Tech Tip

Dealing with Metal Adduct Ions in Electrospray: Part 1

The term “adduct ion” is a popular term among liquid chromatography/mass spectrometry (LC/MS) users to describe ions formed by adduction of alkali metal ions to an analyte molecule in positive ion analysis.

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Dealing with Metal Adduct Ions in Electrospray: Part 1

The most commonly observed metal adduct ions in positive-ion electrospray analyses are single-charge sodium and potassium adducts, symbolized [M+Na]+ and [M+K]+ respectively. However, ions in electrospray can form with many other adducting species, e.g., ammonium ions [M+NH₄]+; metal ions abstracted from the electrospray needle at high voltages, for example, [M+Fe]+; adducted solvent molecules such as [M+H+H₂O]+; and, in negative-ion analysis, negative-charged adducting species like [M+Cl]⁻. Adduct species other than protonated molecules may also occur in atmospheric pressure chemical ionization (APCI) interfaces. Adduct ions in APCI must be volatile which means the ammonium, chloride, and water adducts described above can occur in APCI but the metal adduct ions cannot.

How can I recognize these ions?
Table 1 illustrates common adduct ion species seen in electrospray and their corresponding m/z values. The first three rows show the most common single charge species. Figure 1 shows the analysis of a single analyte with a molecular mass of 212 u in the presence of sodium and potassium ions. The peak at m/z 213 represents the protonated molecule [M+H]+. The peak at 22 m/z units above the protonated molecule is the sodium adduct and the one 38 units above is the potassium adduct. Analysts who routinely use electrospray must train their eye to look for one or both of these metal adduct species when developing LC/MS methods. In some cases, the protonated molecule may be completely absent and only metal adduct species are present. We will discuss a real-world example of this problem in next month’s instalment of MS Solutions.

The last four rows of Table 1 illustrate variations on multiple-charge ions. In these cases note that each adducting species contributes one positive charge and the mass spectral peak shows up at an m/z value corresponding to the mass of the adduct species divided by the total number of charges on the ion. Highly charged species, such as intact polypeptides, are characterized by charge heterogeneity, i.e., the mass spectrum shows multiple peaks each representing a different charge state. Charge heterogeneity in the presence of alkali metal ions can lead to a completely unusable mass spectrum in which there is a peak at nearly every mass-to-charge ratio.

For this and other reasons it is important for
analysts to understand the source of metal adduct ions and to know how to control their formation.

Why me?
The first question many users ask is “Why do I see these ions?” Analysts are baffled by the fact that they have not added any sodium- or potassium-containing reagents to their sample or mobile phase and yet they still see a significant amount of ion current corresponding to the $m/z$ value of these adduct ions.

The most common source of this metal contamination is laboratory glassware. Various salts are used in the glass manufacturing process and can leach from the glass in the presence of aqueous solvents. For this reason, plasticware is often substituted for glass in the electrospray laboratory. Plasticizers and other organic molecules may leach from plasticware but are generally less of a problem than metal ion adducts because they occur at known and fixed $m/z$ values and can therefore be discounted when interpreting mass spectra.

Another common source of sodium and potassium contamination is the analysts themselves. Simply touching lab ware with an ungloved hand can transfer enough salt to cause a significant appearance of metal adduct ions.

Biological samples, of course, often have a high endogenous concentration of various salts while other salts may be added during the sample preparation process. Clinical patient samples such as urines or plasmas will vary widely in salt concentration and the greater and lesser degree of metal adduct ion formation from one sample to another can lead to inaccurate quantitative results.

Many existing HPLC methods use sodium- or potassium-containing reagents for pH control or ion-pairing. It is important to modify these methods to eliminate these metal ion salts when moving the method to LC/MS.

What can I do about it?
Regardless of the source, it is important to understand and control formation of metal adduct ions particularly for quantitative applications. There are two strategies which can be employed:

1. Use alternative solvents: Switching to organic solvents that do not leach metal ions from labware can help reduce adduct ion formation.

2. Modify sample preparation: Pre-treat samples with ion-exchange resins or other methods to remove excess metal ions before analysis.

Table 1: Common Adduct Ions in Electrospray

<table>
<thead>
<tr>
<th>Adduct Ion Composition</th>
<th>Observed $m/z$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]$^+$</td>
<td>M+1</td>
</tr>
<tr>
<td>[M+Na]$^+$</td>
<td>M+23</td>
</tr>
<tr>
<td>[M+K]$^+$</td>
<td>M+39</td>
</tr>
<tr>
<td>[M+xH]$^x$</td>
<td>(M+x)/x</td>
</tr>
<tr>
<td>[M+xNa]$^{x+23}$</td>
<td>(M+23x)/x</td>
</tr>
<tr>
<td>[M+xK]$^{x+39}$</td>
<td>(M+39x)/x</td>
</tr>
<tr>
<td>[M+xNa+yK+zH]$^{x+23y+39z}$</td>
<td>(M+23x+39y+z)/(x+y+z)</td>
</tr>
</tbody>
</table>

Table 1: Common Adduct Ions in Electrospray

*M is the molecular mass of the analyte molecule

Figure 1: Typical electrospray mass spectrum with Na and K adduct ions
work reliably for dealing with these ions:

(1) Lower the pH: The preferred method for dealing with unwanted alkali metal adducts is to lower the pH of the mobile phase with a simple organic acid such as formic acid. This provides an excess of protons relative to metal ions which in turn drives all or a major portion of the ion formation to the protonated molecule \([M+H]^+\). Compared with an unmodified mobile phase, lowering the pH should improve ionization efficiency and thus provide a better LOD for the protonated molecule.

(2) Add potassium or sodium acetate to the mobile phase. Just as the large excess of protons in the previous example drives formation of protonated species, the large excess of metal ion drives the nearly exclusive formation of the metal adduct ion species. You should go to this strategy if approach (1) does not eliminate most of the metal adduct species. You may also find that response is better (improved LOD), for metal adduct ions than for protonated species although this can only be determined by experiment. Note that it is important to use acetates or other volatile reagents. Involatile salts will precipitate in the interface and may plug the entrance to the MS.

Fred Klink is a trainer and consultant to the pharmaceutical, biotech, and chemical industries as well as law enforcement and other government laboratories. Fred’s specialty is HPLC, LC/MS, and solid-phase extraction technologies.

Fred received a degree in biochemistry from Northwestern University and completed graduate studies and an internship in forensic chemistry at the University of Illinois. After graduation, Fred entered the analytical instruments industry where he spent seventeen years in varying positions from applications chemist, development project manager, and manager for strategic planning. Fred has been teaching highly regarded MS and LC/MS courses and providing consulting services since 1996.

Fred is the author of several journal articles and book chapters including the LC/MS entry in the Wiley Encyclopedia of Analytical Chemistry. He is a member of the American Chemical Society and American Society for Mass Spectrometry.
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Dealing with Metal Adduct Ions in Electrospray: Part 2

Last month we discussed the fundamental issue of metal adduct ion formation in electrospray LC/MS including method development strategies for dealing with adduct ions. This month we will examine a real-world application which employs these strategies.

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Dealing with Metal Adduct Ions in Electrospray: Part 2

Last month we discussed the fundamental issue of metal adduct ion formation in electrospray LC/MS including method development strategies for dealing with adduct ions. This month we will examine a real-world application which employs these strategies. A few years ago our laboratory developed a method for penicillin G by electrospray LC/MS. The structure of the compound is shown below:

The molecular mass of penicillin G is 334 Da. The molecule contains two amides and a carboxylic acid moiety, therefore, both positive and negative ion methods may work well. From the known fragmentation pattern shown in the figure it is clear that either nitrogen may be protonated because both fragments $F_1$ and $F_2$ produce expected MS/MS fragmentation.
intense peaks in positive ion MS/MS. With two possible sites for protonation we thought positive ion may be the better choice and decided to begin our investigation there.

Unexpected Initial Results

We expected to find the protonated molecule ([M+H]+) at m/z 335 and, knowing that our standard was a potassium salt, we also expected the potassium adduct ion at m/z 373. Upon running the sample, we obtained the spectrum shown in Figure 1. The expected peak at 335 was completely absent, however a very intense peak at 373 was noted and identified as the potassium adduct ion peak and confirmed based on its isotope peak pattern (see below for further description of isotope peaks).

A second very intense peak was present at m/z 411. This m/z value represents M+77. It is theoretically possible to have a doubly charged ion of penicillin G because two nitrogens are available as charge loci. Two potassiations though would result in a mass of 412 (M+78), not 411. More importantly, a doubly potassiated ion would be a double-charge ion ([M+2K]+2) and would thus show up in the spectrum at m/z 412÷2 or 206.

Our next step was to examine the 411 peak in more detail. Examination of Figure 2 shows there is a very intense X+2 peak, i.e., a peak whose m/z value is 2 units higher than the main peak at 411. This indicates the presence in the ion of elements which have a good abundance of a naturally occurring stable isotope whose mass is two mass units greater than the integer nominal mass of the element. Common elements of this type seen in electrospray LC/MS are oxygen, chlorine, bromine, sulfur, and potassium. Sulfur and oxygen are known to be present in penicillin G but cannot account for all of the

<table>
<thead>
<tr>
<th>Ion Type and m/z</th>
<th>No additive</th>
<th>0.2% formic acid</th>
<th>50 μM potassium acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]+</td>
<td>335</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>[M+K]+</td>
<td>373</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>[M+2K-H]+</td>
<td>411</td>
<td>23</td>
<td>100*</td>
</tr>
</tbody>
</table>

Table 1: Results of method development experiments for Penicillin.
*All results normalized to this peak height

Figure 1: Electrospray LC/MS spectrum of Penicillin G sample.
X+2 peak intensity. However, if two potassium atoms are present then the intensity of this peak can be explained. (A future MS Solutions column will explain how to perform these isotope ratio calculations.)

Having already eliminated the possibility of a double-charge metal adduct ion we were left with only one conclusion and that is a potassium adduct ion of a potassium salt. This ion takes the form [M+2K-H]+. One potassium displaces the acidic proton on the carboxylic acid moiety of the penicillin G and the other adducts to one of the nitrogens. Thus the total mass is M+2K= 411 and the resultant charge is +1.

We have identified both potassium adduct ion species but this method is not acceptable for quantitative analysis because we are not controlling the ionization process. If one patient sample has more potassium compared with another quantitative results may vary because of the efficiency of ion formation rather than actual physical amount of the drug in the sample. Our next step was to take the two approaches described in last month’s column: (1) reduce the pH with an organic acid to provide a great excess of protons and thereby drive formation of the protonated molecule, and (2) modify the mobile phase with potassium acetate to drive formation of only one metal adduct ion species.

**Adduct ion gives the best result:** Table 1 summarizes our observations. The first column shows the symbol for each ion type and the expected m/z value. The remaining three columns summarize the experimental results. The first of these shows the results with no mobile phase additives, i.e. just acetonitrile/water. These results are also shown in Figure 1 as discussed above. The second column shows the result of lowering the pH of the mobile phase with 0.2% formic acid. As expected, the [M+H]+ ion is the dominant species with only minimal amounts of the two potassium adducts present and the intensity of the [M+H]+ is greater than either peak in the first analysis. This may, in fact, be an acceptable quantitative method depending on the required limits-of-detection (LOD).

The third column shows the results after addition of 50 μM potassium acetate to the mobile phase. Again, we have successfully forced nearly all of the ion current into a single ion type, in this case the [M+2K-H]+. Not only that, but we observe that this ion gives us the most intense signal (all results in the table are normalized to this peak height). As a result, the method using ACN/water with 50 μM potassium acetate is the preferred method and [M+2K-H]+ is chosen as our quantitation ion.

We initially believed that...
[M+2K-H]⁺ is a very unusual ion. However, since then we have encountered this ion in many different applications such as peptide and protein analysis and drug synthesis. Any analyte compound containing an amine (or amide), and a carboxylic acid functional group has the potential to form a metal adduct ion salt. Other possible forms of this ion, namely [M+2Na-H]⁺ (m/z M+45), and [M+Na+K-H]⁺ (m/z M+61), have also been observed in our lab.