

Analytix

Issue 3 • 2008



Sigma-Aldrich New Product Branding



- Fluka becomes THE brand for Analytical Reagents
- Titrimetric and IC reference standards certified by BAM
- HybriScan™ Rapid Test Systems for microorganism in food
- Ready-to-use media and agar plates
- Solvents for speciation analysis
- Coulometric KF reagents

SIGMA-ALDRICH®

Product Branding

Making it easier to find and access our products



Trevor Jones
Marketing Manager Europe

Dear Colleague,

Over the years our product offering has grown through innovation, acquisitions and partnerships. Concurrently, our technology realms, production facilities, quality control systems, catalogues and brand names have also increased.

Inevitably, this long-term expansion has resulted in some duplication and misalignment of products in the different Sigma-Aldrich catalogues. So, with a goal of providing a logical structure to our product offering, we decided to perform some housekeeping to streamline and realign our products and brand names, as outlined below.

First step: Alignment under scientific field

First, we grouped our products according to the very broad customer groups we serve. Recognising some overlap exists, these groups are chemistry, life science and analytical. This created the primary structure for our reorganisation efforts.

Second step: Assigning a brand

Next, we realised that certain brands have a stronger presence in some markets than in others. We took advantage of this brand recognition and assigned the Aldrich® brand to our chemistry products and the Sigma® brand to our life science products. We have two particularly strong brands containing products for analytical techniques. Fluka® has specialty solvents, reagents and standards for analytical procedures; products specifically for chromatography and separations are aligned under Supelco®. Solvents and reagents for general use by all customer groups carry a Sigma-Aldrich® label.

Third step: Combining brands and catalogues

We are starting to move products between brands and our catalogues reflect these changes. The Sigma catalogue will contain all products for life science, the Aldrich catalogue, chemistry, and the Fluka catalogue, analytical reagents and standards. The Supelco catalogue continues in its current form. One notable change is the disappearance of the Riedel-de Haën® brand name. Riedel-de Haën laboratory reagents remain, but will now carry the Fluka brand. This was a difficult decision, but the high degree of overlap between Riedel-de Haën and Fluka products causes confusion with many users. Both names are synonymous with quality and reliability. Fluka is globally recognised as such and the Riedel-de Haën products are well placed here, with more general reagents receiving the Sigma-Aldrich label.

Whatever else may have changed, the important aspects of our products and our philosophy stay:

- Our dedication to quality exceeding expectations
- Our emphasis on innovation
- Our drive to provide customised services

Our feature article in this edition of Analytix has more information on our realignment efforts. The main rationale behind these changes is so you can more easily find the right product for your particular application from our extensive offering. We sincerely hope these changes have that intended result. Please let us know how we are doing!

Kind regards,

Trevor Jones
Marketing Manager Europe

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Sigma-Aldrich Product Branding

With some 130,000 products in total, Sigma-Aldrich is proud to have the widest range of chemicals, biochemicals and reagents for laboratory use on the market, but the larger our product range grows, the more difficult it can be to navigate and find the products one needs.

To address this, we have decided to group our products into clearly defined groups aligned with the scientific groups that we think are most interested in them.

We are widely recognised for our specialty products and they fall into 3 main categories:

- Analytical
- Biochemistry & Life Science
- Chemistry

We also have a comprehensive range of essential products, for example many solvents and reagents, that are to be found in many laboratories, regardless of the industry or discipline. Alongside our established laboratory products, our materials for specialty production and manufacturing are multiplying rapidly.

We now categorise all our products according to the primary discipline in which they are used, and we are aligning each product group under one of our core brands – the one that best describes its primary field of use.

Sigma – innovative kits & products for life science

Aldrich – comprehensive range of chemicals for research

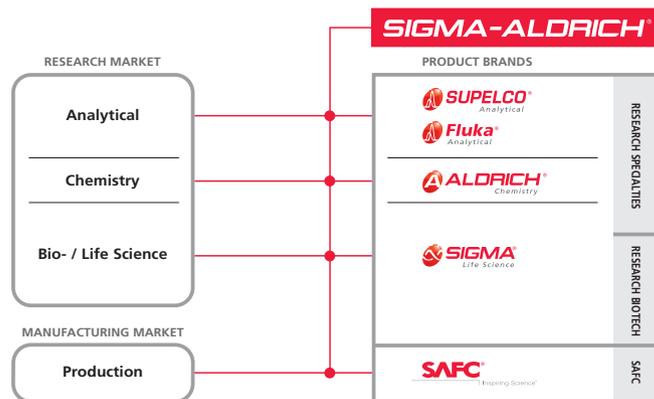
Fluka – high quality, specialty reagents & standards for analysis

Supelco – innovative products for chromatography & separations

Sigma-Aldrich – essential products for laboratory use

SAFC – leading edge materials for demanding production

This categorisation means you will start to see some products appearing with a label different to that you may have been used to. For example, laboratory reagents from Riedel-de-Haën are now being produced and packaged under the Fluka label as they are primarily used in Analytical applications. You will notice that the Fluka label itself has transitioned from its traditional blue form to a new, modern red image, in keeping with Sigma-Aldrich's corporate



design. Some Riedel-de Haën laboratory reagents will be packaged as Sigma-Aldrich products, for example those reagents that are used in a wider variety of procedures and laboratories in numerous disciplines. The decision to move these products to the Fluka and Sigma-Aldrich names was not taken lightly.

Riedel-de Haën laboratory reagents have been a part of Sigma-Aldrich since 1997. The products, services and technology are now intimately woven into Sigma-Aldrich's infrastructure and portfolio. We continue to make, package and distribute them in the same way. The products are still manufactured in the same facilities in Germany and across Europe. Most importantly of all, they remain subject to our stringent Quality Assurance & Management processes. As with all our products, we ensure that they are of the best quality and always meet the demands in the applications for which they are intended. Sigma-Aldrich still has all the factories, laboratories, warehouses and staff to continue supply research and quality control; and as we better align our products with the scientists we serve, so we can better focus our future investments accordingly.

- Easier navigation of our printed and on-line catalogues
- Easier and quicker to search-and-find products
- Easier to access on-line and technical service
- Easier to decide which product and grade is most appropriate

Our product range, dedication to quality and reliable service remain the same, it is only the names on individual products that change. Ultimately we want to maintain a product range that has breadth, reliability and structure that makes it easier for you to navigate through the Sigma-Aldrich world and readily find the products and services that you need.

Opening Doors to a World of Science

BAM Certified Reference Materials for Titrimetry and Ion Chromatography

A decade of fruitful cooperation between BAM and Sigma-Aldrich

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It is the responsibility of certain federal agencies and metrological institutes to assign traceable values, providing the ultimate link to the SI unit by performing a primary measurement. However, not every agency may be adequately equipped for the efficient production of standards and calibration solutions. Often

commercial producers are better equipped for this task. An ideal situation is to combine these complementary skills; like the decade-long cooperation between the Sigma-Aldrich production facility in Buchs, Switzerland, and the Swiss Federal Institute for Materials Science and Technology (EMPA) and Federal Institute for Materials Research and Testing (BAM) for the certification of titrimetric standards and calibration solutions. In this partnership, Sigma-Aldrich was responsible for the supply and production of different reference materials. When EMPA terminated its activities in chemical metrology in 2004 and no longer acted as a national metrological institute, BAM and Sigma-Aldrich intensified their close collaboration and launched the current series of certified anion standards.

Certified reference materials (CRM) for titrimetry

Although cutting-edge analytical instruments like spectrometers, AAS, ICP-OES, etc. are now commonplace in analytical laboratories, volumetric titration is still widely used because it is fast, inexpensive and very precise. The most important titrimetric CRMs offered by Sigma-Aldrich and their typical use are summarised in **Table 1**.

For the standardisation of titrants, a primary standard should be used to assure traceability to a well-defined reference. A primary standard should have:

- 1) Well-defined content and high purity (>99.8%) with a stated measurement uncertainty
- 2) Traceability to SI unit
- 3) Good stability for handling under ambient conditions and during storage (shelf life)
- 4) High equivalent weight for optimal weighing
- 5) Good solubility, especially important for titrimetry

Industries like pharmaceutical or semiconductor may need to work with certified standards with higher qualification to comply with GMP, GLP or ISO guidelines. Labs working in a regulated environment usually have to prove traceability of their measurements, evaluate measurement uncertainty and validate their analytical methods.

The increasing trend of using CRMs for regular use is reinforced by official regulations of the European Community within the guideline 96/23/EG, paragraph 3.1.1.2. which states:

“The determination of trueness (one component of accuracy) is described: trueness can only be established by means of CRMs. A CRM should be used whenever available.”

The U.S. Food and Drug Administration (FDA) demands in their Inspection Guide the use of reference standards in analytical methods.

Even when a laboratory performs measurements under no special regulations, it is often advantageous to use a CRM when methods need to be verified or instruments qualified. This explains the increasing demand for CRMs certified by an independent metrological institute.

Table 1 BAM-certified reference materials for titrimetry including the new CRM potassium dichromate (no. 42019, available in 50 g package size) with their typical use

Titration method	Standard for volumetric titration	Cat. no.	Examples of use
Acid-base titration	Benzoic acid	12353	Acidimetric CRM for standardisation of bases in non-aqueous solution
	Potassium phthalate monobasic	60357	Acidimetric CRM for standardisation of bases in aqueous solution
	Sodium carbonate	71363	Basimetric CRM for standardisation of acids in aqueous solution
	Tris(hydroxymethyl)aminomethane (Tris base, Trizma® base)	93440	
Redox titration	Arsenic trioxide	17971	Reductometric CRM for standardisation of iodine solution
	Sodium oxalate	71804	Reductometric CRM for standardisation of permanganate solution
	Potassium dichromate	42019	Reductometric CRM for standardisation of thiosulfate solution
	Potassium iodate	60386	
Complexometric titration	Calcium carbonate	21067	Complexometric standard for standardisation of EDTA solution
Precipitation titration	Sodium chloride	71387	Argentometric standard for standardisation of silver solution

(continued on page 6)

Each BAM-certified standard for titrimetry is certified according to ISO/IEC 17025 and accompanied by a printed certificate fulfilling ISO Guide 31^[1]. The following parameters are provided in each certificate:

- Certified property value and its uncertainty
- Detailed analysis report of BAM, including traceability statement and measurement procedure
- Intended use, storing and handling instructions
- Expiry date based on stability studies
- Homogeneity and the recommended minimal amount to use

The label on each bottle contains the following information:

- Certified property value with uncertainty
- Sigma-Aldrich catalogue number and product name
- Batch number
- Expiry date

Please visit www.sigma-aldrich.com/titrimetry for more information.

Certified standards for ion chromatography

After the successful introduction of the BAM-certified titrimetry CRMs, Sigma-Aldrich and BAM launched a series of CRM for ion chromatography. With PRIMUS two ready-to-use Primary Multi-ion Solutions were introduced and the outstanding characteristics of these CRM were described in an article of "Metrohm Information"^[2]. In addition, a series of mono-anion CRM were launched which are described in more detail here (**Table 2**): To guarantee the highest reliability of the 1000 mg/L anion standards, they are certified by two independent certification bodies. The batch solution of the reference material is prepared gravimetrically, using well-characterised high-purity starting materials (SM)^[3] in a double-accredited laboratory under ISO/IEC 17025 and ISO Guide 34^[4]. The SM content is calculated by 100% minus all impurities. The calculated SM content is verified by a comparison with an appropriate standard reference material from the National Institute of Standard & Technology (NIST). If contamination and loss of material during the whole production procedure is strictly prevented, this approach allows high accuracy and small uncertainties. The first certified value of the bottled CRM is based on this approach and is therefore directly traceable to the SI unit kilogram.

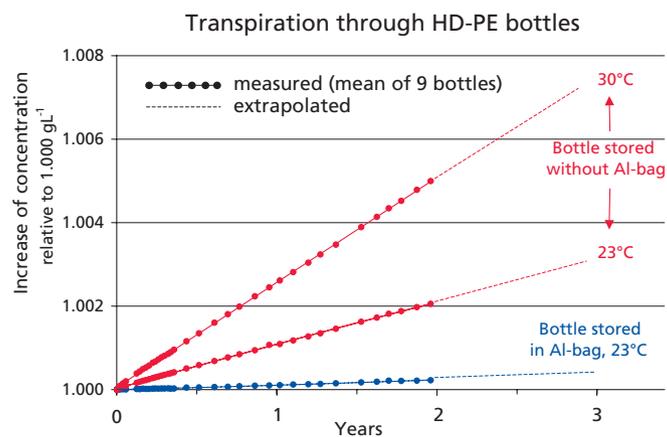
After bottling the batch solution under clean-room conditions and random sampling, a second certified value is produced by BAM using volumetric titration or precipitation analysis. The measurements are traced directly to SI unit or to a Standard Reference Material from NIST. Both property values, the certified value from BAM and from

Sigma-Aldrich, are combined to the certified value of the anion standard solution.

The equipment and materials used to package the standard solution must maintain the quality instilled by the certification procedure. Easy handling, low impurities and good storage stability are essential. The use of HDPE bottles with aluminium-coated bags extends the shelf life of the unopened CRM up to four years and in addition this special packaging leads to smaller uncertainties. Only this combination of high-precision analytical measurement and packaging know-how leads to uncertainties of the certified values down to 0.2% (expanded uncertainty). **Figure 1** shows a comparison between storage with and without additional packaging. Detailed data on trace impurities and data from transpiration studies are given in the printed certificate.

Please visit www.sigma-aldrich.com/ic for more information.

Figure 1 Aqueous standards in tightly closed plastic bottles (HDPE, LDPE, etc.) lose a significant percentage of solvent at room temperature within a storage time of several years. Aluminized bags and also storing at low temperature (i.e. refrigerator) will reduce this transpiration rate to an acceptable level.



References

- 1] ISO Guide 31, Reference materials – Contents of certificates and labels, 2000.
- 2] PRIMUS – Certified standard solutions for ion chromatography, Metrohm Information, Vol. 33 (2004) No. 1 (available at www.metrohm.com/company/metrohm/2004/mi2004_1e.html).
- 3] TraceCERT® – Traceable Certified Reference Materials. Part 3: Challenges in the characterisation of high purity starting materials, Analytix, Vol. 2, 2007 (available at www2.sigmaaldrich.com/analytix).
- 4] ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories, 2005.
ISO Guide 34, General requirements for the competence of reference material producers, 2000.

Table 2 BAM and Sigma-Aldrich certified reference materials for ion chromatography

Cat. no.	Constituents	Starting material	Quality	Package size
87969	Bromide	NaBr	Certified reference materials for ion chromatography	100 mL HDPE bottle sealed in aluminised bag
87603	Chloride	NaCl		
80373	Fluoride	NaF	Certified by BAM and gravimetric assay	
86576	Nitrate	NaNO ₃		
81193	Phosphate	Na ₂ HPO ₄		
80218	Sulphate	Na ₂ SO ₄	1000 mg/L in water, exact content and uncertainty on label and enclosed certificate. Shelf life: four years.	

Nitrogen and protein determination according to Kjeldahl method: the use of modern automated digestion & distillation systems and operational quality check procedure using reference materials

An overview of the Kjeldahl method including state-of-the-art equipment and a new traceable standard for IQ and OQ

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Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

The Kjeldahl method

For almost 130 years the determination of nitrogen using the method developed by Danish chemist Johan Gustav Christoffer Thorsager Kjeldahl (1849–1900) has been an internationally accepted standard. He introduced the method in 1883 at a meeting of the Danish Chemical Society as a means to determine nitrogen in barley and yeast. The method, named after its inventor, has since found widespread application in agricultural, environmental, chemical, biochemical and pharmaceutical markets. Today, the Kjeldahl method is applied to the determination of nitrogen, primarily as a means to calculate the protein content, in dairy products, meat, beer, cereals and many other samples.

Kjeldahl process

When determining protein according to the Kjeldahl method, the sample is first treated (digested) with concentrated H_2SO_4 , which leads to the formation of ammonium sulphate. Through alkalisation with NaOH, the ammonia is displaced from the ammonium sulphate and over-distilled into a boric acid receiver via steam distillation. This solution is then titrated with HCl or H_2SO_4 to determine the nitrogen content (and hence protein) in the sample.

Today, the Kjeldahl method can largely be automated by half- or fully automated Kjeldahl Distillation Units.

The three primary steps in the Kjeldahl method:



New for Kjeldahl: KjelFlex K-360

Büchi Labortechnik AG, a market leader in analytical laboratory solutions, has recently developed the KjelFlex K-360, the latest innovation in a wide range of Kjeldahl digestion and distillation instruments.



Thanks to its unique modular design, the KjelFlex K-360 offers flexibility to meet changing laboratory needs. By means of four add-on modules, the user can adjust the K-360 according to each individual application. The K-360 can be delivered completely with all modules; the modules can also be purchased separately and installed at any time for easy upgrading.

The basic configuration provides automatic dosage of boric acid, NaOH and water as standard. If needed, the unit can be equipped with an additional acid-resistant pump for the dosage of stronger acids. The application range is extended by a number of non-Kjeldahl applications, such as phenol distillation and the determination of formaldehyde, sulphur dioxide and volatile acids.

By connecting an external titrator (Mettler, Metrohm, Schott, Radiometer, for example), the K-360 capabilities are extended to provide an automatic system for nitrogen and protein determination. With such a configuration, manual work is reduced to an absolute minimum. The titration starts automatically when the distillation is finished. After titration, the contents of the sample tube and receiving vessel are drawn off automatically.

Besides the unique modularity, the K-360 offers a variety of additional features, including storage capacity of up to 50 customer-specific methods or 500 results, password security, data printout using a USB interface, multilingual software and numerous safety functions.

(continued on page 8)

Qualification of the KjelFlex K-360

The qualification of laboratory instruments is an important aspect when following GMP/GLP regulations. The Installation Qualification (IQ) and Operational Qualification (OQ) assure that the instrument is delivered and operates according to the manufacturer's specifications. Büchi and Sigma-Aldrich have also collaborated to develop a complete service package that comprises Installation Qualification, Operational Qualification, reference substance for the validation and several certificates. Visit www.buchi.com for more information on the K-360.

NIST-Traceable Analytical Standard for KjelFlex K-360 from Sigma-Aldrich

Sigma-Aldrich has developed new Fluka-brand reference material for KjelFlex K-360: an ammonium phosphate monobasic analytical standard for nitrogen determination according to the Kjeldahl method. Its nitrogen content is traceable to NIST SRM 194.

Please visit www.sigma-aldrich.com, and type in the word "Kjeldahl" under Keyword (full text) in the search engine to see our complete offering of products and literature on the Kjeldahl method.

Table 1 Ammonium phosphate standard for Kjeldahl method from Sigma-Aldrich

Cat. no.	Brand	Description	Package size
88431	Fluka	Ammonium phosphate monobasic, Analytical standard, for nitrogen determination according to Kjeldahl method, $\geq 99.5\%$	25 g

Upcoming Events ... HYDRANAL® seminars



HYDRANAL seminars in 2008

As a service to the scientific community, we routinely offer seminars to provide training on the chemistry behind the Karl Fischer technique and information specific to the HYDRANAL product line. In 2008, seminars in many cities around the world are planned. Please visit www.sigma-aldrich.com/events to see our most up-to-date schedule.

July

1 st	Newbury, Berkshire, UK
3 rd	Runcorn, Cheshire, UK

September

25 th and 26 th	Buenos Aires, Argentina
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October

1 st	Rio de Janeiro, Brazil
2 nd	Sao Paolo, Brazil
13 th	Helsinki, Finland
14 th	Turku, Finland
16 th	Gothenborg, Sweden

17 th	Stockholm, Sweden
21 st	Karlsruhe, Germany
23 rd	Zofingen, Switzerland
tbd	South Africa
tbd	Bologna, Italy

November

10 th	Lund, Sweden
11 th	Copenhagen, Denmark
12 th	Jylland, Denmark
13 th	Oslo, Norway
25 th -26 th	Seelze, Germany (2-day seminar)

For registration and additional information please contact:

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Fluka is an Authorised Distributor of the Institute for Reference Materials and Measurements (IRMM) Geel, Belgium

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- **fast reliable service**
- **attractive product prices**
- **expert technical service**



Fluka-brand physical properties standards and reference materials

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

Sigma-Aldrich offers a large collection of Fluka-brand reference materials and certified reference materials with highly specified parameters for the most important areas of physical property measurement. We are also an authorised distributor of the complete range of physical properties certified reference materials from the IRMM (Institute for Reference Materials and Measurements).

Sigma-Aldrich offers standards for the following physical properties:

Colour
Density
Melting point
Conductivity
MS (molecular weight) markers
Particle size
pH calibration
Redox
Turbidimetry
X-Ray

Colour reference solutions

Colour reference solutions are used to measure the degree of coloration of liquids in the red, yellow, green, blue and brown colour ranges as specified by the European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP) and by federal institutes such as the American Society for Testing and Materials (ASTM).

We were the first to offer the most commonly used colour reference solutions according to Ph. Eur., USP and ASTM/APHA. These solutions are used to estimate the colour of a liquid against established international and standardised liquid colour values. The two convenient package sizes, 2 mL or 10 mL, are sealed in airtight ampuls under argon. Each set is supplied with a certificate of analysis with the expiration date clearly indicated on the label.



Proper use of colour standards

The perception of colour and similarities between colours depends to a great extent on the viewing conditions and quality of illumination. Determinations should be made under diffuse, uniform illumination and conditions that minimise shadows and non-spectral reflectance. Liquids should be compared in matched colour comparison tubes against a white background. The colours of the standards should be as close as possible to those of the samples when quantifying colour differences.

Colour reference solutions acc. to Ph. Eur.

Ph. Eur. solutions are available as a complete set of 37 solutions, divided into five colour series: red (R1-R7), greenish-yellow (GY1-GY7), yellow (Y1-Y7), brownish-yellow (BY1-BY7) and brown (B1-B9). Each colour series is also sold separately.

Table 1 Colour reference solutions acc. to Ph. Eur.

Cat. no.	Brand	Description	Package size
83952	Fluka	Colour reference solutions B, BY, Y, GY, R acc. to Ph. Eur., set	Set of 2 mL ampuls
83951	Fluka	Colour reference solution B acc. to Ph. Eur., set	Set of 2 mL ampuls
86293	Fluka	Colour reference solution BY acc. to Ph. Eur., set	Set of 2 mL ampuls
83883	Fluka	Colour reference solution Y acc. to Ph. Eur., set	Set of 2 mL ampuls
82995	Fluka	Colour reference solution GY acc. to Ph. Eur., set	Set of 2 mL ampuls
87448	Fluka	Colour reference solution R acc. to Ph. Eur., set	Set of 2 mL ampuls
90232	Fluka	Colour reference solutions B, BY, Y, GY, R acc. to Ph. Eur., set	Set of 10 mL ampuls
92936	Fluka	Colour reference solutions B acc. to Ph. Eur., set	Set of 10 mL ampuls
72666	Fluka	Colour reference solutions BY acc. to Ph. Eur., set	Set of 10 mL ampuls
83967	Fluka	Colour reference solutions Y acc. to Ph. Eur., set	Set of 10 mL ampuls
90269	Fluka	Colour reference solutions GY acc. to Ph. Eur., set	Set of 10 mL ampuls
95872	Fluka	Colour reference solutions R acc. to Ph. Eur., set	Set of 10 mL ampuls

Colour reference solutions acc. to USP

The USP prescribes twenty different colour solutions, marked A-T, covering the range red, yellow, green, blue and purple. These solutions are available in one complete set.

Table 2 Colour Reference Solutions acc. to USP

Cat. no.	Brand	Description	Package size
87576	Fluka	Colour reference solutions acc. to USP, set	Set of 2 mL ampuls
87574	Fluka	Colour reference solutions acc. to USP, set	Set of 10 mL ampuls

Colour Reference Solutions acc. to ASTM/APHA

ASTM lists a standard method for the visual measurement of the colour of light-coloured liquids using the platinum-cobalt scale. This method is referred to by many as APHA colour. The preparation of these platinum-cobalt standards was originally described by A. Hazen. Consequently, this test is also termed "Hazen Colour." The complete APHA set comprises 20 standard solutions, marked with different colour standard numbers: 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 100, 150, 200, 250, 300, 350, 400, 450 and 500.

Table 3 Colour Reference Solutions acc. to ASTM/APHA

Cat. no.	Brand	Description	Package size
72599	Fluka	Colour reference solutions acc. to APHA, set	Set of 2 mL ampuls
77147	Fluka	Colour reference solutions acc. to APHA, set	Set of 10 mL ampuls

Density CRMs from H&D Fitzgerald

Density standards are used to calibrate density meters. Although calibration with air and water is common practice, using reference standards has benefits with regard to GLP compliance and traceability of the calibration to recognised national standards. We offer a line of liquid density standards from respected density metrologists H&D Fitzgerald. ISO 9001 and 17025 compliant, the standards are also supplied with a UKAS-accredited calibration certificate, linking them to recognised national standards of mass, temperature and pressure. They are produced using a UKAS-accredited hydrostatic weighing system, and supplied in 10 mL sealed ampuls with a certificate of analysis.

Table 4 H&D Fitzgerald density standards

Cat. no.	Brand	Description*	Package size
36232	Fluka	Density Standard, 1251 kg/m ³	10 mL
12156	Fluka	Density Standard, 1623 kg/m ³	10 mL
15889	Fluka	Density Standard, 692 kg/m ³	10 mL
44964	Fluka	Density Standard, 998 kg/m ³	10 mL
89353	Fluka	Density Standard, 1191 kg/m ³	10 mL
76081	Fluka	Density Standard, 749 kg/m ³	10 mL
76731	Fluka	Density Standard, 870 kg/m ³	10 mL

* The precise density (at a given temperature) uncertainty is provided with each sample. Density standards have an uncertainty of ± 0.01 to ± 0.03 kgm⁻³ over the stated temperature range.

Melting point standards

Melting point is used to identify compounds and estimate purity. We offer a range of melting point standards to help ensure reliable performance of melting point instruments. Replicate measurements allow reporting of an uncertainty value, $\pm 0.3^\circ\text{C}$ or $\pm 0.5^\circ\text{C}$, for melting points above 150°C . Measurements are made in the thermodynamic mode, with traceability to primary reference material.

Table 5 Fluka-brand melting point standards

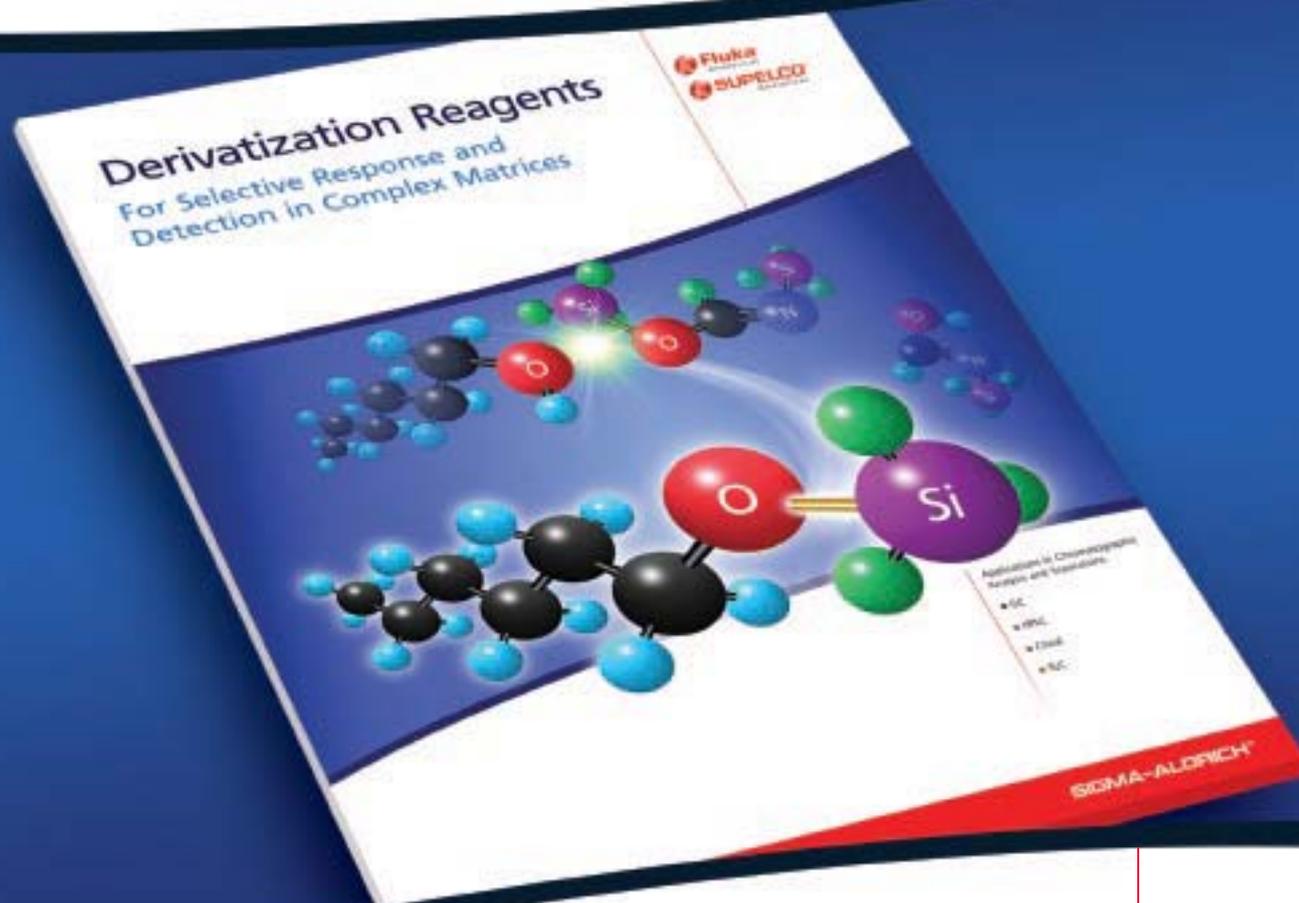
Cat. no.	Brand	Description	Package size
76170	Fluka	Melting point standard 121-123°C (Benzoic acid)	5 g
42183	Fluka	Melting point standard 182-184°C (p-Anisic acid)	250 mg, 1g
41019	Fluka	Melting point standard 235-237°C (Caffeine)	1 g, 5 g
67372	Fluka	Melting point standard 283-286°C (Anthraquinone)	250 mg, 1g
50296	Fluka	Melting point standard 47-49°C (Benzophenone)	1 g, 5 g
01422	Fluka	Melting point standard 79-81°C (Naphthalene)	250 mg, 1g

For further information on these and our other standards for physical property measurements, please visit our web page www.sigma-aldrich.com/physicalproperties

Derivatization Reagents

 **Fluka**
Analytical

 **SUPELCO**
Analytical



Many compounds must be derivatized to improve or even permit their analysis, in particular for GC, HPLC and TLC. Derivatization reagents for nearly every type of compound and application can be found in our comprehensive product offering, which includes chiral derivatization reagents for enantiomer analysis. Visit our website to view our products and obtain a copy of our new Derivatization Reagents brochure.

www.sigma-aldrich.com/derivatization



Ready-to-use, pre-prepared growth media in flasks and plates in blister packs

New forms of ready-to-use growth media are a time-saving and safer alternative for all microbiology laboratories

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

Ready-to-use, pre-prepared growth media in flasks

Sigma-Aldrich, through our Fluka brand, now offers flasks containing different types of popular growth media, both broths and agars, in a convenient, ready-to-use form. The new product line saves an enormous amount of time in both preparation and sterilisation. They are also much safer to use since they require no autoclaving. To use, simply melt the flasks of solid media in a microwave without losing or changing any promotional growth characteristics. Flasks of liquid media can be used without any further steps. The flasks and metallic caps are completely microwave safe (**Figure 1**).

Table 1 Fluka pre-prepared media in flasks

Cat. no.	Brand	Description	Package size
Liquid media & broths:			
94792	Fluka	Lactose Broth	10 x 100 mL
75717	Fluka	MacConkey Broth purple	10 x 100 mL
94217	Fluka	Peptone Water, phosphate-buffered	1 x 500 mL
43592	Fluka	Tryptic Soy Broth	1 x 800 mL
07507	Fluka	Tryptone water	10 x 100 mL
Solid agars:			
94216	Fluka	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts	10 x 100 mL
01477	Fluka	Nutrient Agar No. 2	10 x 100 mL
68414	Fluka	Plate Count Agar according to Buchbinder et al.	10 x 200 mL
51684	Fluka	Potato Glucose Agar	10 x 100 mL
55277	Fluka	Sabouraud 4% Glucose Agar	10 x 100 mL
79872	Fluka	Tryptic Soy Agar	10 x 100 mL
79873	Fluka	Violet Red Bile Glucose Agar	10 x 200 mL

Ready-to-use, pre-prepared agar plates in blister packs

As a further way to help microbiologists save time, we now offer pre-cast, ready-to-use agar plates. The formulations follow pharmacopeial and ISO regulations for testing the microbial content of liquid samples, like water and beverages, using the membrane filtration technique. The plates have a diameter of 55 mm and contain 10 mL of medium for accurate results after longer incubation times. Each box contains 30 plates in five blister packs (**Figure 2**).

Table 2 Fluka pre-cast 55 mm agar plates

Cat. no.	Brand	Description	Package size
14521	Fluka	Cetrimide Agar Plates	1 box of 30 plates
38259	Fluka	Enterococcus Selective Agar Plates (Slanetz-Bartley Agar; ISO 7899-2:2001)	1 box of 30 plates
19958	Fluka	mFC Agar Plates	1 box of 30 plates
09166	Fluka	Mannitol Salt Agar Plates	1 box of 30 plates
44776	Fluka	Nutrient Agar Plates	1 box of 30 plates
00464	Fluka	Plate Count Agar Plates	1 box of 30 plates
40376	Fluka	Sabouraud 4% Glucose Agar Plates	1 box of 30 plates
57994	Fluka	Tryptic Soy Agar Plates	1 box of 30 plates

Advantages:

- Long shelf life
- Store at room temperature
- Tightly sealed caps
- Sterility tested
- Reliable growth formulations
- Saves time
- Autoclaving or sterile filtration not needed

Figure 1 Flasks of pre-prepared media



Advantages:

- Long shelf life (6 months)
- Store at room temperature
- Each plate is sterile and individually packed
- Sterility tested
- Bubble-free surface
- Reliable growth formulations
- Saves time
- Autoclaving not needed

Figure 2 Pre-cast agar plates in blister packaging



HybriScan™ Rapid Test Systems

Rapid detection, identification and quantification of microorganisms in beverages, food and water

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The new **HybriScan** Test System, which uses sandwich hybridization, provides fast, sensitive and reliable detection, identification and quantification of spoilage and pathogenic microorganisms in beverages, food and water. It is ideal for the comprehensive and reliable routine control of raw materials and concentrates in all production steps up to the quality check of finished goods. **HybriScan** is a simple, time-saving assay that can be performed with standard laboratory equipment.

Benefits over conventional detection methods and PCR

HybriScan has significant time- and labour-saving benefits over traditional methods. It also has benefits over PCR and real-time PCR, which, although highly sensitive, are susceptible to experimental interferences, like template inhibition from insufficient purification, and lack quantification accuracy due to biases associated with PCR and reverse transcription reactions. In contrast, the **HybriScan** method is nearly independent of the influences of sample matrix and is able to distinguish between live and dead cells. It also permits the detection of non-culturable microbes. **Table 1** compares the benefits and disadvantages of the various methods.

Table 1 Advantages of **HybriScan** over other detection techniques

Detection technology	Advantage	Disadvantage
HybriScan	<ul style="list-style-type: none"> differentiates live/dead cells minimal interference by sample matrix high specificity low cross-reactivity easy handling cost-efficient read-out devices quantitative and qualitative high sample throughput (96-microwell plates) detects non-culturable microbes 	<ul style="list-style-type: none"> no differentiation of serotypes or subspecies limited probe design (rRNA target)
PCR	<ul style="list-style-type: none"> high sample throughput sensitive quantitative 	<ul style="list-style-type: none"> no live/dead-cell differentiation sensitive to matrix interference susceptible to polymerase inhibition
ELISA	<ul style="list-style-type: none"> differentiation of serotypes or subspecies high sample throughput (96-microwell plates) quantitative and qualitative 	<ul style="list-style-type: none"> low sensitivity low specificity, higher cross-reactivity slow and expensive assay development
Conventional cultivation-based methods	<ul style="list-style-type: none"> relatively inexpensive simple specific widely accepted method 	<ul style="list-style-type: none"> time-consuming (up to 10 days) no detection of non-culturable microbes low sample throughput laborious

Principles of the HybriScan method

The **HybriScan** method is based on the detection of rRNA via hybridization events and specific capture and detection probes (**Figure 1**). Specificity is achieved by targeting conserved or unique rRNA sequences. A labelled capture probe is used to immobilise the target sequence on a solid support plate (coated microtiter plate). A labelled detection probe provides an enzyme-linked optical signal read out. Detection results from application of antibody labelled enzyme. The bound complex is visualised by chromogenic substrate. Photometric data are measured at 450 nm and compared with standard solutions. The **HybriScan** software enables easy measurement and data analysis.

Sensitivity, specificity, flexibility and applicability of HybriScan technology

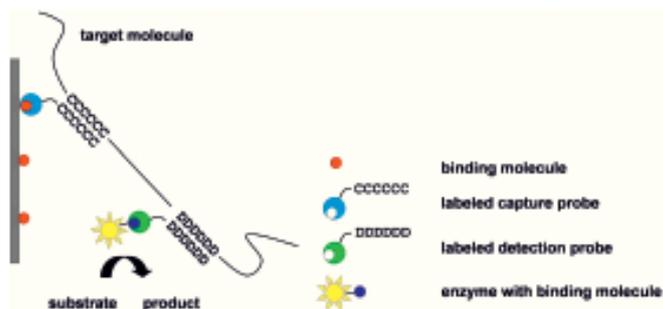
Sandwich hybridization is very sensitive, detecting attomoles of the respective target rRNA molecules.^[1] The ideal hybridization target for bacteria and yeast is rRNA. These cells contain a large number of rRNA-containing ribosomes; a single cell therefore contains several thousand copies of rRNA but only one DNA. Sandwich hybridization also provides sensitivity in crude biological samples because it is not susceptible to matrix interference.

By using specific probes, **HybriScan** allows flexible group- and species-specific detection. It is applicable to many analytical fields, including monitoring the microbial content of beer, wine, non-alcoholic beverages, drinking water, a wide variety of foods and wastewater. **HybriScan** rapidly and accurately identifies, detects and quantifies many important pathogenic species, including *Salmonella*, *Campylobacter*, *Listeria* and *Legionella* including the most relevant species *L. pneumophila*.^[2,3]

HybriScan *Listeria monocytogenes*: Rapid and innovative test system

One of the most important foodborne pathogens is *Listeria monocytogenes* (**Figure 2**), which poses a health threat in foods that have long, refrigerated shelf lives. Listeriosis, caused by ingestion of foods contaminated with *Listeria monocytogenes*, has increased

Figure 1 Principle of the **HybriScan** sandwich hybridization assay.



dramatically in recent years, causing a great deal of distress and even death. Milk, cheese, ice cream and meat contaminated with this pathogen have led to recent outbreaks of listeriosis. *L. monocytogenes* proliferates at refrigeration temperatures and is able to grow over a wide pH range from 4.39 to 9.40.

Figure 2 HybriScan Listeria mono Confirmatory Agar Fluka 92302 In front *Listeria monocytogenes*. *Listeria monocytogenes* permits rapid identification of suspect colonies within one hour.



Conventional culture-based methods to detect *L. monocytogenes* generally involve selective enrichment followed by culturing on selective medium, isolation and biochemical identification. This laborious and time-consuming approach often takes several days to show results. Also, compared to molecular biological and immunological methods, culture-based methods often give false negatives.

HybriScan *Listeria monocytogenes* is an excellent alternative to lengthy culture-based methods. It is as reliable and comprehensive as classical methods, but permits rapid detection and quantification with results available within 48 hours. The species-specific probe permits direct detection of *L. monocytogenes*, thereby eliminating false positives caused by other *Listeria* species. Even more compelling, suspected single colonies can be identified within one hour using the **HybriScan I** identification kit without need for further cultivation.

Figure 3 shows the validation results of **HybriScan** *Listeria monocytogenes*. Food samples were analysed with the **HybriScan** method and compared to the culture-based method according to § 64 LFGB. Five different food categories were tested. Results of validation showed a relative accuracy of 99.2%, relative specificity of 98.5% and relative sensitivity of 99.6%.

Two versions are available. **HybriScan I** *Listeria monocytogenes* is used for the extremely rapid, sensitive and economical identification of suspect colonies of *L. monocytogenes*. **HybriScan D** *Listeria monocytogenes* is used for the detection, identification and quantification of *L. monocytogenes* in different food matrices.

HybriScan kits are the result of a joint project between Sigma-Aldrich and Scanbec GmbH. For more details please visit us at www.sigma-aldrich.com/hybriscan

Figure 3 Validation of **HybriScan** *Listeria monocytogenes*. 355 food samples were analysed and compared to culture-based method according to § 64 LFGB. The blue values are the number of *L. monocytogenes* positive food samples in each category. Validation was according to ISO 16140:2003 (ASU L00.00-22).

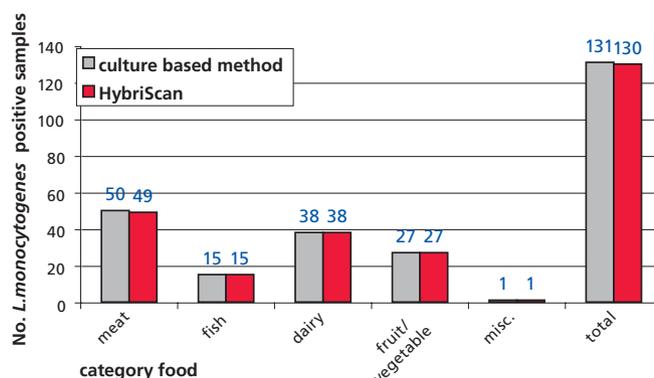


Table 2 **HybriScan** products (**HybriScan D** = detection kit; **HybriScan I** = identification kit)

Cat. no.	Brand	Description	Reactions
62533	Fluka	HybriScan D Beer	96
68301	Fluka	HybriScan D Drinks	96
96343	Fluka	HybriScan D <i>E. coli</i>	96
59744	Fluka	HybriScan D Lactobac	96
16593	Fluka	HybriScan D <i>Legionella</i>	96
07190	Fluka	HybriScan D <i>Legionella pneumophila</i>	96
55661	Fluka	HybriScan D <i>Listeria</i>	96
49699	Fluka	HybriScan D <i>Listeria monocytogenes</i>	96
55662	Fluka	HybriScan D <i>Salmonella</i>	96
02349	Fluka	HybriScan D Total Bacterial Count	96
04447	Fluka	HybriScan D Waste Water <i>Microthrix parvicella</i>	96
78436	Fluka	HybriScan D Waste Water Total Bacterial Count	96
61397	Fluka	HybriScan D Yeast	96
79742	Fluka	HybriScan I <i>Brettanomyces</i>	48
19503	Fluka	HybriScan I <i>Candida albicans</i>	48
76545	Fluka	HybriScan I <i>E. coli</i>	48
75724	Fluka	HybriScan I <i>Lactobacillus brevis</i>	48
80065	Fluka	HybriScan I <i>Lactobacillus buchneri</i>	48
86827	Fluka	HybriScan I <i>Lactobacillus lindneri</i>	48
49417	Fluka	HybriScan I <i>Legionella pneumophila</i>	48
77007	Fluka	HybriScan I <i>Leuconostoc</i>	48
49712	Fluka	HybriScan I <i>Listeria monocytogenes</i>	48
42875	Fluka	HybriScan I <i>Megasphaera</i>	48
89384	Fluka	HybriScan I <i>Pectinatus cerevisiiphilus</i>	48
73582	Fluka	HybriScan I <i>Pectinatus frisingensis</i>	48
67289	Fluka	HybriScan I <i>Pediococcus damnosus</i>	48
44492	Fluka	HybriScan Software	

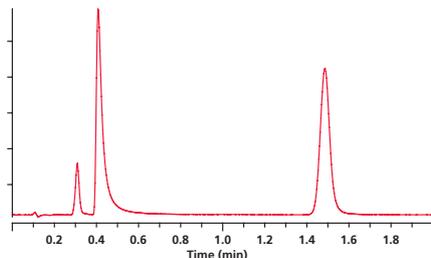
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New solvents for trace metal speciation analysis

TraceSELECT® solvents for LC-ICP-MS applications

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The toxicity of trace metals in food, clinical chemistry, biology and environmental sciences is an area of increasing interest. In the environment many diverse species of an element can be present, and different species of the same element can possess very different degrees of toxicity.

Monitoring elemental species requires an analytical method that is sensitive and specific enough to resolve and quantify the individual species at ultra-trace levels, since an individual toxic species may constitute only a fraction of an element's total concentration in a sample. The coupling of the two well-established analytical techniques, HPLC and inductively coupled plasma-mass spectrometry (ICP-MS), is straightforward primarily because the flow rates commonly used with LC are compatible to conventional liquid sample introduction systems, such as those based on pneumatic nebulisation. Thus, the outlet of the LC column is directly connected to the ICP-MS nebuliser. This technique is especially useful in carrying out automated, high-throughput speciation analysis.

Sigma-Aldrich has recently developed new high-purity TraceSELECT solvents for speciation analysis by LC-ICP-MS. TraceSELECT solvents undergo rigorous purification procedures followed by UV spectroscopy, IC and ICP-MS testing to assure high chemical purity and high UV-transmittance. The blank values for metal traces are in the ppb range, or even lower.

Definition of speciation analysis

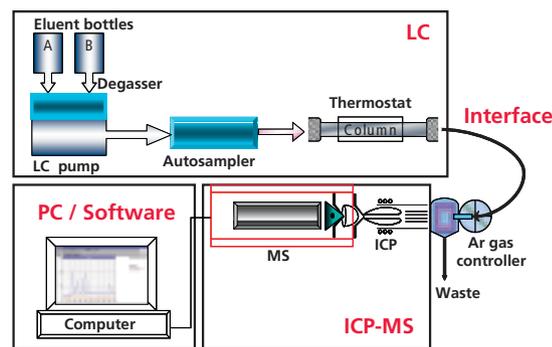
The definition of speciation analysis set by The International Union of Pure and Applied Chemistry (IUPAC) is "analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample". A chemical species is a "specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure."^[1]

In speciation analysis, the objective is usually to determine the identity and/or concentration of one or more chemical species in a sample, which is often of natural origin and therefore potentially contains many different species. Care must be taken to choose and execute the analysis to maximise sensitivity and specificity. Performing a speciation analysis in a complex mixture involves separation, identification and characterisation of various forms of elements in the sample. The most commonly used strategy for speciation analysis is to perform a separation step before a generic detector.

LC separation coupled with ICP-MS

The hyphenated technique, HPLC-ICP-MS, is a robust, sensitive element-selective method capable of giving a complete picture of the elemental species in a solution. The elemental response is usually independent of species, so it is often possible to quantify a species even when its structure is unknown (assuming good HPLC recovery). Identification, however, is based solely on retention time matching, and hence a compound in the sample can be identified only by comparison with a standard.

Figure 1 HPLC-ICP-MS Coupling (published with kind permission of PerkinElmer, Inc.)



Applications

ICP-MS is a superior detection technique for trace elements in general, but especially for interesting elements, such as arsenic, selenium, cadmium, iodine and others in chromatographic eluents. Some specific examples from the literature appear below.

Organotin speciation analysis^[2]

Because organotins have biological and fungicidal properties, they are often used as pesticides and other agricultural chemicals, wood preservatives, marine antifouling paints and as polymer additives. In this application, organotin analytes were leached into a diluted methanolic solution and then injected into the HPLC. Chromatographic separation was achieved in ten minutes with excellent resolution.

Selenium speciation analysis^[3,4]

Selenium exists in several oxidation states and a variety of inorganic and organic compounds, such as selenocysteine, selenomethionine, selenoethionine, selenite (Se(IV)) and selenate (Se(VI)). The chemistry of selenium is complex in both the environment and living systems: it is an essential element at trace levels and toxic at higher levels. Interest in speciation analysis for selenium has grown rapidly in the last decade, facilitated by the LC-ICP-MS technique.^[3] Complete characterisation of selenium compounds is necessary to understand selenium's significance in metabolic processes, clinical chemistry, biology, toxicology, nutrition and the environment. Gómez-Ariza^[4] described a method for extraction of the chemical species from native yeast. The extracted selenium compounds were analysed by liquid chromatography (LC) – hydride generation (HG) – atomic fluorescence spectrometry (AFS).

Arsenic speciation analysis^[5]

Arsenic, like selenium, appears in both organic and inorganic forms, and from natural and anthropogenic sources. Applying speciation analysis to fish and other seafood, it was shown that the naturally occurring organic arsenic species are less toxic than inorganic arsenic. This data has caused a reassessment of arsenic, and other metals, as it applies to food analysis.

Chromium speciation analysis^[6,7]

Chromium is a naturally occurring metal found in small quantities, usually associated with other metals. Due to its extensive use in industrial processes, like steel and other alloys, bricks in furnaces, dyes and pigments, chrome plating, leather tanning and wood preserving, it is a widespread environmental contaminant. While Cr(III) is an essential nutrient, Cr(VI) is a known mutagen and carcinogen. It is also more soluble and, therefore, more

mobile than Cr(III). The separation of chromium species is accomplished isocratically by ion-pair chromatography using tetrabutylammonium hydroxide (TBAH, Fluka no. 86832) as the ion-pair reagent. The detection limit by ICP-MS is about 50 ng/L for each chromium species.

Figure 2 Example of an HPLC-ICP-MS System for Speciation analysis (with kind permission of PerkinElmer Inc.)



Table 1 TraceSELECT® solvents ideal for speciation analysis

Cat. no.	Brand	Description	Package size
01324	Fluka	Acetonitrile, TraceSELECT® (≥99.9%)	1 L
42105	Fluka	Methanol, TraceSELECT® (≥99.9%)	1 L
14211	Fluka	Water, TraceSELECTUltra®	1 L

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Complexometric titration with aminopolycarboxylic acids (EDTA and analogs)

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By using complexing agents, most cations and some anions can be determined by direct or indirect titration. The right choice of complexing agent, indicator, pH, titration method and masking agent enables various selective determinations.

Sigma-Aldrich offers a range of selected aminopolycarboxylic acids (EDTA and analogs) as reagents for complexometric titration under the product line IDRANAL®. In complexometric titration, metals in the sample react with complexing agents (chelating agents) to create complexes. Typically, a colour change determines the end point of the titration. Analytical applications include the determination of water hardness and special titrimetric determinations of metal ions.



Sigma-Aldrich's reagents for complexometric titration are available as solids, ready-to-use volumetric solutions and as concentrates in FIXANAL® ampuls (see **Tables 1-3**). In addition, we offer buffer solutions, masking agents and indicators specially designed for complexometric titration.

Complex formation

IDRANAL I is predominantly used as a disodium salt for complexometric-alkalimetric titration of heavy metals. For making a 0.1 mol/L solution of IDRANAL I, 19.11 g of IDRANAL I are diluted in a volumetric flask in 500 mL water and about 200 mL 0.1 mol/L sodium hydroxide solution. The prepared mixture is adjusted to the colour change of methyl red and filled up to 1000 mL.

Many of the complexing agents are free acids and poorly soluble in water. Therefore, their sodium salts are used for producing the volumetric solutions. IDRANAL II, for example, is nearly insoluble in water. Solutions of its alkaline salts are used for titration. The disodium, trisodium and tetrasodium salts are readily soluble in water.

When determining water hardness with EDTA- Na_2 , the colour change of Eriochrome® Black T is not very clear. Therefore, the volumetric solution of EDTA- Na_2 is spiked with different amounts of IDRANAL II Zinc. These mixtures are supplied as ready-to-use solutions and concentrates of IDRANAL A and IDRANAL B. A 1 mL IDRANAL A solution shows 5.6° German hardness when titrating a 100 mL sample, a 1 mL IDRANAL B solution shows 1° German hardness when titrating a 100 mL sample.

The complex-forming properties of IDRANAL IV (DCTA) are very similar to EDTA, with a few differences. The complex-forming constants of DCTA are slightly higher, which proves especially useful for determination of alkaline earth elements. The formation of Al-complexes proceeds more easily, and the usual heating during back-titration can be eliminated. The Ni-DCTA-complex is highly stable and cleavage of this complex with cyanide is difficult, therefore a separation of Ni from Zn, Cd and Cu is possible. The complex formation with DCTA progresses more slowly than with EDTA, therefore the titration speed should be slower, or the titration should be carried out at elevated temperatures.

IDRANAL IV is insoluble in water and has to be dissolved in alkaline solutions for titration. The solutions of its different sodium salts show different pH-values:

- DCTA- Na_2 pH 5.3
- DCTA- Na_3 pH 8.2
- DCTA- Na_4 pH 11

Best suited for titration are tri- and tetrasodium salts, as they produce less acid, so smaller buffer concentrations are sufficient.

The application of IDRANAL V is limited to particular cases, e.g. determination of rare earth metals and titration of Ba and Sr.

To prepare a 0.1 mol/L solution of IDRANAL V, 39.33 g IDRANAL V are dissolved in 500 mL 1 mol/L sodium hydroxide solution in a volumetric flask which is filled up to 1000 mL (pentasodium salt). The titer is adjusted with 0.1 mol/L zinc sulphate solution at pH 10 against Eriochrome Black T.

IDRANAL VI is suitable for the simultaneous determination of Ca^{2+} and Mg^{2+} , as the stability constants are very different. IDRANAL VII is particularly suitable for determination of Fe^{3+} ions under acidic conditions.

Table 1 Reagents for complexometric titrations – Powders

Fluka cat. no.	Product name	Description	Pack size
34539	IDRANAL I (NTA)	Puriss. p.a., ≥99%; hardly soluble in water, readily soluble in diluted alkaline solutions	50 g, 250 g
34540	IDRANAL II (EDTA)	Puriss. p.a., ≥99.5%; slightly soluble in water, soluble in diluted alkaline solutions, insoluble in most organic solvents	100 g, 250 g, 1 kg
34553	IDRANAL II Zinc	Puriss. p.a., ≥98%	100 g
33350	IDRANAL II Magnesium	Puriss. p.a., ≥98%	100 g
34549	IDRANAL III (EDTA-Na ₂)	Puriss. p.a., Reag. Ph. Eur., 99-101%, ≤0.1% nitrilotriacetic acid (HPLC)	50 g, 100 g, 1 kg
34588	IDRANAL IV (DCTA)	Puriss. p.a.; soluble in diluted alkaline solutions	25 g, 100 g
34589	IDRANAL V (DTPA)	Puriss. p.a., ≥99%; soluble in diluted alkaline solutions	100 g
34596	IDRANAL VI (EGTA)	Puriss. p.a., ≥98.5%; soluble in diluted alkaline solutions; especially suitable for the determination of Ca in presence of Mg	25 g
34461	IDRANAL VII (HEDTA-Na ₃)	≥99.0%; particularly useful for controlling Fe ³⁺ ions and well suited for use in acidic formulations	100 g

Table 2 Reagents for complexometric titrations – Volumetric Solutions

Fluka cat. no.	Product name	Description	Pack size
35332	IDRANAL II Copper standard solution 0.01 mol/L	Volumetric solution	500 mL
35322	IDRANAL III standard solution 0.01 mol/L	Volumetric solution	1 L
34550	IDRANAL III standard solution 0.1 mol/L	Reag. Ph. Eur., volumetric solution	500 mL, 1 L, 5 L, 10 L
35102	IDRANAL III standard solution 0.2 mol/L	Volumetric solution	1 L, 5 L, 10 L
34547	IDRANAL A standard solution (IDRANAL III solution with zinc complex added)	Solution for water hardness determination; 1 mL = 5.6 German degrees of hardness in 100 mL of water	1 L
34543	IDRANAL 100 solution (IDRANAL III solution)	Solution for water hardness determination; 1 mL = 1 German degree of hardness in 100 mL of water	1 L
34544	IDRANAL B solution (IDRANAL III solution with zinc complex added)	Solution for water hardness determination; 1 mL = 1 German degree of hardness in 100 mL of water	500 mL, 1 L, 5 L, 10 L
34542	IDRANAL C solution (IDRANAL III solution with zinc complex added)	Solution for water hardness determination with measuring tube H DIN 12812; 3.73 mL = 20 German degrees of hardness in 40 mL of water	1 L
35103	IDRANAL IV standard solution 0.1 mol/L	Reag. Ph. Eur., volumetric solution	1 L, 5 L, 10 L

Table 3 Reagents for complexometric titrations – Concentrates

Fluka cat. no.	Product name	Description	Pack size
38057	IDRANAL III concentrate 0.1 mol	Concentrate (FIXANAL), for preparation of volumetric solution (37.224 g EDTA-Na ₂)	1 ampul
38055	IDRANAL A concentrate (IDRANAL III with zinc complex added)	Concentrate (FIXANAL), for preparation of 1 L volumetric solution; 1 mL ready-to-use solution = 5.6°d of hardness in 100 mL of water	1 ampul
38056	IDRANAL B concentrate (IDRANAL III with zinc complex added)	Concentrate (FIXANAL), for preparation of 1 L volumetric solution; 1 mL ready-to-use solution = 1 German degree of hardness in 100 mL of water	1 ampul

Titration methods

Direct titration

A metal salt solution is titrated with a solution of an IDRANAL compound, with an added indicator showing the equivalent point. The pH-value of the titration is chosen according to the metal to be titrated and in consideration of other foreign metals that may be present. Alkali earth metals must be titrated in alkaline solutions. Zn could be titrated at pH 5 where any Mg that might be present is left out of the titration (selective titration). In contrast, a titration at pH 10 would allow for a combined titration of Zn and Mg (sum titration).

Requirements for direct titration:

- Complex stability under chosen conditions
- Fast and stoichiometric course of reaction
- Suitable indicator

Back titration

Direct titration is sometimes impossible, for example if complex formation is too slow (Al), if side reactions take place (precipitation of hydroxide) or if a suitable indicator is not available. In these cases, a back titration can be used. The solution of the metal salt (e.g. Al) is converted with excess IDRANAL, and after complex formation, which may take some time or need another pH value, the excess IDRANAL is back-titrated with another metal (e.g. Zn). The titration conditions have to be adjusted to the requirements of this second metal.

Substitution titration

Alternatively, a substitution titration can be carried out, with Pb for example. Pb is converted with IDRANAL-Mg, where the Mg is replaced by Pb, forming IDRANAL-Pb and an equivalent amount of Mg. The Mg can now be titrated. For this titration, the emerging complex must show higher stability. Ideally suited are IDRANAL II Magnesium, for substitution reactions at pH 10, and IDRANAL II Zinc at pH 5.

(continued on page 20)

Indirect titration

If cations or anions do not form complexes with IDRANAL, a direct determination is impossible. In these cases, a conversion with a titratable metal can enable indirect titration. A well-known example of this is the determination of sulphate through precipitation of barium sulphate. The precipitate is removed by filtration, dissolved, and the amount of barium titrated. Alternatively, the excess barium can be back-titrated after precipitation of sulphate. Analog determination of phosphate or fluoride is possible.

Masking agents

It is possible to protect single metals from reaction with EDTA by prior conversion with an auxiliary complexing agent. These so-called masking procedures considerably expand the field of possible separations. Unmasking of single metals is also possible. Possible changes in pH by addition of masking agents must be considered. Their concentration should be kept low.

The following substances can be used as masking agents:

- Potassium cyanide (Cat. no. 31252)
Forms highly stable complexes in alkaline solutions with Zn, Cd, Hg, Ni, Co, Cu (higher stability than EDTA-complexes)
- Triethanolamine (Cat. no. 33729)
Forms stable complexes with Fe and Al in alkaline solutions

- Fluoride (e.g. Cat. no. 09835 Ammonium hydrogen difluoride)
Fluoride ions can form stable insoluble compounds with Be, Mg, Ca, Al, Ti, Sn or soluble fluoro-complexes, preferred in weakly acidic solutions.
- 2,3-Dimercapto-1-propanol (Cat. no. 38520)
Some Cations (Hg, Zn, Cd, As, Sb, Sn, Pb, Bi) form uncoloured complexes with 2,3-Dimercapto-1-propanol in ammoniacal solutions that are more stable than their corresponding EDTA-complexes.
- Thioglycolic acid (Cat. no. 88652)
Forms stable, uncoloured complexes with Pb, Bi, Cd, Zn, Hg in alkaline solutions. Cu gives a weakly yellow complex. Fe gives a red complex, Ni and Co give intensely coloured complexes with low stability.
- Thiourea (Cat. no. 33717)
Thiourea is preferred for masking Cu in neutral solutions.

Indicators for complexometric titrations

For complexometric titrations, visual indicators are preferred. They are mostly dyes that form intensely coloured metal complexes with different colours, predominantly triphenylmethane and azo dyes. Some of the most important metal titration indicators are shown in

Table 4.

Table 4 Selected indicators for metal titration

Fluka cat. no.	Indicator	Description	Examples for applications
32672	Pyrocatechol violet	colour of aqueous solutions: acidic - yellow, alkaline - red/violet	Bi, Th, In, Ga
21030	Calcein	fluorescent indicator, fluorescence disappears at equivalence point	Ca in presence of Mg in strongly alkaline conditions
32751	Eriochrome® Black T	acidic solution red, weakly alkaline solution (up to pH 11) blue, strongly alkaline solution yellow/orange	Mg, Zn, Mn, Cd, Pb
33460	Methylthymol Blue	strongly acidic solution red, up to pH 7 yellow, pH 7-11 light blue, pH 11-12.7 yellow-grey, above pH 12.7 intensely blue	Bi, Pb, Zn, Sn, Mg, Ca
33414	Murexide	acidic solution below pH 3 not stable, acidic solution pH 3-6 red-violet, above pH 6 blue-violet to blue	Ni, Co, Cu, Ca
89460	Tiron	colour change at pH 2-3 blue/green to yellow	specific for Fe
33825	Xylenolorange	aqueous solution yellow in acidic conditions, in alkaline conditions red-violet	Bi, Th, Hg (pH 2-3) and Cd, Zn, Pb, Al (pH 5-6)
36817	IDRANAL® Indicator buffer	contain Eriochrome Black T and another dye compound for clear colour	Ca, Mg, Zn, Cd, Pb and water
36818	tablets	change from red to green, contain ammoniumchloride as buffer substance	hardness

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Information about our line of titration products and complete product listings can be found by visiting our web site www.sigma-aldrich.com/titration and the online product catalogue.

References:

- 1] Schwarzenbach, G.; Flaschka, H. *Komplexometrische Titration*. Ferdinand-Enke-Verlag, Stuttgart, 1965.
- 2] Jander, G.; Jahr, K.-F. *Maßanalyse*. Walter de Gruyter, Berlin, New York, 2003.

Coulometric water determination according to Karl Fischer

Reagents and examples of applications

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The coulometric Karl Fischer technique is a micro-method that is particularly suitable for samples with low water content, from ~10 µg up to ~10 mg. It uses electrochemically generated iodine, produced from iodide-containing reagents by anodic oxidation at a generator electrode directly in the titration vessel. The amount of consumed iodine and therefore the amount of water from the sample is proportional to the total current consumption (current x time).

Its high precision and the convenient introduction of liquid samples with a syringe make coulometry a viable and easy technique for water determination.

Advantages of coulometric titration include:

- Easy to use
- Detects low concentrations of water
- High accuracy

Coulometric titration vessels

The coulometric technique can be carried out with two different types of generator electrodes: with or without diaphragm. The diaphragm separates the smaller cathode compartment, where protons are reduced to hydrogen, from the larger anode compartment, where iodide is oxidised to iodine. Generator electrodes without diaphragm also have anode and cathode, but the compartments are not separated. Reagents that are specially designed for diaphragm-less cells must be used.

The function of the diaphragm is to enable the exchange of certain anions and cations, and to prevent the diffusion of the generated iodine and its immediate reduction at the cathode, which causes erroneous results. In cells without diaphragm, the cathode construction is modified to prevent any disturbances.

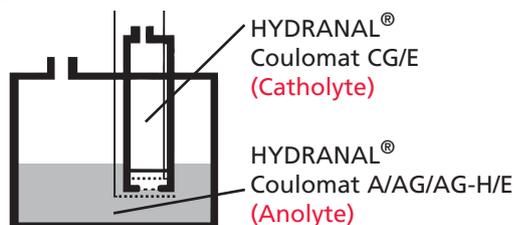
Coulometric Karl Fischer titrations in cells with diaphragm require two reagent solutions, an anolyte and a catholyte. Catholyte solution (5 mL) is added to the cathode chamber, and anolyte solution is added to the same level in the anode chamber (see **Figure 1**). The levels of liquids in the two chambers must be equal to prevent exchange of anolyte and catholyte by pressure compensation. In diaphragmless cells, the anolyte (~100 mL) is used as single solution.

When using reagents containing chloroform and/or xylene, or analysing samples with water content below 50 µg/g, using a generator electrode with diaphragm is recommended to ensure high precision.

Care and maintenance of the coulometric cell

Proper care and maintenance of coulometric cells can help maximise the sensitivity and reliability of Karl Fischer titrations.

Figure 1 Coulometric titration vessel with diaphragm



- Renew catholyte at least weekly, otherwise unpleasant odour, darkened electrodes and yellowish precipitates in the cathode chamber can occur.
- Remove remaining iodine from the anolyte by addition of wet methanol or 2-methoxyethanol.
- Solid samples cannot be analysed with a coulometric system directly, pre-dissolve them and run against blank samples.
- Clean glass anode chamber with water or a suitable solvent, then dry in an oven at 50°C or under a stream of warm air.
- Clean generator electrode with a diaphragm by placing it in a beaker of methanol or, if necessary, in nitric or chromosulphuric acid, then repeat with methanol.
- Clean generator electrode without diaphragm by rinsing it with water or a suitable solvent, if necessary it can be dipped in nitric or chromosulphuric acid, then repeat with water or solvent.

Reagents and Applications

- HYDRANAL Coulomat A type reagents are used as anolytes. They contain iodide and a sulphur dioxide/imidazole buffer in a suitable solvent.
- HYDRANAL Coulomat CG reagents are used as catholytes.
- The non-toxic HYDRANAL Coulomat E can be used as both anolyte and catholyte solution; it is based on ethanol and has a water capacity of over 1000 mg per 100 mL (100 mg per 5 mL when used as catholyte).

Water determination in petrol, unleaded (Application Report L428)

The solubility of petrol in the methanolic medium of the Karl Fischer titration is limited, therefore the use of reagents containing solubilisers is recommended. In 100 mL HYDRANAL Coulomat A, up to 50 mL of petrol can be dissolved. Also, HYDRANAL Coulomat E is suitable, since 100 mL dissolves up to 35 mL of this sample. HYDRANAL Coulomat E (5 mL) is added to the cathode compartment; the anode chamber is also filled with HYDRANAL Coulomat E to the same liquid level as in the cathode chamber. If HYDRANAL Coulomat A is used in the anode chamber, the cathode chamber is filled with 5 mL HYDRANAL Coulomat CG. After titrating to dryness with low and stable drift, the samples can be injected.

(continued on page 22)

HYDRANAL Coulomat AG, AD and AG-H are analytes free of halogenated hydrocarbons, whereas HYDRANAL Coulomat A contains chloroform for better solubility of fat-containing substances. The chloroform content may still be increased, but it should not exceed 30%, otherwise the conductivity will decrease. The minimum conductivity differs from instrument to instrument, so an exact maximum chloroform volume cannot be given.

Many long-chained mineral oils cannot be dissolved completely; they must be analysed in reagents containing solubilisers or dispersion agents. For example, the water content of finely dispersed oil can be determined very easily with reproducible results. Based on Methanol and with addition of aromatic and halogenated hydrocarbons, HYDRANAL Coulomat Oil is very well suited for the analysis of oil samples. It should be used in coulometric cells with diaphragm using HYDRANAL Coulomat CG as catholyte.

Water determination in rapeseed oil (Application Report L319)

Rapeseed oil does not dissolve in a methanolic KF working medium. The addition of chloroform helps to finely disperse the sample in the medium. In addition, it is important to choose a delay time of 30 seconds in the titration software to ensure that all contained water is titrated. HYDRANAL Coulomat Oil, which contains chloroform and xylene as solubilisers, is very well suited for coulometric determination in fats and oils.

HYDRANAL Coulomat CG (5 mL) is added to the cathode compartment of a titration vessel with diaphragm. HYDRANAL Coulomat Oil (~100 mL) or a mixture of HYDRANAL Coulomat A and 30% chloroform are added to the anode compartment up to the same liquid level.

HYDRANAL Coulomat AK is an analyte for the coulometric determination of water in ketones. Its water capacity is approximately 100 mg per 100 mL. HYDRANAL Coulomat CG-K is the corresponding catholyte, free of halogenated hydrocarbons.

Water determination in octamethylcyclotetrasiloxane (Application Report L519)

When determining the water content of octamethylcyclotetrasiloxane coulometrically in a methanol-containing reagent, the drift increases with each injected sample. An esterification takes place as a side-reaction. However, the substance can be determined with stable end points and without increasing drift in a methanol-free reagent.

HYDRANAL Coulomat CG-K (5 mL) is placed in the cathode compartment of the coulometric cell. The anode compartment is filled up to the same level with HYDRANAL Coulomat AK.

Coulometric cells without diaphragm require only one reagent. HYDRANAL Coulomat E, AD, AG, AG-H, AG Oven and AK can be used with diaphragmless cells; no catholyte solution is needed.

Toxicity

Except for HYDRANAL Coulomat AK, which contains 2-methoxyethanol, all HYDRANAL Coulomat reagents are free of pyridine,

carbon tetrachloride and 2-methoxyethanol. In most reagents, methanol is the most hazardous component. Using HYDRANAL Coulomat E, which is based on ethanol, can eliminate even this.

Use of the Karl Fischer Oven

Many substances release their water only at high temperatures, making them inappropriate for direct KF titration.

Water determination in poly-L-lactide, PLLA, with KF oven (Application Report L577)

This sample is an uncoloured granulate, which cannot be dissolved for direct titration in the alcohol-containing media of KF reagents, not even by addition of chloroform.

To evaluate its temperature behaviour, a sample was gradually heated from 50°C to 250°C (see **Figure 2**). At 50°C, the adherent water is released. Above 60°C, the included water is released. Water release is completed at 210°C. According to literature, thermal decomposition occurs at 230°C.

This heating ramp shows that sample heating at 210°C for 10-15 minutes is recommended.

HYDRANAL Coulomat CG (5 mL) is added to the cathode chamber of a coulometric cell with diaphragm; the anode compartment is filled to the same level with ~100 mL HYDRANAL Coulomat AG Oven.

After starting the instrument, it automatically titrates to dryness. Once the drift is low (<10 µg H₂O/min.) and stable, the carrier gas is switched on. As soon as the original stable drift value is reached with the carrier gas, about 0.5 g of the sample, weighed precisely, can be heated.

The water in these substances can be evaporated in a Karl Fischer oven at 100°C to 300°C, depending on the sample. It is then transferred to the KF titration cell by purging with a dry, inert gas. The coulometric titration cell is filled with HYDRANAL Coulomat AG-Oven, which is especially suitable for use with Karl Fischer ovens as it shows high drift stability, or HYDRANAL Coulomat E for non-toxic applications.

This method of water determination can be applied to:

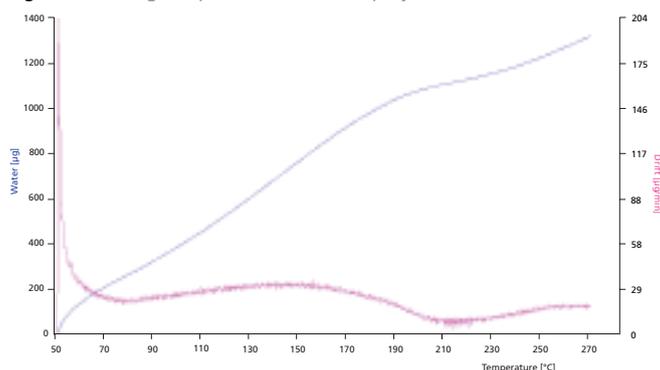
- Insoluble solids that only release their inherent water at temperatures above 60°C (e.g. plastics, salts)
- Solids and liquids that undergo side reactions with conventional Karl Fischer titration reagents (ascorbic acid, mineral oils), assuming that the matrix is not vaporised at these temperatures and that none of the substances decompose to products that can interfere with the Karl Fischer reaction.

Suitable carrier gases are air or nitrogen, with nitrogen preferred when the sample is sensitive to oxidation at temperatures of 100-300°C. The carrier gas must be dried, for example with 34241 HYDRANAL Molecular Sieve 0.3 nm.

The working conditions must be optimised for each product analysed. The temperature chosen depends on the properties of the substance being investigated. Of particular importance is the determination of the optimum oven temperature to remove the water.

The temperature must be high enough to drive off the water in the sample within 10-15 minutes. At the same time, the temperature must be low enough to prevent vaporisation of the sample matrix, which could interfere in the Karl Fischer titration, but again high enough to prevent condensation in the transfer tubing.

Figure 2 Heating ramp from KF oven for poly-L-lactide, 50°C to 270°C



Control of accuracy

In order to control the accuracy of reagents and instrument according to ISO 9000, the use of liquid 34828 HYDRANAL Water Standard 1.00, 1 g (1 mL) contains 1 mg = 0.10% water (at 20°C), and 34847 HYDRANAL Water Standard 0.10, 1 g contains 0.10 mg = 0.01% water, is recommended. As solid standards for the control of KF oven systems, 34748 HYDRANAL Water Standard KF Oven 220°C-230°C and 34693 HYDRANAL Water Standard KF Oven 140°C-160°C are recommended.

Table 1 HYDRANAL reagents for coulometric KF titration

Cat. no.	Brand	Description	Package size
34807	Fluka	HYDRANAL Coulomat A	500 mL
34836	Fluka	HYDRANAL Coulomat AG	500 mL, 1 L
34810	Fluka	HYDRANAL Coulomat AD	500 mL
34843	Fluka	HYDRANAL Coulomat AG-H	500 mL
34739	Fluka	HYDRANAL Coulomat AG Oven	500 mL
34726	Fluka	HYDRANAL Coulomat E	500 mL
34820	Fluka	HYDRANAL Coulomat AK	500 mL
34868	Fluka	HYDRANAL Coulomat Oil	100 mL, 500 mL
34840	Fluka	HYDRANAL Coulomat CG	50 mL
34821	Fluka	HYDRANAL Coulomat CG-K	50 mL

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New Fluka-Brand Drug Standards for Ecstasy and Cannabis

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com



To analysts in the forensic and clinical markets, we are pleased to introduce high-purity standards of phenylethylamine derivatives and THC compounds for the European market. With Switzerland as the country of origin, export is allowed into all EU countries.

MDMA (3,4-methylenedioxyamphetamine, ecstasy) along with MDA, MDEA and others are members of the

phenethylamine class of psychoactive drugs. A popular party drug, especially in the rave culture, MDMA is one of the most commonly used illicit drugs world wide.

THC (Δ^9 -tetrahydrocannabinol), derived from the Cannabis sativa plant, is considered to be the most wide spread illegal drug and, according to the United Nations Office on Drugs and Crime (UNODC) in Vienna, as the most important agricultural raw material worldwide.

Table 1 New Fluka-brand MDMA standards

Cat. no.	Brand	Description	Package size
56296	Fluka	(-)- Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) solution, 1 mg/mL in ethanol	1 mL
91613	Fluka	(-)- Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) solution, 10 mg/mL in ethanol	1 mL
90899	Fluka	Cannabidiol solution, 10 mg/mL in ethanol	1 mL
51853	Fluka	Cannabidiol solution, 1 mg/mL in ethanol	1 mL
39961	Fluka	Cannabidiolic acid	1 mg
39382	Fluka	Δ^9 -Tetrahydrocannabinolic acid A	10 mg
65963	Fluka	(\pm)-3,4-Methylenedioxyamphetamine hydrochloride (MDMA)	10 mg, 50 mg
18087	Fluka	(\pm)-3,4-Methylenedioxyamphetamine hydrochloride (MDMA) solution, 1 mg/mL in methanol	1 mL
56458	Fluka	(\pm)-3,4-Methylenedioxyamphetamine hydrochloride (MDA) solution, 1 mg/mL in methanol	1 mL
50499	Fluka	(\pm)-3,4-Methylenedioxy-N-ethylamphetamine hydrochloride (MDEA) solution, 1 mg/mL in methanol	1 mL
14232	Fluka	(\pm)-4-Bromo-2,5-dimethoxyamphetamine hydrochloride	10 mg

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