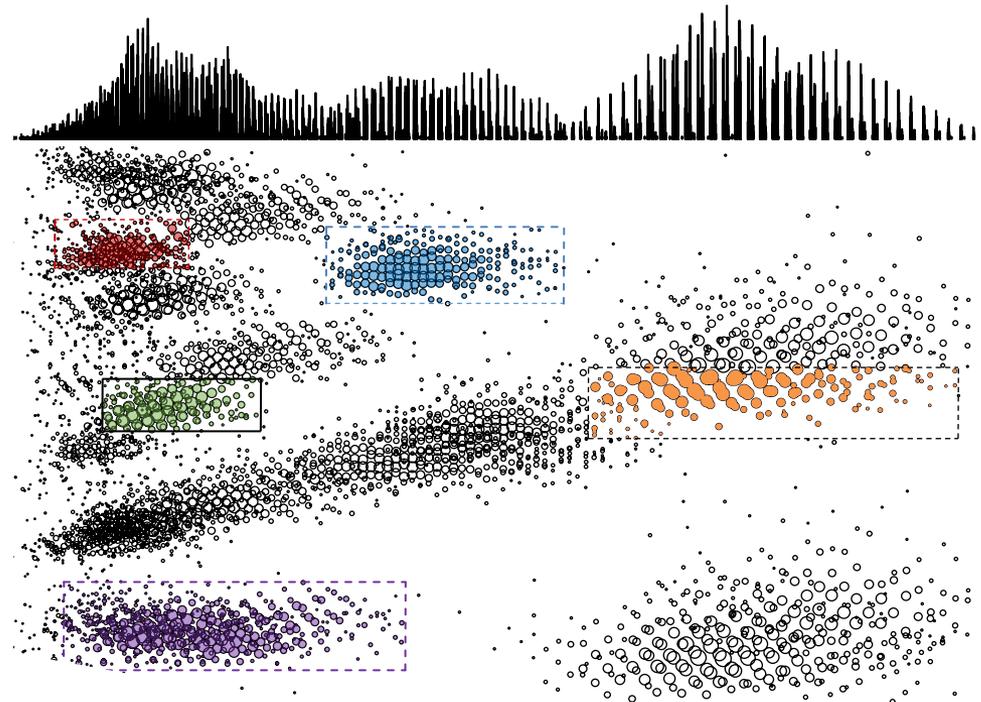


(wicked = great, cool)

KMD for MS: WKD !

T. Fouquet
AIST Tsukuba, Japan



1. Mass defect and Kendrick mass
2. Few examples of KMD analyses
3. What about new terms ?
4. What about a “real-time” KMD plot ?

1. KMD ?

Kendrick Mass Defect

KMD analysis =

data processing tool to visualize mass spectra and/or filter and/or assign ion series using an additional m/z -related dimension

1. Atomic mass defect

Kendrick Mass Defect

Atomic mass constant $m_u = m_a(^{12}\text{C})/12$

$$m_a(^{12}\text{C}) = 12$$

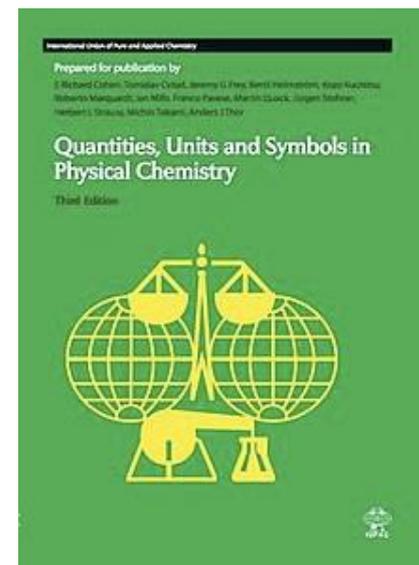
$$m_a(^1\text{H}) = 1.007825$$

$$m_a(^{14}\text{N}) = 14.003074$$

$$m_a(^{32}\text{S}) = 31.972071$$

...

E. R. Cohen, T. Cvitas, J. G. frey et al, Quantities, Units and Symbols in Physical Chemistry (3rd ed) IUPAC, 2007.



1. Atomic mass defect

Kendrick Mass Defect

Atomic mass constant $m_u = m_a(^{12}\text{C})/12$

Mass excess (atom)

Element-dependent

$$\Delta = m_a - Am_u$$

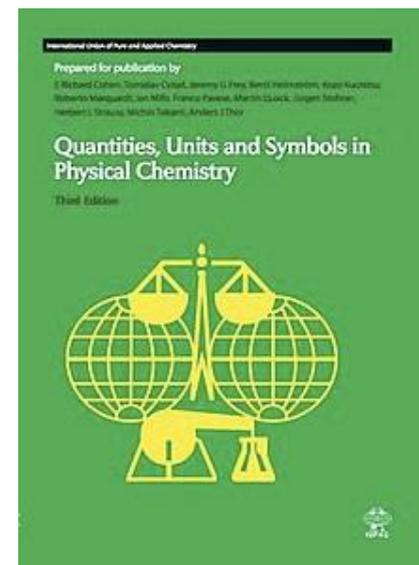
Mass of atom

Mass number
(nucleon number)

Fractional mass (atom) $= \text{round}(m_a) - m_a$

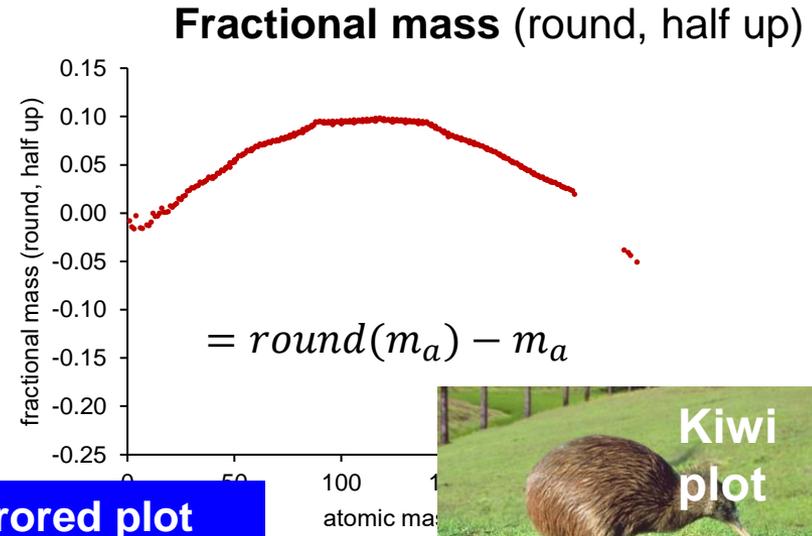
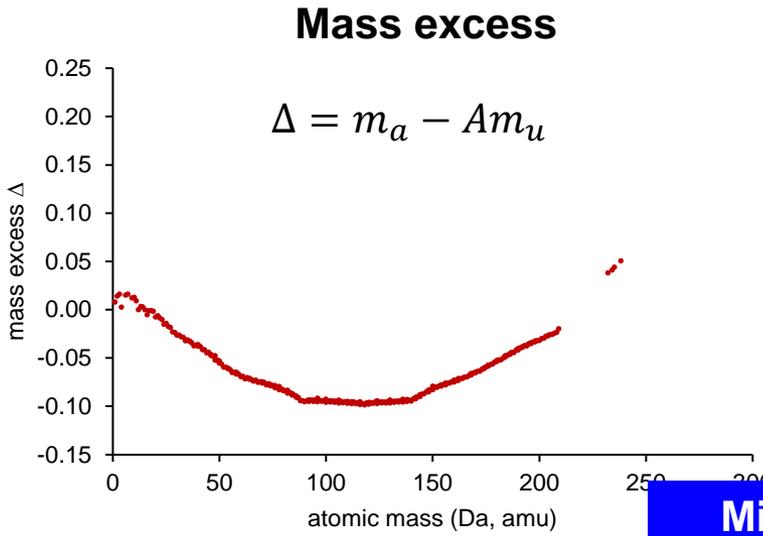
Element-independent

E. R. Cohen, T. Cvitas, J. G. frey et al, Quantities, Units and Symbols in Physical Chemistry (3rd ed) IUPAC, 2007.



1. Atomic mass defect

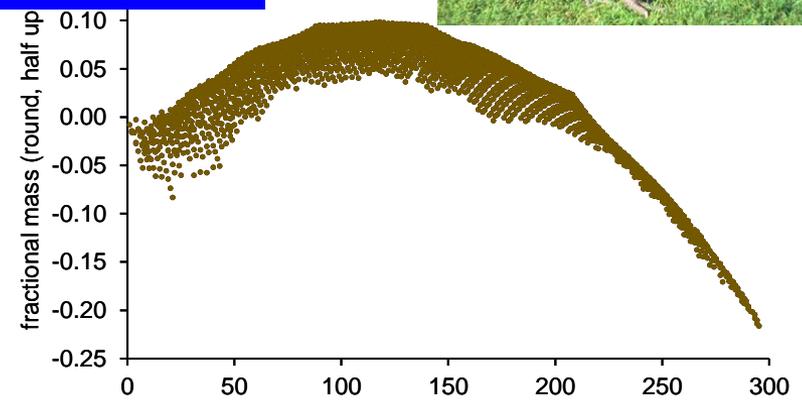
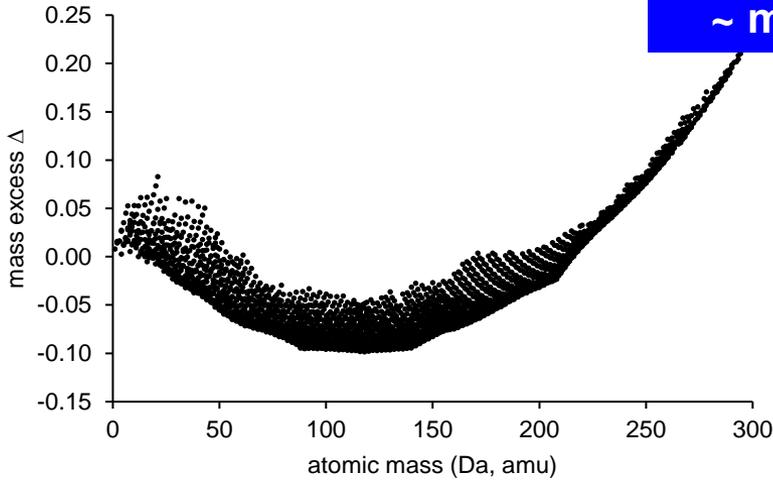
287 stable
Isotopes
from 82
elements



**Mirrored plot
~ mass defect**



“All” 3120
Isotopes
from all
elements



“mass defect plot”

1. Molecular mass defect

Kendrick Mass Defect

Nominal mass

Mass excess (molecule)

composition-dependent

$$\Delta = m - A$$

Monoisotopic mass

Polystyrene
 $C_4H_9(C_8H_8)_{100}H$
 $A=10458$
 $m=10464.338$
 $\Delta=6.338$

Fractional mass (molecule) = $round(m) - m$

Composition-independent

Fractional mass=-0.338

S. Pourshahian. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 1836.



Education Society for Mass Spectrometry, 2017



J. Am. Soc. Mass Spectrom. 28(17):1836-1841
 DOI: 10.1002/jmsl.20171836

CRITICAL INSIGHT

Mass Defect from Nuclear Physics to Mass Spectral Analysis

Sohel Pourshahian

Alvie BioPharma, Inc. Part of the Johnson Pharmaceutical Companies, South San Francisco, CA 94080, USA



Abstract: Mass defect is associated with the binding energy of the nucleus. It is a fundamental property of the nucleus and the principle behind nuclear energy. Mass defect has also entered into the mass spectrometry terminology with the availability of high-resolution mass spectrometry and has found application in mass spectral analysis. In this application, isotopic masses are differentiated and identified by their mass defect. What is the relationship between nuclear mass defect and mass defect used in mass spectral analysis, and are they the same?

Keywords: Mass defect, Mass excess, Delta mass, Fractional mass, Mass defect filter, Peaking function, Nuclear binding energy, High-resolution mass spectrometry

Received 17 April 2017; Revised 29 May 2017; Accepted 13 June 2017; Published Online 23 July 2017

Introduction

Mass defect and binding energy of the nucleus are two related fundamental properties of atoms. Even though they are often discussed in the context of nuclear energy, mass defect and binding energy are concepts with wider applications. Mass defect exists universally in bound systems of all sizes in which the components are bound together by force. It is applicable to small systems such as the nucleus of an atom as well as large systems such as the solar system [1]. A bound system has a lower potential energy and mass than its components in an unbound state. The difference between the mass of a bound system and its constituents in an unbound state is referred to as mass defect. Binding energy is the energy equivalent of mass defect according to Einstein's theory of mass-energy equivalence, and is more pronounced in the atomic nucleus than the solar system due to its small size and enormous amount of energy involved. Nuclear binding energy is the source of energy of the sun and nuclear power plants.

The emergence of the concepts of nuclear mass defect and binding energy goes back to the early 20th century after atomic weights were determined accurately by chemical methods and the derivation of atomic masses from which numbers were investigated by mass spectrograph [2–4]. Early research in mass spectrometry was primarily focused on determining the accurate mass and isotopic compositions of elements. However, by the 1940s this work was largely complete and mass

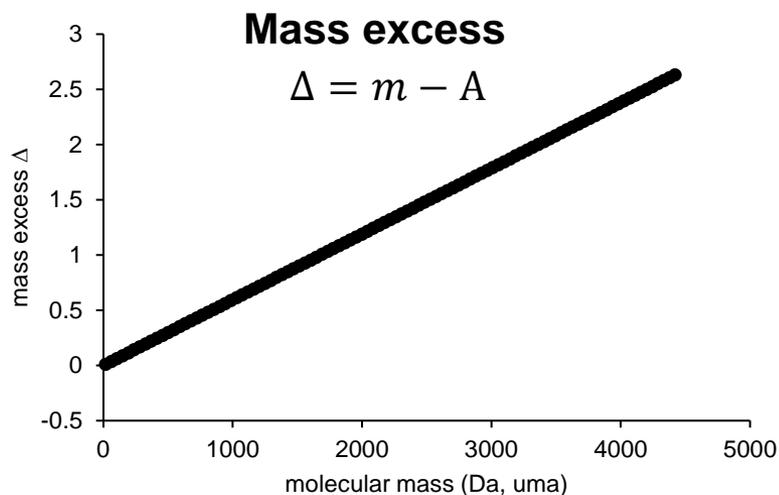
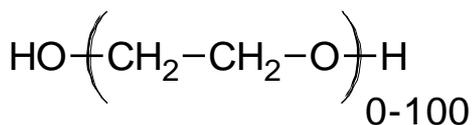
spectrometry moved from academic laboratories into research and development facilities in the petroleum and chemical industry [5]. Resolution and accuracy of the instruments increased over the years and the accurate mass of molecules made determination of their empirical formula possible. Mass defect re-enters scientific literature this time for mass spectral analysis and applied to the identification of molecules rather than analysis of atoms [6, 11]. In order to make the following discussion clear, application of mass defect in nuclear physics and mass spectral analysis are referred to as nuclear and chemical mass defect, respectively, even though the distinction does not exist in the literature. Chemical mass defect, defined as the difference between the monoisotopic mass and the nominal mass, became a useful criterion for sorting through a crowded mass spectrum from a complex sample in order to identify compounds of interest among many unrelated ion peaks. It was first utilized to visualize and identify different classes of compounds in petroleum samples, and later found applications in drug metabolism and pharmaceutical analysis and identification of endogenous compounds in complex biological samples [12].

Nuclear mass defect and binding energy are often discussed in connection with chemical mass defect and mass spectral analysis and are incorrectly considered to be the same [13–15]. Referring to both nuclear and chemical mass defect simply to mass defect can cause confusion, especially when discussed among a broader audience from different disciplines. For example, the nuclear mass defect for carbon ^{12}C is 0 (mass unit), which when converted to energy is equal to the binding energy per nucleon (protons and neutrons in the

Correspondence to: Sohel Pourshahian, email: sohel@alviebio.com

1. Molecular mass defect

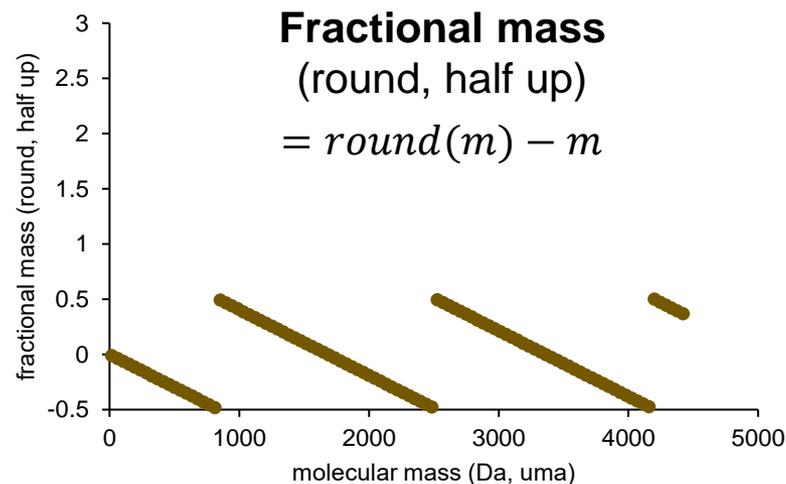
“mass excess plot”



$$m(\text{C}_2\text{H}_4\text{O}) = 2 * 12 + 4 * 1.007825 + 15.994915 = 44.026215$$

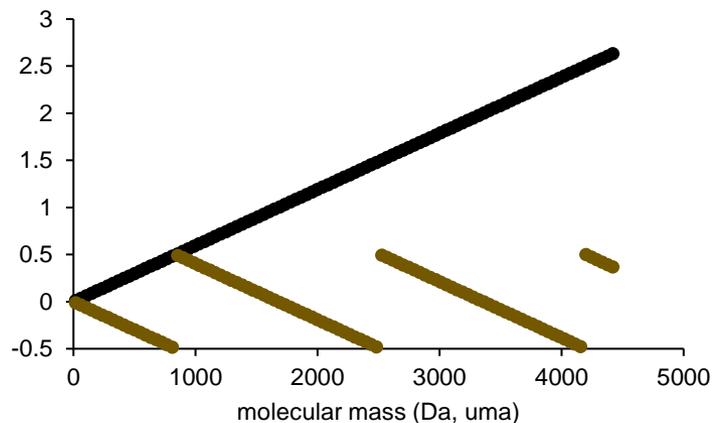
$$A(\text{C}_2\text{H}_4\text{O}) = 2 * 12 + 4 * 1 + 16 = 44$$

$$\Delta(\text{C}_2\text{H}_4\text{O}) = +0.026215$$



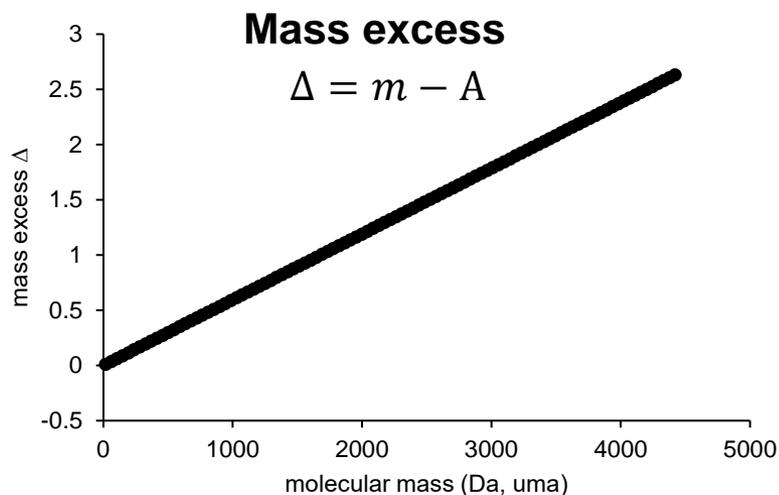
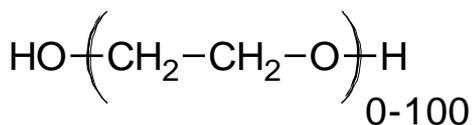
Mirrored plot
~ mass defect !

“mass defect plot”



1. Molecular mass defect

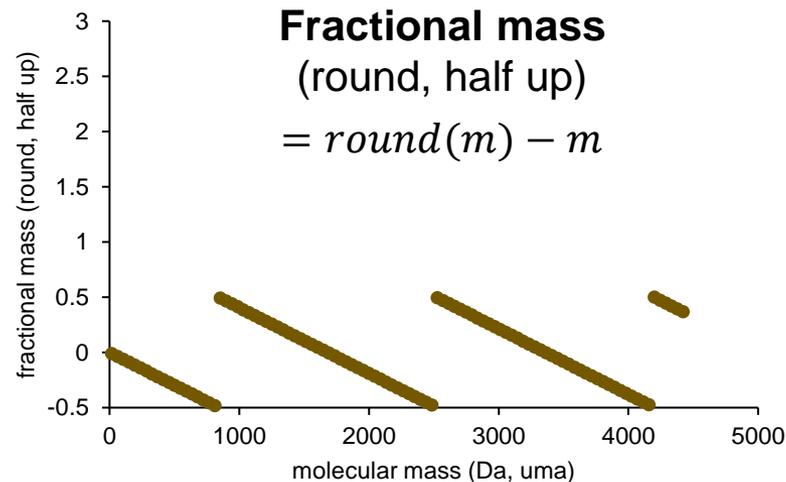
“mass excess plot”



$$m(\text{C}_2\text{H}_4\text{O}) = 2 * 12 + 4 * 1.007825 + 15.994915 = 44.026215$$

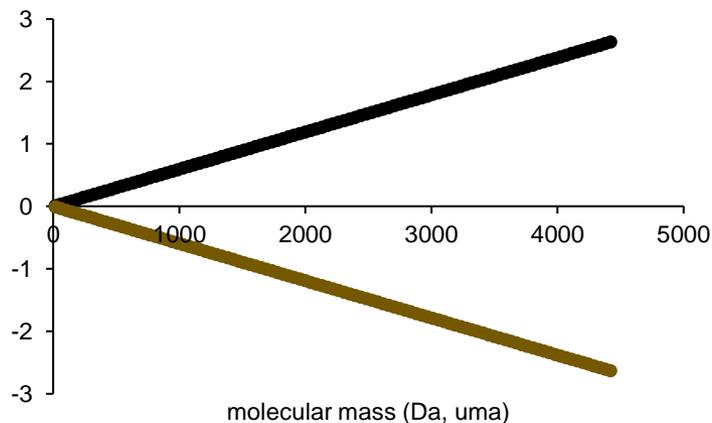
$$A(\text{C}_2\text{H}_4\text{O}) = 2 * 12 + 4 * 1 + 16 = 44$$

$$\Delta(\text{C}_2\text{H}_4\text{O}) = +0.026215$$



Mirrored plot
~ mass defect !

“mass defect plot”



1. Kendrick mass

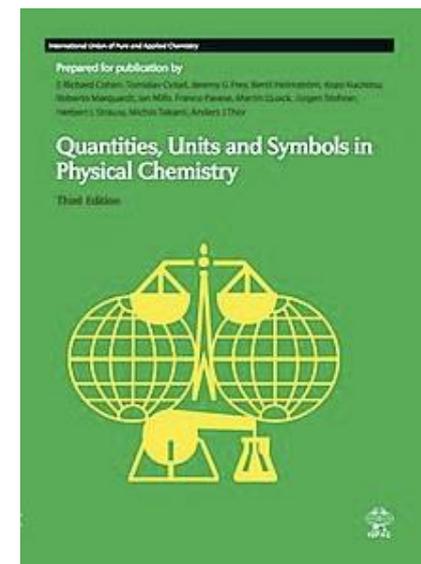
Kendrick Mass Defect

Atomic mass constant $m_u = m_a(^{12}\text{C})/12$

$$m_a(^{12}\text{C}) = 12$$

$$m(^1\text{H}_2^{12}\text{C}) = 14.015650$$

E. R. Cohen, T. Cvitas, J. G. frey et al, Quantities, Units and Symbols in Physical Chemistry (3rd ed) IUPAC, 2007.



1. Kendrick mass

Kendrick Mass Defect

Change of mass scale

$$m(^1\text{H}_2^{12}\text{C}) = 14.015650$$



$$m(^1\text{H}_2^{12}\text{C}) = 14$$

E. Kendrick. *Anal. Chem.* **1963**, *35*, 2146

A Mass Scale Based on $\text{CH}_2 = 14.0000$ for High Resolution Mass Spectrometry of Organic Compounds

EDWARD KENDRICK¹

Analytical Research Division, Esso Research and Engineering Co., Linden, N. J.

► The problems of computing, storing, and retrieving precise masses of the many combinations of elements likely to occur in the mass spectra of organic compounds are considerable. They can be significantly reduced by the adoption of a mass scale in which the mass of the CH_2 radical is taken as 14.0000 mass units. The advantage of this scale is that ions differing by one or more CH_2 groups have the same mass defect. The precise masses of a series of alkyl naphthalene parent peaks, for example, are 127.9195, 141.9195, 155.9195, etc. Because of the identical mass defects, the similar origin of these peaks is recognizable without reference to tables of masses. Tables of the mass defects for combinations of H, C¹³, C¹⁴, N, O, S³⁴, and S³² are presented.

DOUBLE focusing mass spectrometers with resolutions of one part in 2000 or better are now coming into use as analytical instruments. Commercial instruments after the original design by Mattauach and Herzog (7-9) have been described by Voorhies and coworkers (5) and by Craig and coworkers (6). Another instrument based on a design by Nier and Roberts (4, 10-12) has been described by Craig and Ervick (3). Beynon (1) has pioneered the application of high resolution mass spectrometers to organic chemistry.

The high resolution of these instru-

ments makes possible precise mass measurement to a few parts in 10⁵ or better. This is sufficient to distinguish between ions having the same mass number, but different chemical composition. The precise mass of an ion is the sum of the masses of its constituent elements and the molecular formula can usually be found from the mass determination. This ability to determine the chemical composition of an ion from a measurement of its mass gives mass spectrometry a new dimension.

With the advent of the high resolution mass spectrometer have come the problems of computing, storing, and comparing large amounts of mass data. The masses of the isotopes of most elements are known to very high accuracy, but the number of combinations of these elements in organic chemistry is extremely large. Published tables cover only a fraction of the combinations. Comprehensive tables of precise masses are required to give the precise masses of ions used for internal calibration standards in high resolution mass spectra and to convert the precise mass measurements made on samples into molecular formulas. Such tables are indispensable for these tables. No more than four atoms of oxygen, nitrogen, sulfur or carbon-13 and no more than two atoms of sulfur-34 are allowed in any combination. The total number

of compounds having masses as high as 1000, or even higher.

The table of precise masses most widely used at present is by Beynon (1), which lists, in addition, the relative abundances of isotope peaks. This table, which is based on $\text{O}^{16} = 16.00000$, covers combinations of elements up to a mass number of 250, and is limited to those containing no more than four oxygen atoms, four nitrogen atoms, and a total of no more than six atoms other than carbon and hydrogen. Beynon's table has about 6000 entries. Beynon's table has recently been extended to mass 500 and based on the standard $\text{C}^{12} = 12.0000$ (2). The inclusion of sulfur (S^{32} and S^{34} isotopes) and C^{13} would increase the size of the table enormously. Over one and a half million entries would be required to cover the ions up to mass 600.

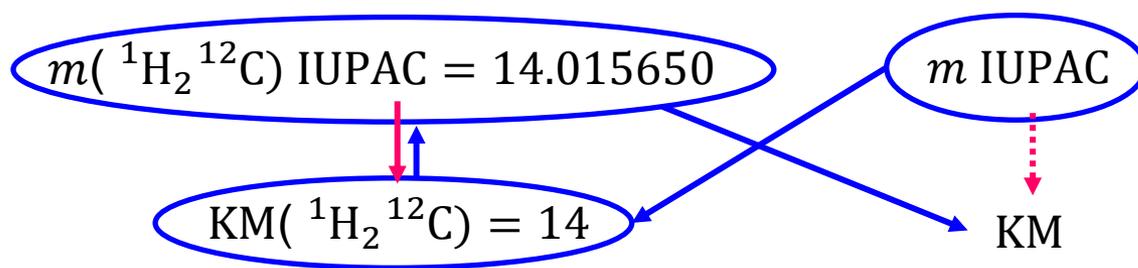
A mass scale with $\text{CH}_2 = 14.00000$ enables the same data—i.e., up to mass 600—to be expressed in less than 100,000 entries. This reduction is a consequence of the same defect's being repeated at intervals of 14 mass units—i.e., one (CH_2) group. Restrictions similar to those imposed by Beynon have been used for these tables. No more than four atoms of oxygen, nitrogen, sulfur or carbon-13 and no more than two atoms of sulfur-34 are allowed in any combination. The total number

¹ Present address: Esso Research, Ltd., Abingdon, Berkshire, England.

1. Kendrick mass

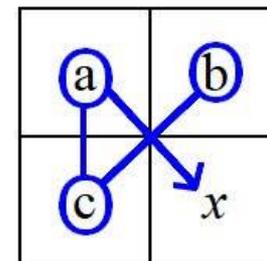
Kendrick Mass Defect

$$KM = m_{IUPAC} \cdot \frac{14}{14.015650}$$



Change of mass scale

$$m(^1\text{H}_2\text{ }^{12}\text{C}) = 14$$



the Rule of 3

1. Generalized Kendrick mass

Nominal mass

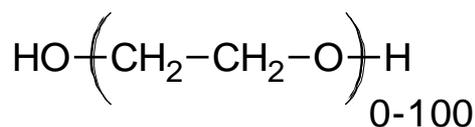
↓

$$KM = m_{IUPAC} \cdot \frac{\text{round}(R)}{R}$$

↑

Exact mass (IUPAC) of any moiety

1. Kendrick “mass defect” plot



Repeating unit: C₂H₄O

R = 44.02621 (IUPAC)



R = 44

$$\text{KM} = m_{\text{IUPAC}} \cdot \frac{44}{44.02621}$$

fractional m = round(m) - m ~ mass defect (MD)

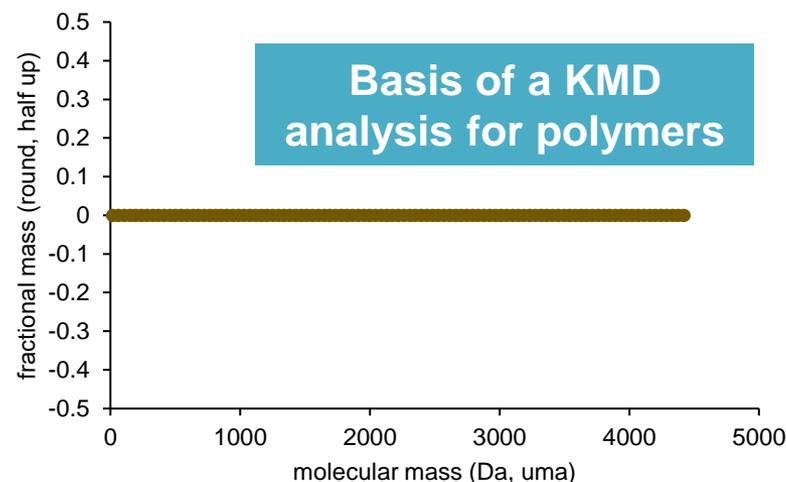
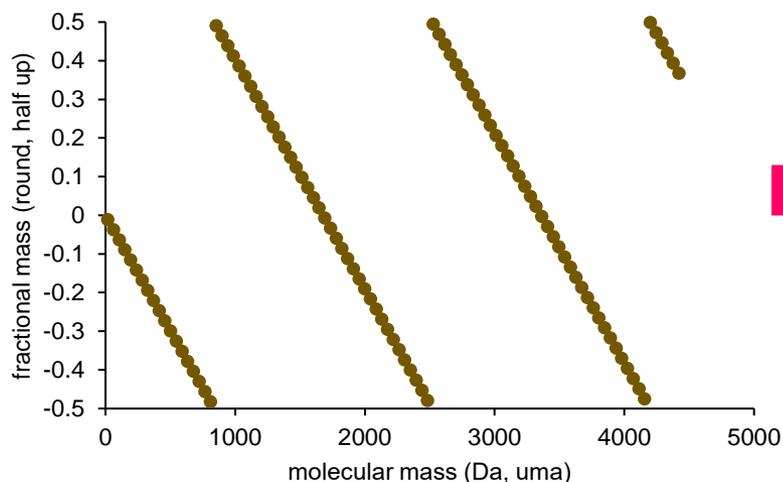
fractional KM = round(KM) - KM ~ Kendrick mass defect (KMD)

“mass defect” plot

Kendrick “mass defect” plot

$$\text{round}(44.026215) - 44.026215 = -0.026215$$

$$\text{round}(44) - 44 = 0$$



1. Generalized Kendrick mass (2)

Kendrick mass

$$KM = m_{IUPAC} \cdot \frac{\text{round}(R)}{R}$$



Kendrick mass-to-charge ratio

$$KM = \frac{m}{z} IUPAC \cdot \frac{\text{round}(R)}{R}$$



= 1

Charge-dependent resolution-enhanced Kendrick m/z

$$KM = Z \cdot \frac{m}{z} \cdot \frac{\text{round}(R/x)}{R/x}$$

$\text{round}(2R/3) < x < \text{round}(2R)$
“recommended range”

“Divisor” for enhancement of resolution



$$KM(R, x, Z) = Z \cdot \frac{m}{z} \cdot \frac{x}{R}$$

$$KMD(\text{fractional mass} \sim \text{mass defect}) = \text{round}(KM) - KM$$

1. KMD analysis

Anal. Chem. 2017, 89, 2682

$Z = 1$
 $x = \text{round}(R) \pm 1, 2, 3 \dots \in \mathbb{N}$
Resolution-enhanced

(Z, x)
 $Z = \{z, z + 1, z + 2 \dots\}$
 $x \in \mathbb{R}$

**Pseudo-continuous
 resolution-enhanced**

Rapid Commun Mass Spectrom. doi:
 10.1002/rcm.8500

$Z = z$
 $x = \text{round}(R) \pm 1, 2, 3 \dots \in \mathbb{N}$
**Charge-dependent
 resolution-enhanced**

J. Am. Soc. Mass Spectrom. 2014, 25, 1346

$Z = 1$
 $x = \text{round}(R)$
Regular

**+ combination in
 sequential analysis**

J. Am. Soc. Mass Spectrom.
 2018, 29, 1611

$Z = z$
 $x = \text{round}(R)$
Charge-dependent

$$\text{KM}(R, x, Z) = Z \cdot m/z \cdot \frac{x}{R}$$

Main point

$$\text{KMD}(\text{fractional mass} \sim \text{mass defect}) = \text{round}(\text{KM}) - \text{KM}$$

Minor issue

Major asset

KMD analysis done using

Any spreadsheet

Excel spreadsheet with macro and VBA: Kendo
+ several R programs



Open source
mMass
MZMine

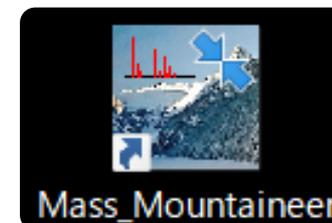
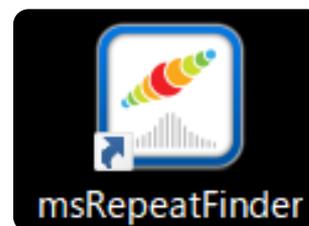


 MZmine 2.40

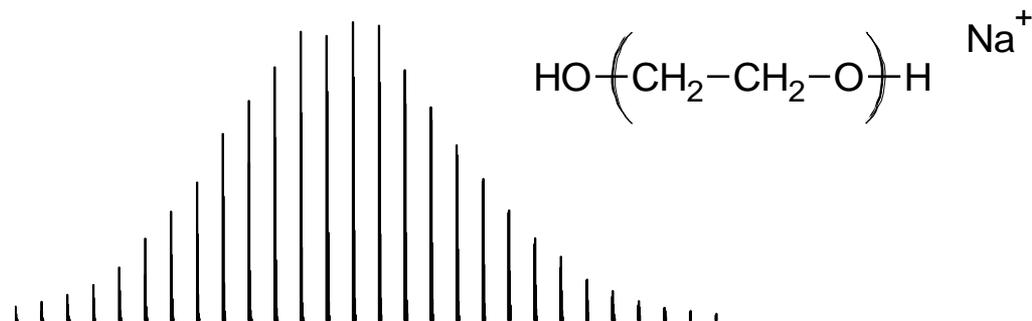


Commercial programs

Mass Mountaineer, msRepeatFinder,
Spectrascope



2. Examples: KMD plots



Example 1:

MALDI-MS, PEO

Rotating KMD plot
Enhancement of resolution (isotopic pattern)

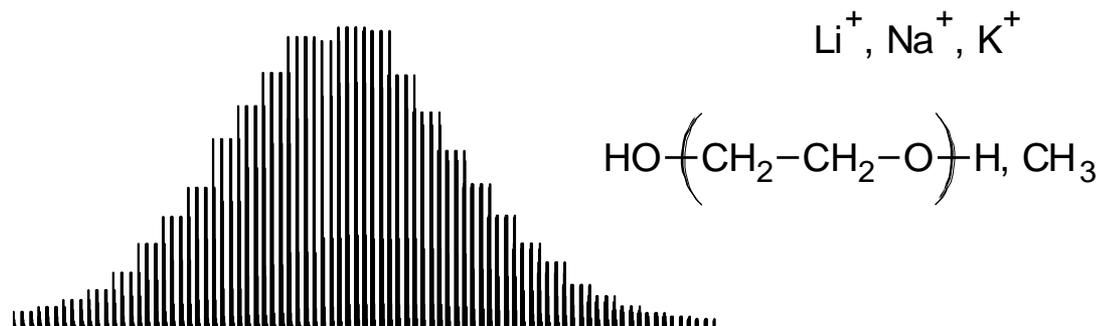
RB Cody, T Fouquet. *Anal. Chem.* **2018**, 90, 12854

H Sato, S Nakamura, K Teramoto, T Sato. *J Am Soc Mass Spectrom.* **2014**, 25, 1346

T Fouquet, H Sato. *Mass Spectrom. (Tokyo)* **2017**, 6, A0055

T Fouquet, H Sato. *Anal Chem.* **2017**, 89, 2682

2. Examples: KMD plots



Example 2:

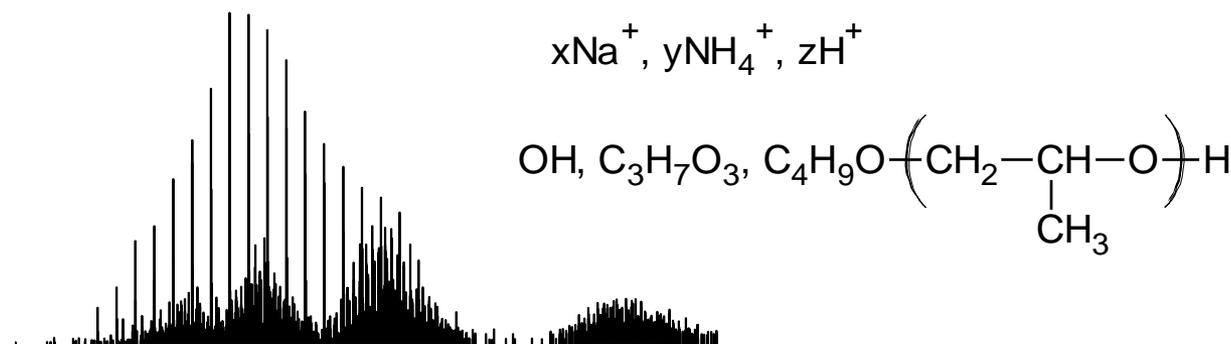
MALDI-MS, PEO

(pseudo-continuous) Enhancement of resolution
(end-groups, adducted ion)

S. Nakamura, RB Cody, H Sato, T Fouquet. *Anal Chem.* 2019, 91, 2004

Fouquet T, Nakamura S, Sato H, Cody R. *Rapid Commun Mass Spectrom.* just accepted

2. Examples: KMD plots



Example 3:

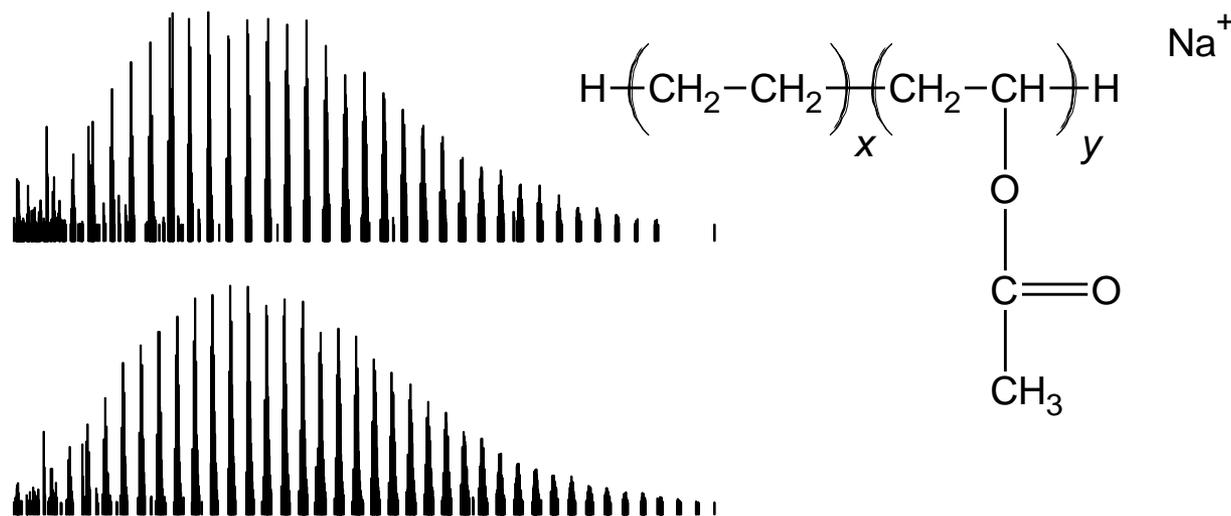
ESI-MS, PPO

Charge state distribution (regular KMD plot)
 Charge-dependent KMD plot
 Enhancement of resolution (end-groups)
 Simulator

Q Zheng, Y Zhang, W Diono, T Fouquet, X Zeng, H Kanda, M Goto. *Fuel* **2019**, under review

T Fouquet, RB Cody, Y Ozeki, S Kitagawa, H Ohtani, H Sato. *J. Am. Soc. Mass Spectrom.* **2018**, 29, 1611

2. Examples: DP plot



Example 4:

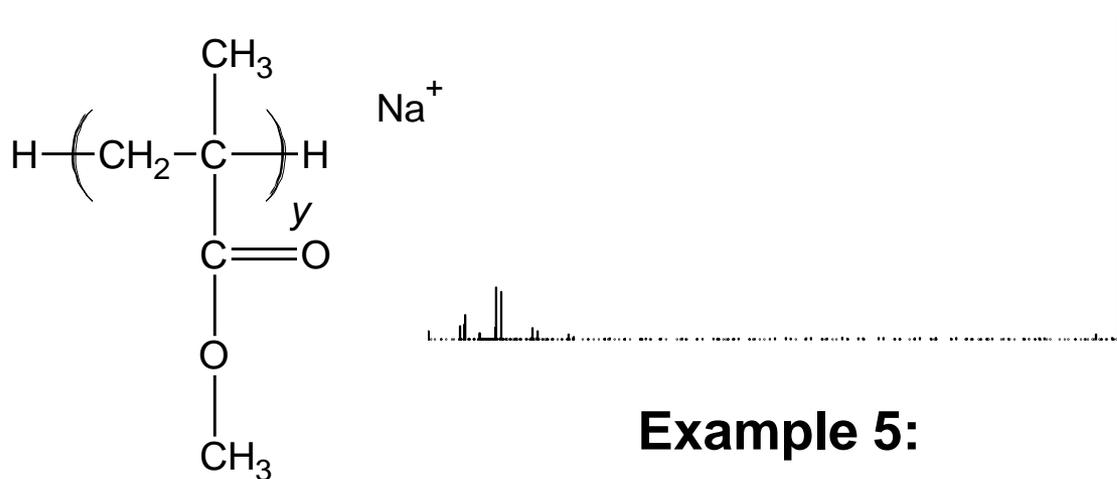
MALDI-MS, EVA40 vs EVA25

Allied plot: “degree of polymerization” plot (DP plot)

T Fouquet, RB Cody, H Sato. *J. Mass Spectrom.* **2017**, 52, 618

T Fouquet, S Nakamura, H Sato. *Rapid Commun. Mass Spectrom.* **2016**, 30, 973

2. Examples: Remainders plot



Example 5:

MALDI-TOF/TOF MS, PMMA

Allied plot: “remainders of KM” plot (RKM plot) for low-resolution data

T Fouquet, T Satoh, H Sato. *Anal Chem.* **2018**, *90*, 2404

T Nagy, A Kuki, M Zsuga, S Kéki. *Anal Chem.* **2018**, *90*, 3892

3. What about new terms ?

- Kendrick mass analysis
- Kendrick mass defect analysis
- KMD analysis
- Kendrick mass defect spectrum
- Kendrick mass defect plot
- KMD plot
- Kendrick mass plot
- Kendrick mass defect diagram
- Kendrick plot
- resolution-enhanced
- charge-dependent
- pseudo-continuous enhancement

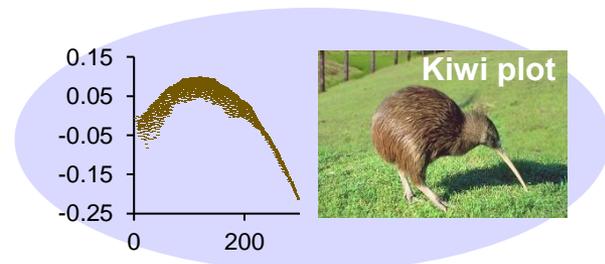


Google で検索または URL を入力



- Mass defect
- mass excess
- fractional mass ~ mass defect
- mass vs. mass-to-charge

Kendrick plot or **K-plot** ?
Implied function of R, x, Z



4. What about a real-time K(MD)-plot ?

Instant computation of KMD plot while recording data ?

The screenshot displays the JEOL software interface for the MSTornado instrument. The main window shows a 'SpecTrace' plot with 'Intensity (%)' on the y-axis (0 to 8) and 'Spectrum Number' on the x-axis (0 to 200). The plot is titled 'MSTornado (JEOL)'. Below it is an 'Accumulated Spectrum' plot with 'Intensity (%)' on the y-axis (0.0 to 3.0) and 'm/z' on the x-axis (800 to 4000). The interface includes a menu bar (File, Load Method, Save Method, DataSet, Tools, Help), a control panel on the left with buttons for 'Main', 'Auto Acquisition', 'Data Processing', and 'Instrument Monitor', and a right-hand control panel with 'Auto Stage Drive' and 'Laser Intensity (%)' controls. At the bottom, there are 'Data Acquisition' and 'SpecTrace' settings panels.

KMD plot =

- not CPU-intensive
- from m/z only

4. What about a real-time K(MD)-plot ?

Instant computation of KMD plot while recording data ?

The screenshot displays a software interface for a mass spectrometer. The main window is divided into several sections:

- Control Panel (Top):** Includes menu items (File, Load Method, Save Method, DataSet, Tools, Help), Control Method (Spiral-FOG), Process Method (Spiral-dfprm), Current Calibration (0001-SP-2000-df.spcm), and HV Switch (OFF).
- Left Panel:** Contains buttons for Main, Auto Acquisition, Data Processing, and Instrument Monitor. It also shows Target Plate ID (not loaded), DataSet (501-0000000, 304 Spot HST Plate), and Vacuum Gauge status (EVAC Ready).
- Top Graph (SpecTrace):** A plot of Intensity (%) vs. Spectrum Number (0 to 200).
- Bottom Graph (Accumulated Spectrum):** A plot of Intensity (%) vs. m/z (800 to 4000).
- Right Panel:** Features a Laser Intensity (0) control with a value of 61, and a Base unit section with Delay Time (250 ns) and Detector (57) settings.
- Bottom Panel:** Contains Spectrum Setting (Mass Range: 500-3000, Sampling Interval: 0.5 ns) and SpecTrace (Sampling Mass Range: 500-3000, Accumulation Intensity: 0-20) controls.

Two red-bordered boxes on the right side of the interface highlight the following features:

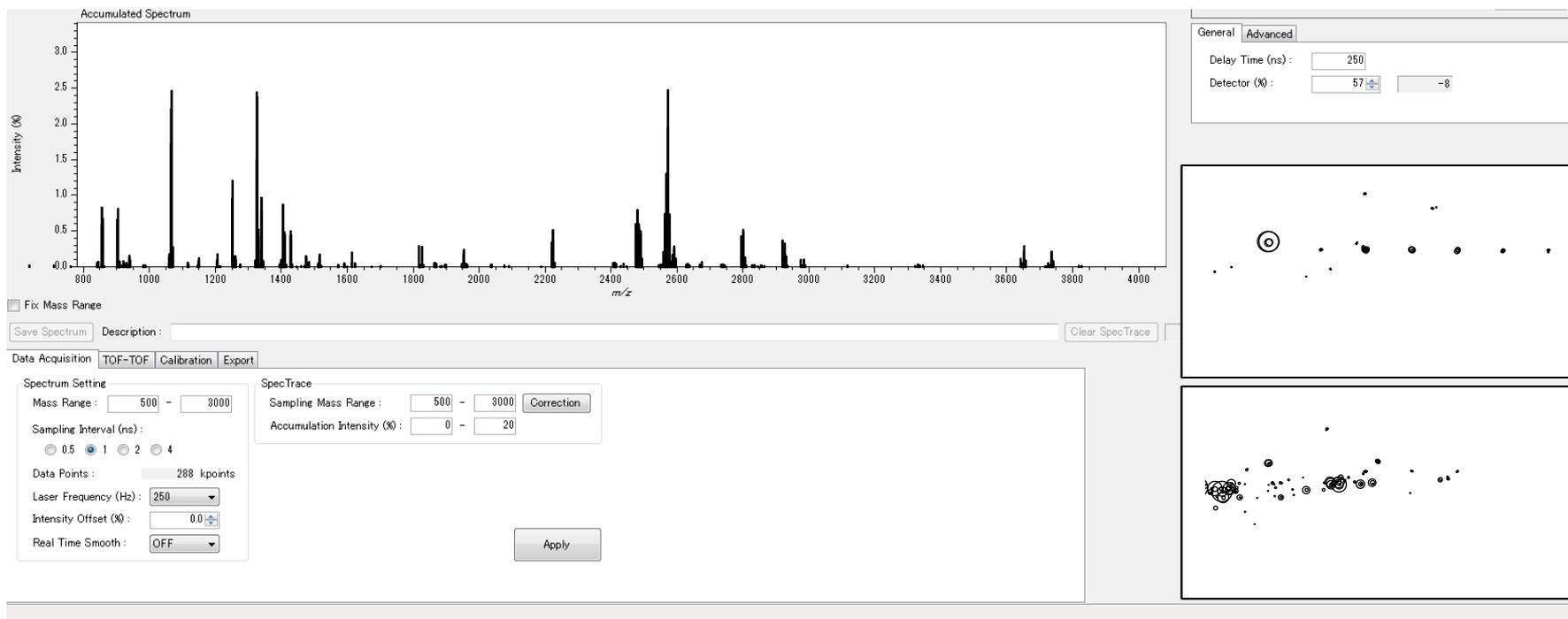
- Base unit**
- KMD plot scan by scan**
- KMD plot sum**

4. What about a real-time K(MD)-plot ?

Instant computation of KMD plot while recording data ?

Examples of use:

- Tracking of sweet spots

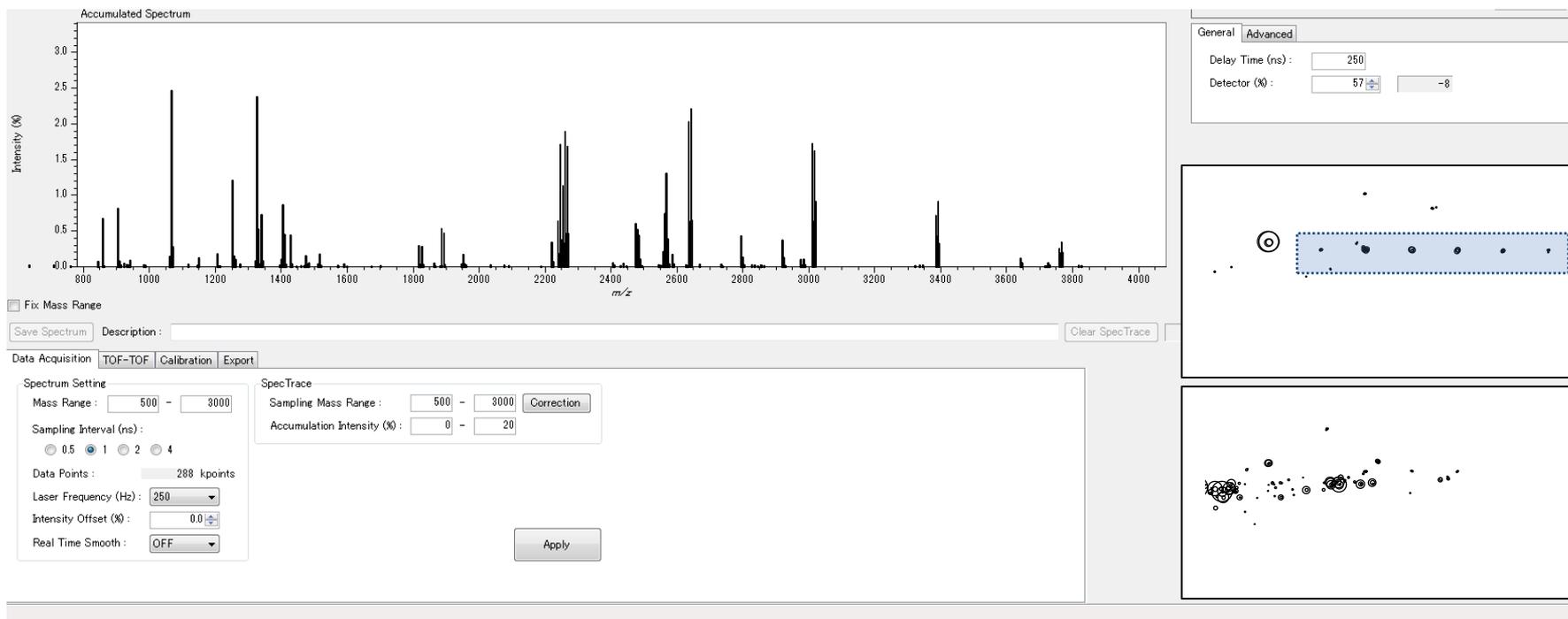


4. What about a real-time K(MD)-plot ?

Instant computation of KMD plot while recording data ?

Examples of use:

- Tracking of sweet spots
- Mass defect filtering

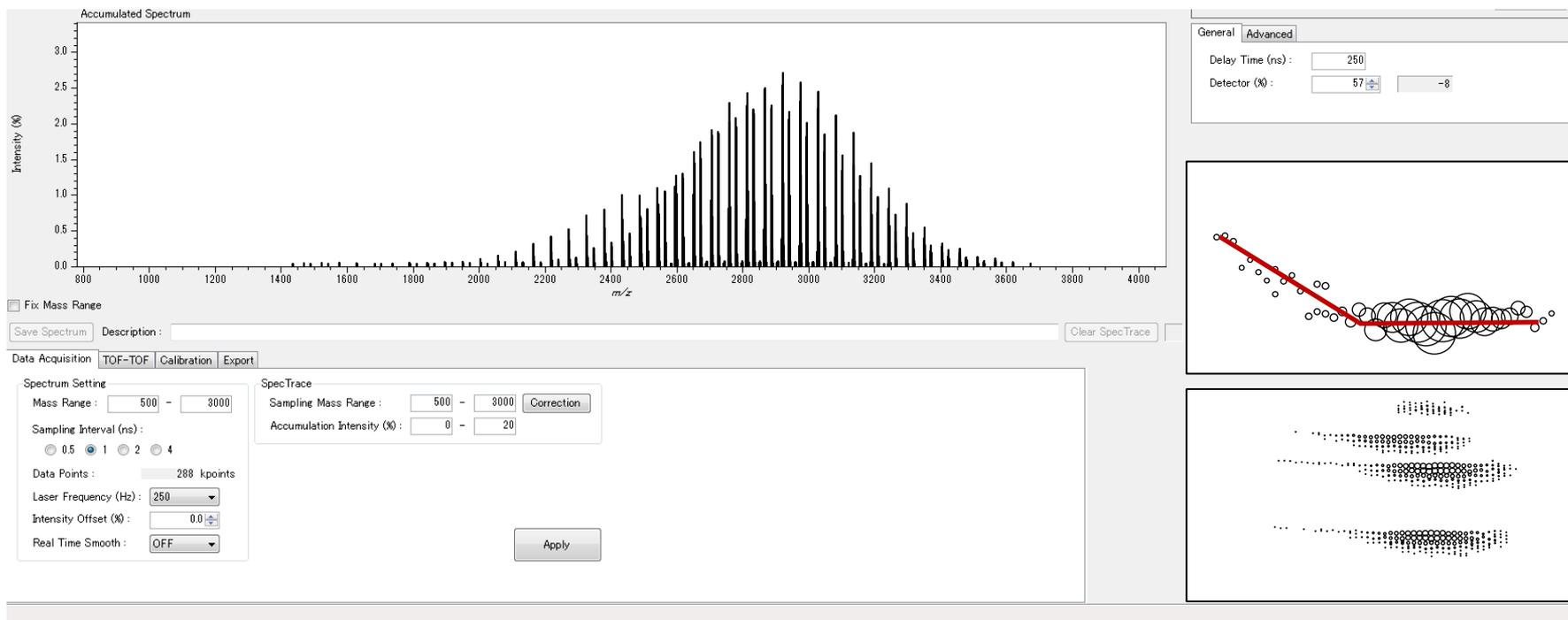


4. What about a real-time K(MD)-plot ?

Instant computation of KMD plot while recording data ?

Examples of use:

- Mass defect filtering
- Tracking of sweet spots
- Miscalibration



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