

Analytix

Advances in Analytical Chemistry

Issue 1 • 2007

 **Fluka**

Riedel-deHaën®

Basic Mobile Phase Additives for LC-MS

Standards

- *TraceCERT*™ Certified Reference Materials
- Trans Fat Analysis
- Mycotoxin Internal Standards

Analytical Microbiology

- Chromogenic Media for Enterobacteria

Sensorics

- Dyes for Optical Chemosensors

Titration

- Role of pH in Karl Fischer Titrations

Chromatography

- Flash Performance Upgrade

New Product Corner

- New Chemical Reference Standards



SIGMA-ALDRICH

TraceCERT™ Swiss precision meets analytical competence



Picture Michael Weber,
Manager Research & Development /
Europe

Dear Colleague,

During my time as the national Swiss delegate for metrology in inorganic chemistry, I saw the myriad ways that people deal with metrological themes such as traceability, uncertainty and certification of reference materials. All too often, these concepts are neither understood nor applied properly and consequently many customers are confused by them. Hence we decided to write a series of articles to answer the most frequently asked questions related to these topics. After an introduction in the last issue of Analytix, the first technical article „What Traceability Really Means“ appears in this issue.

To help clarify the fundamental metrological themes, we've begun a series of articles associated with the market introduction of our new **TraceCERT™** products. These traceable and certified reference materials are produced with special and unique equipment that is tailor-made for the production of metrological intercomparison samples. As you may already know, the Swiss Federal Institute for Materials Science and Testing (EMPA) terminated their activities as a national metrological institute for metrology in chemistry in 2005. As the former head of the metrological research group at EMPA, I had the unique opportunity to bring this metrological know-how to Sigma-Aldrich. Recognizing that properly certified reference materials are a strategic market opportunity, Sigma-Aldrich decided to acquire most of the equipment from EMPA and build a new, dedicated laboratory especially designed for this purpose.

The first **TraceCERT™** calibration solutions for ICP, AAS and IC manufactured in our new facility are now available. These solutions are characterized by:

- Unique (metrological) level of accuracy, uncertainty and documentation
- Traceability to at least two independent references (i.e. NIST, BAM or SI unit kg)
- Lot-specific values, expiry dates, storage data
- Certification according to ISO Guide 35
- Highest-purity starting materials available
- ICP standards list up to 70 trace impurities and are packaged in light- and gas-tight aluminum foil bags
- Competitive pricing

With more than 25,000 products for analytical chemistry and over 8,000 standards, Sigma-Aldrich is the worldwide leader in this market. With our new **TraceCERT™** series we demonstrate that we are also the No. 1 supplier of highly sophisticated products such as certified reference materials.

We sincerely hope you will enjoy the upcoming series of technical articles. With the introduction of **TraceCERT™** reference materials, we are confident of bringing more accuracy and less uncertainty to your measurement results.

Kindly,

Michael Weber

Manager Research & Development / Europe
michael.weber@europe.sial.com



Picture

Front Cover of Analytix Issue 5 2006

Feature Article

- 04 *Mobile Phase Additives for LC-MS. Part 5: The Bases – Reverse Buffering, Negative and Reverse Ionization*
Benefits of and procedures for using bases as LC-MS mobile phase additives

Standards

- 07 *TraceCERT™ – Traceable Certified Reference Materials. Part 2: What Traceability Really Means*
Theory of and background information on traceability as well as the new „triple traceability“ approach with *TraceCERT™* products is presented
- 10 *Trans Fat Analysis*
Complete offering of calibration standards, GC columns and sample preparation devices
- 14 *Mycotoxin Internal Standards from Sigma-Aldrich*
Fully ¹³C-isotope labeled internal standards for the LC-MS analysis of mycotoxins

Analytical Microbiology

- 16 *Chromogenic Media for Enterobacteriaceae from Sigma-Aldrich*
Rapid, reliable and specific technique for the isolation, differentiation and identification of Enterobacteriaceae in water, food, environmental and biological samples

Sensorics

- 18 *Selected Dyes for the Preparation of Optical Chemosensors*
Chromoreactands, fluororeactands and chromionophores for optical detection of alcohols, amines, bisulfite and pH

Titration

- 20 *Karl Fischer Titration*
Influence of pH on reaction rate and how to eliminate pH-related problems

Chromatography

- 22 *Flash Performance Upgrade*
Enjoy the benefits and cost-savings of VersaPak® pre-packed cartridges on your existing flash system

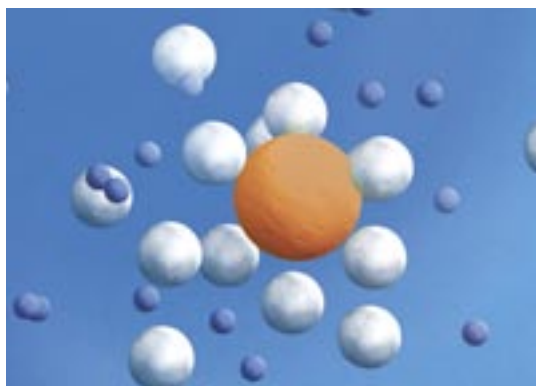
New Product Corner

- 23 *New Mycotoxin, Pesticide and Forensic Standards*

Mobile Phase Additives for LC-MS. Part 5: The Bases – Reverse Buffering, Negative and Reverse Ionization

This is the fifth article in a five part series on mobile phase additives for LC-MS to appear in each issue of *Analytix* in 2006 and the first issue in 2007

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



LC-MS analysis most often is run in positive ion mode using additives that support it, like organic acids and their ammonium salts. However, surprising possibilities exist outside this conventional approach. Basic additives are among the most interesting yet well-kept secrets in LC-MS. Basic pH is a good precondition for negative ionization (forming anions), but positive ionization (forming cations) is also possible. Under basic conditions, positive ionization would be the “reverse ionization,” whereas negative ionization here is the “straight ionization.” When the chromatographic resolution is best under acidic conditions, but sensitivity better in negative ion mode under basic conditions, the so-called “reverse buffering” can be useful, wherein a basic additive is added post-column via T-piece. Basic additives offer a much wider range of ionization capabilities than other additives, although they are somewhat compound-specific. Also, care must be taken in choosing an HPLC column that can withstand the high pH values that may occur when the basic additive is contained in the mobile phase.

For all figures
red trace is raffinose,
green is bradykinin, dark
red is digoxin, blue is
reserpine and dark
green is propazine.

Figure 1 EIC of test compounds with 0.1% w/v ammonium bicarbonate as mobile phase additive (positive ion mode = reverse ionization)

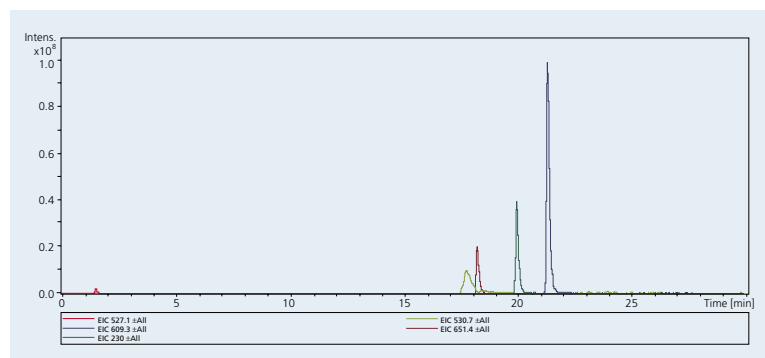


Table 1 List of Sigma-Aldrich LC-MS additives

Cat. No.	Brand	Description*	Package Size	Packaging
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	10 x 1 mL	Glass ampuls
56302	Fluka	Formic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49199	Fluka	Acetic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49916	Fluka	Propionic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
55674	Fluka	Ammonium formate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
49638	Fluka	Ammonium acetate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
61333	Fluka	Sodium citrate tribasic dihydrate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
40867	Fluka	Ammonium bicarbonate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
44273	Fluka	Ammonium hydroxide solution 25%, puriss p.a., eluent additive for LC-MS	100 mL	HDPE bottle
65897	Fluka	Triethylamine, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle

*“puriss” quality grade is defined as >98.5% assay, <0.1% ash, and specification n + 0.001, d + 0.001 with no extraneous color and a homogeneous appearance. “p.a.” or pro analysi denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

Table 2 pH values of basic (alkaline) blends

Solvent	additive	pH
Water	0.1% ammonium bicarbonate	8.0
Acetonitrile	0.1% ammonium bicarbonate (8% water)	10.2
Water	0.1% ammonia	11.2
Methanol	0.1% ammonia (0.3% water)	10.9
Acetonitrile	0.1% ammonia (0.3% water)	12.1
Water	0.1% triethylamine	11.4
Acetonitrile	0.1% triethylamine	12.4

Figure 2 EIC of test compounds with 0.1% w/v ammonium bicarbonate as mobile phase additive (negative ion mode = straight ionization)

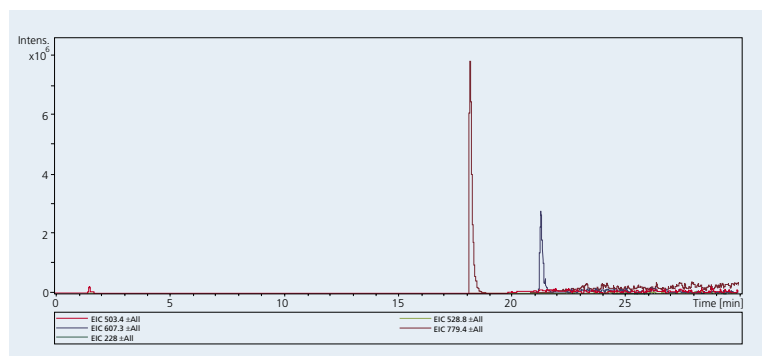


Figure 3 EIC of test compounds with 0.1% w/v ammonia as mobile phase additive (positive ion mode = reverse ionization)

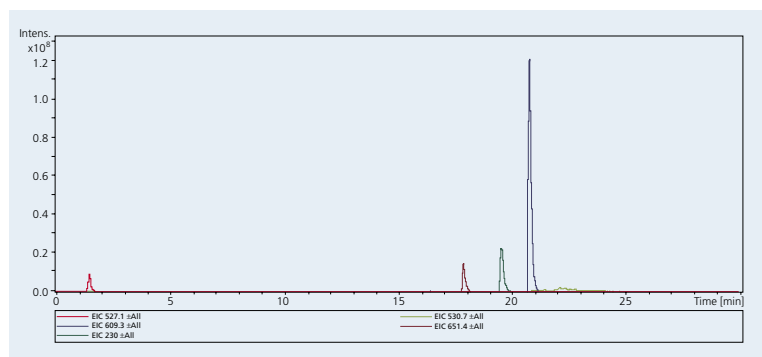


Figure 4 EIC of test compounds, no additive in HPLC, 10% w/v ammonia added via T-piece post-column (negative ion mode = straight ionization)

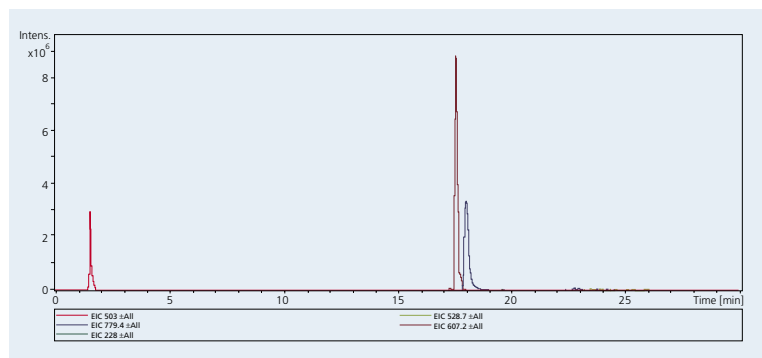
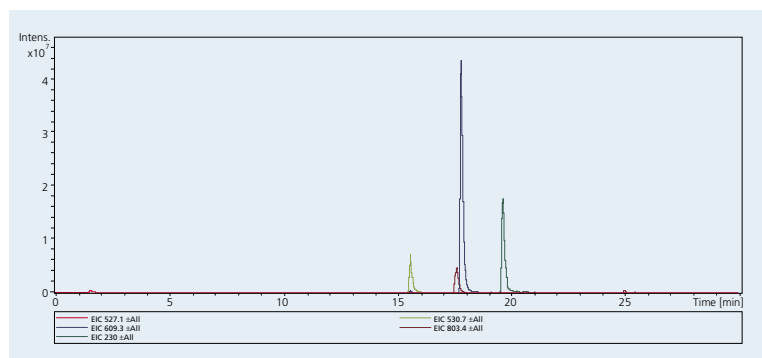


Figure 5 EIC of test compounds, 0.1% FA as additive in HPLC, 10% w/v ammonia added via T-piece post-column (reverse buffering, positive ion mode = reverse ionization)



In this article we will describe some basic additives and experimental setups for their introduction. LC-MS-quality grades of three basic (alkaline) additives are offered by Sigma-Aldrich: ammonium bicarbonate, ammonium hydroxide solution (ammonia) and triethylamine (**Table 1**), listed in order of increasing basic strength (**Table 2**). Ammonium bicarbonate is used for separation and detection of amines and polar compounds under mildly basic conditions. Ammonia solution (ammonium hydroxide) is used for alkaline separation and for post-column addition via T-piece. Triethylamine is mainly used to obtain alkaline conditions for lipophilic compounds. As in previous articles in this series [1-4], we used the test mixture of the five model compounds, the same HPLC instrument (Agilent 1100) and MS detector (Bruker Daltonics esquire3000plus ion trap), and an electrospray-compatible flow rate of 0.4 mL/min through the column. For separations under alkaline conditions we used high pH-stable columns with bridged C18 material, for separations under neutral or acidic conditions (with post-column addition of basic additives) a Supelco Discovery® C18, 15 cm x 2.1 mm I.D., 5 µm, the latter giving the best separation (selectivity and resolution). Additionally an accelerated separation of narcotic drugs (opiates) with ammonium bicarbonate as mobile phase analyzed on a Waters Quattro Micro API Triple Quad MS is discussed.

Under the mildly-basic conditions of ammonium bicarbonate, the elution order of the test compounds is shifted (**Fig. 1**), but, surprisingly, the sensitivity in positive ion mode (reverse ionization) is one of the highest achieved in the entire series, especially for reserpine which is normally measured with addition of formic acid. Raffinose and bradykinin exhibit poor chromatography under these conditions (small or broad peak). In negative ion mode, only digoxin and reserpine are visible and raffinose is very small (**Fig. 2**). Similar results are obtained using ammonia as mobile phase additive, with reserpine giving once again a higher signal than ever achieved in positive ion mode (reverse ionization) and a good signal for raffinose in both modes (**Fig. 3**). Ammonia as an additive and negative ion mode (straight ionization) are the optimal conditions for raffinose and digoxin, especially, but not suitable for the other three test compounds. For these it may be useful to perform the separation under neutral conditions and to add the basic additive post-column via T-piece (**Fig. 4**). However, because the contribution of the flow from the column is much higher than that from the syringe, the additive has to be much more concentrated (here 10%). Under these negative ion mode conditions, raffinose exhibits an especially good signal.

Another interesting experimental setup is to perform the separation under acidic conditions, and change to

Figure 6 EIC of test compounds, 0.1% formic acid as additive in HPLC, 10% w/v ammonia added via T-piece post-column (reverse buffering, negative ion mode = straight ionization)

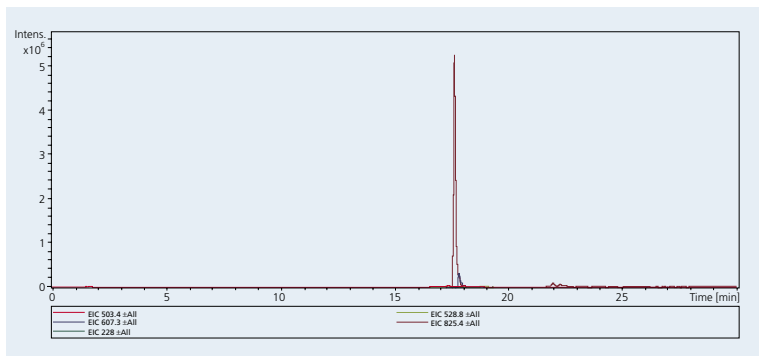


Figure 7 Mass spectra of bilirubin, 0.1% formic acid as additive in HPLC, 10% v/v TEA in acetonitrile added via T-piece post-column (reverse buffering, pos. and neg. mode)

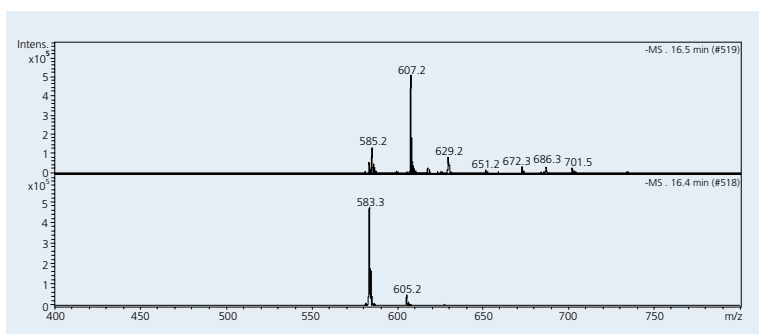
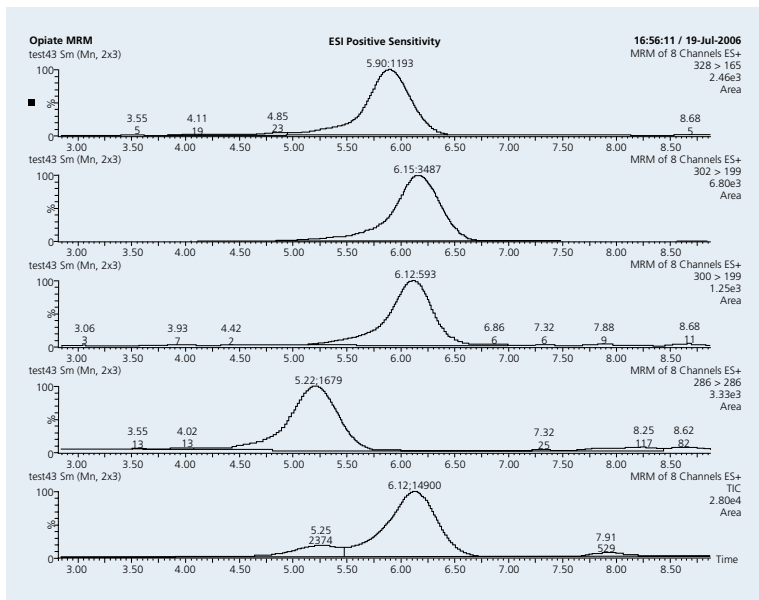


Figure 8 MRMs and TIC of opiates, 90% 10mM ammonium bicarbonate in water (pH=10), gradient to 90% methanol; heroine (328→165), dihydrocodeine (302→199), codeine (300→199) and morphine (286); last track is TIC (positive ion mode = reverse ionization)



alkaline conditions post-column before the flow enters the MS-interface (reverse buffering). This can be combined with negative ion (straight) or positive ion (reverse) ionization (**Figs. 5 and 6**). Care must be taken when choosing the right mass for monitoring in EIC. Under these conditions, digoxin forms the $[M+Na]^+$ ion in positive ion mode and the $[M+formate]^-$ ion in negative ion mode ($M+45!$).

Chromatographic separation of the different bilirubin isomers can be achieved only under acidic conditions, but the MS signal is poor. Bilirubin elutes with a high percentage acetonitrile, so the best way to increase the pH is to add triethylamine (TEA) dissolved in acetonitrile (10% v/v). With this setup, mass spectra can be obtained in both positive and negative ion modes (reverse buffering with reverse or straight ionization, **Fig. 7**). The mass of bilirubin is 694.2 Dalton.

The rapid measurement of narcotic drugs is essential in emergency overdose situations. An experimental setup that permits the simultaneous determination of opiates is shown in **Fig. 8**. The elution is very fast under mildly basic conditions with ammonium bicarbonate as additive. Although the compounds co-elute, quantification is achieved by use of the different MRM-tracks of the molecules. This is a typical triple quad application, where the capabilities of the instrument were combined with optimal chromatographic conditions, which also allow a sufficient ionization in MS.

These various examples show the broad applications for basic additives in LC-MS, which, although not so well known and often underestimated, offer a variety of possibilities over the more-commonly used acidic additives. With this article, we close our series on LC-MS additives. We sincerely hope that we have provided you with some practical hints for the use of these "little helpers" in your daily work with modern, high-tech LC-MS, and have demonstrated that advances in LC-MS are not only driven by the physics of the instrument, but also the chemistry of the column and the mobile phase.

References

- [1] "Mobile Phase Additives for LC-MS. Part 1: Acids – The Most Common Choice" **Analytix** 2006/2, 8-9.
- [2] "Mobile Phase Additives for LC-MS. Part 2: How to Overcome Suppression Effects of TFA" **Analytix** 2006/3, 16-17.
- [3] "Mobile Phase Additives for LC-MS. Part 3: The Neutral Salts" **Analytix** 2006/4, 9-11.
- [4] "Mobile Phase Additives for LC-MS. Part 4: Special Case – Sodium Adduct Formation" **Analytix** 2006/5, 6-7.

Past and current issues of *Analytix* can be viewed and downloaded at: <http://www.sigma-aldrich.com/analytix>

TraceCERT™ – Traceable Certified Reference Materials. Part 2: What Traceability Really Means

This is the second article of a series on Certified Reference Materials to appear in Analytix

By Michael Weber, Manager R&D Europe, Sigma-Aldrich Switzerland ... michael.weber@europe.sial.com
and Jürg Wüthrich, Senior Scientist R&D Europe, Sigma-Aldrich Switzerland ... jwuethrich@europe.sial.com

Figure 1

The international prototype of the kilogram, an artifact made of platinum-iridium, is kept at the International Bureau of Weights and Measures in Paris.



Establishment of SI units and NMIs: A historical perspective

Prior to standardization of weights and measures, enormous variation existed between nations, regions and even individual vendors. For example, in earlier times people could not get the same amount of potatoes when they bought them at different market stands because there was not an established reference for mass. The situation between nations became very confusing, and resulted in the Meter Convention being signed in Paris in 1875 by seventeen nations [1]. The purpose of this treaty was to ensure world-wide uniformity of measurements and their traceability to the International Systems of Units (SI), ultimately established in 1948. Today, fifty-one member states signed this diplomatic treaty. After the adoption in 1954 of the first six base units, the mole was introduced in 1973 as the base unit for amount of substance. Currently, all units of measurement on earth are derived from this set of seven base units (Table 1).

Table 1

The seven base units of the International Systems of Units (SI)

Property	Base Unit	Symbol
Mass	Kilogram	kg
Length	Meter	m
Time, Duration	Second	s
Electric Current	Ampere	A
Temperature	Kelvin	K
Luminous Intensity	Candela	cd
Amount of Substance	Mole	mol

To provide oversight, each member country established a National Metrological Institute (NMI), one per country with a few exceptions. NMIs have responsibility within the respective country for the realization of SI base units and to ensure comparability of measurement results between the member nations. The supply of national standards and reference materials is therefore of crucial importance. Although there are many NMIs that provide the highest level of reference materials and certification services, perhaps the most well-known are the US National Institute of Standardization and Technology (NIST), the European Institute of Reference Materials and Measurement (IRMM) and the German Federal Institute for Materials Research and Testing (BAM).

Metrological background of the traceability concept

The International Vocabulary of Basic and General terms in Metrology (VIM) defines traceability as the “*property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties* [2].” Analysts often report their results as traceable to NIST or to another NMI. This is not literally correct, for, as one consequence of the above definition of traceability, a measurement result is not traceable to an NMI but to a reference material certified by an NMI. Because of the Mutual Recognition Arrangement (MRA) a certified reference material issued by one NMI (e.g. NIST SRM 915, CaCO₃) must always be comparable, within the stated degree of equivalence, to a second NMI (e.g. BAM RS3, CaCO₃). One is not more traceable than the other.

Practical realization of traceability in chemistry

Compared to other base units there is one big difference in the realization of the mole: there is no extant prototype of the mole since it is defined as a multiple of the mass of carbon-12 atoms! So how can we realize traceability in chemistry in practice? Traceability to the SI system can be achieved by traceability to one of the other six base units. Such measurements are called “primary methods.” The following methods are accepted as primary: isotope dilution with mass spectrometry (IDMS), coulometry, gravimetry, titrimetry and determination of freezing point depression [3]. The result of a primary method (analytical technique) is not expressed in moles but in one of the other six base units.

Coulometry, for example, gives a direct traceability from the amount of a certain species (i.e. the analyte concentration in a solution) to the SI base unit ampere.

Another appropriate way to achieve traceability to the SI system is by the weighing of high purity compounds. This follows from an almost unique feature of chemical measurement: 100% purity forms a natural reference value which cannot be exceeded. Coupled with widely available and excellent reference data for atomic and molecular weight, and often with additional data on physical parameters such as density, a high purity

material represents a local, practical realization of concentration units through conversion of mass to molar quantity. Calibration with materials of well-established purity is accordingly a valid means of establishing traceability [4].

Key comparisons and the limits of analytical measurements

In addition to the supply of reference materials, it is also important to evaluate the trans-national comparability of measurement results. Toward this end, periodically the NMIs are invited to participate in so-called Key Comparisons. Key Comparisons are proficiency tests, also called round robin tests, on the highest metrological stage. Only NMIs are invited to participate.

Since the results of these intercomparisons represent national measurement capabilities, the NMIs often expend intense effort in terms of technical equipment and time employing multiple techniques and many replicates. As a consequence, a single result from such a high precision measurement often takes days or weeks.

Round robin results are posted by the Bureau International des Poids et Mesures (BIPM) at <http://kcdb.bipm.org>. A closer look into this database reveals the limits of analytical measurements for many different applications [5-7]. For simple analytical tasks (matrix-free elemental or anion solutions at 1 g/L) the uncertainties of the NMIs are in the range of 0.1-0.4%, with the best values having uncertainties below 0.05%. It is important to remember that these data are collected at the highest metrological stage, which you certainly do not want to pay for when buying a simple elemental standard! Ergo: With very few exceptions it is quite normal to obtain combined, expanded uncertainties of 0.5% and higher under ordinary laboratory conditions (Figure 2).

Figure 2 Increasing uncertainty along the traceability chain. When individual parts of an uncertainty budget are calculated together analysts end up easily with a measurement uncertainty of >0.5%.

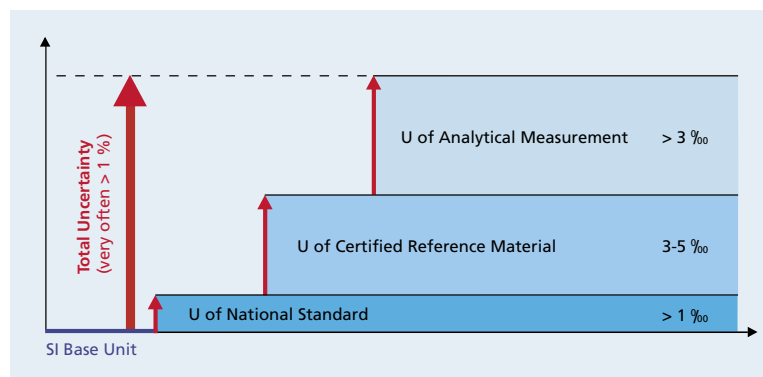
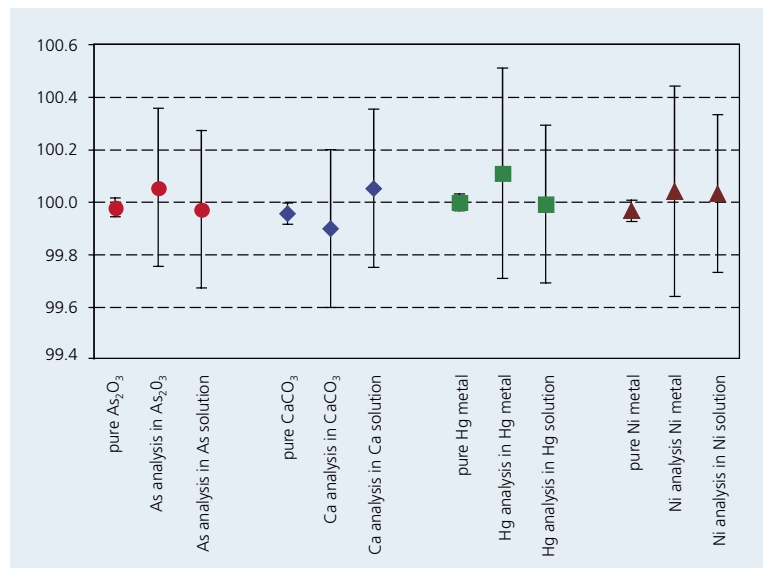


Figure 3 Examples of the purity of *TraceCERT*[™] starting materials (arsenic trioxide, calcium carbonate, mercury and nickel metal) determined by calculation 100% minus all traces (analyzed using ICP-MS, ICP-OES, AAS and IC). The values “X analysis in X” show the purity of the starting materials by direct titrimetric measurement traceable to NIST SRM. The values “X analysis in X solution” show direct titrimetric measurements (traceable to BAM CRM) of *TraceCERT*[™] standard solutions and are relative to the certified content of the gravimetrically-produced standard.



The new „triple traceability“ approach with *TraceCERT*[™]

There are many approaches to establish traceability to different reference standards in analytical chemistry. For *TraceCERT*[™] reference materials and calibration standards, we decided to establish a new concept called “triple traceability.” Standards made according to this concept are characterized by independent traceability chains to three different references (Figure 3):

Traceability chain No. 1:

The starting material is measured against a certified reference material (e.g. NIST) followed by gravimetric preparation of the solutions using balances calibrated with SI-traceable weights. Consequently the value calculated by this unbroken chain of comparisons is

traceable to the reference to which the starting material is compared.

Traceability chain No. 2:

The bottled **TraceCERT™** solution is directly compared to a second reference (e.g. BAM or EMPA) which is independent from the first reference.

Traceability chain No. 3:

Since only comprehensively characterized starting materials of highest purity are used (>70 trace impurities checked in **TraceCERT™**) the calculation of the gravimetric value using the molar mass of the analyte leads to direct traceability to the SI base unit kilogram. As previously discussed, this value is the most accurate; uncertainties of less than 0.2% can be achieved only with this method. Of course, during the preparation of all **TraceCERT™** solutions we prevent material loss and contamination and all balances are certified by DKD (Deutscher Kalibrierdienst) and calibrated with OIML (International Organization of Legal Metrology) Class E2 (up to 12 kg) and F1 (up to 64 kg) weights.

Last but not least, an analysis of variance (ANOVA) is made with the whole dataset. Only when all the values overlap is the **TraceCERT™** batch certified. Further details about the uncertainty calculation and traceability concept are found in the certificates of analysis provided with our **TraceCERT™** products, an example of which is shown in **Figure 4**.

References

- [1] The International System of Units (SI), 8th edition 2006, Organisation Intergouvernementale de la Convention du Mètre. http://www.bipm.org/utis/common/pdf/si_brochure_8_en.pdf (accessed Jan. 5, 2007).
- [2] International vocabulary of basic and general terms in metrology. ISO, Geneva, 1993.
- [3] Kaarls R.; Quinn T.J. The Comité Consultatif pour la Quantité de Matière: a brief review of its origin and present activities. *Metrologia* **1997**, *34*, 1-5.
- [4] Traceability in Chemical Measurement. Eurachem/CITAC Guide, 2003.
- [5] Weber M.; Felber H. Ion Analysis at the Highest Stage – The Metrological Approach by National Metrological Institutes. *Microchimica Acta* **2004**, *146(2)*, 91-95.
- [6] Felber H.; Weber, M. Final report on key comparison CCQM-K8 of mono-elemental calibration solutions. *Metrologia* **2002**, *39*, Tech. Suppl., 08002.
- [7] Weber M., Wüthrich, J., Key comparison CCQM-K29: Anion calibration solutions: final report. *Metrologia* **2006**, *43*, Tech. Suppl., 08012.



Figure 4

Packaging and certificate of analysis included with the new **TraceCERT™**Ultra (ICP) standards. Certificates for **TraceCERT™** (AAS) solutions are available as internet downloads.

Special Offer: 50% off
on your first order on
TraceCERT™Ultra ICP Standards

Please quote promotion code X91 when placing your order.
Valid until April 30th 2007.

An up-to-date list of **TraceCERT™** standards can be found at www.sigmaaldrich.com/tracecert

Trans Fat Analysis Look to Sigma-Aldrich for the complete offering of calibration standards, GC columns and sample preparation devices for reliable identification and quantification

Vicki Yearick, Chemical Standards Product Manager ... vyearick@sial.com



Current interest in trans fats

As analytical chemists, we are cognizant of the impact of our work on nearly every aspect of modern life, from the environment to consumer products of all market categories. However, it is rewarding when our efforts can be directly applied to topics of current interest to the general population. One area where analytical chemistry and public interest broadly overlap is food safety and nutrition. Today, obesity, diabetes and cardiovascular disease with their related complications are epidemic in America, Europe and increasingly in other parts of the world. Although lifestyle and heredity contribute, a clear link between diet and these maladies has been firmly established, specifically the intake of different types of fats.

Nutritionally, two types of fats are of particular concern: saturated fats and trans unsaturated fats (or trans fats), both referring to the structure of the fatty acid moieties. In nature, fats with cis conformation dominate and, correspondingly, enzymes have evolved to efficiently digest and metabolize them with a high degree of specificity. Naturally-occurring trans fats are relatively rare, but because of their texture- and stability-enhancing properties, they have become predominant synthetic additives to processed foods, especially fried foods and baked goods. However, because the number, position and conformation of double bonds influence the function and metabolism of fatty acids, trans fats interfere with

the natural metabolic process and result in an imbalance of the LDL:HDL ratio and increased lipoprotein(a) and the associated health-related problems [1-3].

Because trans fats have adverse health consequences and no known nutritional benefits over other fats, consumer groups have pressured manufacturers and restaurants for their elimination. Regulatory agencies worldwide, including the US FDA [4], now require content labeling to inform buyers of trans fat levels of foods and some dietary supplements.

Sigma-Aldrich products for trans fat analysis

Reliable analytical methods are necessary to monitor levels of trans fats in foods and to ensure regulatory compliance. Sigma-Aldrich, in keeping with our commitment to offering relevant and high quality products for analytical chemistry, has all the components necessary for the successful analysis of trans fats in all matrixes. These products include:

- **Discovery® Ag-Ion Solid Phase Extraction (SPE):** Specifically developed for the fractionation by argentation chromatography of FAMES based on cis/trans conformation and the degree of unsaturation.
- **Supelco SP-2560 Capillary GC columns:** The bis(cyanopropylsiloxane) phase is the benchmark in the Food & Beverage industry for cis/trans fatty acid analysis. Supelco SP-2560 has unsurpassed efficiency and is offered in traditional 100 meter and 75 meter lengths for fast GC.
- **Calibration Standards:** Mixes and single components with high purity, extensive testing and quality documentation.

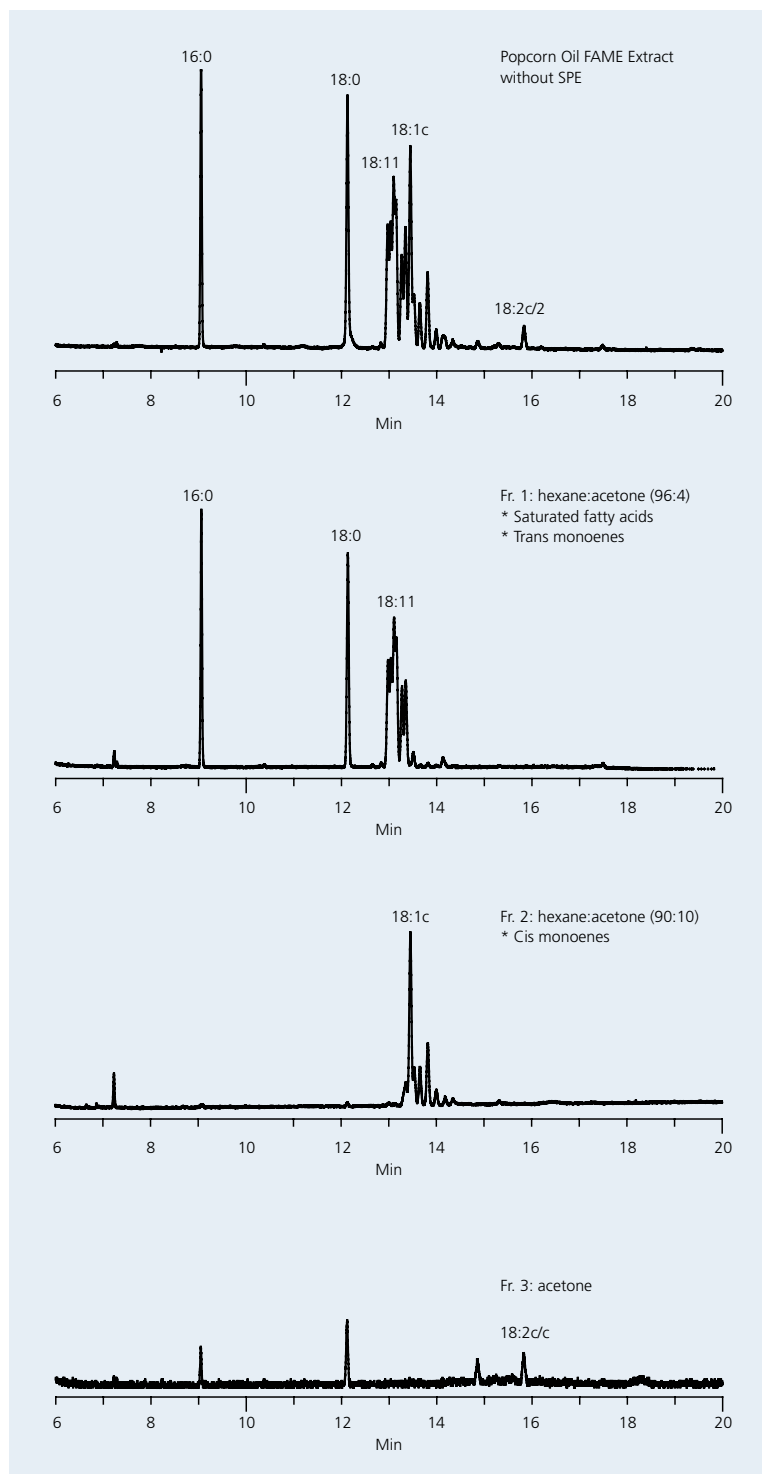
Effective cis/trans fractionation using discovery Ag-Ion SPE: reduce complexity of capillary GC analysis

Fats are typically removed from the food sample by extraction with an organic solvent, like petroleum ether,

Table 1 Recovery distribution of FAMES fraction of popcorn oil using Discovery Ag-Ion SPE

Fraction No.	Eluent (v/v)	Vol. (mL)	18:0	Trans 18:1	Cis 18:1	Cis/Cis 18:2
1	Hexane:Acetone (96:4)	6	100%	100%	2%	
2	Hexane:Acetone (90:10)	4			98%	
3	Acetone	4				100%

Figure 1 Improved resolution of FAMES from microwave popcorn oil sample by using Ag-Ion SPE prior to GC analysis



GC Conditions

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 μ m (23348-U)
oven: 180 $^{\circ}$ C, isothermal
inj.: 220 $^{\circ}$ C
det.: FID, 220 $^{\circ}$ C
carrier gas: hydrogen, 40 cm/sec. at 180 $^{\circ}$ C
injection: 0.5 μ L, 100:1 split
liner: 4 mm I.D., split, cup design

concentrated and reconstituted in hexane (see AOAC Method 996.06 [5]). After conversion to the corresponding methyl ester, the FAMES are separated using capillary GC using a polar stationary phase, like Supelco SPTM-2560. Although the extraction method yields a clean FAME profile, the monounsaturated octadecenoic (C18:1) fatty acids, which are the most common trans fats in food [6], are difficult to resolve by capillary GC. To reduce the sample complexity and improve resolution, silver-ion solid phase extraction (Ag-Ion SPE) is used to fractionate cis/trans isomers and other FAMES prior to capillary GC analysis.

Discovery[®] Ag-Ion SPE was developed specifically for the fractionation of FAMES based on degree of unsaturation and for the resolution of cis/trans isomers. The Ag-Ion SPE phase is silver-loaded using a proprietary procedure to offer optimal resolution, performance and capacity. The silver ions act as electron acceptors to form polar complexes with double bonds of unsaturated FAMES. Fractionation occurs because strength of the interaction increases with the number of double bonds, and cis FAMES interact more strongly than trans due to steric accessibility. As a result, cis FAMES are more strongly retained on Ag-Ion SPE than trans FAMES. These differences can be exploited, allowing for FAME fractionation to reduce sample complexity prior to GC analysis.

The improvement in trans FAME analysis by using Discovery Ag-Ion SPE is demonstrated by comparing the chromatograms in **Figure 1**. Classes of FAMES were fractionated using the elution scheme described in **Table 1**. Note the cleaner profile and high recovery as a result of the Ag-Ion fractionation prior to GC analysis.

Discovery Ag-Ion SPE is available in two convenient formats: a 750 mg bed weight, 6 mL SPE tube and a 750 mg bed weight, 1 mL Rezorian[™] cartridge. Both have a high capacity (1 mg) for FAMES. Rezorian cartridges are small polypropylene barrels capped at both ends with Luer-Lok[™] fittings. The hardware is designed for low positive pressure applications, which can also be adapted for use with vacuum manifolds. Regardless of the format, each lot of Discovery Ag-Ion SPE is tested and quality controlled for cis/trans FAME resolution.

For more information, please request Technical Report "Discovery[®] Ag-Ion SPE for FAME Fractionation and Cis/Trans Separation (T406062)," available on-line at www.sigma-aldrich.com or by contacting your local Sigma-Aldrich office or by returning the overleaf and ticking the box with the code IRV.

Table 2 Product Listing

Discovery® Ag-Ion Solid Phase Extraction (SPE)

Cat. No.	Brand	Description	Package Size
54225-U	Supelco	Discovery Ag-Ion SPE, 750 mg/6 mL SPE Tubes	30 tubes
54226-U	Supelco	Discovery Ag-Ion SPE, 750 mg/1 mL Rezorian™ Cartridges	10 tubes

Supelco SP-2560 Capillary GC Columns

Cat. No.	Brand	Description
23348-U	Supelco	SP-2560, 75 m x 0.18 mm I.D. x 0.14 µm df
24056	Supelco	SP-2560, 100 m x 0.25 mm I.D., 0.20 µm df

Calibration Standards - Cis/Trans Mixes

Cat. No.	Brand	Description	Package Size
47885-U	Supelco	37-Component FAME Mix (each ampul contains 10 mg/mL of the FAME reference standard mix in methylene chloride)	1 mL
18916	Supelco	C18:0 - C20:0 FAME Mix	100 mg
18917	Supelco	C14:0 - C:22:0 FAME Mix	100 mg
18919	Supelco	C:4 - C24:1 FAME Mix	100 mg
18920	Supelco	C8:0 - C22:0 FAME Mix	100 mg
O5632	Sigma	Linoleic acid, conjugated methyl ester (A mixture of <i>cis</i> - and <i>trans</i> -9,11- and -10,12-octadecadienoic acid methyl esters)	250 mg, 1 g
47791	Supelco	Linoleic Acid Methyl Ester Mix, <i>cis/trans</i> , 10 mg/mL (total weight) in methylene chloride	1 mL
47792	Supelco	Linolenic Acid Methyl Ester Isomer Mix, 10 mg/mL (total weight) in methylene chloride	1 mL

Single Isomers

Cat. No.	Brand	Description	Package Size
47571 -U	Supelco	<i>cis</i> -5,8,11,14,17-Eicosapentaenoic acid methyl ester, 10 mg/mL in heptane	1 mL
46902-U	Supelco	<i>cis</i> -9-Octadecenoic acid methyl ester, 10 mg/mL in heptane	1 mL
76117	Fluka	<i>trans</i> -9-Hexadecenoic acid methyl ester	100 mg
83914	Fluka	<i>trans</i> -12-Hydroxy-9-octadecenoic acid methyl ester	100 mg
47199	Supelco	<i>trans</i> -6-Octadecenoic acid methyl ester, 10 mg/mL in heptane	1 mL
46903	Supelco	<i>trans</i> -9-Octadecenoic acid methyl ester, 10 mg/mL in heptane	1 mL
45119	Fluka	<i>trans</i> -9-Octadecenoic acid methyl ester	1 mL, 5 mL
46905-U	Supelco	<i>trans</i> -11-Octadecenoic acid methyl ester, 10 mg/mL in heptane	1 mL
V1381	Sigma	<i>trans</i> -11-Octadecenoic acid methyl ester	25, 100 mg, 500 mg, 1 g
46951-U	Supelco	<i>trans-trans</i> -9,12-Octadecadienoic acid methyl ester, 10 mg/mL in heptane	1 mL
62155	Fluka	<i>trans,trans</i> -9,12-Octadecadienoic acid methyl ester	100 mg
70055	Fluka	<i>trans</i> -9-Tetradecenoic acid methyl ester	100 mg

References

- [1] Ascherio, A.; Willett, W. Health effects of *trans* fatty acids. *Am. J. Clin. Nutr.* **1997**, *66*(suppl), 1006S-1010S.
- [2] Stender, S.; Dyerberg, J. Influence of *Trans* Fatty Acids on Health. *Annals of Nutrition and Metabolism* **2004**, *48*(2), 61-66.
- [3] American Heart Association Web Page, <http://www.americanheart.org/presenter.jhtml?identifier= 1728> (accessed Jan. 4, 2006).
- [4] Federal Register, July 11, 2003 (Volume 68, Number 133), 21 CFR Part 101: Food Labeling; *Trans* Fatty Acids in Nutrition Labeling, <http://www.cfsan.fda.gov/~lrd/fr03711a.html> (accessed Jan. 4, 2006).
- [5] Official Methods of Analysis of AOAC International, 17th edition, Revision 1, 2002.
- [6] US Food and Drug Administration Web page. Questions and answers about *trans* fat nutrition labeling. <http://www.cfsan.fda.gov/~dms/qatrans2.html> (accessed Jan. 4, 2006).

TraceCERT™

Swiss Precision Meets Analytical Competence

Traceable Certified Reference Materials for Analytical Chemistry

- Unique level of accuracy & lot specific certified values
- Traceable to at least two independent references (*i.e.* NIST, BAM or SI unit kg)
- Certified according to ISO Guide 31 and 35 including uncertainty statement
- Highly sophisticated packaging & documentation with expiry date
- ICP standards list up to 70 trace impurities
- Production capabilities fulfilling highest metrological requirements

Ordering Information

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

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



Mycotoxin Internal Standards from Sigma-Aldrich Fully ^{13}C -isotope labeled internal standards for the LC-MS analysis of mycotoxins

By Nicole Amann, Product Manager Analytical Standards ... namann@europe.sial.com



Mycotoxins, toxins produced by mushrooms, molds and yeasts, are ubiquitous contaminants in food and feedstuffs. Mycotoxin detection is often accomplished using rapid techniques such as ELISA, but detailed analysis of individual mycotoxins in multitoxin samples using HPLC with MS detection (LC-MS) is becoming increasingly prevalent. Recently, the determination of up to 40 distinct mycotoxins in a single run has been reported using LC-MS [1].

To take advantage of the sensitivity and resolving power of LC-MS, sample matrix effects, which can suppress MS ionization, must be kept to a minimum. One technique to compensate for matrix effects is to employ appropriate internal standards (IS). IS addition also accounts for variations during sample preparation and cleanup, as well as during ionization, and permits the

development of methods with especially high analytical accuracy and precision.

The ideal IS is a stable, non-radioactive isotopically-labeled version of the target analyte. In this scenario, both the IS and analyte have identical molecular structures and physicochemical properties. Additionally, the ionization potentials are nearly the same, but because of the higher molecular weight of the IS (due to the incorporated isotopes), clear distinction between it and target analyte is facilitated. Ideally, isotopically-labeled analogues must have a large enough mass difference to nullify the effect of the natural abundance of heavy isotopes in the analyte. This mass difference will depend generally on the molecular weight of the analyte itself; with molecules ranging from 200 to 500 amu, a minimum of three extra mass units is typically required.

Isotopically-labeled mycotoxin standards supplied by Sigma-Aldrich are fully labeled, thus providing an optimum mass unit difference between labeled standard and target analyte. For example, the [$^{13}\text{C}_{15}$]-DON (deoxynivalenol) standard, which is available as 25 $\mu\text{g}/\text{mL}$ liquid calibrant, was thoroughly characterized by Häubl et al. [2] with regard to purity and isotope distribution and substitution, the latter being close to 99%. Fortification experiments with maize proved the excellent suitability of [$^{13}\text{C}_{15}$]-DON as an IS; a correlation coefficient (r^2) of 0.9977 and a recovery of 101% \pm 2.4% were obtained. The same analysis without an internal standard resulted in $r^2=0.9974$ and a recovery

Table 1 Labeled Mycotoxin Internal Standards from Sigma-Aldrich

Cat. No.	Brand	Description	Package Size
34155	OEKANAL (Riedel-de Haën)	Deoxynivalenol- d_1 , 100 $\mu\text{g}/\text{mL}$ in acetonitrile	2 mL
34128	OEKANAL (Riedel-de Haën)	[$^{13}\text{C}_{15}$]-Deoxynivalenol, 25 $\mu\text{g}/\text{mL}$ in acetonitrile	1 mL
34129	OEKANAL (Riedel-de Haën)	3-Acetyl- d_3 -deoxynivalenol, 100 $\mu\text{g}/\text{mL}$ in acetonitrile	2 mL

Table 2 New Products Coming Soon

Cat. No.	Brand	Description	Package Size
33892	OEKANAL (Riedel-de Haën)	[$^{13}\text{C}_{24}$]-T-2 toxin, 25 $\mu\text{g}/\text{mL}$ in acetonitrile	1 mL
33705	OEKANAL (Riedel-de Haën)	Ochratoxin A- d_5 , 100 $\mu\text{g}/\text{mL}$ in acetonitrile	1 mL
33621		[$^{13}\text{C}_{34}$]-Fumonisin B1*	
33416		[$^{13}\text{C}_{20}$]-Ochratoxin A*	

* Currently in development, available summer 2007.

Figure 1
Molecular structure of fully ^{13}C -labeled mycotoxins for use as internal standards

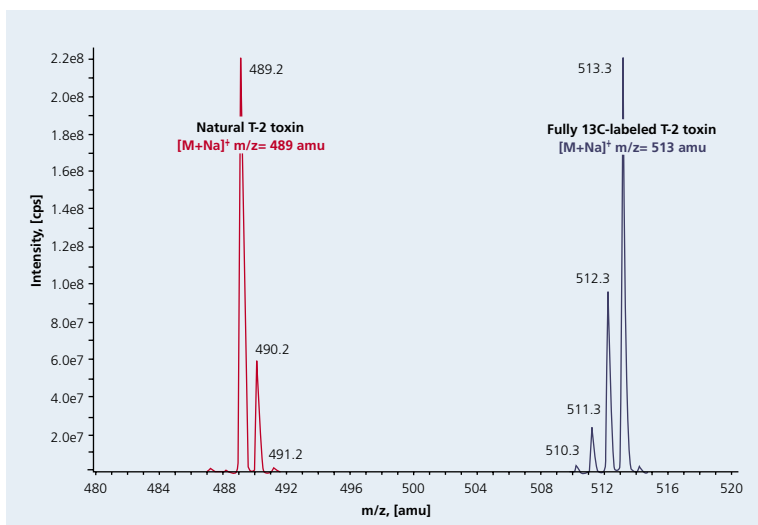
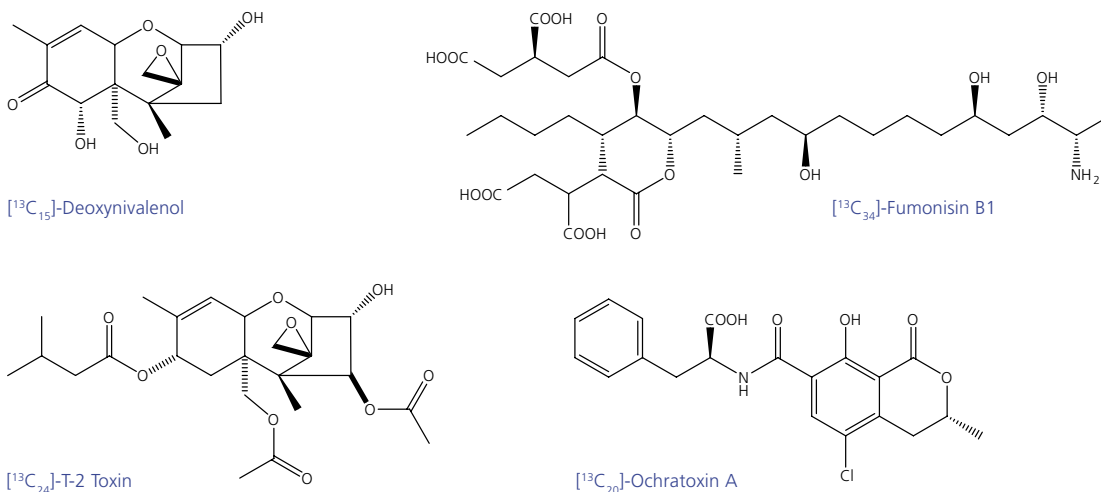


Figure 2 Sodium adducts (+23 amu) of natural T-2 toxin and fully ^{13}C -labeled T-2 toxin

of only 76% \pm 1.9%, underscoring the successful compensation for analyte loss during sample preparation and ion suppression effects by using isotopically-labeled internal standards [3].

Sigma-Aldrich is pleased to offer fully labeled $[^{13}\text{C}_{15}]$ -deoxynivalenol, deoxynivalenol- d_1 , 3-acetyl- d_3 -deoxynivalenol, $[^{13}\text{C}_{24}]$ -T-2 toxin and ochratoxin A- d_5 to enable the reliable quantification of the corresponding naturally occurring toxic secondary mycotoxin metabolites. Additional labeled internal standards, $[^{13}\text{C}_{34}]$ -fumonisin B1 and $[^{13}\text{C}_{20}]$ -ochratoxin A, are currently under development and will be introduced in the near future.

References

- [1] Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2649-2659.
- [2] Häubl, G.; Berthiller, F.; Krska, R.; Schuhmacher, R. Suitability of a fully ^{13}C isotope labeled internal standard for the determination of the mycotoxin deoxynivalenol by LC-MS/MS without clean-up. *Anal. Bioanal. Chem.* **2006**, *384*(3), 692-696.
- [3] Häubl, G.; Berthiller, F.; Rechthaler, J.; Jaunecker, G.; Binder, E. M.; Krska, R.; Schuhmacher, R. Characterisation and application of isotope-substituted $[^{13}\text{C}_{15}]$ -deoxynivalenol (DON) as an internal standard for the determination of DON. *Food Add. Contam.* **2006**, *23*(11), 1187-1193

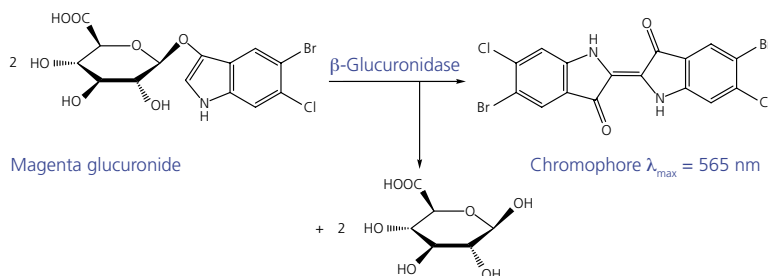
Acknowledgements

This article was developed in close cooperation with DI. Georg Häubl from the Christian Doppler Laboratory for Mycotoxin Research, Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Applied Life Sciences Vienna, Austria

Chromogenic Media for Enterobacteriaceae from Sigma-Aldrich Rapid, reliable and specific technique for the isolation, differentiation and identification of Enterobacteriaceae in water, food, environmental and biological samples

By Jvo Siegrist, Product Manager Microbiology ... isiegris@europe.sial.com

Figure 1
Presence of
 β -glucuronidase
activity using Magenta
glucuronide-containing
chromogenic media



Enterobacteriaceae is a large family of bacteria that has great impact on the safety and quality control of water and food. Some members of this family are normal inhabitants of the digestive tracts of humans and animals. Others, however, like *Salmonella* and enterohaemorrhagic *Escherichia coli*, are serious pathogens that have been linked to outbreaks of food- and water-borne illnesses and deaths. Having rapid, reliable and specific techniques to identify Enterobacteriaceae is important in order to test for the pathogens in foodstuffs before they enter the food chain and to identify the source of outbreaks.

Enterobacteriaceae are gram-negative and oxidase-negative rod-shaped bacteria. They are facultative anaerobes, exhibit both respiratory and fermentative metabolism, and have optimal growth between 22 and 37°C. Most Enterobacteriaceae can grow on D-glucose as the sole carbon source. Enzymes like tryptophanase, phenylalanine deaminase, urease, β -glucuronidase and other enzymes give the organisms a characteristic biochemical profile.

This unique biochemical profile can be used to identify members of Enterobacteriaceae and is the basis behind chromogenic media. Chromogenic media contain substrates that produce a chromophore when they undergo a reaction with a specific enzyme. An example is shown in **Figure 1** where a chromophore is produced by action of β -glucuronidase on Magenta glucuronide substrate in the media. The color change indicates enzyme activity and this in turn identifies or confirms the presence of the specific pathogen or species of pathogen.

Besides providing reliable and accurate Enterobacteriaceae identification, additional benefits of chromogenic media are time savings by elimination of further confirmation steps, such as the indole reaction. The range of chromogenic media for Enterobacteriaceae

available from Sigma-Aldrich is listed on the next page. Additional chromogenic media, instructions and further information can be found in Analytix 1/2005 or on our website at www.sigma-aldrich.com/microbiology.

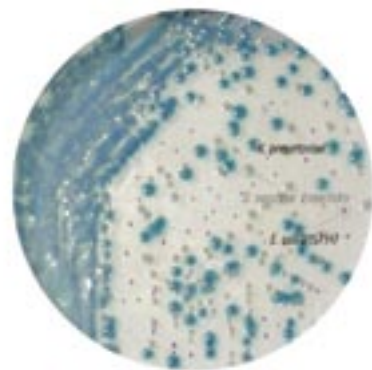


Figure 2 *E. coli* 0157:H7 (dark purple-magenta), *Klebsiella pneumoniae* (green with blue halo) and *Salmonella* serotype *Enteritidis* (pale turquoise) on HiCrome™ EC O157:H7 Selective Agar without supplement.

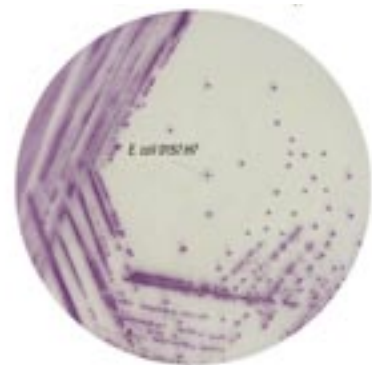


Figure 3 *E. coli* 0157:H7 (dark purple-magenta) on HiCrome™ EC O157:H7 Selective Agar.

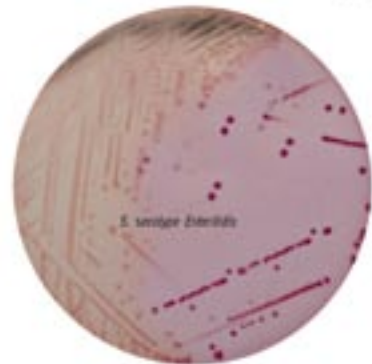





Figure 4 *Salmonella* serotype *Enteritidis* on HiCrome™ Salmonella Agar, Improved.

Special Offer: Test the smallest pack size of one of our  Chromogenic Media from the list below for free! Quote promotion code Y02 when placing your order. One order per customer. Offer expires March 31st 2007.

Table Chromogenic Media for Enterobacteriaceae Detection from Sigma-Aldrich

Cat. No.	Brand	Media	Description
81938	Fluka	HiCrome™ Coliform Agar Supplement: Novobiocin (Fluka 74675)	A selective chromogenic medium recommended for simultaneous detection of <i>E. coli</i> and total coliform in water and food samples.
95207	Fluka	HiCrome™ E. coli Agar A	Recommended for the detection and enumeration of <i>E. coli</i> in foods without further confirmation.
70722	Fluka	HiCrome™ E. coli Agar B	Recommended for the detection and enumeration of <i>E. coli</i> in foods without further confirmation.
39894 	Fluka	HiCrome™ EC O157 Agar Optional Supplement: 1% potassium tellurite solution (Fluka 17774)	A chromogenic medium for isolation and differentiation of <i>E. coli</i> O157 from food and environmental samples.
72557 	Fluka	HiCrome™ EC O157:H7 Selective Agar, Base (see Figure 2 + 3) Supplement: HiCrome ECO157:H7 Selective Supplement (Fluka 44931)	A selective agar recommended for selective isolation and facile detection of <i>E. coli</i> O157:H7 in food samples.
73009	Fluka	HiCrome™ ECC Agar	A differential medium recommended for the presumptive identification of <i>E. coli</i> and other coliforms in food and environmental samples.
85927	Fluka	HiCrome™ ECC Selective Agar Supplement: Cefsulodin (Fluka 22126)	A selective medium recommended for the simultaneous detection of <i>E. coli</i> and other coliforms in water and food samples.
09142 	Fluka	HiCrome™ ECD Agar with MUG	For the detection of <i>E. coli</i> in water and food samples by using a combination of chromogenic and fluorogenic substrates.
83339	Fluka	HiCrome™ Mac Conkey Sorbitol Agar Supplement: Tellurite-Cefixime Supplement (Fluka 77981)	Recommended for selective isolation of <i>E. coli</i> O157:H7 from food and animal feed.
00563 	Fluka	HiCrome™ MM Agar	Recommended for identification and differentiation of <i>Salmonella</i> and non-salmonella <i>Citrobacter</i> from water samples.
51489	Fluka	HiCrome™ Rapid Coliform Broth	For detection and confirmation of <i>E. coli</i> and coliforms on the basis of enzyme substrate reaction in water samples using a combination of chromogenic and fluorogenic substrates.
78419	Fluka	HiCrome™ Salmonella Agar	A selective media used for simultaneous detection of <i>E. coli</i> and <i>Salmonella</i> in food and water.
05538 	Fluka	HiCrome™ Salmonella Agar, Improved (see Figure 4)	A selective chromogenic media used for simultaneous detection of <i>Salmonella</i> and <i>E. coli</i> in food and water samples.
16636	Fluka	HiCrome™ UTI Agar, modified	Chromogenic differential media for identification, differentiation and confirmation of enteric bacteria from specimens such as urine, water or food which may contain large number of <i>Proteus</i> species as well as potentially pathogenic gram-positive organisms.
39734 	Fluka	Membrane Lactose Glucuronide Agar	A chromogenic media for the differentiation and enumeration of <i>E. coli</i> and other coliforms, which simplifies the membrane filtration technique for <i>E. coli</i> and coliforms by reducing the number of filtration stages required from two to one and by reducing the need for further confirmation steps.
84369	Fluka	Salmonella Chromogen Agar Supplement: Salmonella Chromogen Agar Supplement (Fluka 38589)	A differential diagnostic agar for the detection of <i>Salmonella</i> in food and clinical samples.
92435	Fluka	TBX Agar	Chromogenic agar for the detection and enumeration of <i>E. coli</i> in foodstuffs, animal food and water without further confirmation.

References

- [1] Manafi, M.; Kneifel, W. *Zentralbl. Hyg.*, **1989**, 189, 225.
- [2] Rambach, A. New Plate Medium for Facilitated Differentiation of *Salmonella* sp. from *Proteus* sp. and other Enteric Bacteria, *Environm. Microbiol.*, **1990**, 56, 301.
- [3] Murray, P.; Traynor, P.; Hopson, D. Evaluation of microbiological processing of urine specimens: comparison of overnight versus two-day incubation, *J. Clin. Microbiol.*, **1992**, 30, 1600.
- [4] Benett, A. R., et al., Evaluation of Rambach Agar and Salmosyst Enrichment for the isolation of *Salmonella* from foods., Congress-Poster, RAMI, London, September **1993**.
- [5] Draft International Standard ISO/DIS 16649-2, Microbiology of food and animal feeding stuffs, Horizontal method for the enumeration of presumptive *Escherichia coli*, Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (**1999**).
- [6] Manafi, M. New developments in chromogenic and fluorogenic culture media, *Int. J. Food Microbiol.*, **2000**, 60, 205.

Selected Dyes for the Preparation of Optical Chemosensors Optical detection of alcohols, amines, bisulfite and pH using commercially available chromoreactands, fluororeactands and chromoionophores from the research group of Dr. Gerhard J. Mohr

By Gerhard J. Mohr, Institute of Physical Chemistry, Friedrich-Schiller University Jena, Germany ... gerhard.mohr@uni-jena.de



The detection of electrically neutral analytes is often accomplished by using enzyme-based optical sensors. However, by using chromo- and fluororeactands, it is possible to measure amines [1-3] and alcohols [1,4,5] directly. The chemosensor dyes are embedded in lipophilic polymer layers and perform reversible chemical reactions with the analyte, thus causing changes in absorbance or fluorescence.

Amines and alcohols can be continuously monitored both in the aqueous and gaseous phase. The analysis of thiols and phenols is possible as well. Similarly, bisulfite anion is detected via a reversible chemical reaction in lipophilic polymer sensor layers [6]. The measurement of pH, in contrast, is performed with a pH indicator dye covalently immobilized to hydrophilic polymer matrices such as cellulose or hydrogel [7].

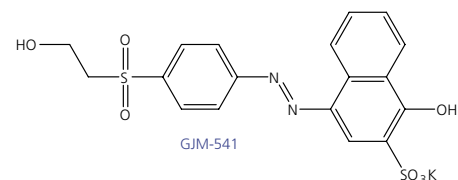
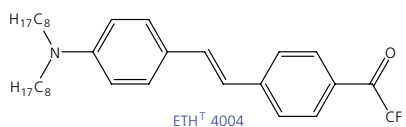
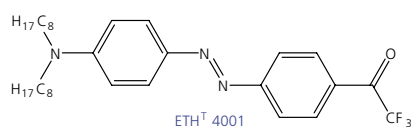
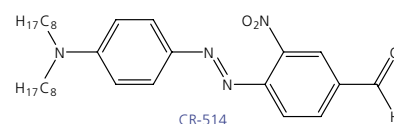
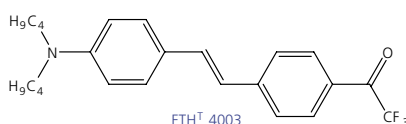
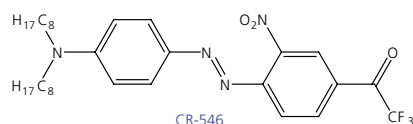


Table 1 Applications and chemical properties of chemosensor dyes

Analyte	Indicator	Additives	Polymer matrix	Sensitive range (analyte in aqueous buffer)	Shift in maxima (absorbance or fluorescence)	Response time	Ref.
Amines	CR-546		PVC/NPOE	0.5 – 50 mM 1-propylamine	556 nm → 482 nm	5 – 10 min	1
Alcohols	CR-546	TDMACI	PVC/NPOE	0.1% (v/v) - 15% (v/v) ethanol	501 nm → 482 nm	15 – 30 min	1
Alcohols	CR-546	TDMACI, S-13	PVC/NPOE	0.1% (v/v) - 15% (v/v) ethanol	Excitation 500 nm, change in intensity at 535 nm and 575 nm	20 – 40 min	1
Amines	ETH ^T 4001		PVC/DOS	4 – 400 mM 1-propylamine	497 nm → 424 nm	5 – 10 min	2,3
Alcohols	ETH ^T 4001	TDMACI	PVC/DOS	1% (v/v) – 40% (v/v) ethanol	495 nm → 433 nm	10 – 30 min	4
Amines	ETH ^T 4003 ETH ^T 4004		PVC/DOS	10 – 1000 mM 1-propylamine	450 nm → 364 nm Emission: 576 nm → 426 nm	5 – 10 min	2
Alcohols	ETH ^T 4003 ETH ^T 4004	TDMACI	PVC/DOS	2% (v/v) – 50% (v/v) ethanol	453 nm → 373 nm Emission: 576 nm → 420 nm	10 – 15 min	5
Bisulfite	CR-514	Proton ionophore III	PVC/NPOE	0.02 – 30 mM bisulfite at pH 4.9	524 nm → 484 nm	10 – 20 min	6
pH	GJM-541		The pH indicator can be covalently linked to –OH containing polymers (e.g. cellulose, hydrogels, Sephadex)	(pH 6 - 9; pK _a = 7.5)	481 nm → 541 nm	2 – 5 min	7

Table 2 Product Listing – Dyes for Preparation of Optical Chemosensors

Cat. No.	Brand	Description	Package Size
08709	Fluka	4- <i>N,N</i> -dioctylamino-4'-trifluoroacetyl-2'-nitroazobenzene (Chromoreactand CR-546)	10 mg, 50 mg
18596	Fluka	4- <i>N,N</i> -dioctylamino-4'-trifluoroacetylazobenzene (Chromoionophore XV, ETH'4001)	50 mg, 250 mg
28218	Fluka	4- <i>N,N</i> -dibutylamino-4'-trifluoroacetylstilbene (Chromoionophore IX, ETH'4003)	10 mg, 50 mg
28222	Fluka	4- <i>N,N</i> -dioctylamino-4'-trifluoroacetylstilbene (Chromoionophore X, ETH'4004)	10 mg, 50 mg
95647	Fluka	4- <i>N,N</i> -dioctylamino-4'-formyl-2'-nitroazobenzene (Chromoionophore CR-514)	10 mg, 50 mg
19684	Fluka	1-Hydroxy-4-[4-(2-hydroxyethylsulfonyl)-phenylazo]-naphthalene-2-sulfonic acid, potassium salt (Chromoionophore XVII, GJM-541)	100 mg
91661	Fluka	Tridodecylmethylammonium chloride (TDMACl) Selectophore	100 mg, 1 g
16459	Fluka	<i>N,N'</i> -bis(1-hexylheptyl)perylene-3,4:9,10-bis(dicarboximide) (S-13)	1 mg, 10 mg
95298	Fluka	<i>N,N</i> -Dioctadecylmethylamine (Proton Ionophore III) Selectophore	50 mg, 250 mg
73732	Fluka	2-Nitrophenyl octyl ether (NPOE) Selectophore	5 mL, 25 mL, 100 mL
84818	Fluka	Bis(2-ethylhexyl)sebacate (DOS) Selectophore	1 mL, 5 mL, 25 mL
81392	Fluka	Poly(vinyl chloride) (PVC) Selectophore, high molecular weight	1 g, 10 g, 50 g

A complete product list of our ion sensor materials can be found at www.sigma-aldrich.com/sensoric.

References

- [1] Mohr, G. J. *Anal. Chim. Acta* **2004**, *508*, 233-237.
 - [2] Mohr, G. J.; Demuth, C.; Spichiger, U. E. *Anal. Chem.* **1998**, *70*, 3868-3873.
 - [3] Mohr, G. J.; Zhylyak, G.; Nezel, T.; Spichiger-Keller, U. E.; Kerness, N.; Brand, O.; Baltes, H.; Grummt, U.-W. *Anal. Sci.* **2002**, *18*, 109-112.
 - [4] Mohr, G. J.; Citterio, D.; Spichiger, U. E. *Sens. Actuators B* **1998**, *49*, 226-234.
 - [5] Mohr, G. J.; Spichiger-Keller, U. E. *Anal. Chim. Acta* **1997**, *351*, 189-196.
 - [6] Mohr, G. J. *Chem. Commun.* **2002**, *22*, 2646-2647.
 - [7] Mohr, G. J.; Wolfbeis, O. S. *Anal. Chim. Acta* **1994**, *292*, 41-48.
- *Reactand is a chemical with the properties of a chemical reagent and a selective ligand.

NEW Ionophore Web page

Use our interactive sensorics Web page to find the right Selectophore® product for your particular application.

Move the pointer over the Periodic Table to see the data sheets for the ionophores we offer for each element. You can also follow the links below the Periodic Table to view our complete list of Selectophore® products and information about our equipment for pH and ion-selective electrodes and the preparation of optode membranes. The Web page is frequently updated with the newest ionophores from Sigma-Aldrich.

www.sigma-aldrich.com/sensoric

Karl Fischer Titration Influence of pH value on reaction rate and how to eliminate pH-related problems

By Helga Hoffmann, HYDRANAL®-Technical Center ... hhoffman@europe.sial.com



Influence of pH on the Karl Fischer reaction

Performance of a Karl Fischer (KF) titration is often measured in terms of duration of titration and end point stability. Many factors influence the performance, including the type of reagents used and the pH in the titration vessel. At pH 1 and below, the KF reaction does not take place and it is impossible to titrate the water content of any sample under these conditions.

Figure 1, the effect of pH on log K, shows that reaction

rate increases with increasing pH, reaching an optimum performance range between pH 5 - 7. In this range, the reaction rate changes negligibly with pH. Indeed, the best pH to perform a titration is pH 6. Above this optimal range, under alkaline conditions, decomposition of the reagent occurs and the titration conditions required for an end point are never met.

The pH in the titration vessel

Figure 2 compares the time it takes to titrate 40 mg of water at three different pH values. Depending on which buffer is used and whether the medium in the titration vessel is buffered or not, titration at lower pH can take ten-times longer than at pH 6, the optimum value for the KF reaction. Short titration time is not only more convenient for the analyst, it also typically gives better accuracy than titrations with longer duration. Short titration times are less susceptible to errors caused by drift from the slow infiltration of atmospheric humidity into the titration vessel.

Figure 1

Dependence of the reaction rate constant (K) on pH

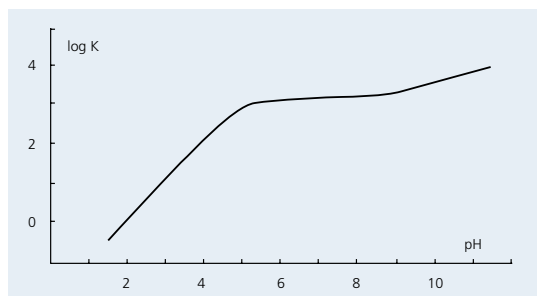
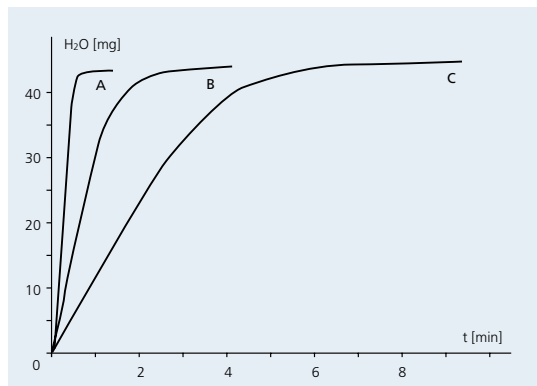


Figure 2

Titration time under different pH conditions



Curve A: Titration at pH 6 with HYDRANAL®-Titrant 5 using HYDRANAL®-Solvent in the titration vessel.

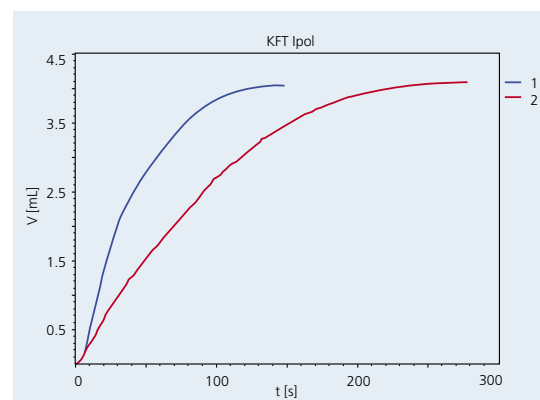
Curve B: Titration at pH 5 with HYDRANAL®-Composite 5 using methanol in the titration vessel.

Curve C: Titration at pH 3 with a reagent containing pyridine using methanol in the titration vessel.

In **Figure 3** the titration time for 20 mg of water in the one-component reagent HYDRANAL®-Composite is shown. Titration time is strongly influenced by using a buffered reagent in the titration vessel. Depending on the amount of water to be titrated, a dedicated volume of HYDRANAL®-Composite is added to the vessel. The titration time can be shortened substantially by using HYDRANAL®-Methanol Rapid, which contains an accelerant for the KF reaction.

Figure 3

Titration time of 20 mg water using HYDRANAL®-Composite 5 with and without accelerants in the working medium



Titration of 20 mg water using HYDRANAL®-Composite 5

Curve 1: Titration in HYDRANAL®-Methanol Rapid

Curve 2: Titration in HYDRANAL®-Methanol dry

The pH of the sample

Even if the working reagents are maintained in the optimum pH range, the sample can influence the reaction by altering the pH or by actually reacting with the iodine in the KF reagent. For example:

- Phenols, such as naphthol, pyrocatechol, o-cresol, guaiacol or 8-hydroxyquiniline, react with iodine at pH 6. The titration of water content in samples containing these substances has to be performed at a lower pH or by adding benzoic acid before pre-titration.
- Penicillin samples often contain residual penicillic acid which reacts with iodine. Addition of benzoic acid or salicylic acid before pre-titration ensures that only the moisture of the penicillin is titrated.
- Hydrazine hydrate solution reacts with iodine and requires lowering the pH as well.
- Erythromycin A requires a working pH 6; the lower the pH the stronger the side reaction with iodine.

Acidic Samples

Because **weak acids**, such as carboxylic acids, do not significantly influence the pH in the titration vessel, they can be titrated following standard procedures. However, **strong acids** may cause the pH to drop below pH 2 and therefore suppress the KF reaction rate and increase the titration time. Strong acids can be investigated by adding a suitable buffer, such as **HYDRANAL®-Buffer Acid**, before pre-titration to neutralize the system.

Usually the addition of 5 or 10 mL of **HYDRANAL®-Buffer Acid** is sufficient; however, adding an excess of the buffer to the titration vessel does not disturb the reaction. Highly concentrated sulfuric acid causes an esterification in methanol and has to be neutralized before titration. (See Laboratory Report L 049.)

1 mL **HYDRANAL®-Buffer Acid** neutralizes 5 mmole acid

Basic Samples

Weak bases, such as nitrogen-containing compounds, do not influence the pH in the titration vessel, but **strong bases** do. If the pH increases outside the optimal range by adding the sample, the end point is vanishing or the titration cannot be completed. The iodine in the reagent will decompose at high pH, and conditions needed for a clear end point are never met. We investigated numerous nitrogenous compounds (e.g. Laboratory Report L 288). The adverse influence on the pH can be eliminated by using **HYDRANAL®-Buffer Base** in the titration vessel or by adding an acid, such as **HYDRANAL®-Salicylic acid** or **HYDRANAL®-Benzoic acid**, before pre-titration.

1 mL **HYDRANAL®-Buffer Base** neutralizes 1 mmole base

Many **pharmaceutical compounds** contain nitrogen in their chemical structure. Like other basic compounds, they should be titrated in **HYDRANAL®-Buffer Base** or one of the above-mentioned acids for accurate titrations.

Table Product Listing

Cat. No.	Brand	Description	Package Size
34805	Riedel-de Haën	HYDRANAL®-Composite 5	500 mL, 1 L, 2.5 L, 5 L
34806	Riedel-de Haën	HYDRANAL®-Composite 2	500 mL, 1 L, 2.5 L, 5 L
34827	Riedel-de Haën	HYDRANAL®-Composite 1	500 mL, 1 L
34801	Riedel-de Haën	HYDRANAL®-Titrant 5	500 mL, 1 L, 2.5 L
34732	Riedel-de Haën	HYDRANAL®-Titrant 5E	500 mL, 1 L, 2.5 L
34811	Riedel-de Haën	HYDRANAL®-Titrant 2	500 mL, 1 L, 2.5 L
34723	Riedel-de Haën	HYDRANAL®-Titrant 2E	1 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
34800	Riedel-de Haën	HYDRANAL®-Solvent	1 L, 2.5 L
34730	Riedel-de Haën	HYDRANAL®-Solvent E	1 L, 2.5 L
34804	Riedel-de Haën	HYDRANAL®-Buffer Acid	500 mL
37859	Riedel-de Haën	HYDRANAL®-Buffer Base	500 mL, 1 L
34847	Riedel-de Haën	HYDRANAL®-Water Standard 0.10 (1 g contains 0.10 mg H ₂ O)	40 mL
37865	Riedel-de Haën	HYDRANAL®-Salicylic acid	500g
37862	Riedel-de Haën	HYDRANAL®-Benzoic acid	500g
34828	Riedel-de Haën	HYDRANAL®-Water Standard 1.00 (1 g contains 1.0 mg H ₂ O)	40 mL
34849	Riedel-de Haën	HYDRANAL®-Water Standard 10.0 (1 g contains 10 mg H ₂ O)	80 mL
34241	Riedel-de Haën	HYDRANAL®-Molecular sieve 0.3 nm	250 g, 1 kg
34788	Riedel-de Haën	HYDRANAL®-Humidity Absorber	500 g, 1 kg

Confirming the accuracy of the titrations

The capability of the KF apparatus to deliver accurate titrations can be established by injecting a certified water standard such as **HYDRANAL®-Water Standard 0.10**, **HYDRANAL®-Water Standard 1.00** or **HYDRANAL®-Water Standard 10.0**, which contain 0.01%, 0.1% and 1.0% water, respectively.

The **HYDRANAL®-Laboratory reports** listed below describe the titration procedures and troubleshooting for a number of samples. These and many more can be found on our Web site: www.sigma-aldrich.com/hydranal

List of HYDRANAL®-Laboratory reports

Aminophylline	L 468
Cephalexin monohydrate	L 473
Folic acid	L 383
Hydrazine hydrate solution	L 435
Nitrogen bases	L 288
Perchloric acid 70%	L 406
Sulfuric acid, conc.	L 049
Trifluoroacetic acid	L 380

Flash Performance Upgrade Enjoy the benefits and cost-savings of VersaPak® pre-packed cartridges on your existing flash system

Dan Vitkuske, Sample Handling Product Manager ... dvitkuske@sial.com



Figure 1
50 gram VersaPak® cartridge installed in an Isco flash system

Flash is a type of preparative chromatography used for the rapid, crude purification of compounds of wide ranging polarity, often reaction mixtures where the target (synthesized) molecule must be separated from excess reagents and reaction by-products. The most common flash adsorbent is silica and, while the majority of flash separations are performed on hand-packed cartridges, pre-packed, disposable cartridges designed for use on semi-automated flash systems are gaining popularity. However, a significant limitation to these systems is that the flash cartridges are not interchangeable, thereby limiting researchers' choices in cartridge design and packing materials.

Supelco entered the flash market by introducing the VersaFlash® system. Elegant in its simplicity and versatility, one of the keystone innovations of VersaFlash is the high efficiency VersaPak® pre-packed cartridge design. Current VersaPak users enjoy these benefits:

VersaPak® Feature	Benefit to User
Simple, versatile design	Wide choice of sample size, sample types, adsorbents, separation modes, sample loading techniques, flow rates and flow directions
Spherical, 20-45 µm silica packing	Efficient, uniformly-packed bed gives higher resolution, higher sample loading capacity, higher yields than larger, irregular particles, samples elute in more concentrated fractions requiring less time to remove solvent
Variety of packing materials	Pre-packed with silica, C18-silica, and custom-packed with your particular adsorbent
Wide range of cartridge volumes	More easily match cartridge volume to sample volume or mass, less possibility of excessive sample dilution or overload
Solid sample cartridges	Empty cartridges allow flash processing of solid or viscous samples
Pre-compressed cartridges	No compression barrels
Symmetric end fittings	Stackable, reversible, allows direct-loading

Adapt the VersaPak® Cartridges to Isco® or Biotage® Flash Systems

Now, the unique features and benefits of the VersaPak® pre-packed cartridge design are available to users of any flash system, including Isco® and Biotage®.

Connecting VersaPak® Cartridges to Isco® systems:

Reusable adapters attach VersaPak® cartridges quickly and easily to Isco® flash systems (**Figure 1**). For large cartridges, use the VersaFlash Station described below.

Use VersaFlash® Station to connect VersaPak® cartridges to any system:

Place the VersaPak® cartridge of your choice in the VersaFlash® Station, connect the outlet of the flash system pump to the inlet of the VersaFlash® Station and its outlet to the inlet to the detector or fraction collector (**Figure 2**). The VersaFlash® Station essentially becomes a replacement cartridge holder for the flash system.

To learn more about adapting VersaPak® to your system, request our VersaFlash® Brochure (T403110B, FWL) by contacting your local Sigma-Aldrich office or visiting our Web site: www.sigmaaldrich.com/versafash.



Figure 2 Using the VersaFlash® Station to connect large volume VersaPak® cartridge to an Isco CombiFlash® system. The same design can connect VersaPak® to any flash system.

New Mycotoxin, Pesticide and Forensic Standards for LC-MS Analysis

By Nicole Amann, Product Manager Analytical Standards ... namann@europe.sial.com



Mycotoxin Standards

Sigma-Aldrich offers high-purity mycotoxin standards for LC-MS. *Fusarium* moulds are a large group of phytopathogenic fungi which are able to infect cereal crops in the field and contaminate them with toxic secondary metabolites. Concerning its rate of occurrence and mean concentration, deoxynivalenol (DON) appears

to be the most important *Fusarium* mycotoxin in cereal commodities. In recent years LC-MS/MS has become the method of choice for the analysis of mycotoxins as it offers high sensitivity and specificity and permits the determination of many different analytes within one run.

Cat. No.	Brand	Description	Concentration	Package Size
34134	Riedel-de Haën, OEKANAL	B-Trichothecene mix, (DON, NIV, 3-AcDON, 15-AcDON)	100 µg/mL in acetonitrile	2 mL
34135	Riedel-de Haën, OEKANAL	Deepoxy-deoxynivalenol solution	50 µg/mL in acetonitrile	2 mL
34136	Riedel-de Haën, OEKANAL	HT-2 Toxin solution	100 µg/mL in acetonitrile	2 mL
34137	Riedel-de Haën, OEKANAL	Diacetoxyscirpenol solution	100 µg/mL in acetonitrile	2 mL
34138	Riedel-de Haën, OEKANAL	Neosolaniol solution	100 µg/mL in acetonitrile	2 mL
34139	Riedel-de Haën, OEKANAL	Fumonisin B1 solution	50 µg/mL in acetonitrile	2 mL
34142	Riedel-de Haën, OEKANAL	Fumonisin B2 solution	50 µg/mL in acetonitrile	2 mL
34143	Riedel-de Haën, OEKANAL	Fumonisin Mixture	50 µg/mL in acetonitrile:water	2 mL

Labeled Pesticide and Forensic Standards

Sigma-Aldrich is pleased to offer new deuterated standards for MS analysis. PESTANAL® products are high-purity standards of pesticides and their metabolites, the product line contains more than 1200 analytical standards for food and environmental applications. The

VETRANAL® product line contains high-purity standards of the active ingredients of veterinary drugs. More than 200 analytical standards - belonging to the chemical classes of antibiotics and hormones are offered for forensics and residue analysis in the food sector and in environmental analytics.

Cat. No.	Brand	Description	Package Size
33853	Riedel-de Haën, PESTANAL	Thionazin-d ₁₀	10 mg
34209	Riedel-de Haën, PESTANAL	Pirimicarb-d ₆	10 mg
34165	Riedel-de Haën, PESTANAL	Triforine-d ₈	10 mg
34170	Riedel-de Haën, PESTANAL	Imidacloprid-d ₄	10 mg
34207	Riedel-de Haën, VETRANAL	HMMNI-d ₃	10 mg
34196	Riedel-de Haën, VETRANAL	Dimetridzol-d ₃	10 mg



Isofenphos-Methyl Standard

Sigma-Aldrich now offers PESTANAL® grade isofenphos-methyl, a highly-effective, broad-spectrum organophosphorus insecticide used to control soil- and ground pests such as mole crickets, grubs, cutworms and click beetles in many types of crops. Likely produced in Asia, its use in the EU is not permitted; it is not mentioned in 91/414/EEC, the Plant Production Products Directive.

Beginning in late 2006, the CVUAS (Chemisches und Veterinäruntersuchungsamt Stuttgart) tested pepper samples from different countries and found isofenphos-methyl traces in 60% of the Spanish samples (40% had levels of 0.01 mg/kg or more). Although Isofenphos-methyl toxicity has not been firmly established, a similar compound, Isofenphos, is a neurotoxin and regulated according to 2076/2002/EC. Since its health risks cannot be ruled out, having high quality analytical standards is critical to establish its presence in food, agricultural and environmental samples.

Cat. No.	Brand	Description	Package Size
33436	Riedel-de Haën	Isofenphos-methyl	50 mg

Argentina

SIGMA-ALDRICH DE ARGENTINA S.A.
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia

SIGMA-ALDRICH PTY LTD.
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria

SIGMA-ALDRICH HANDELS GmbH
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium

SIGMA-ALDRICH NV/SA.
Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil

SIGMA-ALDRICH BRASIL LTDA.
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada

SIGMA-ALDRICH CANADA LTD.
Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

China

SIGMA-ALDRICH (SHANGHAI)
TRADING CO. LTD.
Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic

SIGMA-ALDRICH S.R.O.
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark

SIGMA-ALDRICH DENMARK A/S
Tel: (+45) 43 56 59 10
Fax: (+45) 43 56 59 05

Finland

SIGMA-ALDRICH FINLAND OY
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 9255

France

SIGMA-ALDRICH CHIMIE S.à.r.l.
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 00
Fax: (+33) 474 95 68 08

Germany

SIGMA-ALDRICH CHEMIE GmbH
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Greece

SIGMA-ALDRICH (O.M.) LTD.
Tel: (+30) 210 994 8010
Fax: (+30) 210 994 3831

Hungary

SIGMA-ALDRICH Kft
Ingyenes zöld telefon: 06 80 355 355
Ingyenes zöld fax: 06 80 344 344
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9050

India

SIGMA-ALDRICH CHEMICALS
PRIVATE LIMITED
Telephone
Bangalore: (+91) 80 6621 9600
New Delhi: (+91) 11 4165 4255
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 6684 5488
Fax
Bangalore: (+91) 80 6621 9650
New Delhi: (+91) 11 4165 4266
Mumbai: (+91) 22 2579 7589
Hyderabad: (+91) 40 6684 5466

Ireland

SIGMA-ALDRICH IRELAND LTD.
Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: (+353) 1 404 1900
Fax: (+353) 1 404 1910

Israel

SIGMA-ALDRICH ISRAEL LTD.
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4100
Fax: (+972) 8 948 4200

Italy

SIGMA-ALDRICH S.r.l.
Numero Verde: 800 827018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan

SIGMA-ALDRICH JAPAN K.K.
Tokyo Tel: (+81) 3 5796 7300
Tokyo Fax: (+81) 3 5796 7315

Korea

SIGMA-ALDRICH KOREA
Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Malaysia

SIGMA-ALDRICH (M) SDN. BHD
Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico

SIGMA-ALDRICH QUÍMICA, S.A. de C.V.
Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: 52 722 276 1600
Fax: 52 722 276 1601

The Netherlands

SIGMA-ALDRICH CHEMIE BV
Free Tel: 0800 022 9088
Free Fax: 0800 022 9089
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand

SIGMA-ALDRICH NEW ZEALAND LTD.
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway

SIGMA-ALDRICH NORWAY AS
Tel: (+47) 23 17 60 60
Fax: (+47) 23 17 60 50

Poland

SIGMA-ALDRICH Sp. z o.o.
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal

SIGMA-ALDRICH QUÍMICA, S.A.
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia

SIGMA-ALDRICH RUS, LLC
Tel: +7 (495) 621 6037
Fax: +7 (495) 621 5923

Singapore

SIGMA-ALDRICH PTE. LTD.
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

South Africa

SIGMA-ALDRICH
SOUTH AFRICA (PTY) LTD.
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain

SIGMA-ALDRICH QUÍMICA, S.A.
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden

SIGMA-ALDRICH SWEDEN AB
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland

SIGMA-ALDRICH CHEMIE GmbH
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

United Kingdom

SIGMA-ALDRICH COMPANY LTD.
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313
SAFC (UK) Free Tel: 0800 71 71 17

United States

SIGMA-ALDRICH
P.O. Box 14508
St. Louis, Missouri 63178
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Call Collect: (+1) 314 771 5750
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Internet

sigma-aldrich.com

World Headquarters

3050 Spruce St., St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052

Technical Service (800) 325-5832 • sigma-aldrich.com/techservice

Development/Bulk Manufacturing Inquiries SAFC™ (800) 244-1173

The SIGMA-ALDRICH Group

©2007 Sigma-Aldrich Co. All rights reserved.

SIGMA, SAFC, SIGMA-ALDRICH, ISOTECH, ALDRICH, FLUKA, and SUPELCO are trademarks belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, L.P. Riedel-de Haën® trademark under license from Riedel-de Haën GmbH. Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.



SIGMA-ALDRICH

*Accelerating Customers' Success
through Leadership in Life Science,
High Technology and Service*