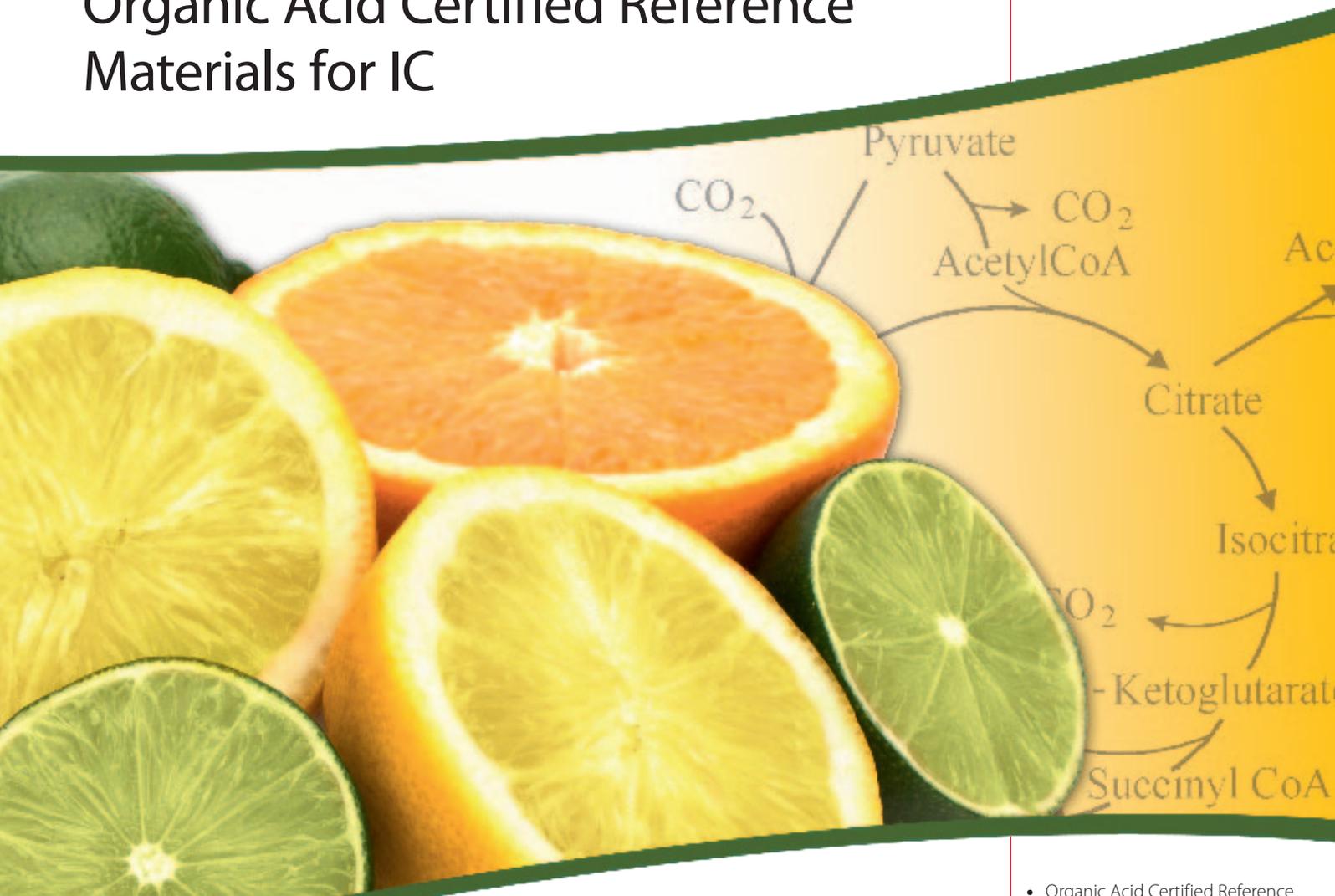


Analytix

Issue 2 • 2012



Organic Acid Certified Reference Materials for IC



- Organic Acid Certified Reference Materials for IC
- Standards for Traditional Indian Herbal Medicine
- Pesticide and Mycotoxin Standards
- Ultra-High Purity Acids
- Detection of Yersinia Species
- LC-MS Blends
- Solvents for Metal Speciation
- Ethanol-based Karl Fischer Reagents

What does our Daily Bread have to do with Reference Materials?



Jürg Wüthrich
Senior Scientist R&D Europe

Dear Reader,

Each of us consumes a wide variety of goods and each purchases many things during his life. But none of this affects us, in the truest sense of the word, more than our daily food. Many of us stock up on a variety of food several times a week. It may be that we purchase it from a local farm, a mom-and-pop grocery store around the corner, or from a global supermarket chain. We might even produce fruits and vegetables in our own garden. However, we are all consumers relying on the food industry and its quality control.

From a biological perspective, we need to maintain all cellular and organ functions by consuming water, carbohydrates, fats, amino acids, proteins, and a number of micronutrients (vitamins, minerals, antioxidants, etc.). But does that tell us anything about a good diet?

My colleague, who is a long-distance triathlete, has a clear opinion about what the "right" food is. In addition to optimal training, nutrition is a key factor to improve athletic performance and achieve good results in a competition. Food is simply the fuel! Of course, not everyone wants to align their daily nutrition to a sport. For decades there has been a controversial debate about a balanced diet or "good" nutrition. This is also reflected very nicely by the varying diet recommendations of government health organizations of the USA, Switzerland and Japan (see pictures below).



Besides the fact that we have to consume important elements, we also want to be sure that our food is safe. Food should not be contaminated with toxins, harmful additives, or food coloring. Some of us prefer organically produced food and certainly don't want genetically modified products on our tables.

But since we consume a meal with all of our senses, food also has to taste good, smell nice, and look attractive. Simply said, the product needs to be of good quality. For some manufacturers, such as internationally operating food companies, it is also important that the product can be manufactured all over the world and still taste the same everywhere. This, however, is only possible if the important ingredients can be measured correctly and when the analytical data are comparable between the different quality control labs.

It is a central task of certified reference materials (CRMs) to ensure the accuracy and comparability of measurement results by providing a reference, which is traceable to the SI base unit. The feature article (page 4) is dedicated to CRM for ion chromatography and describes the most important aspects of certification and production. Many of these new standards are not only important analytes in the food industry but also are used in other application areas as well. We believe that we can achieve a new and improved quality level by realizing the traceability of the starting materials' content using quantitative NMR, which is a primary measurement method.

Remember the next time that you enjoy a tasty cookie or sip a cup of your favorite coffee, this pleasure is partially due to good certified reference materials.

Kind regards,

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

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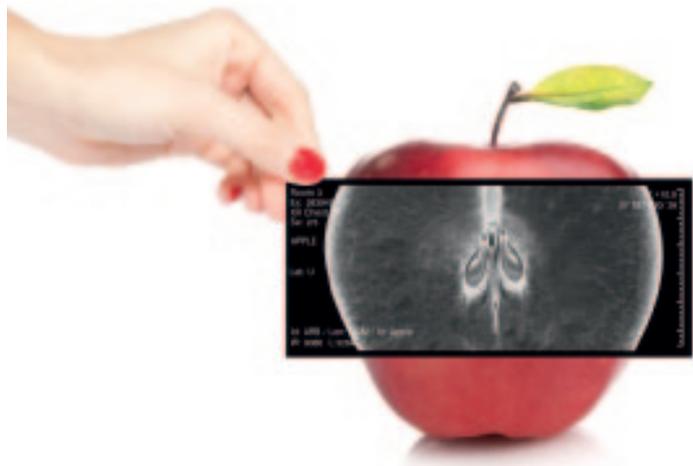
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Get a Closer View with Better Standards

New Organic Acid Standards for Ion Chromatography



Today, ion chromatography (IC) is used in many different industries such as food and beverage, environmental, life sciences, pharmaceutical, power generation, electronics and many more. The huge number of applications includes analytes like inorganic anions and cations, organic acids, carbohydrates, amino acids, proteins, fatty acids, polysaccharides, polyphosphates, surfactants, and more.

Unfortunately IC is not a primary method of measurement and therefore there is an obvious need for certified reference materials (CRMs) for calibration and quality control standards. CRMs play an important role because they serve as reference points that are traceable to another internationally accepted standard, such as a reference material from a metrological

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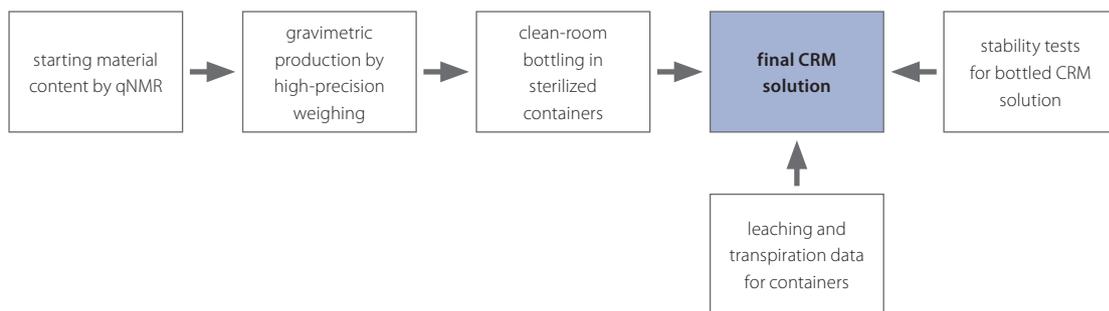
institute or, even better, directly to an SI unit. CRMs should not only be traceable to an accepted reference, but also be of well-defined purity and have a properly calculated measurement uncertainty. Because the precision of measurement results is directly affected by the quality of the CRMs used, the choice of the right CRM producer is crucial. This is, for most scientists, a matter of trust.

This article describes the most important technical aspects of the production and certification of a new group of organic standards for IC under the brand **TraceCERT®**. These CRMs are water-based, single analytes in 1g/L concentrations and cover the most common carboxylic acids.

How to Bring Trust in the Bottle

One cornerstone of the technical and administrative competence of a CRM producer is inspection and accreditation by an independent authority. Sigma-Aldrich in Buchs (Switzerland), where these standards for IC are produced, is accredited by the Swiss Accreditation Service according to ISO Guide 34 (General Requirements for the Competence of Reference Material Producers). We also received accreditation under ISO/IEC 17025 (General Requirements for the Competence of Testing and Calibration Laboratories) for several analytical measurement methods (e.g. gravimetry, qNMR®, ICP-OES, titration, density). The combination of ISO/IEC 17025 and ISO Guide 34 is the highest achievable level of quality assurance and is also called the “Gold Standard” in accreditation for CRM producers.

These accreditation requirements ensure that the CRM production follows a stringent concept and have validated processes. We found the steps described below to be the most important for the production and certification of organic aqueous standards.



Only when major sources for influencing the analyte concentration or potential contaminations are identified and, wherever possible, eliminated, one can call a production process robust and properly validated. Because we have tremendous expertise with regard to the full certification process, we can guarantee that customers can trust every single product bottle they receive.

Certification of Starting Materials by qNMR®

Quantitative nuclear magnetic resonance spectroscopy (qNMR) offers many advantages over other analytical techniques with regard to quantification or purity determination of organic substances. The most outstanding attribute of qNMR is that it is a relative primary method; the signal intensity is in direct proportion to the number of protons contributing to the resonance. Thus, the structures of the chemical substances are completely irrelevant. In addition, no significant empirical factors or unknown biases contribute to the ratio of signals. In other words, the direct response of a qNMR experiment is of highest accuracy, leading to certified values with low uncertainties (see also ANALYTX 02-2010 “Certified Standards for Quantitative ¹H-NMR” and ANALYTX 03-2010 “Launch of a New Generation of Organic CRMs”).

With a few exceptions, like sodium oxalate where the compound carries no protons, the starting materials for our new IC standard solutions are certified by qNMR. All measurements are done under ISO/IEC 17025 accreditation and the certified values are directly traceable to NIST Standard Reference Materials.

Gravimetric Production, Homogenization and Bottling

Gravimetric production, which is the most accurate production technique, is based on high-precision weighing capabilities under ISO/IEC 17025. It is clear that high-precision balances are essential tools for accurate weight measurements. However, the environment around the balances as well as operator technique and know-how are also of critical importance.

We built a special weighing room where the balances stand on three-point supported granite tables weighing up to 1000 kg. Vibrations cannot affect the weighing results and maximum performance is therefore obtained from the balances. Because static electricity can be a source of weighing errors, we use a high-voltage static charge dissipater to help ensure accurate weight measurement. Ambient conditions also affect weighing accuracy. We closely monitor temperature, humidity and barometric pressure in the weighing room since this data is necessary to calculate the air buoyancy bias. The air buoyancy bias can affect the weighing result by up to 0.1%. All high-precision balances are periodically checked and calibrated by a third party and certified according to DAkkS guidelines (DAkkS = Deutsche

Akkreditierungsstelle GmbH, which is the national accreditation body for the Federal Republic of Germany). Only by maintaining all of these conditions and continually improving our infrastructure can we cover the full weight range from 1 mg to 65 kg with very low uncertainties (usually about 0.01%).

After high-precision weighing, the starting material is quantitatively transferred into a 60L PVDF container. While the mixing container is standing on the balance, the batch is filled with high-purity water (specific conductivity of 18 MΩ·cm, total organic carbon at low ppb level and 0.2 μm filtered) until the calculated total mass of the final solution is reached. This gravimetric approach allows a precise adjustment of the final concentration of the calibration solution. The solution is then homogenized by overhead tumbling of the container. With this technique it can be assured that the solution has no measurable inhomogeneity.

Last but not least, the solution must be placed into the final high-density polyethylene (HDPE) bottles without any contamination during the transfer process. This is accomplished by bottling the standards under clean-room conditions using PTFE-tubing and an inert peristaltic pump. Because microbiological contamination is by far the biggest threat to many organic analytes in terms of quality and stability, we take special precautions. The HDPE bottles are x-ray sterilized and the bottled solution is additionally stabilized with sodium azide (about 5 mg/L) and filtered through a 0.2 μm membrane.

Storage and Stability

The ideal container for standard solutions is totally inert, will not adsorb analyte, does not leach impurities into the solution, is impermeable toward the solvent and atmosphere, and is easy to handle and store. It is likely that no container material will ever meet all of these requirements and still be affordable. We found the most suitable material available today was HDPE bottles which fulfill the demand for the absence of most trace impurities. Some contamination (e.g. calcium, sodium, fluoride, acetate, formate or glycolate) might be found at low μg/L levels, which is not typically a problem for 1 g/L standards (**Figure 1**).

HDPE bottles are known to lose solvent through transpiration through the container wall. The rate and extent of loss depends on temperature, thickness of the wall, its shape and surface. Solvent is depleted more rapidly when the surface-to-volume ratio is high. The solvent transpiration rate of HDPE bottles has been investigated comprehensively under various storage conditions during a series of stability studies. This data is considered in the uncertainty budget, and is also used to calculate the expiry date and the maximum storage temperature for the product.

(continued on page 6)

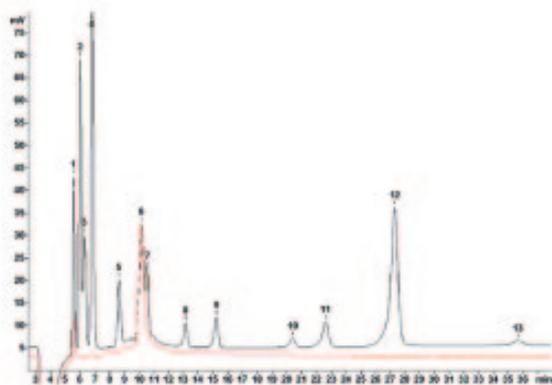


Figure 1 Red line = leaching test sample of water in HDPE bottle. Black line = mixture of 1 µg/kg each: (1) fluoride, (5) chloride, (7) nitrite, (8) bromide, (9) nitrate, (10) phosphate, (11) sulfate, (13) iodide and 10 µg/kg each: (2) glycolate, (3) acetate, (4) formate, (12) oxalate; (6) system peak

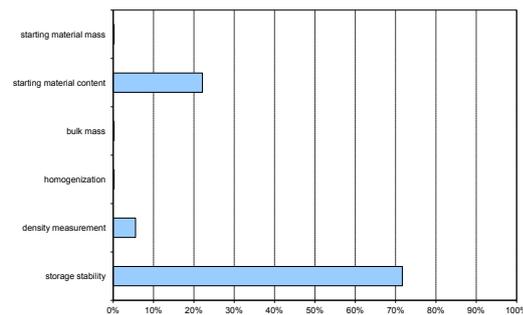


Figure 2 The chart shows the typical uncertainty contributions in %. The expanded uncertainty (95% confidence level) for a 1000 mg/L standard solution (100 mL HDPE bottle) is usually 4 mg/L for 3 year shelf life

Measurement Uncertainty and Certificate

When working in an accredited laboratory under ISO/IEC 17025, the measurement uncertainty of an analytical result needs to be calculated or at least estimated. It is therefore necessary that the uncertainty coming from the reference standard used for calibration is also known. It is of crucial importance that CRM producers consider all relevant uncertainty contributions, which eventually provides a more reliable measurement uncertainty for the analyte content in your sample.

Some CRM producers provide certified values with the statement “value at the date of certification” ignoring the fact that the concentration in the bottle can shift depending on storage conditions and type of container (as described above). This would lead to unrealistically low uncertainties.

The **TraceCERT®** standards are different! We include this potential concentration shift during storage in the final

uncertainty because we want to guarantee the certified concentration independent of whether a customer buys the CRM one month or 2 years after the CRM was produced (**Figure 2**).

A comprehensive certificate (according to ISO Guide 31) is established for these new IC standards. It is available electronically on our web page using product and lot number and shows:

- lot-specific concentration value
- expanded measurement uncertainty (95% confidence level)
- traceability of the certified value
- recommended storage conditions and use
- shelf life and composition

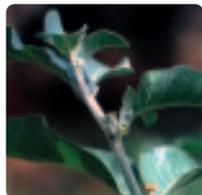
Because we will regularly expand this product line, a comprehensive and up-to-date listing can be found on our website at sigma-aldrich.com/ic

Name	Composition	Cat. No.	Package Size
Acetate Standard for IC	Acetic acid, NaOH, water	51791	100 mL
Benzoate Standard for IC	Benzoic acid, water	40497	100 mL
Butyrate Standard for IC	Sodium butyrate, water	08089	100 mL
Citrate Standard for IC	Citric acid, water	96068	100 mL
Formate Standard for IC	Calcium formate, water	44293	100 mL
Maleate Standard for IC	Maleic acid, water	06908	100 mL
Malonate Standard for IC	Malonic acid, water	42412	100 mL
Oxalate Standard for IC	Sodium oxalate, water	73139	100 mL
Phthalate Standard for IC	Potassium phthalate monobasic, water	90677	100 mL
Propionate Standard for IC	Sodium propionate, water	51716	100 mL
Tartrate Standard for IC	L-(+)-tartaric acid, water	43484	100 mL

Table 1 First series of organic certified reference materials for ion chromatography with analyte concentrations of 1000 mg/L-1

New *Withania somnifera* Analytical Standards and an Improved HPLC Method

Matthias Nold, David S. Bell and Hugh Cramer matthias.nold@sial.com



Fresh Plant



Dried Root

The dried roots of *Withania somnifera* are the source for Ashwagandha, one of the most popular remedies in traditional Indian medicine (Ayurveda). This traditional Indian herbal medicine is becoming increasingly popular in the western world, too, as reflected by the existence of a United States Pharmacopeia (USP) monograph for powdered Ashwagandha roots [1] and a British Pharmacopeia (BP) monograph for *W. somnifera* root for use in Traditional Herbal Medicinal Products (THMP) [2].

The traditionally benign health effects are very diverse and include aphrodisiac, rejuvenative and life-prolonging properties [3]. Beneficial effects are also supported by recent bioactivity studies: Withaferin A has been shown to have significant anticancer activity in animals [4].

Sigma-Aldrich recently added several standards of *W. somnifera* constituents to our herbal medicinal product portfolio for the characterization and quantification of Ashwagandha. An HPLC column was also identified for providing a fast and efficient separation of the key constituents.

Withania Constituents

The characteristic constituents belong to a group of steroidal lactones consisting of a steroidal scaffold attached to a six-membered lactone ring. The structures are shown in **Figure 1**.

HPLC Analysis of *Withania* Constituents Using Ascentis® Express Columns

As part of a large number of natural health product studies recently conducted, many of the main constituents of *Withania* were screened on several modern Fused-Core® stationary phases. The results from the screening effort showed that the Ascentis Express F5 and the Ascentis® Express Phenyl-Hexyl phases provided improved selectivity over the C18 stationary phase. Both phases, presumably due to their intrinsic rigidity, are known to provide enhanced shape selectivity. The shape selectivity component is often found to be useful for the separation of closely related compounds with rigid structures.

Figure 2 shows a comparison of *Withania* standard constituents separated using the USP method to an optimized separation with Ascentis Express Phenyl-Hexyl. The USP method calls for a long, 40-minute gradient and the use of a 25 cm x 4.6 mm C18 column. Even with this lengthy sys-

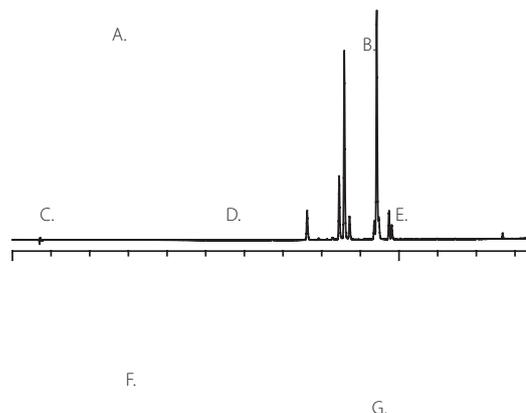


Figure 1 Structures of Selected *Withania* Constituents

- | | |
|------------------------------|-------------------|
| A. 12-Deoxywithastramonolide | E. Withanone |
| B. Withaferin A | F. Withanoside IV |
| C. Withanolide A | G. Withanoside V |
| D. Withanolide B | |

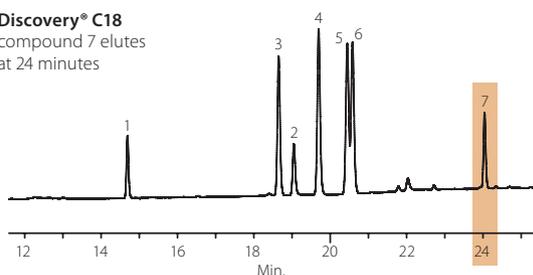
tem, only marginal separation of Withanolide A and Withanone is obtained. Conversely, a 15-minute gradient utilizing a shorter 10 cm x 2.1 mm phenyl-hexyl phase provides baseline separation of all components.

Figure 3 shows the separation of Ashwagandha extract constituents using both systems. The use of the phenyl hexyl column is again shown to provide improved resolution in a shorter period of time and with approximately 3x greater sensitivity. Note that only those components that could be confidently identified are noted. Similar results were obtained utilizing the Ascentis Express F5 stationary phase (data not shown).

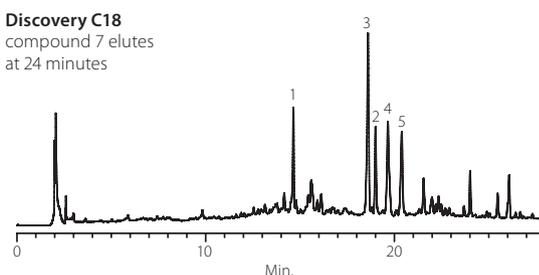
Conclusion

Analyses of complex matrices such as the assay of natural product components may be greatly facilitated through the availability and use of quality standards and modern analytical separation tools. In this case, a fast method with full resolution of 7 constituents from the Ashwagandha root was achieved on an Ascentis Express Phenyl-Hexyl Fused-Core particle column.

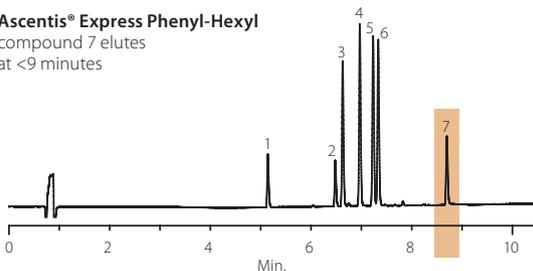
Discovery® C18
compound 7 elutes
at 24 minutes



Discovery C18
compound 7 elutes
at 24 minutes



Ascentis® Express Phenyl-Hexyl
compound 7 elutes
at <9 minutes



Ascentis Express Phenyl-Hexyl
compound 7 elutes
at <9 minutes

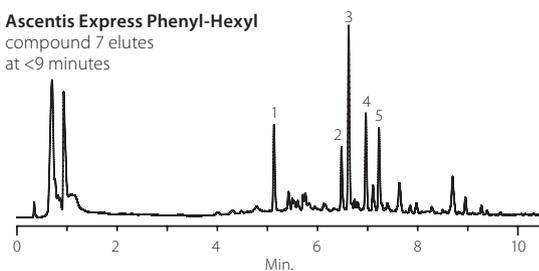


Figure 2 Comparison of *Withania* Standard Separation Using a Standard C18 vs Optimized Ascentis Express Phenyl-Hexyl Method

Figure 3 Comparison of Ashwagandha Extract Component Separation Using a Standard C18 vs Optimized Ascentis Express Phenyl-Hexyl

Discovery C18 Conditions for Figures 2 and 3

column:	Discovery C18, 25 cm x 4.6 mm I.D., 5 µm (504971)	
mobile phase A:	phosphate buffer*	
mobile phase B:	acetonitrile	
gradient:	Min.	%A %B
	0.0	95.0 5.0
	18.0	55.0 45.0
	25.0	20.0 80.0
	28.0	20.0 80.0
	30.0	95.0 5.0
	40.0	95.0 5.0
flow rate:	1.5 mL/min.	
temp.:	27 °C	
det.:	227 nm	
injection:	20 µL	
samples:	Standard: 20 µg/mL each in 80:20	
water:	methanol	
	Extract: As per USP (1)	

* Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Ascentis Express Phenyl-Hexyl Conditions for Figures 2 and 3

column:	Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm (53336-U)	
mobile phase A:	water	
mobile phase B:	acetonitrile	
gradient:	Min.	%A %B
	0.0	80.0 20.0
	10.0	0.0 100.0
	10.5	0.0 100.0
	11.0	20.0 80.0
	15.0	20.0 80.0
flow rate:	0.3 mL/min.	
temp.:	35 °C	
det.:	227 nm	
injection:	5 µL	
samples:	same as Discovery C18	

- | | |
|----------------------|------------------|
| 1. Withanoside IV | 5. Withanolide A |
| 2. Withanoside V | 6. Withanone |
| 3. Withaferin A | 7. Withanolide B |
| 4. Withastramonolide | |

Description	Package Size	Cat. No.
12-Deoxywithastramonolide	10 mg	94187
Withaferin A	10 mg	89910
Withanolide A	10 mg	74776
Withanolide B	10 mg	94284
Withanone	10 mg	90896
Withanoside IV	10 mg	94186
Withanoside V	10 mg	66042
Ascentis Express Phenyl-Hexyl Column 10 cm x 2.1 mm I.D., 2.7 µm	1	53336-U

Sigma-Aldrich offers a wide range of more than 250 analytical standards, primary standards and certified reference materials for constituents of herbal medicinal products for the use in quality control in the phytopharmaceutical industry.

On our website sigma-aldrich.com/medicinalplants these products can be browsed either alphabetically, by substance class or by genus of a large number of medicinal plants.

Acknowledgement

Photographs of *W. somnifera* were provided by Dr. Amit Agarwal, Director, Natural Remedies Pvt. Ltd., Bangalore, India.

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- [1] USP 34, Dietary Supplements, Ashwagandha 1079.
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New Analytical Standards for Pesticides and Mycotoxins

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

New High-Purity Standards for Pesticide Residue Analysis



Through our PESTANAL® line we offer a large portfolio of over 1500 high-purity pesticide and pesticide metabolite standards including isotope-labeled compounds for food and environmental analysis. This portfolio is continuously expanded. Please find the most recent additions of new products in the list below. A complete product listing can be found on our website

sigma-aldrich.com/pesticides

New Mycotoxin Standards



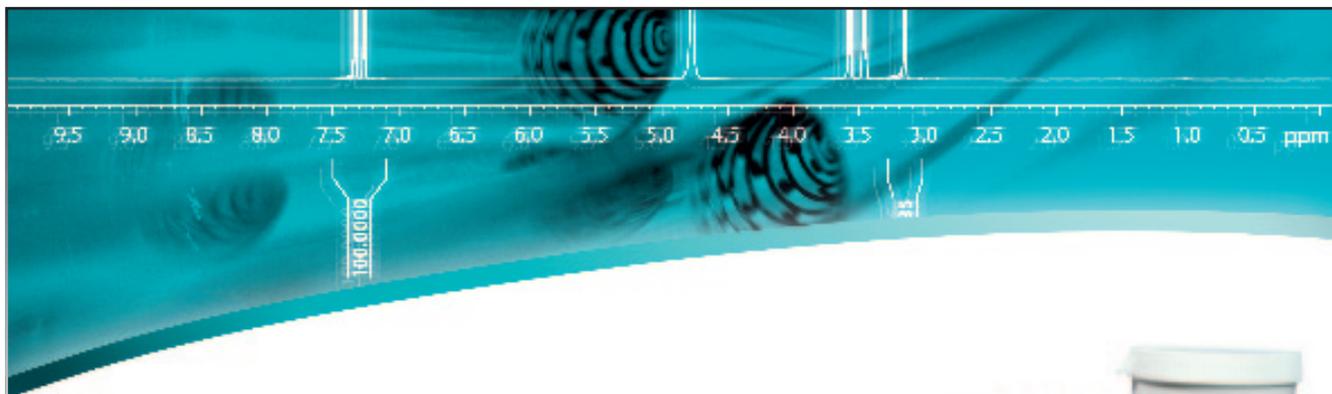
Fungal infection of crops can lead to mycotoxin contamination of human food, either directly or through their use as livestock feed. Sigma-Aldrich offers a comprehensive range of analytical standards of mycotoxins and isotope-labeled mycotoxins for food residue analysis. The table below lists the most recent additions to this portfolio. For a complete list of mycotoxin standards, please visit our website sigma-aldrich.com/mycotoxins

Brand	Cat. No.	Description	Pack Size
Fluka®	32471	4-(3,6-Dimethyl-3-heptyl)phenol-ring- ¹³ C ₆ solution	1 mL
Fluka	35371	Acibenzolar acid	50 mg
Fluka	32461	Ametoctradin	50 mg
Fluka	32457	Aminopyralid	25 mg
Fluka	32849	Amisulbrom	25 mg
Fluka	32442	Amitraz Metabolite BTS 27271	10 mg
Fluka	32474	Benzofenap solution	2 mL
Fluka	32456	Chloretoxyfos	25 mg
Fluka	35376	Cuelure	25 mg
Fluka	37023	Cumyluron	25 mg
Fluka	35978	Dimethirimol	10 mg
Fluka	35352	Dioxabenzofos	10 mg
Fluka	32475	Fenothiocarb	100 mg
Fluka	35372	Fluazifop-P	25 mg
Fluka	37038	Flumorph, mixture of E and Z isomers	25 mg
Fluka	32462	Fluopyram	50 mg
Fluka	32464	Fluthiacet-methyl	25 mg
Fluka	32458	Glycarbylamide	100 mg
Fluka	35378	Haloxifop-P	25 mg
Fluka	35373	Hydramethylnon	100 mg
Fluka	37007	Iminoctadine triacetate	10 mg
Fluka	35351	Inabenfide	10 mg
Fluka	37009	Isouron	10 mg
Fluka	32473	Metoclopramide	100 mg
Fluka	32440	N-(2,4-Dimethylphenyl)-N'-methyl-d ₃ -formamidine	10 mg
Fluka	37005	Oxazioclofene	10 mg
Fluka	35346	Pyraflufen-ethyl	10 mg
Fluka	32459	Pyrazolynate	25 mg
Fluka	32437	Pyrazoxyfen	50 mg
Fluka	35999	Pyrimidifen	10 mg
Fluka	35369	Simeconazole	25 mg

Table 1 New Pesticide Standards

Brand	Cat. No.	Description	Pack Size
Fluka	35758	Alternariol	0.1 mg
Fluka	35762	Alternariol 9-methyl ether	0.1 mg
Fluka	37012	Beauvericin	0.1 mg
Fluka	35878	Citreoviridin A	0.1 mg
Fluka	35410	Citrinin solution, 100 µg/mL in acetonitrile	1 mL
Fluka	37017	Fumagillin solution, 100 µg/mL in acetonitrile	1 mL
Fluka	35598	Gliotoxin solution, 100 µg/mL in acetonitrile	1 mL
Fluka	35970	Meleagrins	0.1 mg
Fluka	37013	Moniliformin sodium salt solution, 100 µg/mL in acetonitrile/water	1 mL
Fluka	35516	Patulin- ¹³ C ₆ solution, 25 µg/mL in acetonitrile	1.2 mL
Fluka	35417	Paxilline solution, 100 µg/mL in acetonitrile	1 mL
Fluka	35976	Stachybotrylactam	0.1 mg
Fluka	35977	Tentoxin	0.1 mg
Fluka	37016	Verruculogen solution, 100 µg/mL in acetonitrile	1 mL
Fluka	35441	Wortmannin solution, 100 µg/mL in acetonitrile	1 mL
Fluka	35405	α-Zearalanol-solution, 10 µg/mL in acetonitrile	1 mL
Fluka	35406	α-Zearalanol-solution, 10 µg/mL in acetonitrile	1 mL
Fluka	35407	β-Zearalanol-solution, 10 µg/mL in acetonitrile	1 mL
Fluka	35409	β-Zearalanol-solution, 10 µg/mL in acetonitrile	1 mL

Table 2 New Mycotoxin Standards



Do you want reliable quantitative results?



Try our NEW organic **TraceCERT**[®] CRMs!

- Products for **HPLC, GC** and **qNMR**[®]
- Certified content measured by **high-performance quantitative NMR** (HP-qNMR)
- Superior level of **accuracy**, calculated **uncertainties**, and **lot-specific values**
- Traceability to **NIST Standard Reference Material**
- Production and certification in accordance with **ISO/IEC 17025** and **ISO Guide 34**

Our product range currently comprises over 100 standards including: Amino Acids, PAHs, Antibiotics, Pesticides, Fatty Acids, and FAMES, Natural Compounds, Organic Acids etc.

We are continuously working on the expansion of this portfolio in order to offer you reliable and traceable reference materials for the analytes you need.

For more information and an up-to-date product list, please visit our website at sigma-aldrich.com/organiccrm

Custom Chemical Standard Services

The extensive product offering of the Sigma-Aldrich family allows you to access thousands of chemicals that can be customized and engineered to fit your standard or reference solution needs.

We can formulate, test, and package custom chemical standard mixtures to your exact specifications. Our custom standard chemists will gladly discuss stability and solubility concerns with you, and make suggestions where needed to maximize the quality of your standard.

These Standard Solutions include:

- Raw materials and solvents screened for identity and purity
- Your choice of gravimetric, qualitative, or quantitative testing
- Packaging choices from ampules to bottles
- Manufacturing processes following ISO 9001/2000 guidelines
- Documentation and Material Safety Data Sheets
- Strict adherence to all shipping regulations
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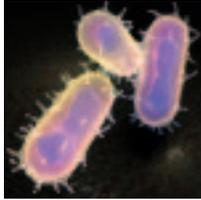


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Genus *Yersinia*



Detection, Identification, Differentiation and Cultivation of *Yersinia* species

Today *Yersinia* is not a major problem but at least three species occasionally appear as pathogens and have potential for severe outbreaks. For example, in March 2011, the Norwegian Institute of Public Health identified twenty-one cases of *Yersinia enterocolitica* 0:9 which presumably could be traced back to bagged salad mix containing radicchio rosso. The producer voluntarily recalled the batch.

Yersinia is a genus of Gram-negative rod-shaped bacteria which are facultative anaerobes belonging to the family of Enterobacteriaceae. It has a fermentative metabolism, is oxidase-negative, mannitol-positive, glucose-positive and lactose negative. *Yersinia* is an organism related to *Salmonella*, but on the Bismuth sulfite Agar (Fluka 95388) it can be differentiated because it is not able to produce hydrogen sulfide (see also further media and tests in **Tables 1 to 4**). It is a psychrophilic organism, surviving and proliferating at low temperatures of 0 to 4 °C (e.g. for on food products in a refrigerator). Some *Yersinia* species are also relatively highly heat resistant, but at 37 °C they grow slower than other Enterobacteriaceae (show only as small colonies). However, they can be quite easily inactivated by oxidizing agents such as hydrogen peroxide and potassium permanganate. Pigs, rodents, rabbits, sheep, cattle, horses, dogs, and cats are the natural sources of *Yersinia*. At the moment, most cases of human illness caused by *Yersinia* originate from *Y. enterocolitica*, but not all biovars shows pathogenic characteristics. This organism is the cause of yersiniosis, an infectious disease with symptoms such as fever, abdominal pain, and diarrhea. Of the eleven known species from the genus *Yersinia*, just two other species are of clinical importance. These are additionally *Y. pseudotuberculosis* (symptoms like *Y. enterocolitica* except in most cases no diarrhea is seen) and *Y. pestis* (organism responsible for the bubonic plague). Most infections are acquired through contaminated food, like raw or undercooked pork products, game, seafood, vegetables, unpasteurized milk or untreated water. However, infections may also occur after contact with infected animals or feces, or through transmission by fleas.

The bacteria received its name from A. E. J. Yersin, a Swiss microbiologist, who discovered the *Yersinia pestis* bacterium in 1894 in Hong Kong.

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

Scientific Classification of *Yersinia*:

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

Species	Differentiation properties
<i>Y. enterocolitica</i>	catalase-positive, most strains are ornithine-positive, motile at 22–26 °C, non-motile at 37 °C, urea-positive, sorbitol- and cellobiose-positive, most strains are sucrose-positive, red "bulls-eye" on CIN Agar
<i>Y. pseudotuberculosis</i>	motile at 22–26 °C, non-motile at 37 °C, urea-positive, esculin-positive, ornithine decarboxylase negative, indole negative, rhamnose positive
<i>Y. pestis</i>	produces two antiphagocytic components (antiphagocytic slime), catalase positive, non-motile at 22–26 °C and 37 °C (except for the <i>Yersinia</i> species), esculin-positive, indole negative, ornithine decarboxylase negative

Table 1 Some differentiation properties of the important *Yersinia* species

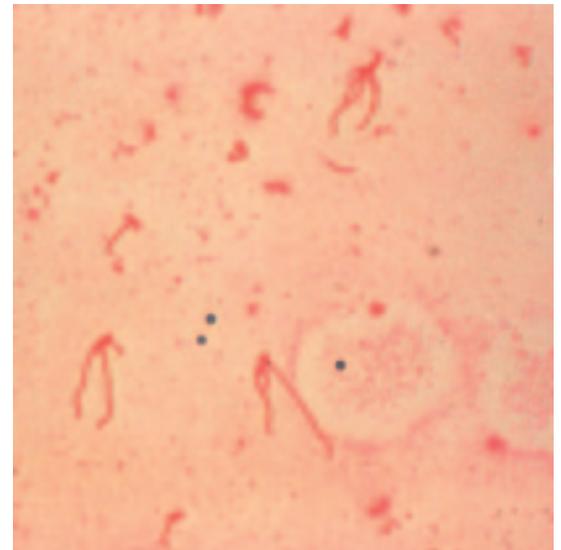


Figure 1 A photomicrograph of *Yersinia enterocolitica* using flagella staining technique (source: CDC 1980).



Nonselective Broths	Cat. No.
Brain Heart Broth	53286
Peptone Water, phosphate-buffered, Vegetone	40893
Peptone Water, phosphate-buffered	77187
Selective Enrichment Broths	Cat. No.
ITC Broth (Base)	17156
Mossel Broth	69965
Peptone Sorbitol Bile Broth	17192
Basal Media for Carbohydrates Utilization	Cat. No.
Andrade Peptone Water	A0715
Andrade Peptone Water, Vegetone	28943
Medium for Autoagglutination	Cat. No.
Methyl Red Voges Proskauer Broth	39484
Nonselective Agars for Differentiation & Confirmation	Cat. No.
Christensen's Urea Agar	27048
Kligler Agar	60787
Motility Indole Ornithine Medium	M1428
OF Test Nutrient Agar	75315
Ornithine Decarboxylase Broth	O5386
Urea Broth	51463
Selective Agars for Detection and Isolation	Cat. No.
<i>Yersinia</i> Selective Agar (CIN Agar)	95760
Selective Agars with Differential System for Differentiation, Detection and Isolation	Cat. No.
Bile Esculin Agar	48300
Bismuth Sulfite Agar	95388
DCLS Agar	70135
DCLS Agar No. 2	90035
Mac Conkey Agar No 1	70143
Violet Red Bile Glucose Agar without Lactose	17213
Violet Red Bile Glucose Agar without Lactose, Vegetone	53605
VRB MUG Agar	95273

Table 2 Media for *Yersinia*

Supplements	Cat. No.
Ferrioxamine E	38266
<i>Yersinia</i> Selective Supplement	75258
Ticarcillin Supplement	17778
Potassium Chlorate Supplement	17777

Table 3 Selective and growth supplement for *Yersinia*

Test for <i>Yersinia</i> Diagnostics	Cat. No.
Bile Esculin Disks	80507
Catalase Test	88597
Cellobiose Disks	56481
Dextrose (Glucose) Disks	63367
DMACA Indole Disks	05686
DMACA Reagent	49825
Kovac's Reagent for Indoles	67309
Kovac's Reagent Strips	78719
Lactose Disks	28816
Mannitol Disks	94438
Methyl Red Solution	08714
Oxidase Reagent acc. Gaby-Hadley A	07345
Oxidase Reagent acc. Gaby-Hadley B	07817
Oxidase Reagent acc. Gordon-McLeod	18502
Oxidase Strips	40560
Oxidase Test	70439
Rhamnose Disks	93999
Salicin Disks	92971
Sorbitol Disks	93998
Sucrose Disks	94309

Table 4 Test for identification and differentiation of *Yersinia*

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

MSTFA and MSTFA-D₉ – Essential Tools for the Identification of Compounds by GC/MS

Dieter Urbach BKA, KT 12 – Zentrale Analytik II 65173 Wiesbaden

Introduction

Derivatization, especially silylation, has become a common and very useful tool in identification and quantification of known and unknown compounds using GC/MS. The application is not limited to small molecules. Even large peptides can be modified with this method, especially when the introduction of deuterium is desired [1]. N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) is the most versatile reagent as it allows the derivatization of a large number of common moieties in organic compounds by attaching a non-polar trimethylsilyl (TMS) moiety. In most cases, TMS derivatives have a lower evaporating temperature, which allows the use of lower temperatures and reduces the thermally induced decay of compounds in the injector or on a column. For quantitative analysis, the use of catalyzers is recommended [2–4].

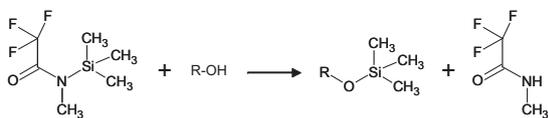


Figure 1 Reaction scheme of the derivatization of hydroxyl moieties by MSTFA (R-X, X=–OH, –COOH, =NH, –NH₂ and –SH).

Using Deuterated MSTFA-D₉ for the Identification of Compounds

In some cases it is unclear how many moieties of a molecule are affected by the derivatization reaction, especially when unknown compounds have to be identified. Using MSTFA-D₉ in an additional reaction helps to overcome this problem, because the molecular mass of the MSTFA-D₉ derivative increases by 9 mass units multiplied by the number of derivatized moieties. Thus, it is possible to calculate the molecular mass of the original compound by taking the mass shift in the MSTFA/MSTFA-D₉ spectra into account.

Figure 2 shows the mass spectrum of amphetamine (α -methylphenethylamine) using GC-MS on SLB-5ms column. Often primary or secondary amino compounds, e.g. amphetamine, show an $[M-1]^+$ ion that could be misleading for the molecular mass and identification of a nitrogen-containing compound. Amphetamine also generates unspecific and ambiguous major fragments (44 m/z can match both amino or aldehyde compound, (**Figure 2**, top)). After MSTFA derivatization, the mass spectrum (**Figure 2**, middle) reveals typical fragments of TMS-derivatives (loss of methyl from TMS), while the target compound, amphetamine, can be calculated based on the fragment mass 192 m/z as intact molecule (192+15 m/z=207 m/z, TMS-amphetamine).

amphetamine, can be calculated based on the fragment mass 192 m/z as intact molecule (192+15 m/z=207 m/z, TMS-amphetamine).

Using MSTFA-D₉, 192 m/z fragment ion is shifted by 6 mass units to 198 m/z and 9 mass units to 201 m/z (**Figure 2**, bottom). The first shift indicates the attachment of one TMS group. A shift of 9 mass units is associated with the cleavage of the α -methyl bond in the aliphatic chain of amphetamine structure and delivers additional information about the fragmentation mechanism.

Method Derivatization

- 1 mg sample dissolved in 270 μ L MSTFA (or ca. 10 μ g sample in 25 μ L MSTFA-D₉)
 2. Heat for 10 min at 70 °C
 3. Add 30 μ L pyridine
 4. Heat for 10 min. at 70 °C
- Dilute the solution with chloroform, if necessary

GC/MS

Column: semi-polar GC column, e.g. Supelco SLB-5ms, 30 m x 0.25 mm, 0.25 μ m (28471-U)
Carrier Gas Flow: 1 ml/min (helium)
Temp. Grad.: 40–300 °C in 26 min, hold for 10 min

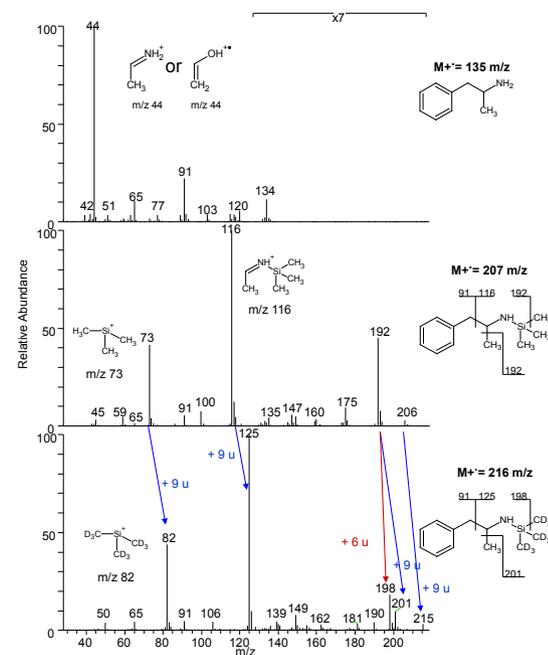


Figure 2 Mass spectra of amphetamine (top), TMS-amphetamine (middle) and TMS-D₉-amphetamine (bottom). The mass spectrum of amphetamine results in only unspecific and misleading fragments. 44 m/z and 134 m/z do not indicate a nitrogen compound or the right molecular mass. This becomes obvious after the derivatization with MSTFA/MSTFA-D₉.

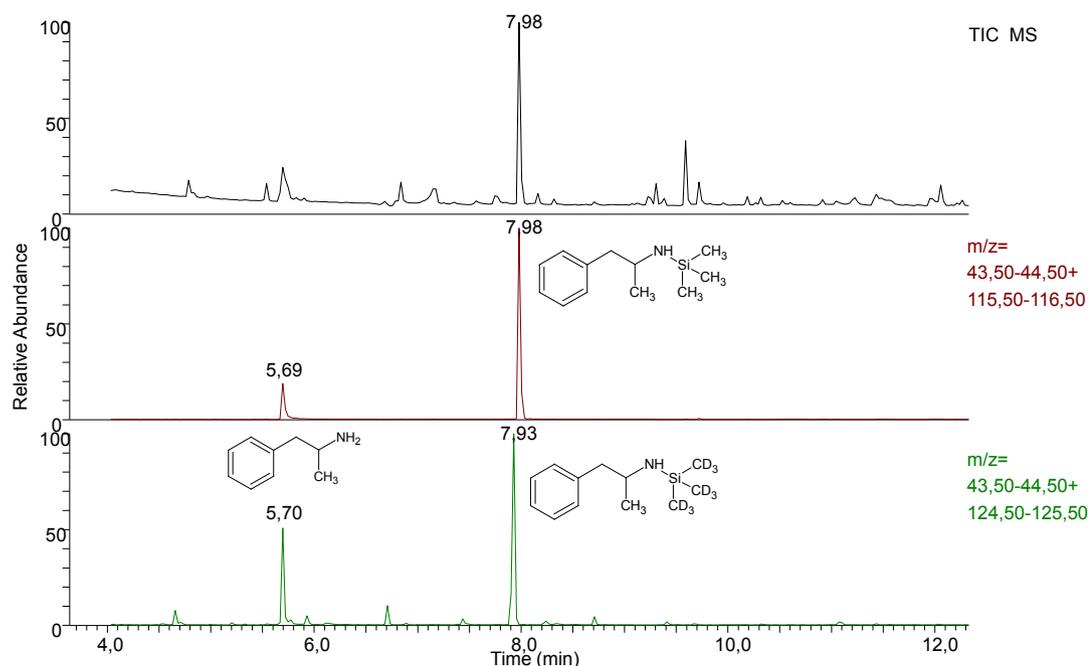


Figure 3 GC/MS chromatogram (TIC, EIC) of derivatized and non-derivatized amphetamine.

Figure 3 shows the effect of derivatization on the chromatography on an SLB-5ms™ column. Unlike other drugs, underivatized amphetamine elutes first, followed by its TMS derivative. This example shows how derivatization can be used to alter retention or selectivity to see unknown compounds that might normally coelute with the sample matrix or other components of the sample.

Summary

The majority of the current GC/MS systems are equipped with single quadrupole mass analyzers with a limited mass resolution. They do not offer the ability to acquire MS/MS or MSⁿ spectra like triple quadrupole or ion trap mass analyzers. These simple GC/MS systems especially profit from the described derivatization method using MSTFA/MSTFA-D₉, as there is no need to change the ionization technique such as chemical ionization to obtain the molecular mass or apply additional MS/MS experiments to elucidate the structure and composition of a fragment or molecule.

Another advantage of MSTFA and MSTFA-D₉ is the comparability of chromatographic properties of their corresponding derivatives. TMS and TMS-D₉ derivatives elute in the same time frame. Deuterated compounds may elute a few seconds earlier. Thus, the retention time can be used to find and identify a target compound even at low concentration levels.

The New Derivatization Reagents brochure lists products for GC, HPLC, Chiral and TLC derivatization. To order a free copy, visit sigma-aldrich.com/derivatization

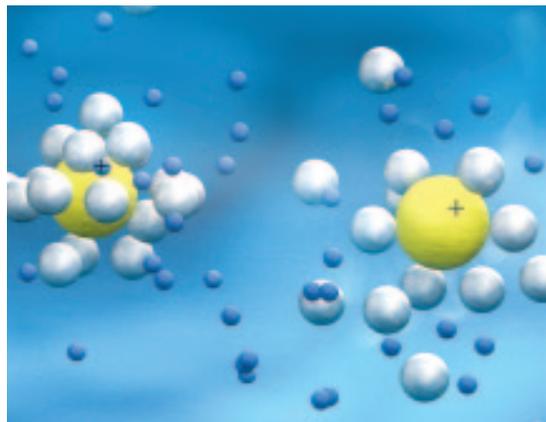
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Brand	Description	Cat. No.
Additional GC derivatization agents		
Fluka®	MSTFA for GC derivatization	69479
Fluka	MSTFA-D ₉ for GC derivatization	68769
Additional GC columns		
Supelco®	Supelco SLB-5ms Capillary-GC column, 30 m x 0.25 mm, df=0.25 µm	28471-U
Additional standards, deuterated standards		
Fluka	Amphetamine, analytical standard for drug analysis	94777
Fluka	Amphetamine-D ₁₁ solution in methanol, analytical standard	610666

LC-MS CHROMASOLV® Water, Solvent Blends and Additives

High purity for accurate analysis



Solvent impurities are the most common cause of extraneous peaks and unstable LC-MS baseline. Solvent-derived impurities do not condition out over time. Most common contaminants include in-organic ions, decomposition products, microbes and their excretion prod-

ucts and particulate matter. These impurities can interfere in the analysis in multiple ways, such as: a) collect on head of HPLC column and elute as a distinct peak or as baseline rise, b) cause general elevation in baseline, lowering sensitivity of analysis, c) foul or damage sensitive instrument components and d) cause cluster ion formation that prevents reliable identification and quantification. Sigma-Aldrich offers LC-MS CHROMASOLV solvents, pre-blended solvents and additives specified for LC-MS requirements.

CHROMASOLV Water is a high-purity product with quality suitable for both gradient HPLC and MS applications, and offers tremendous advantages over other non-gradient grade water available in the market. It can be used in both UV and MS detection methods without any compromise.

Pre-Blended LC-MS Solvents

The mobile phase composition plays a critical role in the success of an LC-MS experiment. Precise formulations provide accurate and reproducible results. Sigma-Aldrich offers pre-blended solutions of most commonly used LC-MS mobile phases, prepared with precision and unsurpassed attention to quality. A special formulation assures that no precipitation or decomposition of the additive occurs under normal laboratory conditions. These pre-blended solvents offer:

- 1) time saving
- 2) accurate composition
- 3) minimized baseline and artifacts and
- 4) ensured high quality.

CHROMASOLV Mobile Phase additives are selective chemicals that are commonly added to the mobile phase or introduced post-column prior to the interface to influence

Shyam Verma, Market Segment Manager shyam.verma@sial.com

analyte ionization. Most often, the objective is to improve the signal quality. On the other hand, some additives are used to suppress unwanted signals or selectively enhance the signal of specific compounds in a mixture, for example glycosidic species in a mixture of peptides.

For minimizing the background and artifacts in LC-MS analysis, highly specified solvents are spiked with ultra-pure salts and acids. These additives improve the chromatographic peak shape and optimize ionization in the MS interface. The most commonly used additives include trifluoroacetic acid (TFA), formic acid, acetic acid and ammonium acetate. Volatile and low molecular weight organic acids improve ionization and resolution of a wide range of molecules.

Two fundamental reasons for use of these additives are:

1. Many chromatography separations benefit in terms of retention and/or peak shape under acidic conditions since any silanol activity is suppressed.
2. Most MS measurements are done in positive ion mode, which is accomplished by the addition of a proton to form the molecular ion $[M+H]^+$. The low molecular weight organic acids mentioned above exhibit necessary acidity and volatility to provide an excess of cations for this purpose.

For additional information about our LC-MS products, please visit our website sigma-aldrich.com/lc-ms

Description	Cat. No.
LC-MS CHROMASOLV Solvents	
Water	39253
Acetonitrile	34967
Methanol	34966
2-Propanol	34965
Ethyl acetate	34972
LC-MS CHROMASOLV Solvents Blends	
Acetonitrile with 0.1% TFA	34976
Methanol with 0.1% TFA	34974
Acetonitrile with 0.1% formic acid	34668
Acetonitrile with 0.1% ammonium acetate	34670
Acetonitrile with 0.1% formic acid and 0.1% TFA	34676
Water with 0.1% TFA	34978
LC-MS CHROMASOLV Mobile Phase Additives	
Trifluoroacetic acid, puriss p.a.	40967
Formic acid, puriss p.a.	56302
Acetic acid, puriss p.a.	49199
Ammonium formate, puriss p.a.	55674

LPLC Purification Media: Custom Solutions to Your Unique Challenges

We offer a broad media portfolio supported by custom services that optimize your purification results



It has been said that the only thing that all purification applications have in common is, that they are all unique. Most users share the common desire to obtain purified material quickly and easily while maximizing purification yields. The special considerations associated with each purification application pose a serious challenge for users in trying to identify the best products to use for their

specific applications. To meet the needs of our customers performing chromatographic purification, Sigma-Aldrich has put together a broad selection of chromatographic media coupled with a range of custom services that allow us to tailor solutions to our customers' individual needs rather than limiting them to just a few, off-the-shelf options.

Media Selection

The selection of chromatographic media is a vital step toward successful chromatographic separation and desirable purification results. Columns packed with a variety of materials, inorganic and organic, are used in various chromatographic modes such as adsorption, ion exchange, hydrophobic interaction and size exclusion in applications ranging from purification and isolation of low molecular weight compounds to more sensitive bio-separations. We offer a full line of resins and media in convenient package sizes from leading manufacturers, including Sigma-Aldrich, Dow/Rohm and Haas, Tosoh, GE Healthcare, Merck KGaA, Grace Davison, Lanxess, Mitsubishi and U.S. Silica. Our product range extends from synthetic polymers to traditional inorganic adsorbents including silica, bonded phase silica, alumina and Florisil®.

Polymeric Resins

Sigma-Aldrich offers many brands and types of organic (polymeric) resins for general and specialized applications that are suitable for all types of purification processes, from laboratory to pilot-scale, including:

- Anion & Cation Exchange
- Mixed Bed, Chelating and Nuclear Ion Exchange
- Adsorption
- Gel Filtration

Inorganic Adsorbents

Sigma-Aldrich's polymeric resins portfolio is further complemented by a broad range of inorganic adsorption media for a wide variety of purification applications. Our offering of inorganic adsorption media includes:

- Silica (bare, C18, C8, aminopropyl, chloropropyl etc.)
- Florisil magnesium silicate
- Activated alumina
- Carboxens™ and carbon molecular sieves

Custom Resin and Media Processing Services

Sigma-Aldrich, through its Supelco® brand, offers custom processing to meet customer-specific purification requirements. Following are just a few of the resin and media processing capabilities we provide.

Cleaning – Our chemists can clean resins using a variety of solvent and solvent-free methods.

Pre-wetting – We can pre-wet the media with solvents or water, making it compatible with your process and easier to handle with less of the media ending up on your laboratory bench and floor.

Drying – Residual moisture can interfere with certain analyses or reduce the resin's capacity or ability to disperse in organic solvents. We can dry nearly any resin to the moisture specifications your application requires.

Ionic form conversion – We can convert and supply media in the correct ionic form for your application.

Custom Packaging

Bulk media or media that we custom process can be supplied to you in nearly limitless formats, from simple storage containers and in-line cartridges to process-specific devices. Single-use, disposable Porozorb™ (250, 1000 and 4000 mL) and Rezorian™ A161 (1 and 5 mL) cartridges are examples of special LPLC media packaging options we can provide to our customers.

Contact Information

For more information on the media and resins with regard to selection and applications or to inquire about custom processing and packaging services, please visit our website at sigma-aldrich.com or contact your local Sigma-Aldrich office to speak with one of our Technical Support Specialists.

Reagents for Trace Analysis Sample Preparation *TraceSELECT*[®]Ultra

Acids, Bases, and Salts



Sample preparation is a critical step for trace analysis. It requires reagents of the highest purity to prevent introduction of foreign trace impurities. Our *TraceSELECT*Ultra products are specifically designed for ultra-trace analysis at *ppb* and even *ppt* levels. These products are produced by sub-boiling distillation. This technique is based on the evaporation of liquid by infrared heating at the surface. It avoids violent boiling and the formation of liquid aerosols that can be transported with the distillate. Sub-boiling is recognized as the best technique for obtaining high-purity acids with the lowest blank values for ultra-trace analysis.

Shyam Verma, Market Segment Manager shyam.verma@sial.com

In order to maintain the high purity of the *TraceSELECT*Ultra products, they are supplied in PTFE PFA (fluoropolymer) bottles. Water and ortho-phosphoric acid are supplied in specially pre-leached HDPE bottles. Recent process improvements have allowed us to reduce our impurity specifications to guarantee the lowest levels of trace impurities in this product line.

The Sigma-Aldrich Quality Management System guarantees consistent quality and safety for all *TraceSELECT*Ultra products. These reagents are produced and bottled under clean-room conditions, and are delivered with a Certificate of Analysis. As a supplier of analytical reagents and standards, quality assurance is of prime importance. We employ a comprehensive Quality Assurance System according to EN 29001 (ISO 9001). Please see our entire selection of high-purity digestion reagents at sigma-aldrich.com/traceselect

Special Offer:

Save 25% on our *TraceSELECT*Ultra products listed in the Table below (except the 5 L pack size). Include Promo No. SCC when placing an order. This promo is valid until May 30, 2012.

Cat. No.	Brand	Product Name	Specification	Pack Sizes
07692	Fluka [®]	Acetic acid	99% (T)	250 mL, 1 L
16748	Fluka	Ammonium hydroxide solution	25% (T)	250 mL, 1 L
23828	Fluka	Hydrobromic acid	44% (T)	250 mL, 1 L
96208	Fluka	Hydrochloric acid	30% (T)	250 mL, 1 L
02658	Fluka	Hydrofluoric acid	49% (acidimetric)	250 mL, 1 L
16911	Fluka	Hydrogen peroxide solution	30% (RT)	250 mL, 1 L, 5 L
02650	Fluka	Nitric acid	-65% (T)	250 mL, 1 L
12415	Fluka	Perchloric acid	67–72% (T)	250 mL, 500 mL, 1 L
64957	Fluka	Phosphoric acid	85% (T)	250 mL, 1 L
77239	Fluka	Sulfuric acid	95% (T)	250 mL, 1 L
14211	Fluka	Water		1 L

Table 1 *TraceSELECT*Ultra reagents

New TraceSELECT® Solvents for Trace Analysis of Pharmaceuticals and Oils

Matthias Drexler, Product Manager, Analytical Reagents matthias.drexler@sial.com



The success of trace metal analysis often greatly depends on the application of the right sample preparation techniques and reagents. In most cases, samples need to be digested either by wet or melting digestion. In contrast, other samples, such as pharmaceutical compounds and various types of oils, can be directly diluted for analysis with AAS or ICP-OES/MS. Avoiding a digestion step generally results in faster (and hence cheaper) analyses, which is particularly useful for routine measurements.

Although some metal-based fuel additives can improve the combustion efficiency of biodiesel, there are several metals (e.g. Cu, Co, Mn) that have detrimental effects on oils, such as catalyzing oxidative degradation or toxicity. Therefore, many official procedures exist for monitoring the trace metal content of vegetable oils and biodiesel. Our new TraceSELECT grade 1-Propanol (Fluka® 09158) is especially suitable for the dilution of vegetable oils and biodiesel without the need for toxic or corrosive digestion reagents.

Due to the toxicity of many heavy metals and their deteriorating effect, the importance of trace metal analysis of pharmaceutical compounds to ensure quality control carries great importance in many official documents. For direct

dilution of pharmaceuticals and fast screening for metal traces, mixtures of organic solvents and water (Fluka 14211) have been used with great success, especially when analyzing for heavy metal traces such as Pd and Pt, which are commonly used in organometallic catalysts for the synthesis of active pharmaceutical compounds or their precursor materials.

All TraceSELECT organic solvents offer very low blank values on up to 70 metal traces, as clearly documented in the certificates of analysis, and are stored in high-quality packaging materials that minimize leaching effects. This results in their suitability for trace analysis even after extended storage.

References

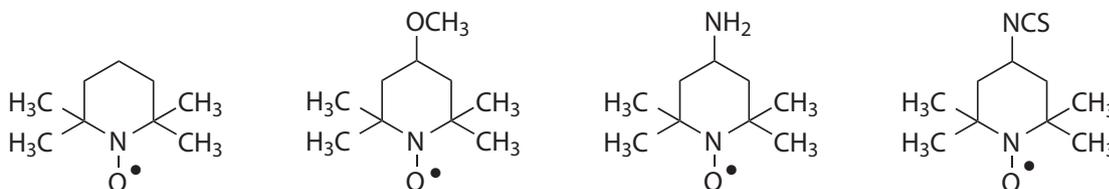
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Cat. No.	Brand	Description	Specification	Pack Sizes
09158	Fluka	1-Propanol	TraceSELECT (>= 99.5%)	500 mL
92328	Fluka	Ethylene glycol butyl ether	TraceSELECT (>= 98%)	1 L
01324	Fluka	Acetonitrile	TraceSELECT (>= 99.9%)	1 L
42105	Fluka	Methanol	TraceSELECT (>= 99.9%)	1 L
14211	Fluka	Water	TraceSELECT Ultra	1 L
43729	Fluka	1-Methyl-2-pyrrolidinone (NMP)	TraceSELECT (>= 99%)	1 L

Product Table Solvents Suitable for Direct Dilution of Samples for Trace Metal Analysis. Please find the complete product list at sigma-aldrich.com/traceselect

Spin Labels for ESR Spectroscopy

Matthias Drexler, Product Manager, Analytical Reagents matthias.drexler@sial.com



ESR spectroscopy has become a valuable method in macromolecular biology, especially for the characterization of protein structure, dynamics and conformational change. In contrast to structure analysis by protein crystallization, ESR allows insight into the structure and conformation in solution. ESR offers some key advantages over NMR spectroscopy including higher sensitivity due to the larger magnetic moment of the electron spin and the much shorter time scale (in nanoseconds) allowing the study of faster dynamics. Recent advances in multifrequency ESR has led to even more detailed characterization of protein dynamics.

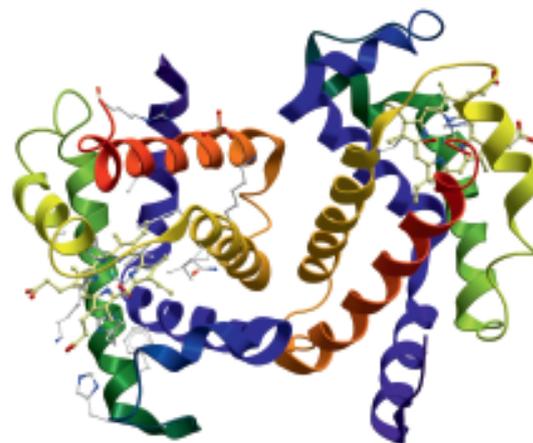
Since most chemical and biological samples of interest lack an inherently stable unpaired electron, the majority of ESR methods rely on the usage of spin-labeling reagents. Commonly, nitroxides such as derivatives of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) are used as they offer high stability of the unpaired electron and exceptional ESR sensitivity, combined with facile and versatile moieties for binding to the sample through chemical reactions. Sigma-Aldrich offers a comprehensive array of spin labels suitable for ESR spectroscopy in high purities to allow for easy interpretation of the analytical results.

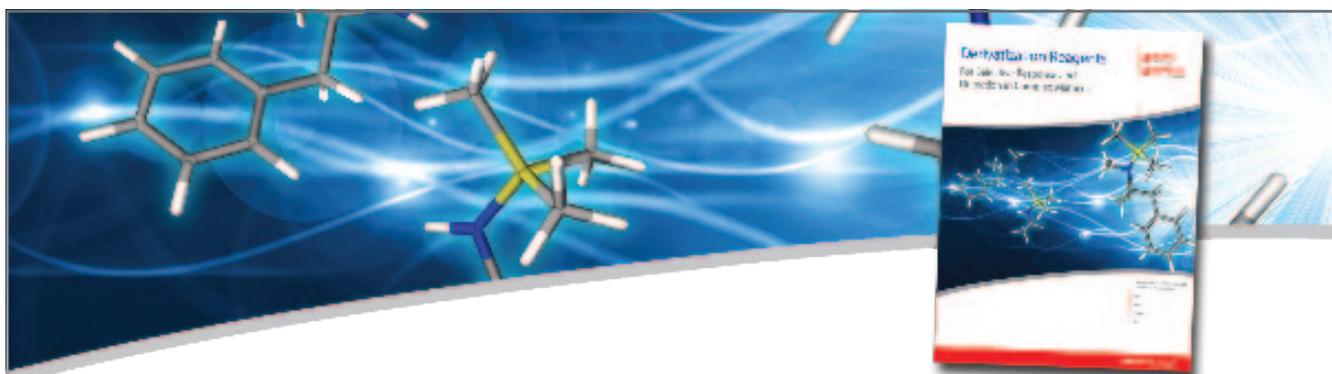
Part. No	Brand	Description	Specification	Pack Sizes
43967	Fluka®	4-Amino-TEMPO	for ESR-spectroscopy, ≥98%	250 mg
42777	Fluka	4-Hydroxy-TEMPO	for ESR-spectroscopy, ≥99%	1 g
76381	Fluka	4-Isothiocyanato-TEMPO	for ESR-spectroscopy, ≥97%	250 mg
42619	Fluka	4-Methoxy-TEMPO	for ESR-spectroscopy, ≥99%	250 mg
42442	Fluka	TEMPO	for ESR-spectroscopy, ≥99.5%	250 mg

Product Table Selected Spin Labeling Reagents for ESR-spectroscopy. Please find the complete product list at sigma-aldrich.com/esr

References

- [1] P. P. Borbat et al., Electron Spin Resonance in Studies of Membranes and Proteins, *Science* (2001), 291, 266–269.
- [2] Wayne L. Hubbell et al., Identifying Conformational Changes with Site-directed Spin Labeling, *Nature Structural & Molecular Biology*, (2000), 7, 735–739.
- [3] Linda Columbus and Wayne L. Hubbell, A New Spin on Protein Dynamics, *Trends in Biochemical Sciences* (2002), 27, 288–295.
- [4] Yuri E. Nesmelov and David D. Thomas, Protein Structural Dynamics Revealed by Site-directed Spin Labeling and Multifrequency EPR, *Biophys. Rev.* (2010), 2, 91–99.





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Seminar Dates Karl Fischer Titration 2012

As a service to the scientific community, we routinely offer seminars to provide training on the chemistry behind the Karl Fischer technique and information specific to the HYDRANAL® Karl Fischer reagents line. For 2012, seminars have been scheduled in different cities around the world. Please visit our website at sigma-aldrich.com/hydranal_seminars for regular updates of schedules and registration.

Date	Location
May 24	Cologne, Germany
tbd	Czech Republic, Slovakia
June tbd	Brazil
July 12	Freiburg, Germany
September tbd	South Korea
October 23	Hamburg, Germany



HYDRANAL® E-type Reagents for Karl Fischer Titration

Instrument parameters for endpoint indication with ethanol-based reagents

Andrea Felgner, Market Segment Manager HYDRANAL andrea.felgner@sial.com



A central focus of our HYDRANAL line of pyridine-free Karl Fischer (KF) reagents is the reduction or elimination of toxic components. One such component is methanol, which is widely used as a single solvent in the titration vessel and as a solvent for other KF reagents. Methanol is an excellent compo-

nent for the KF reaction; however, is also classified as toxic according to chemical regulations in the European Union.

With the goal of improving laboratory safety and reducing environmental toxicity, Sigma-Aldrich offers the HYDRANAL E-type reagents for reliable KF titrations for both volumetric and coulometric KF titration.

Eliminating Methanol: HYDRANAL E-type Reagents

The HYDRANAL E-type reagents of Fluka® contain ethanol instead of toxic methanol. Not only can ethanol-based reagents replace methanol-based reagents in many KF applications, but they also offer advantages for dissolving hydrophobic samples. The solubility for long-chained hydrocarbons in ethanol-based HYDRANAL-CompoSolver E is improved over methanol and methanol-containing reagents. Furthermore, alcoholic side-reactions with ketones are often less pronounced in ethanol than in methanol. Consequently, the water content of certain ketones, including acetone, can be determined using HYDRANAL-CompoSolver E in combination with HYDRANAL-Composite titrating agents. NOTE: This is valid only for weakly reactive ketones, and sample sizes should be small. For more reactive ketones, dedicated HYDRANAL media for ketones and aldehydes are recommended.

By using ethanol-based reagents in several applications, we have also been able to eliminate the need for halogenated hydrocarbons like chloroform, dichloromethane and carbon tetrachloride as solubilizing agents.

Examples of applications where the use of ethanolic reagents is preferred over methanol-based reagents:

- L539 Surface preservative, wood decking protector
- L540 5-Hydroxy-1-methylpyrazole
- L456 Peppermint oil and spearmint oil
- L452 Lacquer

Features and Benefits of HYDRANAL E-type reagents:

- Reduced toxicity over methanol-based reagents
- Available for both volumetric and coulometric titrations
- Increased reaction rate and conductivity over pure ethanol by additives
- Endpoint color appears more intense compared to methanolic reagents
- Compatible with all titration equipment (indication parameters may need adjusting)
- Improved solubility for long-chained hydrocarbons/hydrophobic samples
- Enables titration of weakly reactive ketones like acetone without interfering side reaction
- Pyridine-free like all HYDRANAL reagents

Endpoint Indication Techniques for KF Titration

Karl Fischer himself had to use visual detection when he carried out his first titration, but fortunately today we can rely on more accurate standard techniques. Double platinum wire electrodes are generally used for endpoint indication in KF titration. For direct KF titration, two indication methods are primarily used: biamperometric and bipotentiometric (or bivoltametric) indication. A constant voltage or current, respectively, is applied between the two electrodes, and the resulting effect upon the response parameter is detected. Before reaching the equivalence point of the reaction, the working medium does not contain free iodine, so the current needed to maintain the constant voltage is low (or the voltage needed to maintain the constant current is high). As soon as there is a slight amount of excess iodine present, it depolarizes the electrodes and a drastic drop in voltage (or a jump of the current) occurs and indicates the endpoint of the titration.

Biamperometric indication

A constant voltage is applied to the double platinum indicator electrode and the resulting electrical current is recorded. As long as there is an excess of water in the working medium (beginning of the titration), the current necessary to maintain the set voltage is low. However, toward the end of the titration and at the equivalence point, the excess iodine present depolarizes the electrode, and the current rises significantly. The titration curve is usually shown as current [μA] vs. reagent volume [mL].

Bivoltametric or bipotentiometric indication

A constant current is applied to the indicator electrode, and the resulting voltage is recorded. At the beginning of the titration, the voltage is high; at the end of the titration, however, excess iodine molecules decrease the electrical resistance of the solution (cathodic reduction of I_2/I_3^- and anodic oxidation of I⁻), making it possible to maintain the constant current at a much lower voltage. Hence, the voltage drops suddenly at the equivalent point of the titration. The titration curve is usually shown as voltage [mV] vs. reagent volume [mL].

Practical Experiences with HYDRANAL® E-type Reagents

When carrying out titrations with methanol or methanol-based KF reagents, a current of up to 50 μ A is usually programmed as a parameter for endpoint indication (depending on the instrument). However, when using ethanol-based reagents, over-titration may occur at this current level. It can be recognized by a color change to dark yellow or brown at the endpoint, instead of light yellow. Depending on the instrument that is used, the current level should be reduced.

After changing from methanol-based reagents to ethanol-based HYDRANAL-CompoSolver E, Solvent E, or Coulomat E, over-titration may occur only in the first measurement, even if the current was reduced. In this case, the platinum pins of the electrode need to be wiped clean once with a soft paper tissue. Caution needs to be taken so that the pins are not bent or twisted. Afterwards, the electrode is sensitized for accurate endpoint indication.

For accurate endpoint indication, special switch-off parameters must be defined in the method. For example, selecting too low a switch-off voltage can lead to over-titration and erroneous results. Detailed information about the correct indication parameters for ethanol-based reagents can be obtained from the instrument manufacturer.

Reference Standards for Titer Determination in Ethanol-based KF Reagents

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

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

Take advantage of our expertise gained from over thirty years of experience and our extensive applications database on Karl Fischer titration.

On our website sigma-aldrich.com/hydranal-e-types we provide a list of available application reports for use of ethanol-based reagents. To obtain an application report or for any questions, help, or feedback, please contact our HYDRANAL specialists at hydranal@sial.com

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Brand	Description	Cat. No.
Reagents for volumetric titration (one-component technique)		
Fluka®	HYDRANAL-Composite 5 Water equivalent approx. 5 mg H ₂ O/mL	34805
Fluka	HYDRANAL-Composite 2 Water equivalent approx. 2 mg H ₂ O/mL	34806
Fluka	HYDRANAL-Composite 1 Water equivalent 0.7–1 mg H ₂ O/mL	34827
Fluka	HYDRANAL-CompoSolver E To be used with HYDRANAL Composite	34734
Reagents for volumetric titration (two-component technique)		
Fluka	HYDRANAL-Titrant 5 E Water equivalent 5.00 ± 0.05 mg H ₂ O/mL	34732
Fluka	HYDRANAL-Titrant 2 E Water equivalent 2.00 ± 0.02 mg H ₂ O/mL	34723
Fluka	HYDRANAL-Solvent E To be used with HYDRANAL Titrant	34730
Reagents for coulometric titration		
Fluka	HYDRANAL-Coulomat E Ethanol-based reagent, suitable as anolyte and catholyte	34726
Standards for titer determination		
Fluka	HYDRANAL-Water Standard 10.0 Standard for volumetric KF titration (1 g contains 10.0 mg = 1.0% H ₂ O)	34849

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