Isotope Pattern Evaluation for the Reduction of Elemental Compositions Assigned to High-Resolution Mass Spectral Data from Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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The number of possible chemical formulae assigned to an accurate determined mass was significantly reduced by comparing spectral and theoretical isotope patterns based on mass measurement obtained with an ultrahigh-resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (ESI-FTICR-MS) at high field intensity (7 T). Reduction is performed by rating congruency between experimental and theoretical pattern intensity and mass, and filtering out compositions with insufficient user-definable results. The methods used for isotope pattern simulation, peak searching, and comparison will be briefly described and evaluated on molecule ion signals of 25 compounds (300–1000 Da) applying a mass accuracy of ± 5 ppm, a set of eight elements with constant constraints ($C_{0-200}H_{0-1000}N_{0-15}O_{0-15}S_{0-2}Cl_{0-2}Br_{0-2}Ru_{0-1}$), natural isotope abundances and experimental resolution (full width at half maximum). (J Am Soc Mass Spectrom 2006, 17, 1692–1699) © 2006 American Society for Mass Spectrometry

Determination of elemental composition (EC) from high-accuracy mass measurement is a widely accepted method for gaining information on organic molecules determined in mass spectrometry [1, 2]. In theory, every distinct elemental composition has a unique mass that permits unique identification of a compound just from this exact mass [3]. In reality, accuracy in mass detection is limited, and more than one composition is usually found for molecules above 300 Da—even with the high precision afforded by FTICR-MS over a wide mass range [4, 5]. In fact, the number of elemental compositions increases exponentially with mass, and many attempts have therefore been made to reduce the number of elemental compositions. Most of these are based on further chemical knowledge available for samples derived from other experiments and sample origins, and more constraint building blocks are used for combinatorial assignment instead of single elements; for example, amino acids or nucleotides are combined for large biomolecules [6, 7]. Even very complex mixtures of

compounds from refinery streams and humic acids have been successfully characterized by accurate mass measurement, taking into account the chemical relationship between constituents of mixtures and classification into a homologous series by Kendrick mass analysis [8–10]. The amount of a single element such as carbon can be determined by mass comparison of a ^{13}C -enriched species against a species of the same compound that is naturally abundant, thus leading to a significant reduction in possible sum formulae [11].

High-resolution mass spectra contain a second informational dimension beyond the exact mass, peak intensity, or relative ion abundance. The spectral isotope distribution resulting from natural isotope abundance and elemental composition can be used to rate and exclude empirical compositions. Several methods have been developed to calculate isotope distributions of arbitrary composition with differences in efficiency and accuracy in mass and intensity [12–14]. Spectral properties such as resolution and system peak shape must be taken into account to obtain an accurate simulation. The intensity correlation between experimental spectra for the simulated isotope pattern of an elemental composition expressing the congruence between both patterns is determined and used to exclude elemental compositions with poor spectral representation. Use of this

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method for determining the content of bromine and chlorine in unknown substances and validating expected compounds from spectral data generated on a quadrupole instrument has been reported earlier. Recently, Kind and Fiehn described the identification of metabolites using this approach [16].

In this paper, we will demonstrate the application of the isotope pattern simulation and comparison method to high-resolution FTICR-MS mass spectral data. A known problem is that precision in intensity determination in organic mass spectrometry is no better than a few percent, and that additional difficulties occur in the determination of relative abundances of closely spaced cyclotron frequencies in FTICR-MS [17]. For this reason, the number of simulated isotopic peaks that have a spectral representation within the mass accuracy applied is used along with the intensity correlation to increase the filter effect. For nonoverlapped distributions, one would expect that the whole isotopic distribution would be determined with high mass accuracy, and the simulation should therefore not only match the isotope pattern in intensity, but also in exact mass expressed by the matched peak criteria.

Experimental Materials and Methods

Samples

A group of 25 synthesis products of known elemental composition within a mass range of 300 to 1000 Da was used for demonstration purposes. The synthesis products originated from catalysis and pharmaceutical research. The solvent was either a mixture of HPLC-grade isopropanol/water (50:50), acetonitrile/water (50:50) with 1% formic acid, or methanol/water (50:50) with 1% formic acid. Sample concentrations ranged from 1 to 2 mg/ml.

FTICR-MS+ Data Analysis

Mass spectra were obtained using a commercial 7 T FTICR mass spectrometer (Apex III, Bruker Daltonik, Bremen, Germany) equipped with an external micro-electrospray source. A 5 μ l sample was directly injected with a CTC-PAL autosampler (CTC, Zwingen, Switzerland) coupled with a syringe pump (Cole-Parmer Vernon Hills, IL) at a flow rate of 2 μ l/min and sprayed at 20 lb/in using a nitrogen nebulizer gas stream. In positive ion mode, the voltage between grounded sprayer needle and metal capped glass capillary entrance was -4.5 kV and 80 V to capillary exit. Transfer capillary was heated by nitrogen drying gas to 150 °C. Prior detection ions were external accumulated within a hexapole ion guide for 0.3 to 0.6 s, transferred to the analyzer cell and excited by frequency sweep pulse [4]. The system's mass range was set to 97,729–2000 Da, resulting in a sampling rate of 2×1.11111 MHz. In broadband mode, 1024 k data points were acquired, thus leading to a detection time of 0.472 s for a single

Figure 1. Flow chart of processes and input data applied to isotope pattern evaluation.

scan. Thirty-two single scans were summarized per spectrum. Analyzer cell pressure during detection was about 1.5×10^{-10} mBar. After acquisition, mass spectral raw data were post-processed using XMASS acquisition and processing software (Bruker, Bremen, Germany). Data were apodized by multiplication with a half-period sine function zero-filled to 2048 k data points, applied to Fourier transform and magnitude calculation. External calibration was performed before acquisition using single-charge caesium iodide clusters $(\text{Cs}_n\text{I}_{(n-1)})^+$ between 132.9 ($n = 1$) and 1172.1 Da ($n = 5$) as reference masses, and a second-order polynomial as the calibration function between frequency and mass. Peak lists generated with XMASS as well as spectral resolutions according to FWHM criteria were taken as input data for calculations.

Theory

The information needed for the evaluation of high resolved mass spectral data consists of the exact masses and intensities for each peak (peak list), the set of elements to be considered including exact isotopic masses, abundances and valences, resolution for each peak, and absolute or relative mass accuracy (σ m or σ m/m) [3]. Figure 1 summarizes the information flow

and processes applied during the evaluation of a single peak from a spectrum. After generating elemental combinations for an accurate mass within a tolerance interval of $\pm \sigma m$ as described elsewhere [7, 18, 19], two filters are used to prescreen the list of empirical formulae and reduce the solution set before calculating the isotope pattern for each remaining EC, and comparing the result with the experimental spectrum. The resulting correlation values are applied to a third filter that removes ECs with poor spectral representation.

The first filter calculates the number of rings and double bonds (double-bond equivalent, DBE) for an elemental composition referring to eq 1, where A_1 – A_6 are the amounts of atoms with valences of 1–6 [20].

$$DBE = -\frac{A_1}{2} + \frac{A_3}{2} + A_4 + \frac{3}{2}A_5 + 2A_6 + 1 \quad (1)$$

A negative DBE expresses an oversized number of monovalent atoms in the EC that do not carry any chemical meaningful structural representation and therefore can be excluded from the list, with the exception of ECs with a DBE of -0.5 , which can occur due to addition of one hydrogen or other monovalent atom to a saturated compound ($DBE = 0$) as happens during ES ionization, for example. Optionally, the filter function can be expanded by restricting the minimal and maximal DBE to user-defined values.

The second filter takes into account the anticipated ion state, which means the number of valence electrons in the composition. Neutral, nonradical compounds always have an even-numbered pair-wise arrangement of binding electrons signaled by an integer DBE value. In soft ionization techniques (ESI, APCI, FAB, MALDI), charge is often fed by adding or removing a monovalent ion such as H^+ , Na^+ , or Cl^- ; the resulting EC of the molecule ion ($M + 1A_1$) $^+$ will have an odd number of binding electrons and a fractional DBE (X.5); thus, ECs with integer DBE values or an even number of binding electrons can be removed from the solutions set for a supposed molecule peak if the corresponding ionization methods are applied.

The evaluation of the spectral isotopic distribution (Figure 1: dotted box) can be divided into two main parts: simulation of the theoretical spectrum of a sum formula and comparison between simulated and spectral patterns resulting in a correlation rating. The simulated isotopic distribution for an elemental composition $A_a B_b \dots$ containing the elements A, B, ... is determined by expanding the following expression

$$(A_1 + A_2 + A_3 + \dots)^a (B_1 + B_2 + B_3 + \dots)^b \dots \quad (2)$$

where A_1 is the first isotope of element A, B_1 is the first isotope of element B, and $a, b, \dots$ is the content of element A, B, ... in the respective sum formula [12]. Each resulting unique permutation by the "polynomial" method represents one isotope configuration with the abundance

$$P = \left(\frac{a!}{a_1! a_2! a_3! \dots} P_{A_1}^{a_1} P_{A_2}^{a_2} P_{A_3}^{a_3} \dots \right) \left(\frac{b!}{b_1! b_2! b_3! \dots} P_{B_1}^{b_1} P_{B_2}^{b_2} P_{B_3}^{b_3} \dots \right) \dots \quad (3)$$

where a_1 is the content, and P_{A_1} is the abundance of the first isotope of element A in the isotope configuration, and the mass

$$m = a_1 m_{A_1} + a_2 m_{A_2} + a_3 m_{A_3} + \dots + b_1 m_{B_1} + \dots \quad (4)$$

with m_{A_1} as the mass of the first isotope of element A. For efficiency reasons, only unique permutations for each element (referring to one term in a bracket in eq 3) are calculated separately. Pruning is also applied during this process, removing permutations with abundances smaller than a pruning threshold from further calculations, thus reducing calculation time and memory demands but introducing a slight distortion. Isotope configurations are formed afterwards by combining remaining unique permutations of the elements with each other. The result of the polynomial method is a list of isotopic peaks (P_i, m_i) with infinite resolution. Many configurations have nearly the same exact masses and would overlap in an experimental spectrum due to the finite resolution of the mass spectrometer. To produce a representative simulation, isotopic peaks referring to a sum of unit impulse sequences ($\Delta(0) = 1$) $IP(m) = \sum_i P_i \Delta(m - m_i) = P_1 \Delta(m - m_1) + P_2 \Delta(m - m_2) + \dots$ are convoluted with a peak shape function that should properly match the system's peak shape. The operator $*$ refers to the mathematical convolution defined by $f_1(t) * f_2(t) = \int_{-\infty}^{\infty} f_1(\tau) f_2(t - \tau) d\tau$.

In FTMS, the peak shape approximates to Gaussian after apodization and, has therefore been selected (normalized to 1) for these calculations

$$PS(m) = \exp\left(-\frac{m^2}{\sigma^2}\right) \quad (5)$$

The unit impulse sequence behaves as a neutral element within the convolution integral, thus simplifying to a sum of peak shape functions with maximal intensity π at mass m_i

$$\begin{aligned} I_P(m) &= PS(m) * IP(m) = \sum_i PS(m - m_i) P_i \\ &= P_1 \exp\left(-\frac{(m - m_1)^2}{\sigma^2}\right) \\ &\quad + P_2 \exp\left(-\frac{(m - m_2)^2}{\sigma^2}\right) + \dots \end{aligned} \quad (6)$$

Parameter σ , which complies with the standard deviation of the Gaussian function, is calculated with regard to the spectral resolution $R = \Delta m_{50\%}/m$ (FWHM) at peak mass m . To equalize the spectral and simulated resolution, the peak width of the shape function at half maximum (0.5) must also be $\Delta m_{50\%}$. Taking this pair of variates ($\Delta m_{50\%}/2, 0.5$) and inserting them into eq 5

yields $\sigma = \Delta m_{50\%} (-4 \ln 0.5)^{-0.5}$. The spectral resolution is assumed constant over the relative small mass range that an isotopic distribution occupies, and σ is therefore a constant. For computational calculations, the desired mass range must be mapped to a number of data points, μ . Let us assume that we have w data points within a mass area of $\Delta m_{50\%}$ with the data point density towards $w/\Delta m_{50\%}$. The mass range ($m_{\min}$ – $m_{\max}$) in the calculation is determined by the lightest and heaviest mass of simulated isotope configurations rounded down to the nearest integer and decremented ($m_{\min}$) or incremented ($m_{\max}$) by one. Because the heaviest and lightest isotope configurations of an EC depend on the pruning threshold set and the elemental composition itself, mass range varies and is recalculated for each simulation. Wider mass ranges are possible, but not necessary. The number of data points (NP) results from the product of data point density, and mass range and is rounded up to the nearest integer. Data point $\mu = 0$ is mapped to $m_{\min}$ and data point $\mu = NP - 1$ to $m_{\max}$, respectively. The mass on data point μ is therefore

$$m(\mu) = m_{\min} + \mu(m_{\max} - m_{\min})/(NP - 1) \quad (7)$$

The higher parameter w is set, the more precise the peak shape will be approximated and the smaller the spectral distortion introduced by discretization will be. On the other hand, the number of data points increases linearly with w , as do calculation time and memory use. For the calculations presented here, $w = 7$ data points have been chosen since no significant changes in the results were observed using values above 7. The resulting isotopic distribution over NP data points can be calculated using eqs 6 and 7. Due to the rapid drop in the Gaussian peak shape function, not every isotopic shape over the whole mass range ($m_{\min} \leq m_i \leq m_{\max}$) needs to be computed. Within the interval of $\pm 10 \sigma$ around a center mass m_i , the intensity falls to the $\exp(-100) = 3.72 \times 10^{-44}$ -th part of peak intensity P_i ; the calculation interval for a isotopic peak around m_i can therefore be restricted to $(m_i - 10 \sigma) \leq m_i \leq (m_i + 10 \sigma)$ without causing a significant error.

Peak picking must now be performed to obtain the resulting isotopic peak mass and intensity pairs out of the calculated array of NP data points; the local intensity maxima [$I_P(\mu_{l\max-1}) < I_P(\mu_{l\max}) > I_P(\mu_{l\max+1})$] are determined, and the resulting maxima are interpolated by a second-order polynomial using the three points [μ , $I_P(\mu)$] around a local maximum as grid points of the polynomial [21]. The respective data point is calculated setting the first deviation of the polynomial to zero; the maximum in intensity is found by solving the polynomial with this maximal data point. Other methods for finding the real top point from a peak shape are possible (weight average, simple maximum), and should be selected with regard to the method used with experimental spectra.

After simulation, the resulting isotopic peaks of an EC are searched within spectral data and the intensities are compared to each other. In this paper, only the most intensively simulated isotopic peaks per nominal mass have been considered and used for intensity correlation; possibly resolved isotopic fine structure therefore remains unevaluated. A mass window resulting from instrument mass accuracy around each remaining simulated peak is searched for spectral peaks. If no spectral peak, i , is located within this search window, the corresponding spectral intensity $I_s(i)$ is set to zero. In cases where more than one spectral peak is found inside the search window, the most intensive spectral peak is taken for correlation, as it should correspond to the most intensive isotopic peak that we are searching for, provided that both resolutions are equal (spectral and simulated) and no overlapping patterns occur.

To determine the intensity correlation, the intensities of the isotope pattern $I_p(i)$ are normalized to spectral intensities $I_s(i)$ by the least-square fit method, solving the first deviation of $\sigma^2 = \sum_i (I_s(i) - \alpha I_p(i))^2$ for $\sigma'(\alpha) = 0$, yielding the normalization factor

$$\alpha = \frac{\sum_i I_s(i) I_p(i)}{\sum_i I_p(i)^2} \quad (8)$$

and intensity correlation I_{cor}

$$I_{cor}(\%) = \left(1 - \left(\frac{\sum_i (\alpha^{-1} I_s(i) I_p(i))^2}{\sum_i I_p(i)^2} \right)^{0.5} \right) \cdot 100\% \quad (9)$$

as presented by Siegel et al. [15].

Each simulated isotopic peak that has a spectral representation ($I_s(i) > 0$) is marked as a matched peak. The isotope filter will remove all ECs with intensity correlation and number of matched peaks lower than user-defined values, thus reducing the remaining solution set.

Results and Discussion

The algorithms described have been implemented in the MassSpectral software package and used for the evaluation of the ESI-FTICR mass spectral data gained for 25 compounds of known elemental composition. Signals resulting from single charged molecule ions ($(M + H)^+$, $(M + Na)^+$, M^+) were selected from peak lists. Element restrictions are chosen taking all compounds into account, which included hydrogen and carbon, both unlimited, oxygen and nitrogen ranging from 0 to 15 each, sulphur, bromine, and chlorine limited to a maximum of two and a maximum of one ruthenium, as there were five ruthenium complexes included. One sodium was required where the sodium abduct ion was evaluated. Elemental compositions were assigned for the mono isotopic peaks at a mass accuracy of ± 5 ppm, and only lightest isotopes of each

element were combined. Double-bond equivalence was limited from -0.5 to 250 and the expected ion state according to molecule ion was used for filtering. The pruning threshold for isotope pattern simulation was set to 10^{-5} . Minimal intensity correlation expected for an EC was 80% ; at least three simulated peaks had to be found in spectra for correct identification (matched peak criteria) in nonruthenium compounds. Due to the widespread isotope pattern of ruthenium, at least ten matches were expected for the complexes. A PC equipped with a 1.2 GHz processor and 512 MB RAM was used for calculation. Calculation time increased with the number of elemental compositions directed to isotope pattern evaluation, and ranged from some ms to 16 s for the heaviest compound with 612 ECs. The time taken forming and prefiltering the ECs was negligible, as it was always less than 80 ms. Table 1 summarizes the calculation results for all compounds.

None of the proper sum formulae showed an intensity correlation beyond 80% and were thus eliminated from the solution set, and only three showed a correlation beyond 85% . The reduction effect is shown in Figure 2. Requiring a minimal correlation of 80% and at least three matched peaks (ten for the last five compounds), the number of possible sum formulae could be reduced by 90% on average.

Figure 3 shows the measured and the simulated isotopic distribution of $(C_{27}H_{23}N_4O_3S_1Br_1 + Na)^+$ (Compound 15) at a resolution of $52,000$. Intensity correlation was determined at 94.8% , as there was no 100% optical congruity, and the spectral peak from nominal mass 589 Da did not lie within the mass window of 5 ppm around the simulated peak. Furthermore, simulated peaks from nominal mass 590 and 591 Da were not contained in spectral data, as they lay beyond the noise level and spectral intensities were consequently set to zero. From 121 elemental compositions assigned to measured mass $585.05, 683$ Da, only 16 showed an intensity correlation above 85% with at least three simulated peaks in the spectral data (Table 2).

For all of the 20 noncomplex compounds, the content of halogens in the remaining ECs was assigned exactly with the exception of Compound 14 containing two bromine atoms (Table 1), as ECs containing either Cl_2Br_1 or Br_2 remained. The influence of Cl_2Br_1 and Br_2 on the isotopic distribution was similar both in intensity and mass shift of isotopic peaks, where the resolution was not adequate to resolve isobar isotopic peaks resulting in similar isotope patterns; for example, the proper composition of Compound 14 ($C_{22}H_{26}N_2O_5Br_2 + Na$) showed an intensity correlation of 92.3% with seven matched peaks, while an adequate correlation of 86.6% and six matched peaks was found for another composition from a solution set containing Cl_2Br_1 ($C_{17}H_{23}N_6O_6Cl_2Br_1 + Na$). For the five halogen-free complexes, chlorine containing sum formulae remained, but these ECs had fewer matching peaks [10] within the spectral data than the actual compositions (Table 1).

Assuming sufficient intensity, the presence or absence of ruthenium was accurately detected since only elemental compositions including ruthenium had 10 or more isotopic peaks as required in the case of ruthenium containing compounds. Where ruthenium was not contained within a compound, compositions with ruthenium showed an intensity correlation beyond 80% and were also filtered out. As shown for Sample 15 in Table 2, the number of carbons could not be reliably estimated from ESI-FTICR spectral data since the abundance of carbon isotope C^{13} was not high enough to indicate any change in intensity correlation beyond minimal correlation value with unsubstantial changing carbon numbers in the ECs [9]. Nevertheless, estimation was slightly better in nonhalogen and noncomplex substances as the carbon spread only ranged from -35.4 to 4.5% around the actual value, compared with -68 to 30% and -55.8 to $+22\%$ for the complexes in halogenated compounds. Generally speaking, the higher the influence of an element (depending on amount and isotope abundances) to the resulting isotopic contribution is, the more accurately it will be detected, since it has more influence on intensity correlation. The isotope species of nitrogen and oxygen as well as of hydrogen only slightly contribute to the isotope pattern due to their low isotope abundances and/or low content and, thus, could not be estimated. The content of sulphur was only implicitly evaluated where the abundance of isotope configuration $^{12}C_n^{32}S_{c-1}^{34}S_1$ (4.2% for $c = 1$) was higher than contributions from other elements, such as the abundance of configuration $^{12}C_{n-2}^{13}C_2^{32}S_1$ (Compounds 1, 2, and 3, $n < 20$). In these cases, the presence of the $R^{34}S$ isotopic peak, the third most abundant simulated nominal peak, was determined in spectral data and, if present, all compositions without sulphur were excluded (matched peak criteria). The peaks from the isotopic fine structure doublet in Figure 4 were nearly equal in intensity, and although two successive experimental spectra did show slight differences in intensity, peaks of the doublet could not be interchanged since the mass difference between $R^{12}C_{n-2}^{13}C_2^{32}S$ and $R^{12}C_n^{34}S$ isotope configuration is about 11.7 mDa, sufficient for resolved isotopic peaks not to be interchanged at up to 1000 Da given a ± 5 ppm mass accuracy as they were not within the same search window. Commonly, the applied search and comparison algorithm excluded resolved but low-abundance isotopic peaks contributed by sulphur, and no sulphur content was detected (Table 2). Where the resolution was sufficient for separating $^{12}C_{n-2}^{13}C_2^{32}S$ and $^{12}C_n^{34}S$ configurations ($R > m/11.7$ mDa), the evaluation of isotopic fine structures was expected to increase the filter effect based on the matched peak criteria, as referring to Figure 4 for ECs with sulphur, there would be four spectral peaks detected instead of three.

If peaks contributed by sulphur were overlapped by, for example, carbon ^{13}C or bromine ^{81}Br , only the high

Table 1. Results for compounds investigated: spectral resolution measured FWHM^a, monoisotope mass of actual composition^b, number of elemental compositions without isotope pattern evaluation^c, intensity correlation^d, and number of matched peaks for actual composition^e, remaining compositions with correlation above 85 or 80% and relative reduction of amount of EC's related to $I_{\text{cor}} > 80\%$ ^f. Note only in three cases intensity correlation of actual composition was lower than 85%^g

No.	Sum formula								Resolution ^a	Mol. ion	Δm (mu)	$\Delta m/m$ (ppm)	Mex.(u)	Mthe.(u) ^b	#EC ^c	I_{cor} ^d	#Peaks ^e	# $I_{\text{cor.}} > 85\%$ ^f	# $I_{\text{cor.}} > 80\%$ ^f	Reduct. (%) ^f	Calc. time (ms)
	C	H	N	O	S	Cl	Br	Ru													
1	18	18	0	3	0	0	0	0	100,000	(M + Na) ⁺	0,452	1.5	305.115268	305.114816	5	90.82	3	1	1	80.00	60
2	15	15	1	5	1	0	0	0	90,000	(M + Na) ⁺	0,13	0.4	344.056448	344.056316	18	88.5	4	1	2	88.89	130
3	21	22	2	3	1	0	0	0	80,000	(M + H) ⁺	1,7	4.4	383.144066	383.142390	22	94.74	4	1	1	95.45	150
4	20	20	2	2	1	1	0	0	80,000	(M + H) ⁺	1,3	3.4	387.094162	387.092853	32	87.6	4	4	5	84.38	170
5	24	23	2	2	1	0	0	0	80,000	(M + H) ⁺	1,22	3	403.148692	403.147475	25	91.8	4	1	1	96.00	150
6	28	28	2	2	1	0	0	0	70,000	(M + H) ⁺	0,92	2	457.195349	457.194425	29	96.4	5	2	2	93.10	180
7	28	52	2	4	0	0	0	0	65,000	(M + H) ⁺	−0,8	−1.7	481.399182	481.399984	5	96.6	4	2	3	40.00	70
8	28	26	4	3	1	0	0	0	60,000	(M + Na) ⁺	0,66	1.2	521.162429	521.161783	49	85.7	3	5	6	87.76	360
9	27	23	5	5	1	0	0	0	52,000	(M + H) ⁺	1,2	2.2	530.150456	530.149267	73	83.71 ^g	3	5	7	90.41	670
10	34	30	2	2	1	0	0	0	60,000	(M + H) ⁺	0,07	0.1	531.210153	531.210075	57	87	3	4	5	91.23	420
11	28	26	4	4	1	0	0	0	60,000	(M + Na) ⁺	−0,06	−0.1	537.156633	537.156698	65	84.2 ^g	3	5	7	89.23	430
12	27	23	4	3	1	1	0	0	59,000	(M + Na) ⁺	0,08	0.1	541.107242	541.107161	83	88	4	7	10	87.95	730
13	36	32	2	2	1	0	0	0	57,000	(M + H) ⁺	0,13	0.2	557.225855	557.225725	63	92.2	3	2	4	93.65	540
14	22	26	2	5	0	0	2	0	55,000	(M + Na) ⁺	2,6	4.5	579.012658	579.010070	140	92.3	7	19	24	82.86	1,500
15	27	23	4	3	1	0	1	0	52,000	(M + Na) ⁺	0,2	0.3	585.056825	585.056646	121	94.8	4	16	21	82.64	1,330
16	39	33	5	2	0	0	0	0	52,000	(M + H) ⁺	−0,34	−0.6	604.270359	604.268015	69	88.2	4	5	6	91.30	650
17	40	36	2	2	1	0	0	0	47,000	(M + Na) ⁺	1,4	2.2	631.240331	631.238970	84	88	3	6	8	90.48	830
18	48	49	5	3	0	0	0	0	43,000	(M + H) ⁺	−1,1	−1.5	744.389721	744.390816	104	80 ^g	4	4	7	93.27	1,350
19	44	50	2	9	0	0	0	0	37,000	(M + H) ⁺	−2,7	−3.6	751.359571	751.362280	127	90.4	3	11	16	87.40	1,800
20	51	41	5	2	0	0	0	0	42,000	(M + Na) ⁺	0,9	1.1	774.342329	774.341461	129	92.9	3	9	13	89.92	1,950
21	46	36	6	6	0	0	0	1	37,700	M ⁺	1,5	1.7	864.178194	864.176683	501	86.2	12	13	23	95.41	9,850
22	50	44	6	6	0	0	0	1	35,000	(M + H) ⁺	1,3	1.4	921.248397	921.247108	523	90	10	16	18	96.56	11,300
23	52	48	6	6	0	0	0	1	32,000	M ⁺	1,2	1.3	948.271810	948.270583	550	93.2	12	31	37	93.27	13,000
24	54	52	6	6	0	0	0	1	32,000	M ⁺	1,2	1.3	976.303120	976.301883	564	88.1	12	20	39	93.09	14,000
25	55	52	6	7	0	0	0	1	32,000	M ⁺	2,9	2.9	1004.299691	1004.296798	612	90.9	12	23	36	94.12	16,000

Figure 2. Number of elemental compositions without and with isotope pattern filtering.

content would have a significant influence on the isotopic distribution and, as in the cases of oxygen, nitrogen, and carbon, sulphur cannot be reliably detected, but it has been shown that isotopic fine structure contributed by carbon and sulphur can be resolved for proteins up to 16 kDa with FTICR-MS [22].

Figure 3. ESI-FTICR-MS (left) and simulated (right) spectrum of Compound 15 ($C_{27}H_{23}N_4O_3S_1Br_1 + Na_1$)⁺.

Table 2. Elemental compositions assigned to Compound 15 at mass 585.05683 Da with mass accuracy of 5 ppm, minimal intensity correlation of 85% and at least three isotope peaks recognized in spectral data

No.	Sum formula								$m_{the.}$ (u)	Δm (u)	$\Delta m/m$ (ppm)	DBE	I_{cor} (%)	#Peaks
	H	C	N	O	Na	S	Cl	Br						
1	19	35	2	1	1	0	0	1	585.057297	−0.000472	−0.8	26.5	91.01	5
2	15	20	14	2	1	0	0	1	585.0578	−0.000975	−1.7	19.5	95.17	5
3	19	19	10	6	1	0	0	1	585.056464	0.000361	0.6	14.5	93.86	5
4	23	23	4	8	1	0	0	1	585.05915	−0.002325	−4	13.5	97.29	5
5	23	18	6	10	1	0	0	1	585.055128	0.001697	2.9	9.5	92.46	5
6	27	22	0	12	1	0	0	1	585.057814	−0.000989	−1.7	8.5	95.98	5
7	19	23	10	1	1	1	0	1	585.05396	0.002865	4.9	18.5	95.19	4
8	23	27	4	3	1	1	0	1	585.056646	0.000179	0.3	17.5	94.82	4
9	27	26	0	7	1	1	0	1	585.05531	0.001515	2.6	12.5	97.37	5
10	23	11	12	8	1	1	0	1	585.055813	0.001012	1.7	5.5	85.87	4
11	27	15	6	10	1	1	0	1	585.058499	−0.001674	−2.9	4.5	88.98	4
12	31	14	2	14	1	1	0	1	585.057163	−0.000338	−0.6	−0.5	87.52	4
13	23	20	10	1	1	2	0	1	585.057331	−0.000506	−0.9	13.5	91.05	4
14	27	19	6	5	1	2	0	1	585.055995	0.00083	1.4	8.5	90.37	4
15	31	23	0	7	1	2	0	1	585.058681	−0.001856	−3.2	7.5	91.85	4
16	31	18	2	9	1	2	0	1	585.054659	0.002166	3.7	3.5	89.46	4

Figure 4. Resolved isotopic fine structure of Compound 11 ($C_{28}H_{26}N_4O_4S_1 + Na_1$)⁺ at resolution 60,000.

Conclusions

Isotope pattern evaluation led to an average relative reduction of elemental composition assigned to 25 compounds ranging from 300 to 1000 Da of 90.6% and was thus found to be a valuable tool in the evaluation of high-resolution mass spectral data, although it was not possible to identify the content of each involved element accurately or to gain a unique composition since isotopic species of the elements involved such as hydrogen, oxygen, and nitrogen only have low natural abundances and therefore only a slight influence on the resulting pattern (or no influence if only one isotopic species exists). For example, the number of possible elemental compositions assigned to an ion at mass 751.35,957 Da (Sample 19) and an experimental mass accuracy of ± 5 ppm could be reduced from 127 to 11. One requirement is sufficient intensity for obtaining a peak/information-rich distribution. A fixed set of eight elements ($C_{0-200}H_{0-1000}N_{0-15}O_{0-15}S_{0-2}Cl_{0-2}Br_{0-2}Ru_{0-1}$) was

used for calculation (apart from sodium), as this could be used for automated interpretation of unknown compounds without user interaction. Filtering was based on intensity correlation and the number of simulated peaks found in spectrum within the same error interval $\pm \sigma_m$ used for combinatorial determination of empirical formulae. Further improvements are expected by evaluating the isotopic fine structure.

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