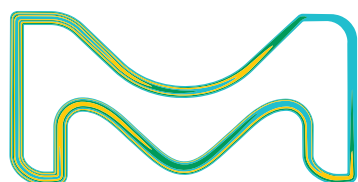


LC-MS Grade Solvents and Reagents

for accurate, brilliant results



K123456789
2018/12/07
2.5 l
1.00029.2500
Supelco
LiChrosolv®
Acetonitrile
hypergrade for LC-MS
Acetonitril
Acetonitrilo
Acétonitrile
Made in Germany
CAS-No. 75-05-8
Merck KGaA, 64271 Darmstadt
Germany, Tel. +49(0)6151 72-2440
EMD Millipore Corporation
400 Summit Drive, Burlington MA 01803
USA, Tel. +1-978-715-4321
sigmaaldrich.com
© 2018

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Supelco®
Analytical Products

LC-MS: Raising the bar on purity

Liquid chromatography - mass spectrometry (LC-MS) is fast becoming a routine fixture in today's well-equipped analytical laboratory. Along with the increased use of LC-MS comes 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 detection, but have raised the bar on the purity expectations of reagents used for sample preparation, mobile phases, and as additives. Some notable examples of how the purity and composition of the chemicals used in LC-MS that affect the analysis include:

- Polymers—including biopolymers such as proteins and DNA—form adducts with inorganic salts, leading to complex mass spectra and a broad distribution of multiply-charged sodium, potassium and chloride adducts.

- Salts can suppress ionization in ESI sources, even with small molecules.
- Reagents, solvents and devices used in sample preparation along with additives always present a risk of contamination.

Some particular compound classes that can be problematic are alkali ions, plasticizers and surfactants, as they are widespread and interfere strongly with LC-MS by forming adducts and causing higher background noise as well as leading to signal suppression. Because of the integral part that chemistry plays in a successful LC-MS analysis, we have developed and introduced a broad portfolio solvents, additives and reagents which are designed specifically to meet the requirements of high purity and consistency. This brochure contains a compilation of articles on LC-MS additives and the advantages of high purity solvents for both small and large molecule analysis.



Table of Contents

Solvents and Blends for LC-MS	4
LC-MS Reagents and Additives	7
Chemical Derivatization Reagents for LC-MS.	9
UHPLC-MS Analysis of Insulin Analogs	10

Solvents and Blends for LC-MS

Superior resolution and sensitivity

Why use LC-MS grade solvents?

- No ghost peaks
- Full reproducibility
- Extends the lifetime of your columns
- Optimized and tested for LC-MS applications
- Minimal background ion suppression
- Global availability

Why Pre-Blended?

- Reduces the risk of contamination
- Saves time
- No cleaning of glassware or filtration
- Less exposure to hazardous chemicals

As LC-MS is a highly sensitive analytical technique, impurities in your solvents can have an impact on the accuracy and reproducibility of your analytical results. Have confidence in your analysis by using our high purity solvents and blends designed to meet the demanding requirements of LC-MS applications, ensuring baseline stability, lowest impurity levels and, in addition, high UV transmittance.

Developed specifically for LC-MS, we have introduced a range of high purity solvents pre-blended with acetic acid, formic acid or trifluoroacetic acid, to provide convenient ready-to-use mobile phases for accurate LC-MS.

This complete product portfolio sets the standard for accurate, reproducible and high-resolution analytical separations for superior performance and sensitivity.



Product No.	Name	Description	Package Size
159004.2500 159004.4000	Acetonitrile + 0.1% Acetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL
159002.1000 159002.2500 159002.4000	Acetonitrile + 0.1% Formic acid (v/v)	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
159014.2500 159014.4000	Acetonitrile + 0.1% Trifluoroacetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL
159007.2500 159007.4000	Water + 0.1% Acetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL
159013.2500 159013.4000	Water + 0.1% Formic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL
480112.2500 480112.4000	Water + 0.1% Trifluoroacetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL
100029.1000 100029.2500 100029.4000 100029.9010 100029.9030 100029.9185	Acetonitrile	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL 10 L ST** 30 L ST** 185 L ST**
106035.1000 106035.2500 106035.4000	Methanol	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
115333.1000 115333.2500 115333.4000 115333.9010 115333.9030	Water	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL 10 L ST** 30 L ST**
103649.1000 103649.2500 103649.4000	Ethyl acetate	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
103701.1000 103701.2500 103701.4000	Hexane	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
103654.1000 103654.2500 103654.4000	Heptane	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
102781.1000 102781.2500 102781.4000	2-Propanol	hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL

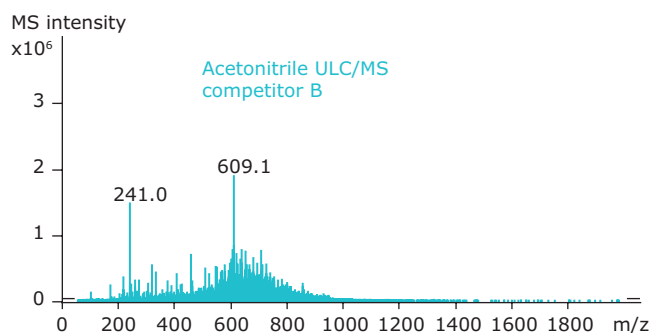
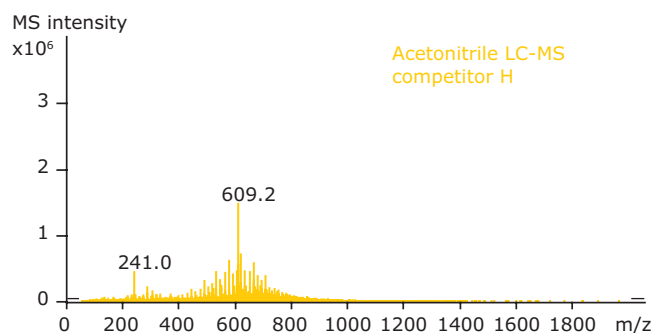
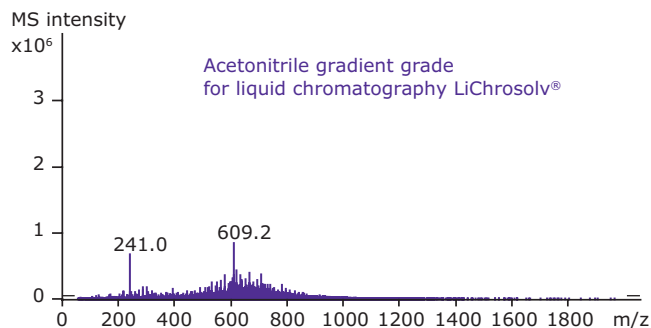
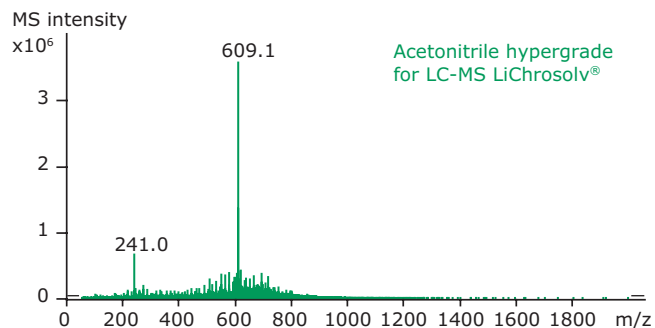
All bottle: special treated amber glass bottle.

** mandatory returnable

All solvents are filtered through 0.2 µm. | GL = glass bottle

ST = stainless steel returnable barrel; additional withdrawal systems necessary; only available in European Union

Why your choice of solvent matters



MS conditions

System	Bruker Esquire 3000+ ion trap MS
Detection	Pos. ESI-MS, m/z range 50 – 2000
Flow rate	0.2 mL/min via syringe pump
Temperature	25 °C
Sample	Reserpine (m/z 609.1), internal standard (m/z 241.0)

Mass spectra displaying the results of reserpine comparing different acetonitrile qualities from Merck and two alternative competitors.

The mass spectra of these four different acetonitrile grades clearly shows the variation in the intensity of the reserpine signal ($[M+H]^+ = 609$), as well as the extent of the background signals. The differences in the intensity of the reserpine signal are caused by ion suppression. This effect occurs due to interfering trace contaminants that can be present in acetonitrile, which can be avoided using the correct high grade solvent for this purpose.

LC-MS Reagents and Additives

Features:

- LC-MS application tested for consistent quality according to the reserpine test
- Optimized to improve ionization and resolution
- Extremely low levels of inorganic and organic impurities
- Manufactured specifically for accurate and fast LC-MS
- Highest quality acids, bases & salts - specified in the certificate of analysis

Introduction

It is common practice in LC-MS to add certain reagents to the mobile phase, or to introduce them post-column prior to the interface to influence analyte ionization. Most often the goal is for an improvement in the analyte signal. In addition, some additives may be used to suppress unwanted signals, or selectively enhance the signal of particular compounds in a mixture. For example, glycosidic species in a mixture of peptides.

To help you obtain the highest quality analysis, we offer a wide range of high purity mobile phase additives for LC-MS applications. The LC-MS portfolio includes the most commonly used acids, bases and volatile salts of high purity tested for LC-MS applications.

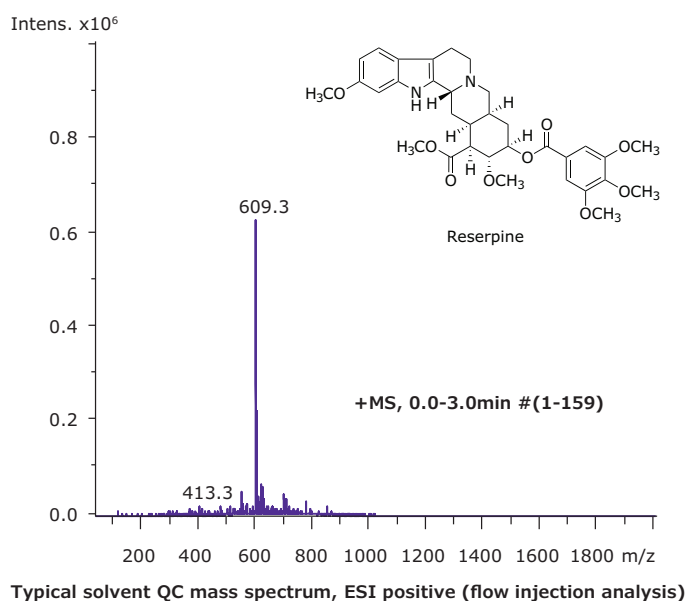
Impurities, such as alkali ions, plasticizers or surfactants, that can be commonly found in lower-grade solvents are particularly problematic as they interfere strongly with LC-MS, resulting in

higher background noise and formation of adducts. Only ultrapure reagents enable high signal-to-noise ratios, which results in the highest and most reliable performance for small and large molecule applications.

Reserpine test

All of our LC-MS solvents and reagents are specified using the standard reserpine test. Reserpine (608.68) is used as the reference substance to quantify possible impurities in the LiChropur® LC-MS reagents. It is performed by diluting 2.5% (v/v) acid, base or 2.5% (w/v) salt in 50/50 (v/v) acetonitrile/water. Every lot produced is analyzed via flow injection analysis mass spectrometry (FIA-MS). The dissolved reagent and the appropriate reserpine reference solutions are introduced into the MS ion source syringe pumps. The total ion chromatogram (TIC) is accumulated during three minutes. The relative intensities of the detected masses are compared with the reserpine signal.

For electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive mode, the specified amount of reserpine is 2 ppb for acids and bases, and 20 ppb for salts. In the negative mode, the specified amount of reserpine is 20 ppb for both.



Acid additives

Volatile, low molecular weight organic acids such as formic and acetic acid or novel difluoroacetic acid (DFA) improve ionization and resolution of a wide range of molecules. Addition of organic acids to the mobile phase can help to overcome the ionization-suppressing effect of trifluoroacetic acid (TFA) present in the mobile phases used for the analysis of proteins and peptides.

Neutral salts

Neutral volatile salts, such as ammonium acetate or ammonium formate are typically used as buffer compounds to control the ionization state of the analytes (and phases), which has a strong influence on the LC-MS separation and performance.

Sodium adduct formation

Alkali adducts diminish instrument sensitivity. When adduct formation tendency is strong, often the addition of defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

Cat. No.	Substance	Description	Package Size
5.33001.0050	Acetic acid	100% for LC-MS LiChropur®	50 mL
5.33002.0050	Formic acid	98-100% for LC-MS LiChropur®	50 mL
5.33003.0050	Ammonia solution	25% for LC-MS LiChropur®	50 mL
5.33004.0050	Ammonium acetate	for LC-MS LiChropur®	50 g
5.33005.0050	Ammonium hydrogen carbonate	for LC-MS LiChropur®	50 g
51197	Sodium formate solution	Suitable for LC-MS LiChropur®	100 mL
00922	Difluoroacetic acid	for LC-MS LiChropur®	1 mL, 10x1 mL, 50 mL
80457	Trifluoroacetic acid	Eluent additive for LC-MS LiChropur®	10x1 mL, 10 mL, 50 mL
18370	2,2,2-Trifluoroethanol	Eluent additive for LC-MS LiChropur®	1 mL, 10x1 mL, 50 mL
18127	1,1,1,3,3,3-Hexafluoro-2-propanol	Eluent additive for LC-MS LiChropur®	10 mL, 50 mL

Extensive QC testing ensuring highest specification

Residue on ignition (evaporation residue) tests show the low content of insoluble matter in the reagent. This provides confidence that your eluents have the low particle content needed for accurate LC-MS measurement.

Sodium and Potassium ions are particularly likely to form adducts with the analyte molecules. This leads to complex mass spectra leading to time-consuming data evaluation. The content of trace metals is in the low ppb range for LiChropur® LC-MS reagents to minimizing the risk of adduct formation in the ion source for cleaner results.

Our LiChropur® LC-MS reagents are stored in borosilicate bottles to prevent leaching of alkali ions out of the glass. The content of the potentially complex forming ions aluminum, copper and iron is also specified.

Full specification can be found in the certificate of analysis for each of our LC-MS grade products.

Specification (Acids/Bases)

Assay (acidimetric)	≥ 98,0%
Colour	≤ 10 Hazen
Residue on ignition	≤ 2 ppm
Al	≤ 5.0 ppb
Ca	≤ 10.0 ppb
Cu	≤ 1.0 ppb
Fe	≤ 5.0 ppb
K	≤ 5.0 ppb
Mg	≤ 2.0 ppb
Na	≤ 5.0 ppb
NH ₄ ⁺	≤ 10 ppm
LC-MS Suitability ESI Positive (Reserpine Test)	≤ 2 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine
LC-MS Suitability ESI Negative (Reserpine Test)	≤ 20 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine

Chemical Derivatization Reagents for LC-MS

Modern mass spectrometry techniques such as APCI or ESI are highly successful in providing valuable structural information, and allow the detection of very low analyte concentrations in various sample matrices. For certain samples e.g. non-polar compounds, and in research areas, such as clinical metabolomics and forensics analytics, there are many cases where such methods can be insufficiently sensitive.

Derivatization reactions in mass spectrometry are used to improve ionization efficiency [1-4]. The derivatization reagents have functional groups possessing high proton (cation) affinity that stabilize a positive charge. Of similar importance when derivatizing is the improvement of qualitative analysis by modifying fragmentation behavior to form unique product ions and the shifting. Finally, derivatization can enhance precise quantitative analysis for profiling of relatively small analyte molecules, particularly in metabolomics.

References

1. Zaikin V, Halket J, 2009. A handbook of derivatives for mass spectrometry. Chichester: IM Publications LLP,
2. Santa T. 2013. Derivatization in liquid chromatography for mass spectrometric detection *Drug Discov. Ther.* 7:9-17
3. Santa T. 2011. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry. *Biomed. Chromatogr.* 25:1-10
4. Santa T, Al-Dirbashi OY, Fukushima T. 2007. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry for biomedical analysis. *Drug Discov. Ther.* 1:108-118.

Cat. No.	Derivatization Reagent	Analyte Functional Group	Typical Application
05689	Diethyl ethoxymethylenemalonate	Amine	Amino acids
29208	(N-Succinimidylloxycarbonylmethyl) tris(2,4,6-trimethoxyphenyl)phosphonium bromide	Amine	Protein sequence analysis
61224	N-Succinimidyl 4-(dimethylamino)benzoate	Amine	Glycerophosphoethanolamine lipids
73177	1-Fluoro-2,4-dinitrobenzene	Amine	Prim./sec. aliphatic amines
73103	Dibenzyl ethoxymethylenemalonate	Amine	Amino acids
03334	Dansylhydrazine	Carbonyl	—
4465962	Amplifex Keto Reagent Kit	Carbonyl	—
5037804	Amplifex Diene Reagent Kit	Diene	—
65562	2-Picolylamine	Carbonyl	Steroids
89397	Girard's reagent T	Carbonyl	Nucleosides
93742	Pentafluorophenylhydrazine	Carbonyl	Oligosaccharides
79291	4-[2-(N,N-Dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole	Carboxylic acid	Fatty acids
42579	4-Phenyl-1,2,4-triazoline-3,5-dione	Diene	Vitamin D
97622	2-Mercaptoethanol	Double bond	Microcystins
00721	4-(Dimethyl-d ₆ -amino)benzoyl chloride	Hydroxy	Deuterium mass shift
03641	Dansyl chloride	Hydroxy	—
05022	N,N-Dimethylglycine	Hydroxy	Cholesterol
06696	3-Amino-9-ethylcarbazole	Hydroxy	Sugars
67954	4-(Dimethylamino)benzoyl chloride	Hydroxy	17β-Estradiol
72702	3,5-Dinitrobenzoyl chloride	Hydroxy	Tetrahydrocorticosterones
41368	p-Toluenesulfonyl isocyanate	Hydroxy	Steroids
49432	Pyridine-3-sulfonyl chloride	Hydroxy	Steroids
55952	Fusaric acid	Hydroxy	Steroids
93535	N-(Propionyloxy)succinimide	Amine	Histones

UHPLC-MS Analysis of Insulin Analogs on BIOshell™ A160 Peptide C₁₈ Using Difluoroacetic Acid as a Mobile Phase Modifier

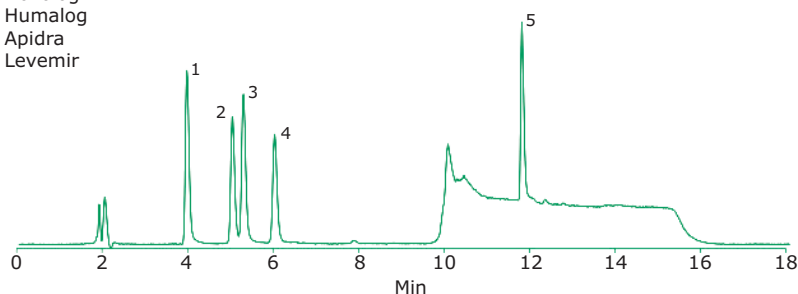
Insulin is an approximately 6000 Da peptide hormone used to regulate the glucose concentration in blood. An insulin analog is a synthetic form of insulin, different from any occurring in nature, but still able to mimic the human body's pattern of insulin release to control glycemia.

A quick and reliable chromatographic technique was developed to identify and detect a mixture of insulin analogs in an unknown sample. This is of utmost importance to pharmaceutical quality control (QC) labs. With this application the ability to resolve these insulin analogs (which sometimes only differ by one or two amino acid substitutions) with high sensitivity in a complex matrix (rat plasma) is demonstrated. The use of difluoroacetic acid (DFA) has less of an effect on ion suppression than trifluoroacetic acid (TFA), thus allowing for mass spectral detection of the insulin analogs.

Experimental Methods

A mixture of five insulin analogs (Lantus, Novolog, Humalog, Apidra, Levemir, 20 µg/mL, 6:3:1 methanol:water:acetic acid containing 0.1% rat plasma) was analyzed chromatographically on a BIOshell™ A160 Peptide C₁₈ HPLC column using a Thermo Ultimate™ BioRS 3000 UHPLC (UV detection) and an Agilent® 1290/6530 Q-TOF system (mass spectral detection). All analyses were performed in triplicate and the elution order of the insulin analogs was confirmed by mass spectral analysis.

1. Lantus
2. Novolog
3. Humalog
4. Apidra
5. Levemir



column	BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.0 µm particles (67243-U)
column temp.	75 °C
mobile phase	[A] 75:25 (0.1% DFA in water: 0.1% DFA in acetonitrile); [B] 50:50 (0.1% DFA in water: 0.1% DFA in acetonitrile)
gradient	23% B for 7 min; 23% B to 100% B in 1 min; hold at 100% B for 7 min
flow rate	0.2 mL/min
pressure	2500 psi (172 bar)
sample	Analog Mixture, 20 µg/mL, 6:3:1 methanol:water:acetic acid containing 0.1% rat plasma
injection	0.5 µL
detector	ESI(+), TIC 100-3000 m/z

Description

Analysis Note	BIOshell™ A160 Peptide C18 is an ideal column for reversed-phase separations of small proteins to large peptide sequences. Due to the sequence similarity between these insulin analogs in the sample; the separation is achieved through the subtle differences in the polarities of surface residues on the peptides. The use of difluoroacetic acid (DFA) has less of an effect on ion suppression than trifluoroacetic acid (TFA), thus allowing for mass spectral detection of the insulin analogs.
Categories	Analytical Chromatography, Proteins, Peptides
suitability	application for UHPLC

Materials

Cat. No.	Description
5.33001	Acetic acid 100% for LC-MS LiChropur®
1.00029	Acetonitrile hypergrade for LC-MS LiChrosolv®
66905-U	BIOshell™ A160 Peptide C18, 2.7 µm HPLC Column 2.7 µm particle size, L x I.D. 15 cm x 2.1 mm
00922	Difluoroacetic acid for LC-MS LiChropur™, ≥97.5% (GC)
1.06035	Methanol hypergrade for LC-MS LiChrosolv®
1.15333	Water for chromatography (LC-MS Grade) LiChrosolv®

High-purity UHPLC-MS LiChrosolv® solvents for rapid and reliable results.

Discover how our new range of advanced UHPLC-MS LiChrosolv® solvents raise the standard for low baseline noise and clean mass spectra.

The new range of advanced UHPLC-MS solvents have been developed to exceed all expectations, providing rapid and reliable results in both ESI/APCI positive and negative ionization modes.

Thanks to their lowest level of background noise and ion suppression, this quality ensures the optimum ionization efficiency to enable the highest sensitivity. With these features, use of these solvents can also help to extend column lifetime.

To ensure that you have confidence in your results, we specify the lowest possible limit of polyethylene glycol (PEG) impurities in all our UHPLC-MS solvents.

Our advanced UHPLC-MS LiChrosolv® solvents have been designed to meet the highest requirements of UHPLC-MS in research and quality control, including proteomics and metabolomics as well as environmental, clinical, food or industrial testing applications.

Ordering Information

Description	Pack Size	Cat. No.
Acetonitrile for UHPLC-MS LiChrosolv®	1 L	1.03725.1002
	2 L	1.03725.2002
Methanol for UHPLC-MS LiChrosolv®	1 L	1.03726.1002
	2 L	1.03726.2002
Water for UHPLC-MS LiChrosolv®	1 L	1.03728.1002
	2 L	1.03728.2002



Supelco®

Analytical Products

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

© 2020 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. Merck, the vibrant M, Supelco, LiChrosolv, LiChropur, and Bioshell are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed

Information on trademarks is available via publicly accessible resources.

MK_BR5591EN Ver. 1.0
31056
04/2020