

Analytix

Advances in Analytical Chemistry

Issue 2 • 2007



New IRMM Certified Reference Materials for GMO Detection

Standards

- Fatty Acid Standards
- Nutritive and Non-Nutritive Sweetener Standards
- Aldehyde and Ketone Air Pollutants
- New PBDE Flame Retardant Standards
- TraceCERT™

Analytical Microbiology

- Clostridia Diagnostic

Titration

- HYDRANAL® Application: Fats and Fat Products
- VOLPAC® Volumetric Solutions

Sensorics

- Selectophore® Products

New Product Corner

- LC-MS Rinsing Solutions
- Luff-Schoorl Reagent



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Picture Ingo Haag,
Marketing Manager Analytical

Dear Colleague,

Today's analytical chemist has an increasingly wide array of techniques, instruments, separation devices and consumables to choose from in order to meet their particular analytical challenges. Some of these are designed for a specific analysis, while others are more general purpose. Irrespective of the specificity or complexity of the analytical method, most analyses require a well-characterized standard to identify or quantify the sample and to calibrate the instruments.

Along with other important consumables for analytical chemistry and chromatography, Sigma-Aldrich offers one of the world's most comprehensive selections of high quality analytical standards and reference materials, primarily through our Fluka/Riedel-de Haën and Supelco brands. Besides offering a wide selection of standards, we strive to remain up-to-date in our offering by bringing you standards and reference materials for today's most relevant analyses.

This year, we are pleased to announce the release of our 2007-2008 Analytical Standards Catalog that lists over 8,000 standards, reference materials and mixtures for many analytical chemistry disciplines, including chromatography, spectroscopy, electrophoresis, titrimetry, physical properties and thermal analysis. Covering environmental, food & beverage, agricultural, occupational hygiene, pharmaceutical, life science, forensic, veterinary, petroleum, water analysis and many other markets, this catalog is sure to be one of the most valuable resources on your laboratory bookshelf.

The catalog has been updated with over 200 new products, including:

- Biodiesel – Calibration standards, internal standards and kits for ASTM D6584
- **TraceCERT™** – Traceable Certified Reference Materials for AAS and ICP have traceability to at least two independent references, among many other benefits

We've also made it easy to find the perfect standard for your application. With our Web-based Standards Explorer search engine, www.sigmaaldrich.com/standards_explorer, our complete line is at your fingertips.

Our new Analytical Reference Standards CD and Catalog are available by contacting your local Sigma-Aldrich office, or on-line at www.sigmaaldrich.com/standards_catalog. Of course, our standards and reference materials are backed by the high quality and service level you expect from Sigma-Aldrich.

I hope you find the articles in this issue of Analytix both interesting and useful.

Kind regards,

Ingo Haag
Marketing Manager Analytical

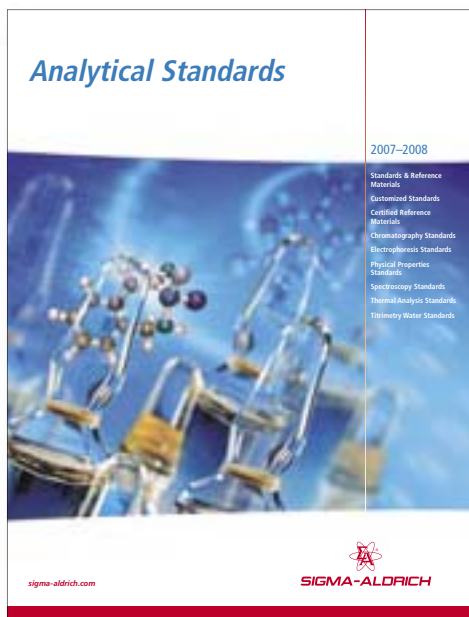


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New IRMM Certified Reference Materials for GMO Detection Additions to our GMO standards product range

By Kurt Vorburger, Manager of Product Management and Innovation ... kvorburger@sial.com



Any genetically modified organism (GMO), whether used as feedstuffs or for human consumption, and its seeds must receive authorization before they are permitted on the EU market. Tight safety and regulatory standards, designed to give freedom of choice for consumers and farmers, took place in all 25 EU Member States beginning in 2004.

The GMO Compass (<http://www.gmo-compass.org>) currently lists 87 GMO crop plants, including cotton, flowers, maize (corn), potato, rapeseed, rice, sugar beets and soybean. Many genetically modified plants have already been approved for use in food and feed in the EU, and decisions on many more are pending. GMO plants are designed to give higher yields, primarily by having herbicide, insect or disease-resistant genes inserted in their genome, or altered composition to improve the nutritional or commercial value of the crop. The introduction of new GMO events has been continuous, and an increasing percentage of acreage worldwide is now used to grow GMO crops.

Whatever their benefits, the use of genetically modified organisms (GMO) is still a widely debated topic. Consumers, especially in Europe, are wary of GMOs and many retailers are reluctant to stock products containing GMO-derived ingredients.

Labelling and traceability of GMOs are critical to help ensure compliance and to measure the release of GMOs into the environment. Analytical methods for detection must accompany introduction of all new GMO events. This analytical challenge is ongoing, and success is dependent on the ready availability of well-characterized GMO standards.

Sigma-Aldrich, as an authorized distributor for the reference materials of the IRMM (Institute for Reference Materials and Measurements, Geel, Belgium), supplies GMO standards to our customers worldwide. We have recently expanded our IRMM GMO product range with new standards for sugar beet, potato, cotton seed and three varieties of maize-derived GMOs.

Maize 1507 – Genetically modified to make it resistant to certain insect pests, like the European corn borer and pink stalk borer.

Maize MIR604 – A genetically modified maize with field protection against corn root worms.

Maize 59122 – Insertion of a gene that codes for a Bt-toxin protects the plant against many common Coleopteran insects.

Sugar Beet H7-1 – Developed for glyphosate tolerance by introduction of a gene that codes for a glyphosate-insensitive version of a critical protein.

Potato EH92-527-1 – An introduced gene increases the desirable characteristics of this GM potato for the starch industry.

Cotton Seed 281-24-236 – A historically successful cotton cultivar parent, *Gossypium hirsutum*, that has been genetically modified for resistance to certain Lepidopteran insect pests.

These matrix standards were prepared by the IRMM by quantitative mixing of different mass fractions of non-genetically modified powder with genetically modified powder produced from ground seed. A dry-mixing technique minimizes DNA and protein degradation during production. Like the GMO CRMs we introduced previously, Sigma-Aldrich again offers each of the maize and cotton seed CRMs in a set containing different GMO mass fractions.

These new CRMs are offered in 1 g quantities packed under argon atmosphere.

Table 1 Maize 1507 Standards (ERM-BF418)

Cat. No.	Certified Value 1507 maize mass fraction (g/kg)	Uncertainty ¹ (g/kg)	Package Size
ERMBF418a-1G	< 0.5	-	1 g
ERMBF418b-1G	1.0	- 0.2 ; + 0.6	1 g
ERMBF418c-1G	9.9	- 0.6 ; + 0.8	1 g
ERMBF418d-1G	98.6	- 1.7 ; + 2.0	1 g
49516	Set of the four maize standards; <0.5, 1.0 , 9.9 , 98.6	-	1 set

Table 2 Sugar Beet H7-1 Standards (ERM-BF419)

Cat. No.	Certified Value H7-1 sugar beet mass fraction (g/kg)	Uncertainty ¹ (g/kg)	Package Size
ERMBF419a-1ea	0	0	1 g
ERMBF419b-1ea	1000	0	1 g

Table 3 Potato EH92-527-1 Standards (ERM-BF421)

Cat. No.	Certified Value Number fraction of EH92-527-1 potato / total number of potatoes [%]	Uncertainty ¹ (g/kg)	Certified property Identity	Package Size
ERMBF421a-1VL	0%	not applicable	potato without the EH92-527-1 event	1 g
ERMBF421b-1VL	100%	not applicable	EH92-527-1 potato	1 g

Table 4 Cotton Seed 281-24-236 standards (ERM-BF422)

Cat. No.	Certified Value 281-24-236 x 3006-210-23 cotton seed mass fraction (g/kg)	Uncertainty ¹ (g/kg)	Package Size
ERMBF422a-1ea	< 0.5	-	1 g
ERMBF422b-1ea	> 979	-	1 g
ERMBF422c-1ea	10.0	1.7	1 g
ERMBF422d-1ea	100	16	1 g
44138	Set of the four cotton seed standards; <0.5, >979 , 10.0 , 100	-	1 set

Table 5 Maize MIR604 Standards (ERM-BF423)

Cat. No.	Certified Value MIR604 maize mass fraction (g/kg)	Uncertainty ¹ (g/kg)	Package Size
ERMBF423a-1G	< 0.9	-	1 g
ERMBF423b-1G	1.0	- 0.3 ; + 1.0	1 g
ERMBF423c-1G	9.8	- 0.9 ; + 1.3	1 g
ERMBF423d-1G	98.5	- 2.6 ; + 2.9	1 g
40982	Set of the four maize standards; <0.9, 1.0 , 9.8 , 98.5	-	1 set

Table 6 Maize 59122 Standards (ERM-BF424)

Cat. No.	Certified Value 59122 maize mass fraction (g/kg)	Uncertainty ¹ (g/kg)	Package Size
ERMBF424a-1G	< 1.2	-	1 g
ERMBF424b-1G	1.0	- 0.2 ; + 1.2	1 g
ERMBF424c-1G	9.9	- 0.8 ; + 1.4	1 g
ERMBF424d-1G	98.7	- 5.8 ; + 5.9	1 g
41740	Set of the four maize standards; <1.2, 1.0 , 9.9 , 98.7	-	1 set

¹ The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor $k = 2$, corresponding to a level of confidence of about 95%.

Essential Fatty Acid Standards A collection of ω -3, ω -6 and other essential fatty acid standards obtainable from one source

By Pat Myers, Technical Service Chemist ... pat.myers@sial.com

Essential fatty acids are nutrients that must be obtained from the diet because humans lack the anabolic processes for their synthesis. There are two closely related groups of essential fatty acids, the ω -3 and ω -6 fatty acids. Both are unsaturated fatty acids with the initial double bond between the third and fourth carbons or the sixth and seventh carbons as measured from the methyl end, respectively.

Essential fatty acids serve multiple purposes in the body, including:

- Production of eicosanoids, which affect inflammation and cellular function
- Production of endogenous cannabinoids, which affect mood and behavior
- Production of lipoxins and resolvins, which affect inflammation
- Influence cell signaling
- Regulation of blood pressure, blood clotting, lipid levels, immune response and gene expression



Seed oils are the primary dietary source of ω -6 fatty acids, while the ω -3 fatty acids are found in fish oils and some nut oils. Before the advent of agriculture, human diets are thought to have consisted of an equal amount of ω -6 and ω -3 fatty acids. In contrast, the current western diet has a ratio of ω -6 to ω -3 of 7:1. Low levels of the essential fatty acids, and in some cases an altered ratio of ω -6 to ω -3 fatty acids, play a key role in a number of human diseases.

Analytical standards for these compounds are necessary for monitoring their concentration in food, beverage and pharmaceutical products. Standards are also required for academic and industrial research into the safety and health effects of fatty acids and toward understanding their fundamental biochemistry.

Sigma-Aldrich offers many essential fatty acids as individual compounds or standards. Each product comes with a Certificate of Analysis that includes a purity determination. Standards are prepared gravimetrically using NIST traceable weights. The availability of small package sizes eliminates the need to buy bulk material as standards.

Cat. No.	Brand	Description	Package Size
62160	Fluka	α -linolenic acid	1 mL, 5 mL
43959	Fluka	stearidonic acid, methyl ester	10 mg
A8105	Sigma	ω -3 Aarachidonic Acid	1 mg, 5 mg
17266	Fluka	<i>cis</i> -5,8,11,14,17-Eicosapentaenoic acid methyl ester	100 mg
17269	Fluka	<i>cis</i> -7,10,13,16,19-Docosapentaenoic acid methyl ester	50 mg
43938	Fluka	<i>cis</i> -4,7,10,13,16,19-Docosahexaenoic acid	25 mg, 100 mg
62230	Fluka	Linoleic acid	5 mL, 25 mL
62174	Fluka	γ -Linolenic acid	100 mg, 500 mg
E3127	Sigma	<i>cis</i> -11,14-Eicosadienoic acid	25 mg, 100 mg, 500 mg
44875	Fluka	<i>cis</i> -8,11,14-Eicosatrienoic acid	10 mg
47572-U	Supelco	<i>cis</i> -5,8,11,14-eicosatetraenoate methyl ester solution	1 mL
10929	Fluka	<i>cis,cis,cis,cis</i> -5,8,11,14-Eicosatetraenoic acid	100 mg, 1 g
D4159	Sigma	<i>cis</i> -13,16-Docosadienoic acid	100 mg
49557	Fluka	<i>cis</i> -7,10,13,16-Docosatetraenoic acid	10 mg
18566	Fluka	all- <i>cis</i> -4,7,10,13,16-Docosapentaenoic acid	10 mg

Nutritive and Non-Nutritive Sweetener Standards Small package sizes eliminate the need to buy bulk material as standards

By Pat Myers, Technical Service Chemist ... pat.myers@sial.com



Sweeteners are classified into two broad groups, caloric (nutritive) and non-caloric (non-nutritive). Nutritive sweeteners, which include sugars and sugar alcohols, are metabolized by the body for energy. Non-nutritive sweeteners are either not metabolized or are used in such small amounts that their contribution to caloric intake is inconsequential. The non-nutritive sweeteners are a diverse group of synthetic and semi-synthetic compounds that includes acesulfame, aspartame, cyclamate, neotame, saccharin and sucralose.

The increased use of non-caloric, non-nutritive or low-caloric sweeteners in recent years has coincided with growing public awareness of the adverse health effects of excess consumption of simple carbohydrates. More products containing non-nutritive sweeteners are made available to consumers each year as the supply of sweeteners increases and their cost decreases.

Non-caloric, non-nutritive sweeteners are used in products designed to meet three consumer needs:

- Weight control
- Management of diabetes mellitus or other diseases of carbohydrate metabolism
- Prevention of tooth decay

The availability of high quality analytical sweetener standards is essential for their monitoring in food products, and for both applied and basic research aimed at developing new sweeteners and understanding their biological effects.

Sigma-Aldrich, through its Supelco brand, offers individual standards and multi-component kits for both nutritive and non-nutritive sweeteners. Each standard comes with a Certificate of Analysis that includes a purity determination. The standards are prepared gravimetrically using NIST traceable weights. The availability of small package sizes eliminates the need to buy bulk material as standards.

Cat. No.	Brand	Product	Package Size
47134	Supelco	Acesulfame K	1 gram
47135	Supelco	Aspartame	500 mg
47829	Supelco	D-(+)-Glucose	1 gram
47840	Supelco	Saccharin, Hemicalcium	1 gram
47827	Supelco	Sodium Cyclamate	1 gram
47839	Supelco	Sodium Saccharin	1 gram
47841	Supelco	D-Sorbitol	1 gram
47844	Supelco	Xylitol	1 gram
47267	Supelco	Monosaccharides Kit, D-(-)-Arabinose, Fructose, D-(+)-Galactose, D-(+)-Glucose (mixed anomers), D-(+)-Mannose (mixed anomers), D-(-)-Ribose, D-(+)-Xylose 500 mg each, packaged separately.	1 Kit
47268-U	Supelco	Disaccharides Kit, Isomaltose (mixed anomers) (100 mg), α -Lactose (500 mg), Maltose (500 mg), Sucrose (500 mg), packaged separately.	1 Kit
47266	Supelco	Sugar Alcohols Kit, Ribitol (Adonitol), Maltitol, iso-Erythritol, Glycerol, Dulcitol (Galactitol), D-Sorbitol, D-Mannitol, D-(+)-Arabitol 500 mg each, packaged separately.	1 Kit

Fast and Reliable Environmental Analysis of Aldehyde and Ketone Air Pollutants

Sigma-Aldrich presents new GC-MS standards of the derivatization reagent PFBHA and its derivatives with aldehydes and ketones

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

Introduction

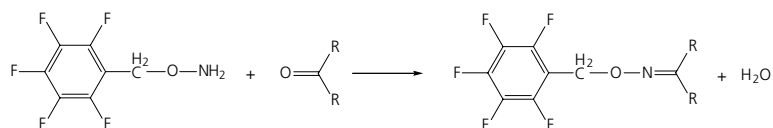
Aldehydes and ketones are ubiquitous air pollutants. Along with esters and ethers, they are major components of indoor air pollution and are therefore important for industrial hygiene applications. Analytical methods must provide reliable quantification to meet today's requirements, as well as keep pace with newer and stricter regulations.

Derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) followed by GC analysis provides a fast and reliable way to quantify aldehydes and ketones in ambient air. As with any analytical method, the availability of high quality analytical standards is important to ensure precision and accuracy of the analysis. However, until now the PFBHA derivatives of aldehydes and ketones have not been commercially available. To meet this need, Sigma-Aldrich now offers these derivatives as high purity standards for GC-MS.

PFBHA: Advantages over derivatization with 2,4-DNPH

Derivatization with PFBHA avoids the disadvantages of 2,4-DNPH. PFBHA reacts quantitatively, even with conjugated aliphatic aldehydes. The PFBHA derivatives do not decompose at an elevated temperature. Neither do they require a time-consuming cleanup step. The resulting oximes can be easily resolved by GC.

Scheme Reaction of PFBHA with aldehyde or ketone to form an oxime



Other Products for Aldehyde and Ketone Analysis from Sigma-Aldrich

Besides the PFBHA reagents, Sigma-Aldrich offers products for both collection, preparation and analysis of aldehydes and ketones.

SPME (Solid Phase Microextraction)

SPME with on-fiber PFBHA derivatization has been applied to environmental monitoring of aldehydes and ketones. The references cited here use a PDMS-DVB fiber coating, which, because of its high affinity for amines, retains the oxime derivatives well. The procedure is simple: soak the fiber in the diluted derivatizing

reagent and expose it to the sample headspace or to the air for TWA analysis.

radiello® passive/diffusive samplers

The radiello® passive/diffusive samplers are ideal for long- and short-term sampling of aldehydes and ketones, along with many other analytes, in both indoor and outdoor air. The radial design offers higher capacity and faster uptake/sampling rates than traditional passive monitors. www.sigma-aldrich.com/radiello

Supelco capillary GC columns

For GC analysis of the PFBHA derivatives according to EPA Methods 556 and 556.1 "Determination of Carbonyl Compounds in Drinking Water", we recommend a Supelco SLB™-5ms capillary GC columns (30 m x 0.25 mm I.D. x 0.25 µm df) as a primary column, and an Equity-1701 of the same dimensions as a confirmation column. These columns provide excellent resolution and fast, reproducible separations.

Recommended reading

PFBHA derivatives can be analyzed via LC with UV detection. A sensitive and selective method for common aldehydes and ketones in water and air is suggested in:

- 1) Synthesis of the *O*-(2,3,4,5,6-Pentafluorobenzyl)Hydroxylamine Oximes of Selected Carbonyl Compounds and Their Determination by Liquid Chromatography with Ultraviolet Detection. K. Wiesenthal, A. Jehlar, S.S. Que Hee; *Journal of AOAC International* **2000**, 83, 859.

The combination of SPME (solid phase microextraction) and PFBHA derivatization in environmental monitoring can be used for a large number of compounds. In most cases, on-fiber derivatization has been used. See the following references:

- 2) Formation and reaction of hydroxycarbonyls from the reaction of OH radicals with 1,3-butadiene and isoprene. J. Baker, J. Arey, R. Atkinson; *Environ. Sci. Technol.* **2005**, 39, 4091-4099.
- 3) Comparison of extraction techniques for GC determination of volatile carbonyl compounds in alcohols. W. Wardencki, J. Namiesnik, J. Orlita; *Fresenius' J. Anal. Chem.* **2001**, 369, 661-665.
- 4) Determination of aldehydes in drinking water using penta fluorobenzylhydroxylamine derivatization and solid-phase microextraction. F. Ventura, B. Cancho, M. T. Galceran; *Journal of Chromatography A* **2002**, 943, 1-13.
- 5) Sampling and Determination of Formaldehyde Using SPME with On-Fiber Derivatization. P. Martos, J. Pawliszyn; *Anal. Chem.* **1998**, 70, 2311-2320.

- 6) Field Sampling and Determination of Formaldehyde in Indoor Air with SPME and On-Fiber Derivatization. J. Koziel, J. Noah, J. Pawliszyn, *Environ. Sci. Technol.* **2001**, *35*, 1481-1486.
- 7) Gas chromatographic determination of glutaraldehyde in the workplace atmosphere after derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine on a solid-phase microextraction fibre. G. Pieraccini, G. Bartolucci, P. Boccalon, S. Dugheri, L. Focardi, M. Pacenti, *Journal of Chromatography A* **2002**, *955*, 117-124.
- 8) Time-weighted average sampling of airborne n-valeraldehyde by a solidphase microextraction device. S.-W. Tsai, T.-A. Chang, *Journal of Chromatography A* **2002**, *954*, 191-198.

Table Product listing

PFBHA derivatives and PFBHA Hydrochloride

Cat. No.	Brand	Description	Package Size
15875	Fluka	Acetaldehyde-O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
44114	Fluka	Acetone O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
65819	Fluka	Acrolein O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
42094	Fluka	Crotonaldehyde-O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
41558	Fluka	Formaldehyde O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
03718	Fluka	Glutaraldehyde bis-(O-pentafluorophenylmethyloxime)	10 mg, 50 mg
43508	Fluka	Propionaldehyde O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
66156	Fluka	Valeraldehyde-O-pentafluorophenylmethyl-oxime	10 mg, 50 mg
76735	Fluka	O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride, derivatization grade	250 mg, 1g

Capillary GC columns for aldehyde and ketone analysis by EPA Methods 556 and 556.1

Cat. No.	Brand	Description
28471-U	Supelco	SLB-5ms, 30 m x 0.25 mm I.D. x 0.25 µm d _f
28465-U	Supelco	SLB-5ms, 10 m x 0.10 mm I.D. x 0.10 µm d _f
28372-U	Supelco	Equity 1701, 30 m x 0.25 mm I.D. x 0.25 µm d _f
inquire	Supelco	Equity 1701, 10 m x 0.10 mm I.D. x 0.10 µm d _f

SPME fibers and accessories for aldehyde and ketone analysis

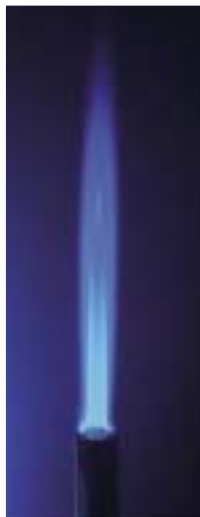
Cat. No.	Brand	Description	Package Size
57317	Supelco	PDMS/DVB fiber, 24 ga, StableFlex™ (for autosamplers)	3 fibers
57310-U	Supelco	PDMS/DVB fiber, 24 ga, fused silica (for manual holder)	3 fibers
57330-U	Supelco	SPME Fiber Holder, for use with manual sampling	each

radiello® passive/diffusive samplers for aldehyde and ketone sampling

Cat. No.	Brand	Description	Package Size
RAD165	Supelco	Adsorbent Cartridge for sampling aldehydes, matrix SS net with 2,4-DNPH coated Florisil®	20
RAD1201	Supelco	Diffusive Blue Body, configured for sampling light sensitive compounds	20
RAD121	Supelco	Triangular Support Plate	20

New PBDE Flame Retardant Standards Sigma-Aldrich offers all components for chromatographic analysis of these harmful substances

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



Use and concern over PBDEs

Polybrominated diphenyl ethers (PBDEs) are flame retardant chemical additives to polymers (plastics) that are used in the manufacture of printed electronic circuit boards, clothing, drapery, upholstery, cushioning foams for furniture, batteries, automotive interiors, boats and many other consumer products. The flame retardant activity is a result of the effective electron-scavenging action of bromine. PBDEs comprise a large number of congeners that are often used in combinations; the choice of individual congener or blend depends on the polymer formulation and its end use.

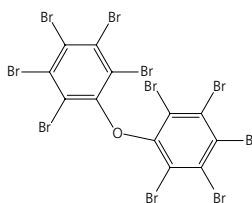


Figure 1

Decabromodiphenyl ether.
Flame retardant still permitted under current EU regulations.

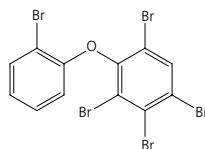


Figure 2

Pentabromodiphenyl ether.
A EU-banned flame retardant substance.

Although their life-saving benefits and value as flame retardants are indisputable, leaching of PBDEs and workplace exposure pose environmental and health threats [1, 2]. Exacerbated by their ubiquitous use, they are persistent organic pollutants prone to bioaccumulation. Measured levels of PBDEs in human tissue have been doubling every 4 to 5 years since 1970. Studies have shown high PBDE levels in neonatal blood matching the levels found in the mother's blood [3], and substances related to Penta-BDE have been found in breast milk and other tissues [4]. Besides concern over the effects of PBDEs on humans, other studies show that there may be additional environmental dangers, including causing thin shells in Peregrine falcon eggs [4, 5]. Although their specific health consequences have not been fully elucidated, they are similar structurally to thyroid hormones and exhibit a wide spectrum of toxicity in laboratory animals [6, 7].

Risk Assessment

Many PBDE-based flame retardants have been phased out, but because of the importance of PBDEs to both manufacturers and consumer safety and the lack of ideal alternatives, it was deemed unwise to eliminate them completely as a class without further study. To date, all commercial PBDE products have undergone evaluation

under the Existing Substances Regulation 793/93/EEC [8]. Based on this risk assessment, the EU has banned the use of Penta- and Octa-BDE, but permitted Deca-BDE. A summary of progress is available on the Web site www.cefic-efra.com under "Library". Summarizing [9]:

"Penta-BDE and Octa-BDE. For both these products, the Risk Assessments concluded that their use presented no risk to consumers' health through contacts with treated products, but raised questions concerning possible effects on industry workers handling the pure chemical and concerning possible bioaccumulation. Therefore neither of these products are used today in the EU.

Deca-BDE. After ten years of extensive studies, the final Risk Assessment was completed in 2004 and confirmed in 2005. The study concludes that the use of the product presented no risk for human health, neither through consumer contact with treated products, nor to workers using the product in industry, nor for indirect exposure of the population via presence of the product in the environment. Also, the report concludes that the product presents no identified risk for the environment, via air, water, soil, sediments or sewage sludges, but that environmental monitoring and further studies should be continued."

Regulations

The primary regulatory methodologies regarding the analysis of PBDEs include:

- EPA Method 527. Determination of Selected Pesticides and Flame Retardants in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography / Mass Spectrometry (GC/MS). Includes, besides various pesticides, PBDE-47, PBDE-99, PBDE-100, PBDE-153.
- Draft EPA Method 1614. Brominated Diphenyl Ethers in Water, Soil, Sediment and Tissue by HRGC/HRMS. Eight congeners of primary interest are listed: PBDE-28, PBDE-47, PBDE-99, PBDE-100, PBDE-154, PBDE-153, PBDE-183, PBDE-209.
- ISO 22032:2006. Water quality. Determination of selected polybrominated diphenyl ethers in sediment and sewage sludge. Method using extraction and gas chromatography/mass spectrometry. PPBDE congeners determined: PBDE-47, PBDE-99, PBDE-100, PBDE-154, PBDE-153, PBDE-183, PBDE-209 (does not include PBDE-28).

Reliable Analytical Methods using Sigma-Aldrich Products

Underlying the ongoing deliberation over the regulation of PBDEs is the need for reliable analytical methodologies. Three components are important to a successful analysis of PBDEs:

- PBDE standards
- Solid phase extraction tubes
- Capillary GC columns

Sigma-Aldrich's analytical brands, Supelco and Fluka/Riedel-de Haën, carry all of the necessary components. Recently, we completed our offering of PBDE standards by introducing sixteen new congeners (see Table). Shown in **Figure 3** is the GC-MS analysis of fourteen PBDE compounds on a Supelco SLB-5ms capillary GC column, which is ideally suited for this sensitive analysis.

Table 1 Product Listing of PBDE Standards

Ampuls of 50 µg/mL PBDE standard in isoctane, except as noted. All products are Riedel-de Haën brand; pack size is 1 mL

	Cat. No.	Name	Description
NEW	33661	PBDE 3	4-Bromodiphenyl ether
NEW	33662	PBDE 15	4,4'-Dibromodiphenyl ether
NEW	33663	PBDE 28	2,4,4'-Tribromodiphenyl ether
NEW	33664	PBDE 36	3,3',5'-Tribromodiphenyl ether
	34123	PBDE 37	3',4',4'-Tribromodiphenyl ether
NEW	33670	PBDE 47	2,2',4,4'-Tetrabromodiphenyl ether
NEW	33671	PBDE 49	2,2',4,5'-Tetrabromodiphenyl ether
	34119	PBDE 66	2,3',4,4'-Tetrabromodiphenyl ether
	34118	PBDE 71	2,3',4',6-Tetrabromodiphenyl ether
	34116	PBDE 75	2,4,4',6-Tetrabromodiphenyl ether
	34115	PBDE 77	3,3',4',4-Tetrabromodiphenyl ether
	34114	PBDE 85	2,2',3,4',4-Pentabromodiphenyl ether
NEW	33676	PBDE 99	2,2',4,4',5-Pentabromodiphenyl ether
NEW	33681	PBDE 100	2,2',4,4',6-Pentabromodiphenyl ether
	34121	PBDE 119	2,3',4,4',6-Pentabromodiphenyl ether
NEW	33682	PBDE 126	3,3',4,4',5-Pentabromodiphenyl ether
	34122	PBDE 138	2,2',3,4,4',5'-Hexabromodiphenyl ether
NEW	33683	PBDE 153	2,2',4,4',5,5'-Hexabromodiphenyl ether
NEW	33684	PBDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether
NEW	33685	PBDE 181	2,2',3,4,4',5,6-Heptabromodiphenyl ether
NEW	33686	PBDE 183	2,2',3,4,4',5',6-Heptabromodiphenyl ether
NEW	33687	PBDE 203	2,2',3,4,4',5,5',6-Octabromodiphenyl ether
NEW	33688	PBDE 205	2,3,3',4,4',5,5',6-Octabromodiphenyl ether
NEW	33689	PBDE 206	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether
	33617	PBDE 207	2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether, 10 µg/mL in isoctane
	34120	PBDE 209	Decabromodiphenyl ether, 50 µg/mL in isoctane:toluene (9:1)

Continue to look to Sigma-Aldrich for high quality, relevant products to meet today's most pressing analyses.

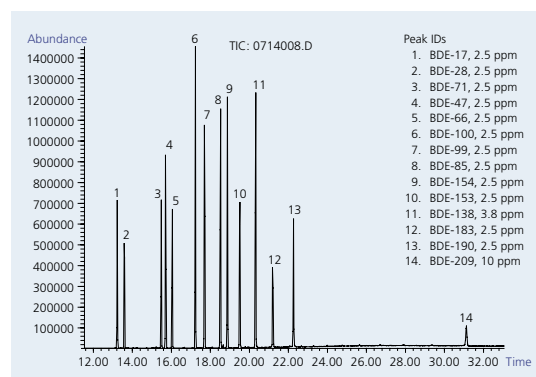


Figure 3 Decabromobiphenyl ethers by GC-MS

Conditions: Capillary GC column: Supelco SLB™-5ms, 30 m x 0.25 mm I.D. x 0.25 µm (Cat. No. 28471-U); Oven: 125 °C (1 min.), 10 °C/min. to 340 °C (15 min.); Injector: 300 °C, MSD interface: 340 °C; Scan range: SIM; Carrier gas: Helium, 1.5 mL/min., constant; Injection: 1 µL, splitless, pulsed (30 psi until 0.2 min.); Liner: 4 mm I.D. single taper.

Sample: PBDE standards, 2.5-10 µg/mL

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For Additional Information

BSEF - Bromine Science and Environmental Forum: www.bsef.com
EFRA - European Flame Retardants Association: www.cfec-efra.com

TraceCERT™ – Traceable Certified Reference Materials. Part 3: Challenges in the characterization of high-purity starting materials This is the third article of a series on Certified Reference Materials to appear in Analytix.

By Michael Weber, Manager R&D Europe, Sigma-Aldrich Switzerland ... michael.weber@sial.com
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Figure 1
Selected high purity
metals as starting
materials for
TraceCERT™ standard
solutions



How to determine >99.9% purity

In the last article of this series about CRMs (Certified Reference Materials) we discussed the almost unique feature of chemical measurement that a 100% pure material would form a natural reference value which cannot be exceeded [1, 2]. Therefore, the calibration with high purity materials is accordingly a valid method of establishing traceability in analytical chemistry [3]. It goes without saying that with this traceability approach, a comprehensive characterization of the starting material is of crucial importance. So, when a chloride reference is made from sodium chloride, the exact sodium content of the salt can be determined with very high accuracy. However, the purity determination of a substance with more than 99.95% purity (salt or metal)

requires an analytical method with much less than 0.05% measurement uncertainty. This is practically impossible for most analytical techniques.

As a consequence, the common sense method for characterization of high purity materials is the so-called impurity approach whereby as many impurities as possible are measured in a certain material by as many different analytical techniques as necessary. The sum of all the impurities (and also the contribution from the potential impurities below their detection limit) is then subtracted from the maximum purity of 100%. With this approach, it is possible to assign a reliable purity statement to high purity materials, even for very high purity grades (>99.99 %). Of course, this approach gives reliable results only when much effort is put into the impurity investigations. For example, it makes no sense to look for the thirty most common metallic impurities in a high purity sodium chloride, while at the same time overlooking the major impurities, bromide and water. How the various types of impurities contribute to the overall starting material purity is diagrammed in **Figure 2**.

From m6N to t3N materials or about apples and oranges

When considering high purity materials, one should always look closely at the details, especially how the purity is defined. The following nomenclature has been established: When a material is assigned to be >99.99% pure based on metallic impurities only, this is declared as an m4N material. Here, the „m“ signifies that only metallic impurities are identified, and the “4N” stands for the „four nines“ (99.99%) purity of the material. In some cases, such „m-characterized“ materials are also defined as „metals base.“ It is important to remember that the number of investigated elements is of great importance. All too often, certificates of analyses report only a very few number of trace metals.

A more reliable purity statement is provided when not only metallic impurities are identified, but also nonmetals, anions, oxides and residual water. In this case a „t“ (for total) replaces the „m“ (for metals only). It should come as no surprise that in many cases the reported purity (number of nines) is lower when the sum of all impurities, not only the metals, is reported. A material declared as a 99.9999% (m6N) purity can be

Table Selected starting materials used for TraceCERT™ standard solutions

Starting material	Purity (%)	Uncertainty (%)	Detected major impurities (ppm)
NaBr	99.881	0.052	Cl (932), K (44), F (43), SO ₄ (23)
NaCl	99.980	0.015	K (5), Br (30)
CaCO ₃	99.954	0.033	Sr (44), Mg (40), Na (36)
Zinc metal	99.975	0.012	K (5)
Cobalt metal	99.951	0.018	Ni (420), P (30), Fe (5), Sn (5)
Magnesium metal	99.991	0.010	Zn (40), Mn (18), Fe (10), Al (9)

„downgraded“ to a 99.9% (t3N) when also taking nonmetal impurities into account [4]. Of course, the total impurity approach requires more analytical effort and is more costly. On the other hand, the approach leads to greater reliability.

When we source high quality starting materials for **TraceCERT™** calibration solutions, we often find that the purity is actually lower than what is reported on the supplier's certificate of analysis. This is due to the fact that manufacturers typically test for only a few impurities, and the contribution of nonmetals and anions to purity is often overlooked. We have concluded that it is not a trivial pursuit to find starting materials on the market with accurately reported purity values of >4N or >5N.

Challenges particular to high purity salts and metals

There are many challenges and also knock-out criteria during the process of evaluating and choosing appropriate starting materials.

Problem No. 1: Water

When using NaCl as the starting material for a chloride calibration solution, drying at 110 °C for 4 hours followed by cooling over anhydrous magnesium perchlorate is not sufficient to ensure complete dryness of the salt. Investigations with TGA-MS show that only above 450 °C does NaCl completely lose its water. Since the amount of water can be as high as 20,000 ppm (0.2%), failure to remove it has a significant impact on the purity results. To guarantee complete dryness, NaCl has to be dried at 500 °C for at least 4 hours [5].

Problem No. 2: Stoichiometry

Na_2HPO_4 is used as starting material for phosphate calibration solutions. During the evaluation of a



Just as with high-precision watches, our CRM customers expect attention to detail in all quality parameters.

particular batch of Na_2HPO_4 , HR-ICP-MS, ICP-OES (for metals), TGA-MS (for water), IC (for anions) and combustion analysis (for carbon) were used to measure purity. Even though 73 different impurities were identified, one significant impurity was missed. When a second IC method was run, significant levels of the dihydrogen analog, NaH_2PO_4 , were found in the starting material. This affected the total content of phosphate in the starting material due to the different stoichiometry of the impurity.

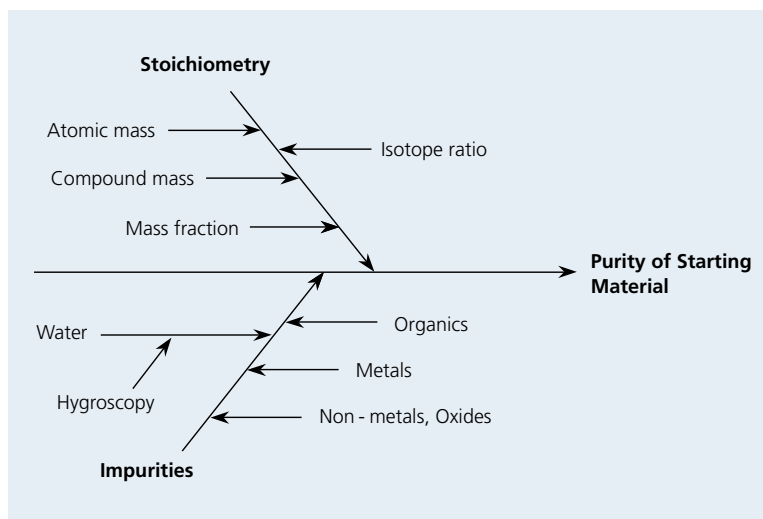
Problem No. 3: Isotope ratio and atomic mass

Most elements, rather than being naturally monoisotopic, exist in a natural isotopic ratio. Since this ratio is not constant, it leads to significant variation in the atomic mass for different materials. The most important example might be lithium where the natural isotope ratio of ${}^6\text{Li}$ to ${}^7\text{Li}$ is approximately 7.6% to 92.4%. When using Li_2CO_3 as a starting material for a lithium calibration solution, this can lead to variation of the Li content up to 0.6% in the final solution.

Problem No. 4: Oxides

With pure metal starting materials, it is usually necessary to take into account oxide impurities. Oxygen, often at a few hundred ppm but rarely exceeding 1000 ppm, is found associated with many high purity metals. In terms of the „tXN“ nomenclature, this means that a t4N purity (>99.99%) is mostly not possible unless the standard is produced under an inert, oxygen-free atmosphere. On the other hand, for certain types of metals the content of oxide impurities is not relevant. However, more often than not their existence must be considered. Since the quantitative determination of oxide impurities in metals is rather difficult, it may be acceptable to not account

Figure 2
Visualization (cause-effect diagram) of relevant contributions to starting material purity



for the oxygen content itself, but to introduce an estimated contribution into the uncertainty budget of the purity calculation.

Implications for the evaluation of *TraceCERT*TM starting materials

The problems outlined previously drive home a few important lessons with regard to evaluating high purity starting materials.

1. Whenever possible and feasible, choose a pure metal over a salt as a starting material. Metals avoid problems with stoichiometry, residual water and hygroscopy. Also, because of their manufacturing process, metals normally do not contain appreciable amounts of nonmetallic, anionic or organic impurities. Another advantage of using metals is that they can be weighed more easily than salts and powders and there is no risk of electrostatic discharges during the weighing step. Even if the surface leaching and the dissolution of metals in acids take longer, using pure metals normally leads to higher accuracy.
2. If a salt must be used as a starting material, it is of utmost importance to eliminate all of the above-mentioned problems. Check for traces of residual water, control hygroscopy, know the stoichiometry and conduct comprehensive analysis of metallic, nonmetallic and anionic impurities. With this information, it is possible to properly characterize the salt. However, for the above-mentioned reasons, high purity salts tend to have higher uncertainties than metals.

*TraceCERT*TM purity assurance

Each starting material undergoes specific pre-treatment procedures. For metals this usually involves pre-cleaning with several different solvents followed by acid etching of the surface and drying over an argon atmosphere. Non-metallic compounds such as salts, carbonates, oxides, etc. are dried by individual drying procedures before the high-purity material is brought to the high-precision weighing room.

$$\text{Purity} = 100\% - \sum w(I_{\text{measured}}) - \sum \frac{1}{2} \cdot \text{DL}(I_{\text{unfound}}) - \sum w(I_{\text{estimated}})$$

Figure 3 Equation for the calculation of a purity statement of high-purity materials: Quantified impurities expressed as mass fraction (*w*) are subtracted from 100% (*I_{measured}*). In addition, contributions for „investigated but not found“ impurities (those below detection limit, *I_{unfound}*) and expected but not investigated impurities (*I_{estimated}*) are considered in the purity assessment. This conservative approach leads to more reliability and surety of *TraceCERT*TM calibration standards and reference materials.

For most of the *TraceCERT*TM starting materials, more than 70 metallic impurities are analyzed using either ICP-OES or ICP-MS in combination with AAS with flame or hydride generation. In addition, several anions are determined using ion chromatography or wet chemical methods. Water-containing materials are dried to absolute dryness by individual drying conditions up to 600 °C. When drying is impossible due to decomposition, water is determined by high-precision KF-titration.

The purity of the material is calculated as 100% minus the sum of the impurities that are actually found (*I_{measured}*). For non-detected impurities, a contribution of half of the detection limit is estimated and also subtracted (*I_{unfound}*). Last but not least, an estimated contribution (also as a mass fraction) by impurities that were not investigated is also considered (*I_{estimated}*).

Classification of materials by this conservative method (**Figures 2 and 3**), may yield pessimistic purity results and the purity may be actually higher than reported. Consequently, only very few materials are found on the market with a proven purity of more than 99.9% (t3N). However, for reference materials, the reliability of the certified value is the most important issue and we therefore decided to apply this approach for all *TraceCERT*TM starting materials without accepting any compromises.

For more information, please visit our *TraceCERT*TM Web site: www.sigma-aldrich.com/tracecert

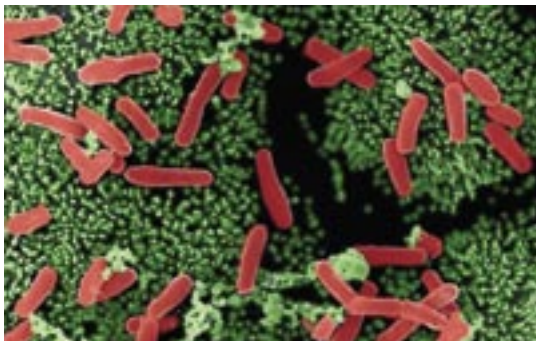
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Clostridia Diagnostic Detection, identification and differentiation of Clostridia species

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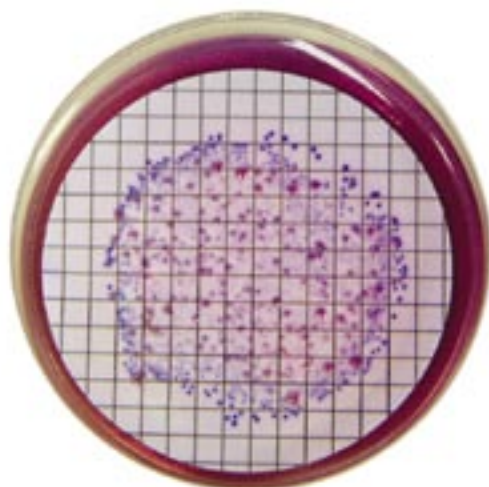
Figure 1
Clostridium difficile
adhering to the
microvilli of the gut



© Dr. J.A. Hobot, School of Medicine, Cardiff University, Medical Microscopy Sciences

Clostridia are relatively large, Gram-positive, rod-shaped bacteria that can undergo only anaerobic metabolism. Most Clostridia cannot grow under aerobic conditions and even can be killed by exposure to O₂, but they form endospores that are able to survive long periods of exposure to air and other adverse environmental conditions. The natural sources of Clostridia are anaerobic habitats with organic nutrients, particularly soils, aquatic sediments and the intestinal tracts of animals. Their fermentation of organic compounds, like sugars, produces large amounts of CO₂ and H₂ as well as volatile organic compounds like acetic and butyric acid, acetone and butanol. Metabolism of substrates like amino acids and fatty acids results in foul-smelling degradation products. Clostridia also have an extended range of extracellular enzymes that degrade large biological molecules in the environment into fermentable compounds. Although there are non-pathogenic Clostridia, this genus produces some of the most potent biological toxins. Three particularly bad actors in this

Figure 2
HiCrome™ M-CP
Agar Base Agar after
development with
ammonia



group are *C. perfringens*, which is responsible for cooked meat-associated food poisoning and wound and surgical infections that lead to gas gangrene, and *C. tetani*, which is responsible for deadly tetanus infections, and *C. botulinum*, which causes botulism.

Below are the most well-known pathogenic Clostridia species with their typical properties and occurrence:

Clostridium perfringens

- produces a huge range of invasins and exotoxins
- enzymes: hemolysins (β-hemolysis), lecithinase, extracellular proteases, lipases (phospholipase-C), collagenase, hyaluronidase, saccharolytic enzymes and is able to reduce sulphite to sulphide
- enterotoxins causes food poisoning
- found in improperly sterilized canned foods (germination of endospores) and water
- nonmotile

Clostridium difficile

- produces two enterotoxins toxin A and toxin B (lethal cytopathic toxin)
- enzymes: hydrolytic enzymes, *p*-hydroxyphenylacetate decarboxylase, ferments mannitol
- formation of *p*-cresol as the main fermentation product of tyrosin

Clostridium tetani

- toxin: tetanospasmin (causative tetanus)
- obligate anaerobe (sensitive to oxygen)
- sensitive to heat
- flagella give limited motility
- terminal spore (resistant to heat and most antiseptics)
- typical Gram-positive, may stain Gram-negative or Gram-variable, especially in older cells

Clostridium botulinum

- seven subtypes (A-G) produces different botulinum toxin (types C and D are not pathogenic)
- grow best in low-oxygen conditions
- subterminal endospores (resistant to boiling without pressure)
- occurrence: soil, aquatic sediments, decaying vegetation, found in improperly sterilized canned foods (germination of endospores)
- acidity, high concentration of sugar, very low levels of moisture or high levels of oxygen inhibits the growth
- enzyme: lipase production on egg yolk agars

Table 1 Media for Clostridia

Non-selective media	Brand	Cat. No.	Description
AC Agar	Sigma	A3340	Supports the growth of aerobic, anaerobic and microaerophilic microorganisms.
AEA Sporulation Broth (Base), modified	Fluka	17170	For early sporulation of <i>C. perfringens</i> from foods.
Alternative Thioglycollate Medium	Sigma	A0465	Recommended for sterility testing with certain biological products which are turbid or viscous.
Brain Heart Infusion Agar	Fluka	70138	A solid medium for the cultivation of fastidious pathogenic bacteria such as Streptococci and <i>Neisseria</i> .
Brewer Agar	Fluka	15997	For the culture of <i>Clostridium</i> species and other anaerobic microorganisms in surface culture.
Brewer thioglycollate medium	Sigma	B2551	Used for testing the sterility of biological products.
Brucella Agar with Hemin and Vitamin K	Sigma	B2926	Used for the isolation and subculture of anaerobes.
Casein peptone Lecithin Polysorbate Broth	Fluka	22089	For the enumeration of samples from pharmaceutical, cosmetic industry.
Clostridial Nutrient Medium	Fluka	27546	For the cultivation and enumeration of Clostridia and other anaerobes.
Clostridium Agar	Fluka	27548	For the cultivation and enumeration of Clostridia and other anaerobes in food, clinical specimens and other material.
Columbia Agar	Fluka	27688	For the cultivation of fastidious microorganisms.
Cooked Meat Broth	Fluka	60865	For the primary cultivation of aerobic, microaerophilic and anaerobic bacteria from clinical specimens.
Liver Broth	Fluka	61724	For preliminary testing and for the enrichment of Clostridia and other anaerobes from meat, food and other material.
Reinforced Clostridial Agar	Sigma	R0898	Used for the cultivation and enumeration of Clostridia.
Skim Milk Agar, modified	Fluka	17175	For cultivation and enumeration of microorganisms encountered in dairy industry.
Thioglycolate Broth	Fluka	70157	A medium for sterility tests and the cultivation of microaerophilic and anaerobic organisms.
Thioglycolate Broth with Resazurine	Fluka	90404	For cultivation of aerobic and anaerobic organisms, and for sterility testing. Any increase in the oxygen content is indicated by a color change of redox indicator resazurin.
Tryptone Soya Broth without Dextrose	Sigma	T3938	Recommended for the cultivation of anaerobes from root canals and blood.
Differential media	Brand	Cat. No.	Description
Blood Agar (Base)	Fluka	70133	A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms.
Clostridial Differential Broth	Fluka	27544	For the MPN enumeration of all Clostridia in food and other material.
Gelatin Iron Agar	Sigma	G0289	Used for detecting gelatin liquefaction and hydrogen sulphide production.
Lactose Gelatin Medium (Base)	Fluka	61348	For the detection of lactose and gelatin metabolizing microorganisms (<i>C. perfringens</i>).
Meat Liver Agar	Fluka	46379	For the cultivation of anaerobic microorganisms.
Motility Nitrate Medium	Fluka	14305	Differential medium for motile nitrate-utilizing microorganisms (<i>P. aeruginosa</i> , <i>C. perfringens</i>).
Nutrient Gelatin	Fluka	70151	Nutrient gelatin is recommended for the determination of gelatin-liquefying microorganisms and enumeration of proteolytic organisms in water.
Nutrient Gelatin special grade	Fluka	70198	For electronic microcolony counting (Coulter Counter) in the bacteriological examination of milk.
Sulfite Iron Agar	Fluka	86128	For the detection and enumeration of <i>Clostridium</i> species in meat and meat products.
Tributyryn Agar	Fluka	91015	For the detection and enumeration of lipolytic microorganisms in food and other material.
Selective media	Brand	Cat. No.	Description
Clostridium difficile Agar (Base)	Fluka	17145	Used with supplement for cultivation of <i>C. difficile</i> from food and certain pathological specimens.
Wilkins Chalgren Anaerobic Agar	Sigma	W1761	Used for the isolation of anaerobic bacteria.
Selective differential media	Brand	Cat. No.	Description
HiCrome™ M-CP Agar Base	Fluka	75605	Selective chromogenic media recommended by the Directive of the Council of the European Union 98/83/EC for isolation and enumeration of <i>C. perfringens</i> from water (see Figure 1).
SPS Agar	Fluka	85627	For the isolation and enumeration of <i>C. perfringens</i> and <i>C. botulinum</i> in food.
SPS Agar, modified	Fluka	17231	For the selective isolation and enumeration of <i>C. perfringens</i> from foods.
TSC Agar	Fluka	93745	For the isolation and enumeration of vegetative forms as well as spores from <i>C. perfringens</i> in food, clinical specimens and other material.
TSN Agar	Fluka	93735	Highly selective medium for the detection and enumeration of <i>C. perfringens</i> in food and other material.

Identification of Clostridia is an important first step toward the control and eradication of this potent pathogen. To aid in the diagnosis, Sigma-Aldrich has developed a broad range of selective media (**Table 1**), tests (**Tables 2 and 3**) and anaerobic equipment (**Table 4**) for the detection, identification and differentiation of Clostridia.

Further details about the media, tests and equipment for Clostridia and many other pathogens can be found on our Web site:

www.sigma-aldrich.com/microbiology

Table 2 Tests for identification and differentiation of Clostridia

Test for Clostridia Diagnostics	Brand	Cat. No.	Description
Aminopeptidase Test	Fluka	75554	For the detection of L-alanine-aminopeptidase which is found primarily in Gram-negative microorganisms.
Mannitol Disks	Fluka	94438	Used to differentiate bacteria on the basis of mannitol fermentation.
Nitrate Reagent Disks	Fluka	08086	Used to detect an organism's ability to reduce nitrate.
Tributyryn-Strips	Fluka	75744	The test principle is hydrolysis of tributyrin. This reaction causes color change of acidobasic indicator.

Table 3 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 Anaerobic equipment

Anaerobic Equipment	Brand	Cat. No.
Anaerobe Atmosphere Generation Bags	Fluka	68061
Anaerobe Indicator Test	Fluka	59886
Anaerobe Jar Insert for Petri Disks	Fluka	68886
Anaerobic Jar	Fluka	28029

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HYDRANAL® Application: Fats and Fat Products Improving solubility of oily samples by use of specially designed HYDRANAL®-reagents

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



Figure 1
fats and butter products

Generally, the water content in fats and fat products can easily be determined using the Karl Fischer titration. The most important task, in order to be able to detect the complete water in these samples, is to dissolve them as completely as possible in the Karl Fischer working medium. Most fats and oils show limited solubility in alcohols like methanol and ethanol, which are generally used as working media.

In order to enhance the solubility of fats and fat products in the Karl Fischer reagents to obtain at least a dispersion, solubilizing agents can be added to the working medium, such as long-chained alcohols or chlorinated hydrocarbons. Our specially designed HYDRANAL®-reagents offer different possibilities to substitute or complement the Karl Fischer working media:

For volumetric one-component determinations:

- HYDRANAL®-LipoSolver CM; chloroform-based working medium for water determination in non-polar samples, fats and oils
- HYDRANAL®-LipoSolver MH; chloroform-free working medium for water determination in non-polar samples, fats and oils
- HYDRANAL®-Solver (Crude) Oil; working medium containing xylene and chloroform for water determination in oils
- Mixture of HYDRANAL®-Methanol dry/HYDRANAL®-Methanol Rapid with HYDRANAL®-Chloroform (1:2)

For volumetric two-component determinations:

- HYDRANAL®-Solvent CM; solvent for water determination in oils, containing chloroform
- HYDRANAL®-Solvent Oil; solvent for water determination in oils, free of halogenated hydrocarbons
- Mixture of HYDRANAL®-Solvent with HYDRANAL®-Chloroform (1:2).

For coulometric determinations:

- HYDRANAL®-Coulomat AG-H; anolyte for titration of long-chained hydrocarbons, free of halogenated solvents (for cells with and without diaphragm)
- HYDRANAL®-Coulomat Oil; anolyte for the titration in oils containing chloroform and xylene as solubilizer; to be used in cells with diaphragm only
- Mixture of HYDRANAL®-Coulomat A with HYDRANAL®-Chloroform (2:1)

In some cases it can be advisable to additionally carry out the titration at elevated temperatures (max. 50 °C) in order to improve titration conditions for poorly soluble samples.

Being able to determine the exact water content of for example butter is of high importance for both food analysis and food production. There are guidelines and regulations about the water and fat content in butter which have to be followed, e.g. the Swiss regulation about animal foodstuff, the UK milk and milk products regulations or the German regulation for butter.

Water Determination in Butter (Laboratory Application 104*)

As butter is insoluble in methanol, a suitable solubilizer needs to be added to the working medium. The titration vessel is filled with 40 mL HYDRANAL®-Solvent CM. HYDRANAL®-Titrant 5 is used for titration to dryness. Approximately 0.5 g of butter are weighed in. The sample can be weighed in by using a syringe or directly by means of a tared PTFE weighing spoon, which remains in the solvent until the end of titration. After dissolution of the sample the water content is titrated, the titration time is about 2 minutes. The sample should be homogenized before analysis.

The titration vessel can also be filled with 40 mL HYDRANAL®-LipoSolver CM or a mixture of 20 mL HYDRANAL®-Methanol dry and 20 mL HYDRANAL®-Chloroform. In this case HYDRANAL®-Composite 5 is used as titration agent.

We are pleased to offer additional Laboratory Applications* for the determination of water in fats and fat products:


L 104	Butter
L 458	Butter fat
L 457	Fat emulsifier mixture
L 434	Fatty alcohol sulphate sodium salt
L 470	Fish oil silage
L 477	Grease with additives based on polyglycole
L 098	Hard fats
L 486	Linseed oil
L 083	Margarine
L 084	Mayonnaise
L 164	Peanut oil
L 319	Rape seed oil
L 075	Sunflower oil
L 544	Borage oil
L 063	Vegetable oil

* To request Laboratory Applications, please contact our HYDRANAL® Laboratories at helga.hoffmann@sial.com

Table Product list HYDRANAL® reagents

Cat. No.	Brand	Product	Package Size
34805	Riedel-de Haën	HYDRANAL®-Composite 5	500 mL, 1 L, 2.5 L, 5 L
34741	Riedel-de Haën	HYDRANAL®-Methanol dry	1 L, 2.5 L
37817	Riedel-de Haën	HYDRANAL®-Methanol rapid	1 L, 2.5 L
37855	Riedel-de Haën	HYDRANAL®-LipoSolver CM	1 L
37856	Riedel-de Haën	HYDRANAL®-LipoSolver MH	1 L
34697	Riedel-de Haën	HYDRANAL®-Solver (Crude) Oil	1 L, 2.5 L
34801	Riedel-de Haën	HYDRANAL®-Titrant 5	500 mL, 1 L, 2.5 L
34800	Riedel-de Haën	HYDRANAL®-Solvent	1 L, 2.5 L
34812	Riedel-de Haën	HYDRANAL®-Solvent CM	1 L, 2.5 L
34749	Riedel-de Haën	HYDRANAL®-Solvent Oil	1 L
37863	Riedel-de Haën	HYDRANAL®-Chloroform	1 L
34843	Riedel-de Haën	HYDRANAL®-Coulomat AG-H	500 mL
34868	Riedel-de Haën	HYDRANAL®-Coulomat Oil	100 mL, 500 mL

For more details about HYDRANAL® reagents, please visit our website www.sigma-aldrich.com/hydranal



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VOLPAC® Volumetric Solutions Newly supplied with adapters for direct connection to titration tubes

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

Figure 1
Specially designed adapter for VOLPAC® containers



Our popular 10 liter containers are now also available as 5 liter containers for volumetric solutions ready for use. A 5 L or 10 L-VOLPAC®-container consists of a flexible polyethylene bag with an outlet tap and a cubic cardboard supporting frame.

Due to the composition of the bag no volume compensation by access of air is necessary during the emptying process. VOLPAC®-containers can be drained to the dregs without contamination!

Advantages of VOLPAC®-containers:

- high quality of content and packing
- easy handling
- no contamination upon emptying
- reduced storage place
- reduced packing material
- easy disposal of empty containers

NEW All our VOLPAC®-containers are now supplied with a specially designed adapter to allow direct connection of your titrating tube to the VOLPAC®-container.

www.sigma-aldrich.com/titration

Table 1 VOLPAC® Volumetric Solutions by Riedel-de Haën

Cat. No.	Product name	VOLPAC® Size
33643	Buffer solution pH 4.0 (20 °C); citric acid / sodium hydroxide solution / sodium chloride; solution ready for use with fungicide	5 L, 10 L
33665	Buffer solution pH 4.0 (20 °C); citric acid / sodium hydroxide solution / sodium chloride; solution ready for use with fungicide; red colored	5 L, 10 L
33646	Buffer solution pH 7.0 (20 °C); potassium dihydrogen phosphate / di-sodium hydrogen phosphate; solution ready for use with fungicide	5 L, 10 L
33666	Buffer solution pH 7.0 (20 °C); potassium dihydrogen phosphate / di-sodium hydrogen phosphate; solution ready for use with fungicide; green colored	5 L, 10 L
33648	Buffer solution pH 9.0 (20 °C); borax / hydrochloric acid; solution ready for use	5 L, 10 L
33667	Buffer solution pH 9.0 (20 °C); borax / hydrochloric acid; solution ready for use; blue colored	5 L, 10 L
33668	Buffer solution pH 10.0 (20 °C); borax / sodium hydroxide solution; solution ready for use; violet colored	10 L
34544	IDRANAL® B; IDRANAL® III solution with zinc complex added; for water hardness determination (1 mL = 1 German degree of hardness in 100 mL water)	5 L, 10 L
35102	IDRANAL® III standard solution; 0.2 M	5 L, 10 L
34550	IDRANAL® III standard solution; Ph Eur 0.1 M	5 L, 10 L
35103	IDRANAL® IV standard solution; Ph Eur 0.1 M	5 L, 10 L
35328	Hydrochloric acid standard solution; Ph Eur 1 M	5 L, 10 L
35329	Hydrochloric acid standard solution; Ph Eur 0.5 M	5 L, 10 L
35335	Hydrochloric acid standard solution; Ph Eur 0.1 M	5 L, 10 L
34631	Potassium dichromate standard solution; for COD determination according to DIN 38409, part 41; 0.02 M	5 L, 10 L
35116	Potassium hydroxide standard solution; 0.5 M	5 L, 10 L
35175	Potassium iodide standard solution; 3 M	5 L, 10 L
35375	Silver nitrate standard solution; Ph Eur 0.1 M	5 L, 10 L
34277	Sodium carbonate standard solution; 0.5 M	5 L, 10 L
34247	Sodium fluoride standard solution; 40 g/L	10 L
35256	Sodium hydroxide standard solution; Ph Eur 1 M	5 L, 10 L
35257	Sodium hydroxide standard solution; 0.5 M	5 L, 10 L
33337	Sodium hydroxide standard solution; 0.28 M	10 L
35260	Sodium hydroxide standard solution; 0.25 M	5 L, 10 L
35261	Sodium hydroxide standard solution; 0.2 M	10 L
35263	Sodium hydroxide standard solution; Ph Eur 0.1 M	5 L, 10 L
35262	Sodium hydroxide standard solution; 0.01 M	10 L
34246	Sodium oxalate solution; 25 g/L	10 L
35233	Sodium thiosulfate standard solution; 0.2 M	5 L, 10 L
34449	Sodium thiosulfate standard solution; 0.05 M	10 L
35224	Sodium thiosulfate standard solution; for determination of iodine adsorption number according to ASTM D 1510; 0.0394 M	5 L, 10 L
35245	Sodium thiosulfate standard solution; Ph Eur 0.01 M	5 L, 10 L
35348	Sulfuric acid standard solution; 2.5 M	5 L, 10 L
35354	Sulfuric acid standard solution; Ph Eur 0.5 M	5 L, 10 L
35355	Sulfuric acid standard solution; Ph Eur 0.25 M	5 L, 10 L
35357	Sulfuric acid standard solution; Ph Eur 0.1 M	5 L, 10 L
35358	Sulfuric acid standard solution; Ph Eur 0.05 M	5 L, 10 L



SPECIAL OFFER: 30% OFF ON YOUR FIRST ORDER ON VOLPAC® VOLUMETRIC SOLUTIONS listed in Table 1.

Please quote promotion code Y63 when placing your order. Valid until May 31st 2007.

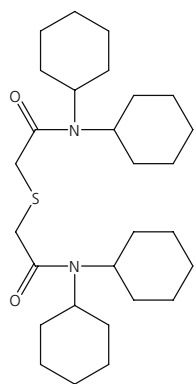
Selectophore® Products Materials for the preparation of potentiometric & optical sensors and ion-selective electrodes

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

Introduction

Ion-selective electrodes provide accurate, rapid, sensitive and non-destructive analyses, often at much lower cost compared to other analytical methods. Ion-selective electrodes employ ionophores, chemicals that function to transport small molecules across a membrane. When used in potentiometric or optical sensors, ionophores and auxiliary components (polymers, plasticizers, additives, etc.) have special requirements compared to when they are used in other applications. Sigma-Aldrich's Selectophore® products, the most comprehensive line on the market today, fulfill the stringent quality requirements for use in the preparation of sensor membranes for ion selective potentiometric and optical devices.

The development of new ion-selective electrodes and innovative applications continues to be an interesting area of analytical research. The four ionophores described in this article are recent additions to our Selectophore® line.



Copper Ionophore IV, Selectophore®

Function tested (Fluka 50242)

N,N,N',N'-Tetracyclohexyl-3-thiaglutaric diamide

Drinking water can become contaminated with Cu(II) ions from additives designed to control algal growth and from contact with copper plumbing and copper-containing fixtures. If corrosive water remains motionless in the plumbing system, copper levels may exceed 1,000 µg/L (1.6×10^{-5} M). Long-term exposure (more than 14 days) to copper in drinking water at those levels has been linked to kidney and liver damage in infants.

The U.S. Environmental Protection Agency (U.S. EPA) has determined that copper levels in drinking water should not exceed 1,300 µg/L. According to the European Drinking water directive (Council Directive 98/83/EC) on the quality of water intended for human consumption, the allowable limit is 2,000 µg/L.

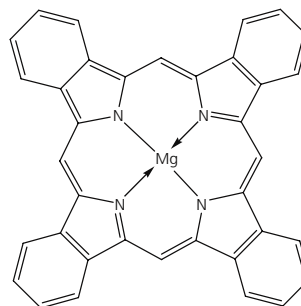
Copper Ionophore IV is especially suitable for monitoring drinking water since Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} do not interfere with the measurement, even if they are present in very high concentrations.

Characteristics

Linearity:	2×10^{-7} to 1×10^{-1} M in drinking water ($\text{Cu}(\text{NO}_3)_2$)
Slope (sensitivity):	33 mV/dec
Detection limit:	2×10^{-9} M (low ionic background) 1×10^{-7} M (high ionic background, typical of drinking water)
Shelf life:	>2 months

Reference

Szigetti, Z.; Bitter, I., Tóth, K.; Latkoczy, C.; Fliegel, D.; Günther, D.; Pretsch, E. *Anal. Chim. Acta* **2005**, 532, 129-136.



Cyanide Ionophore II, Selectophore®

Function tested (Fluka 40585)

Magnesium phthalocyanine, CAS# 1661-03-6

Cyanides are used extensively in many industrial processes. Frequently they occur in toxic wastes and wastewater of diverse origin, and in streams that receive uncontrolled and unpurified industrial effluents.

The Cyanide Ionophore II sensor is ideal for determining cyanide ions after a prior conversion into the dicyanoargentate $[\text{Ag}(\text{CN})_2]^{-}$ anion with the addition of small quantities of silver cyanide.

Table Product Table

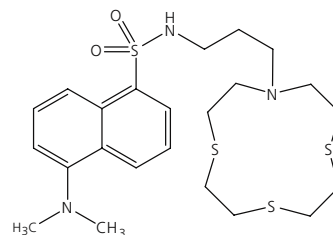
Cat. No.	Brand	Description	Package Size
50242	Fluka	Copper(II) Ionophore IV (<i>N,N,N',N'</i> -Tetracyclohexyl-3-thiaglutaric diamide)	10 mg, 100 mg
72017	Fluka	Tetrakis[3,5-bis(trifluoromethyl)phenyl] borate (NaTFPB)	10 mg, 50 mg, 500 mg
73732	Fluka	2-Nitrophenyl octyl ether (NPOE)	5 mL, 25 mL, 100 mL
81392	Fluka	Poly(vinyl chloride) high molecular weight	1 g, 10 g, 50 g
86643	Fluka	Tetraethylammonium nitrate (Et_4NNO_3)	5 g
87369	Fluka	Tetrahydrofuran	10 mL, 100 mL, 500 mL

Characteristics

Linearity: 1×10^{-5} to 1×10^{-2} M ($K[Ag(CN)_2]$)
 Slope (sensitivity): -55 mV/dec
 Detection limit: 7×10^{-6} M
 Shelf life: >2 months
 pH range: 5-7

Reference

Hassan, S.; Marzouk, S.; Mohamed, A.; Badawy, N. *Electroanalysis* 2004, 16(4), 298-303.

**NEW Fluoroionophore for Silver, Selectophore®**

10-[3-(Dansylamido)propyl]-1,4,7-trithia-10-azacyclododecane (Fluka 89998) $[12]aneNS_3$

Optical sensors (optodes) are based on substances which change their optical properties on interaction with the analyte. Optodes have increased in popularity because of improvements that have been made to their optical and electronic components.

The silver-selective fluoroionophore consists of an Ag^+ -ionophore and a signaling fluorophore connected by a flexible spacer. This fluorophore can be used for the direct determination of silver in waste material, drinking water, medical radiological film, photographic fixing solutions, bleaching solutions and other samples.

Characteristics

Analyte: Ag^+
 Indicator: $[12]aneNS_3$
 Additive: KTpClB
 Polymer matrix: PVC/NPOE
 Sensitive range
 (analyte in pH 6.5 buffer): 5.0×10^{-7} to 1.7×10^{-2} M
 LOD: 1×10^{-7} M
 λ_{ex} : 416 nm in H_2O (340 nm in MeCN)
 λ_{em} : 520 nm H_2O (512 nm in MeCN)
 Response time: <40s

Reference

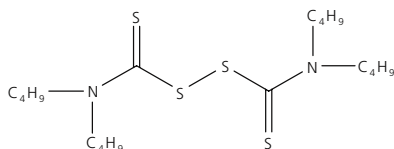
Shamsipur, M.; Alizadeh, K.; Hosseini, M.; Caltagirone, C.; Lippolis, V. *Sens. Actuators B* 2006, 113, 892-899.

Table Product Table

Cat. No.	Brand	Description	Package Size
89998	Fluka	10-[3-(Dansylamido)propyl]-1,4,7-trithia-10-azacyclododecane ($[12]aneNS_3$)	25 mg
60591	Fluka	Potassium tetrakis(4-chlorophenyl)-borate	100 mg, 1 g, 5 g
73732	Fluka	2-Nitrophenyl octyl ether (NPOE)	5 mL, 25 mL, 100 mL
81392	Fluka	Poly(vinyl chloride) high molecular weight	1 g, 10 g, 50 g
87369	Fluka	Tetrahydrofuran	10 mL, 100 mL, 500 mL

Table Product Table

Cat. No.	Brand	Description	Package Size
40585	Fluka	Cyanide Ionophore II (Magnesium phthalocyanine)	50 mg
91661	Fluka	Tridodecylmethylammonium chloride (TDMAC)	100 mg, 1 g
73732	Fluka	2-Nitrophenyl octyl ether (NPOE)	5 mL, 25 mL, 100 mL
81392	Fluka	Poly(vinyl chloride) high molecular weight	1 g, 10 g, 50 g
379166	Aldrich	Potassium dicyanoargentate	10 g, 50 g
184535	Aldrich	Silver cyanide	10 g, 50 g
87369	Fluka	Tetrahydrofuran	10 mL, 100 mL, 500 mL

**Zinc Ionophore I, Selectophore®**

Function tested (Fluka 96491)
 Tetra-n-butyl thiuram disulfide, CAS# 1634-02-2

Zinc is widely used in electroplating, paint, chemical and pharmaceutical operations. The detection of zinc ions by conventional analytical methods is difficult and complicated. The use of ion-selective electrodes dramatically simplifies the analysis.

Characteristics

Linearity: 1×10^{-6} to 1×10^{-1} M ($Zn(NO_3)_2$)
 Slope (sensitivity): 28.0 mV/dec
 Detection limit: 4.2×10^{-7} M
 Response time: 2-10s
 pH range: 3.5-6.5

Reference

Kojima, R.; Kamata, S. *Anal. Sci.* 1994, 10, 409-412.

Table Product Table

Cat. No.	Brand	Description	Package Size
96491	Fluka	Zinc Ionophore I (Tetra-n-butyl thiuram disulfide)	100 mg, 500 mg
60591	Fluka	Potassium tetrakis(4-chlorophenyl)borate	100 mg, 1 g, 5 g
73732	Fluka	2-Nitrophenyl octyl ether (NPOE)	5 mL, 25 mL, 100 mL
81392	Fluka	Poly(vinyl chloride) high molecular weight	1 g, 10 g, 50 g
87369	Fluka	Tetrahydrofuran	10 mL, 100 mL, 500 mL

Rinsing Solutions - The Afterwork Partners for Your Instrument

By Rudolf Koehling, Applications Development R&D, Sigma-Aldrich Switzerland ... rudolf.koehling@sial.com
and Joachim Emmert, LC-MS Specialist, Klinkner & Partner ... joachim.emmert@onlinehome.de



By daily work, your instrument is contaminated with sample matrix, salts etc. Especially bad are lipophilic and ionophoric or ion-pairing compounds. But in real samples this cannot be avoided all of the time. So it is necessary to clean the instrument from time to time or in certain cases, where sensitivity has dropped down dramatically. Sigma-Aldrich offers two rinsing solutions with different properties for different tasks. Rinsing solution I (water / 2-Propanol 50/50 %) has bipolar capabilities and can be used in combination with the connected HPLC-system. It removes mainly lipophilic and partly ion-pairing compounds. Rinsing solution II (water with 8% formic acid) is strongly acidic and cannot be used with connected HPLC. At least the column must be removed to avoid destruction of the

packing material (hydrolysis!). It is best directly infused into the MS with a syringe at low flow rates of about 5-10 µl/min. It helps best, when salts or other ionic species influence the MS-signal with low sensitivity, spikes and/or black-outs. Both solutions are prepared ready-to-use in tested high LC-MS grade quality.

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Valid until May 31st 2007.

Table LC-MS Rinsing Solutions

Cat. No.	Brand	Description	Package Size	Packaging Material
34689	Riedel-de Haën	Rinsing Solution I (water / 2-propanol 50/50 v/v)	1L	White glass bottle
34692	Riedel-de Haën	Rinsing Solution II (water with 8% formic acid)	1L	White glass bottle

Luff-Schoorl Reagent for the Determination of Reducing Sugars

By Andrea Felgner, Product Manager ... andrea.felgner@sial.com



For determination of reducing sugars in different matrices the method according to Luff-Schoorl can be used, whereby reducing monosaccharides like glucose, fructose and galactose as well as reducing disaccharides like lactose and maltose can be determined. This method can be applied for samples like sugar solutions, sugar containing foods like jams and confectionary products, fruit juices and nectars, vegetable products, vine, cereals and bakery products, and for determination of lactose in animal feeds.

Standard methods according to Luff-Schoorl are given e.g. by the International Commission for Uniform Methods of Sugar Analysis ICUMSA GS1-5 (1998) and the Swiss Federal Office of Public Health SLMB 28A/5.1.1.

Our new stable and ready for use Luff-Schoorl reagent is a solution of 24.9-25.1 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, containing Cu^{2+} ions in a carbonate basic solution at a pH value of 9.3-9.4. After a suitable sample preparation, which should include a Carrez clarification, the Luff-Schoorl reagent is added to the sample and immediately brought to a boil. As reducing sugars form up as open chain aldehydes or ketones in basic solutions, they are now oxidized while Cu^{2+} is reduced to Cu^+ and precipitates as Cu_2O . After addition of potassium iodide and sulfuric acid, excess Cu^{2+} can be titrated iodometrically using a sodium thiosulfate standard solution.

Table Product Listing

Cat. No.	Brand	Product	Package Size
34400 NEW	Riedel-de Haën	Luff-Schoorl Reagent	1 L
30315	Riedel-de Haën	Potassium Iodide	100 g, 250 g, 500 g, 1 kg, 5 kg
30743	Riedel-de Haën	Sulfuric acid 95-97 %	1 L, 2.5 L, 5 L
35245	Riedel-de Haën	Sodium thiosulfate solution 0.1 M	1 L, 5 L, 10 L
85655	Fluka	Starch solution 1 % in H_2O	100 mL, 500 mL

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Fax: 52 722 276 1601

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