Mobile Phase Additives for LC-MS. Part 2: How to Overcome Suppression Effects of TFA ..... This is the second installment in a five-part series on mobile phase additives for LC-MS to appear in each of the issues of Analytix in 2006.

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Mobile phases for HPLC of proteins and peptides usually contain trifluoroacetic acid (TFA) to control the pH and improve peak shape and resolution. TFA enhances retention by ion pairing with the peptide and improves peak shape by reducing silanol interactions (1). However, TFA has adverse effects on MS detection. Its high surface tension prevents efficient spray formation and TFA ions in the gas phase ion-pair with the peptide's basic groups suppressing their ionization and reducing the MS signal (2, 3, 4). When TFA cannot be avoided, its effects can be mitigated by additional use of other acids, like formic or propionic acid, either post-column or as so called triple blends (**Tables 1 and 2**).

Table 1 ..... 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 Ampoules
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 ..... Selection of LC-MS solvents and blends

Cat. No.	Brand	Solvent or Blend Description	Package Size	Packaging
34965	Riedel-de Haën	2-Propanol Chromasolv LC-MS	1 L, 2.5 L	Amber glass bottle
34677	Riedel-de Haën	Water with 0.1% formic acid and 0.01% TFA	2.5 L	Amber glass bottle
34676	Riedel-de Haën	Acetonitrile with 0.1% formic acid and 0.01% TFA	2.5 L	Amber glass bottle

Table 3 ..... Components of the peptide mixture

Cat. No.	Brand	Component	Molecular Mass	Mol. ion / Charge
B4181	Sigma	Bradykinin fragm. 1-7	756.4	$[M+H]^+$ / 1
A8846	Sigma	Angiotensin II	1045.5	$[M+2H]^{2+}$ / 2
P2613	Sigma	$P_{14}R$	1532.9	$[M+2H]^{2+}$ / 2
A8346	Sigma	ACTH fragm. 18-39	2464.2	$[M+3H]^{3+}$ / 3
I 6154	Sigma	Insulin oxid. B chain	3493.7	$[M+3H]^{3+}$ / 3

All analytical conditions and test compounds were the same as already described in the first article (5), using TFA as additive instead or the triple blends as solvents respectively. Propionic acid was added post column / pre electrospray via T-piece as a 10% solution in 2-propanol. For additional experiments, a peptide mixture (pepmix) was prepared to study the specific influence on this kind of separation. The test compounds and the pepmix were both separated on a Supelco Discovery HS C18 column, 15 cm x 2.1 mm ID, 5 µm particle size; the 5 components (peptides) of the pepmix are listed in **Table 3**. MS-EIC traces are the same for all chromatograms.

Figure 1 shows the separation without any additive. Under these conditions, the basic peptide bradykinin is barely distinguishable from the baseline. Its mass spectrum can still be obtained (**Figure 2**, lower), showing the doubly-charged molecular ion $[M+2H]^{2+}$ with m=1061.6 or m/z = 530.8. Raffinose is unaffected by adding TFA or other organic acids. Its spectrum (**Figure 2**, upper) shows the H^+ (505 m/z) and NH_4^+ adducts (522.1 m/z) and the high abundance Na^+ adduct (527.1 m/z).

Addition of 0.1% TFA (**Figure 3**, top) causes all five test compounds to elute as well separated and sharp peaks. However, note that sensitivity drops almost 10-fold. The suppression effect is reduced by using 0.1% TFA and adding propionic acid (10% in 2-propanol) post-column (**Figure 3**, middle), an effect described in detail by Apffel et al. (2). Using the triple-blend of 0.1% formic acid/0.01% TFA (**Figure 3**, lower) greatly improves the signal, but with a compromise. Compared to TFA alone, resolution is poorer; and compared to formic acid alone (5), sensitivity is poorer.

The three additives can be used in synergy, by balancing their benefits and limitations. Add small amounts of TFA to formic or propionic acid to improve peak shape;

Figure 1 .....

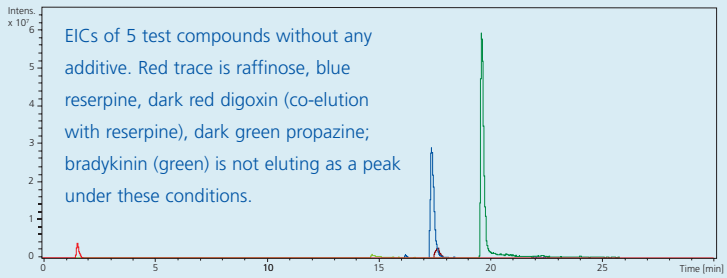


Figure 2 .....

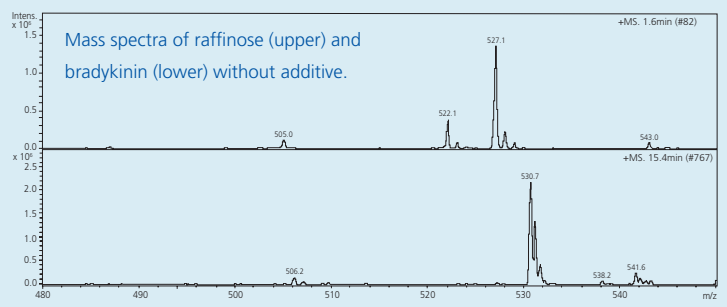
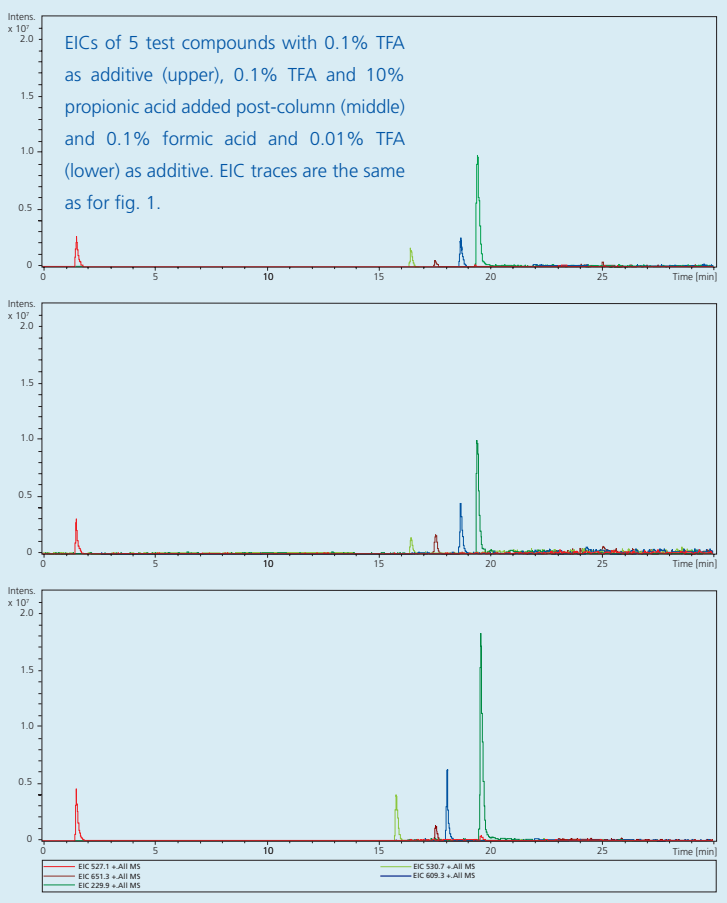


Figure 3 .....

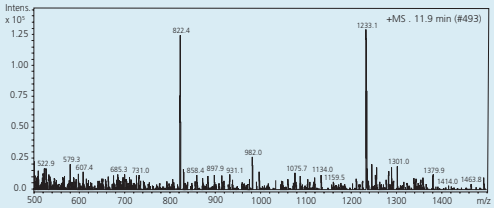


reduce TFA and add formic or propionic acid to improve the MS signal. Other MS and chromatographic parameters also influence this decision, including analytes, column packing material and dimensions, length of mixing zone, flow rate, etc. (1, 2). This is especially true for peptide separations. The charge state of the molecular ion is not affected by this and varies in the pepmix between singly-charged (Bradykinin fragment 1-7) and triply-charged (insulin oxidized B chain) (**Table 3**). Depending on instrument and conditions it may be the case that one peptide appears in more than one charge state, i.e. doubly- and triply-charged in one spectrum (**Figure 4**).

In summary, the ionization-suppressing effects of TFA can be partly overcome by addition of other LC-MS compatible organic acids, like formic or propionic acid. For convenience and to guarantee reliable composition, Sigma-Aldrich offers pre-blended LC-MS mobile phases that contain acidic additives in high purity LC-MS CHROMASOLV® grade solvents. Our triple blends contain TFA with formic acid to provide both MS sensitivity and chromatographic performance.

Figure 4 .....

Mass Spectrum of ACTH fragm. 18-39 with 0.1% TFA as additive and 10% propionic acid post-column. Doubly (m/z 1233.1) and triply (m/z 822.4) charged molecular ions.



References

[1] "Eliminate TFA and Improve Sensitivity of Peptide Analyses by LC-MS" Supelco Application Note 168 (T302168).

[2] A. Apffel, A.; Fisher, S.; Goldberg, G.; Goodley, P. C.; Kuhlmann, F.E.; *J. Chromatogr. A*, 1995, 712, 177-190.

[3] Mirza, U. A.; Chait, B. T.; *Anal. Chem.* 1994, 66, 2898-2904.

[4] Wang, G; Cole, R. B.; *J. Am. Soc. Mass Spectrom.*, 1996, 7(10), 1050-1058.

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