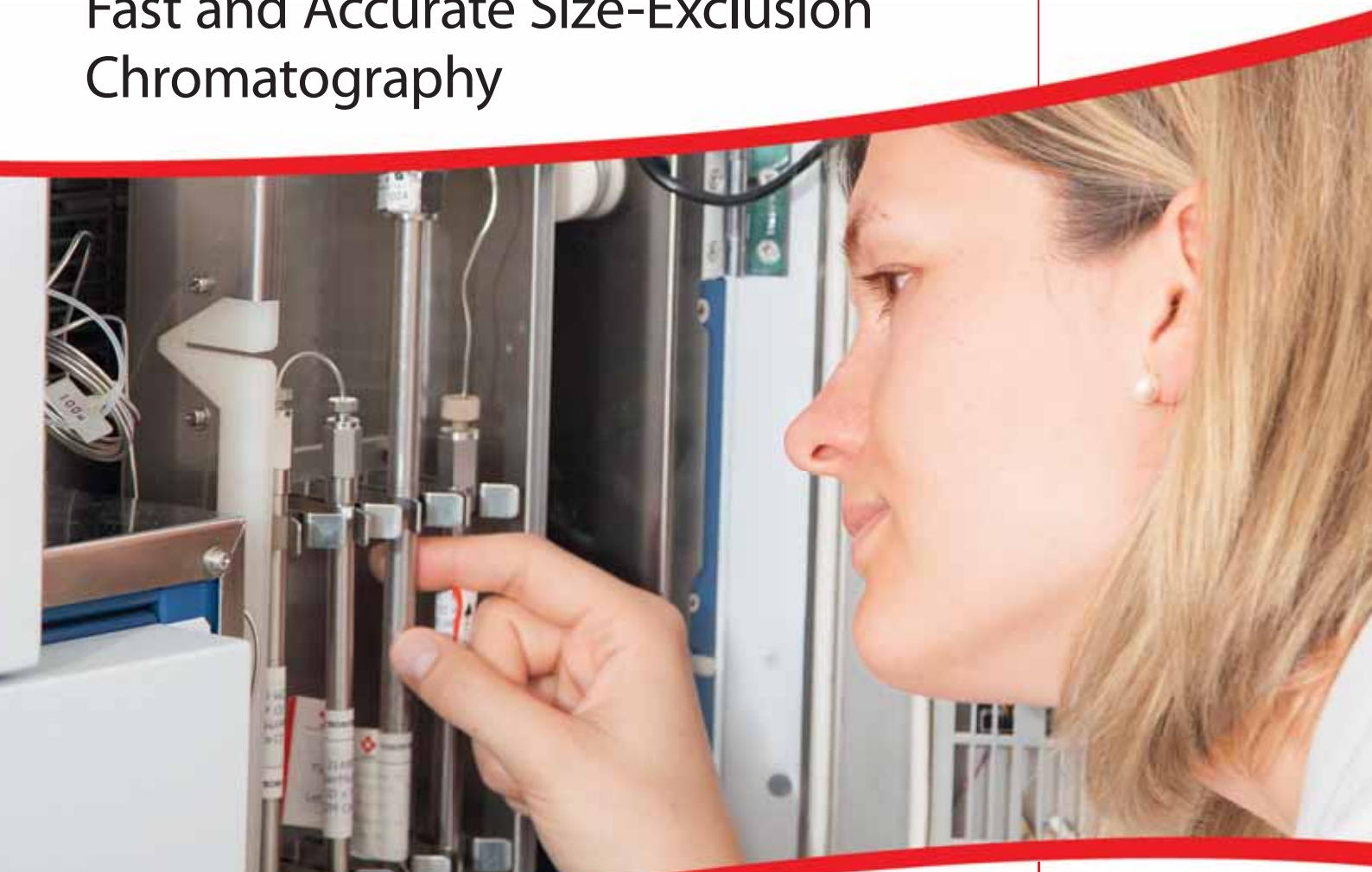
Analytix

Issue 1 • 2014

Fluka Analytical

Riedel-de Haën

Fast and Accurate Size-Exclusion Chromatography



- Columns and Analytical Standards for SEC of Protein Samples
- ***New*** Analytical Standards for Carotenoids
- ***New*** Certified Reference Materials from IRMM
- Determination of Water Content in Candies
- Micro-Arrays for Mass Spectrometry (MAMS)
- Titration Reagents

Editorial

Sigma-Aldrich®'s Distribution and Fruitful Collaboration for Analytical Standards and CRMs

Dr. Eva Katharina Richter
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Dear Reader,

In a highly competitive world, we believe that the key to success lies in producing high-quality products and by gaining input from fruitful collaborations. Therefore, we place great value on working with selected partners to expand our broad standards portfolio continuously.

Sigma-Aldrich is proud of the collaboration it has with the Tosoh Corporation. In recent months, we have developed a protein mix standard for calibrating Tosoh's size exclusion columns used for protein analysis. This saves analysts the time-consuming preparation of calibration mixes.

Learn more about these ready-to-analyze size-exclusion chromatography tools on pages 4 – 6.

Our in-house competence centers for the production of analytical standards and certified reference materials (CRMs) are located at different sites in Europe and the US. In addition, we also distribute standards and CRMs for several suppliers.

We constantly add new CRMs from the Institute of Reference Materials and Measurements (IRMM) to our portfolio. For the newest additions, please see the product table on page 15 – 17. Our range of physical properties standards offering also grew significantly after adding Paragon Scientifics CRMs to our portfolio in 2013.

Thanks to all of these generous scientific exchanges, we are able to offer our customers high-quality analytical standards, developed to meet all needs of analysts.

Please find the comprehensive analytical standards portfolio in our up-to-date online product catalog at **sigma-aldrich.com/standards**. It covers many different application areas and market segments, ranging from standards for the food and beverage industry (including environmental analysis), to those used in the cosmetic, petrochemistry and pharmaceutical industries. For most groups of standards and CRMs, we offer corresponding brochures and flyers, which can be ordered free of charge at **sigma-aldrich.com/lit-request**

With best regards,

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Analytix is published five times per year by Sigma-Aldrich Chemie GmbH, MarCom Europe, Industriestrasse 25, CH-9471 Buchs SG, Switzerland
Publisher: Sigma-Aldrich Marketing Communications Europe
Publication Manager: Michael Jeitziner
Editor: Daniel Vogler

Feature Article

Standards

Spectroscopy

Titration

Ready-to-Analyze

Columns and Analytical Standards for SEC of Protein Samples

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Aqueous size-exclusion chromatography (SEC) is well established for protein characterization. As the technique has increasingly been used for quality control of biologics since the early days of recombinant protein development for pharmaceutical purposes, various method improvements have evolved. Today, (U)HPLC columns developed for specific separation tasks and ready-to-use standards facilitate method development and increase robustness of SEC methods.

Size Exclusion Chromatography for Molecular Mass Determination

Analytical size-exclusion chromatography (analytical SEC) can be employed whenever a sample contains various molecules that need to be separated and characterized according to their size. The technique uses highly porous particles that are packed into a chromatography column. Depending on the size of a certain molecule in the sample, it may have access to all pores, limited access to some pores or no pore access. Based on this partitioning principle, bigger molecules elute first, followed by sample components of medium and small size. Once a sample has been separated successfully, the sample molecules' sizes can be determined using two basic detection methods. On the one hand, static light scattering offers the opportunity to do molecular mass determinations. In this case, molecular masses are calculated of a light scattering signal and a refractive index signal and are independent of the hydration shell thickness of the molecules. However, the downside of this approach is the equipment cost and a comparably high vulnerability of the light scattering detector to background noise disturbance. Although light scattering has been established successfully for many applications, there are cases where the more classical approach of molecular size determination outperforms static light scattering.

A calibration mix consisting of various molecules of known size is injected onto the same column as used for the sample. The retention time of each component is obtained from the calibration chromatogram and plotted against the logarithmic molecular mass. This results in a calibration curve. **Figure 1** shows calibration curves for three ***NEW*** U(HPLC) columns that were developed for the analysis of immunoglobulin G. **TSKgel® SuperSW mAb HTP** was developed for easy method transfer from HPLC to fast UHPLC analysis, **TSKgel SuperSW mAb HR** provides superior resolution for fragments, monomers, and aggregates in one run, and **TSKgel UltraSW Aggregate** covers higher molecular weights and separates antibody dimers and higher aggregates best. Please find an up-to-date product list and ordering information at **sigma-aldrich.com/tsk**

The calibration curves show a linear correlation between the retention time and a certain range of molecular masses. This range of molecular masses is called the linear calibration range of a column, and acts as the optimal working range for a separation. If a sample does not elute in this frame, the sample will usually require a SEC column with either smaller or larger pores. Also, proper column and eluent choice ensures that secondary interactions that may change the retention time are avoided.

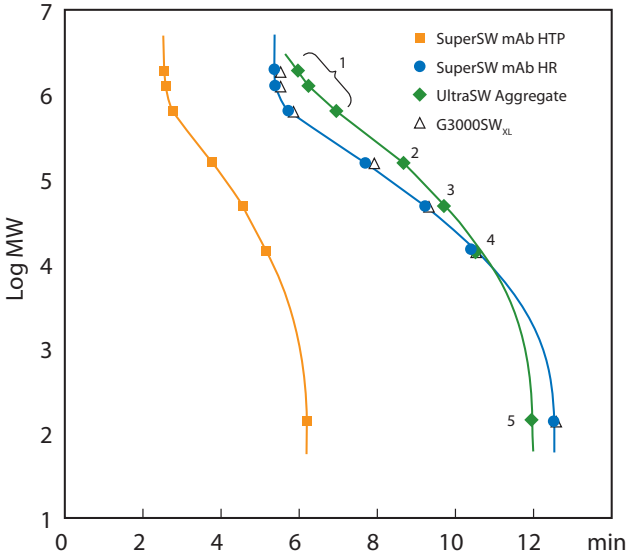

Figure 1 Calibration curves of **TSKgel SuperSW mAb HTP** 4.6 mm ID x 15 cm L, **TSKgel SuperSW mAb HR**, **TSKgel UltraSW Aggregate and TSKgel G3000SWxl**, both 7.8 mm ID x 30 cm L. **Mobile Phase:** 0.2 M sodium phosphate buffer, pH 6.7 and 0.05% sodium azide. **Calibrating proteins** are: thyroglobulin (640 kDa), γ-globulin (155 kDa), albumin (47 kDa), ribonuclease A (13.7 kDa) and p-aminobenzoic acid (0.14 kDa), all contained in **Sigma Aldrich's Protein Standard Mix 15 – 600 kDa. Injection volume:** 10 µl. Flow rate: 0.35 ml/min (4.6 mm ID) or 1 ml/min (7.8 mm ID). **UV detection** @280 nm

NEW Calibration Standard

It is important to note that a calibration mixture should contain molecules that are as similar to the analyzed sample molecules as possible. This is because SEC separates molecules according to their hydrodynamic size, which is in fact dependent on the hydration shell thickness. The degree of molecular hydration in a given solvent is determined by molecular characteristics, such as the polarity. Often, polyethylene glycols are used as standards, because of their monodispersity. The molecules used for calibration should resemble just one molecular mass per peak. Also, polyethylene glycols are quite stable and can be stored at room temperature for years. Unfortunately, for protein analysis, polyethylene glycols have limited usefulness due to their much thicker hydration shell. For the molecular mass determination of proteins in SEC, proteins should serve as a calibration standard. Consequently, calibration standard handling becomes more elaborate, as typically protein-related problems such as protein degradation will emerge. Calibration mixtures based on proteins have limited shelf life, which means that either tiny amounts of standard need to be prepared, or large quantities of the calibration mix will be wasted. Overall, the preparation can be time-consuming. An easy way to avoid the standard preparation is to employ ready-to-use standards. **Sigma Aldrich®'s Protein Standard Mix** for SEC offers full coverage of the molecular weight range from 15 kDa to 600 kDa. This qualifies it for the molecular mass determination of most proteins. The protein mix comes in 2 mL tubes that contain lyophilized protein powder and all necessary buffer ions. Simply add water and inject the calibration standard. This makes quick and easy column calibrations with a freshly prepared standard possible. When SEC is applied in the QC analysis of biopharmaceuticals the main purpose is quantification of sample components, and not determination of the molecular weights. For these types of SEC analysis, the injection of a protein standard solution is often used for system suitability tests. The new protein standard is a convenient tool to facilitate the preparation of these system suitability samples.

Column Calibration

When calibrating a column, it is important to apply the same conditions as used for analysis. Changing buffer systems changes the retention time considerably. For instance, arginine is a frequently-used additive for monoclonal antibody (mAb) aggregate analysis. It prevents secondary interactions of hydrophobic mAb aggregates and the stationary phase [1, 2 and 3] and shortens the retention time in SEC, which might also be due to a slightly altered hydration shell. **Figure 2** illustrates the effect of an addition of 200 mM arginine to the liquid phase in SEC of an aggregated mAb sample.

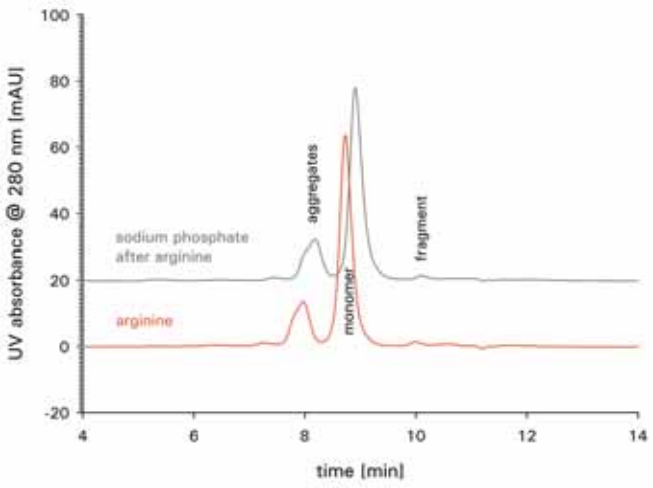

Figure 2 mAb sample on **TSKgel UltraSW Aggregate** with 0.1 M sodium phosphate buffer containing 0.2 M arginine in the mobile phase (red). After 10 injections, the mobile phase was switched to sodium phosphate buffer with an addition of 0.2 M sodium sulfate (grey). For both mobile phases, injection #10 is shown.
Column: TSKgel UltraSW Aggregate. **Flow:** 1 mL/min. Injected volume: 20 µL. **Injected mass:** 100 µg. **Detection:** UV @ 280 nm.

Retention shifts, such as for the arginine additive, would lead to miscalculating the molecular masses, if the calculation referred to a calibration standard separation that does not use arginine in the eluent. Similar effects have been observed for the change from phosphate buffer to HEPES (data not shown). Besides the applied liquid phase, the calibration standard concentration and injection volume are crucial for correct retention times and subsequent molecular mass calculation. Overloading SEC columns causes resolution loss and may lead to shifted retention times. Exemplary injection volumes and standard concentrations were injected onto TSKgel SuperSW mAb HR. Sigma Aldrich's Protein Standard Mix was dissolved in 200 µL, 500 µL and 1000 µL water. Clearly, the highly concentrated calibration sample is not properly resolved. The effect is the most significant for the largest injection volume of 100 µL(**Figure 3**).

(continued on page 6)

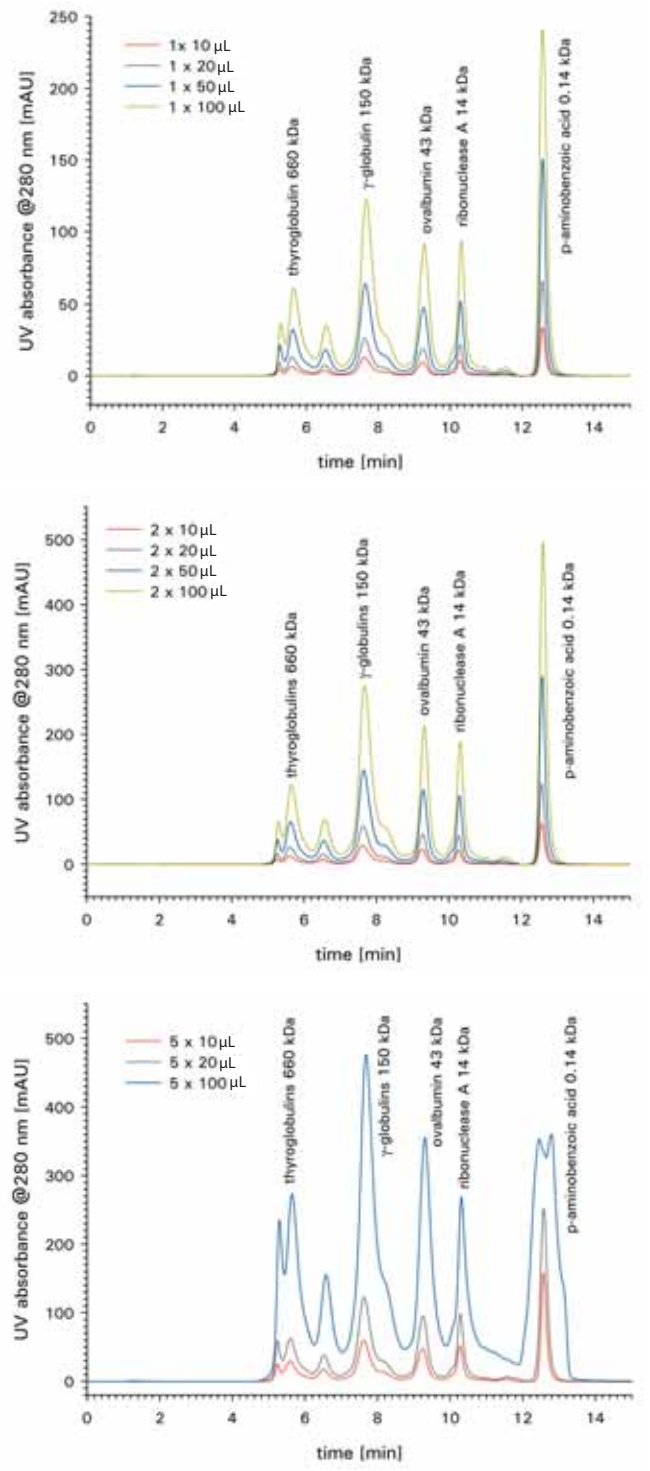

Figure 3 Protein Standard Mix 15 – 600 kDa on TSKgel SuperSW mAb HR 7.8 mm ID x 30 cm L. Injection volumes are varied from 5 to 100 µL. The standard was prepared in different concentrations: 1 x, 2 x and 5 x. **Eluent:** 200 mM sodium phosphate buffer, pH 6.7 and 200 mM sodium sulfate. **Flow:** 1 mL/min. **UV absorbance** @280 nm.

These results show the importance of a proper injection volume and concentration. Only then can reliable molecular masses be calculated. Moreover, permanently overloading the column may lead to early column deterioration. This is also true for the samples. The required amount of sample for a sufficient significant signal needs to be balanced against reduced column life time due to overloading. In general, 7.8 mm ID x 30 cm L columns achieve best results and high lifetimes when injecting 20 µL samples with a concentration of 1–5 mg/mL.

Molecular mass determination using SEC can be an analysis with good accuracy and reproducibility. However, some basic methodical parameters need to be considered. Ready-to-use calibration standards allow removal of potential error sources, such as weighing tiny protein amounts. Easy handling and properly concentrated standards make sure the perfect amount for column calibration or system suitability testing is injected.

Cat. No.	Brand	Description	Package Size
69385	Fluka	Protein Standard Mix 15 – 600 kDa	30 mg, 6x30 mg
822854	Supelco	TSKgel SuperSW mAb HR 4 µm (7.8 x 300 mm)	1EA
822855	Supelco	TSKgel SuperSW mAb HTP 4 µm (4.6 x 150 mm)	1EA
822856	Supelco	TSKgel UltraSW Aggregate 3 µm (7.8 x 300 mm)	1EA

References:

[1] Arakawa, T.; et al. The critical role of mobile phase composition in size exclusion chromatography of protein pharmaceuticals. *J. Pharmaceutical Sciences* 99(4) **2010**: 1674–1692.

[2] Yumioka, R.; et al. Mobile phase containing arginine provides more reliable SEC condition for aggregate analysis. *J. Pharmaceutical Sciences*, 99(2) **2010**: 618–620.

[3] Ejima, D.; et al. Arginine as an Effective Additive in Gel Permeation Chromatography. *J. Chromatography A*, 1094(1–2) **2005**: 49–55.

TOSOH

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Nature's Brightest Colors

New Analytical Standards for Carotenoids

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Carotenoids are responsible for the bright colors of various fruits and vegetables. To give products an appealing shade, the food and cosmetic industry uses synthetically produced carotenoids for coloration. They are also used as feed supplements in aquaculture for salmon, crabs and shrimp and laying hens for egg production.

Both in the EU and USA, carotenoids are regulated as food additives, with major differences regarding natural sources and which foods and feed may be colored. For example, in the USA canthaxanthin (E161g) is generally permitted as a food additive for coloring, but in the EU it is only authorized for a specific sausage (saucisse de Strasbourg).

Apart from coloration, these organic pigments have a positive effect on human health: most carotenoids have antioxidant and radical-scavenging properties. Therefore, carotenoids are believed to decrease the risk of various diseases, cancer in particular.

Cat. No.	Description	Package Size
41659	Astaxanthin	1 mg, 5 mg
32993	Canthaxanthin *(trans)*	10 mg
50887	*α*-Carotene ***New***	1 mg, 5 mg
PHR1239	*β*-Carotene	1000 mg
18804	Crocetin dialdehyde ***New***	10 mg
16337	Fucoxanthin ***New***	1 mg, 5 mg
75051	Lycopene ***New***	10 mg
07168	Lutein ***New***	1 mg, 5 mg
14681	Zeaxanthin	1 mg

Table 1 New Analytical Standards for Carotenoids

Sigma-Aldrich recently extended its range of carotenoid standards for quality control in the food, dietary supplement and cosmetics industries. Find an up-to-date product list at **sigma-aldrich.com/carotenoids**

NEW Brochure for Food Color Additive Standards

A Comprehensive Range of Analytical Standards for Precise Detection of Regulated Food Color Additives

- Neats for Artificial Food Colors
- Neats for Natural Food Colors
- Deuterated Internal Standards

For accurate quality control of more than 25 E numbers and 20 other banned food dyes, please order the brand ***NEW*** **Brochure for Food Color Additive Standards** or visit **sigma-aldrich.com/fooddyes** for an up-to-date product list and ordering information.

Fluka® Analytical

The Importance of Accelerated Stability Tests for the Development of Certified Reference Materials (CRM)

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Over the last two decades, the importance of quantification of organic substances using NMR, especially ^{1}H-NMR, has significantly increased[1]. Today, qNMR is used in a variety of applications in industry as well as for academic research [2,3]. The intensity of an NMR signal is directly proportional to the number of protons that give rise to the signal. Quantification is achieved by measuring the sample peak area of interest with respect to a signal from an appropriate internal standard, such as an internationally accepted primary CRM. Using such a primary CRM, for example a Standard Reference Material™ (SRM) from the US National Institute of Standards and Technology (NIST), leads to traceability to the International System of Units (SI) without the need for a reference standard of the same chemical structure as the sample.

As an accredited producer of certified reference materials (CRM), Sigma-Aldrich® has established a portfolio of organic CRMs, quantified by HP-qNMR® (high-performance quantitative NMR), and in accordance with ISO/IEC 17025 and ISO Guide 34. These CRMs provide an unbroken traceability chain to the SI, as well as extensive homogeneity and stability testing. This article shows the advantages of conducting accelerated stability tests over classical short-term stress tests.

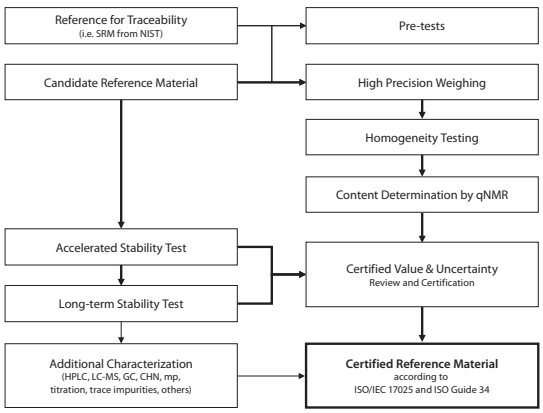

Figure 1 Workflow of the entire certification procedure at Sigma-Aldrich under ISO/IEC 17025 and ISO Guide 34.

Requirements for CRM producers

In addition to organization and management requirements according to ISO 9001, there are several topics that are crucial for ISO/IEC 17025 compliance, including instrument qualification, validation of analytical methods, traceability statement, evaluation of measurement uncertainty, education of personnel and periodic participation in proficiency tests to demonstrate technical capability.

ISO Guide 34 outlines the quality system requirements under which a CRM is produced, and deals with aspects regarding production planning and control, maintenance of a suitable environment, starting material selection and processing, assignment of a property value, their uncertainty and traceability, assessment of homogeneity and stability, assurance of adequate packaging and storage and issuing certificates or documents.

Furthermore, ISO Guide 34 clearly states the requirements for stability testing and also refers to ISO Guide 35[4] for further details. One important aspect regarding stability studies in ISO Guide 35 is the monitoring of the stability. The evaluation of measurement data as described in ISO Guide 35 covers only apparently stable materials. In the case of detectable degradation, both the degradation and its uncertainty shall be included in the stability assessment. In their work, Bremser et al.[5] gave an alternative approach for data handling and a model-based estimation of expiry date for unstable reference materials.

In ISO Guide 35 a distinction is made between "short-term stability" that can be described as the stability under specified transport conditions and "long-term stability" which is the stability under particular storage conditions. The stability data are used for the definition of the shelf life and the recommended storage temperature of a CRM.

Stability study scheme at Sigma-Aldrich

The stability study should be designed so that it yields sufficient data for answering questions about the long-term stability of the CRM at the defined storage temperature, about the maximum impact on the CRM during transport conditions, and provides data for an appropriate estimation of the shelf life.

In contrast to the recommendations in ISO Guide 35, where short-term stability tests are typically carried out only for a few weeks, an accelerated stability test (AST), designed to give results over a prolonged period of time, is used. The advantages of such a design are described in detail by S.R.L. Ellison[6] and he shows that the accelerated studies detect instabilities better than the long-term study at storage temperature. It can also give earlier predictions about the stability model (stable, linear, exponential or autocatalytic degradation).

Stability study samples are analyzed at different points in time, meaning that several months can lie between two measurement values; therefore, the reproducibility of the

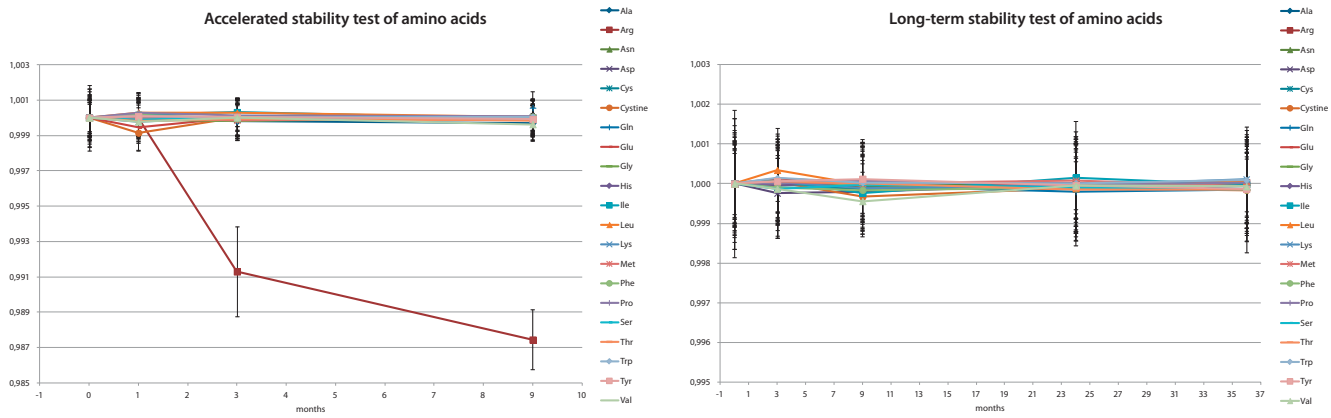

Figure 2 Accelerated stability test (Figure 2a) and long-term stability test (Figure 2b) for different amino acids. The certified values are normalized to 1.0. The bars show the expanded measurement uncertainties ($k = 2$).

measurement method is of primary importance. Because qNMR demonstrates an excellent reproducibility and a low measurement uncertainty, it is chosen as an appropriate analytical method for the determination of the content during stability studies.

Accelerated stability test (AST):

AST is performed at elevated temperature, usually 20 °C above the indicated storage temperature. Duplicate tests are performed at defined times, 1, 3, 9 and in some cases 18 months. If the CRM turns out not to be stable at elevated temperature, this may lead to a reduction of the general storage temperature or particular storage information in the certificate, and to a new start of the AST.

Long-term stability test (LTS):

The long-term stability studies are performed in parallel at the indicated storage temperature and for a longer time period, which typically covers the entire shelf life of a CRM. Typical times are 24, 36 and 48 months. The earlier times (one, three and nine months) are only measured if the corresponding AST indicates a significant instability.

Certification is only accomplished when AST and LTS data indicate a sufficient CRM stability at the recommended storage condition. Under these assumptions, the shelf life can be estimated using AST data and the Arrhenius equation, where a reduction in storage temperature by 10 °C results in a prolongation of the shelf life of two to four times. For a stable CRM, all recorded values should lie within the measurement uncertainty of the certified value and therefore no correction for possible instability is needed. Nevertheless, AST and LTS data are used for the estimation of the uncertainty contribution coming from storage, u_{stab}, which is included in the overall measurement uncertainty. Data from both tests of a previously tested CRM lot are used in addition to decide about a possible prolongation of the shelf life of a second CRM lot. As an example, the set of amino acids illustrates the importance of a prolonged AST for the development of a CRM.

Figure 2a and 2b show the behavior of 21 amino acids under AST and LTS conditions. The AST was conducted at 45 °C and shows the purity of each substance determined by qNMR at various times (t = 0/1/3/9 months). All purity data for t = 0 were normalized to 1.0, and the other data calculated accordingly. Arginine is the only CRM that turned out to be unstable after 3 months at 45 °C. In this case, the storage temperature of Arginine was reduced to 4 °C, and a new AST was started at room temperature. In contrast, all substances were stable in the long-term stability test at room-temperature (approx. 23 °C) over a time period of 3 years.

Using a classical short-term stability test, which typically would only cover the time during transport or shipping, the time might have been too short to detect the instability of Arginine. These data show the importance of a longer testing period at elevated temperature conditions. With regard to different potential degradation kinetics, a period of nine months was chosen as the minimum time interval for AST and 18 months used for particular cases, especially for prolongation of shelf life. In combination with the LTS data, this enables a sound determination of shelf life.

Since qNMR generates accurate and traceable values in combination with low measurement uncertainties, it is an optimal technique for the content determination of organic molecules. qNMR is capable of detecting even small differences in content, and therefore best suited for conducting stability studies.

Sigma-Aldrich offers a comprehensive product range of certified reference materials (CRMs) intended for the use as internal standards for qNMR **sigma-aldrich.com/qnmr**. Also, the range of *Trace*CERT® neat standards, certified using qNMR is steadily growing and comprises over 130 products, including the amino acids mentioned in this article. Find an up-to-date product list on our website at **sigma-aldrich.com/crmneats**

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Water Framework Directive

An Overview of Analytical Standards and Certified Reference Materials for Priority Substances

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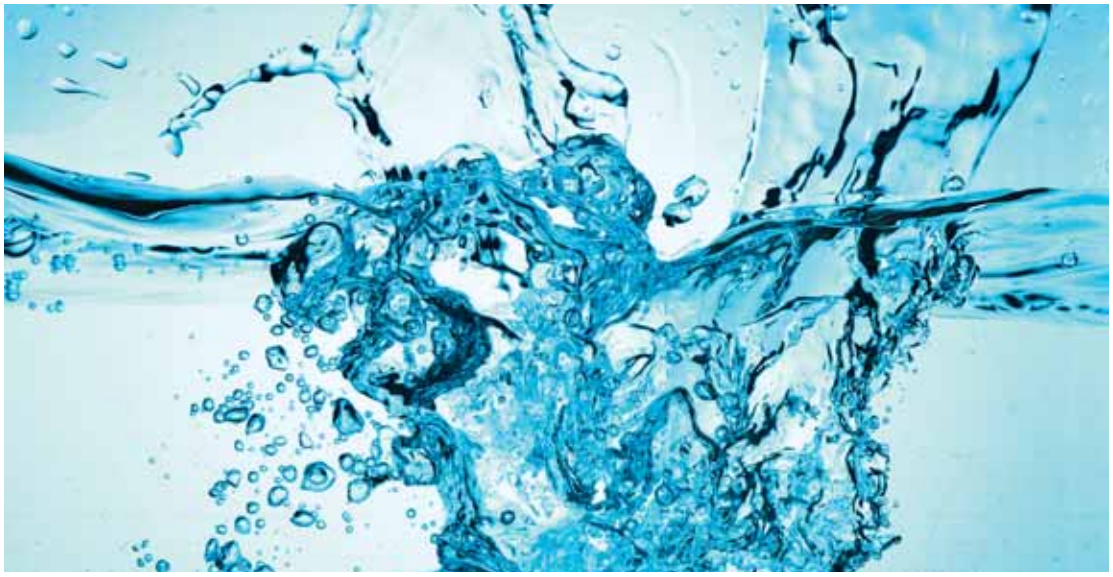

The EU Water Framework Directive (2000/60/EC)[1] was established in the year 2000, committing the member states to achieve "good" qualitative and quantitative status of all water bodies, including rivers, lakes, transitional waters, coastal waters and groundwater by the year 2015. The aim is to protect and restore clean water all across Europe and to ensure its long-term, sustainable use.

The following criteria are taken into account for assessing the ecological and chemical status of surface waters: biological status, hydromorphological quality (e.g. river bank structure), physical-chemical and chemical quality of the water.

In Article 16 of the directive, strategies against chemical water pollution are outlined, with a detailed description of the steps to be taken. Annex X containing the original list of 33 "priority substances" was established in 2001. Since Article 16(4) of the EU Water Framework Directive demands a regular review of the priority substances, in January 2012, 15 additional substances were proposed by the Commission to be added to the list of priority substances. Among these, pharmaceuticals were also proposed for the first time.

The comprehensive portfolio of analytical standards and certified reference materials (CRMs) available at Sigma-Aldrich® also includes basically all 33 priority substances and the 15 newly proposed substances, as shown in the two lists on the next page. Please note that there may also be other qualities for each analyte, but only the highest available quality for each analyte is listed. Thus, whenever a certified reference material is available, it is listed.

For a complete list of all analytical standards and CRMs, please visit **sigma-aldrich.com/standards** and browse our online product catalogue.

References:

[1] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0060:20011216:EN:PDF

Compound	Suggested Product		Quality	Brand	Package Size
Alachlor	45316		analytical standard (PESTANAL®)	Fluka®	250 mg
Anthracene*	07671		certified reference material (*Trace***CERT®**)	Fluka	100 mg
Atrazine	90935		certified reference material (*Trace***CERT**)	Fluka	50 mg
Benzene	12540		analytical standard	Fluka	5 mL, 50 mL
C10-13-chloroalkanes*	*currently no reference material available*				
Cadmium and its compounds*	36379	Cadmium Standard for ICP, 1000 mg/L	certified reference material (*Trace***CERT**)	Fluka	100 mL
Chlorfenvinphos	36551		analytical standard (PESTANAL)	Fluka	250 mg
Chlorpyrifos	94114		certified reference material (*Trace***CERT**)	Fluka	100 mg
Di(2-ethylhexyl)phthalate (DEHP)	67261		certified reference material (*Trace***CERT**)	Fluka	100 mg
1,2-Dichloroethane	02562		analytical standard	Fluka	1 mL
Dichloromethane	02575		analytical standard	Fluka	5 mL
Diuron	45463		analytical standard (PESTANAL)	Fluka	250 mg
Endosulfan*	32015		analytical standard (PESTANAL)	Fluka	250 mg
Flame retardants	*various others*	*visit* ***sigma-aldrich.com/flameretardants***		Fluka	
Fluoranthene	11474		certified reference material (*Trace***CERT**)	Fluka	100 mg
Hexachlorobenzene*	45522		analytical standard (PESTANAL)	Fluka	250 mg
Hexachlorobutadiene*	45525		analytical standard (PESTANAL)	Fluka	250 mg
Hexachlorocyclohexane*	45548	Lindane	analytical standard (PESTANAL)	Fluka	250 mg
Isoproturon	36137		analytical standard (PESTANAL)	Fluka	100 mg
Lead and its compounds	41318	Lead Standard for ICP, 1000 mg/L	certified reference material (*Trace***CERT**)	Fluka	100 mL
Mercury and its compounds*	28941	Mercury Standard for ICP, 1000 mg/L	certified reference material (*Trace***CERT**)	Fluka	100 mL
Naphthalene	91489		certified reference material (*Trace***CERT**)	Fluka	100 mg
Nickel and its compounds	28944	Nickel Standard for ICP, 1000 mg/L	certified reference material (*Trace***CERT**)	Fluka	100 mL
Nonylphenols*	*various*	*visit* ***sigma-aldrich.com/nonylphenols***		Fluka	
Octylphenols	*various*	*visit* ***sigma-aldrich.com/octylphenols***		Fluka/Supelco	
Pentabromodiphenylether*	33676	BDE-99, 50ug/mL in isooctane	analytical standard	Fluka	1 mL
Pentachlorobenzene*	35886		analytical standard (PESTANAL)	Fluka	1 g
Pentachlorophenol	48555-U		analytical standard	Supelco	5000 mg
Polyaromatic hydrocarbons*	*various*	*visit* ***sigma-aldrich.com/pahstandards***		Fluka/Supelco	
Simazine	32059		analytical standard (PESTANAL)	Fluka	250 mg
Tributyltin compounds*	442869	Tributyltin chloride	analytical standard	Supelco	500 mg
Trichlorobenzenes	36627	1,2,4-Trichlorobenzene	analytical standard (PESTANAL)	Fluka	250 mg
Trichlorobenzenes	36555	1,3,5-Trichlorobenzene	analytical standard (PESTANAL)	Fluka	250 mg
Trichlorobenzenes	36742	1,2,3-Trichlorobenzene	analytical standard (PESTANAL)	Fluka	250 mg
Trichloromethane	02487		analytical standard	Fluka	1 mL
Trifluralin	32061	Trifluralin	analytical standard (PESTANAL)	Fluka	250 mg

** = priority hazardous substance*

Table 1 Priority Substances according to 2000/60/EC and suggested analytical standards or CRMs

Compound	Suggested Product		Quality	Brand	Package Size
Aclonifen	36792		analytical standard (PESTANAL)	Fluka	50 mg
17 alpha-ethinylestradiol (EE2)	46263		analytical standard (VETRANAL)	Fluka	250 mg
17 beta-estradiol (E2)	E-060	solution, 100 µg/mL in acetonitrile	certified reference material	Cerilliant	1 mL
Bifenox	31477		analytical standard (PESTANAL)	Fluka	50 mg
Cybutryne	46105	Irgarol®	analytical standard (PESTANAL)	Fluka	250 mg
Cypermethrin	36128		analytical standard (PESTANAL)	Fluka	100 mg
Diclofenac	PHR1144	Diclofenac sodium salt	pharmaceutical secondary standard; traceable to USP, PhEur and BP	Fluka	1 g
Dicofol	36677		analytical standard (PESTANAL)	Fluka	100 mg
Dichlorvos	45441		analytical standard (PESTANAL)	Fluka	250 mg
Dioxin and Dioxin-Like PCBs	*various*	*visit* ***sigma-aldrich.com/pcbstandards***			
Heptachlor	31211	solution, 100 ng/µL in methanol	analytical standard (PESTANAL)	Fluka	2 mL
Hexabromocyclododecane (HBCDD)	51691		analytical standard	Fluka	50 mg
Perfluorooctane sulfonic acid (PFOS)	33607	solution, 100 µg/mL in methanol	analytical standard	Fluka	1 mL
Quinoxyfen	46439		analytical standard (PESTANAL)	Fluka	100 mg
Terbutryn	45677		analytical standard (PESTANAL)	Fluka	250 mg

Table 2 New Priority Substances proposed in 2012

NEW ASTM Reference Gas Oil Ensures Supply Continuity

Vicki Yearick, Product Manager Analytical Standards vicki.yearick@sial.com

Determining the boiling range distribution of petroleum fractions before processing provides insight into the percentage yield of a variety of products that could be obtained from that fraction when processed in the refinery. This information is collected by using test method ASTM D2887-13, "Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography". The method requires a Reference Gas Oil (RGO) sample be used to verify the chromatographic and calculation processes.

Concern that the existing RGO sample lot could someday be exhausted prompted the ASTM D2.04.0H committee to support the development of a second sample. This second RGO sample was tested in an inter-laboratory study to establish the required Accepted Reference Values (ARVs). Final acceptance was then given as a result of the Spring 2013 ASTM D02 Main Committee Ballot, and revisions to test method D2887 were made to include the use of this new material.

Sigma-Aldrich® is pleased to offer both the former and the new Reference Gas Oil calibration standards for our customers. Each product is shipped with a Certificate of Analysis detailing the Consensus Results.

To learn more about all of our standards for petroleum analysis, please visit **sigma-aldrich.com/standards**

Cat. No.	Description	Boiling Point Range	Package Size
40172-U	ASTM D2887 RGO Sample No. 2	115 – 920 °F	1 x 1 mL
40174-U	ASTM D2887 RGO Sample No. 2	115 – 920 °F	6 x 1 mL
506419	ASTM D2887 Reference Gas Oil (RGO), Sample 1, Lot 2	120 – 888 °F	1 x 1 mL
48873	ASTM D2887 Reference Gas Oil (RGO), Sample 1, Lot 2	120 – 888 °F	6 x 1 mL

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- Oximes and DAIH derivatives
- Radiello Calibration solutions and kits

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New Standards for the Analysis of Herbal Medicinal Drugs

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

Sigma Aldrich® offers a broad range of more than 400 standards and certified reference materials for analytes (active ingredients, toxic components or marker compounds) used in quality control of herbal medicinal products. An integral part of this portfolio is the popular product range of primary pharmaceutical reference standards which are manufactured and qualified by HWI Analytik in Rülzheim (Germany)[1]. With the addition of 21 new standards, this group now consists of more than 70 products. The absolute content determination of these products is performed by **quantitative NMR** (qNMR)[2] (see also the article on page 8 f to learn more about our in-house expertise in this technique).

The quantitative analysis of natural products creates several challenges. The most common method is the 100% minus impurities or mass balance approach. In this method, all potential impurities (residual solvents, water, and inorganic impurities) are measured separately and the content of the main component is then calculated by subtraction starting with the chromatographic purity. However, the chromatographic area purity does not necessarily reflect the true ratio of an analyte and its related compounds, because the signal response may vary. Therefore, the higher the purity of the material (ideally >99.5%), the higher the reliability of a mass balance result. However, such high purity is very difficult to achieve for natural products extracted from plant material. Also, since chromatographic methods are usually used for both isolation and analysis, the risk is high that impurities that were not separated during the isolation are also overlooked in the analysis.

By using a "relative" primary method of measurement such as quantitative NMR (qNMR), these kinds of problems can be avoided. qNMR is therefore applied increasingly not only for content assignment of natural products, but also in the pharmaceutical industry in general. The content of the primary pharmaceutical standards produced by HWI Analytik is determined using qNMR. In addition to this content value, the certificates for these products also list the chromatographic purity. The two values may differ since not all impurities are detected by chromatography. Therefore, for quantitative calculations, the qNMR value should be used.

This portfolio is continuously growing. Find below a list of the most recent product additions. A complete listing of the **primary pharmaceutical reference standards** for medicinal herbs, as well as additional information, can be found on our Web page: **sigma-aldrich.com/phytopharma**.
A list of all analytical standards and reference materials for ingredients of medicinal herbs can be found at **sigma-aldrich.com/medicinalplants**.

References:
[1] Förster, G.; Michel, F.; Nold, M. Analytix **1/2010** page 11.
[2] Veith, M. Analytix **1/2010** page 14.

Cat. No.	Description	Package Size
03980585	Apigenin-6,8-diglucosid	10 mg
00890590	Arbutin	50 mg
03520585	Aspalathin	10 mg
00760595	Bilobalid	10 mg
01410590	Camphene	100 mg
00410590	(+)-3-δ-Carene	100 mg
04270590	Carvacrol	50 mg
00290595	(–)-Carvone	100 mg
01940590	Casticin	10 mg
02660590	Eriocitrin	10 mg
01050595	Eugenol	100 mg
01290190	Geranylacetat	100 mg
02250595	Herniarin	10 mg
04280590	Isomenthol	25 mg
04290585	Isorhamnetin	10 mg
00590590	Limonen	100 mg
00350190	Linalool	100 mg
02150595	Osthole	10 mg
00080590	(–)-β-Pinene	100 mg
03420590	(S)-(–)-α-Terpineol	100 mg

20 new additions of primary pharmaceutical reference standards of phytopharmaceuticals

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New Certified Reference Materials from IRMM

Jens Boertz, Product Manager Analytical Reagents jens.boertz@sial.com

The Institute for Reference Materials and Measurements (IRMM) is one of the seven institutes of the Joint Research Centre (JRC), a Directorate-General of the European Commission (EC). One objective of IRMM is the support of EU policies with scientific advice concerning measurements and standards, such as by development of reference methods or certified reference materials.

Figure 1 Bird's-eye view of the IRMM reference material production building opened in 2012 (left), and view into a production hall for certified reference materials. © 2013, European Commission

Sigma-Aldrich® is proud to be an authorized distributor of IRMM´s reference materials. In **Table 1**, the most recent additions to the IRMM product range are listed.

STA-003m – Tetramethylurea

The reliable testing of the authenticity of wines requires the accurate calibration of specific isotope ratio measurements. Therefore, tetramethylurea (TMU) has been certified for its deuterium-to-hydrogen (D/H) amount-of-substance ratio. The D/H (i.e. $^{2}H/^{1}H$) ratio is one of several parameters (among other isotope ratios such as $^{13}C/^{12}C$ (carbon), $^{18}O/^{16}O$ (oxygen), or $^{15}N/^{14}N$ (nitrogen)) which help to determine whether the analyzed sample can be considered authentic or whether it has been adulterated. Hence, the material is important for laboratories authorized to perform those measurements, aiming to fight fraud by verifying the authenticity of wines.

In official wine controls, the measured values are compared with those from the respective authentic wines listed in the European Union (EU) Wine Databank, operated by the European Office for Wine, Alcohol and Spirit Drinks (BEVABS) at the Joint Research Centre (JRC).

STA-003m will be the successor of STA-003k. It is to be used in site-specific natural isotope fractionation – nuclear magnetic resonance (SNIF-NMR) spectroscopy measurements as an internal standard for determining D/H ratios of ethanol

Cat. No.	Material	Certified for	Application Area	Package Size
Sta003m	Tetramethylurea	D/H ratio	Food and Agriculture	500 mL
IRMM471	Cementite grains in carburized pure iron	Carbon mass fraction	Engineering	1 rod of 5 mm diameter
ERMDB001	Human hair	Trace elements	Environment	3.5 g
ERMCA713	Waste water	Trace elements	Environment	100 mL
ERMBF436a	DAS-44406-6 soya	ERMBF436a certified for: 0% GMO	Food and Agriculture	1 g
ERMBF436b	DAS-44406-6 soya	ERMBF436b certified for: 100% GMO	Food and Agriculture	1 g
ERMBF436c	DAS-44406-6 soya	ERMBF436c certified for: 0.1%	Food and Agriculture	1 g
ERMBF436d	DAS-44406-6 soya	ERMBF436d certified for: 1% GMO	Food and Agriculture	1 g
ERMBF436e	DAS-44406-6 soya	ERMBF436e certified for: 10% GMO	Food and Agriculture	1 g
ERMFA013bk	Steel bar	Impact resistance	Engineering	set of 5 bars
ERMBD150	Skimmed milk powder	Trace elements	Food and Agriculture	20 g
ERMBD151	Skimmed milk powder	Trace elements	Food and Agriculture	20 g

Table 1 Newly released CRMs from IRMM

(continued on page 16)

distilled from wines, which constitutes an important measure in wine authenticity testing (according to OIV-MA-AS311-05).

Homogeneity and stability of the material were assessed, and stability is guaranteed within the declared shelf life. The certified value was established by comparing the mean value of 18 samples of the STA-003m, tested together with 18 master batch samples of the CRM IRMM-425 under repeatability conditions. Moreover, these measurements were performed in one laboratory on 3 different NMR appliances by strictly adhering to the SNIF-NMR protocol prescribed in the standard OIV-MA-AS311-05.

IRMM-471 – Cementite Grains in Carburised Pure Iron

Many materials have complex microstructures. Investigation of the carbon mass fraction in microstructures of steel or wear-resistant coating materials gives important information on the materials' properties (for example: ductility, hardness, resistance). Micro-analysis techniques such as electron probe micro-analysis are often employed when analyzing local carbon concentrations in areas of only a few micrometers in diameter in steel and related materials (corrosion and wear resistant coatings). IRMM-471 contains cementite (Fe_3C) grains dispersed in an iron pearlite matrix with an average grain diameter between 20 µm and 50 µm. It is certified for the carbon mass fraction within those cementite grains. IRMM-471 is intended for calibration, quality control and assessment of method performance.

ERM-DB001 – Human Hair

Human hair is frequently used to monitor the exposure of humans to certain metals, as hair keeps a record of past contact due to its sequential growth. Taking samples of hair is pain-free and non-invasive. JRC-IRMM has therefore released ERM-DB001, a new human hair material certified for the mass fraction of the total content of As, Cd, Cu, Fe, Hg, Pb, Se and Zn at the mg/kg range. ERM-DB001 has been processed and certified in accordance to ISO Guides 34 and 35. It is primarily intended for method validation (e.g. trueness estimation) and performance control within laboratories involved in element determinations in human hair or similar matrices.

ERM-CA713 – Waste water

ERM-CA713 was specifically produced to include the four metals cited in the Priority Substances list (Cd, Hg, Ni and Pb) at concentration levels which are targeted to comply with the environmental quality standards (EQSs) referred to in Directive 2008/105/EC.

ERM-CA713 is a waste water effluent of mixed domestic and industrial origin. It has been acidified and fortified with As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn. This wastewater material will serve as a tailored quality control tool for laboratories involved in the mandatory monitoring of the Priority Substances prescribed under the Water Framework Directive. ERM-CA713 will supplement the series of CRMs for water testing available from the JRC Institute for Reference Materials and Measurements (IRMM). ERM-CA713 is primarily intended for method validation (such as trueness estimation) and performance controls. It has been prepared and certified in accordance to ISO Guides 34 and 35.

ERM-BF436 – GMO Material – DAS-44406-6 Soya

Genetically modified organisms (GMOs) need to be authorized before they are allowed to enter the European market. The availability of a reference material is one of the prerequisites for the authorization of a GMO in Europe. Dow AgroSciences (DAS) asked the European Commission's Joint Research Centre, Institute for Reference Materials and Measurements (EC JRC-IRMM) to develop a reference material for DAS-44406-6 soya. The DAS-44406-6 event is designed to confer tolerance to the glyphosate, glufosinate and 2,4-D herbicides.

The EC JRC-IRMM has released a set of five Certified Reference Materials (CRMs) ERM BF436a, b, c, d and e, certified for their DAS-44406-6 mass fractions. These reference materials will enable GMO testing laboratories to carry out measurements and to implement the (EC) No 1829/2003 food and feed labeling threshold.

The CRMs were prepared according to ISO Guide 34 by mixing gravimetrically dried GM and non-GM soya powder. The resulting GM percentages were confirmed by real-time polymerase chain reaction (PCR) analysis after DNA extraction from the powders.

ERM-FA013bk – Charpy Specimens – Low Energy 0 °C

Worldwide, steel producers make millions of impact tests each year to see whether their steels meet the specific strength requirements for applications such as shipbuilding or pipeline construction.

Usually, Charpy impact tests are applied, which consist of breaking a 55 mm bar of steel with a swinging hammer. These Charpy instruments need to be checked a minimum of once a year to assure reliability. The check consists of breaking steel reference samples with known impact resistance.

The new reference material released by the IRMM has certified impact resistance values for tests at 20 °C and at 0 °C because it is easier to cool test samples to 0 °C in an ice water bath than it is to achieve a stable room temperature of 20 °C.

"With this new reference material we now serve the operators of impact test instruments better in industrial settings, where room temperature is not controlled," a JRC scientist said.

JRC-IRMM is the world's second-largest producer of steel reference materials with certified Charpy impact resistance. The new material, with the code ERM-FA013bk, has been processed and certified in accordance to ISO Guides 34 and 35.

ERM-BC150 and ERM-BD151 – Skimmed Milk Powder

The European Reference Materials ERM-BD150 and ERM-BD151 are certified for their amounts of 15 elements (calcium, chlorine, potassium, magnesium, sodium, cadmium, copper, iron, mercury, iodine, manganese, phosphorus, lead, selenium, and zinc) at trace and major constituent levels. They will support the dairy sector (producer and user) and food industry (user) to monitor the nutrient profile of their milk-powder-based products and to comply with limits set in European legislation for some of the trace elements. The materials are intended for quality control and assessment of method performance for measurement of elements in milk and milk products. Reliable testing of milk powder is essential both to establish its quality and to ensure the absence of contamination.

In the EU, hundreds of millions of tons of milk are processed annually. Milk powder is used in a wide range of food products, and its stability and ease of transport make it an important commodity for international trade and humanitarian aid. Reliable testing of milk powder is therefore essential both to assess its quality and to ensure its safety.

These materials are just two of the many reference materials provided by the JRC to laboratories around the world, making the millions of daily measurements reliable to ensure the safety of our food, check the status of our health and prevent major accidents and contamination from the environment. The JRC is the second-largest producer of matrix certified reference materials worldwide.

One material (ERM-BD150) was produced with mass fractions of about one tenth of the regulatory limit for food contaminants in milk and other foodstuffs (EC466/2001 and amendment 1881/2006), and the other material (ERM-BD151) with mass fractions around the regulatory limit. The starting material was fresh raw milk that was spiked with selected elements and converted to fine powders by a conventional industrial process. Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006.

Micro-arrays for Mass Spectrometry (MAMS)

A MALDI-based chip technology for single-cell mass spectrometry (SCMS)

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Heterogeneity is a characteristic attribute of all populations of living organisms. Cell-to-cell variability can be due to a number of reasons; e.g., non-uniformity of the cellular environment, such as a given cell within the cell cycle, genetic differences resulting from mutations or stochastic events in gene expression. However, examination of the underpinnings of intrapopulation variability of cells can only be achieved with single-cell analysis.

Analysis of tiny quantities of small molecules in complex mixtures/matrices is a challenge. Fluorometric approaches generally need the introduction of fluorescent tags; thus, their application to studies of low-molecular-weight compounds in cells and cell extracts is limited to a small number of analytes. Mass spectrometry (MS) is a label-free analytical technique which permits identification of virtually any analyte as well as structural analysis.

During the past decades, the application of mass spectrometry for the analysis of small molecules increased, and MS became the key technique in metabolomics.

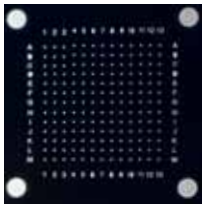

Figure 1 Image of the single cell micro-array square of 13 x 13 spots. Each spot has a diameter of 100 µm. Two arrays are placed on an omniphobic coated ITO slide in dimensions of a standard microscope slide.

Single-cell mass spectrometry (SCMS) is a rapidly growing discipline of analytical chemistry, and many examples of its use for the analysis of cells can be found. A few interesting studies show MS analysis of a couple of metabolites present in single cells. An example of a SCMS study by electrospray ionization (ESI) is the work by Mizuno et al., in which mass spectra corresponding to individual cells obtained from different cell lines were classified. Applications of SCMS based on matrix-assisted laser desorption/ionization (MALDI) for studying biomolecules in single neurons have also been shown.

MALDI-MS is often used in proteomics; however, its potential in metabolomics has recently been demonstrated, as well. MALDI-MS can provide efficient use of the limited amounts of analytes, and its sensitivity with respect to primary metabolites, such as nucleotides is adequate to detect the small levels present in single cells. In addition, MALDI-MS is less dependent on sample matrix composition than other techniques such as ESI-MS.

Here, Sigma-Aldrich is presenting the new MAMS for single-cell analysis. A few products used for the first successful application of the MAMS target are listed in **Table 1**.

Size of MAMS

The MAMS has the dimensions of a standard microscope slide containing two areas of 13x13 spots of 100 µm diameter (in total, 338 spots per slide)

Cat. No.	Material	Application Area	Package Size
50757	MAMS for single-cell mass spectrometry	Single-cell MS	1 slide
92817	9-Aminoacridine	MALDI-MS	1 g
650501	Acetone	CHROMASOLV® Plus, for HPLC, ≥99.9%	1 L
34852	Ethanol	CHROMASOLV absolute	1 L

Table 1 Sigma-Aldrich® products used for single-cell MALDI-MS

Compatibility of MAMS

Experiments were done using a 4800 plus MALDI TOF/TOF analyzer and a TOF/TOF 5800, (both ABsciex, see references). The microarrays were also tested on a Bruker Ultraflex Extreme MALDI Tandem TOF Mass Spectrometer. Please keep in mind that additional adapters for the MAMS slides are needed, e.g., LaserBio Labs Mass Spectrometry Imaging Starter Kit (AB) or Bruker Daltonik MTP Slide Adapter II for the Bruker Ultraflex series. Spot set designs/geometry files, for both systems (ABSciex and Bruker MALDI instruments) can be supplied on request by the instrument manufacturers.

Aliquoting process

Briefly, cells are taken from a liquid culture, quenched using cold solvents (to stop any metabolic activity), after which the supernatant is removed and cells are washed to remove salts. The cell suspension is then spread onto the MAMS target plate. Applying the cell suspension onto the MAMS surface will result in an automated aliquoting of the cell suspension into the hydrophilic reservoirs, without the need for a microspotter.

Depending on the cell concentration used, the number of cells on each hydrophilic reservoir can be between zero and hundreds. The transparency of the MAMS substrate allows for microscopic analysis to determine the number of cells in each reservoir while the cellular metabolism remains quenched because the entire MAMS chip is kept cold in a cryochamber.

9-AA MALDI matrix can be applied by spraying as described in the references below.

References:

[1] Amantonico, A.; Urban, P.L.; Fagerer, S.R.; Balabin, R.M.; and Zenobi, R. Single-Cell MALDI-MS as an Analytical Tool for Studying Intrapopulation Metabolic Heterogeneity of Unicellular Organisms, *Anal. Chem.* **2010**, 82, 7394–7400
[2] Ibanez, A.J.; Fagerer, S.R.; Schmidt, A.M.; Urban, P.L.; Jefimovs, K.; Geiger, P.; Dechant, R.; Heinemann, M.; Zenobi, R. Mass spectrometry-based metabolomics of single yeast cells, *PNAS* **2013**, 110, 8790–8794.
[3] Zenobi, R. Single-Cell Metabolomics: Analytical and Biological Perspectives, *Science*, Vol. 342, DOI: 10.1126/science.1243259

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Determination of Water Content in Candies

Hydranal® Applications: Caramel mass, jelly sweets, fruit gum and fondant

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

Water content determination by Karl Fischer titration is a fast, accurate and reliable method. Especially for food samples, the Karl Fischer (KF) titration shows significant advantages over traditional drying techniques when determining water content. When food samples are heated and dried, the Maillard reaction, a reaction between amino acids and reducing sugars, may occur. During this non-enzymatic browning process, water is formed as a by-product of the reaction. In a drying process, this water would also be detected as weight loss, and the result of the determination would be biased. Furthermore, volatile components contained in the food samples would evaporate and also bias the drying result. KF titration, on the other hand, is not influenced by volatile components. Therefore, KF titration is a broadly applied technique for determining water content, not only in the food industry but also in the chemical, pharmaceutical, and petrochemical industries.

In confectionery products, water content affects stability, texture, and flow properties of the product. Consequently, it is an important parameter which must be closely monitored to hold it within specifications.

For the titration of the total water content, the sample should dissolve or be dispersed in the working medium. Dissolution can be increased by adding a polar solvent such as Hydranal-Formamide, by heating the working medium to about 50 °C using a double-walled titration vessel connected to a circulator, or by introducing a dispersing device to the titration vessel to homogenize, or suspend the sample in the working medium before starting the titration. Instead of methanol as working medium, the ethanol-based Hydranal-CompoSolver E can also be used. However, due to the poorer solubility of sugar-containing samples in this medium, titration times can be longer.

Water content determination in caramel mass

The texture of candies such as soft caramels (toffees) depends on their water content. Soft caramel masses are mainly composed of sucrose, milk, starch syrup, invert sugar, and fat; their average water content is 4 – 8% [1]. Generally, the relationship between water content and texture is that the higher the water content, the softer the toffee. Flavor and quality-defining components such as cream, honey, chocolate, nuts, or vitamins are added to the base mixture just before shaping the toffees.

Caramel masses do not dissolve easily in the methanolic medium of the KF titration at room temperature. Dissolution support is needed here and several possibilities are available:

- Addition of Hydranal-Formamide to the working medium
- Titration at 50 °C in a double-walled titration vessel
- Dispersing device introduced to the titration vessel to homogenize or suspend the sample in the working medium

Complete dissolution of such samples may take up to several minutes. The softened caramel mass may stick to the indicator electrode, disrupting indication, and leading to over-titration. This can cause erroneous results, as the titration may be stopped although the water content has not been completely determined. In this case, the electrode must be cleaned and the titration started over with a new sample.

Application: Caramel mass (L413)
20 mL Hydranal-Methanol dry and 20 mL Hydranal-Formamide dry, or 20 mL Hydranal-CompoSolver E and 20 mL formamide are added to the titration vessel, heated up to 50 °C, and titrated to dryness with Hydranal-Composite 5.

Sample size: approx. 0.25 g. The sample can be easily handled using a syringe without needle.

If an ethanol-based working medium is preferred over methanol, then Hydranal-CompoSolver E can be used. The two-component reagent Hydranal-Titrant (E)/Solvent (E) can also be used.

Water content determination in gum and gelatin candies

For production of jelly, gum, and gelatin candies, aromatized sugar solutions are heated with polysaccharides such as agar, pectin, gum arabic etc., and gelatin. They are formed by pouring the mass into starch moulds and removing the pieces after hardening [1]. The most famous gum candies are probably "gummi bears", traditionally made with gelatin, but also available in vegetarian variations with starch or pectin instead of gelatin. The water content of these candies is relatively high, with an average of 14–18%, resulting in the typical gum-like texture [2].

Samples containing high amounts of gelatin should be chopped very finely for this analysis, using a knife or scissors. The Hydranal service lab recommends adding formamide to the working medium and heating the titration vessel to 50 °C in order to dissolve the sample. Additionally, the use of a dispersing device is recommended and can reduce the titration time to 1–2 minutes.

Application: Jelly sweets (L402)
20 mL Hydranal-Methanol dry and 20 mL Hydranal-Formamide dry are added to the titration vessel, heated to 50 °C, and titrated to dryness with Hydranal-Composite 5.

Sample size: approx. 0.2 g, cut into small pieces

The use of a homogenizer reduces dissolving and titration time. The two-component reagent Hydranal-Titrant/Solvent can also be used.

Application: Fruit gum with sugar crust (L228)
Fruit gum dissolves very slowly in the alcoholic media of the KF reagents. Again, it is recommended to cut the sample into small pieces with a knife or scissors. Addition of formamide to the working medium is required for sample dissolution. If the titration is carried out at 50 °C, dissolution time is around 3 minutes, with an additional 3–4 minutes for titration.

20 mL of Hydranal-Methanol dry or Hydranal-Methanol Rapid and 10 mL Hydranal-Formamide dry are added to the titration vessel, heated to 50 °C, and titrated to dryness with Hydranal-Composite 5.

Sample size: approx. 0.3 g, cut into small pieces

Allow for a dissolution time of around 3 minutes, then titrate with Hydranal-Composite 5. The two-component reagent Hydranal-Titrant/Solvent can also be used.

(continued on page 22)

Water content determination in Fondant

Fondant is a sugar mixture that can be found in various shapes and colors, e.g. egg-shaped during the Easter season, or scented with peppermint aroma at Christmas. It can have a sugar percentage of up to 90%. This sugar is mainly present as undissolved small crystals (these crystals make fondant appear white), in a highly viscous suspension with saturated sugar solution [2]. The water content of Fondant lies at about 10–15% and the mass is still meltable and pourable on heating [1].

Fondant dissolves very slowly in the methanolic medium of Karl Fischer titration; the addition of formamide to the working medium is required. Titration at 50 °C further increases solubility.

Note: If a dispersing device in the titration vessel is used, the addition of formamide and the heating of the titration vessel are not necessary. In our investigations, titration under these conditions took 3 minutes; we obtained similar results with both methods.

Application: Fondant (sucrose and glucose syrup) (L322)

20 mL Hydranal-Methanol dry or Hydranal-Methanol Rapid and 20 mL Hydranal-Formamide dry are added to the titration vessel, warmed to 50 °C, and titrated to dryness with Hydranal-Composite 5.

Sample size: approx. 0.3 g. The sample can be easily handled using a syringe without a needle.

Procedure with dispersing device

40 mL Hydranal-Methanol dry or Hydranal-Methanol Rapid are added to the titration vessel. The homogenizer is switched on and the vessel is titrated to dryness under these conditions.

Sample size: approx. 0.3 g. Titration starts immediately. After dissolution of the sample, the disperser can be switched off.

The two-component reagent Hydranal-Titrant/Solvent can also be used. When using the disperser, 20 mL Hydranal-Solvent and 20 mL Hydranal-Methanol dry are used as the working medium.

Cat. No.	Brand	Description
34805	Fluka®	Hydranal-Composite 5
34741	Fluka	Hydranal-Methanol dry
37817	Fluka	Hydranal-Methanol Rapid
34734	Fluka	Hydranal-CompoSolver E
34724	Fluka	Hydranal-Formamide dry
34801	Fluka	Hydranal-Titrant 5
34732	Fluka	Hydranal-Titrant 5 E
34800	Fluka	Hydranal-Solvent
34730	Fluka	Hydranal-Solvent E

Table 1 Selected Hydranal Karl Fischer reagents

Available application reports:

- L413 Caramel mass
- L402 Jelly sweets
- L228 Fruit gum with sugar crust
- L322 Fondant (sucrose and glucose syrup)

Sigma-Aldrich® offers over 700 application reports. A full list can be found on our website **sigma-aldrich.com/hydranal**. To obtain an application report, or to request assistance with your KF application, please contact one of our Hydranal laboratories (see below).

References

[1] Food Chemistry. 4th revised and extended Edition. Belitz, H.-D.; Grosch, W.; Schieberle, P. Springer-Verlag Berlin Heidelberg, **2009**.

[2] Bundesverband der Deutschen Süsswarenindustrie e.V. ***www.bdsi.de***

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Titration Reagents by Sigma-Aldrich®

Ready-to-Use Solutions and Volumetric Concentrates

Andrea Felgner, Market Segment Manager 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 hence, on the quality of the titration reagents.

Because analysts depend on the quality of the titration reagents, manufacturers of the reagents must adhere to strict quality criteria. High-quality titration products from Sigma-Aldrich include:

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

Concentrates:
Packaged by Quantity of Analyte, Not Concentration

The measured titer (factor) of our ready-to-use solutions is displayed on the certificate of analysis. However, for the concentrates (formerly under the trademark Fixanal®) we take the time to adjust the titer to 1.000 (to a precision of ± 0.1%) during the filling process. With the titer so determined, we then calculate the weight of the volume of liquid corresponding to exactly 0.1 mol of the specified analyte. Using high-precision filling equipment, this exact volume of liquid is added to every ampoule in the lot. The concentration of the liquid inside each ampoule may vary slightly from batch to batch, but the amount of the specified material in each ampoule is exactly the same, for example 0.1 mol HCl (not mol/L).

By diluting the entire content of the ampoule to the desired volume, a solution can be prepared to meet your specific concentration requirement, knowing for certain that it has been tested against a certified reference material.

VOLPAC Solution Containers

Apart from the customary package sizes up to 5 L, the Sigma-Aldrich portfolio also includes special 5 and 10 L VOLPAC containers when larger quantities of ready-to-use solutions are needed. VOLPAC containers consist of a rugged cardboard cube containing a flexible inner polyethylene bag with an outlet tap. As the solution is withdrawn, the polyethylene bag collapses, preventing influx and contamination by laboratory air. Also, because there is no room for volume expansion, the evaporation and condensation of water that increase the error of the titer is prevented. For easy connection to the tubes of the titration equipment, 2 adapters of different sizes are enclosed with each VOLPAC.

Reagents for Complexometric Titration

We offer a selected range of aminopolycarboxylic acids (EDTA analogs) as reagents for complexometric titration (formerly under the trademark Idranal™). In complexometric titration, metals in the sample react with complexing agents (chelating agents) to create complexes. Typically, a color change determines the end point of the titration. Analytical applications include the determination of water hardness and special titrimetric metal determinations. Sigma-Aldrich's complexometric reagents are stable at high temperatures, readily soluble in water, and form stable chelates with most metal ions over a wide range of pH and temperature. They show hydrolytic stability in both acidic and alkaline conditions. These reagents are available as solids, ready-to-use solutions, and concentrates. In addition, we offer buffer solutions, masking agents, and indicators specially designed for complexometric titration.

Fluka reagents for volumetric titration (Selection)	
35263	Sodium hydroxide solution, Reag. Ph. Eur., 0.1 mol/L
35256	Sodium hydroxide solution, Reag. Ph. Eur., 1 mol/L
38210	Sodium hydroxide concentrate for 0.1 mol/L
35071	Wijs solution, 0.1 mol/L
35418	Perchloric acid solution, for titration in non-aqueous medium, 0.1 mol/L in acetic acid
35328	Hydrochloric acid solution, Reag. Ph. Eur., 1 mol/L
35335	Hydrochloric acid solution, Reag. Ph. Eur., 0.1 mol/L
38280	Hydrochloric acid concentrate for 0.1 mol/L

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Date: 01/2014
Lit. Code: PVF