Evaluation of Molecular Isotope Patterns for Elemental Composition Identification on a Unit Resolution Quadrupole Mass Spectrometer

Maria Cristina A. Dancel[1], Yongdong Wang[2] and David H. Powell[1]

[1]Department of Chemistry, University of Florida, Gainesville, FL; [2]Cerno Bioscience, Danbury, CT

OVERVIEW

- Our main goal is to validate elemental composition assignments based on measured accurate masses and their isotope patterns.
- Accurate masses were determined after internal calibration with PFTBA and Calibrated Line Isotope Profile Search (CLIPS) with MassWorks™ (Cerno Bioscience). The latter also provided a theoretical isotope profile for each candidate elemental composition of a given accurate mass.
- Less than 15 ppm mass errors for a molecular ion and its adducts were achieved on the quadrupole MS. Each elemental composition assignment was verified through its isotope pattern.

INTRODUCTION

The accurate mass of a compound's molecular ion or its adduct is often used as proof of chemical composition. In reality, numerous candidates are possible even at sub-parts-per-million tolerance, and the list increases exponentially with mass. For synthetic compounds, constraints based on prior chemical knowledge are applied to limit the selection. The characteristic isotope abundances of bromine and chlorine are often used as qualitative identifier since they produce unique isotope profiles that verify the halogen content. However, the comparison is done visually with most data processing software. In this study, we used a data processing software that determines the congruence between theoretical and measured isotope patterns from a unit resolution quadrupole mass spectrometer after chemical ionization (CI).

METHODOLOGY

Twelve pairs of compounds with the sample same nominal mass, ranging from 363 to 498 Da were collected among 154 samples. Three pairs are reported to illustrate the process of mass spectral interpretation in CI mode and isotope profile evaluation after accurate mass analysis.
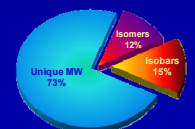
The samples were introduced through a direct introduction probe (DIP) set at 60°C for a minute and then heated up to 340°C at 30°C/min. Each sample underwent CI with methane as reagent gas and the ions formed were detected with a DSQ quadrupole mass spectrometer (ThermoFisher Scientific).

Perfluorotritbutylamine (PFTBA) was introduced into the ion source as internal standard while sample spectra were collected in profile mode. The best line shape for the mass calibrants were generated (Figure 1) using at least three of the ions listed in Table 1 via MassWorks™ (v. 1.2.8). The resulting mathematical function was applied to a sample mass spectrum and the centroid values of the calibrated spectrum were determined. The latter were used as accurate masses for finding possible elemental compositions via the CLIPS feature. The result query also presents a comparison of calibrated and theoretical isotope patterns for each candidate.

Table 1. PFTBA fragments and their monoisotope masses

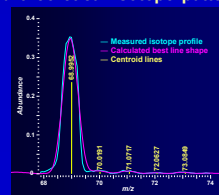| Formula	Monoisotope Mass	Formula	Monoisotope Mass
CF_3	69.9952	$C_8H_{16}N$	413.9775
C_3F_5	130.9920	$C_9H_{20}N$	501.9711
C_4H_9	218.9956	$C_{12}F_{24}N$	613.9648
$C_5H_{10}N$	263.9871	$C_{12}F_{22}N$	651.9616

Figure 1. Measured and caculated isotope profiles for m/z 69 with centroid values

RESULTS and DISCUSSION

Comparison of Isobaric Pairs:

The isobaric compounds are classified into those with and without chlorine Their spectra are shown below where each of the chlorinated compound in Fig. 2 has its counterpart in Fig. 3. The group on the left has an o-chlorobenzyl moiety with acyl (R_1) and cycloalkenyltetrahydopyridine (R_2) substituents. In contrast, the group on the right are quinolinylmethanol ether derivatives .

The CI mass spectra show that the isobaric pairs are easily differentiated through their unique ion profiles. Structures are supported by a few fragment ions while nominal masses are determined through moderate to high intensity protonated molecules and two other methane gas adducts.
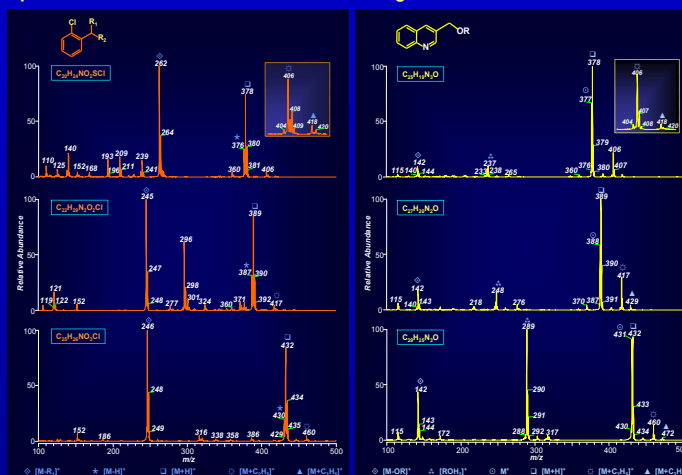

Figure 2. The CI mass spectra above show overlapping [M-H]⁺ and [M+H]⁺ ions, making it appear as if two bromine atom are present. The isotope profiles of the [M+C₂H₅]⁺ and [M+C₃H₅]⁺ ions (*see insert*) confirmed the presence of a chlorine atom.

Figure 3. The CI mass spectra on above show M⁺ and [M+H]⁺ ions which could easily be confused as a combination of [M-H]⁺ and M⁺ ions, respectively. The [M+C₂H₅]⁺ and [M+C₃H₅]⁺ adducts (*see insert*) that appeared 28 and 40 Da higher than m/z 377 established the presence of [M+H]⁺ ion.

Accurate Masses:

Theoretical and measured masses from CLIPS are shown in Table 1 together with reported ppm-errors. The isobars are easily identified through their accurate masses. The mass tolerance for the CLIPS search had to be increased to 0.5 Da in order to see the elemental compositions for an isobaric pair in the same query.

Table 2. Accurate masses of isobaric compounds

Sample's Chemical Formula	[M+H]⁺			[M+C₂H₅]⁺		
	Theoretical mass	Measured mass	Δppm	Theoretical mass	Measured mass	Δppm
$C_{20}H_{24}NO_2SCl$	378.1295	378.1277	-4.6	406.1608	406.1560	-11.7
$C_{22}H_{29}N_2O_2Cl$	389.1996	389.1974	-5.6	417.2309	417.2291	-4.3
$C_{25}H_{35}NO_3Cl$	432.2305	432.2310	1.0	460.2618	460.2592	-5.8
$C_{25}H_{19}N_3O$	378.1606	378.1644	10.0	406.1919	406.1940	5.1
$C_{27}H_{20}N_2O$	389.1654	389.1642	-3.1	417.1967	417.1948	-4.5
$C_{29}H_{25}N_3O$	432.2076	432.2062	-3.1	460.2389	460.2429	4.0

Isotope Pattern Analysis:

Evaluation of isotope patterns from CI mass spectra is simple in the absence of interferences, with accuracies above 95%. However, much lower values were found when [M-H]⁺, M⁺ and/or [M+H]⁺ ion profiles overlap as shown in Figures 2 and 3. Finding the ratio of these interfering profiles manually is very tedious and time-consuming.

An automated comparison of calibrated and theoretical mass spectra with CLIPS addresses the complexity of overlapping ions by providing an ion profile for mixtures. As shown in Fig. 4, the simulations have percent similarity values above 95% after possible elemental formula for interfering ions were also included in the CLIPS parameters.

The software was also very useful when elements with multiple isotope abundances are present and more complex isotope profiles are observed such as in organometallic compounds (data not shown).

Figure 4. The orange and yellow ion profiles above are from the calibrated spectra of C₂₀H₂₄NO₂SCl (Fig. 2) and of C₂₅H₁₉N₃O (Fig. 3). The orange profile at the top-left corner, assigned as [M-H]⁺ & [M+H]⁺ in Fig. 2, overlapped with M⁺ based on the 95.1% similarity of the green profile below it. Meanwhile, the yellow profile at the top-right corner matches the lavender profile for the M⁺ & [M+H]⁺ ions. In the same manner, the [M+C₂H₅]⁺ isotope patterns (bottom) are congruent with the blue profiles.

CONCLUSIONS & RECOMMENDATIONS

- Data acquisition in profile mode on a unit resolution mass spectrometer and processing by means of MassWorks™ provide "best line shape" function for mass standards such as PFTBA.
- The "best line shape" function can be used for internal mass spectral calibration, in order to determine accurate masses and elemental compositions via CLIPS.
- The CLIPS feature also provide an automated comparison of pure and overlapping isotope patterns against theoretical profiles.
- ☑ In the absence of fragment ions, two accurate masses should be reported, either from the molecular ion and its adduct or from two adducts.
- ☑ A theoretical mass spectrum with known percent similarity must be provided when reporting accurate masses for compounds containing elements with complex isotope patterns, such as chlorine, to support elemental composition assignment.
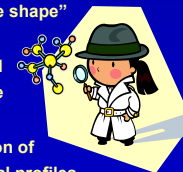
Selected References:

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