

# Analytix

Advances in Analytical Chemistry



**Special Topic: Microbiology**

**Food Analysis:  
Certified Standards & Acrylamide Kit**

**New generation of solvents:  
LC-MS CHROMASOLV®**

**HYDRANAL® Application:  
Pharmaceutical Industry**

[sigma-aldrich.com/analytix](http://sigma-aldrich.com/analytix)



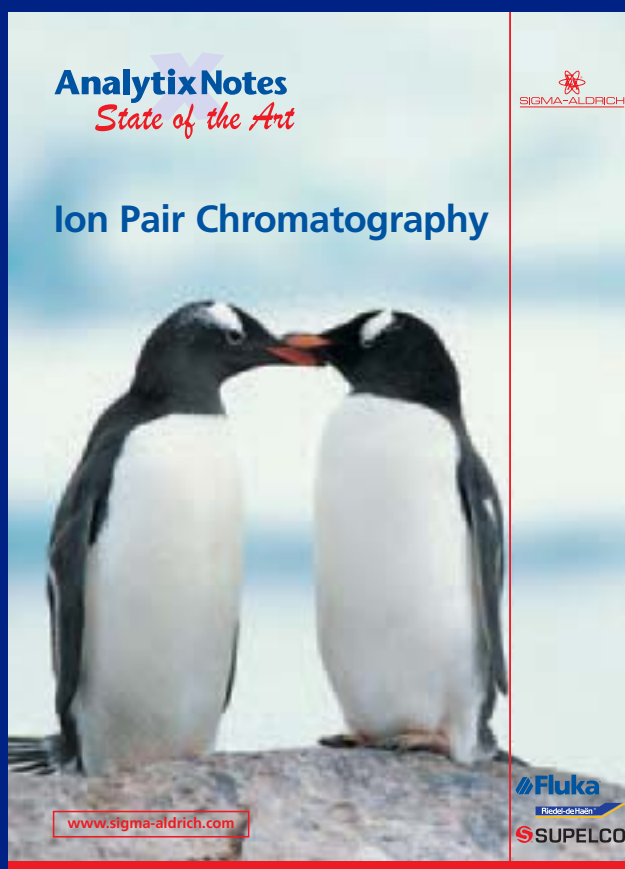
# New!

## AnalytiX Notes on Ion Pair Chromatography

State of the Art on IPC

Two Application Notes

Special Offer Inside (valid until 31.12.2003)



## Available now!

Make sure you'll get your copy

**Just fax**



the ordering form on the reverse side to your  
local Sigma-Aldrich Partner

Please, complete this form and fax it to your Local Service Partner listed on Page 19

### Literature Request – please send me the following items

- |   |   |
|---|---|
| <input type="checkbox"/> Sigma General Catalog (001)        | <input type="checkbox"/> Aldrich Catalog (002)                      |
| <input type="checkbox"/> Fluka Riedel-de Haën Catalog (003) | <input type="checkbox"/> <b>NEW!</b> Material Science Catalog (093) |
| <input type="checkbox"/> Supelco Catalog (013)              | <input type="checkbox"/> <b>NEW!</b> Equipment Catalog (086)        |
- Register for a free subscription of the whole series of the Analytix (NEWA)
- I am interested in buying online from [www.sigma-aldrich.com](http://www.sigma-aldrich.com), please send me further information (FUJ)

### Help us to help YOU – please complete this survey.

#### I use the following techniques:

- |   |  |
|---|--|
| <input type="checkbox"/> HPLC (HLLC, HILC)              | <input type="checkbox"/> X-Ray (XRAY)            |
| <input type="checkbox"/> GC (GASC, GACH)                | <input type="checkbox"/> MALDI-TOF (MALD)        |
| <input type="checkbox"/> Low Pressure LC/FPLC (FPLC)    | <input type="checkbox"/> Electro Spray MS (ESIM) |
| <input type="checkbox"/> Ion Pair Chromatography (IPCH) | <input type="checkbox"/> Quick MS (QTOF)         |
| <input type="checkbox"/> NMR (NMRS)                     | <input type="checkbox"/> Titration (TITR)        |
| <input type="checkbox"/> IR (IRSP)                      | <input type="checkbox"/> Microscopy (MCRO)       |
| <input type="checkbox"/> UV, FI and Lumin. (SPUV)       | <input type="checkbox"/> ICP (ICPS)              |
| <input type="checkbox"/> Raman (SPRA)                   | <input type="checkbox"/> Enzymatic Tests (ENZA)  |
| <input type="checkbox"/> AAS (AASP)                     | <input type="checkbox"/> LC-MS (LCMS)            |
| <input type="checkbox"/> X-Ray (XRAY)                   | <input type="checkbox"/> Others (please specify) |
| <input type="checkbox"/> High Throuput Screening (HTSC) |  |

#### My area of work is best described as:

- |  |  |
|--|--|
| <input type="checkbox"/> Water Analysis (ENVV, DRIN)       | <input type="checkbox"/> Drug Discovery (DRUG)   |
| <input type="checkbox"/> Analytical Chemistry (ANAL, ANLY) | <input type="checkbox"/> Material Science (MAMI) |
| <input type="checkbox"/> Food Analysis (FBSC)              | <input type="checkbox"/> Other (please specify)  |
| <input type="checkbox"/> Biopharmaceutical (BPRM)          |  |

#### Job Function:

- Managing a Lab (LABM)
- Purchasing Supplies (PURA)
- Developing Methods (MTHD)
- Quality Assurance (QAQC)
- Research and Development (RSCH)
- Other (please specify)

#### My organization is best described as:

- Government Research (GOVTR)
- Pharmaceutical (PHARM)
- University (UNIV)
- Biotechnology (BIOT)
- Hospital (HOSP)
- Other (please specify)

**Order now  
your own  
copy free of  
charge!**



- NEW!** AnalytiX Notes IPC (FZI)



- NEW!** Standards and Derivatization Reagents (FMR)



- Microbiology (FDQ)**

Surname \_\_\_\_\_ First Name \_\_\_\_\_

Company \_\_\_\_\_ Department \_\_\_\_\_

Street/No \_\_\_\_\_ Postal Code \_\_\_\_\_

City \_\_\_\_\_ Country \_\_\_\_\_

E-mail \_\_\_\_\_ Fax \_\_\_\_\_ Telephone \_\_\_\_\_

May we contact you by e-mail?  Yes  No

**FY2**

**Thank you for taking the time to complete this short questionnaire.**

Sigma-Aldrich holds customer information in the strictest confidence. We have never shared and never will share information in our Customer Data Base with any 3<sup>rd</sup> party for their marketing use.

# New Products Corner

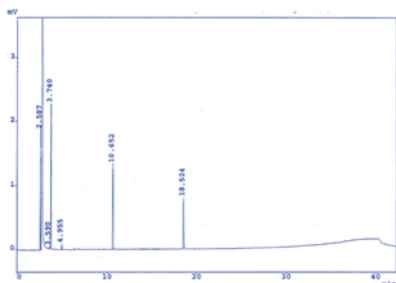
## How deeply coloured is your sample?



In addition to the Colour Reference Solutions of the European Pharmacopoeia, we are now presenting a non-official set of working standards for the B series (brown).

**Cat. No 53116** - Colour reference solutions B0 and BX, Set (2x10 amp.)

## Multicompound or 6 single orders?



Chromatogram included with Cat. No 33418

Time consuming preparation of multi-compound environmental standards belongs to the past!

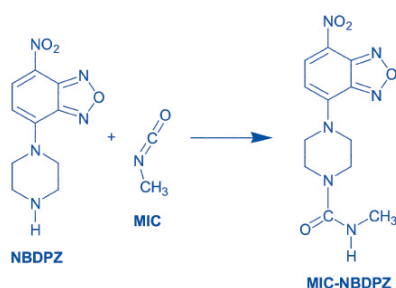
In order to fulfil the requirements of several environmental agencies, two new mixtures for the determination of sulphur and tin compounds were recently added to our catalogue. Both are delivered with a Certificate of Analysis and include a chromatogram.

Do you need a tailor-made customized standard? Please contact [pestanal@sial.com](mailto:pestanal@sial.com)

**Cat No 33433** - Methylmercaptan, methylsulphide, dimethyldisulphide, dimethyltrisulphide, 1000 µg/ml in methanol (5 ml)

**Cat No 33418** - Monobutyltin, dibutyltin, tributyltin, monophenyltin, diphenyltin, triphenyltin, 1000 µg/ml in acetone (2 ml)

## Novel derivatization reagents for isocyanates: NBDPZ



Scheme of the derivatization reaction of NBDPZ with methylisocyanate (MIC) (reproduced by permission of The Royal Society of Chemistry)

**NBDPZ** (4-Nitro-7-piperazino-2,1,3 benzoxadiazole) readily reacts with isocyanates yielding the corresponding urea derivatives, which can be identified by RP-HPLC. NBDPZ provides for increased selectivity due to more favourable detection wavelengths in the visible range.

M. Vogel, U. Karst: Anal. Chem. 2002, 74, 6418-6426; H. Henneken, R. Lindahl, A. Ostin, M. Vogel, J.-O. Levin, U. Karst: J. Environ. Monit., 2003, 5, 100-105

**Cat. No 92614** - 4-Nitro-7-piperazino-2,1,3 benzoxadiazole (100 mg)

[www.sigma-aldrich.com/flukanew](http://www.sigma-aldrich.com/flukanew)



## Analytical News Corner

Dear valued customer,

"Competent partners in analytical challenges" is just more than our motto. The rapid advances in analytical chemistry have given us an ongoing challenge to develop new, innovative products and solutions. We are pleased to highlight a few of them in the latest AnalytiX:

- **Acrylamide Testing:** Ready-to-use Kit for detection of Acrylamide in food.
- **New generation of solvents:** LC-MS CHROMA-SOLV® Water for both gradient HPLC- and MS-applications.

### Innovations in Microbiology

Overview of new methods and solutions to make the detection and differentiation of microorganisms easier and more reliable.

### Karl Fischer Titration

Some helpful inputs to determine water in pharmaceuticals. Benefit from our enormous application know-how in titration!

### Standards

We are continually expanding our product range of **standards and certified reference materials:**

3 articles are dedicated to our outstanding product range.

- In the field of turbidimetry we completed our product range introducing **6 primary standards for turbidimetry** based on polymer suspensions. They offer several advantages in comparison to common formazin solutions.
- Certified Standards for **Food analysis** from IRMM: **Standards of vitamins, oils, fats, aflatoxins and veterinary drugs.**
- A convenient solution for **low level TOC determinations** has been developed: Our **TOC test kit** is used to perform system suitability checks **according USP**. The kit contains standard solution, system suitability solution and reagent water.

Please take your chance and spend a few minutes of your time to evaluate our latest developments.

We would be pleased to hear from you!

Rainer Walz, Ph.D  
Product Manager Analytical Products  
Fluka/Riedel de Haen

## Table of Contents

### Food Analysis

Determination of Acrylamide in Food	2
Certified Reference Materials by IRMM	
Vitamins	3
Oils and Fats	4
Veterinary Drugs and Hormone	
Residues	5
Aflatoxins	6

### Special: Microbiology

New Monotec Test Kit	7
Chromogenic Media	8
Carrying Petri Dishes Around	9
Antimicrobial Susceptibility Discs	10

### LC-MS Chromasolv® Solvents

11

### Turbidimetry Standards

13

### TOC System Suitability Kits

14

### Karl Fischer Titration:

Applications in The Pharmaceutical Industry	16
Seminars: Our Experience at Your Fingertips	18

# Determination of Acrylamide in Food



Acrylamide is a toxic product used in the production of plastics, and has been shown to cause tumors in laboratory animals. It also causes DNA damage and neurological and reproductive effects have been observed at high doses. Last year the Swedish National Food Administration published its concerns regarding the high concentrations of acrylamide detected in foods processed at high temperatures. This discovery was particularly disturbing since the acrylamide-containing foodstuffs includes products that are consumed on a regular basis at rather large quantities, such as potato chips, French fries, roast potatoes, breakfast cereals and crisp bread.

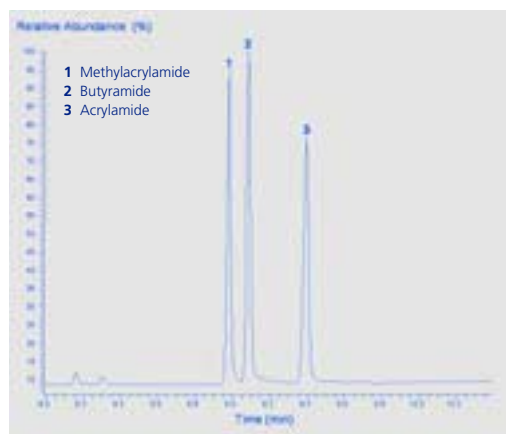
There are two different approaches for the determination of acrylamide in food. One is the Grob-Method, and the other is the one described by EPA. Both methods are laborious and require several high quality reagents and standards that are not available from the same supplier. Sigma-Aldrich has combined efforts and is now presenting a ready-to-use kit that makes acrylamide determination faster and easier. The Acrylamide Kit (Cat. No 72615) contains all the necessary standards and solvents for the extraction and accurate quantification of acrylamide as proposed by Grob<sup>1</sup>.

## GC-MS analysis

The acrylamide determination method developed by Grob[1] includes several extraction steps before GC-MS analysis (**Figure 1**).

A fully detailed protocol, with the standards' GC chromatogram (**Figure 2**) and MS Spectra's is provided with the Acrylamide Kit. You can also find it on the Sigma-Aldrich web page:

[www.sigma-aldrich.com/food\\_analysis](http://www.sigma-aldrich.com/food_analysis).



**Figure 2:** Chromatogram of methacrylamide, butyramide and acrylamide. (Column: Carbowax CW 20 M, 30 m x 0.25 mmID, 0.25 mm. Oven: Gradient starting at 70 °C (1min) until 220 °C at 15 °C/min. Hold 2 min. Injection volume: 1 µl. Carrier gas: helium, 20 cm/sec. Injection on column.)

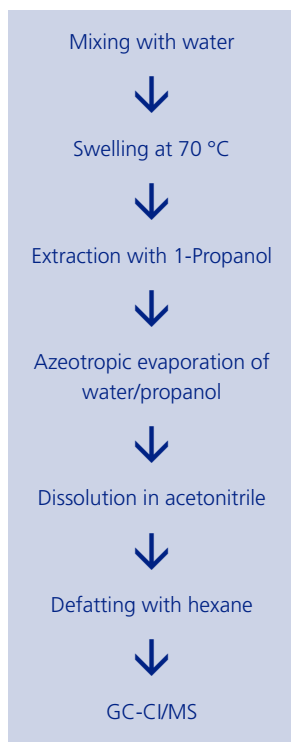
## Your convenient solution: Acrylamide Kit

It was an exciting challenge for us to provide you with a ready-to-use kit for the fast and easy determination of acrylamide. The Kit (Cat. No 72615) contains all reagents and standards needed for 12 determinations. The suitability of all 8 reagents was thoroughly tested. The concentrations and specifications of the Acrylamide, Acrylamide-D<sub>3</sub>- and Methacrylamide-Standard Solutions were specially tailor-made for this method which reduces enormously the time required. The kit contains all the instructions required for sample preparation. **Table 1** provides an overview of the kit and the reagents, their specifications and pack sizes.

If you need more information, chromatograms or MS-Spectra of the standard solutions, please feel free to contact:

Rainer Walz, Ph.D.  
rwalz@sial.com

[1] M. Biedermann, S. Biedermann-Brem, A. Noti and K. Grob; P. Egli and H. Mändli  
Mitt. Lebensm. Hyg. 2002, 93, 638-652



**Figure 1:** Acrylamide determination: extraction steps (taken from Grob<sup>1</sup>)

Product	Description	Pack Size
Acrylamide Standard Solution	500 ppm in acetonitrile	5 ml (Certan® vial)
Acrylamid-D <sub>3</sub> Standard Solution	500 ppm in acetonitrile	5 ml (Certan® vial)
Methacrylamide Standard Solution	500 ppm in acetonitrile	5 ml (Certan® vial)
Butyramide Standard Solution	25 ppm in acetonitrile	5 ml (Certan® vial)
1-Propanol	puriss.p.a., >99.5% (GC)	250 ml bottle glass
n-Hexane	puriss, >99.0% (GC)	100 ml bottle glass
Acetonitrile	for residue analysis, >99.9% (GC)	50 ml bottle glass
Oil	suitable for acrylamide determination	50 ml bottle glass

**Table 1:** Components of Acrylamide Kit (Cat. No 72615). Standard solutions are available in kit only and cannot be ordered individually. The number of possible determinations is 12.

## Certified Reference Materials by IRMM/BCR®

The Institute for Reference Materials and Measurements (IRMM) is a Metrology Institute that belongs to the European Commission. One of the main objectives of IRMM is to produce Certified Reference Materials. More than 500 of these Certified Reference Materials were developed and are now commercially available under the Trade Name of BCR®. Since April 2000, Sigma-Aldrich has been an authorized distributor of IRMM, making BCR® products deliverable worldwide within a few days. The product range covers areas such as Environmental, Food and Agriculture, Industrial Raw Materials, Occupational Hygiene, Physical Properties, Reactor Neutron Dosimetry and Clinical Chemistry. In this article, we shall focus on Certified Reference Materials for the Food Industry.

### The Mission of the Food Safety and Quality Unit

IRMM is divided into several Units, one of them being the Food Safety and Quality Unit (FSQ). The mission of the FSQ is to develop, harmonize and validate analytical methods to monitor chemical, physical and biological parameters and to disseminate the results in the field of food and feed safety and quality. All care is taken to ensure consumer safety by control of the quality and safety of food and feed, as well as to support control laboratories in implementing the EU food and feed legislation. The Unit's underlying research activities endeavor to ensure the protection and promotion of human health. Results are achieved by active interaction with European Institutions and Standardization Bodies and the Food and Feed Industry.



### Vitamins

Accurate and reliable methods for the determination of vitamins in food are essential in Nutritional Research in order to generate data for healthier eating habits. Measurements are also of extreme importance to meet the EU Directives on food labeling and food additives. IRMM has prepared 6 Certified Reference Materials for vitamins in food to aid food-testing laboratories.

### Verifying vitamin measurements

Nearly 50 European laboratories took part in optimization of the measurements processes for each of the vitamins shown in **Table 1**. For vitamin analysis, the first step is extraction from the food sample with a combination of either strong acid or alkali, or with solvent combined with heating. This step is necessary because the measurement techniques do not apply for solid food material. The resulting extract is then submitted to an enzyme treatment to release the vitamins bound to proteins and other components in the food matrix. The vitamins are measured using either chemical, microbiological or high pressure liquid chromatography (HPLC) techniques. Sample clean-up procedures are often used prior to HPLC analysis in order to remove any interfering compounds. **Table 1** gives you an overview on the food matrixes currently available, and the respective certified vitamin content.

Certified Vitamins	BCR-121 Whole meal flour mg/kg	BCR-122 Margarine mg/kg	BCR-421 Milk Powder mg/kg	BCR-431 Brussel sprouts mg/kg	BCR-485 Mixed vegetables mg/kg	BCR-487 Pig's liver mg/kg
B <sub>1</sub> (thiamine)	4.63 +/- 0.39		6.51 +/- 0.48		3.07 +/- 0.34	8.6 +/- 1.1
B <sub>2</sub> (riboflavin)			14.5 +/- 0.6			106.8 +/- 5.6
B <sub>6</sub> (total pyridoxine)	4.10 +/- 1.02		6.66 +/- 0.85		4.8 +/- 0.8	19.3 +/- 2.9
B <sub>12</sub>			0.034 +/- 0.005			1.12 +/- 0.09
C (total ascorbate)			769 +/- 39	4830 +/- 240		
D <sub>3</sub> (cholecalciferol)		0.125 +/- 0.007	0.143 +/- 0.008			
E (tocopherol)		241 +/- 12	99 +/- 6			
Folate (total)	0.50 +/- 0.07		1.42 +/- 0.14		3.15 +/- 0.28	13.3 +/- 1.3
Niacin			68 +/- 2	43 +/- 3		
Trans- $\alpha$ -carotene					10.5 +/- 0.6	
Trans- $\beta$ -carotene					23.7 +/- 1.5	
Total- $\alpha$ -carotene					9.8 +/- 0.7	
Total- $\beta$ -carotene					25.6 +/- 1.2	
Lutein					12.5 +/- 0.8	
Lutein + zeaxanthin					22.3 +/- 1.3	

**Table 1:** Certified Vitamins

Pack Sizes: BCR-121: about 50 g unit size; BCR-122: filled with about 200 g; BCR-421: about 50 g spiked spray-dried powder in food grade laminated sachets under nitrogen; BCR-431: about 20 g lyophilized and powdered material in food grade plastic laminated sachets under nitrogen; BCR-485: about 25 g unit size; BCR-487: about 15 g unit size.

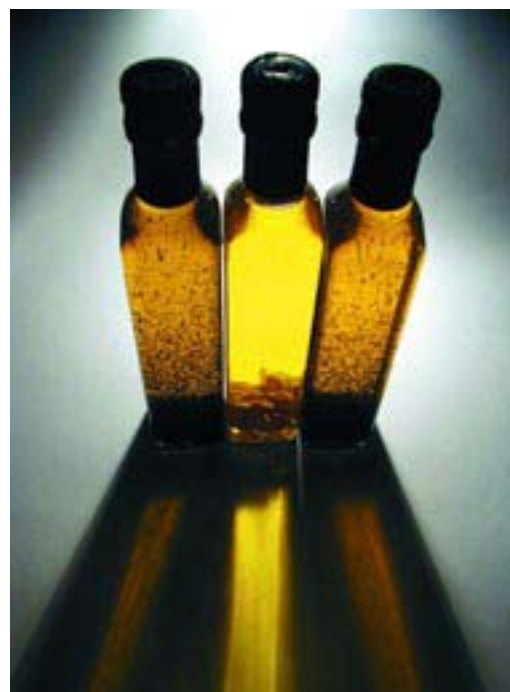
## Oils And Fats

### Edible oils and fats for fatty acid profile determination

The fatty acid methyl ester profiles were determined by gas chromatography using both capillary and packed columns. Although the entire profile is characterized, certified values are only given for the major components (> 2%). Only indicative values are stated for all minor components detected. The Certified Reference Materials may also be used to determine the relative retention times of individual fatty acid methyl esters (**Table 2**). Fatty acids and their methyl esters are also available as working standards for daily use.

You may find a full list, with prices in local currency and availabilities, on the Sigma-Aldrich website. Please follow this link:

[www.sigma-aldrich.com/Standards.html](http://www.sigma-aldrich.com/Standards.html)



Certified Oil blends	BCR-162 Soy maize blend	BCR-163 Beef-pork fat oil blend
Methyl ester of	Mass fraction fatty acid methyl ester/ Total fatty acid methyl ester <sup>1)</sup> (g/100 g)	Mass fraction fatty acid methyl ester/ Total fatty acid methyl ester <sup>1)</sup> (g/100 g)
14:0 n-tetradecanoic acids	(0.1)	2.29 ± 0.04
14:1 $\omega$ 5		(0.3)
15:0		(0.3)
16:0 n-hexadecanoic acids	10.65 ± 0.17	25.96 ± 0.30
16:1 n-hexadecenoic acids		2.58 ± 0.16
16:1 $\omega$ 7 <sup>2)</sup>	(0.1)	
16:1 $\omega$ 9 <sup>2)</sup>		(0.3)
17:0	(0.1)	(1)
17:1	(0.1)	(0.4)
18:0 n-octadecanoic acids	2.87 ± 0.07	18.29 ± 0.16
18:1 n-octadecenoic acids	24.14 ± 0.28	38.34 ± 0.36
18:1 $\omega$ 7 <sup>2)</sup>	(1)	(1-3)
18:1 trans <sup>2)</sup>	(0.5)	(0.5)
18:2 n-octadecadienoic acids	56.66 ± 0.54	7.05 ± 0.17
18:2 trans <sup>2)</sup>	(0.5)	(0.5)
18:2 conj. <sup>2)</sup>		(0.4)
18:3 n-octadecatrienoic acids	4.68 ± 0.21	0.86 ± 0.14
18:3 trans <sup>2)</sup>	(0.5)	(0.5)
20:0	(0.3)	(0.3)
20:1	(0.2)	(0.8)
20:2		(0.3)
20:3		(0.3)
22:0	(0.2)	(0.5)
Sterols	Mass fraction (mg/100 g) in fat	Mass fraction (mg/100 g) in fat
Cholesterol	2.5 ± 0.5	133.6 ± 4.7
$\beta$ -Sitosterol	(399.8)	
Campesterol	(137.4)	
Stigmasterol	(59.5)	
$\Delta^7$ -Avenasterol	(5.6)	
$\Delta^7$ -Stigmasterol	(5.2)	

**Table 2**

Certified Oil Blends

Values in brackets are not certified. Availability: In units of 2.5 mL in dark glass ampoules sealed under nitrogen.

1) Includes any geometric (i.e. cis/trans) and positional isomers, expressed as mass fraction of total fatty acid (methyl esters) derived from triglycerides.

2) These components are included in the Certified Value for this group of fatty acids.

The respective reports give additional indicative values: BCR-162: Iodine, Unsaponifiable Matter, Tocopherols and "Total" Sterol Mass Fraction; BCR-163: Fatty Acids and "Total" Sterol Mass Fraction.



Order the **NEW CD «Standards and Derivatization Reagents»**-free of charge. Just fax the Reply Card inserted in this newsletter to your local Sigma-Aldrich partner!

### Veterinary Drugs And Hormone Residues

Some growth hormones used in meat production have been shown to be human carcinogens, and were therefore banned in the European Community. Policing the meat trade is not an easy task since new compounds that don't show up in the screenings and controls are constantly being introduced in the black market of drugs. The Standards, Measurement and Testing Program has sponsored several projects that resulted into the development of new reference materials to help laboratories to check and optimize their hormone measurement techniques.

### Controlling the animal drug trade

Cattle is tested for growth hormones by taking samples of urine, in a similar approach to the anti-doping control done on athletes. Each country is free to choose its own measurement method, as long as it conforms with the Euro-

pean guidelines for screening and confirming the levels of drugs in urine. For instance, it is required that the laboratory maintains a quality control check on their procedures. Furthermore, it is necessary to prove that the accuracy and correctness of its own measurements and results is verified. This is where the use of Certified Reference Materials is of paramount importance. In **Table 5** you may find 11 certified hormone content BCR® products.

Drugs in bovine tissue				
Cat. No.	Specified residue	Hormones in lyophilized bovine urine Mass concentration in reconstituted sample (µg/L) <sup>(a)</sup>		
BCR-386	Diethylstilboestrol (DES)	< 0.1		
BCR-387	Dienoestrol (DE)	< 0.1		
BCR-388	Hexoestrol (HEX)	< 0.1		
BCR-389	Diethylstilboestrol (DES)	12.8 +/- 2.5		
BCR-390 (RM)	Dienoestrol (DE)	(34 +/- 7)		
BCR-391	Hexoestrol (HEX)	13.3 +/- 3.1		
		Content	Lower 90% Limit	Upper 90% Limit
BCR-502 <sup>(2)</sup>	Clenbuterol	< 0.1		
	Salbutamol	< 0.1		
BCR-503 <sup>(2)</sup>	Clenbuterol	2.5	2.1	2.9
	Salbutamol	2.3	1.7	3.2
BCR-504 <sup>(2)</sup>	Clenbuterol	6.0	5.5	6.7
	Salbutamol	5.6	4.5	7.5
BCR-648-649 <sup>(3)</sup> , set	Clenbuterol	< 0.5 1.12 +/- 0.11		

**Table 5:** Certified Veterinary drugs in tissue

(a) for BCR-648 and -649 mass concentration is given in µg/kg

Remarks on Table: Value in brackets is not certified.

Availability: (1) Units of lyophilized urine equivalent to about 2.0 mL in vials sealed under nitrogen. (2) Units of lyophilized urine equivalent to about 5.0 mL in vials sealed under nitrogen. (3) Units of 10 g of lyophilized bovine liver in vials sealed under argon. The certificates of analysis give additional indicative values for trans- and cis-DES in BCR-389

## Aflatoxins

Aflatoxins are potent human carcinogens. They are naturally occurring toxic metabolites produced by fungi, such as *Aspergillus flavis*, a mold found on foodstuff such as corn and peanuts or peanut butter. Aflatoxins have been associated with various diseases in livestock, domestic animals and humans. Since they represent a risk to both humans and livestock, they have a large economic impact on the food industry and on commercial feed traders. Animal feed coming into the EU is rigorously screened for possible contamination. Taking and preparing samples for this test is critical, since any error done in this step will render the results meaningless. For this reason, IRMM is now introducing food matrixes with certified aflatoxin content.

The limits of aflatoxin M1 in milk powder can be evaluated using BCR-283 and BCR-285. BCR-282 is a near-blank intended for investigation of limits of detection and recovery experiments (Table 6). These Certified Reference Materials can also be used to check the reliability of the immunoassay, a test of growing importance.

Aflatoxin B1 is considered as the most toxic one. It is detected when certain grains, like peanuts, are grown under stressful conditions or incorrectly stored. The levels of aflatoxin B1 in these matrixes may vary from blank or very low to high level (Table 7).

For any further information, please contact

Rainer Walz, PhD  
rwalz@sial.com

## Fluka – Your supplier for BCR® Reference Materials



On the Sigma-Aldrich Web Page ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)), you will be able to find the complete list and descriptions of all BCR® products. Just use the search engine to look for your product. For a complete list of all standards currently available check [www.sigma-aldrich.com/standards.html](http://www.sigma-aldrich.com/standards.html)



«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.»

### Certified Aflatoxin M1

		Aflatoxin M1 (µg/kg)
<b>BCR-282</b>	Whole milk powder (very low level)	< 0.05 <sup>1)</sup>
<b>BCR-283</b>	Whole milk powder (low level)	0.09 + 0.04, - 0.02
<b>BCR-285</b>	Whole milk powder (high level)	0.76 ± 0.05

**Table 6**

Certified Aflatoxin M1. The materials are provided in 25 g units sealed in laminate sachets.

1) Probable content is in the range of 0.01 to 0.02 mg/kg.

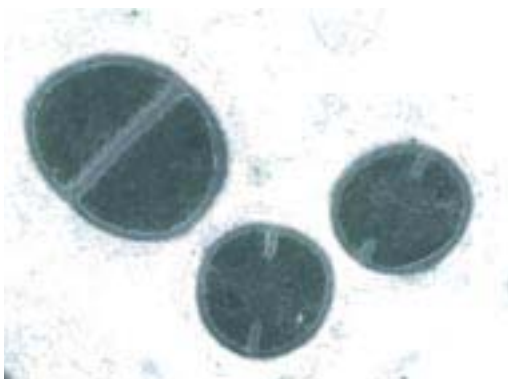
### Certified Aflatoxin B1

Substance	<b>BCR-262</b> Defatted peanut meal (blank)	<b>BCR-263</b> Defatted peanut meal (medium level)	<b>BCR-264</b> Defatted peanut meal (high level)	<b>BCR-375</b> Compound feed (very low level blank)	<b>BCR-376</b> Compound feed (low level)
Aflatoxin B1	Mass fraction (µg/kg) < 3	Mass fraction (µg/kg) 43.3 ± 2.8	Mass fraction (µg/kg) 206 ± 13	Mass fraction (µg/kg) < 1	Mass fraction (µg/kg) 9.3 ± 0.5

**Table 7**

Certified Aflatoxin B1. Sachets sealed under vacuum containing about 50 g (BCR-375 and BCR-376), about 100 g (BCR-262 and BCR-263) and about 150 g (BCR-264) of finely ground defatted peanut meal.

## NEW! Staphylo Monotec Test Kit



Ultramicrography of *Staphylococcus aureus*

*Staphylococcus aureus* is by far the most important human pathogen among the Staphylococci. It is found in the external environment, in food, in the anterior nares and on skin. Although it is usually a part of the human microflora, under the appropriate conditions it may cause significant opportunistic infections [1].

Staphylo Monotec (Cat. No 08986) is a new rapid agglutination test kit for differentiation of *Staphylococcus aureus* from other species of Staphylococcus. Both coagulase and protein A on *Staphylococcus aureus* are detected in one step, making this test highly sensitive and specific (see **Table 1** for performance results). This kit suitability was evaluated for veterinary and food material as well as for clinical specimens [2-3].

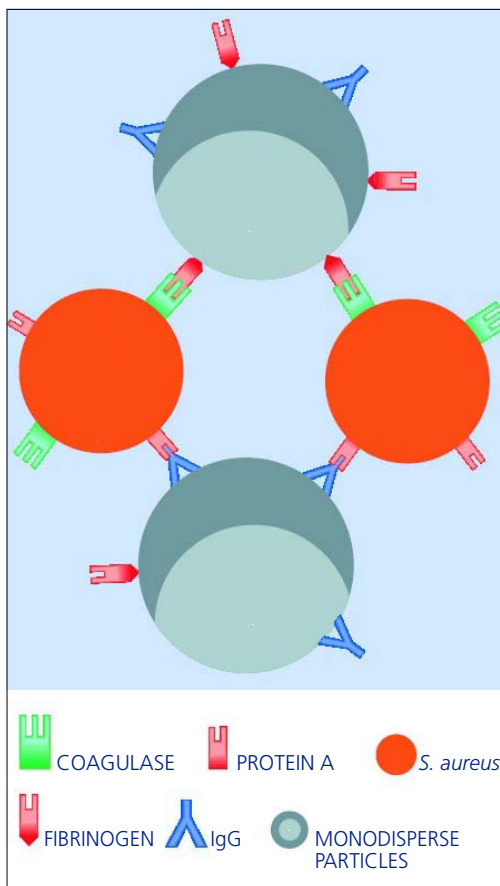
	Human	Veterinary/Food
Sensitivity	98.6%	100%
Specificity	99.0%	98.0%

**Table 1** Performance of the Staphylo Monotec test kit

The principle of Staphylo Monotec kit is the rapid agglutination that occurs when fibrinogen binds to the cell-associated enzyme coagulase at the same time that the Fc part of the IgG binds to protein A (**Figure 1**). The test reagent is a solution made of monodisperse microparticles coated with IgG and fibrinogen. This reagent is mixed with the cultures grown on blood agar or other recommended media. When *Staphylococcus aureus* is put together with the Staphylo Monotec test reagent, there is a rapid agglutination that is immediately seen. The positive tests gives clear white agglutinates against a black background (**Figure 2**). When the agglutination has an ambiguous reading, it can be further checked using a negative control made of monodisperse microparticles coated with bovine serum albumin.

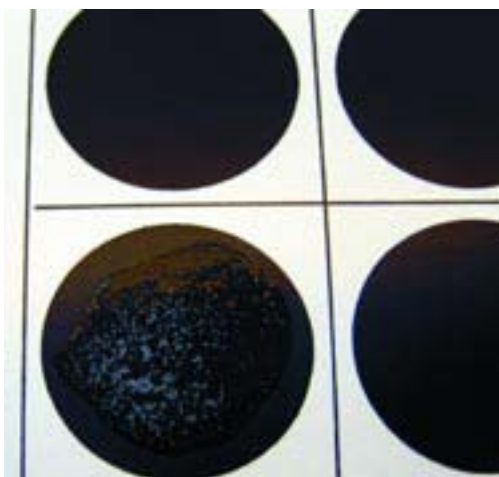
The Staphylo Monotec kit is particularly suitable for use with clinical samples since an average of 97% of human *Staphylococcus aureus* are coagulase positive and more than 95% of all

clinical isolates have protein A in the cell wall. Furthermore, the control reagent that is included with the kit greatly improves its performance.



**Figure 1:**

The principle of the test: When fibrinogen binds to the cell-associated enzyme coagulase at the same time that the Fc part of the IgG binds to protein A, there is rapid agglutination of *Staphylococcus aureus*



**Figure 2:**

Positive test: white agglutinates that are clearly seen against black background are formed

### Cat. No 08986 Staphylo Monotec Test Kit

1 kit is sufficient for 100 tests.  
Components: test reagent, negative control reagent and analysis cards

### References

- [1] Grahams B.S., Snell J.D.J., *Medicine* 62: 384-393 (1983)
- [2] Flesland, Ø., *APMIS sect. B* 95: 83-84 (1987)
- [3] Holme, I.J.R., *Acta Vet. Scand.*, 32, 155-161 (1991)

Photography of *Staphylococcus aureus* by courtesy of Bleiss W., Viveiros M. & Amaral L., Humboldt-University, Berlin, Germany & IHMT, Lisboa, Portugal.

## Chromogenic Media

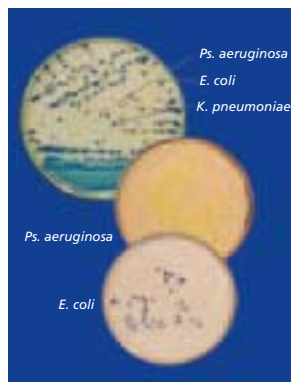
Chromogenic media are based on traditional media modified with chromogenic substrates. The use of such substrates makes the detection and isolation of microorganisms easier, faster and more reliable. We are proud to announce that **NEW!** chromogenic media were added to our line of products, and some more will be coming soon.

The microorganisms are differentiated by detecting the presence of highly specific enzymes. Their activity is measured using chromogenic agents, such as X-glucuronide, ONPG or Salmon-Gal. The chromogenic agent in the growth medium is uptaken by the cell and the intracellular enzyme splits the bond between the chromophore and the sugar residue. The chromophore is then released into the medium,

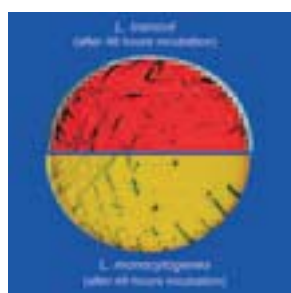
giving coloration to the colonies. More information about the state of the art in Chromogenic Culture Media can be found in Prof. M. Manafi's most recent publications [1-2].

### References

- [1] Manafi M., Int. J. of Food Microbiol., 31, 45-58 (1996)  
 [2] Manafi M., Int. J. of Food Microbiol., 60, 205-218 (2000)



**Figure 1:** HiCrome™ ECC Agar (Cat. No 53707)



**Figure 2:** HiCrome™ Listeria Agar Base, modified (Cat. No 53707)

Cat. No. Pack Sizes	Product	Description
72953 50 g 250 g	Candida identification Agar	Candida Identification Agar is suitable for the selective isolation and identification of <i>Candida albicans</i> from clinical material like stool, urine, skin scurf and swabs. This medium is also used for the isolation and identification of a wide range of Gonococci, yeasts and molds. Supplement with 2 ml/l gentamycine (Cat. No 48755)
87959 27 g	HiCrome™ Coliform Agar	HiCrome™ Coliform Agar is a selective chromogenic medium recommended for simultaneous detection of <i>Escherichia coli</i> and total coliforms in water and food samples. It contains two chromogenic substrates: Salmon-GAL and X-glucuronide. Supplement with 5 mg/l novobiocin (Cat. No 74675)
81938 500 g	HiCrome™ <i>Escherichia coli</i> Agar A	HiCrome™ <i>Escherichia coli</i> Agar A is recommended for the detection and enumeration of <i>Escherichia coli</i> in foods without further confirmation on membrane filter or with indole reagent.
95207 36.6 g	HiCrome™ <i>Escherichia coli</i> Agar B	HiCrome™ <i>Escherichia coli</i> Agar B is recommended for the detection and enumeration of <i>Escherichia coli</i> in foods without further confirmation on membrane filter or by indole reagent.
70722 36.6 g	HiCrome™ ECC Agar	HiCrome™ ECC Agar is a differential medium recommended for the presumptive identification of <i>Escherichia coli</i> and other coliforms in food and environmental samples. It contains two chromogens: X-glucuronide and Salmon-GAL (see <b>Figure 1</b> ).
89823 26.5 g	HiCrome™ ECC Selective Agar	HiCrome™ ECC Selective Agar is a selective medium recommended for the simultaneous detection of <i>Escherichia coli</i> and coliforms in water and food samples. The ingredients help even the sublethally injured coliforms to grow rapidly. It contains two chromogenic substrates: Salmon-GAL and X-glucuronide. Optional supplement: 5-10 mg/l efsulodin (Cat. No 22126)
85927 500 g	HiCrome™ Enterococci Broth	HiCrome™ Enterococci Broth is recommended for identification and differentiation of Enterococci from water samples.
52441 100 g 500 g	<b>new</b> HiCrome™ Listeria Agar Base, modified	HiCrome™ Listeria Agar Base (modified) is a selective and differential agar medium recommended for rapid and direct identification of Listeria species, in particular of <i>Listeria monocytogenes</i> ( <b>Figure 2</b> ) Supplement with 2 vial/l HiCrome™ Listeria Selective Supplement (Cat. No 59688)
53707 50 g 250 g	<b>new</b> HiCrome™ Mac Conkey Sorbitol Agar (SMAC Agar, Sorbitol MacConkey Agar)	HiCrome MacConkey Sorbitol Agar is recommended for selective isolation of <i>Escherichia coli</i> 0157:H7 from food and animal feeding stuffs. Supplement with 2 vial/l Tellurite-Cefixime Supplement (Cat. No 77981)
83339 50.1 g	HiCrome™ Mac Conkey Sorbitol Agar (SMAC Agar, Sorbitol MacConkey Agar)	HiCrome MacConkey Sorbitol Agar is recommended for selective isolation of <i>Escherichia coli</i> 0157:H7 from food and animal feeding stuffs. Supplement with 2 vial/l Tellurite-Cefixime Supplement (Cat. No 77981)
88474 500 g	HiCrome™ Mac Conkey Sorbitol Agar (SMAC Agar, Sorbitol MacConkey Agar)	HiCrome MacConkey Sorbitol Agar is recommended for selective isolation of <i>Escherichia coli</i> 0157:H7 from food and animal feeding stuffs. Supplement with 2 vial/l Tellurite-Cefixime Supplement (Cat. No 77981)

**Table 1**  
Chromogenic Media



# Antimicrobial Susceptibility Discs

We are now proud to announce that the antimicrobial susceptibility discs recommended by the WHO Expert Committee on Biological Standardization are available in our catalog (see **Table 2**). Antimicrobial susceptibility discs are paper discs impregnated with antimicrobial substances. They are used to measure the *in vitro* susceptibility of pathogenic organisms by using techniques such as disc-agar diffusion or disc broth elution. For disc-agar diffusion, the bacterial susceptibility is ascertained by measuring the zone of bacterial inhibition around the discs on an agar surface (**Figure 1**). Disc-broth elution is associated with an automated rapid susceptibility test system and employs fluid mediums, such as the Mueller Hinton Broth (Cat. No 70192). When the disc is placed into the medium, the antimicrobial substance is eluted. The resulting changes in the bacterial growth are then measured by using a photometer. It can be used either to identify a suspect organism or to study resistances and determine the

For any further information, please contact  
Jvo Siegrist  
siegrist@sial.com

**Table 1**  
Range of antimicrobial susceptibility discs

Cat. No	Antibiotic (Disc content)	Colour
42456	Amikacin (30 µg)	yellow/green
16527	Amoxicillin (25 µg)	blue/white
01601	Amoxicillin + Clavulonic acid (20 + 10 µg)	blue/white/orange
08541	Ampicillin (10 µg)	red/blue
68601	Azithromycin (15 µg)	green/orange
52856	Cefachlor (30 µg)	gray/pink
68611	Cefalexin (30 µg)	blue/beige
30321	Cefotaxim (30 µg)	blue/dark green
89867	Ceftazidime (30 µg)	beige/yellow
90424	Ceftriaxone (30 µg)	pink/dark blue
92241	Cefuroxime (30 µg)	white/blue
07651	Chloramphenicol (30 µg)	White
08587	Ciprofloxacin (5 µg)	yellow/gray
93646	Clindamycin (10 µg)	dark blue/white
67237	Erythromycin (15 µg)	Green
14683	Fleroxacin (5 µg)	beige/white
69531	Gentamycin (30 µg)	red/dark green/white
55941	Imipenem (10 µg)	orange/blue
01403	Kanamycin (30 µg)	Red
19607	Lincomycin (15 µg)	blue/dark blue
05577	Methicillin (10 µg)	red/blue/white
39782	Nalidixic acid (30 µg)	Orange
56758	Neomycin (30 µg)	dark green/white
44543	Norfloxacin (5 µg)	pink/gray
42079	Ofloxacin (5 µg)	red/green
54357	Oxacillin (5 µg)	white
16244	Penicillin G (6 µg)	Blue
11435	Pipemidic acid (20 µg)	beige/orange
56383	Piperacillin (100 µg)	green/red/blue
56973	Rifampicin (5 µg)	red/dark green
75139	Streptomycin (30 µg)	Pink
75141	Tetracycline (30 µg)	Yellow
73477	Trimethoprim (5 µg)	gray/white
74794	Trimethoprim (1,25 µg) + Sulfomethoxazole (23,75 µg)	gray/orange/white
75156	Vancomycin (30 µg)	beige/white/dark blue



**Figure 1:** Susceptibility test to of *Staphylococcus ssp.* Isolated from food grown on Mueller Hinton Agar (Cat. No 70191). Disc color code: ampicillin: red/blue; cefalexin: blue/beige; norfloxacin: pink/gray; erythromycin: green; ciprofloxacin: yellow/gray; imipenem: orange/blue.

antibiotic of choice for the treatment of bacterial diseases.

Antimicrobial susceptibility test discs have 9 mm diameter. For better identification, each disc has a different color (see **Table 1**). The discs are manufactured under aseptic conditions, which makes them to be extra stable: 3 to 4 years, at room temperature.

The factors that may affect the assay accuracy and precision are rigorously controlled. Amongst these it is included the preparation of the media, thickness of the agar, inoculum density, and any change in test procedure, such as using a new disc or medium batch, or handling by a different person.

The susceptibility discs can be also used for testing the presence of broad-spectrum  $\beta$ -lactamase, for example in species belonging to the Enterobacteriaceae.  $\beta$ -lactamases hydrolyze penicillins and oxyminocephalosporins (cefotaxime, ceftazidime and ceftriaxone), but are susceptible to certain inhibitors like clavulanic acid [1-2].

Their presence can be detected by performing a synergy test, that combines sensitivity with susceptibility in one single plate. The sensitivity test is done by using Mueller-Hinton medium. For the susceptibility one, ceftazidime, cefotaxime and amoxicillin-clavulanic acid discs are placed at a distance of 1.5 cm from each other. The cultures are incubated overnight at 37 °C. The synergic action of all these factor makes the inhibition areas not to be perfect circles, as in a normal susceptibility test. In this case, the enlargement of the ceftazidime and cefotaxime inhibition areas towards the disc containing amoxycillin and clavulanic acid is an indication of the presence of broad spectrum  $\beta$ -lactamases [3]

## References

- [1] Brown R.P., Aplin R.T., Schofield C.J. Biochemistry. 35(38):12421-32. (1996)
- [2] Philippon L.N., Naas T., Bouthors A.T., Barakett V., Nordmann P., Antimicrob Agents Chemother. 41(10):2188-95 (1997)
- [3] Skov R., Frimodt-Møller N., Espersen F., APMS. 110(7-8):559 (2002)



The combination of classic Liquid Chromatography (LC) with Mass Spectrometry (MS) led to a remarkable development of analytical applications. Over the past decade the improvements done on LC-MS systems convert it into one of the most important techniques for identification and quantification of metabolites in pharmaceutical laboratories, as well as for pharmacokinetics and also protein, peptide and oligonucleotide analysis.

Modern LC-MS ionization methods such as Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI) require very special high quality solvents, fulfilling the special demands of atmospheric pressure interfaces (API). For such solvents, only a very low amount of metal ions, especially sodium and potassium is tolerated. Also the presence of particles, which could clog up the inlet filter of a Nano-LC instrument, must be checked.

We are now proud to present a new generation of LC-MS CHROMASOLV® Solvents, accurately tested for LC-MS suitability and meeting all the above mentioned requirements. In addition, they present a high UV-transmittance and an excellent gradient baseline for combination with UV or Diode Array Detection in line. The LC-MS suitability test is based on the reserpine specification of many instrument suppliers.

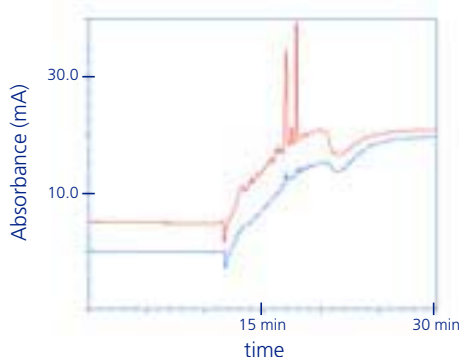
Furthermore, we have developed a new high quality water, suitable for both gradient HPLC- and MS- applications. The LC-MS CHROMASOLV® Water (Cat. No. 39253) offers a tremendous advantage over other grades: It can be used for UV and MS detection, without any compromise. A comparison of LC-MS CHROMASOLV® Water with a non-gradient grade one was made. The difference is evident (Figure 1).

### Investigation of Cluster Formation in Presence of Alkali Ions

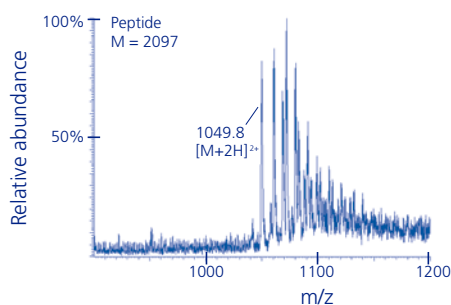
In our R+D laboratories we have investigated the influence of metal ions on the quality of LC-MS spectra. Human gastrin (M=2097) was used as a model peptide. The sample was infused with a

micro syringe thus modeling the conditions of a capillary nano spray.

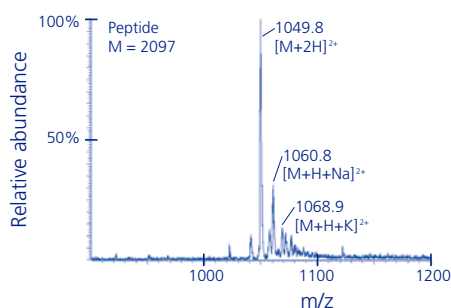
It is known that when operating ESI in positive ion mode, the preferred ionization reaction is the formation of  $[M+H]^+$ -ions. In the case of peptides, normally a protonated double charged  $[M+2H]^{2+}$ -ion is generated. For gastrin, a molecular ion with  $m/z = 1049.8$   $[M+2H]^{2+}$  is obtained. First, human gastrin was dissolved in *normal HPLC water* (containing 0.2% formic acid) having a sodium and potassium content higher than 10ppm. As a result, the metal ions formed clusters with the peptide. The mass spectrum that is obtained is unspecific: the dominating clusters appear as a *fence* of double and multicharged ions (Figure 2). Such an artefact makes it difficult to determine the "true" peak: the protonated double charged molecular ion of gastrin. Furthermore, major problems will occur with the automatic MS/MS mode, where the most abundant peaks are selected for further collisions to give the amino acid sequence.



**Figure 1:** UV gradient at 205nm. Comparison between non-gradient water (red line) and LC-MS CHROMASOLV® Water (Cat. No 39253, blue line)



**Figure 2:** ESI mass spectrum of human gastrin dissolved in water having a sodium and potassium content higher than 10ppm (gastrin molecular ion=1049.8 m/z). A *fence* originated by the metal ion cluster can be clearly noted (peaks between 1051 and 1150 m/z).



**Figure 3:** ESI mass spectrum of human gastrin in LC-MS CHROMASOLV® Water. The gastrin molecular ion can be found at 1049.8 m/z. Only a few clusters are observed (between 1051 and 1100 m/z). Sensitivity was enhanced 10-fold.



When human gastrin was dissolved in LC-MS CHROMASOLV® water, having a very low amount of sodium and potassium ions (< 0.1ppm), only few metal ion clusters appear. The molecular mass peak can be easily determined, isolated and further fragmented to yield the amino acid sequence (**Figure 3**). Only two clusters are observed in a relatively low abundance ( $[M+H+Na]^{2+}$  and  $[M+H+K]^{+2}$ ). Besides that, the sensitivity is enhanced 10-fold. In accordance to these results we decided to

upgrade our already existing LC-MS CHROMASOLV® Solvents to this high quality specifications: very low content of the alkali elements sodium and potassium (see **Table 1**). The only exception made was ethyl acetate, mainly used for sample preparation.

Are you interested to know more about *Cluster Formation in Presence of Alkali Ions*? Please ask Joachim Emmert at [jemmert@europe.sial.com](mailto:jemmert@europe.sial.com) for your copy. For any further information, please contact Frederik Pillong at [fpillong@europe.sial.com](mailto:fpillong@europe.sial.com).

Alternatively, you can find more detailed information in [www.sigma-aldrich.com/lc-ms-solvents](http://www.sigma-aldrich.com/lc-ms-solvents).

**If you want to avoid obstacles in your protein research, check our LC-MS CHROMASOLV® Solvents. Make sure you will only get the TRUE PEAKS!**

LC-MS Solvent	Water	Acetonitrile	Methanol	2-Propanol
Cat. No	39253	34967	34966	34965
Pack Size	1 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L
Assay (GC) (min)	–	99.9 %	99.9 %	99.9 %
Fluorescence at 254nm (max.)	1 ppb	0.5 ppb	1 ppb	1 ppb
Fluorescence at 365nm (max.)	1 ppb	0.5 ppb	1 ppb	1 ppb
Chloride (Cl) (max.)	0.000001 %	–	–	–
Fluoride (F) (max.)	0.000001 %	–	–	–
Nitrate (NO <sub>3</sub> ) (max.)	0.00001 %	–	–	–
Sulfate (SO <sub>4</sub> ) (max.)	0.00001 %	–	–	–
Free acid (max.)	–	0.001 %	0.001 %	0.001 %
Free alkali (as NH <sub>4</sub> ) (max.)	–	0.0002 %	0.0005 %	0.0005 %
Non-volatile matter (max.)	0.001 %	0.0002 %	0.0005 %	0.0005 %
Water (Karl Fischer) (max.)	–	0.01 %	0.02 %	0.05 %
Transmittance at 200nm (min.)	95 %	95 %	–	–
Transmittance at 230nm (min.)	99 %	99 %	75 %	75 %
Transmittance at 260nm (min.)	–	–	98 %	98 %
HPLC gradient (254nm) (max.)	1 mAU	0.2 mAU	2 mAU	2 mAU
Silver (Ag) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Aluminium (Al) (max.)	0.5 ppm	0.5 ppm	0.5 ppm	0.5 ppm
Barium (Ba) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Calcium (Ca) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Cadmium (Cd) (max.)	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm
Cobalt (Co) (max.)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm
Chromium (Cr) (max.)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm
Copper (Cu) (max.)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm
Iron (Fe) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Potassium (K) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Magnesium (Mg) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Manganese (Mn) (max.)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm
Sodium (Na) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Nickel (Ni) (max.)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm
Lead (Pb) (max.)	0.1 ppm	0.1 ppm	0.02 ppm	0.1 ppm
Tin (Sn) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Zinc (Zn) (max.)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Particle test	Pass	Pass	Pass	–
LC-MS suitability test	Pass	Pass	Pass	Pass

**Table 1**  
LC-MS CHROMASOLV® Solvents

# Turbidimetry Standards



## Primary Turbidimeter Standards

Turbidity is defined as the reduction of transparency of a liquid caused by the presence of undissolved matter. The calibration of turbidimeters for this sort of measurement requires specific standards, classified as Primary, Secondary, or Alternative standards. Standard Methods [1] describes Primary Standards as those prepared by the user from traceable raw materials, using precise methodologies and under controlled environmental conditions. Secondary Standards are those certified by either a manufacturer or an independent testing organization to give instrument calibration results equivalent (within certain limits) to results obtained when an instrument is calibrated with a primary standard.

However, *Standard Methods* and the *Environmental Protection Agency* (EPA) differ in their definition for standards. EPA recognizes only 3 types of Primary Standards for approved use in the calibration of turbidimeters: polymer suspensions, formazin, either prepared by the user or commercially produced, and stabilized formazin solutions.

## Polymer Suspension Standards

Formazin was established as the first calibration standard for turbidimeters in the 1950's, and it forms the basis of most measurements. Later on, the EPA approved in 1984 polymer suspensions as calibration standards for turbidimeters.

Polymer suspensions are made of cross-linked copolymer microspheres suspended in ultra pure water. They are better standards for the calibration of surface source water analysing turbidimeters. The primary reason is its particle size distribution, 0.02  $\mu\text{m}$  to 0.2  $\mu\text{m}$  and a mean size of 0.121  $\mu\text{m}$ . EPA dictates a turbidity design configuration to maximize submicron particle detection

less than 1.0  $\mu\text{m}$ . It is therefore advantageous to calibrate a turbidimeter with a standard that most closely matches the size of the particulate it is analysing. Plus the standard is more precise and the size distribution is consistent.

Apart from their consistent size particle, the use of polymer suspensions offers several other advantages. These include, for instance, increased stability down to 0.10 NTU for at least 1 year. Also, no preparation is required as vigorous mixing or agitation will degrade the accuracy of the polymer standards. They are non-toxic, which means can be used in any setting without fear of contamination to the user, equipment, or the environment.

From all the approved standards, the polymer suspensions have unique characteristics. They are

- non-toxic
- stable
- ready to use
- accurate +/- 1% of stated value lot to lot
- submicron in particle size distribution
- values available from 0.10 NTU to 2000 NTU
- size, shape, and particle size distribution is always the same, regardless of lot.
- no special storage requirements
- no special disposal requirements
- can be frozen solid for four hours before degradation of suspension.
- N.I.S.T. Traceable 1690 & 1691

We are now introducing in our catalogue Primary Turbidimeter Standards (**Table 1**), certified by the following regulatory bodies:

- EPA Federal Registry, Vol. 47, #42, March 3 1982
- Standard Methods of Water & Wastewater, APHA-AWWA-WPOCF, 16th, 17th, 18th & 19th Edition
- ASTM, D1889-88a, June 24, 1989

Certified Turbidimeter Standards	
Cat. No	NTU-Value
96867	0.1
88457	0.8
88487	1.8
83083	18
90258	80
85721	180
88899	800
92019	1800

**Table 1**  
Primary Turbidimeter Standards

## Turbidity Measurement

For your turbidity measurements either in field experiments and in laboratories, we now offer you Aquanal®-plus Spectro Turbidimeter (**Figure 1**), a portable and laboratory turbidity meter for both clear and coloured liquids.

The Aquanal®-plus Spectro was designed as a compact, easy to use instrument for the fast and accurate determination of turbidity. A light emitting diode (LED) is used as a light source. The photodetector is positioned to detect light scattered by a sample at 90° to the incident beam. The LED is characterised by maximum long-term stability and monochromatic light emission with minimum power input. The sample chamber, which is the most critical part of any photometers fully sealed so no water can penetrate into the electronic components. The size of the sample chamber ensures easy cleaning of the light entry surfaces. The Aquanal®-plus Spectro may be used as test equipment with software-based calibration adjustment facilities.

- Comfortable measurements
- Infrared light
- Range 0,1 - 2000 TEF/NTU
- Measurement of coloured liquids

<b>Measurement cycle:</b>	approx. 9 seconds
<b>Display:</b>	LCD-display
<b>Optics:</b>	temperature-compensated LED and photosensor amplifier in water protected sample chamber, infrared light
<b>Sample chamber:</b>	Waterproof
<b>Measuring range:</b>	0,1 - 2000 TEF = NTU = NFU
<b>Keypad:</b>	3 key polycarbonate film, splash proof
<b>Power supply:</b>	9 V power pack battery providing 40 hours operation - equivalent to approx. 600 measurement cycles with a cycle of 4 minutes
<b>Auto-OFF:</b>	automatic switch-off approx. 5 minutes after last key press
<b>Housing:</b>	ABS
<b>Dimensions:</b>	190 x 110 x 55 mm (W x D x H)
<b>Weight:</b>	approx. 0.4 kg (basic unit)
<b>Environmental conditions:</b>	Temperature: 0 – 40 °C Rel. humidity: 30 – 90% rel.
<b>CE conformity:</b>	DIN EN 50081-1, VDE 0839 part 81-1: 1993-03 DIN EN 50082-2, VDE 0839 part 82-2: 1996-02

**Table 2**

Technical Data of Aquanal®-plus Spectro (Cat. No 70034)

If you have further questions concerning Turbidimeter Standards or Aquanal® products, please contact us at

Rainer Walz, Ph.D.  
rwalz@sial.com or aquanal@sial.com

**Figure 1**

Aquanal®-plus Spectro Turbidimeter (Cat. No 70034)



## Reference

[1] Standard Methods, 18th edition supplement (1995).

## TOC System Suitability Test Kits



Low level Total Organic Carbon (TOC) determinations are used to monitor the performance of various operations during the manufacture of medicines. Both the European and the United States Pharmacopoeia had set up guidelines for its measurement (USP 23 <643> "TOC and Conductivity" and EP Kap. 2,2,44). The procedures used to qualify the chosen method and the interpretation of results in limit tests were also described.

A standard solution is analysed at suitable intervals, depending on the frequency of measurements; the solution is prepared with a substance that is expected to be easily oxidizable (for example, sucrose) at a concentration adjusted to give an instrument response corresponding to the TOC limit to be measured. The suitability of the system is determined by analysis of a solution prepared with a substance expected to be hardly oxidizable (for example, 1,4-benzoquinone).

According to the suggested protocol, a standard solution is prepared dissolving 1.19 mg of sucrose (reference material grade) per litre yielding a 500 ppb solution. The system suitability solution consists of 0.75 mg 1,4-benzoquinone (reference material) in 1 litre TOC water having a concentration of 500 ppb carbon.

For preparing the standard solution and system suitability solution, suitable TOC water must be used. For TOC highly purified water, the specifications also include a conductivity below  $1.0 \mu\text{S}\cdot\text{cm}^{-1}$  at 25 °C and a TOC content not greater than 0.1 mg/l.

The system suitability test is carried out by the following procedure:

Run TOC water ( $R_w$ ), standard solution ( $R_s$ ) and system suitability solution ( $R_{ss}$ ) solutions and record the responses. Calculate the percentage response efficiency using the expression:

$$\frac{R_{ss} - R_w}{R_s - R_w} \times 100$$

The system is suitable if the response efficiency is not less than 85 per cent and not more than 115 per cent of the theoretical response.

Since the system suitability check has to be carried out in certain short time frames, preparation of the standard solution and system suitability solution have to be done frequently. Furthermore the preparation of the reagents also requires scrupulously cleaning glassware to remove organic matter. Finally, the continuous preparation of the samples is a critical point in all system suitability measurements and means a large effort for laboratory staff.

Fluka has been innovative in this challenge and now offers a convenient, ready-to-use kit for system suitability tests.

The kit (Cat. No 95451, shown in **Figure 1**) contains 5 solutions: 3 x 40 ml reagent water, 1 x 40 ml system suitability solution and 1 x 40 ml standard solution. The solutions are stabilized and filled thoroughly under laminar flow in pre-cleaned vials. These vials are assembled with open-top screw caps and specially designed septa (10 mil of Teflon facing on 90 mil of silicone) The vials are made of low extractable borosilicate glass. The kit is packed in a box containing a holder for the 5 vials for safety and convenient storage (see **Figure 1**); it is delivered at 4 °C and should be stored in a refrigerator until use. Because of their short expiry date of 3 months, all kits are only produced after being ordered.

If you need more details please do not hesitate to contact us:

Rainer Walz, Ph.D.  
rwalz@sial.com



**Figure 1:** Ready-to-use kit for TOC determination (Cat. No 95451). Kit contains 3 x 40 ml reagent water, 1 x 40 ml system suitability solution and 1 x 40 ml standard solution

# Karl Fischer Titration – Applications in the pharmaceutical industry

Water content determination is mandatory for many materials used in the manufacture of medicines. The Karl Fischer titration has long been prescribed as the standard method of analysis by the leading Pharmacopoeias, like the European (Ph.Eur), the United States (USP) and the Japanese (JP). Several approaches to this technique can be made.

In the latest edition of the European Pharmacopoeia (4th Edition, year 2002), the most prescribed method for water determination is the Karl Fischer titration. However, the pyridine-containing reagent that is recommended is not



Acetone	Lidocaine hydrochloride
Adenosine triphosphate disodium	L(+)-Lysine-1-hydrate
Almond oil	Macrogol cetostearyl ether
Amoxicillin trihydrate	Macrogol stearate
Ampicillin sodium	Maleic acid
Ampicillin trihydrate	D-Mannitol
Benserazide hydrochloride	Methanol
Benzalkonium chloride	Methotrexate
Betamethasone sodium phosphate	Methyl-iso-butyl-ketone
1-Butanol	Nikethamide
2-Butanol	Octyldodecanole
Castor oil	Olive oil
Cellulose Acetate Phthalate	Oxytetracycline hydrochloride
Chlorobutanol-hemihydrate	Peanut oil
Citric acid-1-hydrate	Penicilline G procaine
Citric acid anhydrous	Polyethylene glycol 400
Crospovidon	Polyethylene glycol 4000
Cyclophosphamide	Polyethylene glycol 6000
Dexpanthenol	Polyoxyl 40 hydrogen castorol
Dextrometorphan hydrobromide	Polysorbate 20
Dibutylphthalate	Polysorbate 60
Dichloromethane	Polysorbate 80
Diethylether	Polyvinyl pyrrolidone 25
Di-isopropylether	Potassium citrate
N, N-Dimethylformamide	Potassium Clavulanate
Dimethyl sulfoxide	1,2-Propanediol
Doxycycline hydrate	Quinoline sulfate/Potassium sulfate
Erythromycin ethyl succinate	Saccharin sodium
Esculin	tri-Sodium citrate-2-hydrate
Ethyl acetate	di-Sodium hydrogen phosphate-12-hydrate
Folic acid	Sodium nitroprusside
D(-)-Fructose	Sorbic acid
Gentamicin Sulfate	Sorbitan monooleate
D(+)-Glucose	D(-)-Sorbitol
Glucose monohydrate	Sulfacetamide Sodium
Glycerol	Tetrahydrofuran, stabilized
Glycerol 99%	Tetrahydrofuran
Glyceryl monostearate	Theophylline-Ethylendiamine
n-Hexane	Theophylline-Monohydrate
Hexetidine	Thiamine hydrochloride
Isomalt	Toluene
Isopropanol	Triamcinolone acetonide
Isopropylacetate	Triglyceride (medium-chained)
D(+)-Lactose monohydrate	Valerian root dried extract

available on the market. Furthermore, it is being replaced by a pyridine- and methyl glycol (2-methoxy ethanol)-free reagents, which are less toxic and harmful.

Should there be any doubt concerning this matter, the Ph.Eur informs that "the National Pharmacopoeia prescribe exact methods for the analysis of pharmacopoeia products, which may also be carried out with HYDRANAL® reagents (...). Other methods ... may be used ... providing that the same results .... are obtained".

## Comparison tests

Our HYDRANAL®-Laboratories have verified for you the suitability of HYDRANAL®-Composite 5 to determine the water content according to the Ph.Eur requirements. For this test, the pyridine reagent described in the Ph.Eur II/DAB10, was specially produced. More than 100 pharmaceutical products, listed on **Table 1**, were titrated.

## Applications

One of the advantages of using Karl Fischer titration is the possibility of determining not only the surface water but also the bound water. Many substances release their water slowly or only at high temperatures. In this case, the products are not suitable for a direct Karl Fischer titration. An additional problem could be the low solubility of certain samples in alcohols. Also, many pharmaceutical compounds tend to yield a fading end point, or even no end point at all. If these molecules have nitrogen, add HYDRANAL®-Buffer Base or add HYDRANAL®-Salicylic Acid!

**Table 1**  
Pharmaceutical products titrated using HYDRANAL®-Composite 5 and Reag.Ph.Eur II/DAB10. Detailed protocols are available upon request at [hhoffman@europe.sial.com](mailto:hhoffman@europe.sial.com)

## Penicillins

### (Laboratory Application L166E\*)

Penicillins are a group of fungal metabolites used to treat different kinds of infections caused by bacteria. They act by killing the bacteria or preventing their growth.

Analysis of penicillins, for instance penicillin-G (Figure 1), may present some technical difficulties. Samples also contain penicillin-derivatives such as penicilloic acid (Figure 2) and other hydrolysis products. These contaminants are oxidized by iodine, a side reaction that can be suppressed by titrating in weakly acidic conditions.

The coulometric determination of water is carried out as usual. However, 20 g HYDRANAL®-Salicylic Acid should be used to acidify 100 ml HYDRANAL®-Coulomat AG. For the volumetric method, the titration vessel is filled with 30 ml HYDRANAL®-Methanol Dry or HYDRANAL®-Methanol Rapid and titrated to dryness with HYDRANAL®-Composite 2. A sample of approximately 1g is then added and titrated with HYDRANAL®-Composite 2.

Often, penicillins have limited solubility in alcoholic media. This can be a technical difficulty for Karl Fischer titration. For instance, ampicillin (Figure 3) dissolves poorly in the alcoholic medium and tends to form clumps in the titration vessel. However, it can be dissolved gradually during the course of the titration in HYDRANAL®-Buffer Base (Laboratory Application L422\*). Amoxicillin (Figure 4) dissolves only very slowly in the methanolic working conditions of the Karl Fischer titration. No other solvent could be found to accelerate this process. However, the solubility can be increased by addition of HYDRANAL®-Salicylic Acid (Laboratory Application L352E\*).

Other types of penicillins like penicillin-G-procain, penicillin-G-Na, penicillin-G-K, tetracycline-HCl, and benzylpenicillin-procaine were also successfully analyzed.

## Erythromycin

### (Laboratory Application L242\*)

Erythromycin (Figure 5) is considered the prototype of macrolide antibiotics. It has a similar action spectrum to penicillins, that includes many Gram-positive bacteria. Yet, Staphylococci are often resistant to this drug.

Erythromycin dissolves rapidly in methanol and it can easily be titrated using either the one-component reagent HYDRANAL®-Composite or the two-component system HYDRANAL®-Solvent and HYDRANAL®-Titrant. We have also replaced methanol by HYDRANAL®-Working Medium K. The same results were obtained using both working media.

In case the end point fades, or when no end point at all is found, just add HYDRANAL®-Buffer Acid before the pre-titration. Once the pH value is set to 6, any side reaction will be suppressed.

## Aminophylline and Theophylline

### (Laboratory Application L468\*)

Theophylline (Figure 6) and its hemiethylenediamine complex aminophylline are bronchodilators (Figure 7). These drugs can effectively open the lung passages and are used to treat people with asthma.

Aminophylline does not dissolve readily in the alcoholic media used for Karl Fischer titration. It also tends to produce secondary reactions, depending on the pH in the titration vessel. However, the addition of HYDRANAL®-Salicylic acid to the medium gave excellent results: the water is sufficiently extracted and the secondary reactions are suppressed.

## Calcium folinate (Leucovorin calcium)

### (Laboratory Application L366\*)

Calcium folinate (Figure 8) is a B-vitamin used to decrease the harmful blood effects of different medications. It also can be used to treat some kinds of anemia and, in combination with 5-fluorouracil, to fight cancer.

This substance does not dissolve completely in methanol, but heating and amending the medium with HYDRANAL®-Salicylic Acid improves its solubility.

For any further information please contact:

### USA and Canada

Mr. Doug Clark  
HYDRANAL® - Technical Center  
e-mail: dclark@notesgw.sial.com  
Tel: +1-800- HYDRANAL® (toll-free hotline)

### Europe and Rest of the World

Ms. Helga Hoffmann  
Technical Support HYDRANAL®  
e-mail: hhoffman@europe.sial.com

## \*Laboratory Applications

Please contact our HYDRANAL®-Laboratories (hhoffman@europe.sial.com). We will be glad to send you our Laboratory reports by fax or mail.

You can also find the full list on our website [www.sigma-aldrich.com/Hydranal](http://www.sigma-aldrich.com/Hydranal)

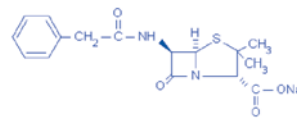


Figure 1  
Penicillin-G Sodium  
(e.g. Cat. No 13752)

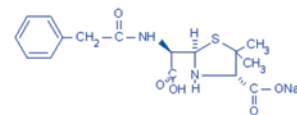


Figure 2  
Penicilloic acid sodium salt

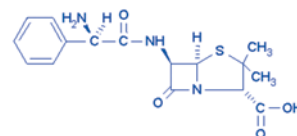


Figure 3  
Ampicillin (e.g. Cat. No 10047)

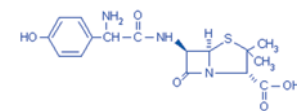


Figure 4  
Amoxicillin (e.g. Cat. No 46060)

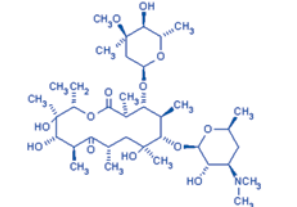


Figure 5  
Erythromycin A (e.g. Cat. No 45673)

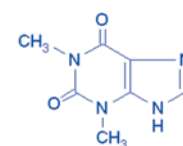


Figure 6  
Theophylline (e.g. Cat. No 88308)

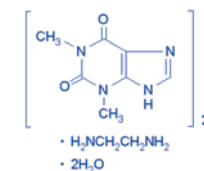


Figure 7  
Aminophylline hydrate  
(e.g. Cat. No 09249)

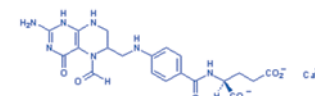


Figure 8  
Calcium folinate  
(e.g. Cat. No 47612)

## Karl Fischer Seminars: Our experience at your fingertips!



Riedel-de Haën\* is the leader in pyridine-free Karl Fischer Titration reagents. Over 24 years of experience has resulted in more than 50 different HYDRANAL® reagents as well as an enormous number of reagents for general use.

Karl Fischer Titration is used by analytical specialists to investigate the water content in products such as pharmaceuticals, chemicals, oils, fats, polymers, cosmetics, foodstuffs and sweets. Their analysis can be quite challenging: secondary reactions take place, solubility problems arise and even moisture in the atmosphere may be significant. Our HYDRANAL®-Laboratory has investigated hundreds of problematic products and worked out suitable applications and troubleshooting protocols.

This experience, together with the knowledge obtained from reagent development, is used to support HYDRANAL® users in many different ways. One of the most helpful ways is with a HYDRANAL® Seminar.

A HYDRANAL® Seminar includes three lectures given by Helga Hoffmann. The first one is *Basics of Karl Fischer Titration*, and it is dedicated to basic procedures and corresponding reagents, equations and the influence of pH. This is followed by *Variants of the Karl Fischer titration*, focusing on problematic samples such as aldehydes, ketones, pharmaceuticals, phenols or strong amines. Finally, *Practical tips for the daily routines* gives an insight on titre determination, influence of drift and sample handling.

The last seminar took place last June 26th at Fluka (Buchs SG, Switzerland). On this occasion other lecturers were also invited. Prof. Dr. Isen-gard from the University of Hohenheim gave a lecture on *Foodstuffs: Sometimes difficult for Karl Fischer titration*. Ms. Regina Schlink (Metrohm Ltd.) lectured about *Automatisation in Karl Fischer titration*. Dr. H.J. Muhr (Mettler-Toledo) presented on *Water determination by coulometric determination*.

During lectures participants are encouraged to ask questions, discuss the analysis of problematic

samples or share their knowledge and experiences. A comment we often hear after a HYDRANAL® Seminar is **Tomorrow I have to try something I learnt today!**

### Next HYDRANAL® Seminars:

October

7. Milan, Italy

9. Roma, Italy

22. Basel, Switzerland

November

5.-6. Seelze, Germany

December

2.-3. Cairo, Egypt

If you are interested in attending a HYDRANAL® Seminar please contact Ms Helga Hoffmann on +49-5137-8238-353 or [hhoffman@europe.sial.com](mailto:hhoffman@europe.sial.com).

### Technical Support

Profit from our experience on Karl Fischer Titration!

We suggest a solution for your analytical problem. If necessary, we can develop an individual method just for you. Our comprehensive application collection makes the daily work easier for HYDRANAL®-users, and is always at your disposal anytime you need.

Please contact us at [hhoffman@europe.sial.com](mailto:hhoffman@europe.sial.com)

\* Riedel-de Haën is a member of the Sigma-Aldrich Family

## Your Sigma-Aldrich Service Partners

### **Austria** Wien

Tel. 01-605 81 10  
Fax 01-605 81 20  
Email: sigma@sigma.co.at

### **Australia** Castle Hill

Free Tel. 1800 800 097  
Free Fax 1800 800 096  
Free Tel. 0800 936 666 (New Zealand)  
Free Fax 0800 937 777 (New Zealand)  
Email: ausmail@sial.com

### **Belgium/Luxembourg** Bornem

Free Tel. 0800 147 47  
Free Fax 0800 147 45  
Email: becustsv@europe.sial.com

### **Canada** Oakville

Free Tel. 1-800-565-1400  
Free Fax 1-800-265-3858  
Email: canada@sial.com

### **Czech Republic** Praha

Tel. 246 003 251  
Fax 246 003 291  
Email: CZECustSV@europe.sial.com

### **Denmark** Vallensbæk Strand

Tel. 43 56 59 10  
Fax 43 56 59 05  
Email: denorder@europe.sial.com

### **Finland** Helsinki

Tel. 09-350 9250  
Fax 09-350 92555  
Email: finorder@europe.sial.com

### **France** St. Quentin Fallavier

Tél. 0800 21 1408 (appel gratuit)  
Fax 0800 03 1052 (appel gratuit)  
Email: fradvsv@europe.sial.com

### **Germany** Taufkirchen

Free Tel. 0800 51 55 000  
Free Fax 0800 64 90 000  
Email: deorders@europe.sial.com

### **Greece** Ilioupoli, Athens

Tel. 210-994 8010  
Fax 210-994 3831  
Email: GRCustSV@europe.sial.com

### **Hungary** Budapest

Tel. (06-1) 269-6474  
Fax (06-1) 235-9068  
Email: info@sigma.sial.hu

### **Ireland** Dublin

Tel. 01-404-1900  
Fax 01-404-1910  
Email: EICustsv@europe.sial.com

### **Israel** Rehovot

Tel. 08-9484-222  
Fax 08-9484-200  
Email: sigisr@sigma.co.il

### **Italy** Milano

Tel. 02-33417-310  
Fax 02-38010-737  
Email: itorder@europe.sial.com

### **Norway** Oslo

Tel. 23 17 60 00  
Fax 23 17 60 10  
Email: nororder@sial.com

### **Poland** Poznań

Tel. 061-829 01 00  
Fax 061-829 01 20  
Email: plcustsv@europe.sial.com

### **Portugal** Sintra

Tel. 800 20 21 80 (Gratuito)  
Fax 800 20 21 78 (Gratuito)  
Email: poorders@europe.sial.com

### **South Africa** Johannesburg

Tel. 011-979 1188  
Fax 011-979 1119  
Email: rsa@sial.com

### **Spain** Tres Cantos, Madrid

Tel. 900 10 13 76 (Gratuito)  
Fax 900 10 20 28 (Gratuito)  
Email: esorders@europe.sial.com

### **Sweden** Stockholm

Tel. 020-35 05 10  
Fax 020-35 25 22  
Email: sweorder@europe.sial.com

### **Switzerland** Buchs

Free Tel. 0800 80 00 80  
Free Fax 0800 80 00 81  
Email: Fluka@sial.com

### **The Netherlands** Zwijndrecht

Free Tel. 0800 022 9088  
Free Fax 0800 022 9089  
Email: nlcustsv@europe.sial.com

### **United Kingdom** Gillingham

Free Tel. 0800 717181  
Free Fax 0800 378785  
Email: ukcustsv@europe.sial.com

### **USA** Milwaukee

Free Tel. 1-800-558-9160  
Free Fax 1-800-962-9591  
Email: aldrich@sial.com

Do you want to receive the whole series?  
Please, subscribe at [www.sigma-aldrich.com/subscription](http://www.sigma-aldrich.com/subscription)

Analytical Standards  
HYDRANAL®



HYDRANAL® Review  
New Standards  
Volumetric Solutions  
Aquanal® Professional

Derivatization Reagents



Food Analysis  
Clinical Chemistry  
Speciality Solvents  
Microbiology  
Coulometry

HYDRANAL®-E



Certified  
Reference Calibration  
Standards

[www.sigma-aldrich.com/analytix](http://www.sigma-aldrich.com/analytix)

The Sigma-Aldrich Family



Biochemicals and Reagents for Life Science Research



Organics and Inorganics for Chemical Synthesis



Speciality Chemicals and Analytical Reagents for Research



Laboratory Chemicals and Reagents for Research and Analysis



Chromatography Products for Analysis and Purification



Promoting Research and Discovery