — | 0.77 | 15.0 | 0.91 | 11.4 | 1.28 | 20.2 |
| PFDA | N.D. | — | 0.51 | 32.1 | 0.95 | 4.6 | 2.44 | 20.7 |
| PFUnDA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFDoDA | N.D. | — | N.D. | — | BLQ | — | N.D. | — |
| PFTrIDA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFTrEDA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFBS | 1.05 | 7.1 | 2.83 | 6.0 | 41.12 | 12.6 | 38.16 | 3.9 |
| PFPeS | 0.11 | 8.7 | 0.17 | 11.8 | 0.39 | 23.7 | 0.34 | 6.2 |
| PFHxS | 0.26 | 10.3 | 0.86 | 5.6 | 4.72 | 17.7 | 4.07 | 3.5 |
| PFHpS | N.D. | — | N.D. | — | N.D. | — | BLQ | — |
| PFOS | 0.34 | 16.4 | 3.40 | 22.4 | 8.17 | 9.4 | 6.17 | 19.9 |
| PFNS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFDS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFDoDS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| GenX | N.D. | — | 0.12 | 8.8 | 0.65 | 15.2 | 0.59 | 5.4 |
| ADONA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| 9C1CPF3ONS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| 11C1CPF3OUdS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| 4_2 FTS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| 6_2 FTS | N.D. | — | N.D. | — | 4.44 | 16.2 | 2.91 | 18.9 |
| 8_2 FTS | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| FOSA | BLQ | — | BLQ | — | BLQ | — | BLQ | — |
| NMeFOSA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| NEtFOSA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| NMeFOSAA | N.D. | — | N.D. | — | 1.16 | 10.7 | 1.26 | 28.1 |
| NEtFOSAA | N.D. | — | N.D. | — | 0.90 | 14.6 | 0.90 | 27.6 |
| NMeFOSE | N.D. | — | N.D. | — | 2.96 | 9.8 | BLQ | — |
| NEtFOSE | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| 3:3 FTCA | N.D. | — | N.D. | — | 4.91 | 21.4 | N.D. | — |
| 5:3 FTCA | N.D. | — | N.D. | — | 88.91 | 18.4 | 3.91 | 4.3 |
| 7:3 FTCA | N.D. | — | N.D. | — | 4.66 | 16.8 | N.D. | — |
| PFMPA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFMBA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| PFEEA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |
| NFDHA | N.D. | — | N.D. | — | N.D. | — | N.D. | — |

Table 2. Detected concentrations of PFAS in each type of water sample and associated %RSD of n=5 replicates. (N.D.) not detected. (BLQ) below limit of quantitation.

Each sample was collected and extracted in five replicates and the average calculated concentration is reported with associated %RSDs for the replicates.

The ground water had the lowest detectable PFAS with nine PFAS detected above the limit of quantitation (LOQ) and ranging from 0.11–7.03 ng/L.

The surface water sample had a slightly larger range of PFAS, with 12 detected above the LOQ in a range of 0.17–15.4 ng/L. The wastewater samples had the largest range and concentrations of PFAS detected. A comparison of the detected PFAS in the influent and effluent water is shown in Figure 5.

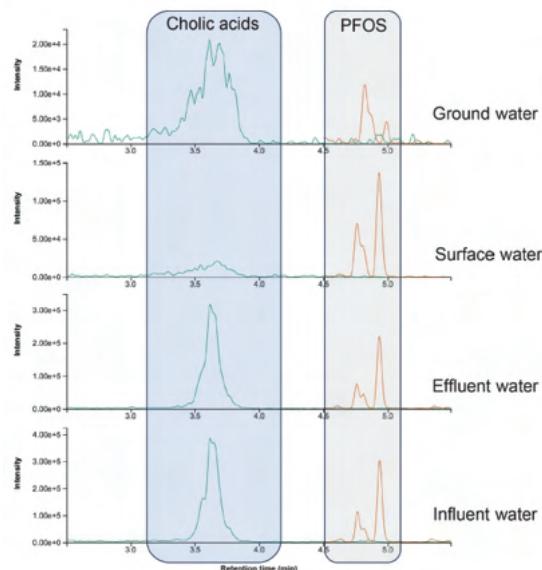


Figure 4. Overlay chromatograms of cholic acids and PFOS MRM channels demonstrating the large cholic acid interference present in wastewater samples.

Application Showcase

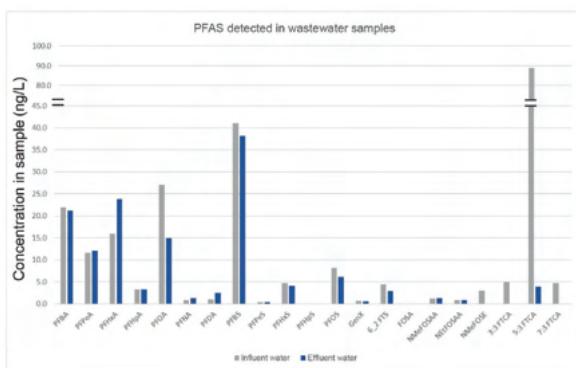


Figure 5. Comparison of the quantified PFAS in influent wastewater (gray) and effluent wastewater (blue). Note the split axis to accommodate the maximum concentration value for 5:3 FTCA.

19 PFAS were quantified in the influent wastewater, whereas 16 were detected in the effluent wastewater, indicating that the water treatment at this site is effective at removing some PFAS. When comparing the concentrations though, most of the PFAS were quantified at approximately the same concentration in both influent and effluent water. NMeFOSE, 3:3 FTCA, and 7:3 FTCA were not present in the effluent water discharged from the treatment plant, and 5:3 FTCA was significantly reduced (from 88.9 ng/L in the influent to 3.9 ng/L in the effluent).

Conclusion

Sample preparation and analysis was performed for several water samples using EPA 1633 procedures. A prototype bilayer dual-phase SPE cartridge containing both WAX and GCB was utilized for the sample extraction and clean up in place of performing the extraction and clean up in two separate steps with dispersive GCB. This cartridge provides a better user experience and reduces time spent in the sample preparation step. All recoveries were within the acceptance criteria ranges with the mean EIS recovery of 20 extractions (including ground water, surface water, influent water, and effluent water) at 91.2%, with a mean RSD of 9.2%. This demonstrates the equivalence of the bilayer dual-phase SPE cartridge as a suitable single step replacement for the multi-step extraction and clean up presented in EPA 1633.

Additionally, a Waters ERA wastewater certified reference material processed and analysed using the same method was easily within the certified reference value range, giving high confidence in method accuracy. Four types of water samples, varying in complexity, were analysed for the 40 PFAS included in EPA 1633 and PFAS were detected in all samples ranging from 0.1 to 88.9 ng/L. The data presented demonstrates that the bilayer dual-phase SPE cartridge in combination with the LC-MS/MS system easily fulfills all requirements for analysis of water samples for EPA 1633.

References

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H-CUBE® PRO

FLOW REACTOR FOR ADVANCED HYDROGENATION



Features

- Hydrogenation without cylinders:** The H-Cube® Pro has two electrolytic cells for the in situ generation of up to 60 NmL/min of hydrogen gas (up to 100 bar)
- Adjustable hydrogen amount:** Users can control the amount of hydrogen gas to be used in a chemical reaction, making the implementation of selective transformations easier and more effective.
- No catalyst filtration:** 30 mm or 70 mm CatCarts® can be used with the system, eliminating the need of catalyst filtration during work-up
- Active cooling system:** The H-Cube® Pro can cool reaction mixtures down to 10 °C
- Extended heating capability:** Reactions can be performed up to 150 °C with the H-Cube® Pro
- User-friendly software:** Monitoring and recording real-time parameters of each reaction

Applications

Reactions with hydrogen gas:

Triple bond full reduction, debenzylation, nitrile reduction, selective nitro reduction, selective triple to double bond reduction, selective double bond reduction, aldose reduction, aromatic heterocycle reduction, aromatic ring reduction

Other reactions:

Deuteration, Sonogashira coupling, Suzuki coupling

| | |
|-------------------------------------|---|
| Temp range | 10 - 150° C |
| Pressure range | atm to 100 bar |
| Flow rates | 0.3 - 3 mL/min |
| Max. H ₂ production rate | 60 NmL/min in 100% of Hydrogen production |
| Water reservoir capacity | 300 mL |
| Water specifications | Deionized water with maximum conductivity of 71 nS/cm (min. resistance 14 MΩcm) |

Application Showcase



Benzyl and Cbz Removal via Catalytic Hydrogenation

Deprotection Reactions Using the ThalesNano H-Cube® Continuous Flow Reactor

Deprotection reactions are fundamental in organic synthesis, enabling the selective removal of protecting groups to facilitate controlled chemical transformations. By temporarily masking reactive functional groups, deprotection allows for precise control over regioselectivity and stereoselectivity, preventing undesired side reactions and enhancing product purity. This application note gives examples of deprotection reactions performed on the H-Cube® from the recent articles.

Keywords: Deprotection, catalytic hydrogenation, H-Cube® (Continuous Flow Reactor), benzyl groups/Cbz groups, organic synthesis, ThalesNano

Introduction

Protecting groups play a central role in modern organic synthesis. The benzyl groups and benzyl carbamate or Cbz groups are some of the most commonly used protecting groups and play a central role in the protection of alcohols, carboxylic acids, and amines. The benzyl and benzyl carbamate groups are removed using catalytic hydrogenation using elevated temperature. The H-Cube® is able to remove benzyl groups from amines, acids, or alcohols very efficiently in one pass through a 10% Pd/C CatCart®. This application note gives examples of deprotection reactions performed on the H-Cube® from the recent articles.

O-Benzyl Deprotection

Parameter Optimization

To find the optimal parameters of catalytic hydrogenolysis, the O-debenzylation of O-benzyl protected N-Boctyrosine (Figure 1.) was investigated by

Application Showcase

Knudsen et al¹. A series of experiments over 10% Pd/C catalyst in EtOH:EtOAc (1:1) solvent were performed to probe the effects of flow rate, temperature and concentration using an automated hydrogenation platform. Results showed a faster catalyst deactivation rate with increasing concentration and flow rate, but at higher temperature they found dramatically increased conversion rates. At room temperature the conversion rate was half of that observed at 60°C.

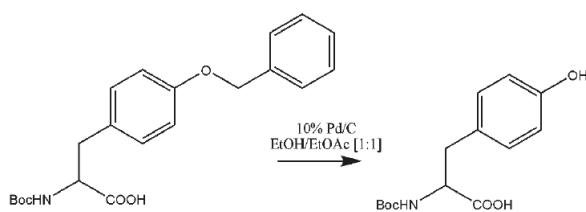


Figure 1: Debenzylation of O-benzyl protected N-Boc-tyrosine

100% conversion was reached, when the concentration of reactant solution was 0.1 M, the applied temperature was 60°C, and the flow rate was 1 mL/min

Catalyst Reactivation

As the catalyst can be poisoned and deactivated over a long reaction time, catalyst reactivation is a key factor in heterogeneous catalysis. To increase the catalyst lifetime the following reactions were performed. Equal amount of reactant was introduced to the system in two different ways: continuously and through repeated injections of small amounts. With the injection method, there was a short period of time between each injection where the solvent regenerated the catalyst by washing off any adsorbed material. This short washing period generated higher conversion rates compared to continuous pumping the material through the catalyst.

N-Cbz Deprotection

Reaction Optimization and Library Synthesis

An N-deprotection reaction was also optimized by Knudsen and co-workers¹ with the reaction shown in Figure 2. After finding the optimized reaction conditions, such as elevated temperature of 80°C, pressure of 1 bar, and solvent concentration of 0.05 M

M using 10% Pd/C as catalyst, a small library of N-protected compounds, including dipeptide and amino acid derivates, was successfully hydrogenated. As seen in the previous reaction series, the temperature also had a significant effect on the conversion rate. The observed yield of isolated products were high in each case and are displayed in Table 1.

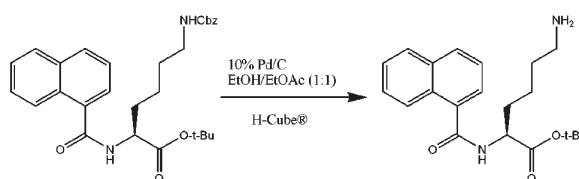


Figure 2: Deprotection of ϵ -[N-Cbz] lysine tert-butyl ester

| Substrate | Conversion [%] | Yield [%] |
|----------------------------|----------------|-----------|
| Coz-Pro-OMe | 99 | 99 |
| Coz-Piperazine | 99 | 80 |
| Coz-Asp(OMe)-OMe | 99 | 77 |
| Coz-(OBn)Tyr-OMe | 97 | 83 |
| Coz-Ser-OMe | 98 | 82 |
| Boc-(N-Coz)-Lys-(ONap) | 95 | 86 |
| Coz-Thr-Tyr(O-t-Bu)-O-t-Bu | 99 | 95 |
| Coz-Pro-tetrazole | 99 | 96 |

Table 1: Deprotection of eight N-Cbz protected compounds

Fraction collection can be performed manually or automatically, with automation often triggered by a UV detector signal. One common automated approach is the peak-based collection, where fractions are collected

Part of New Synthesis Pathway

A practical and safe synthesis of (S)-pyrrolidine-2-yl-1H-tetrazole was developed by Franckevicius and coworkers² to prepare a catalyst which can be used in Mannich and aldol reactions leading to high yields. The synthetic steps eliminate the generation of hazardous materials such as ammonium azide, and the use of non-volatile solvent.

In the first synthetic step a protected pyrrolidine derivate was synthesized, which was subsequently-transformed to the tetrazole derivate of the Cbz protected pyrrolidine with sodium azide.

Application Showcase

Overall yield was 92%. In the last step, Cbz deprotection (Figure 3.) was achieved by the H-Cube® using a volatile solution mixture of EtOAc:EtOH:AcOH = 1:1:1. The method dramatically reduces the reaction time. In batch when 9:1 acetic acid:water solvent mixture was used, the reaction time- and time-consuming work-up extended the total reaction time to 3 days.

While in flow mode 3g of desired compound was synthesized in 3.5 hours. The reaction seen in Figure 4. was performed using full hydrogen mode at a flow rate of 1 mL/min, temperature of 80°C and a starting material concentration of 0.05 M. 10% Pd/C was used as catalyst. Working-up the reaction was carried out by simple evaporation of the solvent.

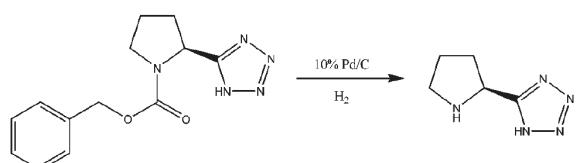


Figure 3: Deprotection step of (S)-5-pyrrolidin-2-yl-1H-tetrazole synthesis

O-Debenzylation

Part of New Reaction Pathway

A debenzylation step was performed by Desai and Kappe to produce dihydropyrimidines (DHPM) C5 carboxylic acids³. The synthesis of the acids can be readily achieved by hydrogenolysis of the corresponding benzyl esters.

The previously reported batch reaction resulted in high yields and used an external hydrogen gas source, while when the H-Cube® was used with 10% Pd/C catalyst there was no need for a gas cylinder.

The reaction scheme and the isolated yield values obtained are seen in Figure 4. and Table 2. Under these circumstances in continuous flow (“CF”) mode 80 – 95% isolated yields of the DHPMs acids were obtained.

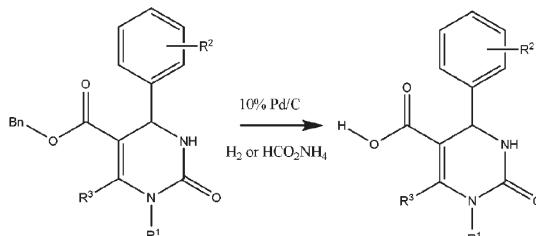


Figure 4: Hydrogenation of DHPM C5 Benzyl Esters

Yields were also obtained from the deprotection reaction of DHPM esters under other conditions. Namely, room temperature catalytic transfer hydrogenation (“rt”) in ammonium formate, and microwave-assisted transfer hydrogenation (“MW”) in ammonium formate⁴. When room temperature hydrogenation was applied, reactions took 8–10 h reaction time and only 56 – 66% yields were obtained. The microwave assisted method did not result in significant enhancement of the yields. The obtained values were between 53 – 62%, but the reaction times were reduced to minutes.

| R ¹ | R ² | R ³ | Conditions | Yield [%] |
|----------------|----------------|----------------|------------|-----------|
| H | H | Me | rt | 66 |
| | | | MW | 62 |
| | | | CF | 95 |
| H | H | Ph | rt | 65 |
| | | | MW | 60 |
| | | | CF | 90 |
| Me | H | Me | rt | 57 |
| | | | MW | 55 |
| | | | CF | 85 |
| H | 4-Me | Me | rt | 56 |
| | | | MW | 59 |
| | | | CF | 85 |

Table 2: Isolated yield values of hydrogenation of DHPM C5 benzyl esters.

Compared to the batch and microwave techniques, H-Cube® gave yields between 80 – 95%. The major advantage as they emphasized that these excellent yields were obtained after a simple evaporation thus this easy work-up makes this method an attractive solution for automated library generation.

Application Showcase

O-Debenzylation

A debenzylation in conjunction with a double bond reduction reaction plays an important role in the synthesis of polyhydroxylated oxamacrolides, as reported by Matos and Murphy⁵ in a multistep synthesis.

In the previous step, before the reduction, a mixture of macrolactones was produced, separated and then introduced into the H-Cube®. Deprotection of the four benzyl groups and the saturation of the double bond (Figure 5.) was carried out at 80 bar pressure, 60°C reaction temperature and a flow rate of 1 mL/min over 5% Pd/C catalyst in ethanol. In the first experiment lower yield was observed due to the low solubility of the product in ethanol. The structure of colourless oil was confirmed by X-ray crystal structure.

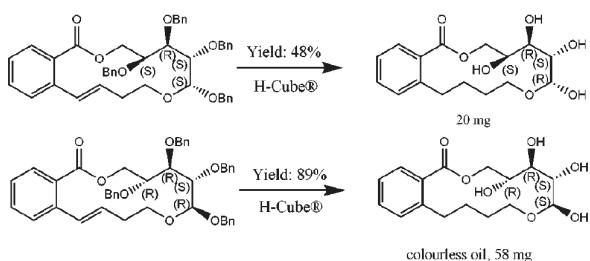


Figure 5: Synthesis of polyhydroxylated oxamacrolides



H-Cube® Continuous Flow Reactor

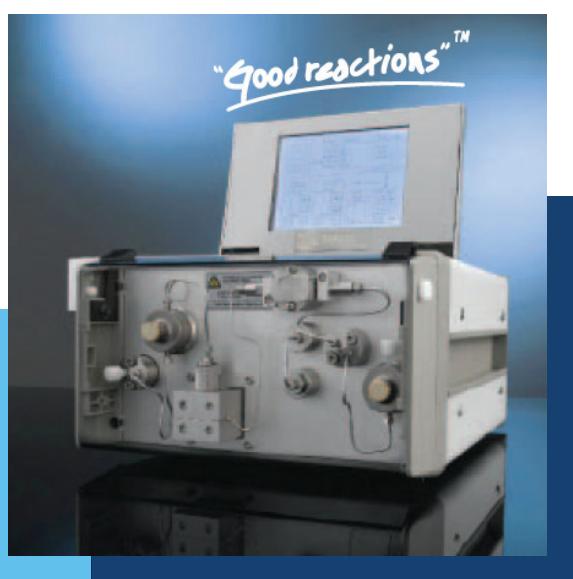
Conclusion

H-Cube® continuous flow reactor is proven to be an ideal tool for performing deprotection catalytic hydrogenation.

The excellent yields in all cases and the easy handling of catalysts suggest this method to be a fast, efficient, and safe way to perform benzyl deprotection compared to batch and microwave methods.

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Application Showcase



Accelerated Drug Discovery

On-line TLC/CMS technique using the Advion Interchim Scientific® expression® CMS and Plate Express™

This application explores the use of alternative chromatography technology for faster, more efficient analysis over traditional LC/MS runs.

Plate Express™ is a device that provides a simple, automated means of obtaining mass spectra directly from TLC plates, combined with the Advion Interchim Scientific® expression® CMS (Compact Mass Spectrometer) creating a technique known as TLC/CMS. Using this technique chemists can quickly and confidently identify products even in complex mixtures without additional sample preparation.

By decoupling the separation from the analysis, medicinal chemists can quickly analyse a developed TLC plate for answers in seconds. Not only less time-consuming, but the process is also much lower cost, with significantly less solvent consumption.

Keywords: Chromatography, LC/MS, TLC/CMS, Advion Interchim Scientific®

Introduction to TLC/CMS vs LC/MS

TLC/CMS for the organic synthetic and medical laboratories

Thin layer chromatography (TLC) is used in many organic synthetic and medical laboratories because it is a simple, cost-effective technique that provides chemists with critical information about their synthetic reactions. Structural characterization of the analytes by TLC is not possible by optical methods such as UV or ELSD. Typically, characterization is performed by GC/MS or LC/MS using sample preparation techniques which involves scraping the TLC spot of interest,

Application Showcase

extraction using suitable solvents, concentration, and then reconstitution in MS appropriate solvents. Here, an on-line TLC/CMS technique is presented using the Advion Interchim Scientific® expression® CMS and Plate Express™ to provide compound structural information without sample preparation after TLC separation.

A Suzuki reaction for the synthesis of 4-aminobiphenyl will be demonstrated. The online TLC/CMS technique provides rapid and accurate determination of reaction mixture components without the need for off-line TLC sample preparation procedures.

Method

Suzuki Reaction Setup

Reactants A and B were mixed at equimolar amounts in a round-bottom reaction flask and stirred at room temperature. 2 mL aliquots were transferred from the flask and spotted onto a Merck TLC Silica gel 60 F254 plate (10 x 10cm). Chemicals were purchased from Sigma- Aldrich with a purity greater than 99%. MS solvent was LC/MS grade.

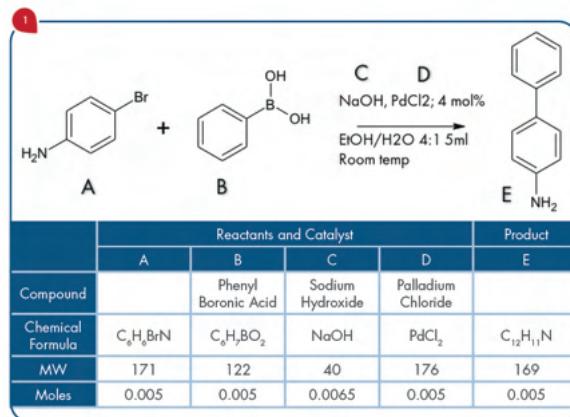


Figure 1: Chemical structure and information of reactants and catalysts for the Suzuki reaction

TLC Plate Preparation

Merck TLC Silica gel 60 F254 on Aluminium, 20 x 20 cm - cut to 10 x 10 cm. TLC plates were baked at 100 °C for 10 minutes to displace moisture. A 2 μ L aliquot of the reaction mixture was spotted onto the TLC plate at the

position of 1 cm away from bottom. The TLC plate was then baked at 80 °C for 5 min. 30 mL Benzene was added to a TLC plate developing chamber at the level of 0.5 cm. The developing chamber was sealed with a glass lid for 30 min. The separation was stopped when the development solvent front reached a position which was 1 cm away from the top of the TLC plate.

The analytes (reactant and product) on the developed plate were observed under UV at 254 nm (Figure 2). The R_f value of the product (4-aminobiphenyl) was 0.1; the R_f of the reactant (4-bromaniline) was 0.15.

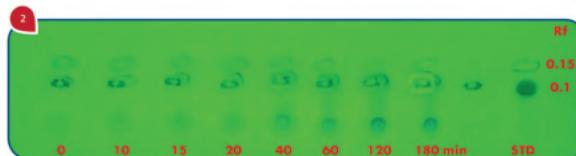


Figure 2: The developed TLC plate with Suzuki reaction mixture.

TLC/CMS Method

The TLC/CMS analysis of the Suzuki reaction mixture at different reaction times was performed on the Advion Interchim Scientific® expression® CMS and Plate Express™. A solvent composed of 0.1% formic in methanol was used for the elution of the analytes from the TLC plate. The eluted analytes were directed to the CMS for acquisition of the corresponding mass spectra for the reactants and products.

Results

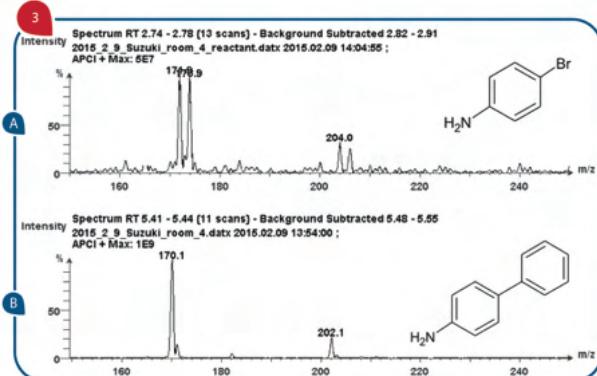
The mass spectra of the reactant and product are shown in Figure 3. The [M+H]⁺ for 4-bromoaniline was observed at m/z 171.9 (⁷⁹Br) and 173.9 (⁸¹Br). Methanol adducts were also detected at m/z 204.0 and 206.0 (Figure 4A). The [M+H]⁺ for 4-aminobiphenyl was observed at m/z 170.1 with a methanol adduct at m/z 202.¹ (Notice the absence of the bromine doublet peaks in the non-brominated product of p-aminobiphenyl, Figure 1B).

The extracted ion current of the product ion at m/z 170.1 (protonated 4-aminobiphenyl) was monitored over different reaction times from 0 to 180 minutes (Figure 3A). The mass spectrum of the product ion at 120 min is

Application Showcase

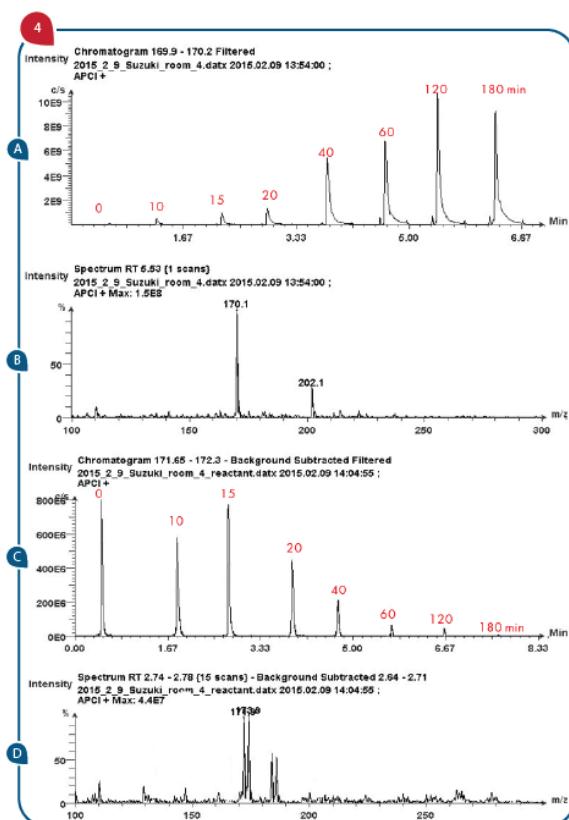
shown in Figure 4B. The extracted ion current (XIC) of the reactant ion at the m/z 171.9 (protonated 4-bromaniline(⁷⁹Br)) is shown in Figure 4C.

The intensity of the m/z 171.9 decreased over the course of the reaction with respect to the product ion at the m/z 170.1. The reactant ion at the m/z 171.9 was no longer detected at 180 minutes, indicating the reaction was complete.



The extracted ion current of the product ion at m/z 170.1 (protonated 4-aminobiphenyl) was monitored over different reaction times from 0 to 180 minutes (Figure 4A). The mass spectrum of the product ion at 120 min is shown in Figure 4B.

The extracted ion current (XIC) of the reactant ion at the m/z 171.9 (protonated 4-bromaniline(⁷⁹Br)) is shown in Figure 4C. The intensity of the m/z 171.9 decreased over the course of the reaction with respect to the product ion at the m/z 170.1. The reactant ion at the m/z 171.9 was no longer detected at 180 minutes, indicating the reaction was complete.



Conclusion

The Advion Interchim Scientific® expression® CMS coupled with the Advion Plate Express offers a simple and fast technique to monitor a Suzuki reaction for the synthesis of 4-aminobiphenyl.

The Advion Interchim Scientific® TLC/CMS system allows the synthetic chemist to monitor the reaction in real-time by evaluating the mass spectra for structural information (i.e., relative intensity of reactants vs. product) directly from the TLC plate.

The compact size allows it to fit into space-limited labs for direct access and immediate results for chemists requiring mass confirmation, reaction monitoring, quality control and purity analysis.

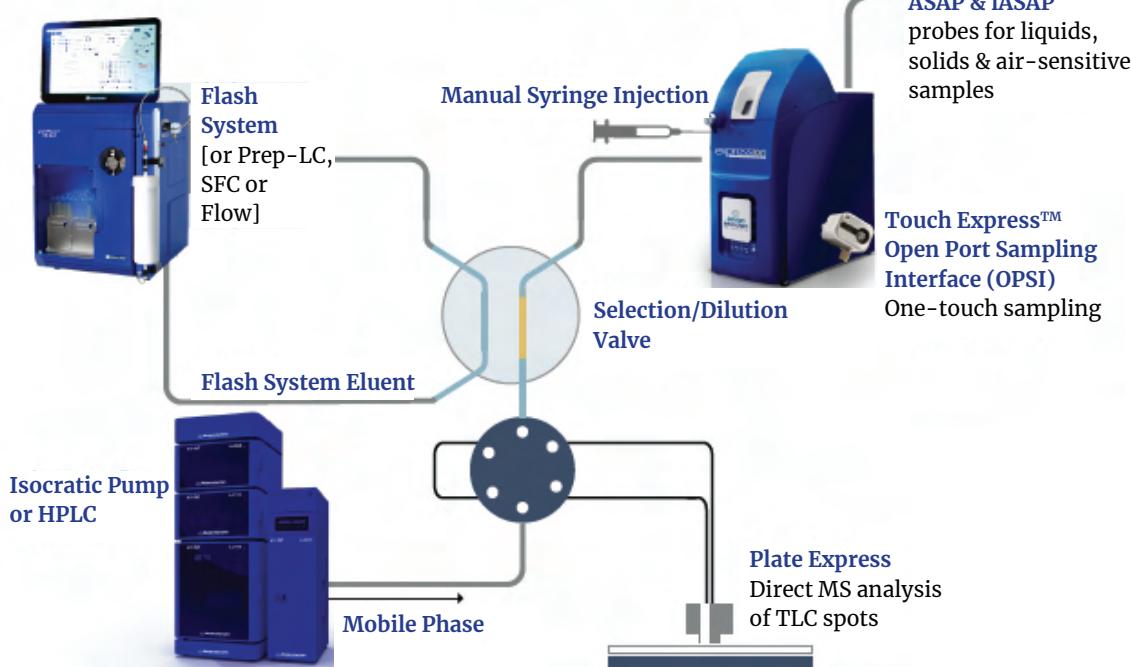
Application Showcase

Multi-mode Mass Spectrometry Simultaneous integration with several techniques

Placed at the center of the chemist's workflow, the expression[®] CMS can provide critical information for many processes, reaction monitoring, separation, purification, and impurity determination.

It can be integrated with several techniques at once and the chemist can move effortlessly between these techniques in seconds without the need to re-plumb or re-configure the system, as below:

- Manual syringe injection
- Direct analysis probe (ASAP and iASAP probes)
- TLC/CMS with the Plate Express
- Flash chromatography, Prep-LC or SFC
- VOCs in air, breath and headspace (vAPCI)
- (U)HPLC



Combined flash chromatography, TLC, (U)HPLC and manual injection interface. No re-plumbing necessary.

From Contamination to Compliance: The Role of Sartorius Lab Water System in PFAS Detection

Reduction of PFAS Contaminants Using Arium® Comfort I UV

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2. Claros Technologies Inc, 1600 Broadway St NE, Suite 100, Minneapolis, MN 55413, USA
3. Novem Scientific, LLC, 2357 Ventura Drive, Suite 110 Woodbury, MN 55125, USA

and combustion ion chromatography to discern how the Arium® Comfort I UV reduces PFAS contaminants concentration below detection limits to up to single-digit ppt or sub-ppt-level (parts per trillion, dependent on the compound) in water. The results show that Arium® Comfort I UV is highly efficient in the reduction of PFAS in water with both the standard and PTFE-free setup.

Per- and Polyfluoroalkyl Substances (PFAS), a family of over 4,700 chemical molecules is known for non-stick and waterproof properties. These versatile compounds find their application in diverse industries, including cosmetics, textiles, and automobiles. Resistance to degradation, they persist in water, soil and atmosphere, contributing to environmental concern.

This application note uses two complementary analytical techniques: liquid chromatography – mass spectrometry

Keywords or phrases: Per- and Polyfluoroalkyl Substances (PFAS), PFAS reduction, Ultrapure water, ASTM Type I water, Arium®, LCMS, CIC TOF

Introduction

PFAS are a group of synthetic chemicals widely used in industrial and consumer products, such as non-stick cookware, waterproof clothing, and firefighting

Application Showcase

foam, due to their unique properties. However, their persistence in the environment and potential adverse health effects have raised concerns about their presence in water sources, including drinking water. Human exposure to PFAS can occur through ingestion of contaminated food and water, inhalation of dust and air, and skin contact with products containing PFAS.

Once released into the environment, PFAS can persist for a long time and can leach into soil and water,

concentration below detection limits to up to single-digit ppt or sub-ppt-level (parts per trillion, dependent on the compound) in water.

Ultrapure Water Purification

System Overview

The Arium® Comfort I UV is a comprehensive water treatment solution designed to produce ultrapure water from in- house potable water for various applications,

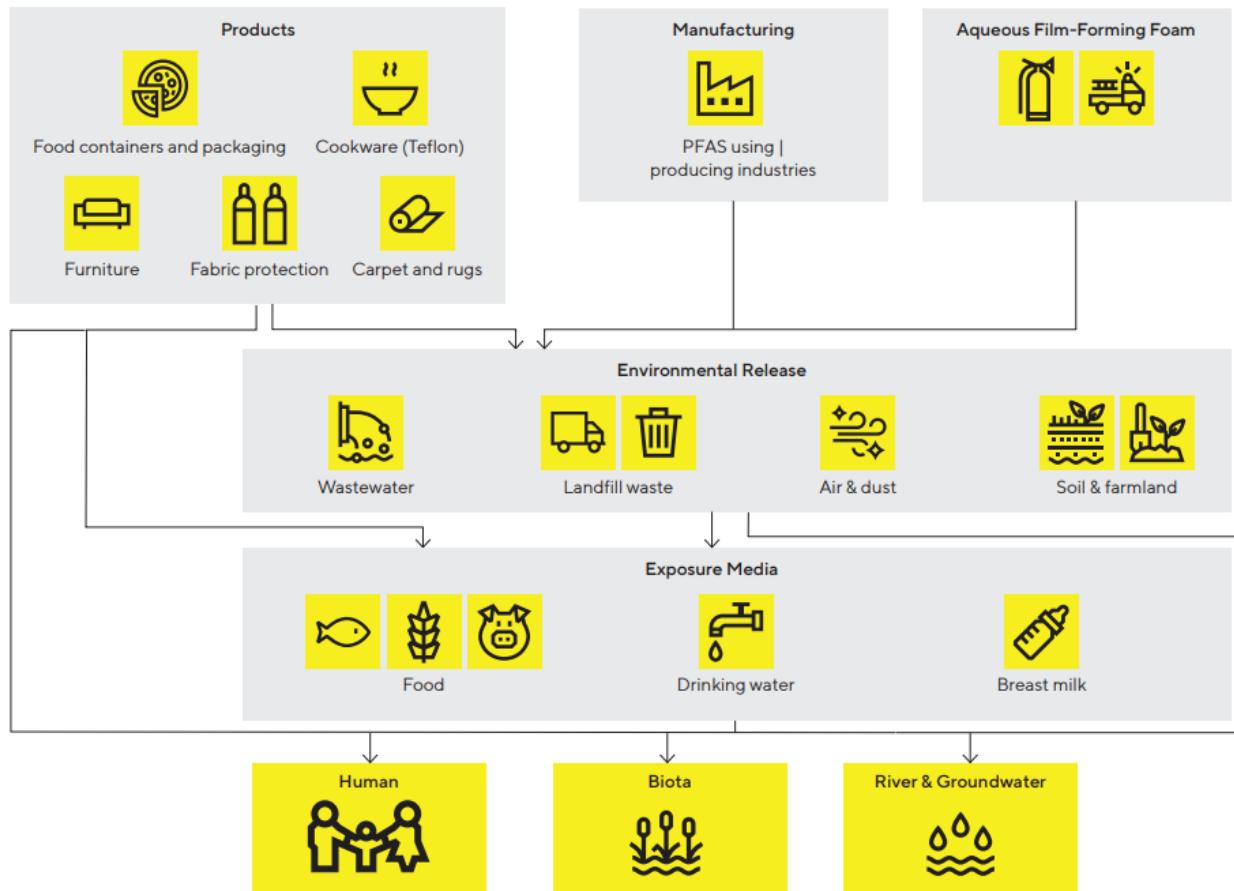


Figure 1: Main PFAS Exposure Pathways for Humans and Biota²

potentially contaminating drinking water sources and harming wildlife (Figure 1).^{1,2}

This application note discusses how the Arium® Comfort I UV effectively reduces PFAS contaminants

ensuring the reduction of a wide range of contaminants, including traces of metal ions, organic contamination and PFAS. The system employs a multi-stage approach combining activated carbon, reverse osmosis technology, and resin ion exchange to achieve efficient PFAS removal.

Application Showcase

PFAS Removal Process

PFAS Removal Process³

Our Arium® Comfort I UV employs a multi-stage process to eliminate PFAS contaminants from potable water, ensuring the highest level of water purity.

Reverse Osmosis (RO) Module

The process begins with the water passing through the RO module. The cornerstone of our PFAS removal strategy lies in the implementation of advanced RO technology.^{4,5} RO has proven to be remarkably efficient in removing PFAS compounds, boasting a removal rate of up to 99% of PFOS from the feedwater.⁶ This includes the drastic reduction of both long-chain and short-chain PFAS molecules (C4 - C14), demonstrating the technology's comprehensive efficacy.

Activated Carbon Filtration

Following RO filtration, the water enters the activated carbon filtration stage. Activated carbon, renowned for its exceptional adsorption properties, further targets PFAS molecules.

This step enhances the purification process by capturing remaining PFAS compounds and other organic impurities.^{7,8}

Resin Ion Exchange

The final stage involves resin ion exchange, a process that further polishes the water by exchanging undesirable ions, including remaining traces of PFAS, with ions on the resin.

This step ensures that even the minute concentrations of PFAS that may have passed through earlier stages are effectively removed.⁴

Achieving Below Detection Limit Removal

The combination of activated carbon, RO treatment, and

resin ion exchange in the Arium® Comfort I UV enables the reduction of PFAS concentrations to below detection limits in tap water. The system ensures the production of high-quality water that is verified to reduce the risk of PFAS contamination from the original water source, meeting the stringent purity requirements of various applications.

Treating Highly Contaminated PFAS Solutions

The effectiveness of the Arium® Comfort I UV is not limited to potable water. The system has been tested and proven to treat highly contaminated solutions containing up to ~4 ppb (parts per billion) of various PFAS compounds. This capability highlights the system's robustness and efficiency in addressing challenging contamination scenarios.

Materials

- Arium® Comfort I UV
- Arium® Bag Tank
- LCMS System (Shimadzu 8060 LCMS and PromoChromSPE03)
- CIC-System (Metrohm Custom Halogen Analyzer)
- Extracting tools and equipment
- Arium® Comfort I UV

Sartorius offers the compact, environmentally friendly, reliable, and easy-to-use Arium®

Comfort I for producing ASTM Type 1 ultrapure water and Type 3 pure water combined in a single system. The system contains state-of-the-art reverse osmosis technology and a unique cartridge specifically to produce the highest ultrapure water quality.

Compared to conventional water systems, the Arium® Comfort I optimizes water consumption using the integrated iJust control unit. This unique touch display with intuitive menu navigation ensures the utmost ease of use.

Application Showcase

With the optionally integrated TOC monitor, its compact design, and the SD card slot, the Arium® Comfort I is the ideal choice for demanding laboratory applications.

Arium® Bagtank

The pure water is stored in the innovative enclosed Arium® Bagtank system. This system protects the prepared pure water against secondary contamination. The Sartorius Bagtank system enables consistent water quality over a prolonged period, thereby ensuring permanent, reproducible results. Unlike conventional water reservoirs, the Arium® Bag offers a high level of user safety and time savings, as there is no need for a complicated cleaning procedure with chemicals.

LCMS Condition

The instrument is modified to reduce PFAS contamination from the instrument components and LC solvents including a delay column. The chromatographic separation is performed with a BEH C18 column (2.1 × 100 mm, 2.1 µm) using a gradient (Solvent A and B follows EPA draft method 1633).

MS source conditions are adjusted to allow optimized performance for all 40 PFAS compounds.

Storage

Samples were stored in 4 °C environment prior to the extraction.

Extraction

Samples were taken out of the refrigerated environment and allow to return to room temperature. Method blanks and lab control samples were created according to the lab procedure. All samples, method blank, and lab control samples contain the same amount of spike of surrogate compounds (extracted internal standards). The samples were mixed by inverting the bottle gently back and forth for approximately one minute. The samples were loaded on PromoChrom SPE03 automatic solid phase extraction system. The 1633 method solid

phase extraction method was used for these samples.

CIC-Condition

Combustion Ion Chromatography (CIC) data was obtained using a customized halogen analyser from Metrohm for the analysis of Total Organofluorine (TOF) data per sample, allowing for a direct comparison with LCMS data. Briefly, a known amount of sample is combusted in a greater than 1,000 °C oven and the gas phase containing total ions is condensed in an absorber solution with subsequent analysis by ion chromatography. This result gives the total fluorine (TF) value of the sample. An Asupp7 column is used in standard conditions (EPA 300.0) to separate and quantify the components which are detected by conductivity.

Simultaneously, the same sample is sent directly to a different IC to analyse for inorganic fluoride (IF, as free fluoride). This result gives the inorganic fluoride value. TOF may now be calculated by TF – IF. The analysis is non-speciated, focusing on the collective impact of PFAS, and offers a streamlined approach with significantly reduced sample preparation. This comprehensive TOF perspective provides prompt insights into unknowns, potentially negating the need for subsequent advanced testing. In this analytical setup, the Custom Halogen Analyzer has been optimized after receipt by Metrohm and features a reporting limit of 7.5 ppb.

Result

LCMS Results

The method of detection limit (MDL) and the method reporting limits (MRL) for LCMS are shown in Table 1. The last complete validation of the LCMS was performed in Q2 2023, and the analysis were performed in Q2 and Q3 2023. Any compounds with T in the analyte indicate that both branched and linear isomers are in quantitation consideration.

Application Showcase

| Analyte | MDL (ppt) | MRL (ppt) |
|------------|-----------|-----------|
| PFBA | 2.1 | 5 |
| PFPeA | 0.399 | 1 |
| PFHxA | 0.375 | 1 |
| PFHpA | 0.419 | 1 |
| PFOA | 0.507 | 2 |
| PFNA | 0.547 | 2 |
| PFDA | 0.274 | 1 |
| PFUnA | 0.399 | 1 |
| PFDoA | 0.554 | 2 |
| PFTrDA | 0.759 | 2 |
| PFTeDA | 0.395 | 1 |
| T-NMeFOSAA | 0.212 | 1 |
| T-NEtFOSAA | 0.514 | 2 |
| PFBS | 0.503 | 2 |
| PFPeS | 0.489 | 2 |
| T-PFHxS | 0.427 | 1 |
| PFHpS | 0.5 | 2 |
| T-PFOS | 0.351 | 1 |
| PFNS | 0.395 | 1 |
| PFDS | 0.373 | 1 |
| PFDoS | 0.748 | 2 |
| 4:2 FTS | 1.389 | 5 |
| 6:2 FTS | 2.215 | 5 |
| 8:2 FTS | 2.55 | 10 |
| PFOSA | 0.445 | 1 |

| Analyte | MDL (ppt) | MRL (ppt) |
|--------------|-----------|-----------|
| HFPO-DA | 0.628 | 2 |
| ADONA | 0.973 | 2 |
| 9Cl-PF3ONS | 0.662 | 2 |
| 11Cl-PF3OUDS | 1.958 | 5 |
| N-MeFOSA | 0.612 | 2 |
| N-EtFOSA | 0.672 | 2 |
| N-MeFOSE | 10.668 | 30 |
| N-EtFOSE | 6.733 | 20 |
| 3:3 FTCA | 0.412 | 1 |
| 5:3 FTCA | 3.099 | 10 |
| 7:3 FTCA | 3.483 | 10 |
| PFMPA | 0.314 | 1 |
| PFMBA | 0.35 | 1 |
| NFDHA | 0.907 | 2 |
| PFEESA | 0.308 | 1 |

Table 1: MDL and MRL Values of the PFAS

Experiment I

Samples of water mimicking a normal lab setting by connecting the system to the tap water. Two different Arium® Systems were chosen for this experiment. The first Arium® Comfort UV I system has the standard setup, which is equipped with virgin PE and PTFE tubing (Setup I).

The second Arium® Comfort I UV system is only equipped with PE tubing (Setup II). The samples were collected at (Figure 2):

- Feed water
- Post RO-treatment and before the water was stored in Arium® Bagtank
- At point of use of the system (without any final filter)

Results I

The Arium® Comfort I UV systems were installed in Woodbury, MN, a location with a historical presence of PFAS in tap water.

The analysis conducted three times and detailed in Table 2 with results reported in ppt, reveals that the PFAS content in the feed water is consistently reduced to levels below the limit of detection after undergoing RO-treatment.

Furthermore, there is no discernible PFAS leakage during the polishing step of the system.

Application Showcase

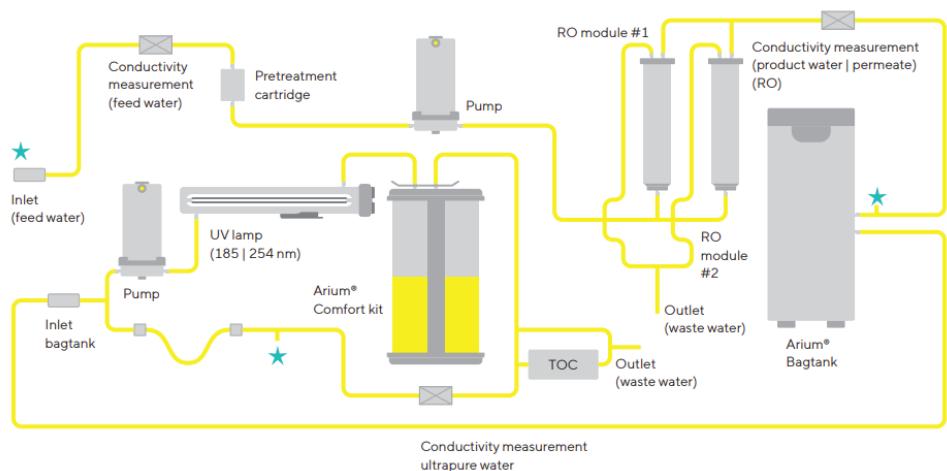


Figure 2: Flowchart of Arium® Comfort I UV. Blue Stars Mark the Point of Sampling

| | Feed Water (ppt) | | Post RO (ppt) | | Point of Use (ppt) | |
|--------------|------------------|--------------------|---------------|----------|--------------------|----------|
| | Average | Standard Deviation | Setup I | Setup II | Setup I | Setup II |
| PFBA | 233.715 | 1.778 | n.d. | n.d. | n.d. | n.d. |
| PFPeA | 5.275 | 0.102 | n.d. | n.d. | n.d. | n.d. |
| PFHxA | 1.796 | 0.026 | n.d. | n.d. | n.d. | n.d. |
| PFOA | 1.982 | 0.087 | n.d. | n.d. | n.d. | n.d. |
| PFBS | 1.374 | 0.055 | n.d. | n.d. | n.d. | n.d. |
| T-PFHxS | 1.451 | 0.277 | n.d. | n.d. | n.d. | n.d. |
| T-PFOS | 0.926 | 0.105 | n.d. | n.d. | n.d. | n.d. |
| PFHpA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFNA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFDA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFUnA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFDoA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFTrDA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFTeDA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| T-NMeFOSAA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| T-NEtFOSAA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFPeS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFHPS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFNS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFDS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFDoS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 4:2 FTS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 6:2 FTS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 8:2 FTS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFOSA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| HFPO-DA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| ADONA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 9Cl-PF3ONS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 11Cl-PF3OuDS | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-MeFOSA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-EtFOSA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-MeFOSE | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-EtFOSE | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 3:3 FTCA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5:3 FTCA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 7:3 FTCA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFMPA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFMBA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| NFDHA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| PFEESA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

Table 2: PFAS Results of the Experiment I. The Table Shows the Average of Three Analysis Content of the Respective PFAS

Application Showcase

Experiment II

Samples for the spiked the 50 L bag filled with reagent water with additional PFAS to test the system's capability were collected at (Figure 3):

- The reagent water, before it was spiked with PFAS
- Post 50 L bag for the spiked PFAS concentration
- Post RO-treatment and before the water was stored in Arium® Bagtank
- At the outlet of the system (without any final filter)

The first Arium® Comfort UV I system has the standard setup, which is equipped with virgin PE and PTFE tubing (Setup I).

The second Arium® Comfort I UV system is only equipped with PE tubing.

Results II

All reported results in Table 3 are in ppt. The PFAS concentration before spiking was below the limit of detection, consequently, is not presented in the table below. Although one 50 L water bag was intentionally spiked with PFAS, the experiments were conducted a week apart, resulting in an intriguing disparity in the initial concentrations. This variance could be attributed to the practical challenges of effectively mixing PFAS with a 50 L water bag, given the surfactant nature of PFAS, potentially resulting in a heterogeneous distribution within the bag. Furthermore, the instability of certain PFAS compounds at room temperature could contribute to the observed variations. These nuances underscore the importance of careful experimental design and consideration of PFAS properties in ensuring accurate and reliable results.

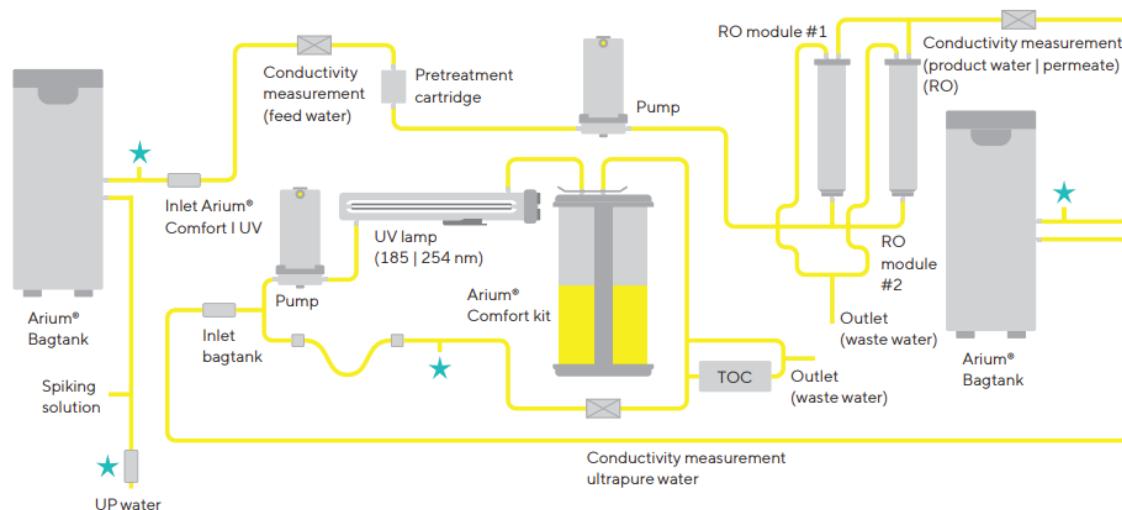


Figure 3: Modified Flowchart for the Spiked Experiment Utilizing Arium® Comfort I UV. Blue Stars Mark the Point of Sampling

Application Showcase

| | Setup I | | | Setup II | | |
|--------------|---------------------|---------------|--------------------|---------------------|---------------|--------------------|
| | Post 50 L bag (ppt) | Post RO (ppt) | Point of Use (ppt) | Post 50 L bag (ppt) | Post RO (ppt) | Point of Use (ppt) |
| PFBA | 1166.33 | n.d. | n.d. | 4587.67 | n.d. | n.d. |
| PFPeA | 1090 | n.d. | n.d. | 4149 | n.d. | n.d. |
| PFHxA | 881.43 | n.d. | n.d. | 4482 | n.d. | n.d. |
| PFHpA | 17.63 | n.d. | n.d. | 106.83 | n.d. | n.d. |
| PFOA | 502.17 | n.d. | n.d. | 2017 | n.d. | n.d. |
| PFNA | 14.4 | n.d. | n.d. | 89.5 | n.d. | n.d. |
| PFDA | 15.1 | n.d. | n.d. | 94.77 | n.d. | n.d. |
| PFUnA | 11.67 | n.d. | n.d. | 94 | n.d. | n.d. |
| PFDoA | 7.33 | n.d. | n.d. | 68 | n.d. | n.d. |
| PFTrDA | 4.73 | n.d. | n.d. | 44.07 | n.d. | n.d. |
| PFTeDA | 2.87 | n.d. | n.d. | 21.27 | n.d. | n.d. |
| T-NMeFOSAA | 8.7 | n.d. | n.d. | 60.37 | n.d. | n.d. |
| T-NETFOSAA | 6.3 | n.d. | n.d. | 37.53 | n.d. | n.d. |
| PFBS | 1387.33 | n.d. | n.d. | 8196 | n.d. | n.d. |
| PPeS | 26.5 | n.d. | n.d. | 293.93 | n.d. | n.d. |
| T-PFHxS | 638.07 | n.d. | n.d. | 4103.33 | n.d. | n.d. |
| PFHpS | 47.5 | n.d. | n.d. | 389.47 | n.d. | n.d. |
| T-PFOS | 636.83 | n.d. | n.d. | 4271 | n.d. | n.d. |
| PFNS | 17.57 | n.d. | n.d. | 200.47 | n.d. | n.d. |
| PFDS | 11.33 | n.d. | n.d. | 181.5 | n.d. | n.d. |
| PFDoS | 4.3 | n.d. | n.d. | 49.37 | n.d. | n.d. |
| 4:2 FTS | 62.07 | n.d. | n.d. | 342.57 | n.d. | n.d. |
| 6:2 FTS | 54.33 | n.d. | n.d. | 280.57 | n.d. | n.d. |
| 8:2 FTS | 52.37 | n.d. | n.d. | 303.23 | n.d. | n.d. |
| PFOSA | 1.87 | n.d. | n.d. | n.d. | n.d. | n.d. |
| HFPO-DA | 12.83 | n.d. | n.d. | 79.6 | n.d. | n.d. |
| ADONA | 12.57 | n.d. | n.d. | 82.2 | n.d. | n.d. |
| 9CI-PF3ONS | 10.87 | n.d. | n.d. | 74.97 | n.d. | n.d. |
| 11CI-PF3OUds | 3.9 | n.d. | n.d. | 94.6 | n.d. | n.d. |
| N-MeFOSA | 0.73 | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-EtFOSA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-MeFOSE | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| N-EtFOSE | 37.07 | n.d. | n.d. | 522.7 | n.d. | n.d. |
| 3:3 FTCA | 343.23 | n.d. | n.d. | 4651.67 | n.d. | n.d. |
| 5:3 FTCA | 131.3 | n.d. | n.d. | 1218.6 | n.d. | n.d. |
| 7:3 FTCA | 24.63 | n.d. | n.d. | 322.03 | n.d. | n.d. |
| PFMPA | 21.4 | n.d. | n.d. | 260.47 | n.d. | n.d. |
| PFMBA | 39.1 | n.d. | n.d. | 523.3 | n.d. | n.d. |
| NFDHA | 35.53 | n.d. | n.d. | 494.13 | n.d. | n.d. |
| PFEESA | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

Table 3: Post 50 L Bag, Post RO Treatment and at the Point of Use Concentrations of the PFAS in the Spiked Experiment

Notable differences in initial concentrations between the two setups distinctly influence the concentrations observed at both the post RO sampling point and the point of use sampling point. Commencing at concentrations in part ppb, the analytes swiftly reach non-detect levels at the point of use. The Arium® Comfort I UV system, producing reagent water, further enhances its significance, providing water of the requisite purity for testing labs engaged in PFAS applications.

TOF Results

The determination of Total Organic Fluorine (TOF) involves the subtraction of Inorganic Fluorine (IF) from the total, resulting in TOF. To calculate TOF, the inputs required are TF and IF ($TOF = TF - IF$). The measurement of free fluoride in the sample typically represents IF (Figure 4).

The process of obtaining TF involves combusting the sample at temperatures exceeding 1,000 °C to break carbon bonds and form ions. The gas containing the ions

Application Showcase

of interest is then condensed into an absorption solution and analysed by Ion Chromatography (IC). Determining IF is achieved by running the sample through IC for free fluoride. The subtraction of these values yields the TOF of the sample.

It is crucial to consider various factors, including matrix effects, contaminant ions, and the presence of dissolved or suspended solids and metals. For solids, a different approach is necessary, requiring the extraction of a solid for IF determination even though TF can be directly determined.

Adhering to the same experimental design and utilizing identical sample points as outlined in the LCMS schema, the chosen methodology involves employing CIC to analyse TOF data on a per-sample basis, allowing for a direct comparison with LCMS data. This non-speciated analysis is specifically focused on evaluating the combined impact of PFAS. A comprehensive examination of TOF provides swift insights into unknown elements, offering a rapid understanding.

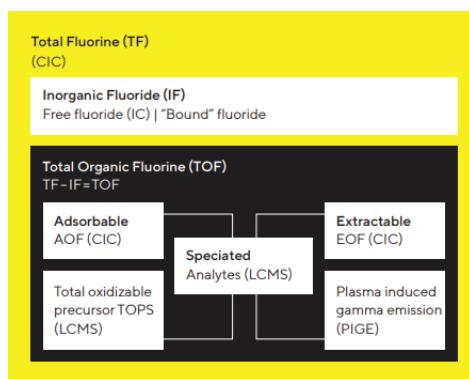


Figure 4: Scheme of a Fluorine Mass Balance Approach Applying Organically Bound Fluorine Sum Parameters

| Sample ID | | Setup I | Setup II |
|---------------|---------------------|---------|----------|
| Experiment I | Tap Water (ppb) | <7.5 | <7.5 |
| | Post RO (ppb) | <7.5 | <7.5 |
| | Point of Use (ppb) | <7.5 | <7.5 |
| Experiment II | Pre-Spiking (ppb) | <7.5 | <7.5 |
| | Post 50 L bag (ppb) | <7.5 | 24.3 |
| | Post RO (ppb) | <7.5 | <7.5 |
| | Point of Use (ppb) | <7.5 | <7.5 |

Table 4: Total Organofluorine (TOF) via Combustion Ion Chromatography (CIC)

The Arium® Comfort I UV system consistently produces high-quality reagent water, making it well-suited for PFAS testing labs. The TOF data serves as a valuable complement to LCMS data, ensuring a more comprehensive analysis. Verified non-detects and the confirmed increase after a 50 L Bag in System 2 reinforce the system's reliability. Importantly, the impact of PFAS contribution is verified, dispelling concerns of hidden analytes driving total organic fluorine levels upward. This underscores the system's transparency and accuracy in PFAS analysis.

Return on Investment

The cost analysis provided over a one-year period, as shown in Figure 5 clearly highlights the advantages of transitioning to in-house treated water. With a daily demand of two litres, the investment in the treatment device starts to yield significant financial benefits in as little as four months, ultimately resulting in substantial cost savings within the course of a year. Furthermore, this approach aligns with sustainability goals by eliminating the need for glass bottles, reducing pollution associated with shipping, and eliminating the necessity for extensive storage space.

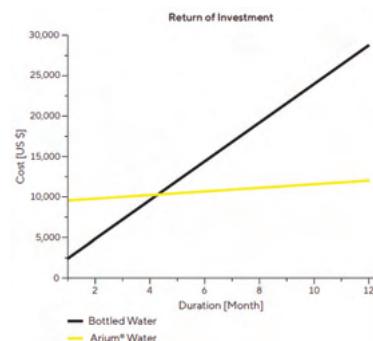


Figure 5: Comparison of Expenses Between In-House Produced Arium®-Water Type I (Ultrapure Water) and Bottled Water (LCMS Grade, With PFAS Content Below Detection Limit)

Conclusion

The Arium® Comfort I UV offers a comprehensive solution for the reduction of PFAS contaminants from tap water, reducing their concentrations to below limit of detection.

Application Showcase

Through a combination of activated carbon filtration, reverse osmosis treatment, and resin ion exchange, the system ensures the production of ultrapure water suitable for a wide range of applications. Additionally, the system's ability to treat highly contaminated solutions (~4 ppb) demonstrates its efficacy in managing even the most challenging PFAS contamination scenario, whether the system is equipped with PE tubing alone or both PE and PTFE tubing (Setup I and II). For analytical laboratory and applications demanding the highest water quality standards, the Arium® Comfort I UV provides a reliable and effective solution for PFAS reduction.

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| Application | Low Viscosity Fluids (e.g., solutions, oils) | Medium Viscosity Fluids (e.g., syrups, lotions) | Medium Viscosity Fluids (e.g., creams, jels) |
|-------------------|---|--|---|
| Pharmaceuticals | 10 - 50 RPM | 1 - 20 RPM | 0.5 - 20 RPM |
| Food and Beverage | 10 - 60 RPM | 5 - 30 RPM | 0.5 - 20 RPM |
| Chemicals | 20 - 100 RPM | 10 - 50 RPM | 1 - 50 RPM |
| Cosmetics | 10 - 50 RPM | 5 - 30 RPM | 0.5 - 20 RPM |



DSC Measurement of Polypropylene

Analysing heat-induced structural changes in polymer crystallinity using the Hitachi NEXTA® DSC 200

Thermogravimetry (TG) is a thermal analysis technique that is useful for evaluating thermal characterization of materials, predicting material lifetimes, and quantifying the composition of samples.

This application investigates the influence of heat treatment on the crystallinity of polypropylene using differential scanning calorimetry. By analysing the melting behaviour and isothermal kinetics of PP in the Hitachi NEXTA DSC200, the significant impact of heat on material properties is highlighted.

Keywords: Crystallinity, polypropylene, heat, thermal, polymer, Hitachi NEXTA® DSC200

Introduction

Polypropylene (PP) is a crystalline polymer that is cheap, excellent formability, resist water, chemical resistant and high strength. Due to these characteristics, it is used as a general-purpose plastic in a wide variety of products, including food packaging, electrical products, medical materials, car parts and synthetic paper.

When PP is formed, its crystal formation changes according to the heat treatment temperature and the conditions of the cooling process. These changes create differences in strength, heat resistance and pressure bonding properties. Food-related products may be thermally sterilized after packaging. Therefore, it is important to understand how to manage the desired crystallized state for the product and the effects of heat treatment on crystallinity.

Application Showcase

In this application, differential scanning calorimetry (DSC) is used to evaluate the crystallinity of PP molds.

Measurement

The samples were commercially available PP sheets. The NEXTA® DSC200 differential scanning calorimeter was used for the measurements.

Measurement condition 1 evaluated the temperature dependence of crystal structure. An untreated sample weighing 0.5 mg was heated from room temperature to 200 °C at 10 °C/min in a nitrogen atmosphere. In addition, thermally treated samples were created by heating them to 110, 115, and 120 °C and the quench them. These samples were then measured under the same conditions as condition 1.

Measurement condition 2 evaluated the temperature dependence of crystallization. Samples weighing 3mg were heated to 200 °C in a nitrogen atmosphere to melt them. Next, they were quenched to 120, 123, 125, 127 or 130 °C and held at that temperature for 15 to 50 minutes.

Results

1. The effects of heat treatment on PP crystallinity

Figure 1 shows the DSC curves for measurement condition 1. All samples showed an endothermic peak due to PP melting around 160 °C. Furthermore, on the low temperature side of the endothermic peak, the untreated sample showed a smooth DSC curve between 110 and 125 °C while the treated samples showed a minute peak.

Figure 2 enlarges the results around 120 °C. The treated samples showed minute endothermic peaks of several tens of μ W near their heat treatment temperatures. Each heat treatment temperature produced a different crystal structure. The peaks in the figure are considered the melting points of these structures during DSC measurement.

concentration below detection limits to up to single-digit ppt or sub-ppt-level (parts per trillion, dependent on the compound) in water.

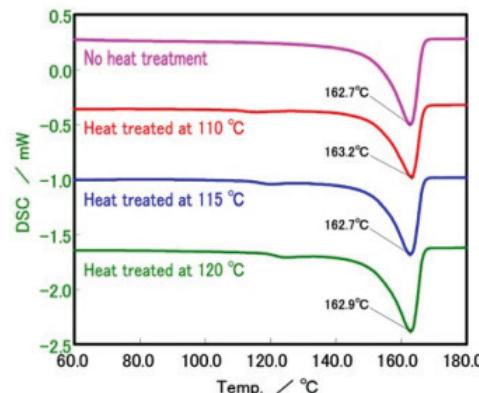


Figure 1: DSC curves for Measurement Condition 1

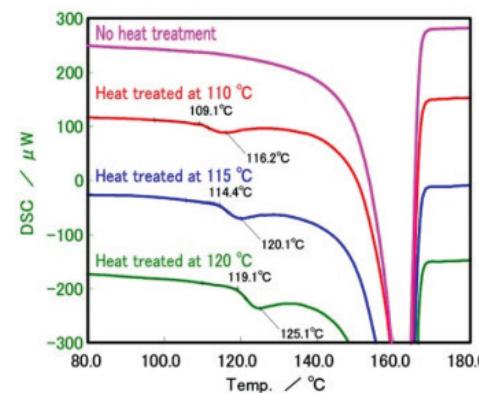


Figure 2: Enlarged views of the DSC curves for Figure 1

2. Measurement of isothermal crystallization

Figure 3 shows the DSC curves for measurement condition 2. PP crystallization produced an exothermic peak at each holding temperature. The lower the holding temperature, the sharper the peak and the earlier the peak top occurred. The higher the holding temperature, the broader the peak and the later the peak top occurred. This occurred because the higher the temperature, the greater the freedom of molecular motion, which makes crystallization more difficult and increases the time

Application Showcase

required for crystallization to be completed. The relation of crystallization temperature and crystallization time can be investigated using these results.

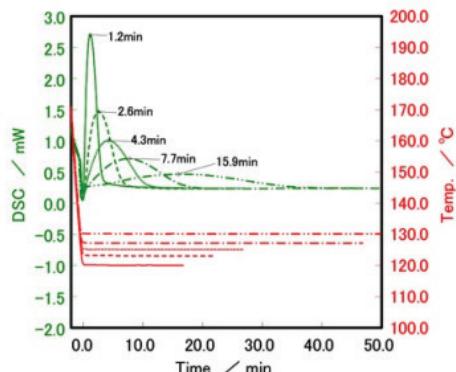


Figure 3: DSC curves for Measurement Condition 2

Conclusion

This study emphasised the impact of heat treatment on the crystallinity of polypropylene. Through differential scanning calorimetry, it is possible to observe and quantify the structural changes induced by varying thermal conditions. Key finding in this analysis highlight the importance of precise thermal analysis in understanding and controlling PP's final material properties.

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Freeze-drying of Active Pharmaceutical Ingredients

Employing the BUCHI Lyovapor™ L-250 for efficient freeze-drying

The synthesis and purification of related substances (RS) of Active Pharmaceutical Ingredients (APIs) are vital steps in drug development, ensuring the production of therapeutic compounds with high purity and yield. This process involves optimizing reaction conditions and employing techniques such as liquid-liquid extraction, filtration, crystallization, chromatography, distillation, and freeze-drying to isolate and purify the API-RS. Given the temperature sensitivity of the samples, freeze-drying is used as a safe and effective method to remove moisture, enabling longer preservation. These techniques are crucial for maintaining the structural integrity of sensitive compounds and ensuring compliance with regulatory standards.

Keywords: Freeze-drying, APIs, BÜCHI Lyovapor™ L-250.

Introduction

The synthesis of small molecules (molecular weight less than 1000 Da or g/mol), such as Active Pharmaceutical Ingredients (APIs) and their related substances (RS), is a pivotal step in drug development. This process involves producing the core chemical compound responsible for the therapeutic effect. It requires a combination of organic chemistry, chemical engineering, and analytical techniques to ensure that the final product meets stringent quality standards.

A crucial aspect of API-RS synthesis is optimizing reaction conditions to achieve consistent and reproducible results. Once the reactions are completed, a first purification isolates the API from by-products, impurities, and unreacted materials.

The workup process typically begins with quenching, where the reaction is stopped by neutralizing reactive intermediates, often using water, an acid, or a base. This

Application Showcase

stabilizes the product and removes inorganic by-products, commonly through liquid-liquid extraction. Following extraction, filtration is employed to remove solid impurities or residual catalysts. Depending on the material and particle size, techniques such as gravity filtration, vacuum filtration, or pressure filtration may be used.

Subsequent purification techniques, including crystallization, distillation, and chromatography, are employed to achieve the desired purity. Crystallization isolates solid APIs by dissolving the crude product in a suitable solvent and inducing crystal formation under controlled conditions. For more complex mixtures, chromatography is used, providing precise separation of the target compound from closely related impurities. Purification is essential for meeting regulatory requirements concerning potency, purity, and stability, as well as ensuring the therapeutic efficacy and safety of the final drug product.

After purifying temperature-sensitive materials using normal-phase or reverse-phase chromatography, it is essential to remove any residual solvents. Freeze-drying is preferred over other drying technologies for this purpose, as it effectively removes solvents while preserving the integrity of the final purified material. Freeze-drying is significant for stabilizing thermally/heat-sensitive products or those that could degrade during traditional drying methods including air drying, vacuum drying or the use of desiccators. The resulting final sample is preserved for further analysis or studies.

Equipment, Chemicals and Materials

- PrepChrom™ C-700, BÜCHI Labortechnik AG.
- C18 column (10 µm).
- Lyovapor™ L-250 Basic, BÜCHI Labortechnik AG.
- Advanced vacuum control.
- Manifold drying chamber with 12 ports.
- Two-stage Rotary vacuum pump.
- Rotary evaporator with Dewar container.
- Acetone.

- Dry ice.
- Liquid Nitrogen.
- Round Bottom flask (50, 100, 200, 500 & 1000mL).
- Acetonitrile 99.9%, Honeywell.
- Ethanol 90%, Merck.
- Methanol 90%, Merck.
- Trifluoro acetic acid 99.9%, Carl Roth.
- Acetone 99.5%, Honeywell.
- Acetic acid 95.9%, Carl Roth.

Purification & Isolation

Preparative Liquid Chromatography (Prep LC) is designed to isolate compounds in quantities and with purity sufficient for further analysis. This process is essential in various fields and aims to achieve a final purity of $\geq 95\%$ for the compound of interest.

A. Modes of Separation

Separation was achieved using reversed-phase chromatography, the most commonly employed technique in liquid chromatography. In this method, the stationary phase is less polar than the eluting solvent, which typically consists of a mixture of water and organic solvents, such as acetonitrile or methanol.

B. Quick Start Method Development

For reversed-phase separations, method development typically starts with a C18 column (10 µm, 30 x 300 mm) to optimize the solvent system for the target compound, considering the use of acidic or basic pH buffers. The mobile phase is prepared in two components: an aqueous phase (designated as Mobile Phase A) and an organic solvent (designated as Mobile Phase B). This phase consists of a mixture of solvents, including acetonitrile (60–80%), methanol (10–60%), trifluoro acetic acid (TFA), acetic acid and water.

C. Fraction Collection

Fraction collection can be performed manually or automatically, with automation often triggered by a UV detector signal. One common automated approach is the peak-based collection, where fractions are collected

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D. Fraction Analysis and Recovery

After collection, fractions are evaluated for purity using HPLC and recovery methods to assess the efficiency and yield of the purification process. Recovery methods, such as UV spectroscopy, mass spectrometry (MS), and nuclear magnetic resonance (NMR), focus on quantifying the amount and verifying the purity of the target compound in the collected fractions. Recovery calculations are performed to determine the yield.

The recovery of a compound is typically calculated as a percentage of the initial amount of the compound applied to the system.

Recovery rate (%) =

$$\frac{\text{Amount of target compound in collected fractions}}{\text{Initial amount of target compound loaded onto the column}} \times 100$$

Freeze-drying

The start-up of Lyovapor™ L-250 instrument begins with the conditioning, including the cooling of the ice condenser and the vacuum pump warm-up. This step requires approximately 30 minutes.

Meanwhile the samples were prepared for freezing. The freezing procedure varies depending on solvent mixture. The samples containing acetonitrile as the main solvent were frozen in a mixture of acetone and dry ice at around -75 °C. For samples containing ethanol or methanol, a rotary evaporator was used to remove these solvents. This step was essential because methanol's low freezing temperature and potential evaporation under reduced pressure could complicate the freeze-drying process. The remaining solvents were frozen using liquid nitrogen at -196 °C.

All sample in the round-bottom flask was frozen using Dewar accessory and a rotary evaporator.

This rotational freezing technique created a uniform, thin layer with a larger surface area, significantly

reducing processing time and facilitating faster sample drying. The drying process was performed at a set vacuum pressure of 0.50 mbar. The samples were loaded onto the manifold valve singularly. After the vacuum reached the set value, or within 15 minutes, the next sample was attached to the manifold. This procedure was repeated for each sample, ensuring low actual pressure in the drying chamber that the samples are exposed to.

Results and Discussion

The following molecules are freeze-dried to remove the solvents and solvent residuals from the sample. The obtaining API-RS can be used in disease treatment as stated in Table 1.

The duration of the process ranged from approximately 4 to 20 hours, depending on the sample volume and the solvent composition. Generally, water requires a longer drying time compared to organic mixtures. No samples showed signs of collapse or melting during the process. The goal of this study to freeze-dry various samples were successfully processed using the Lyovapor™ L-250 Basic.



Application Showcase

| Purpose | API - RS Sample | Removing Solvent |
|---|---|-------------------------|
| Bacterial Infections | Amoxicillin | ACN, water |
| | Cefixime | ACN, water |
| | Cetirizine lactose ester | ACN, water |
| | Cephalexin | Water |
| | Ciprofloxacin | ACN, water |
| | Clindamycin | ACN, water |
| | Doxycycline | ACN, water |
| | Ertapenem | ACN, water, acetic acid |
| | Gentamicin | ACN, water |
| | Linezolid | ACN, water |
| | Minocycline | ACN, buffer, water |
| | Moxifloxacin | ACN, water |
| | Tetracycline | ACN, water |
| | Tigecycline | ACN, water |
| | Oxytetracycline | ACN |
| High blood pressure | Streptomycin | ACN, water |
| | Acetbutolol | ACN, water |
| | Atenolol | Water |
| | Bisoprolol | Water |
| | Diltiazem | ACN |
| | Labetalol | ACN, water |
| Diabetes | Carvedilol | ACN, water |
| | Glimepiride | ACN, water |
| | Linagliptin | ACN, water |
| | Metformin | Water |
| | Sitagliptin | Water |
| | Varicose glucose | ACN, water |
| Depression | Voglibose | Water |
| | Duloxetine | Water |
| | Escitalopram | Water |
| | Fluvoxamine | Water |
| | Vortioxetine | Water |
| Cancer treatment | Vilazodone | ACN, water |
| | Cyclophosphamide | Water |
| | Hydroxyethyl pyrrolidine | Water |
| | Hydroxyurea | Water |
| Bladder treatment | Pazopanib | ACN, water |
| | Mirabegron | ACN, water |
| | Solifenacain | ACN, water |
| Hypocholesterolemia | Atorvastatin | ACN, water |
| | Bempedoic acid | ACN, water |
| Treatment influenza A and B | Laninamivir | ACN, water |
| | Oseltamivir | Water |
| Stomach ulcers | Famotidine | ACN, water |
| Mouth ulcers | Apremilast | ACN, water |
| Anesthetic | Bupivacaine | ACN, water |
| Calcium deficiency | Calcium Gluconate | ACN, water |
| Malarial infections | Chloroquine | Water |
| Blood clots | Dabigatran | ACN, water |
| Eye disease | Dorzolamide | ACN, water |
| Insomnia | Doxylamine | ACN, water |
| Prevent blood from clotting | Ethylenediamine tetraacetic acid (EDTA) | ACN, water |
| Fungal infection | Efinaconazole | ACN, water |
| Anaemia deficiency | Folic acid | Water |
| Treatment for leishmaniasis | Meglumine | ACN, water |
| HIV viral particles | Mercapto benzamide | Water |
| Delay spray | Dapoxetine | ACN, water |
| Improving muscle strength | Neostigmine | ACN, water |
| Eye drops | Nepafenac | Water |
| Pain relief in newborns. | Aminophenol | ACN, water |
| Analgesics | Paracetamol | ACN, water |
| Relief of stuffy nose, sinus and ear symptoms | Phenylephrine | Water |
| Mood disorders | Risperidone | Water |
| Migraine headaches | Rizatriptan | ACN |
| To lower phenylalanine levels in the blood | Sapropterin | Water |
| Prevent organ rejection in transplant | Tacrolimus | ACN, water |
| Glucose deficiency | Teneligliptin | ACN, water |
| Restructure proteins | Urea adducts | Water |
| Asthma | Vilanterol | ACN, water |

Lyovapor™ L-250

The Green Freeze Dryer The standard for Performance and Eco-Friendliness

Lowest ice condenser temperature at 25 °C: -85 °C

EcoStream™ Innovation

- ✓ Natural coolants, with a GWP of 4, significantly reduce environmental impact while also lowering lab heat and noise emissions.

Save Energy, Enhance Performance

- ✓ A smart compressor design, stable ice condenser temperature, and reliable solvent handling enable efficient, cost-effective, and precise freeze-drying of both aqueous and organic samples.

Embrace Efficiency And Elevate Control

- ✓ Infinite-Control™ technology provides real-time process monitoring and sample protection, all within a compact and easily installed instrument.

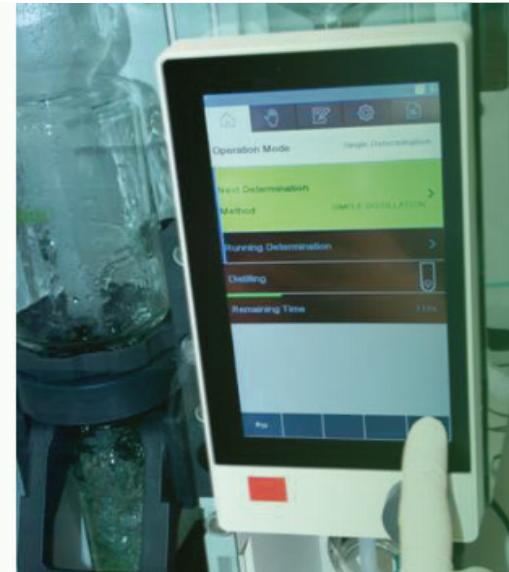
| | |
|---------------------|-----------------|
| Condenser capacity | ≤ 5 kg |
| Condensing capacity | 5 kg / 24 h |
| Applications | Water & organic |
| Eco-Friendly | Yes (low GWP) |
| Automation | Pro version |
| Best For | Green labs, R&D |



Mastering Freeze Drying Excellence

KJEL LINE-STEAM DISTILLATION

The smart way to Kjeldahl Nitrogen and Protein Determination



Features

» **Maximize Accuracy & Performance** thanks to AutoDist function and OnLevel sensor

The Kjel Line enables the highest accuracy for Kjeldahl determinations.

- Automatic recognition of distillation start for perfect reproducibility
- Distillation endpoint is recognized automatically for highest accuracy
- Automated titration with connected titrators for minimum user interaction

» **Save Resources & Time** with the reaction detection sensor and more

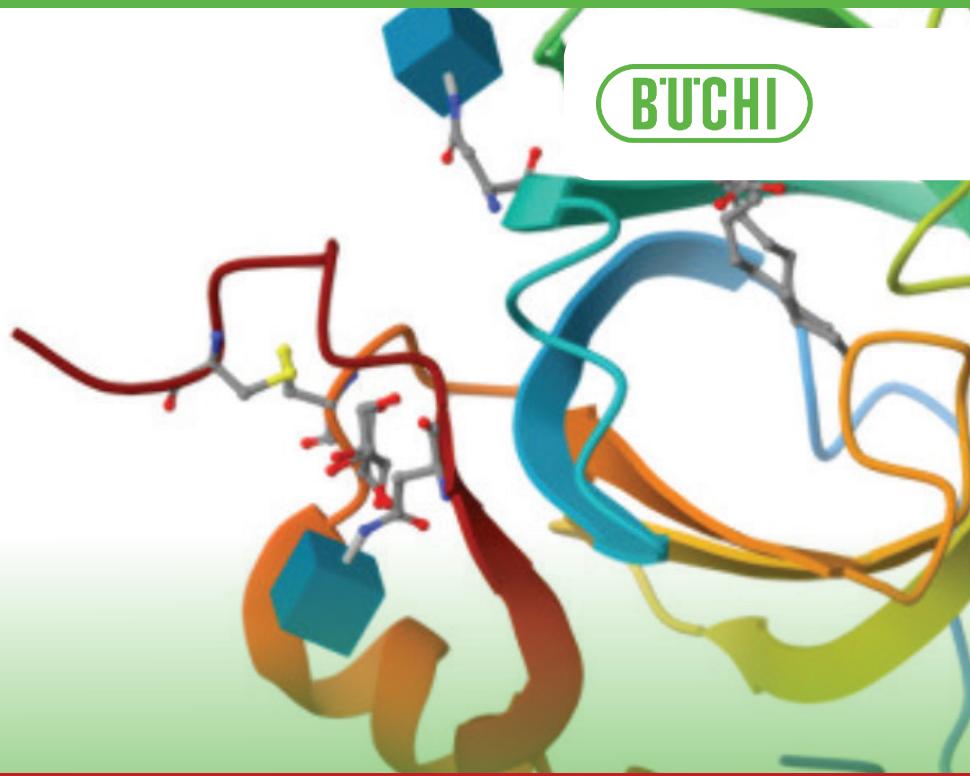
- Optimized alkalization step for saving up to 30% of reagent
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» **Experience Highest Convenience & Safety** with innovative sensor technology Perfect usability and modular upgradeability

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Comparison of different Kjeldahl Tablets for the Determination of Nitrogen and Protein in Milk according to the Kjeldahl Method



Optimised Kjeldahl analysis with the Büchi KjelMaster K-375

This application note introduces a simple and fast procedure for protein determination in milk according to the Kjeldahl method, as described in the DIN ISO 8968-1:2001, LFBG §64 L01.00-10/1, and AOAC 991.20 regulations.

Comparison in the behaviour of the different types of Kjeldahl Tablets and their individual advantages are summarised here. All the samples described in this application were digested together with sulfuric acid and different Kjeldahl Tablets with the SpeedDigester K-439 and then distilled and titrated with the Kjeldahl sampler system K-375/K-376.

1. Introduction

An easy and reliable method for determining nitrogen and protein contents in milk according to the Kjeldahl method, as described in the ISO 8968-1:2001, LFBG §64 L01.00-10/1, and AOAC 991.20 regulations, is introduced in chapter 8. All described samples were digested with the SpeedDigester K-436 and K-439. The distillation and boric acid titration are performed with the Kjeldahl sampler system K-375/K-376. In a first step, the new BUCHI Kjeldahl Tablets for performing standard Kjeldahl were compared with each other in order to learn more about the advantages of each type of tablet. For the experiments tryptophan was selected as a reference substance, whole milk and strawberry milk drink were chosen as sample products.

Keywords or phrases: Protein determination, milk, Kjeldahl method, Kjeldahl tablets, Büchi

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2. Equipment

- SpeedDigester K-436, K-439 (the parameters used for K-436 are also valid for SpeedDigester K-425)
- Scrubber K-415 TripleScrub ECO
- KjelMaster K-375 with KjelSampler K-376
- Analytical balance (accuracy ± 0.1 mg)

3. Kjeldahl Tablets

Three different types of Kjeldahl Tablets are available for performing standard Kjeldahl. Due to the different weight of the tablets, different volumes of sulfuric acid had to be used during the experiments in order to obtain the optimal ratio of catalyst to sulfuric acid. The composition of each tablet, their weight and the used volume of sulfuric acid are summarized in Table 1.

| Weight (g) | Potassium sulfate (%) | Copper sulphate pentahydrate (%) | Titanium (IV oxide) (%) | Sulphuric acid (mL) |
|------------|-----------------------|----------------------------------|-------------------------|---------------------|
| 3.7 | 94.3 | 2.8 | 2.8 | 15 |
| 4 | 99.9 | 0.06 | - | 16 |
| 5 | 99.6 | 0.4 | - | 20 |

Table 1: Composition of the Kjeldahl Tablets Titanium, ECO and Missouri

4. Chemicals and Materials

- Sulfuric acid conc. 98 %, Fluka
- Titanium, BUCHI Kjeldahl Tablet
- Missouri, BUCHI Kjeldahl Tablet
- ECO, BUCHI Kjeldahl Tablet
- Sodium hydroxide 32 %, Brenntag
- Boric acid 4 %, 200 g boric acid, Brenntag diluted to 1 l with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.1 mol/l, Fluka standard solution
- Neutralization solution for the Scrubber: 600 g sodium carbonate, calcined, technical, Synopharm about 2 mL ethanol and a spatula tip of bromothymol blue, Fluka diluted to 3 l with distilled water
- DL-tryptophan (assay >99 %), Alfa Aesar

5. Samples

- Whole milk with a labelled protein content of 3.5 g/100 mL

- Strawberry milk drink with a labelled protein content of 3 g/100 mL

The samples were purchased at a local supermarket.

6. Procedure

The determination of nitrogen and protein in whole milk and strawberry milk includes the following steps:

- Homogenization of the sample by shaking
- Digestion of the sample, using SpeedDigester K-439 or K-436
- Distillation and titration of the sample, using Kjeldahl sampler system K-375/K-376

6.1 Digestion method - tryptophan (verification of the method)

- Place approx. 0.18 g tryptophan in a 300 mL sample tube
- Add two tablets of the to be tested catalyst and the corresponding volume of sulfuric acid (98 %) described in Table 1
- Prepare additional blanks, chemicals without sample
- Carefully suspend the sample by gently swirling the tube
- Connect the Scrubber K-415 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- Insert the rack containing the samples into the preheated unit
- Digest the samples according to the parameters listed in Table 2 or 10, depending on the catalyst type.

6.2 Digestion method - samples

- Place approx. 5 g of the sample in a 300 mL sample tube
- Add two tablets of the to be tested catalyst and the corresponding volume of sulfuric acid (98 %) described in Table 1
- Prepare additional blanks, chemicals without sample
- Carefully suspend the sample by gently swirling the tube

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- Connect the Scrubber K-415 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- Insert the rack containing the samples into the preheated unit
- Digest the samples according to the parameters listed in Table 2 or 10, depending on the catalyst type.

| Step | Temp (°C) | Time (Min) |
|------------|-----------|-----------------------|
| Preheating | 480 | - |
| 1 | 480 | 10 |
| 2 | 55 | 10 |
| 3 | 490 | Variable ¹ |
| Cooling | - | 30 |

Table 2: Temperature profile for the comparison of the Kjeldahl Tablets with the K-439

¹ In order to be able to determine the progression of the digestion, different digestion times were applied in the third digestion step (490 °C), with varying time periods of 65 min and 95 min. In total the digestion time, of step 1 to 3, was of 85 min and 115 min.

6.3 Distillation and titration

Distil the samples according to the parameters listed in Table 3.

| Method parameters KjelMaster K-375 | | | |
|------------------------------------|---|------------------------|--|
| H ₂ O Volume | 60 mL | Titration solution | H ₂ SO ₄ , 0.1 mol/L |
| NaOH volume | 90 mL ² / 60 mL ³ | Sensor type | Potentiometric |
| Reaction time | 5s | Titration mode | Standard |
| Distillation mode | Fixed Time | Measuring mode | Endpoint pH |
| Distillation time | 180 s | Endpoint pH | 4.65 |
| Stirrer speed distillation | 5 | Stirrer speed volume | 7 |
| Steam output | 100% | Titration start volume | 0 mL |
| Titration type | Boric acid | Titration algorithm | optimal |
| Receiving solution volume | 50 mL | | |

Table 3: Distillation and titration with the Kjeldahl sampler system K-375/K-376

² Volume of sodium hydroxide used for the comparison of the Kjeldahl Tablets

³ Volume of sodium hydroxide

6.4 Calculation

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

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

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

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

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

w_N : weight fraction of nitrogen

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

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

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

c : titrant concentration [mol/l]

f : titrant factor (for commercial solutions normally 1.000)

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

m_{sample} : sample weight [g]

1000 : conversion factor [mL/l]

%N : percentage of weight of nitrogen

%P : percentage of weight of protein

PF : sample-specific protein factor (6.38 for dairy products)

%N_{try} : percentage of weight of nitrogen corrected for the purity of reference

substance tryptophan (%)

P : purity of the reference substance tryptophan (%)

7. Results for the comparison of the Kjeldahl Tablets

7.1 Recovery of tryptophan

The results of the nitrogen determination and the recovery of tryptophan (assay > 99 %) after 85 and 115

Application Showcase

min, are presented in Table 4-5. The nominal value of tryptophan is 13.72 % of nitrogen.

| Tryptophan | m _{sample} [g] | V _{sample} [ml] | %N _{try} | Recovery [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|--------------------------|-------------------|--------------|-------------|---------|
| Titanium ^a | 0.1733 | 8.48 | 13.53 | 98.6 | 98.8 | 0.4 |
| Titanium ^a | 0.1858 | 9.39 | 13.623 | 99.3 | | |
| Titanium ^a | 0.1816 | 8.872 | 13.522 | 98.6 | | |
| ECO ^b | 0.1741 | 8.341 | 13.236 | 96.5 | 95.6 | 11 |
| ECO ^b | 0.1805 | 8.581 | 13.143 | 95.8 | | |
| ECO ^b | 0.1939 | 9.08 | 12.963 | 94.5 | | |
| Missouri ^c | 0.182 | 8.778 | 13.335 | 97.2 | 96.8 | 0.3 |
| Missouri ^c | 0.1827 | 8.762 | 13.259 | 96.7 | | |
| Missouri ^c | 0.1786 | 8.566 | 13.253 | 96.6 | | |

Table 4: Results of the recovery of nitrogen in tryptophan with K-439 after 85 min

^a Blank 0.194 mL (n=3)

^b Blank 0.198 mL (n=3)

^c Blank 0.201 mL (n=3)

| Tryptophan | m _{sample} [g] | V _{sample} [ml] | %N _{try} | Recovery [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|--------------------------|-------------------|--------------|-------------|---------|
| ECO ^b | 0.177 | 8.652 | 13.522 | 98.6 | 98.3 | 0.4 |
| ECO ^b | 0.1822 | 8.88 | 13.49 | 98.3 | | |
| ECO ^b | 0.1886 | 9.157 | 13.448 | 98 | | |
| Missouri ^c | 0.1724 | 8.497 | 13.617 | 99.3 | 99.2 | 0.3 |
| Missouri ^c | 0.1916 | 9.381 | 13.558 | 98.8 | | |
| Missouri ^c | 0.1891 | 9.317 | 13.641 | 99.4 | | |

Table 5: Results of the recovery of nitrogen in tryptophan with K-439 after 115 min.

7.2 Protein determination in milk

The results of the determination of nitrogen and protein contents in whole milk and strawberry milk drink after 85 and 115 min are presented in Tables 6-9.

| Milk drink | m _{sample} [g] | m _{sample} [g] | %N | Protein [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|-------------------------|-------|-------------|-------------|---------|
| Titanium ^a | 5.331 | 8.545 | 0.456 | 2.91 | 2.9 | 0.2 |
| Titanium ^a | 5.0626 | 8.392 | 0.454 | 2.89 | | |
| Titanium ^a | 5.0838 | 8.45 | 0.455 | 2.9 | | |
| ECO ^b | 5.0963 | 7.932 | 0.425 | 2.71 | 2.8 | 3.1 |
| ECO ^b | 5.1436 | 8.432 | 0.448 | 2.86 | | |
| ECO ^b | 5.1437 | 8.444 | 0.449 | 2.87 | | |
| Missouri ^c | 5.1347 | 8.467 | 0.451 | 2.88 | 2.9 | 0.2 |
| Missouri ^c | 5.1361 | 8.46 | 0.45 | 2.87 | | |
| Missouri ^c | 5.1399 | 8.449 | 0.45 | 2.87 | | |

Table 6: Results of the recovery of nitrogen and protein in strawberry milk drink with K-439 after 85 min.

^a Blank 0.086 mL (n=3)

^b Blank 0.090 mL (n=3)

^c Blank 0.098 mL (n=3)

The experimental protein content [%] was re-calculated taking the density of (1.030 g/mL)² into account in order to obtain the protein content as g/100 mL.

| Milk drink | m _{sample} [g] | V _{sample} [ml] | %N | Protein [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|--------------------------|-------|-------------|-------------|---------|
| ECO ^b | 5.1212 | 8.529 | 0.456 | 2.91 | 2.9 | 0 |
| ECO ^b | 5.1004 | 8.495 | 0.456 | 2.91 | | |
| ECO ^b | 5.1222 | 8.537 | 0.456 | 2.91 | | |
| Missouri ^c | 5.0566 | 8.415 | 0.455 | 2.9 | 2.9 | 0.1 |
| Missouri ^c | 5.1903 | 8.615 | 0.454 | 2.9 | | |
| Missouri ^c | 5.0821 | 8.459 | 0.455 | 2.9 | | |

Table 7: Results of the recovery of nitrogen and protein in strawberry milk drink with K-439 after 115 min.

^b Blank 0.194 mL (n=3)

^c Blank 0.201 mL (n=3)

With the catalyst Titanium and Missouri a digestion time of 85 min is sufficient to have corresponding protein content to the labelling of the strawberry milk drink of 3.0 g/100 mL. To compare the results, the average in % was multiplied with the density of 1.030 g/mL. With the catalyst ECO an elongation of 30 min is necessary to show a complete digestion.

In Table 8 and 9, the results of the determination of nitrogen and protein in whole milk are presented

| Whole milk | m _{sample} [g] | V _{sample} [ml] | %N | Protein [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|--------------------------|-------|-------------|-------------|---------|
| Titanium ^a | 5.06 | 9.768 | 0.53 | 3.38 | 3.4 | 0.3 |
| Titanium ^a | 5.0499 | 9.783 | 0.532 | 3.39 | | |
| Titanium ^a | 5.0279 | 9.693 | 0.529 | 3.38 | | |
| ECO ^b | 5.0091 | 9.557 | 0.523 | 3.34 | 3.3 | 0.5 |
| ECO ^b | 5.0326 | 9.521 | 0.519 | 3.31 | | |
| ECO ^b | 5.0129 | 9.563 | 0.523 | 3.34 | | |
| Missouri ^c | 5.0486 | 9.617 | 0.522 | 3.33 | 3.3 | 0.1 |
| Missouri ^c | 5.0543 | 9.607 | 0.521 | 3.33 | | |
| Missouri ^c | 5.0223 | 9.554 | 0.522 | 3.33 | | |

Table 8: Results for the recovery of nitrogen and protein in whole milk with K-439 after 85 min

^a Blank 0.194 mL (n=3)

^b Blank 0.198 mL (n=3)

^c Blank 0.201 mL (n=3)

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| Whole milk | m _{sample} [g] | m _{sample} [g] | %N | Protein [%] | Average [%] | RSD [%] |
|-----------------------|-------------------------|-------------------------|------|-------------|-------------|---------|
| ECO ^b | 5.0174 | 9.688 | 0.53 | 3.38 | 3.4 | 0.1 |
| ECO ^b | 5.0396 | 9.736 | 0.53 | 3.38 | | |
| ECO ^b | 5.0645 | 9.803 | 0.53 | 3.39 | | |
| Missouri ^c | 4.9668 | 9.564 | 0.53 | 3.37 | 3.4 | 0.3 |
| Missouri ^c | 5.0572 | 9.725 | 0.53 | 3.37 | | |
| Missouri ^c | 5.0429 | 9.744 | 0.53 | 3.38 | | |

Table 9: Results of the recovery of nitrogen and protein in whole milk with K-439 after 115 min.

^b Blank 0.194 mL (n=3)

^c Blank 0.201 mL (n=3)

To compare the results, the average in % was multiplied with the density of 1.030 g/mL.

The labelled protein content for the whole milk is 3.5 g/100 mL. Just with the catalyst Titanium a complete digestion in 85 min is possible. For Missouri and ECO an elongation of 30 min is necessary to reach the labelled protein content of 3.5 g/100 mL.

7.3 Conclusion

With the reference material tryptophan, a good differentiation between the Kjeldahl Tablets is possible. Titanium is the only catalyst, with a recovery (98.8 %; rsd 0.4 %) within the specification of > 98 %, after a digestion time of 85 min. Missouri and ECO need an elongation of 30 min to reach the specification (Figure 1).

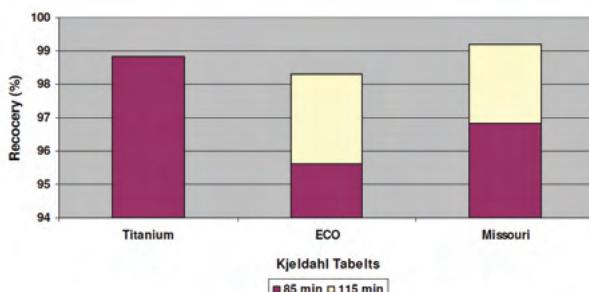


Figure 1: Recovery of tryptophan with different types of Kjeldahl Tablets and digestion times.

In Figure 2 the behaviour of the Kjeldahl Tablets with the sample material strawberry milk drink and whole milk are presented.

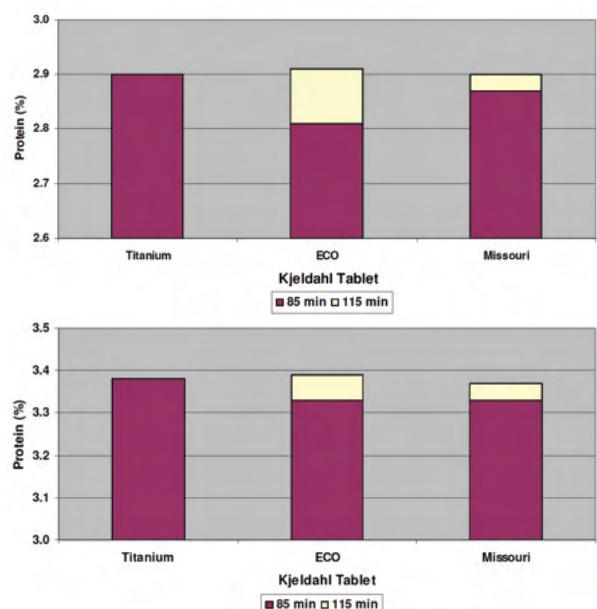


Figure 2: Up: Protein content (%) of strawberry milk drink (n=3) using different Kjeldahl Tablets and digestion times; Below: Protein content (%) of whole milk (n=3) using different Kjeldahl Tablets and digestion times.

The results of the samples are also presented in Table 6-9. The behaviour is similar to the results with the tryptophan. Just the catalyst Titanium is able to digest the samples in 85 min. The catalyst ECO and Missouri need an elongation of 30 min. It is possible, that the combination of copper and titanium helps to digest the ring-bonded nitrogen faster, than the copper alone. In the official regulations¹, a combination of copper sulphate and potassium sulphate, similar to the catalyst Missouri is described.

| Kjeldahl Tablet | Total digestion time [min] | Advantage |
|-----------------|----------------------------|-------------------------------|
| Titanium | 85 | Shortest total digestion time |
| ECO | 115 | Lowermost copper content |
| Missouri | 115 | Similar to AOAC 99120 |

Table 10: Comparison of the Kjeldahl Tablets

For the digestion with the K-439 and the K-436, the fastest Kjeldahl Tablet, "Titanium", was used for the following application.

Application Showcase

8. Results for the digestion conducted with the SpeedDigester K-439 and K-436

The following chapter describes the digestion of the milk samples whole milk and strawberry milk drink which were conducted with the SpeedDigester K-436 and K-439.

The tablet "Titanium" was the only catalyst used for this experiment, the total digestion time was of 85 min.

The preparation of the samples and the calculation of the results are similar to the ones described in Chapter 7. Two tablets "Titanium" and 15 mL of sulfuric acid (98 %) were used (Table 1) for the experiment. The total time of the digestion conducted with the K-439 was of 85 min respective 90 min for K-436. All parameters are specified in Table 11.

| | K- 439 | | K- 436 | |
|------------|------------------|------------|---------------|------------|
| Step | Temperature [°C] | Time [min] | Heating Level | Time [min] |
| Preheating | 480 | - | 8.5 | 10 |
| 1 | 480 | 10 | 8.5 | 10 |
| 2 | 550 | 10 | 9.5 | 15 |
| 3 | 490 | 65 | 8.5 | 65 |
| Cooling | - | 30 | - | 30 |

Table 11: Parameters for the digestion with K-439 und K-436

- If the liquid inside the sample tube is not clear and of a blue-green colour after the digestion, digest for an additional 30 min as described in step 3

- Let the samples cool down to room temperature

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

8.1 Digestion with the Kjeldahl Tablet Titanium and K-439

8.1.1 Recovery of tryptophan

The results of the recovery of nitrogen in tryptophan

(assay > 99 %) are presented in Table 12. The nominal value of tryptophan is 13.72 % of nitrogen. The determined values are within the specification of > 98 %¹.

| Tryptophan | m _{sample} [g] | V _{sample} [ml] | %N _{Try} | Recovery [%] |
|------------|-------------------------|--------------------------|-------------------|--------------|
| Sample 1 | 0.1733 | 8.48 | 13.53 | 98.6 |
| Sample 2 | 0.1858 | 9.139 | 13.623 | 99.3 |
| Sample 3 | 0.1816 | 8.872 | 13.522 | 98.6 |
| Average | - | - | 13.56 | 98.8 |
| Rsd [%] | - | - | 0.1 | 0.1 |

Table 12: Results of the recovery of nitrogen in tryptophan with K-439.

The mean blank volume for this sample was 0.194 mL (n = 3).

8.1.2 Protein determination in milk

The results of the determination of nitrogen in strawberry milk drink and whole milk are presented in Tables 13 and 14.

| Milk drink | m _{sample} [g] | V _{sample} [ml] | %N | %P |
|------------|-------------------------|--------------------------|-------|------|
| Sample 1 | 5.1331 | 8.545 | 0.456 | 2.91 |
| Sample 2 | 5.0626 | 8.392 | 0.454 | 2.89 |
| Sample 3 | 5.0838 | 8.45 | 0.455 | 2.9 |
| Average | - | - | 0.455 | 2.9 |
| Rsd [%] | - | - | 0.2 | 0.2 |

Table 13: Results of the determination of nitrogen and protein in strawberry milk drink with K-439

The mean blank volume for this sample was 0.194 mL (n = 3).

| Whole milk | m _{sample} [g] | V _{sample} [ml] | %N | %P |
|------------|-------------------------|--------------------------|-------|------|
| Sample 1 | 5.06 | 9.768 | 0.53 | 3.38 |
| Sample 2 | 5.0499 | 9.783 | 0.532 | 3.39 |
| Sample 3 | 5.0279 | 9.693 | 0.529 | 3.38 |
| Average | - | - | 0.53 | 3.4 |
| Rsd [%] | - | - | 0.3 | 0.3 |

Table 14: Results of the determination of nitrogen and protein in whole milk with K-439

Application Showcase

The mean blank volume for this sample was 0.194 mL (n = 3).

The experimental protein content [%] was re-calculated taking the density (1.030 g/mL) into account in order to obtain the protein content as g/100 mL. For the strawberry milk drink, the protein content is 3.0 g/100 mL, for the whole milk 3.5 g/100 mL. The values correspond to the labelled ones, described in chapter 5.

8.2 Digestion with Kjeldahl Tablet Titanium and the K-436

8.2.1 Recovery of tryptophan

The results of the recovery of nitrogen in tryptophan (assay > 99 %) are presented in Table 15. The nominal value of tryptophan is 13.72 % nitrogen. The determined values are within the specification of > 98 %¹

| Tryptophan | m _{sample} [g] | V _{sample} [ml] | %N _{Try} | Recovery [%] |
|------------|-------------------------|--------------------------|-------------------|--------------|
| Sample 1 | 0.1755 | 8.632 | 13.597 | 99.1 |
| Sample 2 | 0.1817 | 8.964 | 13.65 | 99.5 |
| Sample 3 | 0.1886 | 9.267 | 13.605 | 99.2 |
| Average | - | - | 13.62 | 99.3 |
| Rsd [%] | - | - | 0.2 | 0.2 |

Table 15: Results of the recovery of nitrogen in tryptophan with K-436

The mean blank volume for this sample was 0.199 mL (n = 3).

8.2.2 Protein determination in milk

The results of the determination of protein in strawberry milk drink and whole milk are presented in Tables 16 and 17.

| Milk drink | m _{sample} [g] | V _{sample} [ml] | %N | %P |
|------------|-------------------------|--------------------------|-------|------|
| Sample 1 | 5.0527 | 8.43 | 0.456 | 2.91 |
| Sample 2 | 5.2099 | 8.659 | 0.455 | 2.9 |
| Sample 3 | 4.9259 | 8.227 | 0.457 | 2.91 |
| Average | - | - | 0.456 | 2.91 |
| Rsd [%] | - | - | 0 | 0 |

Table 16: Results of the determination of nitrogen and protein in strawberry milk drink with K-436.

The mean blank volume for this sample was 0.199 mL (n = 3).

| Whole milk | m _{sample} [g] | V _{sample} [ml] | %N | %P |
|------------|-------------------------|--------------------------|-------|------|
| Sample 1 | 4.9758 | 9.602 | 0.529 | 3.38 |
| Sample 2 | 5.0644 | 9.74 | 0.528 | 3.37 |
| Sample 3 | 5.0349 | 9.756 | 0.532 | 3.39 |
| Average | - | - | 0.53 | 3.38 |
| Rsd [%] | - | - | 0.4 | 0.4 |

Table 17: Results of the determination of nitrogen and protein in whole milk with K-436.

The mean blank volume for this sample was 0.199 mL (n = 3).

The experimental protein content [%] was re-calculated taking the density (1.030 g/mL) into account in order to obtain the protein content as g/100 mL. For the strawberry milk drink, the protein content is 3.0 g/100 mL, for the whole milk 3.5 g/100 mL. These values correspond to the labelled ones, described in chapter 5.

9. Comparison to Standard Methods

The standard methods DIN EN ISO 8968-1:2001 and LFGB §64 L01.00-10/1 are identical. AOAC 991.20 Part "Traditional Method" does not use sucrose and water as blanks. All other parts are identical with DIN EN ISO 8968-1:2001 and LFGB §64 L01.00-10/1. The differences to this application are shown in Table 18.

Application Showcase

| | This application | Standard methods | Notes/Impact |
|---|---|---|--|
| Sample tube | 300 ml | 500-800 ml | No impact |
| Catalyst | 2x 3.7 g Tablets cont. - 47.7 % K ₂ SO ₄ - 2.8 % TiO ₂ - 18 % CuSO ₄ | 15 g K ₂ SO ₄ + 1ml CuSO ₄ Solution ¹ | Easy to handle specially in routine analytics. The choice of catalyst does not influence the result, but the digestion time |
| Sulfuric acid | 15 ml | 25 ml | No impact. Same ratio of sulfuric acid / catalyst |
| Water | 60 ml | 300-400 ml | The K-375 generates steam in a separated vessel; therefore it is not necessary to add such a high amount of water to the digested sample as described in the standard methods. |
| Sodium hydroxide | 60 ml (Conc.: 32 %) | 75 ml (Conc.: 50 %) | No impact. Same ratio of sodium hydroxide /sulfuric acid. Sodium hydroxide 32% is gentler to the pump than higher concentrated alkali. |
| Blank with sucrose for determination of samples | no | DIN EN ISO and LFGB §64 use 0.85 g and 5 ml water for blank in addition to the chemicals. | No difference observed between blanks with and without sucrose. |
| Titration | Boric acid titration | AOAC: back titration | No impact. The results are equal with boric acid titration or back titration. |

Table 18: Differentiation to the standard methods. ¹5 g CuSO₄ x 5 H₂O diluted to 100 mL deionized Water

10. Conclusion

The determination of nitrogen and protein contents in whole milk and strawberry milk drink with the help of the different types of Kjeldahl Tablets provides reliable and reproducible results which correspond to the labelled values of the sample products with low relative standard deviations. The total digestion time can vary between 85 and 115 min depending on the type of Kjeldahl Tablets and digestion unit used and can therefore be adapted to meet the individual needs. The fully-automatic Kjeldahl sampler system, KjelMaster K-375 and KjelSampler K-376, in combination with the unique SpeedDigester models, reduce the time needed for sample analysis significantly.

References

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- DIN EN ISO 8968-1:2001, LFGB §64 L01.00-10/1
- Operation manual of SpeedDigester K-425 / K-436
- Operation manual of SpeedDigester K-439
- Operation manual of Scrubber K-415
- Operation manual of Kjeldahl sampler system K-375/K-376

BUCHI





FT-IR Spectroscopy in Ultrahigh Vacuum

Surface Science Approach for Understanding Reactions on Catalytic Oxide Powders using Bruker VERTEX 80v FT-IR vacuum spectrometer with the external UHV adaption

This application investigates the role of oxygen vacancies on TiO_2 , using a UHV-FTIR system integrated with other surface analysis techniques. By using the Bruker FT-IR spectrometer in ultrahigh vacuum environments, surface science and catalytic reactions on oxide powders have been explored.

Keywords or phrases: FT-IR Spectroscopy, Ultrahigh Vacuum (UHV), TiO_2 , oxygen vacancies, catalytic, surface, density, Bruker VERTEX 80v FT-IR

Introduction

The fundamental advantage of using FT-IR spectroscopy in vacuum is to avoid the absorption of atmospheric moisture and other gas species (CO_2). Vacuum spectrometers provide a better stability and reproducibility in comparison to the dry air/nitrogen purged spectrometers. Furthermore, the UHV adaption allows IR characterization of samples that have to be prepared and stored under UHV conditions without sample-transfer in air and therefore enables the closely combination of FT-IR technique with other UHV experimental methods.

The number of defects on an oxide surface is crucial for its catalytic activity. To understand the role of the defects in particular is the precondition for investigating numerous catalytic reactions in detail. Recently, the oxygen defects on titanium dioxide (TiO_2) have been considered using scanning tunnelling

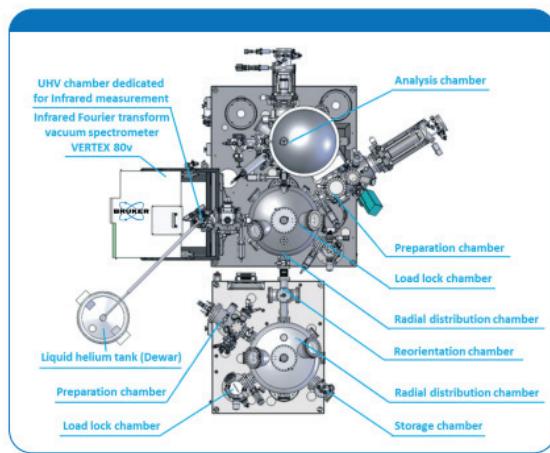
Application Showcase

microscopy (STM) combined with density functional theory (DFT) calculations. Unfortunately, many standard experimental techniques in this research field can only be applied to single crystalline oxides in a straightforward fashion, but not to powders or nanoparticles, which is the technologically most important form of oxide materials. In contrast, FT-IR spectroscopy is not limited to the single crystalline phase only, but can also be applied to powders, nanoparticles etc. with high sensitivity.

Therefore, ultrahigh vacuum FT-IR spectroscopy (UHVFTIRS) was applied to both the well-understood single crystal reference system and the powder particles, in order to determine the density of O vacancies and to demonstrate the role of O vacancies for the surface chemistry of formaldehyde on TiO_2 . For this purpose, CO was used as a probe molecule to identify the defect sites on rutile TiO_2 (r- TiO_2) surface¹.

Experimental

An UHV-FTIRS apparatus dedicated to the spectroscopic characterization of oxides, single crystals as well as powders was developed. The schematic build-up of this innovatively designed system is shown in Figure 1.



of load-lock, preparation, distribution, measurement, magazine, and analysis chambers.

The UHV system enables additional characterizations using combined measurement techniques, such as X-ray photoelectron spectroscopy (XPS), ultraviolet photoelectron spectroscopy (UPS), Auger electron spectroscopy (AES), low energy electron diffraction (LEED), and thermal desorption spectroscopy (TDS). The FTIRS experiments on the r- TiO_2 single crystal and powder samples were performed in the UHV chamber dedicated for IR measurements (UHV-IR chamber, Figure 2), which is internally adapted in the sample compartment of the VERTEX 80v spectrometer. The adaption is realized by vacuum-compliant window flanges which are mounted directly to the window fitting of the UHV chamber. The spectrometer can be demounted from and attached to the UHV-IR chamber by simply sliding backwards and forwards along its supporting frame. The unique feature of this dedicated design is the entire evacuated optical path to avoid absorption of atmospheric moisture and other background signals from gas phase (CO_2), thus providing the possibility to record IR data with highest sensitivity and stability.

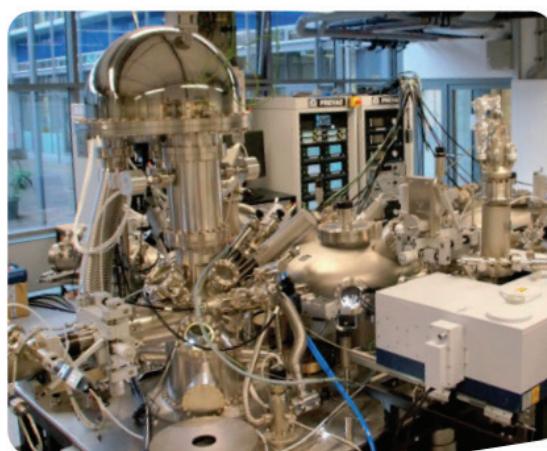


Figure 1: a) Schematic drawing of the UHV system (PREVAC, www.prevac.eu) combined with the Bruker vacuum FT-IR spectrometer VERTEX 80v.
b) A photo of the UHV system at Institute of Functional Interfaces (IFG) in Karlsruhe Institute of Technology (KIT).

It combines a Bruker VERTEX 80v vacuum FTIR spectrometer with a PREVAC UHV system consisting

This is an essential prerequisite for monitoring molecular species adsorbed on oxide surfaces,

Application Showcase

since the optical properties of oxides result in a decreased signal-to-noise ratio (SNR) by one to two orders of magnitude relative to metals. A spectrometer internal motorized off-axis paraboloidal mirror in front of the spectrometer sample compartment together with another motorized 90° off-axis ellipsoidal mirror adjusted to the detector can be driven in two positions respectively for transmission (powders) and IR reflection absorption spectroscopy (IRRAS) measurements at grazing incidence with an incident angle of 80° to surface normal (for flat substrates such as single crystals)².

In order to identify the defects, CO was adsorbed onto perfect and reduced r-TiO_2 surface. The reduction was carried out by controlled sputtering or over-annealing at elevated temperature (900 K) to produce O vacancies. The r-TiO_2 single-crystal measurements were performed using IRRAS and the data for powder samples were recorded in transmission geometry. The low-vacancy (perfect) surface of the powder sample was achieved by depositing the powder to a small amount of oxygen in UHV at 110 K.

Results

Figure 3 presents the surface structure model of r-TiO_2 . As supported by calculations and previous works, the exposed CO molecules will populate only the adsorption sites labelled 1 and 2 in Figure 3 (and sites located further away from the O vacancy) but not bind to the Ti atoms exposed within or next to the O vacancy (labelled 0).

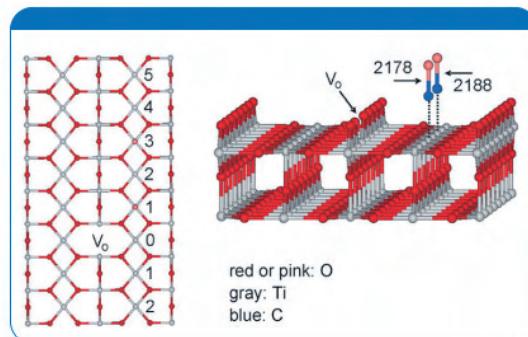


Figure 3: Ball-and-stick model of the r-TiO_2 surface. The CO molecules adsorbed at different Ti sites (labelled 0-5) are sketched¹.

The IRRAS data shown in Figure 4 (left) demonstrate that on a fully oxidized (perfect) r-TiO_2 surface with low density of defects only one absorption band at 2188 cm^{-1} is visible in the CO-stretching regime related to CO adsorbed on perfect parts of the surface (labelled 2-5 in Figure 3), whereas on reduced r-TiO_2 a second band at



Figure 2: a) The UHV-IR chamber which can be directly adapted to the sample compartment by vacuum-compliant window flanges. b) Top view of the internal adapted UHV-IR chamber in the opened Bruker FT-IR spectrometer VERTEX 80v. The adaption positions are indicated. c-d) close-up view of the adaption positions.

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2178 cm^{-1} appears which is assigned to CO bound to Ti cations located on sites labelled 1 in Figure 3. On the basis of the intensity ratio of the two bands and considering the maximum coverage of CO on r-TiO₂ at 110 K, the concentration of O vacancies can be estimated to an amount of around 10%.

The same procedure was applied to the r-TiO₂ powder particles. The FTIR spectra are shown in Figure 4 (right).

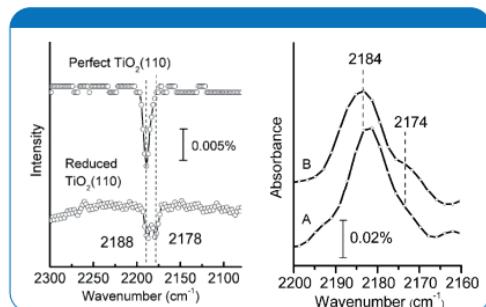


Figure 4: Left: IRRAS data for CO adsorbed on perfect and reduced r-TiO₂ single-crystal surface at 110 K. The absorbance amounts to 10-5 au. Right: UHV-FTIR data for CO adsorbed on oxidized (A) and reduced (B) r-TiO₂ powder particles at 110 K recorded in transmission mode¹.

On oxidized powder particles (A in Figure 4 right) only one absorption band at 2184 cm^{-1} related to CO bound to Ti cations distant to O vacancies or other defects was detected. On reduced powder particles (B in Figure 4 right) a new band appears at 2174 cm^{-1} related to CO adsorbed on site 1 which is in accordance with the IRRAS data or r-TiO₂ single crystal surface. Furthermore, the defect density on the powder particles can be calculated from a comparison of the relative band intensities and amounts to about 8%. To demonstrate the potential of this established method for determining defects densities in a semi-quantitative fashion, the reductive coupling of formaldehyde to ethylene catalysed by TiO₂, an important C-C coupling reaction was investigated. Formaldehyde was exposed to both oxidized and reduced r-TiO₂ powder particles and IR spectra were recorded (data not shown here). Only on the reduced powder the intermediate of the catalysed surface reaction was identified and the yield of ethylene correlates well with the above estimated density of defects on the r-TiO₂ powder particles.

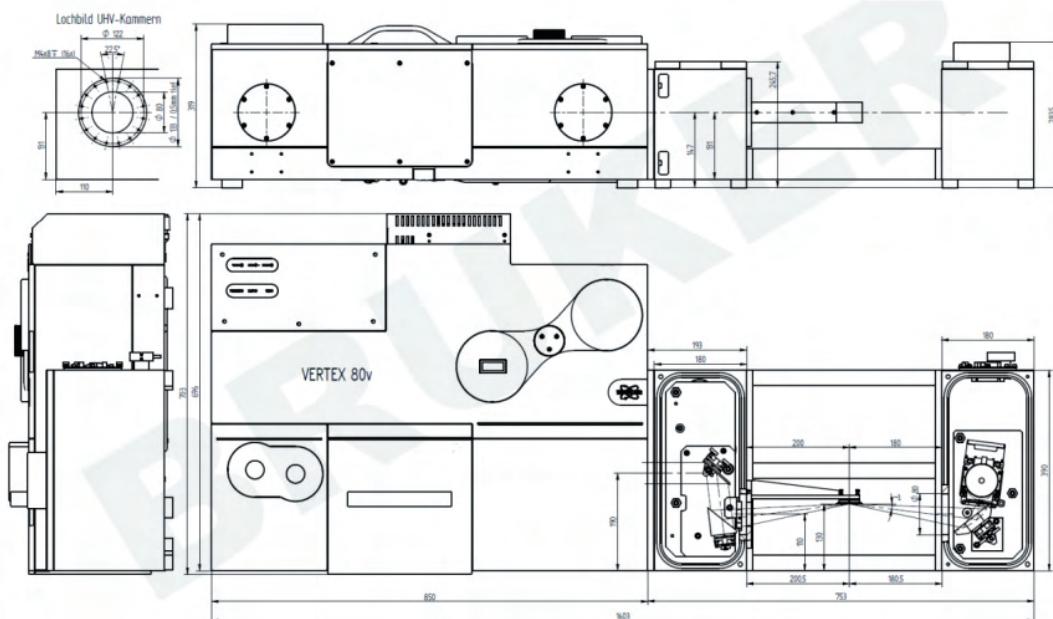


Figure 5: Technical drawing of the Bruker VERTEX 80v FT-IR vacuum spectrometer with the external UHV adaption (W109/UHV).

Application Showcase

Further approaches of UHV-FTIR adaption

An additional possibility to adapt FTIR spectroscopy to an existing UHV system, which has been used more frequently by customers, is to use the vacuum chambers W109/UHV (Bruker) matched to VERTEX 70v/80v consisting of two chambers (Figure 5). One is adapted to the spectrometer and contains a kinematic base plate with transfer optics. The other free-standing chamber includes further transfer optics and detector mount. Both chambers are prepared for a vacuum tight adaption to the UHV system. In this case, the UHV system will be arranged outside the spectrometer sample compartment (external adaption) providing more flexibility and space for voluminous UHV chambers with combined techniques. Also, for this approach transmittance as well as IRRAS configuration is available (Figure 6)

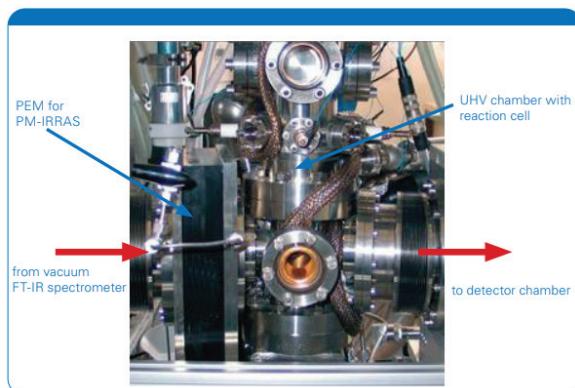


Figure 6: Experimental setup combining a UHV surface analysis chamber with a UHV-high-pressure reaction cell optimized for PM-IRRAS⁷.

Conclusion

Integration of FT-IR Spectroscopy with UHV conditions offer a powerful method for studying the surface of oxides in catalytic reactions. The ability to avoid atmospheric interference significantly improves sensitivity of the results. Bruker's VERTEX 80v vacuum FT-IR Spectrometer opens avenues in various applications in surface science.

References

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FT-IR Spectrometers

VERTEX 80/80v

Inert Gas Glovebox Workstation

Features:

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



GLOVEBOX TECHNICAL DATA

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

Applications:

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



Weighing Metrology Terms Simplified

Effective application of weighing instruments and integrity of measurement data is essential across numerous scientific industries. This necessitates a comprehensive and thorough understanding of weighing metrology.

From “tare” to “calibration”, weighing terminology can often be confusing. This guide¹ provides a systematic explanation of fundamental terms. By providing clarity on these technical concepts, this resource aims to improve measurement practices across all industries.

1 Accuracy

What is it and when is a balance or scale weighing accurately?

Accuracy is how close the measurement value is to the true value.

Three different explanations could be used to answer this question:

1. A weighing instrument is accurate when it meets the process and quality requirements, i.e., when the measurement uncertainty is smaller than the desired weighing tolerances.
2. Accuracy can be achieved when the smallest net weight you are weighing (user requirement) is larger than the minimum weight (derived from calibration).
3. Weighing results are accurate when you are weighing in the safe weighing range.

To assess how accurate your weighing results are, an onsite calibration in the environment where the balance or scale is being used is required before you use the instrument in a weighing process. It is also required on a regular basis to ensure ongoing accuracy.

2 Adjustment

What is it and how does it differ from calibration?

Adjustment is a modification of the instrument so that its reading corresponds more closely to the true value.

Calibration

A balance or scale is calibrated to understand and document how far the indication (measured value) is from the true value.

Adjustment

Adjusting a balance means adjusting the displayed values (ideally on the basis of a calibration).

3 Calibration

What is it and how often does a weighing device need to be calibrated?

Calibration establishes a relation between what you read on the instrument and a measurement standard including the associated measurement uncertainty.

The accuracy of weighing devices deteriorates over time because of normal wear and tear. If not discovered, these changes can lead to inconsistent product quality, reputational damage, and financial and legal consequences.

To avoid these risks, a balance or scale must be calibrated regularly to assess and document how it behaves—that is, how far the measured value (reading) is away from the true value.

To measure device performance including the measurement uncertainty, the device must be calibrated in its actual place of use over its entire weighing range. This is the only way to know how reliable your weighing results are (in other words, if what you see is what you get).

4 Capacity

What is it and is capacity the only thing to consider when choosing a balance or scale?

Capacity limits the largest mass you can weigh on the instrument.

No. When selecting a balance or scale, other factors besides capacity play also an important role and need to be considered to ensure your balance is fit for the intended use. These additional factors include:

Maximum Weight

The maximum load you weigh (including the tare container)

Smallest Net Weight

The smallest load you weigh (excluding the tare container)

Weighing Tolerance and Regulations

The acceptable weighing error, specified as \pm percentage; often derived from standards and/or regulations (e.g., ISO, USP, etc.)

Safety Factor

Considers the environment and external device influences (e.g., vibrations, drafts, number of operators, etc.) By matching device performance to your weighing process requirements, you can select a device fit for its intended purpose.

5 Eccentricity

What is it and why is it an important factor to consider?

Eccentricity describes the error when a load is not placed in the middle of a weighing platform. Why is eccentricity an important factor to consider?

Eccentricity (also known as “corner load”) is tested during calibration, and it is one of the four weighing properties that contribute to measurement uncertainty (along with repeatability, sensitivity and non-linearity).

The eccentricity error is a limiting property of balance performance, and it reviews how different the balance indication is when the test weight is placed in positions other than the center on the weighing pan.

The table shows the results of the eccentricity test of a calibration. The graphic below the table shows the degree to which the measurements of the corners deviate from the value at the center position.

6 Error of Indication

What is it and how is it determined?

Error of indication describes the difference between a measurement and a reference value (e.g., a test weight). How is the error of indication tested?

During calibration, the so-called error of indication is determined for a selected number of test points throughout the weighing range of the device. The test itself involves placing several weights at different fractions of the weighing range onto the balance/scale (from zero to maximum capacity) and measuring the difference between the displayed indication and the test weight value.

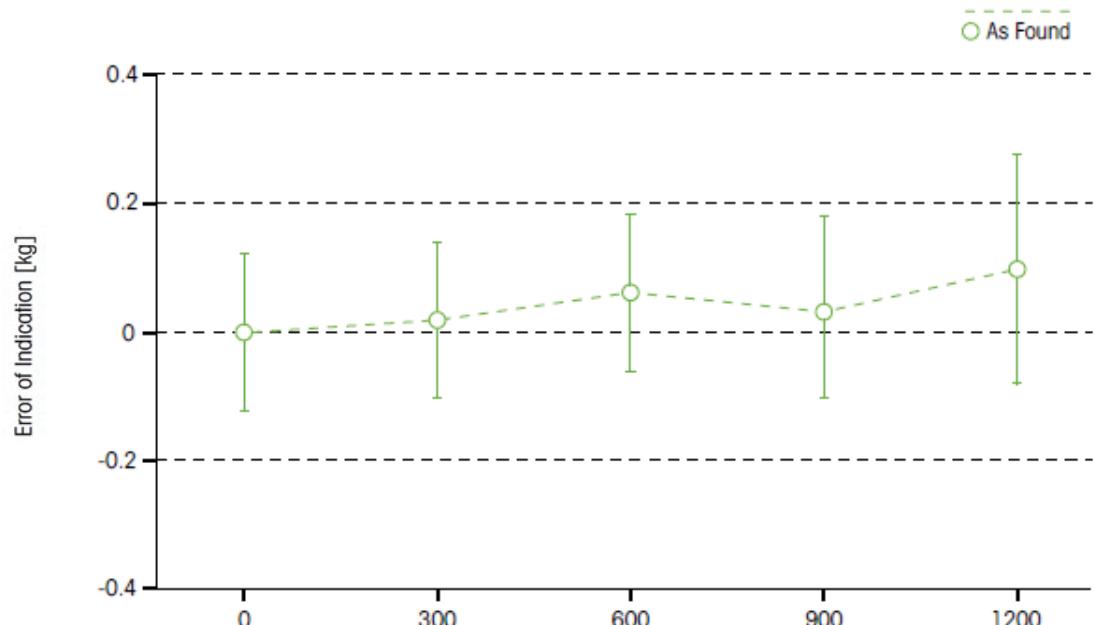
In the figure/ table on the next page, five test points are taken, starting with zero up to a point close to the maximum capacity (in this case, 1,200 kg).

The uncertainty derived from calibration is multiplied by the coverage factor k to provide the expanded uncertainty. The coverage factor k is chosen such that the expanded uncertainty corresponds to a coverage probability of approximately 95%.

For example, in line 2 of the table shown on the next page, with a 95% probability, the error of indication at 300 kg lies

between -0.06 kg and $+0.10$ kg. The statement of the expanded measurement uncertainty is an indispensable part of a calibration certificate.

| | Reference value | Indication | Error of Indication | Expanded Uncertainty | k |
|---|-----------------|------------|---------------------|----------------------|---------|
| 1 | 0 kg | 0.0 kg | 0.00 kg | 0.075 kg | 2.65 kg |
| 2 | 300 kg | 300.02 kg | 0.02 kg | 0.080 kg | 2.26 kg |
| 3 | 600 kg | 600.06 kg | 0.06 kg | 0.10 kg | 2.13 kg |
| 4 | 900 kg | 900.04 kg | 0.04 kg | 0.14 kg | 2.05 kg |
| 5 | 1200 kg | 1200.08 kg | 0.08 kg | 0.17 kg | 2.05 kg |



7 Mass

What is it and how does it relate to weight?

Mass is a measurement that quantifies the amount of matter an object is made of.

Mass

Mass quantifies the physical amount of matter an object has. The mass stays the same, no matter where in the universe the measurement is taken. The unit of the mass is the kilogram or gram.

Weight

Weight is the “heaviness” of an object. It depends on the gravity exerted on the object multiplied by its mass. Therefore, weight is not a constant. It changes from place to place: $\text{Weight} = \text{Mass} \times \text{Gravity}$ (measured in newtons)

8 Measurement Uncertainty

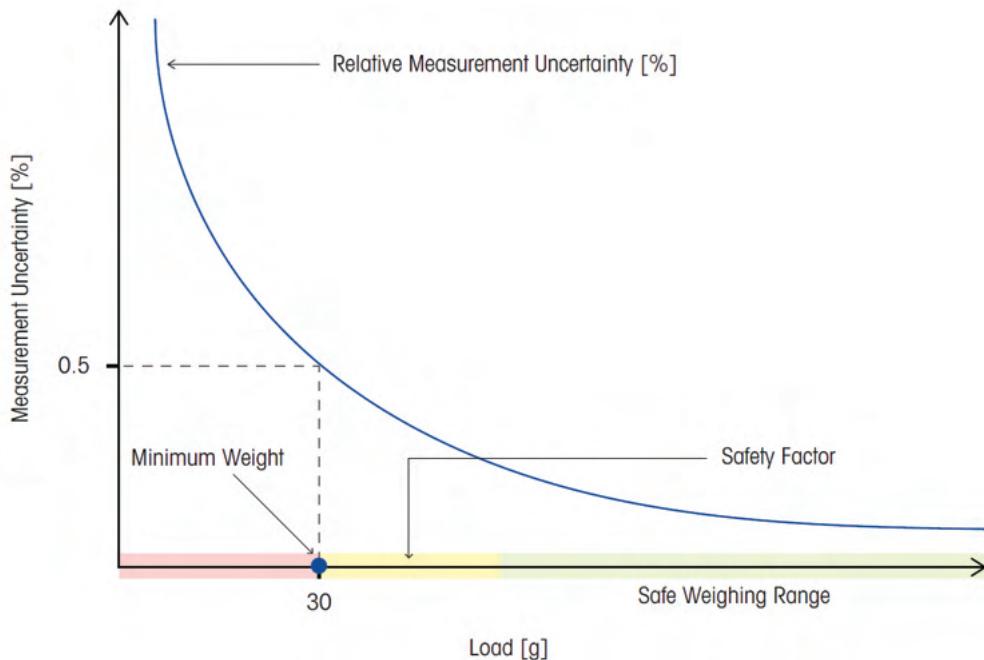
What is it and why is it so important to be determined?

Measurement uncertainty quantifies how accurate a measurement is using plus/minus.

All weighing devices have an inherent measurement uncertainty. Calibration quantifies how accurate the weighing results really are by determining the measurement uncertainty of the device. Without quantifying measurement uncertainty, measuring degrades to guesswork, and can lead to inconsistent product quality, waste and rework.

Here is an example of how measurement uncertainty helps: If your balance indicates 100 g and has a estimated 0.01 g measurement uncertainty, then you can be certain that your value (mass of the weighed object) lies somewhere within 99.99 g and 100.01 g. Is this range “good enough” for your weighing process?

Contributors to measurement uncertainty include the balance or scale itself, the reference weights used for calibration and environmental conditions. Looking at relative measurement uncertainty (see figure), you can see that the smaller a load is, the larger the relative measurement uncertainty gets. If you weigh at the very low end of an instrument’s measurement range, the relative uncertainty can become so high that the weighing result cannot be



9 Minimum Weight

What is it and how does it relate to accuracy?

Minimum weight is the smallest weight that can be accurately weighed on a balance or scale.

The minimum weight varies for different balance types and even between balances of the same type when they are placed in different weighing locations or conditions. This is why the minimum weight must be determined

experimentally (by calibrating the weighing device) onsite. When quantities smaller than the minimum weight are weighed on the device, the relative measurement uncertainty exceeds the tolerance requirement (see figure). In simple words, the weighing result is not accurate enough.

Even after the minimum weight of a balance or scale is defined, a safety factor must be applied to ensure that measurements remain consistent under less-than-ideal conditions. The minimum weight plus an appropriate safety factor defines the lower end of the safe weighing range. This limit must always be smaller than the smallest net weight required by the user/SOP (Standard Operating Procedure) to ensure accurate results.

The upper limit of the safe weighing range is determined by the capacity of the balance or scale.

10 Readability

What is it and upon weighing, do you get what you see?

Readability is the smallest reading difference that can be consistently observed on a weighing instrument.

Because every measurement comes with measurement uncertainty, what you see on the display is not necessarily the true weight of the object being weighed. In order to weigh accurately you need to define the device's measurement uncertainty through calibration.

Hence, the readability of an instrument is not equivalent to its weighing accuracy.

The readability—is only one of many factors that influence accuracy; it's also important to minimize rounding errors common to all digital measuring devices. You can do this by using weighing devices with a finer readability, e.g., 10 g instead of 1 kg. Other balance performance properties that contribute to measurement uncertainty are repeatability, eccentricity, non-linearity and sensitivity.

11 Repeatability

What is it and what happens if a balance or scale does not provide repeatable measurements?

Repeatability is the ability of an instrument to provide nearly identical measurements when the same object is measured repeatedly (expressed as the standard deviation).

Repeatability is one of the main contributors to measurement uncertainty, especially when weighing small loads, and it is a random error tested during calibration. Repeatability refers to a balance or scale's ability to consistently deliver the same indication when a given object is weighed multiple times.

The result of the repeatability test can also be described as precision (closeness of agreement between test results). Example (see figure): A 50 kg weight placed on a scale reads 2×50.00 kg, 2×50.04 kg, and 2×49.98 kg, for a standard deviation of 0.027 kg.

If a weighing device is not precise enough and provides inconsistent readings for the same weight, measurement uncertainty increases. Out of specification results, waste and rework are the likely outcomes.

Example:

Test Load: 50 kg

| | As Found | As Left |
|---|----------|----------|
| 1 | 50.00 kg | 50.00 kg |
| 2 | 50.04 kg | 50.02 kg |
| 3 | 49.98 kg | 50.00 kg |
| 4 | 50.04 kg | 50.02 kg |
| 5 | 49.98 kg | 50.02 kg |
| 6 | 50.00 kg | 49.98 kg |

| | | |
|--------------------|----------|----------|
| Standard Deviation | 0.027 kg | 0.016 kg |
|--------------------|----------|----------|

12 Safe Weighing Range

What is it and how does it help ensure weighing accuracy?

Safe weighing range is the range of a balance/scale where a user meets its accuracy requirements.

The safe weighing range is the range within which the balance or scale provides the required accurate results, including a safety factor. This range lies between a scientifically determined lower accuracy limit (minimum weight determined through calibration) to which a safety margin is added and the maximum weighing limit (capacity) of a balance or a scale.

If the smallest net weight and safe weighing range are defined appropriately, the measurement uncertainty will always be smaller than the allowable weighing tolerance.

Therefore, requirements for weighing accuracy will be met.

The safety factor selected depends on various factors, but in a stable environment with trained operators, a safety

13 Sensitivity

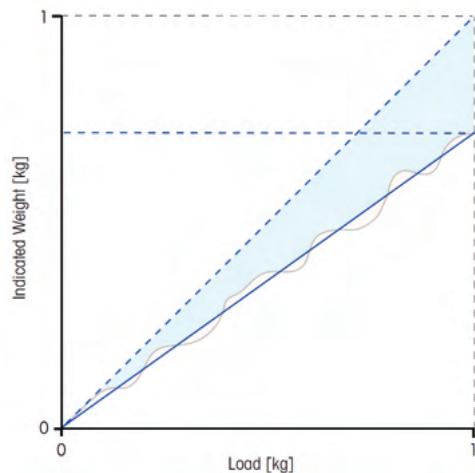
What is it and why sensitivity error should be defined?

Sensitivity is a balance or scale's change in reading divided by the change in load.

Sensitivity error describes the offset between the device indication and a reference weight at the nominal capacity of the device.

Usually, this error is by far the most dominant systematic error for balances, and it should be assessed to determine the accuracy of a device.

In the figure, the dashed line with the associated blue area represents the sensitivity offset of the example instrument with device non-linearity superimposed on it (the grey area indicating the deviation of the characteristic curve from the straight line).



1. GU-metrology-terms (www.mt.com)

Product Highlight



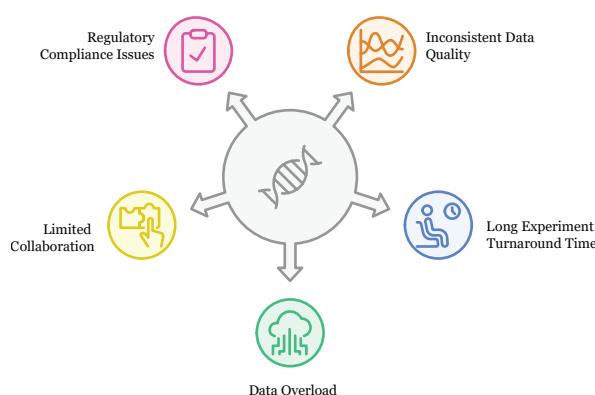
Chemspeed Solutions for R&D and QC Workflows

Modern R&D and QC labs face unprecedented demands. The pressure to accelerate operations while ensuring product quality and maintaining regulatory compliance has intensified. Such research environments face a complex web of challenges that severely hinder efficiency, accuracy, speed and even cost-effectiveness.

In a typical pharma R&D setup, researchers are tasked with generating vast compound libraries, optimizing complex workflows and rapidly screening potential drug candidates. In a similar way, QC labs are intensely scrutinized to maintain stringent quality standards while meeting high-throughput demands. Factors like manual sample preparation, repetitive testing and data management are often cumbersome and prone to errors.

These core challenges demand addressal for driving innovation and efficiency.

R&D Workflow Challenges



Prioritizing QC Challenges for Impact and Urgency



Product Highlight

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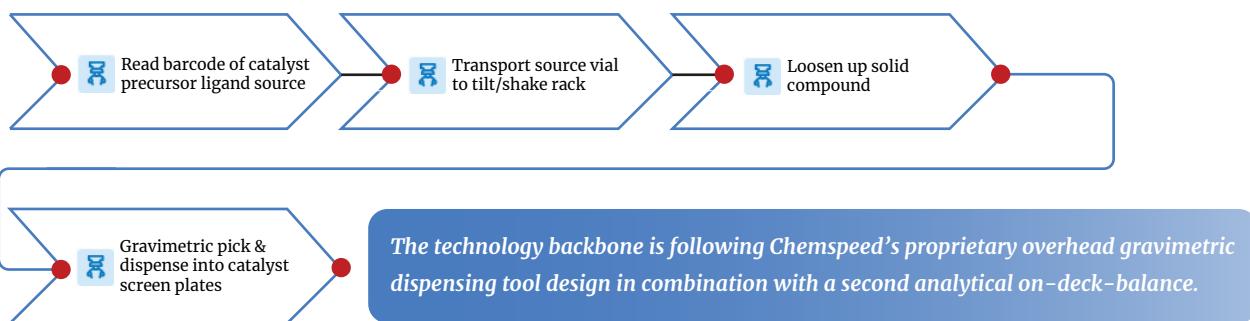
Product Development Cycle

Chemspeed Technologies offers hardware and software for every step in the product development cycle



Example workflow

Below is an example of **matrix-to-matrix gravimetric pick & dispensing of solids for catalyst screening** with gravimetric pick & dispense of ligands, catalyst precursors, catalysts.



Powerful advantages

- Proprietary robotic tool exchange interface.
- Ventilated hood for safety and conditioned operation, trolley.
- SWILE Robotic Tool can be combined with other tools to accommodate multiple workflows, e.g. small-scale reactor for subsequent μ L reaction screening.
- On-deck balance, tilt / shake rack, barcode reader, multi-gripper.
- AUTOSUITE SOFTWARE - drag & drop experimentation with easy interface to data analysis.
- Interface to e.g. DOE, ML, AI, LIMS, ELN, ERP.
- Optimized footprint (3x3 ft, 90x90 cm) versus capacity - all while reducing costs and maximizing return on investment.



CRYSTAL POWDERDOSE

Fully automated gravimetric solid dispensing in a compact and flexible benchtop solution.

FLEX

A compact footprint (90×90 cm / 3×3 ft) to save precious lab space.



SWING / SWING XL

The SWING platform and its "big partner", the SWING XL designed to automate complex laboratory workflows.

FLEXSHUTTLE

Combine and connect as many FLEX units as you like into one integrated.



Automate and digitalize your workflow now

Crystal Powderdose

Benchtop gravimetric solid dispensing with unparalleled flexibility

A fully automated and compact benchtop solution which allows you to optimize and speed up your sample preparation process.



Dispensing characteristics

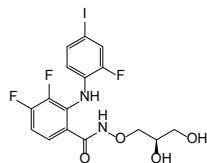
| | |
|----------------------|--|
| Compound type | Solids and powders |
| Powder distribution | One-to-one; many-to-one; many-to-many |
| Technology | Direct gravimetric fine dispensing unit for solids and powders |
| Balance resolution | 10 µg |
| Dispensing range | mg's to 20g |
| Dispensing container | 20 mL |
| Available wellplates | 6 (2x3), 12 (3x4), 24 (4x6), 48 (6x8), 96 (8x12) |

Advantages:

- › Direct dispensing to the vessels
- › Increased efficiency
- › Enhanced traceability
- › Gradient and incremental value dispensing

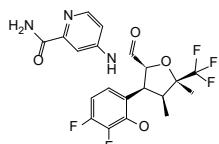


Recent novel FDA approved drugs

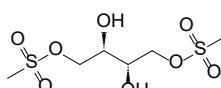


Mirdametinib

A kinase inhibitor, mirdametinib (Gomekli) treats neurofibromatosis type 1 in adults and paediatric patients who have symptomatic plexiform neurofibromas not cooperative to complete resection.



Suzetrigine



Treosulfan

Treosulfan (Grafapex) an alkylating drug is indicated in combination with fludarabine as a preparative routine for allogeneic hematopoietic stem cell transplantation in adult and pediatric patients >1 year old with Acute Myeloid Leukemia or Myelodysplastic Syndrome

Datopotamab deruxtecan (Datroway), an antibody drug conjugate made up of a monoclonal antibody and a topoisomerase inhibitor, used for the treatment of metastatic HR-positive, HER2-negative breast cancer and nonsquamous non-small cell lung cancer (NSCLC), in patients who have received prior systemic chemotherapy.

Industry Buzz

Asia Lab Expo Chandigarh, 2025



MAHE -IIT symposium on Advancements in Microfluidics and Biomarkers in Healthcare- Manipal,2025

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| INK-240DM-10L | 10 | 240 | 40/80 | Amb-100 |
| INK-600DM-22L | 22 | 600 | 40/80 | Amb-100 |



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