

Analytix

Issue 1 • 2009



Headspace Solvents & Standards



- Solvents, Standards and Columns for GC-MS
- Analysis of Melamine in Milk
- ¹³C-marked Mycotoxin Derivatives
- Inorganic Trace Analysis Brochure
- Additional *TraceCERT*[®] Standards
- New Food Dye Standards
- New HYDRANAL[®] Applications

Special application solvents



Michael Kiselewsky
Product Manager
Analytical Reagents

Dear Colleague,

High-purity solvents have become a matter of course, while highly sensitive analytical techniques like LC-MS, GC-MS, UPLC and many others are nowadays mainstream routine business. Moreover, new techniques will shortly gain importance for routine investigations, like LC-NMR, which also have a high-end demand in terms of purity and reliability.

However, a modern solvent needs to match many different requirements. Scientists all over the world take into account that the quality of a solvent can affect instrument downtime, troubleshooting and challenges in analytical business. By using high-purity solvents, which are dedicated for a certain analytical technique or instrument, an operator can optimise a result and bring his instrument to the peak of its performance. A solvent can be important to ideally utilise a certain instrument, and it is also part of the development of the detection limit within method development.

Sigma-Aldrich offers a wide range of solvents and reagents which meet the demands of each of these modern techniques. We will also continue to contribute products that meet the daily demands of the challenging analytical business in the future, with respect to technical development. The portfolio of LC-MS application-tested products is a good example of the successful history of Sigma-Aldrich research. Starting with the introduction of metal-free solvents in order

to cover the special demand of the MS detection environment, we now offer a broad range of solvents, blends and additives.

In addition to the development of instrument manufacturers, the restriction and regulation of the authorities has set new benchmarks in various fields of science and business. For instance, our pharmaceutical customers need to check the residue of volatile solvents that have been used for the manufacturing process of drug production. Unwanted residues can hinder medical approval and cause adverse effects. To match requirements, we recently introduced a new line of products that have been tested for static Headspace GC applications, and we also included the specification requirements given by customer feedback. These low-volatile solvents enable the detection of highly volatile impurities. Details about our new product line are available at sigma-aldrich.com/gc-hs

Future challenges will cause further modification and even new products that we are currently not aware of. We are proud to deal with our customers face to face, and we value all personal contact as it enables us better to meet your requirements in the innovative, dynamic world of modern analytics.

With kind regards,

A handwritten signature in black ink, appearing to read 'M. Kiselewsky', with a stylized flourish at the end.

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

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Residual Analysis Optimised for Static Headspace GC Applications

Solvents, Standards and GC columns meet the requirements of Ph.Eur., USP and ICH guidelines

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Static headspace GC (GC-SH) is a technique used to concentrate volatile analytes prior to analysis. It can improve detection of low levels of volatile analytes and minimises matrix interference by eliminating the need to inject the sample directly. An important application of GC-SH is for the determination of residual volatile organic impurities in active drug substances or excipients in drug formulations. Other consumer-oriented applications include the detection of residual solvents in foods, dietary supplements and packaging materials.

GC-SH is a relatively straightforward technique, and the methodology, as it applies to residual solvents in pharmaceuticals, is described and validated in specific monographs [1-3]. These guidelines recommend both the types of solvents and the acceptable levels of residual solvents in pharmaceuticals and formulations to help ensure consumer safety.

New Headspace Grade Solvents

When developing a GC-SH method, such parameters as sample solvent, extraction temperature, extraction time, sample volume and headspace volume are optimised [4, 5]. Because the composition and purity of the sample solvent have significant effects on the recovery and quality of the chromatogram (see **Figure 1**), we have developed solvents specifically for GC-SH applications. Their purity and handling specifications meet the requirements of European Pharmacopoeia (Ph.Eur.) and United States Pharmacopoeia (USP), as well as ICH guidelines. The new GC-SH line includes water and

three of the most commonly used organic solvents: dimethyl sulphoxide (DMSO), N,N-dimethylformamide and N,N-dimethylacetamide. N,N-dimethylformamide and dimethyl sulphoxide are specified in Ph.Eur. and USP for water-insoluble substances. Water is the preferred solvent for water-soluble solutions, as described in Ph.Eur. and USP monographs. All solvents are microfiltered at 0.2 µm and packed under inert gas for longer shelf life.

USP and Ph.Eur. Residual Solvent Standards

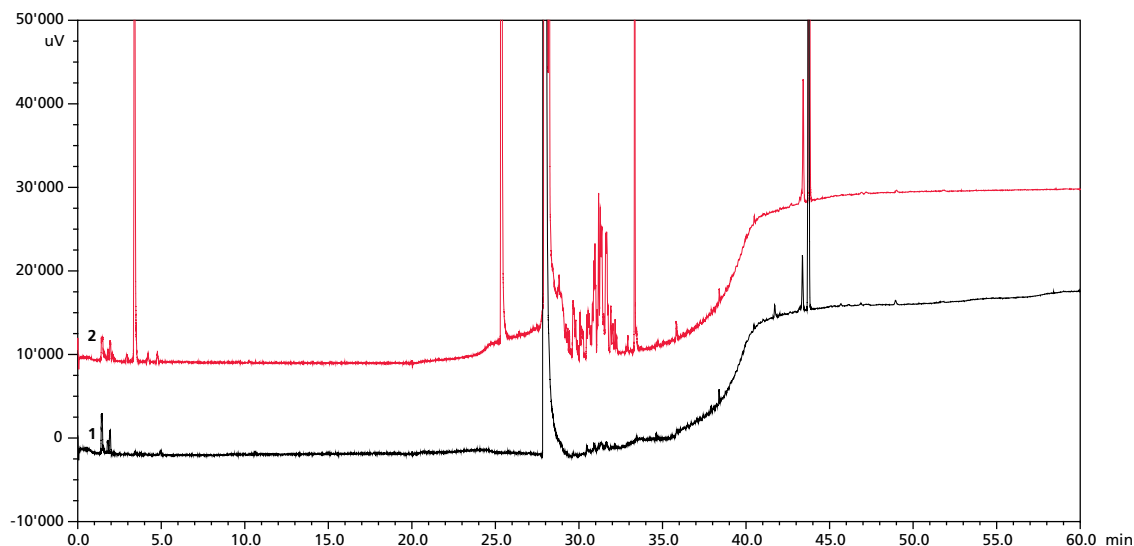
The residual solvents described in the ICH guidelines are analysed by direct injection of three separate mixtures, divided by their individual classes (I, II or III). We have developed calibration standards specifically for this application, and can tailor them in combinations and concentrations to meet specific customer needs through our Custom Chemical Standards Program.

Capillary GC Columns for Residual Solvent Analysis [6]

No single column is capable of separating all 61 solvents listed in the ICH guidelines. Here, we present data indicating the elution order and separation of the 61 listed solvents on the Supelco OVI-G43.

The Supelco GC column **OVI-G43** is specially prepared and tested to meet the requirements of USP Method 467 and the Ph.Eur. general method for determining residual solvents in pharmaceutical preparations. This column will separate residual solvents for research purposes or

Figure 1 Headspace gas chromatogram of two DMSO grades: GC-HS grade (black trace) and conventional grade (red trace) [2]



qualitative analysis. The USP and European Pharmacopoeia methods also specify using a deactivated 5 m guard column. In order to ensure analysis under optimum conditions, we strongly suggest the use of a deactivated guard column (Cat. no. 25339) with the OVI-G43 column. For further information, please consult Supelco Technical Bulletin 933 (sigma-aldrich.com/supelco)

References

- 1] United States Pharmacopeia, 31st Edition (2008), <467> Residual Solvents.
- 2] Ph.Eur. 6.0 (2008) Method 2.4.24, Identification and control of residual solvents.

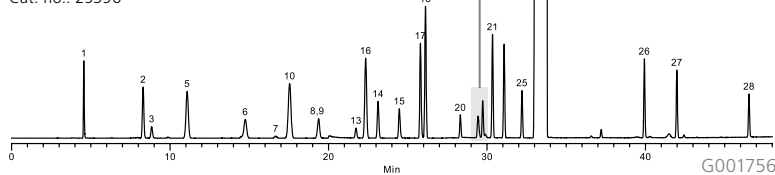
- 3] ICH Guideline Q3C, Impurities: Guideline for Residual Solvents, *The Fourth International Conference on Harmonization*, July 17, 1997.
- 4] Camarasu, C. C. Residual Solvents Determination in Drug Products by Static Headspace-Gas Chromatography. *Chromatographia* **2002**, *56*, S137–S143.
- 5] Lee, C. R.; Nguyen van Dau, C.; Krstulović, A. M. Artefact formation in the determination of residual solvents according to a method of the European Pharmacopoeia. *Int. J. Pharm.* **2000**, *195*, 159–169.
- 6] Supelco Technical Bulletin 933. Capillary GC Column Choices for Residual Solvent Analyses Using Direct Injection or Solid Phase Microextraction (SPME).

Figure 2 Solvents – Pharmaceutical Solvents, Class II, III (GC) using a GC column OVI-G43

Oven: 35 °C, hold 15 min., 5 °C/min to 200 °C
Inj.: 225 °C
Det.: FID, 250 °C
Flow: 30 cm/sec (constant) He at 35 °C
Inject.: 1 µL, 33:1 split
Liner: single taper
Sample: Class I: 1000-5000 ppm In DMSO

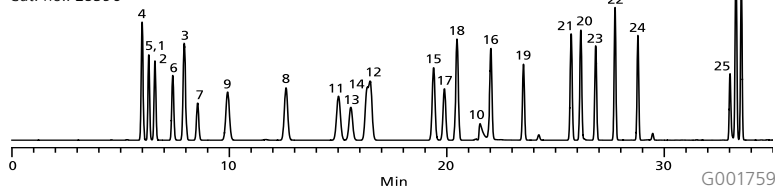
Class 2 Solvents on OVI-G43

Column: 30 m x 0.53 mm ID x 3.0 µm w/5 m intermediate polarity guard
Cat. no.: 25396



Class 3 Solvents on OVI-G43

Column: 30 m x 0.53 mm ID x 3.0 µm w/5 m intermediate polarity guard
Cat. no.: 25396



Class 2 Solvents on OVI-G43

Peak #	Identification
1	Methanol
2	Acetonitrile
3	Methylene chloride
4	Nitromethane
5	Hexane
6	cis-1,2-Dichloroethylene
7	Chloroform
8	2-Methoxyethanol
9	1,2-Dimethoxyethane
10	Cyclohexane
11	Ethylene glycol
12	Formamide
13	Trichloroethylene
14	1,4-Dioxane
15	2-Ethoxyethanol
16	Methylcyclohexane
17	Pyridine
18	Toluene
19	Dimethylformamide
20	Methyl butyl ketone
21	Chlorobenzene
22	Dimethylacetamide
23	m-Xylene
24	p-Xylene
25	o-Xylene
26	n-Methylpyrrolidone
27	Tetralin
28	Sulpholane

Class 3 Solvents on OVI-G43

Peak #	Identification
1	Ethanol
2	Acetone
3	2-Propanol
4	Pentane
5	Ethyl ether
6	Ethyl formate
7	Methyl acetate
8	1-Propanol
9	Methyl-t-butyl ether
10	Acetic acid
11	2-Butanone
12	sec-Butanol
13	Ethyl acetate
14	Tetrahydrofuran
15	iso-Butanol
16	n-Butanol
17	Isopropyl acetate
18	Heptane
19	Propyl acetate
20	Isoamyl alcohol
21	4-Methyl-2-pentanone
22	n-Amyl alcohol
23	Isobutyl acetate
24	Butyl acetate
25	Dimethyl sulphoxide 2
26	Anisole
27	Cumene

Product table for GC-MS solvents

Cat. no.	Brand	Description	Boiling point	Pack size
44901	Fluka	N,N-Dimethylacetamide, puriss. p.a. for GC-MS	166 °C	1 L
51779	Fluka	Dimethyl sulphoxide, puriss. p.a. for GC-MS	189 °C	1 L
51781	Fluka	N,N-Dimethylformamide, puriss. p.a. for GC-MS	153 °C	1 L
NEW 67484	Fluka	1,3-Dimethyl-2-imidazolidinone puriss. p.a., for GC-MS	225 °C	1 L
53463	Fluka	Water, puriss. p.a. for GC-MS	100 °C	1 L

Product table for Residual Solvent Standards (selection)

Cat. no.	Brand	Description	Pack size
861255	Supelco	Class 1 Residual Solvent Standard in DMSO	25 x 2.5 mL
861256	Supelco	Class 2 Residual Solvent Mix in DMSO	25 x 2.5 mL
32978	Fluka	Ph Eur 5.0 GC Calibration Solution for Residual Solvents in DMSO	2 mL
47632-U	Supelco	International USP 467 Mix	1 mL
47545-U	Supelco	USP 467 OVI Mix 1, 24th ed.	1 mL
4M4626-U	Supelco	USP 467 OVI Mix 2 w/o Benzene each component in DMSO	5x1 mL
47546-U	Supelco	USP 467 OVI Mix 2, 24th ed. in DMSO	1 mL

If you are interested in customised standards, please feel free to contact us by e-mail at customstandards@sial.com

Product table for GC columns for Residual Solvent Analysis by headspace GC (selection)

Cat. no.	Brand	Description	Pack size
25396	Supelco	OVI-G43, L x I.D. 30 m x 0.53 mm, df 3.00 µm	1 each
25339	Supelco	OVI-G43 Guard Column, L x I.D. 5 m x 0.53 mm	1 each

Analysis of Melamine in Milk

Analytix Report – November 3, 2008

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Introduction

Recent news stories from China [1] have reported incidents of milk, milk products and infant formula tainted with melamine, a substance used to make plastics and fertilisers. It is not approved for use in foods, but health officials reported that melamine was added to these dairy products to inflate their apparent protein content. Several infants died and thousands of children were hospitalised after suffering kidney failure from ingesting the melamine-adulterated milk. Laboratories worldwide are now developing methods to test products for melamine.

Milk is a complex sample matrix, demanding careful sample prep to ensure valid melamine-content results. Sigma-Aldrich offers high-purity melamine and cyanuric acid chemicals that allow customers to prepare their own standards to meet their particular application needs, as well as Discovery solid phase extraction products to ensure clean samples and Ascentis HILIC columns to give superior chromatographic results.

Discussion

Sample extraction

A 5 mL aliquot of milk was spiked with melamine standard (Fluka Cat. no. 52549). Next, 5 mL of phosphate buffer (100mM, pH 2.5) and 1 mL acetonitrile were added to the sample and set in an ultrasonic bath for 5 minutes. The sample was centrifuged at 3500 rpm for 10 minutes and the middle supernatant separated from the top and the bottom layers.

Sample SPE preparation

Supelco Discovery DSC-SCX 500 mg/6 ml SPE tubes (Cat. no. 52688-U) were used for further sample cleanup. The SPE tubes were conditioned with 3 ml methanol and 3 ml 100mM phosphate buffer (pH 2.5). Then 2.2 mL of the resulting supernatant was loaded into the SPE tube and washed with 3 ml of phosphate buffer (100mM, pH 3) and 3 ml of methanol. The melamine was eluted with 4 ml 5% ammonia in methanol.

Q1	Q3	Declustering potential (DP)	Entrance potential (EP)	Collision energy (CE)	Collision cell exit potential (CXP)
127	85.0	46	4	31	4
127	68.0	41	4	31	4

Other instrument parameters were as follows:

Curtain Gas: 20 Ion Spray Voltage: 5000
Gas 1: 20 Temperature: 350 °C
Gas 2: 40

The elution solvent was evaporated to dryness at 50 °C in a water bath under a flow of nitrogen at 5 psi. The samples were reconstituted into 1 ml of the LC mobile phase A (see conditions below).

LC-MS-MS method

Melamine (**Figure 1**) is a polar molecule with a pKa of 5.6 and a Log P value of -1.37 [2], making it a good candidate for Aqueous Normal Phase (ANP) chromatography. In ANP, a polar hydrophilic analyte partitions between a relatively polar stationary phase and a relatively non-polar mobile phase. ANP is commonly referred to as HILIC, but the term HILIC (Hydrophilic Interaction Chromatography) implies a mechanism that is one of several that may be operative under ANP conditions. Determining melamine content using the HILIC mode, run on Supelco's Ascentis® Express HILIC column (Cat. no. 53939-U), gives better retention of the polar molecule than conventional reversed-phase chromatography.

This HILIC mechanism describes the process of preferential solvation of the polar stationary phase with the aqueous component of the mobile phase and subsequent depletion of the mobile phase of water. This sets up a biphasic system where there is a semi-immobilised layer of water near the surface and an organic-rich mobile phase layer. A polar compound may then partition from the moving organic-rich mobile phase into the stagnant aqueous solvent near the surface. Water then becomes the "strong" solvent and analytes generally elute (assuming HILIC is the dominant mechanism) in order of "decreasing" hydrophobicity (a lower log P, indicating a more polar molecule, and consequently more retention).

Detection for melamine was accomplished using an ABI/MDS Sciex 3200 Q Trap MS/MS with the parameters below. Two Multiple Reaction Monitoring (MRM) transitions were monitored; 127/85 MRM was used for quantitation.

Quantitation

An external calibration curve was used for melamine. External calibration standards were prepared in the mobile phase. No matrix-matched standards were used.

Results and Review

Table 1 Recovery of melamine from milk samples (versus external standards in buffer)

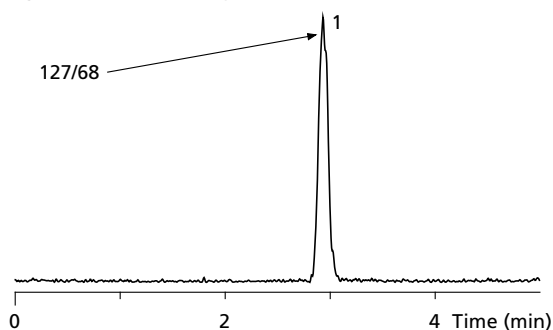
Spiked concentration	Recovery (%)	Standard deviation (n=3)
10 ng/ml	71 ^a	3
100 ng/ml	85	7
250 ng/ml	82	8
500 ng/ml	83	2

^a Corrected for background, the background of 2.4 ng/ml possibly comes from plastic ware.

Because melamine is often present together with cyanuric acid and the two form a very stable complex, we also tested the melamine from milk in the presence of cyanuric acid. Both melamine and cyanuric acid were mixed at 1000 ng/ml in water and the samples kept for 30 minutes. Then the milk was spiked at 100 ng/ml of the mixture, and the subsequent analysis yielded a 94 % melamine recovery rate.

We also examined the ionisation effect of the cleaned sample by spiking melamine into a cleaned blank milk sample, followed by analysis with LC-MS-MS. This test can be an indicator of the effectiveness of sample cleanup; the cleaned sample should have no effect on the ionisation of the analyte of interest. The spiked concentration was 100 ng/mL, and the found concentration was 105 ng/mL, indicating no ionisation effects from the cleaned sample.

Figure 2 LC-MS-MS Analysis of Melamine Extracted from Milk Spiked at 100 ng/mL



LC-MS-MS Conditions:

Instrument: Agilent 1100 HPLC system with ABI/MDS Sciex 3200 Q Trap MS/MS
 Column: Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 μm particles
 Mobile Phase A: 10 mM ammonium formate in 90:10 acetonitrile:water
 Mobile Phase B: 10 mM ammonium formate in 70:30 acetonitrile:water

Product listing

Brand	Cat. no.	Product name
Supelco	52688-U	Discovery DSC-SCX SPE Tube, bed weight 500 mg, volume 6mL, pk of 30
Supelco	53939-U	Ascentis® Express HILIC HPLC Column, 2.7 μm particle size, 10 cm x 2.1 mm ID
Fluka	52549	Melamine standard, 99 % purity
Fluka	16614	Cyanuric acid standard, 98 % purity

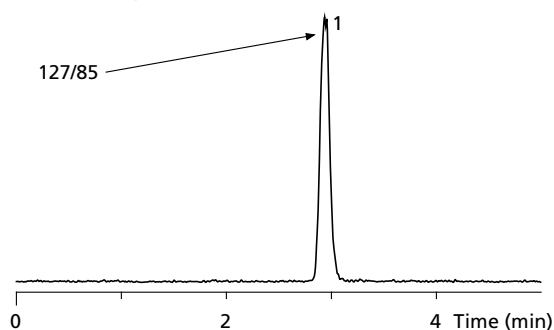
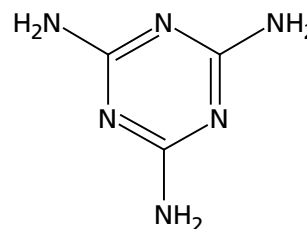
Conclusion

Using Supelco Discovery DSC-SCX SPE cartridges, a sample preparation method for the extraction of melamine from milk was developed. Good recoveries were seen for the melamine at concentrations between 10 ng/mL and 500 ng/mL. Standards developed with our high-purity melamine and cyanuric acid chemicals ensure valid results. Finally, HILIC chromatography with the Ascentis Express HILIC HPLC column gave good retention of the analyte. A further advantage of the HILIC mode is the enhanced MS response due to the high concentration of organic in the mobile phase.

References:

- Spears, L.; Lawrence, D. China Milk Scandal Widens as Melamine Found in Yogurt. *Bloomberg* [Online] 2008, updated Sept 16, 2008. <http://www.bloomberg.com/apps/news?pid=20601087&sid=aPYE0JvTLHQA&refer=home> (accessed Nov 7, 2008).
- ACD/ LogP DB, version 11.01, Advanced Chemistry Development, Inc., Toronto ON, Canada, www.acdlabs.com, 2007.

Figure 1 Structure of Melamine



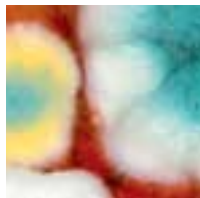
Gradient:

Time	Flow	%A	%B
0	200 μL/min	100	0
5	400 μL/min	0	100
10	400 μL/min	100	0
15	200 μL/min	100	0

Temperature: 30 °C
 Flow Rate: as above
 Injection Volume: 2 μL
 Sample: 100 ng/ml of melamine from milk
 Detection: MS-MS with MRM at 127 -> 85 and 127 -> 68

A New GC-MS Method for Mycotoxin Analysis Using ^{13}C -marked Mycotoxin Derivatives

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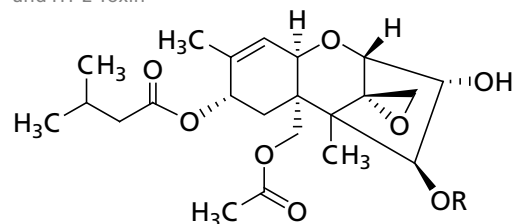


Mycotoxins are toxic metabolites of fungi such as moulds. Fungal infection of crops can lead to mycotoxin contamination of human food, either directly or through its use as livestock feed.

Some mycotoxins show a particularly high toxicity towards humans, such as the type A Trichothecene mycotoxins T-2 toxin and HT-2 toxin (**Figure 1**). For these reasons, regulations governing the mycotoxin content of food are crucial to public health. However, such regulations depend on the availability of sufficiently sensitive and accurate analytical methods for the testing of foodstuffs.

Andreas Breidbach [1] and Wolfgang Brodacz [2,3,4] developed a new GC-MS method using fully ^{13}C isotope-labelled analogues of T-2 toxin and HT-2 toxin that allows for the detection of these toxins at concentrations as low as 2–5 ppb. Both the $^{13}\text{C}_{24}$ T-2 and the $^{13}\text{C}_{22}$ HT-2 toxins are now available from Sigma-Aldrich under our Fluka brand.

Figure 1 Molecular structure of the Trichothecenes T-2 Toxin and HT-2 Toxin

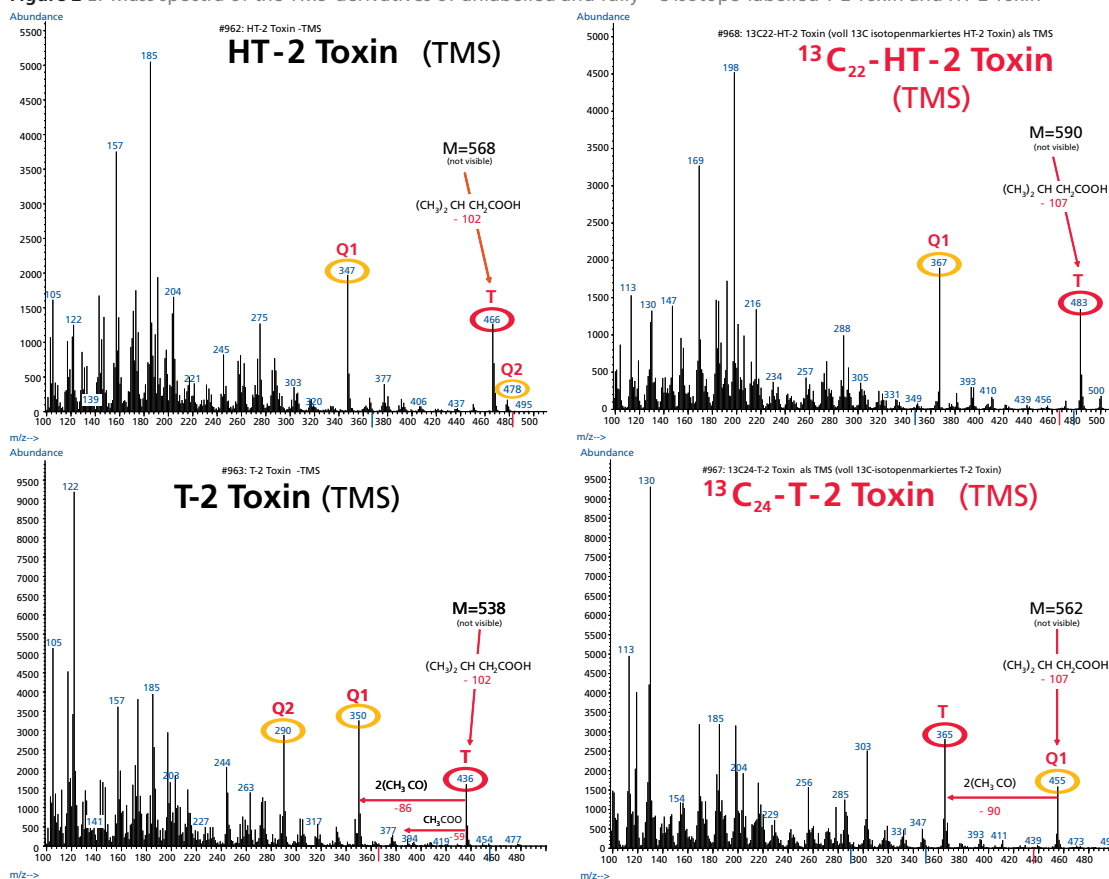


T-2 Toxin: R = Ac
HT-2 Toxin: R = H

^{13}C Isotope-labelled Standards

Fully ^{13}C isotope-labelled mycotoxins open the door for isotopic dilution mass spectrometry (IDMS). In this technique, the precision and accuracy of analytical measurements can be significantly increased, and sample handling is simplified. IDMS takes advantage of the fact that the chemical and physical properties of ^{13}C isotope-labelled analogues are nearly identical to those of non-labelled analytes. This means that their behaviour in sample

Figure 2 EI-Mass spectra of the TMS-derivatives of unlabelled and fully ^{13}C isotope-labelled T-2 Toxin and HT-2 Toxin



workup is essentially the same, but the labelled and non-labelled analogues can still be distinguished by mass spectrometry (**Figure 2**). The standard is added right after the sample extraction, thus matrix effects as well as inaccuracies of the following preparation steps are compensated for.

Silylation Using MSTFA

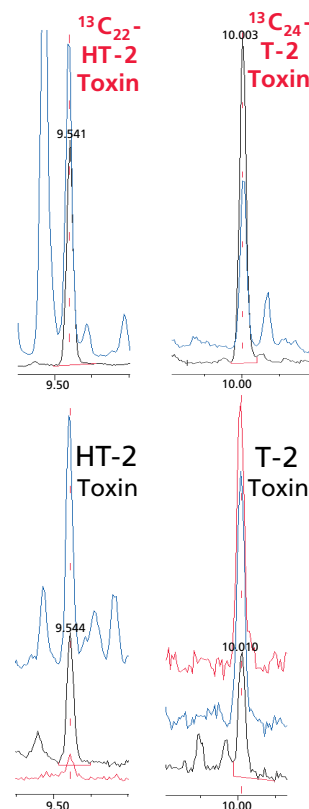
Prior to conventional GC analysis, analytes have to be converted into volatile compounds by silylation. Then the excess of the silylating reagent has to be quenched by water followed by a reextraction of the analyte. However, by using N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing 1 % trimethylchlorosilane as a silylating reagent, one can eliminate the water-quenching and the reextraction steps because the relatively low boiling point (131 °C) allows for the use of MSTFA as a solvent for splitless injection GC.

GC-MS Analysis

For GC analysis, a robust stationary phase with medium polarity such as a Poly(Diphenyl 35 %)/ Dimethylsiloxane 65 %) phase is required to withstand the effects of the silylating agent and heating. A very good separation of T-2 toxin and HT toxin can be achieved with the application of a fast-temperature programme. MS detection with selected-ion-monitoring (SIM) enables detection limits in the range of 2–5 ppb for HT-2 toxin and T-2 toxin, even in complex matrixes. As claimed by EU guideline 96/23/EG, the identities of the toxins were confirmed not only by the retention time but also by the three SIM ions. A qualifier was measured for each internal standard to ensure peak purity (**Figure 3**).

The high price of fully ^{13}C isotope-labelled standards often acts as a deterrent for the use of IDMS. However, the quality of analysis far outweighs the higher price. Furthermore, sample size and quantity of internal standards required can be kept to a minimum with a well-designed method, as quantitative handling after addition of the standard is not critical.

Figure 3 SIM chromatograms of a barley sample contaminated at very low levels. (Target T=black; 1. Qualifier Q1=blue; 2. Qualifier Q2=red). HT-2 Toxin (5.3 µg/kg; T=466 Q1=347 Q2=478); T-2 Toxin (2.4 µg/kg; T=436 Q1=350 Q2=290); $^{13}\text{C}_{22}$ HT-2 Toxin (T=483 Q1=367); $^{13}\text{C}_{24}$ T-2 Toxin (T=365 Q1=455)



References:

- 1] Breidbach, A.; Povilaityte, V.; Mischke, C.; Doncheva, I.; Stroka, J.; „T-2 and HT-2 by GC/MS for official food control – a validated method“; Poster anl. 29. Mykotoxin-Workshop 14.-16.05. 2007 Fellbach, Germany.
- 2] Brodacz, W. Isotopenverdünnungsanalytik von Mykotoxinen mit GC/MSD. *LaborPraxis*. 2007, 9, 46–49.
- 3] Brodacz, W. Entwicklung optimierter GC-Trennungen in der Mykotoxinanalytik. *LaborPraxis*. 2004, 6, 26–28.
- 4] Brodacz, W. Stabilisotopenverdünnungs-GC/MS: Hochtoxischen Mykotoxinen auf der Spur. *Chemiereport*. 2008, 1, 32–33.

Acknowledgements: This article was developed in cooperation with Wolfgang Brodacz of the Austrian Agency for Health and Food Safety (AGES) in Linz, Austria.

Table 1 Suggested Materials

Cat. no.	Brand	Description	Pack size
69478	Fluka	MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) with 1 % trimethylchlorosilane	5 ml, 25 ml
33892	Fluka	T-2 Toxin- $^{13}\text{C}_{24}$ OEKANAL Solution 25 µg/ml in acetonitrile	1 ml
33842	Fluka	HT-2 Toxin- $^{13}\text{C}_{22}$ OEKANAL Solution 25 µg/ml in acetonitrile	1 ml

Table 2 Other ^{13}C -isotope labelled mycotoxins available from Sigma Aldrich

Cat. no.	Brand	Description	Pack size
34128	Fluka	Deoxynivalenol- $^{13}\text{C}_{15}$ OEKANAL Solution 25 µg/ml in acetonitrile	1 ml
33621	Fluka	Fumonisin B1- $^{13}\text{C}_{34}$ OEKANAL Solution 25 µg/ml in acetonitrile	1 ml
33416	Fluka	Ochratoxin A- $^{13}\text{C}_{20}$ OEKANAL Solution 10 µg/ml in acetonitrile	1 ml

New TraceCERT® Single- and Multi-element Standards for AAS, ICP and IC

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Jürg Wüthrich, Senior Scientist R&D Europe juerg.wuethrich@sial.com

The standards of our *TraceCERT* line are developed and produced according to ISO 17025 and ISO Guide 34 [1, 2], the highest achievable quality standard for producers of certified reference materials. Produced using the highest-purity starting materials available, *TraceCERT* standards are traceable to at least two independent references (i.e., NIST, BAM or SI unit kg) and are shipped with comprehensive documentation including a proper uncertainty calculation, expiration date and storing/handling instructions. Our ICP standards have low uncertainties and a shelf life of four years, thanks to our highly sophisticated packaging (coated aluminium bag). The ICP certificates list up to 70 trace impurities.

To better serve our customers, we have expanded our offering of AAS and ICP single-element standards, introducing 30 new spectrometric standards over the last several months, including 15



new elements (see **Table 1**). Furthermore, we present new multi-element solutions for ICP (see **Table 2**) and cation standards for IC (see **Table 3**). For the complete *TraceCERT* product offering and for more information on the *TraceCERT* line, please visit us on the web at sigma-aldrich.com/tracecert

Table 1 New Fluka-branded *TraceCERT* single-element AAS and ICP standards (1000 mg/L)

Cat. no.	Description	Pack size	Cat. no.	Description	Pack size
94117	Antimony Standard for AAS	250 mL	73495	Antimony Standard for ICP	100 mL
41856	Beryllium Standard for AAS	250 mL	51985	Beryllium Standard for ICP	100 mL
76668	Bismuth Standard for AAS	250 mL	05719	Bismuth Standard for ICP	100 mL
53378	Cerium Standard for AAS	250 mL	16734	Cerium Standard for ICP	100 mL
67717	Cesium Standard for AAS	250 mL	96664	Cesium Standard for ICP	100 mL
52874	Gallium Standard for AAS	100 mL	16639	Gallium Standard for ICP	100 mL
42225	Indium Standard for AAS	100 mL	00734	Indium Standard for ICP	100 mL
59916	Lithium Standard for AAS	250 mL	12292	Lithium Standard for ICP	100 mL
67210	Molybdenum Standard for AAS	250 mL	68780	Molybdenum Standard for ICP	100 mL
78437	Palladium Standard for AAS	100 mL	77091	Palladium Standard for ICP	100 mL
47037	Platinum Standard for AAS	100 mL	19078	Platinum Standard for ICP	100 mL
11561	Rhodium Standard for AAS	100 mL	04736	Rhodium Standard for ICP	100 mL
55727	Rubidium Standard for AAS	100 mL	01444	Rubidium Standard for ICP	100 mL
16259	Silicon Standard for AAS	250 mL	15747	Silicon Standard for ICP	100 mL
02334	Vanadium Standard for AAS	250 mL	18399	Vanadium Standard for ICP	100 mL

Table 2 New Fluka-branded ready-to-use TraceCERT Multi-element calibration solutions

Cat. no.	Description	Pack size
90243	Multi-element standard solution I for ICP (in 10 % HNO ₃) Ag, Ba, Ca, Cd, Co, Cu, Fe, Mg, Mn, Sr, Zn: 10 mg/L each Al, B, Cr, Li, Mo, Na, Ni, Ti: 50 mg/L each Bi, K, Pb: 100 mg/L each	100 mL
49596	Multi-element standard solution III for ICP (in 5 % HNO ₃) K: 200 mg/L Mg: 400 mg/L Na: 1000 mg/L Ca: 2000 mg/L	100 mL
51844	Multi-element standard solution IV for ICP (in 10 % HNO ₃) Be, Cd, Co, Mn: 10 mg/L each Cr, Cu, Ni: 20 mg/L each Al, As, Ba, Pb, V: 40 mg/L each B, Fe, Se, Ti, Zn: 100 mg/L each	100 mL
54704	Multi-element standard solution V for ICP (in 10 % HNO ₃) Ag, Al, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mg, Mn, Mo, Ni, Pb, Rb, Sr, Ti, V, Zn: 10 mg/L each Ca, Fe, Ca, Na: 100 mg/L each	100 mL

Table 3 New Fluka-branded TraceCERT single-ion standards for IC (1000 mg/L) in ≤ 0.1 % nitric acid. Na and K are in water

Cat. no.	Description	Pack size
39865	Calcium Standard for IC	100 mL
40786	Copper Standard for IC	100 mL
42151	Strontium Standard for IC	100 mL
42637	Nickel Standard for IC	100 mL
43492	Sodium Standard for IC	100 mL
49594	Cobalt Standard for IC	100 mL
51439	Manganese Standard for IC	100 mL
51777	Lead Standard for IC	100 mL
53337	Potassium Standard for IC	100 mL
59878	Lithium Standard for IC	100 mL
67902	Zinc Standard for IC	100 mL
69679	Cadmium Standard for IC	100 mL
87142	Barium Standard for IC	100 mL
89441	Magnesium Standard for IC	100 mL


References:

- 1) TraceCERT Traceable Certified Reference Materials. *Analytix*, Vol 5, 2006; *Analytix* Vol 1–4, 2007.
- 2) Double Accreditation brings a new class of CRMs. *Analytix*, Vol 2, 2008.



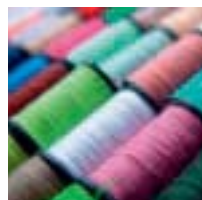
The new LC-MS brochure

All you need to know about mobile phases, blends and additives for LC-MS applications. View our entire portfolio of products specially tested for LC-MS, and learn tips and tricks to help you in your daily tasks. Get your free copy on our website sigma-aldrich.com, or order it by ticking the box on the business reply card of this Analytix, or contact your local sales representative.



Industrial Dyes with Potential for Genotoxicity/Carcinogenicity

New standards for the reliable analysis of these illicit compounds



In Analytix 4 (2008), we introduced a new group of standards, Sudan dyes, which are banned as food additives worldwide [1] but are sometimes still used illicitly to enhance and maintain the colour of food, especially chili and chili-derived products.

Since May 2005, industrial and other kinds of dyes have also been found in food, despite the fact that these compounds are not authorised as food colours by the European Parliament and Council Directive 94/36/EC on colours for use in foodstuff. As such, Sigma-Aldrich offers a wide variety of analytical standards useful for the testing of foodstuffs for these dyes.

The European Commission asked the European Food Safety Authority (EFSA) to carry out a review of toxicological data available for dyes similar to Para Red, Sudan

dyes, Rhodamine B, etc. to determine the extent of the problem. The EFSA split the examined dyes into two groups. The first part, known as Annex 1, included Sudan I to IV, Para Red, Rhodamine B and Orange II dyes. Annex 2, the second and wider part, was to identify and compile a list of other potentially genotoxic/carcinogenic dyes considered harmful because of their structure-activity relationships, based on the Monographs of the International Agency for Research on Cancer (IARC), publications of the U.S. National Toxicology Program (NTP) and other relevant sources. These dyes belong to the group of azo, triphenylmethane or anthraquinone dyes.

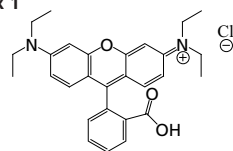
References:

- 1] "Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to review the toxicology of a number of dyes illegally present in food in the EU" The EFSA Journal (2005) 263, 1-71.

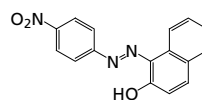
Table 1 Dye Standards

Cat. no.	Brand	Description	Purity	Pack size
79754	Fluka	Rhodamine B	> 97 %	25 mg
40446	Fluka	Para Red	> 97 %	100 mg
69143	Fluka	Orange II	> 98 %	25 mg
49823	Fluka	Acid Red	> 97 %	25 mg
44426	Fluka	Metanil Yellow	> 98 %	100 mg
75768	Fluka	Congo Red	> 97 %	25 mg
73225	Fluka	Butter Yellow	> 98 %	25 mg
91282	Fluka	Solvent Red I	> 96 %	25 mg
49547	Fluka	Naphtol Yellow S		25 mg
69669	Fluka	Leucomalachite Green	> 98 %	25 mg
22308	Fluka	Ponceau MX	> 96 %	25 mg

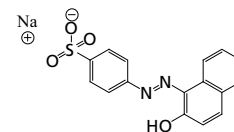
Annex 1



79754 Rhodamine B

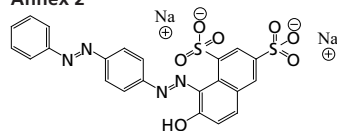


40446 Para Red

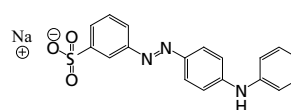


69143 Orange II Sodium salt

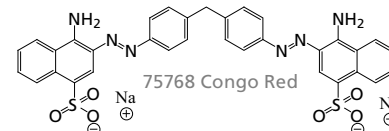
Annex 2



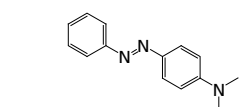
49823 Acid Red 73 Sodium salt



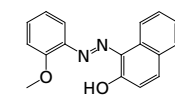
44426 Metanil Yellow



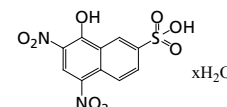
75768 Congo Red



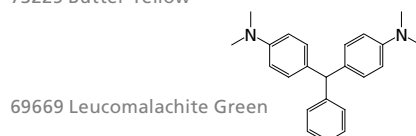
73225 Butter Yellow



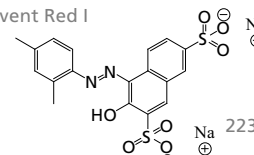
91282 Solvent Red I



49547 Naphtol Yellow



69669 Leucomalachite Green



22308 Ponceau MX

Assessment of the Fatty Acid Status in Blood Lipids

Prof. Dr. Claudio Galli, Department of Pharmacological Sciences, University Milan, Italy claudio.galli@unimi.it



Fatty acids (FA) are major components of the human diet and of plasma and cell lipids as both energy sources and as components of cell membranes. Fatty acids are also a variable component of our diet, both quantitatively and especially qualitatively. As such, Sigma-Aldrich offers a complete kit for the evaluation of FA status in blood (Cat. no. 11312) through our Fluka brand. The kit is designed for easy collection of blood drops as well as their storage, shipment and processing for FA analysis. Information obtained through subsequent analysis of the FA in the blood samples allows the definition of an individual's FA status in relation to the average values in the general population and provides the basis for the development and application of adequate preventative dietary strategies.

Fatty Acids

FA are carbon chains, generally with even numbers of carbon atoms, and with a different number of double bonds. FA with 0 (zero) double bonds are the Saturated FA (SFA), mainly present in animal (especially ruminants and derived products) and some vegetable fats (coconut). FA with 1 (one) double bond are the Monounsaturated FA (MUFA), the most relevant one being oleic acid (C18:1), present in certain vegetable oils such as olive and canola and in animal fat (pork and poultry). Finally, FA with 2 or more double bonds are the polyunsaturated FA (PUFA). These PUFA are further subdivided in two major series: the Omega 6 (or n-6) and the Omega 3 (or n-3), depending upon the distance (number of C atoms) interposed between the methyl end of the FA chain and the nearest double bond. The 18C components of the PUFA class, the so-called essential FA (EFA) that cannot

be synthesised in animals, are generated in plants and mainly found in vegetable fats, while PUFA, with longer chains (>20C) and a higher degree of unsaturation (>3 double bonds), are almost exclusively found in animals. The long chain highly unsaturated FA of the Omega 6 (especially arachidonic acid, AA 20:4) and Omega 3 series (especially EPA 20:5, and DHA, 22:6) play critical roles both as structural and functional components of cell membranes. While some AA is found in lean animal tissues, especially meat, EPA and DHA are found mainly in fatty fish, where they accumulate following the fish's consumption of algae.

Fatty Acids and Health

Extensive literature indicates that optimal intake – generally higher than that provided by typical Western diets – of Omega 3 FA is associated with better health. Further, optimal intake of Omega 3 FA has been cited as a factor in preventing the onset of chronic degeneration, often based on inflammatory processes, of various systems (cardiovascular, respiratory, etc.).

Therefore, the assessment of blood FA profiles is a relevant type of analysis because:

- FA, especially PUFA, are mainly derived from the diet
- intake levels are difficult to assess on the basis of food composition data and questionnaires, especially in the case of the Omega 3 FA because it is usually a minimal component of total dietary fat intake (<0.5 g Omega 3 vs > 100 g total FA /day)
- the FA "status" is an indicator of FA intake and also correlates with various pathophysiological conditions

Assessment of the Fatty Acid Status

In conclusion, the assessment of the FA status of a subject provided by the analysis of blood FA is a useful tool applicable to the optimisation of the intake of different kinds of fats and to the planning of dietary strategies for improving health status and for the prevention and treatment of diseases. General recommendations are aimed to increase Omega 3 FA, especially the long chain EPA and DHA, but it is important to verify the adherence to and the efficacy of these recommendations through adequate monitoring.

Product table

Brand	Prod. no.	Description	Pack size
Fluka	11312	Blood Collection Kit contains 100 x Blood Collection Dipsticks, 50 mL BHT solution, 100 x Desiccant Packs, 100 x Foil-barrier ziplock bags, working instruction sheet	1 kit
Fluka	05904	Lab Kit for the evaluation of FA status in blood (n-3 + n-6 PUFA)	1 kit

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Eliminate baseline anomalies and sensitivity problems caused by impurities in your IPC reagent.

SPECIAL OFFER:

Get 40 % discount on all recommended IPC reagents listed below.

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Offer only applies to pack sizes in the table.

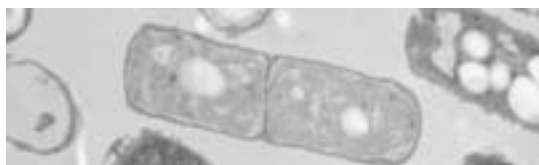
Brand	Prod. no.	Description	Pack size
Fluka	52862	Sodium 1-hexanesulphonate monohydrate	2.5 g
Fluka	51832	Sodium 1-heptanesulphonate monohydrate	2.5 g
Fluka	74882	Sodium 1-octanesulphonate monohydrate	2.5 g
Fluka	30631	Sodium 1-decanesulphonate	2.5 g
Fluka	71726	Sodium dodecyl sulphate	5 g
Fluka	87724	Tetramethylammonium bisulphate	5 g
Fluka	86626	Tetraethylammonium hydrogen sulphate	2.5 g
Fluka	86853	Tetrabutylammonium bisulphate	2.5 g
Fluka	87299	Tetrahexylammonium hydrogensulphate	5 g
Fluka	52864	Sodium 1-hexanesulphonate solution, concentrate, ampule concentrate (~0.33 M) for 6x1 l 0.005 M ready-to-use solution	1 each
Fluka	51834	Sodium 1-heptanesulphonate solution, concentrate, ampule concentrate (~0.33 M) for 6x1 l 0.005 M ready-to-use solution	1 each
Fluka	86847	Tetrabutylammonium bisulphate solution, concentrate, ampule concentrate (~0.33 M) for 6x1 l 0.005 M ready-to-use solution	1 each

Special Offer – Microbiology Products

Testing medium for *Bacillus cereus* differentiation

Brand	Cat. no.	Name
Fluka	70133	Blood Agar (Base)
Fluka	21065	Calcium caseinate Agar
Fluka	22310	Cereus Selective Agar
Fluka	92325	HiCrome™ Bacillus Agar NEW
Sigma	M1928	MYP Agar Base

For a limited time, get a **30 % discount** on our *Bacillus* media and experience the advantages of Sigma-Aldrich quality and experience. Please quote promotion code T06 when placing an order.



Sterility spore indicator strips

Brand	Cat. no.	Name
Fluka	05290	Sterility Indicator (Radiation Sterilisation)
Fluka	74041	Sterility Indicator (Steam Sterilisation)

Bacillus species are also important as an indicator system for sterilisation processes! As spores from *Bacillus stearothermophilus* and *Bacillus pumilus* are highly resistant, they are best suited as indicator organisms. Please test our sterility indicators and get a **40 % discount** for a limited time. Please quote promotion code W30 when placing an order.

Figure *Bacillus cereus*
(electron microscope image;
Photo James W. Brown, NC State
University Raleigh, USA)

Bacillus: A Ubiquitous Bacteria Genus

Some *Bacillus* species play a critical role in helping us understand more about bacteria, while others are dangerous spoiling organisms or even pathogens. All, however, are important in biotechnological research.

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

Bacillus species are Gram-positive, rod-shaped bacteria; they can be either obligate or facultative aerobes and show positive reaction in the catalase test. Members of the genus *Bacillus* are known to form spores under stressful conditions. These endospores are highly resistant to heat and radiation and are viable for extremely long periods.

Kingdom: Bacteria
Division: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: *Bacillus*

B. subtilis is one of the best-understood bacteria, thus it is often used in molecular biology and as a general model organism. Its harmless nature, brilliant genetic amenability and relatively large gene size make the organism a highly valuable tool for science and demonstration purposes. *Bacillus subtilis* has been used to help demonstrate biochemical differentiation, gene/protein regulation and cell cycle events in bacteria.

B. thuringiensis is an important insect pathogen. Its Bt toxin is specifically active against several undesirable species of insects. Therefore, the Cry and Cyt genes are used for the production of biological-based insecticides; in agriculture, the toxin genes are used to modify crops to make them insect-resistant. When insects take up the substance, it is cleaved under the alkaline conditions in their digestive tract, and the toxin becomes active. The protein then inserts itself into the insect's gut cell membranes, forming a pore and resulting in swelling, cell lysis and possible death for the insect.

B. anthracis causes anthrax. The name *anthracis* comes from the Greek *anthrakis*, meaning "coal", because in the most common form of the disease, cutaneous anthrax, the sufferer develops large black skin lesions.

Did you know ... that *B. subtilis* was involved in the testing of the New York subway system's vulnerability to a biological attack? The organism was released to find out how many people would be killed in the event of a bio-warfare attack. The result? The entire system could be contaminated by the release of bacteria in just one train.

It is a relatively big (1–6 µm) facultative aerobe, non-motile organism that can form centrally located ellipsoid spores even under stressful conditions. The cells are usually built in chains. In vivo, the bacterium forms a capsule from polyglutamate, which protects it from phagocytosis. On blood agar cultures, however, the capsule is usually not present. Genotypically and phenotypically, it is very similar to *Bacillus cereus* and to *Bacillus thuringiensis*, but there are some differentiating characteristics. (Table 1)

Table 1 Differentiating characteristics of *B. anthracis*, *B. cereus* and *B. thuringiensis*

Characteristic	<i>B. anthracis</i>	<i>B. cereus</i> and <i>B. thuringiensis</i>
Growth requirement for thiamin	+	-
Hemolysis on sheep blood agar	-	+
Glutamyl-polypeptide capsule	+	-
Lysis by gamma phage	+	-
Motility	-	+
Growth on chloral hydrate agar	-	+
String-of-pearls test	+	-

B. cereus causes a foodborne illness similar to those caused by *Clostridium perfringens* or *Staphylococcus aureus*. *B. cereus* is a facultative aerobe, beta hemolytic soil bacteria that produces exotoxins. There are two types of illness caused by *B. cereus*, depending on the contaminated substance ingested: a diarrhoea type (similar to that caused by *C. perfringens*) and a vomiting type (similar to that caused by *Staphylococcus aureus*). The minimum infectious dose is about 10⁶ germs/g. Infection sources for the diarrhoea type of *B. cereus* infection are sweets (pudding, vanilla sauce), meats

Table 2 Media for detection and differentiation of *B. anthracis*

Brand	Cat. no.	Name	Description
Fluka	55678	PLET Agar Anthraxis-Selective-Supplement (Fluka 72659)	The best selective medium for isolation and cultivation of <i>Bacillus anthracis</i> from environmental specimens, animal products or clinical specimens, inhibiting <i>Bacillus cereus</i> .
Fluka	70133	Blood Agar (Base)	A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms.

(continued on page 16)

(roast, goulash, sausages), vegetables, salads, soups and UHT milk products. The vomiting type of *B. cereus* illness is caused by contaminated cooked rice that has been reheated. The risk for strong propagation and the resulting illness can be minimised by storing foods at <math><5\text{ }^{\circ}\text{C}</math> or $>65\text{ }^{\circ}\text{C}$ and by rapidly cooling down foods, thus lowering the pH value to <math><4.5</math> with a respective a_w -value of <math><0.95</math>. Note: *B. cereus* spores are in most cases not eliminated by heating; in fact, heating activates spore germination. At the same time, however, the spoilage flora is eliminated.

Figure 1 Microscopic picture from *Bacillus anthracis*

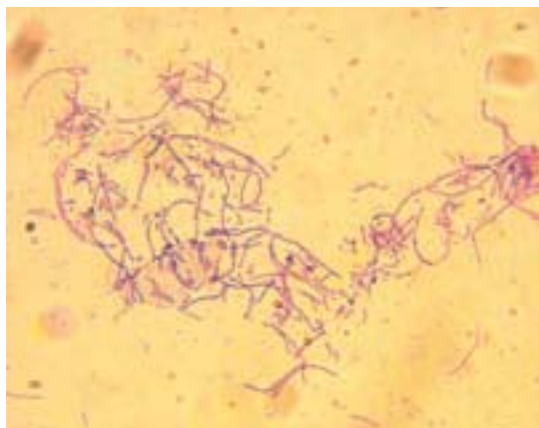


Table 3 Medium for *Bacillus* species

Nonselective media for <i>Bacillus</i> species		
Brand	Cat. no.	Name
Fluka	70181	Antibiotic Agar No 1
Fluka	70182	Antibiotic Agar No 2
Fluka	22089	Casein peptone Lecithin Polysorbate Broth
Fluka	22095	CASO Agar
Fluka	22098	CASO Broth
Fluka	70185	Czapek Dox Agar
Sigma	D3435	Dey-Engley Neutralising Broth
Fluka	70147	Milk Agar
Fluka	72548	Nitrate Broth
Fluka	70148	Nutrient Agar
Fluka	17179	Nutrient Agar pH 6.0 with 0.8 % NaCl
Fluka	44776	Nutrient Agar Plates (Diameter 55 mm)
Fluka	03856	Nutrient Broth No. 4
Fluka	70179	Peptone Water
Fluka	70152	Plate Count Agar
Fluka	88588	Plate Count Agar according to Buchbinder et al.
Fluka	68414	Plate Count Agar according to Buchbinder et al.
Fluka	00464	Plate Count Agar Plates (diameter 55 mm)

Nonselective media for *Bacillus* species (continuation)

Brand	Cat. no.	Name
Fluka	19718	Plate Count Agar, Vegitone
Fluka	80957	Plate Count Skim Milk Agar
Fluka	17175	Skim Milk Agar, modified
Fluka	17274	Thermoacidurans Agar
Fluka	70157	Thioglycollate Broth (USP Alternative)
Fluka	90404	Thioglycollate Broth with Resazurine
Fluka	28976	Thioglycollate Medium with K Agar
Fluka	79872	Tryptic Soy Agar
Fluka	22091	Tryptic Soy Agar
Fluka	57994	Tryptic Soy Agar Plates (Diameter 55 mm)
Fluka	14432	Tryptic Soy Agar, Vegitone
Fluka	22092	Tryptic Soy Broth
Fluka	43592	Tryptic Soy Broth
Fluka	51228	Tryptic Soy Broth No. 2
Fluka	41298	Tryptic Soy Broth, Vegitone
Fluka	51414	Tryptic Soya Agar with Polysorbate 80 and Lecithin
Fluka	70159	Tryptone Glucose Extract Agar
Sigma	T2188	Tryptone Glucose Yeast Extract Agar

Nonselective differential media for *Bacillus* species

Brand	Cat. no.	Name
Fluka	70133	Blood Agar (Base)
Fluka	39212	Blood Agar SLMB
Fluka	21065	Calcium caseinate Agar
Fluka	70136	Deoxyribonuclease Test Agar
Fluka	30787	Deoxyribonuclease Test Agar
Fluka	31415	Dextrose Caseinpeptone Agar
Sigma	G0289	Gelatin Iron Agar
Fluka	16447	Glucose Bromcresol Purple Agar
Sigma	M1928	MYP Agar Base
Fluka	70151	Nutrient Gelatin
Fluka	91015	Tributyryn Agar

Selective differential media for *Bacillus* species

Brand	Cat. no.	Name
Fluka	22310	Cereus Selective Agar
Fluka	92325	HiCrome™ Bacillus Agar

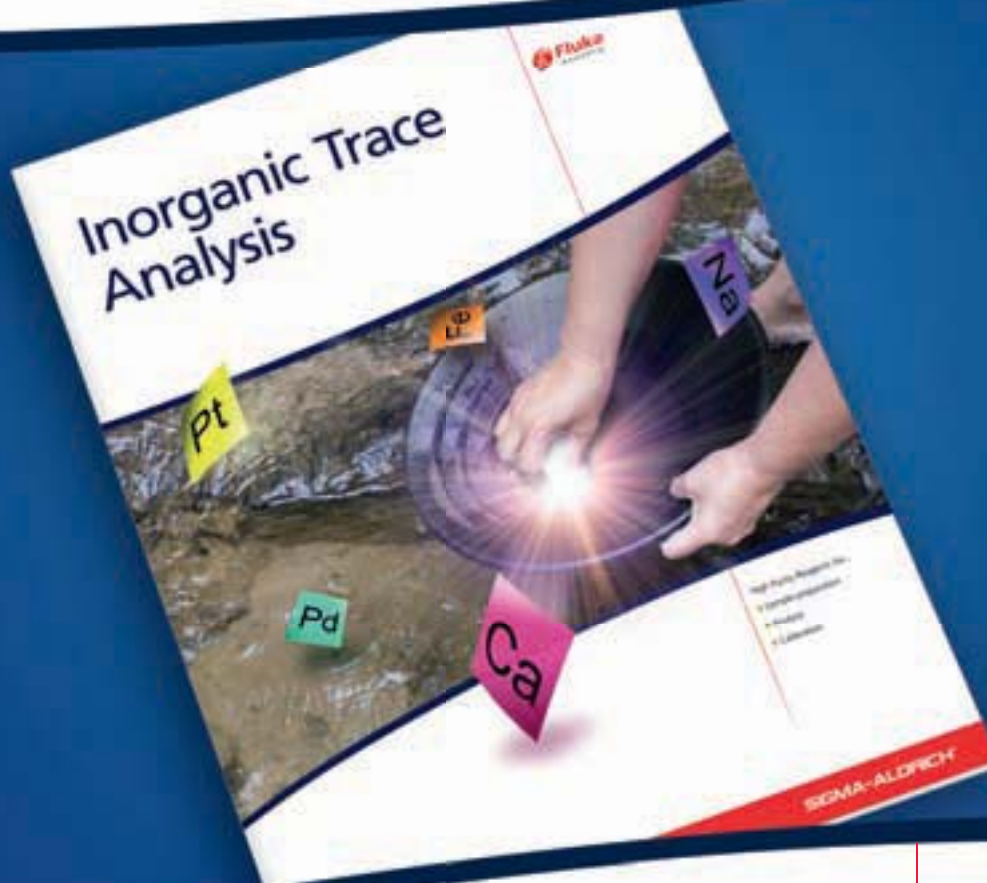
Detection of trace amounts of penicillin in milk using *Bacillus stearothermophilus*

Brand	Cat. no.	Name
Fluka	17186	PM Indicator Agar

Table 4 Products for the identification and differentiation of *Bacillus* species

Brand	Cat. no.	Name
Fluka	88597	Catalase Test
Fluka	77730	Gram Staining Kit
Fluka	04551	Schaeffer and Fulton Spore Stain Kit
Fluka	44378	M'Fadyean Stain Solution

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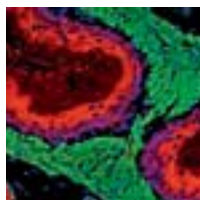
- Digestion Reagents for Trace Analysis
- Auxiliary Reagents for AAS
- Solvents for Trace Metal Speciation Analysis
- High-purity Reagents for Voltammetry
- Certified Eluent concentrates for Ion Chromatography
- Traceable Certified Reference Materials for AAS, ICP and IC

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MALDI Mass Spectrometry

Unique, high-purity ionic MALDI matrixes from Fluka



Sigma-Aldrich offers an extensive line of MALDI matrixes through our Fluka brand to provide scientists with high-purity chemicals for use in the MALDI (matrix-assisted laser desorption/ionisation) technique, as an alternative to common laboratory-grade materials purified onsite.

MALDI analysis involves mixing a sample with a matrix substance and crystallising the mixture out of a small droplet on the MALDI plate. The crystallised sample/matrix mixture is irradiated by laser light, usually UV. As the matrix absorbs the light energy, it evaporates, carrying portions of the sample into the vapour phase, resulting in an indirect ionisation of the sample molecules. MALDI is especially useful for protein and peptide identification using masses alone, as the masses of ions can be determined with high accuracy.

Although there have been numerous attempts to replace solid chemical matrixes by direct MS from solid supports like silicon surfaces or directly from 2-D gels, most MALDI users still mix the samples within a chemical matrix.

Because MALDI is often used for extremely low-level detection, matrixes must first and foremost be free of organic impurities and ions. Organic impurities lead to extraneous peaks, especially in the low mass range. Traces of ions, especially Na⁺ and K⁺, cause adducts of the sample molecules which complicate the MS spectrum, giving it a fence-like appearance; each “post” in the fence differs in mass according to the mass differences between these various positive ion adducts.

At Sigma-Aldrich, we realise the benefits of highly purified MALDI matrix compounds, tailored to the customer’s specific type of sample and analyte. Through the years, we have built up a broad range of matrixes, all extensively purified to meet stringent MALDI specifications and to provide sufficient quality for even the highest sensitivity requirements.

For further information about our high-purity MALDI matrix modifiers, please visit our MALDI web page: sigma-aldrich.com/maldi



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Our 5 L and 10 L VOLPAC solution containers are perfect when larger volumes of ready-to-use titration solutions are needed. VOLPAC containers consist of a flexible polyethylene bag with an outlet tap inside a cubic cardboard supporting frame. Because of VOLPAC’s clever design, air cannot enter the container during dispensing. As a result, VOLPAC containers can be drained completely without contamination for less waste and more reliable analyses.

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- High quality of content and packing
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All VOLPAC containers are now supplied with a specially designed adapter that allows direct connection of the titrating tube to the VOLPAC container.

Please find our complete VOLPAC product listing on our website at sigma-aldrich.com/volpac

HYDRANAL® Karl Fischer Reagents

Determining water content in pesticides

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The water content of any substance or product may affect its quality, shelf life, chemical stability and reactivity. This is especially true of reference materials, as water content is a crucial factor in the purity of the material and must be taken into account for any reference measurements and content calculations. Karl Fischer (KF) titration is a perfectly suitable method for accurate and reproducible determination of water content of all types of substances, including chemicals, oils, pharmaceuticals, pesticides and food.

Water determination in pesticides can be especially challenging, however; special reagents may be needed for improving sample solubility or preventing side reactions. As such, our HYDRANAL laboratory has developed new applications for a series of pesticides using volumetric and coulometric KF titration techniques, which are described below.

Direct Volumetric KF Titration

Substances like the fungicide tolclofos-methyl that dissolve easily in the KF media can be titrated following standard procedures, i.e., the one-component KF titration technique. If the dissolving process of a substance

proves too slow, a stirring time for 60 seconds before titration can prove a useful step, as is indicated for the insecticide fipronil, for example.

Further improvement of sample solubility may be achieved by using the two-component KF technique. The working medium used, HYDRANAL-Solvent, shows dissolving advantages over pure methanol due to its imidazole and sulphur dioxide content. A sample weight of 1 g is recommended for substances with very low water content.

Reagent	One-component technique
Titrating agent	HYDRANAL-Composite 2
Working medium	HYDRANAL-Methanol Rapid or HYDRANAL-Methanol dry
Two-component technique	
Titrating agent	HYDRANAL-Titrant 2
Working medium	HYDRANAL-Solvent

(LabReport no. 616: tolclofos-methyl, no. 612: fipronil)

KF Titration with Solubilising Agents

Solubility of the insecticide imidacloprid in the alcohol media of KF titration is limited. A quantity of 100 mg will dissolve completely in 30 mL of methanol; however, this volume is insufficient for accurate determination due to the material's low water content. Thus, to dissolve 1 g of this sample, a mixture of HYDRANAL-Formamide and methanol is recommended as a working medium, or the two-component technique with HYDRANAL-Solvent, in which a 1 g sample dissolves almost completely.

Reagent	One-component technique
Titrating agent	HYDRANAL-Composite 2
Working medium	HYDRANAL-Methanol Rapid or HYDRANAL-Methanol dry and HYDRANAL-Formamide dry (ratio 1:1)
Two-component technique	
Titrating agent	HYDRANAL-Titrant 2
Working medium	HYDRANAL-Solvent

(LabReport no. 611: imidacloprid)

Other fungicides like procymidone or thiram only dissolve with the addition of chloroform as a solubilising agent for both the volumetric one- and two-component techniques. HYDRANAL-LipoSolver CM as one-component and HYDRANAL-Solvent CM as two-component working medium are perfectly suited for these applications; both contain appropriate mixing ratios of methanol and chloroform. The recommended sample weight is approximately 1–2 g, depending on the water content of the sample.

(continued on page 20)

Reagent	One-component technique
Titrating agent	HYDRANAL-Composite 2
Working medium	HYDRANAL-LipoSolver CM
Reagent	Two-component technique
Titrating agent	HYDRANAL-Titrant 2
Working medium	HYDRANAL-Solvent CM

(LabReport no. 613: procymidone, no. 615 thiram)

Indirect Coulometric Karl Fischer Titration with an Oven

The fungicide mancozeb hardly dissolves at all in the media used in Karl Fischer titration. In addition, a strong side reaction occurs and consumes iodine; this side reaction cannot be suppressed with any kind of auxiliary reagent, hence the indirect method using the KF oven should be chosen for analysis of this sample.

Our HYDRANAL laboratory tested the temperature behaviour of mancozeb by applying a temperature ramp in the KF oven in order to determine a suitable heating temperature for water determination. The sample was gradually heated, with a temperature increase of 3 °C per minute, from 50 °C to 250 °C. **Figure 1** shows the result of this procedure: a water release from the sample was observed between 50 °C and 160 °C. Just above 160 °C, however, the substance began to decompose. Thus, a temperature of 130 °C is recommended for the determination of water content in mancozeb.

Strongly Oxidising Samples

The fungicide chloranil is a strong oxidising agent. The iodide present in the titration vessel (before start of

titration) is oxidised by chloranil; this is visible as a colour change caused by an excess of iodine. Oxidation reactions are also dependent on the pH value in the titration vessel. Acidification of the medium is only able to delay this effect, not eliminate it, so water content of this substance cannot be determined by direct titration.

We tested the indirect method using the KF oven, again observing sample behaviour during a temperature increase of 3 °C per minute from 50 °C to 250 °C. For chloranil, 170 °C is the recommended heating temperature for water determination.

Reagent	Coulometric titration with diaphragm
Anolyte	HYDRANAL-Coulomat AG Oven or -Coulomat AG or -Coulomat AD
Catholyte	HYDRANAL-Coulomat CG
Reagent	Coulometric titration without diaphragm
Anolyte	HYDRANAL-Coulomat AG Oven or -Coulomat AG or -Coulomat AD
Catholyte	not required

(LabReport no. 614: mancozeb, no. 617 chloranil)



Figure 1 Temperature ramp for mancozeb with KF oven, coulometric determination

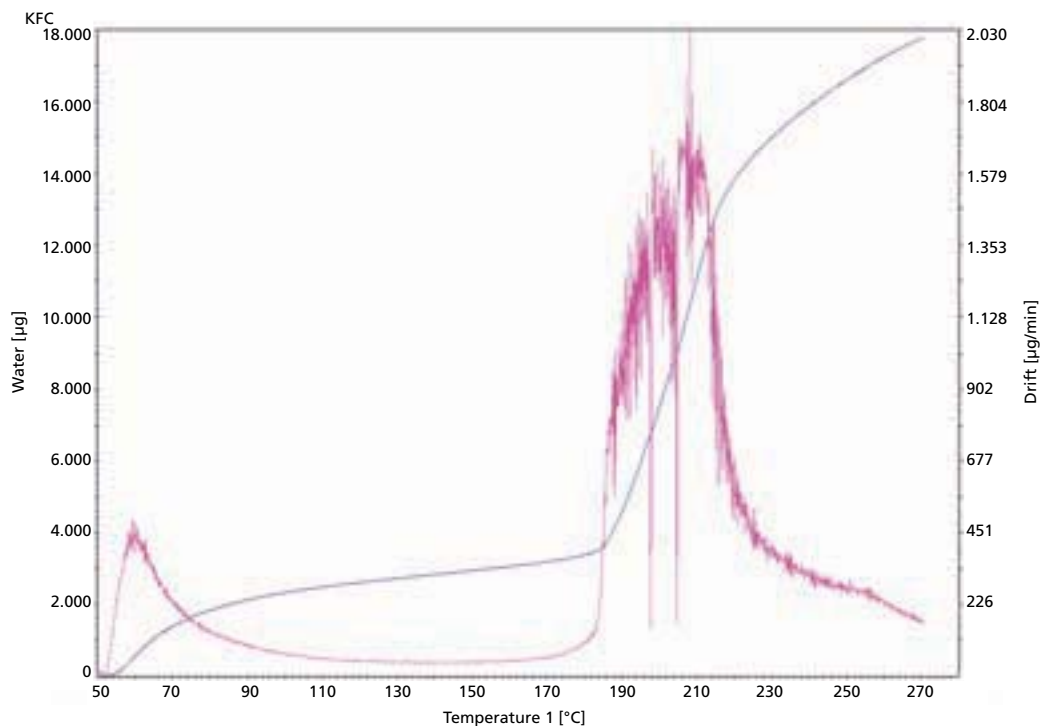


Table 1 Selected HYDRANAL reagents for Karl Fischer titration

Cat. no.	Brand	Description	Pack size
34806	Fluka	HYDRANAL-Composite 2 One-component reagent for KF titration; to be used with HYDRANAL-Methanol dry, Methanol Rapid or CompoSolver E	500 mL, 1 L, 2.5 L
34741	Fluka	HYDRANAL-Methanol dry One-component working medium for KF titration; to be used with HYDRANAL-Composite	1 L, 2.5 L
37817	Fluka	HYDRANAL-Methanol Rapid One-component working medium for KF titration; to be used with HYDRANAL-Composite	1 L, 2.5 L
37855	Fluka	HYDRANAL-LipoSolver CM One-component working medium for KF titration in non-polar substances; to be used with HYDRANAL-Composite	1 L
34811	Fluka	HYDRANAL-Titrant 2 Two-component reagent for KF titration; to be used with HYDRANAL-Solvent	500 mL, 1 L, 2.5 L
34800	Fluka	HYDRANAL-Solvent Two-component working medium for KF titration; to be used with HYDRANAL-Titrant	1 L, 2.5 L
34812	Fluka	HYDRANAL-Solvent CM Two-component working medium for KF titration in oils and fats; to be used with HYDRANAL-Titrant	1 L, 2.5 L
34724	Fluka	HYDRANAL-Formamide dry Solvent for KF titration	1 L
34739	Fluka	HYDRANAL-Coulomat AG Oven Analyte for coulometric KF titration with Karl Fischer-Oven; suitable for cells with and without diaphragm	500 mL
34836	Fluka	HYDRANAL-Coulomat AG Analyte for coulometric KF titration; suitable for cells with and without diaphragm	500 mL, 1 L
34810	Fluka	HYDRANAL-Coulomat AD Analyte for coulometric KF titration; suitable for cells with and without diaphragm	500 mL
34840	Fluka	HYDRANAL-Coulomat CG Catholyte for coulometric KF titration; free of halogenated hydrocarbons	50 mL (10 x 5 mL ampules)

Technical Help

Our HYDRANAL laboratory has worked out more than 600 applications for Karl Fischer titration on a broad variety of sample substances. For details and complete applications, please visit our website sigma-aldrich.com/hydranal or contact our HYDRANAL laboratories:

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SIGMA-ALDRICH®

pH Buffers for Titration

The variety you need for laboratory success

Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com



Sigma-Aldrich offers a wide variety of reagents for acidimetric, alkalimetric, redox and precipitation titration methods. These reagents are available as ready-to-use solutions as well as concentrates, powders and tablets. An important addition to our titration portfolio is the range of pH buffer products; with our buffer solutions, tablets and concentrates, almost all your titration needs for laboratory work are covered!

Our product range of pH buffers comprises:

- pH Buffers from pH 1.00–13.00, available as ready-to-use solutions, tablets and powders as well as FIXANAL® concentrates in ampules
- Colour-coded buffer solutions for pH 4 (red), 7 (green), 9 (blue) and 10 (violet); ideal for instrument calibration
- High-precision buffer solutions according to DIN 19266 for high-precision measurements

Packaging Options:

Ready-to-use solutions in VOLPAC® Solution containers

Apart from the customary package sizes up to 5 L, the Sigma-Aldrich portfolio also includes 5 and 10 L VOLPAC solution containers when larger quantities of ready-to-use solutions are needed. VOLPAC containers consist of a rugged cardboard cube containing a flexible inner polyethylene bag with an outlet tap. With each VOLPAC container, an adapter is provided for direct connection of the outlet tap to a titration tube.

Buffers keep the pH value within a specified range, an important consideration for chemical and biochemical reactions that occur in aqueous solution. Buffers are typically mixtures of weak acids or bases with their salts or the salts of multi-alkaline acids. By dissolving these buffer substances in salt- and carbon dioxide-free water, buffer solutions are created with reproducible pH values that remain stable for long periods.

The accuracy of Sigma-Aldrich buffer solutions is ensured by the careful choice of buffer substances, the use of deionised water with extremely low conductivity and the subsequent calibration of the pH value with standard buffer solutions according to DIN 19266.

Table 1 Accuracy of Sigma-Aldrich buffer solutions at 20 °C

Buffer solution pH value	Accuracy
pH 1–9	± 0.02
pH 10–13	± 0.05

Table 2 Overview of Sigma-Aldrich buffer products

Buffer solutions
Buffer solutions pH 1-13
Buffer standard solutions, colour-coded
Buffer standard solutions, acc. to DIN 19266
Acetate buffer solutions
Phosphate buffer solutions
Buffer tablets & powders
Buffer tablets pH 4.0, pH 7.0, pH 9.2
Buffer powders pH 2-12, pH 6.4, pH 7.4
Buffer concentrates (FIXANAL)
Buffer concentrates pH 1-13, for 500 mL buffer solution

Concentrates in FIXANAL® ampules

pH buffer concentrates are also available in Sigma-Aldrich's FIXANAL ampules: by diluting the entire content of the ampule to the desired volume, a solution can be prepared to meet the customer's specific concentration requirement, with the certainty that it has been tested against a certified reference material.



Table 3 Advantages of ready-to-use solutions and FIXANAL concentrates

Ready-to-use solutions	FIXANAL concentrates
Contain exact amount in terms of concentration (e.g., 1 mol L ⁻¹)	Contain exact amount of substance (e.g., 1 mol)
Titer precision 1.000 ± 0.1 %	Titer precision 1.000 ± 0.2 %
Readily available, no preparation required	Economical and space-saving
Also available in 5 and 10 L VOLPAC solution containers	Final concentration is user-specified

Please visit our website and find our complete product list for titration/pH buffers in our online product catalogue at sigma-aldrich.com/catalog

Table 4 Laboratory chemicals for titration by Sigma-Aldrich

- **Volumetric standard solutions:** acid/base solutions, salt solutions and buffer solutions in various concentrations
- **Indicator solutions and powders** for all types of volumetric titrations
- Volumetric reagents **packaging options:**
 - FIXANAL concentrates** in ampules
 - Ready-to-use solutions**
 - 5 L/10 L VOLPAC solution containers:** easy handling and reduced packaging material for titration without contamination
- **Reagents for complexometric titrations:**
 - Aminopolycarboxylic acids (EDTA/NTA analogs)
 - Buffer solutions, indicators and masking agents

Food Analysis

Luff-Schoorl reagent for determination of reducing sugars

Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com

The Luff-Schoorl reagent solution, Fluka Cat. no. 34400, in addition to the 1 L container is now also available as a 5 L VOLPAC® solution container!

For determination of reducing sugars in different matrices, the method according to Luff-Schoorl can be used: reducing monosaccharides like glucose, fructose and galactose as well as reducing disaccharides like lactose and maltose can be determined. This method can be

applied for samples like sugar solutions, sugar containing foods like jams and confectionary products, fruit juices and nectars, vegetable products, wine, cereals and bakery products, and for determination of lactose in animal feeds. Standard methods according to Luff-Schoorl are given e.g. by the International Commission for Uniform Methods of Sugar Analysis ICUMSA GS1-5 and the Swiss Federal Office of Public Health SLMB 28A/5.1.1.

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