

Analytix

Issue 2 • 2009



HYDRANAL[®] going Green

Non-toxic Karl Fischer titration with
E-type reagents



- FAME Standards for GC
- *TraceCERT*[®] CRMs for Chromatography
- Di- and Tricationic LC/MS Additives
- Food Analysis Assay Kits
- Waste Water Control with HybriScan[®]
- Titration in non-aqueous media

2009/2010 Fluka Analytical Reagents & Standards Catalogue



Trevor Jones
Marketing Director Europe

Dear Colleague,

The name Fluka® has become synonymous with quality and reliability in science.

As a part of the Sigma-Aldrich family, Fluka is now the flagship brand for high-quality Analytical Reagents and Standards available around the world. All products are produced with meticulous care and under closely monitored conditions. We manufacture to specifications designed to match the purpose for which the products are intended. This is what makes them so reliable.

We have released for 2009 our new Fluka Catalogue of Analytical Reagents & Standards, dedicated to all products for analytical chemistry available from Sigma-Aldrich. To provide more space to fully list all our analytical products in detail, the chemical and biochemical listings have been reduced and make reference to our website for those requiring full information. Full listings of chemicals and biochemicals from Sigma-Aldrich, including those previously listed in the Fluka Catalogue are to be found detailed in the Aldrich and Sigma Catalogues respectively.

The new Fluka Catalogue is divided into two sections, Analytical Reagents and Analytical Standards, containing in total over 21,000 products. In the Analytical Reagents section you will find all the specialty reagents that you are likely to need for analytical chemistry techniques and methods. Highlights are the CHROMASOLV® range of solvents for HPLC and other chromatographic techniques and the HYDRANAL® range of reagents for Karl Fischer water determinations. But the complete range extends far beyond that, from basic chemicals and reagents for general laboratory use, through to specialty kits for food testing and environmental analysis.

The new Analytical Standards section details our broad range of standards and certified reference materials. Whereas our reagents are listed alphabetically, our standards are listed by application category to help find the appropriate standard needed for any given procedure. The huge range of PESTANAL® standards for pesticide residue analysis in the environmental field are complemented by diverse standards for use in fields such as forensic, food & beverage and petroleum analysis. A special mention should be made for our range of Certified Reference Materials. The catalogue now lists CRMs for Pharmaceutical/Clinical quality control, determination of physical properties, e.g. particle size, and of course Spectroscopy, plus many more. Our **TraceCERT**® standards for AAS and ICP have become extremely popular due to their precision and accuracy, underpinned by the double accreditation of our laboratories under ISO/IEC 17025 and ISO Guide 34.

You can read about **TraceCERT** CRMs, HYDRANAL Karl Fischer Reagents and more products from the new catalogue in this edition of Analytix. But to fully appreciate the depth and range of what Sigma-Aldrich has to offer the analytical chemist, you need your own copy of the new Fluka Analytical Reagents & Standards Catalogue. You can order online at sigma-aldrich.com/fluka_cat or contact your local Sigma-Aldrich sales office.

With kind regards,

A handwritten signature in black ink, appearing to read 'T Jones', written over a horizontal line.

Trevor Jones
Marketing Director Europe

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HYDRANAL® going Green

Non-toxic Karl Fischer titration with E-type reagents

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Growing health and environmental awareness leads to unease in handling reagents with toxic components; the need to reduce workers' exposure to hazardous and toxic substances is in great demand. With this goal of improving laboratory safety and reducing environment toxicity, Sigma-Aldrich offers HYDRANAL E-line reagents for reliable Karl Fischer titrations without toxic compounds.

Environmental responsibility with non-toxic Karl Fischer titration

HYDRANAL KF reagents were designed to ensure reliable titrations that are free of noxious pyridine by replacing it with safe and odourless substances like imidazole or diethanolamine. Over the years, the skull and crossbones, a symbol for toxicity, was more and more eliminated from HYDRANAL KF reagents by replacing toxic components like methoxyethanol, chloroethanol, chloroform, and carbon tetrachloride with non-toxic ones. The challenge in replacing methanol by a non-toxic compound was that methanol not only serves as a solvent, it also takes part in the KF reaction. Ethanol was the obvious choice as a close relative of methanol, but its low dielectric constant leading to low conductivity and slow reaction rates was an obstacle that had to be overcome first.

Replacement of methanol in Karl Fischer reagents

Over ten years ago, researchers at Sigma-Aldrich were able to successfully replace methanol and launch the first non-toxic KF reagents. By incorporating accelerators to activate ethanol and increase reaction rates, as well as using the most suitable additives to obtain conductivity comparable to methanol-containing reagents, Sigma-Aldrich was able to develop a non-toxic alternative for Karl Fischer. By using ethanol-based reagents, in several applications we have also been able to eliminate the need for halogenated hydrocarbons, like chloroform, dichloromethane and carbon tetrachloride as solubilising agents. As a result of these research efforts, our E-type reagents are now patent protected (by EP 0 933 634 and JP 4440362).

Application Benefits of HYDRANAL E-types for Hydrophobic samples and Ketones

Ethanol-based reagents provide improved solubility for long-chained hydrocarbons compared to methanol-

Features of HYDRANAL E-line:

- Reduced toxicity over methanol-containing reagents
- Better solubility of hydrophobic samples
- Additives increase reaction rate and conductivity of ethanol
- Several ketones can be titrated without side-reactions
- Pyridine-free, like all HYDRANAL reagents
- Suitable for both volumetric and coulometric KF titrations
- Possible replacement for most methanolic KF methods
- Compatible with all titration equipment
- End-point colour appears visually more intense compared to methanolic titrations
- Users fulfil requirements of DIN EN ISO 14001

containing reagents and, as a result, more analyses can be carried out in one charge of working medium-reducing consumption of reagents. The need for solubilising agents may be completely eliminated, depending on the sample. However, if solubility is not sufficient, formamide, chloroform or xylene can be added to the HYDRANAL E-type working medium. Also, the temperature of the titration can be increased up to 50 °C in order to improve sample solubility.

Finally, the water content of several less-reactive ketones, including acetone, can be titrated in HYDRANAL Compo-Solver E in combination with non-toxic HYDRANAL Composite, without unwanted side-reactions. Alcoholic side-reactions with ketones are often less pronounced in ethanol than in methanol. For other more reactive ketones, specially designed working media are required like HYDRANAL Medium K (find more information on sigma-aldrich.com/hydranal).

Reference standards for titer determination

Sodium tartrate-2-hydrate is a common primary standard for volumetric KF titration. However, its solubility in ethanol is very limited. To get reliable, precise and quantitative results when using HYDRANAL E-type reagents, we recommend using one of our liquid HYDRANAL-Water Standards for titer determination. Fluka 34849 HYDRANAL-Water Standard 10.0 and Fluka 34813 HYDRANAL-Standard 5.00 are specifically designed for titer determination of volumetric KF reagents.

Table 1 HYDRANAL E-Type reagents product list

Cat. No.	Brand	Description	Pack Size
Reagents for volumetric titration (one-component technique)			
34734	Fluka	HYDRANAL-CompoSolver E (to be used with non-toxic HYDRANAL-Composite)	1 L, 2.5 L
Reagents for volumetric titration (two-component technique)			
34723	Fluka	HYDRANAL-Titrant 2 E (Water equivalent approx. 2.00 mg H ₂ O/mL)	1 L
34732	Fluka	HYDRANAL-Titrant 5 E (Water equivalent approx. 5.00 mg H ₂ O/mL)	500 mL, 1 L, 2.5 L
34730	Fluka	HYDRANAL-Solvent E	500 mL, 1 L, 2.5 L
Reagents for coulometric titration			
34726	Fluka	HYDRANAL-Coulomat E (to be used as anolyte and catholyte)	500 mL

HYDRANAL E-type applications

We analysed samples from a large range of chemical groups and tested them for accuracy and extraction efficiency. We found that most applications that use methanol-based reagents are readily transferable to HYDRANAL E-reagents without difficulty. In **Table 2** (see next page), we compiled tested methods from our HYDRANAL laboratory to give an overview of the possible applications of our HYDRANAL E-type reagents. 30 mL of either HYDRANAL-Solvent E or CompoSolver E were added to the titration vessel and titrated with HYDRANAL-Titrant 5 E or Composite 5, respectively. We tested the recovery rate of added water, the titration speed and the capacity of the working medium. Generally a total of 20 mL or 20 g of the substance were determined taking 10 mL or 10 g sample sizes for each titration (where smaller samples were taken, see legend for respective reason).

Visit our website for an E-types application list and find out how you can eliminate toxic reagents from your KF application!

Take advantage of our expertise gained from over twenty-five years' experience and our extensive applications database on Karl Fischer titration. On our website

sigma-aldrich.com/hydranal we provide a list of samples where application reports for ethanol-based reagents are available. Find out how your KF application can be transferred to non-toxic reagents! For any questions, help or feedback, please contact our HYDRANAL specialists:

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SPECIAL OFFER

Change from toxic to non-toxic Karl Fischer titration and get 25 % off for each bottle of HYDRANAL E-reagent!

Cat. No.	Brand	Description
34734	Fluka	HYDRANAL-CompoSolver E
34732	Fluka	HYDRANAL-Titrant 5 E
34723	Fluka	HYDRANAL-Titrant 2 E
34730	Fluka	HYDRANAL-Solvent E
34726	Fluka	HYDRANAL-Coulomat E

(Please quote promotional code 965. Offer valid until 31.05.2009)

(continued on page 6)

Table 2 Classes of commonly used substances: Applicability of HYDRANAL E-type reagents and suitable sample amounts

Substance class/Sample	Sample amount (in Titrant 5 E/Solvent E)	Sample amount (in Composite 5/CompoSolver E)	Water content**
Hydrocarbons			
<i>n</i> -Hexane	20 mL	20 mL	0.004 %
Toluene	20 mL	20 mL	0.020 %
Xylene	20 mL	20 mL	0.011 %
Dodecane	1 mL (S)*	15 mL (S)*	0.005 %
1-Tetradecene	20 mL	20 mL	0.005 %
<i>iso</i> -Octane	20 mL	20 mL	0.004 %
Cyclohexane	20 mL	20 mL	0.002 %
Dicyclopentadiene	5 mL (P)*	20 mL	0.003 %
2,5-Norbornadiene	20 mL	20 mL	0.013 %
Halogenated hydrocarbons			
Dichlormethane	20 mL	20 mL	0.006 %
1-Chlorooctane	20 mL	20 mL	0.016 %
4-Chlorotoluene	20 mL	20 mL	0.007 %
1-Bromotetradecane	20 mL	20 mL	0.007 %
Alcohols			
1-Propanol	20 mL	20 mL	0.100 %
2-Propanol	20 mL	20 mL	0.000 %
1-Hexanol	20 mL	20 mL	0.020 %
1-Octanol	20 mL	20 mL	0.015 %
Ketones			
Acetone	not possible	10 mL (R)*	0.230 %
Methyl isobutyl ketone	1 mL (R)*	20 mL	0.032 %
3-Octanone	1 mL (R)*	10 mL (R)*	0.450 %
Cyclohexanone	not possible	2 mL (R)*	0.050 %
Acetophenone	2 mL (R)*	10 mL (R)*	0.019 %
Benzophenone	6 g (S)*	5 g (S)*	0.006 %
2-Pyrrolidone	5 mL (R)*	10 mL (R)*	0.091 %
N-Methylpyrrolidone	10 mL (R)*	10 mL (R)*	0.140 %
Ethers			
1,4-Dioxan	20 mL	20 mL	0.052 %
Polyethylene glycol	10 mL (I)*	5 mL (I)*	0.130 %
Anisole	20 mL	20 mL	0.022 %
1,3,5-Trioxane	1 g (S)*	1 g (S)*	0.007 %
Carboxylic acids			
Acetic acid	20 mL	20 mL	0.026 %
Propionic acid	20 mL	20 mL	0.047 %
2-Ethylhexanoic acid	20 mL	20 mL	0.094 %
Malonic acid	10 g (S)*	10 g (I)*	0.150 %
Benzoic acid	10 g (S)*	10 g (S)*	0.004 %
Salicylic acid	10 g (S)*	8 g (S)*	0.004 %
Dichloroacetic acid	4 mL (R)*	5 mL (R)*	0.019 %
Amines			
<i>n</i> -Propylamine	1 mL (A)*	2 mL (A)*	0.042 %
<i>n</i> -Butylamine	1 mL (A)*	2 mL (A)*	0.048 %
Diethanolamine	3 g (A)*	5 g (A)*	0.017 %
Triethanolamine	3 g (A)*	3 g (A)*	0.022 %
1,2-Diaminoethane	not possible	not possible	-
Diethylentriamine	not possible	not possible	-
Morpholine	2 g (A)*	2 g (A)*	0.011 %
Aniline	not possible	2 mL (A)*	0.011 %
N-Methylaniline	5 mL (R)*	20 mL	0.050 %
Pyridine	10 mL	20 mL	0.013 %
Imidazole	10 g	20 g	0.040 %

* A = addition of 5–7 g salicylic acid for neutralisation of basic amines,
 I = indication,
 S = solubility,
 R = side-reaction,
 P = precipitation

** The water content results we obtained are listed, but these are by no means upper or lower limits.

New *cis/trans* FAME Standard for Optimising GC System Performance

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AOCS and AOAC methods for determining the *trans* fatty acid composition of foods by gas chromatography require the use of a highly polar cyanosilicone capillary column to provide the best peak resolution attainable of the numerous geometric (*cis* & *trans*) and positional isomers. Due to the highly polar nature of these phases, subtle changes in the GC system can alter the chromatographic separation of the positional *cis* & *trans* isomers. Therefore, it is important to continually monitor column performance and correct for unexpected changes in the column flow, oven temperature, column degradation and/or sample concentration as they occur.

AOCS Method Ce 1h-05 recommends using a well-characterised mixture of fatty acid methyl esters (FAMEs) covering the range of fatty acids under investi-

gation to monitor changes in column performance over time. Sigma-Aldrich's new *cis/trans* FAME Column Performance Mix is an excellent choice for monitoring these changes. This qualitative mix is specifically designed to optimise and monitor performance changes per AOCS Method Ce 1h-5 and AOAC Method AOAC 996.06. The mix contains C18:1 *cis/trans* positional isomer of critical importance to the food industry.

Because some *cis/trans* positional FAMEs are difficult to separate and baseline resolution may not be possible, regular use of the Sigma-Aldrich FAME Column Performance Mix is of utmost importance to achieve the required column performance. Small changes in the sample size, sample concentration, or oven temperature may be required to achieve the best resolution between

Figure 1 *Cis/Trans* FAME Column Performance Mix; 2.5 mg/mL in Methylene Chloride

Cat. Nos: 40495-U and 4M0495-U

For qualitative identification only. Relative peak sizes may vary among lots.

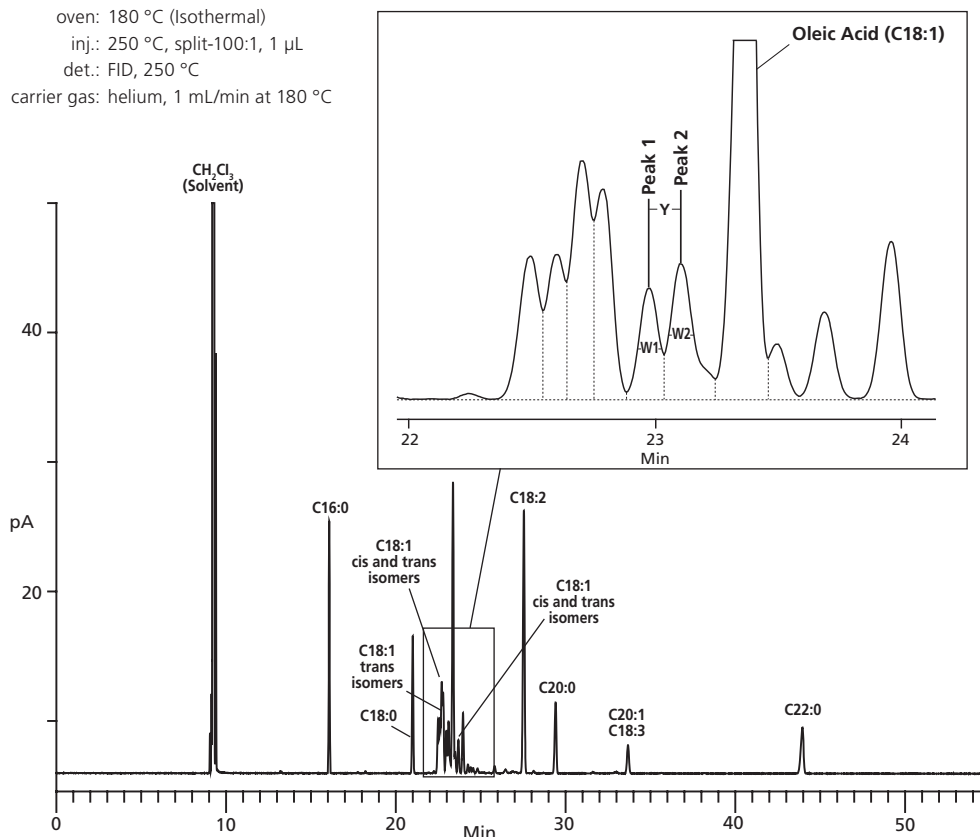
column: SP™-2560 fused silica capillary column (24056),
100 m x 0.25 mm I.D. x 0.2 µm thickness

oven: 180 °C (Isothermal)

inj.: 250 °C, split-100:1, 1 µL

det.: FID, 250 °C

carrier gas: helium, 1 mL/min at 180 °C



G004413,
4419

(continued on page 8)

the C18:1 *cis/trans* isomers. Once optimal resolution for the column is established, weekly analysis of the mix allows monitoring of the column's performance.

The analyst injects the mix, and identifies the peaks labelled as "1" and "2" in **Figure 1**. The resolution between these peaks is then calculated using the equation shown in **Figure 2**. It is important to run the mix upon initial installation of a new capillary column to ensure that conditions are established to optimise resolution. Optimum resolution between two peaks is defined as "baseline" resolution. Full baseline resolution is achieved when the R-value calculated using the equation in **Figure 2** is approximately 1.5 or greater. The example chromatogram shown in **Figure 1** illustrates the measurements required when calculating the resolution value – specifically peak width and retention time. As is evident in **Figure 1**, baseline resolution of peaks 1 and 2 will not be attained under normal testing conditions. The minimum acceptable resolution value will be described in a future revision of AOAC Method Ce 1h-05. For now, laboratories must establish their own criteria.



Figure 2

$$R = 1.18 (t_{R2} - t_{R1}) / (W_{(1/2)1} + W_{(1/2)2})$$

t_{R1} = the retention time of peak 1

t_{R2} = the retention time of peak 2

$W_{(1/2)1}$ = peak width at half height of peak 1 in mm

$W_{(1/2)2}$ = peak width at half height of peak 2 in mm

For example: $R = 1.18 (23.053 - 22.922) / (.0846 + .0912)$
 $R = 0.88$

Featured Product

cis/trans FAME Column Performance Mix, 2.5 mg/mL
 1 x 1 mL, 40495-U
 10 x 1 mL, 4M0495

Related Products

SP-2560, 100 m x 0.25 mm ID, 0.20 μ m 28668-U
 75 m x 0.18 mm ID, 0.14 μ m, 23348-U

At present, no column will fully resolve all C18:1 *cis/trans* positional isomers. By utilising the *cis/trans* FAME Column Performance Mix, analysts can optimise column performance before assaying complex mixtures. Regular use also makes it possible to monitor column degradation and to recognise when to replace the capillary column.

References:

- 1] AOCS Official Method Ce 1h-0. Determination of *cis*-, *trans*-, Saturated Monosaturated and Polysaturated Fatty Acids in vegetable or Non-ruminant Animal Oils and fats by Capillary GLC, 2005.
- 2] AOAC Official Method Ce 996.06. Fat (Total, Saturated, and Unsaturated) in Food, Hydrolytic Extraction Gas Chromatographic Method, 2001.

TraceCERT®

Swiss Precision Meets Analytical Competence



Traceable Certified Reference Materials for ICP, AAS and IC



- Produced and certified in double accredited laboratory according to **ISO/IEC 17025** and **ISO Guide 34**
- Unique level of accuracy & lot-specific certified values incl. uncertainty
- Traceable to at least two independent references (e.g. NIST, BAM or SI unit kg)
- Highly sophisticated packaging & documentation with expiry date
- ICP standards list up to 70 trace impurities

Ordering Information

Find our actual list of TraceCERT® reference materials for calibration and method validation at <http://www.sigma-aldrich.com/tracecert>

For custom standards please contact us at: CustomStandards@sial.com



SIGMA-ALDRICH®

TraceCERT® – Certified Reference Material for the chromatographic determination of hydrocarbon content

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Analytical laboratories working in a regulated environment (e.g. ISO/IEC 17025) usually have to prove traceability of their measurements, evaluate measurement uncertainty and must validate their analytical methods. Therefore Certified Reference Materials (CRM) play an important role for establishing traceability and are part of a reliable uncertainty calculation. Even when a laboratory is producing their measurement under no special regulations, it is very helpful to use a CRM when methods need to be verified or instruments qualified.

The trend for regular use of CRM is furthermore reinforced by official regulations of the European Community within the guideline 96/23/EG: ... paragraph 3.1.1.2., the determination of trueness (one component of accu-

racy) is described: trueness can only be established by means of CRMs. A CRM should be used whenever available. Also the American Food and Drug Administration recommends the usage of reference standards in their inspection guide for analytic methods.

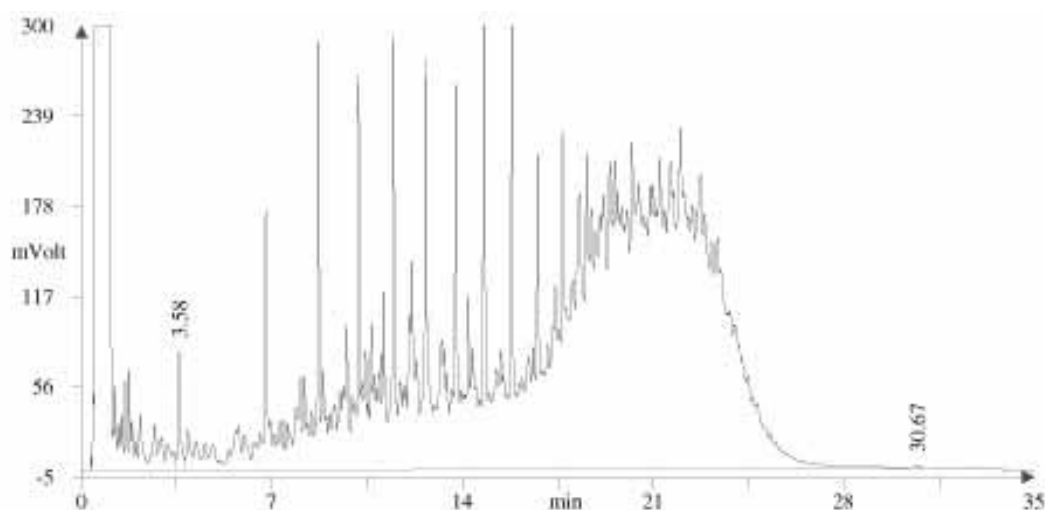
Determination of the hydrocarbon oil index

For the determination of mineral oil content, three different DIN/EN methods are established for water [3], soil [4] and sludge [5] matrices. Each one specifies a specific method for the determination of hydrocarbon oil index through extraction in n-heptane followed by gas chromatography (GC). Hereby the hydrocarbon oil index is the sum of the long-chain and branched aliphatic, alicyclic aromates and alkyl-substituted aromatic hydrocarbons, which are extractable with a solvent with boiling point above 36 °C, which are not adsorbed on Florisil® and have a GC retention time between n-decane (C10) and n-tetracontane (C40). Under the Fluka brand, two kits (Fluka Prod. No. 68172 and 56681) were introduced in 2007 containing all the necessary chemicals to perform these analytical measurements in accordance with the described methods. To complete this, two new Certified Reference Materials have now been introduced to guarantee traceability of these measurements (Figure 1).

Two new CRMs with traceability to BAM

Sigma-Aldrich developed two new CRMs (mineral oil standard mixtures type A and B) whereby the Certified Reference Material BAM-K010 from the Federal Institute

Figure 1 Chromatogram of mineral oil CRM including the retention time markers n-decane (3.58 min) and n-tetracontane (30.67 min) using a high-temperature GC.



for Materials Research and Testing (BAM) in Berlin is used as the starting material. Therefore the certified values are directly traceable to this internationally accepted reference. Both CRMs are produced and certified in an accredited laboratory, fulfilling ISO 17025 (General requirements for the competence of testing and calibration laboratories) and ISO Guide 34 (General requirements for the competence of reference material producers).

Gravimetric preparation is performed using balances certified by "Deutscher Kalibrierdienst" (German Calibration Service, DKD) and calibrated with SI-traceable weights. The bulk solution is homogenised by overhead tumbling and bottled under clean room conditions to ensure the highest purity of these standards. Both CRMs are provided in CERTAN® capillary bottles sealed in aluminium-coated bags to provide maximum shelf life (Figure 2). The certified value, measurement uncertainty and expiration date is given on the label. Further details are provided in the enclosed certificate, which is established according to ISO Guide 31 [1].

Principle of certification and uncertainty evaluation

Gravimetric preparation using well-defined materials (e.g. internationally recognised reference materials) is the most reliable realisation of concentration units, through conversion of masses and mass fraction. If contamination and loss of material during the whole production procedure is strictly prevented, this approach allows maximum accuracy and minimised uncertainties.

The uncertainty is evaluated following international guidelines such as the Eurachem/CITAC Guide [2] for uncertainty calculation. In the cause effect diagram (Figure 3) the key influence parameters are shown for the combined measurement uncertainty of CRM pro-

Figure 2 Mineral oil CRM with optimized packaging. The label carries the certified value, measurement uncertainty and expiration date.

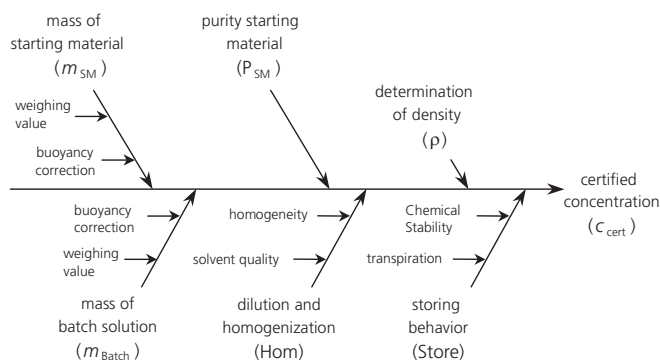


duction, including storing behaviour. Each of the primary parameters (vertical arrows) is composed of a set of secondary parameters (horizontal arrows).

Since the Certified Reference Material from BAM is used as starting material for production, the uncertainty of the BAM CRM directly influences the overall uncertainty of the two new standards. Summarising all the individual uncertainty contributions leads to a final expanded uncertainty U of = 1.8 % ($k=2$).

With the introduction of these two new CRMs, Sigma-Aldrich follows strict protocol to ensure the highest-quality CRM is delivered to customers.

Figure 3 Cause effect diagram of the preparation procedure of the mineral oil CRM. Only first and second order influence parameters are shown.



References

- 1] ISO Guide 31, 1–7, 1st Ed. (1981), "Contents of certificates of reference materials".
- 2] Quantifying uncertainty in analytical measurement, Eurachem/CITAC Guide, second edition, 2000.
- 3] DIN EN ISO 9377-2:2000, Water quality – determination of hydrocarbon oil index.
- 4] ISO 16703, Soil quality – Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography.
- 5] EN 14039, Characterization of waste – Determination of hydrocarbon content in the range of C10 – C40 by gas chromatography.

P/N	Name	Description
39386	Mineral oil standard mixture type A and B for EN ISO 9377-2, Certified Reference Material	Contains 10 g/L mineral oil type A and type B
11499	Mineral oil standard mixture type A and B for ISO 16703 and EN 14039, Certified Reference Material	Contains 8 g/L mineral oil type A and type B

Highly Sensitive Detection of Organic and Inorganic Anions with Di- or Tricationic LC/MS Additives

Rudolf Köhling, Namtso Reichlin, Gregor Wille, R & D, LC/MS Applications rudolf.koehling@sial.com

Since LC/MS became one of the major analytical techniques in liquid chromatography, electrospray ion sources (ESI) were and are still the first choice for the detection of a large number of substances. The most sensitive ESI mode is positive ion detection supported by the addition of formic acid to the mobile phase. Generally the negative ion mode is less sensitive and it is more difficult to enhance the sensitivity by additives, e.g. aqueous ammonia solution. Especially for anions of organic and inorganic acids, the negative ESI mode is preferred, but often the LODs are too insensitive and do not allow trace analysis.

Di- (DC) and tricationic compounds (TC) like alkyl-linked imidazolium salts are very good ion-pairing agents for singly or doubly charged anions and can help to avoid the lack of sensitivity in negative ESI mode [1–2]. **Figure 1** shows the new dicationic compound 75128 (Fluka), which is available as ready-to-use solution (5 μM in water/methanol) allowing the detection of a large variety of different anions, e.g. perchlorate, just by adding the solution post-column to the LC flow. Doubly charged anions can be analysed with the trication fluoride solution 08675 (Fluka) shown in **Figure 2** [3].

A solution of the fluoride salts DCF_2 or TCF_3 is infused to the mobile phase just before entering the MS, leading the formation of stable gas-phase adducts with an anion. The adducts $[\text{M}(\text{anion})+\text{DC}]^+$ still carry a positive charge and can be detected in the positive ESI mode

with a high sensitivity. Analytes like iodide or perchlorate can be found at ppb level even in complex sample matrices, e.g. milk or seawater [4–5].

Qualitative and quantitative analysis of nitrate, nitrite, 2,4-dinitrophenolate and perchlorate

In addition to the post-column infusion, DC and TC solutions can also be added directly to samples. In the following a dilution series of ClO_4^- (**53337 TraceCert Standard**), 2,4-dinitrophenolate (DNP, **34334 Pestanal Analytical Standard**) and $\text{NO}_3^-/\text{NO}_2^-$ (**74246 Nitrate/67276 Nitrite TraceCert Standards**) is prepared and mixed with the **75128 DCF2** solution (3:1, sample:DCF2). The concentrations of the calibration standards are 5, 50, 500, 1000 $\mu\text{g/L}$ except $\text{NO}_3^-/\text{NO}_2^-$, where the linear range of the calibration curve ends at 500 $\mu\text{g/L}$. Two ways of application are tested. For the 1st method the calibrant solutions are infused directly into the ESI source with a syringe pump. The flow rate is 20–30 $\mu\text{L/min}$. The 2nd method is the injection of the calibrant solution in an isocratic HPLC flow of 0.05–0.1 ml/min water/methanol (50/50, v/v) and the application of the dication solution (80–240 $\mu\text{L/h}$) via a T-junction before entering the ESI source. The detection of the dication-analyte adducts are carried out on a Bruker esquire 3000plus ion trap mass spectrometer. Generally the fragmentation of the adducts results in the mass 289.0 Da (DC) and 549.6 Da (TC), which is used for the quantification (see **Figure 3** for DC fragment).

Figure 1 Formation of the detected adduct of dication and perchlorate anion. The resulting singly charged ion can be detected by positive ESI with a very high sensitivity.

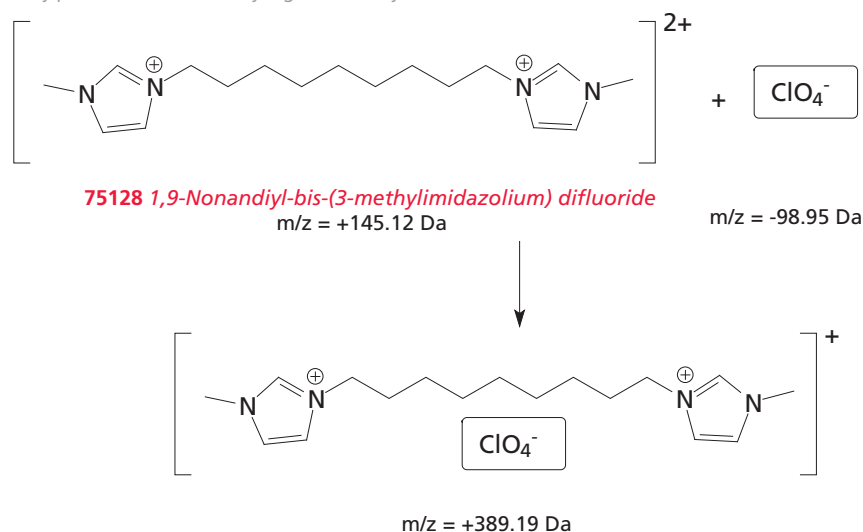
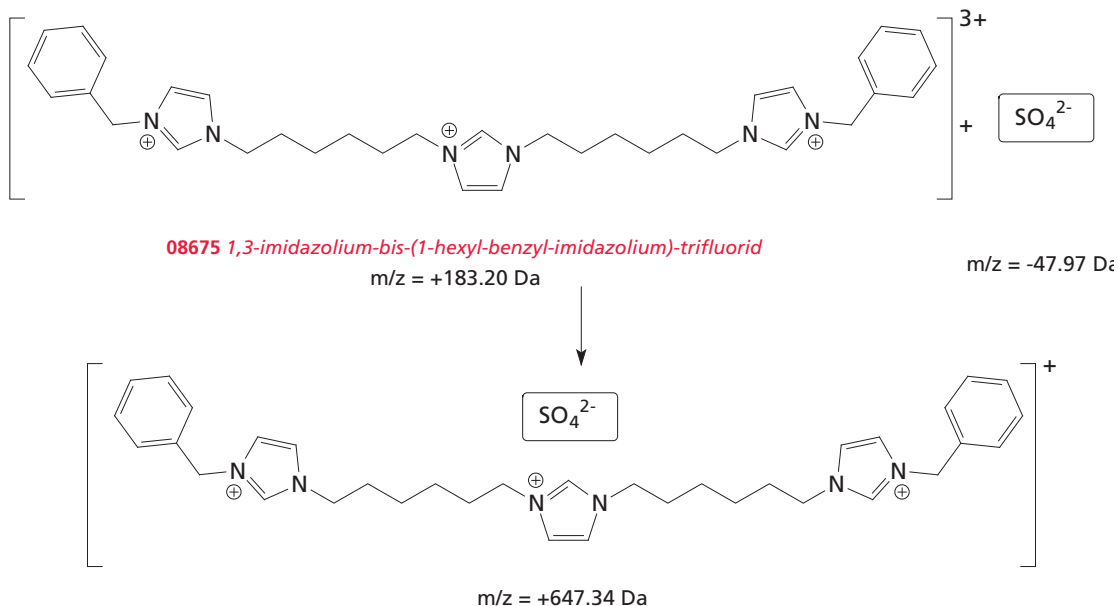
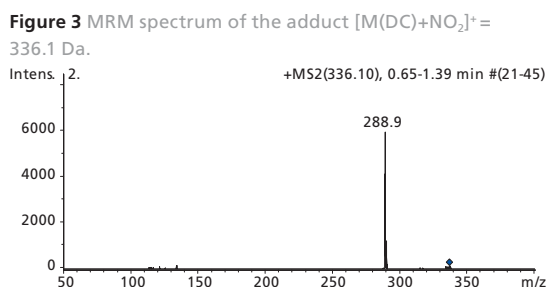


Figure 2 New trication fluoride solution 08675 (Fluka) for the detection doubly charged anions, e.g. sulphate.

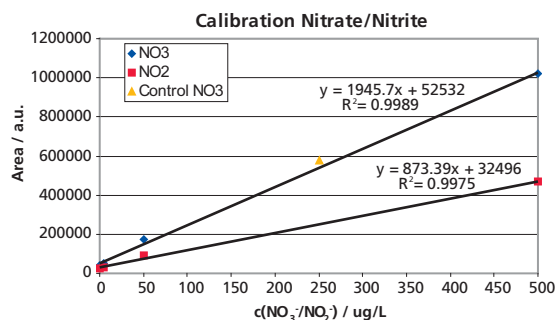


The dication forms adducts with NO_3^- (352.1 Da) and NO_2^- (336.1 Da) and in both cases the MS/MS spectrum (**Figure 3**) results in only the mass of dication (289.0 Da). 1 blank sample and 3 calibration levels (5, 50, 500 $\mu\text{g/L}$) were analysed with the isocratic LC method using an injection volume of 80 μL . The area below the peaks gives the calibration curve in **Figure 4**. The nitrate calibration is checked with the diluted PRIMUS Certified Reference Multielement Anion Standard (**89886**).



Di- and trications can enable and improve the detection of organic and inorganic anions by the formation of adducts. They close a gap in ESI-MS for those compounds which cannot or can hardly be detected in the negative mode. Qualitative and quantitative analysis of anions can be done with high sensitivity and accuracy without a complicated sample preparation, e.g. derivatisation.

Figure 4 Calibration curves of nitrate/nitrite including a control sample at 250 $\mu\text{g/L}$. The sensitivity is very high and allows the detection of sub-ppb concentrations depending on the attached mass spectrometer.



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Enzymatic Food Analysis

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

Enzymatic methods for food analysis are highly specific and offer considerable time and cost savings over other methods, especially from the sample preparation standpoint. Sigma-Aldrich offers a wide variety of convenient kits and reagents for rapid and reliable enzymatic food analysis.

Because of the importance of enzymatic methods in food production and assurance of quality and safety, there are many textbooks, national and international industry associations and government agencies devoted to the subject. Like many other analytical methods, enzymatic analysis was used first in clinical diagnosis, then adapted for the determination of food ingredients. The high specificity of enzymes enables the analysis in complex sample matrixes without complicated sample preparation techniques. This makes enzymatic food analysis a highly valuable tool because it saves time, reduces costs and gives reliable results independent of the sample matrix. Additionally, enzymatic methods use non-hazardous reagents, are environmentally friendly and can be automated for in-line process monitoring.

Applications: Carbohydrate and dietary fibre levels

Qualitative and quantitative analysis in the food and beverage industry is extremely important from quality, storage, nutrition and safety standpoints. The levels of certain **carbohydrates**, like glucose, lactose, fructose, sucrose and starch, affect intolerance conditions, diabe-

Did you know ... that most fructose is produced using enzymes? In the food industry, fructose is commonly produced by digesting cornstarch with α -amylase, glucoamylase and glucose isomerase. The result is a mixture of 42 percent fructose, 50–52 percent glucose, and small amounts of various other sugars.

Figure 1 Enzyme assay



tes and obesity. The presence of unwanted carbohydrates or their hydrolysis products can alter the manufacturing process or reduce product shelf life. They can also indicate microbial contamination (e.g. yeasts) or improper processing (e.g. overheating). For fruit juice and wine, raw materials that have variable sugar content influence the quality of the finished product and should therefore be monitored.

Table 1 Enzymatic assay kits

Brand	Cat. No.	Kit	Pack Size	Detection method & wavelength
Sigma	SCA20	Sucrose Assay Kit	Sufficient for ~20 assays	NADH; 340 nm
Sigma	GAGO20	Glucose (GO) Assay Kit	Sufficient for ~20 assays	H ₂ O ₂ ; 540 nm
Sigma	GAHK20	Glucose (HK) Assay Kit	Sufficient for ~20 assays	NADH; 340 nm
Sigma	FA20	Fructose Assay Kit	Sufficient for ~20 assays	NADH; 340 nm
Sigma	STA20	Starch (GO/P) Assay Kit	Sufficient for ~20 assays	H ₂ O ₂ ; 540 nm
Sigma	SA20	Starch (HK) Assay Kit	Sufficient for ~20 assays	NADH; 340 nm
Sigma	TDF100A	Total Dietary fibre Assay Kit	Sufficient for ~100 assays	gravimetric
Fluka	TDFC10	Total Dietary fibre Assay Control Kit	Sufficient for ≥10 assays	gravimetric

Table 2 Additional required reagents

Brand	Cat. No.	Product	Required for Kit Number
Sigma-Aldrich	258105	Sulphuric acid, ACS reagent	GAGO20, STA20
Sigma-Aldrich	154938	Dimethyl sulphoxide, ACS reagent	STA20
Sigma-Aldrich	459844	Ethyl alcohol, ACS reagent	STA20, TDF100A
Sigma-Aldrich	184519	Petroleum ether, ACS reagent	TDF100A
Sigma-Aldrich	320110	Acetone, ACS reagent	TDF100A
Sigma-Aldrich	S0876	Sodium phosphate dibasic, anhydrous	TDF100A
Sigma-Aldrich	S0751	Sodium phosphate monobasic, anhydrous	TDF100A
Sigma-Aldrich	S2567	Sodium hydroxide, 1.0 M	TDF100A
Sigma	H3162	Hydrochloric acid, 1.0 M	TDF100A

Determination of **dietary fibre** is another important food analysis. Consuming high-fibre foods, like fruits, vegetables, nuts and grains, is recommended to treat or prevent such maladies as constipation, haemorrhoids and diverticulitis. Water-soluble fibre also helps decrease blood cholesterol levels. From a chemical perspective, dietary fibre is a mixture of complex organic substances, including hydrophilic compounds, like soluble and insoluble polysaccharides and non-digestible oligosaccharides, and a range of non-swellable, relatively hydrophobic compounds, like cutins, suberins and lignins. Verifying a high content of dietary fibre in food permits a higher quality grading and access to higher-end product markets.

Figure 2 Natural fruit juice contains dietary fibre and many different sugars



Table 3 Standard Official Methods

Fibre
AOAC Method 920.86: fibre (Crude) in Flour – Ceramic fibre Filter Method, Enzymatic-Gravimetric Method
AOAC Method 985.29: Total Dietary fibre in Foods – Enzymatic-Gravimetric Method
AOAC Method 991.42: Insoluble Dietary fibre in Food and Food Products – Enzymatic-Gravimetric Method, Phosphate Buffer
AOAC Method 991.43: Total, Soluble, and Insoluble Dietary fibre in Food – Enzymatic-Gravimetric Method, MES-TRIS Buffer
AOAC Method 992.16: Total Dietary fibre – Enzymatic-Gravimetric Method
AOAC Method 993.19: Soluble Dietary fibre in Food and Food Products – Enzymatic-Gravimetric Method (Phosphate Buffer)
AACC Method 32-05: Total Dietary Fibre
AACC Method 32-07: Soluble, Insoluble, and Total Dietary fibre in Foods and Food Products
AACC Method 32-21: Insoluble and Soluble Dietary fibre in Oat Products – Enzymatic-Gravimetric Method
SLMB 468: Determination of dietary fibre in special foods – Enzymatic-Gravimetric Method
Sugar (Glucose, Fructose, Sucrose ...)
AOAC Method 969.39 Glucose in Corn Syrups and Dextrose Products – Glucose Oxidase Method
AOAC Method 985.09 Glucose and Fructose in Wine – Enzymatic Method
ICUMSA Method GS 2-4 (2007): The Determination of Glucose + Fructose in White Sugar by the Hexokinase Method
ICUMSA Draft Method No. 8 (2007): The Determination of the Apparent Total Sucrose coming from Sucrose, Glucose and Fructose in Molasses by an Enzymatic Method
ICUMSA Method GS 8/4/6-4 (2007): The Determination of Glucose and Fructose in Beet Juices and Processing Products by an Enzymatic Method
SLMB 305: Determination of D-Glucose, D-Fructose, Saccharose, Lactose and Sorbitol in ice cream – Enzymatic Method
SLMB 840: Determination of diverse sugars in wine – Enzymatic Method
ISO 13965/1998: Meat and meat products – Determination of starch and glucose contents – Enzymatic method
Starch
AOAC Method 2002.02: Resistant Starch in Starch and Plant Materials – Enzymatic Digestion
AOAC-AACC Method 996.11: Starch (Total) in Cereal Products – Amyloglucosidase-alpha-Amylase Method
AACC-AOAC 979.10: Starch in Cereals – Glucoamylase Method
AACC Method 76-13: Total Starch Assay Procedure (Amyloglucosidase/alpha-Amylase Method)
SLMB 467: Determination of starch and starch decomposition products in special foods – Enzymatic Method
ICC Standard Method 128/1: Procedure for the Determination of Starch after Enzymatic Decomposition
ICC Standard Method 164: Measurement of Damaged Starch by Using Enzymatic Kit
ISO 15914/2004: Animal feed stuffs – Enzymatic determination of total starch content
ISO 13965/1998: Meat and meat products – Determination of starch and glucose content – Enzymatic method

Detection principle of enzymatic reactions

Enzymatic methods to determine analyte concentration typically employ photometry to measure the concentration changes of specific products or substrates during the enzyme-catalysed reaction. Concentration of compound of interest is measured using the reaction stoichiometry.

Some commonly employed enzyme systems include:

- **Nicotinamide adenine dinucleotide (NADH/NAD⁺) coenzyme system**

- Activity of dehydrogenases using the NAD⁺/NADH system is

measured by monitoring the changes in absorbance at 340 nm. NADH has an absorption maximum at 340 nm, while the oxidised form NAD⁺ does not absorb at this wavelength.

- **H₂O₂ production (Oxidase/peroxidase system)**

- Oxidases cleave H₂O₂ from molecules (e.g. glucose) and o-dianisidine is oxidised with the enzyme peroxidase and H₂O₂. Oxidized o-dianisidine reacts with sulphuric acid to form a more stable pink-coloured product that can be measured at 540 nm.

(continued on page 16)

Carbohydrate assay kits

The kits for the enzymatic assay of sucrose, fructose and starch are based on the enzymatic determination of glucose. The first step is the conversion or degradation of the carbohydrate to glucose or

glucose derivate. The glucose concentration is then determined according to the two different detection systems: glucose oxidase (GO) or hexokinase (HK) in combination with the glucose-6-phosphate dehydrogenase reaction.

Figure 3 Glucose (GO) assay kit

Detection of glucose via glucose oxidase (GO) and peroxidase, monitored at 540 nm.

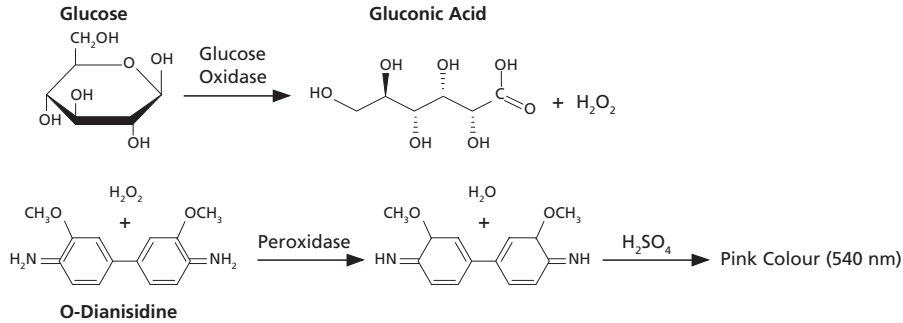


Figure 4 Glucose (HK) assay kit

Detection of glucose via hexokinase (HK) and glucose-6-phosphate dehydrogenase by formation of NADH, monitored at 340 nm.

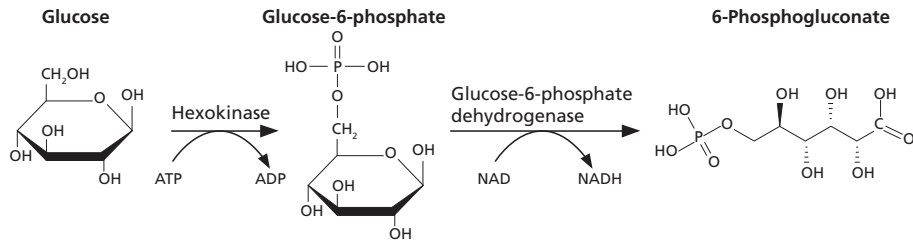


Figure 5 Sucrose assay kit

Sucrose is hydrolysed to glucose and fructose by invertase. Glucose and fructose are phosphorylated with ATP in the hexokinase reaction. Glucose-6-phosphate and NAD are then converted to 6-phosphogluconate and NADH via glucose-6-phosphate dehydrogenase, monitored at 340 nm.

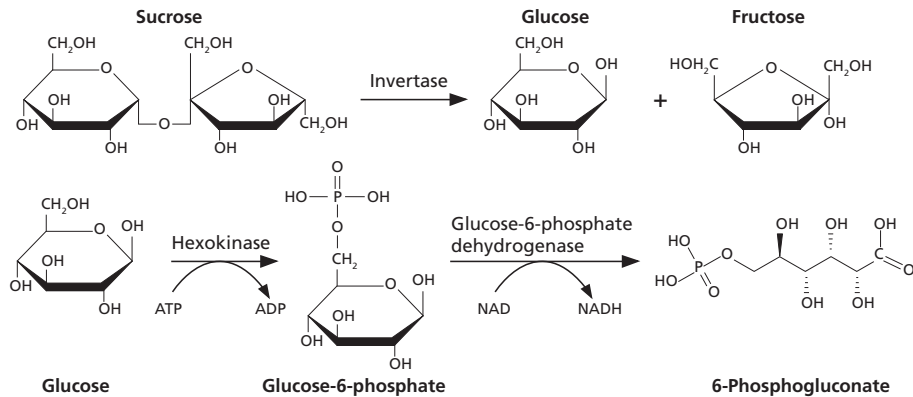


Figure 6 Fructose assay kit

Fructose is phosphorylated by ATP in a reaction catalysed by hexokinase. The resulting fructose-6-phosphate is then converted to glucose-6-phosphate by phosphoglucose isomerase. Glucose-6-phosphate and NAD is converted to 6-phosphogluconate and NADH, monitored at 340 nm.

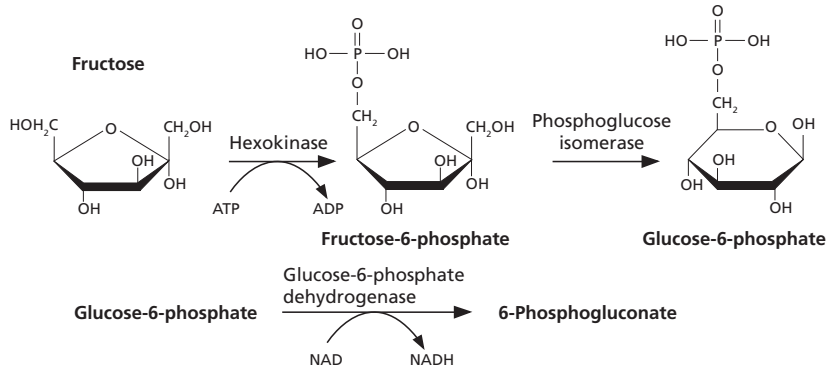
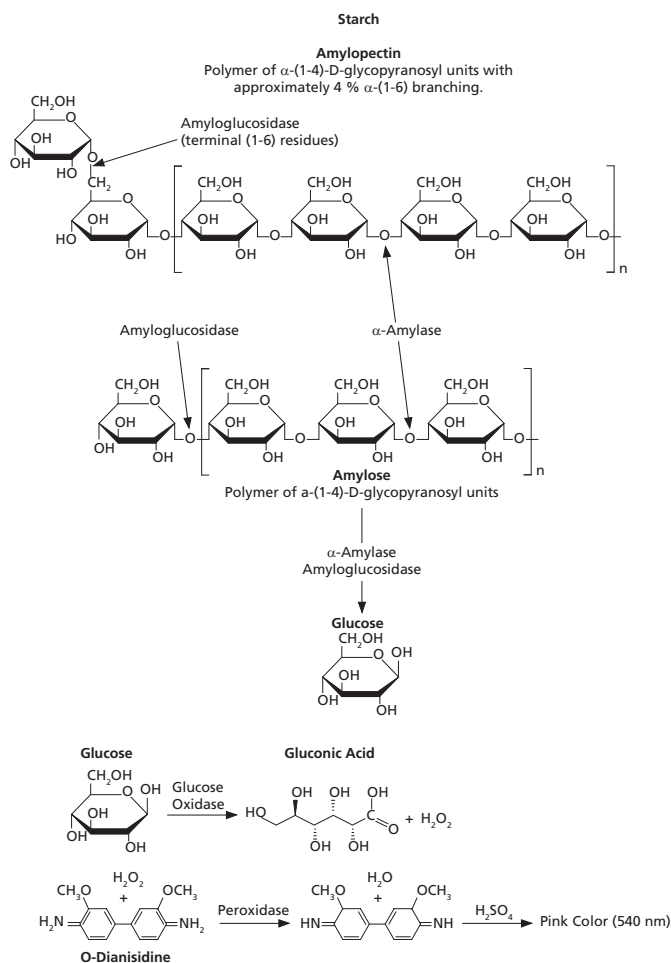
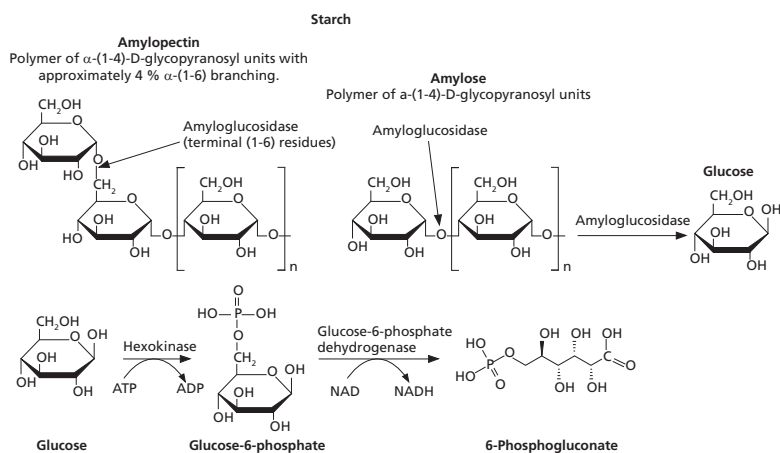


Figure 7 Starch (GO/P) assay kit

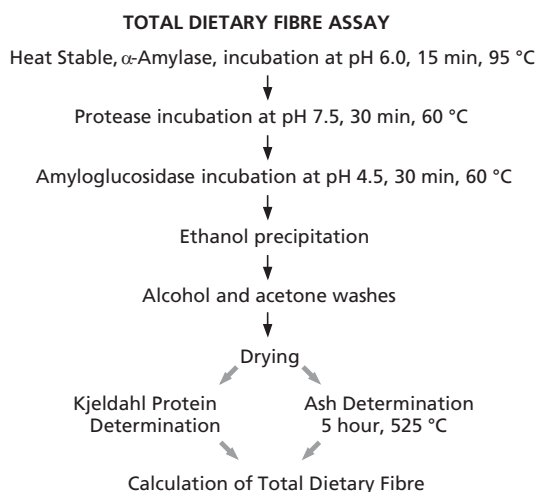
The hydrolysis of starch to glucose is catalysed by α -amylase and amyloglucosidase. Glucose is then converted to gluconic acid and H₂O₂ by glucose oxidase; detection of H₂O₂ via peroxidase reaction at 540 nm (proportional to the original starch concentration).

**Figure 8** Starch (HK) assay kit

The hydrolysis of starch to glucose is catalysed by amyloglucosidase. Glucose is phosphorylated by ATP in a reaction catalysed by hexokinase. Glucose-6-phosphate and NAD is converted to 6-phosphogluconate and NADH; detection of NADH at 340 nm (proportional to the original starch concentration).

**Total dietary fibre assay kit**

The total dietary fibre content is determined by a combination of enzymatic and gravimetric methods. Samples of dried, fat-free foods are gelatinised with heat-stable α -amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch in the sample. Ethanol is added to precipitate the soluble dietary fibre. The residue is then filtered and washed with ethanol and acetone. After drying, the residue is weighed. Half of the sample is analysed for protein and the other half is ashed. Total dietary fibre equals weight of residue minus weight of protein and ash.

Figure 9 Flow chart of Total dietary fibre assay kit

Additional information and instruction bulletins to all our enzymatic assay kits are available on our website sigma-aldrich.com/enzym-food-kits

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Control of *Microthrix parvicella* in the Waste Water Treatment with the HybriScan® Test System

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The sewage treatment plant of Halle-Nord in Germany changed during the years 1994–1997 from the old mechanical system to a new modern complete biological sewage treatment plant for about 300,000 inhabitants.

The activated sludge tank was built as four lines in cascade construction with an upstream denitrification, with two cascades for Bio-P (biological phosphorus elimination) for each line. After the launch in 1997 the plant functioned well without any problems until 1999. However, in September 1999, for the first time, an increase in filament formation was observed under the microscope. At the same time the sludge volume index (ISV) also increased systematically to 150 ml per g dry matter (150 ml/l SV) and even exceeded this value.

With the beginning of the warmer season and the increasing temperature of the wastewater, filamentous bacteria growth rose sharply and became a problem in the activation basins and in the final sedimentation tank. A huge amount of floating sludge accumulated in the sedimentation tanks and removal of the floating layer became a serious problem (see **Figure 1**).

With enormously time-consuming, technical and chemical commitment, the situation was resolved without running beyond the regulated maximum values of the wastewater treatment plant.

The floating sludge was pumped out of the effluent from the activation basins and the surface of the final sedimentation tank with a special high-pressure flushing and suction vehicle for several days. The sludge had to be removed completely from the system and be disposed of. Strongly inhibiting chemicals were used to kill the filamentous bacteria in the activation tank and to avoid foaming in the digestion tower. The manpower

Figure 1 View of the final sedimentation basin with floating sludge



as well as the usage of chemicals created enormous costs for the operation.

By microbial examination, the filamentous bacteria, *Microthrix parvicella* (see **Figure 2**), was relatively easily found to be the causative organism of this disruption. In the following years, the observation of the development of the sludge volume index and the examination of the mud microscopic images was considered to be very important.

For the wastewater technicians, there are two important methods for the observation of the growth of biomass and also the filamentous bacteria. The sludge volume index provides information about the composition of the activated sludge, and the microscopic mud image combined with special staining methods differentiates the species of the filamentous bacteria.

But all these methods do not allow early enough detection of the problematic bacteria population, and are less selective and very time consuming. Microscopic sludge examination is based on the subjective cognition of the observer and should always be performed by the same experienced person. Staining methods need certain biological skills and a dedicated laboratory space. However, that is not usually the case in a sewage plant.

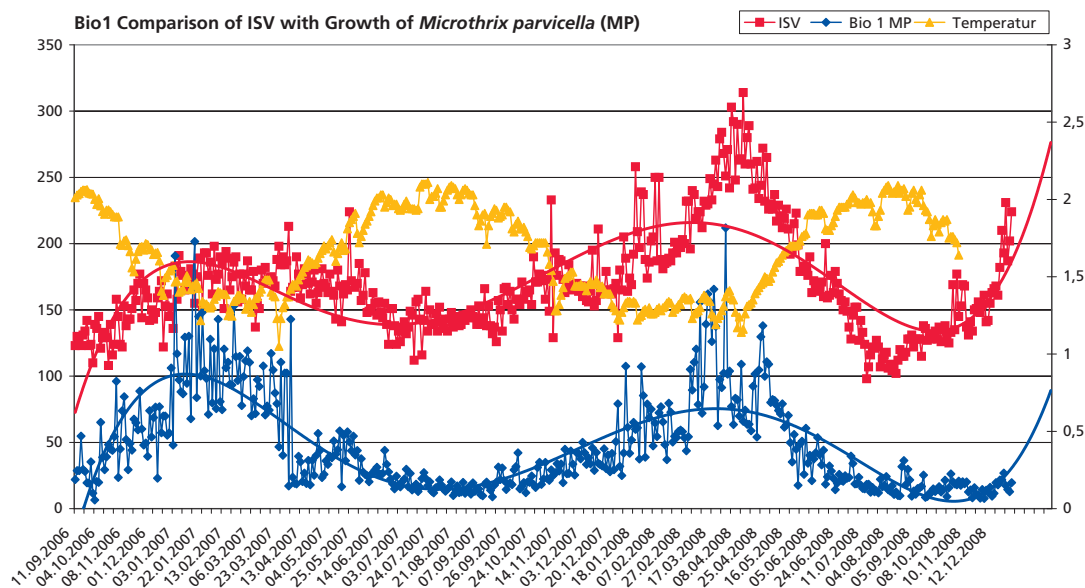
To avoid floating sludge, it is important to keep filamentous bacteria, in this case *Microthrix parvicella*, in low concentration. Therefore, a rapid detection system is essential.

In the summer of 2005, a project was created in partnership with Scanbec from the Technology and Founder Center in Halle/Saale. In this partnership, a rapid test system was developed to detect *Microthrix parvicella*.

Figure 2 Fluorescence-marked *Microthrix parvicella* in activated sludge; Source: © Bayerisches Landesamt für Umwelt



Figure 3 Graph of the sludge volume index compared to the cell concentration of *Microthrix parvicella*



The HybriScan test system for wastewater analysis is based on the detection of microorganism-specific target molecules with a special catcher and detection probe, resulting in a sandwich hybridisation. The method is designed to detect only a certain group or species of problem microorganisms that is guaranteed by using sequence-specific probes for ribosomal RNA. The measured absorption from the addition of substrate that is converted into a blue dye is proportional to the concentration of *Microthrix parvicella*.

HybriScan Waste Water determines both the *Microthrix parvicella* and the total bacterial count. By the parallel determination of the total count, the ratio of *Microthrix parvicella* concentration to the total bacteria number of the sample can be calculated. From the knowledge of the bacterial count, external process-related influences, like rain and variable inflow, can be taken into consideration.

Since September 2006, the laboratory of the sewage plant in Halle/Nord has been using the HybriScan test as a parallel method to observe the sludge volume index (see **Figure 3**).

The existing data provided important information about the influence of different parameters that helped in avoiding disastrous situations (as in the year 2000) without using expensive chemicals.

It was found that the index monitored the increase in cell concentration of *Microthrix parvicella*. The index, however, was not accurate, and the changing population in the activated sludge basin was detected too late. This information is very important for determining the need of dosing inhibiting chemicals to kill *Microthrix parvicella*. With advance information on the growth of

Microthrix parvicella, the precipitating agent could be used more efficiently, since the influence of filamentous bacteria can then be measured directly.

The graph shows a significant seasonal variation of the cell concentration of *Microthrix parvicella*. It was observed that the sludge volume index in the summer months (high temperatures) was swinging at a high level. It was assumed that this was related to the growth of *Microthrix parvicella*. With the HybriScan system this could be denied, as the graph shows a significant decrease of the *Microthrix parvicella* population. This observation is supported by the literature, which suggests that at higher temperatures *Microthrix parvicella* loses its growth advantages compared to other organisms in the biosolids.

For us as operators of the sewage plant, the HybriScan Method is an important tool to detect *Microthrix parvicella* and to keep a stable, well-performing composition of the activated sludge. We were able to see changes in the process parameters of the wastewater plant at an unusually early stage, and were able to implement action needed for maintaining a stable system. In comparison to other known methods, the HybriScan test kit is fast (performed detection in two hours), and specific to the organism providing semi-quantitative results.

For more information: sigma-aldrich.com/hybriscan

Table 1 HybriScan Waste Water kits from Sigma-Aldrich

Brand	Cat.No.	Name	Tests (reactions)
Fluka	78436	HybriScanD Waste Water Total Bacterial Count	96
Fluka	04447	HybriScanD Waste Water <i>Microthrix parvicella</i>	96

Get 25 % off UV Cuvettes (quartz)

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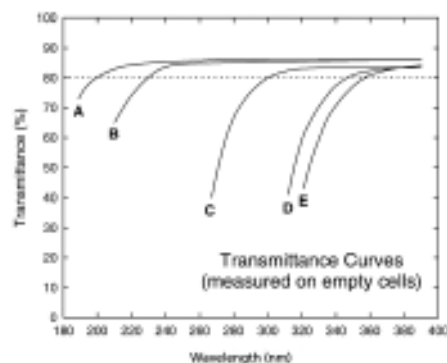
Sigma-Aldrich offers a full line of spectroscopy accessories including highly precise cuvettes for UV, fluorescence and IR spectroscopy. For a complete product listing, please visit our website at sigma-aldrich.com/spectroscopy

UV cuvettes (quartz)

UV cuvettes are manufactured from various types of glass. The most important criterion for the choice of a particular type of glass is the spectral range for which the cuvette is intended. The transmission curves are generally useful at wavelengths where their transmittance is 80 % or greater, using an empty cell. For absorption curve reference, see **Figure 1**.

Unless otherwise indicated, outside dimensions are 12.5 x 12.5 x 45 mm, and the path length is 10 mm. Cuvettes are sold singly. Modern precision-manufacturing methods make pre-matching unnecessary.

Figure 1 Transmission Curves (measured on empty cells)



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Volume	Aperture (mm)	Aperture Center H (mm)	Absorption Curve	Cat. No.
With PTFE cover, two clear sides				
≥3 mL			A	S10C-1EA
With PTFE stopper, two clear sides				
≥3 mL			A	C9417-1EA
Without stopper, two clear sides				
≥3 mL			B	S10-1EA
Semi-micro with PTFE cover				
≥1 mL			A	S10SM-1EA
Semi-micro, with PTFE stopper				
≥1 mL			A	C9542-1EA
Semi-micro, self-masking, with PTFE cover				
≥1 mL			A	C8425-1EA
Semi-micro, self-masking, with PTFE stopper				
≥1 mL			A	C5553-1EA
Micro, with frosted wall and PTFE cover				
≥0.5 mL			A	C5178-1EA
Micro, self-masking, with PTFE cover				
≥0.5 mL			A	C5428-1EA
Micro, short, with frosted wall and PTFE cover				
≥0.4 mL			A	C5053-1EA
Micro, short, self-masking with PTFE cover				
≥0.4 mL			A	C5303-1EA
Ultra-micro				
≥50 µL	2 × 2.5	8,5	A	C1918-1EA
≥50 µL	2 × 2.5	15	A	C9917-1EA
≥100 µL	5 × 2	8,5	A	C2043-1EA
≥100 µL	5 × 2	15	A	C9792-1EA
≥160 µL	8 × 2	8,5	A	C2168-1EA
≥160 µL	8 × 2	15	A	C9667-1EA



C1918



S10C and C9417



S10SM and C9542



C8425 and C5553



C5178 and C5053

Titration in Non-Aqueous Media

Solvents and Titrating Agents for Quantitative Analyses of Acids and Bases

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Quantitative determination of substances via titration is a useful method in laboratories. Most titration applications are performed in aqueous solutions; however, for certain substances this is not possible. When substances are insoluble or not soluble enough in water or too weakly protolysed, water as a solvent prevents titration of weak bases or separate determination of strong acids (e.g. perchloric acid/hydrochloric acid).

Advantages of non-aqueous titration:

- Enlargement of solubility range: many substances that are not soluble in water can be easily titrated in water-free media (e.g. fats and oils)
- Enlargement of application range: weak bases and acids can be easily titrated
- Substance compositions that cannot be separately determined in aqueous media can often be titrated in non-aqueous media

A high accuracy in non-aqueous titrations can be reached by special monitoring of the titration conditions; equivalent points must be clearly identifiable, and standard solutions precisely prepared. Ambient temperature is of special importance for solutions in non-aqueous titrations; organic solvents have a thermal coefficient of extension approximately 10 times higher than water, and a temperature difference of 1 °C may cause an error of 0.3 %.

Titration of bases

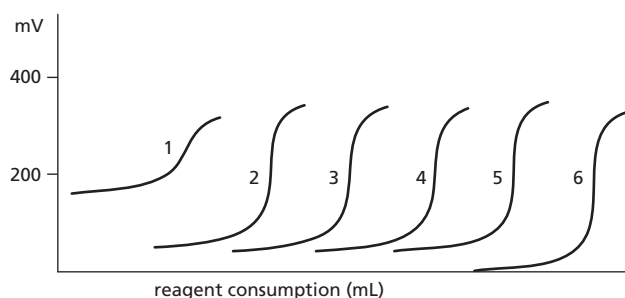
In aqueous solutions, only strong acids with a pK_B -value up to about 7 can be titrated, e.g. aliphatic amines. Weaker bases like aromatic amines or heterocyclic compounds with pK_B -values up to 12 can be titrated in organic solvents. Most common is the titration in glacial acetic acid. As a titrating agent, perchloric acid in glacial acetic acid is commonly used. Characteristic titration curves of different amines are shown in **Figure 1**. Acetic acid is a levelling solvent; therefore nearly all bases show similar types of titration curves.

Solvents for base titration

Choosing the suitable solvent is crucial for the titration to be performed. The acid/base-character and also the purity of the solvent must be suitable, as well as the solubility properties for all reaction products. The dielectric constant should be as high as possible to enable a clear indication of the potential. Sometimes acetylations can occur, e.g. with aromatic amines. Some of the most suitable solvents include the following:

Acetic Acid is the most commonly used solvent, with good solubility properties for most bases; it gives distinct potential curves with clear endpoints and also enables titration of weak bases with pK_B values up to 12. Disadvantageous is the levelling effect on weak bases, therefore simultaneous determinations are not possible.

Figure 1 Titration of different bases in acetic acid with 0.1 mol/L perchloric acid in acetic acid: 1/ pyrazole ($pK_B = 11.51$) 2/ pyridine (8.81) 3/ imidazole (7.00) 4/ diethanolamine (5.12) 5/ iso-propylamine (3.37) 6/ tetramethylammonium hydroxide



Acetic anhydride shows similar properties to acetic acid, but is superior for determination of weak bases, because it is free of water. Primary and secondary amines cannot be titrated; they will be rapidly acetylated.

Methanol shows good solubility properties and does not cause indication problems. Its titration behaviour is very similar to water, and only substances with pK_B up to 9 can be titrated.

Isopropanol is a good solvent because of its high dielectric constant and low acidity. Simultaneous determinations are possible. Not recommended for very weak bases ($pK_B > 10$).

Acetone is often used because it enables a very good differentiation of bases and shows sufficient solubility properties.

Titrating agents for base titration

For acidimetric titration, perchloric acid is mostly used as a titrating agent. When using glacial acetic acid as a solvent, 0.1 mol/L perchloric acid in acetic acid is used. When using differentiating solvents like acetone or isopropanol, acetic acid interferes because of the levelling effect. Instead, 0.1 mol/L perchloric acid in dioxan is recommended; it gives excellent titration results but is not stable for a long time. Once its colour turns to brown, titration curves get shifted. 0.1 mol/L perchloric acid in isopropanol can also be recommended as it gives the same results as perchloric acid in dioxan and is unlimitedly storable.

0.1 mol/L perchloric acid in glacial acetic acid

The FIXANAL ampoule of perchloric acid (Fluka 32046, see product list in **Table 1**) has unlimited storage and can be used for preparation of a standard solution in the necessary solvent. It can be filled up to 1L to form a solution of 0.1 mol/L or filled up to any other volume to give the desired concentration. Temperature must be taken into account when preparing the solution.

(continued on page 22)

0.1 mol/L perchloric acid in dioxan

Before opening the FIXANAL ampoule, fill approx. 500 mL dioxan in a volumetric flask and cool. Then open the ampoule and swirl around the dioxan carefully, so the acid is diluted at once. Rinse the ampoule with dioxan and fill the volumetric flask up to the mark. This solution is limited in storage. It slowly takes on a brownish colour. Addition of 1–2 % water increases stability, but may interfere when titrating weak bases.

0.1 mol/L perchloric acid in isopropanol

In a volumetric flask, fill approx. 500 mL isopropanol. Open the ampoule and immediately swirl around the isopropanol to dilute the acid at once. Rinse the ampoule with isopropanol and fill the volumetric flask up to the mark.

Titration of acids

Acids with pK_A values up to 7 can be titrated in aqueous solutions, if they are sufficiently soluble. Weaker acids with pK_A values up to 12 can be titrated in organic solvents.

Solvents for acid titration

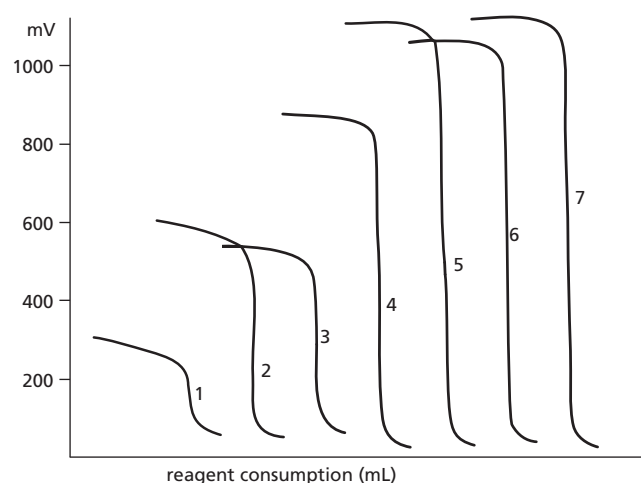
1,2-Ethylenediamine enables determination of all acids up to pK_A 11. Stronger acids are levelled; below pK_A 6 they cannot be differentiated any more. Equivalent points are very distinct. Disadvantages are the unpleasant odour and the possible absorption of carbon dioxide from ambient air. Some of the most suitable solvents include the following:

Dimethylformamide (DMF) is an excellent solvent for salts and for titration of acids. It shows strong differentiation; only strong acids with pK_A 0 are levelled. **Figure 2** shows that the equivalent points are indicated very clearly. Note: after standing for some time, DMF can be hydrolysed by strong acids, causing interferences in the titration curves.

Isopropanol is a good solvent for acids; it differentiates down to pK_A 0. For very weak acids (pK_A 11), this solvent is not recommended.

Acetone is strongly differentiating; even strong acids like hydrochloric or perchloric acid can be separated. It is an ideal solvent for simultaneous determinations and separations.

Figure 2 Titration of different acids in DMF with 0.1 mol/L tetramethylammonium hydroxide in isopropanol: 1/ phenol ($pK_A = 9.95$) 2/ 2-nitrophenol (7.23) 3/ acetic acid (4.73) 4/ 2,4-dinitrophenol (3.96) 5/ picric acid (0.71) 6/ hydrochloric acid (approx. -3) 7/ perchloric acid (approx. -9)



Titrating agents for acid titration

As titrating agents, strong bases are required, soluble in organic solvents. Mainly tetramethylammonium hydroxide or tetrabutylammonium hydroxide are used. Tetramethylammonium hydroxide is adequately soluble in polar organic solvents; but not all solutions are stable for unlimited storage. They decompose under formation of trimethylamine and methanol. Adequately stable are methanolic solutions, which are limited in use due to their levelling effect. Mostly propanol-methanol mixtures are used, which present an acceptable compromise between storage and titration behaviour. Small water content of 1 % increases stability without influencing titration properties. A propanolic solution is preferred as isopropanol has a smaller levelling effect than methanol, which is important especially when titrating very weak acids.

The FIXANAL ampoule Fluka 38335 contains 0.100 mol tetramethylammonium hydroxide dissolved in approx. 20 mL water. Because of the aqueous solution, this FIXANAL ampoule has long storage stability. It can be filled up with the desired solvent to 1 L 0.1 mol/L standard solution (or to any other volume resp. molarity).

Table 1 Sigma-Aldrich reagents for non-aqueous titration (see complete product listing on sigma-aldrich.com/titration)

Brand	Cat. No.	Description	Pack Size
Fluka	35418	Perchloric acid standard solution, ready-to-use, 0.1 mol/L in acetic acid	1 L
Fluka	319228	Perchloric acid standard solution, ready-to-use, 0.1 N in acetic acid	500 mL, 2 L
Fluka	32046	Perchloric acid standard solution, FIXANAL® concentrate, pkg of 0.1 mol (10.046 g HClO ₄)	1 ampoule
Fluka	35317	Trifluoromethanesulfonic acid standard solution, ready-to-use, 0.1 mol/L in acetic acid	1 L
Fluka	45730	Acetic acid, puriss. p.a., for use as solvent (for perchloric acid titration), ≥99.5 %	1 L, 2.5 L, 5 L
Fluka	33638	Pyridine, puriss. p.a., for titration in non-aqueous medium, ≥99.5 %	1 L, 2.5 L
Fluka	35434	Tetrabutylammonium hydroxide standard solution, ready-to-use, 0.1 mol/L in toluene/methanol	1 L
Fluka	35435	Tetrabutylammonium hydroxide standard solution, ready-to-use, 0.1 mol/L in isopropanol/methanol	1 L
Fluka	35436	Tetramethylammonium hydroxide standard solution, ready-to-use, 0.1 mol/L in isopropanol/methanol	1 L
Fluka	38335	Tetramethylammonium hydroxide solution, FIXANAL® concentrate, pkg of 0.1 mol (9.115 g C ₄ H ₁₃ NO)	1 ampoule

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Matthias Nold, Product Manager Analytical Standards matthias.nold@sial.com



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Brand	Part No.	Description	Pack Size
Fluka	32820	Acibenzolar-S-methyl	100 mg
Fluka	32716	Codlemone	100 mg
Fluka	32824	Fluazifop	10 mg
Fluka	34364	Norflurazon	100 mg
Fluka	32821	Pinoxaden	25 mg
Fluka	32719	Quinoclamine	100 mg
Fluka	32713	Spirotetramat	100 mg
Fluka	32721	Thiencarbazone-methyl	100 mg
Fluka	32832	Ditalimfos solution (100 ng/µl in Acetonitril)	2 ml
Fluka	32860	Isofenphos solution (100 ng/µl in Acetonitril)	2 ml
Fluka	32861	Pentanochlor solution (100 ng/µl in Acetonitril)	2 ml

New Veterinary Drug Standards

Matthias Nold, Product Manager Analytical Standards matthias.nold@sial.com



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New veterinary drug standards

Brand	Part No.	Description	Pack Size
Fluka	32841	2-Aminoflubendazole	10 mg
Fluka	32825	Clenpenterole hydrochloride	10 mg
Fluka	32827	Clenproperole	10 mg
Fluka	32826	Hydroxymethylclenbuterole	10 mg
Fluka	32838	Penbutolol hydrochloride	10 mg

New deuterated veterinary drug standards

Brand	Part No.	Description	Pack Size
Fluka	32836	Acepromazine-d6 hydrochloride	10 mg
Fluka	32854	Azaperone-d4	10 mg
Fluka	32819	Carazolol-d7	10 mg
Fluka	32828	Clenproperole-d7	10 mg
Fluka	32853	Crystal Violet-d6 trihydrate	10 mg
Fluka	32839	Flubendazole-d3	10 mg
Fluka	32843	5-Hydroxymebendazole-d3	10 mg
Fluka	32834	Leucocrystal Violet-d6	10 mg
Fluka	32842	Mebendazole-d3	10 mg
Fluka	32837	Propionylpromazine-d6 hydrochloride	10 mg
Fluka	33756	Sarafloxacin-d8 hydrochloride trihydrate	10 mg
Fluka	32844	Tetramisole-d5 hydrochloride	10 mg

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