

Eluent Additives for LC-MS

Maximizing the power of LC-MS LC-MS is one of today's most powerful analytical techniques. It combines the advantages of chromatographic separation with the structural information provided by mass spectrometric detection. Because of the ability of LC-MS to provide high level qualitative and quantitative information, it has become an invaluable analytical tool for biotechnology, as well as pharmaceutical and chemical industries and academia. R&D, analytical and QC laboratories increasingly depend on LC-MS to meet their disparate criteria.

To harness the power of LC-MS and maximize the information it provides, it is essential to use the proper HPLC columns and solvents. Sigma-Aldrich offers an entire family of LC-MS products, including pure solvents, additives, ready-to-use blends and low-bleed HPLC columns from Riedel-de Haën, Fluka and Supelco brands. This brochure describes our high purity mobile phase additives for LC-MS.



Eluent Additives for LC-MS

It is common practice in LC-MS to add certain chemicals to the mobile phase or introduce them post-column prior to the interface to influence analyte ionization. Most often, an improvement of the analyte signal is the goal. However, 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.

Sigma-Aldrich, a leading supplier of chemicals for analytical applications, offers a wide range of high purity additives for LC-MS applications in addition to our pure CHROMASOLV® solvents and ready-to-use blends. Our offering includes the most commonly used acids, bases, volatile salts and a sodium source (see **Table 1**). All are of high purity, usually puriss p.a., and are tested for LC-MS application.

Table 1 Product List of LC-MS additives

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

*"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 a homogeneous appearance. "p.a." or pro analysi denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

The additives are grouped into four blocks: the acids (red), normally used for positive ionization under acidic conditions; the salts (green) used for either positive or negative ionization under neutral conditions; a sodium source (blue) for certain applications, where a specified amount of sodium is required; and the bases (violet), normally the best choice for negative ionization under basic conditions.

The influence of these additives on chromatographic (retention time) and mass spectroscopic behaviour (molecular ion, base peak, abundance) analytes can be shown very instructively with some model compounds which are listed in **Table 2**.

Table 2 Model compounds that show improvement in LC-MS results by using mobile phase additives

Entry	Cat. No.	Brand	Compound	Class	Formula	Molecular Mass
1	83400	Fluka	Raffinose	Saccharides	C ₁₈ H ₃₂ O ₁₆	504.2
2	15859	Fluka	Bradykinin	Peptides	C ₅₀ H ₇₃ N ₁₅ O ₁₁	1059.6
3	37100	Fluka	Digoxin	Glycosides	C ₄₁ H ₆₄ O ₁₄	780.4
4	R0875	Sigma	Reserpine	Alkaloids	C ₃₃ H ₄₀ N ₂ O ₉	608.3
5	45640	Fluka	Propazine	Triazines	C ₉ H ₁₆ N ₅ Cl	229.1

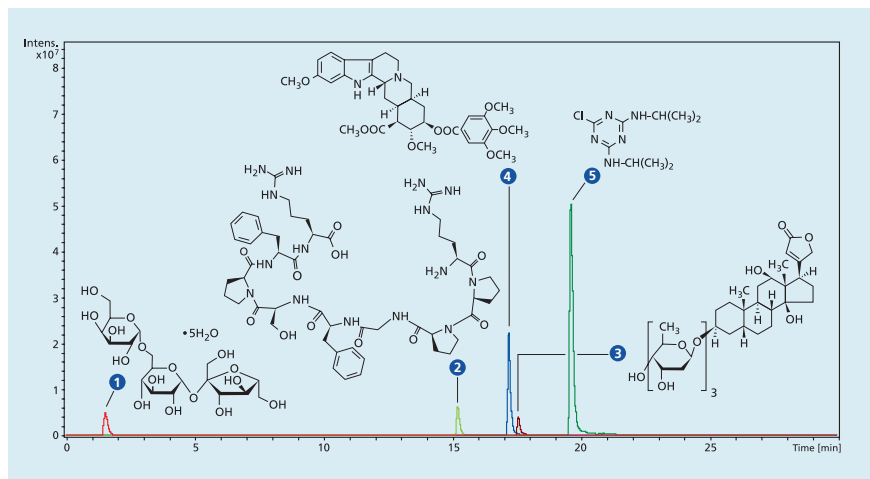


Figure 1 Extracted ion chromatogram of 5 test compounds with 0.1% acetic acid as mobile phase additive; pos. ion mode.

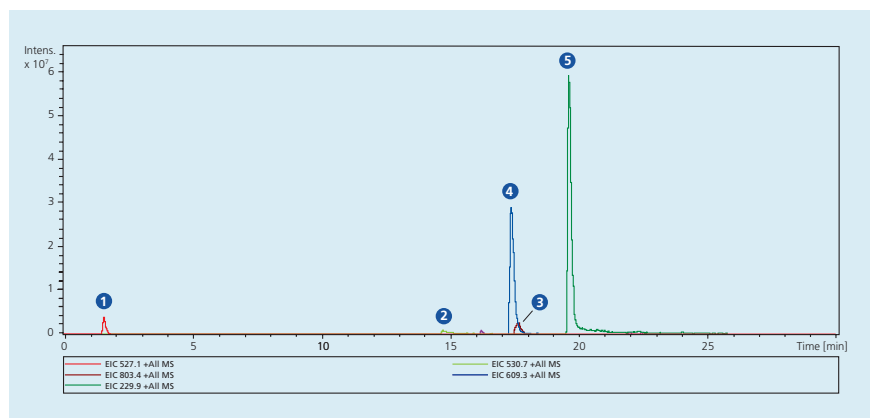


Figure 2 Chromatogram of the 5 test compounds without any additive; pos. ion mode.

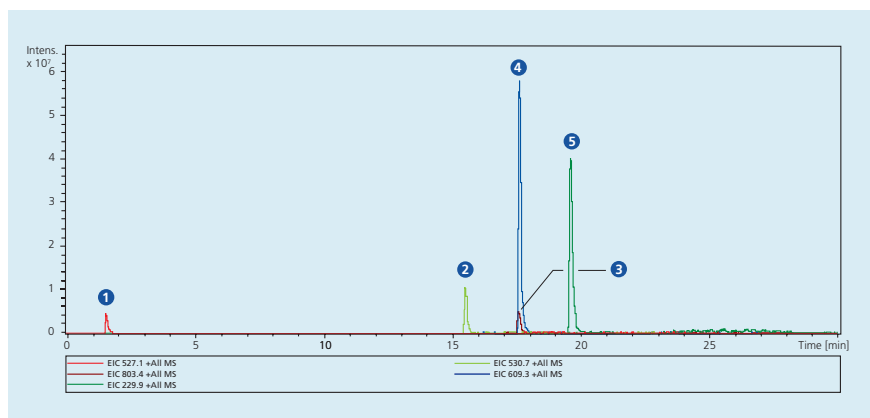


Figure 3 Chromatogram of the 5 test compounds with 0.1% formic acid as additive; pos. ion mode.

The structures of the test compounds and an example chromatogram with acetic acid as additive are shown in **Fig. 1**.

To demonstrate the influence of the additive, all other analytical parameters were kept constant. The concentration of all test compounds was 10 ng/μL, except for reserpine, which was 5 ng/μL. A gradient system with water (LC-MS grade, 39253) and acetonitrile (LC-MS grade, 34967) at a flow rate of 0.4 mL/min was used to separate the test compounds. The additives were either dissolved in both mobile phase eluents at a concentration of 0.1% or added in higher concentration post-column via a T-piece. The column was a Supelco Discovery HS C18, 150 x 2.1mm, 5 μm. The MS was an ion trap (Bruker Esquire 3000+) with electrospray interface (ESI) operated in positive or negative ion mode.

Elution of the test substances without any additive is shown in **Fig. 2**. Note that the peptide bradykinin elutes as a broad peak, barely distinguishable from the baseline. The traces shown are the specific extracted ion chromatograms (EIC) of the analytes, which can vary by the use of different additives. For example when using acids, $[M+H]^+$ is the most often observed high abundant mass peak (base peak).

Acids, like acetic or formic acid, have a positive influence on the chromatography and ionization of many basic analytes which contain one or more nitrogen atoms. The peak shape and resolution are improved and the formation of $[M+H]^+$ ions is supported, making formic acid one of the most popular acidic additives in LC-MS (**Fig. 3**).

Although there are pros and cons to the use of TFA (trifluoroacetic acid) as an acidic additive, it is widely used in HPLC and LC-MS of proteins and peptides. TFA improves the chromatography of these compounds, but it suppresses ionization in the MS interface, thus reducing the signal. Nonetheless, because of its prevalent use, Sigma-Aldrich offers high purity, application-tested TFA as part of our LC-MS additive line. When TFA is necessary, simply adding formic acid (in so called triple blends) or introducing propionic acid via a T-piece prior to the MS-interface, can improve the chromatography without compromising the MS signal.

Under neutral conditions volatile salts, such as ammonium formate or ammonium acetate, work very well as ionization-supporting additives. The pH of their solutions can be adjusted over a wide range with the corresponding acids. Additionally, depending on the analyte, they can be used in both positive and negative ionization modes (**Fig. 4**).

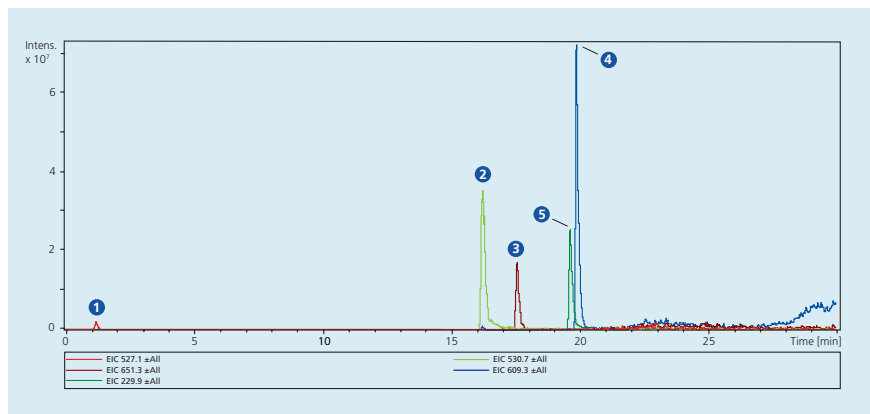


Figure 4 Chromatogram of the 5 test compounds with 0.1% ammonium formate as additive; pos. ion mode. Reserpine is shifted to last position in elution order.

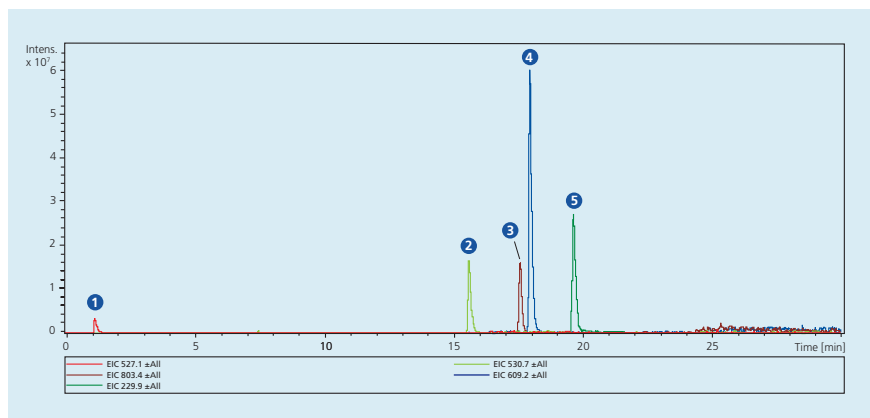


Figure 5 Chromatogram of the 5 test compounds with 0.1% sodium citrate added post-column via T-piece; pos. ion mode.

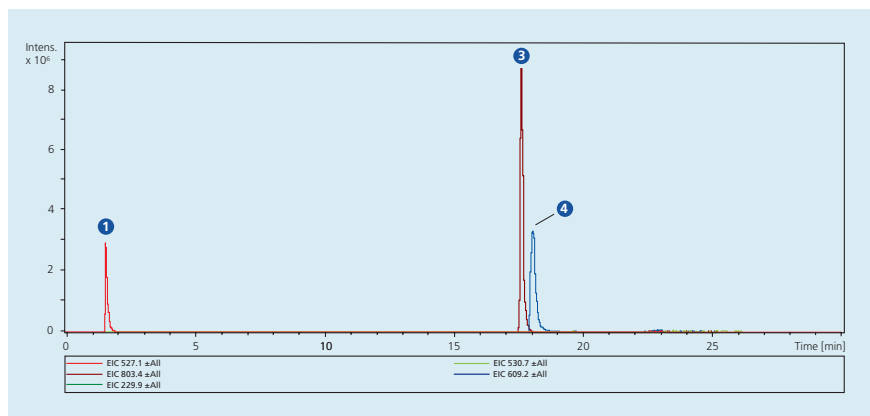


Figure 6 Chromatogram of the 5 test compounds with 10% ammonium hydroxide solution added post-column via T-piece; neg. ion mode.

In positive mode addition of either H^+ or NH_4^+ to the molecular ion is observed, in negative mode subtraction of H^+ or addition of additive anion, i.e. [formate]. Therefore prediction of the expected molecular ion in these cases is sometimes not easy and may change with the pH value.

Sodium citrate as an additive presents a special case. Although sodium and other alkali metals are typically avoided in LC-MS, it can be useful to add sodium salts in cases where the analyte ions have a very high tendency to form adducts with alkali ions. This is especially true for the carbonyl group of sugars and glycosides, in addition to hydroxyl and carboxyl groups. **Fig. 5** shows the influence of sodium citrate on separation and ionization of the test compounds.

The effect of sodium citrate addition on digoxin, a glycoside (third peak), is most pronounced. Some influence is also seen on raffinose, a trisaccharide, and bradykinin, a peptide. Peptides with glycosidic side groups are most susceptible to this effect. The addition of the sodium salt has to be performed carefully and in very low amounts as the optimal concentration range is not as wide as for most other additives.

MS in negative ion mode is often carried out at high pH with basic additives. To prevent damage to the HPLC column, the basic additives are typically added post-column. Under these conditions, the subtraction of H^+ is the most common reaction in the ESI-MS interface and is called reverse buffering with straight ionization/detection. A separation under basic conditions can also be performed on special HPLC columns, followed by MS-detection in positive mode. This is called straight chromatography with reverse detection. **Fig. 6** shows a neutral separation of the test compounds followed by reverse buffering with ammonium hydroxide and straight (negative mode) detection. Only raffinose, digoxin and reserpine can be detected.

Summarizing, the primary function of LC-MS additives is to enhance ionization in the ESI-MS interface. However, different additives are available and which one to use depends on the nature of the analyte and the HPLC and MS conditions. Irrespective of the choice of additive, it must be highly pure, to avoid introduction of extraneous mass signals, and application tested. Sigma-Aldrich's comprehensive line of additives and convenient pre-blended solutions for LC-MS meets the purity and characterization requirements for the most sensitive analyses.



Save time with pre-blended LC-MS solvents

Sigma-Aldrich offers convenient, pre-blended solutions of LC-MS solvents and the most common additives, saving you valuable time!

The mobile phase composition plays a critical role in the success of an LC-MS experiment. Formulations must be precise to provide accurate and reproducible results. However, making these mobile phases can be tedious and time-consuming, especially when you are faced with racks and racks of samples to analyze.

Sigma-Aldrich offers you a solution to this dilemma: pre-blended solutions of the most commonly used LC-MS mobile phases prepared with unsurpassed attention to quality. By using these convenient solutions, you can:

- Save time: Let us do the preparation for you.
- Be certain of accurate composition: Our solutions are tested following stringent QA criteria.
- Minimize baselines and artifacts: We use only the highest purity grades of solvents and additives.
- Ensure quality to the last milliliter: Each bottle is clearly labeled with the expiration date.

Let us take away some of the tedium so that you can concentrate on getting the most out of your LC-MS.

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