Mobile Phase Additives for LC-MS. Part 1: Acids – The Most Common Choice .....

This is the first in a five-part series on mobile phase additives for LC-MS to appear in each issue of Analytix in 2006

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In LC-MS certain chemicals are often added to the mobile phase or introduced post-column prior to the interface to influence analyte ionization. Small organic acids like formic and acetic acid are among the most commonly used additives (see **Table 1**). Their widespread use is derived from two fundamental reasons. First, many chromatographic separations benefit in terms of retention and/or peak shape under acidic conditions. Second, most mass spectrometric measurements are done in positive ion mode, which is accomplished by the addition of a proton to form the molecular ion $[M+H]^+$. The above mentioned organic acids have necessary acidity and volatility to provide an excess of cations for this purpose.

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.

Test conditions

In this article, our aim is to demonstrate the effect on ionization, chromatographic and mass spectral behavior of some common acidic LC-MS mobile phase additives with five test analytes. **Table 2** lists the test compounds, their sum formula and mass in addition to the observed mass and its explanation. The additives were dissolved in both aqueous and organic mobile phase components at a concentration of 0.1%. The alternative method of adding them prior to the interface was not tested in this case. The MS was an ion trap (Bruker Esquire 3000+) operated in positive ion mode. Reliable MS-identification and quantification depends upon using MS-compatible HPLC columns and solvents to minimize background, reduce instrument fouling and maximize sensitivity. Riedel-de Haën LC-MS CHROMASOLV® solvents and Supelco's Ascentis™ and Discovery™ HS columns meet this requirements.

HPLC Conditions

LC-MS column: Supelco Discovery HS C18,
15 cm x 2.1 mm, 5 µm particles (Cat. No. 568502-U)
Mobile phase: A: Water (LC-MS CHROMASOLV®, Cat. No. 39253), B: Acetonitrile (LC-MS CHROMASOLV®, Cat. No. 34967)

Gradient profile:

Time (min.)	%A	%B
0	100	0
10	100	0
20	0	100
30	0	100

Flow rate: 0.4 mL/min
Sample: Raffinose, bradykinin, digoxin, propazine each 10 ng/mL, reserpine, 5 ng/mL
Injection volume: 5 µl

Table 2 ..... Test compounds

Cat. No.	Brand	Compound	Formula	Molecular Mass (monoisotopic)	Observed Mass	Explanation
83400	Fluka	Raffinose	$C_{18}H_{32}O_{16}$	504.2	527.2	$[M+Na]^+$
15859	Fluka	Bradykinin	$C_{50}H_{73}N_{15}O_{11}$	1059.6	530.8	$[M+2H]^{2+}$
37100	Fluka	Digoxin	$C_{41}H_{64}O_{14}$	780.4	803.4 651.3	$[M+Na]^+$ $[M\text{-Digitoxose}]^+$
R0875	Sigma	Reserpine	$C_{33}H_{40}N_2O_9$	608.3	609.3	$[M+H]^+$
45640	Fluka	Propazine	$C_9H_{16}N_5Cl$	229.1	230.1	$[M+H]^+$

Figure 1 ..... EIC without mobile phase additives

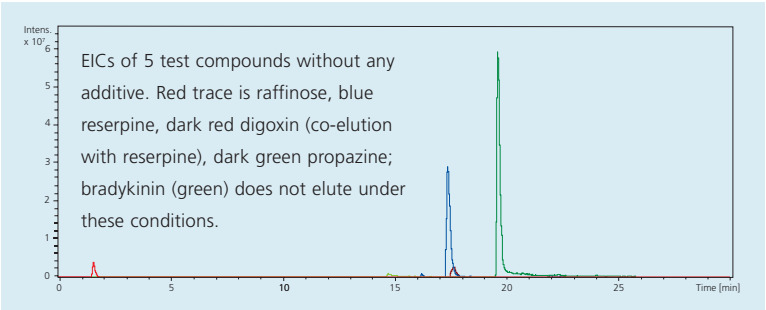


Figure 2 ..... EIC with acetic acid additive

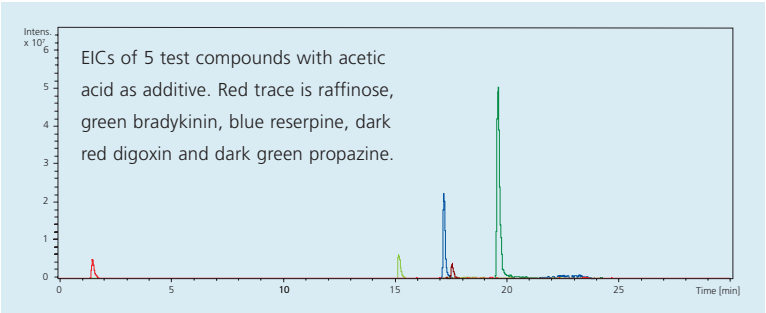


Figure 3 ..... EIC with formic acid additive

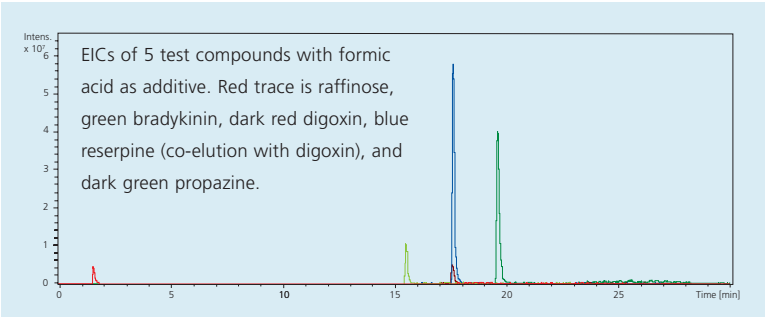


Figure 4 ..... Mass spectrum of propazine with and without acidic additives

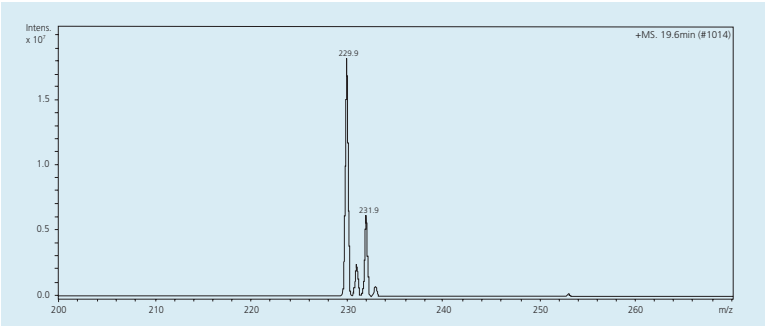
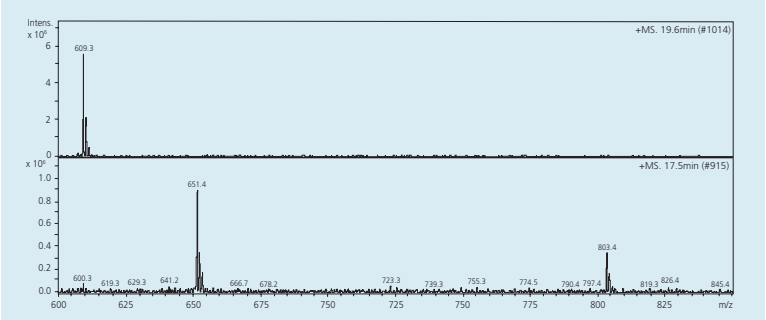


Figure 5 ..... Mass spectra of reserpine (upper) and digoxin (lower) with acetic acid as additive



Effect of acidic additives on chromatography and sensitivity

When used as a mobile phase additive, acids impact the retention behavior of pH-sensitive compounds, especially reserpine where slight pH changes shift its position relative to digoxin and even cause co-elution. The chromatograms always consist of the extracted ion chromatograms (EIC) of the observed mass of each compound. If the observed mass changes due to conditions, the EIC is adjusted accordingly. The y-axis is a measure of the extent of ionization and the achieved sensitivity, and is therefore kept constant in most examples. **Figures 1 – 3** show the effect of acidic additives on the EIC of the test compounds.

Without acidic additives in the mobile phase (**Fig. 1**) elution and ionization of bradykinin and digoxin are insufficient. However, the ionization of propazine is slightly better than under acidic conditions. It will be shown later in the series that neutral conditions are preferred for the ionization of propazine.
Fig. 4 shows the mass spectrum of propazine with the typical chlorine isotopic pattern and the addition of one H^+-ion; the theoretical observed mass is calculated with 230.1 Da. This spectral behavior does not change with the addition of acids, only the extent of ionization.

Resolution of reserpine and digoxin is accomplished by adding acetic acid (**Fig. 2**), which gives a pH of 3.3 to the aqueous portion of the mobile phase. Improved resolution results from a slight retention time shift of reserpine, sharper peaks and a change in the ionization of digoxin. In **Fig. 1** (no additives) the observed mass was the sodium adduct M = 803.4 $[M+Na]^+$, whereas with acetic acid as additive (**Fig. 2**) M = 651.3 $[M-Dig]^+$ is the most abundant mass and is actually a fragment, originating from the removal of one digitoxose unit. Reserpine shows the typical behavior of adding one H^+-ion. **Fig. 5** shows the mass spectra of reserpine (upper) and digoxin (lower) when acetic acid is added to the mobile phase.

Effect of formic acid addition, which gives a pH of 2.7 to the aqueous mobile phase component, is shown **in Figure 3**. Adding formic acid increases the reserpine signal and changes the relative elution of digoxin and reserpine again. The effect on raffinose and bradykinin is only small. More details on this in the next article of the series.

Conclusions

Although volatile, low molecular weight organic acids are commonly used as additives in LC-MS, their effect on ionization is usually not dramatic. Their primary advantage is that they improve ionization and resolution of a wide range of molecules