Guidelines for Comparison of Mass Spectra

1 Scope

This document provides guidelines to use when comparing mass spectra of known and unknown substances. This document does not address identification of unknown substances. Mass spectrometry is only one of many techniques that may be used in an attempt to identify an unknown substance. All acquired data will be considered when identifying an unknown substance. If structural elucidation is achieved with a technique other than mass spectrometry, then the calculation and comparison of ion ratios is not required.

These guidelines are intended for application to full scan, tandem, and selected ion monitoring (SIM) mass spectra acquired in electron impact (EI), chemical (CI), electrospray (ESI), atmospheric pressure chemical (APCI), direct analysis in real time (DART) ionization modes. Other mass spectral techniques are beyond the scope of this document. Any specific mass spectral comparison guidelines in individual General Chemistry standard operating procedures (SOPs) will override any guidelines set forth in this document.

This document applies to Chemistry Unit (CU) personnel that are qualified and authorized to examine General Chemistry evidence.

2 Equipment/Materials/Reagents

Not applicable

3 Standards and Controls

Not applicable

4 Sampling

Not applicable

5 Procedure

Note- DART/time-of-flight mass spectra are limited to the applicable criteria in Section 5.6.4.

5.1 Background-Subtracted Mass Spectrum

Generate a background-subtracted mass spectrum. The background spectra may come before and/or after the peak of interest, and will all be selected from outside the region integrated for determination of ion ratios. This background-subtracted spectrum will be used to establish the base peak and significant ions.

5.2 Significant Ions

Any ion signal greater than ~15% of the most intense ion signal in a background-subtracted mass spectrum will normally be considered a significant ion. An ion that would otherwise be considered significant may be excluded if it can be demonstrated that the ion arises from, or is significantly affected by a chemical interference. This interference can be demonstrated by showing that a reconstructed ion trace for the ion in question does not coincide with the traces for other ions associated with the peak of interest.

5.3 Diagnostic Ions

Diagnostic ions in a mass spectrum are those ions that are characteristic of the chemical compound. Determination of diagnostic ions depends upon knowledge of the chemical structure and assessment of the mass spectrum of reference materials. There is not a universally accepted standard for determining diagnostic ions, however, the following recommendations will be considered.

- Adduct ions will normally be excluded, except that one pseudo-molecular adduct ion may be considered diagnostic.
- Isotopes will be excluded unless they are characteristic of a specific chemical composition (e.g., chlorine, bromine).
- Ions resulting purely from a derivatizing or complexing reagent will normally be excluded from the list of diagnostic ions.
- The molecular (or pseudo-molecular) ion will be considered diagnostic, unless the intensity for that ion is less than ~5%.

5.4 Base Peak

The base peak for the mass spectrum of a reference material is the most intense signal for a diagnostic ion in the background-subtracted mass spectrum. For the purpose of calculating ion ratios, the base peak in an unknown mass spectrum will be defined as the base peak of the reference material spectrum to which it is being compared.

5.5 Calculating Ion Ratios

Ion ratios will be determined by integrating reconstructed ion traces for the selected diagnostic ions. Integrations of reconstructed ion traces from a given spectrum will have comparable start and stop points. Ion ratios are then calculated by dividing the area of each ion trace by the area of the base peak ion trace, and expressing the result as a percentage. In instances where the reconstructed ion traces produce non-integratable data, it is acceptable to substitute ion abundances from the background subtracted spectra.

5.6 Decision Criteria

5.6.1 Full Scan Mass Spectra

a. Every significant ion present in the known spectrum should be present in the unknown spectrum, and vice-versa.

b. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 1 or Table 2. If these limits would produce an acceptable lower bound of less than 1% for a given ion ratio, the lower limit will be set to 1%. Ion ratios for specific diagnostic ions may be excluded from consideration if they meet any of the following criteria:

    1. The ion ratio in the known spectrum is less than 5% (less than 10% for CI, ESI, or APCI spectra).
    2. It can be shown that the signal in either the known or the unknown spectrum is significantly disturbed by an uncorrectable chemical interference. Such interference will normally be demonstrated by showing that a reconstructed ion trace for the ion in question is not coincident with the traces for other ions associated with the component of interest.

If there are more than four diagnostic ions in the known spectrum, then only the ratios for four diagnostic ions (three ratios) need to be evaluated. For compounds with a molecular mass less than 80 Da, or consisting of less than 8 atoms, only three diagnostic ions (2 ratios) need to be

evaluated. The selected ions will normally include the base peak and the molecular (or pseudo-molecular) ion, unless those ions meet one of the exclusion criteria given above. If fewer than three diagnostic ions are available for evaluation, the spectra may still be compared, but information derived from such a comparison is limited.

Table 1: Ion Ratio Ranges for EI Mass Spectra

If the ion ratio in the known spectrum is:	>50%	≥25% and ≤50%	<25%
Then the ion ratio in the unknown spectrum should be within:	10% absolute	20% relative	5% absolute

Table 2: Ion Ratio Ranges for CI, ESI, and APCI Mass Spectra

If the ion ratio in the known spectrum is:	>60%	≥40% and ≤60%	<40%
Then the ion ratio in the unknown spectrum should be within:	15% absolute	25% relative	10% absolute

5.6.2 SIM Mass Spectra

Four diagnostic and significant ions (if available) will normally be selected when setting up a SIM experiment (three ions for compounds with a molecular mass less than 80 Da or consisting of less than 8 atoms). The base peak will normally be one of the chosen ions, and the molecular (or pseudo-molecular) ion will be included if it has an ion ratio greater than ~5% in the known full scan spectrum. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 1 or Table 2.

5.6.3 Tandem Mass Spectrometry (MS/MS)

The limit for determination of significant ions in a tandem mass spectrum is ~10% of the most intense observed ion in the background subtracted spectrum. The high probability of ion association in tandem mass spectrometry means that nearly all ions of reasonable intensity observed in an MS/MS experiment should be considered diagnostic, with the exception of ions resulting purely from the loss of an adduct.

Due to the physical processes involved in precursor ion isolation and fragmentation events in an ion trap mass spectrometer, tandem mass spectra acquired on such an instrument will occasionally show an ion-splitting artifact for a precursor ion returned in a product ion mass spectrum. This is evidenced by the presence of two ions, separated by a fraction of *m/z*, at the

nominal *m/z* of the precursor ion in the product ion spectrum. In instances where this phenomenon is observed, the response for the affected ion will be taken as the total of the response for both components of the split ion signal.

5.6.3.1 Product Ion Experiments

When conducting product ion experiments, the selection of a precursor ion is critical to obtaining useful and reliable information. In most cases, the molecular (or pseudo-molecular) ion of the species under consideration will be selected, if available. It is also acceptable to use a diagnostic isotope of the molecular (or pseudo-molecular) ion, if one is available. If the molecular (or pseudo-molecular) ion is not available, or is not suitable for some reason, then the selected precursor ion should be both significant and diagnostic in the full scan mass spectrum of the substance under consideration. With product ion spectra, it is also important to ensure that the observed fragment spectrum is emerging from the selected precursor ion. For this reason, one of the two following criteria should normally be met for a product ion spectrum:

a. The precursor ion should be observed in the product ion spectrum with an ion ratio of at least 5%.
b. If full scan mass spectral data are collected concurrently with the product ion spectra, the full scan spectrum of the component of interest should show no ions within 1.5 *m/z* of the precursor ion with greater than three times the intensity of the precursor ion.

Following are decision criteria when comparing product ion spectra:

a. Every significant ion present in the known spectrum should be present in the unknown spectrum, and vice-versa.
b. The ion ratios for diagnostic ions in the unknown spectrum should fall within the ranges provided in Table 3. If these limits would produce an acceptable lower bound of less than 1% for a given ion ratio, the lower limit will be set at 0.5%. Ion ratios for specific diagnostic ions may be excluded from consideration if they meet any of the following criteria:

    1. The ion ratio for that ion in the known spectrum is less than 5%.
    2. It can be shown that the signal in either the known or the unknown spectrum is significantly disturbed by an uncorrectable chemical interference. Such interference will normally be demonstrated by showing that a reconstructed ion trace for the ion in question is not coincident with the traces for other ions associated with the component of interest.

If there are more than three diagnostic ions in the known spectrum, then only the ratios for three diagnostic ions (two ratios) need to be evaluated. The three selected ions will include the base

peak and the precursor ion (if present), unless those ions meet one of the exclusion criteria given above. If only a single diagnostic ion is observed in the product ion spectrum, spectra may still be compared, but information derived from such a comparison is limited.

Table 3: Ion Ratio Ranges for MS/MS Product Ion Spectra

If the ion ratio in the known spectrum is:	>40%	≤40%
Then the ion ratio in the unknown spectrum should be within:	25% relative	10% absolute

5.6.3.2 Precursor Ion and Neutral Loss Experiments

The practical information content for precursor ion and neutral loss MS/MS experiments is generally low, but circumstances may still arise in which one of these techniques can provide critical additional information about a given substance. For precursor ion experiments all significant ions present in the known spectrum should be present in the unknown spectrum, and vice-versa. For neutral loss experiments, all significant transition pairs present in the known spectrum should be present in the unknown spectrum and vice-versa.

5.6.3.3 Selected Reaction Monitoring (SRM) Experiments

Two or three diagnostic ion transitions may be chosen for an SRM experiment. Generally, transitions should share a common precursor ion, although it is appropriate to use multiple precursor ions if all are part of a diagnostic isotope cluster in the full scan spectrum of the substance in question. It is desirable that the chosen precursor ion be the molecular (or pseudo-molecular) ion of the substance in question. If this is not possible, or not practical, then the chosen precursor ion should be both significant and diagnostic in the full scan spectrum of the substance in question. When comparing a known SRM spectrum and an unknown SRM spectrum, the ion ratio of the unknown should be within ±10% (relative) of the ion ratio of the known when only two transitions are monitored. When three transitions are monitored, both resulting ion ratios should meet the tolerances specified in Table 3.

5.6.4 Accurate Mass Spectrometry

Ions in an unknown measured accurate mass spectrum should be within 0.005 *m/z* of the ions in the known measured accurate mass spectrum, or the theoretical *m/z* (i.e., exact mass). Any isotope of a molecular (or pseudo-molecular) ion may be considered diagnostic if it meets the 0.005 *m/z* criterion. One additional adduct ion, beyond the pseudo-molecular ion, may also be considered diagnostic if it meets the 0.005 *m/z* criterion. For a SIM experiment, only three ions need to be evaluated if all three ions meet the 0.005 *m/z* criterion.

5.6.5 Library Spectra Comparisons

While mass spectral libraries can be invaluable tools in helping to direct examinations and suggest possible targets for further investigation, there are limitations to their use. Most commercial libraries do not clearly indicate the instrumentation the spectra were acquired on, or at what level of sample loading. In-house library data may have been acquired on the same instrumentation used to obtain a given unknown spectrum, but it is very difficult to ensure that long-term drift in instrument performance has not affected the utility of those library spectra.

Despite these limitations, there may arise instances in which it is necessary to compare an unknown spectrum to a library entry. When such comparisons are conducted, all criteria described above will be utilized. However, ion abundances for the determination of ion ratios will be measured as the intensity of the ion in the spectrum, rather than as the integrated area of a reconstructed ion trace.

6 Calculations

$IR_x = (A_x/A_b) \times 100$, where:

IR_x = the percent ion ratio for ion x
A_x = the integrated area of the reconstructed ion trace for ion x
A_b = the integrated area of the reconstructed ion trace for the base peak ion

(Ion abundances from background subtracted mass spectra may be substituted for integrated areas under certain circumstances detailed in section 5.6.5.)

7 Measurement Uncertainty

Not applicable

8 Limitations

These guidelines are not intended to be exhaustive. Known limitations for specific analytes will be documented in the applicable SOPs.

9 Safety

Not applicable

10 References

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Rev. #	Issue Date	History
0	01/15/20	Original Issue
1	04/01/21	Section 1- added DART to second paragraph; added "and authorized". Section 5- added first sentence.

Approval

Redacted - Signatures on File

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