Clinical Chemistry 49:7
1041–1044 (2003)

Minireview

Ion Suppression in Mass Spectrometry

Thomas M. Annesley

Background: **Mass spectrometry (MS) is being introduced into a large number of clinical laboratories. It provides specificity because of its ability to monitor selected mass ions, sensitivity because of the enhanced signal-to-noise ratio, and speed because it can help avoid the need for intensive sample cleanup and long analysis times. However, MS is not without problems related to interference, especially through ion suppression effects. Ion suppression results from the presence of less volatile compounds that can change the efficiency of droplet formation or droplet evaporation, which in turn affects the amount of charged ion in the gas phase that ultimately reaches the detector.**

Content: **This review discusses materials shown to cause ion suppression, including salts, ion-pairing agents, endogenous compounds, drugs, metabolites, and proteins. Experimental protocols for examining ion suppression, which should include, at a minimum, signal recovery studies using specimen extracts with added analyte, are also discussed, and a more comprehensive approach is presented that uses postcolumn infusion of the analyte to evaluate protracted ionization effects. Finally, this review presents options for minimizing or correcting ion suppression, which include enhanced specimen cleanup, chromatographic changes, reagent modifications, and effective internal standardization.**

Summary: **Whenever mass spectrometric assays are developed, ion suppression studies should be performed using expected physiologic concentrations of the analyte under investigation.**

Mass spectrometry (MS)[1] is a powerful qualitative and quantitative analytical technique that has been introduced into many clinical and research laboratories during the last 5 years. The cost of MS analyzers has dropped to a range that is affordable for a majority of laboratories. Compared with a decade ago, instruments are more robust, computer-interfaced, user-friendly, and easier to maintain. There is a greater awareness by manufacturers of the laboratory applications of MS in the clinical laboratory, so that technical support and assay development are high priorities with these companies. In the clinical laboratory, mass spectrometers are used to measure a wide range of clinically relevant analytes. When applied to biological samples, the power of MS lies in its selectivity toward the identification and quantification of compounds. The combination of gas chromatography or HPLC with MS yields a particularly powerful tool. This is especially so for HPLC-MS or HPLC-tandem MS, which is the reason that this combination is being used by many clinical laboratories.

For many years HPLC separations have been monitored through ultraviolet (UV), fluorescence, or electrochemical detection. In reversed-phase HPLC-UV chromatography, more polar compounds are poorly retained on the column and elute at or near the initial void. This is observed as a peak (or peaks) that will interfere with the detection and quantification of the compound of interest. For this reason, sample cleanup is required, and it is the reason that chromatographic runs may be 10 min long. A trade-off often exists, where more extensive sample cleanup may allow shorter chromatographic runs. In contrast, a mass detector can be configured to isolate and quantify specific ions; therefore, sample cleanup and chromatographic separation can theoretically be reduced compared with other, less selective detection modes. Interferences that are apparent with UV detection are not observed with MS when analyte specific masses or product ions are monitored.

Because of the specificity achieved with MS detectors, misconceptions have evolved that (*a*) chromatographic separation can be minimized or even eliminated, (*b*) the analytical column is needed only as a mechanism to load

Department of Pathology, University of Michigan Medical Center, Ann Arbor, MI 48109-0054.

Address for correspondence: University Hospital, Room 2G332, 1500 East Medical Center Dr., Ann Arbor, MI 48109-0054.

Received February 13, 2003; accepted April 17, 2003.

[1] Nonstandard abbreviations: MS, mass spectrometry; UV, ultraviolet; ESI, electrospray ionization; TFA, trifluoroacetic acid; MALDI, matrix-assisted laser desorption/ionization; and SELDI, surface-enhanced laser desorption/ionization.

sample on the system, (*c*) specimen cleanup can be minimized or eliminated, and (*d*) the use of HPLC-MS or HPLC-tandem MS provides absolute accuracy and specificity. However, mass detection is not completely without problems that can compromise or invalidate results.

One important factor that can affect the quantitative performance of a mass detector is ion suppression. Sample matrix, coeluting compounds, and cross-talk can contribute to this effect. Although ion suppression can have potential deleterious effects on both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), evidence indicates that the electrospray interface is more likely to be impacted *(1)*. Ionization effects can theoretically occur in either the solution phase or the gas phase. Recent experiments involving ESI of biological extracts have shown that the main cause of ion suppression is a change in the spray droplet solution properties caused by the presence of nonvolatile or less volatile solutes *(1)*. These nonvolatile materials (e.g., salts, ion-pairing agents, endogenous compounds, drugs/metabolites) change the efficiency of droplet formation or droplet evaporation, which in turn affects the amount of charged ion in the gas phase that ultimately reaches the detector. The mass and charge of individual analytes are also factors in making a compound a candidate for ion suppression or in making one compound a source of ion suppression for another. It has been shown that molecules with higher mass will suppress the signal of smaller molecules *(2)* and that more polar analytes are more susceptible to suppression *(3)*.

The presence of ion suppression or other deleterious effects can be evaluated via several experimental protocols. The first involves comparison of (*a*) the instrument response for calibrators (including any internal standards) injected directly in mobile phase, (*b*) the same amount of compound added to preextracted samples, and (*c*) the same amount of compound added to specimen matrix before extraction *(4)*. The data for the calibrator in mobile phase provide a relative 100% response value. The data for the same amount of compound added to preextracted samples show the effect of sample matrix on MS response (ion suppression), whereas the response data for extracted samples containing the analyte can highlight whether any loss of signal is attributable to the extraction process or ion suppression. It is important to perform the latter two experiments for several specimens to verify that subtle matrix differences do not contribute to a change in MS response. It is also important to evaluate ion suppression that may result from other specimen types (e.g., EDTA or heparin, uremia, lipemia). Fig. 1 shows an actual example of ion suppression attributable to endogenous matrix components. In this experiment, 0.1-mL aliquots of a deidentified donor serum were subjected to three different extraction techniques: solid-phase extraction using a Varian 60-mg Abselut column (water wash, methanol elution), solvent extraction with 0.8 mL of methylene chloride, and protein precipitation with 0.5 mL of acetonitrile. The three dried extracts were reconstituted with 0.5 mL of a caffeine solution (1 mg/L in 100 mL/L methanol plus 10 mL/L formic acid), vortex-mixed, and analyzed by injection (no analytical column) into the mass analyzer. As can be clearly seen in Fig. 1, all three extracts contained endogenous components that produced ion suppression >90% when compared with a caffeine reference solution of identical concentration.

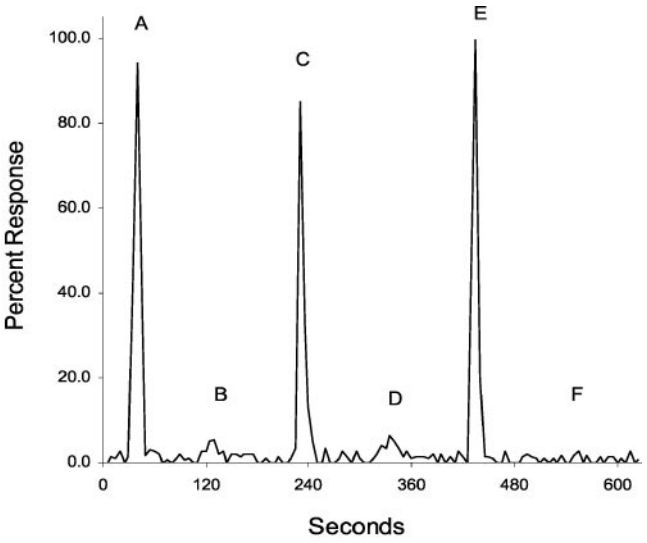

Fig. 1. Signal response comparisons (*m/z* 195) for caffeine added to serum extracts prepared by solid-phase extraction, solvent extraction, and protein precipitation.

Using an HPLC pump at a flow of 0.1 mL/min, I injected 5-μL volumes into PEEK tubing connected directly to the mass analyzer. (*A*, *C*, and *E*), 1 mg/L caffeine solution. (*B*), solid-phase extract with 1 mg/L caffeine added. (*D*), methylene chloride extract with 1 mg/L caffeine added. (*F*), serum protein precipitation extract with 1 mg/L caffeine added. Conditions: mobile phase, 500 mL/L methanol and 10 mL/L formic acid in water; instrument, Waters Micromass LCT; capillary, 3600 V; sample cone, 40 V; RF lens, 180 V; extraction cone, 2 V; desolvation temperature, 200 °C; source temperature, 100 °C; scan duration, 1.0 s; ionization mode, ES+.

The second protocol, which can be viewed as part of interference checks for an assay, involves injection of drugs or metabolites that may also be present in the specimen. Just because a coeluting drug does not produce similar mass fragments does not mean that this compound is incapable of ion suppression.

The third protocol involves postcolumn continuous infusion of compound into the MS detector *(3, 5, 6)*. The instrumental setup includes a syringe pump connected via a "tee" to the column effluent (Fig. 2). Because the compound being tested is introduced into the mass detector at a constant rate, a constant ESI response should ideally be observed (Fig. 3A) if no ionization interferences occur when an extract from a biological specimen, such as serum, is injected into the HPLC portion of the instrument. In actuality it is common to see suppression of the signal at the time point that corresponds to the void volume of the column. The degree of ion suppression and the recovery time to full response can vary from compound to compound *(3)* and from sample to sample, and can also be dependent on the sample preparation method

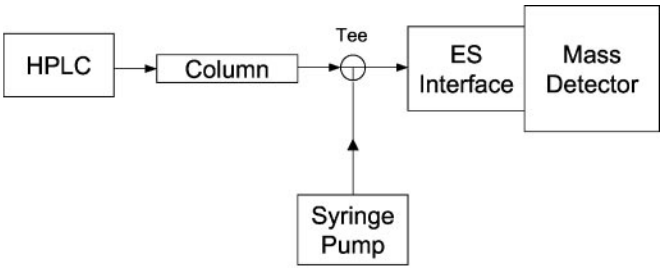

Fig. 2. Postcolumn infusion system.

Mobile phase or specimen extracts are injected into the HPLC system. The analyte being evaluated is continuously infused postcolumn and is mixed with the column effluent through a tee before entering the electrospray (*ES*) interface.

(Fig. 3, B and C). Because endogenous compounds from the specimen matrix can continue to elute at any time during the chromatographic run, ion suppression is not limited to the column void. Ion suppression may not be evident during the initial HPLC-MS analysis but may be present during subsequent injections (Fig. 3C).

The observed degree of ion suppression can also be dependent on the concentration of the analyte being monitored, which relates to the matrix/analyte ratio. For example, when analyzing clenbuterol in urine, van Hout et al. *(7)* reported that the percentage of ion suppression for different concentrations of drug using a C_{18} extraction cartridge ranged from 37% at 93 μg/L to 69% at 45 μg/L. Two important points can be taken from these types of data: (*a*) they highlight the benefits of decreasing the matrix/analyte ratio through more extensive sample cleanup or through better chromatographic separation; and (*b*) they show the importance of performing ion suppression validations, such as those outlined in the

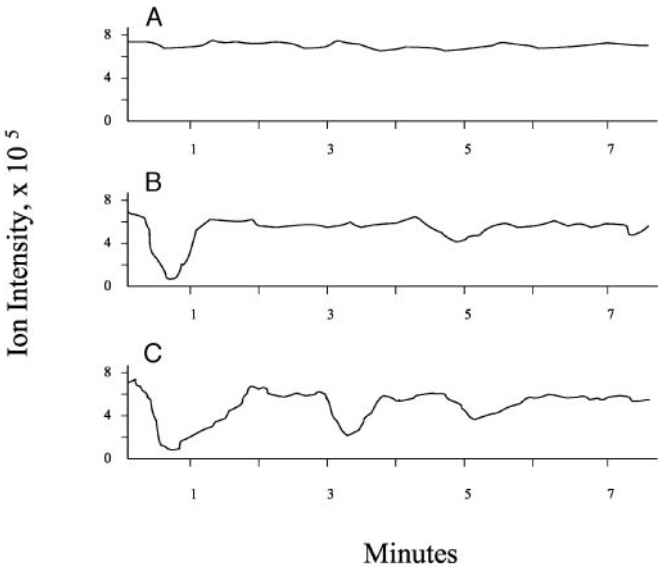

Fig. 3. Infusion chromatograms for hypothetical analyte.

(*A*), mobile phase injection; (*B*), serum liquid-liquid extract injection; (*C*), serum protein precipitation extract injection. These profiles illustrate that ion suppression can be >90%, that a recovery time may exist, and that suppression is not limited to the solvent-front region. For a comprehensive presentation of these types of effects, see Refs. *(3, 5, 6)*.

three protocols above, using concentrations of analyte that reflect those that will be encountered under real conditions.

In addition to the effects of sample matrix components, there are other potential sources of ion suppression to be considered. One is the effect that chromatographic ion-pairing agents may have on signal intensity. For example, trifluoroacetic acid (TFA) has been used in HPLC-UV analyses because it improves peak shape and retention times. Unfortunately, for MS analyses TFA also causes signal suppression and must be dealt with if this acid is used in the mobile phase. Being a component of the mobile phase, this agent will continuously be introduced into the ESI interface, so that signal suppression may be observed throughout the entire chromatographic run.

Fortunately there are several options that can be used to minimize or correct for ion suppression during HPLC-MS analyses. For ion-pairing, other, weaker acids such as acetic, formic, or hexafluorobutyric acid may be successfully substituted for TFA in HPLC-MS analyses. If TFA must be used as an ion-pairing agent to achieve acceptable chromatography and peak shape, it may be possible to decrease the TFA concentration and still obtain adequate separation. The use of surface tension-lowering modifiers in the ESI source has also been reported to be successful when TFA is used as a mobile phase modifier *(8)*. The postcolumn addition of acids and solvent carriers to displace TFA from compounds and aid ionization has also been used successfully. This is a process termed the "TFA Fix" *(9)*. Yamaguchi et al. *(10)* reported the use of 2-(2-methoxyethoxy)ethanol as a signal enhancer to eliminate the ion-suppressive effects of acetate anions; this agent produced an ~100-fold enhancement of the signal for the model drug ibuprofen.

Another way to counter ion suppression is to modify the chromatographic conditions so that the compound(s) of interest elute(s) in a region where ion suppression is not observed. This generally involves increasing the chromatography time, but not always so *(11)*. Because the majority of HPLC assays include an internal standard (stable isotope or structural analog), another approach is to modify the chromatography so that the compound of interest and the internal standard coelute *(12)*. The ion suppression for both compounds should become equal if the peaks chromatographically coincide, thus "correcting" for the degree of suppression. If a stable-isotope internal standard is available, the ion suppression would be identical for the analyte and internal standard. It should be mentioned, however, that the use of an internal standard (even a stable isotope) might not fully solve accuracy or precision problems with the assay if ion suppression exists, as demonstrated in Figs. 1 and 3. Recall that part of the value of MS lies in the high signal-to-noise ratio achievable with this technique. If ion suppression significantly reduces the signal of the analyte or internal standard, the signal-to-noise may be compromised to a point where accuracy or precision becomes

negatively affected. It is for this reason that ion suppression should be evaluated even if an internal standard is included in the assay.

Lastly, because other MS techniques such as Fourier transform (FTMS), matrix-assisted laser desorption/ionization (MALDI), and surface-enhanced laser desorption/ionization (SELDI) are being more widely used, it is worth mentioning that ion suppression is not limited to just HPLC-MS or ESI interfaces, although the discussion in this review has focused on ESI. Arginine-containing peptides have been reported to dominate the peptide pattern for protein digests *(13)*, the extent of which depends on the matrix used. The presence of ionic detergents such as Triton X-100 and Tween 20 has also been shown to cause signal suppression in MALDI experiments, which can be countered by modifications to the matrix *(14)*. Because SELDI also uses the same matrices and ionization process, this technique would be similarly affected.

References

1. King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T. Mechanistic investigation of ionization suppression in electrospray ionization. J Am Soc Mass Spectrom 2000;11:942–50.
2. Sterner JL, Johnston MV, Nicol GR Ridge DP. Signal suppression in electrospray ionization fourier transform mass spectrometry of multi-component samples. J Mass Spectrom 2000;35:385–91.
3. Bonfiglio R, King RC, Olah TV, Merkle K. The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. Rapid Commun Mass Spectrom 1999;13:1175–85.
4. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal Chem 1998;70:882–9.
5. Muller C, Schafer P, Stortzel M, Vogt S, Weinmann W. Ion suppression effects in liquid chromatography-electrospray-ionisation transport-region collision induced dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectral libraries. J Chromatogr B 2002;773:47–52.
6. Hsieh Y, Chintala M, Mei H, Agans J, Brisson J-M, Ng K, et al. Quantitative screening and matrix effect studies of drug discovery compounds in monkey plasma using fast-gradient liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2001;15:2481–7.
7. van Hout MWJ, Hoffland CM, Niederlander HAG, de Jong GJ. On-line coupling of solid-phase extraction with mass spectrometry for the analysis of biological samples. II. Determination of clenbuterol in urine using multiple-stage mass spectrometry in an ion-trap spectrometer. Rapid Commun Mass Spectrom 2000;14:2103–11.
8. Eshraghi J, Chowdhury SK. Factors affecting the electrospray ionization of effluents containing trifluoroacetic acid for high-performance liquid chromatography/mass spectrometry. Anal Chem 1993;65:3528–33.
9. Apffel A, Fischer S, Goldberg G, Goodley PC, Kuhlmann FE. Enhanced sensitivity for peptide mapping with electrospray liquid chromatography-mass spectrometry in the presence of signal suppression due to trifluoroacetic acid-containing mobile phases. J Chromatogr A 1995;712:177–90.
10. Yamaguchi J, Ohmichi M, Jingu S, Ogawa N, Higuchi S. Utility of postcolumn addition of 2-(2-methoxyethoxy)ethanol, a signal-enhancing modifier, for metabolite screening with liquid chromatography and negative ion electrospray ionization mass spectrometry. Anal Chem 1999;71:5386–90.
11. Beato BD, Gray DD, Wong P, Hampsch J, Peters S. Ramifications of pump-switching step gradients for bioanalytical LC/MS/MS assays. Curr Sep 2000;19:55–8.
12. Kitamura R, Matsuoka K, Matsushima E, Kawaguchi Y. Improvement in precision of the liquid chromatographic-electrospray ionization tandem mass spectrometric analysis of 3′-C-ethynylcytidine in rat plasma. J Chromatogr B 2001;754:113–9.
13. Krause E, Wenschuh H, Jungblut PR. The dominance of arginine-containing peptides in MALDI-derived tryptic mass fingerprints of proteins. Anal Chem 1999;71:4160–5.
14. Gharahdaghi F, Kirchner M, Fernandez J, Mische SM. Peptide-mass profiles of polyvinylidene difluoride-bound proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the presence of nonionic detergents. Anal Biochem 1996;233:94–9.