

Analytix

Issue 5 • 2007



New European Pharmacopoeia Regulations for Karl Fischer Titration



- Ketone, Aldehyde and Wine Analysis Standards
- APPI Dopants, HPLC Buffers and Vials
- *Pseudomonas* & *Yersinia* Media and Tests
- Ultra Pure Maldi Matrices
- Titration overview

Isotec® Stable Isotopes

Your Stable Isotope Source



Lisa Roth
Technical Marketing Manager
Stable Isotopes

Dear Colleague,

If you've read recent issues of Analytix, you'll likely know that analytical standards are a very important product area for us. In this issue, I'd like to discuss another important group of standards that you may not be aware that we supply: stable isotopes.

Scientists use stable isotope labeled compounds to probe metabolic pathways and as internal standards for reliable quantitative analysis. Stable isotopes are routinely used in:

- Metabolic research
- Quantitative proteomics
- Protein structure determinations
- Breath test kits and research
- Mass spectrometry
- Positron emission tomography (PET)
- Magnetic resonance imaging (MRI)
- Agricultural research

Isotec®, the Stable Isotope Group of Sigma-Aldrich, is a leading producer of stable isotope labeled compounds and enriched stable isotopes, such as ^{13}C . The most recognizable stable isotope products are NMR deuterated solvents. Other NMR products we offer include:

- NMR solvents
- NMR reference standards
- NMR tubes and accessories
- NMR equipment and software

This is just a small portion of our complete offering, which ranges from isotopically-enriched gases to labeled amino acids to complex biomolecules. Isotec routinely incorporates D, ^{13}C , ^{15}N , and ^{18}O into these compounds to meet our customers' needs.

If you cannot find a labeled compound to meet your requirements, Isotec's R&D Group will work with you to design and synthesize a custom molecule. Our extensive experience, combined with our inventory of basic starting materials, enables us to deliver unique, custom stable isotope labeled compounds.

Along with the other Sigma-Aldrich brands, our commitment to quality and service is found throughout Isotec. Our on-site synthesis, purification and isotopic enrichment facilities and expert Quality Control team and Quality Assurance department provide you with the highest quality products available. Isotec also provides a wide variety of packaging options. We also provide custom packaging on request whenever possible. Our knowledgeable and well-trained Customer Service team handles your requests and answers your toughest questions.

We look forward to serving your needs.

Kind regards,

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New European Pharmacopoeia Regulations for Karl Fischer Titration

Freedom of choice of pyridine-free reagents and other improvements

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The European Pharmacopoeia (Ph. Eur.) specifies Karl Fischer titration to measure the water content of many solvents, chemicals and other substances. Historically, and even until very recently, pyridine-containing KF reagents were specified. This means that for a very long time, the Ph. Eur. did not meet the state-of-the-art in KF technology, since pyridine-free KF reagents have been available and used successfully since 1980. If one wanted to take advantage of the improved safety and other benefits of pyridine-free reagents, tedious comparison tests had to be carried out with both types of reagents. Sigma-Aldrich, an innovator in pyridine-free KF reagents, supported its customers by conducting over 100 of these comparison tests in the **HYDRANAL**® laboratory.

However, the newly released Ph. Eur. 5.7 contains an important change: The use of a pyridine-based KF reagent is no longer compulsory. Chapter 2.5.12 “Water: Semi-Micro Determination” describes in Method A the direct titration of water and in Method B the indirect method. In practice, the direct method, described below, is easier to carry out.

Method A

Introduce into the titration vessel methanol R, or the solvent indicated in the monograph or recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce the substance to be examined rapidly and carry out the titration, stirring for the necessary extraction time.

Several points are important to elucidate:

1. The titration agent is a matter of choice. If methanol is used in the titration vessel, the titrant of choice is the one-component reagent, **HYDRANAL**® Composite.

2. In our earlier comparison tests, we often recommended the addition of a suitable solubilizing agent for the sample. To accommodate this improvement, the new pharmacopoeia now states in reference to the solvent “...or recommended by the supplier of the titrant.”

An example is gentamicin sulphate, for which the Ph. Eur. prescribes methanol to be used in the titration vessel. During suitability tests, we found that gentamicin did not fully dissolve during titration, which resulted in erroneously low water content, and afterwards a too-high recovery of added water. The test results thus did not fulfill the requirements of the suitability test. However, when we used a mixture of **HYDRANAL**® Methanol dry and **HYDRANAL**® Formamide dry in the titration vessel, the gentamicin dissolved well and the results fulfilled the requirements of the suitability test. Both documents are available from us upon request.

3. In addition to the freedom in the choice of titrant, the new Ph. Eur. also permits choices in the medium to be used in the titration vessel, specifically “...methanol R, or the solvent indicated in the monograph or recommended by the supplier of the titrant.”

Acetone provides a perfect example. The Monograph for acetone states that pyridine, which is malodorous and toxic, be introduced into the titration vessel. We have performed successfully the requisite suitability test using both pyridine and odorless **HYDRANAL**® KetoSolver, which requires no hazardous declaration, in the titration vessel. These test results are also available on request.

Method B

This has basically the same requirements as Method A, except after addition of the sample an excess of titrant has to be added. The titrant, which is not consumed by the water in the sample, has to be back titrated using a standard with a known amount of water, like **HYDRANAL**® Water in methanol Standard 5.00.

Suitability

The accuracy of the determination with the chosen titrant must be verified for each substance to be examined. The following procedure, given as an example, is suitable for samples containing 2.5–25 mg water.

The water content of the substance to be examined is determined using the reagent/solvent system chosen. Thereafter, sequential known amounts of water R are added in an appropriate form (at least 5 additions) and the cumulative water content determined after each addition.

1. Calculate the percentage recovery
2. Calculate the regression line of the cumulative water
3. Calculate the percentage mean recovery

The reagent/solvent system is considered to be acceptable if:

- The mean recovery is between 97.5% and 102.5%
- The slope b is between 0.975 and 1.025 (deviation $\pm 2.5\%$)
- The error e1 and e2 are not greater than 2.5%

The suitability test with all necessary calculations is not easy to carry out; neither is the preparation of the necessary documentation. To support our **HYDRANAL**[®] customers, we have put together a test sheet containing all necessary information, including preparation of the titration curve. So far, we have investigated fifteen substances listed in the Ph. Eur. In each case, we used certified **HYDRANAL**[®] Water Standards for the addition of water in an appropriate form.

Using a reagent with titer 5 for very low water content, which is mostly the preferred titer, we were not always able to meet the limits of acceptance. In these cases, we successfully worked out a second procedure using **HYDRANAL**[®] Composite 2. A typical example is the procedure for titration of ethyl acetate, which can also be obtained from us on request.

We have carried out suitability tests on the following substances (other suitability tests may be requested from us):

Acetone (see Table 1)	Glycerol
Citric acid anhydrous	Lactose monohydrate
Dibutyl phthalate	Methanol
Dichloromethane	Olive Oil
Erythromycine	Potassium citrate
Ethyl acetate	2-Propanol
Gentamicin sulphate	di-Sodium hydrogen phosphate dodecahydrate
D(+)-Glucose monohydrate	Trolamine (triethanolamine)

Table 1 Suitability test results for acetone. Titration with **HYDRANAL**[®]-Composite 5 in 30 mL **HYDRANAL**[®]-KetoSolver (after addition of sample, consecutive known amounts of water are added and determined)

	Sample	Water				
		1	2	3	4	5
Sample size (g)	10.0000					
Water added (mg)		11.08	11.1	12.02	7.63	6.31
Water found (mg)	11.2420	11.07	11.01	11.87	7.57	6.28
Water content (%)	0.1124					
Recovery (%)		99.95	99.16	98.77	99.22	99.45
Mean recovery (%):	99.31					
Slope	0.991					
Error 1 (%)	0.88					
Error 2 (%)	1.84					

The German edition of Ph. Eur. 5, Chapter 4.1.2 reference solutions for limit tests contains following information:

Reference solution for Micro determination of Water R	1147300
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Commercially available reference solution for coulometric titration of water delivered with a certified water content in an appropriate solvent.

Suitable **HYDRANAL**[®] reference solutions for coulometric applications are:

34828	HYDRANAL [®] Water Standard 1.00 (1 mg H ₂ O/g = 1000 ppm, exact value stated on CoA)
34847	HYDRANAL [®] Water Standard 0.10 (0.1 mg H ₂ O/g = 100 ppm, exact value stated on CoA)

Table 2 Selected **HYDRANAL**[®] Karl Fischer reagents

34805	HYDRANAL [®] Composite 5
34806	HYDRANAL [®] Composite 2
34741	HYDRANAL [®] Methanol dry
34738	HYDRANAL [®] KetoSolver
34724	HYDRANAL [®] Formamide dry
34849	HYDRANAL [®] Water Standard 10.0
34828	HYDRANAL [®] Water Standard 1.00
34847	HYDRANAL [®] Water Standard 0.10
34802	HYDRANAL [®] Water in methanol Standard 5.00 (for back titration)

Lastly, it has to be pointed out that all other **HYDRANAL**[®] reagents can also be used if the suitability test does meet the requirements.

We will continue to add innovative new products and new applications to our already comprehensive **HYDRANAL**[®] line, keeping it up to date with the latest regulations and sample requirements. To learn more about **HYDRANAL**[®], we invite you to visit our web site: www.sigma-aldrich.com/hydranal. Contact:

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Wine Quality Analysis

Wine analysis stock solutions for the determination of wine components in sweet and dry wines



When applied to the compositional analysis of wine, including naturally occurring compounds, additives and contaminants, analytical chemistry plays an important role in ensuring both the quality of the wine and consumer safety. The notorious 1985 glycol-doping scandal of Austrian and German wines is a prime example.

Acknowledgements

This article was developed in close cooperation with Sertec-Electronics in Olten (Switzerland). We want to thank especially R. Schwarz for providing the measurements and giving us the opportunity to publish these.

Table 1 Comparison of dry and sweet wine composition measured using ion chromatography with RI detection

Peak ID	Compound	Standard (g/L)	Dry wine (g/L)	Sweet wine (g/L)
1	Citric acid (Citronensäure)	0.491	Not detected	Not detected
2	Tartaric acid (Weinsäure)	1.98	1.628	2.874
3	Glucose	1.975	0.507	4.533
4	Malic acid (Apfelsäure)	0.996	1.392	1.464
5	Fructose	1.967	0.371	4.857
6	Succinic acid (Bernsteinsäure)	0.491	0.889	0.819
7	Lactic acid (Milchsäure)	1.975	3.063	1.112
8	Glycerin	4.954	9.411	7.067
9	Acetic acid (Essigsäure)	0.487	0.792	0.472
10	Butanediol	0.494	0.622	0.404
11	Ethanol	101.774	106.669	94.583

Cat. No.	Brand	Description	Pack Size	Components, 100 mL solution contain:
19433	Fluka	Wine Analysis: stock solution I	100 mL	0.5 g Citric Acid, 0.5 g Malic Acid, 0.5 g Succinic Acid, 0.5 g Acetic Acid, 2.0 g Tartaric Acid, 2.0 g Lactic Acid
19065	Fluka	Wine Analysis: stock solution II	100 mL	0.5 g 2,3-Butanediol, 2.0 g Glucose, 2.0 g Fructose, 5.0 g Glycerol
12159	Fluka	Wine Analysis: stock solution IV	100 mL	10.2 g (10 mL) Ethanol

Nicole Amann Product Manager Analytical Standards nicole.amann@sial.com

However, wine presents a complex matrix: Flavors, sugars, carboxylic acids, tannins, phenols, amino acids, alcohols, esters and acetates are among the many typical wine constituents, and many more are present as unwanted contaminants that must be monitored. The composition and ratio of constituents combine to give each wine its unique flavor and texture, a ratio that is easily disturbed by environmental conditions, shipping and storage. Analysts involved in the quality control or regulation of wine require reliable analytical methods and standards for its determination.

To help analysts produce definitive, quantitative results, Sigma-Aldrich offers three Fluka-brand standards for wine analysis. Together, these standards include many of the most commonly analyzed sugars, acids and alcohols in wine.

Ion chromatography (IC) is useful because it permits the simultaneous analysis of many important wine components. The IC chromatograms of the Fluka standards, along with samples of sweet and dry wines, appear in the accompanying figures. These samples were provided by a Swiss vintner that performs in-house QA to show the quality of their wine as a means to help ensure customer confidence in their product. Notable are the elevated sugars in the sweet wine and elevated acids in the dry wine.



Figure 1 Fluka wine standard solutions (see Table 1 for peak IDs)

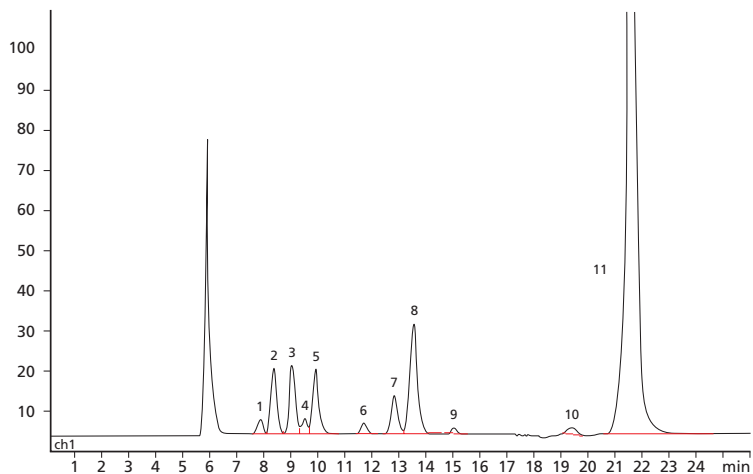


Figure 2 Dry wine sample

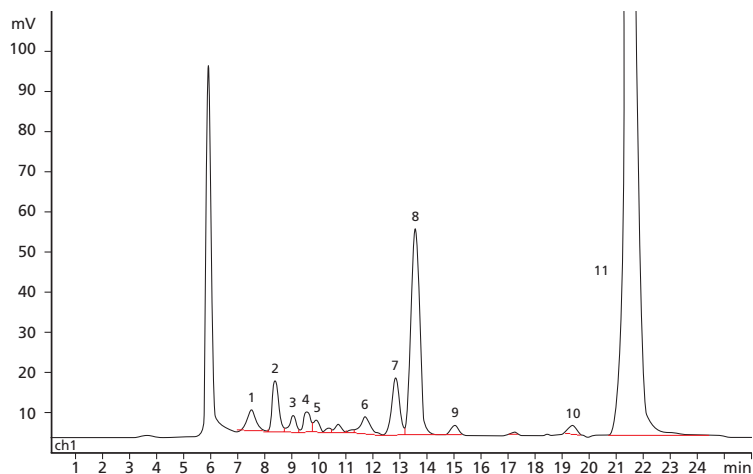
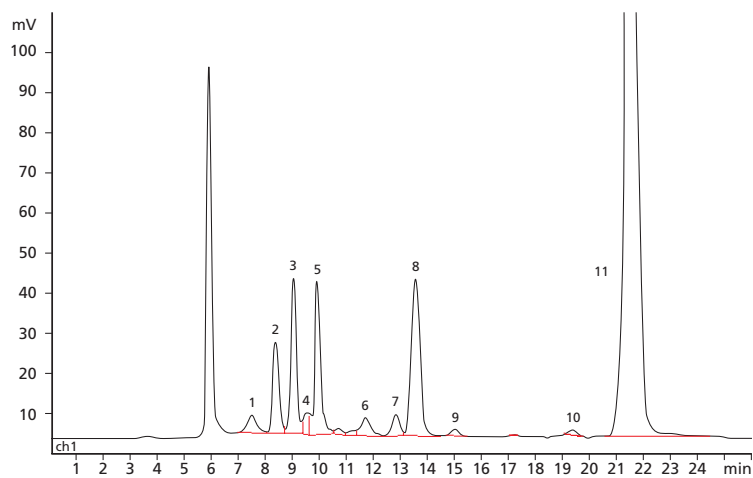


Figure 3 Sweet wine sample



Conditions:

Columns: Sertec-Wine-ST3 300 x 7.8 mm with

Sertec-Wine-ST1, 50 x 4.6 mm precolumn

Mobile phase: 2.5 mM sulfuric acid

Flow rate: 0.65 mL/min.

Temperature: 50 °C

Detection: RI

Injection: 20 μ L, wine sample diluted 1:9 with 10 mM sulfuric acid and filtered

Ketone and Aldehyde Analysis

DNPH-labeled standards for US and European regulations

Ketones and aldehydes are continually released into the atmosphere by motor vehicle emissions, building materials, household products, cigarette smoke and the photo-oxidation of volatile organic compounds. Short-term exposure to carbonyl compounds can result in burning sensations to the eyes, nose and throat, fatigue and nausea. Longer-term exposure may result in cancer as carbonyls are considered probable carcinogens.

Analysts monitoring atmospheric carbonyls follow prescribed methods published by the U.S. Environmental Protection Agency (EPA), the California Resource Board, and the American Society for Testing and Materials (ASTM). These methods call for trapping carbonyls on an adsorbent coated with dinitrophenylhydrazine (DNPH). The captured carbonyls react with the 2,4-DNPH compound to form a more stable dinitrophenylhydrazone derivative that can be analyzed by HPLC with UV detection using appropriate reference standards.

Calibration Mixtures

Table 1 California Resource Board Standards

Cat. No.	Description	Concentration*	Package Size
47650-U	CARB Method 1004 DNPH Mix 1	3 µg/mL each component in acetonitrile	1 mL
47651-U	CARB Method 1004 DNPH Mix 2	30 µg/mL each component in acetonitrile	1 mL
Components:			
	Acetaldehyde-2,4-DNPH	Formaldehyde-2,4-DNPH	
	Acetone-2,4-DNPH	Hexaldehyde-2,4-DNPH	
	Acrolein-2,4-DNPH	Methacrolein-2,4-DNPH	
	Benzaldehyde-2,4-DNPH	Propionaldehyde-2,4-DNPH	
	Butyraldehyde-2,4-DNPH	m-Tolualdehyde-2,4-DNPH	
	2-Butanone-2,4-DNPH	Valeraldehyde-2,4-DNPH	
	Crotonaldehyde-2,4-DNPH		

Table 2 European Standards

Cat. No.	Description	Concentration*	Package size
47672-U	Carbonyl-DNPH Mix 1	20 µg/mL in acetonitrile (except where noted)	1 mL
47671-U	Carbonyl-DNPH Mix 2	2 µg/mL in acetonitrile (except where noted)	1 mL
Components:			
	Acetaldehyde-2,4-DNPH	Formaldehyde-2,4-DNPH (40 µg/mL/4µg/mL)	
	Acetone-2,4-DNPH	Hexaldehyde-2,4-DNPH	
	Acrolein-2,4-DNPH	Methacrolein-2,4-DNPH	
	Benzaldehyde-2,4-DNPH	Propionaldehyde-2,4-DNPH	
	Butyraldehyde-2,4-DNPH	p-Tolualdehyde-2,4-DNPH	
	2-Butanone-2,4-DNPH	Valeraldehyde-2,4-DNPH	
	Crotonaldehyde-2,4-DNPH		

* Concentration expressed as aldehyde equivalent

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Sigma-Aldrich offers more than thirty 2,4-DNPH-carbonyl derivative reference standards for use in the quantification of atmospheric carbonyl compounds. They are available in the form of neat compounds, single component solutions and method-specific mixtures. A Certificate of Analysis stating both the DNPH derivatized and non-derivatized concentration for each carbonyl is supplied with each purchase. Data packets detailing the preparation and testing of both the raw materials and the final product are complementary and available from us upon request. To view a complete listing of all DNPH-derivative products, please visit our web site: www.sigma-aldrich.com/standards.

Table 3 U.S. Environmental Protection Agency (US EPA) Standards

Cat. No.	Description	Concentration*	Package Size
47285-U	TO-11/1P-6A Aldehyde & Ketone DNPH Mix	15 µg/mL each component in acetonitrile	1 mL
Components:			
	Acetaldehyde-2,4-DNPH	Hexaldehyde-2,4-DNPH	
	Acetone-2,4-DNPH	Isovaleraldehyde-2,4-DNPH	
	Acrolein-2,4-DNPH	Propionaldehyde-2,4-DNPH	
	Benzaldehyde-2,4-DNPH	o-Tolualdehyde-2,4-DNPH	
	Butyraldehyde-2,4-DNPH	m-Tolualdehyde-2,4-DNPH	
	Crotonaldehyde-2,4-DNPH	p-Tolualdehyde-2,4-DNPH	
	2,5-Dimethylbenzaldehyde-2,4-DNPH	Valeraldehyde-2,4-DNPH	
	Formaldehyde-2,4-DNPH		

APPI Dopants

Solvents and post-column additives for photo-ionization

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APPI (atmospheric pressure photoionization) dopants enable and enhance ionization in the APPI source. They include substances like toluene, acetone, anisole and chlorobenzene. High gas phase concentrations of the dopants are introduced into the ionization cavity of the APPI source. There, UV radiation readily ionizes the dopant molecules forming a large number of free radicals and molecular ions. Subsequently, other molecules are ionized by the dopants through electron or proton transfer.

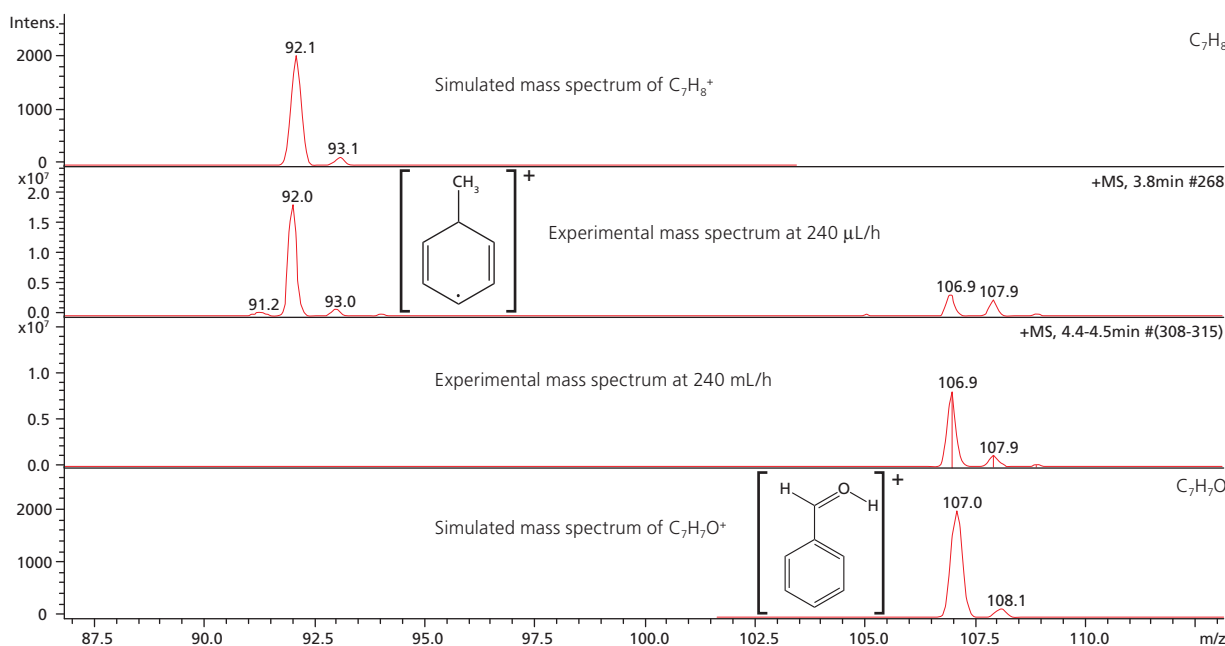
Compounds with higher ionization energy than the energy level of the emitted photons from the UV lamp require dopants, otherwise charge is not generated and these compounds will not drift into the mass spectrometer. However, molecules that can be analyzed directly in the APPI source also benefit from the use of dopants. The intensity and sensitivity can be improved because the dopants increase the number of ionized analyte molecules [1–4].

Using dopants to enhance APPI signal

A typical, yet impressive, application for dopants is the ionization of benzaldehyde dissolved in toluene. Benzaldehyde cannot be ionized directly by ESI and gives poor results with APCI

and APPI without dopant. Thus, the use of a dopant in combination with an APPI source represents the only suitable way to analyze benzaldehyde. To demonstrate the improvement, a benzaldehyde solution was infused via syringe directly into the APPI source. At a low flow rate (240 $\mu\text{L}/\text{hour}$) positively charged toluene radicals ($[\text{M}(\text{C}_7\text{H}_8)]^{+\bullet} = 92.06 \text{ amu}$) are formed, as the upper calculated and observed mass spectra in **Figure 1** demonstrate. Increasing the flow rate to 6 mL/hour causes the formation of uncharged toluene radicals, which are not detectable by MS, and indirectly ionizing benzaldehyde through proton transfer. This in turn leads to the lower calculated and observed mass spectra in **Figure 1**. APPI experiments are optimized in terms of dopant (single components or mixtures), percent dopant in the mobile phase and nebulizer gas (for direct injection into the APPI source). Specific requirements depend on the manufacturer of the mass spectrometer and the specific source design.

Figure 1 Mass spectra of an acetaldehyde solution in toluene at 2 different flow rates



Mass spectra of an acetaldehyde solution in toluene at 2 different flow rates. The solution is directly infused into the APPI source with a syringe pump. The upper spectrum was obtained at a flow rate of 240 $\mu\text{L}/\text{h}$. The primary mass of $m/z=92.0$ amu agrees with a charged toluene radical. Increasing the flow rate to the maximum of 6 mL/h changes the mass spectrum significantly as there is only the protonated benzaldehyde molecule observed. High flow rates support the proton transfer reaction of the charged toluene radical to benzaldehyde and other molecules decreasing the number of the charged toluene molecules nearly to zero.

(continued on page 10)

Dopant purity requirements

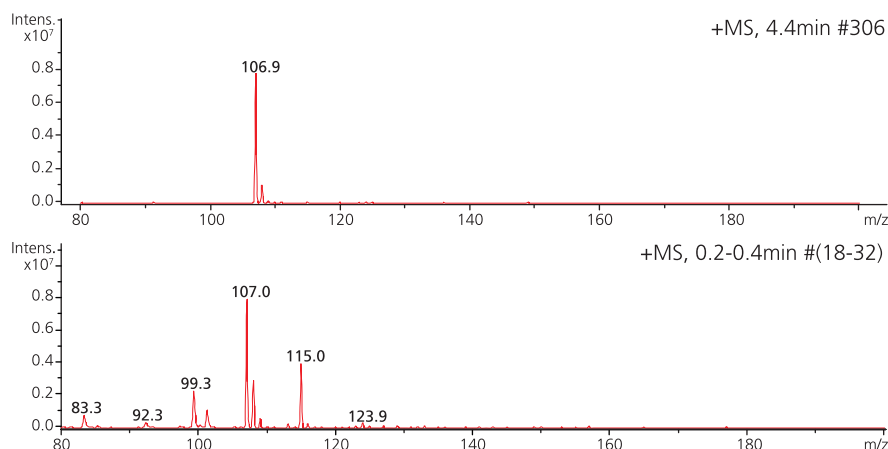
Dopants can enhance signals of analytes in mass spectra, but they also intensify unwanted impurities when present. Small amounts of impurities in a dopant can result in excessive noise or a suppression of the analyte signal. **Figure 2** shows a benzaldehyde solution in suitably pure toluene and in contaminated anisole.

Benefits of dopants in normal phase LC-MS analysis

Electrospray ionization (ESI) is the method of choice for most polar compounds, like drugs and metabolites, and is very sensitive under reversed-phase conditions. However, if normal phase is employed,

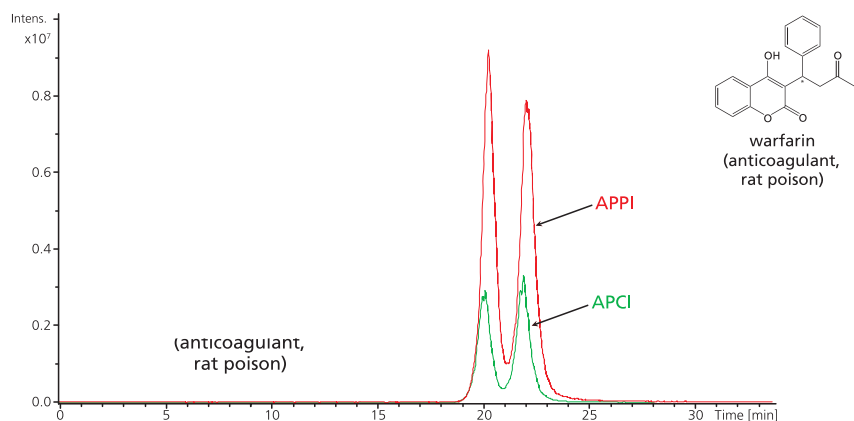
the ion source must be switched from ESI to APCI or APPI because analyte ionization, a key requirement of ESI, does not occur in non-polar organic solvents. Charge on the analyte must be generated by corona discharge or UV radiation. When normal phase in conjunction with MS detection is used, like in the separation of warfarin enantiomers on the P-CAP-DP chiral stationary phase shown in **Figure 3**, the combination of the APPI source with toluene dopant can significantly enhance the signal.

Figure 2 Spectra of benzaldehyde dissolved in toluene (top) and anisole (bottom)



Mass spectra of benzaldehyde dissolved in toluene (top) and anisole (bottom). The concentration is 100 $\mu\text{L/mL}$. In both cases the syringe pump operated at 6 mL/hour, resulting in comparable ionization of benzaldehyde. In the case of anisole, the mass spectrum shows additional signal from contaminants in the dopant.

Figure 3 Separation of warfarin under normal phase conditions



Conditions:

Column: Astec P-CAP DP, 25 cm x 4.6 mm, 5 μm packing (Cat. No. 35024AST)

Mobile phase: heptane:ethanol (0.1% ammonium acetate, formic acid), 95:5

Flow rate: 0.8 mL/min.

Temperature: 50°C

Sample: 3 μL , warfarin 1 mg/mL

Method:

Astec P-CAP DP (4.6–250 mm, 5 μm) heptane/ethanol (0.1% ammonium acetate, formic acid), 95:5, isocratic, T=50°C conc. 1 mg/mL, inject. vol. 3 μL

Product table

Cat. No.	Brand	Description	Package size
650579	Aldrich	Toluene CHROMASOLV® Plus, for HPLC, $\geq 99.9\%$	1 L, 4 L
650501	Aldrich	Acetone CHROMASOLV® Plus, for HPLC, $\geq 99.9\%$	1 L, 4 L
270644	Aldrich	Chlorobenzene CHROMASOLV® Plus, for HPLC, 99.9%	100 mL, 1 L, 2 L, 4 L
96109	Fluka	Anisole puriss. p.a., standard for GC, $\geq 99.9\%$	5 mL, 10 mL

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- 3] Cai, S.; Hanold, K.; Syage, J. *Anal. Chem.*, **2007**, *79* (6), 2491–2498.
- 4] Syage, J.; Hanold, K.; Lynn, T.; Horner, J.; Thakur, R. *J. Chromatogr. A* **2004**, 1050, 137–149.

Selecting the Right Reversed-phase HPLC Buffers

Sigma-Aldrich offers high-purity HPLC buffers as solutions, solids or concentrates



The popularity of reversed-phase HPLC is due in large part to the power it gives the user to control and optimize the separation by altering the mobile phase pH, ionic strength and type of dissolved ions. These choices are especially important when analyzing polar compounds. In all cases, the ionic mobile phase components, salts, buffers and additives, must be of sufficient purity to prevent column and instrument fouling and have negligible contribution to the background absorbance. Through its Supelco brand, Sigma-Aldrich is well-known as a supplier of innovative reversed-phase HPLC columns, but we also offer the other half of the equation: a complete line of high-quality HPLC mobile phase buffers and additives from our Fluka brand.

Mobile phase pH affects retention, resolution, sensitivity and reproducibility

The improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reverse-phase separation of polar and ionizable compounds. Partial ionization of the analyte is one cause of the problems (1). Another source is strong interaction between analytes and residual silanols or other active sites on the stationary phase (2). These, and other problems, can be overcome by proper mobile phase buffering (maintaining the pH within a narrow range) and choosing the right ionic species and its concentration (ionic strength) in the mobile phase. Where LC-MS is concerned, the buffering species must also be chosen based on its ability to maintain, and not suppress, analyte ionization in the MS interface. Sensitive LC-MS separations depend heavily on the correct choice of acid, base, buffering species and other additives (3).

Buffering agents for reversed-phase HPLC

Technically, buffers are solutions of a weak acid and its conjugate base, or a weak base and its conjugate acid. They mitigate the influence of hydrogen/hydronium and hydroxide ions and subsequently reduce the pH

fluctuations, even upon dilution. The typical pH range for reversed-phase on silica-based packings is pH 2 to 8. Buffer concentrations are generally in the 10–100 mM range.

The right buffer system to choose depends on the desired pH and the pKa values of all ionizable species in the analysis, including the mobile phase components. The pKa is the pH at which the concentrations of the ionized and free forms are equal. When a compound has more than one ionizable functional group, it has more than one pKa value.

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Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Phosphate's two pKa values, 2.1 and 7.1, and UV transparency make it ideal for most HPLC separations. It also has a pKa of 12.3 that is suitable for buffering in the pH 11.3–13.3 range. Phosphate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds. With the growth in popularity of LC-MS, volatile buffer systems, such as TFA, acetate, formate and ammonia, are frequently used. Salts without buffering capacity, like KCl or NaCl, are occasionally added to the reversed-phase mobile phases to reduce ionic interactions between analytes, sample and matrix components and stationary phases.

Matching buffer pKa to analyte functionality and target pH range

Selection of a suitable buffer ensures that the ionizable functional group is in a predictable state – whether fully neutralized or fully ionized – to maximize retention reproducibility. It is also important that the buffer has a pKa close to the desired pH since buffers control pH best at their pKa. A rule of thumb is to choose a buffer with a pKa value within 2 units of the desired mobile phase pH (see Table 1).

Table 1 HPLC buffers, pKa values and useful pH range

Buffer	pKa	Useful pH range
Trifluoroacetic acid (TFA)	0.5	<1.5
Chloroacetate	2.9	1.9 – 3.9
Sulfonate	1.8 and 6.9	<1 – 2.8, 5.9 – 7.9
Phosphate	2.1	1.1 – 3.1
Formate	3.8	2.8 – 4.8
Acetate	4.8	3.8 – 5.8
Phosphate	7.1	6.2 – 8.2
Ammonia	9.2	8.2 – 10.2
Phosphate	12.3	11.3 – 13.3

(continued on page 12)

Mobile pH and reversed-phase retention

It is also important to consider the effect of ionization on analyte solubility and retention. Most ionized compounds are highly soluble in aqueous solutions and consequently show low reversed-phase retention. Therefore, when pH is used to increase reversed-phase retention, the pH should be changed in the direction that decreases analyte ionization. For example, organic acids are typically run in phosphate-buffered mobile phases below pH 2.5 where they are primarily in a non-ionized form. Solubility of some analytes is pH-dependent and this must also be taken into account when using pH to adjust retention or resolution.

Guidelines for preparing mobile phases

Because even slight variations in pH and buffer concentration can dramatically affect chromatographic separations of sensitive compounds, it is important to use consistent and specific techniques to prepare buffered mobile phases. A good practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of buffer. The pH of the solution should be adjusted, if necessary, and then diluted to final volume of water prior to adding or blending of organic solvents. Thorough mixing, degassing and filtering prior to use is also strongly recommended.

Table 2 New HPLC-grade buffers and additives from Sigma-Aldrich

Cat. No.	Brand	Description	Package Size
17836	Fluka	Ammonium acetate, puriss. p.a. for HPLC; >99.0% (NT)	50 g, 250 g
17843	Fluka	Ammonium formate, puriss. p.a. for HPLC, >99.0% (NT) (dried material)	50 g, 250 g
17837	Fluka	Ammonium hydroxide solution, puriss. p.a. for HPLC; ~10% (T) in water	100 mL, 1 L
17842	Fluka	Ammonium phosphate monobasic, puriss. p.a. for HPLC; >99.0% (T)	50 g, 250 g
17839	Fluka	Ammonium trifluoroacetate, puriss. p.a. for HPLC; >99.0% (NT)	10 g, 50 g
9676	Fluka	Formic acid, 50%, puriss. p.a. for HPLC; 49–51% (T)	100 mL, 500 mL
9751	Fluka	Formic acid:Triethylamine (2M:1M) concentrate, puriss. p.a. for HPLC	100 mL, 500 mL
9752	Fluka	Formic acid:Triethylamine (2M:2M) concentrate, puriss. p.a. for HPLC	100 mL, 500 mL
17835	Fluka	Potassium phosphate dibasic anhydrous, puriss. p.a. for HPLC; >99.0% (T)	50 g, 250 g
17841	Fluka	Sodium formate, puriss. p.a. for HPLC; >99.0% (NT)	50 g, 250 g
71633	Fluka	Sodium phosphate dibasic dihydrate, puriss. p.a. for HPLC; ~99% (T)	50 g, 250 g
17844	Fluka	Sodium phosphate monobasic anhydrous, puriss. p.a. for HPLC; >99.0% (T)	50 g, 250 g
17840	Fluka	Sodium trifluoroacetate, puriss. p.a. for HPLC; >99.0% (T)	10 g, 50 g
17924	Fluka	Triethylamine, puriss. p.a. for HPLC; >99.5% (GC)	10 x 2 mL ampuls
9746	Fluka	Trifluoroacetic acid:Triethylamine 2M:1M, puriss. p.a. for HPLC	100 mL, 500 mL
9747	Fluka	Trifluoroacetic acid:Triethylamine 2M:2M, puriss. p.a. for HPLC	100 mL, 500 mL

For a complete list of HPLC buffers and additives, please refer to our online product catalog at: www.sigma-aldrich.com

References

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New High-quality Vials and Accessories for Analytical Applications

Sigma-Aldrich expands its range of vials and accessories

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

Vials, caps and septa that come in contact with the sample are critical, but often overlooked, components of a successful analysis. We continually search for the best vials and accessories to add to our already extensive line to make sure you have the vials you need to meet the evolving demands of instruments, samples and measurement techniques. All of our vials have the cleanliness, quality and reproducibility necessary for a successful analysis. The table below contains lists of some of our recently-introduced vials and vial accessories for chromatographic applications. Additionally, if you cannot find the vial, cap, septa or other vial accessory you need, we would be happy to try to find the right product for you or to provide a customized solution.

Table New vials and accessories from Sigma-Aldrich

Cat. No.	Brand	Description	Package Size
TPX Vials			
SU860044, SU860192	Supelco	TopSert™: TPX snap ring vial, 32 x 11.6 mm, clear, with integrated 0.2 mL glass micro insert, 15 mm tip	100, 1000
SU860189, SU860167	Supelco	TopSert™: TPX snap ring vial, 32 x 11.6 mm, amber, with integrated 0.2 mL glass micro insert, 15 mm tip	100, 1000
SU860043, SU860147	Supelco	TopSert™: TPX short thread vial, 32 x 11.6 mm, clear, with integrated 0.2 mL glass micro insert, 15 mm tip	100, 1000
SU860188, SU860166	Supelco	TopSert™: TPX short thread vial, 32 x 11.6 mm, amber, with integrated 0.2 mL glass micro insert, 15 mm tip	100, 1000
Septa for Headspace ND18 Caps			
SU860170, SU860148	Supelco	17 mm septum, silicon blue/PTFE white, 45° shore A, 1.3 mm	100, 1000
SU860171, SU860149	Supelco	17 mm septum, silicon white/PTFE red, 45° shore A, 1.3 mm	100, 1000
SU860173, SU860151	Supelco	17 mm septum, butyl red/PTFE grey, 55° shore A, 1.6 mm	100, 1000
SU860172, SU860150	Supelco	17.5 mm septum, silicon white/PTFE blue, 55° shore A, 1.5 mm	100, 1000
Headspace Caps			
SU860174, SU860152	Supelco	UltraClean™: 18 mm magnetic screw cap, silver closed cap, silicon white/PTFE red, 45° shore A, 1.3 mm	100, 1000
SU860175, SU860153	Supelco	18 mm magnetic cap, silver closed cap, butyl red/PTFE grey, 55° shore A, 1.6 mm	100, 1000
Caps			
SU860176, SU860154	Supelco	MS-Cap, short thread cap, 9 mm, white, single-component polymer	100, 1000
SU860186, SU860164	Supelco	8 mm Polyethylene push-on cap, easy-to-pierce	100, 1000
SU860187, SU860165	Supelco	11 mm Polyethylene push-on cap, easy-to-pierce	100, 1000
SU860180, SU860158	Supelco	11 mm Polyethylene snap cap, blue hole cap, red rubber/PTFE beige, 45° shore A, 1.0 mm	100, 1000
ND 9 caps			
SU860177, SU860155	Supelco	9 mm UltraBond™ polypropylene short thread screw cap, blue hole cap, septum silicon white/PTFE beige, 45° shore A, 1.3 mm	100,1000
SU860178, SU860156	Supelco	9 mm UltraBond™ polypropylene short thread screw cap, blue hole cap, septum silicon beige/PTFE white, 45° shore A, 1.3 mm, with slit	100,1000
SU860190, SU860168	Supelco	9 mm UltraClean™ polypropylene short thread cap, blue closed cap, silicon white/PTFE red, 55° shore A, 1.0 mm	100,1000
SU860179, SU860157	Supelco	9 mm Polypropylene short thread cap, blue hole cap, red rubber/PTFE beige, 45° shore A, 1.0 mm	100,1000
PP vials for Ion Chromatography			
SU860181, SU860159	Supelco	1.5 mL Polypropylene short thread vial, 32 x 11.6 mm, clear polypropylene, graduated	100, 1000
SU860182, SU860160	Supelco	1.5 mL Polypropylene short thread vial, 32 x 11.6 mm, amber polypropylene, graduated	100, 1000
SU860183, SU860161	Supelco	0.7 mL Polypropylene short thread micro vial, 32 x 11.6 mm, clear polypropylene	100, 1000
SU860184, SU860162	Supelco	0.7 mL Polypropylene snap ring micro vial, 32 x 11.6 mm, clear polypropylene	100, 1000
Insert & Microvials			
SU860191, SU860179	Supelco	0.2 mL micro insert, 30.75 x 5 mm, clear glass, flat bottom	100, 1000
SU860185, SU860163	Supelco	0.4 mL crimp type microvial, 30 x 7 mm, amber glass, 10 mm tip	100, 1000

Pseudomonas Media and Tests

Detection, identification, differentiation and cultivation of *Pseudomonas* species

Figure 1 HiFluoro *Pseudomonas* Agar under UV light



Pseudomonas are motile (one or more polar flagella), rod shaped and aerobic Gram-negative bacteria. They are found almost everywhere, in soil, water, plants and animals. In most cases it is not pathogenic and in fact can be beneficial. For example, *P. putida* is used as a bio-scrubber to aid in the biodegradation of diverse organic compounds in polluted air and waste water. However, *P. aeruginosa* is an infamous opportunistic human pathogen most commonly affecting immunocompromised patients. Along with *P. maltophilia*, it accounts for the majority of human infections. Pathogenic *Pseudomonas* are found throughout the body, most commonly in the urinary tract, respiratory tract, blood and wounds [1].

Rugged and opportunistic, *Pseudomonas* use a wide range of nutritional sources, even very simple nutritional environments without any organic compounds. They can remain viable for long periods of time in many different habitats and under very adverse conditions. They are also widespread, being found in water, saline solutions, utensils and even in cosmetics, pharmaceuticals and disinfectants, and many natural and manufactured foods. Psychrotrophic (cold-tolerant) *Pseudomonas* species are a significant food spoilage problem in refrigerated meat, fish, shell fish and dairy products. Because *Pseudomonas* thrive in water systems, they can be the source of contamination in the food and beverage industry [2].

Pseudomonas are not generally fastidious microorganisms. They can grow on very simple media like Kind Agar, for example, which contains a protein hydrolysate, magnesium chloride, potassium sulphate and agar. Analytical microbiology leverages a microbe's unique biochemistry to aid in its identification. For example, selective *Pseudomonas* media use cetrimide, nalidixic acid, cephaloridine, penicillin G, pimaricin,

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malachite green and other inhibitory agents. The proteolytic activity, lipolytic activity, fluorescent pigment formation, nitrate utilisation, glutamate utilisation, hemolytic reaction and other biochemical reactions are used in the media for the identification and differentiation of *Pseudomonas* species.

Pseudomonas gives negative Voges Proskauer, indole and methyl red tests, but a positive catalase test. While some species show a negative reaction in the oxidase test, most species, including *P. fluorescens*, give a positive result (see **Figure 2**). Another feature associated with *Pseudomonas* is the secretion of pyoverdinin (fluorescein, a siderophore), a fluorescent yellow-green pigment under iron-limiting conditions [3]. Certain *Pseudomonas* species may also produce additional pigments, such as pyocyanin (blue pigment, a siderophore) by *P. aeruginosa* [4], quinolobactin (yellow, dark green in presence of iron, a siderophore) by *P. fluorescens* [5], a reddish pigment called pyorubin and pyomelanin (brown pigment). On blood agar a hemolytic reaction can be observed.

Figure 2 Oxidase test



Scientific classification of *Pseudomonas*:

Kingdom: Bacteria	Order: Pseudomonadales
Phylum: Proteobacteria	Family: Pseudomonadaceae
Class: Gamma Proteobacteria	Genus: <i>Pseudomonas</i>

Pseudomonas utilizes sugars as an energy source by using the Entner-Doudoroff pathway with pyruvate as the end product (dissimilation). The reaction utilizes a different set of enzymes from those used in glycolysis and the pentose phosphate pathway. Fermentation catabolism is not observed in *Pseudomonas*, but some species, like *P. aeruginosa*, *P. stutzeri* and *P. denitrificans*, are able to use nitrate as an electron acceptor instead of oxygen. Growth can also occur under anaerobic conditions when the denitrification pathway is used.

Sigma-Aldrich supplies a wide array of products for the detection, identification, differentiation, enumeration and cultivation of *Pseudomonas*, using its biochemical characteristics, including Gram staining kit, and many types of selective growth media (**Table 1**) and diagnostic tests (**Table 2**). Additional information on media and tests for *Pseudomonas* and a wide range of other microbes can be found on our web site: www.sigmaaldrich.com/microbiology.

Table 1 Media for *Pseudomonas*

Cat. No.	Brand	Nonselective Broths	Cat. No.	Brand	Nonselective Agars for Cultivation, Enumeration and Isolation
A0465	Sigma	Alternative Thioglycollate Medium	70147	Fluka	Milk Agar
53286	Fluka	Brain Heart Broth	70148	Fluka	Nutrient Agar
B5051	Sigma	Bushnell Haas Broth	44776	Fluka	Nutrient Agar Plates (Diameter 55 mm)
D3435	Sigma	Dey-Engley Neutralizing Broth	80957	Fluka	Plate Count Skim Milk Agar
63649	Fluka	Membrane filter Rinse Fluid (USP)	17209	Fluka	R-2A Agar
70149	Fluka	Nutrient Broth No 3	17175	Fluka	Skim Milk Agar, modified
03856	Fluka	Nutrient Broth No. 4	51414	Fluka	Tryptic Soya Agar with Polysorbate 80 and Lecithin
70179	Fluka	Peptone Water	70159	Fluka	Tryptone Glucose Extract Agar
77187	Fluka	Peptone Water, phosphate-buffered	T2188	Sigma	Tryptone Glucose Yeast Extract Agar
40893	Fluka	Peptone Water, phosphate-buffered, Vegitone	01497	Fluka	Yeast Extract Agar
70157	Fluka	Thioglycollate Broth (USP Alternative)	Cat. No.	Brand	Nonselective Agars for Differentiation
41960	Fluka	Vegitone Infusion Broth	70133	Fluka	Blood Agar (Base)
Cat. No.	Brand	Selective Enrichment Broths & Biochemical Identification Broths	21065	Fluka	Calcium caseinate Agar
00185	Fluka	Acetamide Nutrient Broth	55420	Fluka	CLED Agar
17129	Fluka	Asparagine Proline Broth	16636	Fluka	HiCrome™ UTI Agar, modified
78886	Fluka	Cetrimide Broth	60788	Fluka	King Agar A
63163	Fluka	Malachite Green Broth	60786	Fluka	King Agar B
39484	Fluka	Methyl Red Voges Proskauer Broth	75315	Fluka	OF Test Nutrient Agar
14305	Fluka	Motility Nitrate Medium	P1852	Sigma	<i>Pseudomonas</i> Agar (for Fluorescein)
72548	Fluka	Nitrate Broth	91015	Fluka	Tributyrin Agar
Cat. No.	Brand	Selective Agars for Detection and Isolation			
11012	Fluka	Cetrimide Nalidixic acid Agar			
22470	Fluka	Cetrimide Agar			
70887	Fluka	Cetrimide Agar			
P2102	Sigma	<i>Pseudomonas</i> Agar Base			
14521	Fluka	Cetrimide Agar Plates (Diameter 55 mm)			
17168	Fluka	Milk Agar, modified according to Brown & Scott			
17208	Fluka	<i>Pseudomonas</i> Isolation Agar			
Cat. No.	Brand	Selective Agars with Differential System for Differentiation, Detection and Isolation			
50875	Fluka	GSP Agar			
78996	Fluka	HiFluoro™ <i>Pseudomonas</i> Agar Base			

Table 2 Test for identification and differentiation of *Pseudomonas*

Cat. No.	Brand	Diagnostic Tests for <i>Pseudomonas</i>
88597	Fluka	Catalase Test
05686	Fluka	DMACA Indole Disks
49825	Fluka	DMACA Reagent
78719	Fluka	Kovac's Reagent Strips
60983	Fluka	Kovac's Reagent for indoles
67309	Fluka	Kovac's Reagent for indoles
08714	Fluka	Methyl Red Solution
70439	Fluka	Oxidase Test
40560	Fluka	Oxidase Strips
07345	Fluka	Oxidase Reagent acc. Gaby-Hadley A
07817	Fluka	Oxidase Reagent acc. Gaby-Hadley B
18502	Fluka	Oxidase Reagent acc. Gordon-McLeod

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Yersinia Diagnostic

Detection, identification, differentiation and cultivation of Yersinia species.

Yersinia are rod-shaped, Gram-negative facultative anaerobic bacteria. They exhibit fermentative metabolism and are oxidase-negative, mannitol-positive. A psychrophilic organism, *Yersinia* survives and proliferates at low temperatures of 0–4°C, for example on food products in a refrigerator. Some *Yersinia* species are also relatively heat resistant. Pigs, rodents, rabbits, sheep, cattle, horses, dogs, and cats are the natural sources of *Yersinia*. Currently, *Y. enterocolitica* is responsible for most cases of human illness caused by *Yersinia*.

Other clinically important species of this genus are *Y. pseudotuberculosis* and *Y. pestis*, the infectious agent of bubonic plague. Most infections are acquired from contaminated food, like raw or undercooked pork products, sea food, vegetables, unpasteurized milk or untreated water. However, infections also occur from contact with infected animals, faeces or transmission by fleas.

Sigma-Aldrich offers a wide range of selective and non-selective media (Table 1) and diagnostic tests (Table 2) for growth and identification of *Yersinia*.

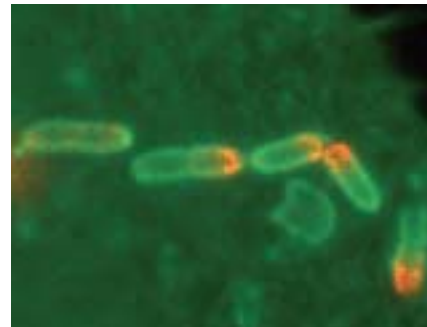
Table 1 Media for *Yersinia*

Cat. No.	Brand	Nonselective Broths
53286	Fluka	Brain Heart Broth
40893	Fluka	Peptone Water, phosphate-buffered, Vegitone
77187	Fluka	Peptone Water, phosphate-buffered
Cat. No.	Brand	Selective Enrichment Broths
17156	Fluka	ITC Broth (Base)
69965	Fluka	Mossel Broth
17192	Fluka	Peptone Sorbitol Bile Broth
Cat. No.	Brand	Basal Media for Carbohydrates Utilization
A0715	Sigma	Andrade Peptone Water
28943	Fluka	Andrade Peptone Water, Vegitone
Cat. No.	Brand	Medium for Autoagglutination
39484	Fluka	Methyl Red Voges Proskauer Broth
Cat. No.	Brand	Nonselective Agars for Differentiation & Confirmation
27048	Fluka	Christensen's Urea Agar
60787	Fluka	Kligler Agar
75315	Fluka	OF Test Nutrient Agar
05386	Sigma	Ornithine Decarboxylase Broth
Cat. No.	Brand	Selective Agars for Detection and Isolation
95760	Fluka	Yersinia Selective Agar (CIN Agar)
Cat. No.	Brand	Selective Agars with Differential System for Differentiation, Detection and Isolation
70135	Fluka	DCLS Agar
90035	Fluka	DCLS Agar No. 2
17213	Fluka	Violet Red Bile Glucose Agar without Lactose
53605	Fluka	Violet Red Bile Glucose Agar without Lactose, Vegitone
95273	Fluka	VRB MUG Agar

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Figure 1 Image of *Yersinia* bacteria.

Yersinia pseudotuberculosis entering a cultured mammalian cell. M. Tang, R.R. Isberg, Howard Hughes Medical Institute, Tufts University School of Medicine, Boston



Scientific classification of *Yersinia*:

Kingdom: Bacteria	Order: Enterobacteriales
Phylum: Proteobacteria	Family: Enterobacteriaceae
Class: Gamma Proteobacteria	Genus: Yersinia

Y. enterocolitica: catalase-positive, most strains are ornithine-positive, motile at 22–26°C, urea-positive, sorbitol- and cellobiose se-positive, most strains are sucrose-positive

Y. pseudotuberculosis: motile at 22–26°C, urea-positive, rhamnose-positive, esculin-positive

Y. pestis: produces two antiphagocytic components (antiphagocytic slime), non-motile at 22–26°C, esculin-positive, responsible organism for the bubonic plague

Table 2 Test for identification and differentiation of *Yersinia*

Cat. No.	Brand	Diagnostic Tests for Yersinia
80507	Fluka	Bile Esculin Disks
88597	Fluka	Catalase Test
56481	Fluka	Cellobiose Disks
94438	Fluka	Mannitol Disks
08714	Fluka	Methyl Red Solution
07345	Fluka	Oxidase Reagent acc. Gaby-Hadley A
07817	Fluka	Oxidase Reagent acc. Gaby-Hadley B
18502	Fluka	Oxidase Reagent acc. Gordon-McLeod
40560	Fluka	Oxidase Strips
70439	Fluka	Oxidase Test
93999	Fluka	Rhamnose Disks
92971	Fluka	Salicin Disks
93998	Fluka	Sorbitol Disks
94309	Fluka	Sucrose Disks

More details about the media, tests can be found on our website: www.sigma-aldrich.com/microbiology.

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Ultra-pure MALDI Matrices

Enabling high spectral quality

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Image kindly provided by Peter Ehmann, e² Werbeagentur

MALDI (matrix-assisted laser desorption/ionization) expands the application of mass spectrometry into the analysis of high molecular weight, non-volatile and thermally labile compounds, such as intact proteins and oligonucleotides. Moreover, it has become an important technique in proteomics research. MALDI requires relatively little sample preparation and is more amenable to topological imaging compared to other forms of MS ionization.

The MALDI technique involves mixing the sample with a matrix substance followed by crystallization by different techniques on the MALDI sample plate. The crystallized sample-matrix mixture is irradiated by laser light, usually UV. As the matrix absorbs the light energy, it evaporates into the gas phase, resulting in an indirect ionization of the sample molecules. Although there have been numerous experiments to simplify MALDI by eliminating the sample-matrix mixing step, like direct MS from solid supports or 2D-gels, using a matrix substance (chemical) is still the most common approach.

Quality of MALDI matrices

A typical MALDI matrix substance is an aromatic acid with a chromophore that absorbs strongly at the wavelength of the incident laser. Choosing a suitable matrix of high quality is the key to the success of a MALDI-MS experiment. Organic impurities can lead to extraneous peaks, especially in the low mass range. Trace levels of ions, especially Na⁺ and K⁺, form adducts with sample molecules. These adducts differ in mass according to the number of positive ions and complicate the MS spectrum. Because of the strict purity requirements for both organic impurities and inorganic ions, many MALDI users have been forced to recrystallize commercially available, but impure, matrix substances.

Ultra-pure MALDI matrix substances

The ever-increasing sensitivity of MALDI-MS instruments and the trend of decreasing sample quantities (e.g. excised spots from 2D-gels in proteomics) requires high purity and high quality MALDI substances, in particular the MALDI matrices. Because of the success of our current offering, we recently developed a process to manufacture MALDI matrix substances with even higher purity, superior to anything that was technically feasible previously.

We are now pleased to offer an ultra-pure line that includes the most commonly-used matrix substances (see Table 1). The strict, application-based specifications for the new, ultra-pure MALDI matrix substances include:

- Purity ≥99.5%
- Large set of trace impurity specifications, typically below 1 mg/kg (<1 ppm)
- Appearance and solubility requirements

Figure 1 Ultra-pure MALDI Matrix, 10 X 10 mg package size



Fluka MALDI matrix substances are extensively purified to meet these specifications and provide sufficient quality to meet the most demanding applications.

(continued on page 18)

Excellent solubility and performance of ultra-pure MALDI matrix substances

One of the most important aspects of the ultra-pure MALDI matrix substances from Sigma-Aldrich is their ability to dissolve rapidly and completely; a brief vortex mixing is typically sufficient. Actual performance of the ultra-pure MALDI substances *in situ* is demonstrated in the accompanying figures with a test mixture of five peptide standards from Sigma's ProteoMass™ line (Table 2). MALDI-MS experiments were performed on a Shimadzu Kratos Axima CFR in Reflectron mode.

The comparison of MALDI mass spectra using the ultra-pure grade HCCA versus standard grade is shown in Figure 2. Note that the use of the ultra-pure HCCA matrix resulted in significantly higher peak resolution. Even when the peptide standard sample was diluted 10-fold (Figure 3), the use of ultra-pure HCCA provides a clear spectrum with resolution (Figure 3a) comparable to the more concentrated sample. In contrast, the standard quality HCCA did not yield a suitable spectrum (Figure 3b).

Table 1 Ultra-pure MALDI Matrices from Sigma-Aldrich

Cat. No.	Brand	Description	Package Size
39468	Fluka	α -Cyano-4-hydroxycinnamic acid (HCCA) puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 x 10 mg
39319	Fluka	2,5-Dihydroxybenzoic acid (Gentisic acid) puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 x 10 mg
49508	Fluka	Sinapic acid puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 x 10 mg
06788	Fluka	2-(4-Hydroxyphenylazo)benzoic acid puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 X 10 mg
69612	Fluka	Succinic acid, puriss. p.a., $\geq 99.5\%$ (T) ultra-pure	10 x 10 mg
41711	Fluka	2',4',6'-Trihydroxyacetophenone monohydrate puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 x 10 mg
05757	Fluka	2',6'-Dihydroxyacetophenone puriss. p.a., $\geq 99.5\%$ (HPLC), ultra-pure	10 x 10 mg

Figure 2 Comparison of MALDI-MS spectra provided by ultra-pure and regular grade HCCA

Figure 2a HCCA Ultra-pure grade (Cat. No. 39468)

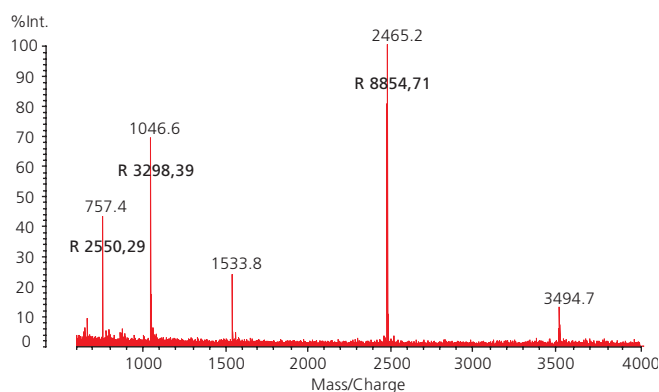
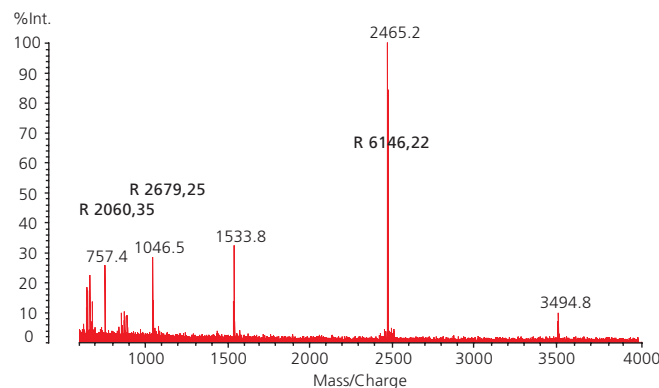


Figure 2b HCCA regular grade



Sample: 0.7 μ L ProteoMass™ peptide mix (Bradykinin (1.5 μ M), Angiotensin II (1.0 μ M), P14R (0.5 μ M), ACTH (1.0 μ M), Insulin B-chain (2.0 μ M), mixed with 0.7 μ L HCCA matrix in AcN/0.1%TFA

MALDI-MS: Shimadzu Kratos Axima CFR, Reflectron mode, laser power 85%

Figure 3 Comparison of MALDI-MS spectra provided by ultra-pure and regular grade HCCA: 10-fold diluted sample

Figure 3a HCCA Ultra-pure grade (Cat. No. 39468)

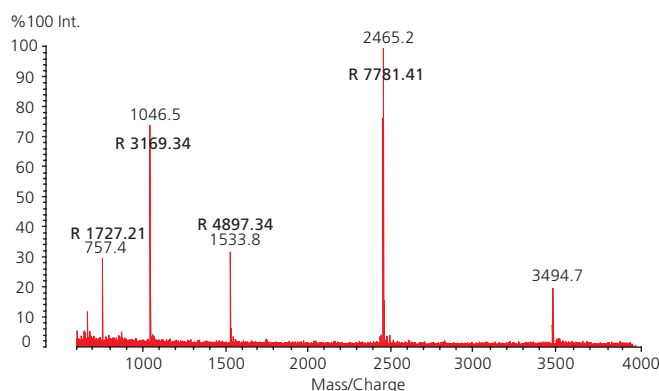
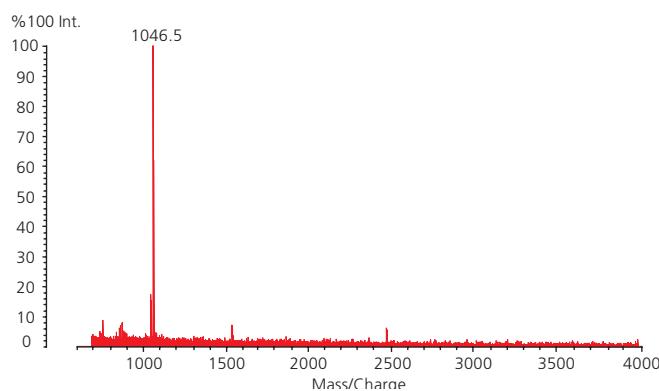


Figure 3b HCCA regular grade



Sample: 0.7 μ L ProteoMass™ peptide mix (Bradykinin (150 nM), Angiotensin II (100 nM), P14R (50 nM), ACTH (100 nM), Insulin B-chain (200 nM)), mixed with 0.7 μ L HCCA matrix in AcN/0.1%TFA

MALDI-MS: Shimadzu Kratos Axima CFR, Reflectron mode, laser power 85%

The perfect MALDI companion: High sensitivity LUCY™ fluorescent dyes

The excellent performance of the ultra-pure MALDI matrix substances toward dilute samples or very small sample volumes, where high sensitivity is required, is of great benefit when analyzing peptides from enzymatic digests separated by 1D- or 2D-gels, as in proteomics experiments. In this case, however, not only must the MALDI matrix be of high purity, but the dyes used to stain the gels prior to excising the spots must allow the visualization of low abundance proteins.

Newly launched LUCY™ fluorescent dyes from Sigma-Aldrich meet this requirement. **Figure 4** shows a typical example. A 100 ng band of β -galactosidase was excised from a 1D-gel which was stained with Lucy-506. After trypsin digestion and peptide extraction using the Trypsin Profile IGD Kit (Cat. No. PP0100), MALDI-MS was performed by mixing peptides with ultra-pure HCCA matrix.

The protein could be identified by database analysis and peptide mass finger print. The resulting MALDI mass spectrum is shown in **Figure 4**.

Conclusion

Sigma-Aldrich offers a complete line of products that meet the sensitivity demands of modern MS analysis, including MALDI-MS. Recently introduced, ultra-pure MALDI matrix substances contain vanishingly low levels of both organic impurities and inorganic ions. A perfect companion, LUCY™ fluorescent dyes improve visualization of peptides in 1D- and 2D-gels compared to competitive dyes. Additionally, the ProteoMass line of peptide standards is ideal for troubleshooting and calibrating sensitive MALDI-MS instruments.

For a complete listing of our MALDI matrix substances and related calibration products, please visit our web site: sigma-aldrich.com/spectroscopy.

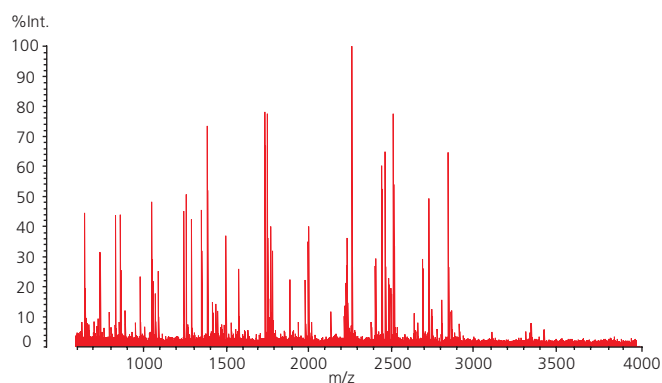
Table 2 ProteoMass™ MALDI-MS Peptide Standards

Cat. No.	Brand	Description	Package Size
P2613	Sigma	ProteoMass™ P14R MALDI-MS Standard (Pro14-Arg) Monoisotopic mol wt 1,532.8582 Da by calculation	5 x 10 nmol
B4181	Sigma	ProteoMass™ Bradykinin Fragment 1-7 MALDI-MS Standard Monoisotopic mol wt 756.3997 Da by calculation	5 x 10 nmol
A8846	Sigma	ProteoMass™ Angiotensin II MALDI-MS Standard Monoisotopic mol wt 1,045.5423 Da by calculation	5 x 10 nmol
A8346	Sigma	ProteoMass™ ACTH Fragment 18-39 MALDI-MS Standard Monoisotopic mol wt 2,464.1989 Da by calculation	5 x 10 nmol
I6154	Sigma	ProteoMass™ Insulin chain B oxidized MALDI-MS Standard Monoisotopic mol wt 3,493.6513 Da by calculation	5 x 10 nmol

Table 3 LUCY™ Fluorescent Dyes

Cat. No.	Brand	Description	Package Size
14149	Fluka	LUCY-506, 5mg / mL in DMSO, 5000 x Stock solution	500 μ L
43772	Fluka	LUCY-565, 5mg / mL in DMSO, 5000 x Stock solution	500 μ L
41629	Fluka	LUCY-569, 5mg / mL in DMSO, 5000 x Stock solution	500 μ L
04297	Sigma	LUCY-Starter Kit (contains all 3 Lucy-dyes)	1EA

Figure 4 Ultra-pure HCCA MALDI-MS spectrum of β -galactosidase digest, excised 1D-gel band following Lucy-506 staining



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Titration Reagents, Solutions and Indicators

An overview of the evolving Sigma-Aldrich portfolio

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Almost every analytical laboratory carries out titrations of some sort, even in this age of highly complex technical instrumentation and trace level analysis. Often, whole manufacturing processes depend on the accuracy of titration results and, hence, on the quality of the titration reagents.

Because analysts depend on the quality of the titration reagents, manufacturers of the reagents must adhere to strict quality criteria. High-quality titration products from Sigma-Aldrich include:

- Ready-to-use volumetric solutions of acids, bases, buffers, salts and complexing agents in different pack sizes, including 5 or 10 L VOLPAC®-containers
- FIXANAL® concentrates for preparation of volumetric solutions
- IDRANAL® reagents for complexometric titrations
- Masking agents, indicators and pH indicator paper and sticks

Certified Volumetric Solutions: Titer determination with primary standards

Assuring the traceability of reagents is an elaborate and often difficult process. In order to help our analytical customers with this laborious task, we offer accurate and traceable volumetric solutions tested against EMPA, BAM and/or NIST standard reference materials. These standard reference materials are primary or titrimetric standards substances with high purity, low reactivity,

Table 1 Major international organizations for reference materials, accreditation and certification

- BAM German Federal Institute for Materials Research and Testing
- EMPA Swiss Federal Laboratories for Materials Testing and Research
- NIST National Institute of Standards and Technology, USA

low hygroscopicity, high solubility and high equivalent weight. Our complete offering can be found on our web site: www.sigmaaldrich.com/titration.

- Throughout their production and quality control, our volumetric solutions are handled with extreme care. The reliability of the test equipment and the quality of the products are continuously monitored by means of a system of in-process controls and final checks.
- FIXANAL® concentrates are packaged with the aid of automated filling lines. For example, to produce a 0.1 mol/L hydrochloric acid solution, a 37% hydrochloric acid is diluted using purified, demineralized water to a titer (factor) of 1.000 ± 0.001 .
- For analysis, we use different titration instruments to adjust the titer against a certified reference material. Whenever possible, we use an EMPA/BAM certified reference material that is regularly compared with a NIST standard reference material during the certification process. This guarantees that the reference material is tested and certified with the know-how of several different institutions with established, worldwide reputations.
- The material that was actually used for the determination of the titer is listed on the certificate of analysis for each lot. A lot is approved only if two analysts have found matching results using different analytical equipment for the measurement of at least two triplicate determinations.

FIXANAL®: Packaged by quantity of analyte, not concentration

The measured titer (factor) of our ready-to-use solutions is displayed on the certificate of analysis. However, for FIXANAL® concentrates we take the time to adjust the titer to 1.000 (to a precision of $\pm 0.1\%$) during the filling process. With the titer so determined, we then calculate the weight of the volume of liquid corresponding to exactly 0.1 mol of the specified analyte. Using high-precision filling equipment, this exact volume of liquid is added to every ampul in the lot. The concentration of the liquid inside each FIXANAL® ampul may vary slightly from lot to lot, but the amount of the specified material in each ampul is exactly the same, for example 0.1 mol HCl (not mol/L).

By diluting the entire contents of the ampul of FIXANAL® concentrate to the desired volume, a solution can be prepared to meet your specific concentration requirement, knowing for certain that it has been tested against a certified reference material.

FIXANAL® concentrates

FIXANAL® ampules are subject to strict production and quality control specifications, both in-process and on the finished product. This extensive testing provides a high degree of reliability for the specified titer. Additionally, the special ampul-sealing process means the titer of FIXANAL® ampules does not change and is guaranteed to be accurate within the specified shelf life. For convenience, the ampules

are supplied with self-adhesive labels that can be easily attached to the storage vessel by the end user.

The ampuls themselves are made of high-resistance polyethylene (PE) and have an integral rinsing funnel, which is used to perforate the diaphragm after twisting the ampul. An integrated deflector accelerates the complete rinsing of the concentrate from the ampul. Depending on the contents, FIXANAL® ampuls may be black-pigmented PE or glass. Glass ampuls do not have the twist-opening mechanism.

Today, the range of FIXANAL® ampuls encompasses concentrates for:

- Reagent solutions
- Buffer solutions
- Complexometric solutions

The majority of Sigma-Aldrich's titration products are offered as both ready-to-use solutions and FIXANAL® concentrates. The increasing automation and the escalating requirements of quality monitoring systems have created a need for ready-to-use solutions for many applications. The choice of FIXANAL® or ready-to-use solution depends on your analytical requirements.

Ready-to-use solutions

- Contain exact amount in terms of concentration (e.g. 1 mol/L)
- Titer precision $1.000 \pm 0.1\%$
- Readily available, no preparation required
- Also available in 5 and 10 L VOLPAC® containers

FIXANAL® concentrates

- Contain exact amount of substance (e.g. 1 mol)
- Titer precision $1.000 \pm 0.2\%$
- Economical and space-saving
- Final concentration is user-specified

Apart from the customary package sizes up to 5 L, the Sigma-Aldrich portfolio also includes special 5 and 10 L VOLPAC® containers when larger quantities of ready-to-use solutions are needed. VOLPAC® containers consist of a rugged cardboard cube containing a flexible inner polyethylene bag with an outlet tap. As the solution is withdrawn, the polyethylene bag collapses, preventing influx and contamination by laboratory air. Also, because there is no room for volume expansion, the evaporation and condensation of water that increase the error of the titer is prevented.

Table 2 Selected FIXANAL® concentrates for preparation of volumetric solutions

Cat. No.	Brand	Description	Package Size
38282	Riedel-de Haën	FIXANAL® hydrochloric acid standard solution	1.0 mol (36.461 g HCl)
38294	Riedel-de Haën	FIXANAL® sulfuric acid standard solution	0.5 mol (49.039 g H ₂ SO ₄)
38224	Riedel-de Haën	FIXANAL® sodium hydroxide standard solution	0.2 mol (7.999 g NaOH)
38090	Riedel-de Haën	FIXANAL® potassium bromide solution	0.1 mol (11.901 g KBr)

Buffers

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

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

Accuracy of Sigma-Aldrich buffer solutions at 20°C is as follows:

- Buffer solutions with pH values 1-9: ± 0.02
- Buffer solutions with pH values 10-13: ± 0.05

Buffers from pH 2 to 7 contain a fungicide additive. For precision measurements and to calibrate pH meters, standard buffer solutions according to DIN 19266 and color-coded buffer solutions for the pH values 4.00 (red), 7.00 (green), 9.00 (blue) and 10.00 (violet) are available.

Table 3 Buffer solutions

- pH 1.00 – 13.00
- Color-coded buffers
- High-precision buffers according to DIN 19 266
- Ready-to-use solutions, tablets, FIXANAL® concentrates

Table 4 Selected Sigma-Aldrich buffer solutions

Cat. No.	Brand	Description	Package Size
33665	Riedel-de Haën	Buffer solution pH 4.0 (20 °C), red colored, with fungicide	500 mL, 1 L, 5 L, 10 L
82574	Fluka	Buffer solution pH 9.0 (20 °C)	50 mL, 1 L
82558	Fluka	Buffer standard solution according to DIN 19266 pH 7.413 \pm 0.010 (25 °C)	500 mL

IDRANAL® reagents for complexometric titration

We offer a selected range of aminopolycarboxylic acids (EDTA analogs) as reagents for complexometric titration under the IDRANAL® trademark. In complexometric titration, metals in the sample react with complexing agents (chelating agents) to create complexes. Typically, a color change determines the end point of the titration. Analytical applications include the determination of water hardness and special titrimetric metal determinations.

(continued on page 22)

Sigma-Aldrich's complexometric IDRANAL® reagents are stable at high temperatures, readily soluble in water, and form stable chelates with most metal ions over a wide range of pH and temperature. They show hydrolytic stability in both acidic and alkaline conditions.

IDRANAL® reagents are available as solids, ready-to-use solutions and FIXANAL® concentrates. In addition, we offer buffer solutions, masking agents and indicators specially designed for complexometric titration.

Special complexometric FIXANAL® concentrates are available for preparation of DEV-volumetric solutions (German standard methods for water, sewage and slurry analysis) for foundry laboratories and titration in non-aqueous solutions to determine water hardness.

pH papers and sticks

The color-changing reactions of pH papers are based on the same reaction mechanisms as the titration indicators. pH papers are absorptive cellulose papers that have been saturated with acid or alkaline indicators. Sigma-Aldrich supplies two types of pH papers: simple indicator papers, such as litmus paper, that determine whether a solution is acidic or alkaline, and pH papers saturated with indicator mixtures that differentiate between pH values. This latter group includes the universal indicator papers and the highly accurate PEHANAL® and PANPEHA® papers.

Table 5 Selected IDRANAL Ready-to-use solutions

Cat. No.	Brand	Description	Package Size
34543	Riedel-de Haën	IDRANAL® 100, IDRANAL® III solution, for water hardness determination (1 mL = 1 German degree of hardness in 100 mL of water)	1 L
34547	Riedel-de Haën	IDRANAL® A solution, IDRANAL® III solution with zinc complex added, for water hardness determination (1 mL = 5.6 German degree of hardness in 100 mL of water)	1 L
34544	Riedel-de Haën	IDRANAL® B, IDRANAL® III solution with zinc complex added, for water hardness determination (1 mL = 1 German degree of hardness in 100 mL of water)	500 mL, 1 L, 5 L, 10 L
34542	Riedel-de Haën	IDRANAL® C solution, IDRANAL® III solution with zinc complex added, for water hardness determination with measuring tube H DIN 12812 (3.73 mL = 20 German degree of hardness in 40 mL of water)	1 L
35322	Riedel-de Haën	IDRANAL® III standard solution, 0.01 M	1 L
34550	Riedel-de Haën	IDRANAL® III standard solution, Reag. Ph. Eur., 0.1 M	500 mL, 1 L, 5 L, 10 L
35102	Riedel-de Haën	IDRANAL® III standard solution, 0.2 M	1 L, 5 L, 10 L
35103	Riedel-de Haën	IDRANAL® IV standard solution, Reag. Ph. Eur., 0.1 M	1 L, 5 L, 10 L

Indicators

Indicators are used primarily in volumetric titrations. They typically are organic compounds that change color in proportion to the concentration of hydrogen ions in the solution. Indicators do not change color precisely at one pH value. Instead, over a specific pH range the color of the indicator continuously changes. The range is different for each indicator. The transition point of an indicator is defined as the point at which the acid and alkaline forms of the indicator exist in equal concentrations.

For titration, Sigma-Aldrich offers indicators as solids, dye solutions and as indicator papers covering different transition ranges.

Sigma-Aldrich indicator papers are supplied in several different forms (see table below). In addition to the test strips, diverse pH papers are also available by the roll. Another specialty are the universal and specific indicator test sticks, in which the color comparison zones are applied to special plastic sticks that have a very low tendency to bleed.

Information about our line of titration products and complete product listing can be found by visiting our web site: www.sigma-aldrich.com/titration.

Table 6 Comparison of Sigma-Aldrich pH-indicating papers and sticks

	Universal Indicator Paper	Specific Indicator Paper	PEHANAL	PANPEHA
Supplied form	Roll and stick	Roll and stick	Roll	Roll
Indication surfaces	1 (roll), 4 (stick)	1	3	8
Range	0–14	Diverse	1–11	0–14
Gradation	1	0.2–0.5	1	0.5

New Product Corner

Ionophore for Quick and Reliable Determination of Benzoate

Michael Jeitziner, Market Segment Manager analytical Reagents & Standards michael.jeitziner@sial.com

Introduction

Benzoic acid and its salts (E210 benzoic acid, E211 sodium salt, E212 potassium salt, E213 calcium salt) are widely used as preservatives in various kinds of food. The methods which are most frequently used for the benzoate determination in food samples are chromatography and spectrophotometry. The direct determination of benzoate in food samples is a fast, reliable and cost-efficient alternative.

Fluka 17384 - Benzoate ionophore I, Selectophore® function tested

Figure Benzoate ionophore I, Selectophore®

N,N',N'',N''',N''',N''''-Hexakisdecyl[dibenzo[klm,z(aa)(ab)]-1,5,9,16,20,24-hexaazacyclotriacontane-1,5,9,16,20,24-hexayl]hexaacetamide, CAS# 227092-22-0

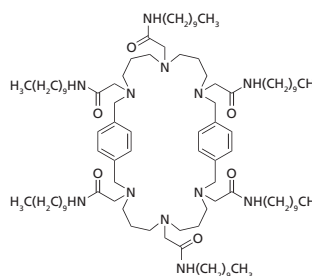
Characteristics

Slope of linear regression:

-45mV/dec (without additive)

-105mV/dec (with 0.18% TDDMACI). (10-2 M MES buffer pH 5.0)

Response time: <3s



Selectivity Coefficients	$\log K_{H, M}^{Pot}$	as obtained by the matched potential method (MPM):
$\log K_{Benzoate, Acetate}^{Pot}$	-2.1	$\log K_{Benzoate, Citrate}^{Pot}$
$\log K_{Benzoate, Chloride}^{Pot}$	-4.1	$\log K_{Benzoate, Sulfate}^{Pot}$
$\log K_{Benzoate, H_2PO_4}^{Pot}$	<<0,	$\log K_{Benzoate, Nitrate}^{Pot}$
	-1.8	-4.2

Membrane composition: 1.0 wt% Ionophore, (0.18 wt% TDDMACI), 66.0 wt% DOP, 33.0 wt% PVC

Table Reagents used for novel membrane sensor

Cat. No.	Brand	Description	Package Size
17384	Fluka	Benzoate ionophore I	25 mg, 50 mg
91661	Fluka	Tridodecylmethylammonium chloride (TDDMACI)	100 mg, 1 g
80030	Fluka	Bis(2-ethylhexyl) phthalate (DOP)	1 mL, 5 mL, 25 mL
81392	Fluka	Poly(vinyl chloride) high molecular weight (PVC)	1 g, 10 g, 50 g

For further information about ionophores, please have a look at www.sigmaaldrich.com/selectophore

References:

- 1] L. Bulgariu, H. Radecka, M. Pietraszkiewicz, O. Pietraszkiewicz, Anal. Letters 2003, 36, 7, 1325-1334.

Natural Compounds Analytical Standards

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

Sigma-Aldrich is pleased to offer seven new Fluka-brand analytical standards to round out our comprehensive portfolio of natural compounds.

Table Sigma-Aldrich portfolio of natural compounds

Cat. No.	Brand	Description	Package Size
65839	Fluka	Ginsenoside Rf	5 mg
68317	Fluka	Ginsenoside Rg1	5 mg
89556	Fluka	Ginkgolide J	5 mg
75741	Fluka	Ginkgolic acid C15:1	5 mg
55822	Fluka	Ginkgolic acid C17:1	5 mg
68527	Fluka	Harpagoside	5 mg
59761	Fluka	Bisabolol oxide A	10 mg

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