

# Analytix

Advances in Analytical Chemistry

Issue 5 • 2006

 **Fluka**

Riedel-deHaën®

## TraceCERT™

Swiss Precision Meets Analytical Competence

### Chromatography

- Mobile Phase Additives for LC-MS
- Lipid Removal Agent
- CERTAN® Capillary Vials
- High Recovery Vials

### Standards

- Sudan Red Dyes
- Hopane Standards
- radiello® Diffusive Air Sampler

### Analytical Microbiology

- Media for Yeasts and Molds

### Titration

- Improving Karl Fischer Titrations

### New Product Corner

- New Chemical Reference Standards



**SIGMA-ALDRICH**

**Fluka/Riedel-de-Haën Catalog 2007/2008** ..... The new catalog is available from December 2006/January 2007



**Picture** ..... Kurt Vorburger,  
Manager Product Management and  
Innovation

**Dear Colleague,**

Although the calendar says it is true, I struggle with the realization that 2006 is nearly over. This last issue of the Analytix for 2006 continues the theme of innovation from Sigma-Aldrich by introducing you to new inorganic trace analysis, chromatography, standards and microbiology products from our Fluka, Riedel-de Haën and Supelco brands, and a new catalog.

In addition to newsletters like Analytix, catalogs are an important communication tool for us. Our extensive collection of analytical reagents, chemicals and standards has been compiled in the new Fluka/Riedel-de Haën catalog, which we are currently in the process of distributing to our customers. I want to take the opportunity here to briefly describe this new catalog to you.

The Fluka/Riedel-de Haën Catalog 2007/2008 contains over 1,200 new products for analytical chemistry, biochemistry and organic chemistry. Many of these products were developed based on customer requests and to keep pace with the latest scientific techniques and regulated methodologies. A few examples of our products for today's most prevalent and/or new scientific applications include:

- High purity LC-MS solvents, additives and convenient blends
- Ionic liquids as innovative performance solvents in organic chemistry
- Standards for new regulations in environmental and food analysis
- Reagents and standards for trace analysis
- Fluorescent antibody conjugates for high sensitive detection methods
- *TraceSELECT*<sup>TM</sup> Ultra high purity samples
- *TraceCERT*<sup>TM</sup> reference materials

#### **Focus on applications**

Recognizing the need for both complete solutions for today's applications and convenience for busy scientists, we supply our products with application-relevant information and provide many in the form of user-friendly kits. A few examples are kits for fluorescence applications in biochemistry, kits for official analytical methods and the wide range of physical and chemical data provided with our new ionic liquids.

#### **The premiere source for chemicals, biochemicals and analytical reagents**

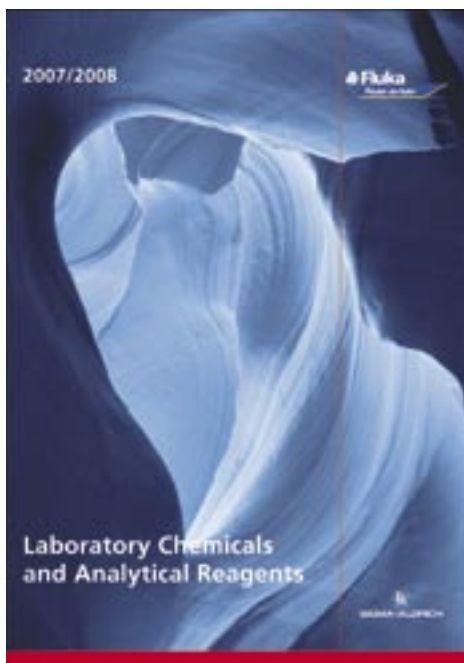
We've taken major strides to make Fluka and Riedel-de-Haën as the premiere brands for analytical reagents and standards within Sigma-Aldrich and in the world market. By listing over 29'000 unique products, the Fluka/Riedel-de-Haën catalog is one of the most comprehensive sources for reagents used in organic chemistry, biochemistry and analytical chemistry. In addition we've broadened its scope by incorporating all analytical reagents and standards from the Supelco, Sigma and Aldrich brands.

Please contact your local Sigma-Aldrich sales office to obtain your personal copy of the new Fluka/Riedel-de-Haën Catalog!

We thank you for trusting us to provide the high quality analytical products you require, and look forward to serving you in the future.

Regards,

Kurt Vorburger  
Manager Product Management and Innovation  
kvorburger@europe.sial.com

**Picture .....**

Front Cover of the 2007/2008 Fluka/Riedel-de-Haën Catalog

**Feature Article**

- 04 *TraceCERT™ Traceable Certified Reference Materials. Part 1: Swiss Precision Meets Analytical Competence*

This is the first of a series of articles to appear on TraceCERT™Ultra calibration standards for ICP and TraceCERT™ calibration solutions for AAS

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## TraceCERT™ Traceable Certified Reference Materials. Part 1: Swiss Precision Meets Analytical Competence .....

This is the first of a series of articles on Certified Reference Materials (CRMs) to appear in the upcoming issues of 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



### Sigma-Aldrich now brings metrological excellence directly to your lab

Due to the increasing demand for certified reference materials (CRM), Sigma-Aldrich established partnerships with several metrological institutes that serve as certifying bodies. For certified elemental and anion calibration solutions, we maintained a long-standing partnership with Swiss Federal Institute for Materials Science and Technology (EMPA). However, EMPA terminated all their activities in chemical metrology in 2004 and no longer acts as a national metrological institute.

Recognizing that EMPA's exit created the need for first-class CRM supplier in the market, Sigma-Aldrich made a strategic decision to acquire the metrological competency from EMPA and transfer it to our Buchs, Switzerland site. Cutting no corners, we began in 2005 to replicate the EMPA metrological environment in order to provide a seamless transition. First, we purchased from EMPA the unique equipment which was custom-designed to produce intercomparison samples of the highest metrological level. Second, we built a special, dedicated laboratory at the Buchs site where the high-precision weighing capabilities, homogenization and clean room

bottling equipment were installed. Third, and perhaps most importantly, we acquired technical know-how and experience by hiring two key EMPA personnel: the former Heads of EMPA's Metrological Reference and Certification Labs.

Now, barely a year after beginning the transfer of EMPA's competency to Sigma-Aldrich, we are proud to introduce the first CRMs developed and produced at our new Buchs facility: thirteen new TraceCERT™Ultra (ICP) and thirteen new TraceCERT™ (AAS) standards (**Table 1**).

### Buying analytical standards is a matter of trust

Any measurement is only as good as the reference standard used to calibrate the system. Although other components of the system contribute to the uncertainty of the analysis, the calibration and reference solutions also contribute. When the accuracy of your measurement matters, it is important to start every analysis with a fresh vial of the highest quality, certified reference or calibration standards. TraceCERT™ standards, an abridgment of Traceable and CERTified, meet these requirements and bring a wealth of security and reliability to your analytical results.

TraceCERT™ products are characterized by

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

**Table 1** ..... Newly-available Fluka TraceCERT™Ultra calibration standards for ICP and TraceCERT™ calibration solutions for AAS\*

| Element   | Starting Material   | Cat. No.<br>TraceCERT™Ultra<br>ICP Standard | Cat. No.<br>TraceCERT™<br>AAS Standard |
|-----------|---|---|--|
| Aluminum  | Al(NO <sub>3</sub> ) <sub>3</sub> × 9H <sub>2</sub> O + HNO <sub>3</sub>          | 61935                                       | 39435                                  |
| Arsenic   | As <sub>2</sub> O <sub>3</sub> + NaOH + HNO <sub>3</sub>                          | 01969                                       | 39436                                  |
| Cadmium   | Cd metal + HNO <sub>3</sub>   | 36379                                       | 51994                                  |
| Calcium   | CaCO <sub>3</sub> + HNO <sub>3</sub>  | 19051                                       | 69349                                  |
| Chromium  | (NH <sub>4</sub> ) <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> + HNO <sub>3</sub> | 68131                                       | 02733                                  |
| Cobalt    | Co metal + HNO <sub>3</sub>   | 30329                                       | 05202                                  |
| Copper    | Cu metal + HNO <sub>3</sub>   | 68921                                       | 38996                                  |
| Iron      | Fe metal + HNO <sub>3</sub>   | 43149                                       | 16596                                  |
| Lead      | Pb(NO <sub>3</sub> ) <sub>2</sub> + HNO <sub>3</sub>                              | 41318                                       | 16595                                  |
| Magnesium | Mg metal + HNO <sub>3</sub>   | 30083                                       | 42992                                  |
| Mercury   | Hg metal + HNO <sub>3</sub>   | 28941                                       | 16482                                  |
| Nickel    | Ni metal + HNO <sub>3</sub>   | 28944                                       | 42242                                  |
| Zinc      | Zn metal + HNO <sub>3</sub>   | 18562                                       | 18827                                  |

\* ICP standards supplied in 100 mL HDPE bottles and sealed in an aluminum bag, including certificate. AAS standards supplied in 250 mL HDPE bottles, except for Hg which is bottled in 100 mL borosilicate white glass bottle.

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

Please quote promotion code T98 when placing your order.  
Valid until January 31<sup>st</sup> 2007.

**Figure 2** .....  
Example of Zinc  
*TraceCERT™Ultra*  
certificate (front  
page out of three  
pages)



Our well-defined approach to CRM production leads to multiple-traceability and, as a further benefit, ensures that significant biases and systematic deviations are excluded to the fullest extent possible.

#### The rationale behind this series of CRM articles

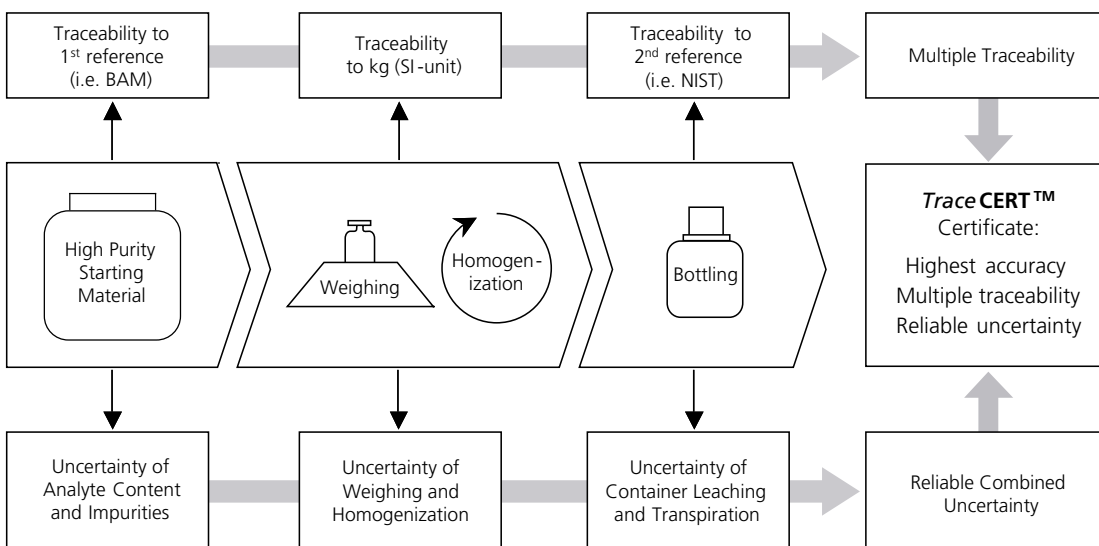
There is much confusion and many misconceptions over traceability, uncertainty calculation and the proper use of reference materials. In the next six issues of *Analytix*, we will attempt to enlighten interested readers on the meaning behind important CRM-related terms and concepts, including

- What does traceability really mean?
- The proper method to calculate uncertainty
- Characterization and purity statement of high-purity starting materials
- Production, liquid handling and packaging on a metrological level
- Certification and accreditation
- Certification of custom standards

To actually produce CRMs that meet all these criteria, the entire development process, beginning from the selection of an appropriate starting material to the choice of a purpose-specific packaging, must be highly sophisticated. **Figure 1** (see below) shows an overview of the production and certification process we employ. Not only is the bottled solution compared to a reference material (NIST, BAM, etc.), the starting material is checked against a second, independent reference. A key step is that the weighing operation leads to direct traceability to the SI unit kilogram. Gravimetric preparation using pure materials is a practical and, in many cases, the most accurate calculation of concentration units, through conversion of mass and mole fraction to mass fraction. As a final step, we provide detailed, lot-specific information in the Certificate of Analysis supplied with every *TraceCERT™* product (**Figure 2**).

Our intention with these articles is to clearly demonstrate that the technical experts at our new facility in Buchs are firmly grounded in the science behind the production of high-quality analytical reference materials. This is the reason why we link our *TraceCERT™* line with the image of high-precision Swiss chronographs (courtesy of IWC Schaffhausen).

We realize that buying and using analytical standards is a measure of your trust in the supplier. In the upcoming series of technical articles, we hope to answer most of your questions about certified reference materials.



**Figure 1** ..... Schematic representation of the production design and certification of Sigma-Aldrich *TraceCERT™* products. This approach was established for the preparation of intercomparison samples at the highest metrological stage.

## Mobile Phase Additives for LC-MS. Part 4: Special Case - Sodium Adduct Formation .....

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

By Joachim Emmert and Tanja Waelti, Applications Development R&D, Sigma-Aldrich Switzerland ... [jemmert@europe.sial.com](mailto:jemmert@europe.sial.com)



Although sodium and other alkali metals are typically avoided in LC-MS, it can be useful to add sodium salts when the analyte ions have a very high tendency to form alkali adducts. This is especially true for the carbonyl group of sugars and glycosides, in addition to hydroxyl and carboxyl groups. These groups often form sodium adducts even under acidic conditions, e.g. with formic acid, because sodium is nearly always present at

trace levels. Adduct formation is associated with a decrease in sensitivity, and often simultaneous addition of  $H^+$ ,  $NH_4^+$  and  $Na^+$  is observed. When adduct formation tendency is strong, addition of small and defined amounts of sodium ions (mostly post-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

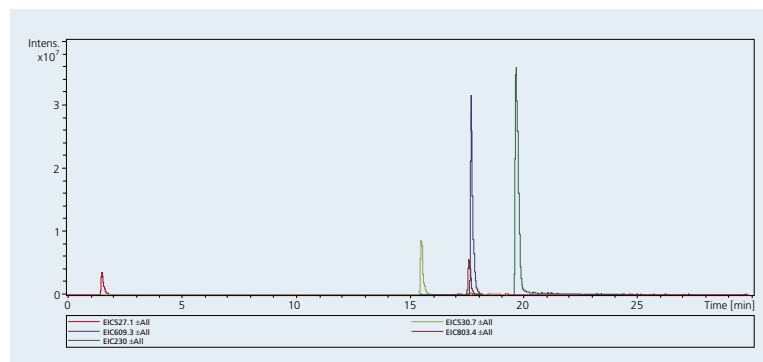
**Table 1** ..... List of Sigma-Aldrich LC-MS additives

| 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              | 10x1 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.

**Figure 1** ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid as mobile phase additive (no sodium added).

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine and dark green is propazine.



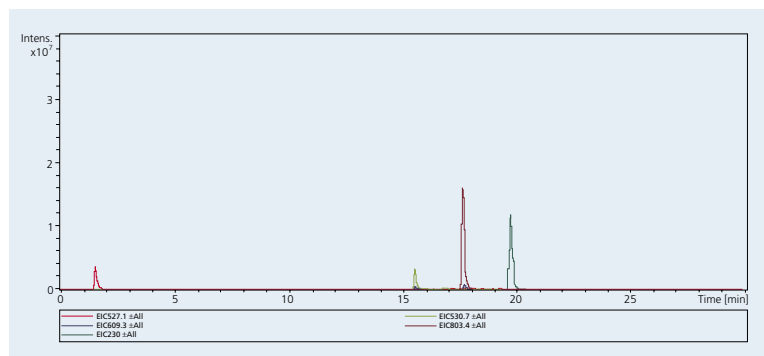
In this article, we will discuss the benefits and practical procedures for using sodium salts as LC-MS mobile phase additives. Riedel-de-Haën LC-MS CHROMASOLV® solvents and Supelco Discovery® HS C18 HPLC columns were used to meet the stringent requirements of LC-MS. These, and the MS conditions, test compounds and mobile phase (water-acetonitrile gradient with 0.1% formic acid) were as described in Part 1 of this series [1]. In this study, an additional series of experiments was run with a concentration of 100 ng/μL raffinose. A 0.1% w/v aqueous solution of sodium, as acetate or citrate salt, was added post-column. The sodium citrate solutions were adjusted to pH 7.8, 5.0 or 3.1. Taking flow rate into account, the total concentration of sodium salt introduced into MS was 0.001% w/v (0.1 mM). It is important to keep the sodium concentration at 1 - 5mM or below, otherwise some MS instruments will be overloaded and show a dramatic loss of sensitivity caused by reversible suppression. The sodium citrate and other high-purity Fluka-brand LC-MS additives from Sigma-Aldrich appear in **Table 1**.

**Figure 1** shows the initial separation of the five test compounds with formic acid as the sole additive. Co-elution of digoxin and reserpine is observed with reserpine having much higher intensity. Raffinose and digoxin exhibit formation of the sodium adduct of the molecular ion in the mass spectrum without any addition of sodium ions.

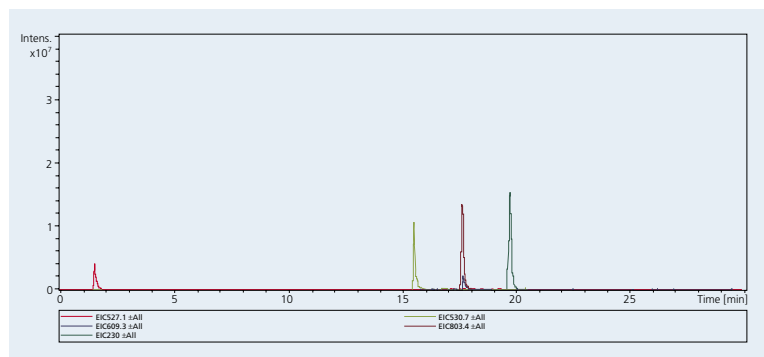
To observe the effect of addition of sodium ions, a 0.1% w/v aqueous solution of sodium acetate was added post-column. Results appear in **Figure 2**. By adding the sodium, the absolute intensity is reduced, and the relative intensity of digoxin and reserpine is reversed. The signal from the glycoside digoxin is boosted by addition of sodium, whereas the other compounds either lose intensity or stay constant.

The selective increase of the glycoside signal is also observed when sodium citrate is added. The influence

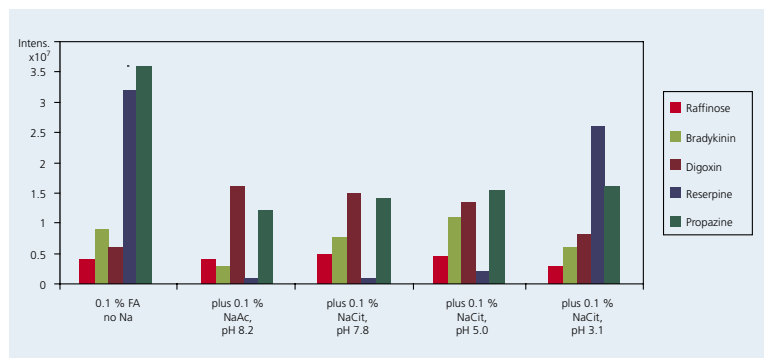
**Figure 2** ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid in the mobile phase, and 0.1% w/v sodium acetate added post-column.



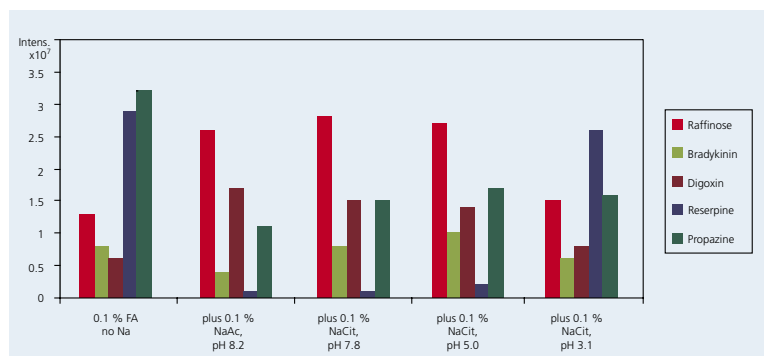
**Figure 3** ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid in the mobile phase, and 0.1% w/v sodium citrate (pH 5.0) added post-column.



**Figure 4** ..... Signal intensities for the 5 test compounds with sodium addition under different conditions (anion and pH). Raffinose at 10 ng/μL.



**Figure 5** ..... Signal intensities for the 5 test compounds with sodium addition under different conditions (anion and pH). Raffinose at 100 ng/μL.



of pH was also studied using sodium citrate. **Figure 3** shows the effect of addition of sodium citrate at pH 5.0. While the digoxin signal is reduced slightly, the bradykinin signal is increased significantly. This is only one example of how pH, together with addition of sodium ions, can influence analyte ionization. The phenomenon is attributed to a competition between the different ions within the electrospray interface.

Further experiments were carried out with sodium citrate solutions at different pH values and two different raffinose concentrations, 10 and 100 ng/μL. At low concentrations (10 ng/μL, **Figure 4**), the raffinose signal shows no dependence on pH. However, at higher raffinose concentrations (100 ng/μL, **Figure 5**), the signal is influenced by pH, exhibiting a maximum at pH 7.8. For digoxin, the maximum signal is obtained in sodium acetate at pH 8.2. Both raffinose and digoxin are highly susceptible to sodium adduct formation. Bradykinin is not susceptible to adduct formation, but its signal is also influenced by pH (hydrogen ion concentration) and the presence of sodium ions. Propazine and reserpine both go through a broad minimum when Na<sup>+</sup> concentration is high and H<sup>+</sup> concentration is low.

But sensitivity is only part of the story. Stability, and perhaps specificity, of the molecular ion are also considerations. The ability to form alkali adducts is useful for quantifying certain classes of molecules, e.g. formation of Cs<sup>+</sup> adducts for digoxin and digitoxin or immunosuppressive drugs [2, 3] and the determination of ionophores by addition of Li<sup>+</sup>, Na<sup>+</sup> or K<sup>+</sup> [4]. It should also be possible to selectively enhance the LC-MS signals of glycopeptides within a peptide mixture with Na<sup>+</sup>. Truly, the use of alkali ions, particularly sodium ions, to enhance LC-MS sensitivity or selectivity has not been explored to the limits as yet.

## References

- [1] "Mobile Phase Additives for LC-MS. Part 1: Acids – The Most Common Choice", *Analytix* 2006/2, 8-9. Downloadable from: <http://www.sigma-aldrich.com/analytix>
- [2] Kaiser, P.; Kramer, U.; Meissner, D.; Kress, M.; Wood, W. G.; Reinauer, H. Determination of the Cardiac Glycosides Digoxin and Digitoxin by Liquid Chromatography Combined with Isotope-Dilution Mass Spectrometry (LC-IDMS) – a Candidate Reference Measurement Procedure, *Clin. Lab.* **2003**, *49*(7/8), 329 – 343.
- [3] Kaiser, P.; Akerboom, T.; Wood, W. G.; Reinauer, H. A new approach for the determination of immunosuppressive drugs using HPLC-MS/MS and Cs adducts. *GMS Ger. Med. Sci.* **2006**, *4*, Doc01 (<http://www.egms.de/de/gms/2006-4/000030.shtml>)
- [4] Schneider, R. P.; Lynch, M. J.; Ericson, J. F. Electrospray ionization mass spectrometry of semduramicin and other polyether ionophores. *Anal. Chem.* **1991**, *63*, 1789 – 1794.

## LRA (Lipid Removal Agent) ..... Synthetic calcium silicate hydrate for the selective removal of lipids, endotoxins and other bio-organic molecules

By An Trinh, Sample Preparation Product Manager ... atrinh@sial.com

### Picture .....

Packaging for LRA



Lipids are naturally-occurring hydrocarbons that are poorly soluble in water and soluble in non-polar solvents such as ether and chloroform. They serve many important functions in cells and other biological systems. For example, lipids serve as structural components in cellular membranes, provide energy reserves (e.g. triglycerides), and are vital in the role of metabolism as vitamins and coenzymes.

However important lipids are to living systems, they can interfere with analytical assays and biopharmaceutical production. For example, the presence of fatty acids in multi-residue pesticide analysis in food products can cause ion-enhancement during MS analysis. Phospholipids can lead to ion-suppression during pre-clinical and clinical studies during pharmaceutical

**Table 1** ..... LRA specifications

|  |            |
|--|------------|
| Moisture (% H <sub>2</sub> O as shipped) | 5.5        |
| Water adsorption, wet endpoint (%)       | 500 – 650% |
| Screen analysis, +325 mesh (%)           | 6.0        |
| Surface area (m <sup>2</sup> /g)         | 120.0      |
| pH of 10% slurry                         | 8.4 – 10.2 |
| SiO <sub>2</sub> (%)                     | ≥ 45.0     |
| CaO (%)                                  | ≥ 4.0      |
| SiO <sub>2</sub> / CaO (%)               | 1.3 – 20.0 |
| Lead (ppm)                               | ≤ 10       |
| Heavy metals (ppm)                       | ≤ 20       |
| Ignition loss (ppm)                      | ≤ 20       |
| Fluoride (ppm)                           | ≤ 10       |

**Table 2** ..... LRA lipid removal capacity

| Lipid                          | Removal Capacity |
|--------------------------------|------------------|
| Cholesterol                    | 40 mg/g LRA      |
| Triglyceride                   | 82 mg/g LRA      |
| Apolipoprotein A1              | 160 mg/g LRA     |
| HDL                            | 73 mg/g LRA      |
| Phosphatidylcholine            | 265 mg/g LRA     |
| Endotoxin (lipopolysaccharide) | 796,000 EU/g LRA |

development. Removal of endotoxins, lipopolysaccharides derived from the cell membrane of gram-negative bacteria (e.g. E. coli), is critical in biopharmaceutical applications involving gene therapy products and transfection studies.

In this report we discuss the use of a new synthetic calcium silicate hydrate called LRA (Lipid Removal Agent) for the selective removal of lipids, macromolecules containing lipid moieties and other biological macromolecules in both analytical and process scale applications.

### What is LRA?

LRA (Lipid Removal Agent) media is a synthetic calcium silicate hydrate developed for the selective removal of lipids in the production of biopharmaceuticals. LRA media is highly specific for lipids, lipopolysaccharides (endotoxins) and lipoproteins in the presence of other biological macromolecules (e.g. albumin, immunoglobulins, and other proteins). LRA is often used for the removal of lipids in biotech process streams and analytical assays that require lipid removal during sample preparation. Please see **Table 1** for detailed specifications of LRA and **Table 2** for LRA's lipid removal capacity.

### Targeted removal of lipoproteins

In this application, bulk LRA was incubated with cryo-reduced source plasma at increasing LRA concentration levels for a period of 3 hours at ambient temperature. Following incubation, the sample was centrifuged and the supernatant was analyzed via nephelometry for removal of protein. **Table 3** shows that proteins, for the most part, are not removed by LRA. Although some non-specific adsorption occurred for plasma ceruloplasmin, IgM and alpha-2 HS glycoprotein occurred at 80 g LRA / L plasma, adsorption to apolipoprotein A1 and B was specific relative to the majority of the other plasma proteins endogenous to the sample.

### LRA effectively removes phospholipids

In this study we demonstrate LRA's high capacity for phospholipids. LRA (40 mg) was packed into a 3 mL SPE tube between two 20 µm PE frits. A 0.5 mL aliquot of a 0.1 mg/mL phosphatidylcholine in 80% acetonitrile was loaded onto the LRA SPE tube with the aid of an SPE vacuum manifold. The resulting eluate was analyzed for phosphatidylcholine breakthrough via LC-UV

**Table 3** ..... Human plasma protein profile following three hour room temperature incubation with LRA media\*

| LRA concentration in plasma    | 0 g/L                                 | 20 g/L      | 40 g/L      | 80 g/L      | Relative adsorption by LRA |
|--------------------------------|---------------------------------------|-------------|-------------|-------------|----------------------------|
| <b>Proteins in supernatant</b> | <b>mg/mL (% from starting plasma)</b> |             |             |             |                            |
| Albumin                        | 30.40 (100)                           | 30.40 (100) | 30.80 (101) | 30.00 (99)  | 0                          |
| Immunoglobulin G               | 6.20 (100)                            | 6.23 (100)  | 6.18 (100)  | 5.80 (94)   | 0                          |
| Immunoglobulin A               | 1.83 (100)                            | 1.85 (101)  | 1.84 (101)  | 1.73 (95)   | +                          |
| Immunoglobulin M               | 0.72 (100)                            | 0.70 (97)   | 0.63 (88)   | 0.51 (71)   | 0                          |
| α-1 acid glycoprotein          | 0.62 (100)                            | 0.61 (98)   | 0.61 (98)   | 0.61 (98)   | 0                          |
| Haptoglobin                    | 0.93 (100)                            | 0.95 (102)  | 0.97 (104)  | 0.97 (104)  | 0                          |
| Transferrin                    | 2.27 (100)                            | 2.30 (101)  | 2.38 (105)  | 2.40 (106)  | 0                          |
| α-1 Proteinase inhibitor       | 1.21 (100)                            | 1.20 (99)   | 1.20 (99)   | 1.20 (99)   | 0                          |
| α-2 Macroglobulin              | 1.03 (100)                            | 1.01 (98)   | 0.96 (93)   | 0.96 (93)   | 0                          |
| α-2 HS Glycoprotein            | 0.81 (100)                            | 0.66 (81)   | 0.66 (81)   | 0.28 (34)   | ++                         |
| Ceruloplasmin                  | 0.29 (100)                            | 0.27 (93)   | 0.24 (83)   | 0.04 (14)   | ++                         |
| Apolipoprotein A1              | 1.11 (100)                            | 0.65 (59)   | <0.25 (<23) | <0.25 (<23) | +++                        |
| Apolipoprotein B               | 1.03 (100)                            | 0.76 (74)   | <0.35 (<34) | <0.35 (<34) | +++                        |

\* Data provided by Advanced Minerals Corporation

analysis. The LRA adsorbent offered 91.4% removal of the phosphatidylcholine with a single pass through the LRA SPE tube (data not shown).

#### Removal of endotoxins

Endotoxin removal is of critical concern in the development and manufacture of biopharmaceutical products. Zhang and coworkers (1) demonstrated that LRA media has an effective endotoxin removal capacity of  $6 \times 10^6$  endotoxin units (EU) per gram LRA, and offered rapid adsorption kinetics as well. For example, over 99.9% endotoxin in a 5000 EU/mL sample was adsorbed onto the LRA media after mixing for only 2 minutes. They further demonstrated the performance of the LRA by comparing it against a number of other commercially-available endotoxin removal agents.

#### Plasmid DNA purification

Although LRA was developed to selectively remove lipids, researchers have exploited LRA's selectivity for other purification purposes. Winters and coworkers (2) characterized the adsorption mechanism of LRA and have developed a cost effective and simple method for removing key impurities from E. coli lysates in the purification of supercoiled plasmid products. In a single adsorptive contact step, they were able to remove genomic DNA and open-circular plasmid whereas supercoiled plasmid remains unbound. When combined with LRA's selective affinity towards detergents and endotoxins, LRA media offers a powerful purification tool in downstream polishing during bioprocess purification.

#### Conclusion

LRA media is a synthetic calcium hydrate developed for the selective removal of lipids in the presence of other

biological macromolecules. Its high capacity and adsorptive properties are ideal for bioprocessing purification, but it can be scaled down for analytical sample prep purposes as well. In this report we showed LRA's capacity and adsorptive efficiency across a range of lipids including endotoxins, phospholipids and lipoproteins. LRA's unique selectivity can also be extended to other applications as well including the purification of supercoiled plasmid DNA.

Although LRA is currently available in bulk packing, LRA media can also be supplied in common LPLC hardware such as SPE tubes, VersaFlash® cartridges, Büchner Funnels, etc.

#### Related information

Customers using LRA media in cGMP production of biopharmaceuticals can request an LRA Regulatory Support Package through Supelco Technical Service. The support package contains detailed information regarding LRA manufacturing and quality control procedures/specifications.

**Table 4** ..... Ordering information

| Cat. No. | Brand   | Description               | Package Size |
|----------|---------|---------------------------|--------------|
| 13358-U  | Supelco | LRA (Lipid Removal Agent) | 100 g        |
| 13360-U  | Supelco | LRA (Lipid Removal Agent) | 500 g        |

#### References

- [1] Zhang, J. P.; Wang, Q.; Smith, T. R.; Hurst, W. E.; Sulpizio, T. Endotoxin Removal Using a Synthetic Adsorbent of Crystalline Calcium Silicate Hydrate. *Biotechnol. Prog.* 2005, 21(4), 1220-1225.
- [2] Winters, M. A.; Richter, J. D.; Sagar, S. L.; Lee, A. L.; Lander, R.J. Plasmid DNA Purification by Selective Calcium Silicate of Closely Related Impurities. *Biotechnol. Prog.* 2003, 19(2), 440-447.

**CERTAN® Capillary Vials** ..... The advantages of a sealed ampul with the flexibility of a screw cap bottle or a septum vial. Ideal for the storage of volatile samples and reference standards.

By Nicole Amann, Product Manager Analytical Standards ... [namann@europe.sial.com](mailto:namann@europe.sial.com)



#### Features

- 1.5 mL, 4.5 mL and 10 mL volumes
- Amber glass
- 1.2 mm I.D. x 28 mm height capillary opening permits sample withdrawal or addition via syringe needle
- PTFE-lined screw-cap gives a sure seal

#### Advantages

- No appreciable change in concentration, even when open
- Secure storage with minimal evaporation
- Minimizes contamination risk
- Sample filling and removal using a standard GC syringe
- Essentially spill-proof

**Table** ..... CERTAN® Capillary Vials (amber glass, PTFE-lined screw-cap closure)

| Cat. No | Brand          | Volume | Capillary Opening | Package Size |
|---------|----------------|--------|-------------------|--------------|
| 44419   | Riedel-de Haën | 1.5 mL | 1.2 x 28 mm       | One vial     |
| 44420   | Riedel-de Haën | 4.5 mL | 1.2 x 28 mm       | One vial     |
| 44421   | Riedel-de Haën | 10 mL  | 1.2 x 28 mm       | One vial     |

#### CERTAN® Vials are intended for these and other important analytical applications

- Storage of liquids removed from conventional glass ampuls
- Storage of stock and standard solutions
- Storage of difficult samples such as volatile halogen compounds or BTEX aromatics
- Archiving of production or QC samples and extracts

**High Recovery Vials** ..... Reduces sample loss during transfer and eliminates need for inserts during micro-sampling. Available in popular closure designs.

By Ron Shawley, Product Specialist ... [rshawley@sial.com](mailto:rshawley@sial.com)



**Figure 1** .....  
Crimp top

**Figure 2** .....  
Screw Top (ABC), R.A.M.™

High Recovery Vials feature a one-piece design with a conical bottom that permits the complete recovery of samples, a feature not possible with typical flat-bottom design. These vials are ideal for carrying out reactions and concentrating samples. They eliminate the need to transfer to another vial or container, a process that often results in the loss of precious samples.

All four High Recovery Vials we offer are composed of high purity, clear, colorless borosilicate glass, with large (6.0 mm) openings.

**Table** ..... High Recovery Vials (clear borosilicate glass)

| Cat. No. | Brand   | Volume | Closure       | Dimensions (OD x height x opening) | Package Size |
|----------|---------|--------|---------------|------------------------------------|--------------|
| 27274    | Supelco | 1.5 mL | Crimp Top     | 12 mm x 32 mm x 6.0 mm             | 100 vials    |
| 29015-U  | Supelco | 1.5 mL | ABC Vial      | 12 mm x 32 mm x 6.0 mm             | 100 vials    |
| 29138-U  | Supelco | 1.5 mL | Snap Seal     | 12 mm x 32 mm x 6.0 mm             | 100 vials    |
| 29152-U  | Supelco | 3.2 mL | 13/425 Thread | 15 mm x 45 mm x 8.25 mm            | 100 vials    |

- Crimp Top: Ideal for HPLC and GC autosamplers
- Screw Top (ABC), R.A.M.™: Screw thread closure with height equaling a crimp vial. Ideal for use with robotic arms
- Snap Seal (Step): Has more glass in the neck area of the vial than other Snap Ring vials
- 13/425 Screw Thread: Larger volume autosampler vial with High Recovery design

## New Sudan Red Dyes from Sigma-Aldrich ..... Standards, deuterated standards and analytical tools for the reliable LC-MS analysis of these carcinogenic compounds in foodstuffs

Carmen T. Santasania, Senior Applications Chemist ... [csantasania@sial.com](mailto:csantasania@sial.com)

### Sudan Red – important azo dyes in science and industry

Besides their use in science as microbiology dyes, Sudan dyes (**Figure 1**) are used legally to give red/orange coloration to leather, fabrics, fats, oils, waxes, polystyrene, cellulose and synthetic lacquers and polishes. Because of their classification as Group 3 carcinogens, they have been banned worldwide in food [1-3].

### Food adulteration with Sudan Red

Sudan dyes are readily available, relatively inexpensive and add a desirable bright red-orange coloration which makes them a temptation to unscrupulous food producers who have been known to add Sudan dyes to products such as chili powder and other ground capsicums, paprika, curcuma, sumac, turmeric, curry powder and palm oil. The intentional contamination of food products with Sudan dyes came to public attention

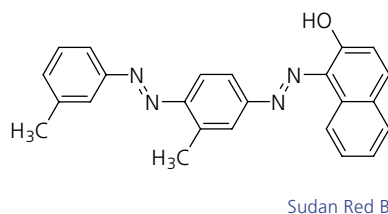
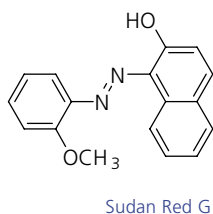
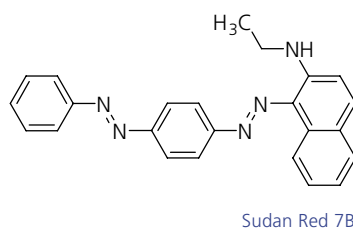
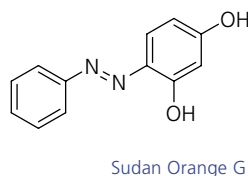
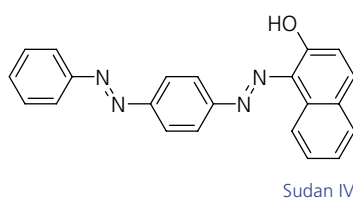
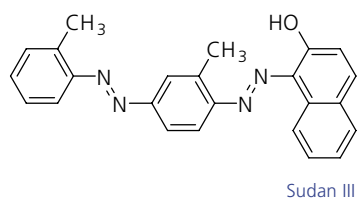
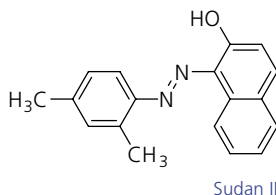
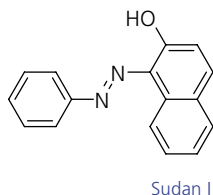
in 2003, when Sudan I was discovered in chili powder imported into Europe from India.

A cascade of regulations ensued. 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. As of this writing, the U.S. Food and Drug Administration (FDA) is monitoring the situation, but has not issued any formal guidance to the food industry [4].

Despite the regulations, problems with adulteration still persist. In 2005, Sudan I contamination triggered the most extensive and expensive food recall ever conducted in the U.K [4-6]. Product recalls due to Sudan contamination also took place in China, Canada and South Africa [4].

Figure 1 .....

Sudan Red  
Compounds



### Challenges of Sudan Red analysis

Sudan dyes are now analyzed worldwide. Since July 2003, all shipments of chili and curry powders entering any EU Member State must carry a certificate of analysis proving they are free of Sudan I. Any shipment without such a certificate is detained for sampling and analysis. Random sampling is also undertaken at ports and by local authorities. In the UK, the Food Standards Agency (FSA) and local authorities randomly sample more than 1000 shipments per year of imported chili products. Any shipment that is shown to contain Sudan I, II, III or IV must be destroyed [6].

Permissible levels of these dyes have not been definitively assigned, and have generally followed the limit of quantification (LOQ). Analysis of Sudan dyes in foodstuffs is usually performed by HPLC with varying forms of detection, including UV, PDA and mass spectrometry (MS). Utilizing LC-UV, the LOQ is around 1 ppm [4]. With more sensitive detection methods, e.g. LC-MS, levels down to 1 ppb and lower should be quantifiable.

### Sigma-Aldrich: offering products for the reliable analysis of Sudan Red

Sigma-Aldrich responded to the need for reliable analytical reference standards of Sudan dye compounds, including deuterated stable isotopes (see Product Listing). Because of the need for lower LOQ, a LC-MS method was sought that could detect Sudan dyes in spiked chili powder. An Ascentis® RP-Amide column



was chosen because of its ability to resolve the compounds and provide clean, interference-free baselines. LC-MS CHROMASOLV® solvents were chosen for their cleanliness, as well as the convenience and reproducibility of the pre-blended 0.1% formic acid solution (see Product Listing).

### Exploratory LC-MS method for analysis of Sudan Red

The purpose of part one of this study was to scout for suitable column, mobile phase and MS conditions that provide adequate resolution and ppm detection of the test compounds. Further studies to optimize sample extraction procedures to maximize sensitivity will be reported in part two of this series.

Table ..... Product listing

#### HPLC Columns

| Cat. No. | Brand   | Description   |
|----------|---------|---|
| 565324-U | Supelco | Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles* |
| 581324-U | Supelco | Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles       |
| 581616-U | Supelco | Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles    |
| 567516-U | Supelco | Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles    |

\* Column shown in this report.

#### CHROMASOLV® solvents

| Cat. No. | Brand          | Description                                   | Package Size |
|----------|----------------|---|--------------|
| 34673    | Riedel-de Haën | Water with 0.1% formic acid LC-MS CHROMASOLV® | 2.5 L        |
| 34967    | Riedel-de Haën | Acetonitrile LC-MS CHROMASOLV®, ≥99.9%        | 1 L, 2.5 L   |
| 34850    | Riedel-de Haën | Acetone CHROMASOLV®, for HPLC, ≥99.8%         | 1 L, 2.5 L   |

#### Sudan Dye Standards

| Cat. No. | Brand          | Description  | Package Size       |
|----------|----------------|--|--------------------|
| 51383    | Fluka          | Sudan I<br>1-Phenylazo-2-naphthol  | 25 mg              |
| 07937    | Fluka          | Sudan II<br>1-(2,4-Xylylazo)-2-naphthol  | 25 mg              |
| 68562    | Fluka          | Sudan III<br>1-[4-(Phenylazo)phenylazo]-2-naphthol   | 25 mg              |
| 67386    | Fluka          | Sudan IV<br>1-[2-Methyl-4-(2-methylphenylazo)phenylazo]-2-naphthol                           | 25 mg              |
| 65450    | Fluka          | Sudan Orange G<br>≥98% (HPLC)  | 1 kg               |
| 46290    | Fluka          | Sudan Red 7B   | 10 g, 50 g         |
| 86010    | Fluka          | Sudan Red B  | 25 g, 100 g, 500 g |
| 17373    | Fluka          | Sudan Red G  | 25 g, 100 g        |
| 34181    | Riedel-de Haën | Sudan I-d <sub>5</sub> solution<br>100 ng/µL in acetonitrile, OEKANAL®, analytical standard  | 2 mL               |
| 34163    | Riedel-de Haën | Sudan IV-d <sub>6</sub> solution<br>100 ng/µL in acetonitrile, OEKANAL®, analytical standard | 2 mL               |

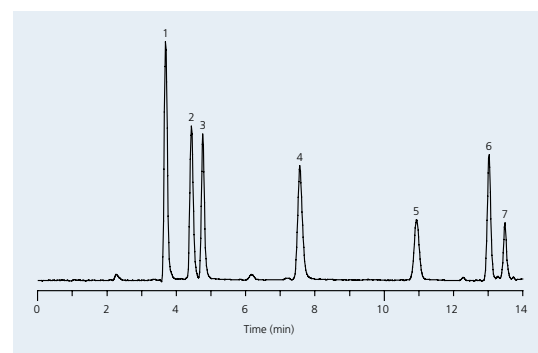
### Sample preparation

Sudan dyes are poorly soluble in polar solvents. Only the Sudan I was soluble in methanol. Stock solutions of other dyes were made in ethyl acetate and diluted to working concentration in acetone. The spiked chili powder sample was prepared by placing 500 mg of chili powder and 10 mL of HPLC grade acetone in a 20 mL vial to which 10 µL of 50 µg/mL Sudan Red Standard solution was added. The vial was shaken vigorously for 3 minutes, solids were allowed to settle and the liquid was decanted. The equivalent concentration of Sudan Red in chili powder was of 1000 µg/kg (1000 ppb). Further work will focus on optimizing the sample extraction and analysis conditions to detect sub-ppb levels.

### HPLC stationary phase

Because Sudan IV and Sudan Red B are geometric isomers, they are not resolved on partitioning HPLC stationary phases. Sudan Red B was not included in the analysis. Separation of the remaining seven dyes was tested on four reversed-phase stationary phases: C18, amide, phenyl and pentafluorophenyl. The best separation was attained on the Ascentis RP-Amide phase (Figure 2).

Figure 2 ..... Total ion chromatogram (TIC) of Sudan Dyes on Ascentis RP-Amide using APCI(+) in selected ion recording (SIR) mode



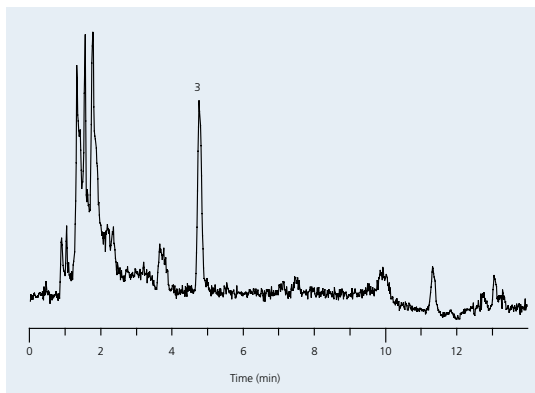
**conditions:** column: Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U); mobile phase A: 0.1% formic acid in water (pH unadjusted) (34673); mobile phase B: acetonitrile (34967); flow rate: 1 mL/min, split to the MS; temperature: 35 °C; detection: MS, APCI(+) in selected ion recording (SIR) mode; injection volume: 15 µL

**sample:** each compound 1 µg/mL (1 ppm) in (30:70), 0.1% formic acid:acetonitrile

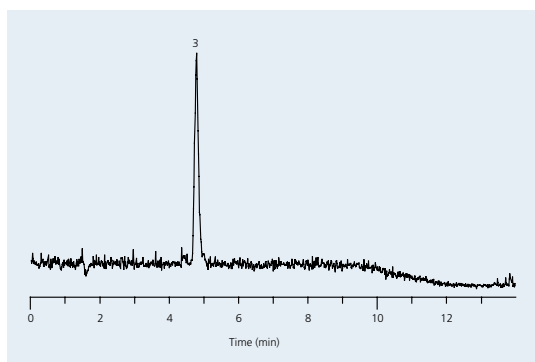
#### peak identification:

1. Sudan Orange G (M+H)<sup>+</sup> = 215.07
2. Sudan Red G (M+H)<sup>+</sup> = 279.10
3. Sudan I (M+H)<sup>+</sup> = 249.09
4. Sudan II (M+H)<sup>+</sup> = 277.12
5. Sudan III (M+H)<sup>+</sup> = 353.13
6. Sudan Red 7B (M+H)<sup>+</sup> = 380.17
7. Sudan IV (M+H)<sup>+</sup> = 381.16

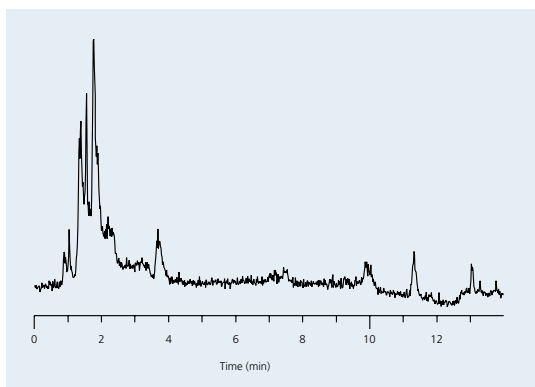
**Figure 3** .....  
 Extracted Ion MS,  
 Sudan I (m/z = 249.09)



Spiked chili powder extract (1 ppm spike, each of seven dye compounds)



Standard mixture containing 1 ppm each dye compound



Blank chili powder extract (no spike)

**conditions:** same as Figure 2

#### Mobile phase conditions

Selectivity was not affected in the study by changing from acetonitrile to methanol as the strong solvent in the mobile phase. Also, omission of the 0.1% formic acid did not change the selectivity. However, it did give slightly better peak shape than pure water.

#### MS conditions

All chromatograms shown in this report are MS with APCI(+) in selected ion recording (SIR) mode. Although ESI(+) was the ionization mode most commonly reported for these compounds in the literature, we found that ESI(+) mode gave a large signal for some of the compounds and a small signal for some others, resulting in a chromatogram with some high and some low responses. APCI(+) detection gave more uniform ionization of the compounds.

#### Results and conclusion

In order to clearly show the individual dyes in the spiked sample, the individual masses were extracted from the Total Ion Chromatogram (TIC). Sudan I (m/z = 249.09) in spiked chili powder and as a standard along with a blank extract appears in **Figure 3** as an example of an extracted ion chromatogram.

This method shows that the new Sudan Dye standards can be separated and analyzed using LC-MS on an Ascentis RP-Amide column with gradient elution and APCI(+) mass spectrometric detection. A spiked sample of chili powder was run showing the method to be feasible on a real sample. Sample extraction procedures to maximize analyte sensitivity and minimize LOQ will be the focus of an upcoming study.

#### References

- [1] 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 Web site (<http://monographs.iarc.fr/ENG/Monographs/vol8/volume8.pdf>) accessed Sept. 25, 2006.
- [2] "EFSA reviews toxicological data of illegal dyes in food" European Food Safety Authority (EFSA) Web site ([http://www.efsa.europa.eu/en/press\\_room/press\\_release/2005/1129.html](http://www.efsa.europa.eu/en/press_room/press_release/2005/1129.html)) 12 September 2005, accessed Sept. 25, 2006.
- [3] "Opinion of the Scientific Panel on Food Additives, Flavours, 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/afc/afc\\_opinions/1127.Par.0001.File.dat/afc\\_op\\_ej263\\_illegaldyes\\_en1.pdf](http://www.efsa.europa.eu/etc/medialib/efsa/science/afc/afc_opinions/1127.Par.0001.File.dat/afc_op_ej263_illegaldyes_en1.pdf), accessed Sept. 25, 2006
- [4] White Paper on Sudan Red and Related Dyes, The American Spice Trade Association Web site ([www.astaspice.org](http://www.astaspice.org)), accessed Sept. 25, 2006.
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- [6] Food Navigator/Europe Web site (<http://www.foodnavigator.com/news/ng.asp?id=66050-sudan-red-colouring-fsa>) accessed Sept. 25, 2006

## New Hopane Reference Standards from Sigma-Aldrich ..... Chemical fossils from ancient bacteria are clues to the source and quality of the underlying oil reservoir and used in oil exploration, site remediation and spill analysis

By Nicole Amann, Product Manager Analytical Standards ... [namann@europe.sial.com](mailto:namann@europe.sial.com)

### Introduction

An interesting application of analytical chemistry is in biomarker analysis: the use of a chemical, a set of chemicals or chemical modifications as clues to answering scientific questions. Sigma-Aldrich researchers and product managers pay close attention to the needs in the biomarker industry, introducing high-quality, relevant reference standards and other analytical tools to the market. One such recent introduction to our line is the hopanoids, lipids that are used by geochemists and environmental chemists as biomarkers in oil exploration and remediation after oil spills or gas leaks.

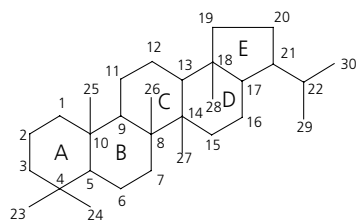
### Hopanoids: ubiquitous lipid compounds

Hopanoids belong to the triterpenoid family, lipids that are resistant to saponification and are abundant in all animal and plant tissues. Sterols are important constituents of cell membranes and have variety of important functions, including controlling membrane permeability, organization and rigidity. In vertebrates, the main sterol is cholesterol, while in prokaryotes, hopanoids dominate.

### Hopanoid nomenclature

Hopanoids are formed by the cyclization of squalane to a pentacyclic triterpanoid with a hopane skeleton (**Figure 1**) which has four cyclohexane rings and one cyclopentane ring. Over 150 distinct, naturally-occurring hopanoids have been identified in soils, sediments and other organic matter. Hopanoids have a fixed stereochemistry and differ in the orientation about Carbon-17 and Carbon-21 ( $\alpha$  or  $\beta$ ) and Carbon-22 (R or S). The projection of the hydrogen in  $\alpha$  isomers is above the plane of the paper, while  $\beta$  isomers project below the plane. Hopanoids also differ in the types of R-groups at Carbon-30 and varying degrees of unsaturation, primarily in rings D and E. Other modifications are also found. Hopanoids present in sediments range from C35 to C27, usually with the C30 isomers as the predominant form.

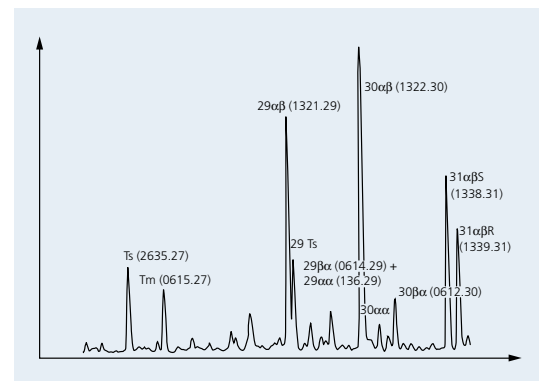
**Figure 1** ..... Hopane characteristic structure



Analysis of hopanes generally begins by fractionation using preparative HPLC followed by GC/MS of the sterol

fraction looking for the characteristic m/z 191 ion. A representative GC/MS chromatogram appears in **Figure 2**.

**Figure 2** ..... GC-MS (m/z 191) of a typical North Sea Oil with identification of the major hopanes. Printed by the courtesy of Chiron AS, Norway ([www.chiron.no](http://www.chiron.no)) and GEUS, Denmark and Greenland Geological Survey, Denmark



|                    |  |
|--------------------|--|
| Ts                 | Chiron AS no. 2635,27                                      |
| Tm                 | 17 $\alpha$ (H), 22,29,30-Trisnorhopane                    |
| 29 $\alpha\beta$   | 17 $\alpha$ (H), 21 $\beta$ (H)-30-Norhopane               |
| 29 $\beta\alpha$   | 17 $\beta$ (H), 21 $\alpha$ (H)-30-Norhopane (Fluka 90102) |
| 29 $\alpha\alpha$  | 17 $\alpha$ (H), 21 $\alpha$ (H)-30-Norhopane              |
| 30 $\alpha\beta$   | 17 $\alpha$ (H), 21 $\beta$ (H)-Hopane (Fluka 90656)       |
| 30 $\alpha\alpha$  | 17 $\alpha$ (H), 21 $\alpha$ (H)-Hopane                    |
| 30 $\beta\alpha$   | 17 $\beta$ (H), 21 $\alpha$ (H)-Hopane (Fluka 08189)       |
| 31 $\alpha\beta$ S | 17 $\alpha$ (H), 21 $\beta$ (H)-22S-Homohopane             |
| 31 $\alpha\beta$ R | 17 $\alpha$ (H), 21 $\beta$ (H)-22R-Homohopane             |

### Hopanoids as petrochemical biomarkers

Important elements for geochemical analysis are maturation, origin and correlation:

*Maturation* is measured as the ratio between a stable and less stable biomarkers and tells whether the sediment is in the oil window, or of the right maturity to produce oil.

*Origin* may be either Algae, bacteria or terrestrial. Origin is important for the quality of the oil.

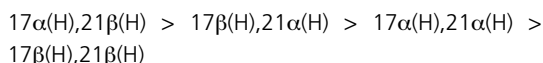
*Source-Oil, oil -oil, and source-source correlation:* The oil and the biomarkers are transported from the source to the reservoir. The biomarker pattern gives important information about the composition of the source for a particular oil field. There can be several sources for one field and one source can give rise to several oil fields.

Coal, oil and natural gas are formed by the compression of ancient biomass under layers of sediment. Abiogenic origins have also been proposed. The quality of the oil is

influenced by the biomass source (bacteria, Archaea, terrestrial plants, algae, etc.) and its age. Reliable biomarkers are valuable as they can help focus expensive exploration efforts on the most lucrative fields. Along with aromatic and low molecular weight hydrocarbons, hopanoids are used as biomarkers for oil, coal and gas exploration and spill remediation. Hopanoids are evidence of bacteria, primarily, but other biomass sources as well. For example, samples with a high concentration of 17 $\alpha$ (H),21 $\beta$ (H)-hopane ( $\beta\alpha$ -hopane, Cat. No. 08189) are usually from non-marine source rocks. Besides rocks and sediment, hopanoids are also used to identify the source of oil seeps that appear on surface waters as indicators of active petroleum systems beneath. Besides petrochemical applications, hopanoids are also used to research the impact of bacteria on other environments.

### Hopanoids and biomass maturation

Hopanoids occur in both recent and ancient sediments, even in rocks as old as 1,800 million years. Not only does the hopanoid profile point to the source of the biomass, the ratio of certain epimers is used to give an age to the oil or sediment. The order of thermodynamic stability of the 17-21 hopane isomers is



With a few exceptions, the thermodynamically unstable  $\beta\beta$ -hopanes are the natural form in living organisms and recent sediments. During the course of diagenesis (the processes that occur during conversion of the sediment to rock) and catagenesis (the natural cracking process that converts the precursor compounds to hydrocarbons), heat and pressure cause the hopanoid to lose its stereospecificity, particularly at the 17 and 21 positions, giving rise to a large array of isomers. The stable  $\alpha\beta$ -

hopanes predominate in crude oils and mature source rock sediments. Smaller amounts of  $\beta\alpha$ - and  $\alpha\beta$ -isomers are present as minor constituents.

Maturity of the oil or sediment is often measured using  $T_m/T_s$ <sup>1)</sup>, which is the ratio of two specific types of trisnorhopanes (18 $\alpha$ (H)-22,29,30-trisnorhopane and 17 $\alpha$ (H)-22,29,30-trisnorhopane, or  $T_s$  and  $T_m$ , respectively).  $T_s$  is more stable undergoes less degradation than  $T_m$  during diagenesis and catagenesis. Other changes also occur during the maturation process, including loss of side chains, oxygenation, cyclization and unsaturation, depending on the environmental conditions during diagenesis and catagenesis.

### Hopanoids in oil field remediation and spill analysis

Hopanoids are important as internal markers in environmental and petroleum remediation studies, due to their persistency and stability. By comparing hopane profiles, the source of the oil spill can be identified. The thermodynamically stable forms 17 $\beta$ (H),21 $\alpha$ (H)-Hopane (Cat. No. 08189) and 17 $\beta$ (H),21 $\alpha$ (H)-hopane (Cat. No. 90656) can be used as internal markers for biodegradation of crude oils. The thermodynamically unstable 17 $\beta$ (H),21 $\alpha$ (H)-Hopane can be used as an internal standard because it is not present in crude oil. Historically, quantitative determination of hopanoid biomarkers has been difficult because of a lack of suitable reference standards. This is no longer an issue with the introduction of many important hopanoids by Sigma-Aldrich's Fluka brand.

<sup>1)</sup> The index most often used is  $T_s/(T_s+T_m)$ . The rearranged  $T_s$  is more stable than the „normal“ series  $T_m$ . It is an index of maturity. It is a topic of discussion whether  $T_m$  actually is converted to  $T_s$ , or whether  $T_s$  is formed faster or  $T_m$  is degraded faster. The ratio may also relate to the source.

**Table .....** Hopane standard solutions from Sigma-Aldrich

Solutions of 0.1 mg/mL in Isooctane, Assay:  $\geq 97.0\%$  (GC/MS) unless otherwise noted.

| Cat. No. | Brand | Description*                                | Molecular Formula               | Molecular Weight | Package Size |
|----------|-------|---|---------------------------------|------------------|--------------|
| 90656    | Fluka | 17 $\alpha$ (H),21 $\beta$ (H)-Hopane       | C <sub>30</sub> H <sub>52</sub> | 412.73           | 1 mL         |
| 08189    | Fluka | 17 $\beta$ (H),21 $\alpha$ (H)-Hopane       | C <sub>30</sub> H <sub>52</sub> | 412.73           | 1 mL         |
| 90102    | Fluka | 17 $\beta$ (H),21 $\alpha$ (H)-30-Norhopane | C <sub>29</sub> H <sub>50</sub> | 398.71           | 1 mL         |
| 41133    | Fluka | (22R)-30-Homohop-17(21)-ene                 | C <sub>31</sub> H <sub>52</sub> | 424.74           | 1 mL         |
| 44133    | Fluka | 30-Norneohop-13(18)-ene                     | C <sub>29</sub> H <sub>48</sub> | 396.69           | 1 mL         |
| 42689    | Fluka | Neohop-13(18)-ene                           | C <sub>30</sub> H <sub>50</sub> | 410.72           | 1 mL         |
| 16411    | Fluka | 22,29,30-Trisnorneohop-13(18)-ene           | C <sub>27</sub> H <sub>44</sub> | 368.64           | 0.5 mL       |
| 11201    | Fluka | Fern-9(11)-ene                              | C <sub>30</sub> H <sub>50</sub> | 410.72           | 1 mL         |
| 04626    | Fluka | Hop-22(29)-ene                              | C <sub>30</sub> H <sub>50</sub> | 410.72           | 1 mL         |
| 03707    | Fluka | 30-Norhop-17(21)-ene                        | C <sub>29</sub> H <sub>48</sub> | 396.69           | 1 mL         |
| 43838    | Fluka | Hop-21(22)-ene                              | C <sub>30</sub> H <sub>50</sub> | 410.72           | 1 mL         |

\* Limited shelf life. Expiry date appears on the label. Store at 2-8°C. Sample mass is measured by relative intensity (TIC) of  $\alpha\beta$ -C<sub>30</sub> hopane using GC/MS or by gravimetry.

## Calibration Solutions and Kits for *radiello*® Diffusive Air Sampler ..... Standard solutions and kits for air sampling of a wide range of air-borne contaminants

By Klaus Buckendahl, European Sales Development Manger ... kbuckend@europe.sial.com



Figure 1 .....  
*radiello* sampler

In 2006, Supelco, a member of the Sigma-Aldrich family, in collaboration with the Fondazione Salvatore Maugeri (FSM), Padova, Italy, partook in a joint venture in which Supelco became the exclusive world-wide distributor of *radiello* products and accessories.

### What is *radiello*?

*radiello* is a patented revolutionary diffusive sampler design. The radial symmetry design gives significantly faster sampling rates, typically 3- to 8-fold faster than common axial diffusive samplers. This speed permits passive sampling for short-term measurements, a feat not attainable before *radiello*.

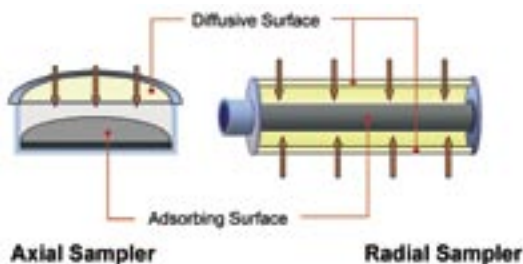
Compared to conventional passive samplers, *radiello* provides:

- **Faster sampling** due to high uptake rates
- **High analyte capacity and broader concentration ranges** due to larger adsorbent bed
- **More reliable results** due to experimentally determined sampling rates
- **Economical use** because of the reusable hardware

Typical applications for *radiello* are indoor sampling, outdoor sampling, and industrial hygiene measurements (work place environments).

The *radiello* diffusive samplers have been designed for the following air-borne contaminants:

- **Aldehydes**
- **VOCs and BTEX** (with both solvent and thermal desorption)
- **Nitrogen and Sulfur Dioxide** (NO<sub>2</sub> and SO<sub>2</sub>)
- **Hydrogen fluoride** (HF)
- **Hydrogen chloride** (HCl)
- **Ozone** (O<sub>3</sub>)
- **Phenols** (with thermal desorption)
- **Hydrogen sulfide** (H<sub>2</sub>S)
- **Ammonia** (NH<sub>3</sub>)
- **Anesthetic Gases and Vapors**



To analyze the loaded *radiello* cartridges after sampling, we now offer calibration kits and solutions. Calibration solutions are available for H<sub>2</sub>S and aldehyde analysis, and calibration kits with pre-spiked cartridges for BTEX and VOC determination. Details appear below.

### Calibration solutions for *radiello* cartridges

#### H<sub>2</sub>S (Hydrogen Sulfide) calibration standard

This standard consists of a methylene blue concentrate that, once diluted 1:50, provides the same absorbance value of hydrogen sulfide at 665 nm at a concentration of 1.145 µg/L sulfide ion. This concentration is the highest absorbance value within the linear range of the spectrophotometer and can be used as a stock solution to prepare standards for the calibration curve. One pack is suitable for preparing 50 calibration curves using the recipe described in the accompanying table. The standard is stable for at least one year.

Table ..... Preparation of H<sub>2</sub>S calibration curve using RAD171

| Solution | Volume (mL)            | Volume (mL) water to add | Equivalent conc. (µg/mL) of S <sup>2-</sup> |
|----------|------------------------|--------------------------|---|
| A        | 2 mL of Stock Solution | 98 mL                    | 1.145                                       |
| B        | 25 mL of A             | 25 mL                    | 0.572                                       |
| C        | 10 mL of A             | 40 mL                    | 0.229                                       |
| D        | 5 mL of A              | 45 mL                    | 0.115                                       |

| Cat. No. | Brand   | <i>radiello</i> Code | Description  | Qty. |
|----------|---------|----------------------|--|------|
| RAD171   | Supelco | 171                  | H <sub>2</sub> S Methylene Blue Calibration Standard, 100 mL | 1    |

#### Aldehyde calibration standard

This standard consists of nine different 2,4-dinitrophenylhydrazones derivatives (2,4-DNPH) diluted in 10 mL of acetonitrile. The concentrations for each compound appear in the accompanying table. Actual concentrations are certified for each lot and provided on the data sheet. The solution is shipped in a crimp cap vial with a pierceable septum, and is stable for at least 4 months when stored at 4°C in the dark.

Table ..... Composition of the aldehyde calibration standard for *radiello*

#### 2,4-DNPH derivatives of below compounds, concentrations given as µg/mL aldehyde

|              |    |             |    |              |    |
|--------------|----|-------------|----|--------------|----|
| Formaldehyde | 50 | Propanal    | 50 | Pentanal     | 50 |
| Acetaldehyde | 50 | Butanal     | 50 | Hexanal      | 50 |
| Acrolein     | 10 | Isopentanal | 50 | Benzaldehyde | 50 |



Figure 2 ..... radiello calibration kit

| Cat. No. | Brand   | radiello Code | Description  | Qty. |
|----------|---------|---------------|--|------|
| RAD302   | Supelco | 302           | Aldehyde Calibration Standard, 10 mL in acetonitrile | 1    |

### Calibration kits

The *radiello* calibration kits can be used to establish a calibration curve, to perform quality control runs and to do system proficiency testing, which gives analysts the opportunity to determine the efficiency of their desorption procedures.

The type of cartridge in each kit is application-dependent. All kits consist of twelve cartridges: Three cartridges are blanks and nine are preloaded with analytes in three different concentration groups. The spiked concentrations are shown in the tables accompanying each kit description. The values shown are indicative; actual concentrations are certified for each lot. The cartridges are stable for at least four months when stored at 4°C.

#### BTEX calibration kit (CS<sub>2</sub> desorption)

This calibration kit is designed for the analysis of BTEX in urban environments followed by chemical desorption with CS<sub>2</sub>. The kit may be used for both routine calibration and quality control. The kit consists of twelve Code 130 Cartridge Adsorbents (Cat. No. RAD130). The preloaded BTEX compounds simulate 7 days exposure (100,080 minutes). The values shown in the following table are representative of the pre-loaded analyte levels.

Table ..... Simulated analyte concentrations (µg/m<sup>3</sup>) of BTEX/CS<sub>2</sub> Kit (equivalent to 7 days exposure)

| Compound     | Group 1 | Group 2 | Group 3 |
|--------------|---------|---------|---------|
| Benzene      | 1       | 10      | 50      |
| Toluene      | 2       | 20      | 100     |
| Ethylbenzene | 1       | 10      | 50      |
| m-Xylene     | 1       | 10      | 50      |
| p-Xylene     | 1       | 10      | 50      |
| o-Xylene     | 1       | 10      | 50      |

| Cat. No. | Brand   | radiello Code | Description                                       | Qty.  |
|----------|---------|---------------|---|-------|
| RAD405   | Supelco | 405           | BTEX Calibration Kit (CS <sub>2</sub> Desorption) | 1 kit |

#### BTEX calibration kit (thermal desorption)

This kit is designed for the analysis of BTEX in urban environments followed by thermal desorption. It can be used for both routine calibration and quality control. The kit consists of twelve Code 145 Cartridge Adsorbents (Cat. No. RAD145). The BTEX preloaded simulate 7 day exposures (100,080 minutes). BTEX is spiked on the cartridges by injecting vaporized BTEX standards in methanol under nitrogen flow. A methanol peak may be visible during chromatographic analysis.

Table ..... Simulated analyte concentrations (µg/m<sup>3</sup>) of BTEX/TD Kit (equivalent to 7 days exposure)

| Compound     | Group 1 | Group 2 | Group 3 |
|--------------|---------|---------|---------|
| Benzene      | 1       | 5       | 25      |
| Toluene      | 2       | 10      | 50      |
| Ethylbenzene | 1       | 5       | 25      |
| m-Xylene     | 1       | 5       | 25      |
| p-Xylene     | 1       | 5       | 25      |
| o-Xylene     | 1       | 5       | 25      |

| Cat. No. | Brand   | radiello Code | Description                               | Qty.  |
|----------|---------|---------------|---|-------|
| RAD407   | Supelco | 407           | BTEX Calibration Kit (Thermal Desorption) | 1 kit |

#### VOC calibration kit (workplace environment)

This kit is ideal for conducting scheduled quality control runs when analyzing VOCs in workplace environments. It comprises twelve Code 130 Cartridge Adsorbents (Cat. No. RAD130). VOCs are spiked on the cartridges by injecting vaporized VOC standards in CS<sub>2</sub> under nitrogen flow. The spiked VOCs represent a broad range of polarity, and the spiked concentrations represent 0.5-, 1- and 2-times the threshold limit values (TLV) for each compound. The VOC cartridges simulate 8 hours exposure (480 minutes).

Table ..... Simulated analyte concentrations (µg/m<sup>3</sup>) of VOC Kit (equivalent to 8 hours exposure)

| Compound              | Group 1 | Group 2 | Group 3 |
|-----------------------|---------|---------|---------|
| Benzene               | 0.1     | 0.2     | 0.4     |
| Toluene               | 19      | 38      | 76      |
| Ethylbenzene          | 12      | 24      | 48      |
| m-Xylene              | 12      | 24      | 48      |
| p-Xylene              | 12      | 24      | 48      |
| o-Xylene              | 12      | 24      | 48      |
| Butanol               | 15      | 30      | 60      |
| 2-Ethoxyethyl-acetate | 2.5     | 5       | 10      |

| Cat. No. | Brand   | radiello Code | Description                                 | Qty.  |
|----------|---------|---------------|---|-------|
| RAD406   | Supelco | 406           | VOC Calibration Kit (Workplace Environment) | 1 kit |

For additional calibration standards and solutions, please refer to the Sigma-Aldrich Analytical Standards Catalog, or visit [www.sigma-aldrich.com/standards](http://www.sigma-aldrich.com/standards).

For more details and information on the radiello diffusive sampler please request the *radiello* brochure or visit our *radiello* web site shown at the bottom right of this page.

## Media for Yeasts and Molds ..... Quality control for yeasts and molds in food, beverage and environmental samples by using specific media

By Jvo Siegrist, Product Manager Microbiology ... [isiegris@europe.sial.com](mailto:isiegris@europe.sial.com)

**Figure 1** .....

HiCrome™ OGYE Agar  
Base: *Aspergillus niger*  
(light blue); *Candida albicans* (green);  
*Saccharomyces cerevisiae* (white)



**Figure 2** ..... A microscopic image of *Aspergillus Niger*



Molds, yeasts and fungi belong to a large group of microorganisms with diverse influences on human life. In the positive sense, they are used to leaven and ferment food and beverages and to provide more stable products. Molds are also very important for the production of life-saving antibiotics like penicillin, cephalosporin and griseofulvin. Many industrial processes are based on mold metabolism, like the production of citric acid. Finally, many enzymes such as amylase, glucoamylase, pectinase, cellulase, protease, lipase, etc. from yeasts and molds are used in processes and research projects.

However, in the negative sense, they are responsible for food spoilage and for serious infections, diseases and poisonings. Some strains of molds produce mycotoxins such as ochratoxin, aflatoxin, deoxynivalenol and fumonisin in many foods including rice, coffee, cocoa and cereals.

The detection of molds and yeasts is still made the classical way, using media and microscopic examination. The steps involved in the classic inspection of food and beverage specimens appears below.

#### Classical steps:

1. Homogenization/Mixing of the sample
2. Dilution series 1:10 ( $10^{-2}$ - $10^{-6}$ )
3. Spread out dilutions on appropriate agar plates or/and inoculation in liquid media
4. Isolate, purify and identify colonies

**Table 1** ..... Chromogenic media for yeasts and molds

| Selective chromogenic Media | Brand | Cat. No. | Organisms/remarks  |
|-----------------------------|-------|----------|--|
| HiCrome™ OGYE Agar Base     | Fluka | 66481    | yeasts and molds; <i>C. albicans</i> shows green and <i>Sacch. cerevisiae</i> colourless colonies. |
| Candida Ident Agar          | Fluka | 72953    | <i>Candida albicans</i> (green), other yeasts (white, pink, beige), Gonococci, molds.              |

For steps three and four, it is important to use an appropriate medium for the detection and identification of molds and yeasts. The range of media is wide and there are, for example, nutrient-rich cultivation media specified for certain molds and yeast species and media with clever approaches to identify and selectively isolate certain microorganisms. Antibiotics, like chloramphenicol, chlortetracycline, gentamicin, oxytetracycline and streptomycin, can be added to inhibit the bacterial growth, yet permit growth of yeasts and fungi. Rose Bengal and dichloran prevent the medium from overgrowth of fast spreading molds.

**Chromogenic media**, one of the selective systems, is a perfect solution for the selective isolation, differentiation and identification of molds and yeasts. With the aide of chromogenic substrates, certain characteristic enzymes are detected while antibiotics inhibit bacterial growth. The differentiation among species is made easy because colonies are colored based on their ability to cleave different chromogenic substrates (see **Table 1** and **Figure 1**).

Common growth media are still used most often for quality control purposes. It is important to know the aim of the control and the type of samples, the number of yeast and mold cells and accompanying bacterial flora. If a high bacterial load in the sample is expected, it is important to use a selective media that restricts bacteria as a first step. This step consists of enumeration followed by the isolation and purification and finally identification based on colony morphology, biochemical metabolism in the media, production of mycelium or pseudo-mycelium and spores, which can be seen by microscope. A choice of common media for yeasts and molds is given in **Table 2**.

#### Reference

- [1] Pitt, J. J.; Hocking, A. D. *Fungi and food spoilage*, 2nd ed.; Aspen Publications; New York, NY, 1999.

**Special Offer: Test  
a chromogenic Media listed in  
Table 1 for free!**

Quote Promotion Code V32 when placing your order for  
66581-100G-F or 72953-50G-F.

Valid Until January 15<sup>th</sup> 2007. One order per customer.

Table 2 ..... Common media for yeasts and molds

| Media   | Brand | Cat. No. | Organisms/remarks  | Isolation | Cultivation | Identification | Selective* | Recommended by                      |
|---|-------|----------|--|-----------|-------------|----------------|------------|-------------------------------------|
| BiGGY Agar  | Fluka | 73608    | <i>Candida albicans</i> , <i>Candida tropicalis</i>  | x         |             | x              | x          |                                     |
| Corn Meal Agar  | Fluka | 42347    | yeasts and fungi, chlamydospore production by <i>Candida albicans</i> , mycel/pseudo-mycelium forming  |           | x           | x              |            |                                     |
| Czapek Dox Agar   | Fluka | 70185    | fungi and yeasts, defined C-source and inorg. N-source   |           | x           |                |            | APHA                                |
| Dichloran Rose bengal Agar (Base)                           | Fluka | 17147    | yeasts and molds   | x         |             | x              | x          |                                     |
| EMB Agar  | Fluka | 70186    | rapid identification of <i>Candida albicans</i> , differentiation and identification of bacteria ( <i>E. coli</i> , <i>Aerobacter aerogenes</i> , Staphylococci) | x         |             | x              |            |                                     |
| Germ count Agar for foodstuffs                              | Fluka | 60646    | yeasts, molds and bacteria   | x         | x           |                |            |                                     |
| Levine EMB Agar   | Fluka | 62087    | rapid identification of <i>Candida albicans</i> , differentiation and identification of bacteria ( <i>E. coli</i> , <i>Aerobacter aerogenes</i> , Staphylococci) | x         |             | x              |            |                                     |
| Lysine Medium   | Sigma | L5910    | wild yeasts in pitching yeasts, defined medium   | x         |             | x              |            |                                     |
| Malt Agar   | Sigma | M9802    | yeasts and molds   | x         | x           |                |            | AOAC, APHA                          |
| Malt Extract Agar   | Fluka | 70145    | yeasts and molds   | x         | x           |                |            |                                     |
| Malt Extract Agar modified, Vegitone                        | Fluka | 38954    | yeasts and molds   | x         | x           |                |            |                                     |
| Malt Extract Broth  | Fluka | 70146    | yeasts and molds   | x         | x           |                |            |                                     |
| OGY Agar  | Fluka | 75310    | yeasts and molds   | x         | x           |                | x          | APHA, ISO 13681:1995, ISO 6611:1992 |
| Orange-serum Agar   | Fluka | 75405    | acid-tolerant spoilage microorganisms  | x         | x           |                |            |                                     |
| Peptone Yeast Extract Agar                                  | Fluka | 77196    | yeasts and molds   | x         |             |                | x          |                                     |
| Potato Glucose Agar   | Fluka | 70139    | yeasts and molds   | x         | x           |                |            | APHA, US FDA                        |
| Potato Glucose Rose bengal Agar (Base)                      | Fluka | 17204    | For promoting ascospore production.  | x         | x           |                | x          | APHA, US FDA                        |
| Potato Glucose Sucrose Agar                                 | Fluka | 17205    | <i>Zygosaccharomyces rouxii</i>  | x         | x           |                |            | APHA, US FDA                        |
| Rice Extract Agar   | Fluka | 83551    | yeasts, differentiation by chlamydospores and morphology   |           | x           | x              |            |                                     |
| Rose bengal Chloramphenicol Agar                            | Fluka | 17211    | yeasts and molds   | x         |             | x              | x          |                                     |
| Sabouraud 2% Glucose Agar                                   | Fluka | 84086    | dermatophytes, fungi and yeasts  | x         | x           |                |            |                                     |
| Sabouraud 4% Glucose Agar                                   | Fluka | 84088    | dermatophytes, fungi and yeasts  | x         | x           |                |            | USP, EP                             |
| Sabouraud 4% Glucose Agar Plates                            | Fluka | 40376    | dermatophytes, fungi and yeasts  | x         | x           |                |            | USP, EP                             |
| Selective Agar for pathogenic fungi                         | Fluka | 84886    | pathogenic fungi, in particular dermatophytes  | x         |             |                |            |                                     |
| Tomato Juice Broth  | Fluka | 17218    | yeasts and other aciduric microorganisms   | x         | x           |                |            |                                     |
| Universal Beer Agar   | Fluka | 17226    | yeasts and organisms which are responsible for beer spoilage (wild yeast, bacteria, molds)   | x         |             |                |            |                                     |
| WL Nutrient Agar  | Fluka | 17222    | yeasts and contaminants (yeasts, bacteria, molds)  | x         |             |                |            |                                     |
| WL Nutrient Broth   | Sigma | W2261    | yeasts and contaminants (yeasts, bacteria, molds)  | x         |             |                |            |                                     |
| Wort Agar   | Fluka | 70196    | yeasts and molds   | x         | x           |                |            |                                     |
| Yeast Agar, buffered  | Fluka | 17136    | yeasts and molds, semisynthetic medium   | x         | x           |                |            |                                     |
| Yeast Carbon Base   | Sigma | Y3627    | yeasts, classification based on nitrogen assimilation  | x         |             | x              |            |                                     |
| Yeast Malt Agar   | Sigma | Y3127    | yeasts, molds and other aciduric microorganisms  | x         | x           |                |            |                                     |
| Yeast Nitrogen Base   | Fluka | 51483    | yeasts, classification based on carbon assimilation  | x         |             | x              |            |                                     |
| Yeast Nitrogen Base without Amino Acid                      | Sigma | Y0626    | yeasts, classification based on amino acid and carbon requirements   | x         |             | x              |            |                                     |
| Yeast Nitrogen Base without Amino Acid and Ammonium Sulfate | Sigma | Y1251    | yeasts, classification based on carbon and nitrogen requirements   | x         |             | x              |            |                                     |
| YGC Agar  | Fluka | 95765    | yeasts and molds in dairy products.  | x         | x           | x              | x          | SLMB, ISO 6611:1992                 |

\* Selective media (low pH of media is not included)

## Improving Karl Fischer Titrations ..... Maintaining the integrity of the titration vessel and other techniques to improve titrations

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It is easy to overlook the importance of the titration vessel in Karl Fischer (KF) titrations. Instead, such factors as the pH of the working medium and sample and the reagent reactivity are usually emphasized. It is also well-known that, because of its pH, the titration speed using the two-component reagent HYDRANAL®-Titrant and HYDRANAL®-Solvent is faster than using the one-

component reagent HYDRANAL®-Composite and methanol. However, irrespective of the type of reagent used, the titration vessel itself, especially how it is assembled and maintained, influences the performance of a titration, the end point stability and the accuracy of the resultant analysis, especially when dealing with samples containing low water content.

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      |
| 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             |
| 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              |
| 34724    | Riedel-de Haën | HYDRANAL®-Formamid dry   | 1 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<br>0.10 (1 g contains 0.10 mg H <sub>2</sub> O) | 40 mL                   |
| 34828    | Riedel-de Haën | HYDRANAL®-Water Standard<br>1.00 (1 g contains 1.0 mg H <sub>2</sub> O)  | 40 mL                   |
| 34849    | Riedel-de Haën | HYDRANAL®-Water Standard<br>10.0 (1 g contains 10 mg H <sub>2</sub> O)   | 80 mL                   |
| 34241    | Riedel-de Haën | HYDRANAL®-Molecular sieve<br>0.3nm                                       | 250 g, 1 kg             |
| 34788    | Riedel-de Haën | HYDRANAL®-Humidity Absorber  | 500 g, 1 kg             |

### Sources of extraneous moisture in the titration vessel

#### Openings – Be certain to create a tight seal

A titration vessel is never completely air- or moisture-tight. The vessel necessarily has openings for reagent and titrant addition, indicator electrodes, removal of waste, the dosage of solid samples, and sometimes for a homogenizer as well. These openings must be sealed effectively, using stoppers, O-rings, Teflon sleeves, or whatever means necessary to provide as much as possible a moisture-tight seal.

The port in the titration vessel where solid samples are added is the same port to which liquid samples are injected. This port is sealed with a septum in order to inject the liquid samples. However, after a finite number of injections, the septum can lose its integrity permitting atmospheric humidity to enter the vessel and a resultant baseline drift.

#### Hygroscopic nature of alcohols – Keep condensation to a minimum

The working medium in the titration vessel always contains an alcohol, typically methanol or ethanol. During stirring, even at room temperature, some of the alcohol vaporizes and condenses in the upper area and

under the head of the titration vessel. Alcohols are very hygroscopic and readily absorb extraneous atmospheric moisture that enters the vessel. Anywhere that alcohol condenses will be a reservoir of extraneous, non-sample derived moisture which consumes reagent and becomes the source of baseline drift. It is important to keep this moisture as low as possible. Slightly shaking the titration vessel from time to time helps to rinse down the condensed alcohol.

#### Maximum acceptable drift

The maximum acceptable drift depends to a great extent on the reagent titer (amount of moisture it will titrate). At a reagent consumption rate of 0.01 mL/min, the maximum acceptable drift (in  $\mu\text{g}$  water/min) for each reagent titer as follows:

Reagent titer 5: 0.01 mL reagent/min = 50  $\mu\text{g}$  water/min

Reagent titer 2: 0.01 mL reagent/min = 20  $\mu\text{g}$  water/min

Reagent titer 1: 0.01 mL reagent/min = 10  $\mu\text{g}$  water/min

#### Use of drying tubes

To significantly reduce the infiltration of atmospheric moisture into the titration vessel, and help remove any moisture that may have entered, we recommend installing a drying tube filled with molecular sieve 0.3nm or Humidity Absorber on the titration vessel.

#### Other vessel-related techniques to improve titrations

- The burette tip should never be placed directly behind the indicator electrode. They should be as far apart as possible to help ensure rapid titrations.
- When a large mass of solid sample is to be titrated, it should be added in smaller portions. This requires frequent opening of the vessel; each opening is an opportunity for moisture to enter the vessel. Since condensed alcohol absorbs moisture, we recommend drying the area around the opening from time to time with a laboratory wipe.
- For the same reasons as above, if many samples are titrated during the work day, the titration vessel will be opened many times and the drift can increase significantly. This is especially true during the summer when ambient humidity is high. As the day progresses, accurate and rapid titrations become more difficult. A slight shaking of the cell helps to rinse down the condensed alcohol together with the adsorbed moisture.
- Titrations at elevated temperatures (40°C or 50°C, for example) cause more alcohol vaporization and condensation than titrations at room temperature.

Dismantling the cell from time to time in order to dry the head of the titration vessel may be necessary under these conditions.

- The stirring speed should not be too slow. A few air bubbles will not disturb the titration or end point indication.
- To ensure a proper end point indication, while the vessel is being stirred the platinum pins of the electrode must be totally covered by the working medium.
- If a reagent with smaller titer, like 1 mg/mL or 2 mg/mL, is used, the titration vessel should be as small as possible in order to minimize the drift.
- The working medium itself can be a source of extraneous moisture. To avoid this, we recommend using very dry media, such as **HYDRANAL**<sup>®</sup>-Methanol dry, **HYDRANAL**<sup>®</sup>-Methanol Rapid and dedicated solubilizing reagents such as **HYDRANAL**<sup>®</sup>-Formamide dry.

#### Control of accuracy

In addition to the steps outlined above, highly accurate and rapid moisture determinations are guaranteed when using high quality **HYDRANAL**<sup>®</sup> reagents. We also recommend routinely checking the accuracy of the system by titrating certified water standards, like **HYDRANAL**<sup>®</sup>-Water Standard 0.10, **HYDRANAL**<sup>®</sup>-Water Standard 1.00 or **HYDRANAL**<sup>®</sup>-Water Standard 10.0, which contain 0.01%, 0.1% and 1.0% water, respectively.



For more information on these or any of the **HYDRANAL**<sup>®</sup> products or applications or to request a Laboratory Report, please contact us at:

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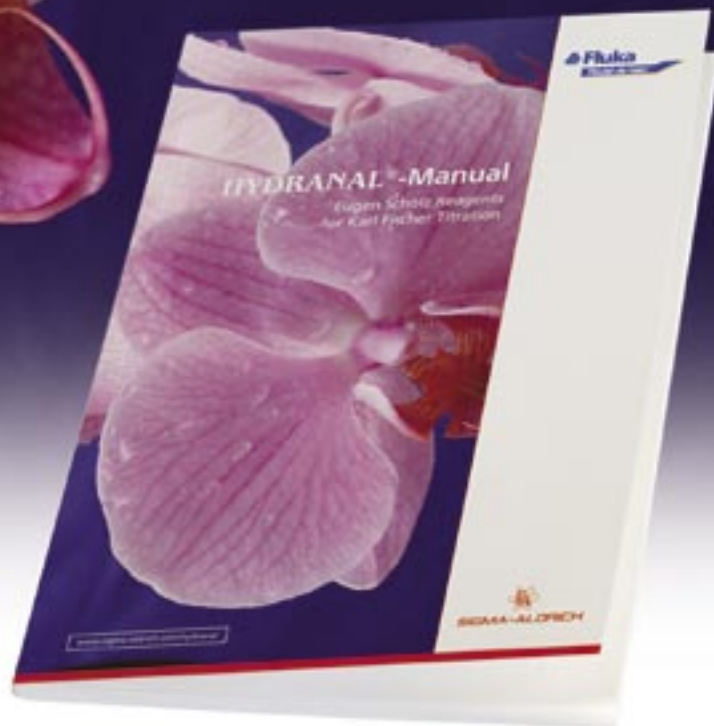
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# HYDRANAL® Manual

## Updated for 2006



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

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

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

This year, we've updated the HYDRANAL® Manual with:

- **New!** HYDRANAL® products, like HYDRANAL® Medium K, a safer solvent for the analysis of Aldehydes and Ketones and HYDRANAL® Solver (Crude) Oil, a solvent for the analysis of Crude Oil samples
- **New!** HYDRANAL® Applications
- **New!** HYDRANAL® Lab Reports
- **New!** HYDRANAL® Literature



Please call or visit our website to obtain your personal copy of our HYDRANAL® Manual.

## New Chemical Reference Standards with Certification for Important Analytes that are Considered Harmful to Humans .....

By Nicole Amann, Product Manager Analytical Standards ... [namann@europe.sial.com](mailto:namann@europe.sial.com)

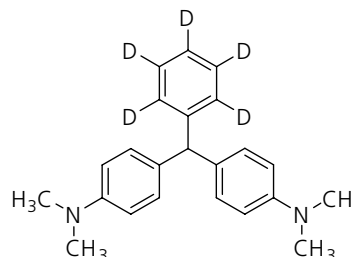


### Leucomalachite green-d<sub>5</sub>

Leucomalachite green is the primary metabolite of malachite green, a widely-used fungicide to suppress fungal growth water. Common uses are pond disinfection and in the ornamental fish business. Leucomalachite green accumulates in fish tissue and is considered to be harmful to humans. Although it is not approved for food applications, its ready availability and low cost make it a temptation to unscrupulous fish farmers, especially in Asian countries.

The level of leucomalachite green in fish tissues is regulated (see European Commission decision 2002/657/

EC). To ensure the stipulated low detection limits of 2 microgram per kilogram fish muscle of leucomalachite green and malachite green are met, labeled standards are necessary. Sigma-Aldrich is pleased to announce the introduction of leucomalachite green-d<sub>5</sub> as a certified OEKANAL® high-purity standard.



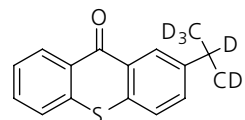
| Cat. No.   | Brand          | Description                         | Package Size |
|------------|----------------|-------------------------------------|--------------|
| 34182-10MG | Riedel-de-Haën | Leucomalachite green-d <sub>5</sub> | 10 mg        |



### ITX-d<sub>7</sub> (2-Isopropyl-d<sub>7</sub>-thioxanthen-9-one)

The compound ITX (isopropylthioxanthone) is used as a photoinitiator for UV-curing of printing inks and some food packaging. Concern was raised when it was detected in foods – infant formula, milk and Norwegian salmon – presumably having leached from the packaging. ITX was placed on the rapid alert notification

list by the European Food Safety Authority (EFSA). The actual health risks associated with ITX are still being debated. To determine safety and set tolerable limits, analytical testing using a reliable reference standard is necessary. Sigma-Aldrich now offers ITX-d<sub>7</sub> as a certified OEKANAL® high-purity standard.



| Cat. No.   | Brand          | Description  | Package Size |
|------------|----------------|--|--------------|
| 33652-10MG | Riedel-de-Haën | ITX-d <sub>7</sub> (2-Isopropyl-d <sub>7</sub> -thioxanthen-9-one) | 10 mg        |

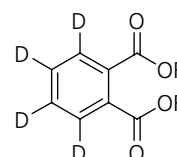


### Labeled phthalate esters

Phthalates are widely-used as plasticizers (softeners) and to provide resistance to water and oil. They are found in food packaging, children's toys, medical devices, time-release medications, nail polish, hairspray, adhesives, carpets and tiles, shoes, electrical insulation

and many other common consumer products. However, phthalates are known to leach from the products and enter the body by absorption through the skin, ingestion and inhalation. Studies in animals show that phthalates present a wide array of adverse health effects and are therefore prohibited in toys and other children's products (see 2004/781/EC) and for colors and adhesives (see 2004/93/EC). To facilitate the analysis of low levels of phthalates, labeled standards are required. Sigma-Aldrich offers the most common phthalates as deuterated, high-purity OEKANAL® standards with certification.

| Cat. No.   | Brand          | Description                           | Package Size |
|------------|----------------|---------------------------------------|--------------|
| 34167-10MG | Riedel-de-Haën | Di-n-hexyl-phthalate-d <sub>4</sub>   | 10 mg        |
| 34169-10MG | Riedel-de-Haën | Di-n-butyl-phthalate-d <sub>4</sub>   | 10 mg        |
| 34185-10MG | Riedel-de-Haën | Diethyl-phthalate-d <sub>4</sub>      | 10 mg        |
| 34187-10MG | Riedel-de-Haën | Dibenzyl-phthalate-d <sub>4</sub>     | 10 mg        |
| 34189-10MG | Riedel-de-Haën | Dimethyl-phthalate-d <sub>4</sub>     | 10 mg        |
| 34194-10MG | Riedel-de-Haën | Di-n-pentyl-phthalate-d <sub>4</sub>  | 10 mg        |
| 34197-10MG | Riedel-de-Haën | Di-n-propyl-phthalate-d <sub>4</sub>  | 10 mg        |
| 34204-10MG | Riedel-de-Haën | Di-iso-butyl-phthalate-d <sub>4</sub> | 10 mg        |



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