

# Analytix

Issue 1 • 2011



## Pharmacopeia Traceable Secondary Standards



- Pharmacopeia Traceable Secondary Standards
- Certified Reference Materials
- Phytopharmaceutical Standards
- Ibuprofen Metabolites
- Premium-Quality Cuvettes
- High-Purity Reagents for AAS
- Solvents for PRA and LC-MS

## Pharmacopeia Traceable Secondary Standards



Dr. Matthias Nold  
Product Manager  
Analytical Standards

### Dear Colleague,

The achievements of modern pharmacy and medicine have certainly had a significant impact on the high standard of living we enjoy today. In view of these strides, it is critical that medicines are free from any impurities that might be harmful for the consumers, and that the quality and safety of medicinal products are meticulously tested. Therefore, pharmaceutical production is highly regulated. Standardized analytical methods and reference standards are provided by national and international pharmacopeia such as the United States Pharmacopeia (USP) or the European Pharmacopoeia (EP).

Sigma-Aldrich has now launched a new product line of certified pharmaceutical secondary standards for application in pharmaceutical quality control. These new standards have multi-traceability to the USP, EP and BP primary standards and in addition are manufactured according to ISO/IEC 17025 and ISO Guide 34 (see page 4 of this issue). Thus, these products save you, as our customer, the time and effort of producing your own secondary standards.

In contrast to synthetic drugs, natural drugs or herbal medicinal drugs have been unregulated for many years. However, concentrations of the active components of medicinal plants may vary significantly. Therefore, efforts are increasing to establish an effective quality control for these products. Analysis procedures require reliable analytical standards of the isolated components. In response, Sigma-Aldrich is continually expanding its portfolio of such standards to meet the growing demand. Several new products are now available and can be found on page 10.

A different aspect of the analysis of pharmaceutical drugs is the consideration of their fate when, after having been consumed as a medicine, they are consequently released into the environment through excretion or the disposal of unused products. The impact that these agents and their metabolites have on ecologic systems is increasingly becoming a topic of concern. To meet the needs of environmental analysis, Sigma-Aldrich is now expanding its offering of analytical standards for the most commonly used drugs and their metabolites, as exemplified by the article on Ibuprofen on page 11.

These topics illustrate the broad and extensive product offering of analytical standards and reagents available from Sigma-Aldrich. In this issue of *Analytix*, you will find these three articles along with several other interesting examples of our innovative thinking and expanding product portfolio. I wish you an enjoyable read!

With best regards,

A handwritten signature in black ink, appearing to read 'Matthias Nold', written in a cursive style.

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

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## Pharmacopeia Traceable Secondary Standards

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With our new product line of certified Fluka® pharmaceutical secondary standards, Sigma-Aldrich provides pharmaceutical laboratories and manufacturers with a convenient and cost-effective alternative to pharmacopeia primary standards. These products are multi-traceable to certified reference standards (CRSs) from various pharmacopeia organizations (United States Pharmacopeia (USP); European Pharmacopoeia (EP) and British Pharmacopoeia (BP)).

The FDA, USP and EP all recognize the use of secondary standards or working standards which are established with reference to the corresponding primary standard. The new Fluka pharmaceutical secondary standards eliminate the effort of preparing your own working standards. In addition, the Fluka secondary standards provide larger quantities at less expense.

### The most important product features are:

- Traceability to United States Pharmacopeia (USP) and (if available) also to European Pharmacopoeia (EP) and British Pharmacopoeia (BP) standards
- Analysis performed on instruments validated according to GMP using pharmacopeia monograph methods
- Certified value according to ISO Guide 34 and ISO 17025 using mass balance approach
- Comprehensive certificate according to ISO Guide 31

The secondary standards offered by Fluka are certified reference materials (CRMs) produced according to ISO/IEC 17025 and ISO Guide 34. Their comprehensive certificates

supply values for the shipped material with reference to USP, EP and BP primary standards (the latter two occurring when a corresponding primary CRS is available).

In addition, an independent certified value is given. This allows the Fluka standards to be also used as reference materials for ISO/IEC 17025 accredited labs.

### Comprehensive certificate

The values on the certificate are always traceable to the current pharmacopeia lots. If a valid pharmacopeia lot changes during the given shelf life of our product, then the corresponding Fluka Pharmaceutical Secondary Standard will be recertified with traceability to the new lot and a new certificate will be made available online. For this reason, the valid certificate always needs to be downloaded from the website prior to use of the material.

### The comprehensive certificate contains the following data:

- Expiry date
- Handling and storage instructions
- Traceability assay results versus pharmacopeia primary standards (see the certificate excerpt shown on the next page)
- Certified value by mass balance (according to ISO 17025)
- Comprehensive analytical data

### Product portfolio

Our initial portfolio of pharmaceutical secondary standards comprises 90 selected products which are listed in **Table 1**. The pharmacopeia standards to which the products are traceable are also shown in this list. This portfolio is continually expanding with the ongoing development of new products. Therefore, we recommend that you visit our webpage at [sigma-aldrich.com/pharmastandards](http://sigma-aldrich.com/pharmastandards) for an up-to-date product listing.

- 1 Product specification
- 2 Reference to pharmacopeia monograph (if available) used for the certification
- 3 Analytical conditions of the used method
- 4 Reference to pharmacopeia lot of the primary standard
- 5 Assay value (traceable to the given pharmacopeia lot)
- 6 Uncertainty of the value

### Traceability Assay Results

Specification: 97.0–103.0% (anhydrous, USP/NF) 1  
 98.5–101.0% (dried substance, EP)

**Determination Method: HPLC (ref., USP32: Ibuprofen)** 2

columns: ProteCol-GP C18 125, 4.6 x 250 mm  
 mobile phase: Acetonitrile/Water/chloroacetic acid (600:600:4)  
 column temp.: 30 °C  
 flow rate: 2 mL/min 3  
 injection: 5 µL  
 detector wavelength: 254 nm

**Assay vs. USP Reference Standard (as is basis)**

Assay Value	Expanded Uncertainty	vs. USP LOT	4
99.5%	± 0.6% k = 2.23	K0J008 Labeled Content = 0.999 mg/mg	

Certificate excerpt of traceability assay results (vs the USP reference standard) for Ibuprofen vs USP (77519)

Cat. No.	Description	Package Size	Traceable to		
			USP #	EP #	BP #
75338	Acetaminophen (Paracetamol)	1 g	1003009	P030000	371
44247	Acetanilide Melting Point Standard	1 g	1004001		
59803	Albuterol Sulfate	1 g	1012633	S0150000	302
42572	Ascorbic Acid	2 g	1043003	A1300000	461
01478	Aspirin (Acetylsalicylic acid)	1 g	1044006	A0200000	617
49858	Avobenzone	1 g	1045337		
57947	Azithromycin	1 g	1046056	Y0000306	
94400	Benzoic Acid	1 g	1055002		
51386	1,4-Benzoquinone	1 g	1056504		
53534	Benzyl Alcohol	1 g	1061901	Y0000167	
87808	Butylparaben (Butyl parahydroxybenzoate)	1 g	1084000	B1217000	
40776	Caffeine	1 g	1085003	C0100000	766
01653	Caffeine Melting Point Standard	1 g	1086006		
49939	Carbamazepine	1 g	1093001	C0450000	
76456	Chlorpheniramine Maleate	500 mg	1123102	C1800000	81
19864	Cimetidine	1 g	1134062	C2175000	475
50371	Cimetidine Hydrochloride	1 g	1134073	C2175500	
91033	Ciprofloxacin Hydrochloride	1 g	1134335	C2190000	
02727	Citric Acid	1 g	1134368	A1202000	
16632	Clarithromycin	500 mg	1134379	Y0000320	833
80328	Clindamycin Phosphate	1 g	1138008	C2269000	
02735	Clotrimazole	1 g	1141002	C2430000	379
80688	Cyclosporine	500 mg	1158504	C2163000	
61377	Dehydrated Alcohol	5 x 1.2 mL	1012772		
51156	Dextromethorphan Hydrobromide	500 mg	1181007	D0740000	
55814	Dextrose (D-Glucose)	1 g	1181302	G0350500	
08502	Diethylene Glycol	1 g	1193265	Y0000217	
55527	Diphenhydramine Hydrochloride	1 g	1218005	D2000000	
68508	Dopamine Hydrochloride	1 g	1225204	D2690000	468
80333	Edetate Disodium	1 g	1233009	D2900000	
16221	Erythromycin	500 mg	1242000	E1305000	794
01420	Ethylene Glycol	1 g	1265515		
75769	Ethylparaben (Ethyl parahydroxybenzoate)	1 g	1267000	E2425000	

(continued on page 6)

Cat. No.	Description	Package Size	Traceable to		
			USP #	EP #	BP #
93146	Famotidine	1 g	1269200	F0050000	653
94402	Folic Acid	1 g	1286005	F0300000	
50618	Fructose	1 g	1286504	F0550000	
09205	Furosemide	1 g	1287008	F0700000	547
07748	Gabapentin	1 g	1287303		
01873	Glycerin (Glycerol)	5 g	1295607	G0400000	
73339	Guaifenesin	1 g	1301007	G0700000	
49413	Homosalate	1 g	1311408		
08213	Hydrochlorothiazide	1 g	1314009	H1200000	186
91506	Hydrocortisone	500 mg	1316004	H1300000	576
51585	4-Hydroxybenzoic Acid	1 g	1609013		
77519	Ibuprofen	1 g	1335508	I0020000	539
55322	Lactose (anhydrous)	1 g	1356676	A1206000	
50495	Lactose monohydrate	1 g	1356701	L0100000	
19543	Lidocaine	1 g	1366002	L0595000	727
43618	Mannitol	1 g	1375105	M0200000	
73530	Mesalamine	1 g	1392705	Y0000297	
04635	Metformin Hydrochloride	500 mg	1396309	M0605000	
91564	Methylparaben (Methyl parahydroxybenzoate)	1 g	1432005	M1650000	
77376	Metoprolol Tartrate	1 g	1441301	M1830000	540
00901	Metronidazole	1 g	1442009	M1850000	603
36405	Naproxen	500 mg	1457301	N0250000	435
05296	Niacinamide (Nicotinamide)	1 g	1462006	N0600000	460
38112	Octinoxate	1 g	1477900		
09133	Octisalate	1 g	1477943		
73821	Octocrylene	1 g	1477411		
12262	Omeprazole	1 g	1478505	O0150000	765
80332	Oxybenzone	1 g	1485001		
44207	Phenacetin Melting Point Standard	1 g	1514008		
11529	Phenylephrine Hydrochloride	500 mg	1533002	P1250000	284
38107	Prednisolone	500 mg	1555005	P2700000	464
67298	Prednisone	1 g	1559006	P2900000	553
01941	2-Propanol	3 x 1.2 mL	1570428		
55670	Propylene Glycol	1.5 g	1576708		
13191	Propylparaben (Propyl parahydroxybenzoate)	1 g	1577008	P3650000	
12334	Pyridoxine Hydrochloride	500 mg	1587001	P4100000	458
44404	Ranitidine Hydrochloride	500 mg	1598405	R0150000	471
77391	Residual Solvents Mixture – Class I	3 x 1.2 mL	1601102	R0250000	
28654	Residual Solvents Mixture – Class IIA	3 x 1.2 mL	1601281		
07078	Residual Solvents Mixture – Class IIB	3 x 1.2 mL	1601292		
13555	Residual Solvents Mixture – Class IIC	3 x 1.2 mL	1601306		
38257	Riboflavin	1 g	1603006	R060000	
49650	Salicylic Acid	1 g	1609002	S020000	775
40814	Sorbitol	1 g	1617000	S100000	
19354	Sucrose	1 g	1623637	S1600000	
02344	Sulfanilamide Melting Point Standard	1 g	1633007		
40719	Sulfapyridine Melting Point Standard	1 g	1635002		
74749	Tetracycline Hydrochloride	500 mg	1650006	T060000	480
92033	Theophylline	1 g	1653004	T080000	327
39499	Thiamine Hydrochloride	1 g	1656002	Y0000467	
00443	$\alpha$ -Tocopherol	500 mg	1667600	T1550000	
04571	$\alpha$ -Tocopheryl Acetate	500 mg	1667701	T1600000	
80329	$\alpha$ -Tocopheryl Acid Succinate	500 mg	1667803	T1610000	
02718	Trimethoprim	1 g	1692505	T220000	344
05194	Valproic Acid	1 g	1708707	V0033000	
59725	Vanillin Melting Point Standard	1 g	1711009		

**Table 1** Fluka® Pharmaceutical Secondary Standards: Initial 90 Products

## New 1 g/L TraceCERT® Single Element Standards for AAS and ICP

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 Jürg Wüthrich, Senior Scientist R&D Europe [juerg.wuethrich@sial.com](mailto:juerg.wuethrich@sial.com)



Our Fluka® brand **TraceCERT** product line includes organic and inorganic certified reference materials (CRMs) for various applications. All **TraceCERT** products are produced according to ISO/IEC 17025 and ISO Guide 34 [1,2], meeting the highest metrological standards.

As a part of the **TraceCERT** product line, the 1 g/L single element solutions for ICP and for AAS include standards for over 50 different elements. **Table 1** lists the most recent additions to this product group. A comprehensive and up-to-date listing may be found on our website at: [sigma-aldrich.com/inorganiccrm](http://sigma-aldrich.com/inorganiccrm). The most commonly used elements are also available as concentrated 10 g/L ICP standard solutions.

### **TraceCERT ICP and AAS standard solutions are characterized by:**

- Unique (metrological) level of accuracy and lot-specific values
- Produced according to ISO/IEC 17025 and ISO Guide 34
- Traceability to at least two independent references (i.e. NIST, BAM or SI unit kg)
- Certification according to ISO Guide 35
- Highest-purity starting materials available
- Comprehensive documentation
- Certificates of ICP standards listing up to 70 trace impurities
- ICP standards packaged in light and gas-tight aluminum foil bags
- Competitive pricing

**TraceCERT** standards are supplied in HDPE bottles, except for Hg, which is bottled in a 100 mL borosilicate white glass bottle. The ICP standards are additionally sealed in an aluminum bag. Due to this sophisticated packaging, the standards have low uncertainties and a shelf life of up to four years.

Our website also lists several multi-element standard solutions for ICP. If you cannot find a standard corresponding to your needs in our portfolio, you can request a **customized standard solution** from our online custom standards platform at [sigma-aldrich.com/csp](http://sigma-aldrich.com/csp). There you can define the matrix, analytes and concentrations according to your specific application with a few simple mouse clicks. After your request has been submitted online, we will promptly provide you a quote through an email reply.

#### References:

- [1] **TraceCERT** Traceable Certified Reference Materials. *Analytix 2006, 5. Analytix 2007, 1–4.*
- [2] Double accreditation brings a new class of CRMs. *Analytix 2008, 2.*

Element (1000 mg/L)	Composition	ICP Standards		AAS Standards	
		Cat. No.	Package Size	Cat. No.	Package Size
Germanium	Ge semi-metal + HNO <sub>3</sub> (HF traces)	05419	100 mL	92685	250 mL
Hafnium	Hf metal + HNO <sub>3</sub> + HF	04617	100 mL	55816	100 mL
Tantalum	Ta metal + HNO <sub>3</sub> + HF	16641	100 mL	40413	100 mL
Tellurium	Te semi-metal + HNO <sub>3</sub> (HF traces)	07193	100 mL	68525	250 mL
Tungsten	W metal + HNO <sub>3</sub> + HF	50334	100 mL	53465	100 mL
Yttrium	Y <sub>2</sub> O <sub>3</sub> + HNO <sub>3</sub>	01357	100 mL	40423	250 mL

**Table 1** NEW **TraceCERT** standard solutions (1 g/L) for ICP and AAS

## New TraceCERT® Organic Certified Reference Materials for Environmental and Food Analysis

Matthias Nold, Product Manager Analytical Standards [matthias.nold@sial.com](mailto:matthias.nold@sial.com)

Alexander Rück, Senior Scientist R&D Europe [alexander.rueck@sial.com](mailto:alexander.rueck@sial.com)

Christine Hellriegel, Senior Scientist R&D Europe [christine.hellriegel@sial.com](mailto:christine.hellriegel@sial.com)

The use of traceable certified reference materials is gaining increased importance, especially for accredited testing labs in the areas of food and beverage analysis as well as environmental analysis. We now present several new CRMs as part of the organic **TraceCERT** line. These products, including pesticides, PAHs (polycyclic aromatic hydrocarbons), fatty acids, and antibiotics are intended for use as chromatography standards.

Recently the innovative new product line of organic **TraceCERT** standards (21 amino acids) was presented [1]. These highest quality certified reference materials (CRMs) are certified in a double accredited laboratory fulfilling both ISO/IEC 17025 and ISO Guide 34 and using **high-performance quantitative NMR** for content determination with direct traceability to NIST and SI.

The organic **TraceCERT** reference materials have several benefits:

- Certified content by quantitative NMR (qNMR)
- Superior level of accuracy, calculated uncertainties and lot-specific values
- Traceability to NIST
- Comprehensive documentation delivered with the product (certification according to ISO Guide 31)

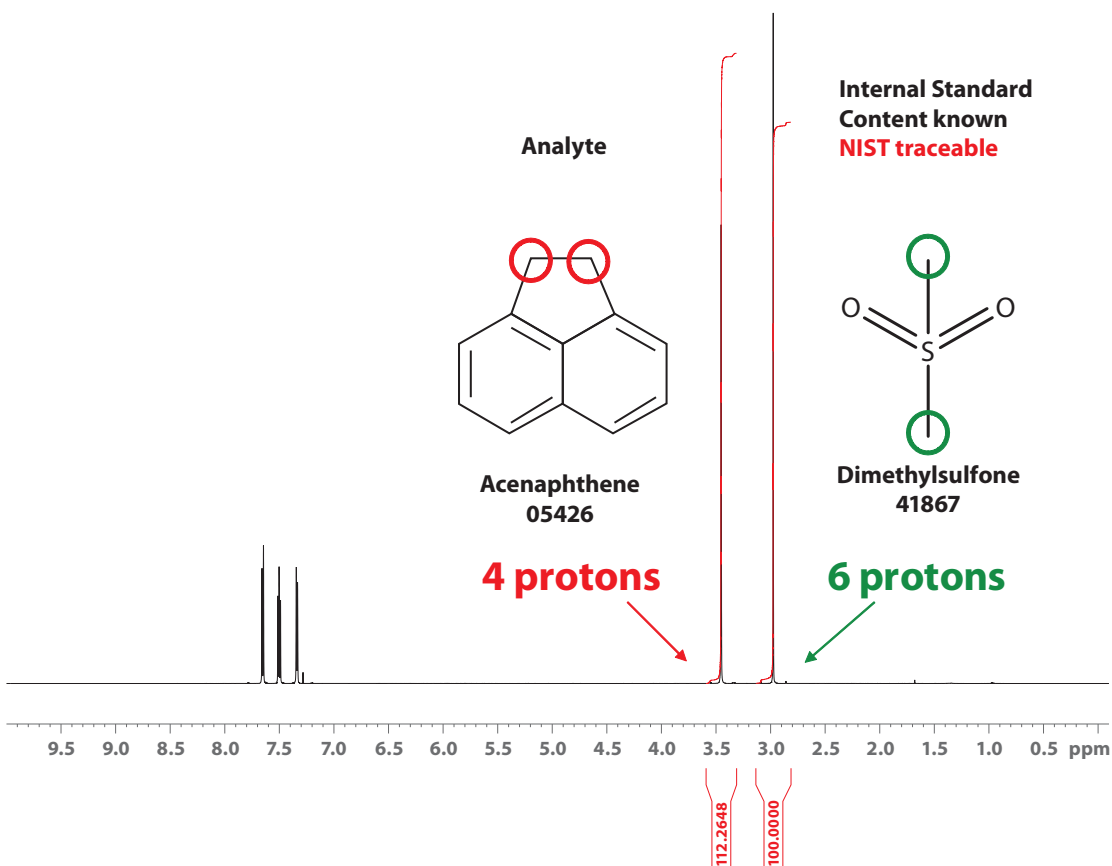
The major advantage of <sup>1</sup>H quantitative NMR as a relative primary method is that the integrals of the proton signals are completely independent of the chemical structure. Therefore, traceability can be established between two different compounds, in contrast to most other analytical techniques.

Thus, with a small set of NIST traceable internal standards, it is possible to certify basically any organic compound by measuring a gravimetrically produced mixture of the analyte and the internal standard. The precise mass-to-mass ratio then allows for the calculation of the analyte content with a very high precision. Typical expanded uncertainties range from 0.5% down to 0.1%.

### Certification of PAHs by qNMR spectroscopy

Polycyclic aromatic hydrocarbons (PAHs), occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of high concern because some of these combustion compounds have been identified to be carcinogenic, mutagenic, or teratogenic. The first PAH CRMs launched as **TraceCERT** products include Acenaphthene, Fluorene, Naphthalene, Phenanthrene and Pyrene.





**Figure 1** qNMR spectrum of Acenaphthene with Dimethylsulfone as an internal Standard

After extensive preliminary tests, the material was quantified by weighing 7–10 separate subsamples together with a suitable reference standard traceable to NIST, dissolved in deuterated solvent, and analyzed by NMR spectroscopy.

**Figure 1** shows the qNMR spectrum of the PAH Acenaphthene using Dimethylsulfone as an internal standard. After the content value has been determined, a comprehensive uncertainty evaluation is performed, leading to an ISO Guide 31 conform certificate associated with the products.

#### New organic TraceCERT® products

**Table 1** lists the most recent product additions, including not only polycyclic aromatic hydrocarbons, but also pesti-

cides, fatty acids / FAME and antibiotics. We are continually working to expand our product portfolio, and new products are added regularly. Please visit our website ([sigma-aldrich.com/organiccrm](http://sigma-aldrich.com/organiccrm)) where you can find an up-to-date product list and download example certificates and technical articles.

#### References:

- [1] TraceCERT Organic Certified Reference Materials, *Analytix*, Vols 2 and 3 2010.

Cat. No.	Description	Package Size	Product Group
05426	Acenaphthene	100 mg	PAH
56849	Fluorene	100 mg	PAH
91489	Naphthalene	100 mg	PAH
73338	Phenanthren	100 mg	PAH
18868	Pyrene	100 mg	PAH
94114	Chlorpyrifos	100 mg	Pesticides
75533	Methylstearate	100 mg	Fatty Acide / FAME
76137	Stearic Acid	100 mg	Fatty Acids / FAME
76177	Sulfamethoxazole	100 mg	Antibiotics

**Table 1** NEW organic neat CRMs TraceCERT

## New Analytical Standards for Phytopharmaceuticals

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Historically, plants have been the most important source for medicinal products. Despite the availability of modern, synthetic drugs, the so-called herbal drugs, or phytopharmaceuticals, are still very popular today. In contrast to synthetic drugs whose production is strongly regulated, herbal drugs have only started being restricted by regulations in recent years. Because their source is natural and they may have been used

traditionally as medicinal products for hundreds of years, the importance of quality control for these products is often not recognized. However, the ingredient profile of a plant extract may differ dramatically across samples, depending on the exact time and

place of the harvest. Thus, also herbal medicinal products require effective control as regards safety and quality. For these reasons, regulative pressure has increased in recent years. In order to monitor the concentrations of active ingredients and other characteristic components, reliable analytical standards are needed. Sigma-Aldrich offers analytical standards, as well as primary reference standards, for active ingredients and marker compounds of medicinal plants. This portfolio is continually expanding. **Table 1** lists some of the most recently added products, including ingredients of the traditional Indian (ayurvedan) medicinal plants curcuma and withania (ashwagandha).

A comprehensive list of our product line can be found on our webpage at [sigma-aldrich.com/medicinalplants](http://sigma-aldrich.com/medicinalplants)

Cat. No.	Description	Package Size	Plant Genus
53653	Santonin-(alpha)	10 mg	Arnica
80098	2',3'-Dehydrosalannol	10 mg	Azadirachta
50878	Procyanidin C1	5 mg	Crataegus, Cinnamomum
90594	Bisdemethoxycurcumin	10 mg	Curcuma
90593	Demethoxycurcumin	10 mg	Curcuma
69727	Hexahydrocurcumin	10 mg	Curcuma
50202	Tetrahydrocurcumin	10 mg	Curcuma
75450	Hydroxycitric acid (Ca salt) (-)	10 mg	Garcinia
44292	Hydroxycitric acid lactone (-)	10 mg	Garcinia
53633	Glabridin	10 mg	Glycyrrhiza
40971	trans-Docosyl caffeate	10 mg	Glycyrrhiza
93169	trans-Eicosanyl caffeate	10 mg	Glycyrrhiza
91595	Chamazulen	20 mg	Matricaria
77347	Matricin	5 mg	Matricaria
18078	4-Allylpyrocatechol	10 mg	Piper
94413	4-Allylpyrocatechol diacetate	10 mg	Piper
97326	Isosilybin A	5 mg	Silybum
95684	Isosilybin B	5 mg	Silybum
59527	Silybin B	5 mg	Silybum
94187	12-Deoxywithastramonolide	10 mg	Withania
89910	Withaferin A	10 mg	Withania
90896	Withanone	10 mg	Withania
94186	Withanoside IV	10 mg	Withania
66042	Withanoside V	10 mg	Withania
74776	Withanolide A	10 mg	Withania, Lycium
94284	Withanolide B	10 mg	Withania, Lycium
89851	Sitosterol-D-glucoside (beta)	10 mg	Withania, Triticum
42630	Gingerol-10	10 mg	Zinigiber

**Table 1** New analytical standards of active ingredients and marker substances for the analysis of herbal medicinal drugs

## Analytical Standards of Ibuprofen and its Metabolites

Matthias Nold, Product Manager Analytical Standards [matthias.nold@sial.com](mailto:matthias.nold@sial.com)

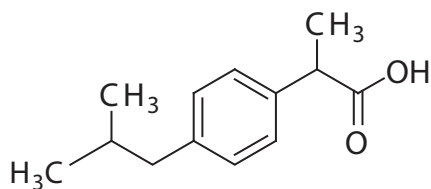
Concerns regarding the environmental effects of pharmaceuticals and personal care products (PPCPs) have been increasing over recent years [1]. These chemical substances are entering the environment through excretion or by disposal of unused medicinal products. Consequently, active components and their metabolites can often be found in sewage treatment plants and in natural aquatic environments.

Moreover, the U.S. Food and Drug Administration (FDA) has published guidelines for metabolism studies and metabolites in safety testing (MIST) [2], establishing new criteria for the testing of drug metabolites. These guidelines underscore the importance of analytical drug metabolite standards for pharmaceutical applications and developments as well.

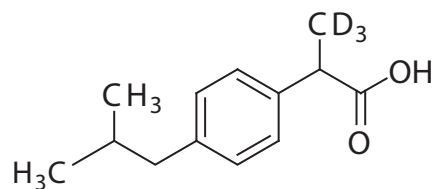
The non-steroidal anti-inflammatory drug ibuprofen is one of the most widely used pharmaceutical drugs worldwide. Therefore, measurable amounts of the drug, as well as of the metabolites, can often be found in the environment. Metabolism of ibuprofen involves oxidation at different sites and glucuronidation [3]. The most relevant metabolites are shown below and are now available at Sigma-Aldrich as analytical standards, in addition to the ibuprofen itself and its deuterated standard.

### References:

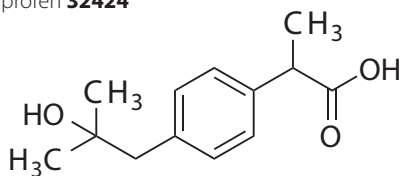
- [1] U.S. Environmental Protection Agency. Pharmaceuticals and Personal Care Products (PPCPs). [www.epa.gov/ppcp/](http://www.epa.gov/ppcp/)
- [2] F.a.D.A. U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER). Guidance for Industry: Safety Testing of Drug Metabolites, (2008).
- [3] Kepp, D. R. et al. *Pharmaceutical Research* 1997, 14, 5.



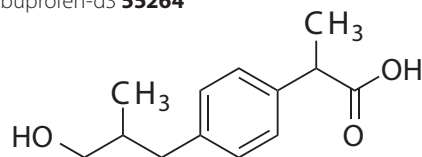
Ibuprofen **32424**



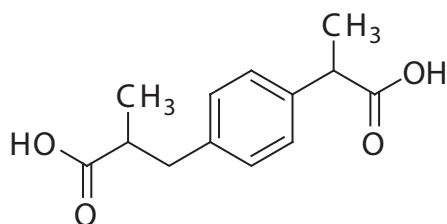
Ibuprofen-d3 **55264**



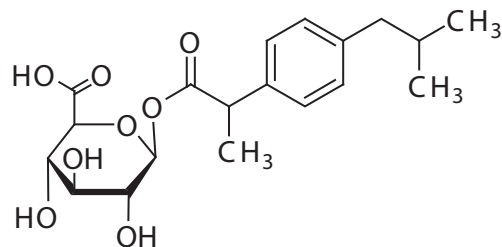
2-Hydroxyibuprofen **32451**



3-Hydroxyibuprofen **50801**



Carboxyibuprofen **32443**



Ibuprofen acyl- $\beta$ -D-glucuronide **68899**

Brand	Cat. No.	Description	Package Size
FLUKA®	32424	Ibuprofen	100 mg
FLUKA	55264	Ibuprofen-d3	50 mg
FLUKA	32451	2-Hydroxyibuprofen	10 mg
FLUKA	50801	3-Hydroxyibuprofen (1 mg/mL in ethanol)	1 mL
FLUKA	32443	Carboxyibuprofen	10 mg
FLUKA	68899	Ibuprofen acyl- $\beta$ -D-glucuronide	5 mg

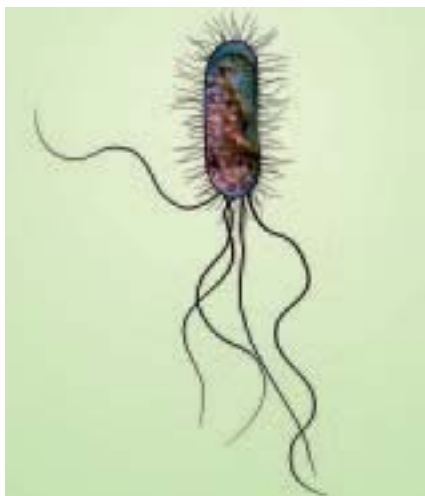
## Enterohemorrhagic *E. coli* (EHEC)

The Shiga-like toxin-producing *E. coli* follows *Campylobacter* and *Salmonella* as the third leading cause of enteritis in humans.

Normally, *E. coli* is a harmless intestinal bacterium. However, some strains are able to produce a Shiga-like toxin, also known as verotoxin, which is able to cleave the n-glycosidic link to adenine in the 28S-rRNA. This iron-regulated reaction leads to cell death, bloody diarrhea, and possible kidney damage [1].

In addition, special core proteins (adhesins) have been found which give the EHEC the ability to adhere to intestinal epithelial cells [5]. In some cases, a heat stable toxin (astA gene) has been found on the bacteria chromosomes [8]. Hemolysin, catalase-peroxidase and the serine protease are also other virulence factors present in EHECs. The genes for these factors have been found on the plasmid pO157, where additional genes for the pathogenic mechanism are likely to be present as well [4, 6, 7].

The pathogenic genes accrue from an infection of *E. coli* from a bacteriophage or from contact with other verotoxin-producing bacteria [2, 3]. The nomenclature of the different EHEC strains is based on the serological differentiation. The “O” from the O157:H7 refers to an antigen present on the cell wall, while the “H” pertains to the antigen found on the flagella.



**Figure 1**  
*E. coli* with flagella

EHEC is found in nature in animals, particularly in cows. Cows, therefore, serve as a source of contamination for raw meat and milk. Ingestion of undercooked meat is the primary route for infection; however, contact infection from animal to human or human to human is possible, since the minimum infection dose is 10–100 cells.

Although *E. coli* O157:H7 is the best-known pathogen causing bloody diarrhea, there are also other noteworthy strains of

EHECs. The most important of these EHEC serotypes are listed in **Table 1**.

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

Serotype	[%]					
	Germany					ECDC 2005
	1999	2004	2005	2006	2009	
O157	34	21	10	17	17	63
O26	20	18	11	16	20	4
O103	4	14	10	17	13	3
O145	4	7	5	6	5	1
O111	3	5	4	3	2	1
O91	6	nd	11	9	11	2
O128	3	nd	1	2	3	1
O146	3	nd	3	2	nd	1
O76	nd	nd	1	nd	nd	0
O55	nd	nd	2	nd	2	0
Orauh	nd	nd	nd	3	3	nd
Non-O157	nd	nd	0	nd	nd	12
untypable	nd	nd	26	5	13	6
others	23	35	16	21	20	8

**Table 1** EHEC serotype distribution in [%] (source: Robert Koch Institute and ECDC)

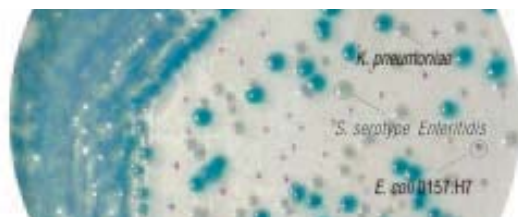
*E. coli* O157:H7 is quite different from other pathogenic and non-pathogenic *E. coli*. It possesses a pilus protein (type of adhesin) for adhesion to the intestinal epithelial cells [9]. *E. coli* O157:H7 is not an enteroinvasive serotype [10] and demonstrates no or limited growth at 44–45.5 °C, particularly in the presence of 0.15% bile salts [11]. It consistently produces EHEC hemolysin [12] and the heat stable toxin EAST1 [13]. There are multiple characteristics which help to differentiate it from other *E. coli* serotypes and from other types of bacteria (see **Table 2**). These features are also incorporated by diverse media to detect and differentiate *E. coli* O157:H7, as shown in **Table 3**.

Selective agents	Bile salts
	Cefixime
	Cefsulodin
	Lauryl sulphate
	Novobiocin
	Tellurite
Differentiating features	Vancomycin
	Typically β-D-glucuronidase negative (MUG, X-Glu)
	Ferments lactose
	Typically not able to ferment sorbitol
	β-galactosidase positive (X-Gal)
	α-galactosidase positive (X-Gal)
Raffinose positive (95%)	

**Table 2** Biochemical characteristics of *E. coli* O157:H7

Media System	Cat. No.	Name	Description
Non-selective + differential system	17178	Mucate Broth	For the identification of enteropathogenic <i>E. coli</i> and <i>Shigella</i> species in milk and milk products (recommended by APHA).
Selective + differential systems	44782	<i>E. coli</i> O157:H7 MUG Agar	A fluorescent selective agar for the isolation and differentiation of enterohemorrhagic (EHEC) <i>E. coli</i> O157:H7-strains from food and clinical material.
	39894	HiCrome™ EC O157 Agar <i>Optional supplement: 0.25 mL/L 1% potassium tellurite solution (Cat # 17774)</i>	A chromogenic medium for isolation and differentiation of <i>E. coli</i> O157 from food and environmental samples.
	72557	HiCrome EC O157:H7 Selective Agar, Base <i>Supplement: 1 vial/L of HiCrome ECO157:H7 Selective Supplement (Cat # 44931)</i>	A chromogenic media recommended for selective isolation and easy detection of <i>E. coli</i> O157:H7 from food samples.
	80330	HiCrome Enrichment Broth Base for EC O157:H7	Recommended for isolation and selective differentiation of <i>E. coli</i> O157:H7 from food and environmental samples by a chromogenic method.
	83339	HiCrome MacConkey-Sorbitol Agar <i>Supplement: 2 vial/L Tellurite-Cefixime Supplement (Cat # 77981)</i>	Recommended for selective isolation of <i>E. coli</i> O157:H7 from food and animal feed. The medium contains sorbitol instead of lactose. <i>E. coli</i> produce purple colonies. The color is due to production of acid from sorbitol and the neutral red indicator and the B.C. indicator, which detects the presence of the enzyme $\beta$ -D-glucuronidase, specific for <i>E. coli</i> . Enteropathogenic strains of <i>E. coli</i> O157 do not possess $\beta$ -D-glucuronidase activity and do not ferment sorbitol, producing colorless colonies.
Selective media	88902	MacConkey-Sorbitol Agar <i>Optional supplement: 2 vial/L CT-Supplement (Cat # 77981)</i>	Recommended medium for selective isolation of pathogenic <i>E. coli</i> O157:H7 which ferments sorbitol but not lactose.
	71882	mEC Broth with Novobiocin	For the selective enrichment of enterohemorrhagic <i>E. coli</i> in food. The medium is used for the isolation and identification of <i>E. coli</i> O157:H7 from meat according to USDA-FSIS methods.
	08069	Modified Tryptone Soya Broth <i>Supplement: 1 vial/L VCC Selective Supplement (Cat # 80704)</i>	Modified tryptone soy broth is recommended as an enrichment medium for the detection of <i>E. coli</i> O157:H7 from food and animal feeding stuffs (recommended by FDA/BAM and CCFRA).
	76704	mTSB Broth with Novobiocin	For the selective enrichment of enterohemorrhagic <i>E. coli</i> in food. The medium meets the requirements of the ISO DIN Norm 10167 for the detection of <i>E. coli</i> serotype O157:H7 in food. It is also recommended by the FDA-BAM as a method for the isolation of enterohemorrhagic <i>E. coli</i> .

**Table 3** List of Sigma-Aldrich media for detection of enteropathogenic *E. coli*



**Figure 2** HiCrome EC O157 Agar (Cat # 39894) not available in USA



**Figure 3** HiCrome EC O157:H7 Selective Agar (Cat # 72557) not available in USA

#### References:

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## High-Purity Reagents for AAS

... auxiliary reagents for interference-free measurements

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



Sigma-Aldrich provides a comprehensive range of reagents and standards for trace and ultra-trace analysis. For a complete list of products, visit our website: [sigma-aldrich.com/traceselect](http://sigma-aldrich.com/traceselect)

### Reducing agents for hydride AAS

Hydride AAS is used for the analysis (especially traces) of arsenic, antimony, tin, selenium, and bismuth. It is used to separate and preconcentrate analytes from sample matrices by a reaction that turns them into their hydride vapors. Fluka® reagents are specifically analyzed to ensure the absence of hydride-generating metals.

Cat. No.	Brand	Product Name	Specification	Package Size
11514	Fluka	Hydroxylamine hydrochloride	TraceSELECT®, ≥99.9995%	100 g
71321	Fluka	Sodium borohydride	for the determination of hydride formers by AAS, ≥99%	25 g, 100 g
44355	Fluka	Tin(II) chloride dihydrate	TraceSELECT, ≥99.999%	250 g, 2.5 kg

**Table 1** Reducing agents for hydride AAS

### Matrix modifiers

Chemical modification should be considered if an analyte is highly volatile, or if the analyte and matrix volatilize at similar temperatures. Such modification would allow ashing at higher (or atomization at lower) furnace temperatures, resulting in elimination of the matrix with no loss of the analyte (or atomization of the analyte, but not the matrix). Depending on the element to be assayed, various substances may be used to perform the chemical modification. Palladium nitrate has emerged as one of the most popular choices. A primary criterion for such substances is a high level of purity guaranteeing the absence of the element to be analyzed. For this reason, the recommended reagents indicated below have been thoroughly tested for their suitability as matrix modifiers.

Cat. No.	Brand	Product Name	Specification	Package Size
428922	Fluka	Ammonium hydrogenphosphate matrix modifier solution	~40% (w/v) in H <sub>2</sub> O	100 mL
428868	Fluka	Calcium matrix modifier solution	2% Ca in 1 wt. % HNO <sub>3</sub>	100 mL
428884	Fluka	Lanthanum matrix modifier solution	5% La in 1 wt. % HCl	100 mL
63043	Fluka	Magnesium matrix modifier	10.0 g/L Mg in ~17 wt. % HNO <sub>3</sub>	50 mL
428906	Fluka	Nickel matrix modifier solution	5% Ni in 1 wt. % HNO <sub>3</sub>	100 mL
76040	Fluka	Palladium matrix modifier	10.0 g/L Pd in ~15 wt. % HNO <sub>3</sub>	50 mL

**Table 2** Matrix modifiers (selection)

### Spectroscopic buffers for flame AAS

In flame AAS, spectroscopic buffers are often used to suppress physical, ionization, and chemical interferences. The Schuhknecht and Schinkel buffer solution, as used in the determination of alkali elements, has become particularly important. A multi-element standard for the elements lithium, sodium, and potassium is also available for this method. The Schinkel buffer solution enables the method to be expanded to include up to 14 mono- and divalent elements, with simple calibration being all that is required for the analysis.

Cat. No.	Brand	Product Name	Specification	Package Size
20980	Fluka	Aluminum nitrate – Cesium chloride buffer for flame atomic absorption spectrometry	Buffer Solution according to Schuhknecht and Schinkel	500 mL
20982	Fluka	Cesium chloride – Lanthanum chloride buffer for flame atomic absorption spectrometry	Buffer solution according to Schinkel	500 mL
95164	Fluka	Cesium chloride – Lanthanum chloride buffer for flame atomic absorption spectrometry	CsCl 100 g/L, LaCl <sub>3</sub> 100 g/L (in water)	250 mL

**Table 3** Spectroscopic buffers for flame AAS

## High-Purity Solvents for Pesticide Analysis

... for LC applications in environmental and food samples

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

**Michael Kiselewsky, Product Manager Chromatography Reagents** [michael.kiselewsky@sial.com](mailto:michael.kiselewsky@sial.com)



Most of the organic pollutants identified in environmental analysis are extracted from matrices, such as water or soil samples, by means of solvents. The abundance of the analytes is quantitatively analyzed following a concentration step. In order to obtain accurate results, it is essential that the solvents used in the quantification process have been established as having very low levels of the substances being analyzed. This precaution allows detection and quantification of the analyte even when it is present at very low concentrations.

The PESTANAL® product line of solvents has been developed specifically for GC/ECD or GC/PND methods in the residue analysis of pesticides and other low-volatile, environmentally

relevant substances. Since polychlorinated biphenyls (PCBs) are also detected in the GC/ECD test, the PESTANAL solvents are suitable for the analysis of this class of substances as well.

These high-purity solvents are manufactured using cutting-edge technology in a modern production facility. They are produced in large, homogenous lots involving multiple purification procedures. The containers are filled under clean-room conditions in contaminant-free, functional packaging materials.

For more information, please visit our website:

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

Method	GC/ECD	GC/PND
Retention range	Pentachlorobenzene to DDT	4-Chloraniline to Coumaphos
Single signals	max. 5 pg/mL	max. 5 pg/mL
Standards	Pentachlorobenzene (Fluka® 35886), Lindane (Fluka 45548), Heptachlor (Supelco® PS78), Aldrin (Fluka 36666), 4,4'-DDD (Fluka 35486), 4,4'-DDT (Fluka 31041)	4-Chloraniline (Fluka 35823), Parathion ethyl (Fluka 45607), Altrazine (Fluka 45330), Coumaphos (Fluka 45403)

**Table 1** Specifications for PESTANAL grade solvents

Cat. No.	Brand	Product Name	Specification	Package Size
34484	Fluka	Hexane	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 4 x 2.5 L
34488	Fluka	Dichloromethane	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 4 x 2.5 L
34480	Fluka	Acetone	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 4 x 2.5 L
31063	Fluka	Ethyl acetate	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 4 x 2.5 L
34496	Fluka	Cyclohexane	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 4 x 2.5 L
34491	Fluka	Acetonitrile	PESTANAL, solvent for residue analysis	1 L, 2.5 L, 6 x 1 L, 4 x 2.5 L

**Product table** PESTANAL solvents (selection)

## LC-MS Solvents and Reagents



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LC-MS is widely becoming a routine fixture in today's well-equipped analytical laboratory. Along with the increased use of LC-MS have come instrumental, chemical and database methods aimed at increasing the sensitivity, specificity and speed of analysis of this invaluable technique. New ion sources, high-resolution LC systems and rapid mass spectrometers with enhanced ion optics and detectors have lowered the limits of detec-

tion, but have raised the bar on the purity expectations of chemicals used for sample preparation, mobile phases and post-column additives.

Alkali ions, plasticizers and surfactants are particularly problematic as they are widespread and interfere strongly with LC-MS by causing higher background noise and the formation of adducts. Because of the integral part that chemistry plays in a successful LC-MS analysis, Sigma-Aldrich has developed and introduced many solvents, additives and reagents that are specifically designed to meet the requirements for high purity and consistency. Find out our latest news on our new webpage under [sigma-aldrich.com/lc-ms](http://sigma-aldrich.com/lc-ms)

## Premium-Quality Cuvettes for Spectrophotometers and Fluorometers

... designed for all common instruments

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

Sigma-Aldrich offers a full line of optical cells made of quartz, glass, and plastics. All cells are manufactured according to high-quality standards and are compatible with all major spectrometry equipment brands.

In order to guarantee a consistent high quality, Sigma-Aldrich offers only quartz and optical glass cells produced by Hellma® Analytics and other selected manufacturers. Every premium-quality optical cuvette is inspected and gauged to meet published specifications. The quartz SUPRASIL® cuvettes provide an excellent spectral performance from below 200 to 2500 nm.



**100-OS**      **101-QS**      **111-QS**      **104-F-QS**

**Figure 1** Various types of glass and quartz cuvettes (for dimensions, see Product Table)

Please visit our website: [sigma-aldrich.com/spectroscopy](http://sigma-aldrich.com/spectroscopy)

Type	Window Material	Wavelength	Dimension H x W x D	Volume	Material Number Sigma-Aldrich	Package Size
100-OS with PTFE lid	Special Optical Glass	320–2500 nm	45 x 12.5 x 12.5 mm	3.5 mL	Z600644-1EA Z600652-2EA	1 cell Box with 2 cells
101-QS with PTFE lid	Quartz SUPRASIL	200–2500 nm	45 x 12.5 x 12.5 mm	3.5 mL	Z600172-1EA Z600180-2EA	1 cell Box with 2 cells
111-QS with PTFE stopper	Quartz SUPRASIL	200–2500 nm	45 x 12,5 x 12.5 mm	3.5 mL	Z600717-1EA Z600725-2EA	1 cell Box with 2 cells
104-F-QS with PTFE lid	Quartz SUPRASIL	200–2500 nm	45 x 12.5 x 12.5 mm	1.4 mL	Z600253-1EA Z600261-2EA	1 cell Box with 2 cells

**Product table** High-precision cuvettes produced by Hellma Analytics (Selection)

## Mobile Phase Comparison Study of LC-MS Solvents

Choosing the right solvents for LC-MS prevents problems

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

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

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

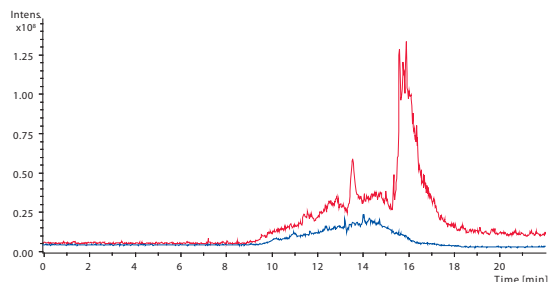
**Figure 2** highlights the difference in sensitivity between the two brands' solvents. This figure shows a chromatogram of a 5 ppm injection of reserpine run at the same gradient used for **Figure 1**'s chromatography. The blue chromatogram illustrates the higher sensitivity and lower interference of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

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

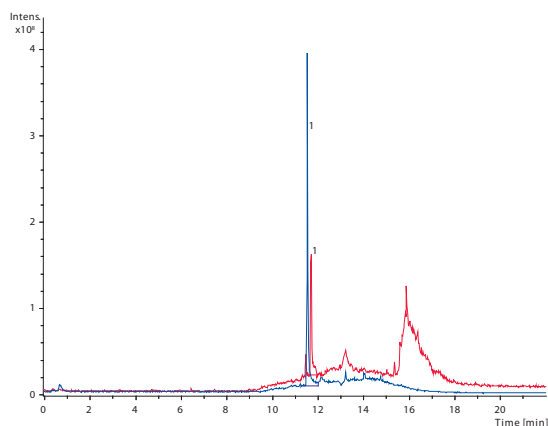
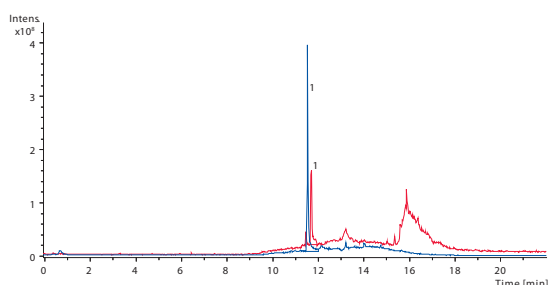
**Figure 4** presents a direct comparison with a leading competitor's Water.

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

Sigma-Aldrich LC-MS CHROMASOLV solvents and blends offer outstanding quality and consistency. They are guaranteed to meet specifications. All products are developed for routine



**Figure 1**



**Figure 2**

analysis, e.g. proteins and peptides (Water/Acetonitrile with 0.1% Formic acid, 0.01% TFA or Water/Acetonitrile with 0.1% TFA) as well as small molecules (Water/Acetonitrile with 0.1% Formic Acid).

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

(continued on page 18)

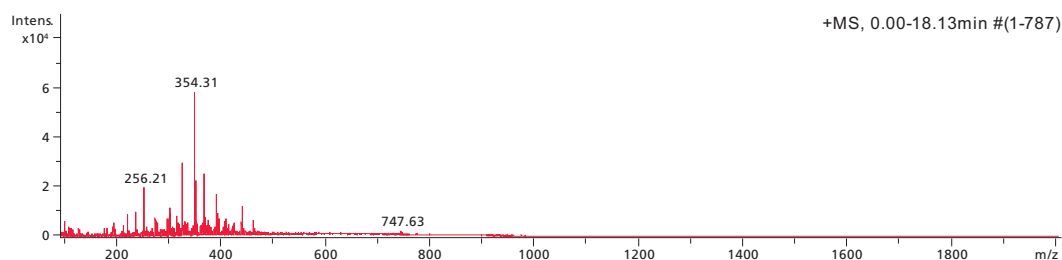


Figure 3

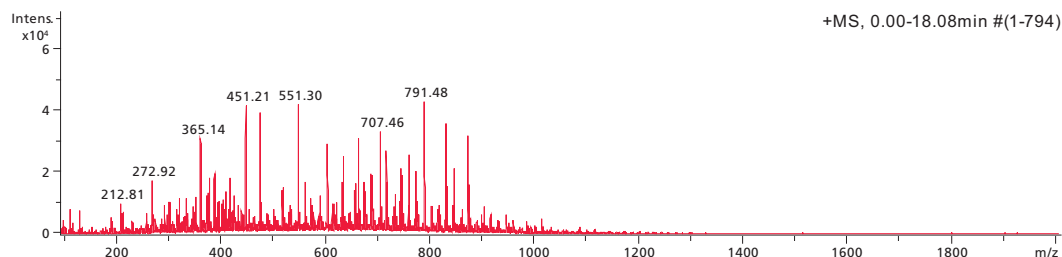


Figure 4

### Solvents

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

### Blends

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

### Additives

Brand	Product	Name	Package Size
Fluka	40967	Trifluoroacetic acid, eluent additive for LC-MS	10 x 1 mL, 50 mL
Fluka	56302	Formic acid, eluent additive for LC-MS	10 x 1 mL, 50 mL
Fluka	49199	Acetic acid, eluent additive for LC-MS	50 mL
Fluka	49916	Propionic acid, eluent additive for LC-MS	50 mL
Fluka	55674	Ammonium formate, eluent additive for LC-MS	50 G
Fluka	49638	Ammonium acetate, eluent additive for LC-MS	50 G
Fluka	61333	Sodium citrate tribasic dihydrate, eluent additive for LC-MS	50 G
Fluka	40867	Ammonium bicarbonate, eluent additive for LC-MS	50 G
Fluka	44273	Ammonium hydroxid solution 25% in water, eluent additive for LC-MS	10 x 1 mL, 100 mL
Fluka	65897	Triethylamine, eluent additive for LC-MS	50 mL
Fluka	34689	Water/2-Propanol 50/50 (v/v), Rinsing Solution I LC-MS CHROMASOLV	1 L
Fluka	34692	Water with 8% formic acid, Rinsing Solution II LC-MS CHROMASOLV	1 L
Fluka	43530	Reserpine standard for LC-MS	4.5 mL
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52038-20EA	Supelco	Silica gel on TLC-Al foils	5 cm x 7.5 cm	254 nm	0.20 mm
23478-50EA	Supelco	Silica gel on TLC-Al foils	5 cm x 10 cm	254 nm	0.20 mm
12606-50EA	Supelco	Silica gel on TLC-Al foils	5 cm x 20 cm	254 nm	0.20 mm
56524-25EA	Supelco	Silica gel on TLC-Al foils	20 cm x 20 cm	254 nm	0.20 mm
55811-20EA	Supelco	Silica gel on TLC-Al foils	5 cm x 7.5 cm	without	0.20 mm
75196-50EA	Supelco	Silica gel on TLC-Al foils	5 cm x 10 cm	without	0.20 mm
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60738-1KG; 60738-5KG; 60738-25KG	Fluka	Silica gel 60Å	for column chromatography	0.035–0.070 mm
60737-1KG; 60737-5KG; 60737-25KG	Fluka	Silica gel 60Å	for column chromatography	0.04–0.063 mm
717177-1KG; 717177-5KG; 717177-25KG	Aldrich	Silica gel 60Å	for column chromatography, technical grade	0.063–0.200 mm
717185-1KG; 717185-5KG; 717185-25KG	Aldrich	Silica gel 60Å	for column chromatography, technical grade	0.04–0.063 mm

Offer valid till 30 April, 2011. Please mention **Promotion Code 954** when ordering, to take advantage of this offer.

## Determination of Water Content in Petrochemical Products

HYDRANAL® Applications: Crude oil and petroleum products

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Water as an impurity in petroleum products can have many negative effects. It accelerates deterioration of lubricants and interferes with lubricant properties. Water causes rust and corrosion of metals, and when combined with dust, may lead to sludge formation. Consequently, water content is an important parameter which must be closely monitored to hold within specifications. To achieve reproducible results for water content using the Karl Fischer (KF) titration method, two fundamental preconditions are required: (1) preliminary homogenization of samples using a homogenizer or ultrasound, and (2) complete dissolution or at least fine dispersion of the sample in the KF working medium for complete extraction of water.

Crude oil and petroleum products mainly consist of long-chained hydrocarbons and have limited solubility in pure methanol. The addition of a solubilizer to the KF working medium, such as chloroform or xylene, improves sample solubility. Allowing for an extraction time before starting the titration can also help to extract the water completely

from the sample. However, problems can be encountered with formulated oils in KF titration because the presence of additives introduces a level of complexity.

### Application recommendations from the HYDRANAL technical service team

Crude oil is basically a mixture of different hydrocarbons containing various amounts of bituminous material. It requires special titration techniques, such as the addition of chloroform to the working medium in order to dissolve the hydrocarbons. Xylene is used to dissolve the bituminous compounds; if these are not finely dispersed, they can stick to the electrode wires and lead to indication problems.

Heavy heating oils also tend to contaminate the electrode wires and vessel walls during KF titration. In this case as well, the addition of chloroform and xylene to the working medium is recommended, as described below in Procedure 1 for crude oil (see also Application L 111).

HYDRANAL®-Solver (Crude) Oil is a practical and reliable product for these applications. It fulfills the requirements of ASTM D 4377-00: in KF analysis using a pyridine-free reagent, a mixture of a KF solvent (for example, HYDRANAL-Solvent) and xylene must be added to the titration vessel (additional international standard methods are presented later in this article). Solubility and titration speed with HYDRANAL-Solver (Crude) Oil are improved in comparison to the solvent mixture. HYDRANAL-Solver (Crude) Oil is recommended for use with both one- and two-component KF techniques; it can be used with HYDRANAL-Composite as well as HYDRANAL-Titrant as titrating agents.

The procedure of water content determination in crude oils is described as follows:

**Procedure 1: HYDRANAL-Solver (Crude) Oil for crude oil samples**

- 30 mL HYDRANAL-Solver (Crude) Oil or
  - 10 mL HYDRANAL-Methanol dry, 10 mL HYDRANAL-Chloroform and 10 mL HYDRANAL-Xylene
- are added to the titration vessel and titrated to dryness with HYDRANAL-Composite/Titrant. A 1–5 g sample of crude oil is then accurately weighed and added using a syringe (weighing by difference). The water content of the crude oil sample is determined by titrating with HYDRANAL-Composite or Titrant.

Oil distillates vary in their composition and thus in their titration demands. Low boiling-point fractions need the addition of chloroform, following titration according to Procedure 2. The coulometric KF method (Procedure 2C) is recommended, since the water content of distillates is typically very low. Standard coulometric reagents can be used: HYDRANAL-Coulomat A, AG-H and Oil already contain solubilizing agents.

Kerosene behaves similarly and has a low water content in the ppm range. To improve the solubility of kerosene, the

chloroform content should be increased for the coulometric determination. A mixture of HYDRANAL-Coulomat A and HYDRANAL-Chloroform should be used (see Procedure 2C); 40 mL of kerosene will dissolve in 100 mL of this mixture (see Application L112).

**Procedure 2: Addition of chloroform as a solubilizing agent**

For an exact determination of water, the methanol content should not be less than 35% by volume, otherwise, the KF reaction will not proceed stoichiometrically.

A) Chloroform addition, volumetric one-component titration

- 30 mL HYDRANAL-LipoSolver CM or
- 10 mL HYDRANAL-Methanol dry, HYDRANAL-Methanol Rapid or HYDRANAL-CompoSolver E and 20 mL HYDRANAL-Chloroform

are added to the titration vessel and titrated to dryness with HYDRANAL-Composite. The sample is then added and titrated in the same manner.

B) Chloroform addition, volumetric two-component titration

- 30 mL HYDRANAL-Solvent CM or
- 10 mL HYDRANAL-Solvent or HYDRANAL-Solvent E and 20 mL HYDRANAL-Chloroform

are added to the titration vessel and titrated to dryness with HYDRANAL-Titrant or HYDRANAL-Titrant E. The sample is then added and titrated in the same manner.

C) Chloroform addition, coulometric titration

The anodic compartment of the coulometric titration cell is filled with

- 100 mL HYDRANAL-Coulomat Oil or
- 70 mL HYDRANAL-Coulomat A and 30 mL HYDRANAL-Chloroform

The cathodic compartment is filled with 5 mL HYDRANAL-Coulomat CG. After titrating to dryness, the sample is injected with a syringe through the septum into the anolyte compartment. Generally, a sample size of 0.5–5.0 mL in liquid form is recommended.

We have also investigated several motor oils and certain additives. The water content of an oil sample without any additives can be determined easily according to Procedure 2, with the use of chloroform. Polyols, such as antifreeze, can be analyzed by following the standard volumetric one-component procedure with HYDRANAL-Methanol dry, Methanol Rapid or CompoSolver E and HYDRANAL-Composite. For dissolution of olefin copolymers, a 4:1 mixture of HYDRANAL-Medium K and HYDRANAL-Formamide



(continued on page 22)



dry should be used as a working medium; the water determination is straightforward under these conditions. For analysis of polybutene, a 3:1 mixture of HYDRANAL®-Chloroform and HYDRANAL-Methanol dry is recommended as a working medium.

Silicone oils present fewer problems. Their analysis requires the addition of HYDRANAL-Chloroform in order to improve the solubility. We recommend following Procedure 2A/2B, modified by using a titrating agent with small water equivalent because of the low water content of the oils (such as HYDRANAL-Titrant 2 (E), Composite 2 or Composite 1). For coulometric determinations, Procedure 2C is recommended. Insulating oils (transformer oils) resemble silicone oils and can be analyzed according to the same procedure.

#### **KF oven method for oils containing additives**

Additives are added to many lubricant products such as engine oils, transformer oils or machine oils to optimize their performance. They improve viscosity, lower the pour point, prevent oxidation, reduce friction, suppress the formation of foam, amalgamate or separate solids, and provide a number of other functions.

Particular attention is needed when analyzing formulated lubricants, since their constituents can interfere with the KF titration. Specific information about the chemical nature

of these additives is usually not available. Experience in our HYDRANAL lab has shown that additives can simulate high water content in the KF titration through side-reactions with methanol or iodine. Consequently, it is not surprising that incorrect values for the water content are obtained by direct KF titration of oils containing additives.

For samples such as these, we recommend the indirect KF oven method, where the sample is heated and thereby releases its water content. The water is then transferred to the titration vessel with a suitable carrier gas (for example, air or nitrogen, where nitrogen is preferred when the sample is sensitive to oxidation at temperatures of 100–300 °C). The sample itself does not get in contact with the KF reagents; therefore, interfering side-reactions can be circumvented. Considering the very low water content of most oil samples, the KF oven is primarily used in combination with a coulometer; however, it can also be used in combination with volumetric KF titration.

Of particular importance is the determination of the optimum oven temperature to remove the water from the sample. It must be high enough to drive off the sample water within 10–15 minutes; however, it must also be kept low enough to prevent vaporization or decomposition of the sample matrix, which could interfere in the KF titration. A suitable temperature must be determined individually for

each sample: a programmed temperature ramp is recorded, where the sample is gradually heated from 50 °C to 250 °C within 50 minutes. The resulting profile shows the water release across the temperature scan which characterizes the release of volatiles in the sample. The suitable temperature for complete release of sample water can thus be determined. An example for investigation of oil samples with additives using the indirect KF method is given in Application Report L531.

### International standard methods

In these standards, the Karl Fischer titration is prescribed for measuring the water content of petroleum and related products:

ASTM D890	Water in Liquid Naval Stores
ASTM D1123	Water in Engine Coolant Concentrate
ASTM D1533	Water in Insulating Liquids
ASTM D4377	Water in Crude Oils (volumetric KFT)
ASTM D4672	Water in Polyols (Polyurethane Raw Materials)
ASTM D4928	Water in Crude Oils (coulometric KFT)
ASTM D5530	Water in Hazardous Waste Fuel
ASTM D6304	Water in Petroleum Products, Lubricating Oils, and Additives
ASTM E203	Standard Test Method for Water Using Volumetric Karl Fischer Titration
DIN 51777-1	Water in petroleum hydrocarbons and solvents (direct KFT)
DIN 51777-2	Water in petroleum hydrocarbons and solvents (indirect KFT)
ISO 10336	Water in crude petroleum (volumetric KFT)
ISO 10337	Water in crude petroleum (coulometric KFT)
ISO 6296	Water in petroleum products (volumetric KFT)
ISO 12937	Water in petroleum products (coulometric KFT)

The following HYDRANAL® application reports for water content determination in crude oil and petroleum products are available upon request:

L108	Crude oil
L109	Engine oil, used (I)
L110	Engine oil, used (II)
L112	Kerosene
L190	Mineral oil with additives
L462	Insulating Oil
L477	Polyglycol-based grease with additives
L534	Oil, cold compressor oil with Freon
L545	Lubricating oil
L585	Used hydraulic fluid

L111	Fuel oil, heavy
L148	Crude oil (coulometric)
L184	Mineral oil without additives
L188	Polybutene
L412	Greases
L381	Hardness oil, used
L531	Oils, with additives
L107	Hydraulic oil

These applications can be obtained from our HYDRANAL experts at [hydranal@sial.com](mailto:hydranal@sial.com). Contact them also for application support, choice of reagents, or any technical question about Karl Fischer titration. A complete list of HYDRANAL Applications is displayed on our website [sigma-aldrich.com/hydranal](http://sigma-aldrich.com/hydranal)

Are you interested in our **HYDRANAL Manual**? It contains detailed information about the application of HYDRANAL reagents and Karl Fischer titration procedures for a wide variety of samples. Check the box on the attached business reply card and receive your free copy!

Cat. No.	Description
<b>Volumetric one-component reagents</b>	
34805	HYDRANAL-Composite 5
34806	HYDRANAL-Composite 2
34827	HYDRANAL-Composite 1
34741	HYDRANAL-Methanol dry
37817	HYDRANAL-Methanol Rapid
34734	HYDRANAL-CompoSolver E
37855	HYDRANAL-LipoSolver CM
34698	HYDRANAL-Medium K
34697	HYDRANAL-Solver (Crude) Oil
<b>Volumetric two-component reagents</b>	
34732	HYDRANAL-Titrant 5 E
34723	HYDRANAL-Titrant 2 E
34801	HYDRANAL-Titrant 5
34811	HYDRANAL-Titrant 2
34730	HYDRANAL-Solvent E
34800	HYDRANAL-Solvent
34812	HYDRANAL-Solvent CM
34697	HYDRANAL-Solver (Crude) Oil
<b>Coulometric reagents</b>	
34807	HYDRANAL-Coulomat A
34843	HYDRANAL-Coulomat AG-H
34868	HYDRANAL-Coulomat Oil
34840	HYDRANAL-Coulomat CG
34726	HYDRANAL-Coulomat E
<b>Auxiliary Reagents</b>	
37863	HYDRANAL-Chloroform
37866	HYDRANAL-Xylene
34724	HYDRANAL-Formamide dry
<b>Recommended water standards</b>	
34694	HYDRANAL-Water Standard Oil
34693	HYDRANAL-Water Standard KF-Oven 140–160 °C
34748	HYDRANAL-Water Standard KF-Oven 220–230 °C

**Table 1** Fluka brand HYDRANAL Karl Fischer reagents

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