

Standards for Biodiesel Analysis

Standards and Derivatization Reagents

- Silylation of Drug Substances
- New IRMM GMO Maize Standards

Analytical Reagents

- LC-MS CHROMASOLV®
- Laboratory Acids and Bases
- Celite® Analytical Filter Aid

Karl Fischer Titration

- Titre of Reagents

Microbiology

- Identification of *Staphylococcus aureus*

New Product Corner

- Everolimus Standard



Reference Materials and Certified Reference Materials... Sigma-Aldrich has responded to the challenge of meeting the increased demand for reference materials by both expanding our product ranges and introducing new research tools.



Picture: Rainer Walz, PhD, Product Manager Analytical, Fluka – Riedel-de Haën

Dear Colleague,

At Sigma-Aldrich we have seen the demand for reference materials and certified reference materials increased rapidly over the last 5 years. Demand comes from all types of analytical and research laboratories across many industry sectors. The dual drivers for increased use of reference materials are increased levels of regulation that require the proper analysis of a substance or property and the adoption of ISO 17025 as an Accreditation Standard by laboratories. The almost universal adoption of ISO/IEC 17025, *The General Requirements for the Competence of Testing and Calibration Laboratories* requires, amongst many other things, that laboratories properly understand, document and manage their use of calibration and reference materials.

First introduced in 1999, the Standard has been used to accredit some 25,000 laboratories worldwide that test products and samples and calibrate precision instruments. With the release of the first major revision to ISO 17025 in 6 years, which reflects the content of ISO 9001: 2000, it is certain that the new ISO 17025:2005 standard will further focus Auditors' attention on the proper use and management of reference materials.

Sigma-Aldrich has responded to the challenge of meeting the increased demand for reference materials by both expanding its range of reference materials and calibration catalog, also available as an easy to use CD ROM.

In this edition of Analytix we describe in some detail a number of new developments that together underpin our commitment to the production and supply of the reference materials and standards that you need.

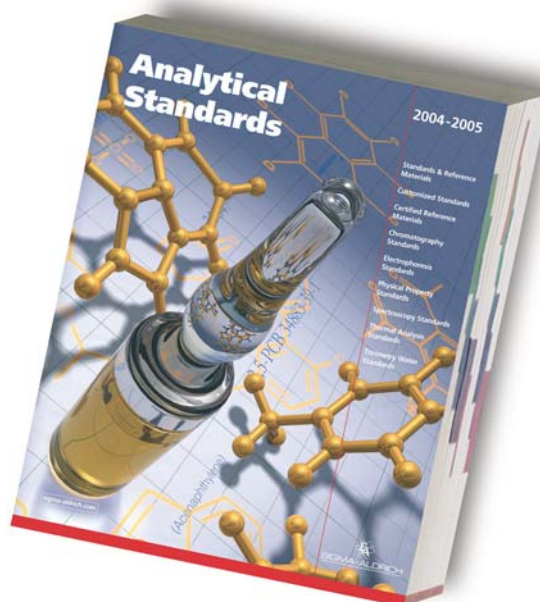
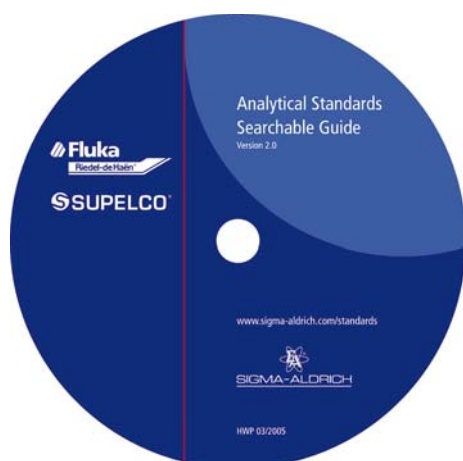
- The IRMM range of GMO Food material standards has been extended by 12 CRMs to facilitate the proper identification and quantification of two new GMO Maize strains from Monsanto, NK603, recently approved by the EU and GA21, for which approval is pending.
- The ready availability of leading edge developments in pharmaceutical active substances is essential to clinical research. Sigma Aldrich is proud to announce that agreement has been reached with Novartis to make available as, a documented analytical standard, their novel proliferation signal inhibitor everolimus (Certican®).
- There is an ever increasing need to both identify and quantify the amount of many prohibited or restricted drugs. To do this reproducibly two things are needed: good reference materials and reliable derivatization reagents. Sigma-Aldrich has recently made available three derivatization reagents: activated N-Methyl-N-trimethylsilylfluoroacetamides, which offer enhanced silylation capability coupled with much lower levels of corrosivity when compared with traditional fluoro-reagents such as BSTFA.

What can we do for you?

If you have any specific reference material need or would like further information about any of the reference materials described in this edition of Analytix please contact our Technical Service Team and put them to the test! Their contact details are on the back page.

Kind regards,

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Feature Article

04 *New Biodiesel Standards for EN 14105 and ASTM D6584*

These guidelines contain the most critical "pass / fail" tests of biodiesel production.

Standards and Derivatization Reagents

06 *Improved silylation of drug substances for GC/MS analysis using activated MSTFA Reagents*

Comparison of activated MSTFA over BSTFA reagents for the silylation of drug substances.

08 *New IRMM Certified Reference Materials for GMO Maize*

Introducing two new GMO standards: Insect Protected Maize YieldGard® MON 863 and MON 810

Analytical Reagents

09 *LC-MS CHROMASOLV® Products*

Mobile phase solvents, blends, additives, and accessories for the most demanding LC-MS application

10 *Special offer on Riedel-de Haën laboratory acids and bases*

Switch to Riedel-de Haën brand acids and bases for high purity and reliable composition

11 *Celite® Analytical Filter Aid (CAFA II)* Solving complex matrix analysis problems

Karl Fischer Titration

12 *Titre of Karl Fischer reagents*

The importance of titre, its determination and what can affect its levels

Microbiology

14 *Identification of Staphylococcus aureus*

Tests, kits and growth media for detection of this potent pathogen by its characteristic enzymes

New Product Corner

15 *Everolimus (Certican™) Analytical Standard*

Partnering with Novartis, Sigma-Aldrich now offers standards of this new immunosuppressant

Upcoming Events

15 *HYDRANAL® Seminars*

New Biodiesel Standards for EN 14105 and ASTM D6584...Sigma-Aldrich now offers standards for the analysis of biodiesel fuels by method ASTM D6584 and similar method EN 14105.

By Steve Cecil, National Specialist, Processing Industries...scecil@sail.com
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Biodiesel

Biodiesel is a renewable, alternative diesel fuel produced from vegetable oils, animal fats and recycled restaurant grease. This non-toxic, biodegradable liquid fuel consists of mono-alkyl esters of long chain fatty acids and may be used alone or blended with petroleum-based diesel fuels.

The most common process for producing biodiesel is the transesterification of fatty acid glycerol esters to methyl esters. Fats and oils are chemically reacted with an alcohol, usually methanol, and a catalyst like sodium or potassium hydroxide to produce chemical compounds known as triglycerides and glycerol. The triglycerides are broken into long chain mono-alkyl esters and glycerol, which are then separated and purified. The esters are retained for fuel, while the glycerol is often sold for use in soaps and other products. Biodiesel is the name given to the esters retained for use as fuel. The resulting biodiesel contains no sulfur or fossil fuel aromatics. Biodiesel is almost 10 % oxygen, making it an oxygenated fuel, which aids combustion in fuel-rich circumstances.

Biodiesel is recognized worldwide as an alternative fuel and qualifies for mandated programs in both the EU and the US. Of the many required tests, producers use the test method EN 14105 and/or ASTM D6584 as the most critical "pass/fail" tests of biodiesel. These critical tests are often the first run. If the fuel does not pass, rework or blending is done before further testing.

Free and total glycerin in B100 biodiesel: Methods EN 14105 / ASTM D6584

Left in the fuel, glycerin can cause clogged fuel systems, injector deposits, filter plugging and build-up in the vehicle's fuel tanks. Therefore, low levels of free and total glycerin are critical to the specifications of biodiesel fuels. High levels of free and total glycerin are caused by the improper or low conversion of oil or fat into the desired mono-alkyl esters.

The biodiesel assay for free and total glycerin is outlined in DIN Methods EN 14105 and ASTM D6584. These methods provide for the quantitative determination of free and total glycerin in 100% biodiesel fuel (B100 methyl esters) by high temperature gas

chromatography after silylating the sample with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). An example of the GC analysis of biodiesel following ASTM D6584 methodology is shown in the figure.

Sigma-Aldrich is pleased to introduce a new line of Supelco brand analytical standards for the analysis of biodiesel fuels by method ASTM D6584 and similar method EN 14105. This new line includes two internal standard solutions, four single component reference solutions, five multi-component reference solutions and two kits. Each biodiesel standard has a certificate of composition. Instructions for sample derivatization are included with each kit.

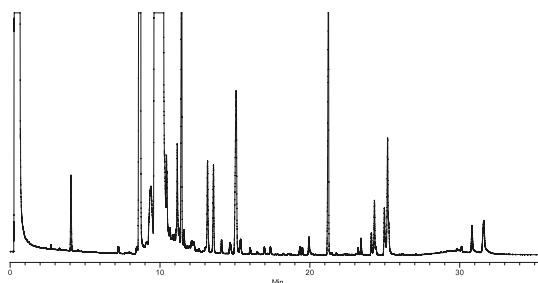


Figure 1: Chromatogram of biodiesel methyl esters and internal standards by ASTM D6584 and similar EN 14105*

Chromatographic conditions

GC Column: HT-5, 12 m x 0.32 mm I.D. x 0.1 µm df, (Supelco, Cat. No. 25002)
Precolumn: HT-5 precolumn, 6 m x 0.53 mm I.D. x 0.1 µm df (Supelco, Cat. No. 25004)
Oven: 50°C, 15°C/min to 180°C, 7°C/min to 230°C, 30°C/min to 380°C, hold 10 min.
Det.: FID, 380°C
Carrier Gas: Hydrogen, 3 mL/min @ 50°C
Injection: 1 µl, cool-on-column
Sample: 12 µg

* Our thanks are extended to George Gonzalez, BSI Inspectorate America Corporation who graciously provided the GC chromatogram of biodiesel MSTFA-derivatives.

Table 1: Biodiesel Standards

Cat. No.	Brand	Description	Package Size
44892-U	Supelco	Glycerin (CAS#: 56-81-5), 500 µg/mL pyridine	1 x 1 mL
44893-U	Supelco	Monoolein (CAS#: 111-03-5), 5000 µg/mL pyridine	1 x 3 mL
44894-U	Supelco	Di olein (CAS#: 2465-32-9), 5000 µg/mL pyridine	1 x 2 mL
44895-U	Supelco	Triolein (CAS#: 122-32-7), 5000 µg/mL pyridine	1 x 2 mL
44896-U	Supelco	Butanetriol (CAS#: 42890-76-6) Internal Standard #1, 1000 µg/mL pyridine	1 x 5 mL
44897-U	Supelco	Tricaprin (CAS#: 621-71-6), Internal Standard #2, 8000 µg/mL pyridine	1 x 5 mL
44898-U	Supelco	ASTM D6584 Individual Stock and Internal Stds Mix Kit Kit contains 1 ea: 44892-U, 44893-U, 44894-U, 44895-U, 44896-U, and 44897-U	1 kit
44899-U	Supelco	ASTM D6584 Standard Solution 1, varied concentration, pyridine Glycerin 5 µg/mL Monoolein 100 µg/mL Di olein 50 µg/mL Triolein 50 µg/mL	1 x 1 mL
44914-U	Supelco	ASTM D6584 Standard Solution 2, varied concentration, pyridine Glycerin 15 µg/mL Monoolein 250 µg/mL Di olein 100 µg/mL Triolein 100 µg/mL	1 x 1 mL
44915-U	Supelco	ASTM D6584 Standard Solution 3, varied concentration, pyridine Glycerin 25 µg/mL Monoolein 500 µg/mL Di olein 200 µg/mL Triolein 200 µg/mL	1 x 1 mL
44916-U	Supelco	ASTM D6584 Standard Solution 4, varied concentration, pyridine Glycerin 35 µg/mL Monoolein 750 µg/mL Di olein 350 µg/mL Triolein 350 µg/mL	1 x 1 mL
44917-U	Supelco	ASTM D6584 Standard Solution 5, varied concentration, pyridine Glycerin 50 µg/mL Monoolein 1000 µg/mL Di olein 500 µg/mL Triolein 500 µg/mL	1 x 1 mL
44918-U	Supelco	ASTM D6584 Standard solution Kit with Internal Standards, Kit contains 1 ea: 44899-U, 44914-U, 44915-U, 44916-U, & 44917-U	5 x 1 mL

Table 2: Derivatization Reagents

Cat. No.	Brand	Description	Package Size
394866-5	Aldrich	MSTFA Derivatization Reagent	5 mL
394865-10	Aldrich	MSTFA Derivatization Reagent	10 x 1 mL
394866-25	Aldrich	MSTFA Derivatization Reagent	25 mL

Improved silylation of drug substances for GC/MS analysis using activated MSTFA reagents... Activated N-methyl-N-trimethylsilylfluoroacetamide (MSTFA) silylation reagents have improved performance over N,O-Bis(trimethyl-silyl)trifluoroacetamide (BSTFA) reagents for the silylation of hydroxylated and amine-containing drug substances.

by Ingrid Hayenga, PhD, Senior Scientist R&D Applications, Fluka...ihayenga@europe.sial.com

Although HPLC and LC/MS are widely used in pharmaceutical analysis, GC and GC/MS have some advantages, including:

- GC/MS very often provides slightly lower detection limits than LC/MS
- GC/MS instruments are more common and less expensive than LC/MS instruments

Irrespective of the benefits of GC/MS, most pharmaceutical compounds and their metabolites cannot be analyzed in their native form without derivatization. Silylation reactions are the most versatile derivatization technique to enhance GC performance. The silylation reaction replaces the active hydrogen on protic functional groups with a trimethylsilyl (TMS) group. The TMS groups reduce dipole-dipole interactions and subsequently increase the volatility of the TMS derivative over the parent compound. The general reaction for the formation of a trimethylsilyl derivative is:



Of the myriad trimethylsilylation reagents available, MSTFA is one of the most important. Its silylation power can be increased by the use of catalysts or additives that scavenge reaction by-products. MSTFA reacts *in situ* with ammonium iodide (NH_4I) to produce trimethylsilyldosilane (TMSI), which has been reported to be the most powerful trimethylsilyl donor available (1). TMSI reacts with adequate speed to produce both trimethylsilyl (TMS) ether and trimethylsilyl enol (TMS enol) ether derivatives (2). Ethanethiol is added to reduce the formed iodine to hydrogen iodide in order to prevent iodine incorporation into the product. As a result, diethyl disulfide is produced during the derivatization reaction (3). Diethyl disulfide formation depends on the amount of ammonium iodide and ethanethiol added to the extract and the chosen experimental conditions such as reaction time and temperature. Imidazole acts as a base catalyst in the MSTFA silylation reaction.

In this short communication, we report the results of the efficacy of BSTFA/TMCS and three activated

MSTFA reagents (Table 1) to form GC/MS-compatible trimethylsilyl derivatives of several important drug classes: cannabinoids, amphetamines and opiates. All derivatization reagents, drug standards and capillary GC columns are available from Sigma-Aldrich.

The results are summarized in Table 2. Although results varied with drug substance tested, one or more of the three activated MSTFA reagents was as effective as or more effective than the BSTFA/TMCS reagent, without the generation of system-damaging corrosive by-products. Therefore, the choice of silylation reagent depends on what drug classes are to be derivatized.

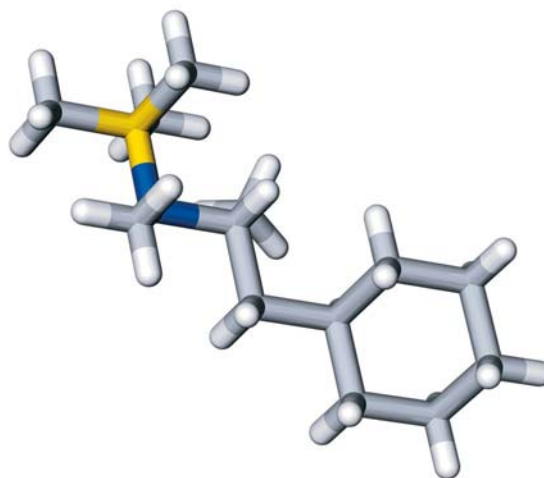


Figure 1: Silylated deoxyephedrin
(blue – nitrogen bonds; yellow – Me_3Si groups)

Table 1: Derivatization (silylation) reagents

Cat. No.	Brand	Description	Remarks	Package Size
50992	Fluka	MSTFA I (N-Methyl-N-trimethylsilyltrifluoroacetamide activated I)	Activated with ethanethiol and ammonium iodide*	5 mL, 25 mL
44156	Fluka	MSTFA II (N-Methyl-N-trimethylsilyltrifluoroacetamide activated II)	Activated with trimethylsilyl-ethanethiol*	5 mL, 25 mL
12124	Fluka	MSTFA III (N-Methyl-N-trimethylsilyltrifluoroacetamide activated III)	Activated with imidazole*	5 mL, 25 mL
441104	Aldrich	BSTFA + 1%TMCS (N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)		10 x 1 mL, 25 mL

* Equivalent silylation power to a 1000:2 iodotrimethylsilane:MSTFA:iodotrimethylsilane:mixture

Table 2: Results of derivatization experiments

Test compounds	Group	BSTFA + 1% TMCS	MSTFA I	MSTFA II	MSTFA III
Cat. No.		441104	50992	44156	12124
11-Nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid	Cannabinoids	Complete silylation	Complete silylation	Complete silylation	Complete silylation
Codeine	Opiates	Complete silylation	Not measured	Not measured	Complete silylation
Hydrocodone (+)-bitartrate salt	Opiates	50:50 mixture of silylated and non-silylated product	Complete silylation	Incomplete silylation	Complete silylation
Ethylmorphine	Opiates	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation
Oxycodone hydrochloride	Opiates	Not measured	Complete silylation (double silylated)	Complete silylation (double silylated)	Double silylated main product and mono-silylated as by-product
Morphine sulfate	Opiates	Complete silylation	Complete silylation	Complete silylation	Complete silylation
(-)-Deoxyephedrine	Amphetamines	Complete silylation	Complete silylation	Incomplete silylation	Incomplete silylation
D-Amphetamine sulfate salt	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation
(\pm)-3,4-Methylenedioxy methamphetamine hydrochloride (Ecstasy)	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation

References:

- [1] Donike, M. and Zimmermann, J., J.Chromatogr. A, 202, 483-486 (1980).
- [2] Van de Kerkhof D. H., van Ooijen, R. D., de Boer, D., Fokkens, R. H., Nibbering, N. M. M., Zwikker, J. W., Thijssen, J. H. H. and Maes, R. A. A., J. Chromatogr. A, 954, 199-206 (2002).
- [3] Opfermann, G., Schänzer, W. In Recent Advances in Doping Analysis (4), Proceedings of the 14th Cologne Workshop On Dope Analysis 17th to 22nd March 1996, W.Schänzer, H.Geyer, A.Gotzmann, U.Mareck-Engelke eds., 247-252, Sport & Buch Strauss, Cologne (1997).

For detailed information on this topic, including methods of analysis and chromatograms, please ask for Analytix Notes: Silylation of Drugs Substances with MSTFA (IEF)

New IRMM Certified Reference Materials for GMO Maize...Two additions to our IRMM product offering of GMO standards.

By Kurt Vorburger, Head Fluka/Riedel-de Haën Product Management...kvorburger@sial.com

One of the most newsworthy applications of analytical chemistry has been toward the identification and environmental tracking of genetically modified organisms (GMO). Reliable, certified standards are required for proper identification and quantification in food, feedstuff and the environment. We have recently expanded our extensive line of IRMM (Institute for Reference Materials and Measurements) standards to include dried flour containing different mass fractions of two varieties of GMO-derived maize, YieldGard® MON 861 and MON 863 insect protected maize varieties developed by Monsanto (St. Louis, MO, USA). MON 861 and MON 863 were developed by Monsanto through genetic modification to express two novel *Bacillus thuringiensis*-derived proteins that have insecticidal activity toward two very damaging pests, corn rootworm and the European corn borer.

These new GMO standards complement other IRMM standards and those required for the 2004 EC guidelines for food safety we already offer. The standards comprise non-GMO dried maize powder containing 0, 0.1, 1 and 10% by weight of the two different GMO maize varieties. The non-GMO raw material and the diluent are from whole kernels of a non-modified maize line. Homogeneity is assured by the use of a dry-mixing technique, which also minimizes DNA and protein degradation during production. The GMO maize CRMs are provided in glass bottles containing 1 g of dried maize powder packed under argon atmosphere.

Table 1: YieldGard® Maize MON 863 Standards (IRMM-416)

Cat. No.	Cert. Ref. Material	% w/w GMO ¹	Mass GMO/kg powder ²	IRMM Ref. No. (ERM® Brand)	Qty.
36394	Maize GMO MON 863 Powder Set	Maize GMO Standard IRMM-416, Set for 0%, 0.1%, 1% and 9.9% MON 863			1 set (1 gram each)
IRMM-416-0	Maize GMO MON 863 Powder	Maize GMO Standard, 0% MON 863	< 1.0 g GMO/kg	IRMM-416-0	1 gram
IRMM-416-1	Maize GMO MON 863 Powder	Maize GMO Standard, 0.1% MON 863	1.0 g GMO/kg	IRMM-416-1	1 gram
IRMM-416-2	Maize GMO MON 863 Powder	Maize GMO Standard, 1% MON 863	9.8 g GMO/kg	IRMM-416-2	1 gram
IRMM-416-3	Maize GMO MON 863 Powder	Maize GMO Standard, 9.9% MON 863	98.5 g GMO/kg	IRMM-416-3	1 gram

Table 2: YieldGard® Maize MON 863 X MON 810 Standards (IRMM-417)

Cat. No.	Cert. Ref. Material	% w/w GMO ¹	Mass GMO/kg powder ²	IRMM Ref. No. (ERM® Brand)	Qty.
8677	Maize GMO MON 863 X MON 810 Powder Set	0%, 0.1%, 1%, 2%, < 9.9% GMO MON 863 X MON 810			1 set (1 gram each)
IRMM-417-0	Maize GMO MON 863 X MON 810 Powder	0% MON 863 X MON 810	< 1.0 g GMO/kg	IRMM-417-0	1 gram
IRMM-417-1	Maize GMO MON 863 X MON 810 Powder	0.1% MON 863 X MON 810	1.0 g GMO/kg	IRMM-417-1	1 gram
IRMM-417-2	Maize GMO MON 863 X MON 810 Powder	1% MON 863 X MON 810	9.8 g GMO/kg	IRMM-417-2	1 gram
IRMM-417-3	Maize GMO MON 863 X MON 810 Powder	9.9% MON 863 X MON 810	98.5 g GMO/kg	IRMM-417-3	1 gram

(1) Prepared by turbula-mixing and dry-mixing of non-modified maize powder (conventional seed line RX670) and MON 863 x MON 810 maize powder.

(2) The certified value is based on the mass fraction of dried non-genetically modified powder and dried genetically modified powder mixed and corrected for the water content. The certified value is traceable to the SI.

LC-MS Products from Sigma-Aldrich... Mobile phase solvents, blends, additives, and accessories for the most demanding LC-MS applications

By Frederik Pillong, Product Manager Fluka / Riedel-de Haën...fpillong@sial.com

To achieve maximum performance of your LC-MS system and to prevent instrument downtime, the background noise arising from the mobile phase and the HPLC column must be negligible. Minimizing the background and artifacts in LC-MS requires highly purified and specified solvents, ultra pure additives and bleed-free HPLC columns.

Scientists at Sigma-Aldrich's Fluka / Riedel de Haën brands have responded to the growing need for high purity solvents and additives. Offering nearly everything but the instrument, our LC-MS consumables line currently comprises:

- **LC-MS CHROMASOLV® Pure Solvents – High purity, extensively tested mobile phase solvents for LC-MS**
- **Solvent Blends – Convenient, accurate and precipitation-free pre-blended solvent and additive solutions for LC-MS**
- **NEW Mobile Phase Additives – Popular mobile phase additives at our highest grade of purity: puriss., p.a., specially tested for suitability under LC-MS conditions**
- **Rinsing Solution – Using this convenient rinsing solution from Sigma-Aldrich will help keep your LC-MS up and delivering reliable results**
- **Dispensing Aid – This proprietary bottle adaptor prevents contamination of high purity LC-MS solvents**

For a complete list of our products for LC-MS, request "LC-MS CHROMASOLV® Product Overview" (IEG)

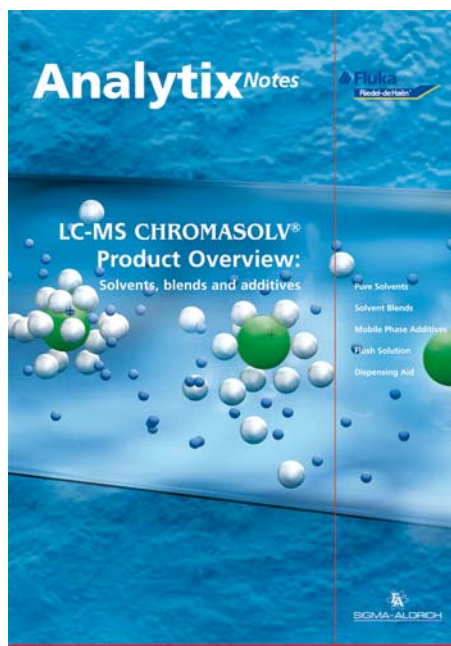


Table 1: LC-MS CHROMASOLV® Pure Solvents

Cat. No.	Brand	Solvent	Package size
39253	Riedel de Haën	Water	1 L
34967	Riedel de Haën	Acetonitrile	1 L, 6 x 1 L, 4 x 2.5 L, 5 L
34966	Riedel de Haën	Methanol	1 L, 6 x 1 L, 4 x 2.5 L, 5 L
34965	Riedel de Haën	2-Propanol	1 L, 6 x 1 L, 4 x 2.5 L, 5 L
34972	Riedel de Haën	Ethyl acetate	1 L, 2.5 L

Table 2: LC-MS CHROMASOLV® Solvent Blends

Cat. No.	Brand	Solvent Blend	Package size
34978	Riedel de Haën	Water with 0.1% TFA	2.5 L
34976	Riedel de Haën	Acetonitrile with 0.1% TFA	2.5 L
34974	Riedel de Haën	Methanol alcohol with 0.1% TFA	2.5 L
34673	Riedel de Haën	Water with 0.1% formic acid	2.5 L
34668	Riedel de Haën	Acetonitrile with 0.1% formic acid	2.5 L
34671	Riedel de Haën	Methanol alcohol with 0.1% formic acid	2.5 L
34675	Riedel de Haën	Water with 0.1% acetic acid	2.5 L
34678	Riedel de Haën	Acetonitrile with 0.1% acetic acid	2.5 L
34672	Riedel de Haën	Methanol with 0.1% acetic acid	2.5 L
34674	Riedel de Haën	Water with 0.1% ammonium acetate	2.5 L
34669	Riedel de Haën	Acetonitrile with 0.1% ammonium acetate	2.5 L
34670	Riedel de Haën	Methanol with 0.1% ammonium acetate	2.5 L
34677	Riedel de Haën	Water with 0.1% formic acid and 0.01% TFA	2.5 L
34676	Riedel de Haën	Acetonitrile with 0.1% formic acid and 0.01% TFA	2.5 L

Table 3: LC-MS CHROMASOLV® Rinsing Solution

Cat. No.	Brand	Rinsing Solution	Package size
34682	Riedel de Haën	Water/2-Propanol 50/50	2.5 L

30%

Special offer on Riedel-de Haën laboratory acids and bases

Switch to Riedel-de Haën brand acids and bases for high purity and reliability.

>> For a limited time, get a **30%** discount off some of our most popular acids and bases (see Table) to technical and a variety of other grades. We can supply you with the right product for your application, no matter how critical or routine. We also strive to supply our acids and bases in packaging materials and package sizes to meet your storage and use requirements.



Visit Simon, our virtual stockroom attendant, at: www.sigma-aldrich.com/virtualstockroom

Cat. No.	Product	Purity	Pack Size
27221	Acetic acid	puriss., 99-100%	1 L, 2.5 L, 4 x 2.5 L, 4 x 5 L
27222	Acetic acid	technical, 99-100%	1 L, 2.5 L, 4 x 2.5 L, 6 x 1 L
05002	Ammonium hydroxide solution	puriss., NH ₃ 30-33% water	1 L, 2.5 L, 4 x 2.5 L, 6 x 1 L
27109	Citric acid	puriss., meets analytical specification of Ph. Eur., BP, USP, E330, anhydrous, 99.5-100.5% based on anhydrous substance, gritty	1 kg, 2.5 kg, 6 x 1 kg, 6 x 2.5 kg
27102	Citric acid monohydrate	puriss., meets analytical specification of Ph. Eur., BP, USP, E330, 99.5-100.5% based on anhydrous substance, gritty	1 kg, 2.5 kg, 6 x 1 kg, 6 x 2.5 kg
27002	Formic acid	purum, > 85%	1 L, 2.5 L, 4 x 2.5 L, 6 x 1 L
03206	Hydriodic acid	> 57%	250 mL, 1L, 6 x 250 mL, 6 x 1 L
07115	Hydrochloric acid	puriss., > 31.5%	2.5 L, 4 x 2.5 L, 5 L
07102	Hydrochloric acid	puriss., meets analytical specification of Ph. Eur., BP, NF, fuming, 36.5-38%	1 L, 6 x 1 L
07102	Hydrochloric acid	puriss., meets analytical specification of Ph. Eur., BP, NF, fuming, 36.5-38%	2.5 L, 4 x 2.5 L (glass bottle)
07102	Hydrochloric acid	puriss., meets analytical specification of Ph. Eur., BP, NF, fuming, 36.5-38%	2.5 L, 4 x 2.5 L (PE bottle)
04423	Phosphinic acid	purum, > 60%	1 L, 6 x 1 L
13020	Lithium hydroxide monohydrate	> 98.5%	100 g, 500 g, 1 kg, 6 x 100 g, 6 x 500 g, 6 x 1 kg
04103	meta-Phosphoric acid	100%, lumps glassy	250 g, 6 x 100 g, 6 x 250 g,
07006	Nitric acid	puriss., 64-66%	1 L, 6 x 1 L
07006	Nitric acid	puriss., 64-66%	2.5 L, 4 x 2.5 L (glass bottle)
07006	Nitric acid	puriss., 64-66%	2.5 L, 4 x 2.5 L (PE bottle)
27728	Oleic acid	puriss., meets analytical specification of Ph. Eur., 65.0-88.0% GC	1 L, 2.5 L, 4 x 2.5 L, 4 x 5 L
03304	Periodic acid	purum, > 99%	25 g, 100 g, 6 x 25 g, 6 x 100 g
04107	Phosphoric acid	puriss., meets analytical specification of Ph. Eur., BP, NF, FCC, 85-88%	1 L, 2.5 L, 5 L, 6 x 1 L, 4 x 2.5 L, 4 x 5 L
04114	Phosphorous acid	> 98.5%	250 g, 1 kg, 6 x 250 g, 1 kg
06005	Potassium hydroxide	puriss., meets analytical specification of Ph. Eur., BP, NF, 85-100.5%, pellets	1 kg, 5 kg, 6 x 1 kg, 4 x 5 kg
05211	Sodium hydroxide solution	purum, > 32%	2.5 L, 5 L, 4 x 2.5 L, 4 x 5 L
06306	Sodium hydroxide	> 98.5%, microprills	2.5 kg, 6 x 2.5 kg
06203	Sodium hydroxide	puriss., meets analytical specification of Ph. Eur., BP, NF, E524, 98-100.5%, pellets	1 kg, 5 kg, 6 x 1 kg, 4 x 5 kg
06213	Sodium hydroxide	purified, > 98%, pellets, white	1 kg, 5 kg, 6 x 1 kg, 4 x 5 kg
07208	Sulfuric acid	puriss., meets analytical specification of Ph. Eur., BP, 95-97%	1 L, 2.5 L, 5 L, 6 x 1 L, 4 x 2.5 L, 5 L
01544	Tetrafluoroboric acid	49.5-50.5%	1 L, 6 x 1 L
27242	Trichloroacetic acid	puriss., meets analytical specification of Ph. Eur., USP 21, 99-100.5% calc. to the dried substance	100 g, 500 g, 1 kg, 2.5 kg, 6 x 500 g, 6 x 1 kg

Please quote promotion code **U13** when placing your order. Offer valid until **31-October-2005**

Celite® Analytical Filter Aid (CAFA II)...Solving complex matrix analysis problems

By Daniel S. Vitkuske, Market Segment Manager...dvitkuske@sial.com

Analytical chemists encounter a wide variety of sample matrices, from relatively simple to highly complex. Examples of difficult matrices include bacterial lysates, biological fluids, vegetable extracts, fruit juices with pulp and other matrices. Generally, the most challenging situations are when the solids content is greater than 1% or when the solids themselves are compressible. Recovery of analytes of interest from these matrices is often very low and variable.

While standard filtration can sufficiently clean up some samples, with complex matrices it is difficult to recover sufficient volume of filtered sample for analysis. Filtration aids made from diatomaceous earth are frequently employed to facilitate the separation of analytes from matrix particulates. Celite® brand diatomite filter aids, manufactured by Advanced Minerals Corporation, are widely utilized in these instances. Celite® filtration aids are employed primarily to serve these two functions:

- **As a dynamic depth filter. This involves suspending the filter aid in the sample matrix prior to filtration. The Celite® diatomites co-migrate with the compressible solids during the filtration process. The rigid and porous diatomite particles act as a pseudo-skeleton, preventing the compressible solids in the sample matrix from forming an impermeable mass.**

- **As a conventional depth filter. Here the Celite® diatomite is simply laid down as a layer in a Büchner funnel prior to filtration. The bed height is typically 3 to 50 mm.**

Supelco division of Sigma-Aldrich has been supplying Celite® materials, including Celite® Analytical Filter Aid (CAFA), to our customers for many years. Recently, Advanced Minerals replaced CAFA with CAFA II, a more highly purified grade of Celite® for analytical and purification applications where filtrate purity is important. The CAFA II manufacturing process was designed to minimize the amount of contaminants that might interfere with the analysis. The physical and chemical properties of CAFA and CAFA II are compared in **table 1 and 2**. Supelco/Sigma-Aldrich is now the sole supplier of research quantities of CAFA II for research and laboratory applications.

Besides CAFA II, other Celite® materials available from Sigma-Aldrich include:

- Celite® 500
- Celite® 501
- Celite® 503
- Celite® 512 Medium
- Celite® 535 Coarse
- Celite® 545 AW Reagent Grade
- Celite® 545 AW Coarse
- Celite® 545 Coarse
- Celite® 577 Fine
- Celite® R566
- Celite® R630

Table 1: Physical Properties

Property	Celite Analytical Filter Aid II (CAFA II)	Celite Analytical Filter Aid (CAFA)
Wet centrifuge density, g/L	240	335
Permeability, D'Arcy	0.2	0.24
150-mesh sieve (105 µm) retains, %	10	3
Median particle size, microns	15	15
Median pore size, microns	3	3.5
Surface area (N ₂), m ² /g	5.1	6

Table 2: Chemical Properties

Property	CAFA II	CAFA
pH, 10% slurry	5.7	6.6
Acid soluble iron, mg/kg	4	60
Citric Acid/methanol soluble Aluminum, mg/kg	3.5	13
Conductivity, µS/cm	7	30
Acid soluble arsenic, mg/kg	0.5	ND
Acid soluble lead, mg/kg	0.1	0.5
Acid soluble substances, %	0.2	0.5
Ignition loss, %	0.1	0.5
Moisture, %	0.15	0.5

Table 3: Ordering Information

Cat. No	Brand	Description	Package size
11484-U	Supelco	Celite® Analytical Filter Aid II (CAFA II)	100 g
11485-U	Supelco	Celite® Analytical Filter Aid II (CAFA II)	500 g
11486-U	Supelco	Celite® Analytical Filter Aid II (CAFA II)	1 kg
11487-U	Supelco	Celite® Analytical Filter Aid II (CAFA II)	15 kg

Titre of Karl Fischer reagents...The importance of titre, its determination and what can affect its level

By Helga Hoffmann, Technical Support **HYDRANAL**® Manager...hhoffman@europe.sial.com
and Michael Jeitziner, Product Manager Analytical Reagents, Fluka / Riedel-de Haën...mjeitziner@sial.com

HYDRANAL® Karl Fischer reagents from Sigma-Aldrich provide reliable, fast, sensitive and quantitative measure of moisture in a wide variety of substances. Although the chemistry behind the Karl Fischer reaction is well characterized and the procedure is widely used, it is useful occasionally to review some of its fundamental principles. In this article, we discuss an important facet of volumetric Karl Fischer reagents, their titre.

“Titre” is defined as the concentration of a substance in solution or the strength of such a substance determined by titration. It also can mean the minimum volume needed to cause a particular result in titration. In the case of Karl Fischer titration, the substance of interest is, of course, water. To calculate the water content of a sample, the titre or “water equivalent” of the reagent has to be known. The water equivalent, or titre, tells how many mg of water have been titrated when exactly 1 mL of reagent is consumed in the titration. Therefore the titre is expressed as mg water per mL reagent, for example 5.125 mg/mL.

Titre determination is performed by titrating a known volume of reagent with a measured mass of water. Pure (neat) water can be used, but the mass of water required is extremely low which can lead to weighing and handling errors. For example, a reagent with titre 2 requires only 10-15 mg of water.

Additionally, instead of pure (neat) water, ISO 760 prescribes using sodium tartrate dihydrate for titre determination. **HYDRANAL**®-Standard sodium tartrate

dihydrate is ideal for this application. Because its water content is 15.66%, the sample size needed to measure reagent titre can be increased accordingly. For the determination of titre 5, 150-200 mg is needed, which definitely reduces the error from weighing. However, a disadvantage of sodium tartrate dihydrate is its limited solubility in methanol.

Influences on titre

Ambient moisture

HYDRANAL® reagents are very stable for long periods in sealed containers. However, when opened, moisture from the air will diffuse into the bottle. Reaction with ambient moisture will consume iodine in the reagent and reduce its effective titre. Since one liter of air contains 12-15 mg water, the reduction in titre from ambient moisture can be significant.

To reduce titre loss from ambient moisture, we recommend strongly that the reagent bottles be fitted with a specially designed drying tube filled with either **HYDRANAL**®-Molecular sieve 0.3nm or the indicating **HYDRANAL**®-Humidity absorber. Molecular sieve should be replaced every six weeks and the humidity absorber as soon as two third of the indicator becomes colorless. Molecular sieve can be regenerated at 300°C for a minimum of 4 hours and the humidity absorber at 140°C until the original (dry) color of the indicator returns.

When the titrator apparatus is not in use, moisture will also migrate through the plastic transfer hoses decreasing the titre in any reagent that is in them. We recommend flushing the apparatus with fresh reagent after periods of inactivity, even overnight, or at least take the possible reduction in titre into account.

Temperature fluctuations

It is important to observe the fact that changes in temperature influence titre. For every 1°C increase in temperature, the titre decreases by 0.1%. This relationship means that all reagents used for titration should be at the same temperature during titre determination.



Influence of the titre on the performance of titration

The condition of the titration vessel influences the results of the titration. A titration vessel is never totally airtight; some moisture always migrates into the upper chamber of the vessel. The consumption of reagent by this moisture appears as drift. After finishing a titration the consumption of reagent should not be greater than 0.01 mL/min. Therefore the maximum drift for different titre values should be:

- Reagent titre 5: 50 µg/min
- Reagent titre 2: 20 µg/min
- Reagent titre 1: 10 µg/min

If the reagent has a titre lower than 5, it is very important that the titration vessel is in extremely good condition. Otherwise, it is very difficult to get a proper end point; the end point fades and the final results are falsely high.

HYDRANAL® Water Standard 10.0 (Cat. No. 34849)

This standard is a certified liquid standard traceable to NIST SRM 2890. Each package contains ten 8-mL glass ampoules and a Certificate of Analysis stating the exact water content of the batch. To determine the titre, an ampoule is opened and 2g portions (exactly weighed) are administered into the titration vessel using a syringe.

Determination of the titre with HYDRANAL®-Standard sodium tartrate dihydrate traceable to NIST SRM 2890 (Cat. No. 34696)

Sodium tartrate forms a dihydrate that under normal conditions remains stable and does not lose or adsorb moisture. The new HYDRANAL®-Standard sodium tartrate contains $15.66 \pm 0.05\%$ water by weight. This value can be ensured by drying the compound at 150°C. HYDRANAL®-Standard sodium tartrate dihydrate is finely-powdered that dissolves much faster in methanol.

The water determination in the HYDRANAL®-Standard sodium tartrate is carried out by several independent methods:

1. The water content is measured by loss upon drying. The result of three determinations with standard deviation is mentioned on the Certificate of Analysis.
2. The titre of HYDRANAL®-Composite 5 is determined using this HYDRANAL®-Standard sodium tartrate dihydrate.
3. The accuracy of titre determination is tested against the certified Standard SRM 2890 (National Institute of Standards, USA).

The HYDRANAL®-Standard sodium tartrate dihydrate certified standard is packed in glass bottle and includes a Certificate of Analysis.

Table 1: Select HYDRANAL® titre products from Riedel-de Haën

Cat. No	Brand	Description	Used for
34241	Riedel-de Haën	HYDRANAL®-Molecular sieve 0.3nm	Drying agent for Karl Fischer application
34788	Riedel-de Haën	HYDRANAL®-Humidity absorber	Drying agent for Karl Fischer application
34849	Riedel-de Haën	HYDRANAL®-Water standard 10.0	Standard for volumetric Karl Fischer titration (1 g contains 10.0 mg = 1% H ₂ O; traceable to NIST SRM 2890)
NEW 34696	Riedel-de Haën	HYDRANAL®- Standard sodium tartrate dihydrate traceable to NIST SRM 2890	Certified standard with $15.66 \pm 0.05\%$ mg/mL water

Special Offer New HYDRANAL®-Standard sodium tartrate dihydrate

Using HYDRANAL®-Standard sodium tartrate dihydrate standard can dramatically improve the accuracy of Karl Fischer reagent titre determination, which ultimately influences the quality of your titration.

To launch this newest member of the HYDRANAL® family, we are offering a special, limited-time discount on NIST SRM 2890 traceable HYDRANAL®-Standard sodium tartrate dihydrate standard.

Take 25% off either the 25 g or the convenient 6 x 25 g package sizes.

Table 1: Ordering Information

Cat. No	Brand	Product	Used for	Package size
34696	Riedel-de Haën	HYDRANAL®-Standard sodium tartrate dihydrate traceable to NIST SRM 2890	Certified standard with $15.66 \pm 0.05\%$ mg/mL water	25 g, 6 x 25 g

>> To take advantage of this special pricing, please specify promotion code U15 when you order. Offer valid until 31-October-05

Identification of *Staphylococcus aureus*...Fluka brand tests, kits and growth media for detection of this potent pathogen by its characteristic enzymes and properties

By Jvo Siegrist, Product Manager Microbiology Fluka, Sigma...isiegris@sial.comm

Staphylococcus aureus, along with *E. coli*, *Listeria*, *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter*, has the dubious distinction of being a member of the microbial community that causes the majority food-borne illness in humans. Staphylococci occur naturally in air, soil and sewage, and in the nasal passages, throat, skin and hair of many animals, including humans. Consequently, staphylococcal contamination is easily spread to water, milk, food and food processing equipment.

Each microbe has its characteristic class of food it most commonly contaminates. For *S. aureus*, meat, egg and egg products, milk, dairy products and all their derivatives are the preferred media. Processes in the food industry where the food is kept at slightly elevated temperatures are frequent targets of staphylococcal food poisoning. Detection of live *S. aureus* in food before it reaches consumers is of utmost importance for scientists and officials working in the food industry.

One way to detect and identify a given organism is by the metabolic products of its unique or distinctive enzymes. *S. aureus* produces diverse enzymes like staphylokinase (a coagulase), protease, phosphatase, lipase, deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). Also, the majority of clinical isolates of *S. aureus* express a unique surface polysaccharide and protein A. Scientists at Sigma-Aldrich's Fluka division have been developing fast and reliable tests and kits to identify *S. aureus* using these characteristics (see Table).

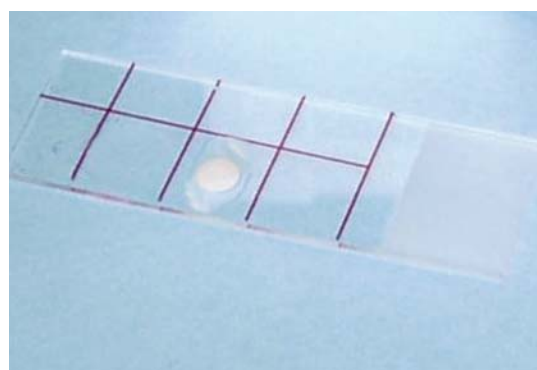


Figure 1: Example of Coagulase Test (Slide)

Table 1: Tests for identification and characterization of *Staphylococcus aureus*

Cat. No	Brand	Kit or Test	Testing feature
88597	Fluka	Catalase Test (Hydrogen peroxide 3%)	Testing for catalase production
75832	Fluka	Coagulase Test (Slide)	Detection of coagulase
74226	Fluka	Coagulase Test (Tubes)	"
07345 combined with 07817	Fluka	Oxidase Reagent acc. Gaby-Hadley A combined with Oxidase Reagent acc. Gaby-Hadley B	Test for presence of oxidase
18502	Fluka	Oxidase Reagent acc. Gordon-McLeod	"
40560	Fluka	Oxidase Strips	"
70439	Fluka	Oxidase Test	"
08986	Fluka	Staphylo Monotec Test Kit	Coagulase and protein A can be detected in one step.

Cat. No.	Brand	Identification Growth Media	Testing features
11705	Fluka	Baird Parker Agar Supplements: Egg-Yolk Tellurite Emulsion (Fluka 75208) or RPF Supplement (Fluka 05939)	Detection of lipolytic and proteolytic activity, ability to reduce tellurite to metallic tellurium (EN-ISO 6888-1: 1999); with RPF Supplement the coagulase activity and the ability to reduce tellurite is detected (EN-ISO 6888-2:2000)
05662	Fluka	HiCrome™ Aureus Agar Base Supplements: Egg-Yolk Tellurite Emulsion (Fluka 75208)	Ability to reduce tellurite to metallic tellurium and detection of lipase and protease
P1227	Sigma	Phenolphthalein Phosphate Agar	Detection of phosphatase
70195	Fluka	Vogel-Johnson Agar Supplements: Potassium Tellurite 1% (Fluka 17774)	Ability to reduce tellurite to tellurium and ability to ferment mannitol

Everolimus (Certican™) Analytical Standard...Partnering with Novartis, Sigma-Aldrich now offers standards of this important, new immunosuppressant drug

By Rainer Walz, PhD...rwalz@sial.com

Certican™ (everolimus) is a novel immunosuppressant introduced by Novartis Pharma AG. This proliferation signal inhibitor was designed to target the primary causes of transplant rejection and late graft loss, including acute rejection, cytomegalovirus (CMV) infection, calcineurin inhibitor (CNI) nephrotoxicity and vascular remodeling (1,2,3). Preventing chronic allograft dysfunction or late graft loss is a major unmet medical need in transplantation (4). Everolimus is based on the macrolide molecule and contains a stable 2-hydroxyethyl chain substitution at position 40 on the sirolimus (rapamycin) structure.

For clinical studies small samples in convenient packaging are needed. To meet this need, Novartis and Sigma-Aldrich collaborated to provide clinical customers worldwide everolimus analytical standards. Each sample is packed in a 10 mg ampoule under argon. An aluminum bag protects the ampoule from damage from light, air and humidity. Each sample is accompanied with a Certificate of Analysis that presents all relevant QC results. Transplant patients and clinical researchers worldwide will benefit from the ready availability of everolimus analytical standards from Sigma-Aldrich.

Cat. No	Brand	Description	Package size
07741	Fluka	Everolimus (Certican™) standard	10 mg ampoule

References:

- [1] Vitko, S. et al., "International, double-blind, parallel group study for the safety and efficacy of Certican™ (RAD) versus mycophenolate mofetil (MMF) in combination with Neoral® and steroids" Am. J. Transplant, Suppl. 1, 474, Abstract 1337 (2001).
- [2] Curtis, J. et al., "One-year results of a multicenter, open-label trial on safety and efficacy of Certican™ (RAD) used in combination with Simulect®, corticosteroids and full or reduced dose Neoral® in renal transplantation" Am. J. Transplant, Suppl. 1, 474, Abstract 1335 (2001).
- [3] Nishimura, T. et al., "40-O-(2-hydroxyethyl-) rapamycin attenuates pulmonary arterial hypertension and neointimal formation in rats" Am. J. Respir. Crit. Care Med., 163, 498-502 (2001).
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Certican™ is a trademark of Novartis AG Corporation, Basel, Switzerland

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Upcoming Events...HYDRANAL® Seminars

When	Where
October 26th	Egerkingen, Switzerland
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November 15th	Barcelona, Spain
November 16th	Barcelona, Spain
November 29th and 30th	Seelze, Germany

For registration and additional information, please contact:

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