Comparison of Isotope Abundance Analysis and Accurate Mass Analysis in their Ability to Provide Elemental Formula Information

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ACCESS

ABSTRACT: Deriving elemental formulas from mass spectra used to be an exclusive feature provided only by expensive high-resolution mass spectrometry instruments. Nowadays this feature can be used on unit resolution quadrupole-based mass spectrometers (MS) combining isotope abundance analysis (IAA) and mass accuracy analysis (MAA) with surprising accuracy that is commonly lower than 1 ppm mass accuracy. In this Article, we assess the usefulness of both MAA and IAA in the elemental formula deriving process performed on unit resolution MS data with constant resolution across the m/z range. The methods’ effective filtration power (EFP) are estimated along with their ability to provide useful elemental information under nonideal experimental conditions. The term effective mass accuracy (EMA) is introduced so that the identification power of IAA can be expressed in a familiar way and compared more readily to MAA. We found that IAA alone commonly has an EMA under 5 ppm. IAA and MAA work well together and provide improved results with median EMA < 1 ppm for calibrated MS or <3 ppm for uncalibrated MS. We have also found that even though these methods cannot be fully trusted to pinpoint the exact elemental formula under poor experimental conditions, IAA can still accurately provide the exact number of several heteroatoms such as sulfur, chlorine, and bromine, while MAA cannot. Under such conditions, a combination of both methods can also provide good insight into the amount of carbon, hydrogen, and other elements in the elemental formula.

KEYWORDS: isotope abundance analysis, accurate mass analysis, elemental formula, mass spectrometry, compound identification

INTRODUCTION

Quadrupole mass spectrometers are sometimes referred to as unit-mass resolution instruments, even though it is clear that they do not deserve this title. From our experience, quadrupole mass analyzers that are used daily, without periodical mass calibration, generate mass spectral peaks that are typically 0.6−0.7 Da wide and provide centralized masses within ±0.14 Da of the actual ones. In the majority of cases, the situation is much better than that of the 26 compounds discussed in this paper that were measured with an uncalibrated GC-MS system, have an average mass error of 0.023 Da with a standard deviation (SD) of 0.011 or 88 ppm (SD = 57). These measurements were obtained with an uncalibrated GC-MS system that was mass tuned in the centroid mode over a year ago. Quadrupole-based instruments that were calibrated with PFTBA in the profile mode can be trusted to yield masses with errors within ±100 ppm, but again the majority of cases provide better mass measurements, and for seven compounds measured in this study with a calibrated instrument, the mean mass accuracy was 30 ppm (SD = 35). This surprisingly good quadrupole mass accuracy, even if much coarser than the estimation provided by the ±2 ppm mass accuracy of expensive high-resolution instruments,1−3 can be utilized by mass accuracy analysis (MAA) algorithms4−7 listing elemental formulas with exact masses that are most similar to the measured mass, under restricted elemental range, to derive estimated elemental formulas.

The abundances of the molecular ion’s isotopologue peaks can also be analyzed using an isotope abundance analysis (IAA) algorithm, which cares for the shape of the pattern with no regard for the exact measured masses.3,8−20 The performance of IAA was shown to be reliable and effective at identifying compound.3,8,17 IAA for this paper was performed using the TAMI software.16

These two physically different algorithms take their data from the same source, a quadrupole-generated mass spectrum, both test the molecular ion and need it to be available, and both yield a list of elemental formulas with their match scores and identification probabilities. But how do they compare to one another? Which one has more filtration power? Which one is more trustworthy? How well do they work together?

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This paper addresses these questions and provides quantitative assessments for their relative roles as well as their combination.

**EFFECTIVE FILTRATION-POWER AND MASS-ACCURACY**

If one takes a broad look at the results provided by isotope abundance analysis or mass accuracy analysis, they both can be considered as filters. For each of these analytical methods, there is a threshold with eligible formulas on one side and irrelevant formulas on the other. The formulas that remain after filtration are the true identification candidates. We define "filtration power" (FP) as the factor by which the total number of all possible elemental formulas (with the same nominal mass, under reasonable user-selected elements and criteria) is reduced via the implementation of the filter. If, for example, there are 1000 identification candidates overall, and after applying the filter, their number is reduced to 50, the filtration power is FP = 1000/50 = 20. A higher FP leads to a lower number of candidates and therefore to higher identification chances and to more trustworthy information.

We found that in a well-calibrated quadrupole GC-MS, with added PFTBA and profile (raw scan) mode scanning, we can consistently measure masses with errors under ±100 ppm, so we can trust a measured mass of ~300 Da, for example, to be within ±30 mDa of the actual mass. We also found that an uncalibrated GC-MS (calibrated in centroid mode long ago) can still be trusted to yield masses with errors within ±140 mDa, which makes this number the minimum mass-filter window to be used when the centroid mass is reported with one point decimal precision. Note that when using the mass accuracy filters, we employ an upper mass error that is much higher than typical errors (which are about 30 ppm for a mass calibrated quadrupole) in order to make sure that the correct compounds remain in the filtered lists.

Figure 1 shows the distribution of exact masses for compounds with a nominal mass of 304 Da. The shaded zone represents a filtration window with a width of ±100 ppm. One can see that most compounds are filtered out by this window. Another observation is that the performance of a mass filter is worse if the measured mass is common, while excelling around rarely seen masses at the edges of the mass defects. The distribution of exact masses of compounds with a nominal mass of 304 Da in Figure 1 was obtained with the TAMI software that provides an elemental formula from quadrupole MS data files.

IAA cannot be assessed in that way, as it does not have a physical measure parameter like MAA. However, there are other ways the effectiveness of IAA and MAA can be tested and, therefore, compared by looking at the actual results they provide. Both methods produce a list of possible elemental formulas, and the position of the correct formula on the list can be examined.

Table 1 compares the performance of IAA and MAA on a variety of real-world compounds analyzed by a mass-calibrated GC-MS (Agilent 5977A, Agilent, Santa Clara, CA, U.S.A.) that was scanning masses in the profile mode using PFTBA at the end of the run. The actual position of the correct elemental formula in the hit-list is presented, along with a new metric called effective filtration power (EFP), specifying the filtration power (ratio of all results to the results left after filtration) of the smallest filter window that would still accommodate this correct result. Effective mass accuracy (EMA) values represent the mass accuracy needed for the same filtration power. The analysis was performed in raw-scan mode (profile) with added PFTBA for the ideal mass calibration and centroiding. The results show how IAA in combination with a default ±0.14 Da MAA (can be used safely even on uncalibrated mass spectrometers with centroid mode) provide great results that are equivalent or better than 3.2 ppm mass accuracy (median = 3.2 ppm) and with very high filtration power values. IAA alone with no mass filters is still very informative and provides results with a median EMA of 5.3 ppm, while MAA with a ±0.14 ppm window has a median EMA of 37 ppm. IAA together with a ±100 ppm MAA combine into a very strong filter and manage to provide the correct elemental formula at the first or second hit, and commonly the first (out of hundreds or thousands of possible candidates), and the median EMA of their combination is lower than 0.3 ppm. In cases where IAA is combined with a mass filter and the result is at first place the values of EFP and EMA were calculated by, respectively, multiplying or dividing by the measured FP of the relative mass window. Nicotine, for example, is already the number one hit with IAA and no mass window, while a ±0.14 Da filter window alone leaves 113 candidates out of 241, this means 2.13 times better, so the EFP we got for the IAA is now multiplied by 2.13 and we get 514, and the EMA divided by 2.13 and we get 2.5 ppm.

The mass spectra of 26 different additional compounds were collected in the centroid mode via an Agilent 5977 GC-MS with a Cold-EI interface that enhances the molecular ion, which was not mass-calibrated (tuned) for over two years, and were analyzed with IAA. The results can be seen in Table 2. The most striking observation is that, for IAA, the correct compound position on the hit-list has a median value of 2. Adding a rough MAA filter of ±0.14 Da, which is the basic way the algorithm is applied when the instrument is operated in the centroid mode (typical GC-MS operation) and not mass-calibrated, brings the median position to 1 and the average EMA to 4.3 ppm (SD = 7.3) with a median of 1.3 ppm.

The benefit of a mass window added to IAA analysis is estimated, in this table, by factoring in the change in mass-range. For example, in the analysis of dimethoate, there are 787 candidates that span a mass defect range of ±943.57 ppm, so we estimated that a mass filter of ±100 ppm will result in a filtration

![Figure 1. A typical histogram of elemental formulas with a nominal mass of 304 Da, obtained by the TAMI software, with no constraints on carbon and hydrogen and the following elemental ranges: O 0–8, N 0–8, S 0–4, P 0–4, Cl 0–3, Br 0–3, F 0–2. The MAA filtration window of ±100 ppm is likely to still contain many elemental formulas, resulting in a low FP. The MAA filter is much more effective for measured masses that reside on the rims of the histogram, but most measured masses do not.](https://doi.org/10.1021/jasms.0c00419)
Table 1. Showing the Performance of Isotope Abundance Analysis (IAA) and Mass Accuracy Analysis (MAA) in the GC-MS Analysis of Seven Compounds Each at 5 ng-
Column Amount, Using PFTBA Calibration in Profile Mode To Improve the Mass Measurements

| cmpd name, formula, and mass | No. of candidates | meas. MAA error (ppm) | IAA hit #, EFP and EMA | MAA of ±100 ppm hit #, EFP and EMA | IAA ± 0.14 Da mass filter hit #, EFP and EMA | IAA ± 100 ppm mass filter hit #, EFP and EMA |
|----------------------------|-------------------|-----------------------|------------------------|-----------------------------------|---------------------------------|---------------------------------|
| benzene, C6H5NO2            | 123.0351 Da       | 78                    | 51                     | 1, EFP > 78, EMA < 8.3 ppm        | 5, FP = 16, EMA = 50 ppm       | 1, EFP > 78, EMA < 8.3 ppm        |
| nicotine, C8H7NO3          | 162.151 Da        | 241                   | 5.1                    | 1, EFP > 241, EMA < 5.3 ppm       | 2, FP = 121, EMA = 5.3 ppm      | 1, EFP > 514, EMA < 2.5 ppm       |
| hexadecane, C16H34         | 226.2655 Da       | 854                   | 37                     | 2, EFP > 427, EMA = 198 ppm       | 1, FP = 854, EMA < 37 ppm       | 1, EFP > 17365, EMA < 4.9 ppm     |
| anthracene, C17H7O7    | 178.0777 Da       | 343                   | 24                     | 1, EFP > 343, EMA < 4.9 ppm       | 6, FP = 57, EMA = 24 ppm        | 1, EFP > 521, EMA < 3.2 ppm       |
| caffeine, C9H4N2O2     | 201.2490 Da       | 479                   | −1.4                   | 1, EFP > 479, EMA < 0.8 ppm       | 2, FP = 240, EMA = 1.6 ppm      | 1, EFP > 755, EMA < 0.5 ppm       |
| methyl stearate, C17H36O2 | 2398              | 95                    | 7                      | 7, EFP > 343, EMA = 141 ppm       | 5, FP = 480, EMA = 95 ppm       | 4, EFP = 600, EMA = 65 ppm        |
| chlorpromazine, C20H21ClN5S | 2896             | 73                    | 4                     | 4, EFP = 724, EMA = 1.5 ppm       | 138, FP = 21, EMA = 73 ppm      | 4, EFP = 724, EMA = 1.5 ppm       |
| descriptive statistics for EMA |                 | avg = 51 ppm, SD = 82 ppm, med = 5.3 ppm | avg = 41 ppm, SD = 35 ppm, med = 37 ppm | avg = 12 ppm, SD = 23 ppm, med = 3.2 ppm | avg = 4.2 ppm, SD = 10 ppm, med = 0.3 ppm |

*Each of the four right-most columns present the results of different analyses, IAA, MAA, IAA + MAA with ±0.14 Da window and IAA + MAA with ±100 ppm window. The position of the correct elemental formula in the resulting hit list is shown, and also the EFP and EMA values that are calculated as explained above. The number of candidates are all possible elemental formulae with the same nominal mass and within the following elemental range: O 0-8, N 0-5, S 0-4, P 0-2, Cl 0-2, Br 0-2 (carbon and hydrogen are not restricted). The number of isotope abundances used in the evaluation was 931.*
Table 2. Twenty-six Compounds, from Different GC-MS Data Files (Obtained with Cold-EI, and All with Centroid Data without Mass Calibration), Ana-lyzed by Isotope Abundance Analysis (IAA), IAA, and Mass Accuracy Analysis (MAA) with a ±0.14 Da Window (Possible Even without Mass Calibration), and an Estimation for the Results of IAA and MAA with a ±100 ppm Window8

| cmpd name, formula, and mass | No. of candidates | IAA result hit #, EFP, and EMA | IAA ± 0.14 Da hit #, EFP, and EMA | estimation for IAA ± 100 ppm, EFP, and EMA |
|----------------------------|------------------|---------------------------|----------------------------------|-----------------------------------------------|
| dimethoate, C₃H₆NO₂⁺S₂P, 228.9991 Da | 787 | #1, EFP > 787, EMA < 1 ppm | #1, EFP > 1215, EMA < 0.6 ppm | EFP > 7426, EMA < 0.11 ppm |
| Quinol ED, C₁₀H₁₄N₂O₂⁺ | 693 | #2, EFP = 347, EMA = 5.1 ppm | #2, EFP = 504, EMA = 3.5 ppm | EFP = 3252, EMA = 0.54 ppm |
| diazinone, C₃H₆N₂O₂⁺, 304.1005 Da | 2141 | #3, EFP = 603, EMA = 4.8 ppm | #3, EFP = 1281, EMA = 2.3 ppm | EFP = 5896, EMA = 0.49 ppm |
| chlorpyrifos, C₁₅H₂₂ClF₃O₂⁺, 348.257 Da | 4266 | #4, EFP = 614, EMA = 1.3 ppm | #4, EFP = 1282, EMA = 0.6 ppm | EFP = 699, EMA = 0.66 ppm |
| methidathion, C₁₃H₁₉N₂O₂PS⁺, 301.9613 Da | 2456 | #8, EFP = 281, EMA = 5.4 ppm | #6, EFP = 584, EMA = 2.6 ppm | EFP = 5944, EMA = 0.13 ppm |
| tebuconazole, C₈H₁₃CIN₂O⁺, 307.9466 Da | 2247 | #8, EFP = 281, EMA = 5.4 ppm | #6, EFP = 584, EMA = 2.6 ppm | EFP = 2660, EMA = 0.57 ppm |
| iprodione, C₁₃H₂₁ClN₂O₂⁺ | 2984 | #30, EFP = 99, EMA = 9.5 ppm | #28, EFP = 348, EMA = 2.7 ppm | EFP = 1479, EMA = 0.64 ppm |
| bifenthrin, C₂₃H₂₂ClF₃O₂⁺, 422.1255 Da | 20440 | #27, EFP = 757, EMA = 1.8 ppm | #19, EFP = 2703, EMA = 0.5 ppm | EFP = 8964, EMA = 0.15 ppm |
| bifenacetate, C₁₇H₂₂N₂O₂⁺, 300.1468 Da | 2385 | #1, EFP > 2385, EMA < 0.7 ppm | #1, EFP > 5128, EMA < 0.3 ppm | EFP = 23920, EMA < 0.07 ppm |
| pyriproxyfen, C₁₇H₂₃NO₂⁺, 321.1359 Da | 2645 | #2, EFP = 1323, EMA = 1.8 ppm | #1, EFP > 4611, EMA < 0.5 ppm | EFP = 20100, EMA < 0.12 ppm |
| prochloraz, C₁₅H₁₆Cl₃N₃O₂⁺, 375.0303 Da | 5447 | #42, EFP = 130, EMA = 5.2 ppm | #36, EFP = 463, EMA = 1.5 ppm | EFP = 1728, EMA = 0.39 ppm |
| tridecane, C₁₃H₂₆⁺, 184.2186 Da | 421 | #1, EFP > 421, EMA < 36 ppm | #1, EFP > 551, EMA < 27.5 ppm | EFP = 4191, EMA < 3.62 ppm |
| eicosane, C₂₀H₄₂⁺, 282.3281 Da | 2001 | #2, EFP = 1001, EMA = 29 ppm | #1, EFP > 2077, EMA < 14 ppm | EFP = 10297, EMA < 2.82 ppm |
| tricantoane, C₁₉H₃₈⁺, 422.4846 Da | 6575 | #13, EFP = 506, EMA = 123 ppm | #3, EFP = 1804, EMA = 34.5 ppm | EFP = 5976, EMA = 10.41 ppm |
| HMPTD, C₂₁H₂₈N₂O₂⁺, 208.069 Da | 648 | #1, EFP > 648, EMA < 0.6 ppm | #1, EFP > 954, EMA < 0.4 ppm | EFP = 6421, EMA < 0.06 ppm |
| xylazine, C₁₇H₂₆N₂⁺, 220.1029 Da | 841 | #2, EFP = 421, EMA = 2.3 ppm | #1, EFP > 654, EMA < 1.5 ppm | EFP = 4159, EMA < 0.23 ppm |
| phenothiazine, C₁₇H₁₉N₂S⁺, 199.045 Da | 444 | #1, EFP > 444, EMA < 0.5 ppm | #1, EFP > 617, EMA < 0.4 ppm | EFP = 4339, EMA < 0.05 ppm |
| propanol, C₃H₇NO₂⁺, 259.1567 Da | 1183 | #1, EFP > 1183, EMA < 1.1 ppm | #1, EFP > 2105, EMA < 0.6 ppm | EFP = 11372, EMA < 0.11 ppm |
| triflupromazine, C₁₇H₂₁F₂N₂S⁺, 352.1216 Da | 11410 | #1, EFP > 11410, EMA < 0.01 ppm | #1, EFP > 40619, EMA < 0.01 ppm | EFP > 161497, EMA < 0.01 ppm |
| promethazine, C₁₇H₂₁N₂S⁺, 284.1342 Da | 1992 | #4, EFP = 498, EMA = 4.1 ppm | #4, EFP = 1051, EMA = 1.9 ppm | EFP = 5178, EMA = 0.39 ppm |
| promazine, C₁₉H₂₆N₂⁺, 284.1342 Da | 1992 | #2, EFP = 996, EMA = 1.4 ppm | #2, EFP = 2102, EMA = 0.7 ppm | EFP = 10355, EMA = 0.13 ppm |
| chlorpromazine, C₁₇H₂₆CIN₂⁺, 318.0952 Da | 2896 | #1, EFP > 2896, EMA < 0.3 ppm | #1, EFP > 6349, EMA < 0.1 ppm | EFP > 27944, EMA < 0.03 ppm |
| haloperidol, C₁₉H₂₁CIN₂⁺, 375.1396 Da | 10955 | #8, EFP = 288, EMA = 3.6 ppm | #30, EFP = 1028, EMA = 1 ppm | EFP = 3835, EMA = 0.27 ppm |
| benzenz, propyl-, C₇H₁₅⁺, 120.0934 Da | 90 | #1, EFP > 90, EMA < 2.9 ppm | #1, EFP > 59, EMA < 4.4 ppm | EFP > 693, EMA < 0.38 ppm |
| propanol, 1,3-dibromo-, C₃H₇Br₂⁺, 199.8831 Da | 574 | #1, EFP > 574, EMA < 7.8 ppm | #1, EFP > 801, EMA < 5.6 ppm | EFP > 5607, EMA < 0.8 ppm |
| fnisteride, C₁₈H₂₈O₂⁺, 372.2771 Da | 4553 | #1, EFP > 4553, EMA < 3.4 ppm | #1, EFP > 16183, EMA < 1 ppm | EFP > 60860, EMA < 0.25 ppm |

8The results for #1 hits in IAA and MAA columns are calculated using the estimated filtration power of the mass window (the ratio of the full mass defect range divided by 100 ppm). The number of candidates are all possible elemental formulae with the same nominal mass and within the following elemental range: O 0-8, N 0-5, S 0-4, P 0-2, Cl 0-2, Br 0-2 (Carbon and Hydrogen are not restricted), except for the following changes: for Chlorpyrifos Cl 0-4, for Bifenthrin F 0-3, for Prochloraz Cl 0-4, for Triflupromazin F 0-3, and for Haloperidol F 0-2. The number of isotopologue peaks used was usually 4, and it was raised when the measured pattern exhibited information in higher masses (multiple chlorine/bromine for example).
intrinsic problem that hinders its performance: the reliance on only one feature, the measured mass of the monoisotopic peak. This feature is not specific enough, as there are many possible elemental combinations that yield similar masses.

A short glance at Table 3, which shows the 10 best matching compounds in terms of mass, all within 5 ppm error compared to diazinon, makes the problem obvious. One can see that these best mass-matching compounds have a widely varying elemental composition. An oxygen atom together with a phosphorus atom, for example, have almost the exact same mass as a carbon atom and chlorine, making the mass of the second elemental formula different by only \(1.8 \times 10^{-4}\) Da from that of diazinon.

IAA, on the other hand, uses several features together: the molecular ion. In terms of identification, IAA as it is commonly used with a wide \(\pm 0.14\) Da mass window provides the correct elemental formula most of the time (median position on the resulting hit-list is \#1\), and is equivalent to mass analysis with under 3 ppm mass accuracy. When IAA is coupled with MAA using a \(\pm 100\) ppm window (on mass-calibrated quadrupoles operated in profile mode), the performance surpasses that of existing high resolution instruments and provides an EMA of under 1 ppm.

IAA was also found to provide more reliable elemental information. The correct number of chlorine, bromine, and sulfur atoms can be found in all eligible results provided by the IAA algorithm, making their determination a near certainty, whereas MAA results vary drastically in this aspect, even when one considers only a few of the best results. The IAA + MAA combination can further reduce the uncertainty about the number of atoms of the elements.

This strong determination of certain heteroatoms favorably affects the determination of other more common elements, as it naturally introduces a strong restriction that greatly reduces the number of possible options (if some heteroatoms are surely present, it leaves a smaller mass range to accommodate other elements into).

It should be noted that both MAA and IAA require that the molecular ion will be present, and IAA unlike MAA relies on the measurement of low abundance isotopologues and thus suffer from statistical fluctuations approximately \(100\times\) more than MAA alone and thus typically require a few nanograms on-column sample amounts or using a second run with a narrow mass spectral window for improved ion statistics. The problem of a weak or missing molecular ion can be overcome via the use of GC-MS with Cold EI that provides significantly enhanced molecular ions yet with full compatibility with NIST library identification.

GC-MS based sample identification usually begins with a NIST library (or another library) search and identification, which provides the sample name and structure and often includes isomer level differentiation, which cannot be deducted from an obtained elemental formula. Therefore, library-based identification is the best tool, when applicable. Unfortunately, the majority of compounds are not included in any library, and in these cases, obtaining the sample elemental formula is the best way for sample characterization. The TAMI software uses IAA to automatically check the library results and alert the user if they seem erroneous (usually since the compound is not recorded in the library) and, in those cases, provide an IAA + MAA alternative, yielding the most probable elemental formulas.

In conclusion, when attempting to obtain elemental formulas with quadrupole MS based data files, it is highly effective to use IAA as the main algorithm, with a coarse \(\pm 0.14\) Da mass accuracy filter, when standard centroid files are analyzed, or \(\pm 100\) ppm if the MS is calibrated in the profile mode. This combination is very likely to help in the determination of the elemental formula in general and to accurately determine the

### DISCUSSION AND CONCLUSIONS

When analyzing mass spectral data, IAA and MAA are both useful techniques, each utilizing a different physical attribute of the molecular ion. In terms of identification, IAA as it is commonly used with a wide \(\pm 0.14\) Da mass window provides the correct elemental formula most of the time (median position on the resulting hit-list is \#1\), and is equivalent to mass analysis with under 3 ppm mass accuracy. When IAA is coupled with MAA using a \(\pm 100\) ppm window (on mass-calibrated quadrupoles operated in profile mode), the performance surpasses that of existing high resolution instruments and provides an EMA of under 1 ppm.

IAA was also found to provide more reliable elemental information. The correct number of chlorine, bromine, and sulfur atoms can be found in all eligible results provided by the IAA algorithm, making their determination a near certainty, whereas MAA results vary drastically in this aspect, even when one considers only a few of the best results. The IAA + MAA combination can further reduce the uncertainty about the number of atoms of the elements.

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In conclusion, when attempting to obtain elemental formulas with quadrupole MS based data files, it is highly effective to use IAA as the main algorithm, with a coarse \(\pm 0.14\) Da mass accuracy filter, when standard centroid files are analyzed, or \(\pm 100\) ppm if the MS is calibrated in the profile mode. This combination is very likely to help in the determination of the elemental formula in general and to accurately determine the

### Table 3. Ten Elemental Formula Most-Mass-Similar to Diazinon (#1)\(^{26}\)

| # | elemental comp. | mass error (ppm) |
|---|-----------------|------------------|
| 1 | C\(_{12}\)H\(_{25}\)N\(_{2}\)O\(_5\)S\(_3\)P\(_1\) | 0.082044 |
| 2 | C\(_{10}\)H\(_{22}\)N\(_2\)O\(_6\)Cl\(_2\)S\(_1\)P\(_1\) | 1.249587 |
| 3 | C\(_{10}\)H\(_{22}\)N\(_2\)P\(_2\) | 2.144028 |
| 4 | C\(_{10}\)H\(_{22}\)O\(_2\)I | 2.726072 |
| 5 | C\(_{10}\)H\(_{22}\)O\(_3\)Si | 2.791840 |
| 6 | C\(_{10}\)H\(_{22}\)O\(_3\)P\(_2\) | 3.291675 |
| 7 | C\(_{10}\)H\(_{22}\)O\(_3\)Cl\(_2\) | 3.291675 |
| 8 | C\(_{10}\)H\(_{22}\)O\(_3\)Si | 4.853539 |
| 9 | C\(_{10}\)H\(_{22}\)O\(_3\)Cl\(_2\) | 4.768161 |
| 10 | C\(_{10}\)H\(_{22}\)O\(_3\)Cl\(_2\) | 5.067404 |

\(^{26}\)Showing the wildly varying number of elements present. Most formulas do not include sulfur or phosphorous (which are present in the correct formula), and some include chlorine (not present). The range of carbon atom numbers is 10—22.

### Table 4. Ten Elemental Formula Most Similar in Their Isotopologue Pattern (IAA) to Diazinon (#1)\(^{26}\)

| # | elemental comp. | IAA error (%) |
|---|-----------------|---------------|
| 1 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.003 |
| 2 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.006 |
| 3 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.011 |
| 4 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.011 |
| 5 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.011 |
| 6 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.011 |
| 7 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.019 |
| 8 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.019 |
| 9 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.021 |
| 10 | C\(_{12}\)H\(_{22}\)N\(_2\)O\(_5\)S\(_3\)P\(_1\) | 0.022 |

\(^{26}\)All show one sulfur atom, none shows chlorine atoms, most show phosphorous, and the number of carbon atoms is 11—13. Comparing to the wildly varying MAA results shown in Table 2, these results are much more cohesive.

shows the 10 best matching compounds in terms of IAA error, and as can be seen, all of them have much in common. Some elements like chlorine, bromine, sulfur, selenium, and silicon have unique effects on the relative abundances of the isotopologue ions that are rarely missed by the IAA algorithm.

Table 5 shows some further specific examples: The elemental spread provided by IAA, MAA, and their combination from the analysis of diazinon, anthracene, caffeine, cholesterol, chlorpromazine, and dibromopropane. In order to see the negative effect brought on by the reliance on only one feature, only the 20 best results were taken into account. This eliminated the difference in Filtration power between the two methods (without this step, MAA would have shown far worse results than IAA). As can be seen, IAA provides clearer results with less uncertainty, and the combination of both methods provides the best results.
TABLE 5. Elemental Spread within the 20 Best Results, Analyzing the Mass Spectrum of Diazinon, Caffeine, Anthracene, Cholesterol, Chlorpromazine, and Dibromopropane Using IAA, MAA, and Their Combination with 100 ppm Mass Accuracy\(^a\)

| Compound      | C  | H  | O  | N  | S  | P  | Cl | Br |
|---------------|----|----|----|----|----|----|----|----|
| *Diazinon*    |    |    |    |    |    |    |    |    |
| actual        | 12 | 21 | 3  | 2  | 1  | 1  | 0  | 0  |
| IAA          | 11–13 | 4–25 | 1–6 | 0–4 | 1  | 0–2 | 0  | 0  |
| MAA          | 8–22 | 12–24 | 0–8 | 0–4 | 0–3 | 0–2 | 0–2 | 0–1 |
| IAA + MAA    | 10–14 | 16–26 | 1–6 | 0–4 | 1  | 0–2 | 0  | 0  |
| *Caffeine*    |    |    |    |    |    |    |    |    |
| actual        | 8  | 10 | 2  | 4  | 0  | 0  | 0  | 0  |
| IAA          | 8–10 | 7–12 | 0–3 | 0–4 | 0  | 0–2 | 0  | 0  |
| MAA          | 5–14 | 10–19 | 0–6 | 0–4 | 0–2 | 0–2 | 0–1 | 0  |
| IAA + MAA    | 8–9 | 10–11 | 2–3 | 2–4 | 0  | 0–1 | 0  | 0  |
| *Anthracene*  |    |    |    |    |    |    |    |    |
| actual        | 14 | 10 | 0  | 0  | 0  | 0  | 0  | 0  |
| IAA          | 12–14 | 6–22 | 0–1 | 0–2 | 0  | 0  | 0  | 0  |
| MAA          | 4–14 | 10–18 | 0–5 | 0–4 | 0–2 | 0–2 | 0–1 | 0  |
| IAA + MAA    | 14 | 10 | 0  | 0  | 0  | 0  | 0  | 0  |
| *Cholesterol* |    |    |    |    |    |    |    |    |
| actual        | 27 | 46 | 1  | 0  | 0  | 0  | 0  | 0  |
| IAA          | 26–28 | 2–46 | 0–3 | 0–4 | 0  | 0–1 | 0  | 0  |
| MAA          | 21–27 | 42–51 | 0–4 | 0–4 | 0–1 | 0–1 | 0–1 | 0  |
| IAA + MAA    | 21–28 | 42–51 | 0–4 | 0–4 | 0  | 0–2 | 0  | 0  |
| *Chlorpromazine* |    |    |    |    |    |    |    |    |
| actual        | 17 | 19 | 0  | 2  | 1  | 0  | 1  | 0  |
| IAA          | 15–19 | 0–31 | 0–2 | 0–4 | 1  | 0–1 | 1  | 0  |
| MAA          | 7–19 | 14–26 | 0–8 | 0–4 | 0–2 | 0–2 | 0–2 | 0–1 |
| IAA + MAA    | 13–21 | 11–27 | 0–4 | 0–4 | 0–2 | 0–1 | 1  | 0  |
| *Dibromopropane* |    |    |    |    |    |    |    |    |
| actual        | 3  | 6  | 0  | 0  | 0  | 0  | 0  | 2  |
| IAA          | 1–3 | 2–6 | 0–1 | 0–2 | 0  | 0  | 0  | 2  |
| MAA          | 0–6 | 0–6 | 0–4 | 0–4 | 0–2 | 0–2 | 0–2 | 0–2 |
| IAA + MAA    | 3  | 6  | 0  | 0  | 0  | 0  | 0  | 2  |

\(^a\)Correct identifications are italic. IAA provides a narrower spread for most elements and therefore greater certainty in the amounts of each of them. The combination yields superior results with smaller uncertainties.

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**REFERENCES**
(1) Xian, F.; Hendrickson, C. L.; Marshall, A. G. High resolution mass spectrometry. *Anal. Chem.* 2012, 84, 708–719.
(2) Hu, Q.; Noll, R. J.; Li, H.; Makarov, A.; Hardman, M.; Graham Cooks, R. The Orbitrap: A new mass spectrometer. *J. Mass Spectrom.* 2005, 40, 430–443.
(3) Lehotay, S. J.; Mastovska, K.; Amirav, A.; Fialkov, A. B.; Martos, P.; Kok, A. d.; Fernandez-Alba, A. R. Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques. *TrAC, Trends Anal. Chem.* 2008, 27, 1070–1090.
(4) Beynon, J. H. Qualitative analysis of organic compounds by mass spectrometry. *Nature 1954*, 174, 735–737.
(5) Bristow, A. W. T. Accurate mass measurement for the determination of elemental formula - A tutorial. *Mass Spectrom. Rev.* 2006, 25, 99–111.
(6) Tyler, A. N.; Clayton, E.; Green, B. N. Exact mass measurement of polar organic molecules at low resolution using electrospray ionization and a quadrupole mass spectrometer. *Anal. Chem.* 1996, 68, 3561–3569.

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**Table 5. Elemental Spread within the 20 Best Results, Analyzing the Mass Spectrum of Diazinon, Caffeine, Anthracene, Cholesterol, Chlorpromazine, and Dibromopropane Using IAA, MAA, and Their Combination with 100 ppm Mass Accuracy**

Presence and number of various heteroatoms even under nonideal experimental conditions. Combining these two algorithms with a library search algorithm (such as NIST’s\(^7\)), when it is applicable, yields a very comprehensive solution to compound identification via quadrupole based mass spectrometry.

The abundances of the various isotopes used by the TAMI software are those recommended by the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC) on 2009.\(^5\)

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**REFERENCES**
(1) Xian, F.; Hendrickson, C. L.; Marshall, A. G. High resolution mass spectrometry. *Anal. Chem.* 2012, 84, 708–719.
(2) Hu, Q.; Noll, R. J.; Li, H.; Makarov, A.; Hardman, M.; Graham Cooks, R. The Orbitrap: A new mass spectrometer. *J. Mass Spectrom.* 2005, 40, 430–443.
(3) Lehotay, S. J.; Mastovska, K.; Amirav, A.; Fialkov, A. B.; Martos, P.; Kok, A. d.; Fernandez-Alba, A. R. Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques. *TrAC, Trends Anal. Chem.* 2008, 27, 1070–1090.
(4) Beynon, J. H. Qualitative analysis of organic compounds by mass spectrometry. *Nature 1954*, 174, 735–737.
(5) Bristow, A. W. T. Accurate mass measurement for the determination of elemental formula - A tutorial. *Mass Spectrom. Rev.* 2006, 25, 99–111.
(6) Tyler, A. N.; Clayton, E.; Green, B. N. Exact mass measurement of polar organic molecules at low resolution using electrospray ionization and a quadrupole mass spectrometer. *Anal. Chem.* 1996, 68, 3561–3569.
(7) Wang, Y.; Prest, H. Accurate mass measurement on real chromatographic time scale with a single quadrupole mass spectrometer. *Chromatography* 2006, 27, 135–140.

(8) Alon, T.; Amirav, A. Isotope abundance analysis methods and software for improved sample identification with supersonic gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 2006, 20, 2579–2588.

(9) Blom, K. F. Enhanced selectivity in determining elemental composition: Concerted precise mass and isotope pattern moment analysis. *Org. Mass Spectrom.* 1988, 23, 783–788.

(10) Bogdanov, V. A.; Vorob’ev, A. V.; Savel’ev, Y. I.; Shchelokov, R. N. Determination of the elemental composition of a substance from low-resolution mass spectra with the aid of a computer. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* 1991, 40, 336–338.

(11) Bogdanov, V. A.; Vorob’ev, A. V.; Savel’ev, Y. I. Determination of the Elemental Composition of Organic Substances from their Low-Resolution Mass Spectra and Data of Proton Magnetic Resonance. *Zhurnal Anal. Khimii.* 1994, 49, 642–644.

(12) Bogdanov, V. A.; Savel’ev, Y. I. Mathematical Aspects of Determination of the Elemental Compositions of an Unknown Substance by the Method of Moments. *Russ. J. Inorg. Chem.* 1994, 39, 1755–1759.

(13) Tenhosaari, A. Computer assisted composition analysis of unknown compounds by simultaneous analysis of the intensity ratios of isotope patterns of the molecular ion and daughter ions in low resolution mass spectra. *Org. Mass Spectrom.* 1988, 23, 236–239.

(14) Tenhosaari, A. Microcomputer program for determining elemental compositions of unknown organic compounds from low-resolution electron impact mass spectra. *Chemos. Intell. Lab. Syst.* 1990, 8, 167–171.

(15) Tenhosaari, A. Determination of molecular formulae from low-resolution mass spectral data by matching experimental and calculated isotope patterns of logical sets of daughter ion candidates. *Anal. Chim. Acta* 1991, 248, 71–75.

(16) TAMI – Molecule Identification Software Suite, http://www.avivanalytical.com/Isotope-Abundance.aspx.

(17) Alon, T.; Amirav, A. Enhancing the Identification Capabilities of EI GC-MS - How Quadrupole GC-MS can compete with High Resolution TOF, http://blog.avivanalytical.com/2013/03/enhancing-identification-capabilities.html.

(18) Blom, K. F. Average mass approach to stable isotope dilution mass spectrometry. *Org. Mass Spectrom.* 1987, 22, 530–533.

(19) Blom, K.; Dybowski, C.; Munson, B.; Gates, B.; Hasselbring, L. Mass Spectral Analysis of Isotopically Labeled Compounds: Average Mass Approach. *Anal. Chem.* 1987, 59, 1372–1374.

(20) Blom, K. F. Elementary composition from moment analyses of the low-resolution isotope pattern. *Org. Mass Spectrom.* 1988, 23, 194–203.

(21) Amirav, A.; Gordin, A.; Poliak, M.; Fialkov, A. B. Gas chromatography-mass spectrometry with supersonic molecular beams. *J. Mass Spectrom.* 2008, 43, 141–163.

(22) Alon, T.; Amirav, A. How enhanced molecular ions in Cold EI improve compound identification by the NIST library. *Rapid Commun. Mass Spectrom.* 2015, 29, 2287–2292.

(23) Mitchell, J. M.; Flight, R. M.; Moseley, H. N. B. Small Molecule Isotope Resolved Formula Enumeration: A Methodology for Assigning Isotopologues and Metabolite Formulas in Fourier Transform Mass Spectra. *Anal. Chem.* 2019, 91 (14), 8933–8940.

(24) NIST mass spectrometry data center, https://chemdata.nist.gov.

(25) Berglund, M.; Wieser, E. M. Isotopic compositions of the elements 2009 (IUPAC Technical Report). *Pure Appl. Chem.* 2011, 83 (2), 397–410.