

Analytix

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Polymer Analysis with LC/MS



- Selective adduct formation in LC-MS
- Monitoring of carcinogenics in foodstuffs
- Kit for measuring non-polar compounds in water
- Selective growth media for coliforms
- Water determination in aldehydes and ketones by KF
- Reagents for microscopy and ion chromatography

Editorial

Higher speed, higher sensibility, better solvents and chemicals the future of LC/MS



Rudolf Koehling
LC-MS Application Scientist

Dear Colleague,

Mass spectroscopy has emerged as one of the most versatile detection methods for routine analysis of all kinds of samples in almost all analytical fields, including clinical, pharmaceutical, forensic, metabolomics, proteomics, and many, many others. In the infancy of the hyphenated MS techniques, GC-MS dominated. Ion sources were very limited. The technique was amenable only to low molecular weight, derivatisable analytes which required extensive sample prep. Although GC-MS remains an important technique, the introduction of electrospray ionisation (ESI) to the wider analytical market opened new paths and made it possible to combine liquid chromatography with mass spectroscopy (LC-MS). As a result, HPLC became much more sensitive, unknown substances could be identified directly and, in most cases, sample preparation could be reduced to a fast and simple liquid-liquid extraction. Time-consuming screenings in pharmaceutical research have benefited in particular from the faster HPLC run times and the simultaneous identification of potentially toxic impurities and metabolites associated with the active ingredient.

Today, a growing number of instrument manufacturers offer affordable spectrometers with different mass analysers that are optimised for small molecules (sector field, ion trap, quadrupole) or large molecules (ToF). The HPLC systems and columns have also evolved to meet the requirements of LC-MS and to shorten the analysis time in order to increase sample throughput.

From the beginning, Sigma-Aldrich has played a part in the development of the fast-moving LC-MS market. First were our Riedel-de Haën solvents, followed by Fluka mobile phase additives and standards. These provide the ultra-high purity and low metal ion traces necessary to guarantee highly sensitive and reproducible LC-MS analyses. Today, MS manufacturers like Bruker-Daltonics recommend our solvents, and place their trust in us for our wide product selection, quality and world-wide distribution, necessary attributes for successful installation, operation and maintenance of their LC-MS systems. With our most recent innovation for LC-MS, Supelco Ascentis Express HPLC columns, we've addressed the need for increased speed and increased sensitivity. Ascentis Express columns take advantage of the technology behind today's fast LC instruments, like Rapid Resolution® and UPLC®.

Our R&D and Product Management teams work hard to keep pace with the evolving LC-MS market by supplying well-suited and well-characterised chemicals, reagents, columns and sample prep products with the quality necessary for the newest LC-MS instruments and techniques. We hope you turn to the Sigma-Aldrich family when you need the highest quality consumables for LC-MS and all of your most routine or demanding analytical applications. We pride ourselves on our expert technical support, so put us to the test! We look forward to working with you.

Kind regards,

Rudolf Köhling
LC-MS Application Scientist

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Selective adduct formation in electrospray ionisation with alkali citrates

The type and quality of the additive dramatically affect the quality of MS-spectra.

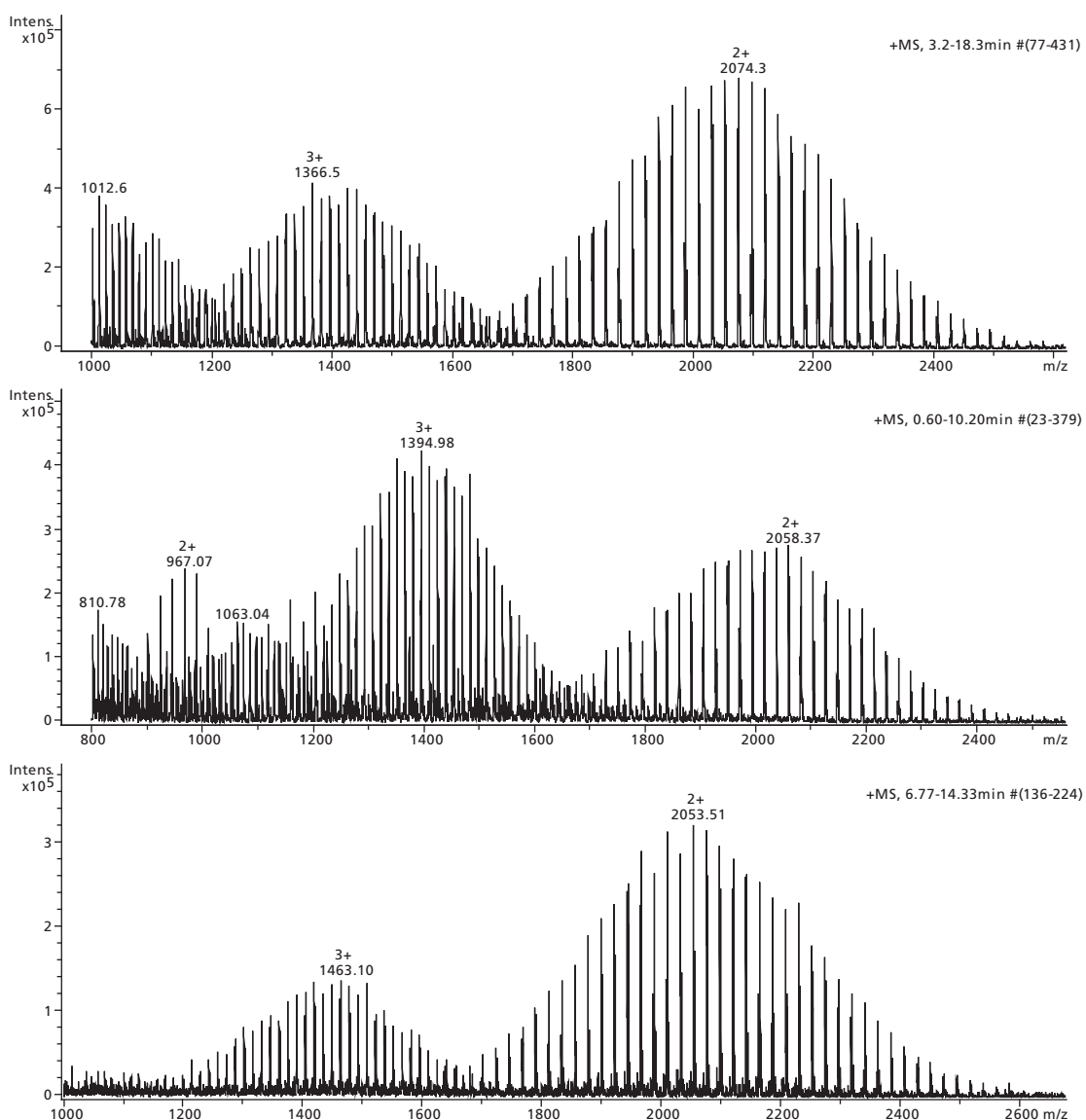
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Although alkali metal ions have a reputation of interfering with LC-MS analysis because they form unwanted adducts with certain analytes, in some cases the formation of $[M+cation]^+$ is desirable. For example, polyethyleneglycols (PEGs) require cations, like alkali metals or ammonium, to form detectable ions. PEGs are not uniform in their chain length and can vary with a more or less narrow distribution around an average chain length. Two or more cations can be attached to the polymer, forming multiply-charged ions. This results in the complex mass spectra shown in **Figure 1**.

Additionally, the type of cation can influence significantly the intensities of m/z distribution in the mass spectra (**Figure 1**). For example, sodium gives more triply-charged adducts whereas potassium and ammonium lead to more doubly-charged adducts.

Because of the influence of alkali metal ions on MS analysis, we offer a large variety of LC-MS additives that can be added directly to the sample to improve analytical results (**Table 1**). These additives are of highest available quality and do not contain traces of any other metal ions.

Figure 1 Mass spectra of PEG-4000 showing multiply-charged adducts with potassium (top), sodium (middle) and ammonium (bottom).



In the PEG system, ammonium formate provides the clearest spectrum, but the multiply-charged ions formed with sodium and potassium citrate can be utilised for mass spectrometers with a limited m/z range, e.g. LC-MS systems with ion traps or quadrupole mass analysers. As can be seen in **Figure 1**, the increase in charge shifts the distribution to lower m/z ratios, making it amenable to ion trap instruments with a maximum m/z of 3000 Da. Even the isotopic pattern of the fourfold-charged methoxy-PEG-5000 can be resolved with the Enhanced Mode of a Bruker Esquire 3000plus used in this study (**Figure 2**). PEGs with an average mass up to 10 kDa ($[M+4 \text{ cation}]^{4+}=2500 \text{ Da}$) can be analysed with this technique.

Our extensive offering of reagents, standards and other products for MS can be found in our convenient on-line product catalogue (sigma-aldrich.com/catalog) under the "Analytical/Chromatography, Mass Spectrometry" section.

Figure 2 Detected (upper) and calculated (lower) mass spectrum of methoxy-PEG-5000 (113 ethyleneglycol units). The high purity of potassium citrate (77843) prevents the formation of sodium or other adducts that appear between the isotopic patterns of the different chain length PEG molecules.

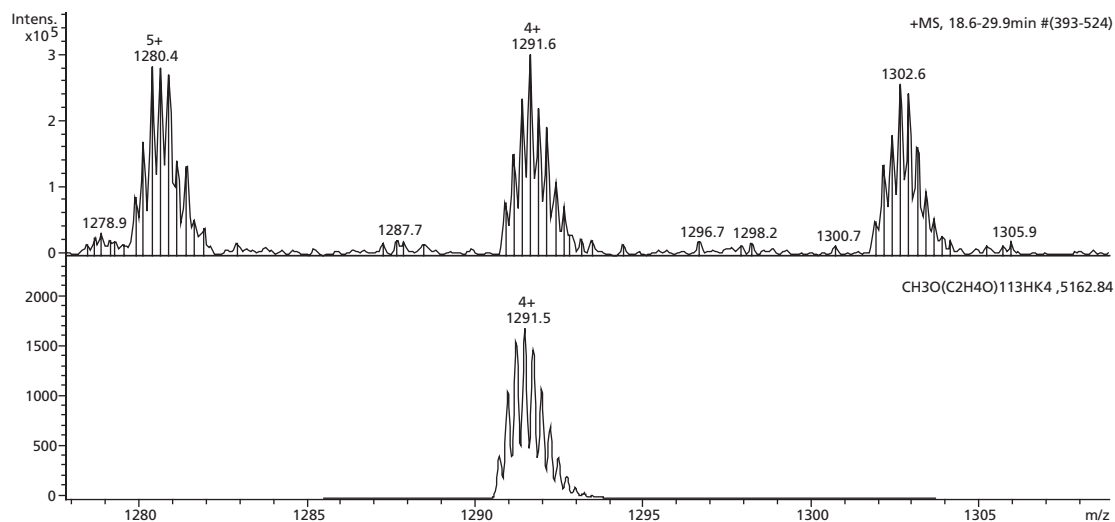


Table 1 Additives for LC-MS

Part No.	Brand	Description	Package Size
77843	Fluka	Potassium citrate for LC-MS	50 g
61333	Fluka	Sodium citrate for LC-MS	50 g
49638	Fluka	Ammonium acetate for LC-MS	50 g
55674	Fluka	Ammonium formate for LC-MS	50 g
81292	Fluka	PEG 4000 Standard	1 g

Sudan Red dye standards

New standards and deuterated standards for the reliable analysis of these carcinogenic compounds in foodstuffs

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Background

Sudan dyes belong to a family of industrial azo-dyes used to give colour to plastics and other materials, including leather, fabrics, fats, oils, waxes, polystyrene, cellulose and synthetic lacquers and polishes. However, problems arise when the Sudan dyes are used illicitly to enhance and maintain the colour of food, especially chili and chili-derived products. In 2003, the dyes were found in chili and chili products imported from India. Since then, Sudan dyes have been detected in foods, including chili and curry powders and the processed foods that contain them, sumac, curcuma and palm oil.

Sudan Red and health

Sudan dyes are classified by the International Agency for Research on Cancer (IARC) as Group 3 carcinogens and are banned as food additives world-wide (1). In 2005 the European Food Safety Authority (EFSA) initiated a review of the toxicology of a number of dyes found illegally in food in the EU. The EFSA came to the conclusion that, especially for Sudan I, there is strong evidence for both genotoxicity and carcinogenicity. Because of structural similarities between Sudan I and the other Sudan dyes, the larger group is presumed to have the same deleterious effects (2,3).



Legislation and limits

The EU issued Decision 2003/460/EC requiring that all hot chili and hot chili products imported to Europe be tested for Sudan I. The Decision was amended in January of 2004 (2004/92/EC) to include Sudan II, III and IV. This requirement remains in effect. Although regulations do not restrict analysts to a particular analytical method, permissible levels of these dyes have generally followed the limit of quantification (LOQ) which is currently 0.5–1.0 mg/kg using LC-UV.

New Sigma-Aldrich Sudan Dye standards

Reliable reference standards are necessary to perform the analysis of Sudan dyes in foodstuffs. We have recently rounded out our line of Sudan dyes standards to include four new standards and two deuterated standards in neat form.

Table 1 Sudan Red dye standards

Part No.	Brand	Description	Purity	Package Size
51383	Fluka	Sudan I	> 96 %	25 mg
07937	Fluka	Sudan II	> 96 %	25 mg
68562	Fluka	Sudan III	> 96 %	25 mg
67386	Fluka	Sudan IV	> 96 %	25 mg
New! 43207	Fluka	Sudan Orange G	> 96 %	25 mg
New! 53373	Fluka	Sudan Red 7B	> 96 %	25 mg
New! 91282	Fluka	Sudan Red G	> 96 %	25 mg
New! 51602	Fluka	Sudan Red B	> 96 %	25 mg
New! 34184	Fluka	d ₅ -Sudan I	enrichment > 98 atom % D	10 mg
New! 34161	Fluka	d ₆ -Sudan IV	enrichment > 98 atom % D	10 mg

References

- 1] "Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to review the toxicology of a number of dyes illegally present in food in the EU" The EFSA Journal (2005) 263, 1–71, online content: http://www.efsa.europa.eu/etc/medialib/efsa/science/afclafc_opinions/1127.Par.0001.File.dat/afc_op_ej263_illegaldyes_en1.pdf, accessed June 27, 2008.
- 2] Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 8: Some Aromatic Azo Compounds, Summary of Data Reported and Evaluation, World Health Organization, International Agency for Research on Cancer (IARC). IARC website (<http://monographs.iarc.fr/ENG/Monographs/vol8/volume8.pdf>) accessed 27 June, 2008.
- 3] "EFSA reviews toxicological data of illegal dyes in food" European Food Safety Authority (EFSA) website (http://www.efsa.europa.eu/en/press_room/press_release/2005/1129.html) 12 September 2005, accessed 27 June, 2008.

Kit for the analysis of oil, grease and other non-polar compounds in water according to US EPA Method 1664

One convenient kit contains all reagents and standards needed for determining HEM and SGT-HEM in surface, saline, drinking and waste water samples.

Ingrid Hayenga, Senior Chemist, R&D Europe ingrid.hayenga@sial.com

Background

US EPA Method 1664 was designed and implemented as part of the United States' on-going efforts to maintain the quality of surface, saline and drinking waters under the Clean Water Act as well as other regulatory programmes. The method describes the gravimetric determination of non-polar, n-hexane extractable materials (HEM), which includes relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related materials, in the water or waste water sample. The method further defines the sample by specifying its treatment with silica gel after the hexane extraction (SGT-HEM) to ensure complete removal of polar substances. Unlike earlier methodology, Method 1664 uses n-hexane instead of Freon-113, a Class I CFC, as the extraction solvent making it compliant with the Montreal Protocol on Substances that Deplete the Ozone Layer.



Summary of method

To determine HEM, the water sample is acidified and serially extracted with n-hexane in a separatory funnel. The hexane layers are combined and dried over sodium sulphate. The HEM is measured gravimetrically after removal of the hexane by evaporation. For the determination of SGT-HEM, the HEM is redissolved in n-hexane and an amount of silica gel proportional to the amount of HEM is added to remove polar materials. The SGT-SEM is also determined gravimetrically. Laboratory water taken through the same extractions serves as a blank.

With a detection limit of 1.4 mg/L and a minimum level of quantitation of 5.0 mg/L, Method 1664 can be used to analyse water samples with HEM and SGT-HEM in the 5 to 1000 mg/L range. By reducing the sample volume, samples with even higher levels of HEM and SGT-HEM can be analysed. The method requires that five percent of all samples from a given sampling site or discharge/waste stream must be spiked to demonstrate recovery and monitor matrix interferences. The spiking solution is stearic acid:hexadecane (1:1) in acetone. A duplicate matrix spike is recommended but is not required.

Reagent and standard kit for Method 1664

To assist analysts performing Method 1664, Sigma-Aldrich offers a convenient kit that contains all necessary reagents and standards for HEM and SGT-HEM determination. The kit is sufficient for the extraction and analysis of 120 HEM samples and 10 SGT-HEM samples with 1000 mg/L HEM.

Table 1 Test kit for EPA Method 1664

Part No.	Brand	Description
80698	Fluka	Kit containing reagents and standards for the analysis of n-hexane extractable material (HEM; oil and grease) and silica gel treated n-hexane extractable material (SGT-HEM; non-polar material) according to US EPA Method 1664.
03286	Fluka	Each kit contains:
85679	Fluka	
52209	Fluka	
320072	Sigma-Aldrich	All other reagents can be ordered directly from Sigma-Aldrich:
320331	Sigma-Aldrich	• Florisil™ anhydrous (for the determination of hydrocarbon), 2x150 g
258105	Sigma-Aldrich	• Stearic acid, 1x500 mg
32293	Sigma-Aldrich	• Hexadecane, puriss. p.a. (> 99.8 %), 1x1 ml in Certan bottle
179124	Sigma-Aldrich	• Water (ACS), 500 ml, 2 l, 4 l
239313	Sigma-Aldrich	• Hydrochloric acid (ACS), 500 ml, 2.5 l
2265918	Aldrich	• Sulfuric acid (ACS), 100 ml, 500 ml, 2.5 l
		• n-Hexane (ACS, puriss. p.a. > 99 %), 1 l, 2.5 l
		• Acetone (ACS, > 99.5 %), 500 ml, 1 l, 2 l
		• Sodium sulphate (ACS, granular, >99.0 % anhydrous), 500 g
		• Microporous carbon boiling chips (silicon carbide), 227 g

TraceCERT®

Swiss Precision Meets Analytical Competence



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- Traceable to at least two independent references (e.g. NIST, BAM or SI unit kg)
- Highly sophisticated packaging & documentation with expiry date
- ICP standards list up to 70 trace impurities

Ordering Information

Find our actual list of TraceCERT® reference materials for calibration and method validation at <http://www.sigma-aldrich.com/tracecert>

For custom standards please contact us at: CustomStandards@sial.com

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Analytical

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Ideal for USP and Ph.Eur. methods for residual solvent determination by headspace GC

Analysis of residual solvents using headspace GC techniques is an important quality control procedure in pharmaceutical and food-related laboratories. With the aim of further ensuring minimal solvent exposure in people taking medications, effective July 1, 2008 USP 467 "Organic Volatile Impurities (OVI)" will change to USP 467 "Residual Solvents". Sigma-Aldrich is pleased to offer Fluka-brand solvents that have been specifically designed and tested for this important application.



Features and Benefits of Headspace-Grade Solvents from Sigma-Aldrich

- Includes most popular GC-HS solvents for wide applicability
- Very high purity to maximise detector sensitivity
- Virtually no background due to volatile impurities on both polar and non-polar GC capillary columns

New GC-HS solvents from Sigma-Aldrich

Part No.	Brand	Description	Boiling Point	Package Size
44901	Fluka	N,N-Dimethylacetamide, puriss. p.a. for GC-HS	166°C	1 L
51779	Fluka	Dimethyl sulphoxide, puriss. p.a. for GC-HS	189°C	1 L
51781	Fluka	N,N-Dimethylformamide, puriss. p.a. for GC-HS	153°C	1 L
53463	Fluka	Water, puriss. p.a. for GC-HS	100°C	1 L

Extensive range of products for Ion Chromatography

Sigma-Aldrich supplies high-purity water, eluent concentrates, validation kits and certified reference materials for highly reliable IC analyses

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

Ion chromatography (IC) is an analytical technique for separating and quantifying very low levels of common anions, like fluoride, chloride, nitrite, nitrate and sulphate, and cations, like 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 high sensitivity of IC, which makes it ideal for low-ppm quantification and trace analysis, requires the use of 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 water suitable for sensitive ion determinations. Designed to save analysts' time and ensure the most sensitive and reproducible IC determinations, our *TraceCERT*[®] standards for IC and pre-made Certified Eluent Concentrates are convenient time- and cost-saving options.

A unique class of IC standards

TraceCERT certified reference materials are produced in a double-accredited laboratory, fulfilling both 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). 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 US National Institute of Standards and Technology (NIST). All details regarding exact content, uncertainty, traceability and expiry date are described in a comprehensive certificate, which is available electronically through our website using product and lot numbers. The complete listing of *TraceCERT* standards can be found at sigma-aldrich.com/tracecert

Table 1 Specifications for IC-grade water (Fluka P/N 00612, Package sizes 2.5 L and 5 L)

Parameter	Specification
Anion traces	
Br ⁻ , Cl ⁻ , F ⁻ , I ⁻ , NO ₃ ⁻ , NO ₂ ⁻ , PO ₄ ³⁻ , SO ₄ ²⁻	≤ 1 µg/kg each
Metal and cation traces	
Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sr, Zn	≤ 5 µg/kg each
Ca, K, Na	≤ 10 µg/kg each
NH ₄ ⁺	≤ 50 µg/kg each
Organic ion traces	
Acetate, formate, glycolate, oxalate	≤ 10 µg/kg each
Conductivity	≤ 2 µS/cm

Figure 1 Sample Certificate of Analysis for sodium bicarbonate solution (Fluka P/N 36486-1L)

Eluent Concentrate
for Ion Chromatography

Certificate

TraceCERT certified reference materials and standards according to ISO Guide 34

Digital identification: Sodium bicarbonate solution 8.1 mmol/L in water
 Fluka Product No.: 36486-1L; 1.000 L; 1000 mL; 1000 mL; 1000 mL
 Composition: Sodium bicarbonate (pure, p.a. AR reagent, 100.0%, Fluka product No. 7980); dissolved in deionized high-purity water (18.2 MΩ, 0.1 µm)

Concentration	Certified value at 20°C	Certified expanded uncertainty (95% k=2)
Sodium bicarbonate	108.9 mmol/L	± 0.4 mmol/L

Intended use: Concentrate for preparation of standards for ion chromatography
Storage and handling: This liquid concentrate should be stored between 5°C and 30°C. To avoid CO₂ uptake the bottle should be equipped with a CO₂ absorber and immediately after opening.
Expiry date: 31 August 2020 (unopened bottle)
Traceability statement: This liquid concentrate solution is traceable to primary standards Sodium Bicarbonate (NIST SRM 910b) and also traceable to SRM and BSM certified elemental reference material (Fluka Prod. No. 8540).
Uncertainty calculation: All concentrations are calculated according to European CEN/ISO Guide 34 and reported as combined expanded uncertainties at the 95% confidence level. Contributions from reference material, gravimetric titration measurements and density effects are included in the reported uncertainty budget.

Certification body	Lot / P-Number	expiry date
	000001-1000000	31 Aug 2020

Fluka, a division of Sigma-Aldrich, is a double-accredited laboratory (ISO/IEC 17025 and ISO Guide 34) for the production of certified reference materials. The complete listing of TraceCERT standards can be found at sigma-aldrich.com/tracecert

Certified and traceable eluent concentrates

Our certified eluent concentrates are traceable by potentiometric titration to NIST Standard Reference Materials and certified in accordance with ISO Guide 31. A Certificate of Analysis, an example of which is shown in **Figure 1**, provides details regarding exact content, uncertainty, traceability and expiry date. The certificates for our Eluent Concentrates are available in electronic form through our website.

High-purity water for ion chromatography

The purity of the water used in IC mobile phases is crucial to permit the ppm to ppb and sometimes even ppt-level determinations of anions and cations. To address this requirement, we have developed a quality grade of water specifically for sensitive IC applications. Produced using state-of-the-art purification technology and analysed with highly sensitive measurement techniques, our IC-grade water is suitable for trace analysis of anions, cations and organic compounds by IC. Some quality parameters of our IC-grade water are shown in **Table 1**. To ensure long-term quality, it is supplied in special containers that have been proven in extended storage tests, as demonstrated by the data presented in **Figure 2**.

Product Table Sigma-Aldrich Certified Eluent Concentrates

Part No.	Brand	Description	Package Size*
50439	Fluka	Perchloric Acid Solution for IC, 0.01 M in water	1 L
36486	Fluka	Sodium Bicarbonate Solution for IC, 0.1 M in water	1 L
56169	Fluka	Sodium Carbonate Solution for IC, 0.1 M in water	1 L
43617	Fluka	Sodium Hydroxide Solution for IC, 0.1 M in water	1 L
50972	Fluka	2,6-Pyridinedicarboxylic Acid Solution for IC, 0.02 M in water	1 L
68487	Fluka	Oxalic Acid Solution for IC, 0.1 M in water	1 L
16355	Fluka	Nitric Acid Solution for IC, 0.1 M in water	1 L
68279	Fluka	Sulphuric Acid Solution for IC, 0.1 M in water	1 L
50433	Fluka	DL-Tartaric Acid Solution for IC, 0.1 M in water	1 L
61699	Fluka	Potassium Hydroxide Solution for IC, 0.1 M in water	1 L
55517	Fluka	Methanesulphonic Acid Solution for IC, 0.1 M in water	1 L

* Packaging: 1L HDPE bottles with 45mm screw thread

Traceable and certified standards for qualification activities

Sigma-Aldrich has developed a new test kit (prod. no. 12674, contains six certified bromide standards from 5 to 1000 mg/kg) that is extremely useful for qualifying ion chromatography systems. The kit is intended for testing detection linearity, injector precision and injector carry-over, and is also part of Metrohms «IQ/OQ Kit for IC», which includes all parts for validation of Professional IC Systems. A special advantage of the kit is that the certified bromide standards can be used with conductivity, UV/Vis and electrochemical detectors.

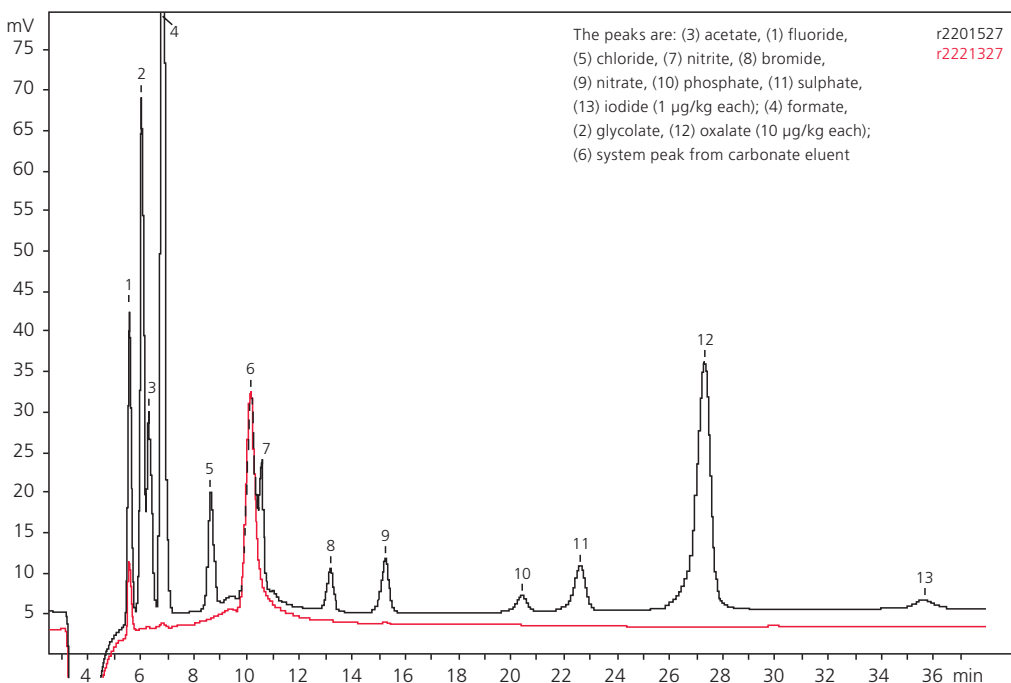
Primary multiion standards (PRIMUS)

Two metrological institutes – Swiss Federal Institute for Materials Science and Technology (EMPA, Switzerland) and Federal Institute for Materials Research and Testing (BAM, Germany) – together certified a set of primary

materials where up to 85 parameters per substance are analysed. The purity was then confirmed by high-precision measurements to additionally demonstrate the accuracy of the certified content and the correct stoichiometry of the compound. Using these primary materials Sigma-Aldrich produces the PRIMUS standard solutions gravimetrically under clean-room conditions into pre-cleaned 50 mL HDPE bottles. PRIMUS standards are primary standards characterised by their traceability to SI as well as a uniquely low degree of expanded uncertainty of <0.2 %. These “ready-to-use” multi-element standards contain 10 mg/kg of each Br⁻, Cl⁻, F⁻, NO₃⁻, PO₄³⁻, SO₄²⁻ (anion solution, P/N 89886) or Ca²⁺, Li⁺, K⁺, Mg²⁺, Na⁺ (cation solution, P/N 89316).

More technical information and product specifications of our IC products can be found at sigma-aldrich.com/ic

Figure 2 Demonstration of long-term product quality of IC-grade water (Fluka P/N 00612). Anion chromatogram from a 4-month leaching test of 2.5L HDPE bottle. The black line shows the anions and organics in the concentration of the specification limits.



Selective growth media for differentiation and detection of *Escherichia coli* and other coliforms

E. coli and other coliforms are important indicator organisms for the presence of pathogens in food, occupational and environmental safety applications, some of the most important areas of analytical microbiology.

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

E. coli: Indicator organism for fecal contamination

Normal constituents of the intestinal flora of animals, coliforms are rod-shaped, Gram-negative, non-spore forming facultative anaerobes. They ferment lactose with the production of acid and gas when incubated at 35–37°C. Although commonly found in lakes, rivers, swimming pools and soil from faecal sources, in most cases coliforms do not cause illness. However, they are used as indicators for other pathogenic organisms of faecal origin. The most common genera of coliforms are *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella* and *Serratia*.

Escherichia coli (Figure 1) is the best-known coliform and an important indicator of faecal contamination because it is found almost exclusively in faeces. Occasional outbreaks of food poisoning have been linked to certain gastroenteritis-causing *E. coli* strains, such as serotype O157:H7. *E. coli* are rod-shaped bacteria, distinguished from most other coliforms by their ability to ferment lactose at 44°C, and by their growth characteristics on certain media. Easy to culture, *E. coli* is often used in molecular biology.

Figure 1 *Escherichia coli*



Selective growth media for *E. coli*

Microbiological media is used not only to grow microorganisms, but also to select or identify a particular type of microorganism based on some unique or distinctive aspect of its biochemistry. Most of this so-called “selective growth” media contain a protein source, often a hydrolysate of casein, and a fermentable sugar, like lactose or glucose. Occasionally an indicator, like neutral red or bromo cresol purple, is added to detect the acid produced by the fermentation process.

A new generation of selective media is available from Sigma-Aldrich that employs chromogenic and fluorogenic substrates (Figure 2). These media indicate *E. coli* by the presence of β -D-glucuronidase (GUD) and other coliforms by the presence of β -D-galactosidase. GUD catalyses the hydrolysis of β -D-glucopyranosiduronic acid into the corresponding aglycones and D-glucuronic acid. GUD is present in 94–96 % of *E. coli* strains, but is also found in some *Salmonella*, *Shigella* and *Yersinia* spp. (1-3). There is a wide range of media available that use different substrates for the detection of *E. coli* and coliforms (Table 1). The media are also supplemented with agents like bile salts, brilliant green, sodium lauryl sulphate and other substances to enhance their selectivity.

Figure 2 HiCrome™ M-TEC Agar for differentiation and enumeration of thermotolerant *E. coli* from water by membrane filtration technique. *E. coli* colonies appear blue because the chromogen, X-glucuronide, is cleaved by the enzyme β -glucuronidase.



For a complete list of our growth and selective media, and our other products and resources for microbiology, please visit our website www.sigma-aldrich.com/microbiology

References:

- 1] Frampton, E. W.; Restaino, L. Methods for *E. coli* identification in food, water and clinical samples based on beta-glucuronidase detection. *J. Appl. Bacteriol.* 1993, 74, 223–233.
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- 3] Manafi, M. Fluorogenic and chromogenic enzyme substrates in culture media and identification tests. *Int. J. Food. Microbiol.* 1996, 31, 45–58.

Table 1 Specific media for the detection, enumeration and identification of coliforms and *E. coli***Key:** red = chromogenic media; blue = fluorescent media; violet = chromogenic and fluorogenic media

Media System	Part No.	Name	Media System	Part No.	Name
Non-selective media used for identification	A0715	Andrade Peptone Water	Selective media for differentiation	83339	HiCrome™ Mac Conkey Sorbitol Agar
	28943	Andrade peptone water, Vegitone		90924	HiCrome™ M-TEC Agar
	70133	Blood Agar (Base)		51489	HiCrome™ Rapid Coliform Broth
	36408	Bromcresol Purple Broth		54232	Lactose TTC Agar with Tergitol®-7
	22520	China Blue Lactose Agar		62087	Levine EMB Agar
	27048	Christensen's Urea Agar		62634	LST-MUG Broth
	55420	CLED Agar		70143	Mac Conkey Agar No. 1
	D2935	Decarboxylase Broth Base, Moeller		19352	Mac Conkey Agar No. 1, Vegitone
	31436	DEV Lactose Broth		94216	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15 % Bile Salts
	31437	DEV Lactose Peptone Broth		M8302	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15 % Bile Salts
	31406	DEV Tryptophan Broth		70144	MacConkey Broth
	16447	Glucose Bromcresol Purple Agar		75717	MacConkey Broth purple
	73009	HiCrome™ ECC Agar		16377	MacConkey Broth purple
	00563	HiCrome™ MM Agar		63014	MacConkey MUG Agar
	16636	HiCrome™ UTI Agar, modified		51405	MacConkey-Agar (without salt)
	60787	Kligler Agar		88902	MacConkey-Sorbitol Agar
	94792	Lactose Broth		39734	Membrane Lactose Glucuronide Agar
	70142	Lactose Broth		85766	m-Endo Agar LES
	19057	Lactose Broth, Vegitone		96961	M-FC Agar
	62915	Lysine Iron Agar		19958	m-FC Agar Plates (55 mm diameter)
	M8802	Malonate Broth		43291	M-FC Agar, Vegitone
	39484	Methyl Red Voges Proskauer Broth		07348	M-Lauryl Sulphate Broth
	69150	Methyl Red Voges Proskauer Saline Broth		M1678	MUG EC Broth
	17171	Mineral-modified Glutamate Broth (Base)		92435	TBX Agar
	M1053	Motility Test Medium		86455	Tergitol®-7 Agar
	17178	Mucate Broth		70188	Violet Red Bile Agar
	17165	MUG Tryptone Soya Agar		42376	Violet Red Bile Agar, Vegitone
	72548	Nitrate Broth		79873	Violet Red Bile Glucose Agar
	75315	OF Test Nutrient Agar		70189	Violet Red Bile Glucose Agar
	51413	Plate Count MUG Agar		17213	Violet Red Bile Glucose Agar without Lactose
	85463	Simmons Citrate Agar		53605	Violet Red Bile Glucose Agar without Lactose, Vegitone
	44940	Triple Sugar Iron Agar		41270	Violet Red Bile Lactose Dextrose Agar
	93657	Tryptone Medium		95273	VRB MUG Agar
07507	Tryptone Water	17215	WL Differential Agar		
39964	Tryptone Water, Vegitone	76721	XLT4 Agar (Base)		
51463	Urea Broth	Selective media	17112	A1 Broth	
16016	BRILA MUG Broth		16025	Brilliant Green Bile Lactose Broth	
31432	DEV ENDO Agar		44653	EC Broth	
44657	ECD MUG Agar		44655	ECD Agar	
70186	EMB Agar		61749	Lauryl sulphate Broth	
E5399	Endo Agar		17349	Lauryl sulphate Broth	
70137	ENDO Agar (Base)		17162	M Endo Broth	
48716	Gassner Agar		17184	M HD Endo Broth with Brilliant Green	
81938	HiCrome™ Coliform Agar		49522	M-Lauryl Sulphate Broth, Vegitone	
70722	HiCrome™ E. coli Agar B		69965	Mossel Broth	
85927	HiCrome™ ECC Selective Agar				
09142	HiCrome™ ECD Agar with MUG				

Detection of enterovirulent *Escherichia coli*

Enterovirulent *E. coli*, like the enterohemorrhagic *E. coli* (EHEC), are known to cause severe disease. Undercooked or raw meat is often responsible for outbreaks. One solution is to detect their presence in food with new and reliable growth media.

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

E. coli are Gram-negative, rod-shaped bacteria belonging to the family of Enterobacteriaceae. Normally a useful organism in the human digestive system, a few *E. coli* strains are capable of causing human illness by several different mechanisms. The enterohemorrhagic *E. coli* (EHEC) strain designated as *E. coli* O157:H7 is the most important of the enterovirulent *E. coli*. It produces large quantities of one or more potent toxins that cause severe hemorrhagic colitis or hemolytic uremic syndrome. These toxins (verotoxin, shiga-like toxin) are identical or closely related to the toxin produced by *Shigella dysenteriae*.

Different types of *E. coli* are known based on their pathogenic mechanisms:

- Enteropathogenic *E. coli* (EPEC). Virulence mechanism is unrelated to the excretion of typical *E. coli* enterotoxins. Causes gastroenteritis (childhood diarrhea).
- Enteroinvasive *E. coli* (EIEC) or Shiga toxin-producing *E. coli* (STEC). Causes a *Shigella*-like dysentery.
- Enterotoxigenic *E. coli* (ETEC). Produce heat-labile (LT) or heat-stable (ST) enterotoxins. Cause traveller's diarrhea.
- Enteroaggregative *E. coli* (EAaggEC) or Enteroadherent *E. coli* (EAEC). Able to attach to tissue culture cells in an aggregative manner and may produce EAST (Enterotoxigenic ST) toxin. Subgroups, like diffusely adhering *E. coli* (DAEC) strains and Cytodetaching *E. coli* (CDEC), are differentiated. Primarily associated with persistent diarrhea in children in developing countries and also traveller's diarrhea

Table 1 Biochemical characteristics of *E. coli* O157:H7

Selective agents	Bile salts
	Lauryl sulphate
	Tellurite
	Novobiocin
	Cefixime
Differentiating features	Typically β -D-glucuronidase negative
	Ferments lactose
	Typically not able to ferment sorbitol
	β -galactosidase positive
	α -galactosidase positive

Figure 1 HiCrome™ EC O157 Agar (Fluka P/N 39894)

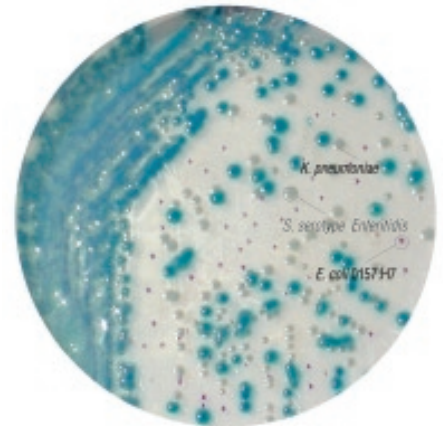


Figure 2 HiCrome™ EC O157:H7 Selective Agar (Fluka P/N 72557)

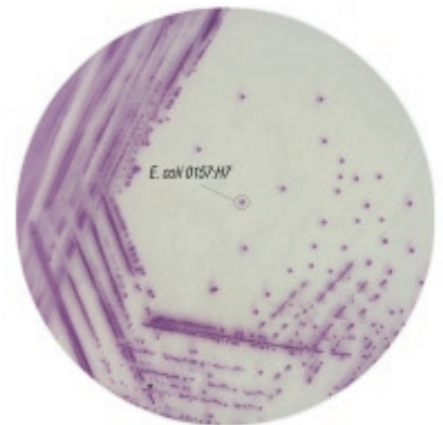


Figure 3 HiCrome™ Enrichment Broth Base for EC O157:H7 (Fluka P/N 80330)



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

Important features used for the detection of *E. coli* O157:H7 from other *E. coli* and other bacteria appear in **Table 1**. These features are also used in our diverse media to detect and differentiate *E. coli* O157:H7 found in **Table 2**.

For a complete list of our growth and selective media, and our other products and resources for microbiology, please visit our website sigma-aldrich.com/microbiology

Table 2 List of Sigma-Aldrich media for enterovirulent *E. coli*

Key: red = chromogenic media; blue = fluorescent media

Media System	Part No.	Name	Description
Non-selective + differential system	17178	Mucate Broth	For the identification of enteropathogenic <i>E. coli</i> and <i>Shigella</i> species in milk and milk products.
Selective + differential systems	44782	<i>E. coli</i> O157:H7 MUG Agar	A fluorescent selective agar for the isolation and differentiation of enterohaemorrhagic (EHEC) <i>E. coli</i> O157:H7-strains from food and clinical material.
	39894	HiCrome™ EC O157 Agar	A chromogenic medium for isolation and differentiation of <i>E. coli</i> O157 from food and environmental samples.
		Optional supplement: 0.25 mL/L 1 % potassium tellurite solution (Fluka 17774)	
	72557	HiCrome™ EC O157:H7 Selective Agar, Base	Recommended for selective isolation and easy detection of <i>E. coli</i> O157:H7 from food samples.
		Supplement: 1 vial/L of HiCrome ECO157:H7 Selective Supplement (Fluka P/N 44931)	
	80330	HiCrome™ Enrichment Broth Base for EC O157:H7	Recommended for isolation and selective differentiation of <i>E. coli</i> O157:H7 from food and environmental samples by a chromogenic method.
	83339	HiCrome™ MacConkey-Sorbitol Agar	Recommended for selective isolation of <i>E. coli</i> O157:H7 from food and animal feed. The medium contains sorbitol instead of lactose. <i>E. coli</i> produce purple colonies. The colour is due to production of acid from sorbitol and the neutral red indicator and the B.C. indicator, which detects the presence of the enzyme β-D-glucuronidase, specific for <i>E. coli</i> . Enteropathogenic strains of <i>E. coli</i> O157 do not possess β-D-glucuronidase activity and do not ferment sorbitol, producing colourless colonies.
		Supplement: 2 vial/L Tellurite-Cefixime Supplement (Fluka P/N 77981)	
	88902	MacConkey-Sorbitol Agar	Recommended medium for selective isolation of pathogenic <i>E. coli</i> O157:H7 which ferments sorbitol but not lactose.
Selective media	71882	mEC Broth with Novobiocin	For the selective enrichment of enterohemorrhagic <i>E. coli</i> in food. The medium is used for the isolation and identification of <i>E. coli</i> O157:H7 from meat according to USDA-FSIS methods.
	76704	mTSB Broth with Novobiocin	For the selective enrichment of enterohemorrhagic <i>E. coli</i> in food. The medium meets the requirements of the DIN Norm 10167 for the detection of <i>E. coli</i> serotype O157:H7 in food. It is also recommended by the FDA-BAM as a method for the isolation of enterohemorrhagic <i>E. coli</i> .

Microscopy reagents and accessories

Comprehensive offering of high-quality products to maximise the information obtained from your microscopy experiments

Jvo Siegrist, Product Manager Biochemicals [ivo.siegrist@sial.com](mailto:jvo.siegrist@sial.com)

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

Transmission microscopy is a routine, but important, tool in biology and medicine. Since the light passes through the specimen before reaching the objective, the specimen must be transparent. Many specimens, like single-celled organisms, algae and fungi, small crustaceans and individual plant and animal cells are naturally transparent. However, other samples, such as plant or animal tissues, are naturally opaque and must be made more transparent before they can be examined with transmission microscopy. This is normally accomplished by mincing or homogenisation, or by producing smears or very thin slices of the sample.

Sigma-Aldrich offers a comprehensive range of products and reagents to facilitate the transfer of the specimen in its original condition to the microscope slide and to maximise the information that is obtained from the sample. These include embedding media to encapsulate the specimen, fixation reagents to conserve tissue sections, immersion oils to increase the resolution of the microscope, mounting media to preserve the sample and stains and dyes to increase contrast. Just a few are described below.

Embedding media

Typically, specimens are applied to the microscope slide in a drop of embedding liquid, compressed and sealed with a cover slip. Water is the most common embedding media; however, since it evaporates quickly the prepared sample is viable for only a short time. We offer a range of embedding and mounting media that extends the lifetime of the preparation, and also improves the microscopy by better matching of refractive index.

Stains

Key attributes of our wide range of dyes and stains for light microscopy are high quality and purity, homogeneity and rapid dissolution. They are also free-flowing in terms of their physical characteristics.

Slides and cover slips

In addition to chemicals for embedding, fixing and staining the sample, we also carry microscope slides and cover slips. Our microscope slides are 3 x 1 inches (75 x 25 mm) and available with or without marking



surfaces (Part Nos. S8902, S8400, S9027). They are also supplied in boxes that serve double duty for storing and drying the slides. The specimen is compressed, sealed and protected on the slide with a wafer-thin square of glass called a cover glass or cover slip. Our cover slips are thin (0.13 mm), 18 mm square and made from high-purity glass for optimum transparency (Part No. CLS286518).

Sample prep accessories

Indispensable tools to prepare samples for microscopy are razor blades, scalpels and needles or pins. We've selected the best among these products, and many more, to offer to our microscopy customers.

The above-mentioned items are just a small sample of our products for microscopy. For further information about microscopy products and reagents from Sigma-Aldrich, please visit our web page sigma-aldrich.com/microscopy

New Selectophore® phthalate ionophore

For phthalate analysis using potentiometric sensors

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

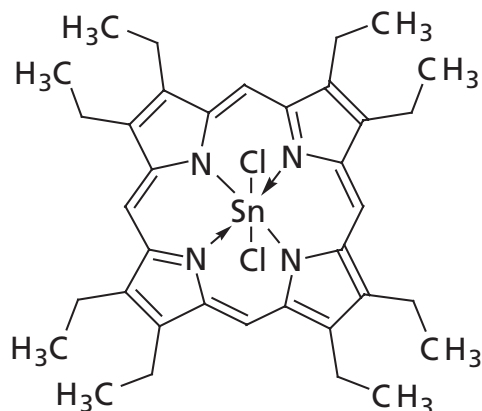


Phthalate acid esters (PAE) increase the flexibility of rigid plastics and are the most common plasticiser used in the production of PVC. Because PAEs are only physically bound to the polymer chains, they can dissociate from the PVC and leach into their surroundings. Plastic containers are ubiquitous and are a major source of PAE in the environment. Humans are exposed to phthalates in multiple ways: from food contaminated during its production, processing, packaging or storage, from cosmetics and from medical devices such as intravenous (IV) storage bags and tubing (1).

Analytical methods using potentiometric detection with ion-selective electrodes (ISE) are a viable alternative to chromatographic techniques for PAE analysis. Potentiometric methods are compatible with field use, environmentally friendly, have good analyte selectivity, provide simple and fast sample preparation and do not require expensive instrumentation (2).

For further information about these and our other ionophores, please visit our sensorics web page sigma-aldrich.com/sensoric

Figure 1 Phthalate ionophore I (Fluka P/N 30513)



References

- 1] Phthalate Information Center™ (www.phthalates.org), accessed June 2008.
- 2] Santos, E.; Araújo, A.; Couto, C.; Montenegro, M. Use of Tin (IV) Porphyrins as Ionophores for the Construction of Phthalate-Selective Electrodes: Influence of the Structure and Membrane Composition on their Response Properties. *Electroanalysis* 2005, 17, 1945–1951.

Characteristics of Selectophore® phthalate ionophore

Slope (sensitivity): -57.8 mV/dec

DL $\log a_{\text{phthalate}}$: < -5, pH range: 2.7-4.8

Selectivity Coefficients $\log K_{\text{NO}_3, X}^{\text{Pot}}$ as obtained by the single solution method (SSM):

$\log K_{\text{Phthalate, Salicylate}}^{\text{Pot}}$	1.1	$\log K_{\text{Phthalate, Nitrite}}^{\text{Pot}}$	-1.9
$\log K_{\text{Phthalate, Thiocyanate}}^{\text{Pot}}$	0.8	$\log K_{\text{Phthalate, Chloride}}^{\text{Pot}}$	-3.0
$\log K_{\text{Phthalate, Nitrate}}^{\text{Pot}}$	-1.2	$\log K_{\text{Phthalate, Acetate}}^{\text{Pot}}$	-3.1
$\log K_{\text{Phthalate, Benzoate}}^{\text{Pot}}$	-1.4	$\log K_{\text{Phthalate, Sulphate}}^{\text{Pot}}$	-4.8

Membrane composition: 1 wt % Ionophore, 0.1 wt % TOABr, 65.9 wt % NPOE, 33.0 wt % PVC

Product table

Part No.	Brand	Description	CAS No.	Package Size
30513	Fluka	Phthalate ionophore I, Selectophore®, function tested	721924-26-1	50 mg
81392	Fluka	Poly(vinyl chloride) high molecular weight (PVC), Selectophore®	9002-86-2	1 g, 10 g, 50 g
87994	Fluka	Tetraoctylammonium bromide (TOABr), Selectophore®	14866-33-2	500 mg, 5 g
73732	Fluka	2-Nitrophenyl octyl ether (NPOE), Selectophore®	37682-29-4	5 mL, 25 mL, 100 mL



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Advantages of VOLPAC containers:

- High quality of content and packing
- Easy handling
- No contamination during dispensing
- Reduced storage footprint and packaging material

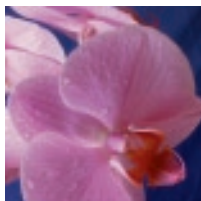
New! All VOLPAC containers are now supplied with a specially designed adapter that allows direct connection of the titrating tube to the VOLPAC container.

Special offer on all VOLPAC solution containers!

Receive 35 % off your first order of any VOLPAC container. Please quote promotion code 985 when placing your order. Offer valid until November 14th 2008.

A complete product listing can be found under sigma-aldrich.com/volpac

Upcoming Events ... HYDRANAL® seminars



HYDRANAL seminars in 2008

As a service to the scientific community, we routinely offer seminars to provide training on the chemistry behind the Karl Fischer technique and information specific to the HYDRANAL product line. In 2008, seminars in many cities around the world are planned. Please visit sigmaaldrich.com/events to see our most up-to-date schedule.

For registration and additional information please contact:

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* This year, Sigma-Aldrich will hold its 25th Karl Fischer seminar in Seelze, Germany. Featuring knowledgeable speakers from different fields, representatives from leading instrument manufacturers and practical demonstrations, the seminar is a must for any practising KF analyst!

September

25th-26th Buenos Aires, Argentina

October

1st Rio de Janeiro, Brazil
 2nd Sao Paulo, Brazil
 6th Johannesburg, South Africa
 8th Durban, South Africa
 10th Cape Town, South Africa
 13th Helsinki, Finland
 14th Turku, Finland
 16th Gothenborg, Sweden
 17th Stockholm, Sweden
 21st Karlsruhe, Germany
 23rd Zofingen, Switzerland
 29th-30th Bologna, Italy

November

10th Lund, Sweden
 11th Copenhagen, Denmark
 12th Jylland, Denmark
 13th Oslo, Norway
 25th-26th **Seelze, Germany
 (2-day seminar)***

HYDRANAL® reagents for Karl Fischer titration of water in aldehydes and ketones

Reagents with performance and safety advantages for both volumetric and coulometric methods

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

Using the Karl Fischer (KF) titration method to determine water in aldehydes and ketones can be challenging. With conventional methanol or methanol-containing KF reagents, aldehydes and ketones can undergo nucleophilic addition reactions that lead to the formation of acetals or ketals and water (see **Figure 1**). The produced water leads to erroneously high results and vanishing end-points. Additionally, a second side reaction, the bisulphite addition, can occur during the titration of aldehydes. Some of the water in the sample, depending on the strength of its bonds, can be consumed by this reaction leading to erroneously low results (see **Figure 2**).

Figure 1 Aldehydes and ketones undergo nucleophilic addition of methanol resulting in formation of acetal or ketal and water

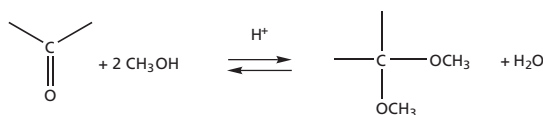
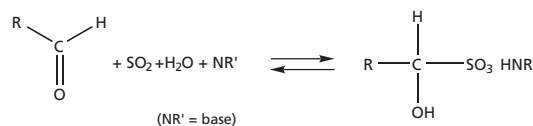


Figure 2 Bisulphite addition reaction consuming water



HYDRANAL K-reagents: Ideal for KF titration of aldehydes and ketones

Addressing this reactivity problem, researchers at Sigma-Aldrich found that certain halogenated alcohols (2-chloroethanol, trifluoroethanol) and 1-methoxy-2-propanol are particularly suitable for suppressing these side reactions. The methanol-free HYDRANAL K-reagents were developed based on these findings. HYDRANAL K-reagents prevent unwanted side reactions, eliminating or significantly reducing them as sources of error.

HYDRANAL Composite 5 K, designed for determination of water in aldehydes and ketones, consists of a solution of imidazole, sulphur dioxide and iodine in diethylene glycol monoethyl ether. We offer three different working media for HYDRANAL Composite 5 K: HYDRANAL KetoSolver, a halogen-free medium, HYDRANAL Medium K, a non-toxic medium and HYDRANAL Working Medium K, a medium that has excellent dissolving properties for difficult samples but contains chloroform and toxic 2-chloroethanol.

Elimination of nucleophilic addition by using a suitable working medium

The elimination of methanol in HYDRANAL KetoSolver, Medium K and Working Medium K presents a two-fold advantage. First, it eliminates unwanted side reactions like nucleophilic addition. Second, it improves the solubility of certain samples. For example, we found that as much as 5 g of 3-benzoylpropionic acid can be titrated in these media with suppression of ketal formation. In some cases, HYDRANAL Medium K can be used in conjunction with the standard volumetric reagent, HYDRANAL Composite 5.

Prevention of bisulphite addition by using HYDRANAL Composite 5 K

Adduct formation during bisulphite addition reactions consume water from the sample causing erroneously low results. This reaction can be avoided by using HYDRANAL Composite 5 K as titrating reagent in combination with HYDRANAL KetoSolver, Medium K or Working Medium K. Compared to HYDRANAL Composite 5, HYDRANAL Composite 5 K gives slightly slower reaction speed in order to maximise the suppression of the bisulphite addition.

The HYDRANAL reagents described above can also be used for the determination of water in substances other than aldehydes and ketones. HYDRANAL Medium K, Working Medium K and KetoSolver can act as a solvent for the determination of water in any substance where methanol can interfere with the titration and therefore must be avoided. Nearly universal in their applicability, they can also be used to titrate samples that do not present interference problems.

HYDRANAL Medium K: Non-toxic Karl Fischer reagent with performance and safety advantages

HYDRANAL Working Medium K is categorised as very toxic (T⁺) because it contains 2-chloroethanol. However, we have replaced toxic 2-chloroethanol with less hazardous alcohols in HYDRANAL Medium K, permitting its categorisation merely as harmful (X_n) due to its percentage of chloroform.

(continued on page 20)

Benefits of HYDRANAL Medium K over HYDRANAL Working Medium K

- Reduced toxicity for improved workplace safety while providing equal reactivity
- No water-producing side reactions
- No water-consuming side reactions when using HYDRANAL Composite 5 K as titrating reagent

HYDRANAL Medium K is a full-fledged substitute for HYDRANAL Working Medium K, providing the same sample capacity, speed and accuracy. It offers important application, safety and transportation benefits; because it is non-toxic, it not only improves workplace safety, it also reduces the amount of packaging material that is required and must be disposed of. In addition, HYDRANAL Medium K offers performance advantages with very reactive aldehydes such as propionaldehyde, butyraldehyde and crotonaldehyde. Capacity and accuracy are also improved for other compounds including salicylaldehyde, acetylacetone, 2,4-dihydroxyacetophenone and 2-benzolpyridine.

HYDRANAL KetoSolver: A halogen-free working medium

Our research into using different alcohols resulted in HYDRANAL KetoSolver, a working medium that is completely free of halogenated hydrocarbons. It can be used with both HYDRANAL Composite 5 or HYDRANAL Composite 5 K, depending on which is best for the sample. The main component of HYDRANAL KetoSolver is 1-methoxy-2-propanol, which suppresses the previously described side reactions, for the most part, and allows simple and reliable water determinations. Aldehydes and certain highly reactive ketones like cyclohexanone, trifluoroacetone and diacetyl require the use of HYDRANAL Composite 5 K as the titration agent.

Coulometric Karl Fischer titration in ketones

HYDRANAL Coulomat AK is an anolyte specially designed for the coulometric water determination in ketones. It contains imidazole, sulphur dioxide and iodide dissolved in a suitable solvent mixture and has a capacity of approximately 100 mg of water per 100 mL. HYDRANAL Coulomat AK can also be used as a single reagent for coulometry without diaphragm. HYDRANAL Coulomat CG-K is the corresponding catholyte. It does not contain halogenated hydrocarbons. The water capacity of 5 mL HYDRANAL Coulomat CG-K is 100 mg.

Recommendations for KF titration in aldehydes and ketones

Aldehydes

Short-chain aldehydes show a strong tendency to form acetals. Aromatic aldehydes tend to undergo the bisulphite addition. To overcome these potential interferences, we recommend using relatively small samples and titrating rapidly to suppress the formation of acetals and bisulphite compounds. Coulometry is not advised, but if it is necessary, the sample volume should be very small. For example, for water determination in aromatic aldehydes the sample volume must be less than 0.5 mL. Aliphatic aldehydes, like acetaldehyde, are highly reactive and tend to rapidly form acetals. Only volumetric titration with Methanol-free K-reagents is recommended and the amount of sample should be kept to a minimum.

Ketones

Ketones have a tendency to form ketals while at the same time forming water. Cyclohexanone and acetone react rapidly, while long-chain ketones and aromatic-substituted ketones show slower reaction rates. Reactive ketones are titrated with methanol-free K-reagents. The coulometric determination is possible by using HYDRANAL Coulomat AK and Coulomat CG-K reagents.

Table 1 Recommended procedures and reagents for Karl Fischer titration in selected aldehydes and ketones

Sample	Recommended KF procedure and respective HYDRANAL reagents		
	Volumetric 1-component technique (HYDRANAL Composite 5 K + HYDRANAL Medium K)	Coulometric technique with diaphragm (HYDRANAL Coulomat AK + HYDRANAL Coulomat CG-K)	Coulometric technique w/o diaphragm (HYDRANAL Coulomat AK)
Acetaldehyde	x		
2-Anisaldehyde	x	x	
Benzaldehyde	x	x	
1,2-Cyclohexane-dione	x	x	x
Acetone	x	x	x
Acetophenone	x	x	x

Table 2 Product listing HYDRANAL K-reagents

Part No.	Brand	Description	Package Size
34816	Fluka	HYDRANAL Composite 5 K	500 mL, 1 L, 2 L
34738	Fluka	HYDRANAL KetoSolver	500 mL, 1 L
34698	Fluka	HYDRANAL Medium K	1 L
34817	Fluka	HYDRANAL Working Medium K	1 L
34820	Fluka	HYDRANAL Coulomat AK	500 mL
34821	Fluka	HYDRANAL Coulomat CG-K	50 mL

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Fastrate™ 10 mg *Volumetric** is a water standard in tablet form to support volumetric Karl Fischer titrations. The tablets are manufactured and packaged in single-use, individually sealed foil pouches and are supplied with a Certificate of Analysis with each carton. Fastrate™ 10 mg *Volumetric* is available in a pack of 30 tablets.

* Licensed from Facet Analytical Services and Technology, LLC
U.S. Patents 7,049,146 and 7,122,376 and foreign equivalents apply

For details and complete applications on HYDRANAL K-reagents and our other high-quality HYDRANAL reagents for pyridine-free water determination by Karl Fischer titration, please visit our website sigma-aldrich.com/hydranal or contact our HYDRANAL laboratories:

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Only the brand has changed

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1-800-493-7262 or visit:
sigma-aldrich.com/rebranding

New High-Purity Standards for Pesticide Residue Analysis

Matthias Nold, Product Manager Analytical Standards matthias.nold@sial.com



The reliable quantification of pesticide residue hinges on using standards of appropriate composition and quality. Our PESTANAL® line of over 1200 high-purity pesticide and metabolite standards for food and environmental applications meets these criteria. Suitable for both routinely monitored and newly introduced pesticides,

this line was recently expanded by the addition of the pesticide standards shown below.

Please visit our website and our on-line catalogue to find the appropriate standard for your application.

Part No.	Brand	Description	Package Size	Part No.	Brand	Description	Package Size
32872	Fluka	Alanycarb	100 mg	32800	Fluka	Nitarzone	100 mg
32874	Fluka	Chlorimuron-ethyl	100 mg	32802	Fluka	Triclabendazole	100 mg
32871	Fluka	Cyclanilid	100 mg	32964	Fluka	Metrafenone	100 mg
33706	Fluka	Spinosad	50 mg	32967	Fluka	Bispyribac sodium	100 mg
33754	Fluka	Nosiheptide	25 mg	32966	Fluka	Metaflumizon isomeric mix	100 mg
32900	Fluka	Benalaxyl-M	10 mg	32904	Fluka	Cefalonium hydrate	100 mg
33746	Fluka	Moxidectin	25 mg	32973	Fluka	Pyrasulfutole	100 mg
32811	Fluka	Thiosultap monosodium	100 mg	32974	Fluka	Forchlorfenuron	100 mg
32808	Fluka	Metalaxyl-M	100 mg	32917	Fluka	Prallethrin	100 mg
32801	Fluka	Flubendiamid	100 mg	32919	Fluka	Imazosulfuron	50 mg
32805	Fluka	Mandipropamid	100 mg	32918	Fluka	Halosulfuron-methyl	50 mg

New Alkylphenol Standards for Water Analysis

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ISO 18857-2 pertains to the analysis of octylphenol, nonylphenol and the mono- and diethoxylates in surface water. Established in Germany in 2007, it was followed by international interlaboratory proficiency testing in 2007 and 2008. In addition to the standards required

for ISO 18857-2, we now offer the non-ionic tensides shown below. These new compounds belong to our OEKANAL® line, which includes over 400 high-purity standards of common pollutants and contaminants for environmental analysis.

Part No.	Brand	Description	Package Size
33701	Fluka	4-(3,6-Dimethyl-3-heptyl)-phenol	10 mg
33707	Fluka	4-(3,5-Dimethyl-3-heptyl)-phenol	10 mg
33708	Fluka	4-(2,6-Dimethyl-2-heptyl)-phenol	10 mg

New Natural Compound Standards

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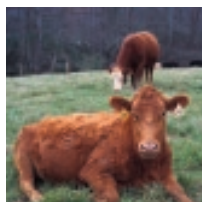


The purported health benefits of theaflavins, polyphenolic phytochemicals found in tea, have stimulated their analysis in food and other substances. We are pleased to offer theaflavin and two theaflavin gallates in reference standard quality to facilitate their analysis. We also offer canthaxanthin, a carotenoid administered to farm-raised fish and used as a colouring agent in tanning pills.

Part No.	Brand	Description	Package Size
55016	Fluka	Theaflavin	1 mg
53963	Fluka	Theaflavin monogallate	1 mg
92223	Fluka	Theaflavin 3,3'-digallate	1 mg
32993	Fluka	Canthaxanthin	10 mg

New Hormone Standards

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Our VETRANAL® product line includes over 200 high-purity standards of the active ingredients of veterinary drugs, primarily antibiotics and hormones. We have added two new anabolic-androgenic steroid hormones, clostebol and desogestrel, to round out the VETRANAL line.

Part No.	Brand	Description	Package Size
33755	Fluka	Clostebol acetate	100 mg
32809	Fluka	Desogestrel	25 mg

New Deuterated Drug Standards

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Mass spectrometry using isotope-labelled internal standards is powerful analytical tool for trace-level detection. Because it can be difficult to obtain labelled standards of

adequate purity for MS analysis, we offer a high-purity line of deuterated drug standards, including two recent additions, ibuprofen-d₃ and ochratoxin-d₅.

Part No.	Brand	Description	Package Size
55264	Fluka	Ibuprofen-d ₃	50 mg
33705	Fluka	Ochratoxin A-d ₅ (100 µg/mL in acetonitrile)	1 mL
32981	Fluka	Sulphamethoxypyridazin-d ₃	10 mg
32982	Fluka	Ciprofloxacin-d ₈ hydrochloride	10 mg
32983	Fluka	Enrofloxacin-d ₅ hydrochloride	10 mg
32985	Fluka	Benzylpenicillin-d ₇	10 mg
32987	Fluka	Difloxacin-d ₃ hydrochloride	10 mg
32998	Fluka	Ofloxacin-d ₃ hydrochloride	10 mg

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