Analytix

Issue 5 • 2008

New Patent for HYDRANAL® Karl Fischer Reagents

Patent Protected!

Continued use of Imidazole by a mixture of Imidazole and 2-Methylimidazole:
- Same performance
- Better stability
- No crystal formation

Improved Formula

- Comparison tests for LC-MS
- High purity water
- Phenols in surface and waste water
- Ionophores in clinical analysis
- Media for Mycobacteria and *E. Coli*
- Water in green coffee beans

SIGMA-ALDRICH®

HYDRANAL® Karl Fischer Reagents

HYDRANAL-Composite is now patent protected!

Andrea Felgner
Product Manager
Analytical Reagents

Dear Colleague,

The basis of HYDRANAL is Karl Fischer titration, a well-known method for water determination in various substances like chemicals, oils, pharmaceuticals and foodstuffs. Seizing on an opportunity to improve the safety and performance of the KF method, pioneering Riedel-de Haën® chemists Eugen Scholz and Helga Hoffmann replaced the noxious pyridine with imidazole. Thus began HYDRANAL, now the worldwide leader in pyridine-free reagents for KF titration, setting the industry standard for quality, capacity, speed, safety and reliability.

Although it was developed nearly 30 years, we have continued to update the line with new, innovative HYDRANAL reagents, techniques and accessories for volumetric and coulometric titrations. Today, we have placed heavy emphasis on the development of environmentally friendly reagents, replacing many toxic and hazardous components with "greener" chemicals. The methanol-free HYDRANAL-E reagent line is a prime example. We have also consciously pursued application-specific reagents and techniques for difficult-to-analyse substances like aldehydes and ketones or fats and oils.

HYDRANAL-Composite, our product line for volumetric one-component Karl Fischer titration, is the most frequently used pyridine-free Karl Fischer reagent. Developments in our HYDRANAL laboratory led to an improved formulation of the HYDRANAL-Composite reagents, leading to better stability of the mixture and elimination of crystal formation while maintaining the same level of performance. The pending patent has now been granted: European Patent no. 1,207,389 and US Patent no. 6,946,298 apply to our HYDRANAL-Composite product line. Please read more in the feature article on pages 4 and 5 of this Analytix issue.

There have been another important change: the Riedel-de Haën logo has been replaced by Fluka®. Along with a large group of other products, our HYDRANAL product line will now be sold under our Fluka brand that stands for specialty solvents, reagents, and standards for analytical procedures. Product performance and stability as well as manufacturing and packaging remain the same; only the label has changed.

As always, our most important HYDRANAL product is our service. Our goal is your 100 % satisfaction and success with water determinations using HYDRANAL reagents.

I hope you find this edition of Analytix interesting and useful.

Kind regards,

Andrea Felgner
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HYDRANAL®-Composite reagents for volumetric Karl Fischer titration

Improved one-component reagent now patent protected

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HYDRANAL-Composite is the world's most frequently used pyridine-free Karl Fischer reagent. This one-component reagent has proven its capabilities in volumetric titration for more than 25 years in a large range of applications in the most diverse fields of research and industry. Because recent development work has yielded significant improvements to this reagent, our HYDRANAL-Composite product line (see **Table 1**) is now patent protected by European Patent no. 1,207,389 and US Patent no. 6,946,298.

Patent protection for improved formulation of HYDRANAL-Composite

In a Karl Fischer reaction, sulphur dioxide, alcohol, iodine, and water react with each other at a stoichiometric ratio. The sulphur dioxide and the acid generated during the reaction are neutralised using a suitable alkali in order to keep the reaction solution within the optimal pH range. The alkali formerly used had been the tried-and-proven imidazole. Our HYDRANAL laboratory has produced an improved formulation based on a mixture of imidazole and 2-methyl imidazole. This new formulation retains the advantageous buffering action of the imidazole and maintains the typical pH range during titration. This adjustment of the composition significantly improves the product's characteristics. All HYDRANAL-Composite reagents contain this new formulation and can be visually identified by the "Improved Formula" stamp on the label.

Enhancement of titer stability

Compared to the composition's old formulation, the new formulation exhibits greater reagent titer stability. Test results showing titer decline of HYDRANAL-Composite formulations on the basis of imidazole (old formulation) and of the mixture of imidazole and 2-methyl imidazole (new formulation) are shown in **Figure 1**. The new HYDRANAL-Composites show better stability, as observed in the smaller titer decline of the new formulation as compared to the old formulation.

Table 1 HYDRANAL-Composite one-component reagents (titrating agents)

Cat. No.	Brand	Description	Package Size
34827	Fluka	HYDRANAL-Composite 1, titer 1 mg H_2O/mL	500 mL; 1 L
34806	Fluka	HYDRANAL-Composite 2, titer 2 mg H_2O/mL	500 mL; 1 L; 2.5 L
34805	Fluka	HYDRANAL-Composite 5, titer 5 mg H_2O/mL	500 mL; 1 L; 2.5 L
34816	Fluka	HYDRANAL-Composite 5 K, for titration of aldehydes and ketones, 5 mg H_2O/mL	500 mL; 1 L; 2.5 L

Figure 1 Results of the comparability test for titer stability

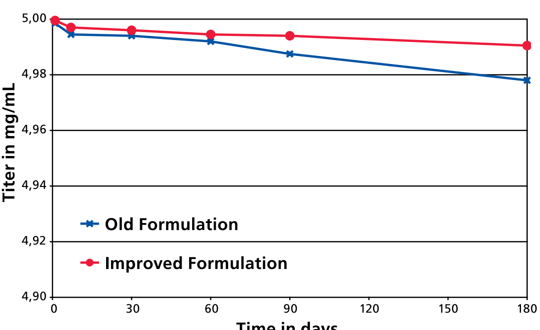

Table 2 Determination of process parameters using HYDRANAL-Water Standard 10.0 (n=10).
The certified water content of this batch of HYDRANAL-Water Standard 10.0 was 10.03 mg/g.

	HYDRANAL-Composite Formulation	
Parameter	**Old**	**Improved**
Mean (mg H_2O)	10.04	10.03
Recovery rate (%)	100.1	100.0
RSD (%)	0.06	0.08

Reduction of crystallisation effects

The improved formulation also prevents the crystallisation effects observed under the influence of airborne moisture and/or when the reagent has been in the cell and tubes for a prolonged time period.

Performance

A process validation for both formulations showed that there are no significant differences in rates of reaction. Ten titrations using 34849 HYDRANAL-Water Standard 10.0 were performed for investigation of other important process parameters such as recovery rate and standard deviation. An extremely high accuracy was determined for both formulations. As shown in **Table 2**, the results for the two reagents are similar.

The HYDRANAL laboratory also performed comparative tests for both formulations using a diverse range of sample materials. Water-content determinations were performed in inorganic substances such as acids, organic substances such as gasoline and diesel, various solvents and ketones, products such as commercial preservation agents and brake fluid, pharmaceutical products, dairy products and confectionery. Both formulations showed the same titration performance in tests on these extremely diverse substances. No significant deviations were observed in the results obtained.

No hazard symbol

Toxicological studies performed under GMP conditions confirm that the use and handling of both formulations can be classified as harmless to human health. The affixing of a hazard symbol on our HYDRANAL-Composite reagents is therefore not necessary on the basis of these studies and the EU directive concerning classification and marking.

Technical support

We will be glad to provide you with support in the analysis of your sample based on our vast experience with Karl Fischer titration. We can suggest a solution to your analytical problem and, if necessary, develop an individual analytical method for you. Our comprehensive application library makes daily work easier for HYDRANAL users.

For complete applications and details on our other high-quality HYDRANAL reagents for pyridine-free water determination by Karl Fischer titration, please visit our website *sigma-aldrich.com/hydranal* or contact our HYDRANAL laboratories.

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Interlaboratory proficiency testing on the analysis of alkylphenols, alkylphenol ethoxylates and Bisphenol A in surface and waste waters according to ISO/CD 18857-2 using new ^{13}C-marked internal standards

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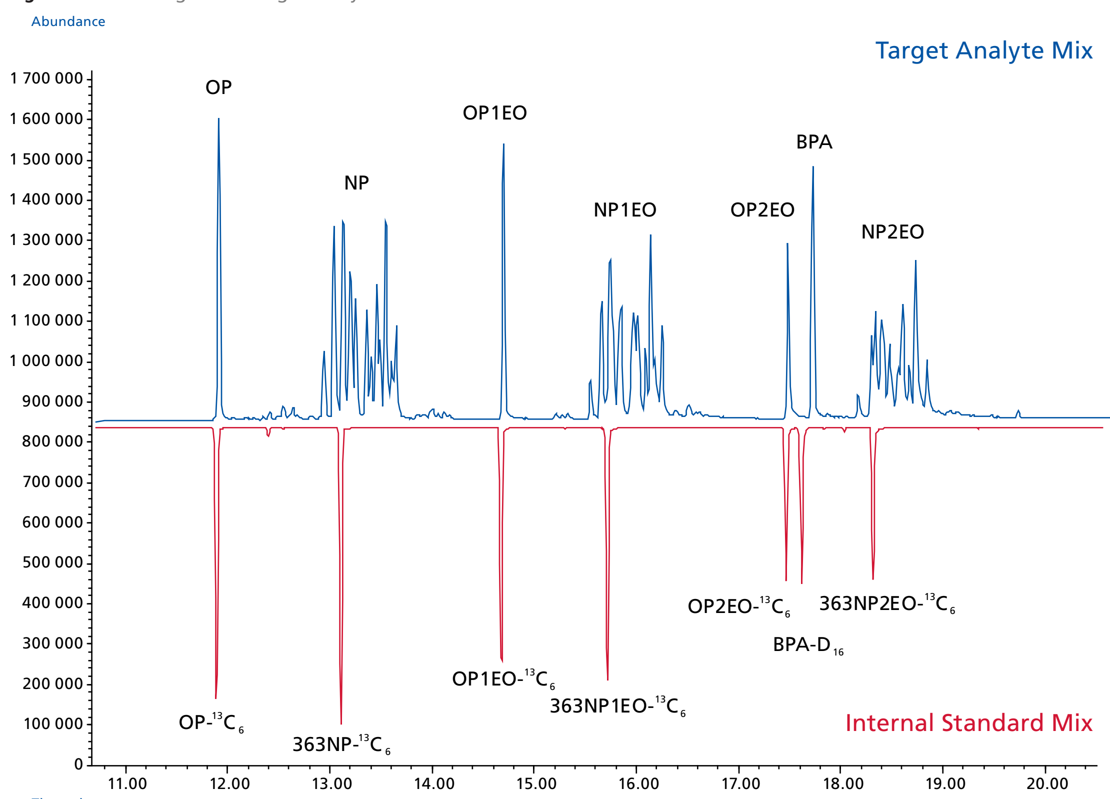
As reported previously [1], alkylphenols and their short-chain ethoxylates have considerable environmental relevance because of their endocrine-disrupting properties. For this reason, they have been introduced into several international measurement programmes. The most important monitoring programmes for surface water in Europe include the "Water Framework Directive" 2000/60/EC and the "OSPAR Convention for the Protection of the Marine Environment" for waters in the northeast Atlantic.

Among the alkylphenols, nonylphenol and its ethoxylated derivates present a particular analytical challenge because they are technical mixtures of isomers. **Figure 1** shows the chromatogram of technical nonylphenol and the mono- and diethoxylate. Their identification is based on the peak pattern (fingerprint) of the chromatogram, although the relation between the individual peaks of the pattern may differ in samples and standards. Substances co-eluting with nonylphenol and its mono- and diethoxylates can interfere in the determination. This may have a large influence on the result, since these three analytes are quantified from the sum of all peaks belonging to the chromatographic pattern. The analyst should use caution to include only those peaks from the sample that are attributable to the multicomponent analyte. In complex environmental samples, such interferences can occur even if GC-MS is used. The difficulty of analysing these compounds is illustrated by the fact that different interlaboratory trials resulted in widely varied results [2, 3]. Another disadvantage in current analytical procedures is the lack of appropriate isotope-marked internal standards.

For these reasons, the German Institute for Standardisation (referred to as DIN) submitted in April 2006 a new work-item proposal to the International Organisation for Standardisation (ISO) to develop an ISO standard method for the determination of alkylphenols, alkylphenol ethoxylates, and Bisphenol A in non-filtered water samples using solid phase extraction and gas chromatography with mass selective detection.

For a final evaluation, the method referred to as ISO/CD 18857-2 [4] had to be validated by an interlaboratory trial in order to assess the performance of the method, to

Figure 1 Chromatogram of target analyte mix and internal standards mix



give information on the comparability of the analytical results attained at different laboratories, and to evaluate critical points such as the suitability of the proposed internal standard for quantifying multi-component analytes (e.g. the technical mixture of nonylphenol isomers) that was specially developed for this analytical approach. The interlaboratory study was evaluated according to ISO 5725 [5] and included two duplicate non-filtered water samples: surface water (2 x 1 L) containing the target compounds in an analyte-concentration range from 0.05 to 0.4 µg/L, and waste water (2 x 100 mL) containing the target compounds in a concentration range from 0.1 to 5 µg/L. All laboratories were asked to follow the procedure exactly as prescribed in the draft standard. Briefly, the method applied consists of the following steps:

(1) Acidification of the water sample to pH 2 with sulphuric or hydrochloric acid.
(2) Addition of ^{13}C-marked internal standards, specified in tab. 2.
(3) Solid phase extraction and drying of the cartridge.
(4) Elution, concentration, and reconstitution of the extract.
(5) Derivatization with MSTFA.
(6) Measurement and quantification of the trimethylsilane derivatives.

Details of the method are given elsewhere [4]. The interlaboratory trial was organized by the Federal Environment Agency (Bad Elster, Germany) and started in November 2007. Fourteen laboratories from four different countries (Austria, Canada, Italy, and Germany) participated. Thirteen laboratories from four countries reported results; one laboratory withdrew.

Table 1 shows a summary of the results from this trial. The accuracy of the method is expressed by the recovery, and the precision of the method is determined by the variance coefficients of the reproducibility CV_R as a measure of the comparability between the different laboratories, and the repeatability CV_R as a measure of internal laboratory precision. The data show that the method developed under ISO/CD 18857-2 represents a robust procedure for the quantitative analysis of octyl- and nonylphenol and their mono- and diethoxlyates in water samples. Bisphenol A can also be determined by

Figure 2 Molecular structures of ^{13}C-labelled octyl- and nonylphenol and corresponding ethoxylates.

OP [$^{13}C_6$] **OP1EO [$^{13}C_6$]** **OP2EO [$^{13}C_6$]**

363NP [$^{13}C_6$] **363NP1EO [$^{13}C_6$]** **363NP2EO [$^{13}C_6$]**

(continued on page 8)

this method. Results obtained were of good accuracy and reproducibility. The isotope-marked standard compounds developed in this context have been proven to be reliable internal standards that allow a precise and accurate quantitation of all compounds specified in ISO/CD 18857-2.

Sigma-Aldrich now offers the entire range of isotope-marked compounds in ISO/CD 18857-2 for the quantitative analysis of octyl- and nonylphenol and their mono- and diethoxylates. **Table 2** lists the available concentrations with the corresponding product numbers. In addition to the ^{13}C-marked compounds for analysis with GC-MS, there are also twice-deuterated compounds that are ideal for analysis with LC-MS/MS.

References

1] Heemken, O. P.; Amann, N., Analytix 2007, 4, pp 6–7.
2] Loos, R.; Wollgast, J.; Castro-Jimenez, J.; et al., TrAC Trends in Analytical Chemistry 2008, 27 (1), 89–95.
3] Sobiecka, E.; Van der Sloot, H.; Hansen, N.; Gawlik, B. M., Project HORIZONTAL Validation Report, Office for Official Publications of the European Communities, Luxembourg 2007, ISBN 978-92-79-07123-2.
4] International Standardisation Organisation, ISO/CD 18857-2, 2008.
5] International Standardisation Organisation, ISO/CD 5725-6, 1994.

Table 1 Performance data from the interlaboratory validation of ISO/CD 18857-2 of octyl- and nonylphenol (OP, NP), the corresponding ethoxylates ($_{1,2}$EO), and Bisphenol A (BPA) in surface waters

Matrix	Compound	No. of labs. *	Results used*	Outliers [%]	Total mean**	Assigned value**	Recovery [%]	CV_R [%]	CV_r [%]
spiked surface water [ng/L]	OP	13	26	0.0	53.7	50.0	107.3	19.0	2.5
	OP_1EO	11	22	15.4	86.2	88.0	98.0	10.0	2.0
	OP_2EO	12	24	7.7	68.7	68.0	101.0	15.9	3.4
	NP	13	26	0.0	216.1	150.0	144.1	29.5	7.8
	NP_1EO	13	26	0.0	234.5	190.0	123.4	23.3	6.2
	NP_2EO	10	20	23.1	306.3	290.0	105.6	13.6	3.1
	BPA	12	24	0.0	82.8	80.0	103.4	23.4	3.8
spiked waste water [µg/L]	OP	11	22	15.4	1.55	1.50	103.6	10.8	3.5
	OP_1EO	12	24	7.7	1.27	1.30	97.3	13.9	2.5
	OP_2EO	12	24	7.7	0.87	0.90	96.5	14.1	4.1
	NP	12	24	7.7	3.80	3.50	108.6	16.8	5.5
	NP_1EO	12	24	7.7	3.91	4.10	95.4	14.5	3.9
	NP_2EO	13	26	0.0	3.84	3.80	101.1	21.9	4.2
	BPA	12	24	7.7	1.18	1.20	98.2	22.5	1.9

* without outliers ** all mass concentrations in ng/l (surface water) or µg/l (waste water)

Table 2 Labelled octyl- and nonylphenol and corresponding ethoxylates from Sigma-Aldrich

Compound	Package size	Cat. no.	Package size	Cat. no.
4-tert-octylphenol (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33565	10 ml / 1 µg/mL	33566
4-tert-octylphenol monoethoxylate (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33563	10 ml / 1 µg/mL	33564
4-tert-octylphenol diethoxylate (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33229	10 ml / 1 µg/mL	33244
4-(3,6-dimethyl-3-heptyl)-phenol (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33574	10 ml / 1 µg/mL	33575
4-(3,6-dimethyl-3-heptyl)-phenol monoethoxylate (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33572	10 ml / 1 µg/mL	33573
4-(3,6-dimethyl-3-heptyl)-phenol diethoxylate (Ring $^{13}C_6$)	1 ml / 10 µg/ml	33207	10 ml / 1 µg/mL	33222
4-tert-octylphenol (Ring D_2)	1 ml / 10 µg/ml	33557	10 ml / 1 µg/mL	33559
4-tert-octylphenol monoethoxylate (Ring D_2)	1 ml / 10 µg/ml	33523	10 ml / 1 µg/mL	33525
4-tert-octylphenol diethoxylate (Ring D_2)	1 ml / 10 µg/ml	33254	10 ml / 1 µg/mL	33257
4-(3,6-dimethyl-3-heptyl)-phenol (Ring D_2)	1 ml / 10 µg/ml	33569	10 ml / 1 µg/mL	33571
4-(3,6-dimethyl-3-heptyl)-phenol monoethoxylate (Ring D_2)	1 ml / 10 µg/ml	33567	10 ml / 1 µg/mlL	33568
4-(3,6-dimethyl-3-heptyl)-phenol diethoxylate (Ring D_2)	1 ml / 10 µg/ml	33249	10 ml / 1 µg/mL	33252
Target analyte mix according to ISO/CD 18857-2			10 ml / 1 µg/mL	33623
Internal standard mix according to ISO/CD 18857-2			10 ml / 1 µg/mL	33627

Mobile phase comparison study of LC-MS solvents

Choosing the right solvents for LC-MS prevents problems

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LC-MS is fast becoming a routine apparatus in the modern analytical laboratory. Increased use of LC-MS technology, new ion sources, high-resolution LC systems, and rapid mass spectrometers with enhanced ion optics and detectors have driven down limits of detection. Moreover, dedicated and application-tested high-purity mobile phases have increased in popularity. Even with the stricter specifications that many commercially available mobile phases offer, there are still differences in purity between brands that affect measurements.

Figure 1 shows a water/acetonitrile gradient comparison between a competitor's LC-MS mobile phase and Sigma-Aldrich LC-MS CHROMASOLV® mobile phases.

The blue chromatogram shows the baseline of an HPLC gradient starting with 100 % Fluka LC-MS CHROMASOLV 39253 Water to 100 % of Fluka LC-MS CHROMASOLV 34967 Acetonitrile. The red chromatogram shows the same gradient run with a competitor's brand of Water for LC-MS and Acetonitrile for LC-MS. Contamination peaks are evident only in the competitor's solvent chromatography.

Figure 2 highlights the difference in sensitivity between the two brands' solvents. This figure shows a chromatogram of a 5 ppm injection of reserpine run at the same gradient used for **Figure 1's** chromatography. The blue chromatogram illustrates the higher sensitivity and lower interference of the Fluka LC-MS CHROMASOLV 39253 Water and Fluka LC-MS CHROMASOLV 34967 Acetonitrile gradient as compared to the red chromatogram of the competition's Water for LC-MS and Acetonitrile for LC-MS.

Figure 3 shows the average mass spectrum of the two Sigma-Aldrich solvents 39253 Water and 34967 Acetonitrile.

Figure 4 presents a direct comparison with a leading competitor's Water.

Here the average mass spectrum of the competitor's solvent versus Sigma-Aldrich 34967 Acetonitrile is shown. Again, contamination is evident in the competitor's solvent.

Figure 1

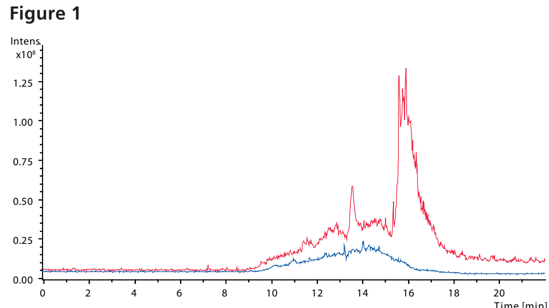
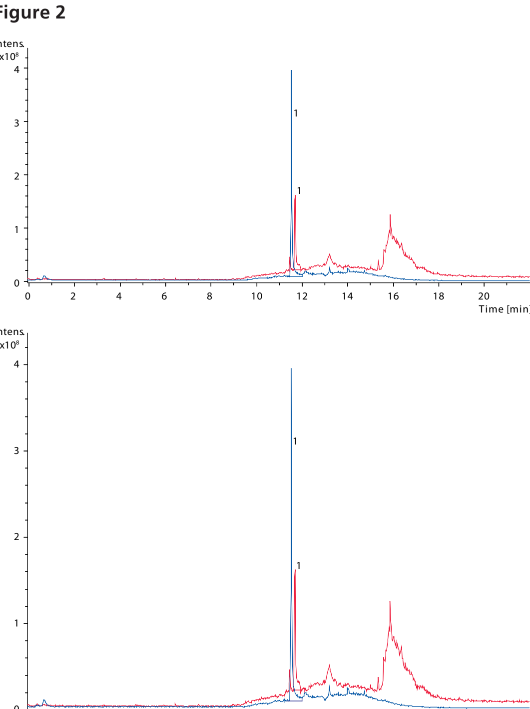


Figure 2



Sigma-Aldrich LC-MS CHROMASOLV solvents and blends offer outstanding quality and consistency. They are guaranteed to meet specifications. All products are developed for routine analysis, e.g. proteins and peptides (Water/Acetonitrile with 0.1 % Formic acid, 0.01 % TFA or Water/Acetonitrile with 0.1 % TFA) as well as small molecules (Water/Acetonitrile with 0.1 % Formic Acid).

LC-MS Chromasolv solvents, blends and additives are of the highest purity and are optimised to meet your special chromatographic needs.

(continued on page 10)

Figure 3

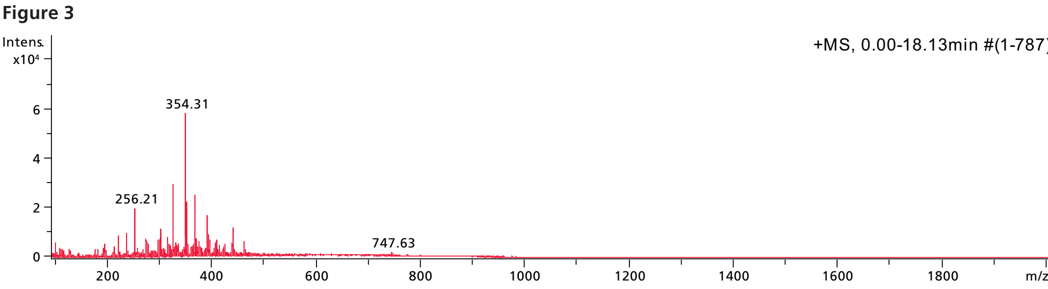
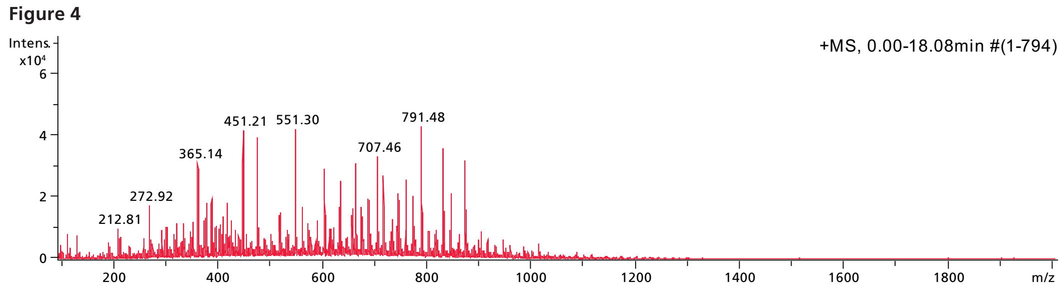


Figure 4



Solvents

Brand	Product	Name	Package size
Fluka	34967	Acetonitrile, LC-MS CHROMASOLV	250 mL, 1 L, 2.5 L
Fluka	34966	Methanol, LC-MS CHROMASOLV	1 L, 2.5 L, 6 x 1 L, 4 x 2.5 L
Fluka	39253	Water, LC-MS CHROMASOLV	1 L
Fluka	34965	2-Propanol, LC-MS CHROMASOLV	1 L, 2.5 L
Fluka	34972	Ethyl acetate, LC-MS CHROMASOLV	1 L, 2.5 L
Fluka	34986	Hexane, LC-MS CHROMASOLV	1 L, 2.5 L
Fluka	34999	Heptane, LC-MS CHROMASOLV	1 L, 2.5 L

Blends

Brand	Product	Name	Package size
Fluka	34978	Water with 0.1 % trifluoroacetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34976	Acetonitrile with 0.1 % trifluoroacetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34974	Methanol with 0.1 % trifluoroacetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34673	Water with 0.1 % formic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34677	Water with 0.1 % formic acid und 0.01 % trifluoroacetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34668	Acetonitrile with 0.1 % formic acid, LC-MS CHROMASOLV	1 L, 2.5 L
Fluka	34676	Acetonitrile with 0.1 % formic acid und 0.01 % trifluoroacetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34671	Methanol with 0.1 % formic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34675	Water with 0.1 % Acetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34678	Acetonitrile with 0.1 % acetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34672	Methanol with 0.1 % acetic acid, LC-MS CHROMASOLV	2.5 L
Fluka	34674	Water with 0.1 % Ammonium acetate, LC-MS CHROMASOLV	2.5 L
Fluka	34669	Acetonitrile with 0.1 % Ammonium acetate, LC-MS CHROMASOLV	2.5 L
Fluka	34670	Methanol with 0.1 % Ammonium acetate, LC-MS CHROMASOLV	1 L, 2.5 L

Additives

Brand	Product	Name	Package size
Fluka	40967	Trifluoroacetic acid, eluent additive for LC-MS	10 x 1 mL, 50 mL
Fluka	56302	Formic acid, eluent additive for LC-MS	10 x 1 mL, 50 mL
Fluka	49199	Acetic acid, eluent additive for LC-MS	50 mL
Fluka	49916	Propionic acid, eluent additive for LC-MS	50 mL
Fluka	55674	Ammonium formate, eluent additive for LC-MS	50 G
Fluka	49638	Ammonium acetate, eluent additive for LC-MS	50 G
Fluka	61333	Sodium citrate tribasic dihydrate, eluent additive for LC-MS	50 G
Fluka	40867	Ammonium bicarbonate, eluent additive for LC-MS	50 G
Fluka	44273	Ammonium hydroxid solution 25 % in water, eluent additive for LC-MS	10 x 1 mL, 100 mL
Fluka	65897	Triethylamine, eluent additive for LC-MS	50 mL
Fluka	34689	Water/2-Propanol 50/50 (v/v), Rinsing Solution I LC-MS CHROMASOLV	1 L
Fluka	34692	Water with 8 % formic acid, Rinsing Solution II LC-MS CHROMASOLV	1 L
Fluka	43530	Reserpine standard for LC-MS	4.5 mL
Fluka	21004	Caesium iodide, standard for high-resolution mass spectroscopy	1 G

Chromatography

The new LC-MS brochure

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

High-purity water for analytical applications: much more than just H_2O

The appropriate combination of water purification, bottle and closure quality, and analytical testing profile is critical to versatile analytical and bioanalytical applications

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Switzerland is famous for having nearly unlimited high-quality spring water resources, but this is not the only reason that water plays an important role at Sigma-Aldrich Switzerland in Buchs. Traditional water quality standards established by ASTM, CAP/NCCLS, or USP are no longer sufficient for today's sophisticated analyses. The increasing sensitivity of analytical techniques creates an ongoing need for new and higher-quality water for analytical use. For gradient HPLC, a common application, total organic carbon (TOC) content is crucial to accurate and consistent results. For other applications such as trace analysis ionic impurities are of highest importance. Obviously, hyphenated techniques such as HPLC-MS or HPLC-NMR demand even tighter specifications for eluents. With these needs in mind, Sigma-Aldrich produces many different water qualities in our multi-step purification plants, where every product is tailored to a specific application. Both extensive knowledge and advanced equipment are needed for the production and distribution of high-purity water. Selecting the right purification techniques, choosing suitable containers and closures, and running an adequately varied set of analytical tests are critical in high-purity water production.

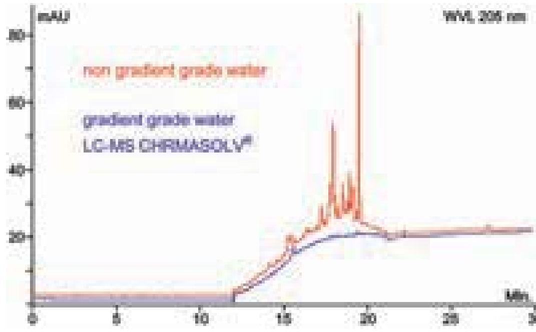
The challenge of water purification

A wide range of easy-to-handle purification systems is available on the market. There are limits to these systems, however; such a compact device is suitable for producing only small amounts of only one quality of pure water, and whether this water quality is fit for the purpose depends on the application. Because many different analytical techniques are used in the typical lab, analysts would obviously need more than one purification system, which may not be cost-effective for many labs, to produce their own high-purity water. In addition, only very few quality parameters (conductivity, perhaps TOC) are actually measured by commercial water purification devices. Any of the unmeasured parameters may negatively influence the application. Even when such devices are GMP qualified, such a qualification reflects only one point in time (the date of qualification), and there is no guarantee of consistent water quality in the future. Further, there is limited or no documentation to support the quality of water drawn from these desktop purification systems.

The production of highest-purity water is a complex process with many factors to consider. For instance, each cleaning step is typically designed to remove certain impurities, but each purification module is also a potential source of contamination. An ion-exchange unit is highly efficient in removing ions, but it also releases small amounts of organics and particles. In addition, many commonly used ion-exchange resins do not remove all type of ions. Therefore, we produce water for trace elemental analysis (such as *Trace*SELECT® Ultra) using special boron removal cartridges. Further, not only must we choose the right purification modules, but we must also combine them in the correct order. At Sigma-Aldrich in Buchs, we employ a modular multi-step water purification system that combines best-in-class commercial devices with self-developed and modified components.

As the "starting material" for all the different water qualities produced in Buchs, a basis quality water is produced through a standard series of treatments: rough and fine pre-filtration, reverse osmosis, electro-deionisation, UV irradiation, activated charcoal filtration, several ion-exchange units, and microfiltration. At this point in the process, the water basis already fulfils ASTM type I, CAP/NCCLS type I, and USP 27 quality requirements. Nevertheless, for ultra-trace analysis, gradient HPLC, LC-MS, or other analytical applications, the quality must be further improved by subsequent purification steps, or the so-called "final polishing." **Figure 1** shows the gradient tests of two different water qualities; the red chromatogram shows the water

Figure 1 Chromatogram of raw quality water (red line), and the final CHROMASOLV grade water for LC-MS (blue line)



before the last purification steps (raw quality), and the blue chromatogram shows the final LC-MS-grade water with no organic impurities detected with 205 nm UV.

Following these steps, we produce many different high-purity waters to meet specifications for any particular application. In **Figure 2**, our purification techniques are listed.

Another crucial issue is the quality of the feed water; the better the source, the better the final quality. High water qualities may be produced by repeating the same purification steps, but this is hardly a cost-effective method. In the case of organic contamination, the feed water quality significantly affects the final product quality. Based on this knowledge, we screened several mountain springs above Buchs and identified and tapped the purest source. Special care was also taken in constructing the pipeline that brings the spring water to our facility.

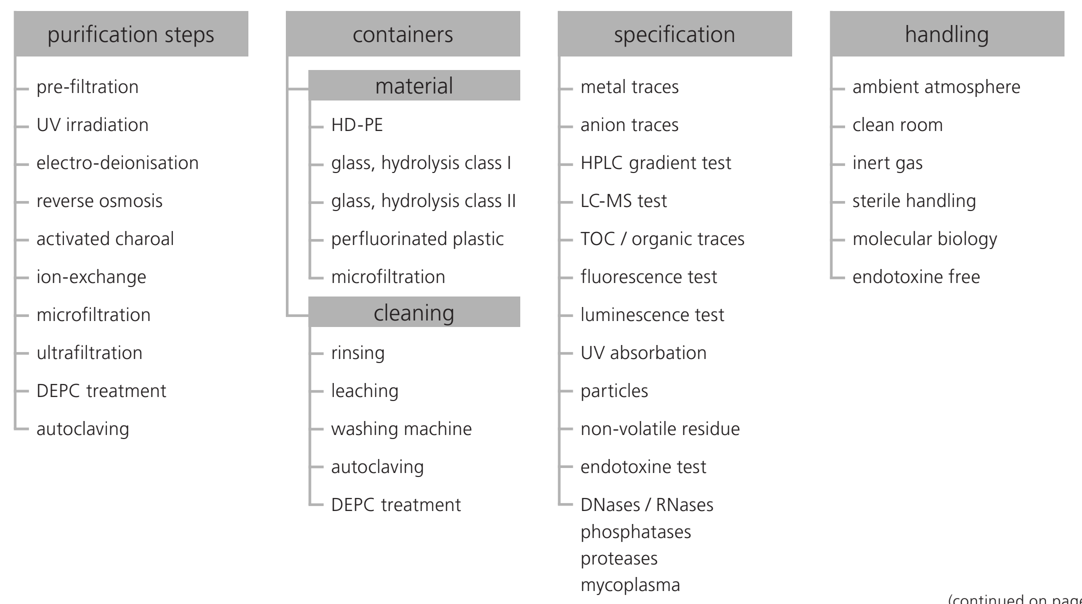
From tap to point of use

Even when the water has undergone the last purification step, the process is still not complete. There is still the matter of transport and distribution of the water to consider.

The water must be transported from the tap to the point of use and/or stored temporarily; therefore, it must be put into a suitable container. Because some containers can show leaching of contaminants in as little as minutes or even seconds, great care must be taken to choose an adequate container to ensure the water is not damaged. The right choice of adequate containers is no less important than product purity itself. One of the fundamental challenges is the fact that there is no "perfect" bottle material. Every material shows leaching of certain traces in the µg/L to ng/L range. Even perfluorinated polymers such as PFA or FEP are not the best solution for every application; they are also quite expensive materials. Plastic bottles are not suitable for HPLC(-UV) water because small organics (acetate, formiate, oligos, plasticizers, etc.) leach from the bottle into the water, contaminating it.

These same factors affect not only the bottle but the caps, pouring rings, and cap inlayers/sealings as well: in short, anything that touches the water. After we conducted several indepth studies, we found that only precleaned borosilicate white glass bottles with Teflon® closures are suitable for LC-MS Chromasolv® water. Other poorer glass qualities showed significant leach (in the ppm range) of alkali ions, leading to cluster formation in the mass spectrometer. Because particulates may also disturb LC-MS performance, clean-room bottling

Figure 2 Diversity of parameters crucial to high-purity water production



(continued on page 14)

of this water grade is required. By the same token, water for ion-chromatography presents its own particular transport and distribution challenges. Minimisation of ionic impurities is the key issue, but small organics and ion traces are also potential contaminants. To combat these problems, HDPE, a material with minimal organic (acetate, formiate, glycolate) leaching, has been developed for use in IC water bottles.

As you can see, the optimal container and closure materials must be selected, and the requirements of the particular analytical application kept in mind. Further, these materials must be tested in long-term studies to ensure proper evaluation of product expiration dates. By combining these production aspects with the adequate filling conditions (sterile, clean room, inert gas), we can offer our customers water qualities of the highest purity to meet well-defined specifications.

Specifications tailored to the application

After a high-purity water has been produced, the proper bottle and cap have been developed, and the water has been bottled under the right conditions, a set of analytical tests is then defined for each particular water grade to guarantee the specifications of the final product. A well-defined set of parameters is assigned to each of the different quality grades suitable for specific applications. In HPLC, for instance, isocratic or gradient elutions with UV detection, or hyphenated techniques such as HPLC-MS, are common. These different chromatographic and detection techniques require particular eluents. Therefore, we offer three different grades of HPLC water, each with its individually tailored set of analytical tests (**Table 1**) to ensure fitness to the application.

Sigma-Aldrich has introduced several different water grades for nearly every analytical application, including water for production and biochemical uses (**Table 2**). Each product lot is continually analysed at several stages of the production process according to its particular set of specifications. To ensure product quality and specifications, all analyses are performed after the filling, in the same type of container in which the water will be shipped to the customer.

When we take all these issues into account, it is easy to see that, although it might seem counterintuitive, $H_2O \neq H_2O$.

Table 1 Specification comparison of three different waters for HPLC.

Specified parameter	Water for HPLC (Prod. no. 95304)	Chromasolv® Plus, for gradient HPLC (Prod. no. 34877)	Water for LC-MS (Prod. no. 39253)
UV-Test	✔	✔	✔
Transmittance	✔	✔	✔
HPLC suitability test	✔	✔	✔
HPLC gradient test	-	✔	✔
No. anions tested	-	4	4
No. elements tested	-	0	17
Luminescence	-	✔	✔
Non-volatile residues	-	✔	✔
LC-MS Application (Reserpine)	-	-	✔
Particles	-	-	✔

Table 2 Selection of Fluka and Aldrich branded water qualities in different bottles (HC= hydrolysis class)

Product no.	Water quality	Package size
39253	LC-MS CHROMASOLV	white glass, borosilicate, HC-I
34877	CHROMASOLV Plus, for gradient HPLC	brown glass, HC-II
95304	for HPLC	brown glass, HC-II
00612	for ion chromatography	HD-PE (precleaned)
53463	for GC-HS	brown glass, HC-II
14211	TraceSELECT Ultra, ACS UltraTrace, for trace analysis	HD-PE (leached)
95305	TraceSELECT	HD-PE (precleaned)
95286	for organic trace analysis	brown glass, HC-II
78533	for TOC analysis	brown glass, HC-II
95280	purified water (Aqua Purificata), Ph Eur	brown glass, HC-II
34478	PESTANAL®, solvent for residue analysis	brown glass, HC-II
95289	BioChemika, for cell biology, free of endotoxins, ultrafiltered and autoclaved	white glass, septa cap
95284	BioChemika, for molecular biology, DEPC-treated and sterile filtered	
95283	BioChemika, for HPCE, for luminescence, for UV-spectroscopy	

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Shyam Verma shyam.verma@sial.com

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Polyaromatic: Amberlite®, Amberchrom®, Diaion®/Sepabeads®/MCI GEL®; Dowex® Optipore
Hydrophobic Interaction Media: Sepharose®, Toyopearl®

Gel Filtration Media

Sephacryl®, Sephadex®, Sepharose, Superdex®, Toyopearl

Ion Exchange Media

Strong and Weak Anion or Cation Resin: Amberlite, Diaion, Duolite®, Dowex, Lewatit®
Mixed Bed Ion Exchange Media: Amberlite, Dowex, Lewatit
Chelating Ion Exchange Media: Amberlite, Diaion, Dow, Duolite, Dowex, Lewatit
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Chromatography

Ionophores used for clinical applications

Serotonin and mexiletine analysis by potentiometric sensors

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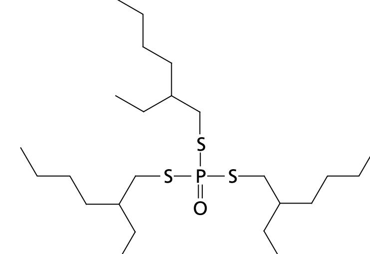
Ionophore-incorporated PVC membrane sensors are well-established analytical tools routinely used for the selective and direct measurement of a wide variety of different analytes in complex biological and environmental samples. S,S,S-Tris(2-ethylhexyl)phosphorotrithioate has proved an effective solvent mediator for constructing a serotonin-selective membrane electrode. This ionophore shows superior selectivity for alkali metal cations, and a lower selectivity for quarternary ammonium ions.

The same solvent mediator can also be used for the detection of mexiletine (sold under the trade name Mexitil®), which is used to treat heart arrythmias or extremely irregular heartbeat. The sensitivity of the electrode is adequate for measuring therapeutic mexiletine levels in saliva.

Figure 1 Solvent mediator (Fluka 30513)



For further information about ionophores, please visit our sensorics web page *sigma-aldrich.com/sensoric*

Characteristics of Serotonin-selective membrane electrode [1]:
Slope (sensitivity): 53.8 mV/dec
Detection limit: 40 µM in physiogical saline containing 150 mM NaCl and 10 mM HEPES-NaOH (pH 7.4)
Composition: 0.5 mg NaHFPB (Fluka 72015), 60 mg solvent mediator (Fluka 11686), 30 mg PVC (Fluka 81392)
Characteristics of Mexiletine-selective membrane electrode [2]:
Slope (sensitivity): 58.8 mV/dec
Detection limit: 2 µM in physiological saline containing 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.4)
Composition: 0.5 mg NaHFPB (Fluka 72015), 20 mg solvent mediator (Fluka 11686), 30 mg PVC (Fluka 81392)

Product table

Part no.	Brand	Description	CAS no.	Package size
11686	Fluka	S,S,S-Tris(2-ethylhexyl)phosphorotrithioate, Selectophore®	181629-03-8	250 mg
81392	Fluka	Poly(vinyl chloride) high molecular weight (PVC), Selectophore®	9002-86-2	1 g, 10 g, 50 g
72015	Fluka	Sodium tetrakis[3,5-bis(1,1,1,3,3,3-hexafluoro-2-methoxy-2-propyl) phenyl]borate trihydrate (NaHFPB), Selectophore®	120945-63-3	50 mg

References

1] Katsu, T.; Hirodo, H. Serotonin-Selective Membrane Electrode Made with the Solvent Mediator S,S,S-Tris (2-ethylhexyl)phosphorotrithioate. Sensor Lett. 2003, 1, 99-101.

2] Katsu, T.; Tsunamoto, Y.; Hanioka, N.; Komagoe, K.; Masuda, K.; Narimatsu, S. S,S,S-Tris(2-ethylhexyl) phosphorotrithioate as an effective solvent mediator for a mexiletine-sensitive membrane electrode. Anal Bioanal Chem. 2007, 387, 2057-2064.

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Mycobacteria – ongoing interest in an old pathogen

The genus Mycobacterium is known and dreaded as the causative agent of serious diseases like tuberculosis *(M. tuberculosis)* and leprosy *(M. leprae)*.

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Markus Auly Product Management Assistant

Mycobacterium avium complex (MAC) infection has gained notoriety recently as a significant cause of death in AIDS patients. After a period where *Mycobacterium*-related diseases were considered to be eradicated – at least in countries with high medical standards – the occurrence of multiresistent strains and a worrisome number of problematic infections in immunocompromised individuals have generated a new interest in research on this genus.

Mycobacteria are aerobic, often microaerophilic, and generally nonmotile bacteria that are characteristically acid-alcohol fast [1]. This is due to their distinctive hydrophobic cell wall, comprised of a thick layer of mycolic acid and outer lipids in addition to the normal peptidoglycan, which gives them considerable protection against acids, alkali and certain antibiotics that attack bacterial cell walls. Mycobacteria are classified acid-fast Gram-positive (because they lack an outer cell membrane), although they do not retain the crystal violet stain as typical Gram-positive bacteria do. Many mycobacteria can survive and grow in nutritionally poor environments such as water puddles and even chlorinated tap water. Other species like *M. leprae* are difficult to cultivate and seem to be obligate parasites.

Mycobacteria's exceptional hardiness and low nutritional demands are the principles of their isolation on such media as the Gruft-modified Loewenstein-Jensen medium (see **Table 1**). The supplemented antibiotics are intended to eliminate all Gram-negative and normal Gram-positive germs and spare only the more resistant *Mycobacteria*. Appropriate staining methods include the procedures according to Ziel-Neelson or Kinyoun as well as the auramine fluorochrome method, all of which are available from Sigma-Aldrich (see **Table 2**). The auramine fluorochrome is a specific stain for Acid Fast Bacilli (*Mycobacterium* sp.) in specimens and in culture. This fluorescent method, which is actually considered the best procedure, stains mycobacteria selectively by binding dye to the mycolic acid of the cell wall. The differentiation of the numerous species and subspecies has in the past been based on a variety of physiological tests [2], but molecular biological methods are gaining in importance [3].

For more details about our products for analytical microbiology, please visit our website *sigma-aldrich.com/microbiology*

Figure 1 Mycobacterium (Scanning electron microscope image; Photo Mazen T. Saleh, Laurentian University)

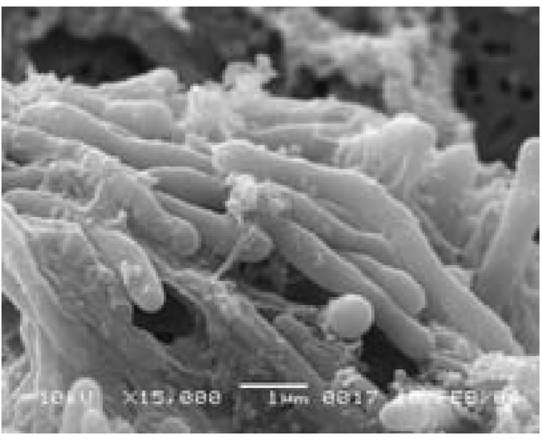

Table 1 Media for detection, isolation, differentiation of mycobacteria

Brand	Cat. no.	Media & supplements
Fluka	63237	TB-Medium Base according to Loewenstein-Jensen
Fluka	51803	Gruft Mycobacterial Supplement
Sigma	M0178	Middlebrook 7H9 Broth Base
Sigma	M0303	Middlebrook 7H10 Broth Base
Sigma	M0428	Middlebrook 7H11 Broth Base

Table 2 Fluka products for staining of mycobacteria

Brand	Cat. no.	Media & supplements
Fluka	21820	Carbol-Fuchsin solution according to Ziehl-Neelsen
Fluka	21819	Carbol-Fuchsin solution according to Kinyoun
Fluka	05151	Fluorescent Stain Kit for Mycobacteria
Fluka	56694	Acid Alcohol solution
Fluka	30503	Phenolic auramine solution
Fluka	81199	Potassium permanganate solution

References:

1] Ryan, K. J.; Ray, C. G., eds. Sherris Medical Microbiology, 4th ed.; McGraw Hill: New York, 2004.
2] Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn, W. C., Jr. Diagnostic Microbiology, 5th ed., Lipincott Williams & Wilkins: Philadelphia, 1997.
3] Parish, T. Making Sense of Mycobacteria. In Mycobacteria: Molecular Biology and Virulence; Ratledge, C., Dale, J., Eds.; Trends in Microbiology, 2000, 8 (5), p 245.

Microbiology

Differentiation of *Escherichia coli* from coliforms

Escherichia coli and coliforms are important indicator organisms for hygiene status. A broad range of biochemical tests is available for differentiation and identification of these organisms.

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In August 2008, the discovery of *E. coli*-contaminated beef in the United States prompted a nationwide recall of beef. The source turned out to be one supplier that had a history of contamination of its beef products. The usual sources of *E. coli* in beef are faeces-contaminated animal carcasses, water supply, and/or other hygiene problems. Even in Switzerland, where drinking water is unusually pure, there are rare cases of faecal contamination by liquid manure. Detection is critical to maintaining hygiene.

E. coli is an aerobe, rod-shaped, motile, Gram-negative intestinal bacterium that ferments lactose and diverse other carbohydrates (see **Table 3**). Detection is possible because the bacterium ferments dextrose (D-glucose) by producing mixed acids (e.g. lactic, acetic and formic acids) that can then be made visible with the addition of the indicator methyl red. There are many other methods of detection to indicate the presence of *E. coli*. For instance, Voges and Proskauer found a test to detect acetoin and 2,3-butanediol produced when *Klebsiella* and *Enterobacter* ferment glucose. The researchers found that under alkaline conditions, these two compounds oxidize themselves into diacetyl. Diacetyl then reacts with creatine (a guanidine derivative) and appears as a pinkish-red compound, or it reacts with α-naphtol and appears cherry-red in colour.

Table 1 Biochemical reactions of *E.coli*
Key: AG/A acid (yellow) and gas formation in butt of tube and acid (yellow) on slant surface

Biochemical test	Reaction
Catalase	+
Citrate utilisation (Simmon's citrate Agar, Fluka 85463)	-
TSI Agar (Fluka 44940)	AG/A
Gelatin liquefaction (Nutrient Gelatin, Fluka 70151)	-
Indole Production	+
Nitrate Reduction	+
Urease (Urea Broth, Fluka 51463; or Christensen's Urea Agar, Fluka 27048)	-
Voges-Proskaur	-
Methyl Red	+
Presumptive test (Lauryl sulphate Broth, Fluka 17349)	+
Phenylalanine deaminase (Phenylalanine Agar, Fluka 78052)	-
Motility (SIM Medium, Fluka 85438; or Tryptone Agar, Fluka 93655)	+
Lysine (LD Broth, Fluka 66304)	+
ONPG (β-galactosidase)	+
Oxidase	-

Figure 1 Kovac's indole reaction (from left to right: blank, negative, positive)

Some other characteristic enzymes can also be detected by their interactions. Tryptophanase cleaves Tryptophan into pyruvate, indol, and ammonia; by using reagents (Kovac's and DMCA), researchers can detect indole production (see **Figure 1**). β-Galactosidase is detected with ONPG (2-Nitrophenyl β-D-galactopyranoside), a chromogenic substrate that turns yellow after cleavage has occurred. Further, the ability to reduce nitrate to nitrite can be detected with the addition of sulphanilic acid and α-naphthylamine, which results in a red precipitate (prontosil). Finally, lysine is degraded by *E. coli* to cadaverine by the lysine decarboxylase. Because this

Figure 2 TSI Agar: From the left, we see the medium without organisms, followed by an extreme reaction in the butt of the tube and on the slant surface; the second tube from left shows the typical reaction when E. coli organisms are present.

is an alkaline reaction, the indicator (bromocresol purple) will change colour from yellow to purple.

Interesting differentiation results are obtained with the inoculation of TSI Agar slants. Due to the formation of acid during fermentation of lactose, sucrose and glucose, the pH level usually drops. However, in the case of oxidative decarboxylation of peptone alkaline products, the pH rises. This increase is indicated by phenol red, which changes colour in acidic surroundings from red-orange to yellow; upon alkalinisation, it turns deep red. *E. coli* shows an acid reaction (yellow) and gas formation in the butt of the test tube and an acid reaction (yellow) on the slant surface.

An overview of the important biochemical reactions of *E. coli* is included in **Table 1**. Sigma-Aldrich products available for differentiation are listed in **Tables 2 and 3**.

Table 2 Tests and reagents for differentiation and identification

Cat. no.	Name	Description (Engl)	Package size
75554	Aminopeptidase Test	For the detection of L-alanine-aminopeptidase in microorganisms. It is found almost exclusively in Gram-negative microorganisms.	50 ea
29333	Barritt's Reagent A	These reagents are used in the Voges-Proskauer test for detection of acetoin production by bacterial cultures.	100 mL
39442	Barritt's Reagent B		100 mL
88597	Catalase Test	A reagent to detect the enzymes catalase and peroxidase.	100 mL
05686	DMACA Indole Disks	Detection of tryptophanase activity.	50 ea
49825	DMACA Reagent		50 mL
96343	HybriScan®***D*** *E. coli* **NEW**	Genetic based detection and identification of *Escherichia coli* in water and food samples.	96 tests
60983	Kovac's Reagent for indoles	*E. coli* is able to split tryptophan into indole and alpha-aminopropionic acid. The reagents listed enable the detection of indole. Fluka 67309 contains isoamylic alcohol as solvent, while Fluka 60983 contains n-Butanol as solvent. Both formulations are more stable than the old formulation with amyl alcohol.	100 mL
67309	Kovac's Reagent for indoles		100 mL
78719	Kovac's Reagent Strips		25 ea
08714	Methyl Red Solution	Differentiates between bacteria based on level of acid production from glucose (high/low/none).	100 mL
38497	Nitrate Reagent A	Reagents, disks and kits for the detection of nitrate reduction by bacteria.	100 mL
39441	Nitrate Reagent B		100 mL
51138	Nitrate Reagent Disks Kit		50 ea
73426	Nitrate Reduction Test		1 ea
07689	O'Meara's Reagent	Used in the Voges-Proskauer test for the detection of acetoin production by bacterial cultures.	100 mL
49940	ONPG Disks	Testing for β-galactosidase.	50 ea
07345	Oxidase Reagent acc. Gaby-Hadley A	Reagents, disks and strips for the detection of cytochrome oxidase activity of microorganisms, an important differentiation step for Gram-negative bacteria.	100 mL
07817	Oxidase Reagent acc. Gaby-Hadley B		100 mL
18502	Oxidase Reagent acc. Gordon-McLeod		100 mL
40560	Oxidase Strips		100 ea
70439	Oxidase Test		50 ea

Table 3 Carbohydrates Differentiation Discs (available in single packs of 25 disks; or package size of 10 x 25 disks).
Key: [+] = positive reaction, yellow color; [-] = negative reaction

Organisms (ATCC)	Adonitol		Arabinose		Cellobiose		Dextrose		Dulcitol		Fructose		Galactose	
	(Fluka 55876)		(Fluka 80372)		(Fluka 56481)		(Fluka 63367)		(Fluka 73044)		(Fluka 53901)		(Fluka 89608)	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Citrobacter freundii (8090)	-	-	+	+	+	-	+	+	-	-			+	+
Enterobacter aerogenes (13048)	+	+	+	+	+	+	+	+	-	-	+	+	+	+
Escherichia coli (25922)	-	-	+	+	-	-	+	+	-	-	+	+	+	+
Klebsiella pneumoniae (13883)	+	+	+	+	+	+	+	+	-	-	+	+	+	+

Organisms (ATCC)	Inositol		Lactose		Maltose		Mannitol		Mannose		Melibiose		Raffinose	
	(Fluka 89614)		(Fluka 28816)		(Fluka 77653)		(Fluka 94438)		(Fluka 94445)		(Fluka 93196)		(Fluka 94226)	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Citrobacter freundii (8090)	-	-	+	+	+	+	+	+	+	+	-	-	-	-
Enterobacter aerogenes (13048)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli (25922)	-	-	+	+	+	+	+	+	+	+	+	+	-	-
Klebsiella pneumoniae (13883)	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Organisms (ATCC)	Rhamnose		Salicin		Sorbitol		Sucrose		Trehalose		Xylose	
	(Fluka 93999)		(Fluka 92971)		(Fluka 93998)		(Fluka 94309)		(Fluka 92961)		(Fluka 07411)	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Citrobacter freundii (8090)	+	+	-	-	+	+	+	+	+	+	+	+
Enterobacter aerogenes (13048)	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli (25922)	+	+	-	-	+	+	-	-	+	+	+	+
Klebsiella pneumoniae (13883)	+	+	+	+	+	+	+	+	+	+	+	+

HYDRANAL® Karl Fischer reagents

Determining water content in green coffee beans

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Coffee is one of the world's most important traded commodities as well as one of the world's favorite beverages. The International Coffee Organisation (ICO) states that in the past year (August 2007 to July 2008), nearly 5.7 million tons of green coffee were exported; the three biggest exporters are Brazil, Vietnam, and Colombia.

The coffee tree belongs to the family Rubiaceae, genus *Coffea*; the most important of approximately 70 known species are *C. arabica* and *C. canephora* var. *robusta*. The seeds of the coffee berries are green coffee beans, which are made into roasted coffee in a multi-step process.

Water content of green coffee beans is critical in most steps of coffee bean processing. If water content remains too high, microorganisms can infest the beans, and their quality will suffer. Beans that are too dry become brittle and may break during hulling and therefore be considered defective.

Table 1 Composition of raw coffee beans [1]

Composition	in % of dry matter
Water	7–13 % (1.5–3.5 % in roast coffee beans)
Soluble Carbohydrates	6–12.5 %
Insoluble Polysaccharides	24 %
Lipids	8–18 %
Chlorogenic acid	6.7–12.1 %
N-based substances	11–15 %
Caffeine	0.8–4 %
Ash	4.1 %

Different methods can be used to determine water content: Gas Chromatography, Nuclear Magnetic Resonance spectroscopy, various drying techniques, or Karl Fischer (KF) titration. In our HYDRANAL Laboratory we compared different variations of the KF titration technique, using HYDRANAL reagents, to the loss on drying (LOD) method, in order to develop a feasible and reliable procedure for determining water content in green coffee beans.

Step one: sample preparation

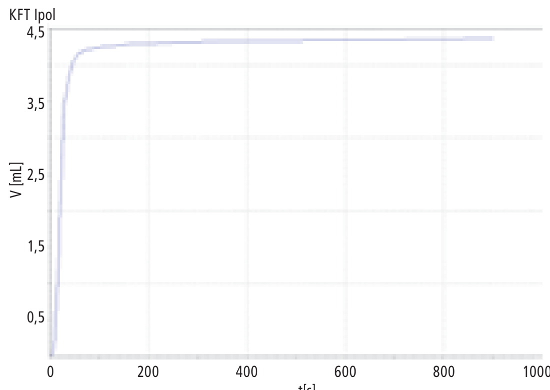
Green coffee beans are too elastic to be pounded using a mortar. A laboratory mill with a cooling jacket is a better choice for obtaining small granules of the coffee beans. The required milling time is approximately five minutes, during which the milled material should be loosened from time to time. If whole coffee beans are used for determinations like KF titration or LOD, only the water from the surface and the outer layers is determined; the water contained inside the bean will not be detected.

Due to the high water content, it is important to handle and store the milled material under air-tight conditions at all times. Tests showed that a coffee bean sample in ambient air can lose 0.8 % of its water within five minutes.

Direct volumetric KF titration in boiling methanol

Various tests showed that it is not possible to extract the water from natural products like green coffee beans at room temperature within reasonable titration times. Therefore, titration in boiling methanol was applied as a method of direct volumetric KF determination. In this procedure, the sample is heated in boiling methanol under reflux and then titrated as usual (please contact Technical Support for more information about this technique).

Figure 1



As can be seen in **Figure 1**, this method yields a regular course of titration for iodine consumption, and no side reaction occurs, which proves that this procedure is highly suitable for ground green coffee beans. **Figure 1a** shows in detail the process of the end-point adjustment; the red line (line 2) indicates the blank value, and the blue line (line 1) is the sample titration curve. The drift peaks in the sample titration show that water is still being released from the sample towards the end of the 15-minute extraction time, meaning that this long extraction time is necessary for determining the total water content of the sample. In a shorter extraction, this residual water would not be sufficiently detected, and the test results would be incorrect.

Figure 1a

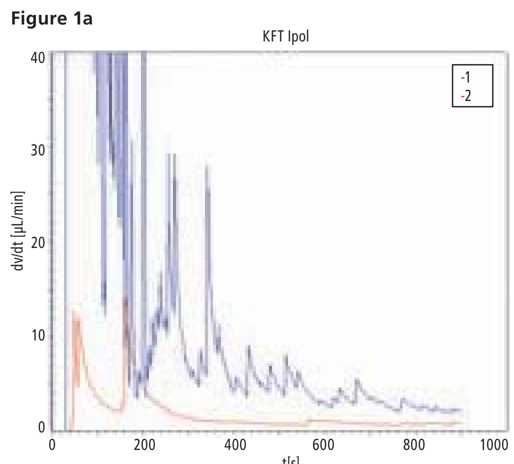
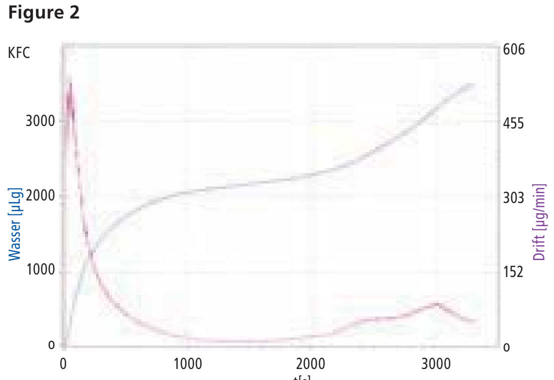


Figure 2



The accuracy of the result is further improved by taking into account the blank value for the duration of the analysis.

Indirect volumetric Karl Fischer titration with an oven

Another series of experiments was carried out using the indirect procedure of titration with the KF oven. Ground green coffee beans were again used as the sample.

In order to investigate the sample characteristics and determine the optimal heating temperature, a temperature ramp in the KF oven was applied to the sample. Starting at 50 °C, the temperature was gradually increased by 4 °C per minute until it reached 250 °C. The water released from the sample was continually transferred to the titration cell by a carrier gas (e.g. nitrogen). The course of this drift (water release shown in µg/min) during the temperature ramp was recorded and analysed (see **Figure 2**). Evaluation showed that most of the sample's water content was released between 50 °C and 90 °C; however, residual water continued to be released between 90 °C and 150 °C. A gradual process of decomposition began above 150 °C. Thus, 140 °C seems to be the ideal temperature for this sample, as a series of determinations with reproducible results showed:

Water content by KF/Oven-Temperature	130 °C	140 °C
Measurement 1	9.01 %	8.93 %
Measurement 2	8.82 %	8.94 %
Measurement 3	8.58 %	8.92 %

Determination of Loss on Drying (LOD)

In practice, the water content of green coffee is often determined by LOD, which is regulated by various standard methods. As a comparison to the KF titration, we analysed the LOD of the ground green coffee bean sample using a repeat determination.

The following parameters were used: 10 g sample dried for 16 hours at 105 °C; calculation of LOD by weight difference before and after drying. The determination was run over a long period of time in order to control the stability of the weight loss after 16 hours:

	Sample A	Sample B
After 16 hours	13.43 %	13.81 %
After 21 hours	13.67 %	14.00 %
After 37 hours	14.04 %	14.36 %
After 53 hours	14.19 %	14.52 %

Considering the heating behaviour of this sample (**Figure 2**), 105 °C is too low a temperature to capture all the water contained in the sample. As proven previously, 140 °C is needed for complete water release. Our investigations have shown that ground coffee samples after determination of LOD still contain approximately 1 % water, as measured by KF titration.

Further, this procedure determines a sample's released water as well as other volatile ingredients, calling the result into question. The result also depends on the drying time: the longer the time, the more weight will be lost; and again this weight includes not only water but other volatile substances. Thus the results of determination of LOD cannot be considered as the true water content for samples known to contain volatile ingredients.

(continued on page 22)

Conclusion

Titration in boiling methanol and indirect determination with a KF oven deliver the most reliable results. From a practical point of view, the KF oven is preferable, as this procedure can be automated for use in industry.

Application: direct volumetric titration in boiling methanol

40 mL of HYDRANAL-Methanol dry is placed in an all-glass titration vessel fixed with a reflux condenser, heated to boiling point, and titrated to dryness with HYDRANAL-Composite 5. A sample of approximately 0.2 g of ground coffee beans is prepared by differential weighing, and the water content is titrated in boiling heat. An extraction time of 15 minutes must be observed to ensure that all residual water is included in the determination.

Application: indirect titration with KF oven, volumetric

50 mL HYDRANAL-Methanol dry is placed in the titration vessel and titrated to dryness with HYDRANAL-Composite 5. The KF oven is heated to 140 °C; the nitrogen carrier gas is connected and again titrated to dryness until the drift is stable. A sample of approximately 0.1 g of ground green coffee beans is prepared by differential weighing and heated to 140 °C. The water that is released is transferred with the carrier gas to the titration cell and titrated using HYDRANAL-Composite 5.

Application: indirect titration with KF oven, coulometric

5 mL HYDRANAL-Coulomat CG is put into the cathodic compartment of a coulometry cell with diaphragm; the anodic compartment is filled to the same level with approximately 100 mL HYDRANAL-Coulomat AG Oven. (Note: if using a cell without diaphragm, only 100 mL HYDRANAL-Coulomat AG Oven is required.) The machine is switched on and automatically titrates to dryness. When the drift is low (< 10 µg water/min) and stable, the nitrogen carrier gas is connected. When the original stable drift value is reached with the carrier gas, approximately 0.02 g of the sample, prepared by means of differential weighing, can be heated to 140 °C and analysed.

HYDRANAL-Coulomat AG Oven, HYDRANAL-Coulomat AG, or HYDRANAL-Coulomat AD (only for cells without diaphragm) can be used in this application.

Literature

1] Belitz, H. D.; Grosch, W.; Schieberle, P., Lehrbuch der Lebensmittelchemie (Handbook of Food Chemistry). Springer: Berlin, 2001.

Technical help

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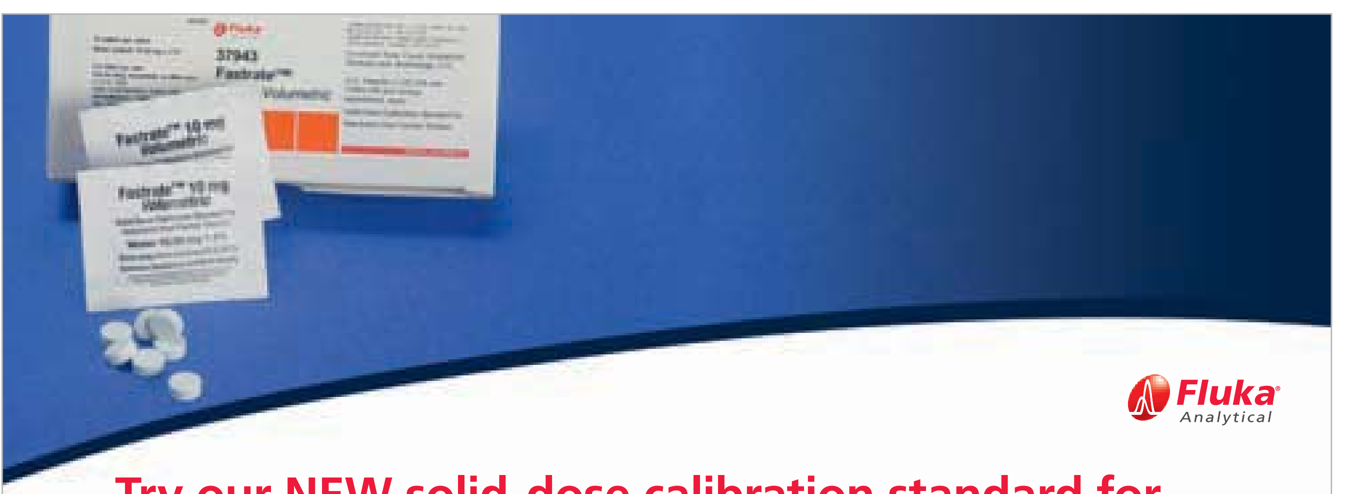
Table 2 Selected HYDRANAL Karl Fischer Reagents

Cat. no.	Brand	Description	Package size
34805	Fluka	HYDRANAL-Composite 5	500 mL; 1 L, 2.5 L
34741	Fluka	HYDRANAL-Methanol dry	1 L; 2.5 L
34724	Fluka	HYDRANAL-Formamide dry	1 L
34739	Fluka	HYDRANAL-Coulomat AG Oven	500 mL
34836	Fluka	HYDRANAL-Coulomat AG	500 mL; 1 L
34810	Fluka	HYDRANAL-Coulomat AD	500 mL
34840	Fluka	HYDRANAL-Coulomat CG	50 mL
34241	Fluka	HYDRANAL-Molecular Sieve 0.3 nm	250 g; 1 kg
34849	Fluka	HYDRANAL-Water Standard 10.0	80 mL
34828	Fluka	HYDRANAL-Water Standard 1.00	40 mL
34693	Fluka	HYDRANAL-Water Standard KF Oven 140 °C-160 °C	10 g



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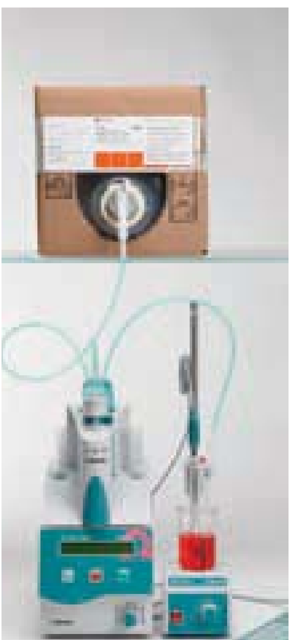

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