

Analytix^{Notes}



LC-MS CHROMASOLV[®] Product Overview: Solvents, blends and additives

Pure Solvents

Solvent Blends

Mobile Phase Additives

Flush Solution

Dispensing Aid



SIGMA-ALDRICH

LC-MS consumables from Sigma-Aldrich LC-MS CHROMASOLV® Solvents, Blends and Additives and Ascentis™ HPLC Columns.

The power of LC-MS comes from its ability to provide structural information and detect compounds at extremely low levels. To achieve this, the background noise arising from the mobile phase and the HPLC column must be negligible. Minimizing the background and artifacts in LC-MS requires highly purified and specified solvents, ultra pure additives and low-bleed columns. LC-MS CHROMASOLV® and Ascentis™ HPLC columns from Sigma-Aldrich offer researchers state-of-the-art purity and stability for the most demanding LC-MS applications.

Introduction

Use of LC-MS is growing, especially in life science, pharmaceutical, as well as food & beverage and environmental applications because of its sensitivity and specificity. However, impurities arising from mobile phase solvents and additives, or from hydrolysis of the HPLC bonded phase are a serious annoyance and can significantly diminish the ability to detect and identify compounds that exist at very low levels. Furthermore, particulate and non-volatile impurities can clog susceptible and delicate hardware components and cause expensive system down-time.

Scientists at Sigma-Aldrich's Fluka/Riedel-de Haën and Supelco brands have responded to the growing need for high purity solvents, additives and low-bleed HPLC columns.

Offering nearly everything but the instrument, our LC-MS consumables line currently comprises:

- **Pure Solvents** – High purity, extensively tested mobile phase solvents for LC-MS
- **Solvent Blends** – Convenient, accurate and precipitation-free pre-blended solvent and additive solutions for LC-MS



Picture 1 LC-MS CHROMASOLV® bottles

- **Mobile Phase Additives** – Popular mobile phase additives at our highest grade of purity: puriss. p.a., specially tested for suitability under LC-MS conditions
- **Flush Solution** – Using this convenient flush solution from Sigma-Aldrich will help keep your LC-MS up and delivering reliable results
- **Dispensing Aid** – Prevent contamination of high purity LC-MS solvents using these adapters
- **Ascentis™ HPLC columns** – Classic and unique selectivity optimized for fast, sensitive LC-MS separations

LC-MS CHROMASOLV® Pure Solvents High purity, extensively tested mobile phase solvents for LC-MS.

- Water LC-MS CHROMASOLV®
- Acetonitrile LC-MS CHROMASOLV®
- Methanol LC-MS CHROMASOLV®
- 2-Propanol LC-MS CHROMASOLV®
- Ethyl acetate LC-MS CHROMASOLV®

Impure mobile phase solvents are the most common source of extraneous peaks and unstable LC-MS baselines. Unlike other sources of contamination, solvent-derived impurities do not condition out over time. Potential LC-MS contaminants include inorganic ions, decomposition products, closely related compounds from the manufacture of the solvent, microbes and the compounds they excrete, particulate matter from improper filtration and precipitation and compounds adsorbed from the atmosphere and packaging materials. Irrespective of the source, impurities in LC-MS mobile phase solvents can:

- Collect on the head of the HPLC column and elute as distinct peaks or as baseline rise when gradient conditions match their elutropic strength
- Cause a general elevation in baseline, lowering the sensitivity of the analysis
- Foul or damage sensitive instrument components
- Cause cluster ion formation that prevents reliable identification and quantification

LC-MS CHROMASOLV® solvents from Sigma-Aldrich undergo 34 distinct and relevant tests to ensure they meet the stringent criteria required for sensitive LC-MS analyses. Some of the most important features are:

- Application-tested for LC-MS using the reserpine test (**Figure 1**)
- Very low level of inorganic and metal ions for high sensitivity spectral interpretation
- Free of particles and non-volatile compounds to maintain system integrity
- Low gradient baseline even with your own optimized protocols

Another important feature of the LC-MS CHROMASOLV® solvents is the significantly reduced levels of omnipresent phthalate contaminants. Phthalates are found in many solvents by leaching from storage containers or by improper handling. To ensure the LC-MS solvent does not become contaminated by phthalates during the transfer process, it is recommended to use Sigma-Aldrich's PTFE Dispensing Aid Adapter, described later in this brochure.

Table 1 lists the specifications for LC-MS CHROMASOLV® solvents.

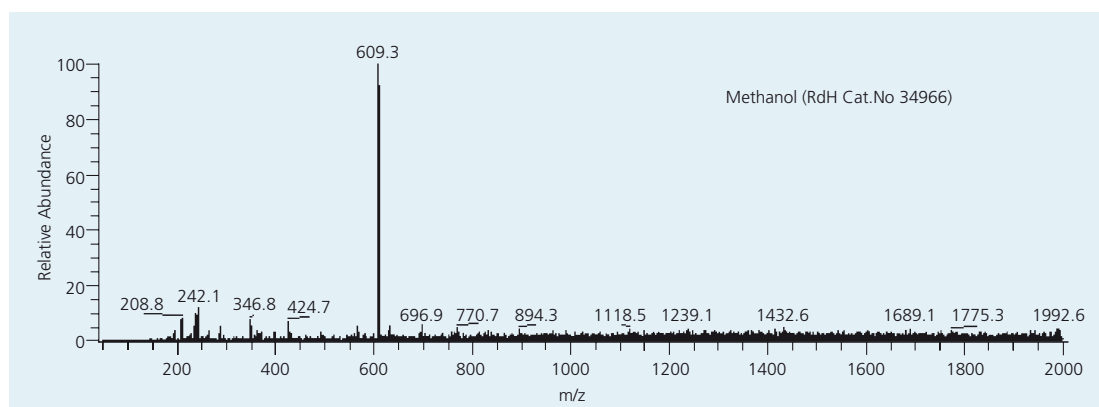


Figure 1 Reserpine Test: reserpine spectrum measured in Methanol (Cat. No. 34966); no signals should be greater than $[M+H]^+ = 609$ (100ppb reserpine; ESI, positive mode).

Table 1 Specifications for LC-MS CHROMASOLV®-Solvents from Riedel-de Haën

	Water	Acetonitrile	Methanol	2-Propanol	Ethylacetate
Cat. No.	39253	34967	34966	34965	34972
Brand	Riedel-de Haën	Riedel-de Haën	Riedel-de Haën	Riedel-de Haën	Riedel-de Haën
Pack Sizes	1 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L
Assay (GC) (min)	-	99.9%	99.9%	99.9%	99.7%
Fluorescence at 254 nm (max)	1 ppb	0.5 ppb	1 ppb	1 ppb	-
Fluorescence at 365 nm (max)	1 ppb	0.5 ppb	1 ppb	1 ppb	-
Chloride (Cl) (max)	0.000001%	-	-	-	-
Fluoride (F) (max)	0.000001%	-	-	-	-
Nitrate (NO₃) (max)	0.00001%	-	-	-	-
Sulfate (SO₄) (max)	0.00001%	-	-	-	-
Free acid (max)	-	0.001%	0.001%	0.001%	-
Free alkali (as NH₃) (max)	-	0.0002%	0.0005%	0.0005%	0.0005%
Non-volatile matter (max)	0.001%	0.0002%	0.0005%	0.0005%	0.0005%
Water (Karl Fischer) (max)	-	0.01%	0.02%	0.05%	0.03%
Transmittance at 200 nm (min)	95%	95%	-	-	-
Transmittance at 230 nm (min)	99%	99%	75%	75%	-
Transmittance at 260 nm (min)	-	-	98%	98%	50%
HPLC gradient (254nm) (max)	1 mAU	0.2 mAU	2 mAU	2 mAU	-
Silver (Ag) (min) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Aluminum (Al) (max)	0.5 ppm	0.5 ppm	0.5 ppm	0.5 ppm	-
Barium (Ba) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Calcium (Ca) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Cadmium (Cd) (max)	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm	-
Cobalt (Co) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Chromium (Cr) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Copper (Cu) (max)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm	-
Iron (Fe) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Potassium (K) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Magnesium (Mg) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Manganese (Mn) (max)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm	-
Sodium (Na) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Nickel (Ni) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Lead (Pb) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Tin (Sn) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Zinc (Zn) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Particle test	+	+	+	-	-
LC-MS suitability test (reserpine test)	+	+	+	+	+

Table 2 LC-MS CHROMASOLV® Solvents

Cat. No.	Brand	Solvent Blend	Package Size	Packaging
39253	Riedel-de Haën	Water LC-MS CHROMASOLV®	1 L	Clear glass bottle
34967	Riedel-de Haën	Acetonitrile LC-MS CHROMASOLV®	1 L, 6 x 1 L, 2.5 L, 4 x 2.5 L	Amber bottle
34966	Riedel-de Haën	Methanol LC-MS CHROMASOLV®	1 L, 6 x 1 L, 2.5 L, 4 x 2.5 L	Amber bottle
34965	Riedel-de Haën	2-Propanol LC-MS CHROMASOLV®	1 L, 6 x 1 L, 2.5 L, 4 x 2.5 L	Amber bottle
34972	Riedel-de Haën	Ethyl acetate LC-MS CHROMASOLV®	1 L, 6 x 1 L, 2.5 L, 4 x 2.5 L	Amber bottle

Cluster Formation in Presence of Alkali Ions

When using mobile phases that are contaminated with alkali metals, particularly sodium and potassium, interpretation of the mass spectra is complicated and sensitivity is decreased. Ideally, the analyte should give molecular ions by forming adducts with protons, $[M+H]^+$. However, when other cations are present as contaminants in the mobile phase, undesirable adducts are formed, e.g. $[M+Na]^+$, making spectral interpretation difficult.

We investigated the influence of metal ions on the quality of LC-MS spectra using human gastrin ($M=2097$) as a model peptide. Human gastrin was dissolved in 0.2% formic acid in standard HPLC-grade water that typically contains >10 ppm sodium and potassium. The high metal content causes formation of metal ion clusters with the peptide. As a result the mass spectrum is difficult to interpret; the dominating clusters appear as a fence of double and multi-charged ions (**Figure 2**). Such artifacts make it difficult to determine the true peak which is the protonated double charged molecular ion of gastrin ($[M+2H]^{2+}$).

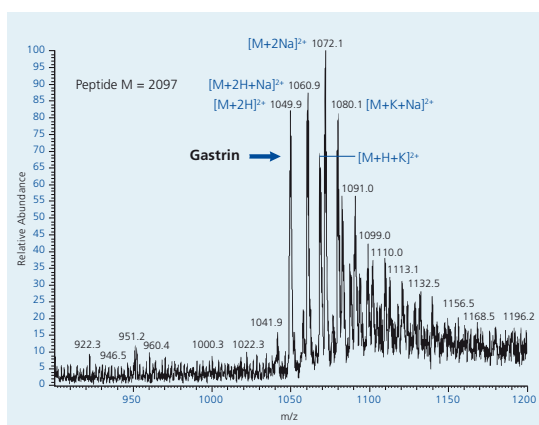


Figure 2 ESI mass spectrum of human gastrin dissolved in water having a sodium and potassium content higher than 10 ppm (gastrin molecular ion = 1049.8 m/z). A fence originated by the metal ion cluster can be clearly noted (peaks between 1051 and 1150 m/z) (samples analyzed with a Thermo Finnigan LCQ Advantage LC-MS ion trap using electrospray ionization and direct syringe infusion).

Additionally, major problems will occur with the automatic MS/MS mode, where the most abundant peaks are selected for further subsequent collisions experiments to give the amino acid sequence.

In comparison, when the HPLC-grade water was replaced with LC-MS CHROMASOLV® water, which has very low concentration of sodium and potassium ions (< 0.1 ppm), very few metal ion clusters were observed (**Figure 3**). Only $[M+H+Na]^{2+}$ and $[M+H+K]^{+2}$ were found in a relatively low abundance. The protonated double charged molecular ion peak of gastrin ($[M+2H]^{2+}$) can be easily discerned, isolated and further fragmented to yield the amino acid sequence.

Not only is the mass spectrum using the LC-MS CHROMASOLV® water easier to interpret, an added benefit to the fewer artifacts is the nearly 10-fold increase in sensitivity compared to the standard HPLC-grade water. The purity and quality of LC-MS CHROMASOLV® solvents allow the LC-MS to operate at its maximum sensitivity and specificity.

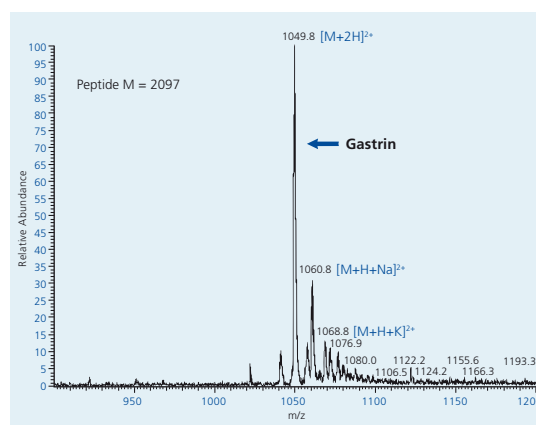


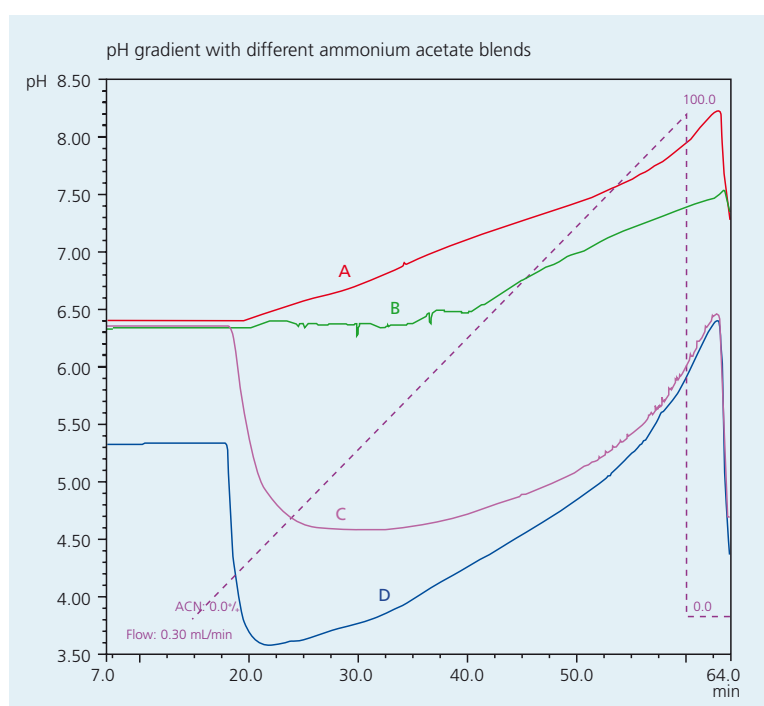
Figure 3 ESI mass spectrum of human gastrin in LC-MS CHROMASOLV® Water. The gastrin molecular ion can be found at 1049.8 m/z. Only a few clusters are observed (between 1051 and 1100 m/z). Sensitivity was enhanced 10-fold (samples analyzed with a Thermo Finnigan LCQ Advantage LC-MS ion trap using electrospray ionization and direct syringe infusion).

LC-MS CHROMASOLV® Solvent blends Convenient, accurate and precipitation-free pre-blended solvent and additive solutions for LC-MS

- **Trifluoroacetic acid**
0.1% in LC-MS CHROMASOLV® water, methanol or acetonitrile
- **Formic acid**
0.1% in LC-MS CHROMASOLV® water, methanol or acetonitrile
- **Acetic acid**
0.1% in LC-MS CHROMASOLV® water, methanol or acetonitrile
- **Ammonium acetate**
0.1% in LC-MS CHROMASOLV® water, methanol or acetonitrile

Minimizing the background and artifacts in LC-MS requires highly specified solvents 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 LC-MS solvents are acetonitrile, methanol and water. Common additives include trifluoroacetic acid (TFA), formic acid, acetic acid and ammonium acetate. Sigma-Aldrich offers ready to use pre-blended solvents and additives specified for LC-MS requirements. Using these precisely blended solvents eliminates time-consuming mobile phase preparation, and can eliminate lost sample information and instrument down-time caused by

Figure 4 Effective pH as a result of changes in water:organic ratio using LC-MS CHROMASOLV® blends



impure mobile phases. A special formulation assures that no precipitation or decomposition of the additive occurs under normal laboratory conditions.

Separation of peptides with LC-MS CHROMASOLV® ammonium acetate solvent blends

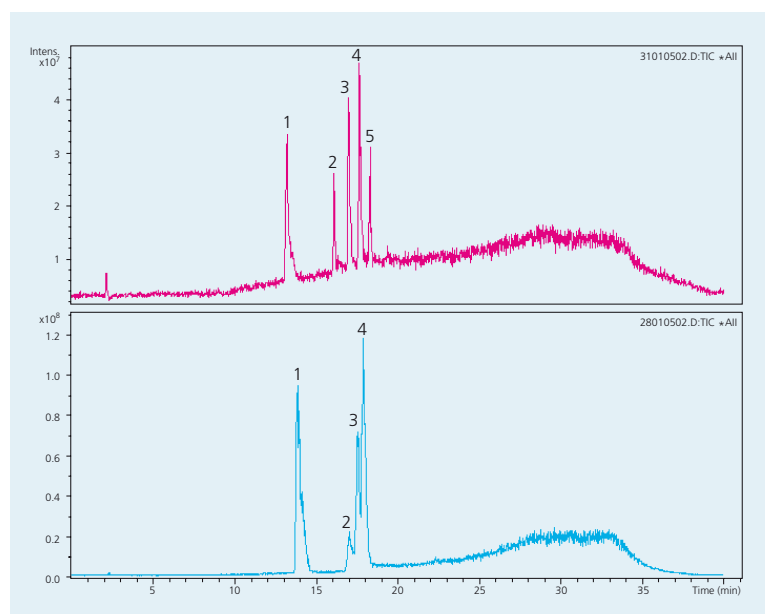
As an LC-MS additive, ammonium acetate can be used in both positive and negative ionization modes at mid-range pH values. The pH values in this range are sometimes necessary to provide proper selectivity, good peak shape or to ensure the compounds of interest are in the right ionization state.

An interesting side effect of using ammonium acetate blended solvents is the generation of a pH gradient parallel to the solution gradient. Depending on its direction this may influence the peak shape, resolution and enable both ionization modes. So far these pH gradients and their influence on peptide separations had not been measured. The use of different solvents, either aqueous, aqueous with acetonitrile, organic or both leads to different pH values and gradients (**Figure 4**).

Figure 4 shows the change in the effective pH value during a gradient from 100% aqueous to 100% organic using different LC-MS CHROMASOLV® solvent + ammonium acetate blends. An increasing pH gradient was obtained using water + 0.1% ammonium acetate and pure acetonitrile (trace A) or methanol + 0.1% ammonium acetate (trace B). This type of gradient is useful when negative ionization is desired, which is often preferred at higher pH values. For positive ionization a more acidic pH is needed, which can be obtained using water + 0.1% ammonium acetate and acetonitrile + 0.1% ammonium acetate (trace C). The use of pure water in this combination (trace D) is not the intended use, but can be helpful when very low pH values during the HPLC run are necessary. The pH curves of the blends are a result of the buffer capacities of these mixtures.

- A: water 0.1% ammonium acetate (Cat. No. 34674) / pure acetonitrile (Cat. No. 34967)
 B: water 0.1% ammonium acetate (Cat. No. 34674) / methanol 0.1% ammonium acetate (Cat. No. 34670)
 C: water 0.1% ammonium acetate (Cat. No. 34674) / acetonitrile 0.1% ammonium acetate (Cat. No. 34669)
 D: pure water (Cat. No. 39253) / acetonitrile 0.1% ammonium acetate (Cat. No. 34669)

Figure 5 Separation of bradykinin (BK) peptides under mild acid conditions (upper) and neutral / slight alkaline conditions (lower).



We compared the separation of a mixture of related peptides using the mild acidic conditions of C (0.1% ammonium acetate in both water and acetonitrile) (**Figure 5**, top chromatogram) and the more neutral / mildly alkaline conditions of conditions B (0.1% ammonium acetate in both water and methanol) (**Figure 5**, bottom chromatogram). Better resolution of the peptides and an impurity peak was obtained using the mildly acidic conditions provided by the 0.1% ammonium acetate in water-acetonitrile gradient.

- 1: BK 1-6
- 2: Lys-Ala3-BK
- 3: BK 4: Des-Arg1-BK
- 4: Des-Arg1-BK
- 5: Impurity

Specifications: Acetonitrile and Methanol Blends

LC gradient testing in UV and MS, metal impurities (Na < 2 ppm, K, Mg, Ca < 0.5 ppm), UV-transmittance, additive content: 0.093-0.107 TFA, FA, AA (v/v), ammonium acetate (w/v). Solvent content: (GC): > 99.0 % (Cat. No. 34669 – acetonitrile with 0.1% ammonium acetate; solvent content (GC) > 98 %)

Specifications: Water blends

LC gradient testing in UV and MS, metal impurities (Na < 2ppm, K, Mg, Ca < 0.5 ppm), UV-transmittance, additive content: 0.093-0.107 TFA, FA, AA (v/v), ammonium acetate (w/v), pH: effective +/- 0.1

Table 3 LC-MS CHROMASOLV® Solvent Blends

Cat. No	Brand	Solvent Blend	Pack size	Packaging
34978	Riedel-de Haën	Water with 0.1% TFA LC-MS CHROMASOLV®	2.5 L	amber bottle
34976	Riedel-de Haën	Acetonitrile with 0.1% TFA LC-MS CHROMASOLV®	2.5 L	amber bottle
34974	Riedel-de Haën	Methanol with 0.1% TFA LC-MS CHROMASOLV®	2.5 L	amber bottle
34673	Riedel-de Haën	Water with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34668	Riedel-de Haën	Acetonitrile with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34675	Riedel-de Haën	Water with 0.1% acetic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34678	Riedel-de Haën	Acetonitrile with 0.1% acetic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34672	Riedel-de Haën	Methanol with 0.1% acetic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34674	Riedel-de Haën	Water with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34669	Riedel-de Haën	Acetonitrile with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34670	Riedel-de Haën	Methanol with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34671	Riedel-de Haën	Methanol with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	amber bottle
34677	Riedel-de Haën	Water with 0.1% formic acid/0.01% TFA LC-MS CHROMASOLV®	2.5 L	amber bottle
34676	Riedel-de Haën	Acetonitrile with 0.1% formic acid/0.01% TFA LC-MS CHROMASOLV®	2.5 L	amber bottle

Mobile Phase Additives for LC-MS Popular mobile phase additives at our highest grade of purity: puriss., p.a., specially tested for suitability under LC-MS conditions

Buffers and other ionic additives are nearly always added to LC-MS mobile phases. The additives serve a variety of purposes, including reducing adsorption, controlling the pH, improving the peak shape, selectivity or recovery and optimizing the ionization at the MS interface. Mobile phase additives that are suitable for UV detection are often unsuitable when MS detection is employed. For example, sodium and potassium phosphate, two of the most common buffers in HPLC with UV detection, cannot be used in LC-MS because they are not volatile and suppress ionization.

Some of the most popular additives for LC-MS are formic acid, acetic acid, ammonium acetate and trifluoroacetic acid (TFA), although it can suppress ionization. Sigma-Aldrich offers these additives as ready to use pre-blended solvents (described above) or as pure chemicals or neat solutions specified for LC-MS requirements.

Table 4 Additives for LC-MS

Cat. No.	Brand	Description*	Pack Size	Packaging
40867	Fluka	Ammonium bicarbonate, puriss., p.a., eluent additive for LC-MS	50 g	HDPE bottle
40967	Fluka	Trifluoroacetic acid, puriss., p.a., eluent additive for LC-MS	10 x 1 mL	Glass Ampoules
40967	Fluka	Trifluoroacetic acid, puriss., p.a., eluent additive for LC-MS	50 mL	HDPE bottle
44273	Fluka	Ammonium hydroxide solution 25%, puriss., p.a., eluent additive for LC-MS	100 mL	HDPE bottle
49199	Fluka	Acetic acid, puriss., p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49638	Fluka	Ammonium acetate, puriss., p.a., eluent additive for LC-MS	50 g	HDPE bottle
49916	Fluka	Propionic acid, puriss., p.a., eluent additive for LC-MS	50 mL	HDPE bottle
55674	Fluka	Ammonium formate, puriss., p.a., eluent additive for LC-MS	50 g	HDPE bottle
56302	Fluka	Formic acid, puriss., p.a., eluent additive for LC-MS	50 mL	HDPE bottle
61333	Fluka	Sodium citrate tribasic dehydrate, puriss., p.a., eluent additive for LC-MS	50 g	HDPE bottle
65897	Fluka	Triethylamine, puriss., p.a., eluent additive for LC-MS	50 mL	HDPE bottle

* puriss. quality grade is defined as >98.5% assay, <0.1% ash, and specification n + 0.001, d + 0.001 with no extraneous color and an homogeneous appearance. p.a. or pro analysi denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

LC-MS Flush Solution Using this convenient flush solution from Sigma-Aldrich will help keep your LC-MS up and delivering reliable results

To maintain minimal baseline and reduce extraneous peaks, it is important to flush the LC-MS system at regular intervals; the length of the interval depends on the nature of the samples. A commonly employed flush solution is 50% isopropanol in water because it solubilizes both hydrophilic and moderately hydrophobic contaminants. Although this solution can be easily prepared from ingredients found in most laboratories, it is critical that the purity of the flush solution be such that it does not add contaminants to the LC-MS system.

To eliminate the possibility of adding contaminants and also reduce the time needed to prepare your own flush solution, we have developed the **CHROMASOLV®** Flush Solution. This solution comprises a 50% v/v mixture of 2-propanol (isopropanol) in water. Both the 2-propanol and the water are of our highest LC-MS **CHROMASOLV®** quality. The mixture has been tested for GC and LC-MS purity, water content by Karl-Fischer titration, organic and non-volatile impurities, UV transmittance and levels of sixteen inorganic ions.

The data in **Figure 6** presents a clear picture on the effect of purity of flush solution on the quality of the MS chromatogram. The chromatogram shows the total ion current (TIC) for full scan 100-1000 dalton obtained with 4µL/min direct syringe injection (no column) of

different 50/50 2-propanol/water mixtures. During the first three minutes a laboratory mixed solution was used, prepared carefully from gradient grade HPLC solvents. Note the high baseline offset and noise (0-3 minutes). Then, while still scanning, the content of the syringe was changed to the **CHROMASOLV®** Flush Solution. After the initial disturbance peaks, the baseline obtained with the **CHROMASOLV®** Flush Solution (6-11 minutes) was at least six-fold lower with far less noise than the gradient grade solvents. To show that this is not a cleaning effect, the process was repeated. The syringe was refilled with the gradient grade solution and injected at 12 min, resulting again in higher baseline and noise (14-20 minutes). Then, the syringe was filled with **CHROMASOLV®** Flush Solution and injected at 21 minutes. The flat baseline after 22 minutes confirmed the earlier observation and the purity of the **CHROMASOLV®** Flush Solution.

Flush solutions are necessary for thorough removal of mobile phase and sample impurities that can interfere with sensitive LC-MS analyses. To ensure your flush solution isn't itself a source of impurities, use **CHROMASOLV®** Flush Solution. The convenience of the preblended mixture and the high purity make it an ideal solution for chemists facing the promises and challenges of both routine and high-throughput LC-MS.

Figure 6 Purity comparison of gradient grade solvents with premixed **CHROMASOLV®** Flush Solution

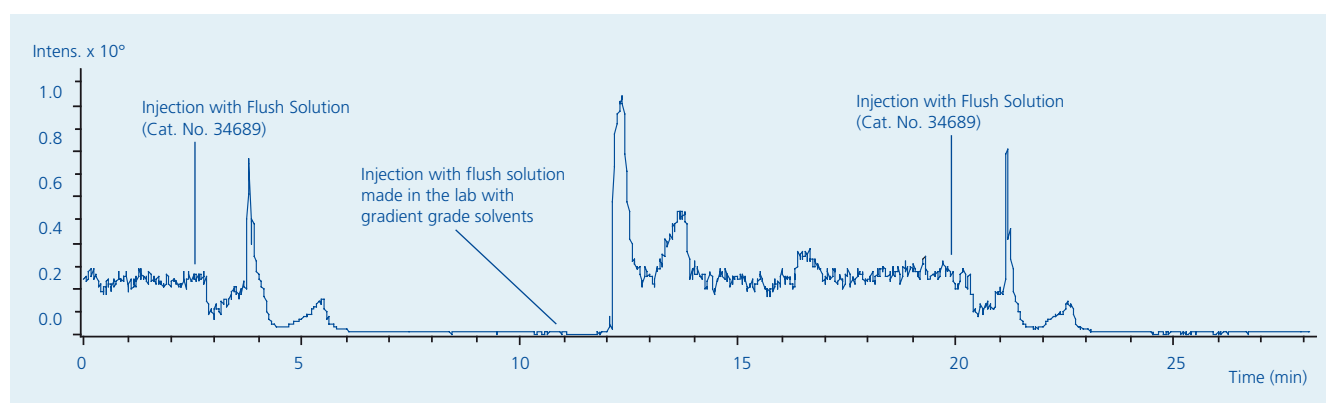


Table 5 **CHROMASOLV®** Flush Solution

Cat. No.	Brand	Description	Package Size	Packaging
34689	Riedel-de Hën	CHROMASOLV® Flush Solution, water:2-propanol, 50:50	1 L	White glass bottle

Dispensing Aid for LC-MS Solvent Bottles Prevent contamination of high purity LC-MS solvents using these adapters

LC-MS CHROMASOLV® solvents are supplied free of dissolved gasses and impurities, both of which can cause erroneous, unreliable LC-MS results and instrument downtime. To avoid the risk of contamination during transfer of the CHROMASOLV® solvent, use our new practical dispensing aid (Cat. No. 79212). The dispensing aid comprises a PTFE adaptor screwed directly to the CHROMASOLV® bottle and four pins that securely attach the connecting tubing thus eliminating the possibility of impurities entering the lines during dispensing operation.



Picture 2 LC-MS bottles PTFE adaptor (Cat. No. 79212)

Table 6 LC-MS Dispensing Aid

Part. No.	Brand	Description	Package Size
79212	Riedel-de Haën	PTFE adaptor for LC-MS solvent bottles	One adapter and connecting pins

Ascentis™ LC-MS Columns Classic and unique selectivity optimized for fast, sensitive LC-MS separations

- Ascentis™ C18, 3 and 5 µm
- Ascentis™ RP-Amide, 3 and 5 µm

Ascentis™ C18 and RP-Amide columns offer:

- Extremely low bleed baselines for better sensitivity and less interference
- Higher hydrophobic retention to allow mobile phases with high organic concentrations for improved sensitivity
- Choices in bonded phase selectivity to improve resolution and enhance polar compound retention

Supelco's proprietary Surface-Optimized Technology bonding chemistry and our unique, revolutionary approach to end-capping combine with high-surface area, high purity silica to produce the Ascentis™ premier line of HPLC columns. Ascentis™ HPLC columns have high bonded phase surface coverage with no bleed under LC-MS conditions. The high surface area permits the use of higher organic mobile phases which improves LC-MS sensitivity by increasing the desolvation rate.

Ascentis RP-Amide: Enhanced polar selectivity without LC-MS bleed

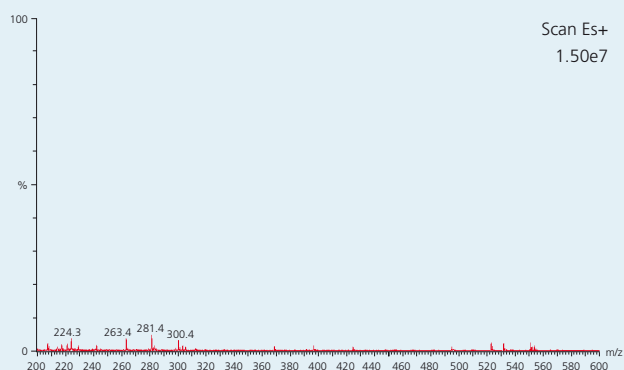
In the past, the choices of LC-MS-compatible HPLC columns have been limited to alkyl-only phases, primarily C18. Other phase chemistries that might offer unique selectivity compared to a C18 were often overlooked for LC-MS applications because of their tendency to undergo hydrolysis. The hydrolyzed bonded phase molecules that are mobilized by the LC-MS gradient show up as characteristic bleed ions in the mass spectrum. Today, however, polar selectivity and LC-MS compatibility are both attainable. The Ascentis™ RP-Amide provides enhanced polar selectivity without the problem of phase bleed. **Figure 7** compares the bleed levels on Ascentis™ RP-Amide to two other popular reversed phase columns with polar embedded groups.

Figure 7 Comparison of LC-MS Detectable Bleed from Ascentis RP-Amide and Competitive Polar-Embedded Phases After Storage in 50:50 CH₃OH:water for 48 Hours

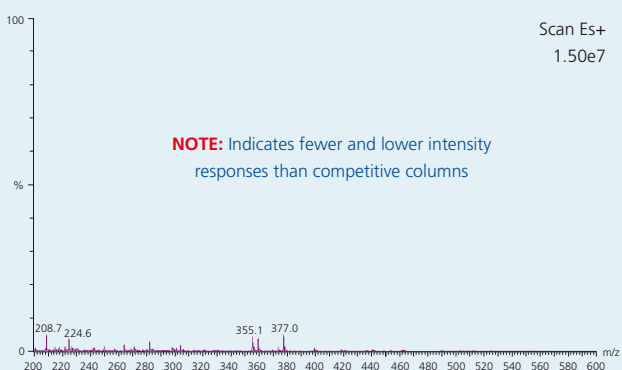
mobile phase: (A) 0.1% formic acid
(B) 0.1% formic acid in CH₃CN
flow rate: 1.0 mL/min
temp: 35 °C
det: MS, ESI (+) mode, Full Scan (m/z 200-600 shown)
Injektion: 0 µL
sample: None

gradient:	Min	A%	B%
	0	95	5
	5	95	5
	20	5	95
	25	5	95
	26	95	5
	30	95	5

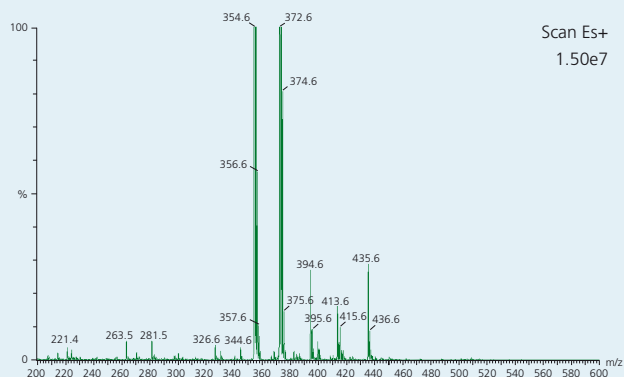
Blank Run Without Column



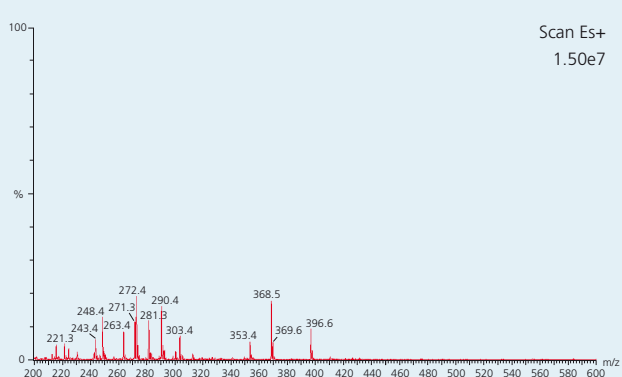
Ascentis RP-Amide



Brand A



Brand B



Check our website:

www.sigma-aldrich.com/ascentis

for applications and to see the complete and continually growing line of Ascentis™ HPLC columns for LC-MS



SUPELCO

Your Sigma-Aldrich Service Partners

Europe

Austria Wien

Tel. 01-605 81 10
Fax 01-605 81 20
E-mail: sigma@sigma.co.at

Belgium/Luxembourg Bornem

Free Tel. 0800 147 47
Free Fax 0800 147 45
E-mail: becustsv@europe.sial.com

Czech Republic Praha

Tel. 246 003 251
Fax 246 003 291
E-mail: CZECustSV@europe.sial.com

Denmark Brøndby

Tel. 43 56 59 10
Fax 43 56 59 05
E-mail: denorder@europe.sial.com

Finland Helsinki

Tel. 09-350 9250
Fax 09-350 9255
E-mail: finorder@europe.sial.com

France St. Quentin Fallavier

Tél. 0800 21 1408 (appel gratuit)
Fax 0800 03 1052 (appel gratuit)
E-mail: fradvsv@europe.sial.com

Germany Taufkirchen

Free Tel. 0800 51 55 000
Free Fax 0800 64 90 000
E-mail: deorders@europe.sial.com

Greece Ilioupoli, Athens

Tel. 210-994 8010
Fax 210-994 3831
E-mail: GRCustSV@europe.sial.com

Hungary Budapest

Tel. (06-1) 269-6474
Fax (06-1) 235-9068
Email: info@sigma.sial.hu

Ireland Dublin

Tel. 01-404-1900
Fax 01-404-1910
E-mail: EICustsv@europe.sial.com

Israel Rehovot

Tel. 08-9484-222
Fax 08-9484-200
E-mail: sigisr@sigma.co.il

Italy Milano

Tel. 02-33417-310
Fax 02-38010-737
E-mail: itorder@europe.sial.com

Norway Oslo

Tel. 23 17 60 00
Fax 23 17 60 10
E-mail: nororder@sial.com

Poland Poznań

Tel. 061-829 01 00
Fax 061-829 01 20
E-mail: plcustsv@europe.sial.com

Portugal Sintra

Tel. 800 20 21 80 (Gratuito)
Fax 800 20 21 78 (Gratuito)
E-mail: poorders@europe.sial.com

Spain Tres Cantos, Madrid

Tel. 900 10 13 76 (Gratuito)
Fax 900 10 20 28 (Gratuito)
E-mail: esorders@europe.sial.com

Sweden Stockholm

Tel. 020-35 05 10
Fax 020-35 25 22
E-mail: sweorder@europe.sial.com

Switzerland Buchs

Free Tel. 0800 80 00 80
Free Fax 0800 80 00 81
E-mail: Fluka@sial.com

The Netherlands Zwijndrecht

Free Tel. 0800 022 9088
Free Fax 0800 022 9089
Email: nlcustsv@europe.sial.com

United Kingdom Gillingham

Free Tel. 0800 717181
Free Fax 0800 378785
E-mail: ukcustsv@europe.sial.com

America

Canada Oakville

Free Tel. 1-800-565-1400
Free Fax 1-800-265-3858
E-mail: canada@sial.com

USA St. Louis, MO

Toll Free 800 325 3010
Free Fax 800 325 5022
E-mail: custserv@sial.com

Oceania

Australia Castle Hill

Free Tel. 1800 800 097
Free Fax 1800 800 096
E-mail: ausmail@sial.com

New Zealand Castle Hill

Free Tel. 0800 936 666
Free Fax 0800 937 777
E-mail: ausmail@sial.com

Africa

South Africa Johannesburg

Tel. 011-979 1188
Fax 011-979 1119
E-mail: rsa@sial.com

Asia

India Bangalore

Tel. 91-80-5112 7272
Fax 91-80-5112 7473
E-mail: india@sial.com

sigma-aldrich.com



The
SIGMA-ALDRICH
Family



Biochemicals and
Reagents for Life
Science Research



Organics and
Inorganics for
Chemical Synthesis



Speciality Chemicals
and Analytical Reagents
for Research



Laboratory Chemicals
and Reagents for
Research and Analysis



Chromatography
Products for Analysis
and Purification

©2005 Sigma-Aldrich Co. Printed in USA Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip. SIGMA and -are registered trademarks of Sigma-Aldrich Co. and its division Sigma-Aldrich Biotechnology LP. Riedel-de Haën®: trademark under license from Riedel-de Haën GmbH.