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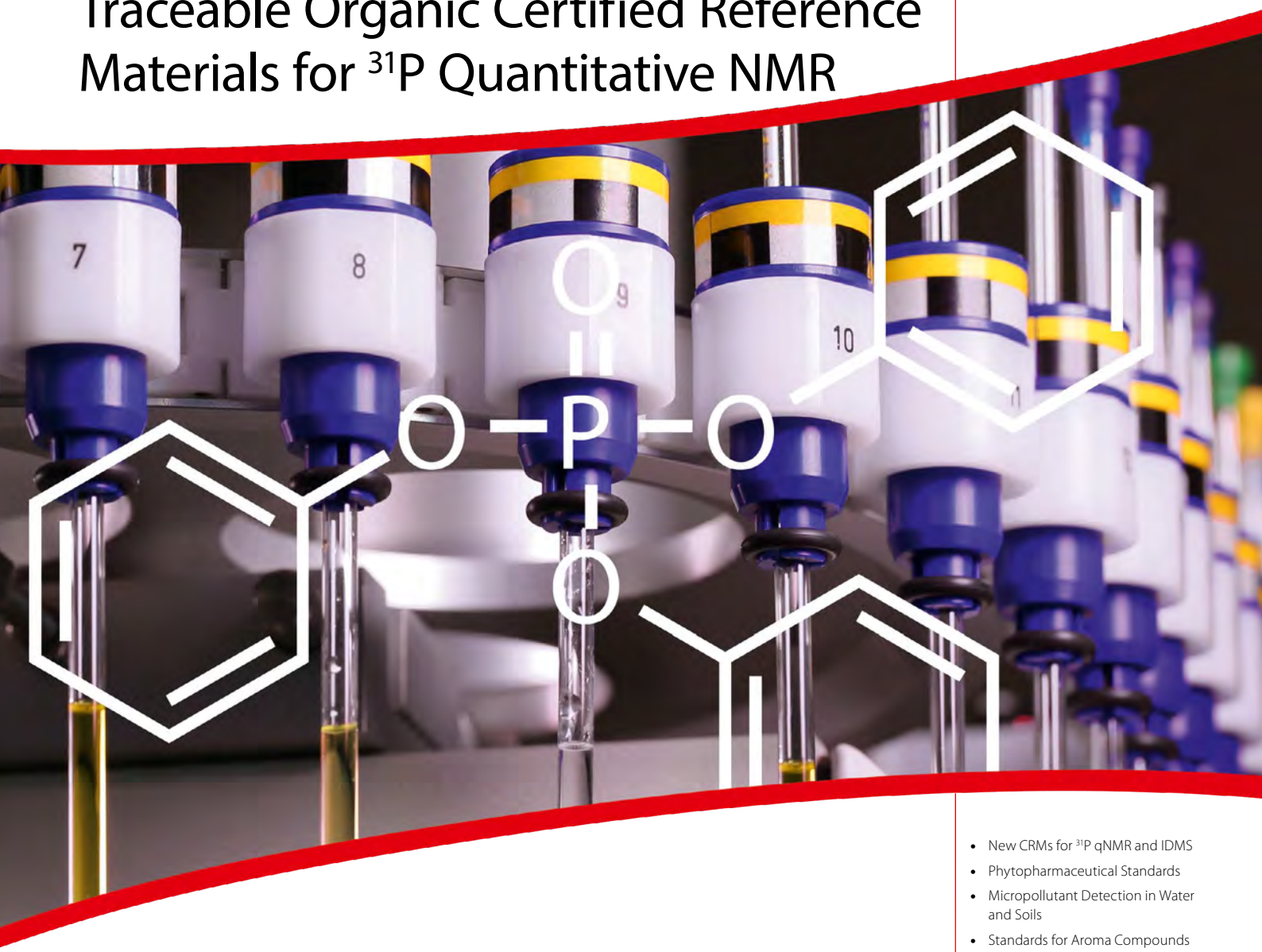
Traceable Organic Certified Reference Materials for ^{31}P Quantitative NMR



- New CRMs for ^{31}P qNMR and IDMS
- Phytopharmaceutical Standards
- Micropollutant Detection in Water and Soils
- Standards for Aroma Compounds
- Pesticide Metabolites Standards
- Antifungal Drug Monitoring
- Monodisperse Calibrants for MALDI-MS

Traceable Organic Certified Reference Materials for ^{31}P Quantitative NMR

Dr. Alex Rück
Supervisor R&D
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Dear Reader,

We all are aware of the physiological importance of phosphorus to various biological processes. It is essential for life, is part of phospholipids in cellular membranes, is part of DNA and RNA, plays a major role in energy metabolism in the form of ATP, and is extremely important in cell signaling. A large number of studies and publications are dedicated to the field of metabolism, accompanied by an increasing need for certified reference materials that can be used for the quantification of phosphorylated compounds and metabolites. Not all analytical methods are suitable for quantification, not every enzyme reaction can be detected by UV, and not everyone likes to work with ^{32}P radioactive detection.

QNMR has already shown its potential as a quantification method via proton NMR, making use of a universal reference standard which is not required to have the same chemical structure as the analyte. This allows the quantification of a large number of organic substances with only a handful of CRMs. The feature article on page 4 shows how this principle can also be applied to ^{31}P qNMR.

We started CRM development via ^{1}H qNMR in 2009, after having undergone a long process of method validation that led to successful accreditation according to ISO/IEC 17025 and ISO Guide 34. The first CRMs were able to be launched in 2010, and ongoing development since then has led to a list of currently 15 CRMs for ^{1}H qNMR and approximately 180 CRMs for chromatography. I recommend viewing the entire list at **sigma-aldrich.com/qnmr** and **sigma-aldrich.com/organiccrm**. We received a lot of feedback from the qNMR user community and many suggestions for new products. We are now able to present two new CRMs for ^{31}P qNMR as a novel alternative for phosphorus quantification. In addition to the qNMR article mentioned here, I trust that you will also enjoy reading the other interesting articles in this edition.

Best regards!

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Traceable Organic Certified Reference Materials for ^{31}P qNMR

New Tools for the Quantification of Phosphorus

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Phosphorus plays an important role in physiological processes since it is part of various significant molecules, such as nucleic acids (e.g. DNA), high-energy phosphates (e.g. ATP) and phospholipids. It is essential in the regulation of metabolism, since phosphorylation and dephosphorylation reactions are rapidly occurring processes at the protein level. Studies of physiological pathways, kinetics, metabolomics, and diseases, as well as biomarker discovery, are important fields of investigation, and there is a need for certified reference materials (CRMs) for the quantification of phosphorylated organic compounds and metabolites. The quantification by NMR offers several advantages as it is based on a signal comparison of the analyte with an internal or external reference standard. In contrast to other methods, such as chromatography, the reference standard is independent of the analyte's chemical structure. Moreover, using very pure primary reference materials, qNMR is a highly accurate method with low measurement uncertainty, which also provides traceability to SI units (Système International d'Unités) and thus offers the possibility of certifying reference materials for ^{1}H or other nuclei, such as ^{31}P. This article presents the concept of value assignment by ^{31}P qNMR measurements for the development of CRMs and describes different approaches to establish traceability to primary Standard Reference Material from the National Institute of Standards and Technology (NIST SRM) [1]. Water-soluble and phosphorus-containing CRMs are widely available, e.g. NIST SRM 194a ($NH_4H_2PO_4$), which can be used as a primary standard for establishing traceability in ^{31}P qNMR measurements. Unfortunately, the situation is different for phosphorus-containing primary CRMs that are soluble in common organic solvents, such as dimethylsulfoxide or methanol. No internationally accepted reference standard, such as a primary CRM from a national metrological institute (NMI), is currently available, and therefore, direct traceability to a NIST SRM is not possible in the case of organic candidates. For that reason, the concept was to perform quantification via the protons using ^{1}H qNMR and use the assigned content to determine the purity of an analyte via the phosphorus using ^{31}P qNMR. Phosphonoacetic acid is analyzed as a water-soluble CRM candidate, whereas triphenyl phosphate is a good candidate for use in organic solvents. The substances contain both nuclei, ^{1}H and ^{31}P. For the validation of the concept, triphenyl phosphate and phosphonoacetic acid have been used as ^{31}P qNMR standards to determine the purity of the analyte tris(2-chloroethyl) phosphate.

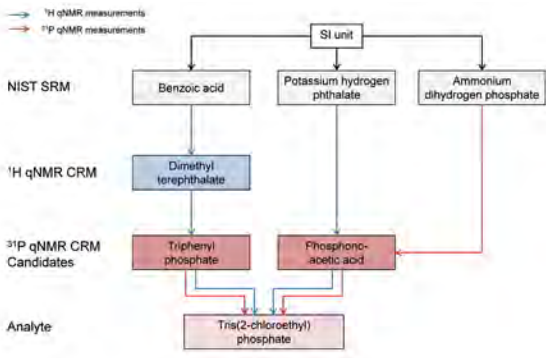

Figure 1 Concept for the quantification of two phosphorus candidate CRMs

Preliminary tests have been performed to check the chemical compatibility between sample and internal standard by acquiring a proton spectrum and, where required, a ^{31}P NMR spectrum of the mixture right after preparation and again after 24 h. It was necessary to exclude potential signal overlaps, as well as reactions between analyte and standard or reactions with the solvent. To ensure that no impurity was lying underneath the peaks of interest, 2D NMR experiments were applied where impurities of less than 0.05% signal intensity portion could be detected. Prior to the quantification measurements, the T1 relaxation times in different deuterated solvents were determined by inversion recovery experiments. Relaxation times should preferably be short, because the relaxation delay is adjusted to at least seven times T1. Since relaxation times may slightly vary in the mixture, the longest value in a mixture is used for calculating the overall relaxation time of the experiment. In a subsequent step, hygroscopy and volatility of the candidate substances were checked, since both attributes have a strong influence on the weighing results, and thus the outcome of the quantification measurement.

Quantification of the Candidate CRM Phosphonoacetic Acid

To prove the concept that no bias in a qNMR measurement is created when the purity is determined using either proton or phosphorus signals, the quantification of phosphonoacetic acid was performed by ^{31}P and ^{1}H qNMR measurements. In the first experiment, the content of phosphonoacetic acid was determined by ^{31}P qNMR using ammonium dihydrogen phosphate (NIST SRM 194a) as a reference, resulting in a purity value of 99.26% ± 0.75%. The second purity determination was carried out by ^{1}H qNMR measurement using potassium hydrogen phthalate (NIST SRM 84L) as a reference, resulting in a purity value of 99.32% ± 0.17%. These two results deriving from two independent traceability chains and via qNMR measurements of different nuclei are fully consistent and overlap within their expanded measurement uncertainties, as shown in **Figure 4**.

Quantification of the Candidate CRM Triphenyl Phosphate

After the ^{1}H and ^{31}P qNMR experiments for the quantification of phosphonoacetic acid proved the independence of the purity value determined from the nucleus, triphenyl phosphate could be quantified by ^{1}H qNMR. Direct application of benzoic acid as standard reference material in proton NMR was not feasible, due to the overlapping of the signals in the aromatic region. Therefore, the traceability chain was achieved by the use of the CRM dimethyl terephthalate (Fluka® 07038) as an internal reference standard that is traceable to NIST SRM 350b, benzoic acid. The content of triphenyl phosphate was determined to be 99.95% ± 0.22%. Although this value was achieved by ^{1}H qNMR measurements, it can be employed in subsequent ^{1}H and ^{31}P qNMR measurements as proven above. To remove any doubt regarding this conclusion, the concept was proven in a further step, in which a sample containing protons and phosphorus was chosen as the sample for the quantification by the two ^{31}P qNMR CRM candidates.

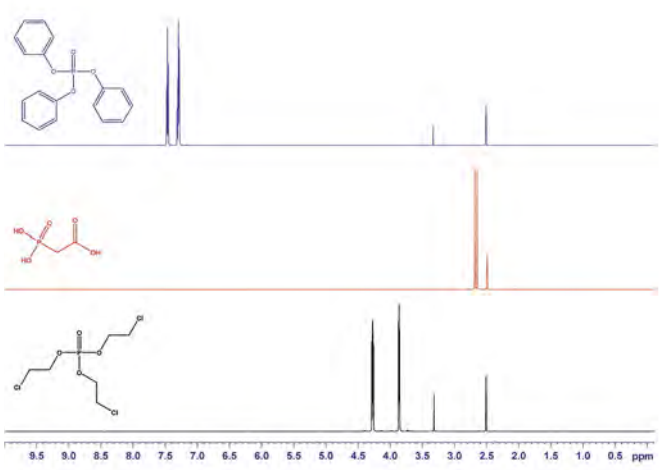

Figure 2 ^{1}H NMR spectra for triphenyl phosphate (blue), phosphonoacetic acid (red) and tris(2-chloroethyl) phosphate (black). All substances were dissolved in DMSO-d_6.

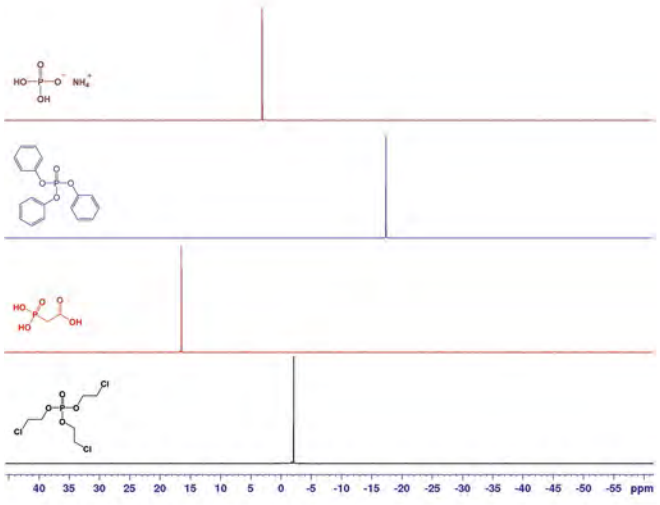

Figure 3 ^{31}P NMR spectra for ammonium dihydrogen phosphate in D_2O (brown), triphenyl phosphate in $CDCl_3$ (blue), phosphonoacetic acid in D_2O (red) and tris(2-chloroethyl) phosphate in $CDCl_3$ (black).

Quantification of Tris(2-Chloroethyl) Phosphate by ^{31}P qNMR

In the certification concept for phosphorus standards described, the chosen candidate compounds contain both ^{1}H and ^{31}P nuclei. After triphenyl phosphate and phosphonoacetic acid have been quantified via their protons and/or phosphorus, the resulting purity values were used for the subsequent purity determination by ^{31}P qNMR experiments. The quantification of tris(2-chloroethyl) phosphate on the basis of ^{31}P qNMR yielded purities of 98.43% ± 0.66% by using triphenyl phosphate as a reference, and 98.45% ± 0.44% by using phosphonoacetic acid as a reference. Additionally, the quantification of tris(2-chloroethyl) phosphate on the basis of ^{1}H qNMR yielded purities of 98.41% ± 0.53% by using triphenyl phosphate as a reference, and 98.88% ± 0.52% by using phosphonoacetic acid as a reference. All these results derived from different nuclei and different traceability chains are summarized in **Figure 5**, and the consistency of the data is demonstrated by the overlap of their expanded measurement uncertainties. A potential combination of triphenyl phosphate and phosphonoacetic acid for quantification within the same NMR tube was not possible, due to their different solubility (polar and non-polar). It is important to note that the experiments were independent of each other, and different measurement systems had to be tested in each case to find a suitable reference standard, selecting the appropriate deuterated solvent and elaborating the appropriate acquisition parameters, such as relaxation times.

Measurement Uncertainty

The quantification of the purity in the experiments shown is based on the so-called internal standard method, where the analyte signal is directly compared to an internal reference signal. This approach can be applied not only for ^{1}H, but also for ^{31}P signals. The CRM content is finally expressed as percent mass fraction. It should be noted that even minor uncertainty contributions from air buoyancy correction were taken into account. Therefore, climate data were recorded during each weighing step. The uncertainty calculation is based on well-established guidelines. The combined standard uncertainty is determined by statistical as well as systematic contributions; the statistical contribution arises from the repeatability of weighing and signal integration. Various systematic contributions, such as the air buoyancy correction, balance parameters, molecular masses, and the purity of the reference (expressed as a mass fraction), were taken into account. Phase correction and the integration of the signals were done manually and may differ slightly with the operator. This individual influence is considered in the overall uncertainty budget as "individual integration contribution". ^{31}P has a relative receptivity of 0.0665, which means it is less sensitive than ^{1}H (receptivity of 1.00), thereby leading to a lower signal-to-noise ratio. This can be compensated for by a higher analyte concentration or a higher number of scans, but it has to be considered that higher analyte concentrations can lead to reduced solubility and therefore higher viscosity or broadening of the signals.

Cat. No.	Brand	Description	Package Size
07038	Fluka®	Dimethyl terephthalate	1 G
96708	Fluka	Phosphonoacetic acid	1 G
05498	Fluka	Triphenyl phosphate	1 G

Table 1 Overview of used *Trace***CERT** CRM

Conclusions

The certification concept for ^{31}P qNMR CRM described has successfully shown how traceability to an SI unit can be established by qNMR using different nuclei. Within this concept, it was proven that purity values of a single material (phosphonoacetic acid (Fluka® 96708)) using ^{1}H or ^{31}P measurements are fully consistent with each other. Taking advantage of this concept, the content of triphenyl phosphate (Fluka 05498) was determined by ^{1}H qNMR, but the substance with its purity value was subsequently used as ^{31}P qNMR CRM. In an additional experiment, the purity of an exemplary analyte (tris(2-chloroethyl) phosphate) was measured following different traceability chains and solvent systems. Since the results are comparable within the range of their measurement uncertainties, the robustness of the certification concept is demonstrated. A possible application for the usage of these ^{31}P qNMR CRMs is given by the measurements of tris(2-chloroethyl) phosphate, a sample analyte. The work described in this article represents an important step towards a successful method validation for ^{31}P qNMR measurements. Find more information and all available CRMs for both ^{1}H and ^{31}P qNMR on our website **sigma-aldrich.com/qnmr**

References:

[1] Weber, M.; Hellriegel, C.; Rueck, A.; Wuethrich, J.; Jenks, P.; Obkircher, M. Method development in quantitative NMR towards metrologically traceable organic certified reference materials used as ^{31}P qNMR standards, *Anal Bioanal Chem*, DOI 10.1007/s00216-014-8306-6.

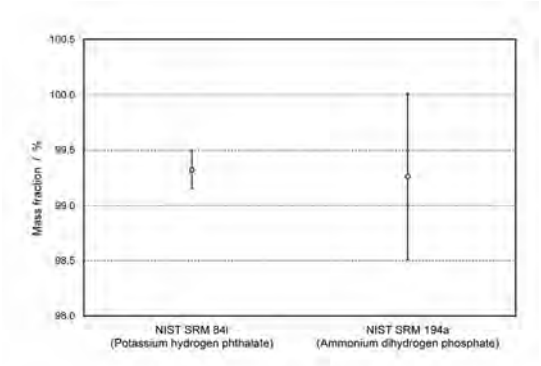

Figure 4 Graphical illustration of purity values and their expanded uncertainties for phosphonoacetic acid: Two different primary references from NIST were used for establishing traceability to SI unit. Potassium hydrogen phthalate was used as internal standard in ^{1}H qNMR measurements and ammonium dihydrogen phosphate in ^{31}P qNMR measurements.

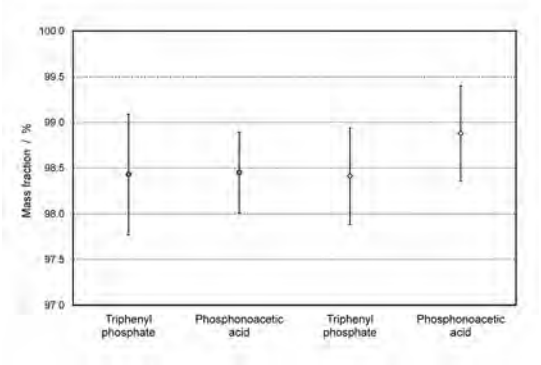

Figure 5 Graphical illustration of purity values and expanded measurement uncertainties for tris(2-chloroethyl) phosphate: Phosphonoacetic acid and triphenyl phosphate are used as internal standards, and the experiments are based on ^{31}P (circle) and ^{1}H (diamond) qNMR measurements.

*Trace*CERT® ICP-MS CRM Solutions

Launch of a New Series of 1mg/L Single-Element Solutions

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

The reliable analysis of elemental residues is of primary importance, not only for environmental testing, but also for quality control in industry. To a large extent, the classical chemical methods have been replaced by more modern spectroscopic methods. This is further demonstrated by the recent introduction of a new ICH Guideline for elemental impurities [1].

With Fluka® brand inorganic *Trace***CERT®** products, Sigma-Aldrich® provides a comprehensive portfolio of single and multi-element solutions for ICP and AAS. These products are highest-quality certified reference materials (CRMs) that are developed and produced according to ISO/IEC 17025 and ISO Guide 34 [2]. For the production, very well characterized highest-purity raw materials are used. *Trace***CERT** CRM are traceable to at least two independent references (i.e. NIST, BAM or SI unit kg) and are delivered together with comprehensive documentation including a proper uncertainty calculation, expiry date and storing/handling instructions.

The current portfolio comprises elemental concentrations of 1000mg/L and 10,000mg/L. This range is now complemented by a series of 1mg/L solutions of the most commonly tested elements. Due to the lower concentration, the delution working step for producing calibration series is much less laborious, which not only saves our customers time and reduces waste, but also minimizes potential error sources.

To guarantee maximum stability and reliability of the certified concentrations, the solutions are filled in 100 mL teflon bottles.

For further technical information on the *Trace***CERT** line and a complete product listing, please visit **sigma-aldrich.com/tracecert** or order our *Trace***CERT** brochure.

References:

[1] http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Step_4.pdf

[2] *Analytix 5*, **2009**, pages 13–15.

Cat. No.	Brand	Element	conc.	Package Size
12313	Fluka®	Cadmium	1 mg/L	100mL
41798	Fluka	Cobalt	1 mg/L	100mL
41621	Fluka	Copper	1 mg/L	100mL
56875	Fluka	Indium	1 mg/L	100mL
75015	Fluka	Lead	1 mg/L	100mL
04488	Fluka	Molybdenum	1 mg/L	100mL
42396	Fluka	Palladium	1 mg/L	100mL
67464	Fluka	Scandium	1 mg/L	100mL
69389	Fluka	Silver	1 mg/L	100mL
96672	Fluka	Strontium	1 mg/L	100mL
44266	Fluka	Titanium	1 mg/L	100mL
75593	Fluka	Vanadium	1 mg/L	100mL

Table 1 First series of 1mg/L elemental standards for ICP-MS

Phytopharmaceutical Standards

New Products for HMP Analysis

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

Sigma-Aldrich® offers more than 500 analytical standards for plant constituents, intended for the analysis of medicinal plants and herbal medicinal products (HMP). The portfolio comprises the most relevant active ingredients and marker substances of a wide variety of medicinal plants. New products are added continuously, and the most recent additions can be found in the table below.

On our website at **sigma-aldrich.com/medicinalplants**, an up-to date list of all phytopharma standards sorted in alphabetical order, by substance class and by plant genus can be found. In addition, the brand new phytopharma standards brochure can be ordered either on our website or using the business reply card of this Analytix issue.

Cat. No.	Brand	Description	Package Size
69139	Fluka®	Benzyl cinnamate	100 mg
67592	Fluka	Boldine	100 mg
43318	Fluka	Citral	1 mL
11532	Fluka	(-)-Dihydroquinine	50 mg
18224	Fluka	Fraxetin	50 mg
78251	Fluka	(Z)-Guggulsterone	10 mg
12448	Fluka	α-Humulene	250 mg
36428	Fluka	Keracyanin chloride	1 mg, 5 mg

Micropollutant Detection in Water and Soils

NEW Analytical Standards for APIs and Metabolites

Eva Katharina Richter, Product Manager Analytical Standards evakatharina.richter@sial.com

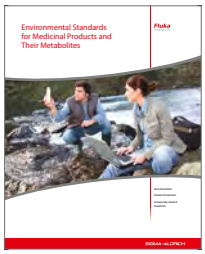

Figure 1 Brochure for API metabolite standards

Water sources such as rivers, lakes and groundwater that are used as drinking water resources have become contaminated throughout the world with a wide range of organic micropollutants. Usually, trace levels of pharmaceuticals do not get removed during wastewater treatment. For many substances, the potential short and long-term effects on humans and in aquatic ecosystems are not clearly understood, since there is not sufficient data available.

However, in 2006 the European Medicines Agency set guidelines for reporting total concentrations of medicinal products (sum of the parent and metabolites) that are being expelled into aquatic or terrestrial environments (EMEA/CHMP/SWP/4447/00). To enable our customers to follow these guidelines, we are pleased to offer a comprehensive portfolio of analytical standards for convenient detection of active pharmaceutical ingredients (APIs, **Table 1**) and their metabolites (**Table 2**) in water and soil. For more information and an up-to-date product list of all APIs and their metabolites, please visit us at **sigma-aldrich.com/pharmametabolites**, or download our brochure (**Figure 1**). There you will find all analytical standards sorted by their parent substance and corresponding metabolites.

Cat. No.	Brand	Description	Package Size
03716	Fluka®	(±)-Lisofylline	25 mg
32386	Fluka	(±)-Ketamine-(methyl-d_3) hydrochloride	10 mg
32388	Fluka	(±)-Metoprolol-(isopropyl-d_7) (+)-tartrate	10 mg
32387	Fluka	(±)-Propranolol-(isopropyl-d_7) hydrochloride	10 mg
32385	Fluka	4-Acetamido-d_3-antipyrine	10 mg
97582	Fluka	Acetazolamide	100 mg
50787	Fluka	Bisoprolol	25 mg
72579	Fluka	Cefaclor	50 mg
93813	Fluka	Cyclophosphamide monohydrate	100 mg
73538	Fluka	DL-4-Hydroxy-3-methoxymandelic acid	25 mg
16616	Fluka	Enalapril maleate salt	50 mg
68459	Fluka	Ethosuximide	100 mg
38701	Fluka	Iopromide	25 mg
78645	Fluka	Levetiracetam	10 mg
92574	Fluka	Mefenamic acid	250 mg
89287	Fluka	Mycophenolic acid	10 mg
76649	Fluka	Nafronyl oxalate salt	25 mg
52848	Fluka	Opipramol	25 mg
55591	Fluka	Oxymetazoline hydrochloride	100 mg
89604	Fluka	Paracetamol sulfate potassium salt	1 mg
90221	Fluka	Retigabine	25 mg
38956	Fluka	Simvastatin	10 mg
91369	Fluka	Sulfamethoxazole hydroxylamine	25 mg
07363	Fluka	Tenivastatin ammonium salt	25 mg

Table 1 *NEW* Analytical standards for APIs

Cat. No.	Brand	Description	Package Size
Allopurinol			
42688	Fluka	Oxypurinol	10 mg
Carbamezepine			
61347	Fluka	10,11-Dihydro-10-hydroxycarbamazepine	25 mg
93203	Fluka	Carbamazepine 10,11-epoxide	25 mg
Clarithromycin			
56291	Fluka	Clarithromycin N-oxide	25 mg
Diclofenac			
18477	Fluka	N-(2,6-Dichlorophenyl)-2-indolinone	100 mg
Dopamine			
11569	Fluka	3,4-Dihydoxyphenylacetic acid	25 mg
69673	Fluka	Homovanillic acid	25 mg
Estradiol			
57451	Fluka	6α-Hydroxyestradiol	1 mg
Estrone			
73532	Fluka	2-Methoxyestrone	25 mg
Fentanyl			
61338	Fluka	Norfentanyl monohydrate	25 mg
Paracetamol/Acetaminophen			
43073	Fluka	Paracetamol β-D-glucuronide	5 mg
Hydroxycarbamide			
56180	Fluka	Urea	1 g
Metamizole			
93574	Fluka	4-Methylaminoantipyrine monohydrate	25 mg
Metoprolol			
80073	Fluka	alpha-Hydroxymetoprolol	25 mg
80774	Fluka	O-Demethylmetoprolol	25 mg
Omeprazole			
43999	Fluka	5-Hydroxyomeprazole	5 mg
36231	Fluka	5-Hydroxyomeprazole sulfone	5 mg
43805	Fluka	Omeprazole 5-carboxylic acid disodium salt	5 mg
05699	Fluka	Omeprazole sulfide 5-carboxylic acid	5 mg
Prasugrel			
75254	Fluka	2-{1-2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl-4-{[2-3-(methoxy-d3) phenyl-2-oxoethyl]thio}-1,2,5,6-tetrahydropyridin-3-yl}acetic acid hydrochloride	1 mg
Primidone			
95923	Fluka	2-Ethyl-2-phenylmalonamide monohydrate	25 mg
Propranolol			
76149	Fluka	1,4-Dihydroxynaphthalene	50 mg
18994	Fluka	3-(1-Naphthalenyloxy)-1,2-propanediol	25 mg
Sulfadimethoxine			
50357	Fluka	N4-Acetylsulfadimethoxine	25 mg
Sulfamethoxazole			
73952	Fluka	4-Nitrososulfamethoxazole	10 mg
Sulfapyridine			
74339	Fluka	N4-Acetylsulfapyridine	25 mg
Sulfasalazine			
18858	Fluka	5-Aminosalicylic acid	100 mg
Tramadol			
94678	Fluka	O-Desmethyltramadol hydrochloride	25 mg
Valproic Acid			
07307	Fluka	(+)-6-Aminopenicillanic acid	100 mg
56298	Fluka	4-Fluorohippuric acid	50 mg
89981	Fluka	2-Propyl-2-pentenoic acid	25 mg
Venlafaxine			
93867	Fluka	O-Desmethylvenlafaxine	50 mg

Table 2 *NEW* API metabolite standards

Ensure Good Flavor and Aroma

NEW Standards for Aroma Compounds

Eva Katharina Richter, Product Manager Analytical Standards evakatharina.richter@sial.com

Sigma-Aldrich® provides a wide range of flavor and fragrance standards. Our current product portfolio of 600+ neat standards and standard solutions, including certified reference materials (CRMs), allows us to cover the extensive analytical needs of the food and cosmetic industry. For an overview, please download our brochure at **sigma-aldrich.com/flavor** or order a hard copy at **sigma-aldrich.com/lit-request**

Our newest additions are listed in **Table 1** and include a standard for Advantame®, a new no-calorie sweetener, which is now approved in the US and EU as a food additive. The substance is a derivate of aspartame and 20,000 times sweeter than sucrose. Visit **sigma-aldrich.com/sweeteners** to find our broad range of analytical standards for natural and artificial sugar substitutes.

In addition, we provide our customers with analytical standards for characteristic compounds of herbs and spices, cheese and other animal products, fruits, grains and alcoholic beverages. Learn more about analysis of phenolic aroma compounds in whiskey in *Analytix 05/2014*.

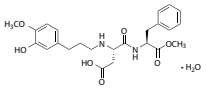

Figure 1 Molecular structure of advantame

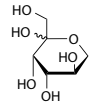

Figure 2 Molecular structure of tagatose

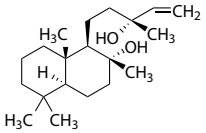

Figure 3 Molecular structure of sclareol

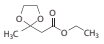

Figure 4 Molecular structure of fructone

Cat. No.	Brand	Description	Package Size
80699	Fluka®	(–)-Myrtenyl acetate	1 mL
42277	Fluka	(±)-2-Octanol	1 mL
80693	Fluka	(+/-)-2-Propyl-4-pentenoic acid	25 mg
09206	Fluka	(±)-alpha-Terpinyl acetate, predominantly alpha-isomer	1 mL
53666	Fluka	1-Furfurylpyrrole	1 mL
90968	Fluka	1,2-Ethanedithiol	1 mL
73537	Fluka	10-Undecenoic acid	1 mL
14643	Fluka	16-Hexadecanolide	100 mg
41513	Fluka	2-Hexanol	1 mL
75018	Fluka	1-Hexanethiol	1 mL
68225	Fluka	1-Octen-3-ol	5 mL
01984	Fluka	1-Penten-3-ol	250 mg
78245	Fluka	2,3,5,6-Tetrachloroanisole	100 mg
14239	Fluka	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	100 mg
57552	Fluka	2-Furoic acid	100 mg
52566	Fluka	2-Methyl-1-butanol	1 mL
03030	Fluka	2-Pentylfuran	1 mL
36333	Fluka	3-Hydroxybenzoic acid	100 mg
75024	Fluka	3-Methoxytyramine hydrochloride	250 mg
90961	Fluka	3-Methylindole	100 mg
62112	Fluka	3-Methylhexanal	100 mg
59702	Fluka	4-(4-Methoxyphenyl)-2-butanone	1 mL
98837	Fluka	4'-Hydroxyacetophenone	100 mg
49722	Fluka	4-Methyl-3-penten-2-one	1 mL
59932	Fluka	4'-Methylacetophenone	1 mL
44191	Fluka	9-Decenoic acid	100 mg
73536	Fluka	Acetovanillone	100 mg
80054	Fluka	Advantame®	100 mg
61982	Fluka	Amyl butyrate, mixture of isomers	1 mL
18018	Fluka	Allyl cyclohexane-propionate	1 mL
78975	Fluka	alpha-Methylbenzyl acetate	1 mL
92656	Fluka	Benzyl formate	1 mL
90966	Fluka	beta-Ionol	1 mL
03633	Fluka	Butyraldehyde diethyl acetal	250 mg
00921	Fluka	Capecitabine	25 mg
74597	Fluka	*cis*-3-Hexenyl acetate	1 mL
16679	Fluka	Cuminaldehyde	1 mL
08268	Fluka	Cyclopentanol	1 mL
75935	Fluka	D-(–)-Tagatose	50 mg
59581	Fluka	Decanal	1 mL
67373	Fluka	Dibenzyl disulfide	100 mg
80865	Fluka	Dibutyl sebacate	1 mL
55688	Fluka	Dihydromyrcenol	1 mL
73605	Fluka	Dimethyl succinate	1 mL
68203	Fluka	Ethyl nicotinate	1 mL
57698	Fluka	Ethyl nonanoate	1 mL, 5 mL
55441	Fluka	Ethyl oleate	250 mg
96374	Fluka	Ethylene brassylate	1 mL
77416	Fluka	Fructone	1 mL
77991	Fluka	gamma-Dodecalactone	1 mL, 5 mL
73105	Fluka	Glyceryl tributyrate	1 mL
77535	Fluka	Hexyl butyrate	1 mL
50803	Fluka	Hexylamine	1 mL
93762	Fluka	Hydrocinnamaldehyde	1 mL
69529	Fluka	Isoamyl butyrate	1 mL, 5 mL
68812	Fluka	Isobutyraldehyde diethyl acetal	1 mL
74598	Fluka	Isopropenyl acetate	1 mL
95197	Fluka	Kojic acid	100 mg
55977	Fluka	Lauric aldehyde	1 mL, 5 mL
41474	Fluka	Levulinic acid	100 mg
18299	Fluka	Maltol	100 mg
68982	Fluka	Methyl 2-octynoate	1 mL
59677	Fluka	Methyl dihydrojasmonate, mixture of *cis* and *trans*	1 mL
42958	Fluka	Methyl phenylacetate	1 mL
30194	Fluka	m-Tolualdehyde	1 mL
05169	Fluka	N-Methyl-2-pyrrolecarboxaldehyde	1 mL
55906	Fluka	Phthalide	100 mg
49944	Fluka	Sclareol	100 mg
90965	Fluka	sec-Butylamine	1 mL
08319	Fluka	Syringaldehyde	100 mg
90628	Fluka	*trans,trans*-2,4-Decadienal	1 mL
76717	Fluka	*trans*-2-Hexen-1-al	500 mg
41284	Fluka	Trimethylamine hydrochloride	100 mg
43341	Fluka	α,α-Dimethylphenethyl acetate	1 mL
91036	Fluka	β-Ionone	100 mg
05089	Fluka	δ-Tetradecalactone	1 mL

Table 1 *NEW* Flavor & fragrances

Pesticide Metabolite Standards

For Food and Environmental Testing

Eva Katharina Richter, Product Manager Analytical Standards evakatharina.richter@sial.com

In agriculture, pesticides are widely used to destroy or mitigate any pest. Active compounds of some pesticides may be converted by the target insects or plants or may be degraded in the environment. This may lead to a large number of metabolites being present at low levels in food and feed. Therefore, pesticide metabolites should also be monitored. In 2012, the EFSA recommended including active substances and all metabolites of toxicological relevance in dietary risk assessment.

We proudly offer our customers a wide range of pesticide metabolite neat standards and solutions in various solvents. Please find our newest additions in **Table 1**.

Cat. No.	Brand	Description	Package Size
34511	Fluka®	Bixafen Metabolite BYF00587	10 mg
34512	Fluka	Flufenacet Metabolite FOE5043	10 mg
32398	Fluka	Metalaxyl Metabolite CGA 62826	25 mg
32399	Fluka	Metalaxyl Metabolite CGA 108906	25 mg
05164	Fluka	(Aminomethyl)phosphonic acid	50 mg
34508	Fluka	Prochloraz Metabolite BTS40348	10 mg
34507	Fluka	Prochloraz Metabolite BTS44595	10 mg
34518	Fluka	Trifloxystrobin Metabolite CGA 321113	10 mg

Table 1 ***NEW*** Pesticide metabolite standards

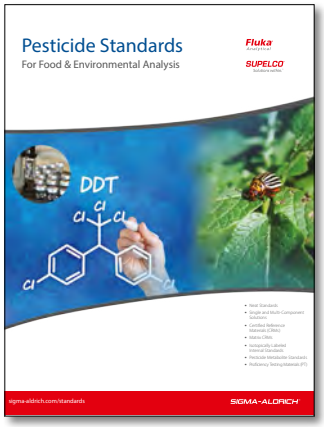

Figure 1 Pesticide Standards Brochure

Please visit us at **sigma-aldrich.com/pesticidemetabolites** and have a look at our broad portfolio. There you will find all analytical standards sorted by their parent pesticide and corresponding metabolites.

Did you know that Sigma-Aldrich® pesticide standards product line is the most comprehensive portfolio available on the market? We proudly offer more than 1300 high-purity pesticide and pesticide metabolite standards and certified reference materials for food and environmental analysis. For an overview, please order our brochure (**Figure 1**) or download at **sigma-aldrich.com/pesticides**

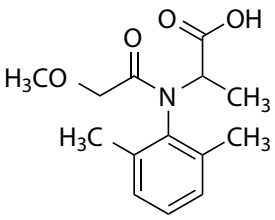

Figure 2 Molecular structure of metalaxyl metabolite CGA 62826

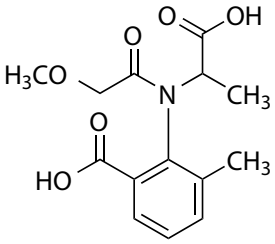

Figure 3 Molecular structure of metalaxyl metabolite CGA 108906

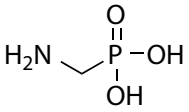

Figure 4 Molecular structure of (Aminomethyl)phosphonic acid

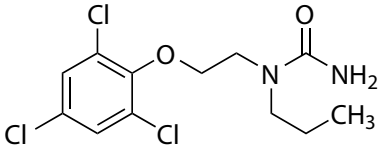

Figure 5 Molecular structure of prochloraz metabolite BTS44595

Therapeutic Drug Monitoring of Antifungal Agents

Antifungal Drug Certified Spiking Solutions®

Derrell Johnson, Manager, New Product Strategy and Tactical Marketing derrell_johnson@cerilliant.com
Maximilian Magana, Technical Marketing Specialist max_magana@cerilliant.com

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Antifungal drugs offer critically ill or immunocompromised patients multiple treatment options against invasive fungal infections. Use of the triazole class of antifungal drugs, such as itraconazole, posaconazole, voriconazole, and fluconazole, presents several clinical challenges in addition to their therapeutic benefits – interactions with co-administered drugs, concentration-dependent toxicity, and variations in drug absorption and metabolism.[1] The impact of triazole antifungals on drug metabolism and drug interactions is a result of their ability to act as a substrate or inhibitor of cytochrome P450 (CYP) metabolic enzymes.[2,3]

Antifungal drug interactions with co-administered drugs can decrease or increase concentrations of the interacting drugs.[3] The former outcome can reduce drug efficacy below therapeutic levels while the latter outcome can increase toxicity of other therapeutic medications.[2,3] Since critically ill or immunocompromised patients – those at highest risk of developing invasive fungal infections – often require multiple drug regimens, any drug interaction must be carefully managed to prevent toxicity and to ensure therapeutic efficacy.[2]

Therapeutic drug monitoring (TDM) is considered for triazole antifungal drugs to avoid adverse clinical outcomes and to ensure effectiveness of the drug regimen. TDM is used to guide dosing levels and is valuable in assessing the effect of a drug-drug interaction between the antifungal and co-administered drugs.[3] It involves quantification of circulating drug concentrations to determine a patient's exposure to the therapeutic drug.[2] Clinical situations that may favor use of TDM include interacting drugs, pharmacokinetic variability, changing pharmacokinetics, poor prognosis diseases, and pharmaceuticals that exhibit narrow therapeutic ranges between efficacy and toxicity.[2,4]

Analytical techniques from bioassay and HPLC/UV to LC-MS/MS have been used to measure serum concentrations of antifungal drugs. While bioassays are inexpensive and simple to perform, they are subject to interferences from other drugs, including other antifungals, and may measure combined activity of the parent drug and metabolites (e.g. itraconazole). HPLC/UV offers a widely available technology with commercially-available assays that can quantify multiple drugs in a single sample. HPLC/UV methods may produce long run times and are also subject to interferences. Despite the greater expense and technical challenges, LC-MS/MS has recently been adopted for antifungal TDM methods by clinical testing laboratories as a result of the technique's higher sensitivity and specificity. LC-MS/MS methods also allow for greater efficiency by quantifying multiple drugs in a single sample.[4]

The development of LC-MS/MS methods for antifungal TDM has increased the need for highly accurate Certified Reference Materials (CRMs) of antifungal drugs and their active metabolites. Accuracy in concentration of the calibrator or control is dependent on proper certification of the reference material. Insufficient characterization and/or use of low-purity research-grade materials in place of CRMs can potentially result in incorrect therapeutic reference ranges and can negatively impact clinical outcomes.[5] Lot-to-lot differences in purity of the reference material or its impurity profile can also impact the test's accuracy and reproducibility if not properly accounted for. Accuracy of the reference standard – and ultimately the test result – is vital to measurement, value assignment, and critical decision making during management of patient care involving antifungal drug treatments.

View Cerilliant's extensive catalog offering of Certified Spiking Solutions® of triazole, imidazole, and other azole antifungals at **sigma-aldrich.com/cerilliant**

Register at **sigma-aldrich.com/registercerilliant** to receive news on the latest reference standards introduced by Cerilliant.

Cerilliant Certified Spiking Solutions® of Antifungals

Cat. No.	Description	Package Size
F-030	5-Fluorocytosine	2.0 mg/mL in Methanol
F-031	Fluconazole	2.0 mg/mL in Methanol
F-035	Fluconazole-$^{13}C_3$	1.0 mg/mL in Methanol
H-110	Hydroxyitraconazole	*Coming Soon*
H-111	Hydroxyitraconazole-D_4	*Coming Soon*
I-015	Itraconazole	2.0 mg/mL in Methanol with 1% 1M HCl
I-021	Itraconazole-D_4	1.0 mg/mL in Methanol with 1% 1M HCl
K-004	Ketoconazole	2.0 mg/mL in Methanol
K-005	Ketoconazole-D_4	1.0 mg/mL in Methanol
M-183	Metronidazole	2.0 mg/mL in Methanol
P-103	Posaconazole	2.0 mg/mL in Methanol
P-108	Posaconazole-D_4	1.0 mg/mL in Methanol
V-032	Voriconazole	2.0 mg/mL in Methanol
V-036	(±)-Voriconazole-D_3	1.0 mg/mL in Methanol

References:

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[5] Marek, B.; Cooper, J.; Dilek, I.; Rettinger, M.; Kirkovits, G.; Chen, W.; Ying, Y.; Sreenivasan, U.; Crawford, M.; Holmquist, B.; Grant, R. Accurate Reference Standards for Accurate Quantitation of Thyroid Hormones: Impact on Clinical Reference Ranges. Presented at Mass Spectrometry: Applications to the Clinical Lab, San Diego, CA, February, **2013**. Accessed 7 January 2015 from: http://www.cerilliant.com/Shoponline/OpenDocument.aspx?DocumentId=177

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Timestrip®

The Smart Way to Measure Time and Temperature

Daniel Weibel, Product Manager Analytical Reagents daniel.weibel@sial.com

In issue 1/2015 of *Analytix,* we introduced 13 new stock products from Timestrip's time and temperature indicator ranges. In this issue, we focus on the temperature range and demonstrate how Timestrip's agile technology can be adapted to create customized products for individual customers or specific markets.

Timestrip PLUS

Timestrip PLUS is a range of ascending temperature indicators. Each one indicates if a maximum temperature threshold has been breached and tracks irreversibly the cumulative time of all breaches (up to 12 hours depending on the threshold).

For example, the Timestrip PLUS 8 °C is chosen to monitor a shipment taking 3 weeks. When the shipment arrives, the indicator shows 4 hours of 8 °C breach. This breach could have happened in one long event or from a series of shorter breach events across the 3 weeks. The Timestrip PLUS 8 °C could have indicated up to 8 hours of temperature breaches during that 3 weeks. They are self-adhesive, 19 x 40 mm and can be kept inert at room temperature until the activation button is squeezed. Currently on stock are five Timestrip PLUS indicators covering a wide range of ascending temperature thresholds: - 20 °C, 0 °C, 8 °C, 25 °C and 30 °C (**Figure 1**).

Figure 1 Timestrip PLUS 8 °C monitors temperature breaches of >8 °C for up to 8 hours.

Customizing Timestrip PLUS

Timestrip have harnessed the PLUS technology to create market-specific stock products such as Timestrip Duo 10 °C & 34 °C. All customized products have activation buttons so they can be stored inert until needed.
Duo cleverly combines a 10 °C and a 34 °C indicator into one unit, and its increased length (19 x 80 mm) means it can monitor longer time periods for the 10 °C threshold: 3, 8, and 14 days of breach, as well as 3 hours for the 34 °C threshold (**Figure 2**).

Duo can be used for other product categories that require the same specifications, but new variants can be made to suit your particular needs. Subject to minimum order quantities and technical criteria, you can change the graphic color, add your brand, choose the temperature thresholds, and change the time markers.

Figure 2 Timestrip Duo 10 °C and 34 °C is a customized version of two temperature breach indicators in one label.

Timestrip has created two graphic concepts to show how Timestrip PLUS could be customized to monitor excessive temperature for a product with a cooling-chain upper limit of 10 °C (**Figures 3a** and **3b**). **They are illustrative concepts only and are not available for purchase from stock.** Each custom product requires full discussion to ensure the specification is technically feasible and appropriate. Activation buttons are on the back.

a)

b)

Figure 3 a) a Custom Timestrip PLUS monitoring breaches of 10 °C for up to 7 days; b) a Custom DUO monitoring breaches of 10 °C for 2, 5, and 7 days and 30 °C for 3 hours.

Timestrip also makes time indicators (reminders from 5 minutes to 2 years). For more information or to suggest new Timestrip applications or products, please contact us at **timestrip@sial.com**

Cat. No.	Brand	Description
93064	Fluka®	Timestrip 1 month
07603	Fluka	Timestrip 3 months
06797	Fluka	Timestrip 6 months
74831	Fluka	Timestrip 12 months
03849	Fluka	Timestrip 3 months, key chain
06929	Fluka	Timestrip 12 months, key chain
06693	Fluka	Timestrip Plus -20 °C
92210	Fluka	Timestrip Plus 0 °C
08168	Fluka	Timestrip Plus 8 °C
92451	Fluka	Timestrip Plus 25 °C
80474	Fluka	Timestrip Plus 30°C
80476	Fluka	Timestrip Plus Duo 10 °C and 34 °C

Table 1 Timestrips available from Sigma-Aldrich in package sizes of 10EA, 100EA and 500EA

For additional information on Timestrip indicators, visit our website **sigma-aldrich.com/timestrip**

Organic *Trace*CERT® Standards as Reference Materials for Isotope Dilution Mass Spectrometry

Characterization of stable isotope labeled caffeine as reference material

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Christine Hellriegel, Senior Scientist R&D christine.hellriegel@sial.com

Introduction

Isotope Dilution Mass Spectrometry (IDMS) is a very precise method to determine concentrations of a given analyte in pure materials or in every type of matrix. This technique is based on the direct proportionality of mass fraction ratio and signal intensity ratio of the natural isotope and an isotopically labeled form of the target analyte. Since the measurement of the signal ratio is directly traceable to an SI unit (mass), this technique is a primary method like qNMR. A closer look at mass spectrometry shows some advantages over NMR or titration, such as higher sensitivity, analysis of matrix reference materials and independence of solvent selection[1–5].

Experimental

Most important for precise IDMS results is the exact determination of the intensity ratio of caffeine and caffeine-$^{13}C_3$. Therefore a large number of single spectra are acquired to minimize the noise caused by the ionization process. Trapping a peak in an injection loop and a slow infusion with a second pump into the ion source allows the acquisition of many more spectra than the direct detection of a chromatographic peak. Peak trapping or direct injection reduces the uncertainty of IDMS data, even of fast scanning mass spectrometers[1–2].

In this particular case, mixtures of labeled and unlabeled caffeine with varying mass fraction ratios were weighed in a sample vial and dissolved in water. Since the labeled caffeine is analyzed as sample, the unlabeled *Trace***CERT** Caffeine (Fluka® 56396) serves as reference standard to determine the content of caffeine-$^{13}C_3$. The total mass of the mixtures was ca. 10 mg. All sample solutions were diluted 1:100 and injected into a 10 µL sample loop and introduced into the ion source with a syringe pump. The mobile phase is water/methanol (50:50, v/v).

^{1}H qNMR data of the labeled caffeine were acquired in parallel to the IDMS experiments using maleic acid as reference material. The resulting caffeine content represents the total amount of all caffeine species.

Results

The technique presented reduces the uncertainty contribution of typical noise of ESI data due to the larger number of acquired spectra and the improved statistics of the calculated intensity ratios of 195/198 m/z (caffeine/caffeine-$^{13}C_3$).

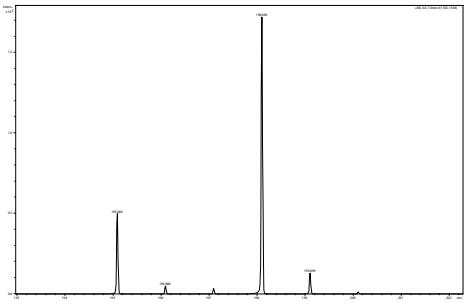

Figure 1 The intensity ratio of 195 and 198 m/z is calculated from this spectrum and compared with the average of the intensity ratios of the single mass spectra.

Calibration Curve of the Different Mass Fractions m(Caffeine)/m(Caffeine-$^{13}C_3$)

Plotting the intensity ratios I(198)/I(195) or I*/I versus the corresponding mass fractions m(caffeine-$^{13}C_3$)/m(caffeine) or m*/m results a straight line (s. **Figure 2**) indicating no interferences between labeled and unlabeled caffeine.

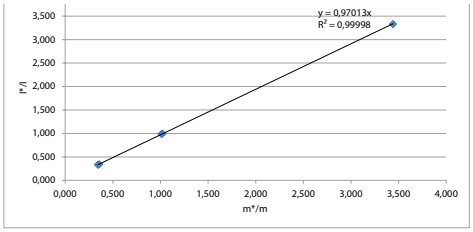

Figure 2 Calibration curve calculated from the 6 individual caffeine/caffeine-$^{13}C_3$ mixtures at 3 m*/m ratios (5 mg/15 mg, 10 mg/10 mg, 15 mg/ 5 mg).

$$\frac{I^*}{I} = \frac{m^* M x^*}{m M^* x}$$

$$\Leftrightarrow x^* = \frac{I^* \cdot m \cdot M^*}{I \cdot m^* \cdot M} \cdot x$$

The unknown content of caffeine-$^{13}C_3$ can be calculated according to equation [1] for each single sample or from the slope of the calibration curve.

Finally the IDMS experiments result in a content of 98.6% +/- 0.5% caffeine-$^{13}C_3$. qNMR results in 98.2% +/- 0.3% and the content according to the Certificate of Analysis of Aldrich 485365 Lot EB0276V is 98.5% caffeine-$^{13}C_3$.

References

[1] Breidbach, A. Improved precision of measured isotope ratio through peak parking and scan-based statistics in IDMS of small organic molecules, *ThOS36-02*, IMSC, Geneva, **2014**.
Breidbach, A. et al. Two-dimensional heart-cut LC-LC improves accuracy of exact-matching double isotope dilution mass spectrometry measurements of aflatoxin B1 in cereal-based baby food, maize, and maize-based feed, **2014**, *Anal. Bioanal. Chem.*, Springer, DOI 10.1007/s00216-014-8003-5.

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SpheriCal is a series of monodisperse calibration standards that offer mass spectrometry users time-saving sample preparation plus highly-accurate calibration. By combining excellent long-term stability with evenly-spaced calibration points, SpheriCal helps users increase the frequency and quality of their MALDI-MS calibrations.

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About Polymer Factory

Polymer Factory is a company with headquarters in Stockholm, Sweden.

Established in 2005, Polymer Factory focuses on developing biocompatible and monodisperse dendrimers and dendron based on 2,2-bis(methylol)propionic acid, where the company has the exclusive right to the production, marketing and sales of such materials. The company provides the world's largest library of advanced dendritic materials, including hyperbranched polymers as well as multifunctional dendritic PEGs.

Polymer Factory supports research and development projects in a broad array of applications, ranging from nanotechnology, medicine, photonic application, catalysis, click chemistry, and MALDI-TOF-MS calibrants to semiconductor materials.

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Provided in five different molecular weight ranges, each product vial contains 5 mg of four different dendritic calibration masses, whose proportions are optimized with respect to the matrix and added cation. Each vial of these "Just Add Solvent" calibrants is sufficient for at least 25 calibrations of your MALDI-MS, based on our recommended protocols.

SpheriCal® Neat

SpheriCal Neat is provided without any matrix or added cation. The freedom of this product line will enable you to explore your own calibration protocols for optimization purposes. SpheriCal Neat is shipped in vials containing 50 µg standard and is sufficient for at least 25 calibrations.

SpheriCal® Aqua Neat

SpheriCal Aqua has proven to be compatible for calibration with conditions commonly used in life-sciences, e.g., MALDI-MS matrices DHB, HCCA or SA and solvents such as water and acetonitrile. SpheriCal Aqua Neat is shipped in vials containing 50 µg standard and is also sufficient for at least 25 MALDI-MS calibrations.

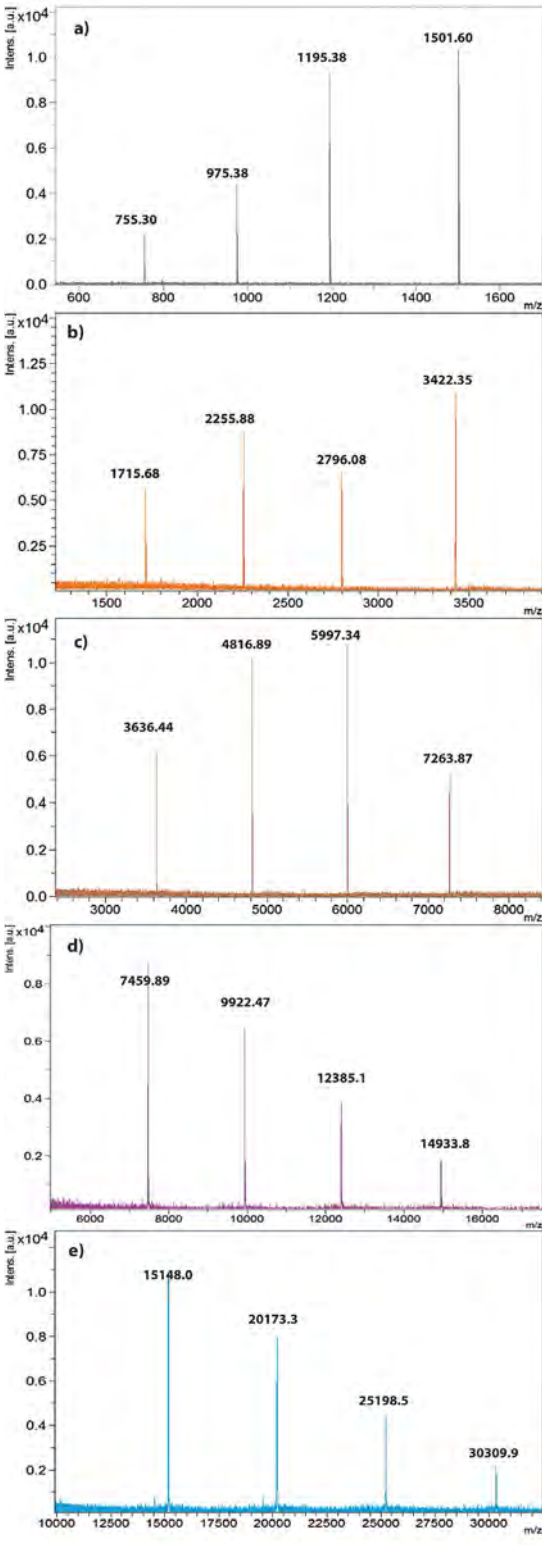
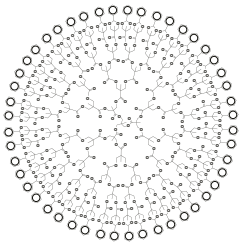


Figure 1 Mass spectra of SpheriCal mass calibrants for specific mass ranges



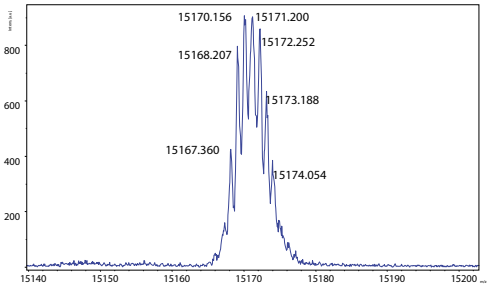
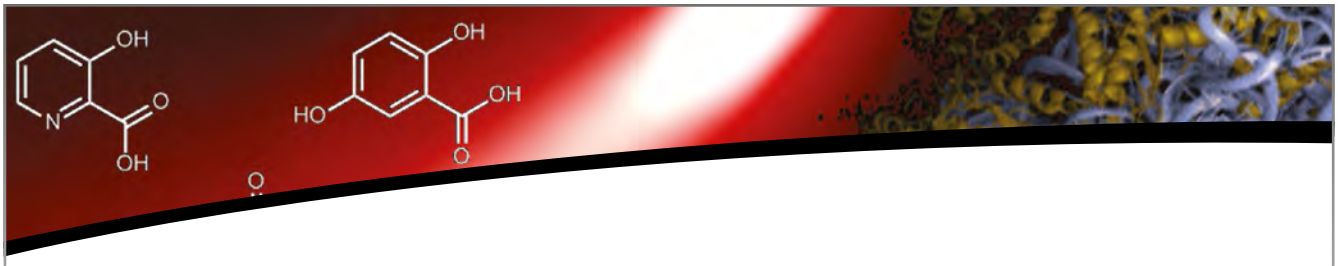


Figure 2 $[M + Na]^+$ of 15,171.2. Mass spectrum obtained using a Bruker Ultraflex MALDI-TOF mass spectrometer

These values are experimental, not calculated.

Cat. No.	Brand	Product Name	Mol. Wt. Range	Description	Package Size
PFS11	Fluka	SpheriCal® Mix - Peptide Low Range	500 – 1600 Da	9-Nitroanthracene:Na^+ (Matrix:Counter Ion)	5 mg
PFS12	Fluka	SpheriCal® Mix - Peptide Medium Range	1600 – 3500 Da		5 mg
PFS13	Fluka	SpheriCal® Mix - Peptide High Range	3500 – 7500 Da		5 mg
PFS14	Fluka	SpheriCal® Mix - Protein Low Range	7500 – 15000 Da		5 mg
PFS15	Fluka	SpheriCal® Mix - Protein Medium Range	15000 – 30000 Da		5 mg
PFS20	Fluka	SpheriCal® Neat - Peptide Low	500 – 1600 Da	Dry Solid	5 mg
PFS21	Fluka	SpheriCal® Neat - Peptide Medium	1600 – 3500 Da		50 µg
PFS22	Fluka	SpheriCal® Neat - Peptide High	3500 – 7500 Da		50 µg
PFS23	Fluka	SpheriCal® Neat - Protein Low	7500 – 15000 Da		50 µg
PFS24	Fluka	SpheriCal® Neat - Protein Medium	15000 – 30000 Da		50 µg
PFS30	Fluka	SpheriCal® Aqua Neat - Peptide Low	500 – 1000 Da	Dry Solid	50 µg
PFS31	Fluka	SpheriCal® Aqua Neat - Peptide Medium	1000 – 2300 Da		50 µg
PFS32	Fluka	SpheriCal® Aqua Neat - Peptide High	2300 – 5200 Da		50 µg
PFS33	Fluka	SpheriCal® Aqua Neat - Protein Low	5200 – 10700 Da		50 µg

Table 1 Sigma-Aldrich's product offering for SpheriCal® mass calibrants



Matrix Substances for MALDI-MS

Choosing the right matrix for the analyte of interest is essential for any successful MALDI analysis. Sigma-Aldrich® offers the most comprehensive selection of pure and ultra-pure matrices for MALDI-MS, covering matrices suitable for many different analytes, such as proteins, peptides, polymers, lipids, oligonucleotides, and many more. Modern Ionic Liquid Matrices (ILMs) extend the unique properties of ionic liquids to MALDI-MS.

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Cobalt(II) *tert*-Butyl-Salophen as Ionophore in Nitrite-Selective Electrodes

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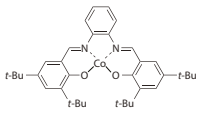

Figure 1
Structure of the nitrite ionophore VI (03721) [1, 2]

Introduction

Nitrogen is primarily found in the atmosphere in its gaseous form. Its incorporation into terrestrial nitrogenous compounds such as ammonia, nitrate and nitrite takes place along a number of different pathways, including microorganisms, plants and man himself through his industrial and agricultural activities (nitrogen cycle, **Figure 2**). The bacterial conversion of ammonia to nitrate (nitrification) proceeds via the intermediate formation of nitrite.

In food, nitrite can be formed *via* the reduction of nitrate in the presence of certain metals, e.g. during the cooking of food in aluminum utensils. Nitrite salts are used as preservatives in food products, especially meat, inhibiting the growth of several undesirable microorganisms. However, the use of nitrites may lead to the formation of carcinogenic nitrosamines. Therefore, nitrite is regulated throughout the world, e.g. nitrite in drinking water is regulated in Europe with a maximum level of 0.5 mg/l [3].

Typically, nitrite is measured spectrophotometrically, either by *Griess reaction* [4, 5] or other colorimetric procedures.

On the other hand, the advantage of a potentiometric sensor is the application ***in-situ***.

Application

The nitrite-selective PVC-based membrane was prepared in the classical manner [6, 7]; for the exact membrane composition see **Table 1**. All solutions are prepared in citrate buffer (5mM, pH 3.1). The experimental conditions are stated in **Table 2**.

The ionophore shows high selectivity against other interfering anions (perchlorate, nitrate, chloride and bromide) as well as a slightly higher interference towards thiocyanate, **Table 3**.

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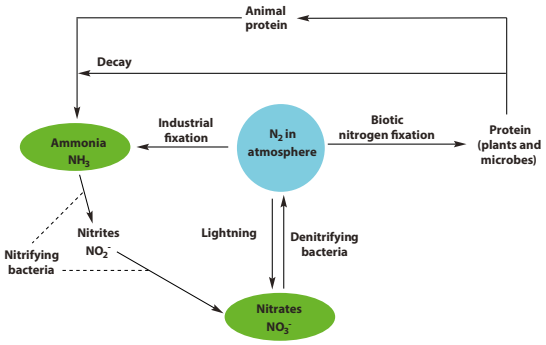

Figure 2 The nitrogen cycle

Cat. No.	Description	Wt%
03721	Nitrite ionophore VI	1.97
84818	Bis(2-ethylhexyl)sebacate	65.08
91661	Tridodecylmethylammonium chloride	0.39
81392	Poly(vinyl chloride)	32.54

Table 1 Membrane composition

Reference electrode Ag, AgCl
Inner electrolyte 3 M KCl outer electrolyte 1 M LiOAc
Ion-selective electrode
Internal solution 0.001 M $NaNO_2$ in 5 mM citrate buffer pH 3.1 + 1 mM NaCl
Conditioning 18h in 0.01 M $NaNO_2$ in 5 mM citrate buffer pH 3.1

Table 2 Experimental conditions

All measurements are performed in citrate buffer, pH 3.1.

Linearity 0.075 – 1.01 x 10^{-4} mol/l NO_2^-			
Slope -59.09 mV/dec			
Detection level 5.08 x 10^{-5} mol/l NO_2^-			
Selectivity coefficients			
$\log K^{Pot}_{NO2,SCN}$	-1.53	$\log K^{Pot}_{NO2,Cl}$	-3.04
$\log K^{Pot}_{NO2,ClO4}$	-2.31	$\log K^{Pot}_{NO2,Br}$	-7.19
$\log K^{Pot}_{NO2,NO3}$	-3.64	$\log K^{Pot}_{NO2,H2PO4}$	-4.23

Table 3 Electrode characteristics and selectivity

References:

[1] Néel, B.; Asfhar, M.G.; Crespo, G. A.; Pawlak, M.; Dorokhin, D.; Bakker E., *Electroanalysis*, **2014**, 26, 473–480.
[2] Peretti, C. L.; Ajiro, H.; Cohen, C. T.; Lobkovsky, E.B. *Journal of the American Chemical Society*, **2005**, 127, 11566–11567.
[3] Council Directive 98/83/EC
[4] Adornato, L.R; Kaltenbacher, E.A.; Villareal, T.A.; Byrne, R.H. Deep Sea Res. Part I: *Oceanograph.Res.Papers*, **2005**, *52*, 543–551.
[5] EPA Method 353.2
[6] Selectophore Brochure Ion Sensor Materials, Sigma-Aldrich, **2011**, *13*.
[7] Malon, A.; Radu, A.; Qin, W.; Qin, Y.; Ceresa, A.; Maj-Zurawska, M.; Bakker, E.;Pretsch, E. *Analytical Chemistry*, **2003**, 75, 3865–3871.

Determination of Osmium with Novel *Trace*SELECT® Reagents

… by Application of Post-Digestion Stabilization in Combination with ICP-MS

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Over the last couple of years, determination of trace-level content of osmium has become increasingly important. In pharmaceutical quality control, this is driven by upcoming changes from both USP and Ph. Eur, and the current draft of the harmonized guideline ICH Q3D [1]. The maximum concentration of osmium allowed in pharmaceuticals will depend on type of exposure (oral, parenteral or inhalation) and daily dose, and can be as low as <0.1 ppm. This requires sample preparation and determination methods that reliably reach sufficiently low determination limits.

Traditionally, pharmaceutical samples are digested by closed-vessel microwave digestion to avoid loss of element prior to instrumental trace-metal analysis, and to get the pharmaceutical sample into a clear solution. Concentrated nitric acid (HNO_3), in combination with hydrogen peroxide (H_2O_2) or hydrochloric acid (HCl), is typically used to mineralize these organic matrices.

Osmium poses some inherent difficulties for traditional inorganic trace analysis with AAS or ICP instruments, as it often forms highly volatile osmium tetroxide (OsO_4). This leads to an overestimation of osmium (recoveries higher than 300%) and loss of osmium during storage of the reference materials used for calibration.

Venzago et al. recently published a method for post-digestion stabilization of osmium which employs a combination of acetic acid, thiourea and ascorbic acid [2]. In this article, we highlight how *Trace***SELECT** reagents, including the recently available high-quality thiourea, can successfully be used following the published method.

After a closed-vessel microwave digestion procedure with nitric acid, all samples, which in this case contained polysaccharides, and all calibration standards were stabilized in a solution containing *Trace***SELECT** grade acetic acid (0.5% v/v), thiourea (0.76 g/L) and ascorbic acid (0.1 g/L). This led to recoveries of osmium from 83–94% at 0.5 ppb level, while performing the same procedure without stabilization resulted in recoveries of over 300%.

All reagents should be used at the highest available purity to minimize the determination limit and to regularly obtain reliable results from the prepared stabilization solution. *Trace***SELECT** grade reagents are ideal as they are specified to contain less than 10 ppb or lower in most individual trace impurities.

Cat. No.	Featured and Related Products	Package Size
30885	***NEW!*** Thiourea, *Trace***SELECT**	5 g
45727	Acetic acid, *Trace***SELECT**	500 mL, 1 L, 2.5 L
05878	Ascorbic acid, *Trace***SELECT**	100 g
84385	Nitric acid, *Trace***SELECT**	250 mL, 500 mL, 1 L, 2.5 L, 5 L
93468	Transition metal mix 3 for ICP, *Trace***CERT** (Ru, Rh, Pd, Os, Ir, Pt and Au, each 100 mg/L)	100 mL
39967	USP 232 Element Impurities Standard 2, *Trace***CERT** (Ir, Os, Pd, Pt, Rh and Ru, each 100 mg/L)	100 mL

References

[1] ICH "Guideline for Elemental Impurities Q3D", step 4 version.
[2] Venzago, C. et al., J. *Anal. At. Spectrom.*, **2013**, 28, 1125.

Water Determination in Chocolate Confectionery

By Karl Fischer Titration with Hydranal® Reagents

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

Cocoa and chocolate have a fascinating history. Pre-Columbian cultures in Latin America cultivated cacao trees over 3,000 years ago. The main use was a chocolate beverage called xocolatl. In Mayan and Aztec cultures the cacao beans were not only used for xocolatl, but also as a very valuable currency. In the early 16th century, Spanish conquerors brought the first cacao beans back in their ships, and chocolate began to conquer Europe.

The largest consumers of chocolate today are the Western countries, while the largest cacao-producing countries are Ivory Coast, Ghana and Indonesia. Nearly 75% of the world's cocoa crop comes from Africa [1]. The tropical cacao tree, Theobroma cacao, probably originated in the northern part of South America, where it grows in a warm, moist climate. Evergreen, it flowers all year round, and it yields 20 to 50 ripe fruits per tree. The cacao beans are the seeds of these fruits [2].

After the ripe cacao pods have been harvested, the cacao beans are removed from the pods and left to ferment for five to seven days. The fermentation process removes all remaining fruit pulp from the beans naturally, while the beans change their color and develop a wealth of aroma compounds. After fermentation, the beans are dried to stop the fermentation process and reduce the moisture content for storage.

Additional processing steps which lead to cocoa powder and chocolate are described in **Figure 1**. The beans' water content is a very important factor and should be between 6 and 8%. If the water content is too high, microorganisms can infest the beans and their quality will suffer. After the beans have been roasted, the water content is further decreased to approximately 3%.

Regulations

Water content is also an important parameter for chocolate and chocolate products, and must therefore be closely monitored. The Swiss Food Register states in method '1010.1 Determination of Water Content of Cocoa and Cocoa Products, acc. to Karl Fischer' that a homogenous sample, if necessary finely grated or melted for two hours at 40 ± 2 °C, can be titrated in a 1:1 mixture of methanol and chloroform as a Karl Fischer (KF) working medium. AOAC International also gives an official method for 'Moisture in Cacao Products, by Karl Fischer method, 977.10'.

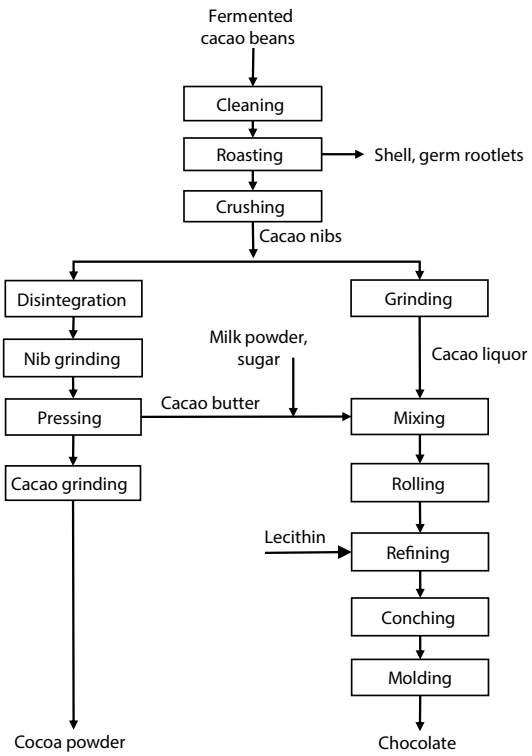

Figure 1 Production of chocolate and cocoa powder [2]

The Hydranal laboratory has developed applications for chocolate and chocolate products, using volumetric KF titration techniques, which are described below.

Application: Water Determination in Chocolate

Chocolate and milk chocolate samples with high fat content need pre-treatment before the water determination can be carried out. Directly before the titration, the chocolate sample should be ground or grated. It should not be left exposed to ambient air once it is grated, otherwise its water content will change according to the room conditions. In order to dissolve the fat and finely disperse chocolate samples in the KF working medium, the addition of chloroform to the working medium is recommended. Alternatively, the titration can be carried out at 50 °C. Recommended sample size is approximately 1 g. Before starting the titration and after sample addition, a stirring time of two to three minutes should be applied. Titration duration is about three minutes, following these procedures:

	Alternative 1	Alternative 2
Reagent	**One-component technique**	**One-component technique**
Titrating agent	Hydranal-Composite 5	Hydranal-Composite 5
Working medium	Hydranal-Methanol Rapid or Methanol dry and Hydranal-Chloroform 1:1	Hydranal-LipoSolver CM
	Two-component technique	**Two-component technique**
Titrating agent	Hydranal-Titrant 5	Hydranal-Titrant 5
Working medium	Hydranal-Solvent: Hydranal-Chloroform 1:1	Hydranal-Solvent CM

Application reports L071 Chocolate, and L079 Milk chocolate

Application: Water determination in chocolate truffles and pralines

Chocolate truffles and pralines are usually composed of different layers that also have different water content. It is therefore crucial to create a homogenous mass and take a representative sample. For example, a single praline can be quickly homogenized using a mortar, and afterwards, the sample should be stored in a tightly sealed container. Larger sample amounts can also be homogenized, using a blender.

When methanol is used as the sole component of the working medium, the chocolate mass disperses very slowly. The addition of formamide for dissolving contained sugars, as well as chloroform for fat content, is highly recommended. Carrying out the titration at an elevated temperature of 50 °C also has a positive effect on the dissolving of the chocolate samples. The duration of the titration may vary from approximately 3–6 minutes, depending on the composition of the sample. In this application, a sample size of approximately 400 mg was used.

Reagent	One-component technique
Titrating agent	Hydranal-Composite 5
Working medium	Hydranal-Methanol dry : Hydranal-Chloroform : Hydranal-Formamide dry 2:1:1
	Two-component technique
Titrating agent	Hydranal-Titrant 5
Working medium	Hydranal-Solvent : Hydranal-Chloroform : Hydranal-Formamide dry 2:1:1

Application report L028 Chocolate truffles and pralines)

Cat. No.	Brand	Description
34805	Fluka	Hydranal-Composite 5
37855	Fluka	Hydranal-LipoSolver CM
34741	Fluka	Hydranal-Methanol dry
37817	Fluka	Hydranal-Methanol Rapid
34724	Fluka	Hydranal-Formamide dry
37863	Fluka	Hydranal-Chloroform
34801	Fluka	Hydranal-Titrant 5
34800	Fluka	Hydranal-Solvent
34812	Fluka	Hydranal-Solvent CM
34849	Fluka	Hydranal-Water Standard 10.0

Table 1 Selected Hydranal KF reagents

References

[1] ICCO – International Cocoa Organization, www.icco.org.

[2] Belitz; Grosch; Schieberle – *Food Chemistry, 4th rev. and ext. Edition*, Springer-Verlag Berlin Heidelberg, **2009**.

Check our list of KF Applications on sigma-aldrich.com/applications

To obtain complete application reports in PDF form, and for more information on Hydranal reagents for pyridine-free water determination by KF titration, visit our website **sigma-aldrich.com/hydranal**, or contact our Hydranal laboratories.

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High-Quality Reagents for Titration by Sigma-Aldrich

Ready-to-use Solutions or Concentrates for Individual Dilution

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

Almost every analytical laboratory carries out titrations of some sort, even in this age of highly complex technical instrumentation and trace level analysis. Often, whole manufacturing processes depend on the accuracy of titration results and, therefore, on the quality of the titration reagents. Consider our broad portfolio of high-quality titration products:

- Ready-to-use volumetric solutions of acids, bases, buffers, salts, and complexing agents in different package sizes, including 5 or 10 litre VOLPAC®-containers
- Concentrates for preparation of volumetric solutions
- Reagents for complexometric titrations
- Masking agents, indicators, pH indicator paper and sticks

Cat. No.	Description
pH Buffer solutions	
82555	Buffer standard solution according to DIN 19266, pH 3.776 (25 °C)
33593	Buffer standard solution according to DIN 19266, pH 4.008 (25 °C)
33594	Buffer standard solution according to DIN 19266, pH 6.865 (25 °C)
33597	Buffer standard solution according to DIN 19266, pH 7.413 (25 °C)
33595	Buffer standard solution according to DIN 19266, pH 9.180 (25 °C)
82454	Buffer standard solution according to DIN 19266, pH 10.012 (25 °C)
33665	Buffer standard solution pH 4.0 (20 °C), red colored
33666	Buffer standard solution pH 7.0 (20 °C), green colored
33667	Buffer standard solution pH 9.0 (20 °C), blue colored
33668	Buffer standard solution pH 10.0 (20 °C), violet colored
33643	Buffer standard solution pH 4.0 (20 °C)
33646	Buffer standard solution pH 7.0 (20 °C)
33648	Buffer standard solution pH 9.0 (20 °C)
33649	Buffer standard solution pH 10.0 (20 °C)
242142	Buffer solution DURACAL pH 4.01 (25 °C), certified buffer solution (500 mL)
242143	Buffer solution DURACAL pH 4.01 (25 °C), certified buffer solution (250 mL)
242221	Buffer solution DURACAL pH 7.00 (25 °C), certified buffer solution (500 mL)
242145	Buffer solution DURACAL pH 7.00 (25 °C), certified buffer solution (250 mL)
242146	Buffer solution DURACAL pH 9.21 (25°C), certified buffer solution (500 mL)
242147	Buffer solution DURACAL pH 9.21 (25 °C), certified buffer solution (250 mL)
242148	Buffer solution DURACAL pH 10.01 (25 °C), certified buffer solution (500 mL)
242149	Buffer solution DURACAL pH 10.01 (25 °C), certified buffer solution (250 mL)

Cat. No.	Description
Reagents for total hardness determination	
34543	Ethylenediaminetetraacetic acid disodium salt solution (1 mL = 1° German hardness in 100 mL water)
34547	Ethylenediaminetetraacetic acid disodium salt solution with zinc complex added (solution A, 1 mL = 5.6° German hardness in 100 mL water)
34544	Ethylenediaminetetraacetic acid disodium salt solution with zinc complex added (solution B, 1 mL = 1° German hardness in 100 mL water)
34542	Ethylenediaminetetraacetic acid disodium salt solution with zinc complex added (solution C, 3.73 mL = 20° German hardness in 40 mL water)
38055	Ethylenediaminetetraacetic acid disodium salt concentrate with zinc complex added (solution A, 1 mL = 5.6° German hardness in 100 mL water)
38056	Ethylenediaminetetraacetic acid disodium salt concentrate with zinc complex added (solution B, 1 mL = 1° German hardness in 100 mL water)
Reagents for complexometric titration	
34588	1,2-Diaminocyclohexanetetraacetic acid monohydrate, DCTA ≥98.5%
32319	Diethylenetriaminepentaacetic acid, DTPA ≥99%
33350	Ethylenediaminetetraacetic acid dipotassium magnesium salt, EDTA-K2 Mg ≥98%
34553	Ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate, EDTA-Na2 Zn ≥98%
34549	Ethylenediaminetetraacetic acid disodium salt dihydrate, EDTA-Na2, 99-101%, Reag. Ph.Eur.
35332	Ethylenediaminetetraacetic acid copper(II) disodium salt solution, EDTA-Na2 Cu 0.01 mol/L)
35322	Ethylenediaminetetraacetic acid disodium salt solution, EDTA-Na2 0.01 mol/L
34550	Ethylenediaminetetraacetic acid disodium salt solution, EDTA-Na2 0.1 mol/L, Reag. Ph. Eur.
35102	Ethylenediaminetetraacetic acid disodium salt solution, EDTA-Na2 0.2 mol/L

Cat. No.	Description
38057	Ethylenediaminetetraacetic acid disodium salt concentrate, EDTA-Na2 (pkg of 0.1 mol)
34461	N-(2-Hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid trisodium salt, HEDTA-Na3 ≥99%
35103	1,2-Diaminocyclohexanetetraacetic acid disodium salt solution, DCTA-Na4 0.1 mol/L, Reag. Ph. Eur.
38146	Magnesium sulfate concentrate (pkg of 0.1 mol)
34276	Magnesium sulfate solution, 0.1 mol/L
34539	Nitrilotriacetic acid, NTA ≥99%
32047	Zinc sulfate concentrate (pkg of 0.1 mol)
35392	Zinc sulfate solution, 0.1 mol/L
Reagents for potentiometric titrations	
35334	Hydrochloric acid solution, 0.01 mol/L
35320	Hydrochloric acid solution, 0.05 mol/L
35335	Hydrochloric acid solution, 0.1 mol/L, Reag. Ph. Eur.
35329	Hydrochloric acid solution, 0.5 mol/L, Reag. Ph. Eur.
35328	Hydrochloric acid solution, 1 mol/L, Reag. Ph. Eur.
38272	Hydrochloric acid concentrate (pkg of 0.01 mol)
38280	Hydrochloric acid concentrate (pkg of 0.1 mol)
38287	Hydrochloric acid concentrate (pkg of 0.2 mol)
38285	Hydrochloric acid concentrate (pkg of 0.5 mol)
38282	Hydrochloric acid concentrate (pkg of 1.0 mol)
35204	Lanthanum nitrate solution, 0.1 mol/L, for determination of fluoride
34294	Silver nitrate solution, 0.01 mol/L
34296	Silver nitrate solution, 0.02 mol/L
35375	Silver nitrate solution, 0.1 mol/L, Reag. Ph. Eur.
38001	Silver nitrate concentrate (pkg of 0.01 mol)
38310	Silver nitrate concentrate (pkg of 0.1 mol)
38311	Silver nitrate concentrate (pkg of 0.5 mol)
35262	Sodium hydroxide solution, 0.01 mol/L
35249	Sodium hydroxide solution, 0.05 mol/L
35263	Sodium hydroxide solution, 0.1 mol/L, Reag. Ph. Eur.
35257	Sodium hydroxide solution, 0.5 mol/L
35256	Sodium hydroxide solution, 1 mol/L, Reag. Ph. Eur.
38227	Sodium hydroxide concentrate (pkg of 0.01 mol)
38210	Sodium hydroxide concentrate (pkg of 0.1 mol)
38217	Sodium hydroxide concentrate (pkg of 0.5 mol)
38215	Sodium hydroxide concentrate (pkg of 1.0 mol)
34449	Sodium thiosulfate solution, 0.05 mol/L
35245	Sodium thiosulfate solution, 0.1 mol/L, Reag. Ph. Eur.
35233	Sodium thiosulfate solution, 0.2 mol/L
35244	Sodium thiosulfate solution, 1 mol/L
38243	Sodium thiosulfate concentrate (pkg of 0.01 mol)
38200	Sodium thiosulfate concentrate (pkg of 0.1 mol)
35358	Sulfuric acid solution, 0.05 mol/L, Reag. Ph. Eur.
35357	Sulfuric acid solution, 0.1 mol/L, Reag. Ph. Eur.
35355	Sulfuric acid solution, 0.25 mol/L, Reag. Ph. Eur.
35354	Sulfuric acid solution, 0.5 mol/L, Reag. Ph. Eur.
38308	Sulfuric acid concentrate (pkg of 0.005 mol)
32043	Sulfuric acid concentrate (pkg of 0.05 mol)
38295	Sulfuric acid concentrate (pkg of 0.25 mol)
38294	Sulfuric acid concentrate (pkg of 0.5 mol)

Table 1 Selected high quality Fluka® reagents for volumetric titration

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