Mobile Phase Additives for LC-MS. Part 4: Special Case - Sodium Adduct Formation .....

This is the fourth article 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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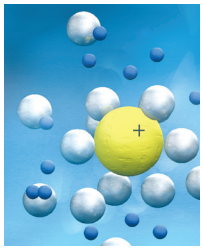


Although sodium and other alkali metals are typically avoided in LC-MS, it can be useful to add sodium salts when the analyte ions have a very high tendency to form alkali adducts. This is especially true for the carbonyl group of sugars and glycosides, in addition to hydroxyl and carboxyl groups. These groups often form sodium adducts even under acidic conditions, e.g. with formic acid, because sodium is nearly always present at trace levels. Adduct formation is associated with a decrease in sensitivity, and often simultaneous addition of H^+, NH_4^+ and Na^+ is observed. When adduct formation tendency is strong, addition of small and defined amounts of sodium ions (mostly post-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

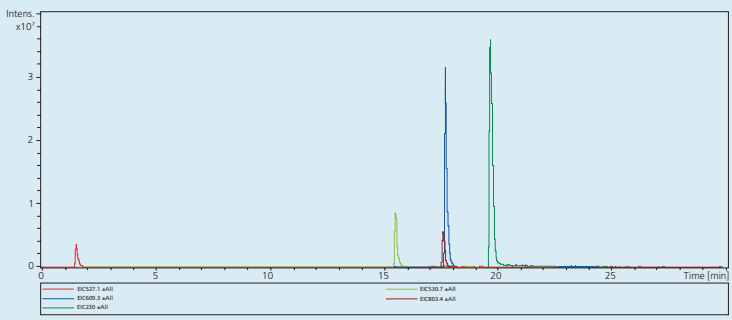
Table 1 ..... List of Sigma-Aldrich LC-MS additives

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	10x1 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.

Figure 1 ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid as mobile phase additive (no sodium added).

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine and dark green is propazine.



In this article, we will discuss the benefits and practical procedures for using sodium salts as LC-MS mobile phase additives. Riedel-de Haën LC-MS CHROMASOLV® solvents and Supelco Discovery™ HS C18 HPLC columns were used to meet the stringent requirements of LC-MS. These, and the MS conditions, test compounds and mobile phase (water-acetonitrile gradient with 0.1% formic acid) were as described in Part 1 of this series [1]. In this study, an additional series of experiments was run with a concentration of 100 ng/µL raffinose. A 0.1% w/v aqueous solution of sodium, as acetate or citrate salt, was added post-column. The sodium citrate solutions were adjusted to pH 7.8, 5.0 or 3.1. Taking flow rate into account, the total concentration of sodium salt introduced into MS was 0.001% w/v (0.1 mM). It is important to keep the sodium concentration at 1 - 5mM or below, otherwise some MS instruments will be overloaded and show a dramatic loss of sensitivity caused by reversible suppression. The sodium citrate and other high-purity Fluka-brand LC-MS additives from Sigma-Aldrich appear in **Table 1**.

Fig. 1 shows the initial separation of the five test compounds with formic acid as the sole additive. Co-elution of digoxin and reserpine is observed with reserpine having much higher intensity. Raffinose and digoxin exhibit formation of the sodium adduct of the molecular ion in the mass spectrum without any addition of sodium ions.

To observe the effect of addition of sodium ions, a 0.1% w/v aqueous solution of sodium acetate was added post-column. Results appear in **Figure 2**. By adding the sodium, the absolute intensity is reduced, and the relative intensity of digoxin and reserpine is reversed. The signal from the glycoside digoxin is boosted by addition of sodium, whereas the other compounds either lose intensity or stay constant.

The selective increase of the glycoside signal is also observed when sodium citrate is added. The influence

Figure 2 ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid in the mobile phase, and 0.1% w/v sodium acetate added post-column.

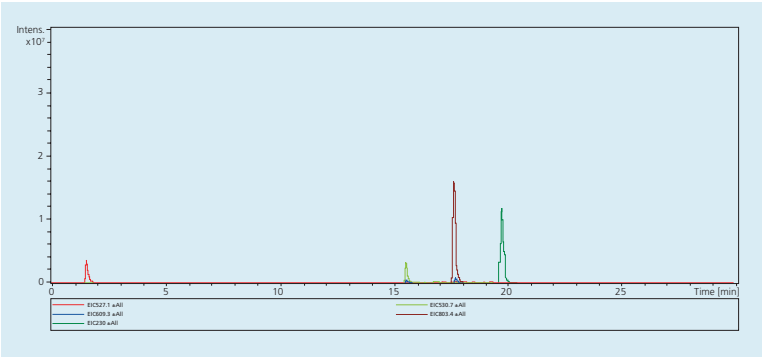


Figure 3 ..... EIC (positive ion mode) of test compounds with 0.1% w/v formic acid in the mobile phase, and 0.1% w/v sodium citrate (pH 5.0) added post-column.

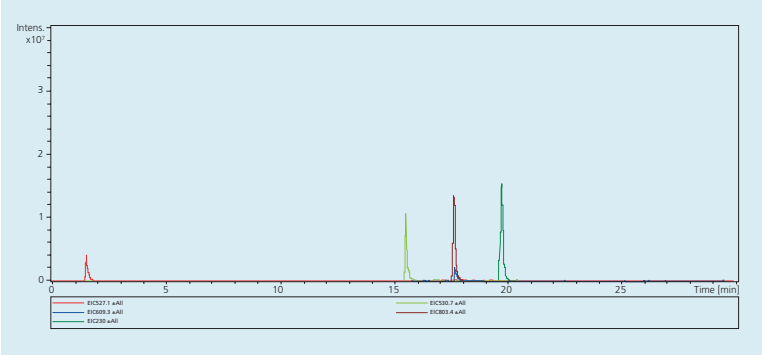


Figure 4 ..... Signal intensities for the 5 test compounds with sodium addition under different conditions (anion and pH). Raffinose at 10 ng/µL.

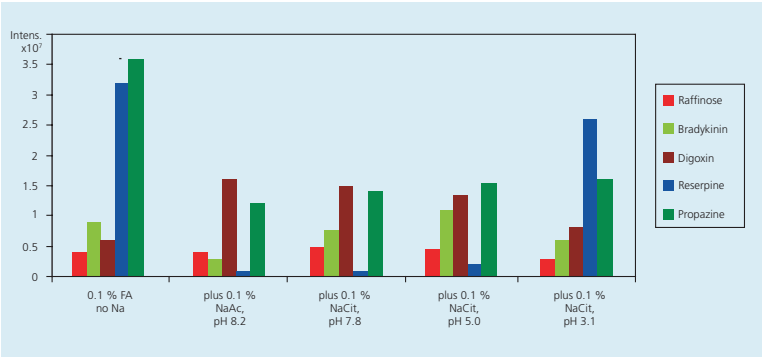
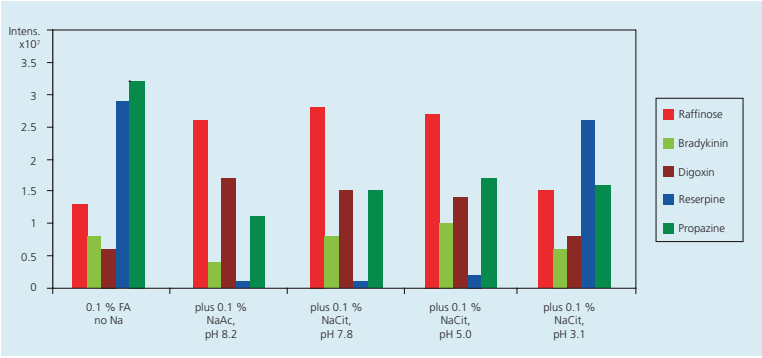


Figure 5 ..... Signal intensities for the 5 test compounds with sodium addition under different conditions (anion and pH). Raffinose at 100 ng/µL.



of pH was also studied using sodium citrate. **Fig. 3** shows the effect of addition of sodium citrate at pH 5.0. While the digoxin signal is reduced slightly, the bradykinin signal is increased significantly. This is only one example of how pH, together with addition of sodium ions, can influence analyte ionization. The phenomenon is attributed to a competition between the different ions within the electrospray interface.

Further experiments were carried out with sodium citrate solutions at different pH values and two different raffinose concentrations, 10 and 100 ng/µL. At low concentrations (10 ng/µL, **Figure 4**), the raffinose signal shows no dependence on pH. However, at higher raffinose concentrations (100 ng/µL, **Figure 5**), the signal is influenced by pH, exhibiting a maximum at pH 7.8. For digoxin, the maximum signal is obtained in sodium acetate at pH 8.2. Both raffinose and digoxin are highly susceptible to sodium adduct formation. Bradykin is not susceptible to adduct formation, but its signal is also influenced by pH (hydrogen ion concentration) and the presence of sodium ions. Propazine and reserpine both go through a broad minimum when Na^+ concentration is high and H^+ concentration is low.

But sensitivity is only part of the story. Stability, and perhaps specificity, of the molecular ion are also considerations. The ability to form alkali adducts is useful for quantifying certain classes of molecules, e.g. formation of Cs^+ adducts for digoxin and digitoxin or immunosuppressive drugs [2, 3] and the determination of ionophores by addition of Li^+, Na^+ or K^+ [4]. It should also be possible to selectively enhance the LC-MS signals of glycopeptides within a peptide mixture with Na^+. Truly, the use of alkali ions, particularly sodium ions, to enhance LC-MS sensitivity or selectivity has not been explored to the limits as yet.

References

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