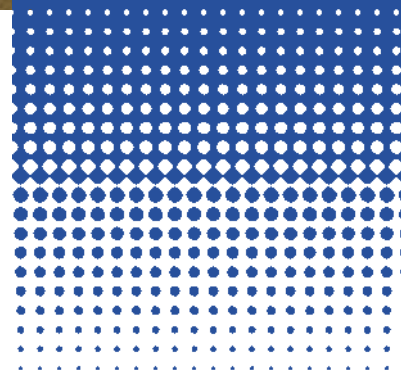


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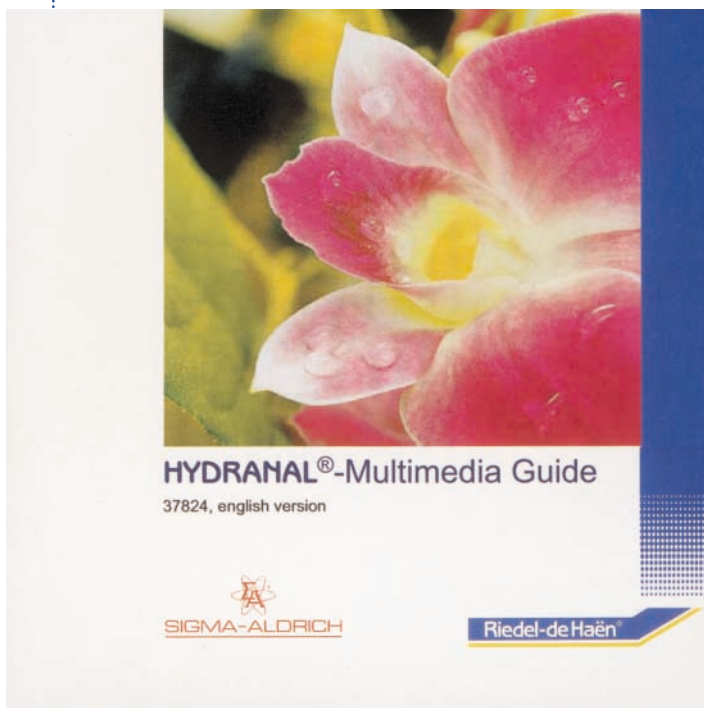
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## Standards

Fluka and Riedel-de Haën have many years of experience with the production and supply of high purity reference standards. Today we provide an exceptional product range for a wide variety of analytical techniques. Due to our commitment to providing the best analytical solutions to our customers, Fluka and Riedel-de Haën have become established partners in the area of certified reference materials.

Our quality assurance policy is based on the ISO standard 9001. Each reference material in our program is provided with guaranteed specifications and has an expiration date. The large variety of modern analytical methods available to us allow to accurately control and analyze each standard. All certified reference materials are supplied with a detailed certification document.

## Definition of Standards

The quality control of all products, e.g., within the chemical industry or the production and control of pharmaceuticals, require the application of chemical-physical methods of analysis and testing. The accuracy of the test results is of critical importance to the analyst and can have far-reaching consequences. In this context, the use of reference substances for the determination of test results is essential.

These materials are referred to by several names: reference substance, reference standard, reference material, standard, certified reference standards, and certified reference material. Depending on the application the standards are used in, either chemical/physical quality control or pharmaceutical quality control/GMP production, different definitions will apply (table 1).

	Chemical/physical application		Pharmaceutical/GMP application				
Base	ISO guide 30		Pharmacopoeia institutions, FDA-guideline, ICH-guide, PIC-guide				
Divided into	Certified Reference Material (CRM)		Reference Material (RM)	Primary standard		Secondary standard/ working standards	Tertiary standards/ working standards
Substructure	Primary standard	Secondary standard		In-house primary standards	Official standards of pharmacopoeias or official authorized institutions (e.g. EMPA, NIST, IRMM, BAM)		
Certification procedure	<ul style="list-style-type: none"> <li>each certified value is accompanied by an uncertainty at a stated level of confidence</li> <li>certificate is included with the product</li> <li>certificate issued by a certifying body</li> </ul>			<ul style="list-style-type: none"> <li>characterization by assay determination, identification test, tests of impurities and by-products</li> </ul>	<ul style="list-style-type: none"> <li>collaborative testing</li> <li>assay determination</li> </ul>	<ul style="list-style-type: none"> <li>assay determination referring to a primary standard</li> </ul>	<ul style="list-style-type: none"> <li>assay determination referring to a secondary standard</li> </ul>
Labeling and marking	in accordance with ISO guidelines		in accordance with official requirements, e.g. guidelines of pharmacopoeia: CRS = chemical reference substance (EP, BP) BRS = biological reference substance RS = reference substance = primary standard (USP)				
Use	<ul style="list-style-type: none"> <li>identification</li> <li>assay determination</li> <li>system suitability test</li> <li>instrument calibration</li> <li>impurity limits</li> <li>method validation</li> <li>purity</li> </ul>	<ul style="list-style-type: none"> <li>system suitability test</li> </ul>	<ul style="list-style-type: none"> <li>identification</li> <li>assay determination</li> <li>system suitability test</li> <li>instrument calibration</li> <li>impurity limits</li> <li>method validation</li> <li>qualification of instruments</li> <li>purity (according to ICH guidelines)</li> </ul>	<ul style="list-style-type: none"> <li>system suitability test</li> </ul>	<ul style="list-style-type: none"> <li>system suitability test</li> </ul>		

The matrix gives an overview of our different standards and their categorization into the above scheme.

Group	Certified Reference Material (CRM)		Reference Material (RM)	Primary standard		Secondary standard/ working standard	Tertiary standard/ working standard
	Primary standard	Secondary standard		In-house primary standard	Official standards of pharmacopoeia*		
<b>Certified reference materials by EMPA/BAM</b>							
Titrimetric substances	●	●	○	○	●	○	○
Cationic standard solutions	○	●	○	○	●	○	○
Anionic standard solutions	○	●	○	○	●	○	○
<b>Certified reference materials by IRMM</b>							
All products	●	○	○	○	●	○	○
<b>GMO reference materials</b>							
IRMM certified	●	○	○	○	●	○	○
Competitors for quantitative GMO detection	○	○	●	○	○	●	○
<b>Trace analysis</b>							
Standard solutions for spectroscopy	○	○	●	○	○	●	○
Standards for ion chromatography	○	○	●	○	○	●	○
<b>Food and beverage standards</b>							
BADGE/BDFGE	○	○	●**	○	○	○	●**
Food irradiation	○	○	●	○	○	○	●
Phytoestrogens	○	○	●	○	○	○	●
<b>Residue analysis standards</b>							
Environmental substances	○	○	●	○	○	○	●
Pesticides	○	○	●	○	○	○	●
Ingredients used by veterinarians	○	○	●	○	○	○	●
<b>Chromatography standards</b>							
GC standards	○	○	●	○	○	○	●
GPC standards	○	○	●	○	○	●	●
<b>Reference substances acc. to pharmaceutical monographs</b>							
GC standards for determining residual solvents	○	○	○	○	○	○	●
Color reference solutions acc. to USP	○	○	●	○	○	●	○
Color reference solutions acc. to PhEur	○	○	●	○	○	●	○
Color reference solutions acc. to APHA	○	○	●	○	○	●	○
<b>Physical properties</b>							
Particle size standards	○	○	●	○	○	●	○
Conductivity standards	○	○	●	○	○	●	○
pH Calibration / buffers	○	○	●	○	○	●	○

\*or official authorized institutions (e.g. EMPA, NIST, IRMM, BAM)

\*\* for qualitative testing

## Certified Reference Material by EMPA and BAM

### Reference Materials as Transfer Standards in the Traceability Chain

The accuracy of a chemical analysis depends very much on the reference material used for the calibration. Private companies, research institutes and metrological institutions offer a great variety of reference materials and reference standards. Often the stated assay is not traceable to the SI or a national metrological normal, and a comprehensible calculation of the assay's uncertainty is not stated. For the validation of an analytical method these details are of eminent and central importance.

More and more private laboratories have to state traceable analytical values and their uncertainties (Norm ISO/IEC 17025). To meet these needs appropriate reference standards or material are needed.

Many federal metrological institutes, while assigning a traceable value very well, are often not well enough equipped for efficient production of these materials. On the other hand, private companies may not be equipped for the assignment of a traceable value but are able to produce reference materials very efficiently on a large scale. To combine both skills in a complementary way, Fluka, EMPA (Swiss Federal Laboratories for Materials Testing, Switzerland) and BAM (Federal Institute for Materials Research and Testing, Germany) have joined in a cooperative effort.

Fluka produces different standards whereas

EMPA is doing the certification by means of traceable measurements. BAM is involved as a partner of EMPA in the certification procedures. BAM and EMPA are accredited according to ISO/IEC 17025. An ISO DIS 9001:2000 certification is in place at EMPA and Fluka. A contract with METAS (Federal Institute for Metrology and Accreditation) entitles EMPA as a national metrological institute.

The results are primary or secondary standards, used for:

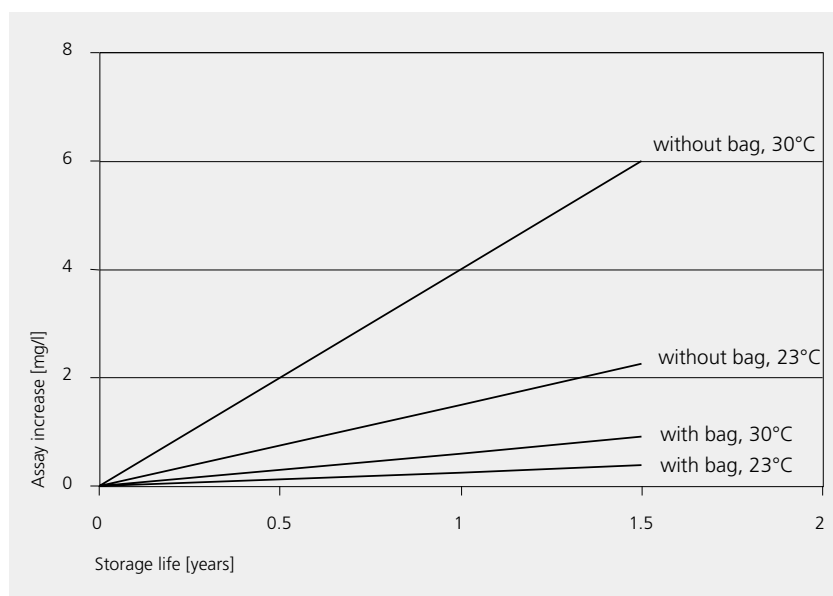
- Method validation
- Qualification of instruments
- Instrument calibration
- System suitability testing
- Identification
- Assay determination

### Production, Value Assignment and Certification

Fluka produces reference standards according to ISO guide 34. The certification analysis is performed by EMPA and BAM applying two different methods of measurement using randomly chosen bottles. The amount of the sample surveys is chosen according the requirements in ISO 3951. The results are combined to yield the certified value and the standard uncertainty. A certificate is provided only if all criteria (correspondent certified value EMPA and BAM, uncertainty, impurities, long term stability) are met.

Figure 2 shows a certification analysis for a monoelemental anionic standard solution.

Figure 1  
Assay increase for cationic/anionic solutions, filled in HD-PE bottles, sealed partly in bag



### Packaging

Due to our commitment to find the best analytical solutions for our customers, Fluka and EMPA have been working together to improve the quality of the packaging of reference materials. In parallel with the certification analyses, long term stability tests have been carried out by EMPA. These investigations resulted in a switch from glass to high density polyethylene (HD-PE) bottles for all standard solutions (cationic and an-

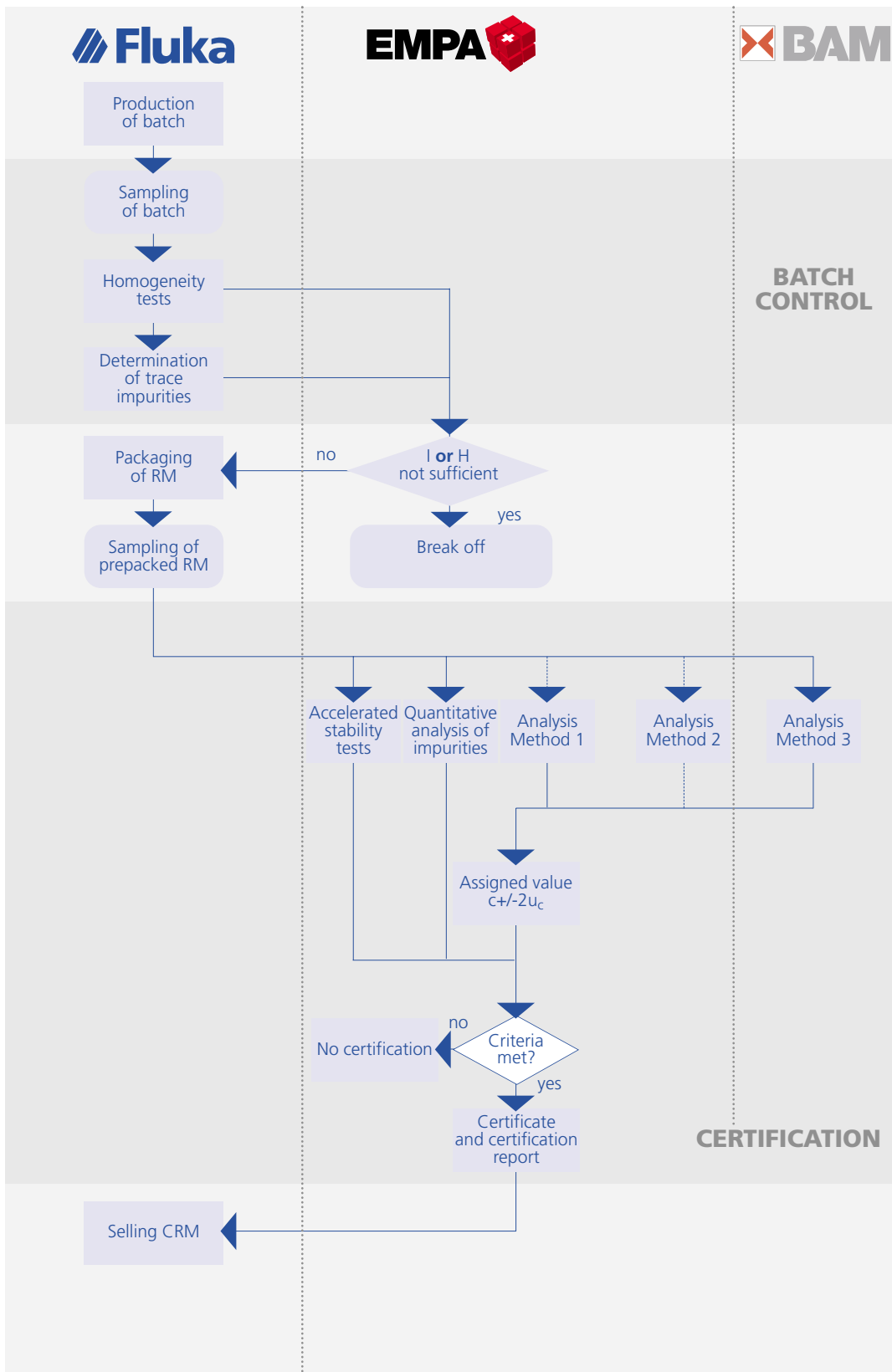


Figure 2: Production and certification of a mono-elemental anionic standard solution used for ion chromatography

ionic), thus avoiding possible contamination by leaching out of the glass. But even for perfectly closed bottles on long term storage, the standard solutions in HD-PE packaging show a measurable loss of water. This is due to the diffusion of water through the plastic and results in an increase of the metal/anion concentration in the standard solution. To minimize the water loss, we have sealed the bottles in airtight multilayer compound bags. Nevertheless, a very small water loss is inevitable

(figure 1). This effect has been included in the calculation of standard uncertainties.

## The Fluka Range of Certified Reference Material

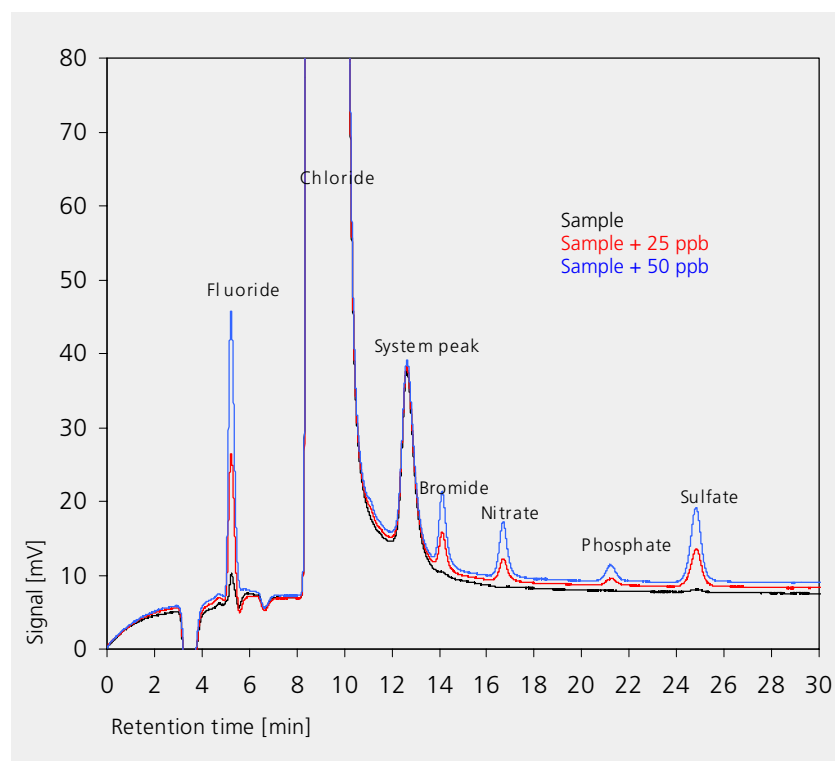
### Example: Certification of a Chloride Standard Solution for Ion Chromatography

EMPA certifies the assay of chloride standard solutions by argentometric titration and ion chromatography, BAM determines it by argentometric titration. Both methods are performed at a high level of precision. The resulting values are combined to the certified value according to common rules. The anionic traces, which are part of the certificate, are determined by ion chromatography by EMPA. Figure 3 shows the ion chromatogram of a chloride standard solution including the different trace impurities.

The results of the long term stability tests are considered in the calculation of the combined standard uncertainty, according to the EURACHEM/CITAG Guide: «Quantifying Uncertainty in Analytical Measurement» (2nd edition, QUAM:2000.P1).

The certificate is issued according to ISO guide 31. Part of the certificate is the certification report including a detailed description of the whole certification process, details of the applied methods and reference materials, the full calculation of values and uncertainties.

Figure 3  
Chromatogram of chloride standard solution (Fluka, Cat. No. 87603)



The Fluka range of certified reference material includes

- Substances for titrimetry
- Cationic monoelemental solutions for spectroscopy (1 g/l)
- Anionic monoelemental solutions for ion chromatography (1000 mg/kg)

#### Titrimetric substances

Cat. No.	Product	Pack Size
17971	Arsenic trioxide	10 g
12353	Benzoic acid	25 g
21067	Calcium carbonate	25 g
15194	Lead nitrate	25 g
60357	Potassium hydrogen phthalate	50 g
60386	Potassium iodate	50 g
71363	Sodium carbonate	50 g
71387	Sodium chloride	25 g
71804	Sodium oxalate	25 g
93440	Tris(hydroxymethyl)aminomethane	50 g

#### Cationic standard solutions for spectroscopy (1 g/l)

06159	Aluminum, Al(III)	50 ml
11078	Arsenic, As(III)	50 ml
20896	Cadmium, Cd(II)	50 ml
21062	Calcium, Ca(II)	50 ml
27014	Chromium, Cr(VI)	50 ml
27571	Cobalt, Co(II)	50 ml
61155	Copper, Cu(II)	50 ml
44907	Iron, Fe(III)	50 ml
15283	Lead, Pb(II)	50 ml
63038	Magnesium, Mg(II)	50 ml
83348	Mercury, Hg(II)	50 ml
72226	Nickel, Ni(II)	50 ml
96460	Zinc, Zn(II)	50 ml

#### Anionic standard solutions for ion chromatography (1000 mg/kg)

87603	Chloride	100 ml
80218	Sulfate	100 ml
87969	Bromide*	100 ml
86576	Nitrate*	100 ml
80373	Fluoride*	100 ml
81193	Phosphate	100 ml

\*available end of 2002

## Certified Reference Materials by IRMM

Certified reference materials (CRM) constitute an essential tool in achieving comparability and traceability of measurements. CRMs enable the optimization of industrial processes, the protection of public health and the environment, the promotion of international trade and the implementation and standardization of European legislation. They play an important role in

- Ensuring comparability of results of trade partners (on a voluntary basis)
- Proper application of written standards and implementation of national legislation or European directives
- Implementation of quality control schemes, laboratory accreditation and mutual recognition of laboratory results
- Reaching the state of the art in industrial technologies where measurements are critical
- Performing measurements which require a common calibrant or reference point

There is an increasing number of CRM producers, and a demonstration of their scientific and technical competence, and the reliability of products is now a basic requirement for ensuring the quality of reference materials.

The demand for new CRMs of higher quality is increasing as a consequence of the increased precision of measuring equipment, and the requirement for more accurate and reliable data in the scientific and technological disciplines.

In Europe, the Bureau Communautaire de Référence (BCR®), a department of the European Commission, publishes guidelines for the production and certification of reference materials which summarize the principles to be applied in certification of BCR® reference materials, the precautions to be taken to ensure the quality of the reference materials, and the information which should be provided to their users.

### IRMM/ BCR® Reference Materials

The IRMM (Institute for Reference Materials and Measurements) is one of the eight institutes of the Joint Research Center, the European Commission's scientific and technical research laboratory. IRMM serves the policies of the European Union by delivering scientific/technical products and services for the European Commission's Directorate Generals (DGs), international organizations, Member States and their institutions, and industry. Since 1995 the IRMM has shared the responsibility for the production and certification of BCR® CRMs. BCR® reference materials are produced and certified in accordance with internationally accepted rules laid down in the various guides by BCR®, ISO and WHO (World Health Organization). These products are produced either on IRMM's own initiative (e.g. to replace exhausted stocks of BCR® reference materials) or



on requests from research, public or industrial organizations and/or partners.

The major steps in the certification process include:

- A feasibility study
- Preparation of the candidate reference material
- Homogeneity testing of the candidate reference material
- Stability testing of the candidate reference material
- Certification measurements employing in general 6 – 15 laboratories

The results of each BCR® certification exercise are evaluated at a technical discussion meeting in which all certifying laboratories participate. The project coordinator then prepares a draft certification report and a draft certificate following the instructions for the certification of BCR® reference materials. The European Commission coordinates the actual certification procedure which involves submission of the certification documents to an independent group of experts. These experts perform, on the basis of these documents, a technical audit to confirm the validity of the certificate. This expert group is referred to as the Scientific Evaluation Group for the Certification of Reference Materials. On the basis of their advice, the European Commission may decide to certify the reference material, which is then made available world-wide through authorized distributors, such as Fluka Chemie GmbH.



«The mission of IRMM is to promote a common European measurement system in support of EU policies, especially internal market, environment, health and consumer protection standards. IRMM prime objectives are to develop and perform specific reference measurements, to produce certified reference materials, to organize international measurement evaluation programs, to establish transnational data bases, and to carry out prenormative research.»

Reference materials can be divided into two categories:

- Pure substances or solutions for the calibration of instruments e.g. standard solutions, mixtures for pesticide analysis, isotopic standards
- Matrix reference materials for the validation of analysis procedures, e.g. heavy metal traces in milk powder, sediments, and sludge; pesticide traces in vegetables and fish.

The BCR® products belong to the last group, except for the environment certified reference materials, which consists of pure compounds and calibration solutions. The whole program contains more than 400 products belonging to various segments such as

#### Clinical Chemistry

- Proteins (plasma, serum)
- Enzymes
- Hormones
- Electrolytes and metabolites (blood, serum)
- Blood cell size reference material

#### Environment

- Pure compounds and calibration solutions
- Environmental matrix materials
  - Major and trace elements
  - Extractable trace elements
  - Speciation

- Polyaromatic hydrocarbons (PAHs)
- Polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs)
- Polychlorinated biphenyls (PCBs)
- Organochlorine pesticides

#### Food and Agriculture

- Food authenticity
- Major and trace elements
- Nutritional properties
- Oil and fats
- Natural toxins
- Veterinary drugs and hormone residues
- Polyaromatic hydrocarbons
- Polychlorinated biphenyls (PCBs)
- Organochlorine pesticides
- Various reference materials

#### Industrial Raw Materials and Products

- Ores and fuels
- Semiconductors
- Glass and ceramics
- Fertilizers
- Plastics

#### Occupational Hygiene

- Chromium species
- Aldehydes as 2,4-Dinitrophenyl hydrazones
- BTX-aromatic compounds

#### Physical Properties

- Heat transmission
- Properties of particulate materials
- Linewidth measurements
- Properties of films and surfaces
- Color measurements
- Flash point closed-cup equilibrium measurements
- Mechanical properties of materials

#### Reactor Neutron Dosimetry (new)

- Activation Monitors
- Fission Monitors

#### Water and Food Microbiology

- Microbiological Reference Materials

Each certified reference material is provided with a comprehensive certificate of analysis stating the certified values along with the associated measurement uncertainty for each certified value. In addition to the certificate the certification report is delivered with each product which provides details on the production, characterization and certification of the material.

#### Distribution of IRMM/BCR® Reference Materials

In April 2000, Fluka became an authorized distributor of the Institute of Reference Materials and Measurements (IRMM) at Geel, Belgium. As a consequence, our customers can now receive more than 400 Certified Reference Materials (CRM) via the Sigma-Aldrich sales organization worldwide. The customers of IRMM/BCR® reference materials can benefit from our fast and reliable service, backed by our technical service.

### Fluka – Your supplier for BCR® Reference Materials



If you browse the Fluka-Website [www.sigma-aldrich/fluka](http://www.sigma-aldrich/fluka) you will be able to find the complete list and descriptions of all IRMM/BCR® in the chapter «Reference Materials».



Order the new Analytical Standard CD – free of charge. Just fax the order form inserted in this newsletter to your local Sigma-Aldrich partner listed on page 19!

## GMO Reference Materials certified by IRMM

An increasing number of genetically modified crops has been approved worldwide during the last several years. However, European consumers expect labeling of foodstuff made from genetically modified plants. In this situation European and worldwide countries have combined the authorization to use GMO crops as food with the obligation to declare products containing ingredients from genetically modified plants. These regulations request labeling of food if an ingredient contains more than a defined limit (1% in the EU and in Switzerland). This has enforced the necessity to develop suitable detection methods and certified reference materials.

In order to meet the tremendous demand for GMO reference materials, Fluka and the Institute for Reference Materials and Measurements (IRMM) developed a series of CRMs for detection of Roundup Ready™ soya bean in 1998. Further GMO reference materials for genetically modified maize were produced later by the IRMM on behalf of Fluka in collaboration with the Institute for Health and Consumer Protection (IHCP) of the Joint Research Center of the European Commission (Ispra, Italy).

### Product Range of GMO Standards

Fluka now offers CRM series for Roundup Ready™ soya and maize MON 810 detection. Sales of CRMs for both maize Bt11 and Bt176 have recently been stopped due to stability problems. Replacement products, manufactured with an improved mixing technology (see «preparation of CRMs») are planned.

All CRMs consist of dried flour containing different mass fractions of powder from genetically modified crops.

### Preparation of CRMs

The first series of CRMs for Roundup Ready™ soya and both Bt176 and Bt11 were prepared by quantitative mixing in an aqueous suspension of non-GMO and 100% GMO crops powder. In order to minimize DNA and protein degradation occurring during this procedure, currently offered CRMs for MON 810 (IRMM-413) and Roundup Ready™ soya (IRMM-410-S replacing IRMM-410R) are now produced by the IRMM with the help of a recently developed dry-mixing technology. New batches of Bt176 and Bt11 are going to be prepared with this new technology in the near future.

The reference materials for Roundup Ready™ soya detection were produced from whole seeds of a non-modified and a genetically modified soya line, whereas those for MON 810 were manufactured from non-genetically modified and 100% MON 810 GMO kernels. They are available in glass bottles containing 1 g powder packed under Argon atmosphere.



### Competitors for quantitative GMO Detection

Methods based on co-amplification of competitors (internal standards) together with target DNA using PCR have been developed to quantify DNA from genetically modified organisms in food samples. Such quantitative PCR (qPCR) methods have been successfully evaluated in a ring study organized by the Swiss Federal Office of Public Health (Bern). Fluka is offering the following internal standards for qPCR:

Literature for the use of certified GMO reference materials and competitors is available on request. Please contact our Fluka technical service: [flukatec@eurnotes.sial.com](mailto:flukatec@eurnotes.sial.com)

#### Roundup Ready™ Soya Standards

Cat. No.	replaces	Product	Description	Pack size
83063 new	53198	Soya Bean Powder, IRMM-410S-0	0% (nominal, < 0.1%) genetically modified soya bean	1 g
72647 new	49232	Soya Bean Powder, IRMM-410S-1	0.1% genetically modified soya bean	1 g
81751 new	57452	Soya Bean Powder, IRMM-410S-2	0.5% genetically modified soya bean	1 g
76913 new	68202	Soya Bean Powder, IRMM-410S-3	1.0% genetically modified soya bean	1 g
78767 new	49215	Soya Bean Powder, IRMM-410S-4	2.0% genetically modified soya bean	1 g
93109 new	44386	Soya Bean Powder, IRMM-410S-5	5.0% genetically modified soya bean	1 g
94162 new	89305	Soya Bean Powder Set, IRMM-410S	0, 0.1, 0.5, 1, 2 and 5% genetically modified soya bean	1 set

#### Maize MON 810 Standards

Cat. No.	Product	Description	Pack size
77119	IRMM-413-0 for 0% MON 810	0% (nominal) genetically modified MON 810 maize	1 g
74411	IRMM-413-1 for 0.1% MON 810	0.1% genetically modified MON 810 maize	1 g
79521	IRMM-413-2 for 0.5% MON 810	0.5% genetically modified MON 810 maize	1 g
71981	IRMM-413-3 for 1% MON 810	1.0% genetically modified MON 810 maize	1 g
79112	IRMM-413-4 for 2% MON 810	2.0% genetically modified MON 810 maize	1 g
76182	IRMM-413-5 for 5% MON 810	5.0% genetically modified MON 810 maize	1 g
78761	IRMM-413-Set for 0%, 0.1%, 0.5%, 1%, 2% and 5% MON 810		1 set

#### Competitors for quantitative GMO Detection

Cat. No.	Product	Description	Pack size
29246	CRRS	DNA competitor for the quantification of genetically modified Roundup Ready™ soya bean material	~100 assays
29247	CP35S	DNA competitor for the quantification of genetically modified foodstuff containing cauliflower mosaic virus 35S promoter	~100 assays
29248	CCRY(Bt176)	DNA competitor for the quantification of genetically modified maize Bt-176	~100 assays
29249	CSL	DNA competitor for the quantification of soya bean lectin gene	~100 assays
29251	CHGMP	DNA competitor for the quantification of maize High Mobility Group Gene	~100 assays

## Food and Beverage Standards

During the last decade, the identification of potentially hazardous residues in food has become a main topic of public concern. Food regulation authorities in the European Community and Switzerland have produced proposals for limits on undesirable residues. Fluka offers a comprehensive product range of specialty standards designed for the food and beverage industry. These include such unique in-house developed items as BADGE (Bisphenol-A-diglycidyl ether) and BFDGE (Bisphenol-F-diglycidyl ether), food irradiation standards and phytoestrogen standards.

### BADGE, BFDGE

Epoxy based resins are used widely for lacquer coatings on food cans and food storage vessels. Studies with canned oily foods have shown that edible oil efficiently extracts BADGE and BFDGE from the coating and prevents the epoxide group from being hydrolyzed even in acidic media. BADGE and/or BFDGE is added to these coatings in order to remove hydrochloric acid by reaction with the epoxide. This results in HCl-adducts, the toxicity of which is largely unknown.

There had been considerable work in Europe to develop and apply methods of analysis for these substances. In 2001, the European Scientific Committee for Food produced a proposal (directive 90/128/EEC) for a limit of 1 mg/kg for BADGE, BFDGE and their adducts and hydrolysates in canned foods.

For performing reliable quality control, standards of BADGE, BFDGE and their derivatives are needed. Fluka offers 9 unique, carefully synthesized standards including: BADGE, BFDGE, the HCl-adducts and hydrolysates. These materials are used by all analytical chemists working within the food industry (control laboratories), producing companies, governmental laboratories, private analytical service laboratories and university laboratories.

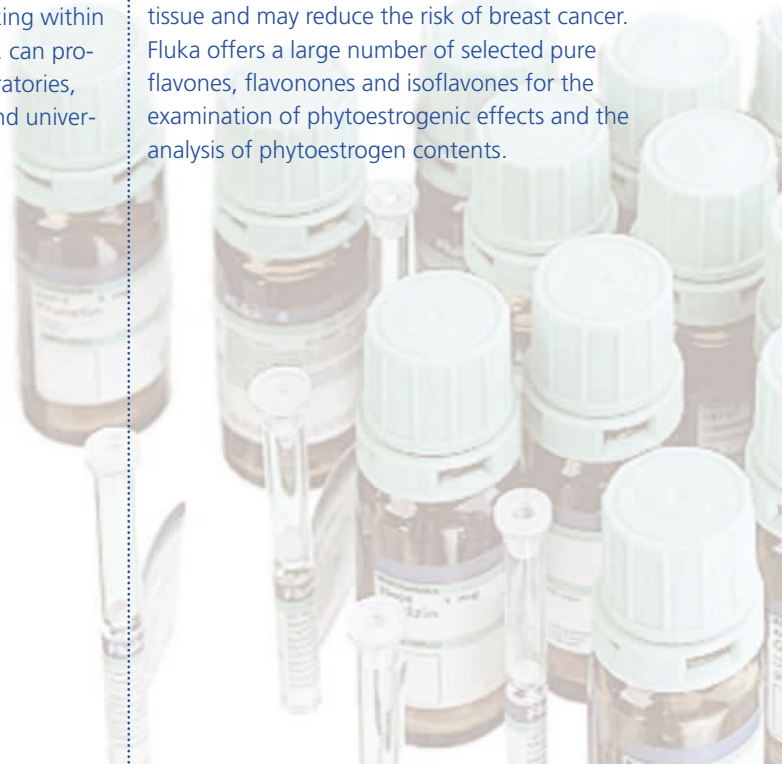
### Food Irradiation

Treatment with ionized radiation is used to eliminate microbiological activity. Such foodstuff, with a few exceptions, may not be sold in certain European countries and need to be declared in many other countries. Irradiation produces certain substances which are absent in untreated food stuff and have been used as indicators of irradiation.

Irradiation of fat-containing foodstuff (e.g. chicken meat, hazelnut) produces free radicals, which partially degrade fat (triglycerides). This process produces alkanes, alkenes, aldehydes and alkylcyclobutanones. Irradiation of water-containing albuminous foodstuffs (meat) produces reactive hydroxyl radicals which react with phenylalanine to produce ortho- and meta-tyrosine. The detection of typical radiation by-products is used to confirm treatment with ionized radiation. Fluka offers selected indicator substances which might be used as standards for GC-analysis.

### Phytoestrogens

It is well established that plants may contain substances with estrogenic effects, so called phytoestrogens. These substances have been found in numerous plant food products, e.g., carrots, peas, lentil, rice, cabbage and soya. They are structurally similar to the mammalian estrogen or estradiol, and show estrogenic properties. However, their estrogenic activity is generally much less than that of human estrogens (estrogenic activity ranges from 1/500 to 1/1000 of the activity of estradiol). Hence, phytoestrogens can act as anti-estrogenic agents by blocking the estrogen receptors and exerting a much weaker estrogenic effect compared with the hormone. As a consequence it has been suggested that they might partly suppress or inhibit normal estrogenic activity in estrogen-responsive tissues such as breast tissue and may reduce the risk of breast cancer. Fluka offers a large number of selected pure flavones, flavonones and isoflavones for the examination of phytoestrogenic effects and the analysis of phytoestrogen contents.



## Residue Analysis Standards

Many harmful and toxic substances have been used in the past for the treatment of soil and plants. Today a more ecologically sensitive attitude is prevalent. Natural processes are complex. For example, where do the active substances in mother's milk come from? How can we accelerate the decomposition of pesticides in the environment? These questions can be answered with help of modern analytical instruments, e.g. GC (Gas Chromatography) and HPLC (High-Performance-Liquid-Chromatography).

In the 1960's, Riedel-de Haën began the development of a broad range of high purity standards especially for those analytical laboratories working on problems involving the environment and food supplies. Today Riedel-de Haën offers more than 1500 residue analysis standards:

### PESTANAL®

«Pesticides», from lat. pestis – infectious illness - and cidere - kill -, is the term for all chemicals used as pest control agents. Among the generic designation «pesticides», are many sub-categories: insecticides, herbicides, fungicides, nematocides, rodenticides and molluscicides. Institutions such as the FDA (Food and Drug Administration) or EC (European Community) have stipulated specified limits of these substances and prescribed analytical methods to ensure consumer safety.

But what does the analyst need? The analyst needs only very small amounts of pesticides of the highest quality for the validation of his analytical instruments! Dependent on individual analytical practices, pesticide standards are used as neat substances or standard solutions. Therefore Riedel-de Haën offers about 900 standards under the tradename PESTANAL® including exemplary substance classes as Atrazines and DDT's.

### VETRANAL®

Beside the pesticides used in agriculture, many pharmacologically-active substances are used in modern stock farming; for therapy or illegal growth promoting. To support analysts worldwide in their endeavor to control food production processes, Riedel-de Haën offers a range of commonly used pharmacological substances under the tradename VETRANAL®.



### OEKANAL®

The third main group of Riedel-de Haën standards is OEKANAL®: chemical substances with environmental damaging properties, e.g., pentachlorophenol (PCB) as preservative for wood decay.

### Neat or Solution?

To meet the different needs of our customers, we offer standards as neat substances or standard solutions. A neat standard has a longer stability while a standard solution needs only a low concentration of the active substance and is more convenient. Standard solutions are delivered in two different pack sizes, 10 ml bottles and in 2 ml glass ampoules. All ampoules are filled under Argon to guarantee stability.

### Certan®-Bottles

To store standard solutions without a change in concentration, the new Certan®-bottles are used. Table 1 shows the advantages of Certan®-bottles for different applications.



Table 1: Advantages of Certan®-bottles

Application	Advantages
Storage of unused standard solutions	No change in concentration even when opened
Storage of working stock	Safety storage
Storage of highly volatile samples	Minimum risk of contamination
Preparation of samples and reference solutions	Easy filling and removal of aliquots with specific GC-syringes
Handling	Impossible to spill; proven usability from -30°C to + 20°C

Custom specific standards?

Please contact  
Mr. Rainer Walz, PhD  
E-mail:  
rwalz@eurnotes.sial.com

High Purity Solvents,  
specially tailored for the  
residue analysis?

Ask for PESTANAL® and  
OEKANAL®!

## Standards for Gas Chromatography

GC (Gas Chromatography) owes its enormous growth in part to its speed, simplicity, relatively low cost, and wide applicability as a separating tool. It is widely used for recognizing the presence or absence of components in mixtures that contain a limited number of possible species whose identities are known.

The simplest method of identification of a chromatographic peak is comparison of its retention time with that of an authentic sample of the suspected compound. The most reliable way to do this is by co-chromatography, in which the authentic sample is added to the unknown.

The most straightforward method for quantitative chromatographic analyses involves the preparation of a series of standard solutions that approximate the composition of the unknown. Chromatograms for the standards are then obtained, and peak heights or areas are plotted as a function of concentration.

Table 1  
Class 1 solvents (should be avoided); class 2 solvents (to be limited)

Cat. No.	Solvents class 1	Concentration limit (ppm)
12540	Benzene	2
02671	Carbon tetrachloride	4
02574	1,1-Dichloroethylene	8
02669	1,1,1-Trichloroethane	1500

Cat. No.	Solvents class 2	Concentration limit (ppm)
45983	Acetonitrile	410
08650	Chlorobenzene	360
02487	Chloroform	60
28920	Cyclohexane	3880
35130	1,2-Dichloroethylene	1870
02575	Dichloromethane	600
72405	1,2-Dimethoxyethane	100
72336	N,N-Dimethylacetamide	1090
72438	N,N-Dimethylformamide	880
76887	1,4-Dioxane	380
79109	2-Ethoxyethanol	160
85978	Ethylene glycol	620
52750	Hexane	290
82762	Methanol	3000
88907	2-Methoxyethanol	50
02473	Methylbutylketone	50
66294	Methylcyclohexane	1180
78769	N-Methylpyrrolidone	4840
02484	Nitromethane	50
02486	Pyridine	200
88965	Sulfolane	160
95462	Tetralin	100
89680	Toluene	890
02667	1,1,2-Trichloroethylene	80
95660	o-Xylene	2170
95670	m-Xylene	2170
95680	p-Xylene	2170

GC standards are mainly used in

- Pharmaceutical industry
- Environmental laboratories
- Flavor & fragrance companies
- Petrol industry

For all purposes, high purity standards are required. Fluka's GC product range includes more than 400 standards belonging to various organic structure classes.

- Alkanes
- Alkenes
- Aromatic hydrocarbons
- Cyclic hydrocarbons
- Fatty acids- and fatty acid methyl esters (FAME)
- Halogenated hydrocarbons
- Ketones
- Lactones
- Terpenes

Fluka's GC standards can be used for different tasks, e.g.:

### Pharmaceutical Analysis

GC reference standards are used to determine residual solvents which are listed in the European Pharmacopoeia, (Ph.Eur.). Residual solvents in pharmaceuticals are defined as organic volatile impurities (OVI) that are used or produced in the manufacture of active substances or in the preparation of medicinal products. Since there is no therapeutic benefit from residual solvents, they should be removed to the extent possible to meet GMP requirements. Therefore, testing should be performed for residual solvents when production or purification processes are known to result in the presence for such solvents. The European Pharmacopoeia subdivides these into the three categories listed below. According to the European Pharmacopoeia, residual solvents have been determined by GC (Gas Chromatography). Fluka offers the complete range of GC reference substances of class 1, class 2 and class 3 solvents.

#### Class 1 Solvents

These solvents should not be employed in the manufacture of active substances and medicinal products because of their unacceptable toxicity. However, if their use is unavoidable in order to produce a medicinal product with a significant therapeutic benefit, their levels should be restricted as shown in table 1.

#### Class 2 Solvents

These solvents should be limited in pharmaceutical products because of their inherent toxicity. The concentrations given in table 1 are the nearest to 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

### Class 3 Solvents

These Solvents listed in table 2 can be regarded as less toxic and of lower risk to human health. This class includes no solvent known as hazardous to human health at levels normally accepted in pharmaceuticals. Amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice.

### Water Analysis

In 2001, the European Commission of standardization introduced a new method for determination of hydrocarbons in water which is defined in the guideline EN ISO 9377-2. The method is based on extraction of the hydrocarbons by a light hydrocarbon solvent, followed by cleanup to remove polar substances, gas chromatographic separation and flame ionization detection (FID). The carbon numbers to be detected by the method is from C10 to C40. By ordering hydrocarbon GC standards from Fluka you benefit from a complete product range of hydrocarbon standards up to C40. Beyond this, we are proud to offer unique in-house developed long-chained alkanes up to C60.

On request all GC standards are provided with our GC method including a chromatogram stating the purity which is usually >99.0%. Each product has an expiration date on the label to make sure that you can get always the latest lot. Our convenient package size makes it easy for you to purchase only what you need.

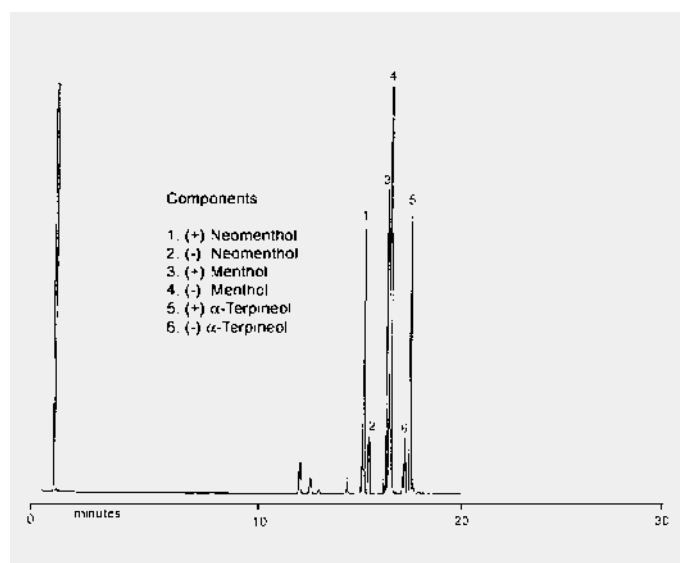
### Analysis of Phytochemicals

GC analysis of phytochemicals is often concerned with the assay of terpenes, which can be found in almost any plant life and have a beneficial function within plants themselves. The analysis of terpenes in plants involves separating and identifying very complex mixtures, the components of which are present at very low concentrations. For example, the origin of many herbs and spices can often be identified from the peak pattern of the chromatograms from their head space analysis (figure 1 shows the GC Analysis of menthol oil). Terpenes are mainly used as reference standards for GC analysis of phytopharmacas, flavor & fragrances and therapeutic agents. Fluka offers a unique range of terpene standards, thoroughly characterized by GC, optical rotation and refractive index.

Cat. No.	Solvents class 3
71251	Acetic acid
00570	Acetone
96109	Anisole
19422	1-Butanol
96870	2-Butanol
73285	Butyl acetate
38911	Methyl ether
28220	Cumene
94563	Dimethyl sulphoxide
02483	Ethanol
58958	Ethyl acetate
91238	Ethyl ether
88554	Ethyl formate
51730	Heptane
94823	Isobutyl acetate
90871	Isopropyl acetate
45997	Methyl acetate
59092	3-Methyl-1-butanol
02469	Methylethylketone
02474	Methylisobutylketone
82059	2-Methyl-1-propanol
76870	Pentane
77597	1-Pentanol
96566	1-Propanol
91237	2-Propanol
40858	Propyl acetate
78445	Tetrahydrofuran

Table 2  
Solvents which should be limited by GMP or other quality-based requirements

Figure 1  
GC analysis of Menthol oil



## Color Reference Solutions

Color reference solutions are used to control the degree of coloration of liquids in the range red, yellow, green, blue and brown. This examination is described in the European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP), or by federal institutes, e.g. American Society of Testing and Material (ASTM).

Fluka is now the first to offer you the most commonly used color reference solutions according to Ph. Eur., USP or APHA (American Public Health Association). Using these solutions, it is possible to compare the international and standardized coloration of liquids. You can choose between two package sizes, 2 or 10 ml. Every solution is sealed in airtight ampoules under Argon. Every set comes with a certificate of analysis and the expiration date printed on the label.

### Color Reference Solutions acc. to Ph. Eur.

Ph. Eur. solutions are available as a complete set of 37 solutions, divided into the five color series, red (R1-R7), greenish-yellow (GY1-GY7), yellow (Y1-Y7), brownish-yellow (BY1-BY7) and brown (B1-B9). Each color series can also be obtained separately (table 1).



### Color Reference Solutions acc. to USP

The USP prescribes twenty different color solutions, marked A – T, covering the range red, yellow, green, blue and purple. These solutions are available as one set (table 2).

### Color Reference Solutions acc. to ASTM/APHA

The ASTM issues a standard method for the visual measurement of the color of essentially light colored liquids using the platinum-cobalt scale. This test method is referred to by many as APHA color. The preparation of these platinum-cobalt standards was originally described by A. Hazen. Subsequently the term «Hazen Color» often occurs in combination with this test. The complete APHA set comprises 20 standard solutions, marked with different color standard numbers - 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 100, 150, 200, 250, 300, 350, 400, 450, 500 (table 3).

### Handling

The perception of color and color matches is dependent on conditions of viewing and illumination. Determinations should be made using diffuse, uniform illumination under conditions that reduce shadows and non-spectral reflectance to a minimum. Liquids should be compared in matched color-comparison tubes, against a white background. Colors of standards should be as close as possible to those of test specimens for quantifying color differences.

Cat. No.	Product
<b>Color Reference Solutions acc. to Ph. Eur.</b>	
83952	Color reference solutions B, BY, Y, GY, R acc. to Ph Eur, set (amp. with 2 ml)
83951	Color reference solution B acc. to Ph Eur, set (amp. with 2 ml)
86293	Color reference solution BY acc. to Ph Eur, set (amp. with 2 ml)
83883	Color reference solution Y acc. to Ph Eur, set (amp. with 2 ml)
82995	Color reference solution GY acc. to Ph Eur, set (amp. with 2 ml)
87448	Color reference solution R acc. to Ph Eur, set (amp. with 2 ml)
90232	Color reference solutions B, BY, Y, GY, R acc. to Ph Eur, set (amp. with 10 ml)
92936	Color reference solutions B acc. to Ph Eur, set (amp. with 10 ml)
72666	Color reference solutions BY acc. to Ph Eur, set (amp. with 10 ml)
83967	Color reference solutions Y acc. to Ph Eur, set (amp. with 10 ml)
90269	Color reference solutions GY acc. to Ph Eur, set (amp. with 10 ml)
95872	Color reference solutions R acc. to Ph Eur, set (amp. with 10 ml)
<b>Color Reference Solutions acc. to USP</b>	
87576	Color reference solutions acc. to USP, set (amp. with 2 ml)
87574	Color reference solutions acc. to USP, set (amp. with 10 ml)
<b>Color Reference Solutions acc. to APHA</b>	
72599	Color reference solutions acc. to APHA, set (amp. with 2 ml)
77147	Color reference solutions acc. to APHA, set (amp. with 10 ml)
<b>Techware</b>	
72671	Prescored ampoules, 10 ml, packages a 144 amp.
66946	Prescored ampoules, 2 ml, packages a 100 amp.

## HYDRANAL® - Pyridine-free Reagents for Karl Fischer Titration

The determination of water content is used to monitor product quality and to ensure chemical and physical properties in a wide variety of substances, e.g., chemicals, oils, pharmaceuticals and food. Over 20 years ago Eugen Scholz at Riedel-de Haën improved the Karl Fischer titration by replacing pyridine with imidazole – the beginning of **HYDRANAL®**, the leading pyridine-free reagents for Karl Fischer titration. In the following years Riedel-de Haën launched a wide range of reagents for volumetric and coulometric titrations. Recent developments at the **HYDRANAL®**-Laboratory have led to ethanol-based and non-toxic Karl Fischer reagents.

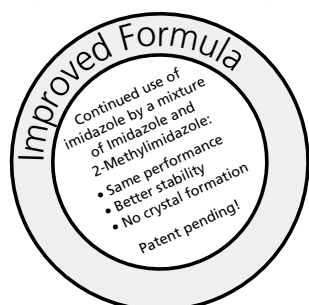
### HYDRANAL®-Composite Improved One-Component Reagent for Volumetric Karl Fischer Titration

**HYDRANAL®**-Composite is the world's most frequently used pyridine-free Karl Fischer reagent. This single-component reagent has proven its capabilities in volumetric titrations for more than twenty years in a large range of applications in diverse fields of research and industry. Recent developmental work has resulted in significant improvements to this reagent.

### Development of the Reagent

Sulfur dioxide, alcohol, iodine and water react at stoichiometric ratios in the Karl Fischer reaction. The sulfur dioxide, and the acid generated during the reaction, are neutralized using a suitable alkali in order to keep the pH of the reaction solution within the optimum range. The alkali used has, up to now, been the tried and proven imidazole.

The Riedel-de Haën development laboratory has now produced for **HYDRANAL®** a new and improved formulation based on a mixture of imidazole and 2-methylimidazole. The advantageous buffering action of the imidazole is retained, as well as the typical pH range during titration. This new composition also resulted in significant improvements to the product. All **HYDRANAL®**-Composite reagents from batch 11820 onward contain this new formulation and can be visually identified by the «Improved Formula» stamp.



### Advantages of the improved Formulation

Humidity, and reagents remaining in the KF titrator's hose system for a prolonged time period, often lead to crystallization effects. The new 'improved formula' protects your Karl Fischer system from such undesirable reactions. In addition, the new formulation has an improved titer stability relative to the former composition.

### Checking of Comparability

A process validation has been performed for both formulations. It was observed that the two formulations did not exhibit any significant differences in rates of reaction. Ten titrations using **HYDRANAL®**-Water Standard 10.0 were performed for the investigation of other important process parameters, such as recovery rate and standard deviation. This standard can be traced back to NIST standard SRM 2890. An extremely high accuracy was obtained for both formulations. As you can see in table 1, the two reagents performed almost identically.

The **HYDRANAL®**-Laboratory performed comparative tests for both formulations using specimen materials from a diverse range of applications. For this purpose, water tests were performed in inorganic substances such as acids, organic substances such as gasoline, diesel, commercial preservation agents, brake fluid, pharmaceutical products, dairy products, and confectionery. Both formulations exhibited the same titration performance in tests on these extremely diverse substances. No significant deviations were observed in the results obtained. The product range of **HYDRANAL®**-Composite is shown in table 2.

Table 1  
Determination of process parameters using **HYDRANAL®**-Water Standard 10.0 (n=10).

Parameter	HYDRANAL®-Composite	
	Old Formulation	Improved Formulation
Mean in mg H <sub>2</sub> O	10.04	10.03
Recovery rate in %	100.1	100.0
RSD in %	0.06	0.08

The effective water content of the **HYDRANAL®** Water Standard 10.0 used is 10.03 mg/g.

## Toxicological Studies

Toxicological tests performed under GMP conditions confirm that the use and handling of both formulations can be classified as harmless to the health. The affixing of a hazard symbol to our **HYDRANAL®-Composite** reagents is therefore not necessary on the basis of these studies and the EU directive concerning classification and marking.

## HYDRANAL®-Water Standards



**HYDRANAL®-Water Standards** are sealed in glass ampoules under Argon. All of them can be traced to NIST SRM 2890. The certificate of analysis and instructions for usage according to guidelines ISO 9001-9004 are enclosed.

## HYDRANAL®-Coulomat Oil

### A new Reagent for Coulometric Water Determination in Oils

Oils are used in many different technical applications. In practice, oils have to pass extensive analytical tests to meet continuously increasing quality requirements. Some of the more stringent require tests on samples containing less than 10 ppm water. This is the reason why Riedel-de Haën developed a new reagent for coulometric water determination in oils called **HYDRANAL®-Coulomat Oil**.

For the determination of water contents in the low ppm range, coulometry is the method of choice. Besides its high precision, this method has distinguished itself due to the ease of introduction of liquid samples with a syringe. With the publication of the ASTM method, D 4928-96 «Standard test methods for water in crude oils by coulometry», the coulometric determination of water in crude oils was established as an accepted procedure.

## Development of the Reagent

For a typical Karl Fischer titration, the preferred medium is an alcohol, in which, due to the differences in polarity, non-polar ingredients of oil are insoluble. The new reagent is based on methanol, to which defined quantities of aromatic and halogenated hydrocarbons have been added to increase the solubility of these non-polar ingredients. Also in **HYDRANAL®-Coulomat Oil**, samples of oil in suspension may be analyzed. Nevertheless, the determination of water contents can be performed without any difficulties. The addition of xylene results in a higher solubility of tar, significant amounts of which are contained in crude oils. The formation of tar layers on the indicator electrode can be eliminated and no disturbances are to be anticipated for the identification of the end points.

This optimally designed formulation meets all requirements of customary coulometers with regard to the conductivity of the reagents used. This new reagent is intended for use as an anolyte for coulometric cells with a diaphragm in combination with the catholyte **HYDRANAL®-Coulomat CG**.

## Applications

Some applications of the determination of low water content in oils can be mentioned here. In transformer oils, the water content is decisive for determining the service life of the oil. Within the framework of process controls, lubricating oils have to be tested on a regular basis. The water content of cosmetic products is a critical factor for their shelf life, and also for cooking oils in the food industry. The water content also has a major influence on raw material costs – especially when dealing with crude oils.

## Validation

Our **HYDRANAL®-Laboratory** performed an extensive validation of this new reagent using coulometers of various manufacturers. In all of the validations, a 5 ml charge of **HYDRANAL®-Coulomat CG** was used as the catholyte, and 100 ml of **HYDRANAL®-Coulomat Oil** was filled in the anode compartment.

For various oils the water contents were determined in samples of 2 ml each (n = 10). Furthermore, the rate of recovery (n=3), as well as the reproducibility, was determined with

Table 2  
Product range of  
**HYDRANAL®-Composite**

Cat. No.	Product	Description	Pack Size
34805	HYDRANAL®-Composite 5	One-component reagent, titer: 1 ml ~ 5 mg water	500 ml; 1 l; 2.5 l
34806	HYDRANAL®-Composite 2	One-component reagent, titer: 1 ml ~ 2 mg water	500 ml; 1 l; 2.5 l
34827	HYDRANAL®-Composite 1	One-component reagent, titer: 1 ml ~ 1 mg water	500 ml; 1 l
34816	HYDRANAL®-Composite 5 K	One-component reagent for the titration of aldehydes and ketones, titer: 1 ml ~ 5 mg water	500 ml; 1 l
34849	HYDRANAL®-Water Standard 10.0	Standard for volumetric KF titration, 1 g contains 10.0 mg = 1.00% water, contains 10 glass ampoules of 8 ml, traceable to NIST SRM 2890	80 ml

**HYDRANAL®-Water Standard 1.00.** This standard can be traced to the reference material NIST SRM 2890. Some validation results are shown in table 3.

Due to the high absorption capacity of **HYDRANAL®-Coulomat Oil**, the determination of water in many samples, even with high water contents, can be performed with exceptional reproducibility. Regardless of the oil samples to be tested, rates of recovery exceeding 99% with a simultaneously high reproducibility can be obtained.

Table 4 gives an overview of the **HYDRANAL®-Coulomat Oil** product range.

### Interferences

**HYDRANAL®-Coulomat Oil** has been formulated with the optimum blend of solubilizers for the majority of oil samples. However, there may be that one unique sample where extra chloroform is required. We have found that an additional 10% chloroform, by volume, may be added without affecting the results.

Oils blended with additives may experience side reactions that simulate water contents and yield results that are too high. These samples have to be tested according to the indirect method with the KF oven. For this procedure **HYDRANAL®-Coulomat E** or **HYDRANAL®-Coulomat AG Oven** is suitable as analyte.

When using coulometric cells without a diaphragm, the quantities of solubilizers contained in the reagent lead to side reactions. The observed results will exceed the real value by 5–10%.

Sample type	Water content		Recovery rate	
	mg/kg	RSD / %	Rate / %	RSD / %
Crude oil BCF	225	5.0	99.3	2.4
Crude oil FRT	109	2.8	99.5	3.0
Fuel unleaded	73.3	2.2	99.3	0.8
Oil Baysilon M 50 EL	79.6	2.3	99.8	0.4
Salad oil	300	2.1	99.9	0.9
Silicon oil AK 20	75.0	3.4	99.6	1.4
Silicon oil TR 50	76.5	2.5	99.7	0.5
Transformer oil	140	3.1	99.0	2.5
Transformer oil Teresso 46	27.5	0.7	99.7	1.2



Table 3  
Determination of the water contents (n=10) and the rates of recovery (n=3) of different oil samples.

### HYDRANAL®-Multimedia Guide

#### A new Tool to support Customers working with Karl Fischer Titration

Riedel-de Haën recently released a new **HYDRANAL®-Multimedia Guide** providing customers with an introduction to Karl Fischer titration. The guide contains 9 different subjects, and features a video assisted description of volumetric and coulometric titration and a complete product listing. In addition, there are more than 500 applications for Karl Fischer titration and more than 300 laboratory reports for special samples which can be ordered at our **HYDRANAL®-Laboratory**. The **HYDRANAL®-Multimedia Guide** (Cat. No. 37824) is available in English and German language.

Order now to get your **HYDRANAL®-Multimedia Guide at a 40% discount.**

To get your **HYDRANAL®-Multimedia guide**, please fax the inserted ordering form to your local Sigma-Aldrich service partner listed on page 19.

Table 4  
Product range of **HYDRANAL®-Coulomat Oil**

Cat. No.	Product	Description	Pack Size
34868	HYDRANAL®-Coulomat Oil	Anolyte for titration of oils	100 ml, 500 ml
34840	HYDRANAL®-Coulomat CG	Catholyte, free of halogenated hydrocarbons, 25 ml bottle or 10 x 5 ml ampoules	25 ml, 50 ml
34828	HYDRANAL®-Water Standard 1.00	Standard for coulometric KF titration, 1 g contains 1.00 mg = 0.10 % water, contains 10 glass ampoules of 4 ml, traceable to NIST SRM 2890	40 ml
34847	HYDRANAL®-Water Standard 0.10	Standard for coulometric KF titration, 1 g contains 0.10 mg = 0.01 % water, contains 10 glass ampoules of 4 ml, traceable to NIST SRM 2890	40 ml

## HYDRANAL®-E Types

### The first non-toxic Karl Fischer Reagents

During the last two decades, the need for environmental protection and safety regulations has increased significantly and has become a topic of public discussion. Today, the main focus of the Riedel-de Haën R & D laboratory is the development of reagents without hazardous substances. Our focus is on the continuous replacement of the toxic methanol, which is widely used as a solvent in the titration vessel or as a solvent for Karl Fischer reagents, by the non-toxic ethanol (table 5). Changing to the higher homologous ethanol has turned out to be a challenging task. Ethanol has not been used in the past for Karl Fischer reagents for two important reasons. The speed of the Karl Fischer reaction in pure ethanol is very slow and the electrochemical behavior varies – the conductivity of ethanol is lower. The first problem could be solved by adding accelerators to the ethanol which do not interfere with the Karl Fischer titration. The additives accelerate the reaction and the titration can be carried out in a shorter time than in methanol. Second, the conductivity for a coulometric reagent can be adjusted. A patent is pending for these developments. As a result of this continuous research work, the new **HYDRANAL®-E** types represent the first non-toxic Karl Fischer reagent line for the volumetric and the coulometric titration.

### Properties of the non-toxic Reagents

In volumetric one-component titration **HYDRANAL®-CompoSolver E** can be used instead of methanol. The non-toxic reagent shows distinct advantages. For example, the titration time is much shorter than in pure methanol. Additionally ketones like acetone or methyl-isobutylketone can also be investigated and the solubility for long chained hydrocarbons is improved so that further solubilizers aren't necessary. The **HYDRANAL®-two-component** system consisting of **HYDRANAL®-Titrant** and **HYDRANAL®-**

Table 5  
Declaration of **HYDRANAL®**-reagents based on methanol and the **HYDRANAL®-E** types.

Reagents based on methanol	Non-toxic <b>HYDRANAL®-E</b> types
<b>Volumetric titration: one-component reagents</b>	
HYDRANAL®-Composite, declaration is not demanded used in conjunction with HYDRANAL®-Methanol dry, toxic	HYDRANAL®-Composite, declaration is not demanded used in conjunction with HYDRANAL®-CompoSolver E
<b>Volumetric titration: two-component reagents</b>	
HYDRANAL®-Solvent, toxic	HYDRANAL®-Solvent E, harmful
HYDRANAL®-Titrant 5, toxic	HYDRANAL®-Titrant 5 E
HYDRANAL®-Titrant 2, toxic	HYDRANAL®-Titrant 2 E
<b>Coulometric titration</b>	
HYDRANAL®-Coulomat AD, toxic	
HYDRANAL®-Coulomat AG, toxic	HYDRANAL®-Coulomat E, harmful

Every **HYDRANAL®**-reagent except **HYDRANAL®-Composite** is additionally declared as flammable. These declarations have been provided according to the European law, adapting to technical progress for the 25th time Council Directive 67/548/EEC.

Solvent is distinguished by a high rate of titration, a stable end point and a high accuracy of the results obtained. The characteristics of the **HYDRANAL®-E** types are very similar to those of the methanol-based Solvent / Titrant combination. The shelf life and the titer of the **HYDRANAL®-Titrant E** reagents is identical to the Titrant reagents. Additionally there is no difference between the reaction speed of **HYDRANAL®-Solvent E / -Titrant E** compared with **-Solvent / -Titrant**. For coulometric titration Riedel-de Haën offers a non-toxic reagent with **HYDRANAL®-Coulomat E**. This reagent contains the same ingredients as **Coulomat AG**, but methanol is replaced by ethanol. An additional benefit is its use as both anolyte and catholyte for the cell with a diaphragm. In this case a special dedicated catholyte is no longer necessary. Furthermore **HYDRANAL®-Coulomat E** can also be used in the cell without diaphragm. The product range of all **HYDRANAL®-E** types are shown in table 6.

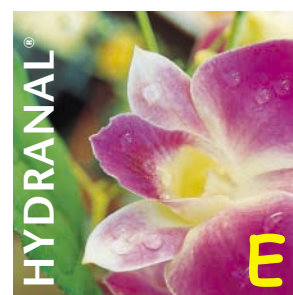


Table 6  
Product range of **HYDRANAL®-E** types

Cat. No.	Product	Description	Pack Size
34734	HYDRANAL®-CompoSolver E	Methanol free working medium for volumetric one-component titration	1 l; 2.5 l
34732	HYDRANAL®-Titrant 5 E	Methanol free titrant to use with HYDRANAL®-Solvent E or HYDRANAL®-Solvent, titer: 1 ml = 5.00 ± 0.02 mg water	500 ml; 1 l; 2.5 l
34723	HYDRANAL®-Titrant 2 E	Methanol free titrant to use with HYDRANAL®-Solvent E or HYDRANAL®-Solvent, titer: 1 ml = 2.00 ± 0.02 mg water	1 l
34730	HYDRANAL®-Solvent E	Methanol free solvent to use with HYDRANAL®-Titrant E or HYDRANAL®-Titrant	500 ml; 1 l; 2.5 l
34726	HYDRANAL®-Coulomat E	Ethanol based reagent to use as anolyte and catholyte	500 ml

### Technical Support

We'll be glad to provide you with support in the analysis of your sample based on our twenty years of experience with Karl Fischer titration. We can suggest a solution for your analytical problem and, if necessary, develop an individual analytical method. Our comprehensive application collection makes daily work easier for **HYDRANAL®** users, and is always at your disposal anytime via our web site [www.sigma-aldrich.com/analytical](http://www.sigma-aldrich.com/analytical).

Just call us!  
We'll be pleased to answer any questions on regarding sample analysis using the Karl Fischer method, and on **HYDRANAL®-reagents**.

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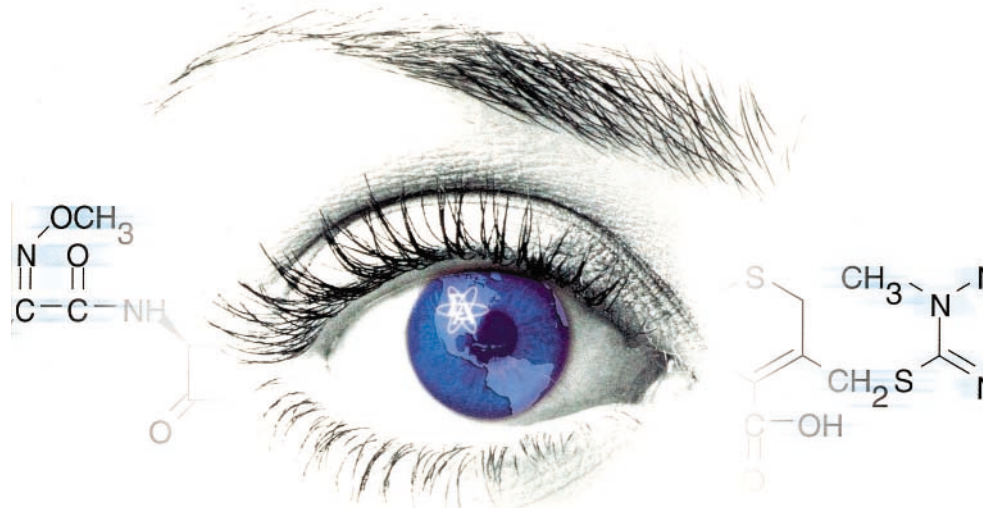
HYDRANAL®-E – non-toxic Karl Fischer Titration



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