

Analytix

Issue 1 • 2008



New chromogenic media



- Campylobacter Diagnostic
- Ion Pair Reagents
- IC: Validation Kit and *TraceCERT*[®] Standards
- Alkaline Digestion with high purity TMAH
- Titration: **HYDRANAL**[®]-E-types and mineral oil standards

Microbiology



Jvo Siegrist
Microbiology Product Manager

Dear Colleague,

As I was contemplating this editorial, I started thinking about the times at social gatherings when I have been asked the inevitable question, "And what do you do?" Even though the fields of analytical and bioanalytical chemistry have been glamorized on television crime dramas so popular today, in reality it is difficult to convey to a non-scientist the satisfaction of what we do. Whether it is by helping pharmaceutical companies develop new therapeutic drugs, forensic chemists measure doping agents and toxins in body fluids, or environmental engineers monitor pollution remediation programs, scientists impact all facets of modern society.

Nowhere is that impact felt more strongly than when it is applied to detecting pathogenic microbes in our food, livestock, living spaces, institutions and environment. Outbreaks have caused immense human suffering and loss of life, while the proliferation of multiple drug-resistant strains presents a serious, growing concern.

At Sigma-Aldrich, we pay close attention to the needs of analytical microbiologists, and have responded by developing products and methodologies designed to be rapid, sensitive and specific to a wide variety of relevant pathogens. One example is our chromogenic media, which uses the target microbe's unique biochemistry to aid in its selective growth or identification. Our recent additions to this important product line are the subject of the featured article in this issue of Analytix.

In addition to the analytical tools themselves, we have also realized that a fundamental service we can supply is to provide information on how to choose, prepare and use the appropriate analytical microbiology product from among our broad offering of media, ingredients, supplements, reagents, discs, test strips and kits. To this end, we have produced three valuable resources, a CD with a comprehensive listing of our microbiology products, a dedicated website, and a Microbiology Manual.

If you are interested in analytical microbiology, I hope you will take a moment to read the featured article in this issue of Analytix, and visit our website to get your copy of the Microbiology CD and manual. As always, please contact us if we can help you with a difficult analytical challenge. We look forward to hearing from you!

Kind regards,

A handwritten signature in black ink, appearing to read 'Jvo Siegrist', written in a cursive style.

Jvo Siegrist
Microbiology Product Manager

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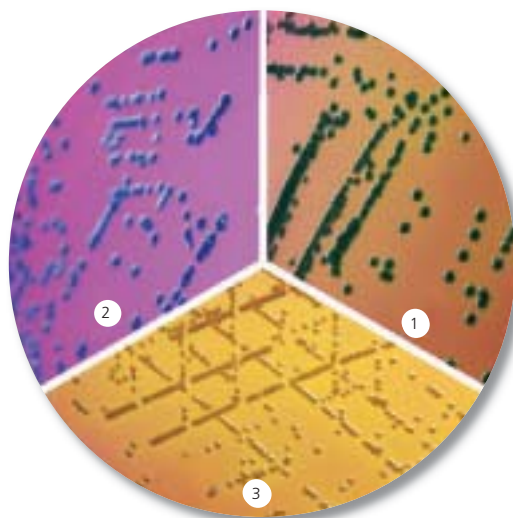
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- Residue analysis**
New Fluka analytical standards

New chromogenic media

Chromogenic differentiation media for bacteria and yeasts

Figure 1 HiCrome™ *Enterobacter sakazakii* Agar with easy differentiation of *Enterobacter sakazakii* and other Enterobacteriaceae



1. *E. aerogenes*
2. *E. sakazakii*
3. *K. pneumoniae*

Figure 2 HiCrome™ Enrichment Broth Base for EC O157:H7



1. Control
2. *E. coli* O157:H7
3. *E. coli*
4. *Ent. sakazakii*
5. *K. pneumoniae*

Jvo Siegrist, Product Manager, Microbiology ivo.siegrist@sial.com

Chromogenic growth media are important tools used by microbiologists to aid in the identification of certain bacteria, yeasts and fungi. These microbes may be pathogenic and monitored for food safety and clinical applications, or beneficial and monitored for the quality assurance of foods and beverages that rely on fermentation or other food culture processes. The principle behind chromogenic media is the use of a characteristic enzyme to identify the presence of the microbe of interest. Chromogenic media contain enzyme substrates linked to a chromogen that is cleaved by the target enzyme. If the enzyme, and hence the microbe, is present, a visually observed color change occurs.

Chromogenic media are very popular primarily because results take less time compared to other detection methods; some media can give confirmed results within 24 hours. They are also popular because the results are visual, easy to interpret and, in most cases, no further confirmatory testing is required.

Sigma-Aldrich has long supplied a broad range of chromogenic media through our Fluka brand. We have recently added new agar-based chromogenic media for detection of many important pathogens (see **Table** on next page). For microbial food analysis, we have new media for detection and differentiation of *Bacillus cereus*, *E. coli*, *Enterobacter sakazakii* (**Figure 1**), *Enterococcus faecium*, *Klebsiella sp.*, *Salmonella sp.* and *Vibrio* species. *Enterobacter sakazakii* has made the news recently as the causative agent behind the deaths of some newborns. We also offer chromogenic media to detect the presence of *E. coli*, *Enterococcus faecium*, *Klebsiella*, *Salmonella sp.* and *Vibrio sp.* in water samples. For clinical applications, we now have special chromogenic media for MRSA and *Candida* detection and differentiation.

To see our complete range of chromogenic media, please visit our website: www.sigma-aldrich.com/chromogenic_media.

Table 1 New additional chromogenic media**HiCrome™ chromogenic media from Sigma-Aldrich**

Organism	Name	Brand	Cat. no.	Description
<i>Bacillus cereus</i>	HiCrome™ Bacillus Agar	Fluka	92325	A differential medium recommended for rapid identification of <i>Bacillus</i> species from a mixed culture by chromogenic method.
<i>Candida</i> sp.	Candida Ident Agar, modified	Fluka	94382	For the selective isolation and identification of <i>Candida</i> species from clinical material like stool, urine, skin scurf and swabs.
<i>E. coli</i> O157:H7	HiCrome™ Enrichment Broth Base for EC O157: H7	Fluka	80330	Recommended for isolation and selective differentiation of <i>E. coli</i> O157:H7 from food and environmental samples by chromogenic method.
<i>E. coli</i> (thermotolerant)	HiCrome™ M-TEC Agar	Fluka	90924	Recommended by the U.S. Environmental Protection Agency (USEPA) for differentiation and enumeration of thermotolerant <i>E. coli</i> from water by membrane filtration technique.
<i>Enterobacter sakazakii</i>	HiCrome™ Enterobacter sakazakii Agar, modified	Fluka	14703	Recommended by ISO Committee for the isolation and identification of <i>Enterobacter sakazakii</i> from milk and milk products.
<i>Enterobacter sakazakii</i>	HiCrome™ Enterobacter sakazakii Agar	Fluka	92324	Recommended for isolation and identification of <i>Enterobacter sakazakii</i> from food, water, sewage, urine and feces samples.
<i>Enterococcus faecium</i>	HiCrome™ Enterococcus faecium Agar Base	Fluka	90919	Recommended for chromogenic identification of <i>Enterococcus faecium</i> from feces, sewage and water supplies.
<i>Klebsiella</i> sp.	HiCrome™ Klebsiella Selective Agar Base	Fluka	90925	For selective isolation and easy detection of <i>Klebsiella</i> species from water and other sources. This medium can also be used in membrane filtration procedure.
<i>Salmonella</i> sp.	HiCrome™ RajHans Medium, Modified	Fluka	90918	Recommended for identification and differentiation of <i>Salmonella</i> species from the members of Enterobacteriaceae, especially <i>Proteus</i> species.
<i>Salmonella</i> sp.	Salmonella Chromogen Agar Set	Fluka	01993	A differential diagnostic agar for the detection of <i>Salmonella</i> in food and clinical material.
<i>Staphylococcus aureus</i> , methicillin resistant (MRSA)	HiCrome™ MeReSa Agar Base	Fluka	90923	Recommended for the isolation and selective identification of Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA) from clinical isolates.
<i>Vibrio</i> sp.	HiCrome™ Vibrio Agar	Fluka	92323	Recommended for isolation and selective chromogenic differentiation of <i>Vibrio</i> species from seafood.

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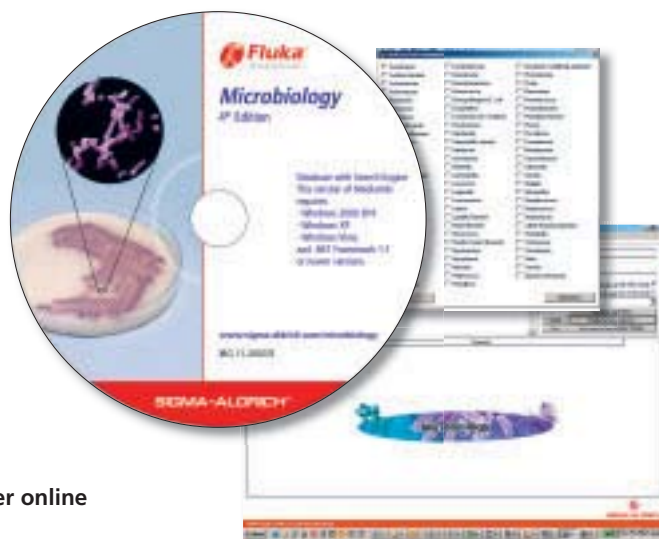
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www.sigma-aldrich.com/microbiology_cd



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TraceCERT® – traceable certified reference materials

New certified standards for ion chromatography

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Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

Ion chromatography (IC) is an analytical technique that is used to separate and quantify ppm-levels of common anions (e.g. fluoride, chloride, nitrite, nitrate and sulfate) and cations (e.g. ammonium, alkali and earth alkali ions) in aqueous samples. Separation occurs via differential interaction with an ion-exchange resin. Conductivity is the most common detection method. The sensitivity of IC makes it ideal for low-ppm level quantification and also for trace analysis. Subsequently, IC requires high-purity eluents and well-defined standards. As a leading supplier of high-quality products for all areas of analytical chemistry, Sigma-Aldrich has expertise in production of standards, eluents and high-purity water suitable for sensitive ion analysis.

Figure 1 First page of a certificate of a TraceCERT® iodide standard

Concentration	Certified value at 20°C ^{*)}	Expanded uncertainty (k=2)
Iodide	1000 mg/L [†]	4 mg/L [†]
	1001 mg/kg [†]	4 mg/kg [†]

1. SCOPE OF CERTIFICATION AND TRACEABILITY STATEMENT

To guarantee the reliability of the values for the TraceCERT® certified reference material three independent procedures were followed. The value here is given in the range of that parameter, and the equally certified value for the parameter conversion has been stated (quantified value).

1. Gravimetric preparation using pure materials in a precisely measured volume of deionized water through conversion of mass to amount of substance (n). In the course of the reaction is demonstrated and a concentration and mass of iodide is exactly determined this approach allows highest accuracy and small uncertainties. The certified value of TraceCERT® reference material is based on this approach and directly traceable to the SI unit kilogram. Therefore commensurately characterized materials of high purity are used. All reference are certified in SI and additional with OIML Class E2 top or 11 top (NIST) (up to 99.99 weight).
2. The starting material is measured against a certified reference material (e.g. NIST 1542 or 1549) followed by gravimetric preparation using deionized distilled water of 10 traceable weight. Consequently the value calculated by the unitless (ratio of components) attributed to the reference to which the starting material is compared.
3. Reference against the certified TraceCERT® iodide is compared to a second reference (e.g. from NIST) (ratio of 1000) which is independent from the reference.

2. Purity of Starting Materials

The high purity material of 99.99% by mass (approximate) of pure substance is compared to 100.00% by mass (approximate) of the pure substance. The purity is given in the range of that parameter, and the equally certified value for the parameter conversion has been stated (quantified value).

Designed to save IC analysts time and ensure the most sensitive and reproducible IC determinations, our line of TraceCERT® standards for ion chromatography is a convenient time- and cost-saving option. These certified reference materials, listed in the Table on page 7, are produced in a doubly accredited laboratory, fulfilling ISO/IEC 17025 and ISO Guide 34.*

The certified value of the reference material is directly traceable to the SI unit kilogram and also measured against a certified reference material from the National Institute of Standards and Technology (NIST). All details regarding exact content, uncertainty, traceability and expiry date for the materials are described in a comprehensive certificate, which is available electronically from our web page, www.sigma-aldrich.com, by entering the product number and corresponding lot number. Additionally, we supply a number of trace ion solutions measured by IC or ICP-OES. An IC chromatogram showing anion traces in an IC iodide standard (1000 mg/L) is shown in Figure 1.

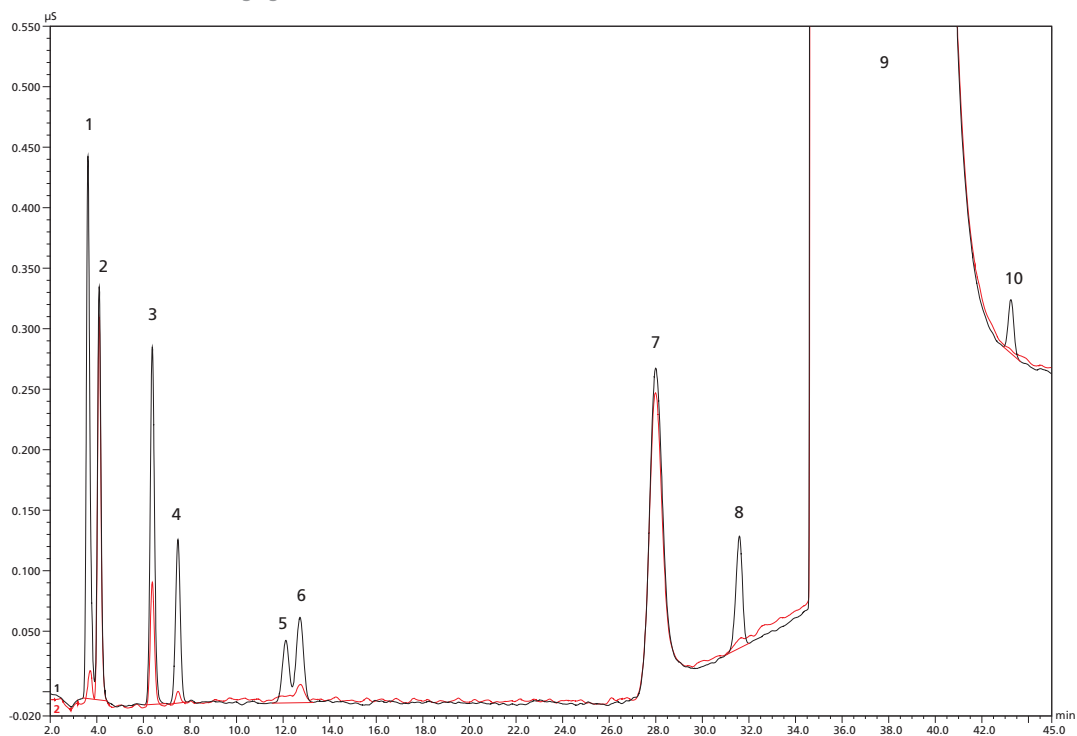
To complement your demanding IC analyses, we also offer a comprehensive line of certified eluent concentrates, high-purity water especially developed for IC analysis, certified multielement standard (PRIMUS) and a new test kit for IQ, OQ or PQ activities.

To see our complete line of TraceCERT® products, please visit our website: www.sigma-aldrich.com/tracecert



* ISO/IEC 17025: General Requirements for the Competence of Testing and Calibration Laboratories. ISO Guide 34: General Requirements for the Competence of Reference Material Producers.

Figure 2 Anion chromatogram of a *TraceCERT*[®] iodide standard (red line, diluted to 200 mg kg⁻¹)



The black line shows the sample with an addition of anion mixture (10 µg kg⁻¹ each).

The peaks are:

- 1] fluoride
- 2] unknown
- 3] chloride
- 4] nitrite
- 5] bromide
- 6] nitrate
- 7] system peak from carbonat eluent
- 8] sulfate
- 9] iodide
- 10] phosphate

Table *TraceCERT*[®] standards for ion chromatography

Cat. no.	Brand	Description	Package size
59755	Fluka	Ammonium Standard for IC, <i>TraceCERT</i> [®]	100 mL
43147	Fluka	Bromide Standard for IC, <i>TraceCERT</i> [®]	100 mL
39883	Fluka	Chloride Standard for IC, <i>TraceCERT</i> [®]	100 mL
40121	Fluka	Chromate Standard for IC, <i>TraceCERT</i> [®]	100 mL
77365	Fluka	Fluoride Standard for IC, <i>TraceCERT</i> [®]	100 mL
41271	Fluka	Iodide Standard for IC, <i>TraceCERT</i> [®]	100 mL
74246	Fluka	Nitrate Standard for IC, <i>TraceCERT</i> [®]	100 mL
67276	Fluka	Nitrite Standard for IC, <i>TraceCERT</i> [®]	100 mL
38364	Fluka	Phosphate Standard for IC, <i>TraceCERT</i> [®]	100 mL
90071	Fluka	Sulfate Standard for IC, <i>TraceCERT</i> [®]	100 mL

Quality management for ion chromatography systems

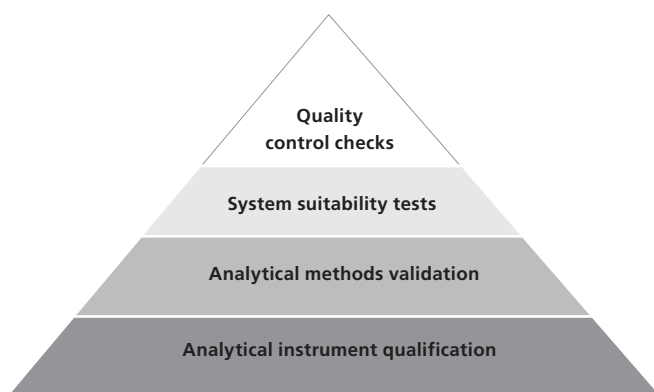
Traceable and verified standards for IQ, OQ and PQ activities

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Markus Läubli, Manager Marketing Support IC, Metrohm AG, Switzerland lae@metrohm.com

Validation and qualification processes

Today, analysts spend a great deal of time and effort ensuring that their analytical data is reliable and in compliance with specific regulations. For laboratories working in regulated environments (e.g. GMP), there are several standards that deal with the validation and qualification of processes and computer-assisted instruments. The most important guidelines are the EU-GMP Guide, DIN ISO 9001, GLP, ISO/IEC 17025, USP General Chapters <1058>, GAMP Good Practices Guide on Validation of Laboratory Computerized Systems and FDA 21CFR Part 11.

Figure 1 The main components for good data quality [1]



The quality of analytical data can be demonstrated by different validation and qualification tools, such as in-process quality control checks, system suitability tests and the use of validated methods. However, before the method can be validated, it is necessary to ensure that the analytical system itself is qualified (see **Figure 1**). In this context it is widely agreed that processes are “validated” and instruments are “qualified.”

Analytical Instrument Qualification (AIQ)

According to the draft of United States Pharmacopeia (USP) General Chapter <1058> “Analytical Instrument Qualification,” instruments are qualified in a stepwise process following four phases: design qualification, installation qualification, operational qualification and performance qualification [2]. This is graphically depicted in **Figure 2**.

Design Qualification (DQ): The AIQ process timeline begins with the DQ phase at the vendor’s site where the instrument is developed, designed and produced in a validated environment (e.g. ISO 9001). The user usually does not need to repeat DQ. However, users should ensure that instruments are suitable for their intended applications and that the manufacturer has adopted a quality system for developing, manufacturing and testing. Users should also establish that the manufacturer provides installation support, service and training.

Installation Qualification (IQ): The following activities and documentation are associated with IQ to guarantee completeness and operability of the new installed system: providing detailed descriptions of the analytical system, ensuring that all components have arrived as purchased and are undamaged, verifying that the installation site meets the requirements, assembling and installing the system, and performing diagnostics and testing after installation.

Operational Qualification (OQ): OQ is performed after initial system installation, after modification of the existing system or repair of a specific module. The instrument is tested according to the manufacturer’s specifications in the user’s environment. Therefore, the manufacturer must define test conditions and specifications. OQ can be executed by testing all individual components or the entire system.

Performance Qualification (PQ): PQ tests conclude the qualification process of a newly installed instrument and verify that the system has performance in line with its intended use. After the successful completion of PQ, the system can be handed over to the customer.

The user should perform PQ at regular intervals and not just after installation of a new instrument. The tests can be similar to those performed during the OQ phase, but the specifications are usually less restrictive. When PQ tests fail to meet specifications, the instrument requires maintenance or repair. Each laboratory should also have SOPs in place that define the period of use, calibration procedures and proper maintenance.

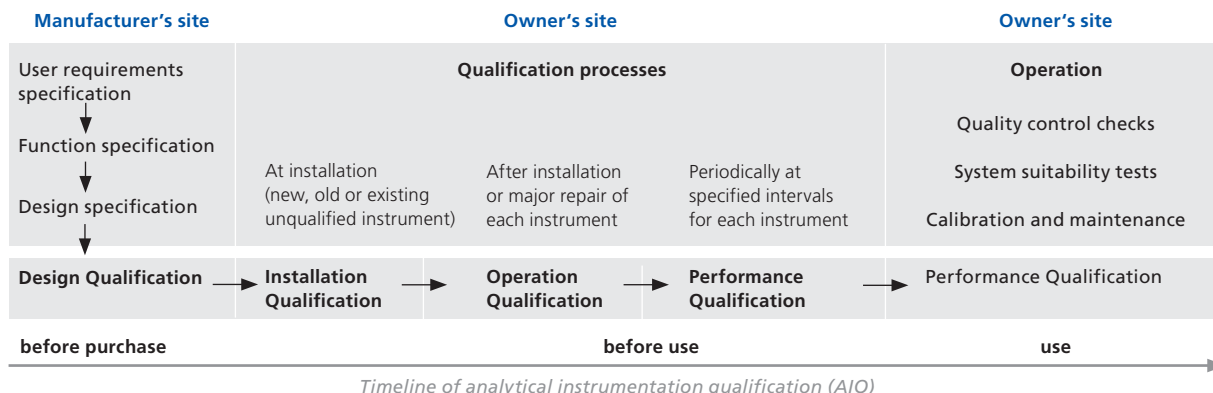
IQ, OQ and PQ for Metrohm ion chromatography instruments

Metrohm AG (Herisau, Switzerland) and Sigma-Aldrich Production GmbH (Buchs, Switzerland) share a fruitful cooperation with a focus on developing customer solutions for ion analysis. While Metrohm’s emphasis is on developing and producing powerful ion chromatography systems, Sigma-Aldrich is a strong partner for the supply of chemical standards, solutions and certified reference materials.

Metrohm has increased its efforts in quality management and documentation following the latest international guidelines. The recent publication “Quality Management with Metrohm – IQ/OQ for Professional IC Systems” provides detailed documentation and guidance [3].

When installing a new Professional IC Instrument, the Metrohm service engineer will perform the following inspections during IQ: completeness of delivery, check of installation site and media supply, documentation of all instruments and detailed tests on hardware and software installations.

Figure 2 Qualification processes during the lifecycle of an analytical instrument



After completion of IQ, the following OQ and PQ tests are carried out: IC pump flow (accuracy and reproducibility), noise and drift as parameters for pump and detector stability, injector precision, injection carry-over, detector linearity, suppression, IC gradient (accuracy, reproducibility and noise of gradient steps) and temperature of the column thermostat.

The parameters described above are only examples of the primary tests; Metrohm performs several other test procedures during IQ, OQ or PQ to ensure the optimum system performance.

Qualify new and existing IC systems with IQ/OQ/PQ Kit from Sigma-Aldrich

Chemicals, including the standards, reagents and solutions, are integral parts of testing procedures to measure the IC system's performance. Sigma-Aldrich has developed a new IQ/OQ/PQ Kit (prod. no. 12674) that is extremely useful for qualifying ion chromatography systems. The kit is intended for testing detection linearity, injector precision and injection

carry-over, and is part of Metrohm's "IQ/OQ Kit for IC" (6.5333.000), which includes all parts for validation of Professional IC Systems.

The kit consists of six certified bromide standards (5, 10, 20, 50, 100 and 1000 mg kg⁻¹). The calculated correlation coefficient for the 5 to 100 mg kg⁻¹ standards is provided. The 50 mg kg⁻¹ standard is used for repeatability tests (injector precision) and the maximum carry-over during injection is verified with the 1000 mg kg⁻¹ standard. The 5 to 100 mg kg⁻¹ standards are used to prove the detector linearity. A special advantage of the kit is that the certified bromide standards can be used with conductivity, UV/Vis and electrochemical detectors.

The kit's certified standards are produced in a double-accredited laboratory, fulfilling ISO/IEC 17025 (general requirements for the competence of testing and calibration laboratories) and ISO Guide 34 (general requirements for the competence of reference material producers). Appropriate documentation is an important quality characteristic. A detailed certificate, showing the traceability to a NIST SRM and SI unit kg and measurement uncertainty of the certified values, is included with each kit.

Figure 3 Professional IC 850 Instrument from Metrohm



Table 1 Product table. IQ/OQ/PQ Kit for IC Systems

Cat. no.	Brand	Description	Package size
12674	Fluka	IQ/OQ/PQ Kit Contains six certified bromide standards (5, 10, 20, 50, 100 and 1000 mg kg ⁻¹) and a certificate	1 kit

References

- 1] AAPS PharmSciTech (2004), 5 (1), article 22.
- 2] Pharmacopeial Forum (2005), 31(5), 1453–63.
- 3] 6.9988.503 Quality Management with Metrohm – IQ/OQ for Professional IC Systems.

Ion pair reagents from Sigma-Aldrich

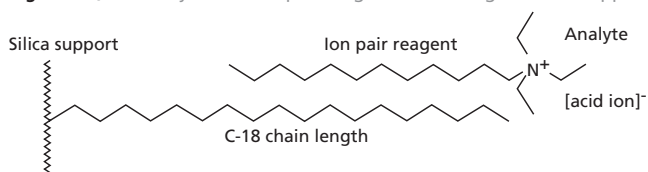
Highest purity for the most demanding IPC methods

Michael Jeitziner, Market Segment Manager, Analytical Reagents & Standards michael.jeitziner@sial.com

Introduction

Ion pair chromatography (IPC) reagents are added to the mobile phase to improve the retention and/or resolution of ionic and other highly polar substances by HPLC (Figure 1). Sigma-Aldrich offers an outstanding range of tailor-made Fluka-brand ion pair reagents: quaternary ammonium and phosphonium salts for anionic analytes and alkanesulfonates for cationic analytes.

Figure 1 Quaternary amine ion pair reagent interacting with C18 support



Advantages of IPC

Although IPC cannot be used in all situations, for example with mass spec detection, it is a well-established and reliable technique that can provide:

- Optimized elution time by selecting the right IP reagent
- Highly reproducible results
- Sharper peak shapes
- Enhanced retention of ionic and other highly polar compounds on C18 columns

There is a wide choice of ion pair additives, mostly differentiated by varying the alkyl chain length, available to improve and optimize the IPC separation.

The importance of IP reagent purity

Every mobile phase component, including the solvents, water, buffers, salts and ion pair reagents, must be stringently controlled so as not to contribute impurities that can reduce the sensitiv-

ity and reliability of the analysis. A common complaint with IPC reagents has been impurities that not only lead to unstable baselines and interferences, but also to early column death from fouling. Highly pure additives mean better reproducibility and reliability along with improved accuracy and longer column lifetime. Only additives that have been carefully analyzed and tested for performance in situ will guarantee a problem-free analysis.

IPC reagents from Sigma-Aldrich are of the highest purity and have minimal absorbance in the low-UV region. For example, our tetrabutylammonium hydrogen sulfate (TBAHSO₄) shows excellent transparency down to 200 nm, at even high concentrations, compared to competitive grades (Figure 2). In addition, our IP reagents are tested for the absence of insoluble matter and non-absorbing impurities like redox traces that can interfere with the sample and foul the column. The final QA check is performed with an application test using gradient analysis.

To view the complete list of Sigma-Aldrich products for IPC, please visit www.sigma-aldrich.com/ipc

Figure 2 Effect of IP reagent purity on low-UV HPLC gradient baseline

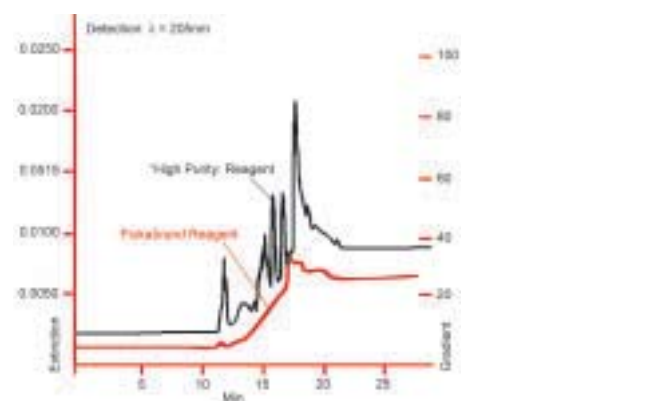


Table Product table (selection)

Brand	Prod. no.	Description	CAS no.	Pack sizes
Fluka	52862	Sodium 1-hexanesulfonate monohydrate	207300-91-2	2.5 g, 10 g, 50 g
Fluka	51832	Sodium 1-heptanesulfonate monohydrate	207300-90-1	2.5 g, 10 g, 50 g
Fluka	74882	Sodium 1-octanesulfonate monohydrate	207596-29-0	2.5 g, 10 g, 50 g
Fluka	30631	Sodium 1-decanesulfonate	13419-61-9	2.5 g, 10 g, 50 g
Fluka	71726	Sodium dodecyl sulfate	151-21-3	5 g, 10 g, 50 g
Fluka	87724	Tetramethylammonium bisulfate	80526-82-5	5 g, 10 g, 50 g
Fluka	86626	Tetraethylammonium hydrogen sulfate	16873-13-5	5 g, 10 g, 50 g
Fluka	86853	Tetrabutylammonium bisulfate	32503-27-8	2.5 g, 10 g, 50 g
Fluka	87299	Tetrahexylammonium hydrogensulfate	32503-34-7	2.5 g, 5 g, 25 g
Fluka	52864	Sodium 1-hexanesulfonate solution, concentrate, ~0.33 M in ampuls for 6 x 1 L of 0.005 M ready-to-use solution	2832-45-3	6 ampuls, ~1 mL each
Fluka	51834	Sodium 1-heptanesulfonate solution, concentrate, ~0.33 M in ampuls for 6 x 1 L of 0.005 M ready-to-use solution	22767-50-6	6 ampuls, ~1 mL each
Fluka	86847	Tetrabutylammonium bisulfate solution, concentrate, ~0.33 M in ampuls for 6 x 1 L of 0.005 M ready-to-use solution	32503-27-8	6 ampuls, ~1 mL each

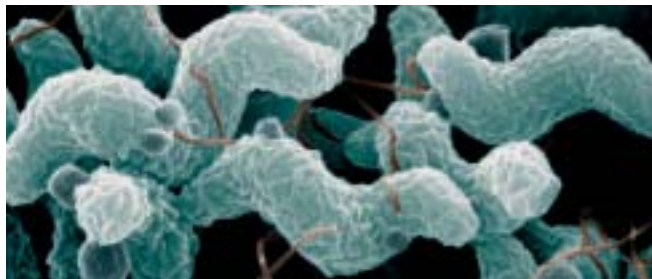
On the trail of *Campylobacter*

Detection, identification, differentiation and cultivation of *Campylobacter*

Jvo Siegrist, Product Manager, Microbiology ivo.siegrist@sial.com

Figure 1 Scanning electron microscope image: shows the characteristic spiral, or corkscrew, shape of *C. jejuni* cells.

Photo by De Wood; digital colorization by Chris Pooley, USDA/ARS



The *Campylobacter* is one of the leading causes of human gastroenteritis. Common *Campylobacter* species *C. jejuni*, *C. coli*, and *C. lari* are responsible for most cases of campylobacteriosis [1, 2]. However, other species, like *C. fetus*, which causes spontaneous abortions, have also been associated with human illness [2–6].

Campylobacter are Gram-negative, spiral-shaped, microaerophilic and motile bacteria with uni- or bi-polar flagella (see **Figures 1, 2 and 3**). The size of a cell is roughly 0.2–0.8 x 0.5–5 µm and, interestingly, in culture they can undergo morphological change from spiral to spherical shape. Most species are catalase- and oxidase-positive (7), with the exception of the catalase-negative *C. sputorum*, *C. concisus*, *C. mucosalis* and *C. helveticus*. The metabolism of *Campylobacter* is chemoorganotrophic, with amino acids and intermediates of the citric acid cycle serving as energy sources; typical carbohydrates cannot be used. *Campylobacter* reduce nitrate to nitrite, obtaining oxygen for their metabolism by

this pathway. These distinctive metabolic reactions can be used for the differentiation and identification of *Campylobacter* species (**Table 1**). Commercially available tests from Sigma-Aldrich appear in **Tables 2 and 3**.

Campylobacter are generally very fastidious microorganisms and grow only on complex media that have been amended with diverse essential amino acids and supplements, such as pyruvate, α-ketoglutarate, hemin, formate and other essential ions. For selective isolation of *Campylobacter*, the growth media can be supplemented with antibiotics like cefoperazone, vancomycin, trimethoprim, amphotericin, cycloheximide, rifampicin, cefsulodin and polymyxin B sulfate. Sigma-Aldrich offers a broad range of specific agars and broths for the detection, identification, differentiation, enumeration and cultivation of *Campylobacter* (**Table 4**).

For more details about our products for analytical microbiology, please visit our website www.sigma-aldrich.com/microbiology

Scientific classification of *Campylobacter*:

Kingdom: Bacteria
 Phylum: Proteobacteria
 Class: Epsilon Proteobacteria
 Order: Campylobacterales
 Family: Campylobacteraceae
 Genus: *Campylobacter*

Table 1 Table of differentiating characteristics of *Campylobacter* species and subspecies

	Species	<i>C. jejuni</i>		<i>C. coli</i>	<i>C. lari</i>	<i>C. fetus</i>	
		subspecies <i>jejuni</i>	subspecies <i>doylei</i>			subspecies <i>fetus</i>	subspecies <i>veneralis</i>
Growth at	25°C	no	no	no	no	yes	yes
	37°C	yes	yes	yes	yes	yes	yes
	42°C	yes	no	yes	yes	partial	no
Biochemical Test	Oxidase	yes	yes	yes	yes	yes	yes
	Catalase	yes	yes	yes	yes	yes	yes
	Nitrate Reduction	yes	no	yes	yes	yes	yes
	Hippurate Hydrolysis	yes	yes	no	no	no	no
	Indoxyl Acetate Hydrolysis	yes	yes	yes	no	no	no
	H ₂ S Production	no		no	yes	yes	no
Antibiotic Sensitivity	Nalidixic acid	S	S	S	R	R	R
	Cephalotin	R	S	R	R	S	S
	Penicillin	R	R	R	R	R	S
	TTC*	R	R	R	S	S	S

(* triphenyltetrazolium chloride)

(continued on page 12)

Figure 2 Scanning electron micrograph of the single polar flagellum and corkscrew shape of *C. jejuni*. These morphologic characteristics contribute to the characteristic darting motility of *C. jejuni* in the viscous mucous layer of the intestinal lumen.

Sean F. Altekruse National Cancer Institute, Rockville

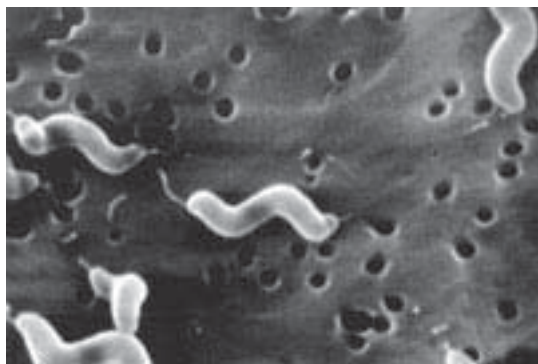


Figure 3 Electron microscope image of Campylobacters by Jochen Reetz, Bundesinstitut für Risikobewertung (BfR), Germany



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- 6] Tee, W., Anderson, B. N., Ross, B. C., Dwyer, B., Atypical campylobacters associated with gastroenteritis. *J. Clin. Microbiol.* (1987), 25, 1248–54.
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Table 2 Sigma-Aldrich tests for identification and differentiation of *Campylobacter*

Campylobacter test	Brand	Cat. no.
Catalase Test	Fluka	88597
Hippurate Disks	Fluka	40405
Hydrogen Sulfide Test Strips	Fluka	06728
Indoxyl Strips	Fluka	04739
Nitrate Reagent A	Fluka	38497
Nitrate Reagent B	Fluka	39441
Nitrate Reagent Disks	Fluka	08086
Oxidase Test	Fluka	70439
Oxidase Strips	Fluka	40560
Oxidase Reagent acc. Gaby-Hadley A	Fluka	07345
Oxidase Reagent acc. Gaby-Hadley B	Fluka	07817
Oxidase Reagent acc. Gordon-McLeod	Fluka	18502

Table 3 Sigma-Aldrich Gram staining kit and single solutions

Gram stain	Brand	Cat. no.
Gram staining kit	Fluka	77730
Gram's crystal violet solution	Fluka	94448
Gram's decolorizer solution	Fluka	75482
Gram's fuchsin solution	Fluka	87794
Gram's iodine solution	Fluka	90107
Gram's safranin solution	Fluka	94635

Table 4 Media for *Campylobacter* (detection, differentiation, and identification)

Nonselective media	Brand	Cat. no.
Tryptic Soy Broth, Vegetone	Fluka	41298
Columbia Agar	Fluka	27688
Tryptic Soy Agar, Vegetone	Fluka	14432
Tryptic Soy Broth	Fluka	22092
CASO Agar	Fluka	22095
Tryptic Soy Broth No. 2	Fluka	51228
Tryptic Soy Agar	Fluka	22091
Tryptic Soy Agar Plates (Diameter 55 mm)	Fluka	57994
CASO Broth	Fluka	22098
Nonselective media with differential system	Brand	Cat. no.
Blood Agar Base No. 2	Sigma	B1676
Hippurate Broth	Fluka	53275
OF Test Nutrient Agar	Fluka	75315
Selective media with differential system	Brand	Cat. no.
MacConkey Agar No. 1	Fluka	70143
Campylobacter selective media	Brand	Cat. no.
Bolton Broth Base	Fluka	67454
Karmali <i>Campylobacter</i> Agar (Base)	Fluka	17152
Brucella Broth Base	Sigma	B3051
<i>Campylobacter</i> Selective Agar (Base)	Fluka	21378
Park and Sanders Enrichment Broth (Base)	Fluka	17189
Blood-free <i>Campylobacter</i> Selective Agar Base	Sigma	B2426

Assessment of the fatty acid status in blood lipids

Kits provide means for easy sample collection and derivatization

Prof. Dr. Claudio Galli, Department of Pharmacological Sciences, University of Milan, Italy

Valuable diagnostic information can be obtained from the fatty acid profile of a single drop of blood.



Fatty acids perform diverse biological functions. They are important as dietary energy sources, and as components of plasma, cell lipids and cell membranes. In the diet, their content varies both in amount (quantitatively) and in composition or profile (qualitatively).

Classifications of fatty acids based on degree of unsaturation

Fatty acids (FA) are carbon chains, generally with an even number of carbon atoms and a varying number of double bonds. FA with zero double bonds are the saturated FA (SFA). These are found primarily in animals, especially ruminants and derived products, and in some vegetable fats, like coconut and palm oils. FA with one double bond are the monounsaturated FA (MUFA). The most important MUFA is oleic acid (C18:1), which is present in both vegetable oils (olive and canola) and animal fat (pork and poultry). FA with two or more double bonds are the polyunsaturated FA (PUFA). The 18C components of the PUFA class are the so-called essential FA (EFA) that cannot be synthesized by animals. EFA are synthesized by plants and are therefore found in vegetable fats. PUFA with longer chains (>20 carbon atoms) and a higher degree of unsaturation (>3 double bonds) are almost exclusively animal derived.

The PUFA are further subdivided in two major series: the omega-6 (or ω -6) and the omega-3 (or ω -3), depending upon the number of carbon atoms between the methyl end of the FA chain and the nearest double bond. The long-chain, highly unsaturated omega-6, especially arachidonic acid (AA, C20:4), and omega-3, especially eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), play important structural and functional roles in cell membranes. While some AA is found in lean animal tissues, especially meat, EPA and DHA are mainly found in fatty fish where they accumulate following the consumption of algae.

Fatty acids and health

There is a vast amount of literature suggesting that high intake of omega-3 FA is associated with higher health status. However, the optimal omega-3 levels are generally higher than provided by the typical Western diet. The correlation with high omega-3 intake and health is thought to be due to prevention of the onset of chronic degenerative, inflammatory processes of the cardiovascular and respiratory system, among other benefits. Because of the health benefits of the presence of "good" FA, and the deleterious affects of high levels of "bad" FA, the FA status in blood is commonly measured.

(continued on page 14)

The following three relevant points are notable:

- FA, especially PUFA, are mainly derived from the diet.
- FA intake is difficult to assess on the basis of food composition data and questionnaires, especially the omega-3 FA because of their low levels in the diet (<0.5 g omega-3 vs. >100 g total FA/day).
- The FA status is an indicator of FA intake and also correlates with various patho-physiological conditions.

Recommended dietary strategies for improving the health status and prevention and treatment of diseases are typically aimed at increasing omega-3 FA levels, especially the EPA and DHA.

Uncomplicated collection of blood samples for assessment of fatty acid status

Monitoring a patient's FA profile is important to optimize fat intake and verify the adherence to and the efficacy of dietary strategies aimed at improving health. Sufficient data to make such assessments can be obtained from only a small drop of fingertip blood [1].

Sigma-Aldrich has created a convenient kit for the easy collection of blood drops, their storage, shipment and processing for FA analysis. A separate derivatization kit is available for the GC analysis of the sample. Information obtained through subsequent analysis of the FA in the blood samples allows the definition of the FA status of an individual in relation to the average values in the population and provides the basis for the development and application of adequate preventive dietary strategies.

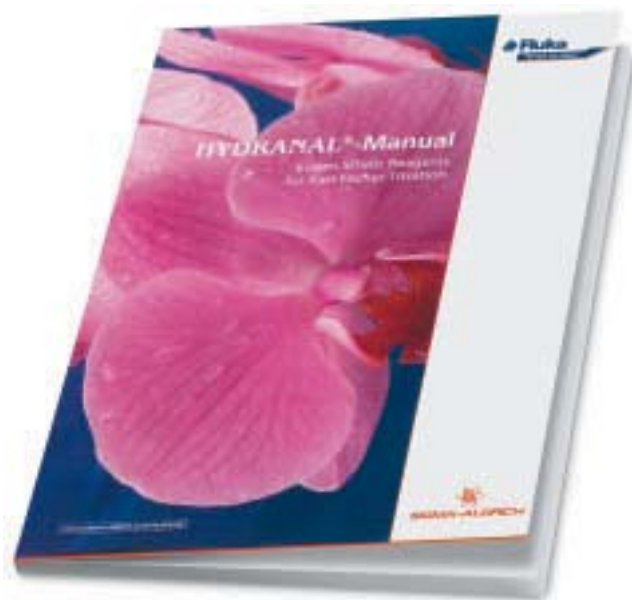
Table 1 Valuable diagnostic information can be obtained from the fatty acid profile of a single drop of blood.

Cat. no.	Brand	Description	Pack size
11312	Fluka	Blood Collection Kit Includes blood collection dipsticks, desiccant packs, foil-barrier ziplock bags, 50 mL BHT solution and complete instructions. Enough for 100 tests.	1 kit
05904	Fluka	Derivatization Kit Includes methanolic HCl solution (1.25 M), saturated KCl solution, distilled water and working instruction sheet. Enough for 100 tests.	1 kit

Reference

1] Marangoni F., Colombo C., Galli C., *Anal. Biochem.* (2004), 326, 267–72.

HYDRANAL® Manual



- **HYDRANAL®** is the gold standard reagent line for pyridine-free water determination by Karl Fischer titration.
- The **HYDRANAL®** Manual is your one-stop reference for everything you need to know about **HYDRANAL®**.

As always, the **HYDRANAL®** Manual laboratory handbook provides practical, up-to-date information on Karl Fischer titration, including:

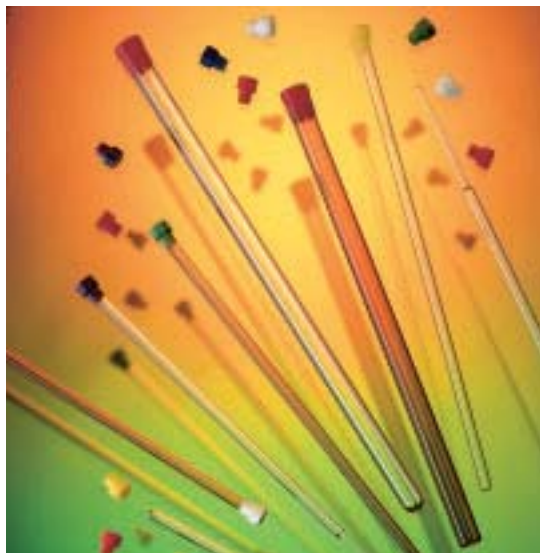
- Principles of Karl Fischer titration
- Standard procedures for volumetric and coulometric titration
- Selection of appropriate solvents for different samples
- Sample handling techniques
- Complete **HYDRANAL®** product listing

Please visit our website to obtain your personal copy of the **HYDRANAL®** Manual: www.sigma-aldrich.com/HYDRANAL_manual

Special promotion – Aldrich premium-quality NMR tubes



Figure 1 Aldrich premium-quality NMR tube



Choose Aldrich premium-quality NMR tubes for your most demanding NMR analyses.

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Offer valid through 31 March, 2008.

Table 1 Product table. Aldrich NMR tubes

Grade	L (in.)	Wall uniformity (in., TIR)	Camber (in.)	Cat. No.
3 mm Microsample NMR tubes – 0.012 inch wall thickness (package of 5 tubes)				
Series 300	7	0.0020	0.0010	Z412902
"	8	0.0020	0.0011	Z412910
Series 400	7	0.0010	0.0005	Z412929
"	8	0.0010	0.0006	Z412937
Series 500	7	0.0005	0.00025	Z412945
"	8	0.0005	0.00035	Z412953
5 mm NMR tubes – 0.015 inch wall thickness (package of 50 tubes)				
Series 60	7	-	-	Z543888
"	8	-	-	Z555908
5mm Thrift NMR tubes – 0.01475 inch wall thickness (package of 5 tubes)				
Series 200	7	0.002	0.0005	Z555894
"	8	0.002	0.0006	Z556092
Series 300	7	0.0015	0.001	Z556106
"	8	0.0015	0.001	Z556114
Series 400	7	0.0005	0.00025	Z556122
"	8	0.0005	0.00035	Z556130
Series 500	7	0.0005	0.00025	Z556149
"	8	0.0005	0.00035	Z556157
5 mm Precision NMR tubes – 0.01475 inch wall thickness (package of 5 tubes)				
Series 80	7	0.003	0.002	Z412783
Series 200	7	0.0025	0.002	Z412791
Series 300	7	0.002	0.005	Z412805
"	8	0.002	0.0006	Z412813
"	9	0.002	0.0007	Z412821
Series 400	7	0.001	0.0005	Z412848
"	8	0.001	0.006	Z412856
Series 500	7	0.0005	0.00025	Z412864
"	8	0.0005	0.00035	Z412872
Series 800	7	0.0002	0.00015	Z412880
"	8	0.0002	0.00015	Z412899
Series 900	7	0.0002	0.00015	Z550868
"	8	0.0002	0.00015	Z548243

AQUANAL® Ecotest Water Laboratory

For fast measurement of ecologically relevant parameters in water

Michael Jeitziner, Market Segment Manager, Analytical Reagents & Standards michael.jeitziner@sial.com



The AQUANAL® Ecotest tests are fast and the instructions complete and easy to follow. Each reagent is color coded, the color corresponding to the color produced in the particular test. The level of the test substance is determined by comparison with the included color chart. The individual reagents are environmentally safe, require no hazardous labeling and pose no risk to the user or to the environment.

Water analysis is one of the most frequently encountered applications of analytical chemistry. Sigma-Aldrich's AQUANAL® Ecotest Water Laboratory contains tests for the following six water parameters:

- Hardness
- Nitrate
- Nitrite
- Phosphate
- pH value
- Ammonia

AQUANAL® Ecotest Water Laboratory has many applications, but is particularly well suited for the private user or hobbyist, or for schools, where it can be used as part of a project to monitor water quality in local bodies of water.

The substances that can be determined by the AQUANAL® Ecotest Water Laboratory and their levels appear in **Table 1**. The two separate kits are described in **Table 2**. Refill packs can be ordered separately. If you have further questions, please contact your local Sigma-Aldrich technical service office, or send an email to: aquanal@sial.com

Table 1 Water parameters measured using AQUANAL® Ecotest Water Labs

Parameter	Measuring range	
	Ecotest Water Lab Riedel-de Haën 37557	Ecotest Water Lab (Low level) Riedel-de Haën 37543
Ammonium (NH ₄ ⁺)	≤0.05 – 10 mg/L	≤0.05 – 10 mg/L
Nitrate (NO ₃ ⁻)	10 – 80 mg/L	0,5 – 50 mg/L
Nitrite (NO ₂ ²⁻)	0.02 – 1.0 mg/L	0.02 – 1.0 mg/L
Phosphate (PO ₄ ³⁻)	0.5 – 6.0 mg/L	0.02 – 0.4 mg/L
pH	pH 5.0 – 9.0	pH 5.0 – 9.0
Total Hardness	1 drop = 7.19 mg/L CaCO ₃	1 drop = 7.19 mg/L CaCO ₃

Table 2 AQUANAL®-Ecotest Water Laboratory products from Sigma-Aldrich

Cat. no.	Brand	Product	Description
37557	Riedel-de Haën	AQUANAL®-Ecotest Water Laboratory	Original Case with tests for Ammonium, Nitrate, Nitrite, Phosphate, pH-value and Total Hardness, color chart and reaction tubes 50-60 test/parameter
37543	Riedel-de Haën	AQUANAL®-Ecotest Water Laboratory (Low level)	Original Case with tests for Ammonium, Nitrate, Nitrite, Phosphate, pH-value and Total Hardness, color chart and reaction tubes 50 – 60 test/parameter

High-purity TMAH for alkaline digestion of biological samples

Ideal for wet digestions and trace analysis applications in the ppm and ppb level

Michael Jeitziner, Market Segment Manager, Analytical Reagents & Standards michael.jeitziner@sial.com

Analysis of trace compounds in complex matrixes like meat requires digestion to solubilize cells, proteins and other solids



Tetramethylammonium hydroxide (TMAH) is a strong base and a very efficient solubilizing agent for animal tissues and other food stuffs. Compared to conventional alkaline sample digestion procedures using NaOH or KOH, TMAH allows more accurate analysis because it has a lower matrix effect. TMAH has been used as a digestion reagent for diverse biological samples. **Table 1** shows a selection of applications using a TMAH solution for solubilizing samples for trace analysis applications.

Table 1 Examples of applications using high-purity TMAH

Sample matrix	Analytes	Condition summary	Ref.
Whole blood	Total Hg	Aliquots of whole blood are diluted 1:1 with TMAH solution, incubated for 1 hour at room temperature and then further diluted 1:4 v/v with HCl solution	1
Food stuff	Iodine	Iodine was extracted quantitatively by TMAH at 90°C	2
Milk powder, bovine liver and muscle	Cd, Pb, Ni, Cr, Cu, Ag	Samples are mixed with 25% TMAH solution, heated to 60–70°C yielding a stable, homogeneous slurry in ~2 hours	3, 4
Coffee and milk	Ca, Cu, Fe, K, Mg, Mn, P, Se, Sn, Zn	350 mg sample incubated with 500 µL 25% TMAH for 10–30 mins. at 80°C	5
Bovine liver and muscle	Si	Digestion procedure using 25% TMAH in an autoclave microwave oven	6
Animal liver tissue	Cu	Digestion of sample in TMAH solution at room temperature for 48 hours	7

High-purity TMAH solution from Sigma-Aldrich

Sigma-Aldrich has developed a new, high-purity aqueous 25% TMAH solution for wet digestions and trace analysis applications in the ppm and ppb level. With production under clean-room conditions, the blank values for metals are below 1 µg/L. We also recommend our *TraceSELECT*[®]Ultra water, should your analysis require solution preparation or sample or reagent dilution. The complete listing of Fluka *TraceSELECT*[®] and *TraceSELECT*[®]Ultra high-purity acids, bases, water and salts designed specifically for trace analysis can be found at www.sigma-aldrich.com/traceselect

Table 2 Product table. TMAH solution and water

Cat. no.	Brand	Description	Pack sizes
68556	Fluka	Tetramethylammonium hydroxide solution, 25% v/v <i>TraceSELECT</i> [®]	250 mL
14211	Fluka	Water, <i>TraceSELECT</i> [®] Ultra	1 L

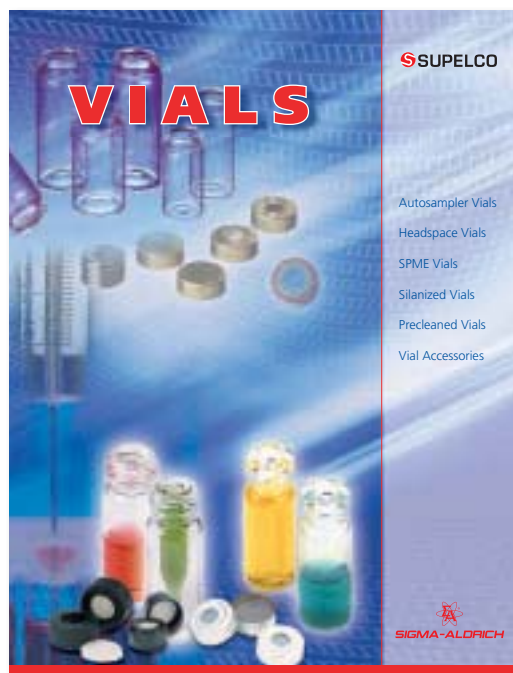
References:

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TopSert™ TPX® autosampler vials

A new addition to our line of vials for demanding analytical applications

Nicole Amann, Product Manager, Analytical Standards nicole.amann@sial.com



(IXH)

Important but often overlooked, the vials, caps and septa that come in contact with the sample are critical components of a successful analysis. Our goal is to continually expand our product range to include vials and accessories with the specifications, quality and cleanliness needed to meet the evolving demands of instruments, samples and measurement techniques.

TopSert™ TPX® vials, a new introduction to our line, consist of a polymethylpentene (PMP) shell and an integrated, 0.2 mL glass insert. The 11.6 mm x 32 mm size fits all popular autosamplers. TopSert™ TPX® vials are available in clear and amber PMP and with either 9 mm short thread or snap top design.

Table TopSert™ TPX® vials from Sigma-Aldrich

Cat. no.	Brand	Description	Package size
SU860043	Supelco	TopSert™ short thread vial, clear PMP	100
SU860147	Supelco	"	1000
SU860044	Supelco	TopSert™ snap top vial, clear PMP	100
SU860192	Supelco	"	1000
SU860188	Supelco	TopSert™ short thread vial, amber PMP	100
SU860166	Supelco	"	1000
SU860189	Supelco	TopSert™ snap top vial, amber PMP	100
SU860167	Supelco	"	1000

(TPX® is a registered trademark of Mitsui Petrochemical Industries, Ltd.)



Sensorics new nitrate-ionophore from Sigma-Aldrich

Eliminate interference from competing nitrite, chloride and sulfate ions

Michael Jeitziner, Market Segment Manager, Analytical Reagents & Standards michael.jeitziner@sial.com

Ion-selective electrodes have long been utilized to provide a real-time measurement of nitrates in water and food stuffs. However, their effectiveness has been restricted due to interference by several anions, including chloride, nitrite and bicarbonate. These ions

are abundant in salted foods such as meats and cheese. Under exclusive license, Fluka chemists have introduced a novel membrane sensor, first described by Kong Thoo Lin, et al [1], that is based on Nitrate ionophore III and shows excellent nitrate selectivity over nitrite, chloride, acetate and sulfate ions.

Nitrate ionophore III molecular structure

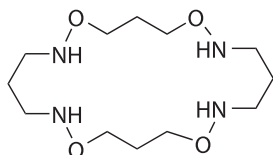
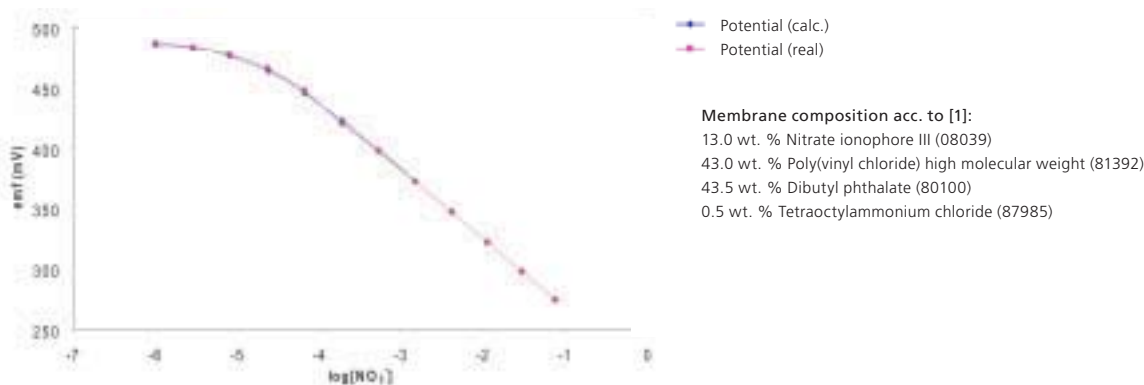


Figure 1 EMF as a function of nitrate concentration for ISE membranes



Nitrate ionophore III characteristics

Slope (sensitivity): 57.9 mV/dec.

Response time: <12 sec.

Selectivity Coefficients $\log K_{NO_3, X}^{Pot}$ as obtained by the single solution method (SSM):

$$\log K_{NO_3, NO_2}^{Pot} -1.5, \log K_{NO_3, Cl}^{Pot} -2.3, \log K_{NO_3, Acetate}^{Pot} -3.4, \log K_{NO_3, SO_4}^{Pot} -4.1$$

Nernstian electrode response (1.5×10^{-5} to 10^{-1} M KNO_3)

Detection limit: $\log(aNO_3^-) \sim -5.0$

Product table Fluka brand ionophore and auxiliary reagents

Prod. no.	Brand	Description	CAS no.	Package size
08039	Fluka	Nitrate ionophore III (1,7,11,17-Tetraoxa-2,6,12,16-tetraazacycloeicosane), Selectophore® (Exclusive license for patent CA138-010965/01)	235093-03-5	50, 250 mg
81392	Fluka	Poly(vinyl chloride) high molecular weight, Selectophore®	9002-86-2	1, 10, 50 g
80100	Fluka	Dibutyl phthalate, Selectophore®	84-74-2	5, 25 mL
87985	Fluka	Tetraoctylammonium chloride, $\geq 97.0\%$ (AT)	3125-07-3	1 g

Reference

1) Kong Thoo Lin, P., Araujo, A. N., Montenegro, M. C. B. S. M, Pérez-Olmos, R., *J. Agric. Food. Chem.* (2005), 53, 211–15.

HYDRANAL® E-type reagents

Ethanol-based reagents for non-toxic Karl Fischer titrations

Helga Hoffmann, Technical Service HYDRANAL® Manager helga.hoffmann@sial.com
 Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com



Karl Fischer titration is a universally accepted method for measuring water content in all types of substances, including chemicals, oils, pharmaceuticals and food. Sigma-Aldrich is a pioneer in the supply of safe, reliable and easy-to-use **HYDRANAL®** Karl Fischer reagents. With the goal of improving laboratory safety and reducing toxicity, we offer **HYDRANAL®** E-type reagents that permit reliable Karl Fischer titrations without toxic methanol.

Advantages and properties of HYDRANAL® E-type reagents:

- Reduced toxicity over methanol-containing reagents
- Better solubility of hydrophobic samples
- Additives increase reaction rate and conductivity of ethanol
- Several ketones can be titrated without side-reactions
- Pyridine-free, like all **HYDRANAL®** reagents
- Suitable for both volumetric and coulometric KF titrations
- Possible replacement for most methanolic KF methods
- Compatible with all titration equipment
- End-point color appears visually more intense compared to methanolic titrations
- Users fulfill requirements of DIN EN ISO 14001

Environmental responsibility and “green” chemistry

The chemical industry has responded to the need to reduce workers' exposure to hazardous and toxic substances, and to reduce and ultimately eliminate the release of these substances into our communities and environment. Because Karl Fischer titration is so

prevalent across many industries worldwide, improving its safety can have a dramatic positive impact on industrial hygiene and the environment.

From the very inception of the line, all **HYDRANAL®** reagents were designed to ensure reliable titrations that are free of noxious pyridine by replacing it with safe and odorless imidazole or diethanolamine. In a second significant improvement, Sigma-Aldrich researchers successfully replaced toxic methanol with ethanol, thereby forming our **HYDRANAL®** E-types product line. By using ethanol, we have also been able to eliminate the need for halogenated hydrocarbons, like chloroform, dichloromethane and carbon tetrachloride as solubilizing agents.

Replacement of methanol in Karl Fischer titrations

Historically, ethanol was avoided in Karl Fischer reagents for two important reasons: very slow KF reaction rates and low conductivity in ethanol. **HYDRANAL®** E-reagents solve these problems by incorporating accelerators to increase reaction rates and by using the most suitable additives to obtain conductivity comparable to methanol-containing reagents. As a result of these research efforts, our E-type reagents are now newly patent protected (by EP 0 933 634).

HYDRANAL® E-type applications

The vast number of sample-specific procedures for Karl Fischer titration makes it impossible to name all advantages and disadvantages for every situation. During the development of the **HYDRANAL®** E-product line, we analyzed samples from a large range of chemical groups and tested them for accuracy and extraction efficiency. We found that most applications that use methanol-based reagents are readily transferable to ethanol-based **HYDRANAL®** reagents without difficulty.

Benefit of HYDRANAL® E-types for hydrophobic samples

Besides having reduced toxicity, **HYDRANAL®** E-reagents provide improved solubility of long-chained hydrocarbons compared to methanol-containing reagents. For example, only 4 mL of iso-octane dissolve in methanol-based reagents, whereas 18 mL dissolve in **HYDRANAL®** Solvent E and over 30 mL in **HYDRANAL®** CompoSolver E. As a result, more analyses can be carried out in one charge of working medium. The addition of a solubilizing agent, like chloroform, is almost entirely unnecessary. However, if ethanol is not sufficient, solubilizing agents such as formamide, chloroform or xylene can be added to the **HYDRANAL®** E-type working medium. Also, the temperature of the titration can be increased up to 50°C in order to improve sample solubility.

Table 1 Possible combinations of HYDRANAL® E-type reagents for non-toxic Karl Fischer determinations

Volumetric one-component technique	
Titrating agent:	HYDRANAL® Composite 1 / 2 / 5
Working medium:	HYDRANAL® CompoSolver E
Volumetric two-component technique	
Titrating agent:	HYDRANAL® Titrant 2 E / 5 E
Working medium:	HYDRANAL® Solvent E
Coulometric technique	
Anolyte:	HYDRANAL® Coulomat E
Catholyte:	HYDRANAL® Coulomat E

Composition of HYDRANAL® E-type reagents

HYDRANAL® Titrant 2 E and HYDRANAL® Titrant 5 E are two-component titration reagents based on ethanol and iodine. They can be used with HYDRANAL® Solvent or HYDRANAL® Solvent E.

HYDRANAL® Solvent E contains imidazole and diethanolamine as bases, as well as sulfur dioxide and ethanol.

HYDRANAL® CompoSolver E contains ethanol as solvent and provides distinct advantages over methanol-based solvents, including: much shorter titration times than in pure methanol, suitability for water determination in ketones like acetone, and higher solubility for long-chained hydrocarbons.

HYDRANAL® Coulomat E contains the bases imidazole and diethanolamine, as well as sulfur dioxide and ethanol. It can be used as both anolyte and catholyte for coulometric cells with and without diaphragm.

Reliable, precise and quantitative reference standards for titer determination

Sodium tartrate-2-hydrate is a common primary standard for volumetric KF titration. However, its solubility in ethanol is very limited. Therefore, when using HYDRANAL® E-type reagents, we recommend using one of our HYDRANAL® Water Standards for titer determination. HYDRANAL® Water Standard 10.0 and HYDRANAL® Standard 5.00 are specifically designed for volumetric KF determinations.

Applications

L510 Ethosuximide (3-Ethyl-3-methyl-2,5-pyrrolidinedione)

The sample is a waxy, solid mass, which is not easily homogenized. We recommend titration in HYDRANAL® CompoSolver E, as the sample dissolves well in this ethanolic medium and titration times are very short (less than one minute). 30 mL HYDRANAL® CompoSolver E are added to the titration vessel and

Table 2 HYDRANAL® E-Type reagents and suitable water standards

Cat. No.	Brand	Description	Package Size
34723	Riedel-de Haën	HYDRANAL® Titrant 2 E (2.00 ± 0.02 mg H ₂ O / mL)	1 L
34732	Riedel-de Haën	HYDRANAL® Titrant 5 E (5.00 ± 0.02 mg H ₂ O / mL)	500 mL, 1 L, 2.5 L
34730	Riedel-de Haën	HYDRANAL® Solvent E	500 mL, 1 L, 2.5 L
34734	Riedel-de Haën	HYDRANAL® CompoSolver E	1 L, 2.5 L
34726	Riedel-de Haën	HYDRANAL® Coulomat E	500 mL
34813	Riedel-de Haën	HYDRANAL® Standard 5.00 Water content 5.00 ± 0.02 mg/mL (20°C)	100 mL, 500 mL
34849	Riedel-de Haën	HYDRANAL® Water Standard 10.0 Box contains 10 ampuls of 8 mL each Water content 10.00 mg/g CoA included in each box, tested against NIST SRM 2890	80 mL

pre-titrated with HYDRANAL® Composite 5. About 2 g of the sample, exactly weighed using differential weighing, are added and the water content titrated with HYDRANAL® Composite 5.

L514 Hydroxyzine pamoate

This substance is a fine powder, but it is insoluble in methanol. The sample was dissolved during titration in the non-toxic medium HYDRANAL® CompoSolver E, titration duration less than 3 minutes. The sample also dissolved very quickly in the reagents of the two-component technique and titration was completed within 1–2 minutes. 30 mL HYDRANAL® Solvent E / CompoSolver E are added to the titration vessel and pre-titrated with HYDRANAL® Titrant 5 E / Composite 5. A 1 g sample is exactly weighed using differential weighing, and its water content titrated with HYDRANAL® Titrant 5 E / Composite 5.

L537 Brake fluid

The tested brake fluid, consisting of glycol ether, borate ester and inhibitors, dissolved easily in the alcoholic media for Karl Fischer titration. 30 mL of HYDRANAL® CompoSolver E / Solvent E is placed in the titration vessel and pre-titrated with HYDRANAL® Composite 2 / Titrant 2 E. Approximately 5 g of the sample, determined by differential weighing, are added to the cell and the water content is titrated with HYDRANAL® Composite 2 / Titrant 2 E. Using a coulometry cell with diaphragm, 5 mL of HYDRANAL® Coulomat E are filled into the cathodic compartment and the anodic compartment is filled to the same level with HYDRANAL® Coulomat E. For a coulometry cell without diaphragm, approximately 100 mL of HYDRANAL® Coulomat E are added to the cell. The cell automatically pre-titrates. When the drift is low and stable, 0.5–1 g of the sample, determined by differential weighing, are weighed precisely and injected into the cell using a syringe.

(continued on page 22)

Contact us for expert technical assistance

Take advantage of our expertise gained from over twenty-five year experience and our extensive applications database on Karl Fischer titration. We can suggest a solution to your analytical problem and, if necessary, develop an individual analytical method for you. Additional information can be found on our website: www.sigma-aldrich.com/hydranal

For answers to all your KF titration questions, please contact our **HYDRANAL**® specialists:

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SPECIAL OFFER!

Change from toxic to non-toxic Karl Fischer titration and get 25% off each bottle of **HYDRANAL**® E-reagent! Please quote promotion code 989 when placing your order.

Offer valid until 31 March 2008.

34734	RdH	HYDRANAL ® CompoSolver E
34732	RdH	HYDRANAL ® Titrant 5 E
34723	RdH	HYDRANAL ® Titrant 2 E
34730	RdH	HYDRANAL ® Solvent E
34726	RdH	HYDRANAL ® Coulomat E

Laboratory chemicals for titration

New and improved website for Sigma-Aldrich titration products: www.sigma-aldrich.com/titration



The Sigma-Aldrich analytical product range includes a wide array of ready-to-use solutions of the many different types of titration, including acidimetric/alkalimetric, redox, argentometric and complexometric titrations, as well as acid, base, buffer and salt concentrates for dilution.

For complete product listings of these solutions and other laboratory chemicals for titration, please visit our revised titration website and our online product catalog: www.sigma-aldrich.com/catalog

New 34694 HYDRANAL® Water Standard Oil

Mineral oil matrix standard for Karl Fischer titration

Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com



Measuring the water content of oily samples can be a challenge. Their inherently low water content and compatibility of the titration technique, reagents and instruments with this type of sample are complicating factors. Additionally, for accurate determinations, the water content standards should have a similar composition to the sample.

To help analysts maximize the accuracy and the ease of water determination of oily samples by Karl Fischer

titration, Sigma-Aldrich developed mineral oil-based **HYDRANAL®** Water Standard Oil. The water content of each lot is analysed using several procedures applied in practice: direct volumetric and coulometric Karl Fischer titration as well as the indirect procedure using a Karl Fischer oven. Actual water content of the standard is reported on the Certificate of Analysis included with each box. **HYDRANAL®** Water Standard Oil is tested against NIST SRM 2890.

Table HYDRANAL® Water Standard Oil

Cat. no.	Brand	Description	Package size
34694	Riedel-de Haën	HYDRANAL® -Water Standard Oil. Tested against NIST SRM 2890.	80 mL (10 ampuls per box, each containing 8 mL)

Residue analysis

New Fluka analytical standards

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com



Sigma-Aldrich is pleased to introduce new standards for residue analysis from our Fluka brand.

PESTANAL® comprises over 1200 high-purity pesticide and metabolite standards for food and environmental applications.

VETRANAL® includes over 200 high-purity analytical standards of the active ingredients of veterinary drugs, primarily antibiotics and hormones.

OEKANAL® products include over 400 high-purity standards of common pollutants and contaminants that are analyzed environmental samples.

Cat. no.	Brand	Description	Package size
PESTANAL® pesticide and metabolite standards			
33197	Fluka	Haloxifop-P-methyl	100 mg
32999	Fluka	Aminoethoxyvinyl glycine hydrochloride	25 mg
34225	Riedel de-Haën	Topramezone	100 mg
33767	Fluka	cis-Mevinphos solution, 100 µg/mL in acetonitrile	2 mL
33765	Fluka	trans-Mevinphos solution, 100 µg/mL in acetonitrile	2 mL
33799	Fluka	Isoxadifen-ethyl	100 mg
33797	Fluka	Fluoxastrobin	100 mg
32994	Fluka	Thiosultap Disodium	100 mg
VETRANAL® veterinary drug standards			
32971	Fluka	Valnemulin	25 mg
32992	Fluka	Aspoxicillin	25 mg
32991	Fluka	Lanconazole	25 mg
32996	Fluka	Sulfadimethoxine-d ₆	10 mg
32997	Fluka	Sulfadoxine-d ₃	10 mg
32995	Fluka	Levothyroxine sodium hydrate	25 mg
OEKANAL® pollutant and contaminant standards			
33842	Riedel de-Haën	HT-2 Toxin- ¹³ C ₂₂ solution, 25 µg/mL in acetonitrile	1 mL
33759	Fluka	Microcystin-LY solution, 5 µg/mL in methanol	1 mL
33578	Riedel de-Haën	Microcystin RR-YR-LR solution, 5 µg/mL in methanol	1 mL
33763	Fluka	Microcystin-LW solution, 8 µg/mL in methanol	1 mL
32978	Riedel de-Haën	Ph Eur 5.0 GC Calibration Solution for Residual Solvents in DMSO	2 mL

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