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

Zhe-ming Gu, Dawei Zhou, Xian-guo Zhao, and Jinn Wu

XenoBiotic Laboratories, Inc. 107 Morgan Lane, Plainsboro, New Jersey 08536

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: CH₃CN
B: 25 mM NH₄OAc, or NH₄OOCH, or NH₄OAc with 1% of HCOOH, with or without 0.5 mM NaOAc in H₂O

Isocratic: A: 100 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

Solvent ions with molecular weights over 250 Da, e.g., multimer ions of water, can be detected only under modest ion source conditions [1]. Thus, interference from these solvent ions is barely observed during LC/MS analysis when high source temperature and high drying gas flow rate are employed. The sodium adduct solvent ions, however, are relatively stable.

- Stable sodium adduct solvent clusters from HCOONH₄ solution were observed in negative ion mode (Figure 1). The MS peaks were revealed to be Na₈F₂⁺ (F⁻=HCOO⁻) by MS/MS product ion scan.
- Stable sodium adduct solvent clusters from NH₄OAc solution were observed in both positive and negative modes (Figure 2). The MS peaks were revealed to be Na₈F₆⁻ (F⁻=HCOO⁻) for negative mode, and Na₈F₆₂⁺₂⁻ for positive mode.
- When 0.01% HCOOH was added to 25 mM NH₄OAc containing 0.5 mM NaOAc solution, 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⁻, and CH₃COO⁻ (Table 1). The ion intensities could be estimated statistically with binomial coefficients.

In the LC/MS quantitation analysis, part of the background comes from similar ion transition of the solvent adducts and an analyte. NH₄OAc with a trace of Na⁺ and HCOOH mobile phase, therefore, would possibly cause high background.

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 NH₄OAc buffer with trace of Na⁺ ions 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, KClO₄, and AgNO₃, were added to the HPLC aqueous mobile phase consisting of 25 mM NH₄OAc and CH₃CN.

References