

Informative articles, technical advice and the latest product updates, as well as techniques and equipment for testing or analysis.



FOCUS: FOOD & BEVERAGE

2024-2025



Welcome to FOCUS: Food & Beverage magazine

Welcome to our FOCUS Food & Beverage, your premier magazine tailored for professionals in the food and beverage industry.

Within these pages, you'll discover a wealth of informative articles, technical insights, and the newest product releases.

Whether you're involved in food production or operate within a laboratory setting, our content covers techniques, equipment and analysis methods pertinent to your field.

We invite you to delve into this edition and trust it will serve as a valuable resource for your endeavors.

Your feedback is invaluable as we strive to enhance future editions to better meet your needs.

SERVICING THE FOOD & BEVERAGE MARKET



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- Cooling packs

INCUL

- Temperature loggers
- Transport boxes



AVANTOR® MICROBIOLOGY





RAPID TESTS

- Hygiene monitoring systems
- PCR and qPCR solutions
- Rapid lateral flow tests
- Microbiology test systems





Navigating disinfection control in food and beverage manufacturing

Saskia Neubacher, Product Manager, Point of Use MilliporeSigma

AN OVERVIEW OF DISINFECTION CONTROL

Disinfection is the process of cleaning using compounds that either eliminate bacteria and other disease-causing organisms or reduce them to levels not harmful to health. Disinfection control is a key process in food manufacturing and comprises several steps: ensuring the correct concentrations of disinfecting reagents are used, checking that disinfection has been completed sufficiently, and testing that it has been accomplished without leaving any contaminating residues. Disinfection control makes use of the chemical reactions caused by disinfectants to determine their absence or presence, or more precisely their concentration.

It is important to note that, in contrast, hygiene monitoring is a related and sometimes overlapping process that is generally preventative. Hygiene monitoring also involves ensuring that cleaning procedures have been implemented adequately and that no contaminating residues remain, but additionally can involve allergen monitoring and prevention of microbial growth.

Both of these processes are critical in the food and beverage industry where preventing food-borne illnesses is a key objective in maintaining safe manufacturing. Here, we focus on disinfection control and how to navigate the numerous options in its methods and instrumentation.

CONSIDERATIONS IN CHOOSING DISINFECTANT

One of the primary concerns in selecting a disinfectant is to maintain the quality of the final manufactured product. As such, a disinfectant in the food and beverage industry must not be toxic or leave toxic residues. It should also be fast-acting in order to minimise disruption to the manufacturing process. Additionally, it should not leave any odour or taste residue that could affect the end product. In order to facilitate the removal of any residues, it is for instance helpful if a disinfectant has good solubility so that it may be rinsed away after disinfection is complete.



The compatibility of the disinfectant with the equipment and methods used in manufacturing is also important. For example, it should work well on the type/material of the surface being disinfected without damaging the equipment. It should also be compatible in terms of other physical factors such as temperature and pH, remaining effective under the conditions it is used in.

Finally, the method of disinfection control required for a given disinfectant can also affect which disinfectant is chosen.

DISINFECTION CONTROL WITH PHOTOMETRY

A number of photometric methods can be used to assess commonly used disinfectants such as peroxide, peracetic acid, chlorine, quaternary ammonium compounds, ozone and more. There are two options for instrumentation in these types of measurements: high-tech benchtop instruments like the Spectroquant® Prove spectrophotometer, or convenient mobile instruments like the Spectroquant® Move family of colorimeters.





TEST STRIP METHODS FOR DISINFECTION CONTROL

An alternative to wet chemistry is using disinfectant test strips. Both of the methods discussed here can be conducted on-site, and share this advantage with Spectroquant[®] Move Colorimeters. Additionally, test strip analysis circumvents the need for handling liquid chemicals or using glass vials in the field to enhance safety, which can be an important factor in choosing a disinfection control procedure.



RQflex[®] 20 reflectometer for disinfection control

Reflectometry with Test Strips

Disinfection control using Reflectoquant® test strips and the RQflex® 20 reflectometer for read-out can be an attractive option because it can be done in the field, is easy to conduct, produces little waste and yields quantitative results. An additional benefit is that tests are barcoded with batch-specific calibration information for accuracy and precision, and results are traceable. Test strips are available for 23 different parameters, and the portfolio includes compounds commonly investigated in the food and beverage industry.



MQuant[®] test strips also available for disinfection control

Test Strips as a Standalone Method

Visual tests using a colour chart for comparison with test strips are also an option for semi-quantitative disinfection control. The MQuant[®] line of test strips can be used for this type of analysis and has the benefit of being the most economical of the methods discussed here. Test strips offer the additional advantage of not requiring any instrumentation, and consequently no electricity, to carry out analyses. MQuant[®] test strips are robust, easy to use and safe.

Benchtop Spectrophotometers

For disinfection control conducted in a laboratory setting, benchtop spectrophotometers like the Spectroquant® Prove can be used. One key advantage of these instruments is that they have a broad measuring range. The Spectroquant® Prove 600, for example, can detect ultra low concentrations if that is required by a disinfection control method. They also use Live ID codes which automatically detect the test method, lot number, expiry date and calibration updates to streamline analysis and documentation.



Spectroquant® Move Colorimeter for disinfection control

Portable colorimeters for On-the-Spot Analysis

Often the convenience of portable instruments is important, especially when it would be most appropriate to conduct disinfection control in the field. Spectroquant[®] Move colorimeters are portable instruments that allow analysis directly at the sampling site.

These instruments are robust. They are waterproof according to IP68 standard and are therefore suitable for use in wet or dusty environments. They also give fast results and are easy to use. The Spectroquant® Move 100 is designed for use with over 100 pre-programmed parameters for versatility. The Spectroquant® Move DC, on the other hand, is designed specifically for disinfection control and can measure five essential parameters: chlorine, ozone, chlorine dioxide, cyanuric acid and pH. A smartphone app, MQuant[®] StripScan, is available to perform colour comparison if desired in order to improve precision, as well as provide documentation of results and traceability.

A CLOSER LOOK AT COMMON DISINFECTANTS Peracetic Acid

Peracetic acid is often used to disinfect drinking water bottles and surfaces in production. Its determination by titration is complex, with many steps requiring the use of glassware. It is also time-consuming, taking about 15 minutes for each measurement.

MQuant[®] test strips, in contrast, can give a semi-quantitative result within a minute, thereby cutting down the analysis time significantly. For a quantitative and documented result, Reflectoquant[®] test strips can be used to get a result in just a few simple steps and comparably a short period of time.

Hydrogen Peroxide

Another disinfectant frequently used in food and beverage manufacturing is peroxide. Tests for its levels are available in the Spectroquant[®] line as well as in test strips both for use with the RQflex[®] 20 reflectometer and for the standalone MQuant[®] method. Several different concentration ranges are available for all of the methods.

Ozone

Ozone is a powerful antioxidant and is also used as a disinfectant. Spectroquant® test kits can be used for a quantitative determination of ozone. Alternatively, MQuant® rapid liquid test kits with colour cards or colour disk comparators can also be used to determine ozone concentrations.

Chlorine

Chlorine is used in the food and beverage industry for its ability to quickly eliminate bacteria and other microbes in water. It is used in potable water disinfection and washing vegetables, among other things. Spectroquant[®] kits can be used for quantitative chlorine testing, whereas MQuant[®] test strips and rapid liquid tests are for semi-quantitative and fast results.

RELATED PRODUCTS

Description	Cat. No.
Prove 100 Spectroquant®	1.73016.0001
Prove 300 suitable for UV/Vis spectroscopy, Spectroquant®	1.73017.0001
Prove 600 suitable for UV/Vis spectroscopy, Spectroquant®	1.73018.0001
Colorimeter Move 100	1.73632.0001
Move DC	1.73635.0001
Reflectometer RQflex® 20 , Reflectoquant®	1.17246.0001

Spectroquant[®] tests, photometric kits to use with instruments

Description	Cat. No.
Chlorine test (Free), 0.010-6.00 mg/L (Cl ₂)	1.00598.0001
Chlorine test (Total), 0.010-6.00 mg/L (Cl ₂)	1.00602.0001
Hydrogen Peroxide test, 0.015-6.00 mg/L (H ₂ O ₂)	1.18789.0001
Hydrogen Peroxide Cell Test, 2.0-20.0 mg/L (H ₂ O ₂)	1.14731.0001
Ozone test, 0,010 - 4,00 mg/L O ₃	1.00607.0001

Reflectoquant[®] test strips, to be used with RQflex[®]20

Description	Cat. No.
Peracetic acid test, 1.0-22.5 mg/L (peracetic acid)	1.16975.0001
Peracetic acid test, 20.0-100 mg/L (peracetic acid)	1.17956.0001
Peracetic acid test, 75-400 mg/L (peracetic acid)	1.16976.0001
Chlorine test, 0.5-10.0 mg/L (Cl ₂)	1.16896.0001
Peroxide test, 0.2-20.0 mg/L (H ₂ O ₂)	1.16974.0001
Peroxide Test, 20.0-100 mg/L (H ₂ O ₂)	1.17968.0001

Mquant[®] test strips for visual control

Description	Cat. No.
Chlorine test, 0-20 mg/L (Cl ₂)	1.17925.0001
Peracetic acid test, 5-50 mg/L (peracetic acid)*	1.10084.0001
Peracetic acid test, 20-160 mg/L (peracetic acid)	1.17976.0001
Peracetic acid test, 100-500 mg/L (peracetic acid)	1.10001.0001
Peracetic acid test, 500-2000 mg/L (peracetic acid)	1.17922.0001
Peroxide test, 0.5-25 mg/L (H ₂ O ₂)*	1.10011.0001
Peroxide test, 0.5-100 mg/L (H ₂ O ₂)	1.10081.0001
Peroxide test, 100-1000 mg/L (H ₂ O ₂)	1.10337.0001

*can be used with MQuant® StripScan App



Environmentally preferable production supplies from Avantor® Make every day Earth Day

Protecting the health, safety and welfare of your people and the integrity of your processes is always the priority, but looking after the future of our planet is also essential. At Avantor® we would like to help you make more sustainable product choices, so we have curated a selection of more environmentally friendly safety and cleanroom supplies and services. We are actively working with our manufacturing locations and key suppliers to add more products and services to this list.

Together we can go green! VISIT OUR WEBSITE



OUR ENVIRONMENTALLY PREFERABLE PRODUCT (EPP) METHODOLOGY

When we identify more environmentally preferable solutions, they are given an (EPP) green leaf designation on our website, so they can be easily identified.

When searching for an article, you can filter to show those that are EPP.



Products must incorporate one or more of the attributes below to qualify for the EPP green leaf.

- Energy efficient
- Water efficient
- Waste reducing (uses less material, recyclable, reusable, compostable or refillable)
- Sustainable materials (>30% post-consumer, renewable or bio-based material)
- Low manufacturing impact (made with >50% renewable energy or 20% less water than standard)
- Safer human and environmental health (refrigerants, chemicals, solvents, low VOC, etc.)
- Sustainable packaging (100% recyclable, or made from >30% recycled or renewable content, no single-use plastics, no packaging)
- Product transparency and disclosure (has verified life cycle assessment (LCA), environmental product declaration (EPD), health product declaration (HPD), ACT label or other third party verified environmental impact assessment)



Verification by a government agency or recognised third party certification is also accepted. For example:

ACT Label **Biodegradable Products** Institute (BPI) **BioPreferred** Blue Angel California Air Resource Board (CARB) **CDPH Standard Method** v1.1 2010 Cradle to Cradle Certified Declare EcoLogo **ENERGY STAR EPEAT** EU Ecolabel Forest Stewardship Council (FSC) German AgBB **Global Recycled Standard**

GreenCircle Certified GREENGUARD **GreenScreen For Safer Chemicals Green Seal** LEVEL by **BIFMA** Nordic Ecolabel NSF International Programme For The Endorsement Of Forest Certification **RoHS** Compliant Safer Choice **SCS Global Services** South Coast Air Quality Management District (SCAQMD) Sustainable Forestry Initiative (SFI) **TCO** Certified WaterSense

Visit our 'Sustainable Solutions' web page to see the environmentally preferable solutions available now. www.vwr.com/sustainable-production-supplies





Ensuring purity from production to plate: Bürkle's innovative disposable samplers



From liquids to solids, dry to highly viscous substances) (particularly in the food industry), the spectrum of raw materials couldn't be more diverse. Whether assessing the consistency of dairy products, the texture of grains, or the viscosity of sauces, the distinct properties of foods demand specific tools and techniques to obtain a representative and accurate sample for analysis and quality control.

Moreover, the purity of the product is an extremely critical issue in industries as sensitive as the food industry. Any form of contamination throughout the production process – from the quality control of the raw material to the final packaging – must be excluded completely to ensure a high-quality product and comply with strict and constantly increasing requirements such as EU food and FDA guidelines.

To meet these criteria, the use of reusable stainless-steel tools, including samplers, has become prevalent. However, cleaning these samplers poses a significant disadvantage and risk factor. Even with non-viscous raw materials that do not strongly adhere, making cleaning challenging or even impossible, a simple wipe after use unfortunately does not suffice to prevent cross-contamination. Validating the critical cleaning processes of reusable tools is a challenging task that often leads to issues.

Single-use Samplers:

- + highest standards regarding purity & sterility
- + complying with EU food requirements & FDA regulations



To avoid these problems, the use of single-use items is an optimal solution. Bürkle's disposable samplers and tools are produced in class 7 cleanrooms, individually packaged, op-tionally sterilised (SAL 10⁻⁶), and compliant with EU food and FDA guidelines. The use of these single-use products ensures complete exclusion of crosscontamination.

With the "DispoSamplers", Bürkle offers a wide range of disposable samplers covering various raw materials from liquids to powders, providing an ultra-pure alternative to conventional reusable stainless-steel samplers, addressing the different needs of the food and beverage industry.

Take Samples – but do it right! Make your daily work comfortable, reliable, and avoid contaminations with Bürkle disposable items.









Peltier technology explained



Peltier technology, also known as thermoelectric technology, is commonly used in laboratory heating and cooling applications. The technology is based on the Peltier effect, which was discovered by Jean Charles Athanase Peltier in 1834. The Peltier effect describes the phenomenon where a temperature difference is created at the junction of two different conductive materials when an electric current is passed through them.

In Peltier devices, typically called thermoelectric modules or coolers, there are two types of semiconductor materials connected in series. These materials are usually made of bismuth telluride or other similar materials with good thermoelectric properties. When a direct current (DC) electric current is applied to the module, one side of the module absorbs heat, while the other side releases heat. This results in one side becoming cooler and the other side becoming hotter.

In laboratory applications, Peltier devices are commonly used for both heating and cooling purposes:

COOLING

The side of the Peltier module facing the sample or the area to be cooled is known as the cold side. This side absorbs heat from the surroundings, providing a cooling effect.

Peltier coolers are often used in applications where precise temperature control is required, such as maintaining a stable temperature for biological samples, chemical reactions, or electronic components.

HEATING

The opposite side of the module, facing away from the sample or the area to be heated, is known as the hot side. This side releases the absorbed heat.

By reversing the direction of the electric current, the Peltier module can be used for heating applications. This is useful for maintaining a specific elevated temperature, such as in the case of incubators or temperature-controlled reactions.



ADVANTAGES OF PELTIER TECHNOLOGY IN LABORATORY HEATING AND COOLING INCLUDE:

Precision: Peltier devices offer precise temperature control, making them suitable for applications that require tight temperature regulation.

Compact size: Peltier devices are often compact and lightweight, allowing for integration into small laboratory equipment.

Low maintenance, long lifespans: Peltier devices are solid-state and have no moving parts, which means they have lower maintenance requirements and longer lifespans compared to systems with mechanical components. **No refrigerants:** Unlike traditional cooling systems that use refrigerants, Peltier devices are solid-state and do not involve any fluids. This eliminates the need for refrigerant maintenance and reduces the risk of leaks.

Lower energy consumption: Peltier devices with heating and cooling function have a much lower energy consumption than comparable compressor cooled systems as also no separate heating elements are required.

Lower noise level: Peltier devices have a much lower noise level than comparable compressor cooled systems and operate almost vibration free.

The choice between Peltier devices and compressor cooled systems depends on the specific requirements, constraints and operating conditions of the intended application.



VWR[®] Cooled incubators with Peltier technology, INCU-Line® ILPR PREMIUM

PELTIER ADVANTAGES:

- Excellent temperature stability and uniformity
- Energy efficient and environmentally friendly
- Low noise level
- Almost vibration-free as no compressor is installed
- No dehumidification of samples due to the closed chamber

ADVANTAGES OF THE INCU-LINE ILPR PREMIUM SERIES

- Equipped with a multi-functional microprocessor controller with 7"full colour touchscreen, which gives overview of set and current parameters while running
- Access control via password login and administrator function to manage 5 user accounts
- 40 user-program memory, quick and easy change of parameters during a running program
- Adjustable over- and under-temperature safety thermostat Class 3.3 (DIN 12880) protects samples and the incubator
- Supplied with Premium Control Software (can be downloaded from internal memory onto USB stick) and manufacturer test certificate at 37 °C





Intuitive menu and user-friendly software. The units can be connected to Ethernet network for remote control from any computer, being one of the greatest advantages.

Available in 3 different chamber volumes: 56, 112 or 245 L Temperature range: 0 °C ... +70 °C (max. 20 °C below ambient temperature)



USB port for data download to USB stick and for uploading programs.



Quick change mode for set temperature during a running program.



Quick change mode for time during a running program.



Access port Φ 30 mm with a silicon cap

Internal LED light



Internal glass door



Handle with door lock

Please find all detailed information on our product page.





VWR[®] CPR Premium series climate chambers

Excellent performance - boosted with Peltier elements cooling system

CPR Premium series climatic chambers feature a stainless steel (DIN 1.4301) inner chamber, stainless steel housing, solid outer door and internal glass door. They are designed for long-term stability testing of drugs and medicinal products (in terms of temperature and humidity conditions), in accordance with ICH (Q1A) standard guidelines. These chambers can also be used for testing the stability of cosmetics, food, as well as for various types of durability tests.

Available in 2 different chamber volumes: 245 or 749 L

Working Temperature range (with humidity): +5 °C to +70 °C (max. 20 °C below ambient temperature)

Humidity range: 10 to 90%



Advantages of CPR Premium series with Peltier technology

AS SMART AS YOUR SMARTPHONE

Highly advanced Premium controller manages the unit. It also has a large 7" touchscreen plus easy and intuitive operation.

PERFECT PERFORMANCE

The cooling system, based on the Peltier element, features excellent temperature variation and fluctuation. It also improves the temperature recovery time (eg., after door opening). The humidity inside the chamber is extremely stable.

ENVIRONMENTALLY FRIENDLY

Elimination of compressor and refrigerants ensure environmental protection.





Peltier-cooled chamber

LOW WATER CONSUMPTION

Compressor-cooled chamber

The external 6 L water tank is mounted on the side wall for easy access.

Water consumption for CPR 240 is so low that you only need to refill the water tank only every second month. Thanks to the installation of a heater in the wastewater pan, the chamber is maintenance-free and can be used in any location.



NO VIBRATION & MORE QUIET OPERATION

Compared to compressor-cooled climate chambers, the CPR Premium does not vibrate, and the noise level is significantly lower.

LIGHTER & SMALLER

The cooling system based on Peltier modules allows reduction in the dimensions of the unit and its weight (compared to compressor-cooled chambers).

ENERGY SAVING

When operating the unit at temperatures close to ambient, energy costs are reduced on average by 40% compared to compressor-cooled chambers.





State-of-the-art 7" full colour touchscreen controller

The CPR Premium constant climate chamber is equipped with an innovative controller and a large (7") colour touchscreen. It displays all important information about the program settings, current parameters, alarms, and program status in a very clear, easy to understand way. The controller uses icons for easy navigation. All information is legible, and programming the unit is extremely intuitive.



Diagram of temperature-humidity range Climate testing points according to ICH Q1 A (R2) guidelines

VWR® CPR Premium constant climate chambers are designed for long-term stability testing of drugs and medicinal products (in terms of temperature and humidity conditions), in accordance with ICH (Q1A) standard guidelines. These chambers can also be used for testing the stability of cosmetics, food, as well as for various types of durability tests.

Stability study	Storage conditions	Minimum time period	Testing frequency
Long-term (choice of storage conditions)	25 ±2 °C / 60 ±5% RH or 30 ±2 °C / 65 ±5% RH	12 months	Each 3rd month 1st year, each 6th month 2nd year, annually thereafter
Intermediate (if long-term conditions are 25 ±2 °C / 60 ±5% RH)	30 ±2 °C / 65 ± ±5% RH	6 months	Minimum 3 time points
Accelerated	40 ±2 °C / 75 ±5% RH	6 months	Minimum 3 time points
Long-term (only semi-permeable containers)	25 ±2 °C / 40 ±5% RH or 30 ±2 °C / 35 ±5% RH	12 months	Each 3rd month 1st year, each 6th month 2nd year, annually thereafter
Accelerated (only semi- permeable containers)	40 ± 2°C / not more than ±25% RH	12 months	Minimum 3 time points



Remotely controlled constant climate chamber with Premium Control sofware

VWR® CPR Premium constant climate chambers can be connected to the Ethernet network and controlled remotely using the Premium Control software supplied. This software has been designed to meet customers' needs to facilitate the operation and control of the VWR® Premium models. Premium Control software can be downloaded from the unit's internal memory to a USB stick.

For more detailed information download the brochure

Introducing SICS technology: Enhancing precision and efficiency in your laboratory



INTRODUCTION

Recognising the crucial significance of precision and efficiency in laboratory environments, we are excited to announce that our touchscreen precision balances now feature the cutting-edge SICS (Standard Instrument Command Set) technology.

SICS, a standardised command set, has been crafted to optimise communication and control functions within precision balances. Offering a universal language, it facilitates smooth integration with laboratory systems, software (such as LIMS -Laboratory Information Management Systems), and instrument networks spanning multiple laboratories.

Through precision balances equipped with SICS, you achieve unmatched authority and adaptability in your weighing protocols. From basic taring tasks to calibration procedures, SICS commands empower you to enhance your workflow effortlessly and accurately.





BENEFITS OF SICS-ENABLED PRECISION BALANCES

Enhanced productivity

Enhance the efficiency of your operations and reduce downtime through the effective control and automation features provided by SICS commands.

Accuracy

Achieve unparalleled accuracy and consistency in your measurements, thanks to precise calibration and data acquisition facilitated by SICS technology.

Seamless integration

Effortlessly incorporate your precision balance into laboratory systems and software, including LIMS and networks of instruments spanning multiple laboratories.

Improve interoperability and simplify data management across your entire organisation.

Simplified operation

Simplify intricate weighing tasks and procedures using intuitive SICS commands, allowing for centralised control and remote monitoring.

In brief, the SICS command set empowers you with remote control over your precision balance, transforming how you oversee and enhance your laboratory workflow and industrial process automations.

Grinding of boneless chicken leg







PRODUCT

MultiDrive control (Cat. No. 412-0325) MI 400 grinding chamber (Cat. No. 431-0304) MI 400.2 cutting blade (Cat. No. 431-0318)

INDUSTRY Food

OVERVIEW

The requirement was to grind boneless chicken legs into a paste for food formulation.

Pate is a cooked meat paste commonly consumed in Europe as a savoury appetizer. It is normally eaten together with bread and it can be made from poultry, liver or other types of ingredients. Other condiments and vegetables can be added to the recipes for more flavour. SAMPLE MATERIAL Boneless chicken leg

EXPERIMENTAL SETUP / SETTINGS

Stability study	Storage conditions
Mill	MultiDrive control
Grinding chamber	MI 400
Cutting blade	MI 400.2
Speed	From 3,000 to 20,000 rpm
Grinding time	1 minute
Sample quantity	350 g



RESULTS

1. Pre-cutting of the boneless chicken leg into 3x3 cm pieces is recommended before grinding to reduce the required grinding time.



Before grinding

During grinding

2. The sample is recommended to be mixed and ground with dry ice (1:1) in the grinding chamber to prevent any temperature increase during the high speed grinding process and to achieve a fine, smooth and homogenous paste.



During grinding

After grinding

Regardless of whether samples are hard, soft or fibrous – the IKA MultiDrive crusher can perform a wide range of crushing tasks involving coarse and fine crushing, thanks to the variety of vessels available. MultiDrive control mixes, grinds and is also fitted with a dispersion vessel and a disposable tube. There is a USB interface available for easy actuation and documentation respectively. Excellent crushing performance is guaranteed by a combination of variable rotational speeds, ranging from 3000 rpm to 20 000 rpm, and a 1000 Watt output. It also offers temperature measurement and includes a weighing function as well as integrated cooling in the milling cup.

Paperless temperature control with Bluetooth

Thermometers with wireless interface

Roland Trübswetter, Product Manager for Measurement Technology Food, ebro Electronic, Xylem Analytics Germany Sales GmbH & Co. KG



Everyone who produces, transports or stores food faces a challenge: Everything must be done to ensure that the food is still edible when it ends up on the plate. At the same time, work must be done efficiently and cost-effectively because the price competition is so fierce. Measuring technology, such as thermometers, also finds itself in precisely this field of tension. These are essential to control compliance with the cold chain. With the current state of the art, you had the choice between simple hand-held thermometers, temperature recording devices (so-called data loggers) and automatic temperature monitoring systems.

CONVENTIONAL TEMPERATURE MEASUREMENT

The hand-held thermometers are the most cost-effective variants, but require a lot of effort on the part of the measuring staff: The measurement data must be recorded manually because the devices do not save anything and do not have an interface to the PC. This means that the examiner would actually need three hands: for a clipboard, pen and thermometer. This measurement takes a relatively long time and the data is difficult to evaluate afterwards. Temperature monitoring systems have the advantage that everything from recording and saving measurement data, to evaluation, to alerting and reporting can be automated.

This is particularly true if they contain wireless data loggers. But they are significantly more expensive. Conventional data loggers in combination with PC software are somewhere in between, both in terms of price and level of automation and effort. Each technology has its advantages and disadvantages, although for cost reasons hand-held thermometers are usually used, at least when monitoring storage rooms, cold rooms, refrigerated cabinets, etc.

NEW TECHNOLOGIES

This situation has not changed for a long time. However, there is currently a change in thinking: More and more companies are realising that they can use current wireless technologies such as Bluetooth and special software not only to reduce the effort, but also to achieve a much higher degree of control over temperature events. Thermometers with a Bluetooth interface are able to send the data to an app on a mobile device. From there they can be forwarded via WiFi, for example a cloud software.

The app can also be designed to guide the measuring staff step by step through the measuring process. Alternatively, the thermometer can send the measurement data to a PC software via a Bluetooth receiving device. Either way, the data is permanently stored in a database and this enables any evaluation at any time – which was almost impossible with hand-held thermometers. At the same time, handling during measurements is significantly improved because you no longer need a clipboard and a pen. This also saves paper – the environment and your wallet will thank you. If a rechargeable battery is used instead of normal batteries, the sustainability of the measuring system is significantly increased.



DIVERSE PROPERTIES

The measurement is even easier if the thermometer can also temporarily store the measured values, as it is the case with the new thermometer TLC 750 BT from the ebro® brand. A tour of several measuring points can then be completed quickly, and the measured values are then transferred to the software at once.

In the past, dual thermometers have proven to be the best, as they are suitable for both incoming goods inspections and warehouse monitoring, and are now very sophisticated in terms of handling and equipment. The TLC 750 BT is the further development of such a thermometer and is therefore one of the top products on the market.

SIMPLE SOFTWARE EDC

For customers who focus on the efficient collection and storage of measurement data, ebro[®] has developed a new software: EDC, short for Easy Data Collector. This software makes it possible to easily read out the measured values from the TLC 750 BT, saving them in the database in the same step. The data can then optionally be evaluated and put into report form. The operation is self-explanatory, as the user interface is clearly structured and does not require a lot of additional bells and whistles. It may seem contradictory, but the goal during development was very clear: to allow the user to spend as little time as possible with the system, so that they can concentrate fully on their actual tasks.

PRACTICAL INTERFACE IF 50

The EDC software is sold as a set together with the IF 50 interface. This interface is a combination device with various



Interface IF 50

functions that are intended to further simplify the operation of the system. It serves as a battery charging station for the TLC 750 BT, practical storage location, but also as an interface for accessing the TLC 750 BT remotely. In many cases the thermometer will be used in places where there is no PC nearby. Without an interface, the thermometer would then have to be carried across several rooms or buildings to the PC on which the software was installed. Since the IF 50 interface has an Ethernet interface, it can communicate with the PC via the LAN while receiving the thermometer data via the Bluetooth interface. This means that you can place the interface where you are measuring, and the software can be on a computer that is in a different location and still communicate with the thermometer or thermometers. Although it sounds complicated, it is not in practice. Once set up, you no longer work with the interface directly, but only with the measuring device and later the software.

TLC 750 BT DUAL INFRARED / FOLD-BACK-THERMOMETER WITH BLUETOOTH

Contamination free surface measurement and core temperature measurement in one device

- Compact pocket size for everyday use
- Temperature measurement range from -50 °C to +250 °C
- HACCP and DIN EN 13485 compliant including calibration certificate
- Display with backlight for reading in dark environments
- Waterproof case (IP 65)
- °C / °F switchable
- Exchangeable battery

APPLICATIONS:

- Incoming goods inspection, transport and storage
- Cold chain monitoring
- Ideal for retail, catering and the food industry
- Contactless and contamination-free surface measurement with infrared
- Core temperature measurement with fold-out penetration probe



Detection of gluten in industrial bakery

Study carried out by a French industrial bakery



CONTEXT

The number of gluten-free consumers has increased greatly in recent years and more and more food industries are trying to develop a range of gluten-free products in order to fulfill the need.

This study is aimed at evaluating the level of gluten contamination in a 2000 m³ production workshop in an industrial bakery. The objective was to create a specific area in the production workshop dedicated to the production of gluten-free products.

The Coriolis[®] Micro has been used to perform air sampling at various points in the bakery during production and also when the machines were stopped. The samples have been then analysed by ELISA.

MATERIALS & PROTOCOLS

Materials

- Air sampler: Coriolis® Micro, sterile cones
- Collection liquid: 15 ml of distilled water

Protocols

- First sampling area (A1)

This area has been emptied out of all products containing gluten before the sampling, but gluten residues still remain in the air. Sampling at 300 L/min for 10 and 20 min when the machines were stopped and during production time

- Second sampling area (A2)

Raw materials containing gluten are continuously used in this area. Sampling at 300 L/min for 10 and 20 min and at 200 L/min for 5 min during production time

Liquid air samples analysed by R5-Mendez ELISA (external laboratory)





RESULTS

Results of the air sampling performed in the first area

Sampling parameters	Gluten (mg of gluten/ kg of liquid collection)	Gluten (mg of gluten/ m³ of air)	Results
Control (distilled water)	<5 ppm	-	ОК
A1-300 L/min-20 min Machines stopped	<5 ppm	< 0,075 mg of gluten/6000 L of air < 12,5 µg of gluten/m³ of air	
A1-300 L/min-10 min Machines stopped	<5 ppm	< 0,075 mg of gluten/3000 L of air < 25 µg of gluten/m³ of air	Low gluten contamination
A1-300 L/min-20 min Production time	<5 ppm	< 0,075 mg of gluten/6000 L of air < 12,5 µg of gluten/m³ of air	Low gluten contamination
A1-300 L/min-10 min Production time	8 ppm	0,12 mg of gluten/3000 L of air 40 μg of gluten/ m³ of air	Increase of the level of gluten during the production

The level of gluten detected with Coriolis[®] Micro in this area is at the detection limit of the analysis method. An increase of the gluten concentration is observed when the sampling is performed during the production. This moderate increase has to be confirmed by further trials.

Description	Model	Pk	Cat. No.
Air sampler	Coriolis® µ	1	710-1973
Cones and caps, single-use, autoclavable		10	710-1999

Not available through Avantor in UK



Results of the air sampling performed in the second area

Sampling parameters	Gluten (mg of gluten/ kg of liquid collection)	Gluten (mg of gluten/ m³ of air)	Results
A2-300 L/min-20 min Production time	35 ppm	0,525 mg of gluten/6000 L of air 87,5 µg of gluten/m³ of air	Detection of
A2-300 L/min-10 min Production time	54 ppm	0,81 mg of gluten/3000 L of air 270 μg of gluten/ m³ of air	gluten at a high concentration -> the production
	44 ppm	0,66 mg of gluten/ 3000 L of air 220 µg of gluten/m³ of air	of gluten-free products close to this area is not
A2-200 L/min-5 min Production time	18 ppm	270 μg of gluten/m³ of air	possible

During the production, the level of gluten detected in the area is high. The installation of a gluten-free production line close to this area is not possible because of the high risk of cross-contamination.

CONCLUSION

Thanks to its high flow rate, Coriolis® Micro is a valuable tool for the collection of gluten even in a low-contaminated environment. Coriolis® Micro can be used by food industries for quality control, in particular, to check the level of gluten in the atmosphere of gluten-free production areas.

VWR[®] Modified Scholten's media

ISO 10705-2: Counting and isolating coliphages in potable, environmental and wastewater samples.



Isolation and enumeration of somatic coliphages in water using the double-layer technique, by incubating the sample with a suitable bacterial strain.

- Concentrated, diluted or direct sample is mixed with a small volume of semisolid agar and a culture of the host bacterial strain. This mixture is poured into a nutrient agar plate
- After adequate incubation, all lysis plaques present in the gelled medium are counted
- Results are expressed as PFU plaque-forming units (particles) per unit volume (PFU/100 ml)
- Somatic coliphages and host bacterium are not pathogenic to humans or animals. Handle according to normal microbiology laboratory safety procedures

Follow specific incubation and culture conditions described on ISO 10705 to obtain accurate and reproducible results. Avoid cross-contamination and external contamination during the entire process of coliphage isolation and enumeration.

ORDERING TABLE

Description	Cat. No.
Modified Scholten's Broth (MSB)	376234ZA / 456230ZA / 92429.0500
Modified Scholten's Semisolid Agar (ssMSA)	306233ZA / 92430.0500
Modified Scholten's Agar (MSA)	106100ZAL / 406103ZA / 92428.0500



PROCEDURE:



to concentrate it.

NOTES:

(N1): Nalidixic acid is recommended when

there is a suspicion of high accompanying microbes.

(N2): CaCl₂ favors phage-bacteria

interaction. Use in samples where low phage counts are expected.

MSB: Modified Scholten's Broth.

MSA: Modified Scholten's Agar.

(N3): Minimum 2 replicates. For samples suspected of low phage count,

it is recommended to increase replicates or

ssMSA: Modified Scholten's Semisolid Agar.

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- Not sure which growth or culture medium you need for your analysis? Try searching by microorganism and browse through our range
- Discover test protocols in the technical sheets to optimise your testing conditions
- Do you already know which medium you are looking for? Then don't hesitate to use our product search box instead

Filter		Filter	
Selective to be used for	<u></u>	Product Type	
Aciduric organisms		140 mm plates	
Acinetobacter		55mm plates	
Actinomycetes		90mm plates	
Aerobic mesophilic bacteria		Bags	
Aerobic plate counts		Bottles	
Aeromonas spp.	-	Contact plates	-

Please visit our leading page for more information: www.vwr.com/microbiology



Regulatory news 2023

New EN ISO 15213 series for the enumeration and detection of *Clostridium spp.* in the food chain

The International Organisation for Standardisation (ISO) is publishing the three-part EN ISO 15213 series, which specifies the enumeration and detection of sulfite-reducing *Clostridium spp.*, including *C. perfringens*, in a broad range of foods, pet food and animal feed, in samples from the primary production stage and in environmental samples in food and feed production and handling.

NEW EN ISO 15213-1:2023 AT A GLANCE ...

- Describes the horizontal method for the enumeration of Clostridium spp. by colony-count technique
- Scope of the method has been changed from "sulphite-reducing bacteria" to "sulphite-reducing Clostridium spp." and includes samples from the primary production
- Typical colonies on Iron sulfite agar (ISA) are confirmed by anaerobic growth and no growth under aerobic conditions
- Concentration of sulfite in the Iron sulfite agar (ISA) has been reduced from 1.0 to 0.5 g/L
- Ten-minute heat treatment at 80 °C for selection of spores has been made optional
- Option to use tubes for inoculation and option to incubate the samples at 50 °C for enumeration of thermophilic sulphite-reducing bacteria have been removed
- A special protocol for the enumeration of sulphite-reducing *Clostridium spp.* in feed has been added in an informative
- Performance characteristics of the method, determined in an interlaboratory study, have been added
- The main technical changes are significant and have major impact on the performance characteristics of the method

Part 1: Enumeration of sulphite-reducing *Clostridium* spp. by colony-count technique. It has replaced ISO 15213:2003. **Part 2:** Enumeration of *Clostridium* perfringens by colony-count technique.

It is set to replace EN ISO 7937:2004.

Part 3: Detection of *Clostridium perfringens*. This part will be newly published.

PART 1: ENUMERATION OF CLOSTRIDIUM SPP. ACC. TO NEW EN ISO 15213-1:2023

Procedure step



PART 2: ENUMERATION OF CLOSTRIDIUM PERFRINGENS ACC. TO NEW EN ISO 15213-2:2023





PART 3: DETECTION OF CLOSTRIDIUM PERFRINGENS ACC. TO NEW EN ISO/TS 15213-3:2023





As a worldwide leading provider of a broad range of granulated and ready-to-use culture media for food, beverage and water microbiology, we closely track and support the development of relevant standards aimed at increasing consumer confidence and safety.

Our GranuCult[®] granulated media, Readybag[®] pre-weighed granulated media in ready-to-use bags, ReadyPlate[™] and ReadyTube[®] ready-to-use media are all compliant with EN ISO 11133:2014+Amd1:2018+Amd2:2020. All information regarding compliance with additional reference standards are displayed on the product label and in the product's technical information available on our website.

COMPLIANCE WITH THE NEW EN ISO 15213 SERIES We are implementing all the requirements as described in the new EN ISO 15213 series.

DEHYDRATED CULTURE MEDIA – FROM BASIC TO PRIME To best suit your needs, we offer two media formats: superior granulated GranuCult[®] and powdered NutriSelect[®] culture media. The added designations basic, plus or prime indicate the quality control level, from basic quality control, to standard QC plus, to prime for full regulatory compliance.



Clostridium perfringens WDCM 00174 on 111972 TSC agar

Ordering Information

Description	Pack size	Cat. No.
GranuCult® prime Iron Sulfite Agar (ISA) acc. ISO 15213-1	500 g	1108640500
GranuCult® prime Columbia Agar (base) acc. ISO 10272 and EP/USP/JP	500 g	1002140500
GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM	500 g	1119720500
TSC (Cycloserine) Selective supplement	1 x 10 vials	1008880010
GranuCult® prime Rapid Perfringens Medium (RPM) acc. ISO 15213-3	500 g	1167510500
GranuCult® prime SIM (Sulfite Indole Motility) Agar acc. ISO 15213	500 g	1054700500
Bactident® Indole (Kovac's indole reagent) acc. ISO and FDA-BAM	30 ml	1113500001
Kovac's Indole Reagent acc. ISO and FDA-BAM	100 ml	1092930100
Products for anaerobic cultivation		
Anaerotest® strips for microbiology (to indicate an anaerobic atmosphere)	50 strips	1323710001
Anaerocult® A mini (gas generator system for 1-4 Petri dishes)	25 x 1 set	1323690001
Anaerocult® P (reagent for generation of an anaerobic atmosphere for 1 Petri dish)	25 x 1 set	1323820001
Anaerocult® A (reagent for generation of an anaerobic atmosphere in an anaerobic jar)	10 x 1 piece	1323690001
Anaerobic Jar (2.5 liter volume) for microbiology	1 unit	1136810001
Anaeroclip® for use in providing a mechanical closure of Anaerocult® products	25 units	1142260001



Analysis of pesticide residues in pistachios

Using QuEChERS extraction and cleanup with Supel™ QuE Z-Sep+

Kathy Stenerson, Principle R&D Scientist, Megan Wesley, 2016 R&D Summer Intern



INTRODUCTION

Pistachios are popular and enjoyed for both taste and health benefits such as decreased cholesterol, weight management, protection against diabetes and hypertension, and improved digestion^[1]. These nuts are grown in the United States (specifically, California), Italy and countries in Central Asia like Iran, Turkey, Afghanistan and Syria. Pesticide tolerances set by the US EPA for pistachios range from 0.01 - 0.7 μ g/g before harvest to 3 - 200 μ g/g after harvest, depending on the pesticide^[2]. Testing for pesticide residues then requires a method which will allow for low level and accurate determination. The "quick, easy, cheap, effective, rugged and safe" (QuEChERS) approach has been used to analyse multiple pesticide residues found in pistachios^[3]. Pistachios contain approximately 45% fat, which can result in a significant amount of co-extracted matrix in the acetonitrile extract generated using the QuEChERS procedure. The use of a cleanup sorbent which can reduce this fat is essential

to prevent fouling of LC-MS/MS and GC-MS/MS systems, and minimise ion suppression, thus allowing low level detection. In this application, Supel[™] QuE Z-Sep+ sorbent was used as part of the QuEChERS method in the analysis of pesticide residues in pistachios. Z-Sep+ is a zirconia and C18 functionalised silica sorbent which acts to retain fatty constituents through both Lewis acid/base and hydrophobic interactions. The selectivity of the zirconia present in Z-Sep+ offers retention of a wider range of fats than C18 alone. In this application, QuEChERS extraction and cleanup using Z-Sep+ sorbent were used before the LC-MS/MS and GC-MS/MS analysis of pesticide residues in pistachios. The targeted analyte list included pesticides relevant to pistachios^[4,5].

EXPERIMENTAL

Pistachios were purchased from a local grocery store. They were frozen with liquid nitrogen (shells on), ground, and spiked at 10 ng/g with the pesticides listed in Table 2 and 4, and allowed to equilibrate for 1 hour. Samples were then subjected to QuEChERS extraction and cleanup with Z-Sep+following the procedure in Figure 1. A 100 µl aliquot of the final extract was diluted to 1 ml with 5 mM ammonium formate/0.1% formic acid in water, and analysed by LC-MS/ MS using the conditions shown in Table 1. The remaining acetonitrile extract was analysed directly by GC-MS/MS using the conditions shown in Table 3. Spiked samples were quantitated against 5-point matrix-matched calibration curves prepared in unspiked pistachio matrix blanks (after cleanup). No internal standard was used.



Table 1. LC-MS/MS analysis conditions

Column	Ascentis® Express RP-Amide, 10 cm × 2.1 mm I.D., 2 μm (Cat. No. 51576-U)
Mobile phase	[B] 5 mM ammonium formate, 0.1% formic acid in 95:5 acetonitrile:water
Gradient	5% B held for 1 min; 5 to 100% B in 12 min; held at 100% B for 1.5 min; 100 to 5% B in 0.5 min; held at 5% B for 1.5 min
Flow rate	0.4 ml/min
Column temp.	30 °C
Detector	MS, ESI (+), MRM (see Table 2)
Injection	5 µl

Table 2. MRMs used for quantitation, LC-MS/MS

Compound	CAS No.	MRM	Frag (V)	CE
Aclonifen	74070-46-5	265/182.1	115	28
Aldicarb	116-06-3	208.1/89.1	70	12
Aldicarb-sulfone	1646-88-4	223.1/86.1	80	8
Bifenazate	149877-41-8 301.1/170.1	95	16	
Butocarboximsulfoxide				
34681-24-8	207.1/132	65 0		
Carbendazim	10605-21-7	192.1/160.1	105	16
Carbofuran	1563-66-2	222.1/165.1	80	20
Chlorantraniliprole	500008-45-7 483.9/452.9	105	16	
Etrimfos	38260-54-7	293.1/125	120	28
Flufenoxuron	101463-69-8 489.1/158	100	20	
Isoxathion	18854-01-8	314.1/105	135	12
Malathion	121-75-5	331/126.9	80	5
Methabenzthiazuron	18691-97-9	222.1/165.1	90	12
Methomyl	16752-77-5	163.1/106	50	4
Neburon	555-37-3	275.07/57.1	100	20
Omethoate	1113-02-6	214/109	80	24
Pyraflufen-ethyl	129630-19-9 413/339	120	25	
Quinalphos	13593-03-8	299/163	90	20
Rotenone	83-79-4	395/213.1	145	20
Spinetoram	187166-40-1 748.5/142.2	206	32	
Spiromesifen	283594-90-1 388/273	110	10	
Thiacloprid	111988-49-9 253/126	100	16	
Thiophanate-methyl	23564-05-8	343/151	90	20
Triazophos	24017-47-8	314.1/162.1	110	16
Trichlorfon	52-68-6	256.9/109	80	12

Table 3. GC-MS/MS analysis conditions

Column	SLB [®] -5ms, 20 m × 0.18 mm l.D., 0.18 µm	
Oven	50 °C (2 min), 15 °C/min to 320 °C (5 min)	
Inj. Temp.	250 °C	
Carrier gas	helium, 1.2 ml/min constant flow	
Detector	MSD, scan and MRM (see Table 4)	
Msd interface	325 °C	
Injection	1 μl, splitless (0.75 min)	
Liner	4 mm I.D. FocusLiner™ with taper	

Table 4. MRMs used for quantitation; GC-MS/MS

Compound	CAS #	MRM	CE	
Chlorpyrifos-methyl	5598-13-0	286/93	20	
Tolclofos-methyl	57018-04-9	265/250	15	
Fenthion	55-38-9	278/169	15	
MGK-264	18691-97-9	164/98	10	
Endosulfan sulfate	1031-07-8	274/239	15	
Etoxazole	153233-91-1	141/63	30	

RESULTS AND DISCUSSION

Background

Initially, cleanup using Z-Sep+ sorbent was compared to PSA/C18, a common QuEChERS cleanup sorbent for fat-rich samples. A visual comparison of the QuEChERS extracts (in acetonitrile) is shown in Figure 2. Both cleanups removed some green colour, resulting in similar light yellow extracts. GC-MS-scan comparisons (Figure 3) show lower background after Z-Sep+ cleanup compared to PSA/C18. The predominant peaks present in the uncleaned extract are fatty acids and monoglycerides. While PSA/C18 only reduced the levels of these compounds, almost none were detected after Z-Sep+ cleanup.

Pesticide recovery

Table 5 shows the average %Recovery and %RSD for n=3replicates of spiked pistachio samples. The majority of the pesticides were analysed by LC-MS/MS; and those without sufficient response were analysed by GC-MS/MS. Out of the 30 pesticides analysed, 22 had recoveries within the generally accepted range of 70-120%. Reproducibility was good, with RSD values < 20% for all 30 pesticides, and < 10% for many. Two pesticides, etoxazole and trichlorfon, had recoveries < 50%. Trichlorfon was most likely retained by the Z-Sep+ sorbent during the cleanup step. This could be due to the Lewis base character of the phosphate group present in its structure. Etoxazole, on the other hand, does not contain a phosphate group. It is a very lipophilic pesticide, indicated by its log P value of 5.6. Extraction efficiency of this compound from the fatty pistachio matrix was probably very poor using acetonitrile. Spinetoram, with a log P of 6.3, also showed lower recovery (56%) than a majority of the pesticides studied. This trend of decreased recovery for high log P pesticides has been observed by others for high fat matrices^[6]. Recovery of both of these compounds may be increased by addition of a less polar solvent such as ethyl acetate for the extraction; however, an increase in the level of co-extracted background can be expected.


Figure 2. Comparison of pistachio extracts; before and after cleanup.







Z-Sep +

No cleanup

Figure 3. GC-MS-scan comparison of pistachio extracts with (a) No cleanup,

(b) PSA/C18 cleanup, and (c) Z-Sep+ cleanup; all the same Y-scale



Table 5. Pesticide recoveries from pistachios using Z-Sep+ Cleanup, Spike level of 10 ng/g

Pesticide	Avg. % (n=3)	% RSD	Analysis
Aldicarb	102%	3%	LC-MS/MS
Aldicarb-sulfone	108%	1%	LC-MS/MS
Bifenazate	88%	4%	LC-MS/MS
Butocarboximsulfoxide	83%	5%	LC-MS/MS
Carbendazim	71%	4%	LC-MS/MS
Carbofuran	104%	4%	LC-MS/MS
Chlorantraniliprole	90%	5%	LC-MS/MS
Chlorpyrifos-methyl	66%	10%	GC-MS/MS
Endosulfan sulfate	58%	6%	GC-MS/MS
Etoxazole	45%	9%	GC-MS/MS
Etrimfos	90%	7%	LC-MS/MS
Fenthion	72%	9%	GC-MS/MS
Flufenoxuron	62%	15%	LC-MS/MS
Isoxathion	92%	3%	LC-MS/MS
Malathion	102%	4%	LC-MS/MS
Methabenzthiazuron	84%	3%	LC-MS/MS
Methomyl	106%	5%	LC-MS/MS
MGK-264 (avg. 2 isomers)	57%	17%	GC-MS/MS
Neburon	92%	7%	LC-MS/MS
Omethoate	66%	2%	LC-MS/MS
Pyraflufen-ethyl	97%	18%	LC-MS/MS
Quinalphos	104%	7%	LC-MS/MS
Rotenone	100%	3%	LC-MS/MS
Spinetoram	56%	10%	LC-MS/MS
Spiromesifen	83%	4%	LC-MS/MS
Thiacloprid	100%	2%	LC-MS/MS
Thiophanate-methyl	100%	3%	LC-MS/MS
Tolclofos-methyl	71%	10%	GC-MS/MS
Triazophos (avg. 2 isomers)	89%	3%	LC-MS/MS
Trichlorfon	14%	13%	LC-MS/MS

CONCLUSIONS

Pistachios, which contain 45% fat, present a challenging matrix when doing pesticide residue analysis. If using QuEChERS extraction, some fat will be co-extracted with the analytes of interest. Thus, the cleanup step must be able to reduce this background. In this application, the use of Supel[™] QuE Z-Sep+ was demonstrated for the effective cleanup of these extracts prior to LC-MS/MS and GC-MS/MS analysis. Fatty acid and monoglyceride background were significantly reduced using Z-Sep+, and compared to PSA/C18 cleanup, the resulting extract had lower background; as evidenced by GC-MS-scan data. Pesticide recovery was within the acceptable range of 70-120% for 22 out of 30 targeted pesticides, with excellent reproducibility demonstrated for spiked replicates.

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unseen in the green

Ensuring sufficient cleanup and sensitivity.

Supel[™] QuE Verde for sensitive and reliable pesticide determination by QuEChERS.

- Remove >95% of pigment interferences
- Attain >70% recovery of even the most challenging planar pesticides

Featured products

Description	Cat. No.
Supel [®] QuE QuEChERS products	
Empty centrifuge tube, 50 ml, pk of 50	55248-U
Supel [™] QuE citrate extraction tube, 12 ml, pk of 50	55227-U
Supel [™] QuE Z-Sep+, 2 ml, pk of 100	55414-U
Supel [™] QuE Z-Sep+, 15 ml, pk of 50	55486-U
Capillary GC Column	
SLB®-5ms, 20 m × 0.18 mm l.D., 0.18 μm	28564-U
HPLC Column	
Ascentis® Express RP-Amide, 10 cm × 2.1 mm I.D., 2 µm	53913-U
Accessories	
QuEChERS shaker and rack starter kit, USA compatible plug	55278-U
QuEChERS shaker and rack starter kit, EU Schuko plug	55438-U

Related Products

Description	Cat. No.
Accessories	
Inlet liner, split/splitless type	
Single Taper FocusLiner [™] design (wool packed)	2879901-U
Molded Thermogreen® LB-2 Septa, solid discs, pk of 50 28676-U	
Thermo-O-Ring [™] Inlet Liner O-Ring, pk of 10	21003-U
Gold-plated inlet seal (straight design), pk of 2	23318-U
Capillary column nut for Agilent®MS, pk of 5	28034-U
Vials	
Certified vial kit, low adsorption (LA), 2 ml, amber, w/slit caps pk of 100	29654-U
Certified vial kit, low adsorption (LA), 2 ml, clear with marking spot,	
PTFE-silicone septa w/slit. pk of 100	29652-U

Supelco® Analytical Products



Managing hydroxymethylfurfural (HMF) in food processing

INTRODUCTION

Hydroxymethylfurfural (HMF) can be formed in many carbohydrate-containing foods during thermal treatment like cooking, frying or baking, but can also increase during storage and transportation.

While thermal treatment guarantees food safety and can improve sensory properties (e.g. flavor, colour and taste) of foodstuffs, HMF can be used as a quality marker and adulteration indicator in specific foods, such as juices, milk, sauces, honey or cereals. It can also be found in alcoholic beverages or vinegar. The level of HMF in baked goods (e.g. bread and cookies), roasted coffee, caramelised sugars and others can vary depending on factors such as cooking time, temperature, and ingredients. The substance is formed during the thermal decomposition of carbohydrates as a product of the Maillard reaction. While HMF can provide caramel-like flavor and a brown colour, which is desired in some foods, excessive levels of HMF can negatively impact taste, aroma and overall quality of others.

As a constituent of processed foods, HMF has both profoundly adverse and beneficial effects on human and bee health^[1]. According to Codex Alimentarius, honey must meet the 40 mg/ kg HMF limit after processing and blending, to ensure that the product has not undergone extensive heating during processing and is safe for consumption (except for honey that originated from tropical climates with a higher limit of 80 mg/kg).

While HPLC or spectrophotometric methods can be labour intensive, reliable fast alternatives are of high interest. See how our application note HMF in honey and the Reflectoquant[®] HMF test provide the first rapid test method for the accurate determination of HMF content in just a few minutes.





REFLECTOMETRIC DETERMINATION AFTER CONVERSION WITH A BARBITURIC ACID DERIVATIVE AND AN AMINOPHENAZONE DERIVATIVE

Reagents:

Description	Cat. No.
Reflectoquant® Hydroxymethylfurfural (HMF) test	1.17952.0001
Reflectometer RQflex® 20	1.17246.0001
	1.16754.4000
Water for analysis	1.16754.5000
	1.16754.9010
Reflectoquant [®] Blank strips	1.16730.0001
Test vessels	1.17989.0001

This application note pertains to RQflex[®] 20 and all discontinued instruments (RQflex[®] 10, RQflex[®] plus).

SAMPLE PREPARATION

Weigh 2,5 g of honey into a test vessel, dissolve with approx. 4 ml of water for analysis, transfer quantitatively to a 10 ml volumetric flask, and make up to the mark with water for analysis. Prepare larger sample volumes according to the instructions as necessary.

BLANK

Every type of honey gives rise to a more or less intense blank value depending on its intrinsic colour. This blank value is determined by immersing a blank strip into the prepared honey sample solution and measuring it in the reflectometer. Select the HMF method beforehand by means of the bar code. It is advisable to take the blank value into consideration even when light-coloured types of honey are being investigated.

ANALYSIS

Press the START button of the reflectometer and at the same time immerse both reaction zones of the test strip in the prepared sample (15 - 30 °C) for 1 second. Allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel.

Approx. 10 seconds before the end of the reaction time, insert the test strips all the way into the strip adapter with the reaction zones facing the display. After the end of the reaction time, read off the result from the display in mg/l hydroxymethylfurfural.

The result [mg/l] is automatically stored. (Please refer to the RQflex® operating instructions and the package insert of the Reflectoquant® Hydroxymethylfurfural (HMF) Test.)

CALCULATION

HMF content [mg/kg] = (measurement result [mg/l] - blank [mg/l]) x 4 / 1.4

MEASUREMENT RESULTS

A series of tests with 50 different types of honey were measured with the Reflectoquant[®] HMF Test. The results were compared with those obtained using the official DIN methods DIN 10751-3 (HPLC). The factor of 1.4 used in the calculation formula is used to balance effects of the honey matrix on the test.



Figure. HMF Reflectoquant versus HPLC

References

 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health, Shapla et al. Chemistry Central Journal (2018)



Quantification of ethylglyoxal in Manuka honey – A simple HPTLC based approach

Markus Burholt, Scientist, Instrumental Analytics R&D Michaela Oberle, Technical Project Manager, Instrumental Analytics R&D Michael Schulz, Head of HPLC & Instrumental Analytics R&D Frank Michel, Analytical & Chromatography Scientific Advisor Monika Bäumle, Global Product Manager Thin-Layer Chromatography

INTRODUCTION

Honey, a natural product, is one of the most frequently tested food products. In recent years, manuka honey has gained popularity because of its high antibacterial activity^[1]. Methylglyoxal (MGO) has been identified as one of the major contributors to its antibacterial activity. MGO is present in high concentrations in manuka honey and is directly responsible for its potency. This makes the manuka honey exclusive and highpriced as compared to the other traditional kinds of honey. Manuka honey from New Zealand usually contains 40 to 800 mg/kg of MGO but can even contain up to 1900 mg/kg^[2]. To avoid adulteration of manuka honey products, a strict quality regulation regarding its origin, purity and quality need to be fulfilled and is a prerequisite for the UMF[™] (Unique Manuka Factor) grading^[2]. It mostly reflects the MGO amount in the honey but also considers other authenticity markers.

In the following application, we focus on the MGO quantification using High-Performance Thin-Layer chromatography (HPTLC) with subsequent substance confirmation by MS measurement. The high viscosity and high sugar content of honey makes it a very complex and matrixrich sample for an analysis. Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are convenient, fast, and efficient separation techniques that enable the development of analytical methods without the need for complicated sample preparations or high investments. Low cost and short analysis time per sample are given by the parallel analysis of many samples on one plate. Furthermore, the high matrix tolerance of TLC offers additional opportunities to existing routine methods.

EXPERIMENTAL

Six different commercially available manuka honey samples were analysed. MGO shows a mesomeric effect and reacts immediately with water to form either methylglyoxal monohydrate or methylglyoxal dihydrate in aqueous environments^[3]. Only a small amount of around 1% MGO remains unreacted. Direct detection of MGO in manuka honey is found to be difficult using conventional methods. In this application, MGO is converted to stable 2-methylquinoxaline by derivatising it with 1,2-phenylenediamine (Figure 1)^[4].

The stable form is then used as the reference. For confirmation of the method and determination of the recovery rate regular honey samples have been spiked with MGO and 1,2-phenylenediamine. Other derivatization options were tested but the reaction with 1,2-phenylenediamine performed best. Water and honey matrix were tested to confirm, that the optimzed reaction conditions provide reproducible results for both matrices.

A calibration curve of 2-methylquinoxaline was calculated based on 3 different standard volumes (Table 1 and Figure 2).

H ₃ C H +	NH2 NH2	 CH N
0	✓ NH ₂	N N

Methylglyoxal (MGO) 1,2-phenylenediamine

Figure 1. Reaction scheme of MGO with 1,2-phenylenediamine

Table 1. Calibration Curve

Spots	Application volume µl	Amount (µg)	Mean Area
1, 10, 19	0.3	0.045	4080.52
2, 11, 20	1.5	0.225	11120.91
3, 12, 21	3.0	0.451	15677.39





A recovery study was performed using regular honey to simulate honey matrix. It was spiked with a known amount of MGO standard solution, followed by the addition of 1,2-phenylenediamine. The measured (and calculated) MGO amount allowed for the correlation of the actual amount of MGO in the Manuka honey samples. The experimental details of the recovery rate study can be found in Table 2, Table 3, Figure 3 and Figure 4.

Table 2. TLC data of recovery rate: In total, nine regular honey samples were applied and one MGO standard sample. Seven honey sample (4-10) were spiked with MGO and 1,2-phenylenediamine.

Spots	Application volume µl	Amount (µg)
1	1.0	Methylglyoxal standard 0.15 mg/ml (water) with 0.2% 1,2-diphenylenediamine
2	5.0	Regular honey, 100 mg/ml in water/ethanol 3:2
3	5.0	Regular honey, 100 mg/ml in water/ethanol 3:2 + 0.2% 1,2-phenylenediamine
4	5.0	Regular honey, 100 mg/ml in water/ethanol 3:2 + 0.2% 1,2-phenylenediamine spiked with methylglyoxal 0.024 mg/ml

Table 3. Quantification of methylglyoxal in the seven honey samples

"Area AU"
6759.37
6665.00
6911.29
6756.10
7055.36
7059.58
7014.80
6888.79
2.35
0.108
0.12
90.05



Figure 3. Visualization of the plate under visible light (white light); a) matrix compounds after staining with anisaldehyde sulfuric acid (black areas); b) 2-Methylquinoxaline (blue spot at hRf 80), (reaction product of Methylqlyoxal with 1,2-phenylenediamine)



Figure 4. Scan of spiked honey tracks (sample 4 – 10) at 480 nm with CAMAG TLC Scanner 3.

All TLC analyses were performed using HPTLC Silica gel 60 F254. The plates were pre-washed with the mobile phase (up to 7 cm) and dried before use.

The standards were prepared by dissolving 100 μ l of ~40% aq. MGO solution (exact content known) diluted in 20.0 ml water. 800 μ l of this stock solution was further diluted with water to 10.00 ml volume and 0.2% (20 mg) of the reactant 1,2-phenylenediamine was added. All standard solutions were stored at 8 °C for two days before use to achieve reproducible reaction of MGO with 1,2-phenylenediamine. Longer storage times (>3 days) lead to partly degradation of 2-methylquinoxaline.

Honey sample solutions of 100 mg/ml in case of sample numbers 1, 3, 5, and 150 mg/ml in case of honey samples 2, 4 and 6 were applied with a higher volume due to the expected lower amount of MGO. To each sample 0.2% of 1,2-phenylenediamine was added, eg., sample 1, 4.0 g honey diluted in 40 ml solution of water / ethanol in 3:2. To the solution 0.2% (80 mg) of the reactant 1,2-phenylenediamine was added. Before using the samples, they needed to be stored at 8 °C for two days to complete the reaction.



The samples and standards were applied as spots in an area of 5 x 3 mm². This step is necessary because of the high matrix and high application volumes of the honey samples. The plate was developed, dried, and then derivatized by dipping in an anisaldehyde-sulphuric acid reagent. Blue spots of 2-methylquinoxaline (product of the reaction of MGO with 1,2-phenylenediamine) appeared at hRf 80. Daylight examination and scanning of the plate at 480 nm were carried out for quantification. Experimental results are shown in Figure 5 and Table 4.



Figure 5. Visualisation of the plate under visible light (white light); a) matrix compounds after staining with anisaldehyde sulphuric acid (black areas); b) 2-methylquinoxaline (blue spot at hRf 80), (reaction product of methylglyoxal with 1,2-phenylenediamine)

Table 4. TLC data: In total 27 samples were applied. Track numbers with applied samples and volumes and obtained hRf values are summarised here (details of tracks 1-3, 10-12 and 19-21 for calibration are given in Table 1):

Manuka samples	Application position	Conc. sample (mg/ml)	Application volume (µl)	Mean area (AU)	Mean amount (µg)	%RSD	MGO in honey (mg/ kg)	Expected amount MGO in honey according to information on product label (mg/kg)
1	4, 13, 22	100.0	5.0	11225.00	0.228	2.68	507.4	600.0
2	5, 14, 23	150.0	9.0	4548.80	0.055	2.84	45.3	nd
3	6, 15, 24	100.0	5.0	8002.84	0.136	3.10	301.4	300.0
4	7, 16, 25	150.0	8.0	6031.58	0.088	2.48	81.4	80.0
5	8, 17, 26	100.0	5.0	9674.57	0.181	3.06	401.8	400.0
6	9, 18, 27	150.0	8.0	7578.22	0.125	3.14	115.6	nd

* The expected MGO concentrations in sample 2 and 6 were not specified by the supplier

A separate plate without staining was used for MS measurement. The coupling to MS was performed on an elution-based approach, that utilised a TLC-MS interface. This enabled the dissolution of the analyte from the silica plate at the zone of hRf 80 by a solvent and a transfer to the mass spectrometer in the liquid phase. This additionally confirmed the spot identification of the MGO derivative 1-methylquinoxaline^[5].

EXPERIMENTAL CONDITIONS

Plate	HPTLC Silica Gel 60 F254 20 x 10 cm (Cat. No. 1.05642.0001)
Application volume	0.3 – 9.0 μl, area application 5 x 3 mm with CAMAG ATS 4
Detection	480 nm
Chamber	20 x 10 chamber without filter paper
Mobile phase	Ethyl acetate/Acetonitrile 85:15 (v/v)
Staining	Anisaldehyde-sulfuric acid reagent (0.5 ml p-anisaldehyde, 85 ml methanol, 10 ml glacial acetic acid, 5 ml sulfuric acid 98%)
Migration distance	5 cm
hRf	80
Drying	0° C
Standard preparation	100 µl of ~40 % aq. methylglyoxal solution (exact content known) diluted in a 20.0 ml volumetric flask and filled up with water. 800 µl of this stock solution is diluted again in a 10.0 ml volumetric flask and made up to the mark with water. Addition of 0.2 % (20 mg) of the reactant 1,2-phenylenediamine. Before the standard is ready for use it is refrigerated at 8 °C for two days to complete the derivatization reaction.
Sample	Solutions of 100 mg/ml of sample nos. 1, 3, 5, and 150 mg/ml of sample nos. 2, 4, 6 were prepared. To every sample 0.2 % of 1,2-phenylenediamine was added. eg, sample 1: 4.0 g honey diluted in 40 ml solution of water/ethanol in 6:4. To the solution 0.2 % (80 mg) of the reactant 1,2-phenylenediamine was added. Before the samples are ready to use, they are refrigerated at 8 °C for two days to complete the derivatization reaction.
MS measurement	The samples are extracted with the Plate Express and measured with the single-quadrupole expression compact mass spectrometer (CMS) from Advion.
Extraction solvent	Acetonitrile/Water 95:5 (v/v) + 0.1% formic acid

RESULTS AND DISCUSSION

As demonstrated, MGO can be identified and quantified in different honey samples within the concentration range of 50 mg to 600 mg/kg. The conversion of MGO into the more stable compound 2-methylquinoxaline allows for an easy evaluation of the MGO content. The recovery study showed a detectable MGO amount of around 90%. The correlated MGO amount in manuka samples was calculated accordingly. One of the samples (sample 1) showed a lower MGO content than indicated by the supplier. This might be because of the degradation of the MGO during storage. Sample 2 and sample 6 only showed MGO concentrations of 50 and 100 mg/kg. These manuka honey samples are considered of lower quality. Although no indication of MGO concentration was provided by the supplier.

CONCLUSION

The analysis of MGO in a complex and challenging food matrix like honey was described. Target analyte could be easily separated and detected without time-consuming and laborintensive sample preparation. The flexible set-up enabled a combination with MS measurements.

Screening and method development capabilities were shown by the application of 27 tracks on one plate (honey samples and standard solutions). The study clearly differentiated various honey qualities (referring to MGO content) on the market. Though the analysis of MGO is challenging, MGO content could be well quantified in the expected range.

To summarise, a fast, cheap, and simple quantification of methylglyoxal can be accomplished with HPTLC. This application demonstrates the main advantages of the method, such as quick sample preparation, high matrix tolerance, and high-throughput.

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FEATURED PRODUCTS

Description	Cat.No.
HPTLC Silica gel 60 F254 20 x 10 cm	1.05642.0001
Methylglyoxal solution ~40% in H_2O	M0252-25G
1,2-Phenylenediamine ≥99%	694975-1G
Ethyl acetate, for liquid chromatography LiChrosolv®	1.00868.2500
Acetonitrile, gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030.2500
Ethanol, gradient LiChrosolv®	1.11727.2500
Methanol, gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007.2500
Sulfuric acid, ACS reagent, 95.0-98.0%	258105-2.5L
Acetic acid, glacial, ACS reagent, ≥99.7%	695092-500ML
p-Anisaldehyde 98%	8.22314.0250
TLC-MS	
Acetonitrile, for UHPLC-MS LiChrosolv®	1.03725.2002
Water, for UHPLC-MS LiChrosolv®	1.03702.2500
Formic acid 98% - 100% for LC-MS LiChropur™	5.33002.0050
Millex LCR PTFE Syringe filter	SLCRBZ5NZ

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...that we offer MS-grade TLC and HPTLC plates for rapid identification and superior characterisation using mass spectrometry (MS)?

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VIEW BROCHURE



Determination of total glucose and xylose in instant coffee by reverse phase HPLC-UV

Eddy Tan, Application Scientist, Lee May May, Senior Application Scientist, Singapore Applications Laboratory



This is a method for the determination of total glucose and xylose in coffee samples by reverse phase HPLC- UV. The limit of detection for glucose and xylose is 53.2 and 33.8 ppm respectively for freeze dried coffee.

INTRODUCTION

Coffee is an indispensable beverage for many people. The adulteration of coffee with coffee husks, cereal grains and soy beans to raise the profit margin is well known. Typical markers for such adulteration include glucose and xylose. Instant coffee is considered to be adulterated if it contains more than 2.46% total glucose and 0.45% total xylose^[1,2].

As sugars lack UV chromophores, their determination is typically accomplished by HPLC-RID (Refractive Index Detector) ^[3] or by anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD)^[1,4] The RID is less sensitive compared to the UV detector and often requires a longer time to stabilise. It is therefore not the detector of choice for many HPLC users. The HPAEC-PAD is a more expensive setup with a limited set of applications and separation columns. It is therefore not a common instrument.

Although there are established methods for sugar determination in coffee eg. AOAC Method 995.13 and ISO Method 11292:1995, they all require the HPAEC- PAD instrument. Here, we demonstrate the determination of total glucose and xylose using a procedure to release the sugars from the coffee followed by an SPE cleanup. The released sugars are next derivatized with a UV tag, 1-phenyl-3-methyl-5-pyrazolone (PMP)^[5,6,7]. A final clean up by liquid-liquid extraction with dichloromethane is done before HPLC injection. The standard addition technique was chosen as it corrects for varying levels of matrix interferences with different coffee samples.

INSTRUMENTS & SAMPLES

The analysis was performed on a Thermo Dionex UltiMate 3000 UHPLC. An ultrasonic bath was used to dissolve the coffee samples. For sample digestion and derivatisation, a water bath and vortex mixer were used. The Visiprep[™] vacuum manifold with a vacuum pump was employed for the SPE cleanup. A bench and a mini centrifuge were used to spin down the samples.

Freeze dried coffee and milk coffee mixture samples were purchased from a local grocery store. Freeze dried coffee refers to pure and instant soluble coffee granules or powder whereas milk coffee mixture would have sugar, milk, emulsifier, flavouring agents etc. compounded with instant coffee powder.

METHOD

Glucose and xylose standard solutions

Prepare 1 L of 1 M hydrochloric acid. Weigh 100 mg of glucose and 100 mg of xylose into a 10 ml volumetric flask. Add 6 ml of hot 1 M hydrochloric acid (~80 °C) and swirl gently. Sonicate for 10 minutes to dissolve completely before topping up to the mark with 1 M hydrochloric acid. Mix well before use.

Coffee sample solutions and reagent blank

Weigh 1.5 g of the freeze dried coffee sample into a 10 ml volumetric flask. For milk coffee mixture sample, use 0.5 g. Add 6 ml of hot 1 M hydrochloric acid (~80 °C) to both. Swirl gently and sonicate for 10 minutes. Ensure all solids are dissolved (milk solids will remain insoluble) before topping up to mark with 1 M HCl.

Table 1. Glucose and xylose spiking for freeze dried coffee sample

Identity	Freeze dried coffee, µl	Glucose, µl	Xylose, µl	1M HCl, µl	Dilution factor	Glucose ppm	Xylose ppm
А	2000	0	0	3000	Nil	0	0
В	2000	200	200	2600	25.00	400	400
С	2000	400	400	2200	12.50	800	800
D	2000	800	800	1400	6.25	1600	1600

Spike in glucose and xylose at 400, 800 and 1600 ppm for freeze dried coffee samples as in Table 1. Do a reagent blank using water in place of sample. For milk coffee samples, use 500 μ l of sample solution instead.

Acid digestion

Incubate all spiked solutions at 80 ±2 °C for 3 hours. Then cool to room temperature, spin down contents and filter through a Millex PTFE hydrophilic 0.45 μ m filter into a new tube.

SPE cleanup

This SPE cleanup is necessary to remove oils, fats and other organics present in the coffee samples. Set up the SPE cartridges (LiChrolut® RP-18 200 mg/3 ml PP SPE tubes) on the Visiprep[™] SPE vacuum manifold system. Connect this to the vacuum pump. Condition the SPE cartridges first with 2 x 3 ml methanol followed by 2 x 3 ml 1 M HCl. Next, place a 15 ml centrifuge tube as a receiver for each of the SPE cartridges. Transfer 1 ml of the filtrate from the acid digestion step into the SPE cartridge. Control flowrate for a dropwise elution.

Derivatization: Tagging the sugars with PMP (UV label)

Prepare 10 ml of 0.5 M PMP in methanol and 10 ml of 1.2 M sodium hydroxide. Pipette 200 μ l from the SPE cleanup step into a 2 ml microcentrifuge tube. Add 200 μ l of 1.2 M sodium hydroxide and vortex for 30 seconds. Pipette 100 μ l into a 5 ml microcentrifuge tube. Add 100 μ l of 0.5 M PMP and vortex for 1 minute. Spin down and incubate at 70 ±2 °C for 100 minutes in a water bath. Cool to room temperature for the next step.

Cleanup of sample for HPLC

Prepare 10 ml of 0.2 M hydrochloric acid. Add 100 μ l of 0.2 M hydrochloric acid to the tagged sample. Vortex for 30 seconds and spin down contents. Add 1800 μ l of water and 1500 μ l of dichloromethane to it. Vortex for 1 minute and centrifuge for 2 minutes at 7000 RCF. Draw off the top aqueous layer into another 5 ml tube. Discard the dichloromethane. Repeat extraction of the aqueous layer with 1500 μ l of dichloromethane twice more. Filter the aqueous layer through a 0.22 μ m 13 mm Millex PTFE hydrophilic filter into a 2 ml HPLC vial. Seal vials and proceed to HPLC injection. Chromatographic conditions are in Table 2.

Table 2. Chromatographic conditions

column	Purospher® STAR RP-18e, 15 cm x 3 mm, 3 µm (Cat. No. 1.50750) with guard cartridge, 4-4 mm (Cat. No. 1.50270) and pre-column holder (Cat. No. 1.16217)
mobile phase	 [A] 200 mM ammonium acetate (pH 6.8 ± 0.05); [B] acetonitrile; (78% A / 22% B; isocratic elution). All filtered through hydrophilic PTFE, 0.2 µm
flow rate	0.4 ml/min
column temp	35 °C
detector	UV, 245 nm
pressure	~200 bar
injection volume	20 µl

RESULTS & DISCUSSION

Both glucose and xylose peaks were symmetrical and eluted at ~9.8 and ~11.5 minutes respectively (Figure 1). The freeze dried coffee has a more complex HPLC profile compared to the coffee mixture sample. See Figure 2 for spiked freeze dried coffee sample.

The freeze dried coffee samples 1 and 2 had a total xylose content >0.42% w/w (Table 3). The coffee mixture samples 1 and 2 had a high glucose content.



Figure 1. Coffee mixture and freeze dried coffee with glucose and xylose standards

Sample	Peak	Compound	Retention time (min)	Resolution	Peak symmetry
Freeze dried coffee	1	Glucose	9.79	5.75	0.97
	2	Xylose	11.52	-	0.93
0.55	1	Glucose	9.83	5.72	1.03
Coffee mixture	2	Xylose	11.55	-	0.93



Figure 2. Freeze dried coffee sample spiked with glucose and xylose standards





0 6.1962 404.08 14.1098 80816 22 4116 1616.32 37.5630 Slope 0.0194 STEYX 0.3132 LOD 53.2 ppm LOQ 161.2 ppm

Figure 3. Standard addition calibration plots and data for freeze dried coffee

>40% w/w as both have sugar and glucose syrup listed as ingredients.

Table 3. Results for coffee samples

Sample	Glucose (% w/w)	Xylose (% w/w)
Freeze dried coffee 1	0.35	4.10
Freeze dried coffee 2	0.55	3.23
Coffee mixture 1	43.31	0.68
Coffee mixture 2	46.59	0.13

CONCLUSION

We can determine total glucose and xylose in coffee by Reversed Phase HPLC-UV. This is a sensitive isocratic separation that can be completed by fifteen minutes with the Purospher® STAR RP-18e fully porous particle column. The method can be modified using Fused-Core® or Chromolith® columns for even faster separation while still applicable to conventional HPLC and to UHPLC instruments.

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Linearity - Xylose

Concentration (ppm)	Mean area
0	56.0414
403.64	68.0976
807.28	80.0099
1614.56	102.9951
Slope	0.0290
STEYX	0.2978
LOD	33.8 ppm
LOQ	102.5 ppm

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FEATURED MATERIALS

Description	Cat. No.
HPLC columns	
Purospher® STAR RP-18e, 3 µm, 150-3 mm, Hibar® RT	1.50750
Purospher® STAR RP-18e 4-4 mm guard cartridge	1.50270
Pre-column holder for LiChroCART [®] cartridges 4-4 for capillary connection	1.16217
Sample prep, reagents and accessories	
LiChrolut® RP-18, 200 mg, 3 ml SPE PP tube, Pk.50	1.02014
Visiprep [™] SPE vacuum manifold	57030-U
Millipore® Chemical duty pump, 220 V/50 Hz	WP6122050
Acetonitrile isocratic grade for liquid chromatography LiChrosolv®	1.14291
Ammonium acetate for analysis EMSURE® ACS, Reag. Ph Eur	1.01116
Dichloromethane for liquid chromatography LiChrosolv®	1.06044
Hydrochloric acid fuming 37%, for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1.00317
Methanol for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1.06009
Methanol for liquid chromatography LiChrosolv®	1.06018
3-Methyl-1-Phenyl-2-Pyrazoline-5-one (PMP), 99%	M70800
Sodium hydroxide pellets for analysis EMSURE®	1.06498
Omnipore® 0.2 µm 47mm membrane filters	JGWP04700
Millex [®] - LCR 0.22 µm 13 mm filter unit, hydrophilic PTFE	SLCR013NL
Millex [®] - LCR 0.45 µm 33 mm filter unit, hydrophilic PTFE	SLCR033NB
HPF Millex [®] - LCR 0.45 μm 33 mm filter unit	SLLGM25NS
Reference materials	
D-(+)-Glucose, Pharmaceutical Secondary Standard	PHR1000
D-(+)-Xylose, Pharmaceutical Secondary Standard	PHR2102

Analysis of plant-based proteins pH measurement using inlab sensors



The complex matrix, diverse physical properties, and varied chemical composition of ingredients in alternative protein products often interfere with pH measurement, posing significant challenges. This application note shares valuable tips and hints on how to obtain reliable and accurate results when measuring the pH of plant-based proteins.

As demand for plant-based proteins continues to grow, manufacturers face increasing pressure to ensure their products are of high quality and meet customer expectations. Accurate pH measurement is essential for ensuring the products' safety, quality, texture, and flavor. However, measuring the pH of such protein samples can be challenging, even more with conventional pH sensors. Many would face issues such as junction clogging, insufficient sample interaction or electrolyte contamination, which can lead to inaccurate pH readings. Using METTLER TOLEDO's InLab® sensors provides a reliable solution for pH measurement of viscous and solid plantbased protein samples.

These measurements require specialized sensors and systems to ensure precise pH readings. This application note provides in-depth guidance on overcoming these challenges and improving the pH measurement results for plant-based proteins using METTLER TOLEDO's InLab® sensors. It also emphasises the importance of following good electrochemistry practices and shares valuable tips on sensor maintenance and storage.

METTLER TOLEDO

The right sensor choice: InLab® Power Pro-ISM and InLab® Solids Pro-ISM



Figure 1. InLab Power Pro-ISM and InLab Solids Pro-ISM sensors.

No.	Sample type	Challenge	InLab Power Pro-ISM	InLab Solids Pro-ISM	Advantage
1	Viscous	Sensor clogging	Pressurised SteadyForce [™] reference system	-	Ensures continuous electrolyte flow in viscous samples and prevents sample ingress.
2	Solid			Open junction	Makes the InLab Solids Pro-ISM sensor maintenance-free, and the open junction prevents clogging related issues.
3	Viscous	Insufficient sample contact	Hemispherical sensing membrane	-	Provides greater surface area and minimises gradient effects that may exist in the sample, thus providing stable readings.
4	Solid		-	Spear shaped sensing membrane	Allows easy sample insertion for solid and semi-solid samples.
5	Viscous and solid	Contamination	Easy-to-clean sensing membranes reduce risk of contamination		Improves accuracy of sensors.
6	Viscous and solid	Frequent monitoring and refilling of electrolytes	Maintenance free sensors		Improves productivity due to reduced downtime.
7	Viscous and solid	Inconsistencies in results caused by varying temperatures	Integrated temperature probe (and ATC functionality of METTLER TOLEDO pH meters)		Integrated temperature probe accurately notes sample temperature and yields a precise slope by compensating for temperature effects.
8	Viscous and solid	Secure and easy data handling	ISM® (Intelligent Sensor Management)		Provides maximum traceability and data security by keeping a track of important information such as the latest calibration data and maximum temperature exposure.

Table 1. Features and advantages of the InLab Power Pro-ISM and InLab Solids Pro-ISM sensors.

Applying good practices for consistent and high-quality results

A. Calibration and verification

- Calibrate the sensor regularly using METTLER TOLEDO pH buffer solutions before measuring the first sample of the day. If the number of samples exceeds 30 per day, the calibration must be repeated after completing half of the samples
- Use at least two pH buffers for calibration, bracketing the expected sample pH, e.g., use pH buffers 4 and 7 for calibration if the expected sample pH is 6.22
- A slope of 95–105% and an offset of 0 mV ± 20 mV confirm a good state of the electrode and hence ensure reliable measurements
- Verify the calibration by measuring a pH buffer of known pH value between the calibration range, like pH buffer 5, in case of point 2 above.
 For details on pH calibration, refer to our pH Essentials Guide
- In case of more than 10 samples, perform a verification measurement after every 10 samples.
 If the verification result is outside of the specified tolerance, perform a new calibration
- Always use fresh pH buffers of high quality and never re-use them

B. Sample preparation and measurement

To evaluate the pH of plant-based proteins such as soy milk, protein powder, plant-based liquid egg, or plant-based chicken nuggets, samples were used as representatives of such food types.

1) Soy milk, plant-based liquid egg, and protein powder

- Soy milk and plant-based liquid egg can be directly measured without any additional preparation.
- Disperse 10% (w/v) protein powder in deionised water or as required.
- Dip the InLab® Power Pro-ISM sensor in the sample, making sure that the junction is fully immersed. Samples with soy milk and protein powder require stirring.
- Record the pH along with the sample temperature. Perform triplicate measurements to obtain consistent results.
- Thoroughly clean the sensor with deionized water after each measurement.
- The InMotion Autosampler could also be used for measuring these thick samples. When using an InMotion Autosampler for the pH measurement of thick samples, additional rinse cycles with deionised water are required.

2) Plant-based chicken nuggets

- Select a small, representative portion of plant-based chicken nuggets for pH measurement.

- Gently insert the InLab Solids Pro-ISM sensor into the sample, ensuring proper contact between the pH sensor tip (including the junction) and the sample
- Record the pH along with the sample temperature
- Perform triplicate sample measurements to obtain consistent results
- Thoroughly clean the sensor with deionised water after every measurement

C. Maintenance and storage of pH sensor

- Repetitive pH measurements of protein-rich samples can result in protein accumulation and contamination of the sensor membrane, leading to delayed response. To avoid slow behaviour, proper electrode maintenance is essential
- Keep the InLab Power Pro-ISM sensor upright for a minimum of 15 minutes prior to measurement
- For thorough cleaning wash the electrode with ethanol and then rinse it with deionized water. Avoid rubbing the sensor surface; instead, gently blot excess water using tissue paper
- Soak the sensor in Pepsin/HCl solution for one hour after completion of measurements
- Periodically recondition the sensor in 0.1 M HCl for InLab Power Pro ISM sensor and 0.01 M HCl for InLab Solids Pro-ISM sensor. The frequency of reconditioning depends on the number of samples analysed per day and the results of the calibration
- The sensor must be re-calibrated after reconditioning
- Store the sensor in the wetting cap filled with InLab storage solution during periods of non-use
- Never store the sensor dry or in distilled water, as this may damage the pH-sensitive glass membrane and shortens the electrode lifetime

RESULTS AND DISCUSSION

The pH of samples like soy milk, plant-based liquid egg, and aqueous solution of plant-based protein powder were measured using the InLab Power Pro-ISM sensor, while the pH of plant-based chicken nuggets was measured using the InLab Solids Pro-ISM pH sensor, both at 25 °C. All the pH measurements were carried out using a SevenExcellence benchtop meter. Additionally, LabX software and an InMotion Autosampler were used to automate the measurement of liquid/emulsion samples. All the samples were measured in triplicates. The standard deviation and average response time were noted as enlisted in Tables 2 and 3.

The standard deviation of the results was less than 0.05 pH, which confirms the reliability of the pH measurement data. An average response time of

METTLER TOLEDO



Figure 2. pH measurement of soy milk using InLab Power Pro-ISM sensor and SevenExcellence S400 meter.

Sr. no.	Sample	Method	pH value	Standard deviation (n≥3)	Average response time (s)
1	Sou mille	Standalone	7.22	0.01	20.3
i Soy mlik	InMotion	7.22	0.02	13.7	
2	Liquid egg	Standalone	6.96	0.01	15.3
2		InMotion	6.97	0.01	9
2	Protein powder	Standalone	6.96	0.03	20.7
3		InMotion	6.99	0.02	11

Table 2. pH values of soy milk, protein powder in aqueous solution and plantbased liquid egg measured with InLab Power Pro-ISM sensor.

around 20 seconds was observed for measuring these samples. Using automation enabled time efficiency both in response time and susequent sensor cleaning after each measurement. In contrast to general aqueous samples, thick samples require sufficient rinse cycles with deionised water for effective cleaning.

CONCLUSION

For measuring the pH of liquid plant-based samples comprising proteinaceous emulsions or suspensions, the InLab Power Pro-ISM sensor is a reliable choice. Its pressurized SteadyForce[™] reference system ensures that the sensor junction does not clog, even in thick or semisolid samples. It is also possible to maximise the sample throughput by using automation in the case of liquid samples.

For the pH measurement of solid protein samples, the InLab Solids Pro-ISM is a suitable sensor with a clog-



Figure 3. pH measurement of plant-based chicken nuggets using InLab Solids Pro-ISM sensor and SevenExcellence S400 meter.

Sr. no.	Sample	Method	pH value	Standard deviation (n≥3)	Average response time (s)
1	Plant-based chicken nuggets	Standalone	5.85	0.02	21

Table 3. pH values of plant-based chicken nuggets measured with InLab Solid Pro-ISM sensor.

free open junction. Regular cleaning and reconditioning as advised in the note ensure a well-performing pH sensor. It is possible to maximise the sample throughput by using automation in the case of liquids. With the right sensor and meter setup, the use of good practices for calibration and measurement, and proper sensor maintenance, reliable pH results can be achieved.

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Supporting quality control in food processing OHAUS scales and moisture analysers help ensure safety and compliance.

FOOD SCALES

Food processing is a highly scrutinised industry with rigorous government oversight and regulations to ensure consumer and worker safety. Manufacturers must rely on quality control measures for their products and processes to remain compliant with the strict standards set by regulatory agencies. More importantly, quality control guidelines allow food manufacturers to minimise contamination risk and provide safe, quality food to customers.

Manufacturers implement standard operating procedures and incorporate the highest quality measuring instruments to ensure reliable and recorded results and product consistency across batches. Since government agencies are paying such close attention, the product's quality status must be reviewed and documented carefully throughout all stages of the production process. The two most common sets of requirements for oversight are GMP (Good Manufacturing Practices) system and HACCP (Hazard Analysis and Critical Control Point). OHAUS keeps both standards in mind when developing precision weighing and measuring instruments for the food industry.





ENSURING QUALITY

Quality control is integral to every step of food processing, including procurement, development, production and distribution. After carefully selected raw materials are approved, the production team will formulate the finished product by following standard operating procedures for ingredients, recommended weights, batch size and process time. Not only does the SOP need to be carefully documented, but so does the ongoing manufacturing process to ensure consistency and compliance, and to identify the cause should an issue arise.

One of the final steps – labeling – is also heavily regulated as what is indicated on the outside of the package must be matched by the finished product that is on the inside from formulation and consistency to weight and other factors. Instruments supporting food quality control are needed both at the front of the building (bench scales, moisture analysers) and in the back processing area (floor scales, washdown scales).

To support food manufacturer needs, OHAUS offers a wide range of durable, reliable measuring instruments to suit many applications in processing, research and development, testing, packaging, warehousing, shipping and more. We have worked with the food processing industry for decades to help support safety, efficiency, throughput and profitability. We offer solutions for improving consumer safety, product consistency and quality control.

OHAUS commercial instruments are designed to help you meet demands for food safety, quality and regulatory compliance. Our scales and moisture analysers provide efficient solutions for nearly all aspects of food processing - material receiving, formulation, quality control, filling, batching, basic weighing and checkweighing. Our precision weighing and measuring instruments for food are dependable, easy to set up and use, and reliably precise to help you maximise yield and minimise waste.

SELECTING THE RIGHT INSTRUMENTS

OHAUS offers a range of precision and analytical balances designed specifically for the food industry. They are durably constructed and designed for ease-of-use and high precision results. We offer models that are certified by NSF to support HACCP systems and are also USDA-AMS accepted. These OHAUS scales are designed with smooth, unobstructed surfaces that are easy to clean and free of areas where material can build up and cause contamination. Here are just a few of the high-performance food scales and moisture analysers we stand behind.

Food scales for high capacity, heavy duty food weighing up to 150 kg OHAUS offers the Defender[™] 6000 series of bench scales. Specifically designed for food processing, Defender









6000 manages the most demanding applications with a 316 stainless steel platform and frame and durable 316 stainless steel or food grade polycarbonate indicator. Defender 6000 bench scales are available in a range of platform sizes, weighing capacities, readability capacity and special features to suit your needs.

Defender 6000 bench scales are designed to handle high-pressure washdowns and harsh cleaning agents and meet metrological standards for legal-for-trade applications with a laser-sealed stainless steel IP68 and IP69K load cell.

Defender 6000 bench scales feature large bright displays and colour checkweighing indicators for easy readouts and efficient work. Software modes include counting, percent weighing and even filling. Communication is simple with a range of connectivity options and GLP/GMP data output with real-time clock for processing tracking and traceability.

Defender 6000 is loaded with special features for convenience and safety, including 150% overload capacity protection, menu and key lock, selectable environmental and auto-print settings, stability indicator, overload/underload indicators, auto shut-off, auto tare and adjustable rubber feet.

To further support quality control and industry compliance, OHAUS offers a range of compact moisture analysers with intuitive features to help you monitor quality and consistency through moisture content analysis. With halogen lamp heaters, durable construction, and easy-to-read displays, our MB Series of Moisture analysers offer a range of capacities, readability, and pan sizes to suit your application needs.



Measuring total, permanent and chill haze in beer

SUMMARY

The formation of haze in beer can be problematic, as it affects the quality of the end product. Beer consists of various ingredients such as proteins, carbohydrates, polyphenols, fatty acids, nucleic acids, amino acids, etc. These ingredients can precipitate, and a haze or turbidity is formed. Most beers are clear at room temperature. If there are haze-producing proteins and tannins (both primarily from malt) suspended in the beer, haze particles don't form because of the warm temperature. When beer is chilled, these proteins and tannins react to clump into larger particles that are big enough to reflect light.

In this procedure you will measure total and permanent chill in beer and the difference between the two values is the chill haze value.

THE APPLICATION

- Hach® Portable 2100Q or Benchtop TL2310 TL2360 ISO Turbidimeter
- Hach Sample Cells
- Ice
- Salt
- 95% Ethanol
- 2 pitchers/beakers or sonication device
- 1 x 500 ml Erlenmeyer flask
- 10 ml pipette

APPLICATION: DETERMINING HAZE IN BEER

Procedure

- **1.** Obtain a beer sample.
- 2. Degas beer sample.
 - a. Passing back and forth up to 70 times b. Ultrasonic
 - c. Blowing gas through sample with air stone
- 3. Measure out 200 ml of degassed beer into 500 ml Erlenmeyer flask.
- 4. Bring to room temperature.
- 5. Add 14 ml of 95% ethanol to the 200 ml of degassed beer and mix thoroughly.
- 6. Let stand for 20 minutes.



- 7. Obtain Hach cuvettes and fill with the beer ethanol mixture past the white line.
- Take initial turbidity reading using the Hach portable 2100Q or benchtop TL2310/TL2360 ISO Turbidimeter.
 a. The result of this reading is the permanent haze
- 9. Prepare an ice water bath with salt added and allow to stand until a minimum temperature of -5 °C has been reached.
- **10.** Chill samples for 1 hour in ice bath in a refrigerated environment.
- **11.** Taking chilled sample reading:
 - a. Remove sample from the ice bath b. Invert once
 - o. Invertionce

c. Quickly wipe down with paper towel d. Once dry, wipe with a Kimwipe to remove finger prints, lint from paper towel, condensation and smudges from the cuvette

e. Condensation can be an interference so it is important to be in a dry/AC lab environment f. Properly oiled cells also help with condensation

- **12.** Place sample in the turbidimeter and take turbidity reading
- **13.** Take turbidity readings as quickly as possible a. This measurement is the total haze reading



CALCULATING CHILL HAZE Chill haze = Total haze – Permanent haze

BEER SAMPLES

	1 EBC	1 NTU/FNU	1 ASBC	Beer sample	Total haze	Permanent haze
EBC	1	0.25	0.014	Pilsner	13.5 NTU	5.48 NTU
NTU/FNU	4	1	0.057	Amber ale	59.3 NTU	2.55 NTU
ASBC	70	17.5	1	Porter	84.1 NTU	8.04 NTU
				Stout	31.9 NTU	14.1 NTU

Key

EBC: European Brewing Convention ASBC: American Society of Brewing Chemists NTU/FNU: Formazine Nephelometric Unit



DR6000 in the brewing industry: Important methods in accordance with MEBAK and ASBC

INTRODUCTION

Compliance and consistent high quality are two of the key goals within the beverage industry. Hach® provides support for these goals through comprehensive analyses of water and beer.

The DR6000[™] UV-VIS Spectrophotometer supports many of the analytical measurements necessary for monitoring throughout the entire brewing process–from raw materials to final product. The DR6000 brewing-specific software has been expanded to include the most important parameters from both MEBAK ^[1] and the American Society of Brewing Chemists (ASBC)^[2]. This means that the DR6000 can be used to measure beer quality around the globe.

THE KEY METHODS IN DETAIL

Beer Colour

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 185 ff. ASBC Beer 10-A

The EBC and ASBC units are used throughout Europe and the United States to describe the colour (more specifically: The colour intensity) of beer and beer wort. The value stipulated by the European Brewery Convention (EBC) or ASBC indicates how much light is absorbed by beer of a certain content of original wort. The actual colour of each beer is nothing more than gradations of a brown tone, which decreases in concentration through red, copper, and amber colours, through to golden yellow and light yellow.

In addition to malt colour and original wort, the colour intensity of the finished beer still depends on many other factors, such as the wort preparation, the pH value and the fermentation process. The measurement of the colour may seem trivial, but it is the first impression that the customer gets before the consumption of the beer. The compliance of the beer colour is therefore an important issue that can be monitored throughout the entire fermentation process.

The absorbance of the beer is measured at a wavelength of 430 nm. Historically, the beer colour in EBC units is 10x absorbance at 430 nm measured in a 1 inch (2.54 cm) cuvette. However, for MEBAK a 1 cm (10 mm) square cuvette is stipulated. Accordingly, the following calculation applies for the determination of the beer colour in accordance with MEBAK:

Absorbance of the beer at 430 nm \times 25 = Colour in EBC units.

Historically, the beer colour in ASBC units is 10 x absorbance of the beer at a wavelength of 430 nm and with the use of a ½ inch (1.27 cm) cuvette. With the use of an intermediately stipulated 1 cm (10 mm) cuvette, the following applies in accordance with ASBC method Beer-10A:

Absorbance of the beer at 430 nm \times 12.7 = Colour in EBC units

Additionally, the turbidity of the sample is checked in the ASBC method by means of an absorbance measurement at 700 nm. A sample is not classed as turbid if the 700 nm absorbance is $\leq 0.039 \times 430$ nm absorbance.

In the DR6000, the programmes for the measurement of the beer colour are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

MEBAK Beer Colour	Programme 2006	0–60 units
ASBC Beer Colour*	Programme 2020	0–60 units

The following scale of beer colours is useful for orientation:

EBC	Example	Beer colour
4	Pale Lager, Witbier, Pilsener, Berliner Weisse	
6	Maibock, Blonde Ale	
8	Weißbier	
12	American Pale Ale, India Pale Ale	
16	Weißbier, Saison	
20	English Bitter, Extra Special Bitter	
26	Biere de Garde, Double IPA	
33	Dunkles Lager, Märzen, Amber Ale	
39	Brown Ale, Bock, Dunkelbier, Dunkelweizen	
47	Irish Dry Stout, Doppelbock, Porter	
57	Stout	
69	Foreign Stout, Baltic Porter	
79	Imperial Stout	

(Source: http://de.wikipedia.org/wiki/EBC_(Bier))

BITTERNESS UNITS

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 234 ff. ASBC Beer-23, Wort-24

The concentration of the bitters is a key quality feature of the beer. Bitters emerge during boiling due to the isomerisation of a-acids from the hops. Bitters are extracted with isooctane from the acidified sample and the absorbance is measured spectrophotometrically at a wavelength of 275 nm.

The MEBAK and ASBC methods differ only minimally in their execution. While in the MEBAK, 6 N HCl is used to acidify the samples, the ASBC uses only 3 N HCl. After extraction, the absorbance is measured in a 10 mm quartz cuvette against a blank of isooctanol of the same quality.

In accordance with the definition of MEBAK and ASBC, the results are calculated as follows:

Beer: Absorbance 275 nm × 50 = Bitterness in Bitterness Units Wort: Absorbance 275 nm × 100 = Bitterness in Bitterness Units

The various calculations result from the dilutions of beer and/or wort samples specified in the procedure.

The standard values in accordance with MEBAK are 10–40 BU (bitterness units) for beer and 20–60 BU for wort. In accordance with ASBC, the measurement range for beer is up to 100 units (wort 200) and is reported in IBU (International Bitterness Units).

In the DR6000, the programmes for the measurement of the bitter units are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Bitterness units, beer	Programme 2001	10-40 BU
Bitterness units, wort	Programme 2003	20-60 BU
ASBC bitterness units, beer*	Programme 2021	10–100 IBU
ASBC bitterness units wort*	Programme 2011	20–200 IBU

Note:

For the analysis of bitterness units, the Hach cuvette test LCK241 (only available in Europe) can also be used. Through the introduction of the chemicals in pre-manufactured cuvettes, both time and costs for chemicals (above all, high-quality isooctane) can be saved.

Beer type	Bitterness units	mg iso-alpha acids/L beer
Wheat	15 – 20	15 – 20
Vollbier	18 – 24	18 – 24
Märzen	20 – 25	20 – 25
Export	22 – 26	22 – 26
Bock	28 - 36	28 – 36
Pils	30 - 38	30 - 38
Alt	35 – 50	35 – 50

Table 1. Bitter units of the most popular beer types (from Brauerei-Forum, VLB)

ISO-A- AND B-ACIDS

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 237 ff.

The humulones (or a-hop bitter-acids) from the hops give the beer the bitter taste. During beer production (wort boiling), the bitter iso-a-acids emerge from the hops. Therefore, the iso-a-acid content is a key factor in the taste of the beer. The β -acids also contribute to the bitter taste and are recorded with this measurement.

After the bitters (see above) have been extracted from the sample with isooctane, and after further sample washing, the iso-a- and β -acid content is determined by the measurement of the sample absorbance at 255 nm and 360 nm^[1]. A 10 mm quartz cuvette is used, and both acid types are determined in a combined measurement at two wavelengths.

The standard values according to MEBAK are:

Beer: 10–40 mg/L iso-α-acids and less than 2 mg/L β-acids Wort: 15–50 mg/L iso-α-acids and less than 1–15 mg/L β-acids

In the DR6000, the programme for the measurement of the iso- α - and β -acids is available for the measurement in accordance with MEBAK.

Iso-α- and β-acids Programme 2013 0–60 mg/L iso-α-acids and 0–80 mg/L β-acids

FAN (FREE AMINO NITROGEN)

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 84 ff. ASBC Beer-31, Wort-12

The sum of the bioavailable nitrogen components in the wort is represented by the free amino nitrogen (FAN). An excessive FAN content can lead to problems, both in the taste and in the microbiological stability of the beer. Brewer's yeast and wild yeast ferment excess amino acids into long-chain alcohols (propanol, isobutanol). FAN levels are also a good indicator of



when fermentation is complete. Monitoring the FAN level with the DR6000 will help to turn over tanks faster once the FAN level is low enough. The typical FAN content is 200–250 mg/L in the wort and 10–120 mg/L in the beer (MEBAK).

The methods for both MEBAK and ASBC are identical. The prepared beer or wort are mixed with a colour reagent (based on ninhydrin) and the absorbance is measured at a wavelength of 570 nm in a 10 mm cuvette.

This absorbance is compared with the colour produced by a 2 mg/L glycine standard as reference. For a more precise determination, the blank value, the glycine standard, and the sample are measured in triplicate and the average value is calculated. Due to the differing sample preparation of beer and wort, internal factors of 50 (for beer) or 100 (for wort) are required.

For dark beers and worts, the MEBAK method makes provision for the measurement of a sample blank value, in addition to the usual reagent blank value in order to take into account the intrinsic colouration of the sample. The measurement process and the concentration calculation for dark beers and worts are stored in the DR6000 as separate programmes. In the DR6000, the programmes for the measurement of free amino nitrogen are available both for the measurement in accordance with MEBAK and for the measurement in accordance with ASBC.

FAN, light beer	Programme 2008	0-400 mg/L FAN
FAN, light wort	Programme 2007	0-400 mg/L FAN
FAN, dark beer	Programme 2016	0-400 mg/L FAN
FAN, dark wort	Programme 2015	0-400 mg/L FAN
ASBC FAN, beer*	Programme 2024	0-400 mg/L FAN
ASBC FAN, wort*	Programme 2025	0-400 mg/L FAN

TOTAL POLYPHENOLS

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 223 ff. ASBC Beer-35

Phenolic compounds from malt and hops come into the beer in differing quantities dependent on production techniques. Dependening on the structure and molecule sye, they have a strong influence on various beer characteristics such as colour, taste, taste stability, foam and chemical-physical stability¹. Polyphenols also have an especially large impact on the final appearance of the beer. High polyphenols levels lead to a hazy beer.

The methods in accordance with MEBAK and ASBC are identical. The polyphenols in the samples react with iron(III) ions in alkaline solution forming coloured iron complexes. Their absorbance is measured spectrophotometrically in a 10 mm cuvette at a wavelength of 600 nm.

The calculation is performed as follows: Absorbance at 600 nm × 820 = mg/L total polyphenols

The calculation is performed as follows: Absorbance at 600 nm × 820 = mg/L total polyphenols Standard values in beer are 150–200 mg/L total polyphenols. The measurement range of the saved programmes reaches up to 800 mg/L.

In the DR6000 the programmes for the measurement of total polyphenols are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Total polyphenols	Programme 2006	0-800 mg/L phenols
ASBC total polyphenols*	Programme 2020	0-800 mg/L polyphenols

VICINAL DIKETONES

MEBAK brew-technical analysis methods, 4th Edition, 2002, page 134 ff. ASBC Beer-25 B

The current MEBAK^[1] describes the gas chromatographic measurement of diacetyl and 2,3-pentanedione.

In the older MEBAK^[3] and in the ASBC two different photometric methods for the determination of the vicinal diketones are provided.

During yeast metabolism, 2-acetolactate and 2-acetohydroxybutyrate emerge in the course of fermentation. These are converted through oxidation into the vicinal diketones diacetyl and 2,3-pentanedione.

However, diacetyl can also occur as a characteristic metabolic product of certain micro-organisms¹. With too high a vicinal diketone content the beer obtains an offflavour. This often causes a butterscotch flavour, or oily mouth feel, which is unpleasant for the consumer.

Following the MEBAK method, the two diketones diacetyl and 2,3-pentanedione react with 1,2-phenylenediamine to form a coloured end product, whose absorbance is measured in a 2 cm quartz cuvette at 335 nm. This frequently used method for operational analytics is clearly faster than the gas chromatographic method, but allows no differentiation between diacetyl and 2,3-pentanedione.

Using the calibration performed by MEBAK, the content of vicinal diketones is calculated as follows:

Absorbance at 335 nm × 1.2 = mg/kg VDK (vicinal diketones)

The target value for light beer is less than 0.15 mg/kg.

The method in accordance with ASBC is described in the method Beer-25 B under the title "Diacetyl – Broad spectrum method for VDK". This method also does not record the diacetyl separately, but rather all present vicinal diketones.

Following ASBC Beer-25 B, diacetyl (and 2,3-pentanedione) reacts with a naphthol solution forming a colour complex, which is measured at a wavelength of 530 nm. The method

was calibrated by Hach with diacetyl standard solutions, and the corresponding factor stored in the programmeming. A user measurement of diacetyl standards for the recording of a calibration curve can then be omitted.

Using the calibration performed by Hach, the content of vicinal diketones is calculated as follows:

Absorbance at 530 nm \times 3.7 = mg/L diacetyl (vicinal diketones).

In the DR6000, the programmes for the measurement of vicinal diketones are available both for the measurement in accordance with MEBAK and also for the measurement in accordance with ASBC.

Vicinal diketones	Programme 2014	0–1 mg/kg VDK
ASBC diacetyl*	Programme 2023	0–1 mg/L diacetyl

Note:

Just as for the determination of the bitters, there is also a pre-manufactured cuvette test from Hach under the number LCK242 (only available in Europe) or TNT819 (available in U.S.) for the determination of vicinal diketones.

Reducibility MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 204 ff.

The reducibility of the beer is a key issue for taste and the biological, chemical, and physical stability of beer. Reducing compounds arising from the malt and the hops prevent and/ or minimise oxidative processes in the beer. All fast-reducing compounds present in the beer are summarised as reducibility. They are measured by their reducing effect on the Tillmann's reagent (DPI). The decolouration of this reagent in the presence of the beer sample is measured at a wavelength of 520 nm, and compared with the original colouration of the reagent. The reducibility is expressed in a dimensionless number. It indicates what percent of the reagent is reduced by the beer sample.

In the evaluation of the reducibility of beers, the following scale applies in accordance with MEBAK1:

60	Very good
50-60	Good
45-50	Satisfactory
< 45	Poor

In the DR6000, the programme for the measurement of the reducibility is available for the measurement in accordance with MEBAK.

Reducibility	Programme 2004	0–100

THIOBARBITURIC ACID NUMBER (TAN)

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 55 ff.

The thiobarbituric acid number is a summary characteristic. It indicates the thermal load of malt and wort. Alongside 5-hydroxymethylfurfural (HMF), a large number of substances that arise from the Maillard reaction (heat promoted reaction of sugars and amino acids) react with thiobarbituric acid.

In the MEBAK test, the substances to be measured react with thiobarbituric acid and form a yellow colour complex that is photometrically analysed at a wavelength of 448 nm.

The standard values in the brewing process are (in relation to 12% original wort):

Light kettle full wort:	< 22
Light cast wort:	< 45
Light cold wort after wort cooling:	< 60

A new approach to this analysis uses a test called TBARS (thiobarbituric acid reactive substance), which essentially records malondialdehyde. Here too, the extent of the thermal load of the wort through the effect of heat is recorded by the measurement.

In the DR6000, the programme for the measurement of TAN is available for measurement in accordance with MEBAK.

TAN in beer/wort	Programme 2011	0-100 TAN (diluted 1/10)
TAN in congress wort	Programme 2012	0–100 TAN (diluted 1/5)

ANTHOCYANOGENS

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 226 ff.

Anthocyanogens, or also leucoanthocyanidins, are a special form of anthocyanidins. Anthocyanidins are the colour-giving part of anthocyanins, a group of plant colourants with a phenolic basis. The anthocyanogens (leucoanthocyanidins from the hops) are converted by hot hydrochloric acid into the redcoloured anthocyanidins.

In the measurement the anthocyanogens are first adsorbed onto polyamide and then converted by hot hydrochloric acid into a red solution. The measurement is made at a wavelength of 550 nm in a 10 mm cuvette.

The standard values in accordance with MEBAK in the beer are 50–70 mg/L, dependent on production techniques. When the stabilised. with PVPP the standard values are correspondingly lower.

In the DR6000, the programme for the measurement of the anthocyanogens is available for the measurement in accordance with MEBAK.

AnthocyanogensProgramme 20050-100 mg/L ATC

PHOTOMETRIC IODINE SAMPLE

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 52 ff.

After malt has been produced from grains, mostly barley, the malt is ground. The actual brewing process begins with



mashing. In this process, water is heated to approximately 60 °C, then the ground malt is added and the resulting mash is heated under constant stirring to approximately 75 °C, dependent on the process. With different roasting temperatures, enzymes convert the starch from the malt into malt sugar. Alternatively, parts of the mash are boiled, which leads to a physical gelatinisation of the starch. An iodine sample it is then measured to determine whether the dissolved starch is completely saccharified.

Dextrins and starch from worts or beer are precipitated, dissolved in phosphate buffer, and mixed with iodine solution. The red-to-blue colouration is measured in the spectrophotometer at a wavelength of 578 nm in a 4 cm cuvette. The standard values (in wort) per MEBAK are < 0.45.

In the DR6000, the programme for the measurement of the anthocyanogens is available for the measurement in accordance with MEBAK.

lodine sample	Programme 2010
	-

IRON

MEBAK, Wort, Beer, Beer-Based Beverages, 1st Edition 2012, page 423 ff.

Iron can enter the beer through the raw materials as well as through filter agents and/or fining agents. It can also be picked up from apparatus, lines, or cans, or be contained in beer foam

0–1 iodine value

stabilising agent. Iron negatively effects the colloidal stability, taste, foam, and gushing tendency of the beer.

Alongside AAS, iron in beer can also be determined spectrophotometrically. Trivalent iron is first reduced to bivalent iron. The bivalent iron reacts with FerroZine to form a violetcoloured complex. The method stored in the DR6000 for iron determination already contains the absorbance coefficient for iron. The increase of the calibration curve is 0.037/µg/L Fe²⁺. Thus, the user of this programme is not required to generate a proprietary iron standard series for the calibration. The reference values in beer are 0.200 mg/L.

In the DR6000, the programme for the measurement of iron in accordance with MEBAK is available.

Iron Programme 2017 0–1 mg/L iron

References

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Analysis of additives in caffeinated energy drinks by HPLC

Caffeinated energy drinks contain a wide range of additives which may include vitamins, preservatives and sweeteners. Food and beverage manufacturers are required to clearly label products with any additives that are used in their product ranges. Therefore, there is a requirement for robust analytical methods for the determination of various ingredients for quality control and to substantiate label claims.

This article discusses the development of a method for the analysis of a range of commonly occurring ingredients in energy drinks that could be used for the routine analysis of the finished drinks product in a quality control environment. Due to the potential complexity of samples and the range in hydrophobicity of the sample components, a highresolution method capable of retaining polar and more hydrophobic analytes is required.

Initially a standard test mix containing the analytes of interest was screened using a generic 5-95% B reversedphase gradient on six Avantor® ACE® novel selectivity phases (**vwr.com/ace**) using a VWR® Hitachi Chromaster HPLC system. Several of the ACE® novel phases provided good retention and selectivity for this set of compounds, with the Avantor® ACE® C18-Amide providing the most promising result. The method was then optimised to provide the separation shown in Figure 1.



Figure 1: Separation of analyte standards commonly found in energy drinks using the Avantor® ACE® C18-Amide.



The method was then applied to the analysis of two commercially available energy drinks (Figure 2) and was proved to be suitable for detection of all eight analytes without interference from other sample peaks. Energy drink samples were decarbonated and filtered, prior to direct injection onto the HPLC system. Caffeine was found to be present in the largest quantity in both energy drinks. The standard amount of caffeine found in energy drinks is 80 – 150 mg per serving, with anything containing over 150 mg/L needing to be stated as having a "high caffeine content" and being unsuitable for consumption by children and pregnant women. The two drinks analysed in this study contain approximately 300 mg and 120 mg/L of caffeine respectively. The average daily caffeine consumption for adults is recommended not to exceed 400 mg on a regular basis.

Both energy drinks analysed also contained two of the most common artificial sweeteners; aspartame and acesulfame K. These sweeteners are approximately 200x sweeter than natural sugars and are added to soft drinks to induce a sweet taste, without the associated calories of natural sugars such as sucrose. Sweeteners also do not increase blood sugar levels after consumption and so are suitable for people with diabetes. The first energy drink analysed contained two B vitamins (B5 and B6) whereas the second soft drink contained only vitamin C (ascorbic acid). All vitamins are present in very small amounts. Citric acid, benzoate and sorbate were also detected and are added to energy drinks as preservatives to increase the product shelf-life.

CONCLUSION

This short article presents a reversed-phase method for the analysis of analytes commonly found in energy drinks. The developed method was applied to two commercially available energy drinks and successfully identified the analytes, where present, with no co-elution with other sample components observed. This method is suitable for the routine analysis of energy drinks for quality control purposes and could easily be scaled to UHPLC to provide a high-throughput solution for large sample numbers or if rapid turnaround is required.



Figure 2: Analysis of two commercially available energy drinks using

the method conditions as stated in Figure 1. Analytes: 1. Pyridoxine (B6),

^{2.} Citric acid, 3. Pantothenic acid (B5), 4. Acesulfame K, 5. Caffeine,

^{6.} Aspartame, 7. Sorbate, 8. Benzoate.



Analysis of sugars using the Avantor[®] ACE[®] NH₂ and VWR[®] Hitachi Chromaster

Carbohydrates are one of the most important components of foodstuffs and beverages that contribute to taste and nutritional value. The determination of carbohydrates in beverages is important for nutritional information, quality control and can be useful in the detection of adulteration. The carbohydrate component of drinks is typically comprised of simple sugars including mono- and di-saccharides, which are readily analysed in HILIC mode using an amino bonded stationary phase. The Avantor® ACE® NH₂ phase is an ultra-inert phase, bonded with a proprietary amino propyl ligand, which is ideal for the analysis of sugars. The high silica purity and proprietary bonding technology provides high robustness with excellent peak shape and column lifetime.

Figure 1 shows example separations of both monosaccharides (a) and disaccharides (b), the structures of the sugars analysed are presented in Figure 2. Full separation of both standard mixes was readily achieved on the Avantor® ACE® NH₂ with excellent resolution of the sugar components obtained. One of the challenges associated with sugar analysis is the lack of a UV chromophore, thereby eliminating the use of the ubiquitous UV detector. In this application, detection is readily achieved using a VWR® Hitachi Chromaster HPLC system equipped with a 5450 Refractive Index (RI) detector. The design of the detector provides a stable signal and minimal baseline drift which is often associated with this type of detector.



Figure 1: Separation of a) monosaccharides and b) disaccharides on the Avantor® ACE® NH2.



Figure 2: Structures of mono- and di-saccharides chromatographed in Figure 1.

Figure 3 shows the application of the ACE NH₂ and VWR® Hitachi Chromaster HPLC system for the analysis of sugars in three soft drinks; orange juice, cola and diet cola. Fructose, glucose and sucrose are found naturally in fruit juices and are also commonly added to sweetened soft drinks. The cola and orange juice samples were shown to contain sucrose as the main sugar component, along with its constituent monosaccharides glucose and fructose. These analytes were then demonstrated to be absent in the diet cola sample. This simple method is useful for routine screening of food and beverage samples for carbohydrate components and may also be used as a good starting point for the determination of other mono- and di-saccharides.



Figure 3: Separation of sugars in different soft drinks on the ACE 3 NH2.



Get more secure and correct analysis of food and beverage samples



By analysing pH, conductivity, oxygen, and turbidity, manufacturers can monitor and optimise the production processes, ensure product consistency and maintain quality and safety standards.

Digital sensor systems can reduce or often even avoid failures during this analysis. The analysis is much better documented than with traditional systems. Failures like not or wrong calibrated equipment can practically not happen. Digital systems (IDS) are also far better supporting GLP demands. More details below.

pH measurement is crucial in food and beverage analysis as it affects the taste, texture, microbial safety and shelf life of products. pH levels can indicate the presence of spoilage microorganisms, fermentation processes, or chemicals.



 Reactions that may affect product quality. For example, low pH levels in acidic pH also tells a lot about the quality and freshness of meat and cheese. The quality of meat, cheese or other semisolid food can be determined by using a spear type pH electrode like SenTix® Sp-T 900. The pH of juice and other beverages like milk can be perfectly measured by using a SenTix® 980 with the platinum diaphragm. There are more types of pH sensors eg., for surfaces or micro for small volumes.



2. Conductivity measurement provides information about the presence of dissolved ions in a sample. In food and beverage analysis, it helps to assess the content of salts, minerals, or contaminants that may impact product quality and safety. Conductivity is often employed to monitor the cleanliness of processing equipment and the degree of water hardness, which can affect the taste and texture of final products. The salt concentration in sauces, ketchup or mustard can be measured accurate with a TetraCon® 925/LV. The fork design makes cleaning easy; the four-electrode technology is insensitive against pollution.



3. Oxygen plays a critical role in food quality and shelf life. Oxygen can cause oxidative reactions, leading to the deterioration of flavours, colours, and nutritional integrity. Monitoring oxygen levels is particularly important in perishable foods prone to oxidation, such as oils, fats and beverages like beer and wine. The FDO® 925 extends below 1 mg/l the resolution up to three digits. This allows a trend display also in the very low range down to extreme low concentrations. Analysing oxygen content helps ensure product stability and extend shelf life by implementing proper packaging and storage conditions.



4. Turbidity refers to the cloudiness or clarity of a liquid caused by suspended particles. In food and beverages, turbidity analysis helps assess the quality and cleanliness of products. It can indicate the presence of unwanted particles like sediments, microbial contaminants, or other impurities. Turbidity measurement is crucial in industries such as brewing, where clarity is an essential quality attribute. In juice and other drinks, it is sometimes desirable to have an idea about pulp. With a turbidity sensor VisoTurb[®] 900-P it is easy to measure the turbidity and draw conclusions on the pulp content.



a xylem brand



IDS, the digital system, is perfectly suited for supporting the quality in food and beverage analysis. IDS means:

Intelligent: IDS sensors are smart. They log on automatically, transfer description, serial number, calibration record and calibration history as well as their complete parameters.

Digital: IDS sensors process the sensitive signals into digital signals and transfer them fail-safe and error-free to the meter. There is no difference when using cable or wireless modules.

Sensor: IDS sensors are based on proven and continuously enhanced WTW[®] sensors. They cover almost any application in pH, conductivity, dissolved oxygen and turbidity measurement.

The IDS system is based on two components: digital sensors and corresponding field and benchtop meters. The outstanding innovation: The measurements are processed in the sensor, not in the meter: One meter for all parameters: pH – conductivity -dissolved oxygen – turbidity. All measured results are saved together with meter and sensor data for easy GLP supporting assignment and tracking, also a user administration is possible.

In conventional systems there are meters with certain sockets for defined parameters, like a pH meter with a socket for a pH sensor, another meter with a different socket to connect eg., a conductivity cell and so on. Digital (IDS) removes all these restrictions and at the same time strongly improves the documentation quality. The user can connect any type of IDS sensor to the digital meter, with cable or even wireless, using the same meter socket. They can change not only simply between different pH sensors without new calibration. They can also change to any available parameter. There is no manual setting of parameters or calibration required at all.

All this makes the digital system IDS clearly to the best choice, not only for Food & Beverage application.

For more information visit our page 'Digital Intelligent Measurement for more Accuracy and Flexibility'



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AUSTRIA

VWR International GmbH Graumanngasse 7 1150 Wien Tel.: +431970020 info.at@vwr.com

BELGIUM

VWR International bv Researchpark Haasrode 2020 Geldenaaksebaan 464 3001 Leuven Tel.: +32 (0) 16 385 011 vwr.be@vwr.com

CZECH REPUBLIC

VWR International s. r. o. Veetee Business Park Pražská 442 C7 - 281 67 Stříbrná Skalice Tel.: +420 321 570 321 info.cz@vwr.com

DENMARK

VWR International A/S Tobaksveien 21 2860 Søbora Tel.: +45 43 86 87 88 info.dk@vwr.com

FINI AND

VWR International Oy Valimotie 17-19 00380 Helsinki Tel.: +358 (0) 9 80 45 51 info.fi@vwr.com

FRANCE

VWR International SAS Immeuble Estréo 1-3 rue d'Aurion 93114 Rosny-sous-Bois cedex Tel.: 0 825 02 30 30* (national) Tel.: +33 (0) 1 45 14 85 00 (international) info.fr@vwr.com * 0,18 € TTC/min + prix appel

GERMANY

VWR International GmbH Hilpertstraße 20a D - 64295 Darmstadt Tel.: 0800 702 00 07* (national) Tel.: +49 (0) 6151 3972 0 (international) info.de@vwr.com *Freecall

HUNGARY

VWR International Kft Simon László u 4 4034 Debrecen Tel.: +36 52 521130 info.hu@vwr.com

IRELAND

VWR International Ltd Orion Business Campus Northwest Business Park Ballycoolin Dublin 15 Tel.: +353 (0) 1 88 22 222 sales.ie@vwr.com

ΙΤΔΙ Υ

VWR International S.r.l. Via San Giusto 85 20153 Milano (MI) Tel.: +39 02 3320311 info.it@vwr.com

THE NETHERLANDS

VWR International B.V. Postbus 8198 1005 AD Amsterdam Tel.: +31 (0) 20 4808 400 info.nl@vwr.com

NORWAY

VWR International AS Brynsalleen 4, . 0667 Oslo Tel : +47 22 90 00 00 info.no@vwr.com

POLAND

VWR International Sp. z o.o. Limbowa 5 80-175 Gdansk Tel.: +48 58 32 38 200 info.pl@vwr.com

PORTUGAL

VWR International -Mat. de Laboratório, Soc. Unipessoal, I da Edifício Ramazzotti Avenida do Forte 6, P-1.09 e P-1.10 2790-072 Carnaxide Tel.: +351 21 3600 770 Info.pt@vwr.com

SPAIN

VWR International Eurolab S.L.U. C/Tecnología 5-17 A-7 Llinars Park 08450 - Llinars del Vallès Barcelona Tel.: +34 902 222 897 info.es@vwr.com

SWEDEN

VWR International AB Esbogatan 16 164 74 Kista Tel.: +46 08 621 34 20 info.se@vwr.com

SWITZERLAND

VWR International GmbH Lerzenstrasse 16/18 8953 Dietikon Tel.: +41 (0) 44 745 13 13 info.ch@vwr.com

UK

VWR International Ltd Customer Service Centre Hunter Boulevard - Magna Park Lutterworth Leicestershire I F17 4XN Tel.: +44 (0) 800 22 33 44 uksales@vwr.com

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CHINA

VWR (Shanghai) Co., Ltd Bld.No.1, No.3728 Jinke Rd, Pudong New District Shanghai, 201203- China Tel.: 400 821 8006 info_ching@vwr.com

INDIA

Avantor Performance Materials India Private Limited 19th Floor, Building No. 5, Tower C, DLF Cyber City, Phase- III, Gurugram - 122002, Haryana, India Tel.: +91-124-4656700 csindia@avantorsciences.com

KOREA

Avantor Performance Materials Korea 1 td 2F ACE Gwanggyo Tower I, Daehak 4ro 17 Yeonatona-au Suwon, Korea 16226 Tel: +82 31 645 7256 saleskorea@avantorsciences.com

MIDDLE EAST & AFRICA

VWR International FZ-LLC Office 203, DSP Lab Complex, Dubai Science Park, Dubai, United Arab Emirates Tel: +9714 5573271 Info.mea@vwr.com

SINGAPORE

VWR Singapore Pte Ltd The Metropolis Tower 1, #05-03 9 North Buona Vista Drive Singapore 138588 Tel: +65 6505 0760 sales.sg@vwr.com

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