Certified Microbiology Standards and Proficiency Testing Services

- Vitroids™ – Microbial CRMs
- Reference Materials for Air Monitoring
- New High Purity Capsaicin Standard
- New Derivatisation Reagents for HPLC
- Reagents for Vitamin D₃ Quantification
- GC Headspace Solvents
- Water Determination in Crude Oil
Dear Colleagues,

I can remember my early days as a student when I was told that microbiology is not a precise science and that the difference of an order of magnitude is not a big issue. Back then, we also tried to standardize our methods and handling to get a reproducible result and create positive and negative controls. Nowadays, however, the trend is quite obviously going more and more in the direction of officially controlled processes and performance. The philosophy of organizations such as ISO is the same in the field of microbiology as it is for analytical chemistry. The same sample analyzed at different times by different analysts and from different laboratories should exhibit the same results. In recent years, we at Sigma-Aldrich saw that even for micro-biology, it is possible to do testing on a quantitative level far below an order of magnitude. The microorganism standards which we developed showed impressive results. I saw many deviations below 10%.

But don’t just take my word for it. Please convince yourself by taking a look at our Vitroids™ (certified reference material of microorganisms). Labs would also be interested in our quality control sets, which could be used to test internally before a proficiency testing round. Of course, this is the same technique that enabled us to provide the complete service of microbiology proficiency testing.

Kind regards,

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

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Vitroids™ – The Microorganism Standards
Testing the Performance of the Microbiology Media or Test Methods is an Important Issue Today.

Sigma-Aldrich extends the ISO 17025 accreditation to microbiology with a new patented technology which allows the production of stable and highly reliable certified microorganisms. The small discs, called Vitroids, contain a certified number of colony-forming units (CFU) from defined bacteria, molds, and yeasts from a recognized culture collection like ATCC and NCTC. The discs are easy to use since they can be placed directly in water, diluent, broth, or even on agar plates. The Vitroids, contain highly viable bacteria and when placed in contact with media, they dissolve rapidly and start to grow without a lag phase. The viability of the CFU in a disc is stable for at least one year (for most organisms, more than two years) when kept under refrigeration. If the Vitroids are kept at -20 °C then stability can be prolonged even longer. It is also not a problem if the product is transported at ambient temperature. The discs are produced under the ISO guideline 34 and are certified according ISO 17025. Each disc is packed in an individual tube with some desiccant and the tubes are then packed in mylar foil. Each package comes with a comprehensive certificate of analysis reporting the CFU and standard deviation.

All of the above-mentioned features help microbiologists to have reliable results, save a lot of time (labor, documentation), and lower costs.

Reference microorganisms in certified and defined colony-forming units (CFU)
- **Standards** in concentrations of 30–50,000 CFU per disc
- **Produced** acc. ISO Guide 34
- **Certified** acc. ISO 17025
- **Delivered** with detailed certificate of origin
- **Reference** strains from ATCC, NCTC, etc.
- **Minimum** 1 year shelf life at 4 °C (usually 2 years)
- **No** lag-phase
- **Amazingly** little standard deviation (e.g. 100 CFU +/- 3%)

**Preparation**
Rehydrate the disc with a common phosphate buffer, or place the disc onto a solid or into a liquid medium. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a loop. Liquid media may simply be shaken to dissolve the disc. The discs can be rehydrated in as little as 100 µL of water or added into larger volumes, e.g. 100 mL, for general water testing methods (MF, MTF, Quanti-Tray, etc). It is also possible to add the disc to the media for pour plate techniques.
<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Origin</th>
<th>Strain No.</th>
<th>CFU</th>
<th>Cat. no.</th>
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</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>ATCC</td>
<td>16404™</td>
<td>80</td>
<td>RQC15003</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC</td>
<td>6633™</td>
<td>80</td>
<td>RQC16003</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC</td>
<td>6633™</td>
<td>10000</td>
<td>RQC02258</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC</td>
<td>10231™</td>
<td>80</td>
<td>RQC14003</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>NCTC</td>
<td>10240</td>
<td>30</td>
<td>RQC02351</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>NCTC</td>
<td>10240</td>
<td>500</td>
<td>RQC20106</td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>ATCC</td>
<td>19404™</td>
<td>80</td>
<td>RQC19003</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>ATCC</td>
<td>13048™</td>
<td>50</td>
<td>RQC01652</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>ATCC</td>
<td>13048™</td>
<td>200</td>
<td>RQC01655</td>
</tr>
<tr>
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<td>ATCC</td>
<td>13048™</td>
<td>1000</td>
<td>RQC01657</td>
</tr>
<tr>
<td>Enterococcus cloacae</td>
<td>ATCC</td>
<td>35030™</td>
<td>50</td>
<td>RQC012102</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>ATCC</td>
<td>19433™</td>
<td>50</td>
<td>RQC011772</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>ATCC</td>
<td>19433™</td>
<td>200</td>
<td>RQC011774</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>ATCC</td>
<td>19433™</td>
<td>500</td>
<td>RQC011775</td>
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<tr>
<td>Enterococcus faecalis</td>
<td>ATCC</td>
<td>19433™</td>
<td>1000</td>
<td>RQC011777</td>
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<tr>
<td>Escherichia coli</td>
<td>ATCC</td>
<td>11775™</td>
<td>50</td>
<td>RQC01702</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC</td>
<td>11775™</td>
<td>200</td>
<td>RQC01705</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC</td>
<td>11775™</td>
<td>1000</td>
<td>RQC01707</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>ATCC</td>
<td>8739™</td>
<td>80</td>
<td>RQC11003</td>
</tr>
<tr>
<td>Heterotrophic Organisms</td>
<td>NCTC</td>
<td>11368</td>
<td>50000</td>
<td>RQC02908</td>
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<tr>
<td>Legionella bozemanii</td>
<td>NCTC</td>
<td>12821</td>
<td>50000</td>
<td>RQC02008</td>
</tr>
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<td>Legionella pneumophilia</td>
<td>NCTC</td>
<td>19115™</td>
<td>30</td>
<td>RQC01901</td>
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<td>Listeria monocytogenes</td>
<td>ATCC</td>
<td>9027™</td>
<td>30</td>
<td>RQC02202</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC</td>
<td>9027™</td>
<td>50</td>
<td>RQC12002</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC</td>
<td>9027™</td>
<td>100</td>
<td>RQC02204</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC</td>
<td>9027™</td>
<td>200</td>
<td>RQC12005</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC</td>
<td>9027™</td>
<td>1000</td>
<td>RQC12007</td>
</tr>
<tr>
<td>Salmonella enterica subsp. Enterica serovar Abony</td>
<td>NCTC</td>
<td>6017</td>
<td>80</td>
<td>RQC18003</td>
</tr>
<tr>
<td>Salmonella enterica subsp. Enterica serovar Typhimurium</td>
<td>ATCC</td>
<td>14028™</td>
<td>50</td>
<td>RQC17002</td>
</tr>
<tr>
<td>Salmonella gallinarum</td>
<td>NCTC</td>
<td>13175</td>
<td>30</td>
<td>RQC02301</td>
</tr>
<tr>
<td>Staphylococcus aureus susp. Aureus</td>
<td>ATCC</td>
<td>6538™</td>
<td>50</td>
<td>RQC13002</td>
</tr>
<tr>
<td>Vitroids™ Blank</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>RQC0001</td>
</tr>
</tbody>
</table>

Many of RTC’s Vitroids microbiological standards are offered as Quality Control microbiology products under the American Type Culture Collection (ATCC®) Licensed-Derivative™ program. Look for the ATCC Licensed-Derivative™ emblem for products derived from ATCC cultures. The ATCC Licensed Derivative emblem signifies ATCC-derived products are endorsed by ATCC. Products displaying the emblem are the only ones for which the quality of the ATCC ingredient can be properly assured. ATCC does not endorse products including ATCC ingredients from companies that are not members of the LDP program. The ATCC Licensed Derivative emblem, the ATCC Licensed Derivative word mark, and the ATCC catalog marks are trademarks of ATCC. RTC is licensed to use these trademarks and to sell products derived from ATCC cultures.
Parabens are esters of para-hydroxybenzoic acid. These compounds are widely used as antimicrobial agents and preservatives in different application fields because of their antifungal properties. The most important members of the parabens are Methyl-, Ethyl-, n-Propyl-, n-Butylparaben.

In the pharmaceutical industry, parabens serve as preservatives of medicinal products. Although there is no upper limit for the allowed concentration of parabens in pharmaceuticals, they must be declared. In the area of cosmetics, parabens are used as preservatives for personal care products such as creams, lotions, make-up, lipsticks, aftershaves, soaps, sunscreens, depilatories and shampoos. Directive 76/768 EWG VI as well as cosmetics directive appendix 6 both allow a concentration of 0.4% for single components and 0.8% for paraben mixtures. As food supplements, parabens are designated with the numbers E214 to E219. In addition to all these uses, parabens are also applied as preservatives of technical oils, fats, glues and shoe polish.

Although the preservational properties of the parabens are desired for all the above-mentioned applications, this characteristic turns into a drawback because the preservation continues after the molecules are absorbed either orally or through the skin, then spread throughout the body by the bloodstream and finally end up in organs or tissues. Parabens have been found in breast tissue, mother’s milk [Ye et al. 2008], urine [Ye et al. 2006] and in blood serum [Janjua et al. 2007]. Since parabens have also been found in cancer breast tissue [Darbre et al. 2004], they are under suspicion to cause cancer. Although a study of the BfR did not confirm this concern, most studies claim that more research has to be done to rule out a potential connection between cancer and parabens.

Because of their widespread use, parabens can nowadays be found almost everywhere, for example in house dust [Canosa et al. 2007], and waste water [Blanco et al. 2009], and since they are not fully decomposed in sewage plants [Nieto et al. 2009], parabens have also been found in surface water [Canosa et al. 2006]. This is of especial concern since parabens have been found too in vitro bind to estrogen receptors and in vivo it could be confirmed that they have estrogen-like properties [Routledge et al. 1998], although several studies showed a $10^3$ to $10^4$ times weaker estrogen activity than the physiological hormone 17-$\beta$-estradiol. The strongest estrogen-like activity showed the butyl paraben while the methyl paraben which is most widely used in cosmetic products showed a much weaker activity. Furthermore, in vitro experiments showed a weak affinity to the androgen receptor. Also, an influence on the quality of human fertility could be demonstrated [Kang et al. 2002]. Therefore, parabens are considered as endocrine disrupting chemicals (EDCs).

Analysis

The regulation of parabens in food products, as well as in cosmetical formulations, requires a precise and reliable analytical quantification. The use of isotopically labeled internal standards can minimize errors when mass spectrometrical methods are applied. With GC-MS, adsorption effects and analyte discrimination at active spots of the injector or in the column might occur. Matrix-caused suppression is often a problem when LC-MS-MS is used. Both cases lead to too low measured values of the target analytes.

By addition of a defined amount of internal standards, these problems can be avoided since losses of the target analyte can be eliminated by calculation. This compensation is particularly accurate if the used internal standards’ chemical and physical properties are very similar to the target analytes. This is particularly the case for isotopically labeled internal standards. The similarity of internal standards and target analytes leads to almost identical retention times in chromatography. Figure 1 shows the $^{13}$C$_6$ ring labeled form of the most common parabens.
In Figure 2, a chromatogram of methyl, ethyl, propyl, butyl and benzyl paraben and their corresponding $^{13}$C labeled internal standards is shown. Sigma-Aldrich offers Ring $^{13}$C labeled standards (Table 1) as well as non labeled standards (Table 2) for the most common parabens. The labeled standards are available as solutions in acetone in concentrations of 10 µg/mL and 50 µg/mL. The non-labeled standards are available as certified reference materials (CRMs) as 1 g packages. These neat standards are produced under ISO/IEC 17025 and ISO Guide 34 double accreditation and are traceable to USP and EP reference materials.

**GC Conditions:**
- **Instrument:** Agilent GC 7890A
- **Detector:** Agilent MS 7000 QQQ
- **Column:** HP-5MS, 5% phenyl polydimethylsiloxane, 25 m x 0.25 mm, 0.25 µm (L x ID, FT)
- **Oven Program:** 760 °C (1 min), 10 °C/min to 200 °C, 15 °C/min to 280 (10 min)
- **Carrier Gas:** helium, 1.2 mL/min
- **Injection:** 2.0 µL splitless
- **Injector:** Gerstel CIS

**Figure 1** Molecular structures of $^{13}$C ring labeled parabens

**Figure 2** Chromatogram of parabens and $^{13}$C-labeled internal standards
### Table 1 ¹³C labeled paraben standard solutions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cat. no.</th>
<th>Concentration</th>
<th>Package size</th>
<th>Cat. no.</th>
<th>Concentration</th>
<th>Package size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylparaben (Ring-¹³C₆)</td>
<td>32557</td>
<td>50 μg/mL in acetone</td>
<td>1 mL</td>
<td>32556</td>
<td>10 μg/mL in acetone</td>
<td>5 mL</td>
</tr>
<tr>
<td>Ethylparaben (Ring-¹³C₆)</td>
<td>32559</td>
<td>50 μg/mL in acetone</td>
<td>1 mL</td>
<td>32558</td>
<td>10 μg/mL in acetone</td>
<td>5 mL</td>
</tr>
<tr>
<td>n-Propylparaben (Ring-¹³C₆)</td>
<td>32562</td>
<td>50 μg/mL in acetone</td>
<td>1 mL</td>
<td>32561</td>
<td>10 μg/mL in acetone</td>
<td>5 mL</td>
</tr>
<tr>
<td>n-Butylparaben (Ring-¹³C₆)</td>
<td>32564</td>
<td>50 μg/mL in acetone</td>
<td>1 mL</td>
<td>32563</td>
<td>10 μg/mL in acetone</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

### Table 2 Non labeled paraben standards

<table>
<thead>
<tr>
<th>Description</th>
<th>Components</th>
<th>Cat. no.</th>
<th>Concentration</th>
<th>Package size</th>
<th>Cat. no.</th>
<th>Concentration</th>
<th>Package size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraben Internal Standard Mix-Solution</td>
<td>Methylparaben Ring-¹³C₆</td>
<td>32124</td>
<td>10 μg/mL each component in acetone</td>
<td>5 mL</td>
<td>32125</td>
<td>50 μg/mL each component in Aceton</td>
<td>1 mL</td>
</tr>
<tr>
<td>Paraben Target Analyte Mix-Solution</td>
<td>Methylparaben Ethylparaben n-Propylparaben iso-Propylparaben n-Butylparaben iso-Butylparaben Benzylparaben</td>
<td>32126</td>
<td>10 μg/mL each component in acetone</td>
<td>5 mL</td>
<td>32127</td>
<td>50 μg/mL each component in Aceton</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

### References

Oxime Derivatives as Air Monitoring Standards

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

In the fields of atmospheric chemistry and occupational medicine, the analysis of aldehydes and ketones in air samples is an important task [1]. Because of their reactivity, they should be stabilized prior to analysis. One of the main derivatization reagents is 2,4-Dinitrophenylhydrazine (DNPH), its method having been introduced as a national and international standard by several standardization bodies [2–4].

However, for unsaturated aldehydes such as acrolein, which is considered one of the greatest non-cancerous health risks of all organic air pollutants, these methods are of limited use. They result in the formation of unstable derivatives, coelution of similar compounds, long sample collection times, and ozone interferences which give poor sensitivity, selectivity and reproducibility.

This problem can be overcome by reacting carbonyls with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) whereby oxime derivatives are formed. The resulting compounds provide much more stable carbonyl derivatives than the DNPH ones for working at high temperatures. These derivatives are appropriate for gas chromatography applications e.g. gas chromatography/electron capture negative ionization mass spectrometry (GC/ECNI MS) [5, 6].

Sigma-Aldrich offers five different aldehyde and ketone PFBHA derivatives analytical standards as neat substances.

![Figure 1 Derivatization of carbonyl compounds with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA)](image)

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Product name</th>
<th>Assay</th>
<th>Package size</th>
</tr>
</thead>
<tbody>
<tr>
<td>44114</td>
<td>Acetone O-pentafluorophenylmethyl-oxime</td>
<td>≥98.0%  (GC)</td>
<td>10 mg</td>
</tr>
<tr>
<td>65819</td>
<td>Acrolein O-pentafluorophenylmethyl-oxime</td>
<td>≥95.0%  (GC)</td>
<td>10 mg</td>
</tr>
<tr>
<td>41558</td>
<td>Formaldehyde O-pentafluorophenylmethyl-oxime</td>
<td>≥98.0%  (GC)</td>
<td>10 mg</td>
</tr>
<tr>
<td>03718</td>
<td>Glutaraldehyde O-pentafluorophenylmethyl-oxime</td>
<td>≥98.0%  (GC)</td>
<td>10 mg, 50 mg</td>
</tr>
<tr>
<td>43508</td>
<td>Propanaldehyde O-pentafluorophenylmethyl-oxime</td>
<td>≥98.0%  (GC)</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

References

New Certified Reference Material from the IRMM

The IRMM (institute of reference materials and measurements), is one of the seven institutes of the Joint Research Center, a Directorate-General of the European commission. Within this structure, the IRMM supplies certified reference materials (pure and matrix materials) for various applications including environmental analysis, food analysis, clinical chemistry, physical properties or industrial applications. Sigma-Aldrich is proud to be an authorised distributor of IRMM reference materials. In Table 1, most recent new additions to the IRMM product range are listed.

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Material</th>
<th>Certified for</th>
<th>Application area</th>
<th>Package size</th>
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</thead>
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<tr>
<td>ERMDA474/IFCC</td>
<td>Human Serum</td>
<td>Protein Content</td>
<td>Clinical</td>
<td>1 mL</td>
</tr>
<tr>
<td>ERMF3304</td>
<td>Colloidal Silica</td>
<td>Diameter</td>
<td>Engineering</td>
<td>9 mL</td>
</tr>
<tr>
<td>ERWCC141</td>
<td>Loam Soil</td>
<td>Element Content</td>
<td>Environmental</td>
<td>24 g</td>
</tr>
<tr>
<td>ERMB8386</td>
<td>Bovine Urine</td>
<td>diethylstilboestrol (blank)</td>
<td>Food and Agriculture</td>
<td>1 vial</td>
</tr>
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<td>ERMB8389</td>
<td>Bovine Urine</td>
<td>diethylstilboestrol (positive)</td>
<td>Food and Agriculture</td>
<td>1 vial</td>
</tr>
<tr>
<td>ERMB8430</td>
<td>Pork Fat</td>
<td>Organic Pollutants</td>
<td>Food and Agriculture</td>
<td>5 g</td>
</tr>
<tr>
<td>ERMB600</td>
<td>Whole Milk Powder</td>
<td>Vitamins</td>
<td>Food and Agriculture</td>
<td>100 g</td>
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<tr>
<td>ERMBF430A</td>
<td>AM04-1020 Potato</td>
<td>GMO content (blank)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
</tr>
<tr>
<td>ERMBF430B</td>
<td>AM04-1020 Potato</td>
<td>GMO content (1%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
</tr>
<tr>
<td>ERMBF430C</td>
<td>AM04-1020 Potato</td>
<td>GMO content (4%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
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<tr>
<td>ERMBF430D</td>
<td>AM04-1020 Potato</td>
<td>GMO content (10%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
</tr>
<tr>
<td>ERMBF430E</td>
<td>AM04-1020 Potato</td>
<td>GMO content (100%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
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<tr>
<td>ERMBF432A</td>
<td>DAS-68416-4 Soya Seed</td>
<td>GMO content (blank)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
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<tr>
<td>ERMBF432B</td>
<td>DAS-68416-4 Soya Seed</td>
<td>GMO content (0.5%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
</tr>
<tr>
<td>ERMBF432C</td>
<td>DAS-68416-4 Soya Seed</td>
<td>GMO content (1.0%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
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<tr>
<td>ERMBF432D</td>
<td>DAS-68416-4 Soya Seed</td>
<td>GMO content (10.0%)</td>
<td>Food and Agriculture</td>
<td>1 g</td>
</tr>
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</table>

Table 1 IRMM products

Reference Substances from Medicinal Plants

Analytical Standards and Primary Reference Standards for Herbal Medicinal Products, Nutraceuticals and Research

Sigma-Aldrich offers an extensive and rapidly growing portfolio of analytical standards of active ingredients and marker compounds for a wide range of medicinal plants. The portfolio also includes several primary reference standards for QC, in-process control and stability testing of herbal medicinal products.

A complete and continuously updated product listing can be found on our webpage sigma-aldrich.com/medicinalplants

The products are listed by: alphabetical order, structure class and plant genus.
Do you want reliable quantitative results?

Try our NEW organic TraceCERT® CRMs!

- Products for HPLC, GC and qNMR
- Certified content measured by high-performance quantitative NMR (HP-qNMR®)
- Superior level of accuracy, calculated uncertainties, and lot-specific values
- Traceability to NIST Standard Reference Material
- Production and certification in accordance with ISO/IEC 17025 and ISO Guide 34

Our product range currently comprises over 100 standards including: Amino Acids, PAHs, Antibiotics, Pesticides, Fatty Acids/FAMEs, Natural Compounds, Organic Acids etc.

We are continuously working on the expansion of this portfolio in order to offer you reliable and traceable reference materials for the analytes you need.

For more information and an up-to-date product list, please visit our website at sigma-aldrich.com/organiccrm
Capsaicin is the primary active component of spicy chili peppers, the fruits of plants belonging to the genus *Capsicum*. It is an irritant for mammals, including humans, and produces a sensation of burning in any tissue with which it comes into contact. Capsaicin is used as a food additive in various spicy cuisines, for therapeutic purposes to treat a number of peripheral painful conditions, as a pesticide, in pepper sprays, and as an ingredient in cosmetics [1, 2].

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a crystalline, lipophilic, colorless and odorless alkaloid with the molecular formula C_{18}H_{27}NO_{3}. Capsaicin displays cis/trans isomerism (see Figure 1). Naturally occuring capsaicin is always found in the trans form [3].

Therefore the Z-Capsaicin (cis form) can only be found in synthetically produced capsaicin, which should not be confused with synthetic capsaicin (Nonivamide) (see Figure 2).

Pure Capsaicin isolated from Capsicum peppers usually contains impurities of other capsaicinoids, such as dihydrocapsaicin and nordihydrocapsaicin. Sigma-Aldrich has been able to produce and offer a superior quality of pure E-Capsaicin, with a guaranteed assay of ≥99.0% by HPLC.

References


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<th>Description</th>
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Table 1 Product information

A comprehensive list of our product line can be found on our webpage at [sigma-aldrich.com/medicinalplants](http://sigma-aldrich.com/medicinalplants)

---

**Figure 1** The structure of E- and Z-Capsaicin

**Figure 2** ^H-NMR spectra of synthetically produced E-Capsaicin contaminated with Z-Capsaicin; Source: Syndeco GmbH, Switzerland
The interest in isolating, identifying, and quantifying carboxylic acids such as fatty acids from biological samples has steadily increased in recent years due to their physiological importance and wide distribution in nature. HPLC offers some distinct advantages over other analytical methods such as GC or TLC, including lower temperature than GC, higher sensitivity and specificity, and higher speed of analysis. Since many naturally occurring carboxylic acids do not possess inherent UV- or fluorescent active chromophores, derivatization is generally required in order to perform HPLC analyses coupled with UV or fluorescence detection [1]. Recently, we have expanded our comprehensive selection of high purity derivatization reagents.

1,2-Benzo-3,4-dihydrocarbazole-9-ethyl-p-toluenesulfonate (BDETS) has been shown to be highly suitable for derivatization of fatty acids in edible oils for analysis via reverse-phase HPLC with fluorescence detection [2]. The same reagent is also useful for determination of bile acids in serum [3]. As reported by Qiu et al. and Suna et al., 2,4’-Dibromoaceto-phenone (BPB, α-Bromophenacyl bromide) proves to be the reagent for rapid and reliable derivatization during determination of fatty acids and very low levels of perfluorocarboxylic acids [4,5].

References

Product Table Derivatization reagents for carboxylic acids. Please find the complete product list at sigma-aldrich.com/derivatization
Vitamin D₃ Quantification – Effect of Unsuitable Solvents on Electrospray Detection (LC-MS)

In routine analysis, modern LC-MS methods are optimized to increase the number of samples. Although many improvements have increased the sensitivity of LC-MS, there are still constraints, e.g. suppression effects. The quantitative analysis including the sample preparation should be easy to use, robust, and cost effective. Solvent quality can be an important factor in the calculation of the cost per sample. Lesser grade solvents that may contain impurities, and are not tested for LC-MS applications, result in interferences that will raise the cost of each analysis. The results presented in this article shows the effect of a lesser grade solvent on the sensitivity of the MS detection (signal suppression effect). ESI, the most common ion source, is especially impacted by this effect when high amounts of a contaminated solvent are introduced into the source. Here we present a typical example in clinical diagnostics, showing the effect of ionic impurities in acetonitrile on the quantitative analysis.

### Experimental

#### ESI Suppression Effect

Vitamin D₃ and its metabolite 25-hydroxyvitamin D₃ (deuterated, D₆) are dissolved in methanol (LC-MS Ultra Methanol, Fluka 14262). The final standards give a concentration of 5 µg/mL (vitamin D₃) and 0.5 µg/mL (25-hydroxyvitamin D₃). The UHPLC system (Dionex Ultimate 3000 RSLC binary) is set up with three different solvents. Channel B contains LC-MS Ultra Acetonitrile, channel C contains LC-MS Ultra water/0.1% formic acid (LC-MS Ultra) at pH=3.5 and channel D contains acetonitrile of a competitor (gradient grade quality for biological and environmental analysis). The system is flushed with an LC-MS Ultra quality solvent (100% C) and six injections of vitamin D₃ are acquired using the high-quality solvent first. The system is then flushed with a gradient grade quality solvent and again six injections of vitamin D₃ are acquired. Flushing back with LC-MS Ultra will clean the system and the whole procedure is repeated for 25-hydroxyvitamin D₃.

#### Gradient Test of UHPLC Solvents

A steep gradient starting from 95% water and ending at 95% acetonitrile is used to test different acetonitrile solvents of varying quality. LC-MS Ultra water and acetonitrile serve as reference. The runs are repeated until a constant UV and MS (BPC/TIC) baseline is obtained.

### Table 1

<table>
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<tr>
<th>Mass spectrometer</th>
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<tr>
<td>Injection Volume:</td>
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<td>UV Wave Length:</td>
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Table 1 LC-MS Method for Vitamin D₃/Hydroxyvitamin D₃

### Gradient Test of UHPLC Solvents

A steep gradient starting from 95% water and ending at 95% acetonitrile is used to test different acetonitrile solvents of varying quality. LC-MS Ultra water and acetonitrile serve as reference. The runs are repeated until a constant UV and MS (BPC/TIC) baseline is obtained.

#### Table 2

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<td>Gradient:</td>
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Table 2 Gradient method
**Results**

**Ion Suppression by Unsuitable Solvents**

All UV chromatograms of vitamin D₃ and 25-hydroxyvitamin D₃ are not affected by the change of the solvent quality. Peak position, size and shape remain the same after exchanging LC-MS Ultra acetonitrile with the gradient grade quality of a competitor (Figure 1). The UV data indicates the robustness of both the chromatographic separation and the precision of the UHPLC-MS system. At first glance both solvents seem to be suitable for this kind of application. However, after having a closer look at the mass spectroscopic data, one can observe a significantly lower signal intensity of the \([M+H]^+\) ion with gradient grade acetonitrile, although all EICs should have the same peak size as the UV chromatograms (Figure 2). This allows only one conclusion: the impurities in the gradient grade do not absorb UV radiation but lead to a significant ion suppression in ESI source of the mass spectrometer. Unfortunately, ionic impurities, e.g. alkali metals, are not specified for the gradient grade, but the average mass spectrum of the background shows the typical pattern of sodium formate clusters (Figure 5).

![Figure 1](image1.png)

**Comparison of Different UHPLC Solvents**

The example of vitamin D₃ shows the severe effect of solvents without application test. But even solvents for UHPLC-MS differ considerably in their MS chromatograms as can be shown with a steep gradient method. Running this test under UHPLC conditions will unveil many more details about impurities in both solvents, especially with two combined detections systems. Figures 3 and 4 show the UV data and ESI(+) data of different UHPLC/UHPLC-MS acetonitrile qualities. Most of them seem suitable for UV detection and UHPLC methods, but the MS data shows additional non-UV absorbing impurities and additional noise in all chromatograms except LC-MS Ultra.

![Figure 2](image2.png)

**Conclusion**

High purity and sensitive UV gradient methods can give only a rough picture of a solvent and its suitability for MS detection. Ions of any kind influence the sensitivity of the ESI source significantly as can be seen in Figure 2. The analysis of the background of these runs is shown in Figure 5, which is the average mass spectrum of the spectra from 3.2 to 4.7 min and overlaps with the vitamin D₃ peak. The
pattern is typical for sodium formate clusters and the intensity is very high. The resulting suppression effect can lower the limit of quantification (LOQ) and has a negative influence on the lifetime of an instrument. The wrong solvent quality may lower the cost for the sample, but it leads to higher costs by counteracting the work of an extensive sample preparation, which was introduced to increase the LOQ.

References


Reagents and Standards for Ion Chromatography

Shyam Verma, Market Segment Manager Analytical Reagents shyam.verma@sial.com

Reagents
Quantification of low levels (mg/L) of cations and anions via ion chromatography commands the following sample preparation requirements:

- high-purity reagents to assure low blank values
- digestion reagents free of any metal ions or other impurities
- complete sample decomposition for reproducible and accurate results

Our high-purity reagents include eluent concentrates traceable to NIST Standard Reference Materials (certified to ISO Guide 31), salts and additives, and high-purity water.

Proficiency Testing for Ion Chromatography
For your IC accreditation needs, Proficiency Testing (PT) samples are manufactured under ISO Guide 34 accreditation, and tested under ISO/IEC 17025 accreditation. The PT program is operated according to ISO/IEC 17043 and NELAC PT provider accreditation. PT samples are available for both anions and cations in wastewater, drinking water and soil matrices. For additional information, contact RTC@sial.com and request IC flyer (code: OSB). You can also visit our website sigma-aldrich.com/ic

Certified Reference Standards, TraceCERT®
These standards include inorganic cation or anion, and organic anion standard solutions, certified multi-ion standard solutions, and TraceCERT multi-ion standards. They are carefully prepared to meet the following guidelines:

- produced in a double accredited laboratory fulfilling ISO/IEC 17025 and ISO Guide 34
- certified value directly traceable to the SI unit (kg), also measured against NIST, BAM and other internationally recognized reference materials
- traceability and uncertainty information on the certificate as per ISO Guide 31. The inorganic CRMs also list the most relevant trace impurities
- each IC standard is offered at a concentration of 1000 mg/L in 100 mL in HDPE bottles. The organic CRMs are stabilized by sodium azide
- certificates are available on the web using product and lot numbers

sigma-aldrich.com/ic
New Headspace Grade Solvents
... for the Analysis of Organic Volatile Impurities

Static headspace GC (GC-HS) is a technique used to concentrate volatile analytes prior to analysis. It can improve detection of low levels of volatile analytes and minimize matrix interference by eliminating the need to inject the sample directly. An important application of GC-HS is for the determination of residual volatile organic impurities in active drug substances or excipients in drug formulations. Other consumer-oriented applications include the detection of residual solvents in foods, dietary supplements, and packaging materials.

GC-HS is a straightforward technique and the methodology, as it applies to residual solvents in pharmaceuticals, is described and validated in specific monographs [1–3]. These guidelines recommend both the type of solvents and the acceptable levels of residual solvents in pharmaceuticals and formulations to help ensure consumer safety.

New Headspace Grade Solvents
When a GC-HS method is being developed, such parameters as sample solvent, extraction temperature and time, sample...
volume, and headspace volume are optimized [4, 5]. Because the composition and purity of the sample solvent have significant effects on the recovery and quality of the chromatogram (Figure 1), we have developed solvents specifically for GC-HS applications. Their purity and handling specifications meet the requirements of European Pharmacopoeia (Ph.Eur.) and United States Pharmacopoeia (USP), as well as ICH guidelines. The new GC-HS solvent line includes water and three of the most commonly used organic solvents: dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF) and N,N-dimethylacetamide (DMAC). DMF and DMSO are specified in Ph.Eur. and USP for water-insoluble substances. Water is the preferred solvent for water-soluble analytes, as described in Ph.Eur. and USP monographs. All solvents are microfiltered (0.2 µm) and packed under inert gas for longer shelf life.

The organic synthesis grade showed many peaks caused by impurities. The GC-HS grade produced a cleaner headspace blank and showed no major interference peaks in the elution range of the target analytes (Figure 1).

Additional purity of both, headspace and organic synthesis grades, DMSO was also tested using Headspace SPME and GC-MS in order to make tentative identifications of impurities eluting in the primary range of OVI's [6]. Total ion chromatograms (TICs) from the analysis of headspace and organic synthesis grade DMSO are presented in Figure 2. The scale of both TICs is the same, and the elution range prior to DMSO is shown. Headspace SPME and GC-MS detected and tentatively identified compounds in the organic synthesis grade DMSO that were not present in the HS-GC grade. Two of these compounds were solvents listed in the ICH guidelines, USP Method <467>, and EP Method 2.4.24.

![Figure 2 Fluka’s High Purity Headspace Grade vs an Organic Synthetic Grade – Using GC-MS and SPME on DMSO](image)

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<td>Water, for GC-HS</td>
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**Product Table** Solvents for GC Headspace Analysis

For additional information, please visit our website sigma-aldrich.com/gc-hs

References

Determination of Water Content in Petrochemical Products
HYDRANAL® Applications: Crude Oil and Petroleum Products

Andrea Felgner, Market Segment Manager HYDRANAL andrea.felgner@sial.com

Water as an impurity in petroleum products can have many negative effects. It accelerates deterioration of lubricants and interferes with lubricant properties. Water causes rust and corrosion of metals, and when combined with dust, may lead to sludge formation. Consequently, water content is an important parameter which must be closely monitored to hold within specifications. To achieve reproducible results for water content using the Karl Fischer (KF) titration method, two fundamental preconditions are required: (1) preliminary homogenization of samples using a homogenizer or ultrasound, and (2) complete dissolution or at least fine dispersion of the sample in the KF working medium for complete extraction of water.

Crude oil and petroleum products mainly consist of long-chained hydrocarbons and have limited solubility in pure methanol. The addition of a solubilizer to the KF working medium, such as chloroform or xylene, improves sample solubility. Allowing for an extraction time before starting the titration can also help to extract the water completely from the sample. However, problems can be encountered with formulated oils in KF titration because the presence of additives introduces a level of complexity.

Application Recommendations from the HYDRANAL Technical Service Team
Crude oil is basically a mixture of different hydrocarbons containing various amounts of bituminous material. It requires special titration techniques, such as the addition of chloroform to the working medium in order to dissolve the hydrocarbons. Xylene is used to dissolve the bituminous compounds; if these are not finely dispersed, they can stick to the electrode wires and lead to indication problems.

Heavy heating oils also tend to contaminate the electrode wires and vessel walls during KF titration. In this case as well, the addition of chloroform and xylene to the working medium is recommended, as described below in Procedure 1 for crude oil (see also Application L 111).

sigma-aldrich.com/hydranal
HYDRANAL®-Solver (Crude) Oil is a practical and reliable product for these applications. It fulfills the requirements of ASTM D 4377-00: in KF analysis using a pyridine-free reagent, a mixture of a KF solvent (for example, HYDRANAL-Solvent) and xylenet must be added to the titration vessel (additional international standard methods are presented later in this article). Solubility and titration speed with HYDRANAL-Solver (Crude) Oil are improved in comparison to the solvent mixture. HYDRANAL-Solver (Crude) Oil is recommended for use with both one- and two-component KF techniques; it can be used with HYDRANAL-Composite as well as HYDRANAL-Titrant as titrating agents.

The procedure of water content determination in crude oils is described as follows:

**Procedure 1: HYDRANAL-Solver (Crude) Oil for crude oil samples**
- 30 mL HYDRANAL-Solver (Crude) Oil or
- 10 mL HYDRANAL-Methanol dry, 10 mL HYDRANAL-Chloroform and 10 mL HYDRANAL-Xylene are added to the titration vessel and titrated to dryness with HYDRANAL-Composite/Titrant. A 1–5 g sample of crude oil is then accurately weighed and added using a syringe (weighing by difference). The water content of the crude oil sample is determined by titrating with HYDRANAL-Composite or Titrant.

Oil distillates vary in their composition and thus in their titration demands. Low boiling-point fractions need the addition of chloroform, following titration according to **Procedure 2**. The coulometric KF method (Procedure 2C) is recommended, since the water content of distillates is typically very low. Standard coulometric reagents can be used: HYDRANAL-Coulomat A, AG-H and Oil already contain solubilizing agents.

Kerosene behaves similarly and has a low water content in the ppm range. To improve the solubility of kerosene, the chloroform content should be increased for the coulometric determination. A mixture of HYDRANAL-Coulomat A and HYDRANAL-Chloroform should be used (see Procedure 2C); 40 mL of kerosene will dissolve in 100 mL of this mixture (see Application L112).

We have also investigated several motor oils and certain additives. The water content of an oil sample without any additives can be determined easily according to **Procedure 2**, with the use of chloroform. Polyols, such as antifreeze, can be analyzed by following the standard volumetric one-component procedure with HYDRANAL-Methanol dry, Methanol Rapid or CompoSolver E and HYDRANAL-Composite. For dissolution of olein copolymers, a 4:1 mixture of HYDRANAL-Medium K and HYDRANAL-Formamide dry should be used as a working medium; the water determination is straightforward under these conditions. For analysis of polybutene, a 3:1 mixture of HYDRANAL®-Chloroform and HYDRANAL-Methanol dry is recommended as a working medium.

**Procedure 2: Addition of chloroform as a solubilizing agent**
For an exact determination of water, the methanol content should not be less than 35% by volume, otherwise the KF reaction will not proceed stoichiometrically.

A) Chloroform addition, volumetric one-component titration
- 30 mL HYDRANAL-LipoSolver CM or
- 10 mL HYDRANAL-Methanol dry, HYDRANAL-Methanol Rapid or HYDRANAL-CompoSolver E and 20 mL HYDRANAL-Chloroform are added to the titration vessel and titrated to dryness with HYDRANAL-Composite. The sample is then added and titrated in the same manner.

B) Chloroform addition, volumetric two-component titration
- 30 mL HYDRANAL-Solvent CM or
- 10 mL HYDRANAL-Solvent or HYDRANAL-Solvent E and 20 mL HYDRANAL-Chloroform are added to the titration vessel and titrated to dryness with HYDRANAL-Titrant or HYDRANAL-Titrant E. The sample is then added and titrated in the same manner.

C) Chloroform addition, coulometric titration
The anodic compartment of the coulometric titration cell is filled with
- 100 mL HYDRANAL-Coulomat Oil or
- 70 mL HYDRANAL-Coulomat A and 30 mL HYDRANAL-Chloroform
The cathodic compartment is filled with 5 mL HYDRANAL-Coulomat CG. After titrating to dryness, the sample is injected with a syringe through the septum into the anolyte compartment. Generally, a sample size of 0.5–5.0 mL in liquid form is recommended.

(continued on page 22)
Silicone oils present fewer problems. Their analysis requires the addition of HYDRANAL-Chloroform in order to improve the solubility. We recommend following Procedure 2A/2B, modified by using a titrating agent with small water equivalent because of the low water content of the oils (such as HYDRANAL-Titrant 2 (E), Composite 2 or Composite 1). For coulometric determinations, Procedure 2C is recommended. Insulating oils (transformer oils) resemble silicone oils and can be analyzed according to the same procedure.

**KF Oven Method for Oils Containing Additives**

Additives are added to many lubricant products such as engine oils, transformer oils or machine oils to optimize their performance. They improve viscosity, lower the pour point, prevent oxidation, reduce friction, suppress the formation of foam, amalgamate or separate solids, and provide a number of other functions.

Particular attention is needed when analyzing formulated lubricants, since their constituents can interfere with the KF titration. Specific information about the chemical nature of these additives is usually not available. Experience in our HYDRANAL lab has shown that additives can simulate high water content in the KF titration through side-reactions with methanol or iodine. Consequently, it is not surprising that incorrect values for the water content are obtained by direct KF titration of oils containing additives.

For samples such as these, we recommend the indirect KF oven method, where the sample is heated and thereby releases its water content. The water is then transferred to the titration vessel with a suitable carrier gas (for example, air or nitrogen, where nitrogen is preferred when the sample is sensitive to oxidization at temperatures of 100–300 °C). The sample itself does not get in contact with the KF reagents; therefore, interfering side-reactions can be circumvented. Considering the very low water content of most oil samples, the KF oven is primarily used in combination with a coulometer; however, it can also be used in combination with volumetric KF titration.

Of particular importance is the determination of the optimum oven temperature to remove the water from the sample. It must be high enough to drive off the sample water within 10–15 minutes; however, it must also be kept low enough to prevent vaporization or decomposition of the sample matrix, which could interfere in the KF titration. A suitable temperature must be determined individually for each sample: a programmed temperature ramp is recorded, where the sample is gradually heated from 50 °C to 250 °C within 50 minutes. The resulting profile shows the water release across the temperature scan which characterizes the release of volatiles in the sample. The suitable temperature for complete release of sample water can thus be determined. An example for investigation of oil samples with additives using the indirect KF method is given in Application Report L531.

**International Standard Methods**

In these standards, the Karl Fischer Titration (KFT) is prescribed for measuring the water content of petroleum and related products:

- ASTM D890 Water in Liquid Naval Stores
- ASTM D1123 Water in Engine Coolant Concentrate
- ASTM D1533 Water in Insulating Liquids
- ASTM D4377 Water in Crude Oils (volumetric KFT)
- ASTM D4672 Water in Polyols (Polyurethane Raw Materials)
- ASTM D4928 Water in Crude Oils (coulometric KFT)
- ASTM D5530 Water in Hazardous Waste Fuel
- ASTM D6304 Water in Petroleum Products, Lubricating Oils, and Additives
- DIN 51777-1 Water in petroleum hydrocarbons and solvents (direct KFT)
- DIN 51777-2 Water in petroleum hydrocarbons and solvents (indirect KFT)
- ISO 10336 Water in crude petroleum (volumetric KFT)
- ISO 10337 Water in crude petroleum (coulometric KFT)
- ISO 6296 Water in petroleum products (volumetric KFT)
- ISO 12937 Water in petroleum products (coulometric KFT)

The following HYDRANAL application reports for water content determination in crude oil and petroleum products are available upon request:

- L108 Crude oil
- L148 Crude oil (coulometric)
- L109 Engine oil, used (I)
- L110 Engine oil, used (II)
- L111 Fuel oil, heavy
- L412 Greases
- L381 Hardness oil, used
- L107 Hydraulic oil
- L462 Insulating oil
- L112 Kerosene
- L545 Lubricating oil
- L190 Mineral oil with additives
- L184 Mineral oil without additives
- L534 Oil, cold compressor oil with Freon
- L531 Oils, with additives
- L188 Polybutene
- L477 Polyglycol-based grease with additives
- L585 Used hydraulic fluid

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These applications can be obtained from our HYDRANAL® experts at hydranal@sial.com. Contact them also for application support, choice of reagents, or any technical question about Karl Fischer Titration. A complete list of HYDRANAL Applications is displayed on our website sigma-aldrich.com/hydranal.

Are you interested in our HYDRANAL Manual? It contains detailed information about the application of HYDRANAL reagents and Karl Fischer Titration procedures for a wide variety of samples. Check the box on the attached business reply card and receive your free copy!

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<td>34730</td>
<td>HYDRANAL-Solvent E</td>
</tr>
<tr>
<td>34800</td>
<td>HYDRANAL-Solvent</td>
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<tr>
<td>34812</td>
<td>HYDRANAL-Solvent CM</td>
</tr>
<tr>
<td>34697</td>
<td>HYDRANAL-Solver (Crude) Oil</td>
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</table>

**Coulometric Reagents**

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Description</th>
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<tbody>
<tr>
<td>34807</td>
<td>HYDRANAL-Coulomat A</td>
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<tr>
<td>34843</td>
<td>HYDRANAL-Coulomat AG-H</td>
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<tr>
<td>34868</td>
<td>HYDRANAL-Coulomat Oil</td>
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<tr>
<td>34840</td>
<td>HYDRANAL-Coulomat CG</td>
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<tr>
<td>34726</td>
<td>HYDRANAL-Coulomat E</td>
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**Auxiliary Reagents**

<table>
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<th>Cat. no.</th>
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<tr>
<td>37863</td>
<td>HYDRANAL-Chloroform</td>
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<tr>
<td>37866</td>
<td>HYDRANAL-Xylene</td>
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<tr>
<td>34724</td>
<td>HYDRANAL-Formamide dry</td>
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**Recommended Water Standards**

<table>
<thead>
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<th>Cat. no.</th>
<th>Description</th>
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<tbody>
<tr>
<td>34694</td>
<td>HYDRANAL-Water Standard Oil</td>
</tr>
<tr>
<td>34693</td>
<td>HYDRANAL-Water Standard KF-Oven 140–160 °C</td>
</tr>
<tr>
<td>34748</td>
<td>HYDRANAL-Water Standard KF-Oven 220–230 °C</td>
</tr>
</tbody>
</table>

Table 1 Fluka® brand HYDRANAL Karl Fischer reagents

NEW pH Buffer Solutions DURACAL

Certified buffer solutions traceable to NIST/PTB reference materials, with DKD* certification

- Convenient 250 mL and 500 mL plastic bottles with economical built-in calibration compartment
- No contamination by microorganisms
- Exact buffer value determined by DKD* laboratory, accredited for pH measurement
- Certificate of Analysis with traceability to international standards available for download at sigma-aldrich.com
- Expiration date on the label

Visit our website sigma-aldrich.com/duracal and browse through our portfolio!

* DKD – Deutscher Kalibriedienst DKD-K-06901 (German Calibration Service), Zentrum für Messen und Kalibrieren GmbH, Wolfen, Germany