

Formation of Metal Adduct Solvent Ions or Ion Clusters and the Influence on LC/MS Analysis



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Introduction

Metal adduct ions and protonated ions formed during LC/MS analysis are widely used as an adjunct for determination of molecular weights or characterization of molecular structures. If needed, trace metal ions, e.g., Na^+ or Li^+ , are introduced to enhance intensity and consistency of adduct ions or to form desired adducts. The metal ions added, however, also generate relatively stable metal adduct solvent ions in various combinations. The adduct ions are distributed over the entire mass monitoring range thus making it very difficult to determine the molecular ions of unknown chemical entities. In the multiple reaction monitoring mode, the presence of adduct ions with similar mass units to the molecular ion of an analyte may also significantly increase instrument background. Experiments were designed and conducted to investigate the formation of sodium adduct solvent ions or ion clusters

Methods

HPLC Condition

LC System: Waters 2690 Separations Module
Mobile Phase

A: CH_3CN
B: 25 mM NH_4OAc , or NH_4OCH_3 , or NH_4OAc with 1% of HCOOH , with or without 0.5 mM NaOAc in H_2O .
Isocratic: A : B = 50 : 50
Flow: 0.3 mL/min

Mass Spectrometer Condition

MS System: PE Sciex API-365
Mode: LC/ESI-MS and MS/MS in positive or negative mode
Ion Spray: 5000 V
Turbo Gas Temp: 400 °C
Orifice: 40 Units
Ring: 300 Units
Collision Energy: 15 ~ 40 eV

Results & Discussion

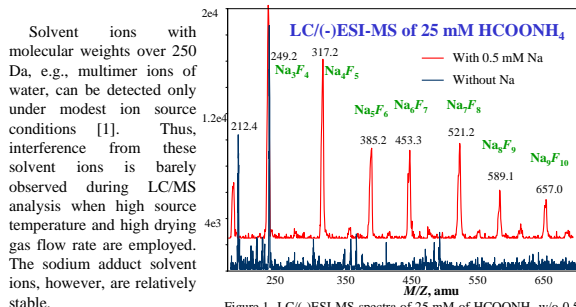


Figure 1. LC/ESI-MS spectra of 25 mM of HCOONH_4 w/o 0.5 mM of NaOAc ; F: formate ion (HCOO^-) with 45 Da

- Stable sodium adduct solvent clusters from HCOONH_4 solution were observed in negative ion mode (Figure 1). The MS peaks were revealed to be $\text{Na}_n \text{F}_{(n+1)}$ ($\text{F} = \text{HCOO}^-$) by MS/MS product ion scan.
- Stable sodium adduct solvent clusters from NH_4OAc solution were observed in both positive and negative modes (Figure 2). The MS peaks were revealed to be $\text{Na}_n \text{A}_{(n+1)}$ ($\text{A} = \text{CH}_3\text{COO}^-$) for negative mode, and $\text{Na}_{(n+1)} \text{A}_n$ for positive mode.
- When 0.01% HCOOH was added to 25 mM NH_4OAc containing 0.5 mM NaOAc solution, ion clusters rather than single ion peaks were observed (Figures 3,4). Each ion cluster consisted of 3-4 ion peaks with a 14 amu difference between two adjacent peaks. MS/MS product ion scans revealed that the ion clusters were formed by various numerical combinations of Na^+ , HCOO^- (F), and CH_3COO^- (A).

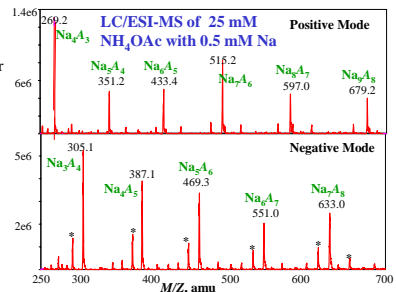


Figure 2. LC/ESI-MS spectra of 25 mM of NH_4OAc with 0.5 mM of NaOAc . *Peaks due to the existence of trace HCOOH in the system (Figures 3 & 4); A: Acetate ion (CH_3COO^-) with 59 Da

The 14 amu difference corresponded to the mass distinction between A and F. The MS/MS product ion scans revealed that the ion clusters were formed by combination of various numbers of Na^+ , HCOO^- (F), and CH_3COO^- (A) (Table I). The peak intensities could be estimated statistically with binomial coefficients.

- In the LC/MS/MS quantitation analysis, part of the background comes from similar ion transition of the solvent adducts and an analyte. NH_4OAc with a trace of Na^+ and HCOOH mobile phase, therefore, would possibly cause high background.

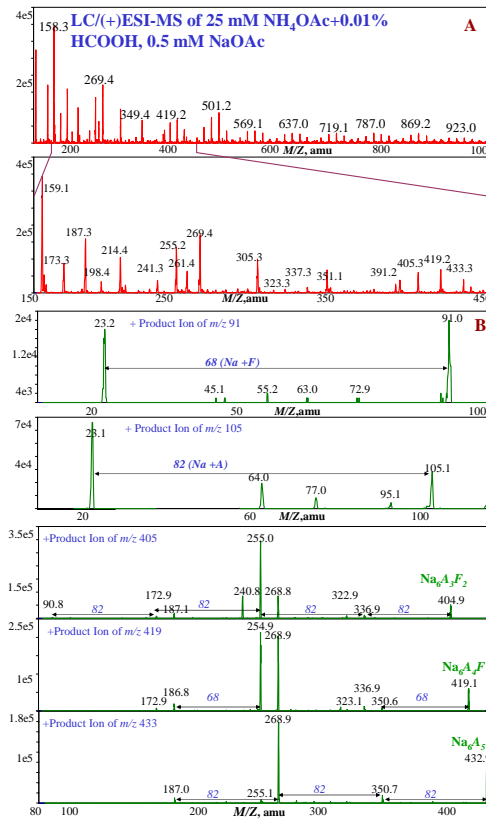


Figure 3. A: LC/ESI-MS spectra of 25 mM of NH_4OAc and 0.01% of HCOOH w/o 0.5 mM of NaOAc ; B: The product ion scans of m/z 91, 105, 405, 419, and 433.

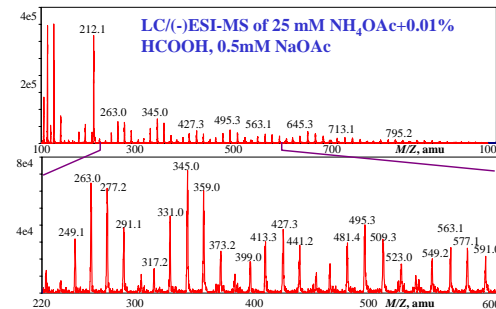


Figure 4. LC/ESI-MS spectra of 25 mM of NH_4OAc and 0.01% HCOOH with 0.5 mM of NaOAc .

m/z	Positive Mode			Negative Mode			
	Cluster	m/z	Cluster	Cluster	m/z	Cluster	
91	$\text{Na}_2 \text{F}$	323	$\text{Na}_3 \text{A}_2 \text{F}_2$	249	$\text{Na}_2 \text{F}_4$	373	$\text{Na}_4 \text{A}_4 \text{F}$
105	$\text{Na}_3 \text{A}$	337	$\text{Na}_4 \text{A}_2 \text{F}$	263	$\text{Na}_3 \text{A}_3 \text{F}_3$	399	$\text{Na}_5 \text{A}_5 \text{F}_5$
159	$\text{Na}_3 \text{F}_2$	351	$\text{Na}_5 \text{A}_4$	277	$\text{Na}_4 \text{A}_2 \text{F}_2$	413	$\text{Na}_6 \text{A}_6 \text{F}_6$
173	$\text{Na}_2 \text{A}_2 \text{F}$	391	$\text{Na}_6 \text{A}_5 \text{F}_3$	291	$\text{Na}_5 \text{A}_3 \text{F}$	427	$\text{Na}_7 \text{A}_7 \text{F}_7$
187	$\text{Na}_3 \text{A}_2$	405	$\text{Na}_6 \text{A}_4 \text{F}_2$	317	$\text{Na}_4 \text{F}_5$	441	$\text{Na}_8 \text{A}_8 \text{F}_8$
241	$\text{Na}_4 \text{A}_2 \text{F}$	419	$\text{Na}_7 \text{A}_6 \text{F}$	331	$\text{Na}_4 \text{A}_2 \text{F}_4$	467	$\text{Na}_9 \text{A}_9 \text{F}_9$
255	$\text{Na}_4 \text{A}_3 \text{F}$	433	$\text{Na}_6 \text{A}_5$	345	$\text{Na}_5 \text{A}_4 \text{F}_2$	481	$\text{Na}_8 \text{A}_8 \text{F}_8$
269	$\text{Na}_4 \text{A}_3$	501	$\text{Na}_7 \text{A}_5 \text{F}_4$	359	$\text{Na}_4 \text{A}_2 \text{F}_4$	495	$\text{Na}_9 \text{A}_9 \text{F}_9$

Table I. Peak assignments of the LC/ESI-MS signals of 25 mM NH_4OAc and 0.01% HCOOH with 0.5 mM NaOAc (Figures 3 & 4); F: Formate ion with 45 Da; A: Acetate ion with 59 Da

Conclusion

- Formation of sodium adduct solvent clusters in different HPLC mobile phases were extensively analyzed by LC/ESI-MS and MS/MS. The results indicate that 25 mM NH_4OAc buffer with trace of Na^+ ion gives very complicated ion clusters over a wide mass range. If Na^+ addition is necessary, selection of HPLC mobile phases should be given carefully attention, so that the interference from the solvent ion clusters can be avoided.
- Similar results were also observed when other metal salts, e.g., KCl , LiCl , KCl , and AgNO_3 , were added to the HPLC aqueous mobile phase consisting of 25 mM NH_4OAc and CH_3CN .

References

1. Ledman, D. W., Fox, R. O., *J Am Soc Mass Spectrom* 1997, 8, 1158-1164.