

Chemical Noise in Mass Spectrometry

Part III — More Mass Spectrometry/Mass Spectrometry

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Kenneth L. Busch assumes that the ions always know what they're doing. From that perspective, MS/MS provides an excellent opportunity for the ions to do what they do, and to thereby distinguish themselves via their reactivity. A well-designed MS/MS experiment reduces ionic fungibility. The opinions and views expressed in this column are solely those of the author, and not those of the National Science Foundation, which awards fungible grants. Readers may e-mail the author at buschken@hotmail.com.

In the first installment of this four-part series on chemical noise in mass spectrometry (MS), attributes of MS/MS that reduce the contribution of chemical noise in a mass spectral measurement were discussed in broad terms. In particular, use of sequential stages of analysis, in which each stage exhibits a greater discrimination against noise than signal, was shown to result in an increased signal-to-noise ratio (S/N) in the final measurement. Implicit in this initial discussion was the assumption that a discriminating factor between signal and noise could be found and exploited, and of course, the better the discriminant, the fewer stages of sequential analysis are needed to achieve the desired S/N.

In the second column in this series (2), the effects of the ionization method selected on chemical noise levels were discussed. It was shown that the traits of the ionization method itself could be used as a discriminant against chemical noise. Chemical ionization was used as an example of an ionization method that can reduce chemical noise observed in a mass spectrum, and fast atom bombardment (FAB) and matrix-assisted laser desorption ionization were presented as examples of ionization methods that, as a direct consequence of how they work, usually actually increase the level of chemical noise. In this

third installment within the series, we now discuss examples of chemical ionization and FAB in conjunction with MS/MS to explore in greater detail how and why chemical noise is reduced. The approach again emphasizes basic overarching principles rather than numerous applications.

In many respects, the properties of chemical ionization are ideal for coupling with MS/MS. The reagent gas can be chosen in such a manner that the proton transfer reaction creates a protonated molecule with very little residual energy that might cause dissociation into fragment ions. Ion current in the mass spectrum is therefore concentrated in the ion signal for the protonated molecule, with that ion appearing at a mass unambiguously related to the molecular mass of the sample. Figure 1 contrasts the situation (simplified for the discussion) achieved between the electron ionization mass spectra of a three-component mixture and the corresponding chemical ionization mass spectrum of the same mixture. We assume that the molecular masses of the compounds are different, and that the chemical ionization reagent gas is chosen to promote a soft protonation. Because mass spectra are additive, the electron ionization mass spectrum is a composite of all the ions formed from each of the components

(and, of course, not conveniently differentiated as in Figure 1). The simplified spectrum achieved with chemical ionization reflects, of course, the same spectral additivity, but here the use of chemical ionization results in a trio of higher-mass ions that are the protonated molecules of the mixture components and the minimum fragmentation expected with the use of chemical ionization. These protonated molecules are usually excellent candidates for selection as parent ions in an MS/MS experiment. Remember that in this MS/MS experiment, the mass-selected parent ion undergoes collision-induced dissociation to produce product ions, which are mass-analyzed to create the product-ion MS/MS spectrum. The product-ion MS/MS spectrum may contain some of the same fragment ions as would the mass spectrum itself, but the spectra are not usually identical, although fragment ions in both instances provide information about the molecular structure of the molecular ion.

Early analytical applications of MS/MS that garnered widespread attention involved the use of chemical ionization MS in natural products analysis. Remember that during the period when MS/MS was being fully developed as a reliable analytical method, improved separations via capillary gas chromatogra-

phy, microcolumn liquid chromatography, and (a bit later) capillary electrophoresis were just entering the analytical armamentarium. Approaches that seemed to minimize the dependence on time-consuming (and sometimes low-resolution) separations, but which still provided the requisite analytical determination, supported the popularization of the get-something-for-nothing character of MS/MS. In these applications, the presence of a low-level component in a complex mixture could be deduced from the match between the product-ion MS/MS spectrum of the protonated molecule of the component culled from the complex spectrum of the mixture, and the product-ion MS/MS spectrum of the protonated molecule of the authentic compound recorded in isolation. Applications to flavor components, drugs in natural matrices, and explosives analysis, among many other needle-in-a-haystack problems, proliferated. The match of the MS/MS spectra, often visually evaluated, was deemed sufficient to establish the presence of a targeted compound within the mixture. The confidence in the match was supported by the often substantial increase in S/N achieved by sequential analyses in MS/MS. Further, the conjoining of chemical ionization with MS/MS was a success because the mass of the protonated molecule was often sufficiently unique such that selection of the parent ion by mass of the protonated molecule was the equivalent of separation of the compound from the other components of the mixture. Selection by mass was more quickly achieved, however, than separation by conventional chromatographic tools, and the separation by mass could be achieved with a variable resolution that could be increased as needed. The analytical success of MS/MS was therefore linked directly to the use of chemical ionization and its ability to concentrate ion current into the signal for the protonated molecule, resulting in an effective separation and isolation of that particular mixture component.

Isolation of the target compound by use of chemical ionization, or any other ionization method that tends to concentrate the signal in a molecular ion, is not perfect. Masses of components can over-

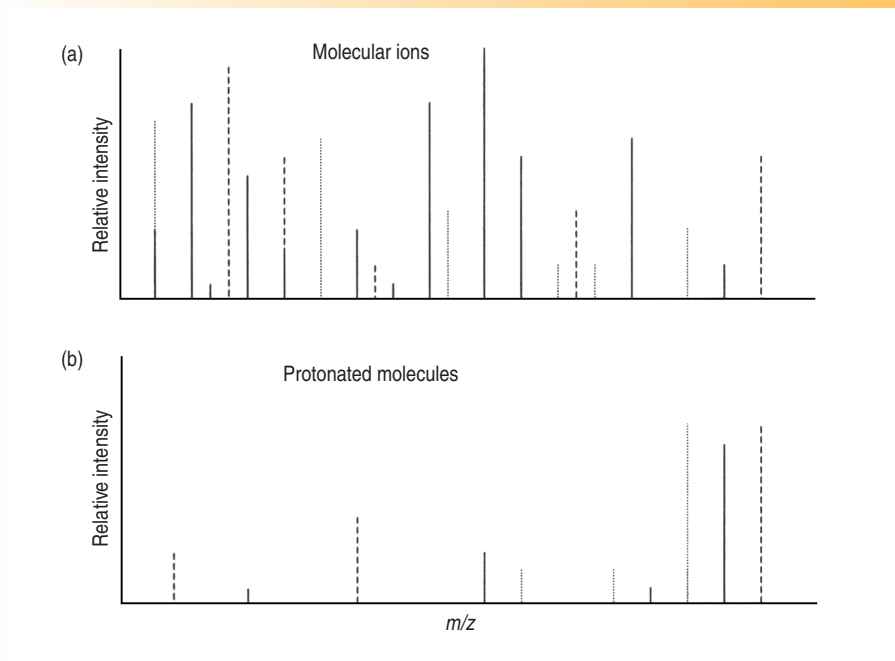


Figure 1. Mass spectra of a multicomponent mixture (solid, dashed, and dotted lines) analyzed by (a) electron ionization and (b) chemical ionization.

lap at either low or high resolution for selection for MS/MS (positional isomers for substituted compounds exemplify such an exact mass overlap), or fragment ions formed from higher mass, higher level mixture components may be similar in intensity to the molecular ions of a lower-mass, lower-level component. The product-ion MS/MS spectrum of the molecular ion can therefore be dissimilar to that recorded for the standard (and the match between them less exact), because (at least two) different parent ions are being examined simultaneously and the product-ion MS/MS spectra are additive. In the examination of mass spectra suspected to arise from the simultaneous presence of several components, a reverse library search can sometimes identify the presence of several discrete components contributing to the mass spectrum by successive examination of differences in the mass spectrum remaining after the ion intensities composing a standard single-component mass spectrum are removed. Similarly, explicit differences between a product-ion MS/MS spectrum measured for a mixture and that recorded for a standard are usually due to the simultaneous presence of a limited number of other components at the same parent-ion mass in the mixture; these components may be

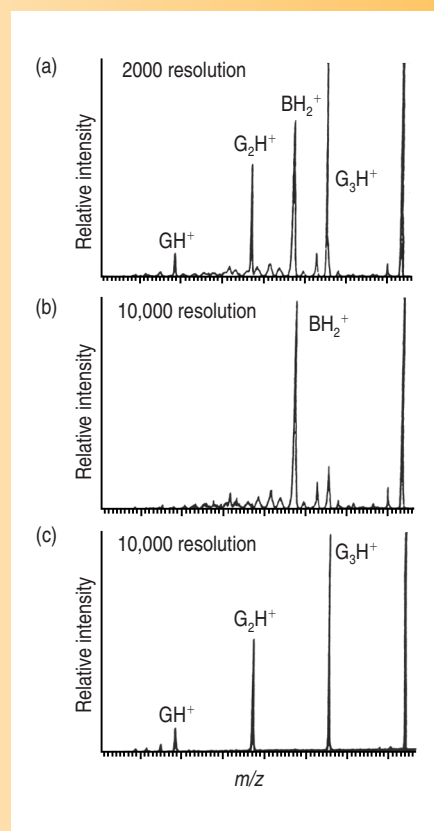


Figure 2. Spectra from (a) composite product-ion MS/MS, (b) composite product-ion MS/MS deconstructed via higher instrumental resolution in parent-ion selection into target signal, and (c) the FAB matrix. This figure is adapted from reference 3.

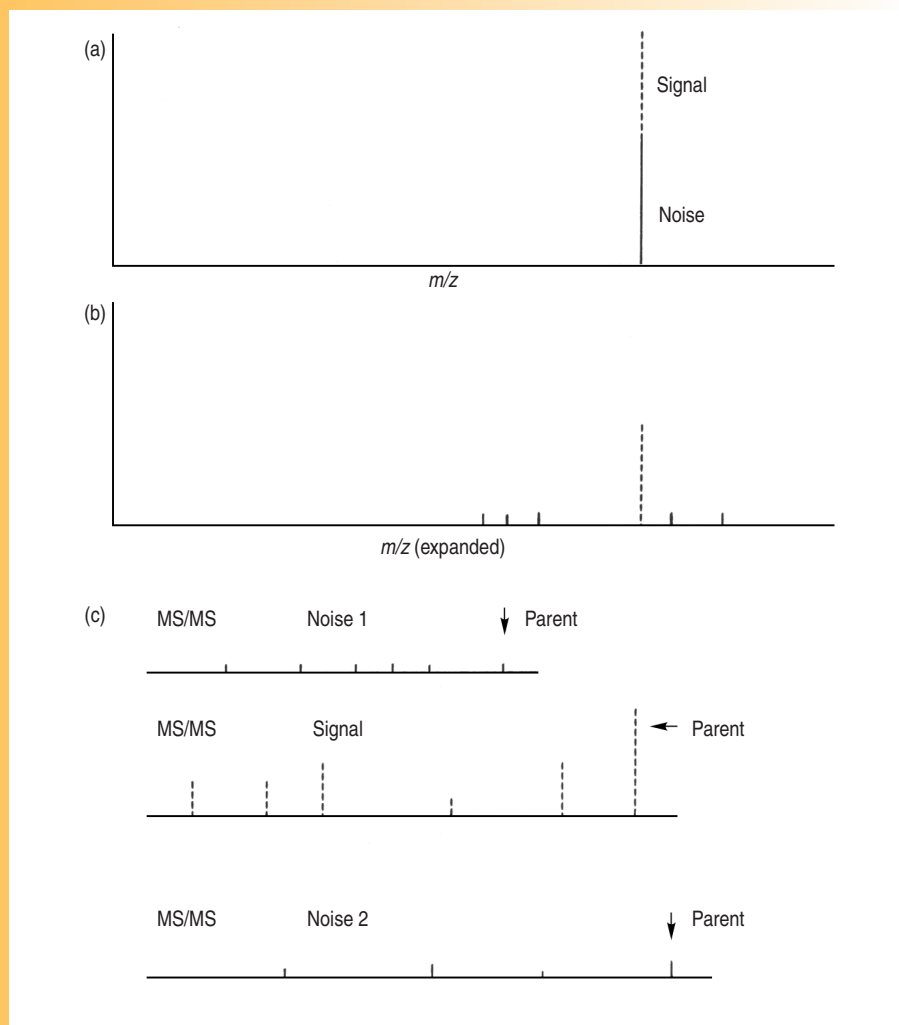


Figure 3. Sequential deconstruction of (a) an initially combined signal (dashed line) and noise (solid line) by first (b) higher resolution mass measurement, and then by (c) MS/MS.

identified by examination of successive differences.

An example of the additive character of MS/MS spectra, and the ability of MS/MS to reduce chemical noise, is given in a first exemplar in Figure 2, adapted from the work of Crow et al. (3). In measurement of the positive ion fast atom bombardment (FAB) mass spectra of 30 nucleosides, mass overlaps between signals from the glycerol (G) matrix and the nucleoside sample ions were observed. Particularly, such mass overlaps occur between ions that represent BH_2^+ , where B is the base cytidine or uridine, and ions derived from the glycerol, namely $(3G-2(H_2O))^+$. Even for the protonated molecules of substituted nucleosides at higher masses (where the problem of mass overlap might be expected to be reduced), such

overlaps occur. The authors document an example in which the mass of the protonated molecule of 2-phenylethyl-tubercidin at m/z 369 overlaps in mass with the strong matrix background signal for the ion $(4G + H)^+$, with G representing glycerol with a mass of 92 Da. With an instrument resolution of 2000 in selection of the parent ion, the signals from the protonated molecule of the sample and the glycerol matrix overlap, and the product-ion MS/MS spectrum is a composite, as shown in Figure 2a. At an instrumental resolution of 10,000 for parent-ion selection and isolation, the signals for the parent ions of two exact masses can be separated, and the individual MS/MS spectra emerge (Figures 2b and 2c). Note that the original MS/MS spectrum (Figure 2a) nevertheless provides evidence for the presence of

the targeted compound despite the overlap in the masses of the parent ions simultaneously selected for collision-induced dissociation, and the presence of a significant level of chemical noise. The difference in the product-ion MS/MS spectra apparent in Figures 2b and 2c is abetted by the relative simplicity of the product-ion MS/MS spectra of the glycerol tetramer $(4G + H)^+$, which dissociates by successive losses of the neutral glycerol molecule (Figure 2c). The masses of the product ions (GH^+ , G_2H^+ , and G_3H^+) do not overlap with the masses of the product ions formed by dissociation of the protonated molecule of the 2-phenylethyl-tubercidin, which, since it has a more complicated molecular structure, produces a more complicated product-ion MS/MS spectrum, and therefore a distinctive pattern of product ions. This is the first exemplar of explicit parent-ion mass overlap in product-ion MS/MS spectra, and the persistent differentiation of signal from noise in the MS/MS spectrum.

We consider two further exemplars, representing different situations for the case of mass overlap of parent ions, and discuss the effect of this overlap on the measured product-ion MS/MS spectra. Each of these examples again uses FAB ionization. For each example, we will again show why the reduction in chemical noise occurs in MS/MS. In the second exemplar, we consider the overlap between the mass of a genuine signal parent ion and a more general contribution of chemical noise at the mass of the parent ion. The background ions from the glycerol matrix contribute to a chemical noise in FAB often described as a "peak at every mass." Caldwell and Gross (2) studied the composition of the background ions formed in the fast atom bombardment process. For a glycerol FAB matrix, the authors used exact mass measurement to determine the empirical formulas of background ions, and then product-ion MS/MS spectra to deduce formation and dissociation chemistry. Caldwell and Gross found that almost every background ion up to m/z 500 resolved to a single-ion empirical formula (other than isotopic overlaps). The noise contribution in glycerol FAB — the "peak at every mass" — was therefore at-

tributable to a single background ion rather than a mixture of ions of different formulas. The product-ion MS/MS spectra of the background ions therefore usually contained predictable ions that reflected the expected dissociation chemistry for these background ions. The chance for a blanketing mass overlap with the product-ion MS/MS signals from a targeted compound of greater molecular complexity is therefore correspondingly reduced. Further, because the background ion signals were of fairly low and constant intensity, the contribution of intensity in the product-ion MS/MS spectrum was also low. Thus, while detection limits for single-stage FAB MS are often limited by the background noise, the use of MS/MS affords much lower detection limits because the background noise does not contribute to any substantial ion intensity in most parts of the product-ion MS/MS spectrum.

Figure 3 demonstrates the third exemplar of how the distribution of ions for signal and for the components of chemical noise at any nominal mass of selected parent ion is used in MS/MS to good effect for the reduction of chemical noise in mass spectral measurements. Figure 3a illustrates the composite parent-ion signal, consisting of equal parts signal from the targeted compound (signal, represented as the dotted line) and signal from all the other components present in the mixture (chemical noise, represented as the solid line). Figure 3b now represents that ion intensity expanded along the horizontal mass axis, corresponding to the exact masses (the ion empirical formula) of all the contributors. The total ion intensity remains the same, but the chemical noise is now seen to be a composite of several contributors. The product-ion MS/MS spectra for the signal, and for two of the noise contributors, are shown in Figure 3c. Not only is the ion intensity for each of the noise contributor parent ions reduced compared to the target ion signal, but the probability of mass overlap in the product-ion MS/MS spectrum is again low. These two attributes — a dilution of intensity and a low mass overlap probability — are the same as in the previous exemplar. The end result is

again a high S/N observed in the product-ion MS/MS spectrum, even with the overlap in the masses of the parent ions.

In the first exemplar, the dilution of intensity for the noise contributor was not germane. However, the constructive use of higher instrument resolution in selection of the parent ion and subsequent MS/MS in that example is immediately apparent. In each of the three cases, chosen to represent all possible cases in which MS/MS might be used, the increase in S/N is intrinsic. We can approach this in simplistic mathematical fashion as well. If the initial signal-to-noise ratio is 1:1, as shown in Figure 3a, the use of higher resolution (Figure 3b) increases S/N (for any of the five different, equally intense noise ions) to 5:1. As there is only a small probability that the product ions in the MS/MS spectrum of the noise-derived parent ions will overlap in mass with those of the target compound, S/N in the product-ion MS/MS spectrum increases to a very high value.

Often, because the noise signal decreases to essentially zero, the spectral S/N becomes limited by electronic noise only. In such instances, the value of the observation of the appropriate pattern of the isotopic product ions becomes apparent. The value of this pattern recognition is considered explicitly in the next and final installment in the chemical noise series.

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