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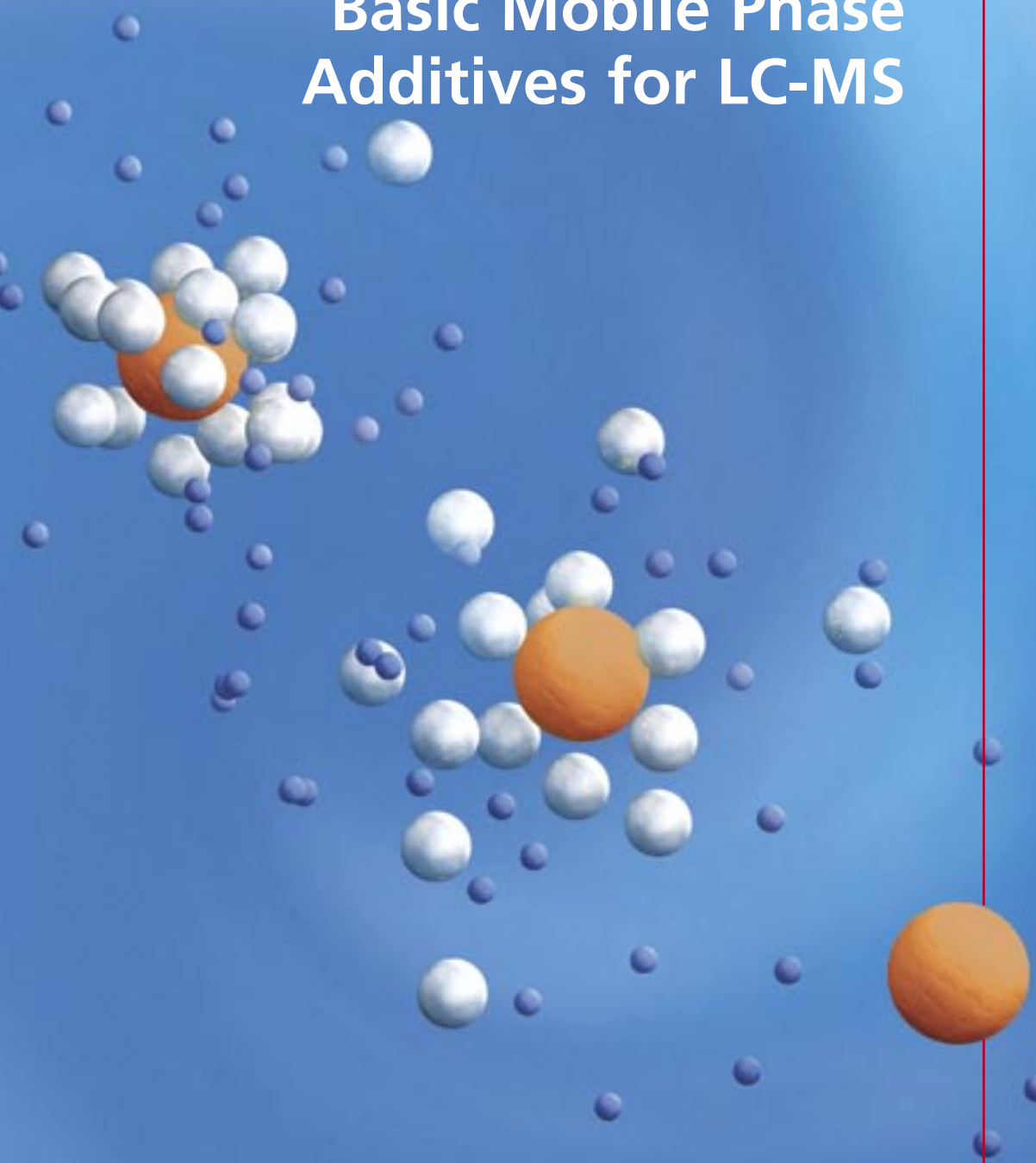
Basic Mobile Phase Additives for LC-MS



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SIGMA-ALDRICH

Mobile Phase Additives for LC-MS. Part 5: The Bases – Reverse Buffering, Negative and Reverse Ionization ..... This is the fifth article in a five part series on mobile phase additives for LC-MS to appear in each issue of Analytix in 2006 and the first issue in 2007

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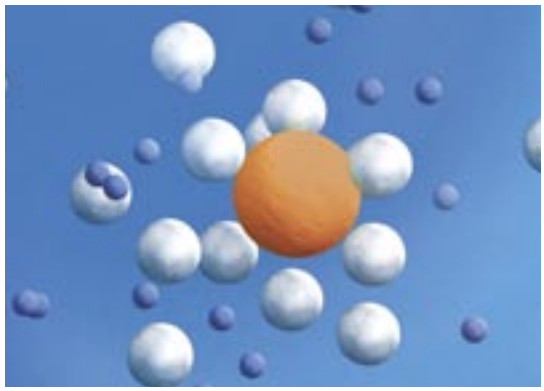


LC-MS analysis most often is run in positive ion mode using additives that support it, like organic acids and their ammonium salts. However, surprising possibilities exist outside this conventional approach. Basic additives are among the most interesting yet well-kept secrets in LC-MS. Basic pH is a good precondition for negative ionization (forming anions), but positive ionization (forming cations) is also possible. Under basic conditions, positive ionization would be the "reverse ionization," whereas negative ionization here is the "straight ionization." When the chromatographic resolution is best under acidic conditions, but sensitivity better in negative ion mode under basic conditions, the so-called "reverse buffering" can be useful, wherein a basic additive is added post-column via T-piece. Basic additives offer a much wider range of ionization capabilities than other additives, although they are somewhat compound-specific. Also, care must be taken in choosing an HPLC column that can withstand the high pH values that may occur when the basic additive is contained in the mobile phase.

For all figures ..... red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine and dark green is propazine.

Figure 1 ..... EIC of test compounds with 0.1% w/v ammonium bicarbonate as mobile phase additive (positive ion mode = reverse ionization)

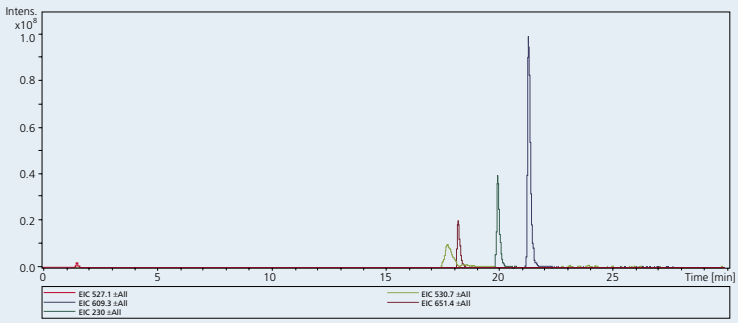


Table 1 ..... List of Sigma-Aldrich LC-MS additives

Cat. No.	Brand	Description*	Package Size	Packaging
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	10 x 1 mL	Glass ampuls
56302	Fluka	Formic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49199	Fluka	Acetic acid, puriss p.a., eluent additive for LC-MS	50 mL	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
49638	Fluka	Ammonium acetate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
61333	Fluka	Sodium citrate tribasic dihydrate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
40867	Fluka	Ammonium bicarbonate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
44273	Fluka	Ammonium hydroxide solution 25%, puriss p.a., eluent additive for LC-MS	100 mL	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 a homogeneous appearance. "p.a." or pro analysi denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

Table 2 ..... pH values of basic (alkaline) blends

Solvent	additive	pH
Water	0.1% ammonium bicarbonate	8.0
Acetonitrile	0.1% ammonium bicarbonate (8% water)	10.2
Water	0.1% ammonia	11.2
Methanol	0.1% ammonia (0.3% water)	10.9
Acetonitrile	0.1% ammonia (0.3% water)	12.1
Water	0.1% triethylamine	11.4
Acetonitrile	0.1% triethylamine	12.4

Figure 2 ..... EIC of test compounds with 0.1% w/v ammonium bicarbonate as mobile phase additive (negative ion mode = straight ionization)

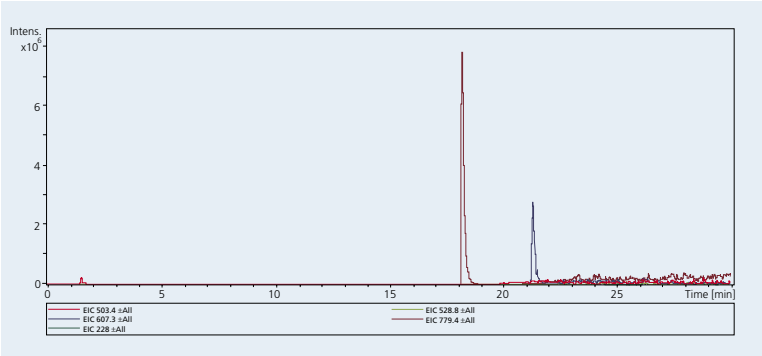


Figure 3 ..... EIC of test compounds with 0.1% w/v ammonia as mobile phase additive (positive ion mode = reverse ionization)

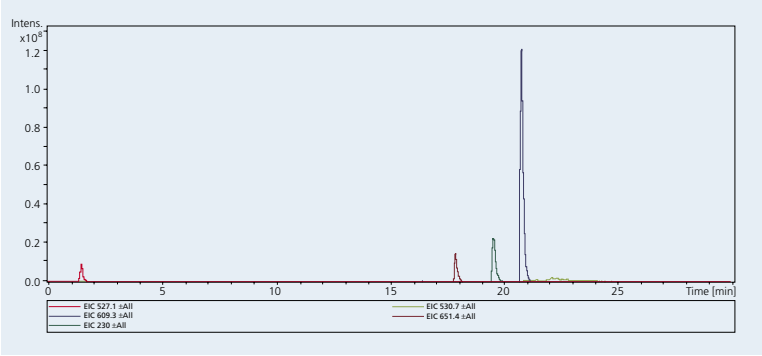


Figure 4 ..... EIC of test compounds, no additive in HPLC, 10% w/v ammonia added via T-piece post-column (negative ion mode = straight ionization)

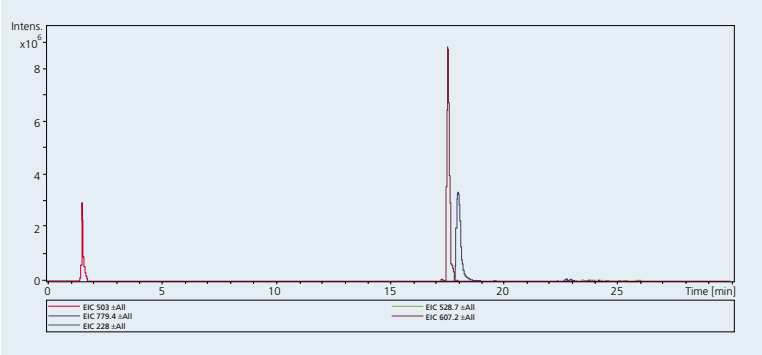
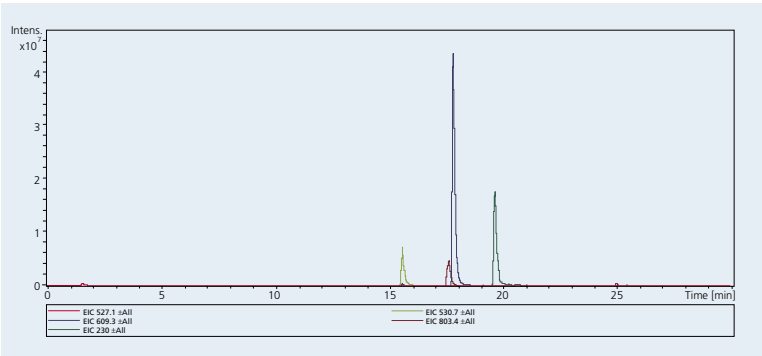


Figure 5 ..... EIC of test compounds, 0.1% FA as additive in HPLC, 10% w/v ammonia added via T-piece post-column (reverse buffering, positive ion mode = reverse ionization)



In this article we will describe some basic additives and experimental setups for their introduction. LC-MS-quality grades of three basic (alkaline) additives are offered by Sigma-Aldrich: ammonium bicarbonate, ammonium hydroxide solution (ammonia) and triethylamine (**Table 1**), listed in order of increasing basic strength (**Table 2**). Ammonium bicarbonate is used for separation and detection of amines and polar compounds under mildly basic conditions. Ammonia solution (ammonium hydroxide) is used for alkaline separation and for post-column addition via T-piece. Triethylamine is mainly used to obtain alkaline conditions for lipophilic compounds. As in previous articles in this series [1-4], we used the test mixture of the five model compounds, the same HPLC instrument (Agilent 1100) and MS detector (Bruker Daltonics esquire3000plus ion trap), and an electrospray-compatible flow rate of 0.4 mL/min through the column. For separations under alkaline conditions we used high pH-stable columns with bridged C18 material, for separations under neutral or acidic conditions (with post-column addition of basic additives) a Supelco Discovery® C18, 15 cm x 2.1 mm I.D., 5 µm, the latter giving the best separation (selectivity and resolution). Additionally an accelerated separation of narcotic drugs (opiates) with ammonium bicarbonate as mobile phase analyzed on a Waters Quattro Micro API Triple Quad MS is discussed.

Under the mildly-basic conditions of ammonium bicarbonate, the elution order of the test compounds is shifted (**Fig. 1**), but, surprisingly, the sensitivity in positive ion mode (reverse ionization) is one of the highest achieved in the entire series, especially for reserpine which is normally measured with addition of formic acid. Raffinose and bradykinin exhibit poor chromatography under these conditions (small or broad peak). In negative ion mode, only digoxin and reserpine are visible and raffinose is very small (**Fig. 2**). Similar results are obtained using ammonia as mobile phase additive, with reserpine giving once again a higher signal than ever achieved in positive ion mode (reverse ionization) and a good signal for raffinose in both modes (**Fig. 3**). Ammonia as an additive and negative ion mode (straight ionization) are the optimal conditions for raffinose and digoxin, especially, but not suitable for the other three test compounds. For these it may be useful to perform the separation under neutral conditions and to add the basic additive post-column via T-piece (**Fig. 4**). However, because the contribution of the flow from the column is much higher than that from the syringe, the additive has to be much more concentrated (here 10%). Under these negative ion mode conditions, raffinose exhibits an especially good signal.

Another interesting experimental setup is to perform the separation under acidic conditions, and change to

Figure 6 ..... EIC of test compounds, 0.1% formic acid as additive in HPLC, 10% w/v ammonia added via T-piece post-column (reverse buffering, negative ion mode = straight ionization)

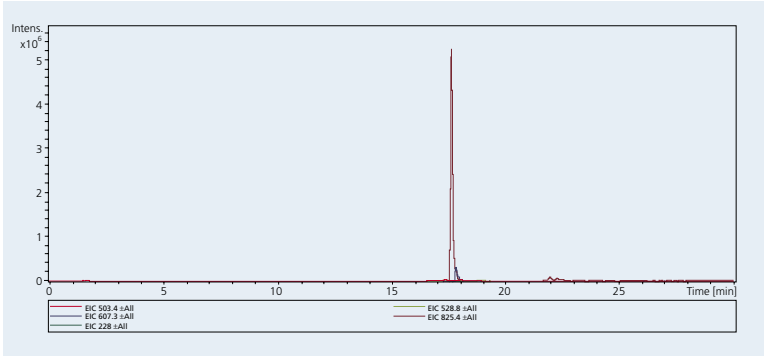


Figure 7 ..... Mass spectra of bilirubin, 0.1% formic acid as additive in HPLC, 10% v/v TEA in acetonitrile added via T-piece post-column (reverse buffering, pos. and neg. mode)

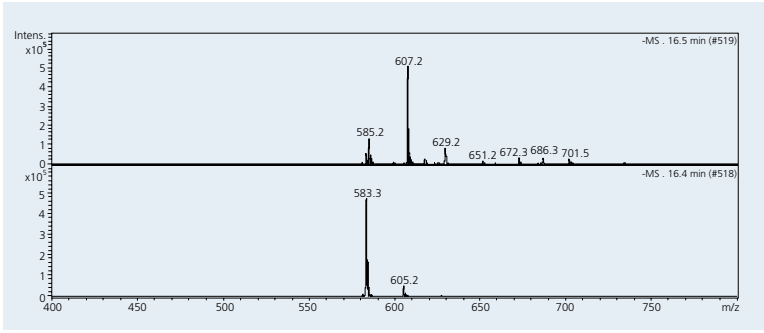
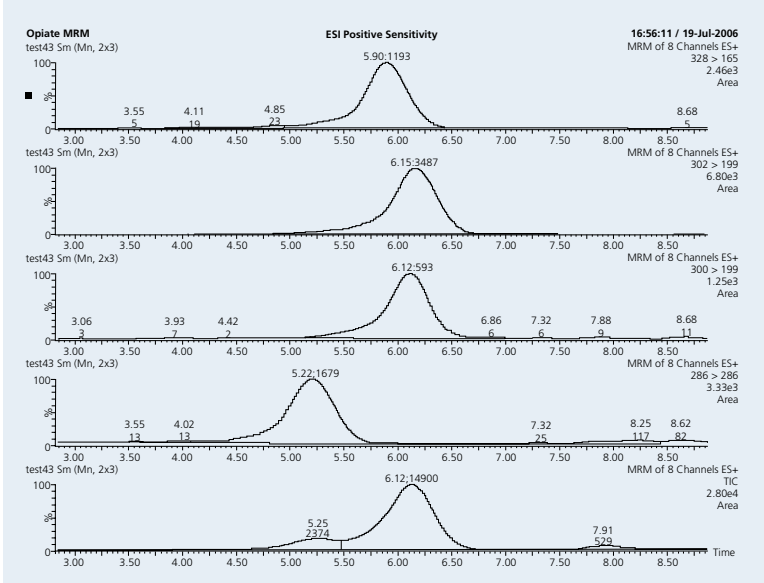


Figure 8 ..... MRMs and TIC of opiates, 90% 10mM ammonium bicarbonate in water (pH=10), gradient to 90% methanol; heroine (328→165), dihydrocodeine (302→199), codeine (300→199) and morphine (286); last track is TIC (positive ion mode = reverse ionization)



alkaline conditions post-column before the flow enters the MS-interface (reverse buffering). This can be combined with negative ion (straight) or positive ion (reverse) ionization (**Figs. 5 and 6**). Care must be taken when choosing the right mass for monitoring in EIC. Under these conditions, digoxin forms the $[M+Na]^+$ ion in positive ion mode and the $[M+formate]^-$ ion in negative ion mode (M+45!).

Chromatographic separation of the different bilirubin isomers can be achieved only under acidic conditions, but the MS signal is poor. Bilirubin elutes with a high percentage acetonitrile, so the best way to increase the pH is to add triethylamine (TEA) dissolved in acetonitrile (10% v/v). With this setup, mass spectra can be obtained in both positive and negative ion modes (reverse buffering with reverse or straight ionization, **Fig. 7**). The mass of bilirubin is 694.2 Dalton.

The rapid measurement of narcotic drugs is essential in emergency overdose situations. An experimental setup that permits the simultaneous determination of opiates is shown in **Fig. 8**. The elution is very fast under mildly basic conditions with ammonium bicarbonate as additive. Although the compounds co-elute, quantification is achieved by use of the different MRM-tracks of the molecules. This is a typical triple quad application, where the capabilities of the instrument were combined with optimal chromatographic conditions, which also allow a sufficient ionization in MS.

These various examples show the broad applications for basic additives in LC-MS, which, although not so well known and often underestimated, offer a variety of possibilities over the more-commonly used acidic additives. With this article, we close our series on LC-MS additives. We sincerely hope that we have provided you with some practical hints for the use of these "little helpers" in your daily work with modern, high-tech LC-MS, and have demonstrated that advances in LC-MS are not only driven by the physics of the instrument, but also the chemistry of the column and the mobile phase.

References

[1] "Mobile Phase Additives for LC-MS. Part 1: Acids – The Most Common Choice" **Analytix** 2006/2, 8-9.

[2] "Mobile Phase Additives for LC-MS. Part 2: How to Overcome Suppression Effects of TFA" **Analytix** 2006/3, 16-17.

[3] "Mobile Phase Additives for LC-MS. Part 3: The Neutral Salts" **Analytix** 2006/4, 9-11.

[4] "Mobile Phase Additives for LC-MS. Part 4: Special Case – Sodium Adduct Formation" **Analytix** 2006/5, 6-7.

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