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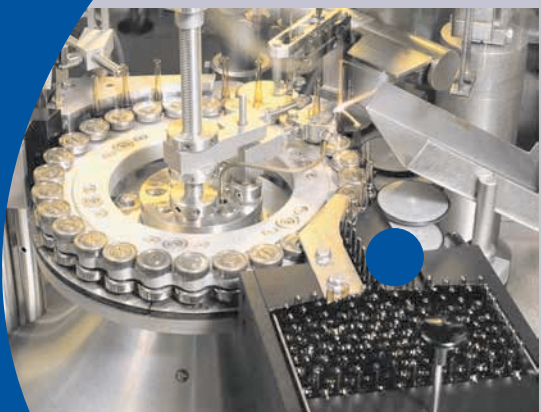
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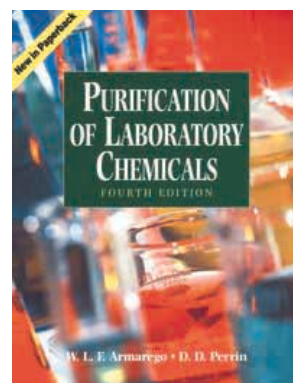
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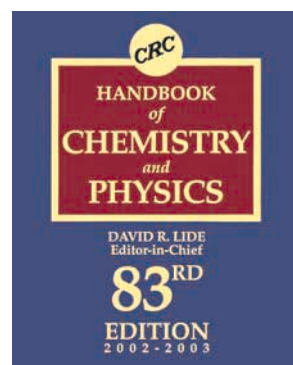
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Yours sincerely,

Gerhard Kudera
Analytical Team Europe

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Nitrofurans in Food

In an investigation that was performed early in 2002, it was discovered that residues of four nitrofuran antibiotics (furazolidone, furaltadone, nitrofurantoin and nitrofurazone) occurred in fishery products like shrimp and catfish originating from South-East Asian countries. Later, intensified efforts revealed that the same residues could also be found in poultry, duck and rabbit imported from this region.

These four drugs have been widely used in the form of food additives for the treatment of gastrointestinal infections. Because furazolidone and similar compounds are classified as mutagenic and genotoxic drugs, the EU prohibited the use of nitrofuran antibiotics. Therefore, the analytical methods have to reach the lowest possible detection limits.

The analysis of residues of nitrofuran drugs is based on the detection of the tissue-bound metabolites of the nitrofuran parent drugs. Since the parent drugs are very rapidly metabolized, they are not detectable shortly after treatment. The metabolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazone are respectively 3-Amino-2-oxazolidinone (AOZ), 5-Methylmorpholino-3-amino-2-oxazolidinone (AMOZ), 1-Aminohydantoin (AH) and semicarbazide (SC) (figure 1).

Screening Methods for Nitrofurans and their Metabolites

W. Leitner, et.al., [1] was the first who developed a LC/MS method for detection of nitrofuran metabolites. After a one-step extraction/derivatization procedure, further sample preparation was improved by solid phase extraction (SPE): The metabolites can be released from protein-bound residues under acidic conditions and subsequent derivatization with 2-nitrobenzaldehyde (NBA). Muscle tissue is homogenized in aqueous hydrochloric acid followed by derivatization with freshly prepared NBA in DMSO. The derivatiza-



tion step serves to isolate the metabolites from the matrix and prevent rebinding to protein following the cleavage step and to produce derivatives which possess chromophores suitable for UV detection. Since many cellular macromolecules also contain reactive amino moieties, a large excess of NBA is required to ensure quantitative derivatization of the metabolites released from the bound residues. The tissue extracts produced according to this procedure contained derivatized AMOZ, AOZ, SC and AH and unreacted NBA.

Tandem mass spectroscopy provides the highest degree of certainty in the analysis of the derivatized metabolites. Detection limits and quantification limits are determined by adding aliquots of blank tissue homogenate to standard solutions of AOZ, AMOZ, SC and AH. Using LC/MS/MS methods, the detection limits of the analytes range from 0.5 to 5 ng g⁻¹.

Most literature references on this subject stated that the metabolites AOZ and AMOZ are not commercially available. Due to this fact, numerous scientists contacted Sigma-Aldrich and requested analytical standards. We responded to the challenge and are now proud to offer all four nitrofuran standards (table 1, figure 2,3). For sample preparation and derivatization we recommend puriss. p.a grade and HPLC reagents: The puriss. p.a. grade guarantees the highest analytical quality. The extensive specifications exceed the American Chemical Society (ACS) or similar standard requirements and gives you complete peace of mind during use. HPLC solvents are produced under cleanroom conditions in laminar flow and by sophisticated distillation. The HPLC grades **CHROMASOLV**® are notable for high transmittance in the UV spectrum and a low level of non-volatile impurities (table 2). Riedel-de Haën has been developing solvents designed especially for LC/MS analysis (table 3): The new LC-MS **CHROMASOLV**® solvent Methanol guarantees a high UV-transmittance and low amount of alkali impurities (see also chapter «LC-MS solvents»).

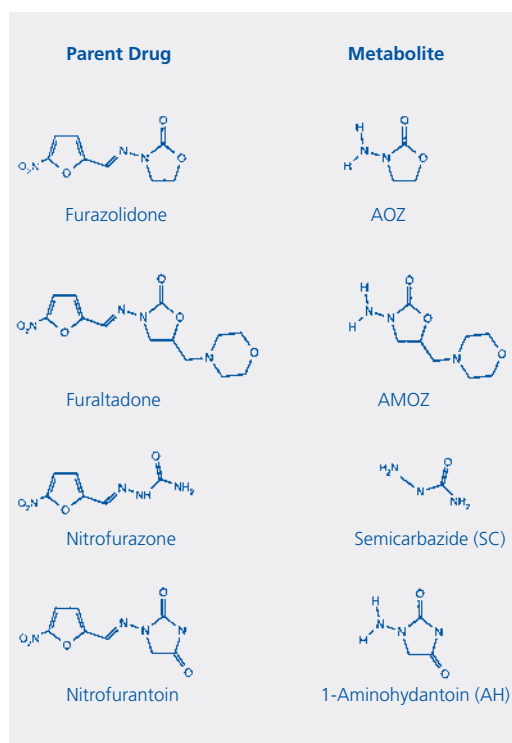
Water for HPLC is produced by Fluka and purified through various stages including UV-irradiation, microfiltration through a 0.2 µm membrane, reverse osmosis and ion-exchange separation, followed by a filling procedure under cleanroom conditions.

Ammonium acetate, HPLC grade, is tested for HPLC applications: solubility tests, filter tests

Custom specific standards?

Please contact
Mr. Rainer Walz, Ph. D.
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Figure 1:
Structures of the parent drugs
and their metabolites



(millipore® 0.45 µm membrane filter), defined maximum UV absorption value, defined pH in aqueous solution and a RP gradient test guarantee HPLC-suitability.

If you need more information about our product range for residue analysis or if you need customized standards, please feel free to contact us at:

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Literature References:

- [1] «Determination of the metabolites of nitrofurantoin antibiotics in animals tissues by HPLC-tandem mass spectrometry», Leitner, P. Zöllner, W. Lindner, J. Chromatogr. A, 939 (2001) 49–58.
- [2] «Determination of the furazolidone metabolite, 3-amino-2-oxazolidinone, in porcine tissues using liquid chromatography-thermospray mass spectrometry and the occurrence of residues in pigs produced in Northern Ireland», R.J MacCracken, D.G. Kennedy, J. Chromatogr. B, 691 (1997) 87–94.
- [3] «Evaluation of the residues of furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in eggs», R. J. MacCracken, D.E. Spence, S.D. Floyd, D.G. Kennedy, Food additives and Contaminants, 18 (2001) 954–959.

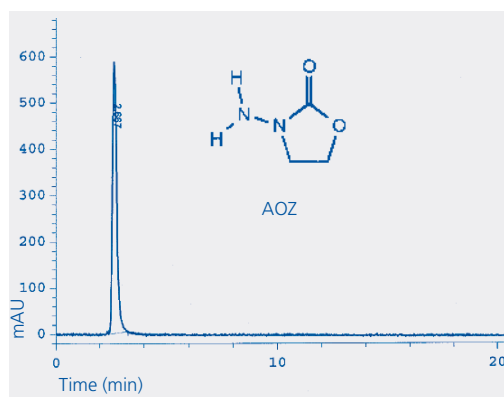


Figure 2: Chromatogram of AOZ
Column: Supelcosil LC8DB L = 250 mm, ID = 4.6 mm, 5 µm
Eluent: Acetonitrile 5%; Water + 0.2 g/l NaH₂PO₄ 95%
Flow Rate :1 ml/min
Detection: UV, 200 nm
Sample : 0.6 mg/ml eluent
Inj. : 10 µl

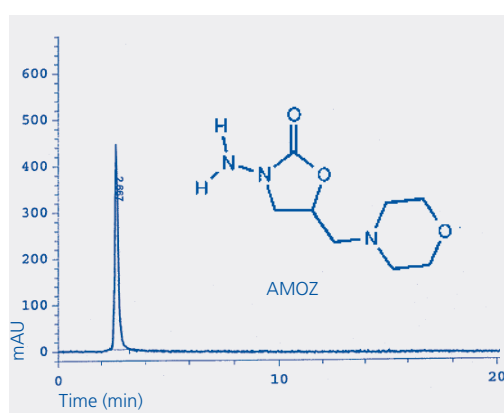


Figure 3: Chromatogram of AMOZ
Column: Supelcosil LC8DB L = 250 mm, ID = 4.6 mm, 5 µm
Eluent: Acetonitrile 20%; Water + 0.2 g/l NaH₂PO₄ 80%
Flow Rate :1 ml/min
Detection: UV, 200 nm
Sample : 0.6 mg/ml eluent
Inj. : 10 µl

Nitrofurantoin Standards

Cat. No.	Brand	Product	Synonym	Description	Pack Size
33347	RdH	3-Amino-2-oxazolidinone	AOZ	> 99.0% (HPLC), VETRANAL®	50 mg
33349	RdH	5-Methylmorpholino-3-amino-2-oxazolidone	AMOZ	> 99.0% (HPLC), VETRANAL®	50 mg
54,595-3	Aldrich	1-Aminohydantoin	AH	> 99.0% (T)	5 g, 25 g
84938	Fluka	Semicarbazide	SC	> 98.0% (AT)	25 g, 100 g, 500 g

Table 1

Reagents for sample preparation and derivatization

Cat. No.	Brand	Product	Description	Pack Size
30723	RdH	Hydrochloric acid	puriss. p.a., >25% (T)	1 l, 2.5 l, 5 l
72780	Fluka	2-Nitrobenzaldehyde	puriss. p.a., >98.5% (HPLC)	10 g, 50 g
34869	RdH	Dimethylsulfoxide CHROMASOLV®	> 99.7% (GC), for HPLC	1 l, 2.5 l
60349	Fluka	Dipotassium hydrogen phosphate	puriss. p.a., >99.0% (T)	50 g, 250 g
06203	RdH	Sodium hydroxide	puriss., > 98.0%, Reag. Ph. Eur.	1 kg, 5 kg
72800	Fluka	4-Nitrobenzaldehyde	puriss. p.a., >99.0% (HPLC)	10 g, 50 g
34858	RdH	Ethyl acetate CHROMASOLV®	>99.7% (GC), for HPLC	1 l, 2.5 l

Table 2

Reagents for LC and LC-MS analysis

Cat. No.	Brand	Product	Description	Pack Size
34966	RdH	Methanol CHROMASOLV® for LC/MS	> 99.9% (HPLC), for LC/MS	1 l, 2.5 l
95270	Fluka	Water, for HPLC	Prepared under cleanroom condition, filtered through a 0.2 µm membrane	1 l
17836	Fluka	Ammonium acetate	> 99.0% (NT), for HPLC,	50 g, 250 g

Table 3

Speciality Solvents

LC-MS CHROMASOLV® from Riedel-de Haën: Solvents for LC-MS Analysis

Over the last decade, LC-MS systems have improved significantly. The combination of classical Liquid Chromatography (LC) with a Mass Spectrometer (MS) has led to an increase in analytical applications. Today LC-MS is an important technique for identification and characterization of metabolites, proteins/peptides and oligonucleotides. LC-MS is becoming the problem solving tool in virtually every field of chemical analysis; particularly for problems in pharmaceutical, environmental, and biotechnological analyses. The well established LC-MS techniques of Atmospheric Pressure Photoionization (APPI) and API-Electrospray require a new generation of solvents whose specifications are adjusted to the special needs of LC-MS.

The new Riedel-de Haën **LC-MS CHROMASOLV®** solvents are developed for tailor-made applications with LC-MS equipment today. This means Riedel-de Haën **LC-MS CHROMASOLV®** solvents guarantee a high UV-transmittance and low amount of alkali impurities (table 1). The high qualities of Acetonitrile, Methanol and 2-Propanol are shown by the mass spectra of the pure solvents in figure 2. As reference compound reserpine (Fluka Cat. No. 83580, structure see figure 3) was used for 1000 µl in following composition:

Composition of Reserpine solution

Solvent*	Methanol	Water	Acetic acid	Reserpine
900 µl	80 µl	20 µl	0.5 µl	1 µg

*Solvent = Acetonitrile (RdH Cat. No. 34967); resp. Methanol (RdH Cat. No. 34966); resp. 2-Propanol (RdH Cat. No. 34965)



Figure 1: Dispensing aid adapter (RdH Cat. No. 79212)

Figure 2: Mass spectra of Acetonitrile, Methanol and 2-Propanol

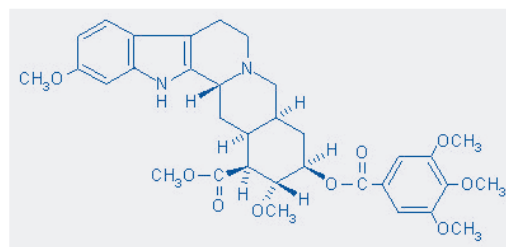
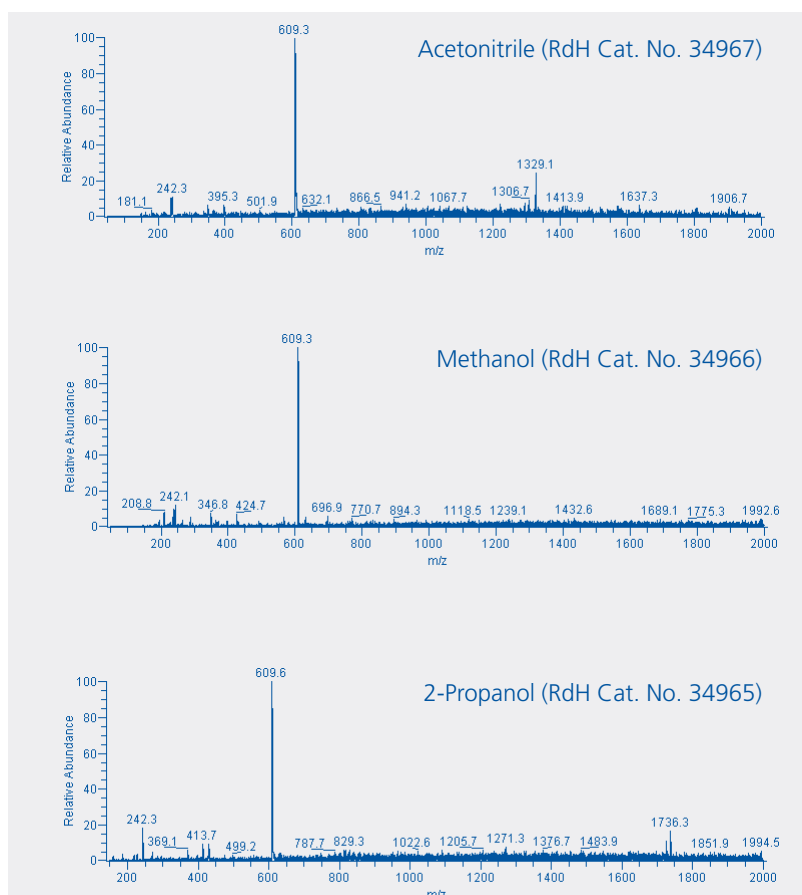


Figure 3: Structure image of Reserpine (Fluka Cat. No. 83580)

Dispensing Aid to avoid Contamination by Impurities

In daily work the analyst often is confronted with the risk of contamination. Degassed solvents are necessary for LC-MS operation. To keep solvents free of gases or other contaminations by contact with the atmosphere, degassing can be achieved by sonification or sparging with an inert gas (Helium, Argon) and storing the solvents under coverage all times.

Riedel-de Haën **LC-MS CHROMASOLV®** solvents are degassed during production and are free of impurities. To maintain this state we offer a practical dispensing aid (RdH Cat. No. 79212) from Riedel-de Haën. It is an PTFE adaptor which must be unscrewed on the bottle. There are 4 pins to attach the connecting tube. So, any impurities can be avoided (figure 1, 4).



Figure 4: Dispensing aid in daily work

Additives for LC-MS Applications

For an optimal separation, common solvents as described above (Acetonitrile RdH Cat. No. 34967, Ethyl acetate RdH Cat. No. 34972, Methanol RdH Cat. No. 34966, 2-Propanol RdH Cat. No. 34965 or Water: Fluka Cat. No. 95270 and RdH Cat. No. 34877) are combined with additives to create gradients and change the pH settings. Commonly used additives are listed in table 2.

Acetic and formic acid are used to reduce the pH of the mobile phase and to enhance the LC separation. Formic acid is more acidic and less is required to reach a desired pH. Normally around 1% of acid is sufficient to adjust the pH of the mobile phase. The addition of organic acids is a possible source of error in negative ion analysis: it can suppress ionization because weakly acidic compounds will not form deprotonated ions in acidic conditions.

If the separating species requires an increase in the pH of the mobile phase, ammonium hydroxide/ammonia solution is suitable. For enhancing the sensitivity for separation of weakly acidic compounds in negative ion mode it is useful to ensure that the mobile phase is acid free. Ammonium acetate is often used to replace phosphate buffers due to their interfering effect in LC-MS systems. It buffers the mobile phase

and improves the separation without changing the MS performance. The optimum is reached by using concentration below 0.1 M.

Trifluoroacetic acid (TFA) is commonly used in the analysis of peptides and proteins. But in concentrations higher than 0.1% the sensitivity in positive ion mode is decreased; in negative ion mode ionisation is completely disabled.

Triethylamine (TEA) is not commonly used. But it gives good ionization of other compounds in negative ion analyses. A disadvantage is the suppression of the ionization of some compounds in positive ion mode.

LC-NMR CHROMASOLV® from Riedel-de Haën: Acetonitrile for LC-NMR Analysis

Although an efficient separation method is available by HPLC, no structure determination is possible when detecting separated compounds by UV-detector. Thus NMR spectroscopy is a better universal detection method. So a combination of both analysis methods was necessary and LC-NMR was born.

Solvents that meet the high requirements of HPLC and proton NMR are needed. But the high throughput of solvents in HPLC excludes the use of expensive deuterated solvents usually required for NMR.

The answer is Acetonitrile LC-NMR **CHROMASOLV®** Cat. No. 34955 from Riedel-de Haën. HPLC Acetonitrile is often contaminated with impurities containing protons such as propionitrile which is not a problem in UV-detection, but is in NMR. Therefore a dramatic decrease of proton-containing contaminants for LC-NMR solvents is essential.

Acetonitrile LC-NMR **CHROMASOLV®** from Riedel-de Haën has been developed to obtain the superior specifications required for LC-NMR.

There are no other signals in ¹H-NMR that exceed the size of the methyl signals (more than 5% at 3.78 ppm NMR of 0.0006% of trimethoxybenzene) found under typical LC-NMR conditions.

LC-NMR Acetonitrile specification

LC-MS solvent	Acetonitrile
RdH Cat. No.	34955
Pack Size	500 ml, 1 l
Assay (GC)	min. 99.9%
Non-volatile matter	max. 0.0002%
Water (Karl Fischer)	max. 0.01%
Free alkali (as NH ₃)	max. 0.0001%
Free acid (as CH ₃ COOH)	max. 0.001%
Propionitrile (GC)	max. 0.0002%

If you need detailed information about these products for LC-MS Analysis or a pricelist in your local currency, please feel free to contact us at:

Frederik Pillong, Ph.D.
Productmanager Fluka/Riedel-de Haën
Fax: 0041/81/755-2824
email:fpillong@eurnotes.sial.com

LC-MS solvents and specifications

LC-MS solvent	Acetonitrile	Ethyl acetate	Methanol	2-Propanol
RdH Cat. No.	34967	34972	34966	34965
Pack Size	1 l, 2.5 l	1 l, 2.5 l	1 l, 2.5 l	1 l, 2.5 l
Assay (GC)	min. 99.9%	min. 99.7%	min. 99.9%	min. 99.8%
Non-volatile matter	max. 0.0005%	max. 0.0005%	max. 0.0005%	max. 0.0005%
Water (Karl Fischer)	max. 0.03%	max. 0.03%	max. 0.03%	max. 0.05%
Free alkali (as NH ₃)	max. 0.0002%	max. 0.0005%	max. 0.0005%	max. 0.0005%
Calcium (Ca)	max. 0.00001%	max. 0.00001%	max. 0.00001%	max. 0.00001%
Potassium (K)	max. 0.00001%	max. 0.00001%	max. 0.00001%	max. 0.00001%
Magnesium (Mg)	max. 0.00001%	max. 0.00001%	max. 0.00001%	max. 0.00001%
Sodium (Na)	max. 0.00001%	max. 0.00001%	max. 0.00001%	max. 0.00001%
Transmittance at 200 nm	min. 50%	–	–	–
Transmittance at 210 nm	–	–	min. 30%	–
Transmittance at 220 nm	min. 90%	–	min. 50%	min. 50%
Transmittance at 240 nm	min. 99%	–	–	–
Transmittance at 260 nm	–	min. 50%	min. 98%	min. 98%
Transmittance at 300 nm	–	min. 98%	–	–

Table 1

Additives for LC-MS applications

Cat. No.	Brand	Product	Description	Pack Size
45727	Fluka	Acetic acid	Trace Select, for trace analysis ≥ 99.0% (T)	100 ml, 500 ml
33015	RdH	Formic acid	puriss p.a., ≥ 98%	500 ml, 1 l, 2.5 l
09676	Fluka	Formic acid solution	puriss p.a., for HPLC, 49–51% in water	100 ml, 500 ml
17837	Fluka	Ammonia solution	puriss p.a., for HPLC, ~10% in water	100 ml, 1 l
09830	Fluka	Ammonium bicarbonate	BioChemika Ultra, ≥ 99.5%	100 g, 500 g, 1 kg
17836	Fluka	Ammonium acetate	puriss p.a., for HPLC, ≥ 99.0% (NT)	50 g, 250 g
17843	Fluka	Ammonium formate	puriss p.a., for HPLC, ≥ 99.0% (NT)	50 g, 250 g
91707	Fluka	Trifluoroacetic acid (TFA)	puriss p.a., for HPLC, ≥ 99.0% (NT), 1 ml ampoules	10 x 1 ml
17924	Fluka	Triethylamine (TEA)	puriss p.a., for HPLC, ≥ 99.5% (NT)	10 x 2 ml

Table 2

Certified Reference Materials by IRMM/BCR®

In Europe, the Bureau Communautaire de Référence (BCR®), a department of the European Commission, publishes guidelines for the production and certification of reference materials. Since 1995 the Institute of Reference Materials and Measurements (IRMM) at Geel, Belgium has shared the responsibility for the production and certification of BCR® certified reference materials. In April 2000, Fluka became an authorized distributor of IRMM. As a consequence, our customers can now receive more than 500 Certified Reference Materials (CRM) via the Sigma-Aldrich sales organization worldwide. The whole program contains more than 500 standards belonging to various segments such as Environment, Food and Agriculture, Industrial Raw materials, Occupational Hygiene, Physical Properties, Reactor Neutron Dosimetry and Clinical Chemistry. This article is dedicated to certified reference materials used in the clinical chemistry.



Standards for the Clinical Chemistry

IRMM has developed unique high technology equipment and expertise for the efficient production of a large variety of highest quality biological reference materials. The Reference Materials laboratory is one of the largest and most sophisticated in the world and hosts a unique multi-func-

Table 1

Enzyme reference materials*				
Cat. No	Brand	Product	Catalytic Concentration	
			U/l	μ .kat/l
BCR-299	Fluka	Creatine kinase BB partially purified, from human placenta	325 \pm 10	5.42 \pm 0.17
BCR-319	Fluka	γ -Glutamyltransferase partially purified, from pig kidney	86.8 \pm 2.1	1.447 \pm 0.035
BCR-371	Fluka	Alkaline phosphatase partially purified, from pig kidney	254 \pm 6	4.23 \pm 0.10
BCR-404	Fluka	Human lactate dehydrogenase isoenzyme 1	642 \pm 26	10.7 \pm 0.5
BCR-410	Fluka	Prostatic acid phosphatase highly purified, from human prostate	28 \pm 0.7	0.466 \pm 0.012
BCR-426	Fluka	Alanine aminotransferase partially purified, from pig heart	129 \pm 4	2.14 \pm 0.05
BCR-476	Fluka	Pancreatic α -amylase (EC 3.2.1.1), from human pancreas	555 \pm 10	9.25 \pm 0.17
BCR-608	Fluka	Creatine kinase CK-MB from human heart	67.2 \pm 1.8	1.12 \pm 0.03
IRMM/IFCC-452	Fluka	γ -Glutamyltransferase partially purified, from pig kidney	114.1 \pm 2.4	1.90 \pm 0.04
IRMM/IFCC-453	Fluka	Human lactate dehydrogenase isoenzyme 1	502 \pm 7	8.37 \pm 0.12
IRMM/IFCC-454	Fluka	Alanine aminotransferase partially purified, from pig heart	186 \pm 4	3.09 \pm 0.07
IRMM/IFCC-455	Fluka	Creatine kinase CK-MB from human heart	101 \pm 4	1.68 \pm 0.07
IRMM/IFCC-456	Fluka	α -Amylase	546 \pm 19	9.1 \pm 0.3

*Description: Sealed glass ampoules of lyophilized material equivalent to about 1 ml of a solution of enzyme stabilized by incorporation in serum albumin matrix of human (BCR-404, BCR-410 and IRMM-453) or bovine (BCR-299, BCR-319, BCR-371, BCR-426, IRMM-452 and IRMM-454) origin kept under dry nitrogen. BCR-476, BCR-608, IRMM-455 and IRMM-456 are provided in sealed ampoules or vials filled with dry nitrogen. Samples are in lyophilized form and equivalent to about 1 ml of a solution of purified enzyme.

Table 2

Certified Thromboplastins				
Cat. No.	Brand	Product	Parameters of the calibration line	Pack size
BCR-148	Fluka	Lyophilized thromboplastin Bovine (OBT/79)	Slope 1.011 \pm 0.015 Intercept - 0.321 \pm 0.025	lyophilized bovine brain thromboplastin equivalent to about 2.2 g bovine brain tissue extract, kept under vacuum in sealed glass ampoules.
BCR-149S	Fluka	Lyophilized rabbit thromboplastin	Slope 1.257 \pm 0.013 Intercept - 0.242 \pm 0.019	sealed in glass ampoules containing the lyophilized form of a 0.5 ml aliquot of the extract of rabbit brain tissue, without calcium ion added.

Table 3

Blood Cell Size Reference Material			
Cat. No	Brand	Product	Parameters of the calibration line
BCR-165	Fluka	Nominal 2 μ m latex (0.02% solids)	2.223 \pm 0.013
BCR-319	Fluka	Nominal 4.8 μ m latex (0.2% solids)	4.821 \pm 0.019
BCR-371	Fluka	Nominal 9.6 μ m latex (1.4% solids)	9.475 \pm 0.018

tional and flexible production laboratory, clean chambers, cryo-grinding, freeze drying, high purity milling, ultrafine classification and levitation melting. This laboratory can quickly adapt its production facilities to respond to urgent requests that arise from changes in policy. Associated with this facility is a very modern analytical laboratory equipped with various spectroscopic methods, neutron activation analysis and gas and liquid chromatography (various PCR and immunochemical techniques are being added) used for in-process control and certification analyses.

Enzymes

Enzyme preparations were produced to help standardize the results of measurements of enzyme catalytic concentrations in serum. For most of the preparations the catalytic concentration of the enzyme is determined according to the method recommended by the International Federation of Clinical Chemistry (IFCC) and is certified. Each CRM is intended to ensure the transferability of the IFCC method and to possibly establish correlations between the results obtained by a particular method and the IFCC method. Table 1 gives an overview of all enzyme reference materials. For the IRMM/IFCC products, detailed Standard Operating Procedures (SOP's) for the measurement of catalytic concentration of enzymes are provided (per example see figure 1, charts for the adjustment and control of pH values of IRMM/IFCC-453, Human lactate dehydrogenase isoenzyme 1.)

Thromboplastins

Two thromboplastin reference materials were calibrated against International Reference Preparations of WHO (thromboplastin human IRP 67/40 and thromboplastin rabbit, plainRBT/90 respectively) which were used as Primary Standard. These certified reference materials can

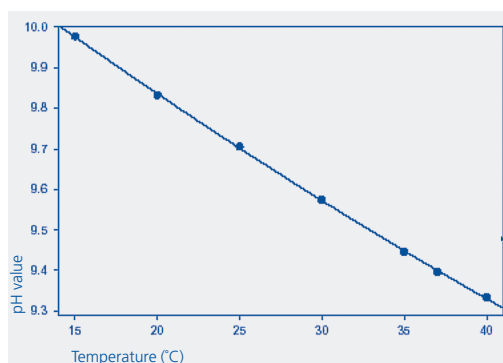


Figure 1: Adjustment and control of pH values of IRMM/IFCC-453, procedure for the adjustment of pH values at temperatures diverging from 37°C.

serve to calibrate any batch of thromboplastin used for the determination of the coagulation time of blood plasma (table 2).

Blood Cell Size Reference Material (Latex Spheres of certified Size)

These CRMs can be used for the calibration of many instruments for particle size measurements and, in particular, for blood cell sizing instruments.

The sizes of the spheres have been selected to correspond approximately to the volume of platelets (thrombocytes), red blood cells (erythrocytes) and white blood cells (leukocytes) respectively. In hematological applications, precautions must be taken, as red blood cells are not rigid spheres and cannot therefore be directly compared to latex spheres. The spheres are suspended in an aqueous solution of stabilizers. In each sample the spheres have a very narrow distribution (99% of the spheres within $\pm 2\%$ of the certified diameter, see table 3). The user should first establish the correlation between the red cell volume determined by the standard manual method [1, 2] and his automated sizing instrument.

Literature References

- [1] J. Clin. Path. 33 (1980) 1.
- [2] Clin. Lab. Haemat. 5 (1983) 83.

Fluka – Your supplier for BCR® Reference Materials



If you browse the Fluka-Website 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 CD «Standards and Derivatization Reagents»-free of charge. Just fax the order form inserted in this newsletter to your local Sigma-Aldrich partner listed on page 15!



«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.»

Table 4

Cortisol reference panel of fresh frozen human sera IRMM/IFCC-451*		
Serum No.	Certified value [nmol/l]	Uncertainty [nmol/l]
1	361	14
2	432	17
3	288	11
4	152	6
5	329	13
6	278	11
7	515	20
8	163	7
9	287	11
10	230	9
11	334	13
12	261	10
13	430	17
14	626	24
15	246	10
16	211	8
17	366	14
18	146	6
19	166	7
20	83	4
21	89	4
22	180	7
23	387	15
24	384	15
25	315	12
26	215	9
27	497	19
28	299	12
29	265	11
30	114	5
31	764	29
32	623	24
33	264	10
34	390	15

* panel of 34 × 1 ml serum in screw capped cryo-vials

Hormones

Cortisol in Human Serum

The lyophilized materials can be used to control and optimize the performance of cortisol assays.

CRM's of Cortisol in Human Serum*

Cortisol concentration in the reconstituted material			
Cat. No.	Brand	($\mu\text{g/l}$)	(mol/l)
BCR-192	Fluka	98.8 ± 2.0	273 ± 6
BCR-193	Fluka	277 ± 5	763 ± 14

* lyophilized material of a 1.25 ml portion of serum kept under nitrogen in sealed glass ampoules

Cortisol Reference Panel of fresh frozen Human Sera IRMM/IFCC-451

The panel is intended to perform method comparison of a routine test system for cortisol in serum with the ID-GC/MS method. This panel should serve the purpose of evaluation/verification of the metrological correctness of values obtained by routine test systems. Besides that, the panel is useful for the manufacturer during test development (e.g., antibody screening) or it can be used to evaluate the suitability of using the method comparison as a basis for recalibration of a tested system that showed sufficient sensitivity and specificity, but incorrect calibration (table 4).

Please feel free to ask for complete product lists of certified reference materials. Order our new CD «Standards and Derivatization Reagents» and visit us under www.sigma-aldrich.com/fluka.

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Thousands of Information-packed Pages:

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2002 Sigma-Aldrich Prize of the Analytical Chemistry Division

The 2002 Sigma-Aldrich prize of the Analytical Chemistry Division, with a value of 915 €, was awarded to **Sébastien Benoit**, for his poster communication during the 2002 SFC Environment Conference. Sébastien Benoit is a Ph.D. student in the Laboratory of Isotopic and Electrochemical Analysis of Metabolism directed by Nabil El Murr (UMR-CNRS 6006) at the University of Nantes. The research of the UMR 6006 is particularly concerned with the following themes:

- The development and optimization of precise and reproducible methods for the quantification of a NMR signal
- An understanding of the origins of the isotopic content of natural products
- The development and optimization of precise, specific and reproducible electrochemical methods of quantification applicable in the food and environmental safety sectors

The poster presented by Sébastien Benoit, and his co-authors Christine Thobie-Gautier, Françoise Lantier and Nabil El Murr, was entitled «A Comparison of the Interactions of a Humic Acid with Different Cations: An Electrochemical Study of the Competitive Complexation». This work forms part of the program of the Electrochemical Methodology Group (EMG) which studies the mechanisms of electron transfer involving at least one biomolecule, a natural or synthetic macromolecule or a microorganism. The applications of this research are directed towards the preparation of new captors or biocaptors, of use in a real context for the analysis of complex samples found in the sectors of food processing, biology and the environment.

The aim of the work presented at the 2002 SFC Environment Conference was the development and optimization of carbon paste electrodes whose mass had been modified by natural macromolecules of the complex-forming humic acid type (EPC-AH). These modified electrodes represent a model close to the natural conditions found at the ground/solution interface. They constitute an interesting tool for examining, by an electrochemical method, the interactions of humic acid, in its solid form, with heavy metal cations in solution. The influence on the electrochemical behaviour of three metallic cations (Cu^{2+} , Pb^{2+} and Cd^{2+}) of different experimental parameters, like pH, the nature of the electrolyte or the accumulation time, were presented. A comparative study of the voltamperometric curves recorded after a complexation stage, in the presence or absence of other metallic salts, showed the existence of competitive complexation reactions of interfering cations in solution. The complexation power of humic acid in relation to the different cations studied followed the decreasing order: $\text{Na}^+ < \text{Mg}^{2+} < \text{Ca}^{2+} < \text{Cd}^{2+} < \text{Cu}^{2+} < \text{Pb}^{2+}$. These results were obtained by the electrochemical determination of the complexation constants.

The application of this work is envisaged for the preparation of amperometric captors, useful for the study of the contamination of water by heavy metals.

Mme Christine Dumas, representing the Sigma-Aldrich company, presented the prize to Monsieur Benoit at the close of the 2002 SFC Environment Conference organized by the Analytical Chemistry Division in November 2002 at the CNAM in Paris.



Determination of Microorganisms with Color

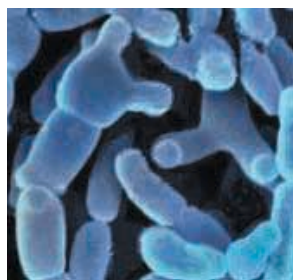


Figure 1:
Bifidobacteria

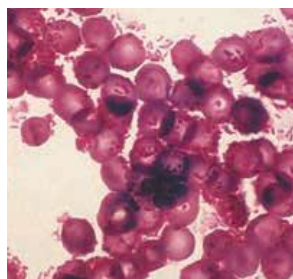


Figure 2:
Gram positive Citrobacter

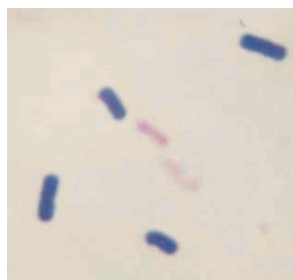


Figure 3:
Gram negative Bacilli

Microscopy in Microbiology

It is possible using microscopy to detect and identify microorganisms and to analyze samples. But in many cases, other methods are required to confirm the results. Modern classification schemes emphasize microscopic morphological features that are stable and exhibit minimal variation (figure 1). Often the morphology of the colony gives first information and in the next step an unstained microscope smear is recommended. Then a stained smear follows, which gives contrast to the microorganism. There are simple stains using a single dye and many other stains that use combinations of dyes. The more complex mixtures make it possible to differentiate different cell components or types of cells. Therefore Fluka provides a broad range of dyes for producing special dyes for every application. The main Fluka catalog also contains some ready-to-use staining solutions (table 2) for the presumptive identification of bacteria and fungi. For microbiologists the most important stain was developed in 1884 by the Danish bacteriologist Hans Christian Gram. Gram Stain allows the morphology to be determined and it also divides bacteria into two large groups. Bacteria that are stained purple are called «Gram Positive». Those that stain pink are called «Gram Negative». This dye provides information about cell wall structure (content of peptidoglycan and lipid) and how it might respond to some antibiotics. Gram Stain is also important for identification of bacteria, and forms the basis for the selection of biochemical tests, (table 1, figure 2, figure 3).

Table 1

Fluka Cat. No.	Product
77730	Gram Staining Kit (figure 4)
94448	Gram's Crystal Violet Solution
90107	Gram's Iodine Solution
75482	Gram's Decolorizer Solution
94635	Gram's Safranin Solution

Table 2

Staining solution for studying bacteria		
Fluka Cat. No.	Product	Description
03981	Bismarck brown solution	Bismarck brown is a metachromatic dye which stains acid mucins, nuclei, and cellulose and inclusion bodies in the cytoplasm. It is generally used as a counterstain, also in the Gram coloration. With methyl violet and crystal violet it is possible to dye metachromatic granules of diphtheria organisms. There is also a method for staining the tubercle bacillus, [1–2].
21819	Carbol-Fuchsin solution according to Kinyoun	Stains used for the demonstration of acid-resistant rod-shaped bacteria, e.g., mycobacteria, especially tuberculosis bacteria.
21820	Carbol-Fuchsin solution according to Ziehl-Neelsen	The acid resistance of certain bacteria is caused by waxy cell walls containing mycolic acid. Carbol fuchsin binds to mycolic acid. Counter-staining with Methylene blue (Fluka Cat. No. 66720) is possible.
45242	Eosin yellowish solution	A simple stain for bacteria. Dyes alkaline cell parts (like cytoplasm) red to pink. It is also often used as a counterstain, e.g., in Gram staining.
Staining solution for studying fungi		
61335	Lactophenol blue solution	The identification of moulds is based on the shape, method of production, and arrangements of spores (conidial ontogeny). Lactophenol Blue Solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi, [3–7].



Figure 4: Gram Staining Kit

Simple Biochemical Color Tests in Microbiology

Often a rapid, simple, low cost method is used for confirming or determining the bacteria in water or food. One type of method is to use chromogenic and fluorescent substrates in combination with selective media (see bioAnalytiX 2/2002 at www.sigma-aldrich.com/analytix or order for free our Microbiology CD). Another method is to use biochemical test reagents. All these biochemical tests are based on the selective detection of characteristic enzyme activity for the different microorganisms. The targets are to differentiate pathogens, indication of specific problems and desired organism present at a certain level. The Fluka catalog contains some of the test reagents often used in microbiology (table 3).

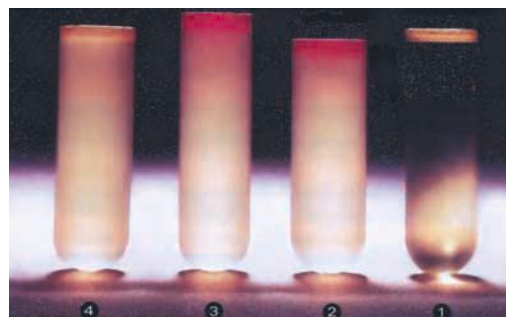


Figure 5: Kovac's reagent, detection of tryptophanase activity: 1. Control; 2. E. coli; 3. Proteus vulgaris (indole positive); 4. Enterobacter aerogenes (indole negative)

Biochemical Color Test

Fluka Cat. No.	Product	Description
11945	Benedict's reagent	Reagent used for the detection of reducing sugars and other reducing substances in urine. The detection limit is 0.01% glucose in water. Sugar reduces cupric ions from the reagent which results in a white colored cuprous thiocyanate, [8].
49825	DMACA reagent	Fast and easy indole spot test for the detection of tryptophanase activity. Tryptophanase cleaves tryptophan to indole and α -aminopropionic acid. Dimethylaminocinnamaldehyde in the reagent reacts with indole to form a blue-purple complex, [9–10].
03891	Ehrlich's solution	For determination of urobilinogen concentration in urine. A cherry red color indicates increased amount of urobilinogen.
60983	Kovac's reagent	In the presence of oxygen, some bacteria with tryptophanase activity, like <i>E. coli</i> , are able to split tryptophan into indole and α -Aminopropionic acid. The indole is detected by a red complex formed with 4-(dimethylamino)benzaldehyde (figure 5). Fluka Cat. No. 67309 contains Isoamyl alcohol and Fluka Cat. No. 60983 n-Butanol as solvent, they are more stable than amyl alcohol.
67309	Kovac's reagent	
72190	Nessler's reagent	Reagent for the determination of bacteria reducing urea with the enzyme urease for detecting the production of ammonia. Also possible to detect arginine dehydrolase by using L-arginine as substrate. The color changes in the presence of ammonia from yellow to dark brown.
80353	TDA reagent	A spot test for identification of Proteus species by detection of tryptophan deaminase activity. Due to the formation of ammonia from the deamination of tryptophan, a brown complex is formed with ferric ions. Can directly be dropped on colonies on the HiCrome UTI Agar, modified (Fluka Cat. No. 16636) or Christensen's Urea Agar (Fluka Cat. No. 27048).
89609	o-Toluidine reagent	For colorimetric estimation of glucose by formation of a green complex between glucose and o-toluidine in glacial acetic acid.

Table 3

References:

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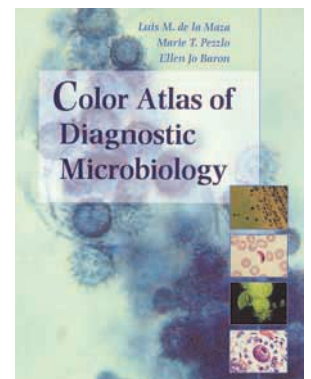
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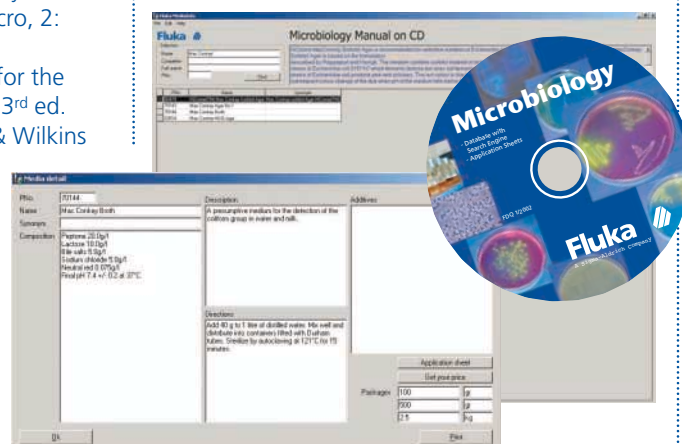
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Coulometric Water Determination according to Karl Fischer

Coulometric titration is based on the electrochemical production of iodine, in comparison to volumetric titration that is based on the addition of a certain volume of a reagent. For this type of water determination it is necessary that the performance of the reagent ensures the correct equation postulated by Eugen Scholz and the formulation has to ensure that no side products will be formed and the current gain is 100% (no possible source of error!). The coulometric titration of water is a titration technique, by which the iodine required for the KF reaction is produced by anodic oxidation of iodide. The current and time required for the oxidation is measured and the instrument converts the electric charge (coulombs) immediately into the amount of water in the sample.



The equation postulated by Eugen Scholz:



The sulfur dioxide reacts with the alcohol and forms an alkyl sulfuric acid which is neutralized by a suitable base. The alkyl sulfurous acid is the reactive component and is already present in the coulometric reagent. The titration of water constitutes the oxidation of the alkyl sulfite anion to alkyl sulfate by the iodine. This reaction consumes water.

The coulometric water determination is a cumulative method with the advantage that many samples of a low water content can be determined in the same reagent with a very high accuracy. The volumetric titration in comparison is limited in this respect.

Applications

HYDRANAL®-Coulomat A and **HYDRANAL®-Coulomat C** were the first reagents for coulometric Karl Fischer titration. They contain halogenated solvents.

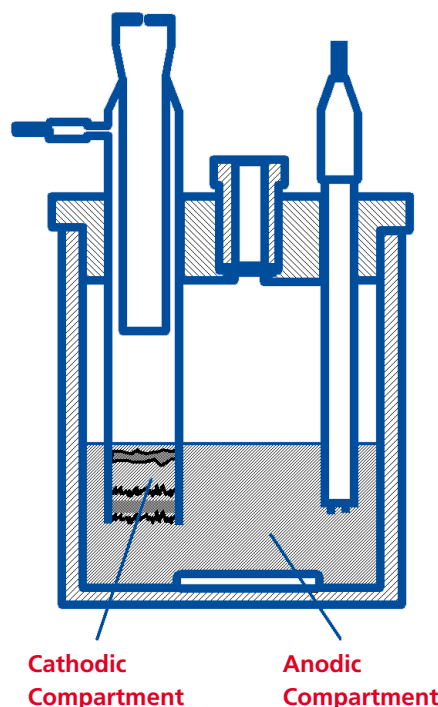
HYDRANAL®-Coulomat AG and **AG-H** are two analytes free of halogenated solvents, **HYDRANAL®-Coulomat CG** is the corresponding catholyte. **HYDRANAL®-Coulomat AD** is a reagent for the diaphragmless cell.

Figure 1:
HYDRANAL®-Coulomat
Reagents



HYDRANAL®-Coulomat E is a new reagent, methanol is replaced by ethanol, it is free of halogenated solvents and can be used in both types of cells.

We offer a comprehensive range of **HYDRANAL®** applications. For further information, please contact our website www.sigma-aldrich.com/hydranal.



Procedure for Coulometric Titration

- Fill the cathodic compartment with **HYDRANAL®-Coulomat CG** or **E**. No need for cells without diaphragm.
- Fill the anodic compartment with **HYDRANAL®-Coulomat E/A/AD/AG/AG-H**.
- Switch on the equipment which automatically titrates the cell to dryness.
- Push the analysis button
- Inject the sample
- Record the water content at the end of analysis.

HYDRANAL®-Coulomat E

HYDRANAL®-Coulomat E is a non-poisonous reagent for use in the coulometric determination of the Karl Fischer titration. It can be used in cells with or without diaphragms.

Conventional KF reagents for the coulometric water determination contain methanol as solvent. They are therefore poisonous and have to be designated by the skull and cross bones hazard symbol. To minimize risk in the work place we have developed KF-Reagents based on ethanol. HYDRANAL®-Coulomat E has been launched for the coulometric analysis like the HYDRANAL®-Coulomat E Types for the volumetric determination. These reagents no longer need the skull and cross bones hazard symbol on the label.

Use

HYDRANAL®-Coulomat E can be used as a one-component coulometric reagent.

In the coulometric cell with a diaphragm, approx. 5 ml of HYDRANAL®-Coulomat E is added to the cathode chamber and then the reagent filled to about the same level in the outer anode chamber. The instrument is switched on according to the instructions for use.

In the cell without a diaphragm, about 100 ml of HYDRANAL®-Coulomat E is poured into the cell and the instrument is switched on according to the instruction for use.

Use as Analyte

The Karl Fischer reaction takes place in the anode chamber. The iodide in the reagent is oxidized to iodine at the anode and this then reacts with the rest of the components of the reagent with the consumption of water. 100 ml HYDRANAL®-Coulomat E have a capacity of over 1000 mg water. Since in coulometry extremely small amounts of water are determined in mg quantities, the water capacity of the reagent is very rarely exhausted. More usually sample related influences require the reagent to be replaced. Typical examples of such influences are solubility limitations or side reactions caused by the sample that prevent an end-point being reached. A build-up of non-polar samples causes a lowering of the conductivity of the reagent and in such cases the instrument displays an error message.

Use as Catholyte

5 ml HYDRANAL®-Coulomat E has a capacity of 100 mg water when used as a catholyte. In order to achieve accurate results, the catholyte must be renewed at least once a week regardless of how much water has been analyzed. Side-reactions that can disrupt results are detected in catholytes that have been left unchanged for longer than this.

Maintaining Accuracy

In order to control the function of HYDRANAL®-Coulomat E and the instrument according to ISO 9000, HYDRANAL®-Water Standard 1.00 and HYDRANAL®-Water Standard 0.10 can be used.

34828 HYDRANAL®-Water Standard 1.00

Standard (1 g contains 1.00 mg water) for general coulometric KF titration. This standard is also traceable to NIST SRM 2890. Although coulometry is thought of as an «absolute» method, not all titrated values are necessarily correct. Air humidity, electrode poisoning, or sample matrix conditions can influence the results. It is therefore necessary to test the system with known water quantities and to check the recovery data. The validation of the instrument should typically be performed once the titration vessel has been charged with the appropriate reagents. To monitor for interferences caused by the sample matrix, a control test could be undertaken immediately after a sample is analyzed. In each case the theoretically added amount of water should correspond to the experimentally titrated amount of water.

34847 HYDRANAL®-Water Standard 0.10

Standards (1 g contains 0.10 mg water) for coulometric titration of small absolute amounts of water. This standard is also traceable to NIST SRM 2890.



Figure 2:
HYDRANAL®-Water Standards sealed glass ampoules under Argon. The Certificate of Analysis is included in the pack.

Technical Support

We will 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 to your analytical problem and, if necessary, develop an individual analytical method for you. Our comprehensive application collection makes daily work easier for HYDRANAL® users, and is always at your disposal anytime.

For additional information please contact:

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Fax: +1-314-771-5765
E-mail: dclark@notesgw.sial.com

Product Range for the Coulometric Titration

Cat. No.	Brand	Product	Description	Pack Size
34807	RdH	HYDRANAL®-Coulomat A	Anolyte for cells with diaphragm, reagent contains chloroform	500 ml
34810	RdH	HYDRANAL®-Coulomat AD	Anolyte for cells without diaphragm	500 ml
34829	RdH	HYDRANAL®-Coulomat AF-7	Anolyte suitable for coulometer AF 7	1 l
34836	RdH	HYDRANAL®-Coulomat AG	Anolyte for cells with and without diaphragm	500 ml, 1 l
34843	RdH	HYDRANAL®-Coulomat AG-H	Anolyte for titration of long-chained hydrocarbons (free of halogenated hydrocarbons)	500 ml
34739	RdH	HYDRANAL®-Coulomat AG-OVEN	Anolyte for determination with a KF oven	500 ml
34820	RdH	HYDRANAL®-Coulomat AK	Anolyte for titration of ketones	500 ml
34840	RdH	HYDRANAL®-Coulomat CG	Catholyte, free of halogenated hydrocarbons, 25 ml bottle, 50 ml contains 10 x 5 ml ampoules	25 ml, 10 x 5 ml
34821	RdH	HYDRANAL®-Coulomat CG-K	Catholyte for titration of ketones, contains 10 ampoules of 5 ml	10 x 5 ml
34868	RdH	HYDRANAL®-Coulomat Oil	Anolyte for titration of oils	100 ml, 500 ml
34726	RdH	HYDRANAL®-Coulomat E	Ethanol-based reagent to use as anolyte and catholyte	500 ml

To check the coulometry

Cat. No.	Brand	Product	Description	Pack Size
34847	RdH	HYDRANAL®-Water Standard 0.10	Standard for coulometric KF titration, 1 g contains 0.10 mg = 0.01% water at 20 °C, contains 10 glass ampoules of 4 ml, traceable to NIST SRM 2890	10 x 4 ml
34828	RdH	HYDRANAL®-Water Standard 1.00	Standard for coulometric KF titration, 1 g contains 1.00 mg = 0.10% water at 20 °C, contains 10 glass ampoules of 4 ml, traceable to NIST SRM 2890	10 x 4 ml
34748	RdH	HYDRANAL®-Water Standard KF-Oven	Solid standard for control of KF ovens, assay of water: 5.55 ± 0.05%	10 g

Important Product Information for Customers, Distributors and Dealers

The production of **34808 HYDRANAL®-Coulomat C** has been stopped.

HYDRANAL®-Coulomat C is used as catholyte for the coulometric Karl Fischer titration. One of the ingredients of the product is carbon tetrachloride. According to the latest environmental regulations the use of carbon tetrachloride has to be stopped soon. Therefore Sigma-Aldrich Laborchemikalien GmbH has decided to do that immediately.

A perfect and innovative replacement for **HYDRANAL®-Coulomat C** is **HYDRANAL®-Coulomat CG**, which does not contain halogenated solvent.

Advantages of 34840 HYDRANAL®-Coulomat CG

- **HYDRANAL®-Coulomat CG** is superior to other catholytes. Its active components are salts of suitable bases which produce only hydrogen during KF titration. These active species do not form by products which could migrate into the anodic compartment. Therefore the obtained results are more accurate than were previously possible.
- Many customers have used the product for up to 10 years and report very good results.
- Because of its superior performance and the high quality of the product we offer two packaging sizes:
25 ml bottle with septum, same as packaging size of **HYDRANAL®-Coulomat C**
50 ml box, packed with 10 ampoules of 5 ml each

The use of ampoules has the advantage that with each single ampoule the right portion is available for filling the cathodic compartment. In the other ampoules the catholyte is protected against moisture from outside.

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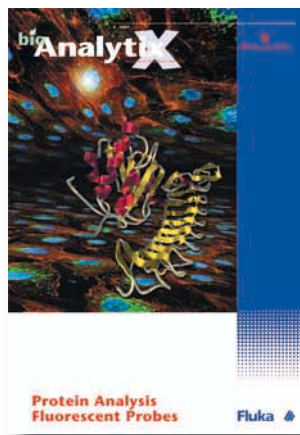
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