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Correlation between the spectrum resolution and the peak location accuracy in the isotopomeric cluster

Andrzej J. Gorączko *

Dept of Inorganic Chemistry, University of Technology and Life Sciences, Seminaryjna 3, 85-7326 Bydgoszcz, Poland

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ABSTRACT

An instrument capable of high resolving power is necessary to assure that the m/z value of the ion being measured is of an ion of a single elemental and/or isotope composition. The accurately determined m/z value of a mass spectral peak may be a weighted average of multiple ions of different isotopes and/or elemental compositions if the resolving power of the instrument used is insufficient to separate very small differences in m/z values. Instruments are operated at high resolution (meaning a resolving power $R = M/\Delta m \geq 10{,}000$), medium resolution ($5000 \leq R < 10{,}000$), or low resolution ($\Delta m \cong 1$). A common mistake made in referring to instruments in the high-resolution mode is that they automatically produce measured accurate mass data. What is the dependence of the equipment resolution (resolving power) and masses of peaks in the mass spectrum on peaks location accuracy? The present paper is an attempt at answering the question.

1. Introduction

The terms resolution, resolution power, high- and low-resolution and accurate mass are commonly used in mass spectrometry. Unfortunately the concordance or sensual similarity is a source of many mistakes and misunderstandings. The IUPAC's recommendation [1] and Sparkman's propositions [2] are sometimes applied inconsistently; many authors do not adhere to these suggestions. The most popular error is using the term 'high-resolution' when referring to 'accurate mass'. The terms are related but are not synonyms. The work presented here attempts to answer the question concerning what conditions high resolution may be adequate to precise locations of mass spectral peaks.

2. Resolving power and resolution

Mass spectrometers are operated according to differences in the m/z values of ions that can be separated. Instruments are operated at a certain resolution, meaning a resolving power (R).

The resolving power R defines the ability of a mass spectrometer to separate ions of two different m/z values, $R = M/\Delta m$, where M is the lower m/z value of two adjacent mass spectral peaks or the m/z value of the peak used to calculate R [3]. The Δm value (often called resolution) is the difference between M and the m/z value of the next adjacent peak and it represents the smallest differences in m/z values that can be separated. The term is used to define the ability of a mass spectrometer to separate ions of two different m/z values. Resolution is the inverse of resolving power and expressed as $\Delta m/M$. Although resolving power is a large number and is associated with a 'valley' or mass spectral peak width, the resolution can be a small number. Resolution is the measure of a separation of two mass spectral peaks and is often reported in terms of parts per million (ppm), which can be misleading, depending on whether or not it is associated with a specific m/z value.

Magnetic sector instruments make use of adjustable slits to set the resolving power of the mass analyzer (like setting the aperture of a camera lens) that may vary from 500 to 100,000 and more. Instruments are operated at $R > 10{,}000$ (high resolution spectrometers), $10{,}000 > R > 5000$ (medium resolution) or $\Delta m = 1$ (low resolution equipment). The resolution Δm can be calculated from these two peaks if separated by a 10% valley and represents the smallest location differences (in m/z). The two peaks will have an overlay (valley) of 10%. In the definition, the height from the baseline to the junction point of the two peaks is 10% of the full height of the two peaks. Each peak contributes 5% to the height of the valley as shown in Fig. 1.

Since it may be difficult to get two mass spectral peaks of equal height adjacent to one another, another method of calculating Δm is to use the full width at half maximum (*FWHM*) of a single m/z value peak [4]. Resolving power calculated using the FWHM method gives values for R that are about twice that determined by the 10% valley method. The resolving power can also be calculated using the full width at 5% full maximum. This will result in an R value close to the 10% valley method. However, it is very dif-

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* Tel.: +48 052 3749049; fax: +48 052 3749005.
E-mail address: andygor@utp.edu.pl

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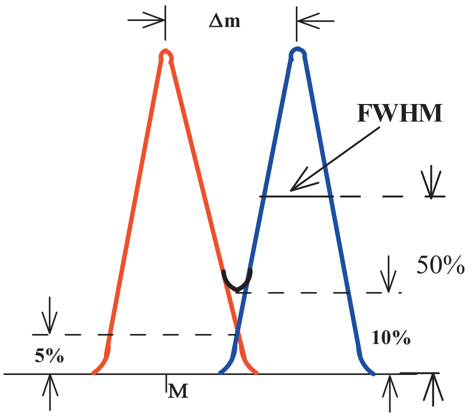

Fig. 1. Parameters for resolving power determination.

ficult because of the background signals. All definitions are shown in Fig. 1.

Quadruple ion traps (QIT) and transmission quadruple (TQ) mass spectrometers operate at constant resolution with increasing resolving power with increasing m/z value. This means that the ability to separate ions at $m/z = 100$ and $m/z = 1000$ is the same. If $\Delta m = 1$ m/z unit at $m/z = 100$, the resolution $\Delta m_{(m/z=100)} = 1$ and resolving power $R = 100$ (100/1). If $\Delta m = 1$ at $m/z = 1000$, the resolution $\Delta m_{(m/z=100)} = 1$ also, however the resolving power $R_{(m/z=100)} = 1000$ (1000/1).

Time-of-flight (TOF) mass spectrometers and instruments using both electric and magnetic fields to separate ions operate at constant resolving power. At a resolving power of 1000 throughout the m/z scale, these instruments will separate ions of $m/z = 1000$ and $m/z = 1001$. In this example $\Delta m = 1$ and $M = 1000$, therefore, $R = 1000$ (1000/1). This property of constant resolving power over the entire m/z scale means that with a resolving power of 1000 values of 0.1 m/z can be separated at $m/z = 100$, e.g. $R = 1000 = M/\Delta m = 100/0.1$. This means that the res-

Table 1
The adjacent peaks locations difference Δm estimated from the instrumental spectrum resolving power R and the ionic mass M.

Resolving power	Masses M [u]									
	100	200	300	400	500	600	700	800	900	1,000
10,000,000	**0.00001**	**0.00002**	**0.00003**	**0.00004**	**0.00005**	**0.00006**	**0.00007**	**0.00008**	**0.00009**	**0.00010**
8,500,000	**0.00001**	**0.00002**	**0.00004**	**0.00005**	**0.00006**	**0.00007**	**0.00008**	**0.00009**	**0.00011**	**0.00012**
7,000,000	**0.00001**	**0.00003**	**0.00004**	**0.00006**	**0.00007**	**0.00009**	**0.00010**	**0.00011**	**0.00013**	**0.00014**
5,500,000	**0.00002**	**0.00004**	**0.00005**	**0.00007**	**0.00009**	**0.00011**	**0.00013**	0.00015	0.00016	0.00018
4,000,000	**0.00003**	**0.00005**	**0.00008**	**0.00010**	**0.00013**	0.00015	0.00018	0.00020	0.00023	0.00025
2,500,000	**0.00004**	**0.00008**	**0.00012**	0.00016	0.00020	0.00024	0.00028	0.00032	0.00036	0.00040
1,000,000	**0.00010**	0.00020	0.00030	0.00040	0.00050	0.00060	0.00070	0.00080	0.00090	0.00100
850,000	**0.00012**	0.00024	0.00035	0.00047	0.00059	0.00071	0.00082	0.00094	0.00106	0.00118
700,000	**0.00014**	0.00029	0.00043	0.00057	0.00071	0.00086	0.00100	0.00114	0.00129	0.00143
550,000	0.00018	0.00036	0.00055	0.00073	0.00091	0.00109	0.00127	0.00145	0.00164	0.00182
400,000	0.00025	0.00050	0.00075	0.00100	0.00125	0.00150	0.00175	0.00200	0.00225	0.00250
250,000	0.00040	0.00080	0.00120	0.00160	0.00200	0.00240	0.00280	0.00320	0.00360	0.00400
100,000	0.00100	0.00200	0.00300	0.00400	0.00500	0.00600	0.00700	0.00800	0.00900	0.01000
85,000	0.00118	0.00235	0.00353	0.00471	0.00588	0.00706	0.00824	0.00941	0.01059	0.01176
70,000	0.00143	0.00286	0.00429	0.00571	0.00714	0.00857	0.01000	0.01143	0.01286	0.01429
55,000	0.00182	0.00364	0.00545	0.00727	0.00909	0.01091	0.01273	0.01455	0.01636	0.01818
40,000	0.00250	0.00500	0.00750	0.01000	0.01250	0.01500	0.01750	0.02000	0.02250	0.02500
25,000	0.00400	0.00800	0.01200	0.01600	0.02000	0.02400	0.02800	0.03200	0.03600	0.04000
10,000	0.01000	0.02000	0.03000	0.04000	0.05000	0.06000	0.07000	0.08000	0.09000	0.10000
8500	0.01176	0.02353	0.03529	0.04706	0.05882	0.07059	0.08235	0.09412	0.10588	0.11765
7000	0.01429	0.02857	0.04286	0.05714	0.07143	0.08571	0.10000	0.11429	0.12857	0.14286
5500	0.01818	0.03636	0.05455	0.07273	0.09091	0.10909	0.12727	0.14545	0.16364	0.18182
4000	0.02500	0.05000	0.07500	0.10000	0.12500	0.15000	0.17500	0.20000	0.22500	0.25000
2500	0.04000	0.08000	0.12000	0.16000	0.20000	0.24000	0.28000	0.32000	0.36000	0.40000
1000	0.10000	0.20000	0.30000	0.40000	0.50000	**0.60000**	**0.70000**	**0.80000**	**0.90000**	**1.00000**
850	0.11765	0.23529	0.35294	0.47059	**0.58824**	**0.70588**	**0.82353**	**0.94118**	**1.05882**	**1.17647**
700	0.14286	0.28571	0.42857	**0.57143**	**0.71429**	**0.85714**	**1.00000**	**1.14286**	**1.28571**	**1.42857**
550	0.18182	0.36364	**0.54545**	**0.72727**	**0.90909**	**1.09091**	**1.27273**	**1.45455**	~~*1.63636*~~	~~*1.81818*~~
400	0.25000	0.50000	**0.75000**	**1.00000**	**1.25000**	~~*1.50000*~~	~~*1.75000*~~	~~*2.00000*~~	~~*2.25000*~~	~~*2.50000*~~
250	0.40000	**0.80000**	**1.20000**	~~*1.60000*~~	~~*2.00000*~~	~~*2.40000*~~	~~*2.80000*~~	~~*3.20000*~~	~~*3.60000*~~	~~*4.00000*~~
100	**1.00000**	~~*2.00000*~~	~~*3.00000*~~	~~*4.00000*~~	~~*5.00000*~~	~~*6.00000*~~	~~*7.00000*~~	~~*8.00000*~~	~~*9.00000*~~	~~*10.00000*~~

□	▤	▦	■
High resolution area	Medium resolution range	Low resolution area	Not applicable range

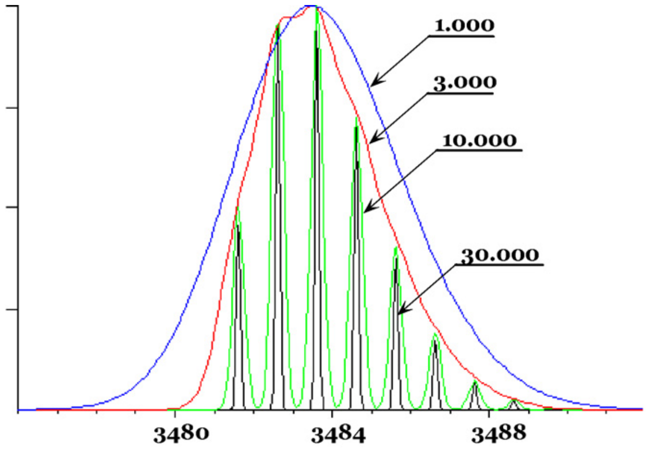

Fig. 2. Molecular ion of glucagon at increased values of resolving power [7].

olution increases with decreasing *m*/*z* values in these types of instruments.

The term **resolving power** (*R*) is a property of the **instrument** (mass spectrometer), and the *resolution* (Δm) is a property of the *mass spectrum* (mass spectral peak) [5].

The spectrum measured with increasing *R* is a source of more precise information, especially for high weight compounds, as shown in Fig. 2. The resolving power value of 10,000 or more can display peaks of the mass spectral cluster. The effect is sufficient for spectral characterization of the compound by spectral base by data searching. The determination of the cluster components also makes it possible for isotopomeric pattern investigation. An instrument of high resolving power is necessary to assure that the *m*/*z* value of the ion being measured is of an ion of a single elemental and/or isotope composition. The accurately determined *m*/*z* value of a mass spectral peak may be a weighted average of multiple ions, of different isotope and/or elemental compositions if the resolving power of the instrument used is insufficient to register very small differences in *m*/*z* values.

3. Accurate location of mass spectral peak (accurate mass)

Accurate mass (recommended – measured accurate mass [6]) is an experimentally determined mass of a molecule, ion or radical that allows the determination of elemental composition, which can be predicted [7]. The term is used to some number of decimal places (commonly 4 or 3). An experimental mass should be presented by measuring precision [8]. Accurate mass measurements are compared against calculated exact mass values for various combinations of elements to determine an elemental composition and so the average mass value is used when the resolution of the data is not sufficient to identify monoisotopic *m*/*z* value. When a measured exact mass value is not favourably comparable with various calculated exact mass values, it may be possible to show that the measured accurate mass peak is actually two mass spectral peaks that have become merged. This can be done by reducing the differences between measured *m*/*z* values (operation at a higher resolving power).

The low resolution spectrum contains peaks located at integer *m*/*z* values which is subject to the isotopic contents, and then $\Delta m = 1$ (actually $\Delta m = 0.1 - 1$). The resolution $\Delta m = 0.0001$ (1 ppm) can be expected as a parameter measured in high-resolution conditions, which does not imply that every high resolution work provides for 0.0001 u accuracy. The absolute inversion of the rule is false: high-resolution spectrum is a synonym for high accuracy in some cases. It causes a misunderstanding; however; under which conditions is it true?

		Peaks location accuracy ***Δm***					
	class	**10⁻⁴**	**10⁻³**	**10⁻²**	**10⁻¹**	**10⁰**	u.
sum in area	from	$< 10^{-4}$	$> 1.5{\cdot}10^{-4}$	$> 1.5{\cdot}10^{-3}$	$> 1.5{\cdot}10^{-2}$	$> 1.5{\cdot}10^{-1}$	m/z
	up to	$< 1.5{\cdot}10^{-4}$	$< 1.5{\cdot}10^{-3}$	$< 1.5{\cdot}10^{-2}$	$< 1.5{\cdot}10^{-4}$	< 1.5	

Scheme 1. Limits of peaks separation *vs.* spectrometer resolution.

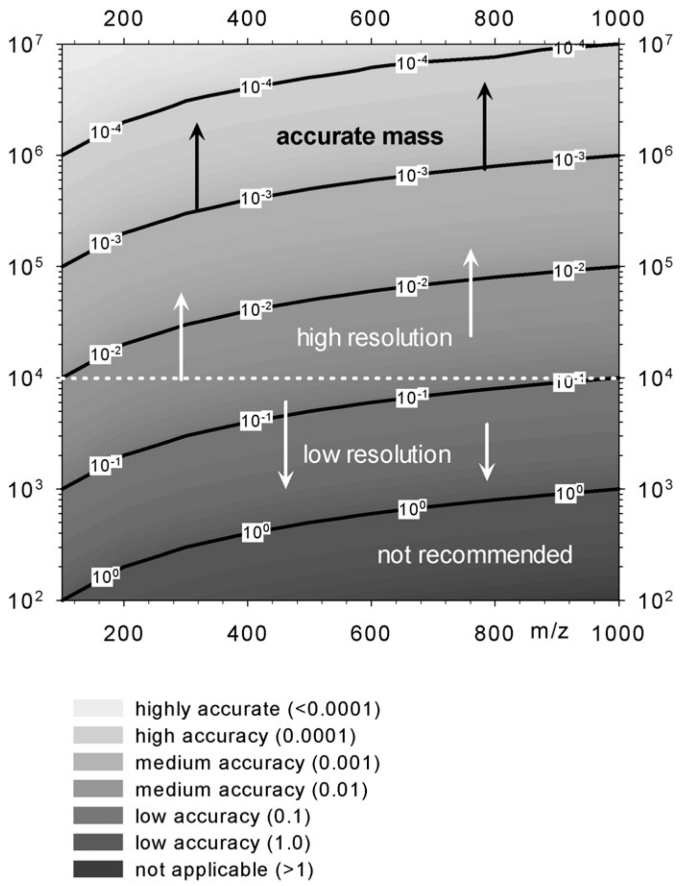

Fig. 3. Modeling of relation $\Delta m = f(R,M)$ of mass spectral resolution, resolving power and peak matching (the part of the surface marked with the light shade of gray above the line denoted with 10^{-3} concerns the high resolution MS and 0.001 Da or higher mass accuracy).

The accurate atomic mass values are not integer; because of the mass defect (negative for light elements up to N, and positive for other nuclides). The accurate location of the mass spectral peak should be in agreement with the exact mass calculated from isotopic contents of the ion and the mass defects corresponding to the nuclides considered. Examples of correct discussion may be found in Smith and Busch [9].

The precisely measured location (0.0001 u or better) of the mass spectral peak, the isotope occurrences and their exact masses calculated with mass defects are fundamentals in the element content determination (elemental composition) [10].

4. Relations of resolving power and ionic mass *vs.* precise location of mass spectral peak

The calculations were based on the resolving power definition: $R = M/\Delta m$. The resolving power from 100 up to 10^7 and ion masses between 100 and 1000 u were tested. All possibilities of the three parameters and results are presented in Table 1.

The results were sorted by a number of classes according to instrumental resolution groups after the manner presented in Scheme 1.

The data presented are transformed as function $\Delta m = f(R,M)$ and will be seen in Fig. 3 as a contour plot. In which region can the accurate mass correspond to high resolution?

The area of $\Delta m < 0.001$ u (10 ppm) presents conditions guaranteeing the high precision in determination of peak location to predict the elemental contents of the involved ion. The area between 0.1 and 1 u (100–1000 ppm) concerns the low resolution.

5. Conclusion

A common mistake made in referring to instruments in the high-resolution mode is that they automatically produce the accurately measured mass data. This term is used when reporting the mass to some numbers of decimal places, and the measured mass should be reported with a precision of measurement. All spectrometers are capable of yielding measured accurate mass data regardless of their resolving power capability through the use of peak matching. In conclusion, it would be appropriate to define two areas of high resolution in mass spectrometry:

1. high resolution which can be applied for isotopomeric modeling of the investigated cluster ($\Delta m > 0.05$ u, above 500 ppm);
2. ultra high resolution for the elemental contents predictions ($\Delta m < 0.001$ u, below 10 ppm).

The area ($0.05 > \Delta m > 0.001$ u, 10–500 ppm) between of these ranges could be applied for rapid investigations of compounds having a poly-isotopic element in the molecule.

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