

# Analytix

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## Double Accreditation Brings a New Class of CRMs



- Double accreditation brings new CRMs
- New standards for tobacco-specific nitrosamines
- New solvents for headspace GC
- Reagents for samples decomposition
- Titration: Titer of Karl Fischer reagents

## The “Gold Standard” – Double accreditation under both ISO/IEC 17025 and ISO Guide 34

The highest possible level of quality assurance for a reference material producer



Jürg Wüthrich  
Head of Accredited Lab  
Senior Scientist R&D  
Sigma-Aldrich Europe

Dear Colleague,

Many aspects of our daily life have changed dramatically over the last few decades. The world is smaller and more connected, the pace of life faster and more frenetic. Globalisation has become an important part of the vernacular. As corporations use geography as an economic level for manufacturing and raw material sourcing, it is critical that we establish and enforce standards on how products and services are characterised to ensure public safety.

Analytical chemistry plays an important role in this process, and certified reference materials (CRMs) play an essential role in analytical chemistry. They serve as reference points that are traceable to another internationally accepted standard, like a CRM from a metrological institute, or, even better, directly to an SI unit. CRMs should not only be traceable to an accepted reference, but also be of well-defined purity and have a properly calculated measurement uncertainty. This information should be reported in a detailed certificate of analysis.

We know that our customers' results are directly affected by the quality of the CRMs they use. The choice of the right CRM producer is a matter of trust. One indicator of the technical and administrative competence of a CRM producer is inspection and accreditation by an independent authority.

Our facility at Buchs, Switzerland, where we produce our certified reference materials, including *TraceCERT*<sup>®</sup> calibration standards, was recently audited and accredited by the Swiss Accreditation Service (SAS) according to ISO Guide 34 “General requirements for the competence of reference material producers.” Because we also received accreditation under ISO/IEC 17025 “General requirements for the competence of testing and calibration laboratories”, we now have double accreditation. This achievement is also called the “Gold Standard” in accreditation for CRM producers.

This combination of ISO/IEC 17025 and ISO Guide 34 is the highest achievable level of quality assurance. With this accreditation we join a select group of institutes and companies worldwide that are working at this level of certified quality.

Buying an analytical standard is a matter of trust. Trust us to supply only the best for all your analytical applications.

Kind regards,

A handwritten signature in blue ink that reads "J. Wüthrich".

Jürg Wüthrich  
Head of Accredited Lab

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## Increasing the reliability and value of analytical results

Sigma-Aldrich achieves “Gold Standard” double accreditation according to both ISO/IEC 17025 and ISO Guide 34, and now offers a unique class of CRM

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Produced in double accredited  
laboratory fulfilling  
ISO/IEC 17025 and  
ISO Guide 34

Following long-lasting cooperation with different metrological institutes, such as Switzerland’s EMPA and Germany’s BAM, Sigma-Aldrich has built up a broad knowledge base in the field of CRM production. In the newly built laboratories equipped with specialized instruments at our Buchs, Switzerland site, we developed a series of CRMs for the analytical market. After two years of continuous development, our Buchs facility was ready to be audited by the Swiss Accreditation Service (SAS) and received the highest achievable quality assurance level for CRM producers: the double accreditation as both a testing lab (ISO/IEC 17025) and a CRM producer (ISO Guide 34). This combination is also called the “Gold Standard” accreditation for CRM producers.

### Accreditation vs. Certification

Confusion often arises over the proper meaning and differences between the terms accreditation and certification. Accreditation means that an authoritative body formally recognises that an organisation or individual is competent to execute a specific service as described in the scope of accreditation. Certification, on the other hand, means that an independent third party has confirmed in writing that a product, procedure or service fulfils the prescribed requirements.

The difference between the two seemingly similar definitions lies in the fact that with accreditation, the formal recognition of competence is based on proven technical knowledge and therefore requires the consultation of a technical expert for the scope to be accredited, while certification primarily involves ensuring conformity to a given norm. For example, certification according to ISO 9001 targets the general management, processes and data-handling within a company. Therefore, ISO 9001

certification is not linked to a technical competence. Although both ISO 9001 certification and ISO/IEC 17025 accreditation do have some overlap, they are more or less independent [1].

### ISO/IEC 17025 for testing labs

The particular aspects of this type of accreditation are described in ISO/IEC 17025 “General requirements for the competence of testing and calibration laboratories [1].” In addition to the basic management requirements of ISO 9001, the following topics are crucial for ISO/IEC 17025 compliance:



- Instrument qualification
- Validation of analytical methods
- Traceability statements
- Evaluation of measurement uncertainty
- Ongoing education of personnel
- Periodic participation in proficiency tests to demonstrate technical capability

Analytical results produced in laboratories fulfilling ISO/IEC 17025 can be labelled with a special quality logo that confirms the reliability of the results. In every country there is at least one official body that is responsible for accreditation, and each has its own design for their quality logos. In Switzerland, it is the Swiss Accreditation Service (SAS) that gives the permission to the accredited laboratories to use the “Swiss Testing” logo. This logo and the individual registration number of the laboratory must appear on the certificate. Furthermore, the scope of each accredited laboratory is published on the web sites of the responsible accreditation bodies.

In most cases, ISO/IEC 17025 is associated with a well-defined analytical technique and a stated measurement range comprising analyte, matrix and concentration range. This is called the scope of the accreditation. For example, Laboratory A is accredited to perform ICP-OES measurements of lead in plastics in the range of 0.1 up to 1000 mg/kg with a stated relative uncertainty of 2 up to 5%. Due to the wide variety of analytical techniques and analytes, there are many laboratories worldwide already having an ISO/IEC 17025 accreditation, each for a very specific analytical scope. Since this accreditation is an assignment of a technical competence, it goes without

saying that it is strictly linked to the specified staff and infrastructure of the accredited laboratory and cannot be moved from one location to another.

### ISO Guide 34 as the relevant guide for CRM producers

A CRM producer is defined as a “technically competent body that is fully responsible for the supply of the CRM and authorises the property values assigned to the CRM.” The most appropriate document dealing with CRM production is ISO Guide 34 “General requirements for the competence of reference materials producers [2].”



This guide outlines the quality system requirements under which reference materials can be produced. It is intended to be used as part of the reference material producer's general quality assurance procedures.

The organisation and management requirements, for example quality system, documentation, services and supplies, preventative and corrective actions, audits and reviews, are usually covered when the laboratory is already certified according to ISO 9001 or accredited under ISO/IEC 17025. In ISO Guide 34, there are a few additional requirements that deal with the production of CRMs:

- Production planning and maintenance of a suitable environment
- Starting materials selection and pre-treatment
- Assignment of a CRM's property values, uncertainty and traceability
- Assessment of CRM homogeneity and stability
- Assurance of adequate packaging and storage
- Certificates that include detailed information

Because many aspects of CRM production require precise measurements, for example homogeneity and stability testing, and proper data evaluation is an integral part of the certification process, a CRM producer under ISO Guide 34 must also comply with ISO Guide 31 (contents of certificates and labels) and ISO Guide 35 (general and statistical principles for certification) [4, 5].

### Double accreditation: The “Gold Standard”

As mentioned previously, ISO/IEC 17025 is the common standard in analytical chemistry, while ISO Guide 34 is more relevant to CRM producers. Nevertheless, the quality of most CRMs also depends on analytical measurement capabilities. Therefore, the double accreditation achieved by following both of these guidelines is the highest achievable quality and confidence level for CRM producers. It is therefore also called the “Gold Standard” for CRM producers. In 2004, the International Laboratory Accreditation Cooperation (ILAC) began recommending this double accreditation for all CRM producers. However, only a very small group of institutes and companies worldwide are working at this level of certified quality today.

### Accreditation @ Sigma-Aldrich

After receiving ISO 9001 certification in 1994, we have systematically improved and expanded our quality management systems and skills. The technical competence for CRM production at Sigma-Aldrich Switzerland is a consequence of the long-standing cooperation between our R&D group and various metrological institutes.

In mid-2007, we achieved ISO/IEC 17025 accreditation for the two scopes “mass determination through high-precision weighing” and “gravimetric preparation of homogeneous solutions from high-purity starting materials.” By the end of 2007, we attained our second accreditation as “Producer of Certified Reference Materials” according to ISO Guide 34 in combination with ISO/IEC 17025. The scope is specified for homogeneous solutions in the concentration range of 0.5 mg/kg to 20,000 mg/kg using gravimetric preparation.

**Figure 1** When climbing up the “quality ladder”, only with ISO Guide 34 (in combination with ISO/IEC 17025) can CRM producers achieve the highest level of reliability.



(continued on page 6)

### TraceCERT® CRM launched

The first products developed and produced under these doubly accredited conditions are our recently launched **TraceCERT** calibration standards for spectrometry and ion chromatography. The name **TraceCERT** stands for Traceability and CERTified, and means that these CRMs are suitable for even the most challenging analytical applications. They also fulfil all the needs of our customers who work in regulated environments. Key characteristics of these new CRMs are:

- Unique level of accuracy and lot-specific values
- Produced in our doubly accredited laboratory that fulfils ISO/IEC 17025 and ISO Guide 34
- Traceable to at least two independent references (NIST, BAM or SI unit kg)
- Sophisticated packaging and comprehensive documentation, including proper uncertainty calculation, expiration date and storage information

The quality of a CRM strongly depends on the quality of the starting materials and, therefore, their characterisation is very important. **TraceCERT** starting materials are characterised by two different approaches: the direct measurement of the purity by the most accurate method (e.g. titrimetry) and the purity assignment by the "100% minus impurities" approach. The combination of these two purity assignments leads to two independent traceability chains, and results in higher reliability of the CRM.

**Figure 2** Only well-characterised and high-purity starting materials are used for **TraceCERT** calibration standards (clockwise from upper left: copper, zinc, nickel and gold).



More information on issues related to CRM quality and production can be obtained by reading the series of Analytix articles we published in 2006 and 2007 [6]. This series covered traceability and uncertainty assignment, handling of high-purity starting materials, production, packaging and stability studies.

#### References

- 1] ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories, 2005.
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- 5] ISO Guide 35, Reference materials – General and statistical principles for certification, 3rd ed., 2006.
- 6] **TraceCERT** – Traceable Certified Reference Materials. Part 1: Analytix, Vol. 5, 2006 and Part 2-5: Analytix, Vol. 1 – 4, 2007, available at [www.sigma-aldrich.com/analytix](http://www.sigma-aldrich.com/analytix)

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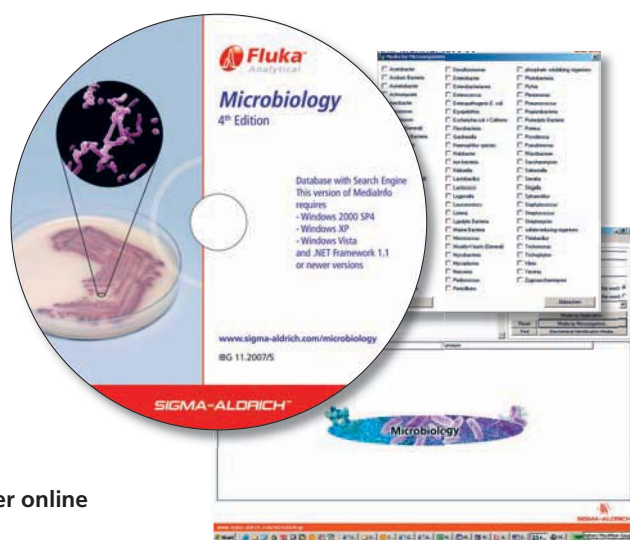
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## Trace analysis of tobacco-specific nitrosamines

New Fluka-brand standards complement SupelMIP™ SPE phases for highly sensitive LC-MS analysis of TSNAs in urine

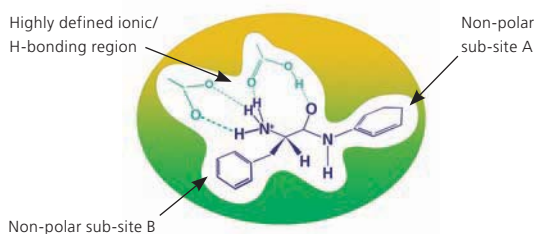
Nicole Amann, Product Manager Analytical Standards [nicole.amann@sial.com](mailto:nicole.amann@sial.com)

There is a perpetual need for sample preparation devices that provide high analyte selectivity and recovery, but do so simply and economically. The need is especially strong when dealing with low-level or trace detection in complex matrixes. Mass spectrometric detection can provide the high sensitivity and specificity, but its effectiveness is dampened by interferences from the sample matrix that suppress ionisation.

### SupelMIP – Highly selective SPE phases

Recently, we addressed this dilemma by introducing SupelMIP solid phase extraction (SPE) products to the analytical market. “MIP” stands for molecularly imprinted polymers. They comprise highly cross-linked polymers that are engineered to extract a single analyte of interest or a class of structurally related analytes with an extremely high degree of selectivity. This is possible because selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities or imprints that are sterically and chemically complementary to the analyte(s) of interest. Figure 1 shows a schematic of the highly selective SupelMIP binding site and representative molecular interactions.

**Figure 1** Schematic of SupelMIP SPE Binding Site



### Lower detection limits

SupelMIP phases offer multiple points of interaction with only the analytes of interest. Consequently, harsher wash conditions can be used that provide cleaner extracts, lower background and better sensitivity than possible with conventional SPE phases.

### Save SPE method development time

There is no need to conduct time-consuming method development. We have developed both the SupelMIP phase and the specific extraction procedure to follow to guarantee the sensitivity, precision and accuracy required from the assay.

### Reduce ion suppression

LC-MS is an invaluable tool for trace analysis. Because the SupelMIP phases are extremely selective only for the target imprinted analytes, matrix interference and resulting ion suppression are not problematic. Even in complex matrixes, such as biological fluids, rapid analysis and high sensitivity are possible with SupelMIP.

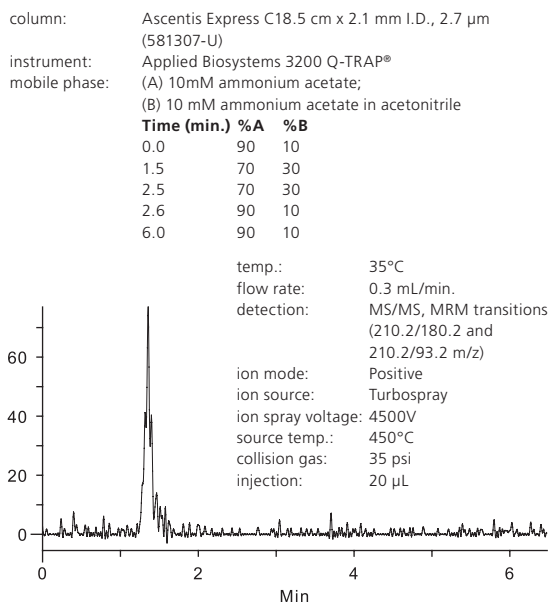
### Featured analysis: Tobacco-specific nitrosamines

One application area that has benefited from the high selectivity of SupelMIP phases is the analysis of tobacco-specific nitrosamines (TSNAs). Two recent Supel Reporter articles and a Technical Bulletin described this application in detail [1-3]. TSNAs are formed through the burning, curing and fermentation of tobacco leaves. They are considered highly carcinogenic. Five TSNAs are of particular interest: N'-nitrosornicotine (NNN), N-nitrosoanabasine (NAB), N-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Total urinary NNAL is a biomarker for monitoring exposure to TSNAs in smokers and in nonsmokers who have been exposed to second-hand smoke.

### SupelMIP SPE phases for NNAL and other TSNAs

Because NNAL is detected in urine at very low concentrations, as low as pg/mL, a highly specific and sensitive assay is called for. Although TSNA extraction and analysis protocols already exist, they require complex and time-consuming sample preparation that takes on the order of days to complete. We eliminated this problem by introducing SupelMIP SPE phases for NNAL and four other TSNAs. By following the clear and simple protocol included with the SupelMIP tubes, higher sample throughput, lower limits of quantitation and greater reproducibility over conventional SPE methods can be achieved, even in the presence of high levels of nicotine.

The chromatogram in Figure 2 shows the LC-MS analysis of NNAL in urine following extraction by SupelMIP NNAL. The high extraction selectivity of SupelMIP NNAL gave low matrix interferences, and permitted a highly sensitive and rapid analysis. With traditional SPE methods, because of their lack of selectivity, non-extracted matrix components reduce sensitivity and make quantification of early-eluting peaks unreliable if not impossible.

**Figure 2** Ion chromatogram of NNAL standard

### A perfect complement: Fluka-brand TSNA standards and deuterated standards

The importance of high-quality analytical standards for reliable and quantitative analysis cannot be overstated. To complement the SupelMIP NNAL and TSNA SPE tubes, we have developed Fluka-brand standards of all five TSNA. Additionally, we provide deuterated NNAL and NNK to enable low detection limits for trace analysis.

For more information on TSNA analysis and SupelMIP in general, including the comprehensive SupelMIP brochure, please use the enclosed reply card, contact our technical service or visit our dedicated website:

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

#### References

(available by download at [www.sial.com/supelmip](http://www.sial.com/supelmip))

- 1] Shimelis, O.; Whilborg, A.; Aurand, C.; Trinh, A.  
 "Trace Level Analysis of NNAL in Urine Using SupelMIP SPE-NNAL and LC-MS-MS." *Supelco Reporter (International)*, 2007, 28, 7-10.
- 2] Boyd, B.; Lundberg, D.; Kronauer, S.; Wihlberg, A.; Trinh, A.  
 "The Extraction of TSNA's using Molecularly Imprinted Polymer SPE." *Supelco Reporter (US)*, 2007, 25.5, 12-14.
- 3] Boyd, B.; Lundberg, D.; Kronauer, S.; Wihlberg, A.  
 "Tobacco-Specific Nitrosamines: Efficient Extraction of Toxic Compounds from Complex Matrices using Molecularly Imprinted Polymers." *Supelco Technical Report (T407117)*.

**Table 1** Product list – TSNA standards

Part No.	Brand	Description	Package Size
75285	Fluka	N'-Nitrosornicotine (NNN)	2 mg
75283	Fluka	N-Nitrosoanabasine (NAB)	2 mg
75281	Fluka	N-Nitrosoanatabine (NAT)	2 mg
59773	Fluka	4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	10 mg
78013	Fluka	4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	10 mg
94454	Fluka	4-(Methyl-d <sub>3</sub> -nitrosamino)-1-(3-pyridyl)-1-butanol (d <sub>3</sub> -NNAL)	2 mg
49578	Fluka	4-(Methyl-d <sub>3</sub> -nitrosamino)-1-(3-pyridyl)-1-butanone (d <sub>3</sub> -NNK)	2 mg

**Table 2** Product list – SupelMIP SPE cartridges for TSNA extraction

Part No.	Brand	Description	Package Size
53206-U	Supelco	SupelMIP NNAL (specific for NNAL) 25 mg sorbent mass/ 10 mL cartridge volume	50 cartridges
53203-U	Supelco	SupelMIP NNAL (specific for NNAL) 25 mg sorbent mass/ 3 mL cartridge volume	50 cartridges
53221-U	Supelco	SupelMIP TSNA's (4 different tobacco-specific Nitrosamines: NNK, NNN, NAB, NAT) 50 mg sorbent mass/ 10 mL cartridge volume	50 cartridges
53222-U	Supelco	SupelMIP TSNA's (4 different tobacco-specific Nitrosamines: NNK, NNN, NAB, NAT) 50 mg sorbent mass/ 3 mL cartridge volume	50 cartridges

## P-I-A-N-O Standards for Detailed Hydrocarbon Analyses

Convenient kits and individual mixes for precise calibration and performance checks of the GC system

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Detailed hydrocarbon analysis (DHA) applications are widely used in the petroleum industry to characterise light petroleum fractions with boiling points up to 450°F/225°C. These applications comply with ASTM methods D 5134, D 6729, D 6730 and D 6733 which use single-column gas chromatography (GC) to group the hydrocarbon components by structure.

DHA is applied to petroleum streams comprising naphtha, alkylate, reformer feed, reformate, isomerate, gasoline and compressed liquids. DHA characterisation of these streams is based on the Kovats Index, and differentiates the composition and concentrations into five groups that are collectively called PIANO:

**Paraffins, Isoparaffins, Aromatics, Naphthenes and Olefins.**

Precise calibration of the chromatographic system is critical for running successful DHA applications. Calibration utilises a set of six complex, quantitative analytical standards known as the PIANO standards. These standards are difficult for most laboratories to prepare due to their complexity and the lack of commercial sources of the many branched-chain hydrocarbons in the mixtures.

To facilitate DHA analysis, Sigma-Aldrich now offers ALPHAGAZ™ PIANO standards as kits and individual mixtures. The kit includes a 139 component quantitative multi-group PIANO mix, plus the five individual quantitative PIANO group mixes. ALPHAGAZ PIANO standards are accurately prepared by weight to three decimal places. Each mixture is shipped with a comprehensive analytical data sheet listing components by weight percent, mole percent, liquid volume percent, retention times and retention indices for each component. A chromatogram of each mixture is also provided. The standards are supplied in crimp-top vials with hole caps and septa.

Utilising these six high-quality analytical standards will ensure the GC system is optimised for proper performance of the DHA analyser. These standards should also be used to perform routine quality assurance checks on the GC system. Checking the system regularly with the Supelco 139-component PIANO mix will reveal the need for maintenance of the GC system, including when it is time to replace the column.

In addition to the standards, we also offer an ideal, petroleum-specific GC column for DHA analysis, the 100-metre Supelco Petrocol™ DH. This column is specifically manufactured for the analysis of complex hydrocarbon mixtures and is ideally suited for DHA systems.

### Quantitative Reference Standards

#### **n-Paraffins Mix, Varied conc. (lot-specific), 0.1 mL, 44585-U**

n-Pentane

n-Hexane

n-Heptane

n-Octane

n-Nonane

n-Decane

n-Undecane

n-Dodecane

n-Tridecane

n-Tetradecane

n-Pentadecane

#### **Isoparaffins Mix, Varied conc. (lot-specific), 0.1 mL, 44586-U**

3,3-Diethylpentane

2,3-Dimethylbutane

2,3-Dimethylheptane

2,5-Dimethylheptane

3,3-Dimethylheptane

3,4-Dimethylheptane (L)

3,5-Dimethylheptane (L)

3,4-Dimethylheptane (D)

3,5-Dimethylheptane (D)

2,2-Dimethylhexane

2,3-Dimethylhexane

2,4-Dimethylhexane

2,5-Dimethylhexane

2,2-Dimethyloctane

2,3-Dimethyloctane

3,3-Dimethyloctane

2,2-Dimethylpentane

2,3-Dimethylpentane

2,4-Dimethylpentane

3,3-Dimethylpentane

3-Ethylhexane

3-Ethylheptane

3-Ethylpentane

2-Methylheptane

3-Methylheptane

4-Methylheptane

2-Methylhexane

3-Methylhexane

2-Methylnonane

3-Methylnonane

2-Methyloctane

3-Methyloctane

2-Methylpentane

3-Methylpentane

Isopentane

2,2,3-Trimethylbutane

2,2,3-Trimethylpentane

(continued on page 10)

**Aromatics Mix, Varied conc. (lot-specific), 0.1 mL, 44587**

Benzene
Butylbenzene
sec-Butylbenzene
tert-Butylbenzene
tert-Butyl-3,5-dimethylbenzene
tert-Butyl-4-ethylbenzene
tert-1-Butyl-2-methylbenzene
1,2-Diethylbenzene
1,2-Dimethyl-3-ethylbenzene
1,2-Dimethyl-4-ethylbenzene
1,3-Dimethyl-2-ethylbenzene
1,3-Dimethyl-5-ethylbenzene
1,4-Dimethyl-2-ethylbenzene
Ethylbenzene
Hexylbenzene
Isobutylbenzene
Isopropylbenzene
2-Methylbutylbenzene
1-Methyl-2-ethylbenzene
1-Methyl-3-ethylbenzene
1-Methyl-4-ethylbenzene
1-Methyl-2-isopropylbenzene
1-Methyl-3-isopropylbenzene
1-Methyl-4-isopropylbenzene
1-Methyl-2- n-propylbenzene
1-Methyl-3- n-propylbenzene
1-Methyl-4- n-propylbenzene
Pentylbenzene
Propylbenzene
1,2,4,5-Tetramethylbenzene
Toluene
1,2,4-Triethylbenzene
1,3,5-Triethylbenzene
1,2,4-Trimethylbenzene
1,3,5-Trimethylbenzene
m-Xylene
o-Xylene
p-Xylene

**Naphthenes Mix, Varied conc. (lot-specific), 0.1 mL, 44588**

n-Butylcyclopentane
Cyclohexane
Cyclopentane
cis-1,2-Dimethylcyclohexane
trans-1,2-Dimethylcyclohexane
trans-1,4-Dimethylcyclohexane
1,1-Dimethylcyclopentane
trans-1,2-Dimethylcyclopentane
cis-1,3-Dimethylcyclopentane
trans-1,3-Dimethylcyclopentane
Ethylcyclopentane
1-Ethyl-1-methylcyclopentane
Isobutylcyclohexane
Isobutylcyclopentane
Isopropylcyclohexane
Isopropylcyclopentane
Methylcyclohexane
Methylcyclopentane
t-1-Methyl-2-(4MP)cyclopentane
t-1-Methyl-2-propylcyclohexane
Propylcyclopentane
1,1,2-Trimethylcyclohexane
1,1,4-Trimethylcyclohexane
cis, trans, cis-1,2,4-Trimethylcyclohexane
cis, trans, trans-1,2,4-Trimethylcyclohexane
cis, cis, cis-1,3,5-Trimethylcyclohexane
cis, cis, cis-1,2,3-Trimethylcyclopentane
cis, trans, cis-1,2,3-Trimethylcyclopentane
cis, trans, cis-1,2,4-Trimethylcyclopentane
cis, trans, trans-1,2,4-Trimethylcyclopentane

**Olefins Mix, Varied conc. (lot-specific), 0.1 mL, 44589**

1-Decene
1-Heptene
cis-2-Heptene
trans-2-Heptene
cis-3-Heptene
trans-3-Heptene
1-Hexene
cis-2-Hexene
trans-2-Hexene
2-Methyl-1,3-butadiene
2-Methyl-1-butene
3-Methyl-1-butene
2-Methyl-2-pentene
4-Methyl-1-pentene
1-Nonene
cis-2-Nonene
trans-2-Nonene
cis-3-Nonene
trans-3-Nonene
1-Octene
cis-2-Octene
trans-2-Octene
1-Pentene
cis-2-Pentene
trans-2-Pentene

**P-I-A-N-O Mix, Varied conc. (lot-specific), 0.1 mL, 44593-U**

139 n-paraffins, isoparaffins, aromatics, naphthenes and olefins  
A single, quantitative mix of the components in the five mixes previously described.

**P-I-A-N-O Kit, one each of the following mixes, 44594-U**

- n-Paraffins Mix (44585-U)
- Isoparaffins Mix (44586-U)
- Aromatics Mix (44587)
- Naphthenes Mix (44588)
- Olefins Mix (44589)
- P-I-A-N-O Mix (44593-U)

**Chromatographic Column**

For PNA, PONA and PIANO-type analyses

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## Optimising the LC-MS analysis of biomolecules

Columns, solvent blends and calibration standards to maximise performance of LC-MS analyses

Rudolf Köhling, Scientist [rudolf.koehling@sial.com](mailto:rudolf.koehling@sial.com)

Mass spectrometry (MS) is an invaluable tool for the analysis of biomolecules like peptides, proteins and structural components, and small molecules like metabolites, lipids, steroids, sugars, amino acids, nucleotides and many others. A combination of a sensitive mass spectrometer and an HPLC system fitted with a suitable column and mobile phase can guarantee a successful analysis of complex biological samples. Sigma-Aldrich, through our Supelco and Fluka brands, offers analysts a comprehensive line of premier MS consumables, including HPLC columns, high-purity solvents, additives and customised solvent blends and standards for calibration and troubleshooting to get the highest sensitivity, precision and resolution out of your HPLC and MS systems.

### Optimising the HPLC column

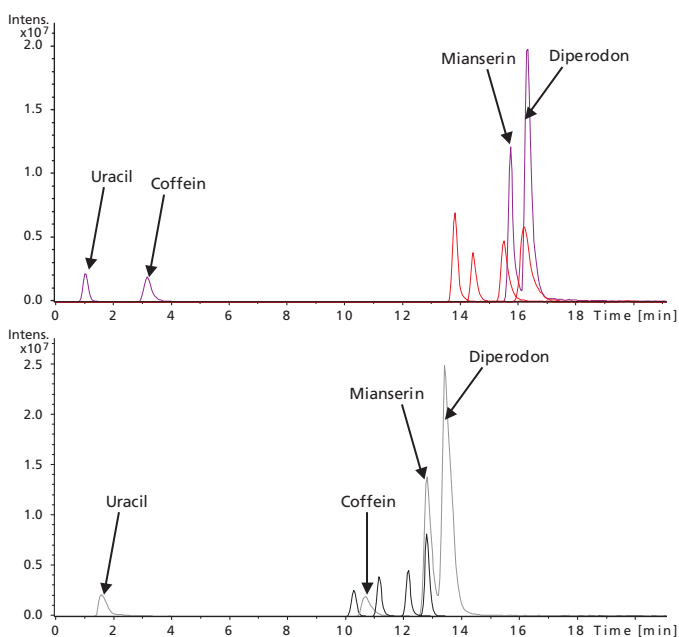
To optimise the column selection for a reversed-phase separation of biomolecules, there are several important factors to consider. First, the pore size of the support particle must be large enough to permit access to the surface area inside the pores. Generally, it is recommended to use a wide pore particle, 300 Å or higher, for peptides above 30 amino acid residues (~3,000 Da). Second, activity of the support particle should be taken into account. Interactions should be limited to those between the analytes and stationary phase, avoiding relatively strong interactions like hydrogen bonding, chelation or ion exchange. This is especially acute with basic compounds, like peptides rich in ARG, HIS or LYS residues. Third, the chemistry of the bonded phase should be chosen to give the desired retention and selectivity.

For peptides below 3,000 Da and other small molecules, Ascentis® HPLC phases offer analysts a highly inert surface for excellent peak shape coupled with the benefit of choices in stationary phase selectivity. High-speed, high-resolution separations are also possible using Ascentis Express. For larger peptides and proteins, silica-based Discovery® BIO Wide Pore phases offer inertness and the necessary pore size, while apHera™ is an excellent 300 Å polymer-based particle. These two phases are both ideal to separate large and small molecules, which is necessary for complex biological samples (Figure 1). Ascentis® and Discovery® columns are available with small inner diameters for micro-flow applications.

### Important qualities of mobile phase additives

We recently reviewed the subject of mobile phase additives for LC-MS in a 5-part series in Analytix [1-5]. Stated simply, the challenge of using ionic mobile phase additives is to balance their positive effects on the chromatography against their detrimental effects on the MS detection. Ionic mobile phase additives like trifluoroacetic acid (TFA) control the pH control complex with oppositely charged ionic groups to enhance reversed-phase retention, or suppress

**Figure 1** Column performance of Discovery BIO Wide Pore (upper) and Astec apHera C18 (lower) toward 4 small test molecules compared to peptide mixture described in Figure 2.



unwanted interactions. However, typical concentrations of TFA (0.1% v/v) have high surface tension and prevent efficient spray formation. Also, TFA ions in the gas phase ion-pair with the peptide's basic groups suppressing their ionisation and reducing sensitivity.

An easy way to minimise suppression effects is realisable with solvent blends containing 0.1% (v/v) formic acid and 0.01% (v/v) TFA. They both offer good separation conditions and high MS sensitivity and are ideal partners for Discovery® BIO Wide Pore and Astec apHera™ columns.

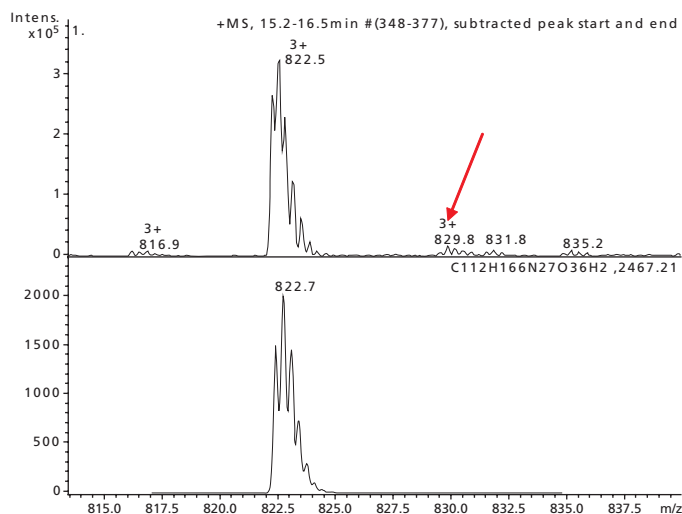
Alternatives to TFA for LC-MS of proteins and peptides include formic and acetic acid (for low pH), ammonium acetate (for neutral pH) and ammonium bicarbonate (for basic pH). We offer a wide choice of high-purity additives, solvents and convenient additive-solvent blends. Their purity, especially the absence of sodium ions, ensures clean baselines and the absence of adducts that complicate the mass spectra (see Figure 2).

### Mass calibration and performance test standards

In addition to columns and solvents, mass spectroscopy/spectrometry requires solutions of well-defined compounds to calibrate the m/z ratio. Molecular weight is often used to aid in protein identification. High-purity calibrants must be used to obtain mass precision in the

(continued on page 12)

**Figure 2** Effect of sodium adduct formation on MS spectral quality. Detected (upper) and calculated (lower) mass spectrum of human ACTH fragment 18-39 m/z ( $[M+3H]^{3+}$ )=822.5 Da. The upper mass spectrum shows a very low abundance of sodium adduct  $[M+2H+Na]^{3+}$  with a m/z ratio of 829.8 Da, demonstrating the purity of the LC-MS CHROMASOLV® solvents.



ppm range, whether these are protein/peptide standards for molecular weight calibration, or small molecules like caffeine, reserpine or uracil, for RP column performance tests. We have designed and

developed standards and test mixtures for HPLC, LC-MS and MALDI to calibrate, maintain and troubleshoot the corresponding instruments. Together, they are powerful tools to calibrate the entire LC-MS system and test the performance and suitability of the HPLC column in the same step.

For further information, please visit our dedicated websites:

Solvents and additives for LC-MS:

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

HPLC columns for biomolecule separations:

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

#### References

- 1] Emmert, J. Mobile Phase Additives for LC-MS, Part 1: Acids – The Most Common Choice. *Analytix*, 2006 (2), 8–9.
- 2] Emmert, J.; Rück, A. Mobile Phase Additives for LC-MS, Part 2: Techniques to Overcome the Ionization Suppression Effects of TFA. *Analytix*, 2006 (3), 16–17.
- 3] Emmert, J.; Leitner, A. Mobile Phase Additives for LC-MS, Part 3: The Neutral Salts. *Analytix*, 2006 (4), 9–11.
- 4] Emmert, J.; Wälti, T. Mobile Phase Additives for LC-MS, Part 4: Special Case - Sodium Adduct Formation. *Analytix*, 2006 (5), 6–7.
- 5] Emmert, J.; Köhling, R. Mobile Phase Additives for LC-MS, Part 5: The Bases – Reverse Buffering, Negative and Reverse Ionization. *Analytix*, 2007 (1), 4–6.

**Table 1** Product table – Selection of analytical standards, calibration kits and test mixtures for HPLC and LC-MS

Part No.	Brand	Description	Package Size
58278	Supelco	<b>Reversed Phase Test Mix 1</b> Acetophenone (7 µg/mL), benzene (750 µg/mL), toluene (775 µg/mL), uracil (7 µg/mL) in methanol:water (60:40)	1 mL
47641-U	Supelco	<b>Reversed Phase Test Mix 2</b> N,N-Diethyl-m-toluamide (600 µg/mL), phenol (700 µg/mL), toluene (4 µg/mL), uracil (5 µg/mL) in acetonitrile:water (58:42)	1 mL
H2016	Sigma	<b>HPLC peptide standard mixture</b> Approx. 0.5 mg each of Gly-Tyr, Val-Tyr-Val, methionine enkephalin, leucine enkephalin and angiotensin II	1 vial
MSCAL1-1KT	Sigma	<b>ProteoMass™ Peptide and Protein MALDI-MS Calibration Kit</b> Configured for analysing complex mixtures of proteins and peptides 700 to 66,000 Da.	1 kit
NEW! 43530	Fluka	<b>Reserpine Standard for LC-MS</b> 5 mg/mL in water/isopropyl alcohol (1:1)	4.5 mL
C6035	Sigma	<b>Caffeine solution</b> 1.0 mg/mL 5% in methanol	1 mL

**Table 2** Product table – Selection of pre-mixed solvent blends for HPLC and LC-MS

Part No.	Brand	Description	Package Size
34677	Fluka	Water with 0.1% FA and 0.01% TFA, LC-MS CHROMASOLV®	2.5 L
34676	Fluka	Acetonitrile with 0.1% FA and 0.01% TFA, LC-MS CHROMASOLV®	2.5 L
34978	Fluka	Water with 0.1% TFA, LC-MS CHROMASOLV®	2.5 L
34976	Fluka	Acetonitrile with 0.1% TFA, LC-MS CHROMASOLV®	2.5 L
34974	Fluka	Methanol with 0.1% TFA, LC-MS CHROMASOLV®	2.5 L

**Table 3** Product table – Selection of HPLC columns for HPLC and LC-MS of biomolecules

Part No.	Brand	Description	Dimensions
65506-U	Supelco	Discovery BIO Wide Pore C18	10 cm x 1 mm I.D., 3 µm
56100AST	Supelco	Astec apHera C18	15 cm x 2 mm I.D., 5 µm

\* Please consult the website [www.sigma-aldrich.com/hplc](http://www.sigma-aldrich.com/hplc) for our complete HPLC column offering.

## New solvents for headspace GC applications

Purity and handling specifications meet the requirement of Ph. Eur., USP and ICH guidelines residual solvents

Michael Jeitziner, Market Segment Manager Analytical Reagents & Standards [michael.jeitziner@sial.com](mailto:michael.jeitziner@sial.com)



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.

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 of the sample solvent and its purity have significant effects on the recovery and quality of the chromatogram (see Figure 1), we have developed solvents specifically for GC-HS applications. Their purity

and handling specifications meet the requirements of Ph. Eur., USP and ICH guidelines. The new GC-SH line includes water and three of the most commonly used organic solvents dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF) and N,N-dimethylacetamide (DMA). N,N-dimethylformamide and dimethyl sulfoxide 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.

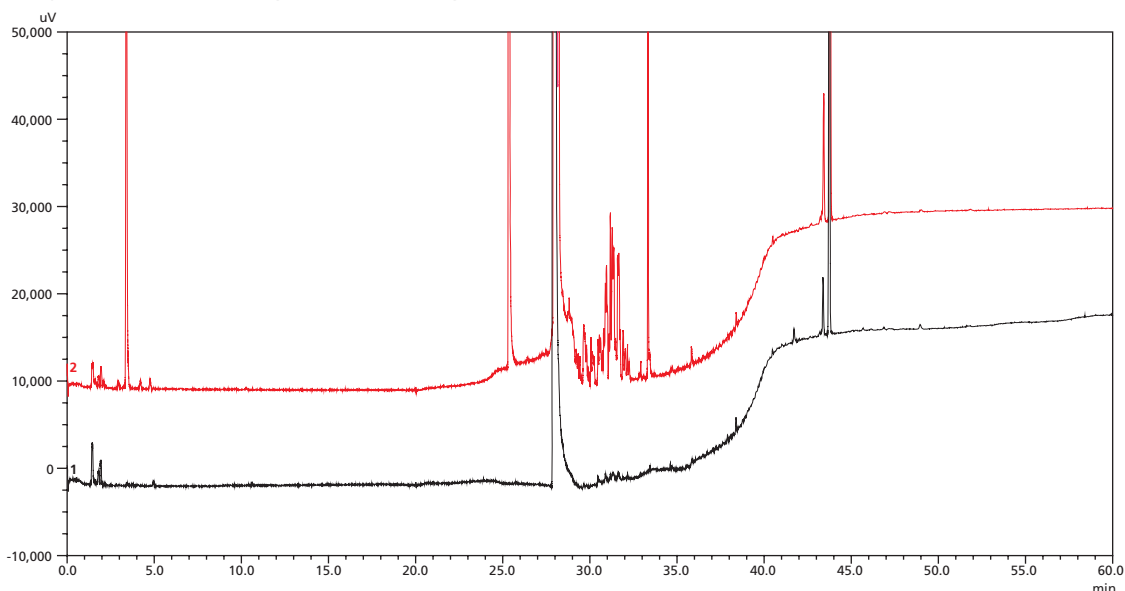
**Table 1** Product table

Part No.	Brand	Description	Boiling Point	Package Size
44901	Fluka	N,N-Dimethylacetamide, puriss. p.a. for GC-HS	166°C	1 L
51779	Fluka	Dimethyl sulfoxide, puriss. P.a. for GC-HS	189°C	1 L
51781	Fluka	N,N-Dimethylformamide, puriss. p.a. for GC-HS	153°C	1 L
53463	Fluka	Water, puriss. p.a. for GC-HS	100°C	1 L

### 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, 137-43.
- 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 Pharmacopeia. *Int. J. Pharm.* 2000, 195, 159-69.

**Figure 1** Headspace gas chromatogram of two DMSO grades: GC-HS grade (black trace) and conventional grade (red trace). Analytical conditions according to Ph. Eur. 6.0 using a SPB™-624 column (Supelco 23323-U) [2]



## Animal-derived peptones

Sigma-Aldrich supplies a wide range of peptones from different sources and production methods

Ivo Siegrist, Product Manager Microbiology [ivo.siegrist@sial.com](mailto:ivo.siegrist@sial.com)

Peptone is a water-soluble protein hydrolysate that contains small proteins, peptides and amino acids. It is produced by the partial enzymatic or acidic hydrolysis of proteins. Peptones are an important nitrogen source and significantly affect the growth and metabolism of microorganisms.

The production process and the protein source give the peptone its particular characteristics. The differences can be seen in the nitrogen and amino acid content, solubility, ash, pH and other parameters (see Table 1).

Further information on peptones can be found on our website [www.sigma-aldrich.com/microbiology](http://www.sigma-aldrich.com/microbiology) or by contacting our technical service at [flukatec@sial.com](mailto:flukatec@sial.com)

Figure 1 4% stock solutions of different N-sources



Table 1 Peptones from different animal sources with specifications and typical values

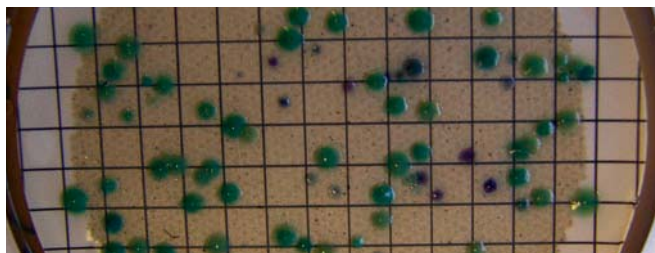
	Part No.	Brand	Description	Total N	Amino N	AN/TN	Ash	Loss on drying	pH (2% in water, 25°C)
Meat-derived Peptones	53283	Fluka	Brain heart infusion	≥12%	≥3.5%	~0.36	≤12%	≤6%	6.5 ± 0.5
	03077	Fluka	Liver hydrolysate	≥10%	≥4%	~0.47	≤18%	≤6%	6.9 ± 0.5
	70164	Fluka	Meat extract	11.5 - 12.5%	3.5 - 4.5%	0.28 - 0.39	≤18%	≤6%	6.0 - 7.0
	77180	Fluka	Peptone from animal proteins	≥10.0%	≥2.5%	~0.22	≤22%	≤6%	7.0 ± 0.5
	70951	Fluka	Peptone from gelatin, enzymatic digest	≥15%	≥2.4%	0.24 - 0.34	≤9%	≤6%	6.5 - 7.5
	70176	Fluka	Peptone from gelatin, pancreatic digest	~16%	2.4 - 3.4%	~0.2	≤10%	~5%	7.0 ± 0.5
	70175	Fluka	Peptone from meat, enzymatic digest	≥10%	≥3%	~0.31	≤12%	≤6%	7.0 ± 0.5
	82962	Fluka	Peptone from meat, enzymatic digest	≥14 %	≥2.5%	0.16 - 0.25	≤6 %	≤6%	5.0 - 6.0
	70174	Fluka	Peptone from meat, peptic digest	≥10%	≥3%	~0.3	≤12%	≤6%	7.0 ± 0.5
	68971	Fluka	Peptone, special	≥12%	≥3.5%	~0.31	≤15%	≤5%	6.5 - 7.5
	77199	Fluka	Peptone, mycological	≥9.0%	≥3.8%	~0.37	≤15%	≤12%	5.0 - 7.0
	82450	Fluka	Proteose-Peptone	≥11%	≥3%	~0.3	≤20%	≤6%	6.0 - 8.0
	70937	Fluka	Tryptose	≥11%	≥3.5	~0.31	≤12%	≤8%	6.2 - 7.2
	93733	Fluka	Tryptose	~14%	~3.2	~0.3	~6%	≤10%	7.0 ± 0.5
	Part No.	Brand	Description	Total N	Amino N	AN/TN	Ash	Loss on drying	pH (2% in water, 25°C)
Fish-derived Peptones	93490	Fluka	Fish peptone	≥10%	≥2%	~0.23	≤27%	≤6%	6.0 - 8.0
	68185	Fluka	Peptone from salmon	≥13 %	≥2%	~0.2	≤10%	≤8%	6.0 - 7.0
	Part No.	Brand	Description	Total N	Amino N	AN/TN	Ash	Loss on drying	pH (2% in water, 25°C)
Milk-derived Peptones	22080	Fluka	Casein from bovine milk	~14%			≤5%	≤15%	
	22078	Fluka	Casein from bovine milk	≥13.5%				≤5%	
	22090	Fluka	Casein hydrolysate	≥11%	≥6%	~0.48	≤8%	≤6%	6.0 - 7.0
	39396	Fluka	Casein yeast peptone (4/1)	~12%	2.5 - 5%	~0.32	≤15%	≤10%	7.2 ± 0.5
	61302	Fluka	Lactalbumin hydrolysate	≥12%	≥4.5%	~0.5	≤6%	≤6%	6.0 - 8.0
	61300	Fluka	Lactalbumin hydrolysate	≥11%	≥5%	~0.5	≤10%	≤6%	6.8 ± 0.5
	70173	Fluka	Peptone from casein and other animal proteins	~14%	3.7%	~0.3	~5%	~5%	7.2 ± 0.2
	70171	Fluka	Peptone from casein, acid digest	≥7%	≥4%	0.56 - 0.93		≤6%	5.5 ± 0.2
	82303	Fluka	Peptone from casein, enzymatic digest	~13 %	3 - 4%	~0.3	≤17%	≤6%	7.0 ± 0.5
	70169	Fluka	Peptone from casein, pancreatic digest	≥12%	≤5.5%	~0.48	≤12%	≤6%	6.8 ± 0.5
	70172	Fluka	Peptone from casein, tryptic digest	≥11%	≥3.5%	~0.38	≤8%	≤6%	6.7 ± 0.5
	82514	Fluka	Protein hydrolysate, Amicase®	~13%	≥8.5%	~0.67	≤5%	~5%	6.0 - 7.0
	82524	Fluka	Protein hydrolysate N-Z-Amine® AS	≥12%	≥5%	~0.48	≤10%	≤8%	6.9 ± 0.5
	70166	Fluka	Skim milk powder	~5.3%			≤10%	≤5%	
	95039	Fluka	Tryptone enzymatic digest from casein	≥12.2%	≥3.5%	0.26 - 0.37	≤17%	≤6%	6.5 - 7.5
	61044	Fluka	Tryptone Plus	≥12%	≥4%	0.32 - 0.45	≤22%	≤8%	6.2 - 7.2

## New CP ChromoSelect Agar

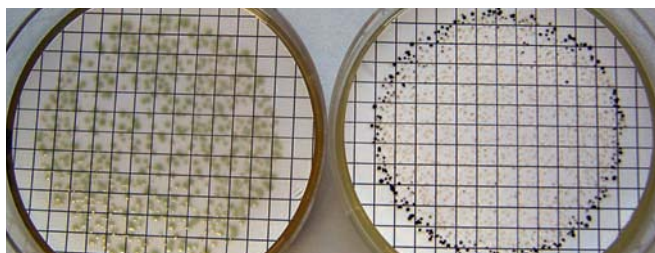
Selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration

*Clostridium perfringens* are anaerobic, Gram-positive, spore-forming rod-shaped bacteria. They are widespread in the environment and also found in the digestive systems of humans and domestic and feral animals. Perfringens poisoning, usually from ingesting undercooked food, especially meat, is one of the most commonly reported food-borne illnesses. Early detection of *Clostridium* in food is important to control outbreaks. To facilitate detection, we have introduced a new chromogenic medium, CP ChromoSelect Agar, for enumeration and differentiation of *Clostridium* sp., in particular *Clostridium perfringens*, in aqueous samples (Figure 1).

**Figure 1** Drinking water sample cultured on CP ChromoSelect Agar. *C. perfringens* appears as distinct green colonies.



**Figure 2** *C. perfringens* ATCC 10873 on CP ChromoSelect Agar (left) and TSC agar (right). Note the false negatives on the TSC agar.



CP ChromoSelect Agar is more reliable and easier to handle than m-CP and TSC agars. The colour does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*. CP ChromoSelect Agar avoids the disadvantages of m-CP agar, including the presence of ammonia, which prevents subculturing the *C. perfringens* colonies, the too-selective nature of m-CP agar, and the evanescence of the red colour of colonies after the addition of ammonia, which makes further confirmation impossible.

CP ChromoSelect Agar also eliminates the excessive and variable blackening of the peripheral colonies encountered with TSC agar, which makes colony counting at lower dilutions difficult and leads to false positives. It is also more reliable at high bacteria counts, where the TSC agar can produce false negatives because of an interference with the other enzymatic mechanisms from acid production and oxygen contact.

Ivo Siegrist, Product Manager Microbiology [ivo.siegrist@sial.com](mailto:ivo.siegrist@sial.com)



Besides having advantages over m-CP and TSC agars, CP ChromoSelect Agar is an ideal growth medium. It contains only vegetable peptones and, together with yeast extract, is an excellent source of nitrogen, carbon, amino acids and vitamin B complex. Sucrose acts as the fermentable carbohydrate and reducing agents lower the redox potential of the medium. Diverse salts provide the required ions for enzymatic reactions. Buffering agents stabilise the pH within the ideal growth range. Inhibitors D-cycloserine and polymyxin B give the medium its selectivity, while further selectivity is achieved by incubation under anaerobic conditions at 44°C. Various promoters and substrates protect injured cells to improve recovery rate and enhance growth. The chromogenic enzyme substrates in the CP ChromoSelect Agar provide the differentiation, for *C. perfringens* in particular (Table 1). A negative indol reaction (Kovac's Reagent) is confirmatory for *C. perfringens*.

**Table 1** *Clostridium* sp. cultural characteristics in CP ChromoSelect Agar

Organisms (ATCC)	Growth	Colony Appearance
<i>Clostridium perfringens</i> (13124)	+++	Green
<i>Clostridium bifermentans</i> (638)	+++*	Dark blue with violet halo
<i>Clostridium sporogenes</i> (8534)	-	-
<i>Clostridium sordelli</i> (9714)	++	Dark green with halo (change to red with Kovac's Reagent)
<i>Enterococcus faecalis</i> (29212)	++	Violet
<i>Escherichia coli</i> (25922)	-	-
<i>Pseudomonas aeruginosa</i> (27853)	-	Colourless
<i>Staphylococcus aureus</i> (25923)	-	-
<i>Bacillus subtilis</i> (6051)	-	-
<i>Salmonella typhimurium</i> (DSM 554)	++	Violet

### References

- 1) Sartory, D. P.; Field, M.; Curbishley, S.M.; Pritchard, A.M. Evaluation of two media for the membrane filtration enumeration of *Clostridium perfringens* from water. *Lett. Appl. Microbiol.* 1998, 27, 323-7.
- 2) Goshu, G.; Evaluation of microbial faecal indicators and quantifying the respective level of pollution in ground and surface water of Bahidar and peri-urban areas. MSc Thesis ES 07.36 Ethiopia UNESCO-IHE Institute for Water Education, Delft, the Netherlands, 2007.

The product can be ordered under the product number Fluka 12398.

## Spectroscopy reagents for sample decomposition

### Kjeldahl and Eschka's reagents for nitrogen and sulphur determination

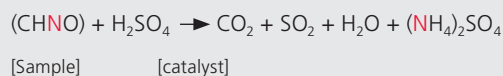
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Many samples destined for analysis require treatment of some form to release compounds of interest from the matrix, or to convert compounds into a form compatible with the analysis method. Digestion is one form of treatment where the sample is completely decomposed, usually by reaction with strong acid or base. Ideally, a digestion reagent should dissolve the sample completely. The purity and required quantity of the digestion reagents are important considerations for trace analysis since this determines the blank value. Sigma-Aldrich offers a wide range of high-purity reagents for various types of chemical digestion.

#### Kjeldahl digestion for nitrogen determination

Kjeldahl digestions convert nitrogen-containing organic compounds, like amino acids, into ammonia compounds by first heating them in concentrated sulphuric acid. A catalyst speeds up the decomposition. Free ammonia is released by adding concentrated sodium hydroxide, which is evaporated by steam distillation. The amount of ammonia present, and therefore the amount of nitrogen present in the original sample, is determined by back titration. The method is described by various official methods such as ASTM D3179, ISO 332 and ISO 333.

Figure 1 Kjeldahl reaction (condensed)



The Kjeldahl method is often used to report the protein content of a sample based on the total nitrogen after applying the so-called Jones factors [1 – 3]. If the sample contains nitrite or nitrate, it must be reduced with Arnd's alloy (Part No. 11066) in weak acid to produce a neutral solution prior to analysis [4].

There are several suitable Kjeldahl catalysts. The mercury- and selenium-free catalyst (Part No. 31835) is popular for environmental and toxicological reasons. The selenium-containing catalyst according to Wieninger (Part No. 36120) is used for very resistant samples such as hetero-aromatic compounds, mineral oils and fats. The Missouri catalyst (Part No. 31831) is an environmentally friendly alternative because it has a low copper content, although the reaction is significantly slower.

#### Eschka's mixture for the determination of sulphur in carbon

The Eschka method is one of the most widely used digestion methods for the determination of sulphur in coal and other carbons. It has been accepted as a standard method in several countries. Sulphur is present in coal either as organically bound sulphur or as inorganic sulphur (iron sulphides pyrite or marcasite and sulphates). The Eschka method has distinct advantages in that the equipment is relatively simple and it uses convenient analytical techniques. The sample is ignited with an Eschka's mixture (Fluka 00166) at 800°C, sulphur is precipitated from the resulting solution as BaSO<sub>4</sub>, filtered, ashed and weighed [5 – 7].

Table 1 Product table – A selection of digestion reagents

Part No.	Brand	Description	Package Size
31835	Fluka	Kjeldahl Catalyst Mercury- and selenium-free Contains Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub> / CuSO <sub>4</sub>	250 tablets (2.5 g)
31831	Fluka	Kjeldahl Catalyst according to Missouri Contains 99.5% K <sub>2</sub> SO <sub>4</sub> and 0.5% CuSO <sub>4</sub>	250 tablets (2.5 g)
36120	Fluka	Kjeldahl Catalyst according to Wieninger Contains 96.5 % Na <sub>2</sub> SO <sub>4</sub> , 1.5 % CuSO <sub>4</sub> and 2.0% Se	250 tablets (2.5 g)
31821	Fluka	Disintegrating mixture for Kjeldahl Contains H <sub>2</sub> SO <sub>4</sub> and Se	1L
11066	Fluka	Arnd's alloy, puriss. p.a. Contains 60% Cu and 40% Mg	50 g
00166	Fluka	Eschka's reagent, puriss. p.a., for sulphur determination in carbon, 38-42% Contains magnesium oxide:sodium carbonate (2:1)	250 g, 1 kg

### High-purity acids for ultra-trace analysis

*TraceSELECT*<sup>®</sup>Ultra grade acids purified by sub-boiling, now with even lower impurity specifications



Sample preparation for trace analysis requires reagents of the highest purity. Our *TraceSELECT*<sup>®</sup>Ultra products for the ultra-trace analysis at ppb and even ppt levels are produced by sub-boiling distillation [8]. Sub-boiling is recognised as the best method to obtain high-purity acid and the lowest blank values for ultra-trace analysis. The technique is based on evaporation of the liquid by infrared heating at the surface. It avoids violent boiling and the formation of liquid aerosols that can be transported with the distillate.

To maintain their high purity, *TraceSELECT*<sup>®</sup>Ultra products are supplied in Teflon PFA bottles. Because of recent process improvements, we have reduced the impurity specifications to further guarantee the lowest levels of trace impurities in our *TraceSELECT*<sup>®</sup>Ultra products.

Please find our complete product range at [www.sigma-aldrich.com/traceselect](http://www.sigma-aldrich.com/traceselect)



**See the quality for yourself!**  
Get 50% off on a trial sample  
of *TraceSELECT*<sup>®</sup>Ultra products.\*

**Table 2** Product table – *TraceSELECT*<sup>®</sup>Ultra acids and basis purified by sub-boiling distillation

Part No.	Brand	Description	Package Size
07692	Fluka	Acetic acid $\geq 99.0\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
23828	Fluka	Hydrobromic acid $\geq 44\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
96208	Fluka	Hydrochloric acid $\geq 30\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
02658	Fluka	Hydrofluoric acid $\geq 49\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
02650	Fluka	Nitric acid $\sim 65\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
12415	Fluka	Perchloric acid 67 - 72%, <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
16748	Fluka	Ammonium hydroxide solution $\geq 25\% \text{ NH}_3$ in $\text{H}_2\text{O}$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL
77239	Fluka	Sulphuric acid $\geq 95\%$ , <i>TraceSELECT</i> <sup>®</sup> Ultra	250 mL

#### References:

- 1] Merrill, A. L. and Watt, B. K. Energy value of foods: Basis and derivation, revised. U.S. Department of Agriculture, Agriculture Handbook 74, 1973.
- 2] Protein (Crude) Determination in Animal Feed: Copper Catalyst Kjeldahl Method. (984.13) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th edition.
- 3] Protein (Crude) Determination in Animal Feed:  $\text{CuSO}_4/\text{TiO}_2$  Mixed Catalyst Kjeldahl Method. (988.05) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th edition.
- 4] Arnd T. Zur Bestimmung des Stickstoffs salpeter- und salpetrigsaurer Salze mit Kupfer-Magnesium. *Angew. Chem.* 1920, 33, 296-8.
- 5] ASTM D3177-02(2007). Standard Test Methods for Total Sulphur in the Analysis Sample of Coal and Coke.
- 6] BS 1016-100:1994. Methods for analysis and testing of coal and coke. General introduction and methods for reporting results.
- 7] ISO 334:1992. Solid mineral fuels - Determination of total sulphur – Eschka method.
- 8] Mitchell, J. W. Purification of Analytical Reagents. *Talanta*, 1982, 29, 993-1002.

\* Trial offer for up to two packs of each of the listed products. Please contact your Local Sales Office and quote promotion code W54 to quality. Offer valid until 31st of May 2008.

## Titer of Karl Fischer reagents

The importance of titer, its determination and ambient influences

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The determination and regular control of the titer of the titration agent is of fundamental importance in Karl Fischer (KF) titration. Despite the fact that the chemistry behind KF reactions is well known and the technique is employed in myriad applications, it is still necessary to take the time to measure the exact titer of KF titration reagents in order to achieve accurate and reliable measurements. Calibration, validation and control of analytical instruments and reagents are central requirements in regular quality management systems in laboratories using KF titration.

### Titer and water equivalent

The titer of a substance is defined in several ways, either as the concentration of the substance in a solution, or as the strength of the substance as determined by titration. Titer can also mean the minimum volume needed to cause a particular result in a titration. In the case of KF titration, the substance of interest is, of course, water, and the volume there needed to determine a defined amount of water is better described as the water equivalent (WE). To calculate the water content of a sample, the water equivalent of the reagent has to be known. WE is calculated by the equation:

$$WE = \frac{\text{Weight of water in mg}}{\text{Consumption of reagent in mL}}$$

Water equivalent gives the amount of water (in mg) that has been titrated when exactly 1 mL of the reagent in question is consumed in the titration. Therefore, the titer (the water equivalent) is expressed as mg water per mL reagent. For example, a titer of 5.125 mg/mL means that 5.125 mg water can be determined with 1 mL of this titration agent.

The titer of a KF titration agent is determined by titrating a defined volume of a reagent that has a known amount of water. Reagents with a known content of water can, of course, be pure water itself or standard substances in which the water content can be precisely and easily controlled. Using pure water requires experience to avoid weighing and handling errors, since the mass of water required is extremely small. For example, a reagent with a titer of 2 requires only 10 – 15 mg of water per measurement, which is only a fraction of a drop produced by the tip of the syringe needle.



### Titer determination with HYDRANAL®-Standard sodium tartrate dihydrate

ISO 760 prescribes using sodium tartrate dihydrate instead of pure water for titer determination of a KF titration agent. Sodium tartrate forms a stable dihydrate that neither loses nor adsorbs moisture under normal conditions. HYDRANAL-Standard sodium tartrate dihydrate is ideal for this application; it contains approximately 15.66% water by weight, the value ensured by drying at 150°C. Although sodium tartrate dihydrate has limited solubility in methanol, it dissolves readily in HYDRANAL-Solvent.

Because of its relatively high water content of 15.66%, when using HYDRANAL-Standard sodium tartrate dihydrate, larger sample sizes can be used, which significantly reduces weighing errors. For example, 150–200 mg HYDRANAL-Standard sodium tartrate dihydrate are used for titer determination of a reagent with a titer of 5.

Finely powdered 34696 HYDRANAL-Standard sodium tartrate dihydrate is a certified reference material directly traceable to the SI base unit kg and tested against NIST SRM 2890. Traceability to a national standard or to an SI base unit is often required in laboratory guidelines. Most HYDRANAL-Water Standards for KF titration are directly traceable to the SI base unit kg and tested against the NIST standard reference material SRM 2890, water saturated 1-octanol (National Institute of Standards and Technology, USA).

In Sigma-Aldrich's HYDRANAL laboratory in Seelze, Germany, the water determination in 34696 HYDRANAL-Standard sodium tartrate dihydrate is carried out by several independent methods in order to control the substance and measure the exact water content of each lot. These methods are:

1. The water content is quantified by loss upon drying. Mean value, number of measurements and uncertainty statement are given on the Certificate of Analysis.
2. The titer of HYDRANAL-Composite 5 is determined using this HYDRANAL-Standard sodium tartrate dihydrate.
3. The water content of HYDRANAL-Standard sodium tartrate dihydrate is verified against NIST SRM 2890.

### Liquid HYDRANAL-Water Standards

One downside of solid sodium tartrate dihydrate is that it does not dissolve in certain media, like ethanol-based reagents or HYDRANAL-Working Medium K and HYDRANAL-Medium K. Liquid standards, like HYDRANAL-Water Standards, are viable alternatives to determine titer in these media. HYDRANAL-Water Standards have a precisely measured water content and are ideal for titer determination of volumetric KF reagents, for control of coulometric KF instruments, for monitoring precision and accuracy and for validation and inspection of KF titrators according to ISO 9000, GMP, GLP and FDA guidelines. They consist of a stable solvent mixture with specific composition and precisely determined water content, supplied in airtight glass ampoules to ensure quality when opened by the end user.

One ampoule of HYDRANAL-Water Standard 10.0 can be used for at least three titer measurements. An ampoule is opened and 1 – 2 g portions, exactly weighed, are added to the titration vessel using a syringe. The standard contains 10 mg water/g; the exact value is stated on the Certificate of Analysis supplied with each package.

HYDRANAL-Standard 5.00 is another liquid standard for KF titration. Available in 100 and 500 mL sizes, 1 mL of HYDRANAL-Standard 5.00 contains  $5.00 \pm 0.02$  mg water (at 20°C); 1 g contains  $5.91 \pm 0.02$  mg water.

**Table 1** Selected HYDRANAL products

Part No.	Brand	Product	Description	Package Size
34849	Fluka	HYDRANAL-Water Standard 10.0	Standard for volumetric KF titration 1 g (1mL) contains 10.0 mg = 1.00% water at 20°C Contains 10 glass ampoules of 8 mL Tested against NIST SRM 2890	80 mL
34813	Fluka	HYDRANAL-Standard 5.00	Non-hygroscopic standard for volumetric KF titration. Water content: $5.00 \pm 0.02$ mg/mL at 20°C	100 mL, 500 mL
34696	Fluka	HYDRANAL-Standard Sodium tartrate dihydrate	Standard for volumetric KF titration Water content: approx. 15.66% (CofA) Tested against NIST SRM 2890	25 g
34803	Fluka	HYDRANAL-Standard Sodium tartrate dihydrate	Standard for volumetric KF titration Water content: $15.66 \pm 0.05\%$	100 g
34241	Fluka	HYDRANAL-Molecular sieve 0.3nm	Drying agent for Karl Fischer titration	250 g, 1 kg
34788	Fluka	HYDRANAL-Humidity absorber	Drying agent for Karl Fischer titration	500 g, 1 kg

(continued on page 20)



### Adverse effect of ambient moisture on titer

HYDRANAL reagents are very stable for long periods in sealed containers. However, when opened, ambient moisture will diffuse into the bottle, consuming iodine in the reagent and reducing its effective titer. Since one liter of air contains 12–15 mg water, the reduction in titer from ambient moisture can be significant.

To reduce titer loss from ambient moisture, we strongly recommend that the reagent bottles are fitted with a specially designed drying tube filled with either a HYDRANAL-Molecular sieve 0.3 nm or a HYDRANAL-Humidity absorber. The molecular sieve should be replaced every six weeks; the humidity absorber should be replaced when colour is lost in two-thirds of the indicator. The molecular sieve can be regenerated at 300°C for a minimum of four hours, and the humidity absorber at 140°C until the original indicator colour is restored.

Ambient moisture can also enter the system by migrating through the plastic transfer tubes of the KF titrator. To prevent loss of effective titer by this mechanism, we recommend flushing the apparatus with fresh reagent after periods of inactivity, even overnight.

### Influence of temperature fluctuations

For every 1°C increase in temperature the titer decreases by 0.1%. Consequently, all reagents should be at the same temperature during measurements to maximise the accuracy of the titration.

### Influence of the titer on the performance of titration

The condition of the titration vessel influences the results of the titration. Because a titration vessel is not completely airtight, ambient moisture can infiltrate through the gaskets and enter the upper part of the vessel. The consumption of reagent by this moisture appears as drift. After finishing a volumetric KF titration, the consumption of reagent should not be higher than 0.01 mL/min. Therefore the maximum drift for different titer values should be:

- Reagent titer 5: max. drift 50 µg/min.
- Reagent titer 2: max. drift 20 µg/min.
- Reagent titer 1: max. drift 10 µg/min.

If the reagent has a titer below 5, it is very important that the titration vessel is in extremely good condition, as airtight as possible and very dry. Otherwise, the endpoint may be fading and final results can be too high.

### Recommendations to maximise titration accuracy

Summarising, to maximise the accuracy of KF titrations measure the titer of reagents using either HYDRANAL-Standard sodium tartrate dihydrate, HYDRANAL-Water Standard 10.0 or HYDRANAL-Standard 5.00, protect opened reagent bottles with HYDRANAL-Molecular sieve 0.3 nm or HYDRANAL-Humidity absorber and change the adsorbents regularly, flush the apparatus

with fresh reagent after periods of inactivity, even if just overnight, keep all reagents at the same temperature and minimise temperature fluctuations, and make sure your titration vessel is dry and airtight, and otherwise in good operating condition.

Further information about water determination according to the Karl Fischer method can be found on our website: [www.sigma-aldrich.com/hydranal](http://www.sigma-aldrich.com/hydranal)

### Expert technical support

Take advantage of our more than twenty-five years of experience with KF titration. We would be happy to help you choose the right HYDRANAL reagent and procedure to meet your titration objectives, taking into account the particular aspects of your sample, and answer any questions you might have. If necessary, we can also develop a method for you. Our comprehensive application collection is a great resource for HYDRANAL users. Please give us a call!

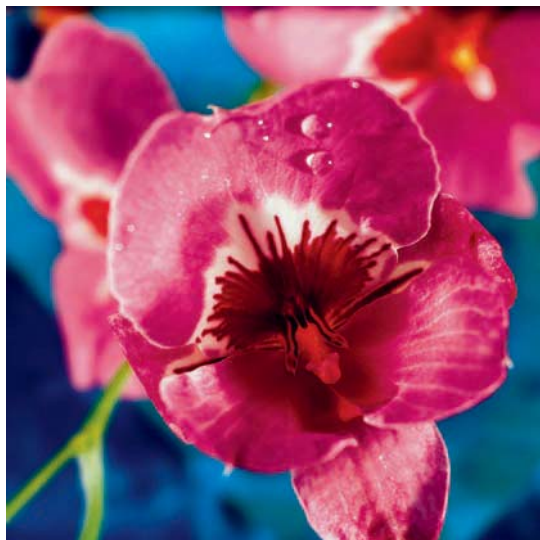
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## Upcoming Events ... HYDRANAL® seminars



### Upcoming HYDRANAL seminars

As a service to the scientific community, we routinely offer seminars to provide training on the chemistry behind the KF technique and information specific to the HYDRANAL product line. In 2008, seminars in the following cities will be conducted:

#### April

22, 2008 Warsaw, Poland

23, 2008 Warsaw, Poland

#### May

13, 2008 Singapore

15, 2008 Kuala Lumpur

#### June

17, 2008 Leverkusen, Germany

#### October

21, 2008 Karlsruhe, Germany

#### November

25 – 26, 2008 Seelze, Germany  
(2-day seminar)

For registration and additional information, please contact:

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

## HYDRANAL® Medium K

Non-toxic Karl Fischer reagent with performance and safety advantages

### HYDRANAL K reagents: ideal for KF titration of aldehydes and ketones

- Eliminate water-producing side reactions with Methanol-free HYDRANAL Medium K
- Maintain reactivity but reduce toxicity by using HYDRANAL Medium K
- Prevent water-consuming side reactions by using HYDRANAL Composite 5K as titrating agent

### HYDRANAL Medium K is an excellent substitute for HYDRANAL Working Medium K

If you are using our original HYDRANAL Working Medium K for KF titration of aldehydes and ketones, HYDRANAL Medium K is the ideal substitute if you want to:

- Improve workplace safety
- Use only non-toxic reagents
- Reduce packaging material waste

HYDRANAL Medium K is a fully fledged substitute for HYDRANAL Working Medium K, providing the same sample capacity, speed and accuracy. In addition, HYDRANAL Medium K offers important application, safety and transportation benefits.

For more details about HYDRANAL Medium K and other high-quality HYDRANAL reagents for pyridine-free water determination by Karl Fischer titration, please visit our website [www.sigma-aldrich.com/hydranal](http://www.sigma-aldrich.com/hydranal)

## Titration ... Concentrates for the preparation of volumetric standard solutions



### FIXANAL concentrates:

- Contain exact amount of substance (e.g. 1 mol)
- Have titer precision of  $1.000 \pm 0.2\%$
- Are economical and space-saving
- Permit user-specified concentration of final solution

**SPECIAL OFFER** on all FIXANAL® concentrates for the preparation of volumetric standard solutions of acids, bases, salts and buffers.

Take 30% off your first order of any FIXANAL ampoule.  
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Offer valid until May 31st 2008, valid for Europe only.

A complete product listing can be found in our online product catalogue section "Analytical/ Titration/ Concentrates":  
[www.sigma-aldrich.com/catalog](http://www.sigma-aldrich.com/catalog)

## New nitrate ionophore

Selectophore® products for nitrate analysis

Michael Jeitziner, Market Segment Manager Analytical Reagents & Standards [michael.jeitziner@sial.com](mailto:michael.jeitziner@sial.com)

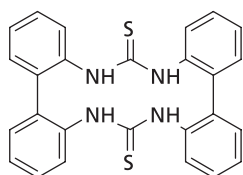


Nitrate is one of the most commonly analysed substances in environmental, food and many other types of samples. In drinking water, nitrate is a major health concern because of its toxicity, especially to young children. The current regulatory standard of 50 mg/L ( $8.1 \times 10^{-4}$  M) nitrate is derived from the standard set forth in the European Union's Drinking Water Directive, which

corresponds to the World Health Organization's guideline value for drinking water.

Sigma-Aldrich is pleased to introduce a new nitrate ionophore with an improved selectivity ( $\log K_{NO_3, X}^{Pot}$  of -3.50) to all known nitrate ionophores [1]. The low detection limit enables the measurement of nitrate in tap water even below the official guideline values.

Figure 1 Nitrate ionophore V (Fluka 39729)



For further information about ionophores, please visit our sensorics web page at: [www.sigma-aldrich.com/sensoric](http://www.sigma-aldrich.com/sensoric)

### Reference

[1] Watts, A. S.; Gavalas, V. G.; Cammers, A.; Andrara, P. S.; Alajarin, M.; Bachas, L.G. Sensors and Actuators B 2007, 121, 200-7.

### Characteristics

Slope (sensitivity): -54.8 mV/dec

Nernstian electrode response ( $2 \times 10^{-5}$  to  $6 \times 10^{-2}$  M  $NaNO_3$ ), DL  $\log aNO_3^-$  ~ -5.3

Selectivity Coefficients  $\log K_{NO_3, X}^{Pot}$  as obtained by the single solution method (SSM):

$\log K_{NO_3, NO_2}^{Pot}$  -1.8

$\log K_{NO_3, Cl}^{Pot}$  -3.5

$\log K_{NO_3, Acetate}^{Pot}$  -4.1

$\log K_{NO_3, SO_4}^{Pot}$  -4.7

Membrane composition: 1 wt % Ionophore, 0.6 wt % TDMACl, 65.6 wt % NPOE, 32.8 wt % PVC

Lipophilicity (Ionophore): log P = 5.6

Table 1 Product table

Part No.	Brand	Description	CAS No.	Package Size
39729	Fluka	Nitrate ionophore V, Selectophore®, function tested	221011-41-2	50 mg
81392	Fluka	Poly(vinyl chloride) high molecular weight (PVC), Selectophore®	9002-86-2	1 g, 10 g, 50 g
91661	Fluka	Tridodecylmethylammonium chloride (TDMACl), Selectophore®	7173-54-8	100 mg, 1 g
73732	Fluka	2-Nitrophenyl octyl ether (NPOE), Selectophore®	37682-29-4	5 mL, 25 mL, 100 mL

## New Sigma-Aldrich standards for residue analysis

Pesticides and veterinary drugs of current analytical interest

Nicole Amann, Product Manager Analytical Standards [nicole.amann@sial.com](mailto:nicole.amann@sial.com)



We've expanded our PESTANAL® and VETRANAL® product lines with standards for several important new analyses. To our PESTANAL® line of high-purity standards of pesticides and metabolites, we've added fluopicolide, a fungicide regularly monitored in agricultural products.

Our VETRANAL® line of analytical standards of antibiotics and hormones for forensic and residue analysis now includes the antibacterials toltrazuril sulphoxide and toltrazuril sulphone, and the fungicide and anti-helmitic hydroxythiabendazole.

Table 1 NEW Fluka brand PESTANAL and VETRANAL standards

Part No.	Brand	Description	Package Size
41132	Fluka	Fluopicolide, PESTANAL®	100 mg
33816	Fluka	Toltrazuril sulfone, VETRANAL®	10 mg
33815	Fluka	Toltrazuril sulfoxide, VETRANAL®	10 mg
33818	Fluka	5-Hydroxythiabendazole, VETRANAL®	10 mg

## New Fluka perfluorinated surfactant standards

For monitoring these widespread and persistent pollutants in water, sediments and food

Nicole Amann, Product Manager Analytical Standards [nicole.amann@sial.com](mailto:nicole.amann@sial.com)



Perfluorinated surfactants are widely used chemicals in food packaging, medicine, electronics, carpeting, paints, coatings and non-stick surface treatments. Chemically and thermally stable with no known biological degradation mechanisms, they are scrutinised because of their widespread presence and persistence in the environment and their purported adverse effects on human health.

Monitoring of perfluorinated surfactants in drinking water, sediments and food, particularly fish, has commenced in Germany, although regulations and critical values have not yet been set. In response, Sigma-Aldrich has introduced new Fluka-brand perfluorinated surfactants standards to facilitate their analysis in a variety of matrices.

**Table 1** NEW Fluka brand perfluorinated surfactant standards

Part No.	Brand	Description	Package Size
33824	Fluka	Pentadecafluorooctanoic acid, OEKANAL®	100 mg
33827	Fluka	Heptadecafluorooctanesulfonic acid, OEKANAL®	100 mg
33829	Fluka	Heptadecafluorooctanesulfonic acid potassium, OEKANAL®	100 mg
33603	Fluka	Pentadecafluorooctanoic acid solution, OEKANAL® 100 µg/mL in methanol	1 mL
33607	Fluka	Heptadecafluorooctanesulfonic acid solution, OEKANAL® 100 µg/mL in methanol	1 mL

## New neat mycotoxin reference materials

Standards and spiked maize matrix facilitate food monitoring

Nicole Amann, Product Manager Analytical Standards [nicole.amann@sial.com](mailto:nicole.amann@sial.com)



Mycotoxins are important food contaminants of fungal origin. Their interest analytically has increased over the past five years, with new methods and new labelled standards making food safety checks faster and easier to perform. Sigma-Aldrich, already well known for its extensive portfolio of mycotoxin standards, now offers

neat standards of the most often tested mycotoxins and matrix reference materials. These standards augment our OEKANAL® product line of high-purity standards of more than 400 common environmental pollutants and contaminants.

**Table 1** NEW Fluka brand perfluorinated surfactant standards

Part No.	Brand	Description	Package Size
32939	Fluka	Zearalenone, OEKANAL®	5 mg
32937	Fluka	Ochratoxin A, OEKANAL®	5 mg
32936	Fluka	Fumonisin B1, OEKANAL®	5 mg
32932	Fluka	Neosolaniol, OEKANAL®	5 mg
32929	Fluka	Nivalenol hydrate, OEKANAL®	5 mg
32928	Fluka	15-Acetyldeoxynivalenol, OEKANAL®	5 mg
32927	Fluka	3-Acetyldeoxynivalenol, OEKANAL®	5 mg
32923	Fluka	Fumonisin B1+B2 in Maize Flour, OEKANAL® FB1 content: 2406 µg/kg ± 630 µg/kg; FB2 content: 630 µg/kg ± 116 µg/kg	100 g
32921	Fluka	Zearalenone in Maize Flour, OEKANAL® Zearalenone content: 60 µg/kg ± 9 µg/kg	100 g
32920	Fluka	Deoxynivalenol in Maize Flour OEKANAL® Deoxynivalenol content: 474 µg/kg ± 30 µg/kg	100 g

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