



# Mass Spectrometry Forum

# Masses in Mass Spectrometry:

# Perfluors and More

Part II of this four-part series on mass calibration looks at the chemical compounds used as mass calibrants in mass spectrometry.

**Kenneth L. Busch**

Corrections belong up front. Part I of this column series included a description of mass scales and units, exhorting readers and users to pay attention to basics. An essential part of basics is good proofreading, a skill that seems to have deserted this author in preparing Part I. Table II in Part I (*Spectroscopy* 19[11], 32–34 [2004]) lists the exact mass for  $^{13}\text{C}$  incorrectly as 13.993355. I considered assigning the blame for such an egregious error to electronic voting machines (it was, after all, an election year, and an election month), or fat fingers on the keyboard, but instead I admit to sloppiness and apologize for the error. The correct value for the exact mass of  $^{13}\text{C}$  is 13.003355. My thanks to the readers who notified me of the error and demanded a recount.

This second installment in the series deals with the chemical compounds used as mass calibrants in mass spectrometry. A preview of this topic appeared in *Mass Spectrometry Forum* in June 1994. While the underlying need for mass calibration in mass spectrometers has not changed, the expansion of more specialized instruments into higher mass ranges, the use of diverse and different ionization methods, and the specific mass accuracy needs of new communities now accessing mass spectral data, all combine to support a more detailed review of mass calibration, including traditional use in lower-mass range instruments (Part II), and extending to new ionization methods and higher-mass, high-throughput biomic systems (Parts III and IV).

**Kenneth L. Busch** is old enough to have 1) used a double-pan analytical balance in chemistry laboratory, and remembers the lecture about draconian consequences should his too-sudden release damage the knife edge, 2) ordered FC-43 directly from that Florida company, and knew where the name came from, and 3) manually tuned the instrument using a scope to set the proper peak shape of the 502 and 614 ions. Contact him at: [wvverners@yahoo.com](mailto:wvverners@yahoo.com).



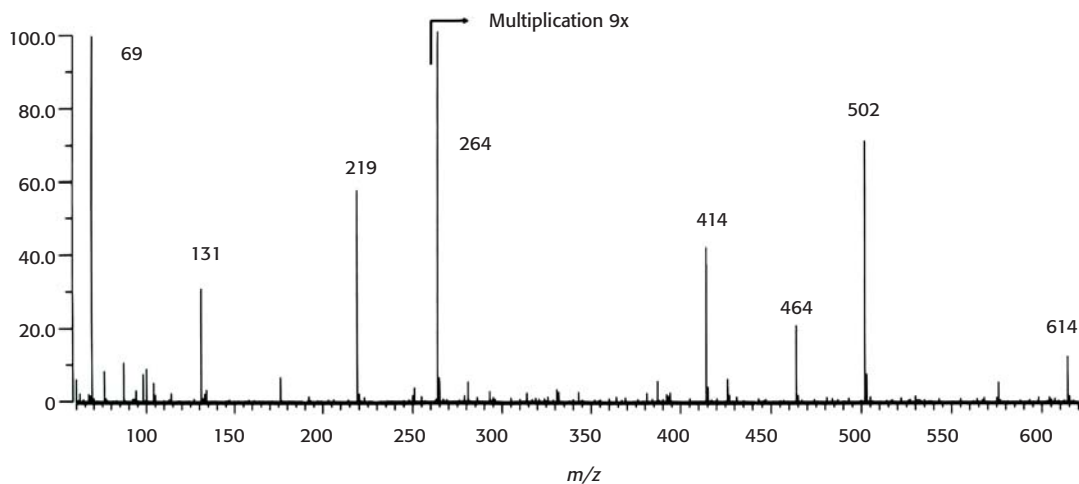
## Certifying Performance

A mass spectrometer is a user-calibrated instrument in which the masses of the ions are determined as relative values (think of a double-pan analytical balance). As a consequence, instrument operation (that is, the particular scan function used) must be certified to provide correct values for the measurement of ion masses. This certification is provided through a process of calibration, which involves examination of the mass spectrum of a mass calibrant. The mass calibrant is a compound for which masses and intensities of ions within its mass spectrum are known. Just as some of us remember using that double-pan analytical balance to compare unknown masses against certified known masses, a calibration of the instrument is performed to demonstrate that the operation of the mass spectrometer, under the specified set of operational parameters, produces a mass spectrum of the calibrant with masses of the ions properly assigned.

“Operation” is a general term that encompasses a multitude of physical and electrical factors. Most of these factors are transparent to the casual operator of the instrument; taken together, the set of parameters constitutes the scan function. As long as the parameters do not change (as in instrumental drift), calibration of the instrument connects the scan function with a calibration file, and the measured masses are assigned correctly. The calibration file contains the mathematical transformation required to assign masses properly to the relative data measured by the instrument. Computer-assisted calibration in modern instruments often also involves instrument tuning such that measured intensities of calibrant-derived ions (or a secondary performance factor compound) fall within a predetermined range. This aspect of tuning is an issue separate from determination of the mass scale, and will be discussed separately in a later series of columns that deals with ion intensities.

## What Calibration Entails

Individual steps in the calibration process should be un-



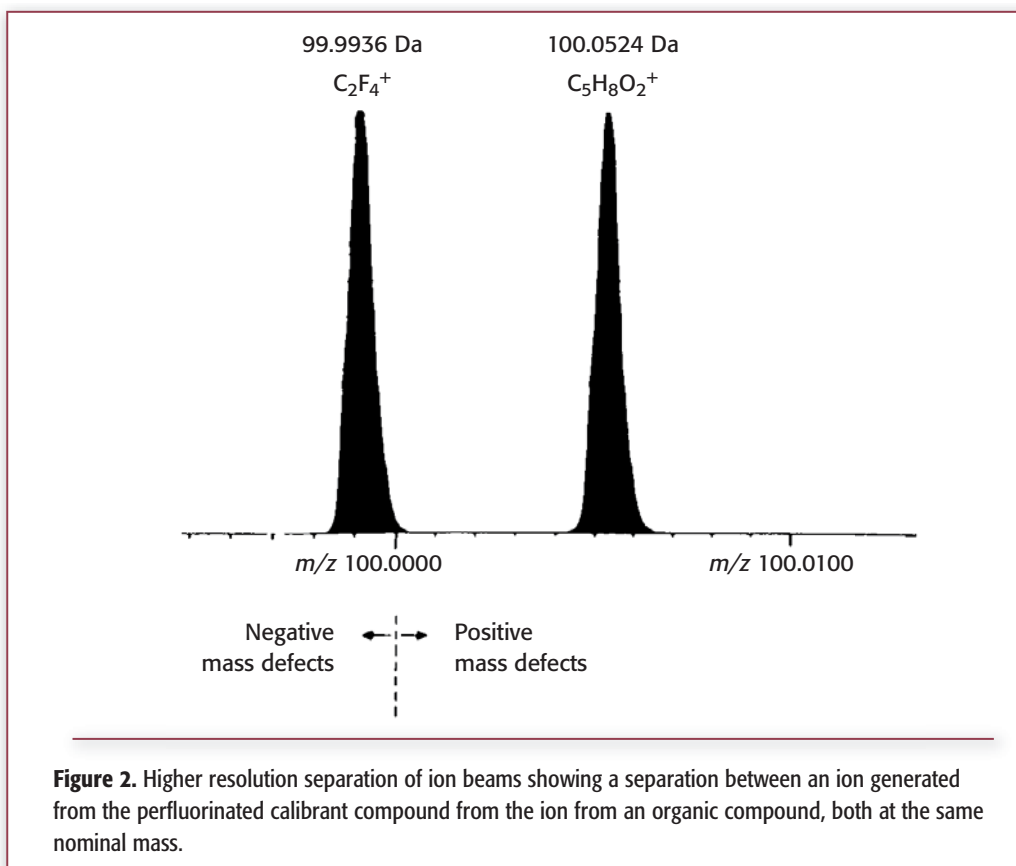
**Figure 1.** Positive ion electron ionization (70 eV) mass spectrum of FC-43.

derstood. Consider the case of calibration by computer in a quadrupole-based gas chromatography (GC)–MS instrument. Data systems for such scanning instruments record data in a time–intensity file under parameters for instrument operation set in the scan function. The time runs from zero at the beginning of the scan to the end of the scan, typically some tenths of seconds later. The measured intensity is the amplitude of the digitized detector response recorded within each successive short time channel during the scan. The scan function controls electronic parameters such as rf/dc values during the scan of the quadrupole mass filter (or current fed to an electromagnetic for a magnetic sector mass analyzer scan). For practical reasons, the scan function starts usually at values that correspond to a higher mass and drops to a lower mass. The relationship between the time–intensity values and the mass scale of the recorded mass spectrum is established by mass calibration. A calibrant compound is introduced into the instrument as a sample of steady concentration. A time–intensity file is recorded for the mass calibrant using the scan function. The data system then determines a mathematical transformation such that its applica-

tion to the time–intensity file measured for the calibrant will provide a mass spectrum in which the ions in the mass spectrum of the calibrant are

assigned properly. Specifically, for a calibrant such as FC-43 (see following paragraphs), a time at which ions of  $m/z$  69 pass through the mass analyzer

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**Table I. Positive ions found in the electron ionization mass spectrum of FC-43.**

Ion Empirical Formula	Exact Mass (Da)	Integral Mass (Da)
CF <sub>3</sub>	68.995210	69
C <sub>2</sub> F <sub>4</sub>	99.993613	100
C <sub>2</sub> F <sub>5</sub>	118.992016	119
C <sub>3</sub> F <sub>5</sub>	130.992016	131
C <sub>4</sub> F <sub>9</sub>	218.985629	219
C <sub>5</sub> F <sub>10</sub> N	263.987100	264
C <sub>6</sub> F <sub>12</sub> N	313.983917	314
C <sub>7</sub> F <sub>12</sub> N	325.983917	326
C <sub>8</sub> F <sub>14</sub> N	375.980743	376
C <sub>8</sub> F <sub>16</sub> N	413.977539	414
C <sub>9</sub> F <sub>16</sub> N	425.977539	426
C <sub>9</sub> F <sub>18</sub> N	463.974335	464
C <sub>9</sub> F <sub>20</sub> N	501.971161	502
C <sub>12</sub> F <sub>22</sub> N	575.967957	576
C <sub>12</sub> F <sub>24</sub> N	613.964722	614

is determined, as well as times for ions at  $m/z$  100,  $m/z$  219, and  $m/z$  614 (ideally, for a linear scan function, the time-to-mass conversion also is linear). After calibration, and in the absence on instrumental drift, subsequent time-intensity taken using the

be linked in the data system to a different calibration file.

### Perfluorinated Compounds as Mass Calibrants

We now consider the nature of the chemical compounds used as mass cali-

**“OPERATION” IS A GENERAL TERM THAT ENCOMPASSES A MULTITUDE OF PHYSICAL AND ELECTRICAL FACTORS, MOST OF WHICH ARE TRANSPARENT TO THE CASUAL OPERATOR OF THE INSTRUMENT.**

same scan function, and linked to that calibration file (the slope of the line connecting time to mass), is transformed into a mass spectrum with the masses assigned correctly. Every data file must be linked to a calibration file, and the data system completes this linkage automatically. Sophisticated mass spectrometer data systems include an automatic function to calibrate *and* tune (set thresholds, resolution, and adjust intensity values as required) the mass spectrometer, but the basic process remains the same. The calibration function often is performed automatically on start up (or at regular intervals throughout continuous operation), along with other automated tuning functions that optimize instrument performance. Note that each different scan function must

brants. For use with electron ionization or chemical ionization (both topics covered previously in this column), the calibrant typically is a liquid compound stored in an internal reservoir adjacent to the ion source. The reservoir (containing a few milliliters of the calibrant liquid) is heated, and a few millitorr of the calibrant gas passes through a calibrated leak valve into the ionization chamber of the mass spectrometer as the calibration process begins. The ideal calibrant compound is pure and inert, nontoxic, inexpensive, and readily available. As a calibrant, it produces a mass spectrum (using the standard conditions of electron or chemical ionization) that contains a number of abundant, evenly spaced ions distributed across the mass range of interest, each with a limited width to

the isotopic envelope in order to maximize the intensity of the ion signal in any one ion. One could argue that the ideal mass calibrant would be composed only of monoisotopic elements; the compounds in common use come close to meeting this ideal.

Several perfluorinated compounds are in common use as mass calibrants in organic mass spectrometry. Perfluorinated compounds replace all hydrogen atoms in the parent compound with fluorine atoms. Examples include perfluorokerosene and perfluorotributylamine (PFTBA, FC-43, "heptacos"). We discuss specifically use of the latter calibrant. The structure of FC-43 ( $(n-C_4F_9)_3N$ ) is such

of fluorine, any isotopic contribution to the mass calibrant ion is due to the presence of carbon alone. Even the highest mass ion tabulated in the table ( $m/z$  614) has only 12 carbons, and therefore the abundance of the ion at  $m/z$  615 is expected to be only 13% of the abundance of the ion at  $m/z$  614 (itself observed with a low abundance relative to the base peak at  $m/z$  69). Ion intensity therefore is maximized in one ion rather than diluted across the mass envelope.

### Peak Matching Experiments

A signature characteristic of the ions derived from the perfluorinated calibration compounds make them useful in

sample — again a relative mass determination against the standard. The separation can be achieved as long as the ion from the calibrant contains no or minimal hydrogen. The exact mass of hydrogen (1.007825) is such that the presence of only a few hydrogen atoms outweighs the negative mass defect contribution of multiple fluorine atoms.

We note that PFTBA (FC-43) is a pure compound, but that perfluorokerosene is not. Reasonably, one might expect that the exact composition of different "batches" of perfluorokerosene produced might vary slightly, with consequential differences in the mass spectrum of the mass calibrant

**THE IDEAL CALIBRANT COMPOUND IS PURE AND INERT, NONTOXIC, INEXPENSIVE, READILY AVAILABLE, AND PRODUCES A MASS SPECTRUM CONTAINING A NUMBER OF ABUNDANT, EVENLY SPACED IONS DISTRIBUTED ACROSS THE MASS RANGE OF INTEREST.**

that starting with the molecular ion, each of the three butyl chains will fragment identically, minimizing the number of different fragment ions in the mass spectrum, and maximizing the intensity of the ion signal at those masses. Finally, these ions are formed with adequate intensity in both the positive and the negative ion mass spectra, and under both electron and chemical ionization conditions.

Figure 1 contains the positive ion electron ionization (EI, 70 eV) mass spectrum of FC-43; the highest mass calibrant ion at  $m/z$  614 was sufficient to cover the mass range accessed most usually in GC-MS. Table I compiles the primary ions in the EI mass spectrum, listing empirical formulas and the exact masses of the ions. Depending upon the instrument and its mode of operation, the calibration of the instrument can use either the nominal mass or the exact mass to generate the transformation contained in the calibration file. Exact masses listed for these ions are the summations for combinations of  $^{12}C$  and for  $^{19}F$ . Because there is only one isotope

peak matching experiments that record data at higher mass resolution. Higher resolution is used to establish the exact mass of ions derived from organic compounds. Each fluorine atom has an exact mass of 18.998405, less than the integral mass of 19. An ion that contains multiple fluorine atoms therefore is found usually at an exact mass less than the integral mass. This difference is sometimes termed a negative mass defect, but use of this term should not be confused with the standard use of the term "mass defect" in atomic physics. In contrast, ions derived from organic compounds containing carbon, hydrogen, nitrogen, and oxygen (in reasonable proportions) will be found with exact masses that are slightly higher than the integral mass, that is, a "positive mass defect."

Figure 2 illustrates this situation for two ions that are each of integral mass 100. Ions from both the mass calibration compounds and the unknown can be present simultaneously, but still are separated. The known mass of the calibrant ion is used to deduce the exact mass of the ion derived from the

standard. The calibration (and tuning) software of the mass spectrometer must accommodate these differences. Additionally, these compounds are synthesized in large batches, which are sufficient to supply the needs of the community for some years, and each batch is shipped with a copy of the electron ionization mass spectrum measured under standard conditions for that particular batch of mass calibrant. Note that the differences are expected primarily in the distribution of intensities of the ions observed in the mass spectrum, and not in the masses of the ions.

### Other Calibrants

Other perfluorinated compounds are in common use as calibrants in GC-MS instruments. The mass spectrum of perfluorokerosene contains a greater number of ions distributed across the same mass range as FC-43. However, the stability and linearity of modern instruments is such that this larger number of ions usually is not needed for the mass calibration of the scan function. Such ions are useful,

however, in peak matching experiments in which the operator would want to use a calibrant ion close in mass to the unknown mass. FC-70 is a compound related to FC-43 developed to provide calibration ions extending to a higher mass range.

A wide range of other compounds has been developed for use in mass spectrometers that used ionization methods other than EI or chemical ionization (CI), and we describe these more in depth in Part III. For EI and CI, triazines (1–3) and fluorinated oils developed for other purposes (as in the Fomblin and Ultramark compounds [4,5]) have been used as mass calibrants that extend the upper limit of the usable mass calibration range. Work continues to develop a calibration compound or mixture of compounds that is as easy to use as FC-43, and exhibits the same desirable characteristics, but which allows calibration to a higher mass limit. Fishman et al. (6) suggest that a mixture of fluorinated compounds can be used for calibration in the mass range 100–3000 Da.

We have focused in this column on mass calibration. GC–MS instruments used in certain analytical protocols must be calibrated, of course, but also must meet performance standards. Decafluorotriphenylphosphine (DFTPP) is a compound used for tuning such instruments to meet the performance standards specified in EPA Methods 625, 1625, 8250, and 8270, covering analysis of volatile and semi-volatile compounds in water. This tuning standard was suggested in 1975 (7), and improved instrument performance has resulted in ion abundance values that are more accurate but at variance with the standard. As a consequence, an artificial transformation must be applied to the measured data to alter the intensities of the measured ions to bring them into compliance with the standard. Mass calibration is unaffected. Similarly, bromofluorobenzene also is a performance standard for mass spectrometric measurements of volatile compounds in water.

In the usual calibration process, perfluorinated compounds used as mass calibrants are allowed to leak into the source for calibration, and then are pumped away. To minimize background in the mass spectra measured for samples, no residual signal is desirable. Both the calibrant compound and the sample can be present in the source simultaneously in peak matching experiments that are used in magnetic sector instruments for exact mass measurements, and in this instance we assume that there is no interaction between the compounds. Ions follow their own rules, of course, and Mosi et al. (8) have shown that several of the ions generated from FC-43 react with polycyclic aromatic hydrocarbons in a quadrupole ion trap mass spectrometer. The relative abundances of the product ions resulting from ion–molecule reactions between the fluorocarbon cations and the hydrocarbons varied with the exact structural isomer, allowing their differentiation.

### Parting Thought

The transparency of the calibration process in a modern au-

tomated mass spectrometer should not result in forgetfulness of the basics: that mass assignment is a process based on a comparison of relative masses; that the hidden reservoir inside contains the calibration compound (and eventually needs to be refilled); that ions, whatever their source, are reactive entities; and that calibration needs for different ionization sources result in development of different calibration compounds. This last point is the focus of Part III of this series.

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