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Detection of symmetrical decomposition of molecules—isotopomeric analysis of the M/2 clusters

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Abstract The interpretation of mass spectra (ms) of molecules containing poly-isotopic elements (e.g. Ge, Se, W, Os, Sn, Te, Zn, Yb) can be difficult due to the occurrence of fragments resulting from isotopomeric composition. MS-clusters located in the range lower than or equal to M/2 are very difficult to interpret. In this area many perturbations may be observed. The coincidence of different fragmentation pathways, the existence of multiply charged ions, background levels, etc. can all contribute to this problem. The present paper reports the application of multi-isotopomeric analysis methods for low-resolution ms. We present a solution that may be useful for detection of the symmetrical decomposition of a molecule and for elucidation of cluster ion genesis. The complex character of the cluster does not perturb determination of the contents of the investigated pattern. In such cases the dominated component is applied in subsequent computations.

Keywords Low-resolution mass spectrometry ·
Computer assisted interpretation · Cluster modelling ·
Symmetrical decomposition · Doubly charged ions

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Introduction

The relative peak intensities of an ion cluster can be calculated from the ionic or molecular formula and the isotopic abundance ratios of the constituent elements [1] by successive expansion of polynomials [2]. Each polynomial represents one element, and the maximal exponent order of each polynomial is equal to the number of atoms of that element in the formula. The isotopomeric character of a molecular ion cluster can be used to explain the elemental formula of the compound being investigated with the help of multi-isotopomeric analysis [3–5]. The interpretation of mass spectra (ms) of molecules containing heteroatoms (e.g. Dy, Gd, Hg, Mo, Nd, Ru, Sm, Sn, Te, Xe, Yb) can be difficult due to the occurrence of fragments resulting from isotopomeric composition.

The occurrence of poly-isotopic elements in a molecule or ion can result in a complex isotopomeric cluster of that ion [6]. This fact creates new interesting possibilities in spectral interpretation [7]. Separately, the ms-clusters located at m/z values of M/2 (M denotes the mono-isotopic molecular mass) or in the range lower than M/2 are very difficult to interpret. In this area (0–M/2) many perturbations may be observed, many more than in the M/2–M area. Sources of the problem can include: the coincidence of different fragmentation pathways, the existence of multiply charged ions, background levels, etc.

The identification of an ion generated by symmetrical fragmentation is very difficult due to overlapping of the ion with the doubly charged ion. The complex character of the cluster investigated can disturb component identification. This problem can be solved by modelling the isotopomeric pattern and comparing it with the appropriate experimental cluster. The most interesting area of the spectrum is located around m/z ≈ M/2; in this region, doubly charged molecular

Table 1 Theoretical identification of the experimental patterns of ions D^+ and d^+

Relationship	Boolean datatype	Suggestions
$D \cong T$	True	The experimental ion cluster D and the theoretical ion pattern T are the same—the experimental ion D^+ is *known* (the experimental ion cluster D is pure)
	False	The experimental ion cluster D and the theoretical ion pattern T are different—the experimental ion D^+ is *unknown* (the experimental ion cluster D is complex)
$d \cong t$	True	The experimental ion cluster d and the theoretical ion pattern t are the same—the experimental ion d^+ is *known* (the experimental ion cluster d is pure)
	False	The experimental ion cluster d and the theoretical ion pattern t are different—the experimental ion d^+ is *unknown* (the experimental ion cluster d is perturbed)

ions and the products of symmetrical fragmentation of the single-charged molecular ion can be observed. Such modelling allows the origin of the interfering patterns to be explained.

Modelling of isotopomeric pattern of doubly charged ions

Interpretation of ms-clusters located at m/z values lower than M/2 is much more difficult than interpretation of other clusters. In this context, many disturbances can be observed, e.g.:

- coincidence of different fragmentation pathways,
- existence of multiply charged ions,
- background level, etc.

The work presented here can provide a solution for multiply (more precisely, doubly) charged ions. The general idea is based on the relationship between two ms-clusters of the experimental mass spectrum:

- cluster D (experimental, 'high-mass' pattern[1]), in which the most abundant peak is located in m_1/z_1, and
- cluster d (experimental, 'low-mass' pattern), in which the main peak is located in m_2/z_2.

For pure conjugated ions (P^+ and P^{2+}) the locations must be $m_1/z_1 = 2 m_2/z_2$. Otherwise, if $m_1 = m_2$ and the charges $z_2 = 2 z_1$, it should be unequivocal that d^+ is not the single charged ion, but is a consequence of the doubly charged ion D^{2+}. These conditions indicate formation of the doubly charged ion during sample ionization. The alternative situation can be illustrated by: $z_1 = z_2$ and $m_1 = 2 m_2$, and suggests the destruction $D = d_1 + d_2$ —symmetrical

$d_1 = d_2$ (symmetrical 'by formula') or unsymmetrical $d_1 \neq d_2$ (symmetrical 'by mass').

Computational methods

The solution suggested is based on the explanation of the relationship between the selected experimental patterns and the calculated patterns. The calculations presented concern four natural clusters[2]:

- two experimental patterns: experimental, 'high-mass' cluster D, and experimental, 'low-mass' cluster d;
- two theoretical patterns calculated using the multi-isotopomericmodelling of ionic pattern (MMIP) [8] method from natural isotope abundance and formulas suggested for the ions D^+ and d^+ [9, 10]: theoretical, 'high-mass' cluster $T - T = \{A_{2a}B_{2b} \cdots Z_{2z}\}$, theoretical, 'low-mass' cluster $t \; t = \{A_a B_b \cdots Z_z\}$.

Simple symmetrical fragmentation

The first stage concerns the identification of experimental clusters assuming that both ions are singly charged. The postulate is true for the 'high-mass' ion. The detailed testing is presented in Table 1.

The agreement of the 'high-mass' experimental ion cluster with the theoretically predicted cluster ($D = T$) proves that both the examined ions are the same and the examined cluster D is 'pure'. In other cases, the hypothesis was false or the experimental pattern was complex $\left(D = \sum_{i=1}^{n} w_i D_i \text{ and } \sum_{i=1}^{n} w_i = 1 \right)$, usually due to dehydrogenation processes. The procedure can be continued after determination

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[1] Cluster = pattern = band denotes well-separated part of the ms-spectrum corresponding to the ms-ion.

[2] In this paper, clusters are denoted without charge, ions are provided with charge; e.g. D is the pattern of the D^+ ion.

of the experimental pattern components, i.e. using the MMIP method [8, 11] based on natural isotope abundances [10] and on the formulas proposed as a working hypothesis. In this case the dominant component is considered.

The second pair examined were 'low-mass' ions—the experimental cluster of d and that predicted for t. A good agreement of these clusters indicates that $d = t (D = d + d)$, i.e. the 'low-mass' experimental ion is known, and it is a singly charged ion. The differences observed suggest a complex cluster of $\left(d = \sum_{i=1}^{n} v_i \, d_i\right.$, and $\left.\sum_{i=1}^{n} v_i = 1\right)$ or the occurrence of the doubly charged ion $d = D/2 \Rightarrow d^+ = D^{2+}$, (but not $D = d + d$). Both possibilities must be verified in subsequent steps. If both relationships listed in Table 2 are true, the formulas of both experimental ions are known, and the 'low-mass' pattern t corresponds to the singly charged ion d^+. The required experimental data may be gathered as a part of the investigation, or obtained from sources such as publications or databases, e.g. from the ms base NIST Mass Spectra Library [12].

The other group of starting data was four transformed patterns:

- two 'doubled' patterns from 'low-mass' bands: from the experimental cluster *[2d]*, and from the theoretical cluster *[2t]*;
- two 'divided' patterns from the 'high-mass' bands: from the experimental, 'high-mass' cluster *[D/2]*, and from the theoretical, 'high-mass' cluster *[T/2]*.

The features of natural patterns and the mode of formation of the transformed bands are given in Schemes 1 and 2. After these initial calculations of the transformed clusters as described above, the next procedure consists of three steps of patterns comparison.

Theoretical possibilities of detection

The second step is prediction of the formulas of the 'high-mass' and 'low-mass' experimental ions. These suggestions are the starting point for convolution [13] of the theoretical clusters T and t of these ions. The element numbers in the tested ion T^+ must be even. For examination the following transformed theoretical pattern [T/2] and [2t] must be calculated as is shown in Scheme 1.

Such patterns may form the basis for consideration of the occurence of doubly charged ions. The solution of the problem is explained in Table 2.

To test the theoretical clusters, the natural patterns T and t are compared with transformed clusters [2t] and [T/2], respectively, and must be significantly different. If the examined bands look similar ($t \approx [T/2]$ or $T \approx [2t]$) then the solution presented cannot determine the occurrence of doubly charged ions in the mass spectrum.

Agreement of the patterns is determined by the variance s^2 calculated from Bieman's equation [14] for the n points $s^2 = \frac{1}{n} \sum_{i=1}^{n} \left(P_{\exp.i} - P_{th.i}\right)^2$ that are relative to the experimental peaks and signals contained in the calculated band (located at the same m/z value). In other words, the s^2 values express the differences between the experimental cluster (peaks intensities $P_{\exp.i}$) and the predicted pattern of the same ion (peak intensities $P_{th.i}$). This criterion was applied in all the comparisons made. The substantial differences ($t \neq [T/2]$ or $T \neq [2t]$) enable the patterns of the mono-charged ion and the corresponding doubly charged ion to be distinguished. Relations 'low' and 'high' are optional and the procedure can be simplified for one of two possibilities. If theoretical bands are distinguishable, the next step of the procedure concerning experimental clusters can be realised, i.e. the theoretical cluster is pure, and any optional convolutions are expandable.

Clusters of doubly charged ions

Transformed patterns connected with the experimental bands obtained as above are needed in the next step of the calculations. The transformation procedure is shown in Scheme 2.

Patterns formed in this way can form the basis for a discussion on whether the 'low-mass' cluster presents the doubly charged ion or not. The similarity of the bands is

Table 2 Relationships between theoretical clusters T and t and their derivatives

Relationship	Boolean datatype	Suggestions
$T \approx [2t]$	True	Theoretical cluster T is similar to the 'doubled' pattern *[2t]*—the detection of doubly charged ion is *impossible*
	False	Theoretical cluster T and the 'doubled' pattern *[2t]* are different—the doubly-charged ion *may be detected*
$t \approx [T/2]$	True	Theoretical cluster t is similar to the 'divided' pattern *[T/2]*—the detection of doubly charged ion is *impossible*
	False	Theoretical cluster t and the 'divided' pattern *[T/2]* are different—the doubly charged ion *may be detected*

Scheme 1 Clusters transformed from theoretical patterns.
a T ⇒ [T/2], **b** t ⇒ [2t]

Clusters transformed from the theoretical patterns

Scheme A.	Scheme B.
$\mathbf{T} \Rightarrow [\mathbf{T/2}]$	$\mathbf{t} \Rightarrow [\mathbf{2t}]$
'high-mass' theoretical cluster $\mathbf{T}$ (T_i, I_i) $\Rightarrow$ 'divided' pattern $[\mathbf{T/2}]$ $(T_i/2, I_i)$	'low-mass' theoretical cluster $\mathbf{t}$ (t_i, i_i) $\Rightarrow$ 'doubled' pattern $[\mathbf{2t}]$ $(2t_i, i_i)$

$$
\begin{bmatrix}
T_1 & I_1 \\
T_2 & I_2 \\
T_3 & I_3 \\
T_4 & I_4 \\
T_5 & I_5 \\
\vdots & \vdots \\
T_{s-4} & I_{s-4} \\
T_{s-3} & I_{s-3} \\
T_{s-2} & I_{s-2} \\
T_{s-1} & I_{s-1} \\
T_s & I_s
\end{bmatrix}
\Rightarrow
\begin{bmatrix}
T_1/2 & I_1 \\
T_3/2 & I_3 \\
T_5/2 & I_5 \\
\vdots & \vdots \\
T_{s-4}/2 & I_{s-4} \\
T_{s-2}/2 & I_{s-2} \\
T_s/2 & I_s
\end{bmatrix}
$$

$$
\begin{bmatrix}
t_1 & i_1 \\
t_2 & i_2 \\
t_3 & i_3 \\
\vdots & \vdots \\
t_{r-2} & i_{r-2} \\
t_{r-1} & i_{r-1} \\
t_r & i_r
\end{bmatrix}
\Rightarrow
\begin{bmatrix}
2t_1 & i_1 \\
2t_1+1 & 0 \\
2t_2 & i_2 \\
2t_2+1 & 0 \\
2t_3 & i_3 \\
\vdots & \vdots \\
2t_{r-2} & i_{r-2} \\
2t_{r-2}+1 & 0 \\
2t_{r-1} & i_{r-1} \\
2t_{r-1}+1 & 0 \\
2t_r & i_r
\end{bmatrix}
$$

related to the pattern of both the 'high-mass' ion and the doubled cluster as well as of the 'low-mass' ion pattern and the divided cluster. The solution of the problem was tested and is shown in Table 3.

The accordance of the 'high-mass' experimental ion cluster with the transformed 'doubled' pattern ($D \approx [2d]$) suggests that both experimental clusters (D and d) have the same (or very similar) structures, i.e. the relationships between the intensities of the appropriate peaks were similar in both patterns tested. This fact can be confirmed

by the relationship of the 'low-mass' ion cluster and the 'divided' pattern ($d \approx [D/2]$).

Problems from overlapping patterns

The complex character of the investigated cluster perturbs the identification of the mass ion belonging to the pattern. In this case isotopomeric modelling of the pattern is an effective solution. Prediction of the 'high-mass' band is simple, the components are singly charged ions and the

Scheme 2 Clusters transformed from experimental patterns.
a $D^+ \Rightarrow [D/2]^+$, **b** $d^+ \Rightarrow [2d]^+$

Clusters transformed from the experimental patterns

Scheme C.	Scheme D.
'high-mass' experimental cluster $\mathbf{D}$ (D_i, A_i) $\Rightarrow$ 'divided' pattern $[\mathbf{D/2}]$ $(D_i/2, A_i)$	'low-mass' experimental cluster $\mathbf{d}$ (d_i, a_i) $\Rightarrow$ 'doubled' pattern $[\mathbf{2d}]$ $(2d_i, a_i)$
$\mathbf{D^+} \Rightarrow [\mathbf{D/2}]^+$	$\mathbf{d^+} \Rightarrow [\mathbf{2d}]^+$

$$
\begin{bmatrix}
D_1 & A_1 \\
D_2 & A_2 \\
D_3 & A_3 \\
D_4 & A_4 \\
D_5 & A_5 \\
\vdots & \vdots \\
D_{n-4} & A_{n-4} \\
D_{n-3} & A_{n-3} \\
D_{n-2} & A_{n-2} \\
D_{n-1} & A_{n-1} \\
D_n & A_n
\end{bmatrix}
\Rightarrow
\begin{bmatrix}
D_1/2 & A_1 \\
D_3/2 & A_3 \\
D_5/2 & A_5 \\
\vdots & \vdots \\
D_{n-4}/2 & A_{n-4} \\
D_{n-2}/2 & A_{n-2} \\
D_n/2 & A_n
\end{bmatrix}
$$

$$
\begin{bmatrix}
d_1 & a_1 \\
d_2 & a_2 \\
d_3 & a_3 \\
\vdots & \vdots \\
d_{v-2} & a_{v-2} \\
d_{v-1} & a_{v-1} \\
d_v & a_v
\end{bmatrix}
\Rightarrow
\begin{bmatrix}
2d_1 & a_1 \\
2d_1+1 & 0 \\
2d_2 & a_2 \\
2d_2+1 & 0 \\
2d_3 & a_3 \\
\vdots & \vdots \\
2d_{v-2} & a_{v-2} \\
2d_{v-2}+1 & 0 \\
2d_{v-1} & a_{v-1} \\
2d_{v-1}+1 & 0 \\
2d_v & a_v
\end{bmatrix}
$$

Table 3 Relationship between experimental clusters D and d and their derivatives

Relationship	Boolean datatype	Suggestions
$D = [2d]$	True	Experimental cluster of ion D^+ is similar to the 'doubled' pattern $[2d]$—d^+ is the *doubly charged ion* from ion D^+
	False	Experimental cluster of ion D^+ and the 'doubled' pattern *[2d]* are different—d^+ is *not the doubly charged ion* from ion D^+ [some (or both) pattern is perturbed—modelling of the complex cluster is recommended]
$d \approx [D/2]$	True	Experimental cluster of ion d^+ is similar to the 'divided' pattern *[D/2]* and d^+ is the *doubly-charged ion* from ion D^+
	False	Experimental cluster of ion d^+ and the 'divided' pattern *[D/2]* are different—d^+ is *not the doubly charged ion* from ion D^+ [some (or both) pattern is perturbed—modelling of the complex cluster is recommended]

MMIP modeling method [8] can be applied. Overlapping in the 'low-mass' ion area generates many more problems. The complex pattern may be composed of both singly- and doubly-charged components. If the dominating component is the doubly charged ion, then the occurrence of D^{2+} can be considered true. Whatever the composition of the ion D^+ cluster, the relationship ($d \approx [D/2]$) can be also observed, thus denoting the occurrence of the doubly charged ion. The situation is more difficult if the 'low-mass' ion cluster is complex. The location of the pattern in the lower part of the spectrum ($m/z > M/2$) facilitates overlapping of the ion pattern with the closely located cluster corresponding to other ions of quite different structure. In this case, modelling can be difficult. For each case the dominating component is applied for consideration in the next step.

A high similarity between the 'low-mass' and the 'higher' patterns can be observed if both clusters are not distorted. If the 'high-mass' ion is complex, the components are usually structurally very similar. The occurrence of a doubly charged ion can also be independently detected by the relationship between experimental clusters whether the 'high-mass' pattern is pure or not.

The agreement found between the 'high-mass' experimental ion cluster and the transformed 'doubled' pattern ($D \approx [2d]$) suggests that both experimental clusters (D and d) have very similar structures, i.e. the intensities of the corresponding peaks are similar in both tested patterns. This fact can be confirmed by the relationship between the 'low-mass' ion cluster and the 'divided' pattern ($d \approx [D/2]$). Both relationships are equivalent: positive verification in either case indicates the presence of doubly charged ion related to the 'low-mass' pattern.

The idea of the solution involves verification of three relationships between the isotopomeric clusters. The first is the possibility of differentiation of the model clusters of singly- and doubly-charged ions. If differences are observed, clusters D^+ and D^{2+} should be distinguishable. The identification of the formulas of both ions ($D \approx T$) and ($d \approx t$) explains the situation—the doubly charged ion is not observed in the spectrum, the appropriate ions are known and their clusters are pure. The other possibility is the occurrence of two ions characterised by the same structure and element content (symmetrical fragmentation = symmetrical by formula), or two ions that differ in their element formulas and structures (asymmetrical fragmentation = symmetrical by mass). The essential relationship is the comparison of experimental clusters. The agreement of the corresponding patterns points to the smaller ion as the doubly charged one. The alternative mechanism consists of symmetrical and asymmetrical fragmentation. Overlapping patterns can perturb any of the steps; however, modelling of the isotopomeric pattern of the considered ion can solve these problems.

The two alternative hypotheses must be verified by testing:

a. Symmetrical fragmentation

$$D^+ = d_1^+ + d_2^+ \quad and$$

$$(m/z)_{d1} = (m/z)_{d2} \; or \; (m/z)_{d1} \cong (m/z)_{d2}$$

The ion D^+ is decomposed (directly or successively) to the ion d^+. If the formation of the ion d^+ proceeds step-by-step, the cluster of the ion may be complex and modelling of the pattern may be required.

b. Doubly charged ion

$$d^+ = D^{2+}$$

The cluster connected to the d^+ ion is, de facto, the pattern of the doubly charged ion D^{2+}. As a result of the +2 charge, the cluster location has the appearance of a single charged ion d^+ with a twofold lower mass for ion D^+.

Example 1 Bis[bis(.eta.-5-cyclopentadienyl)-bis(.mu.-propynyl-zirconium] $C_{26}H_{26}Zr_2$ [15]

The molecular pattern is located near m/z=519 Da (Fig. 1), which corresponds to the average molecular

Fig. 1 Structure (**a**) and mass spectrum (**b**) of bis[bis (.eta.-5-cyclopentadienyl)-bis (.mu.-propynyl-zirconium] $C_{26}H_{26}Zr_2$ [15]
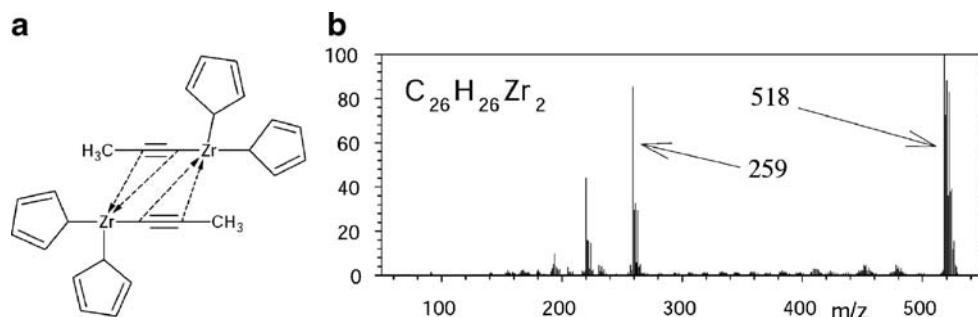
weight of the compound. Two interesting patterns (perhaps interrelated) in the spectrum are observed: a 'high-mass', molecular cluster in the area 515–529 Da (main peak at 518 Da), and a 'low-mass' cluster in the area 284–290 Da (main peak at 259 Da). The patterns are extensive and all possible m/z values in the area are filled. Which is true?

$$P_{518} = P'_{259} + P''_{259}$$ symmetrical fragmentation of the ion $[C_{26}H_{26}Zr_2]^+$

or

$$P_{518} = [2P_{259}]$$ the doubly − charged ion $[C_{26}H_{26}Zr_2]^{2+}$

The element numbers in the formula are even and the molecular structure looks symmetrical. Testing of the experimental and theoretical 'low-mass' and 'high-mass' clusters verified the above hypothesis. Comparison of the 'high-mass' clusters (Fig. 2a) indicates high agreement between the theoretical and experimental patterns ($s^2=2.0$). Similar effects were observed (Fig. 2b) with the 'low-mass' clusters ($s^2=0.93$). This testing allows determination of both experimental clusters, the 'high-mass' as well as the 'low-mass'. Subsequent calculations confirmed the correctness of the earlier suggestions. The doubly charged cluster of ion 518 is detectable (if it exists) because the theoretical clusters of the 'high-mass' and 'low-mass' clusters differ significantly ($s^2=950$, Fig. 2c). Comparison of the experimental patterns also points to an essential difference ($s^2= 857$, Fig. 2d), therefore bands D_{518} and d_{259} concern different ms-ions.

Relation:	$T_{518} = D_{518}$	$s^2=2.0$	True
Conclusion:	The experimental cluster is the same as the theoretical one. $T_{518} = D_{518}$		
Relation:	$t_{259} = d_{259}$	$s^2=0.93$	True
Conclusion:	The experimental 'low-mass' cluster is the same as the theoretical one. $t_{259} = d_{259}$		
Relation:	$T_{518} = [2t_{259}]$	$s^2=950.52$	False
Conclusion:	The isotopomeric patterns corresponding to the 'low-mass' and 'high-mass' ions are different. The cluster of the doubly charged ion can be detected.		
Relation:	$D_{518} = [2d_{259}]$	$s^2=897.01$	False
Conclusion:	$d_{259} \neq D_{518}^{2+}$ The ion located at 259 Da is not the doubly charged ion of the ion situated at 518 Da		

The facts point to ionization of the $C_{26}H_{26}Zr_2$ molecule, being accompanied by 'symmetrical' dissociation and creation of two single-charged $C_{13}H_{13}Zr^+$ ions.

$$D_{518} = d'_{259} + d''_{259} \qquad d'_{259} = d''_{259} \qquad d'_{259} = \left[D_{518}/2 \right]$$

Example 2 1,3,5,7-Tetramethyl 2,4,6,8-tetraseleneadamantane, $C_{10}H_{16}Se_4$ [16]

The molecular pattern is located near m/z=454 Da (Fig. 3), which corresponds to the average molecular weight of the compound. Two interesting patterns (perhaps interrelated) in the spectrum are observed, patterns are extensive and all possible m/z locations in the area are filled: a 'high-mass' molecular cluster in area 443–462 Da (main peak at 454 Da), and a 'low-mass' cluster in area 219–233 Da (dominant peak at 227 Da)

Detailed investigation should point to the right answer:

$$P_{454} = P'_{227} + P''_{227}$$ symmetrical decomposition of the ion $[C_{10}H_{16}Se_4]^+$

or

$$P_{454} = [2P_{227}]$$ the doubly − charged ion $[C_{10}H_{16}Se_4]^{2+}$?

Testing of the experimental and theoretical clusters, and therein the 'low-mass' and 'high-mass' clusters (Fig. 4a–d), may select the appropriate hypothesis. The element numbers in the formula are even, and the molecular structure looks symmetrical thus the existence of a doubly charged ion in the spectrum is possible. Modelling of the isotopomeric cluster by the MMIP method [8] proves that the pattern corresponds to the molecular ion $[C_{24}H_{12}Se_3]^+$. Comparison of the 'high-mass' clusters (Fig. 4a) indicates a high level of agreement between the theoretical and experimental patterns ($s^2=3.50$). The low-mass pattern is still unlike the theoretical pattern calculated for M/2 ($C_5H_8Se_2$). Satisfactory effects are observed for the M/2-H ion (Fig. 4b, $s^2=17.48$). Comparison of theoretical as well as experimental cluster can be regarded as unnecessary in

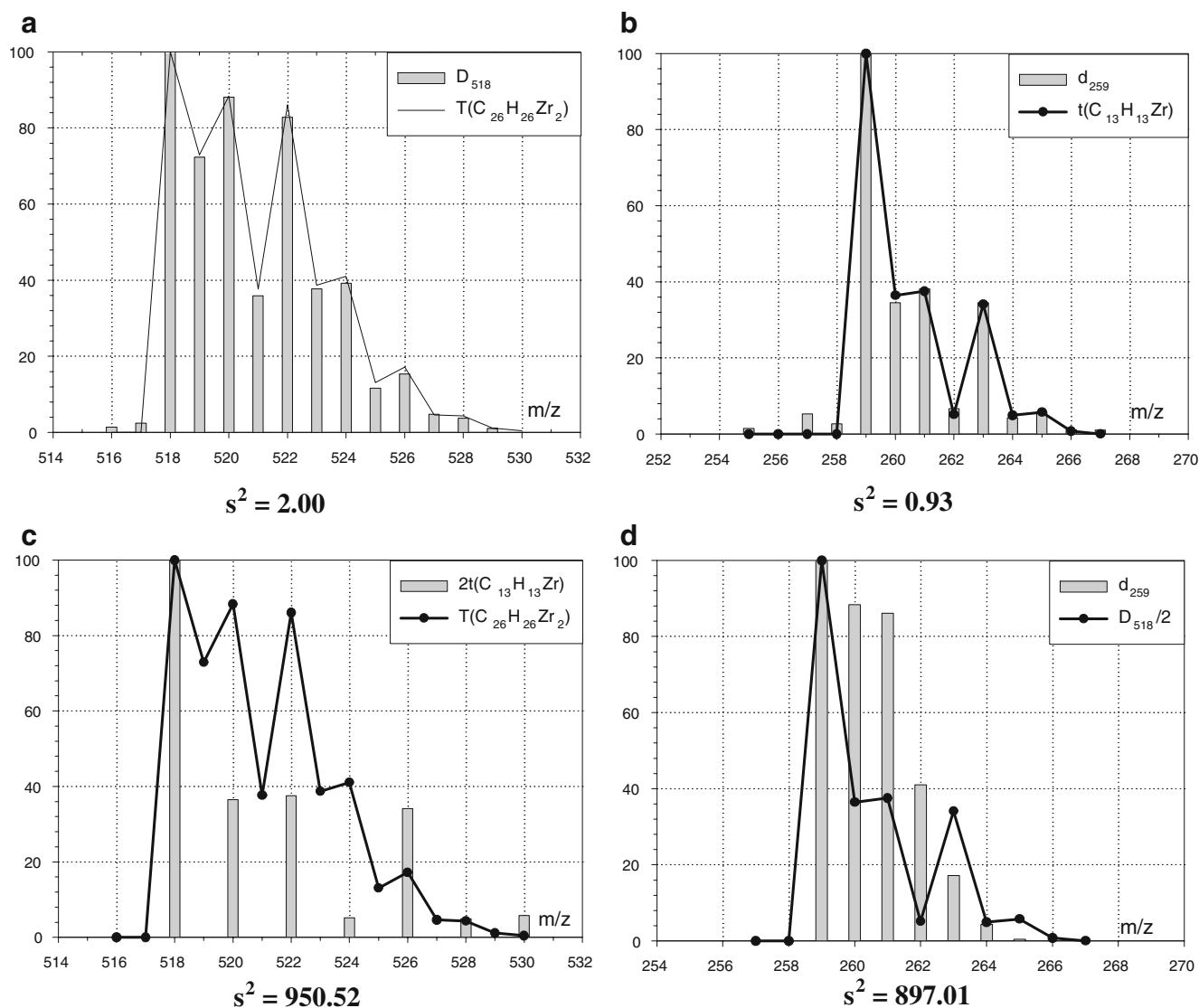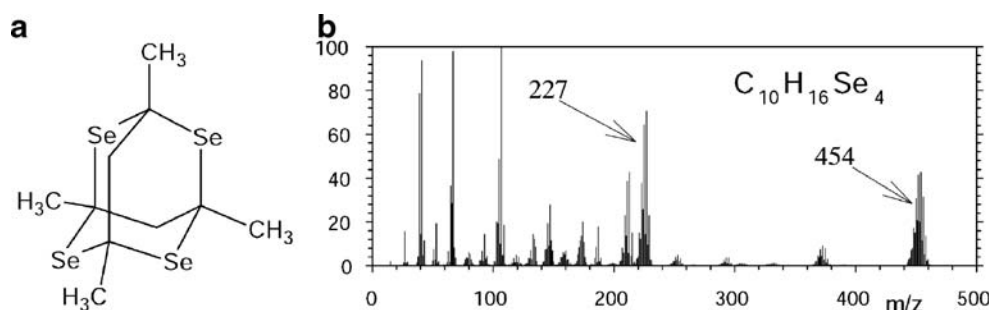**Fig. 2** Identification of the double-charged ion cluster in the bis[bis(.eta.-5-cyclopentadienyl)-bis(.mu.-propynylzirconium) mass spectrum. **a** Identification of the experimental band D_{518}. **b** Identification of experimental band d_{259}. **c** Relationship between theoretical patterns t_{259} vs T_{518}. **d** Differences between experimental patterns D_{518} vs d_{259}

this case. The hypothesis that the theoretical (D and $2d$) and experimental (T and $2t$) clusters are similar is false; the variance values were computed as $s^2 = 796.88$ and $s^2 = 729.29$, respectively.

The agreement between the experimental pattern of the ion d_{227} and the calculated model indicates that the formula of this ion is $[C_5H_7Se_2]^+$. The ion at 227 m/z is the single-charged ion $[C_5H_7Se_2]^+$, which is derived from

Fig. 3 Structure (**a**) and mass spectrum (**b**) of 1,3,5, 7-tetramethyl 2,4,6, 8-tetraselene-adamantane, $C_{10}H_{16}Se_4$ [16]

a $s^2 = 3.50$ (20 points)

b $s^2 = 17.48$ (14 points)

c $s^2 = 796.88$ (points)
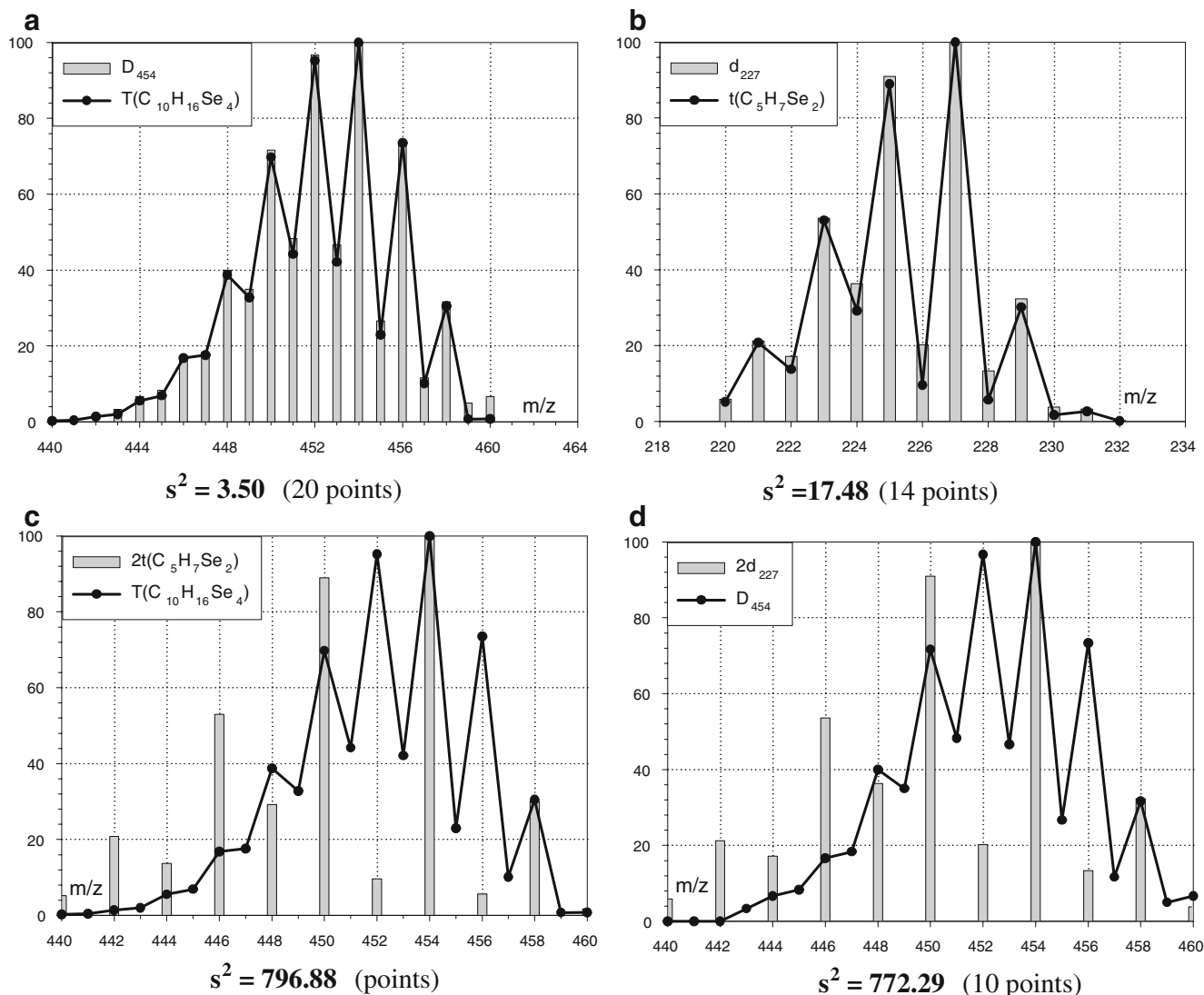
d $s^2 = 772.29$ (10 points)

Fig. 4 Modelling of the mass spectrum clusters of the 1,3,5,7-tetramethyl 2,4,6,8-tetraselene-adamantane. **a** Identification of the experimental pattern $D_{454}^{+}\left(T^{+} = [C_{10}H_{16}Se_4]^{+}\right)$. **b** Identification of the experimental pattern $d_{227}^{+}\left(t^{+} = [C_5H_7Se_2]^{+}\right)$. **c** Comparison of the theoretical ionic clusters $T^{+}=[C_{10164}]$ and $2t^{+}=2[C_{592}]^{+}$. **d** Comparison of the experimental ionic patterns D_{454}^{+} and $2d_{227}^{+}$

the complex decomposition of the molecular ion 452 $[C_{10}H_{18}Se_4]^{+}$.

Example 3 Tris(benzo[b]selenopheno)[2,3:2',3':2',3'] benzene $C_{24}H_{12}Se_3$ [17]

Patterns D_{538} and d_{269} The most intense pattern of the spectrum related to the molecular ion is located in the range between 528 and 545 Da (Fig. 5), which corresponds to the average molecular mass. The cluster has high intensity, and all possible m/z values are filled. The cluster located in the range 265–272 Da also occurs in the spectrum. This location suggests that both clusters are related to the same ion. The 'high-mass' band is related to the single-charged molecular ion M^{+}, and the 'low-mass' pattern may correspond to the doubly charged ion. Is this true or not?

The following detailed investigation should point to the right answer:

$$P_{538} = P'_{269} + P''_{269} \quad \begin{array}{l}\text{single} - \text{charged ion from the}\\ \text{fragmentation of } [C_{24}H_{12}Se_3]^{+}\end{array}$$

or

$$P_{538} = [2\,P_{269}] \quad \text{doubly} - \text{charged ion}[C_{24}H_{12}Se_3]^{2+}?$$

The modelling of the molecular isotopomeric cluster proves that the pattern corresponds to the molecular ion $[C_{24}H_{12}Se_3]^{+}$ $(s^2=2.11)$.

The 'low-mass' pattern contains ten small peaks located nearby; the most intense peak is less than 19%. The element formula prediction of the 'low-mass' ion is precluded—the selenium number is odd and the expected

Fig. 5 Structure (**a**) and mass spectrum (**b**) of tris(benzo[b] selenopheno)[2,3:2′,3′:2′,3′] benzene $C_{24}H_{12}Se_3$ [17]
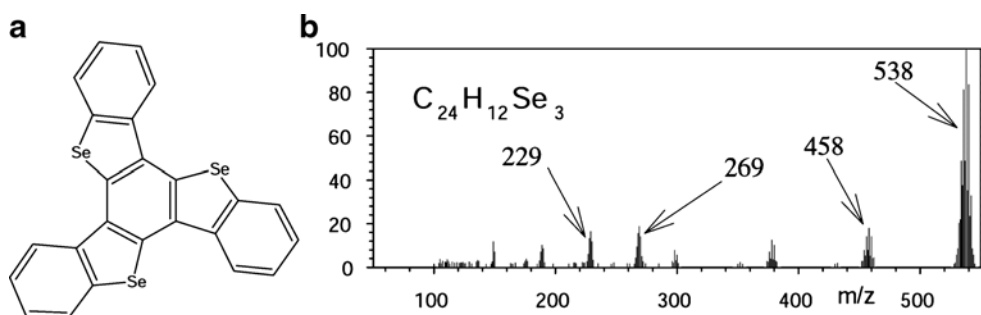
formula cannot be predicted as M/2. The location of the main peak (J. Lamkiewicz, unpublished work) suggests four possible formulas: $C_{22}H_5$, $C_{15}H_9Se$, C_9HSe_2 and $C_2H_7Se_3$, but none of these is correct. This fact indicates that the 'low-mass' cluster could represent the pattern of the

doubly charged ion and this hypothesis must be verified. The effects of this testing are summarised in Fig. 6a–d.

The 'high-mass' clusters are very similar; the theoretical variance is small (Fig. 6a). This fact proves the effective prediction of the experimental 'high-mass' ion cluster.

$s^2 = 2.11$ (18 points)

$s^2 = 25.60$ (8 points)
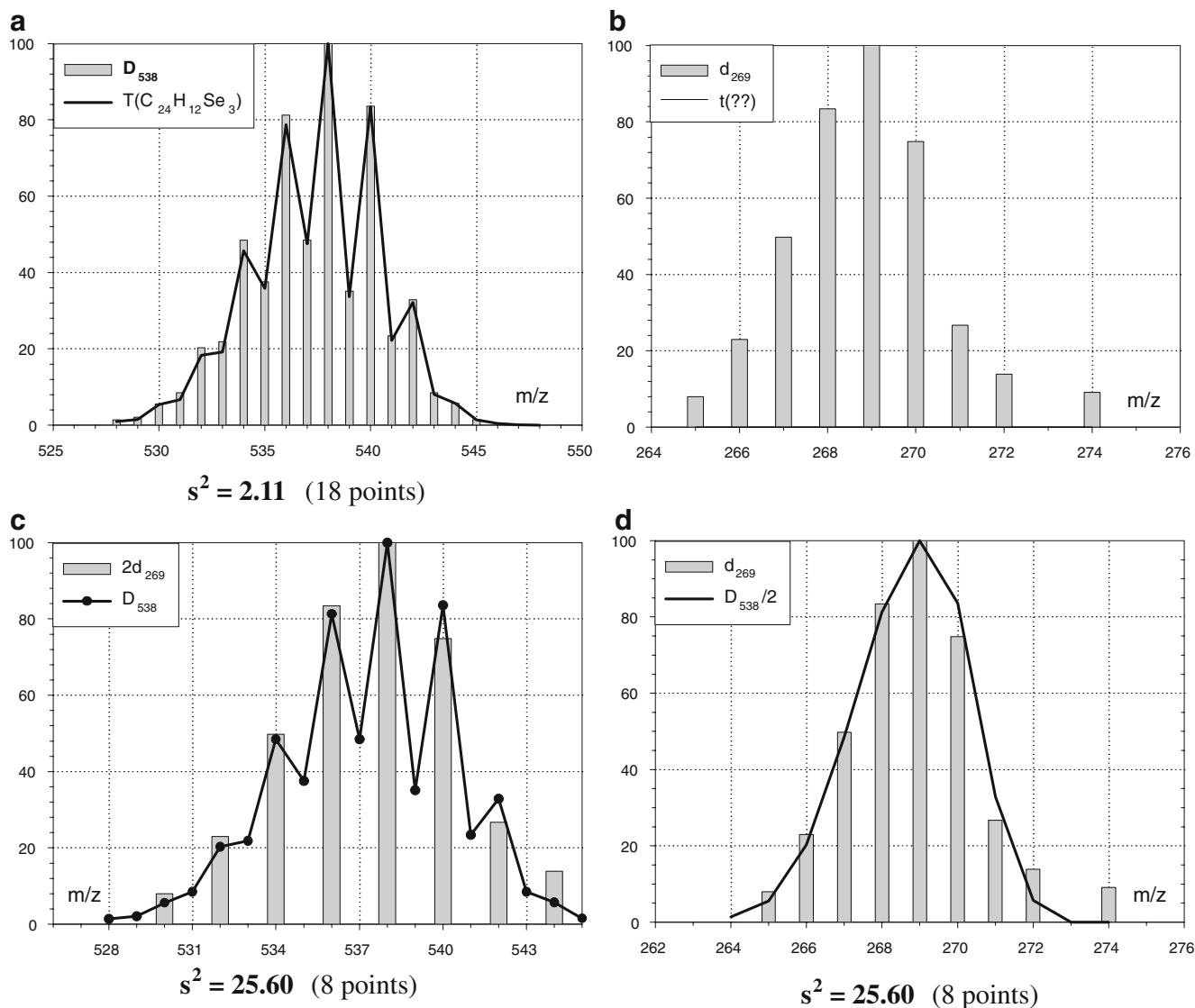
$s^2 = 25.60$ (8 points)

Fig. 6 Identification of the double-charged ion cluster in the tris (benzo-selenopheno)-benzene mass spectrum. **a** Theoretical identification of the experimental band D_{538}. **b** Identification of the

experimental band $d_{269}{}^+$ is impossible. **c** Relationship between experimental patterns: d_{269} vs D_{538}. **d** Comparison of experimental patterns: D_{538} vs d_{269}
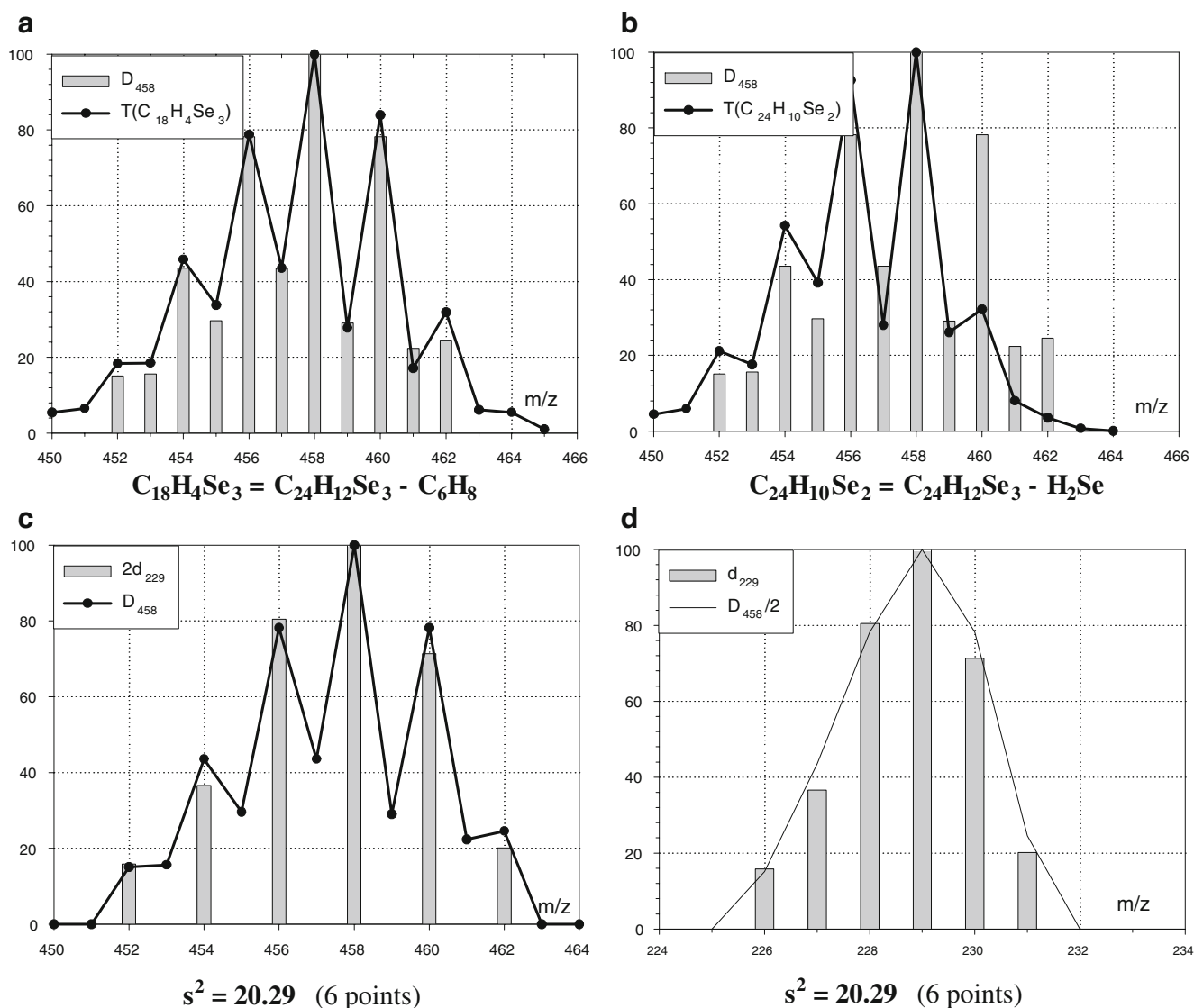
Fig. 7 Identification of the double-charged ion cluster in the mass spectrum of the tris(benzo-selenopheno)-benzene. **a**, **b** Theoretical identification of the experimental band D_{458}. **c** Relationship between experimental patterns: $2 \cdot d_{229}$ vs D_{458}. **d** Comparison of experimental patterns: d_{229} vs $D_{458}/2$

Comparison of the 'low-mass' cluster and transformed 'high-mass' pattern (as well as 'high-mass' cluster and transformed 'low-mass' pattern) indicates that the d_{269} band must correspond to the doubly charged D_{538} cluster. The relatively low value of the variance also confirms the hypothesis $s^2 = 24.95$.

Relation:	$D_{538} = T_{538}$	$s^2 = 2.11$	True
Conclusion:	The cluster of the 'high-mass' ion is complex		
Relation:	$d_{269} \Leftrightarrow ?? t_{269}$		
Conclusion:	The theoretical ions' formula is impossible		
Relation:	$T_{538} \Leftrightarrow [2??]$		
Conclusion:	Impossible to predict		
Relation:	$D_{538}[2d_{269}]$	$s^2 = 25.60$	True
Conclusion:	$d_{269}^+ = D_{538}^+$	The ion corresponding to d_{269} is the doubly charged ion originating from ion D_{538}	

Relative intensities can be similar only in the same isotopomeric compositions, therefore the different locations of such clusters are an consequence of the different charge of the ions corresponding to the patterns investigated. This fact leads solely to the following conclusion: ion 269 is the doubly charged ion derived from ion 538.

The phenomenon responsible for the occurrence of the ion $[C_{24}H_{12}Se_3]^{2+}$ can be related to the presence of three selenium atoms in the molecule, and the persistence of the ion is a consequence of the aromatic character of compounds containing six aromatic rings in the molecule.

Patterns D_{458} and d_{229} The mass spectrum of tris(benzo-selenopheno)-benzene contains other interesting twin

clusters (perhaps interrelated): a 'high-mass' cluster in area 452–462 Da (main peak at 458 Da; 17.9%) comprising 11 peaks, and a 'low-mass' cluster in area 226–231 Da (main peak at 229 Da; 16.4%) with 6 peaks. Two formulas of the 'high-mass' cluster are possible: $C_{18}H_4Se_3$ and $C_{24}H_{10}Se_2$. The formula $C_{24}H_{10}Se_2$ seems most likely because of the probable origin:

$$C_{18}H_4Se_3 = C_{24}H_{12}Se_3 - C_6H_8.$$

The 'high-mass' band is related to the single-charged fragmentation ion $D_{458}{}^+$, but the 'low-mass' pattern may correspond to the doubly charged ion. Is this true or not?

$$P_{458} = P'_{229} + P''_{229}$$ symmetrical by mass decomposition of the ion $[C_{18}H_4Se_3]^+$

or

$$P_{458} = [2\,P_{229}]$$ doubly – charged ion $[C_{18}H_4Se_3]^{2+}$

The effects of testing are summarised in Fig. 7a–d. The 'high-mass' clusters are different; the value of the theoretical variance is high ($s^2 = 315$) suggesting that the pattern is complex and modelling of the experimental cluster is

Scheme 3 Determination of the nature of mass spectral clusters in the range lower than M/2 u
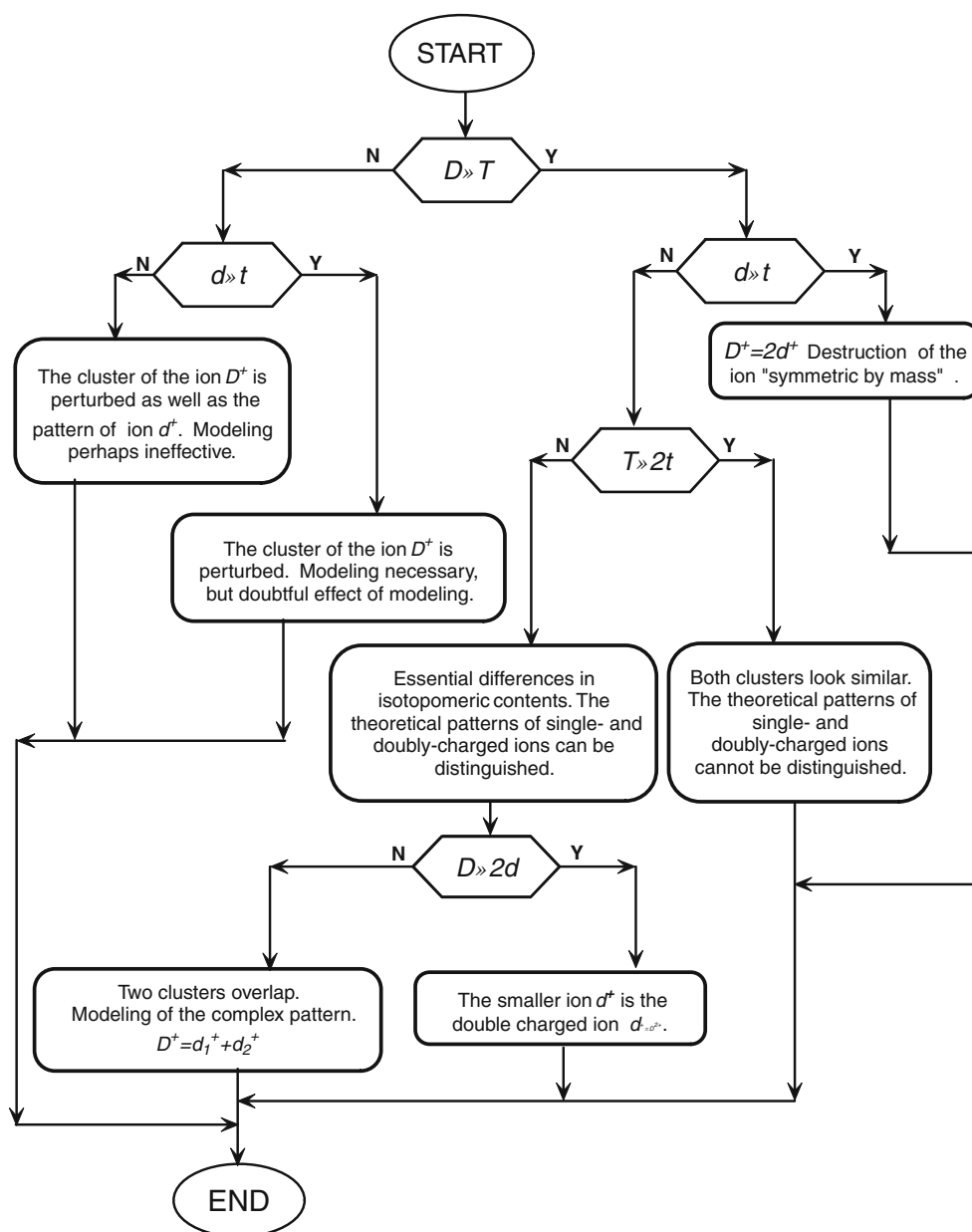
needed (Fig. 7a). The final proof of this thesis was provided by the similarity of the experimental and theoretically calculated model ($s^2=3.16$), with the accordance factor $\alpha = 99\%$. The selenium number in the formula is even, and the simple T/2 ion may be not considered. No possible agreement with $C_{18}H_4Se_3$ is available for the mass 229 u resulting from the mass calculation (J. Lamkiewicz, unpublished work). Therefore, the 'low-mass' cluster could represent the pattern of the doubly charged ion. This hypothesis must also be proved.

Determination of the 'low-mass' band was ineffective ($s^2=1,502$), and no amount of modelling can solve this problem. Fortunately, the next prediction is available. Comparison of the experimental clusters D_{458} and d_{458} showed substantial agreement (Fig. 4c,d), and identification of the doubly charged ion cluster is effective ($s^2=20.29$).

Relation:	$D_{458} = T_{458}$	$s^2=14.21$	True
Conclusion:	The cluster of D_{458} is the pattern of the $[C_{18}H_4Se_3]^+$ ion		
Relation:	$d_{229} \Leftrightarrow ?? \, t_{229}$		
Conclusion:	Theoretical ion t_{229} is impossible—$T_{458} \Leftrightarrow ??2t_{229}$		
Relation:	$D_{458} = [2d_{229}]$	$s^2=20.29$	True
Conclusion:	$d_{229}^+ = D_{458}^{2+}$ The ion d_{229} is the doubly charged ion of D_{458}		

Relative intensities can be similar only in the same isotopomeric compositions, therefore the different locations of such clusters are a consequence of different charges of the ions corresponding to the patterns investigated. This fact leads to only one conclusion: ion 229 is the doubly charged ion derived from ion 458.

Conclusions

Analysis of the isotopomeric structures of clusters can be an effective tool in interpretation of low-resolution mass spectra. The origins of patterns located in the range lower than M/2 can be explained. The identification of symmetrical patterns by formula as well as the symmetrical patterns by mass and doubly charged ion clusters is possible with the help of the method described here. A summary of the procedure is shown in Scheme 3.

The complex character of the clusters investigated may perturb testing, and simple solutions may be ineffective. In this case, modelling of the pattern is needed, with the dominant compound used in further calculations. The complexity of the molecular bands is usually not the problem. Overlapping of the molecular pattern area is generated as an effect of dehydrogenation [18] processes, and their modelling is easy. The components of the complex patterns located in the range M/2 < m/z < M can differ in dehydrogenation level, but the overlapping of ions characterised by significantly different structures is not probable. On the other hand, clusters located in the area m/z < M/2 may also be formed from ions of different chemical structure.

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