
LC Troubleshooting

# Ion Suppression in LC–MS–MS — A Case Study

***Michael D. Nelson and John W. Dolan***

**Sometimes a small change is all it takes.**

Ion suppression in liquid chromatography coupled with mass spectrometry (LC–MS) can occur when a coeluted compound suppresses the ionization of the sample molecules in a mass spectrometer's source. This ion suppression is analogous to the large garbage peak for unretained material common at the beginning of chromatograms monitored by UV detection. Ion suppression, like other chromatographic interferences, compromises quantitative analysis because it can vary from sample to sample. Unfortunately, a tandem mass spectrometer operating in the multiple-reaction monitoring mode is just as susceptible as a single-stage unit. This month's "LC Troubleshooting" examines a case in which ion suppression compromised method performance and shows how the strategy for eliminating ion suppression differs little from addressing other chromatographic interferences.

## Identifying Ion Suppression

Figure 1 illustrates the standard way to screen for potential ion suppression problems. First, infuse a dilute solution of the analyte at a constant rate into the effluent flowing from the LC system to the mass spectrometer to create an elevated but constant baseline. After obtaining a steady baseline, inject a blank sample extract into the LC system. Any eluted material that suppresses ionization in the mass spectrometer will cause a drop in the baseline.

Figure 2a shows a drop in the region between 0.7 and 1.0 min. Ion suppression sometimes can be eliminated by improved sample preparation, but generally it is easier to adjust the chromatographic conditions so the peaks of interest are not eluted in the suppression region, as was the case for the paclitaxel sample in Figure 2b.

## The Current Problem

Our laboratory had developed an LC–MS–MS method to analyze a proprietary pharmaceutical compound and one of its metabolites. The method appeared to be ready for validation until we moved it from one mass spectrometer to another of the same brand and model. At this point, the method performance deteriorated dramatically, especially with lower concentrations of analytes extracted from plasma. Urine extracted in the same manner acted normally. Unfortunately, equipment availability prevented us from moving back to the original LC–MS–MS system.

We suspected ion suppression was the problem source, so we performed the infusion experiment described above with the results shown in Figure 3. The lower trace shows the infusion experiment overlaid on the upper chromatogram of a standard of the three sample components of interest.

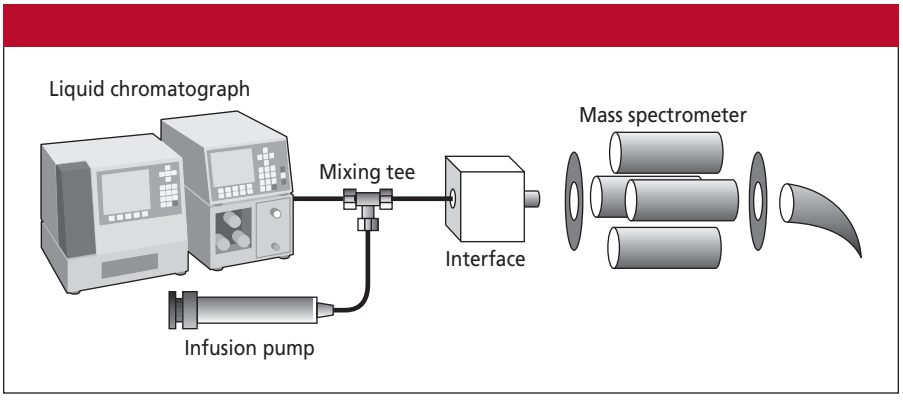


**Figure 1:** Experimental setup for determining ion suppression with LC–MS–MS. See text for details.

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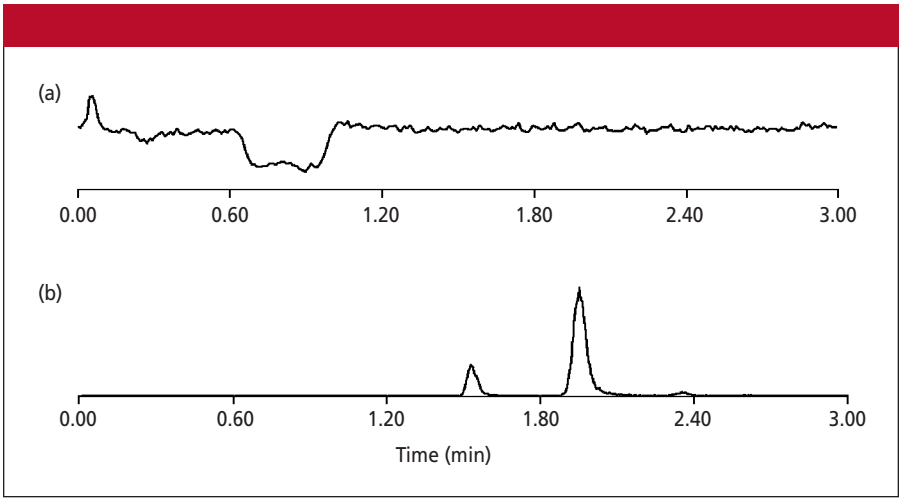


**Figure 2:** Chromatograms showing retention times for (a) the ion suppression region and (b) the analytes of interest in a method for measuring paclitaxel in plasma.

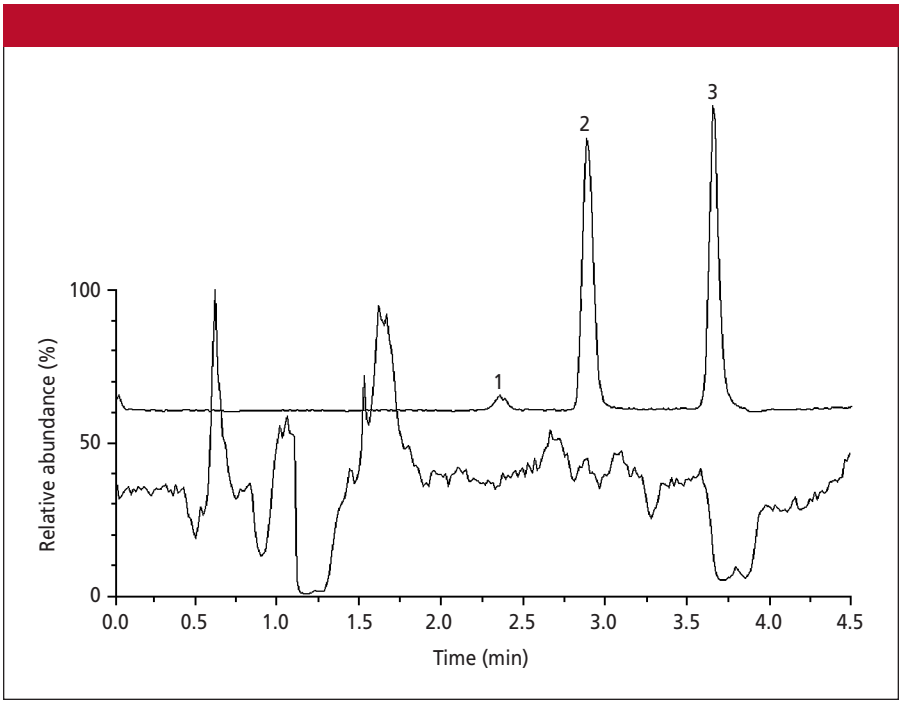


**Figure 3:** Chromatograms obtained using the conditions under which the ion suppression problem was originally discovered. The ion suppression trace is shown on the bottom. Column: 75 mm × 4.6 mm ODS-3; mobile-phase A: 0.05% heptafluorobutyric acid in water; mobile-phase B: 0.05% heptafluorobutyric acid in acetonitrile; gradient: 5–30% B in 4 min; dwell volume: 2.2 mL. Peaks: 1 = metabolite, 2 = internal standard, 3 = parent drug.

The figure shows that the parent drug is eluted at the beginning of a large suppression region. Small changes in retention or variations in the sample matrix easily could change the relative contribution of the suppression and analyte signals and reduce the method performance. We had confirmed that ion suppression was a likely cause of our problem.

## Chromatographic Changes

Although considerably broader than normal chromatographic peaks, ion suppression regions can be moved in the same manner as normal peaks by manipulating chromatographic conditions. We tried several variations in the separation conditions in an attempt to correct the problem.

For gradient elution, a change in gradient slope is analogous to a change in mobile-phase strength in isocratic separations; therefore, it is a powerful way to change chromatographic selectivity. The initial separation (Figure 3) used a

6.25%/min gradient starting at 5% acetonitrile. Changing to a steeper gradient of 11.25%/min moved the main peak further into the suppression region (Figure 4a). A shallower gradient (Figure 4b) caused a similar change. Clearly, gradient steepness alone was an insufficiently powerful tool to solve the current problem.

A change in the mobile phase's organic solvent content can provide dramatic selectivity changes in LC separations, so we thought switching from acetonitrile to

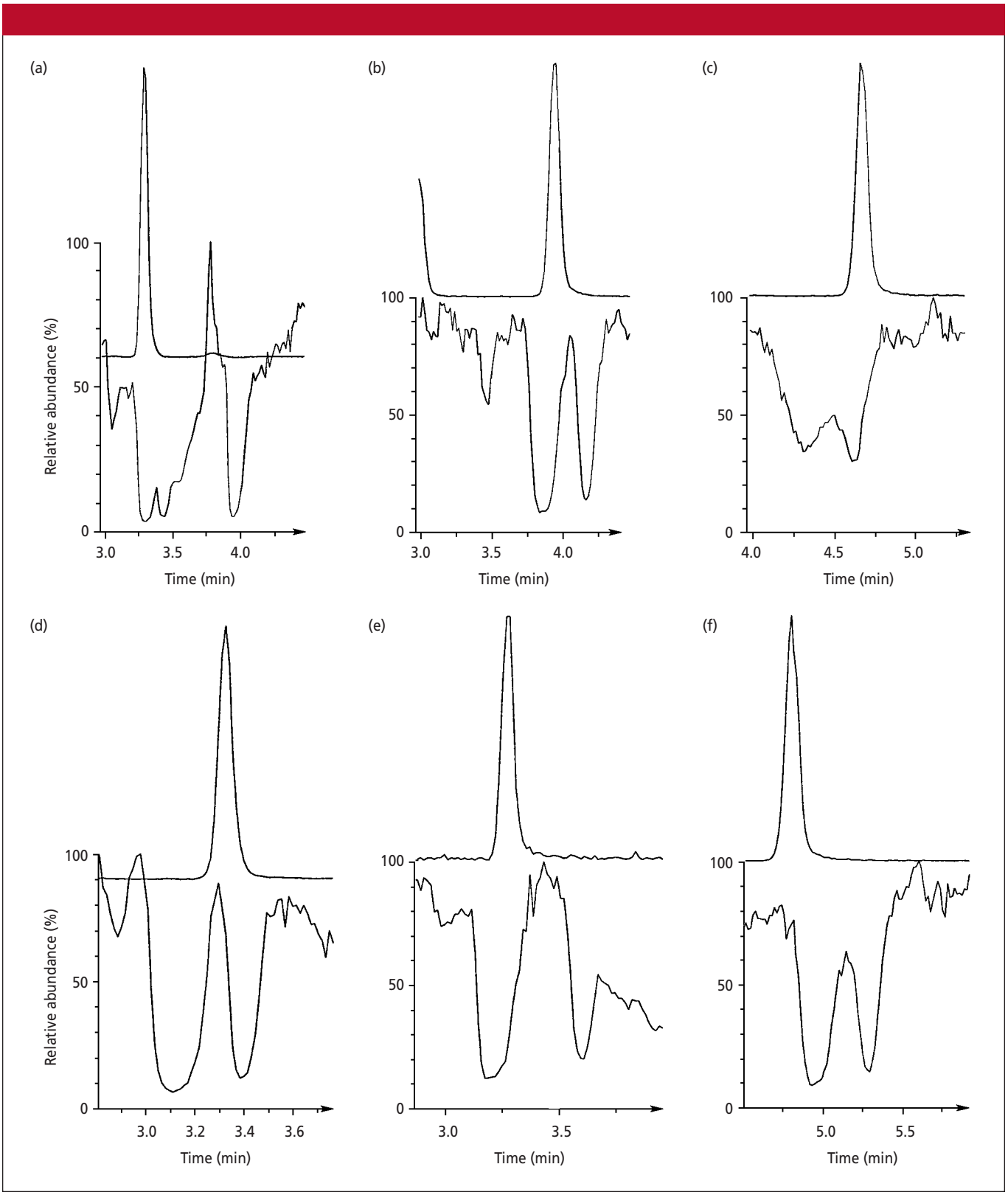


**Figure 4:** Modification of conditions of Figure 3. All conditions were as in Figure 3 except as noted. Shown are results obtained using (a) a steeper gradient, (b) a shallower gradient, (c) methanol instead of acetonitrile as B solvent, (d) 0.025% instead of 0.05% heptafluorobutyric acid, (e) an SB-C18 column, and (f) a 150 mm × 4.6 mm XTerra column.

methanol would be a logical choice to explore this variable. This change moved the main peak to the back edge of the suppression region (Figure 4c), but it also was insufficient to avoid ion suppression.

Heptafluorobutyric acid at a concentration of 0.05% was present in the mobile phase to control the pH and to act as an ion-pairing reagent. We decided to explore the effects of a change in heptafluorobutyric acid concentration. Increasing the heptafluorobutyric acid concentration to 0.1% resulted in all the peaks being eluted in less than 1.5 min and no retention for the metabolite; clearly, it was an undesirable effect. Reducing the heptafluorobutyric acid concentration to 0.025% changed selectivity (Figure 4d), but the main peak still overlapped with the suppression region.

As when developing a new chromatographic method, the next logical step after modifying mobile-phase strength (gradient steepness) and mobile-phase chemistry is to look at the effect of changing the stationary phase. We performed the original separation on a Type B silica C18 column (Inertsil ODS-3, Metachem, Torrance, California). It is best to try a dramatic change in stationary phase for a change in selectivity, so we tried an embedded polar phase (Symmetry Shield, Waters Corp., Milford, Massachusetts) but were unable to find conditions that would give sufficient retention for the polar metabolite.

Although changing from one manufacturer's C18 column to another's generally is a poor way to change selectivity, we were beginning to run out of options. A sterically protected C18 column (StableBond C18, Agilent Technologies, Wilmington, Delaware) changed the profile of the suppression region (Figure 4e) but did not provide enough selectivity change. A low-silica C18 column (XTerra, Waters) provided the best separation so far (Figure 4f). It was very close to the separation we had observed before changing instruments (Figure 5), but it still didn't leave much margin for error. Note that the XTerra column was 150 mm long, whereas the other columns were 75 mm long. For this reason, the gradient time was increased to 8 min to keep the gradient time and flow rate constant and to obtain the same effective gradient steepness. We attribute the change in selectivity to the change in column chemistry and not the change in gradient conditions because of this change.

## What's Left?

After carefully reviewing the data and efforts to change selectivity, we realized that we had failed to examine the effect of system dwell volume on the separation. The dwell volume is the system volume from the point where the solvents are mixed to the point where they reach the inlet of the column. The most common effect of dwell-volume changes is a shift in retention for all peaks accompanied by small changes in selectivity. Large changes in selectivity are less common.

We examined the system records and determined that the original separation (Figure 5) was performed on an LC system using low-pressure mixing (one pump)

***A change in the mobile phase's organic solvent content can provide dramatic selectivity changes in LC separations.***

with a measured dwell volume of 0.88 mL. The remaining chromatograms shown above were obtained with a high-pressure mixing system (two pumps) with a dwell volume of 2.2 mL. Comparing the chromatograms for these two systems (Figures 3 and 5) suggested that a smaller dwell volume moved the main peak forward relative to the suppression region. We previously had used a high-pressure mixing system with this method, and it provided satisfactory results, so the change was a bit surprising. Further digging in the system records, however, showed that the earlier work with the high-pressure mixing system had used a micromixer instead of the standard mixer. We normally use the micromixer for LC–MS–MS work for small columns operated at low flow rates — for example, 50 mm × 2.1 mm columns operated at 0.2 mL/min — but the micromixer is less advantageous for conventional 75 mm × 4.6 mm or 150 mm × 4.6 mm columns operated at higher flow rates (1.5 mL/min, in this case). It appeared that this assumption was wrong for the present case.

To test our hypothesis, we replaced the conventional mixer with a micromixer (0.5 mL) and observed the results shown in Figure 6. Obviously, dwell volume was the key parameter in this situation. The reduced dwell volume had the same effect with the original column (not shown).

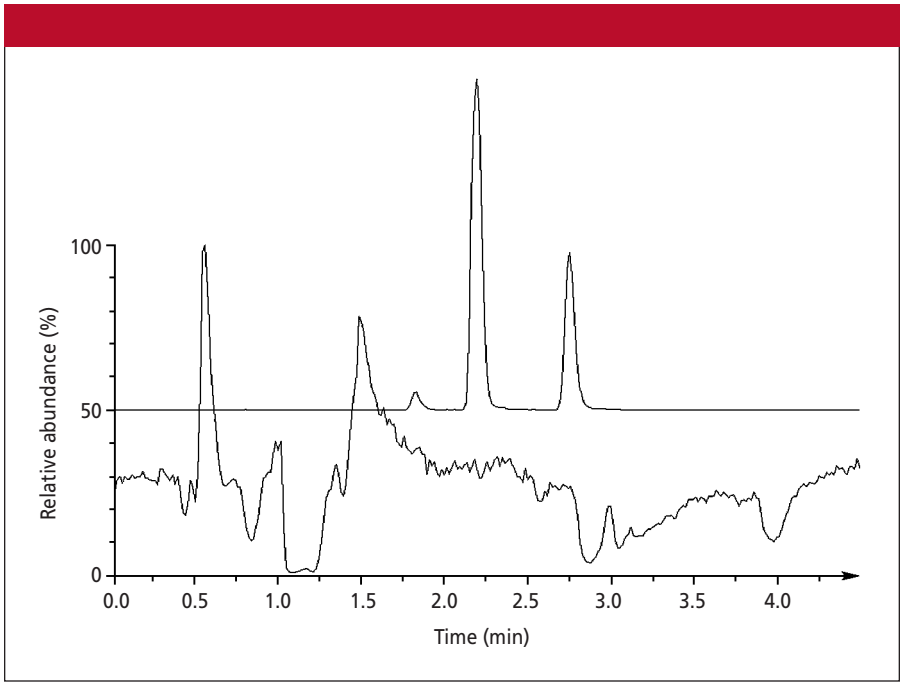


**Figure 5:** Results obtained using the same conditions as in Figure 3 but with a 0.88-mL dwell volume.

## Conclusions

What can we learn from this exercise? When we first discovered the method performance problem, it manifested itself pri-

marily in a fall-off in precision and accuracy at lower analyte concentrations. We were distracted by a suspicion that the freshly prepared standards were diluted improperly. By the time we discovered that the problem was related to ion suppression, we failed to recognize the instrument change as a factor.

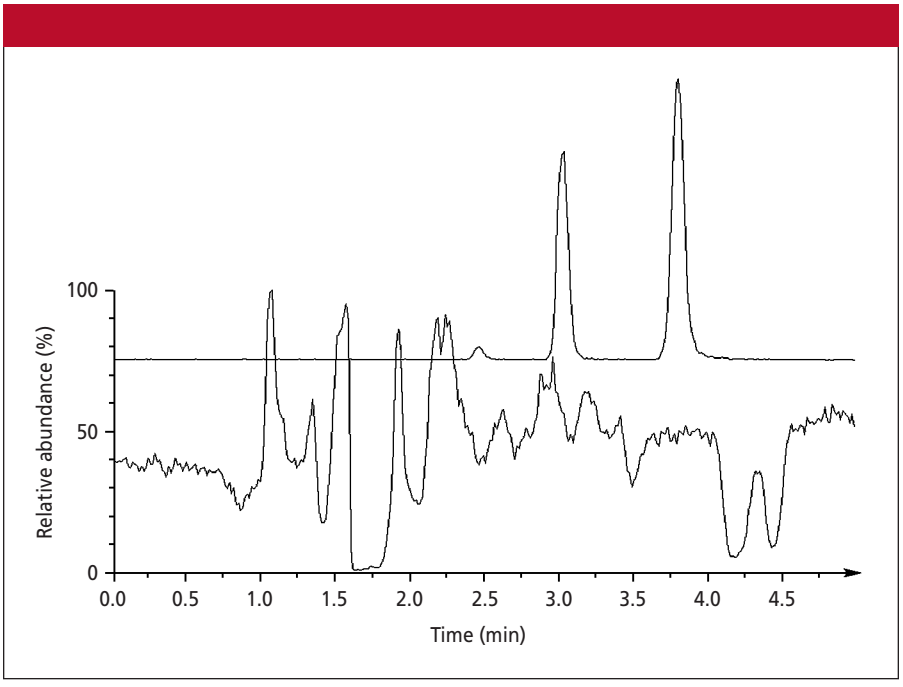


**Figure 6:** Results obtained using the same conditions as in Figure 4f but with a micromixer (0.5-mL dwell volume).

We approached the problem in a logical, stepwise manner: We changed the separation variables using a tried-and-true procedure that took advantage of a combination of the ease of making a change and the leverage, or probability, that the variable would cause a change in selectivity. So, we changed the gradient steepness (equivalent to solvent strength in isocratic elution) first, because it was easy and fairly powerful. Next, we changed the mobile-phase chemistry because chemistry changes are more likely to affect selectivity than are column changes. When we changed columns, it was first to a different type of column (embedded polar phase), which was more likely to change selectivity than a change from one manufacturer's C18 to another.

That the problem was finally traced to a dwell-volume change makes us a bit red-faced — we should have noticed it at the beginning. On the positive side, the exercise produced a nice example of the approach to addressing an ion suppression problem. Eliminating ion suppression problems is no different than dealing with other chromatographic interferences — either eliminate the suppression peak through sample cleanup or modify the chromatographic conditions so it does not cause a problem.

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