Analysis of Labelled Compounds by Mass Spectroscopy in the Presence of Heavy Isotopes at Natural Abundance: The NAIC Program.

K Naveed D Barakzai[a], Brendan J Howlin[a], William J S Lockley[a]*, Konstantinos Sfyrakis.[b]

[a]*Division of Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH, UK.* [b]*Blue Brain Project, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland CH-1015.*

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Introduction

A number of utilities are available which enable the calculation of the relative intensities of the peaks in a mass spectrometric molecular ion cluster from the molecular formula of the analyte. Some of these are integrated into dedicated MS software control and analysis packages.[1] However, there are also excellent web-based open-access programs available.[2] Not all of these packages allow the inclusion of isotopes. Some, however, can be utilised to predict the mass spectrometric ion intensities of compounds labelled at 100% atom abundance with one or more atoms of a heavy isotope, whilst one excellent application[3] has recently been modified to allow for customized isotopic abundance data. Nevertheless, for most isotopic chemists these packages have limited application since synthetic products only rarely contain isotopic atoms at 100% abundance. Indeed, most isotopically labelled products are mixtures of isotopomers containing varying numbers of isotopic atoms, making the analysis of their MS ions a far from trivial task.

This paper describes a program, the Natural Abundance Isotope Correction program (NAIC), designed to address the MS analysis of labelled compounds. An early version of the NAIC program,[4] written in BASIC has been in use since the 1980's. Subsequently the program was ported to a more efficient programming language, significantly refined, extended, validated and distributed for use in several isotopic synthesis laboratories. The modified program runs under the Microsoft Windows operating system and is written in C++. The graphical user interface (GUI) was created using the C++ Builder 6.0 environment and the VCL library.[5] Data input and output is via the GUI with an option to export results to the Excel Microsoft Office® spreadsheet.

The deconvolution of isotopically labelled MS ions has been addressed in the past[6] though the procedures were tedious. Recently an alternative spreadsheet-based and open-source program, IsoPat2, was described and utilized for the deconvolution of the MS ions from isotopomer mixtures generated in the microwave deuteration of ketones.[7] This matrix algebra approach to the deconvolution operation differs from the approach of NAIC which utilises the more conventional algebraic process. However, in our hands the two approaches yield essentially identical results with both synthetic and real MS ion data, prompting us to report here the detailed operation of the NAIC program for the first time.

Discussion

The mass spectrum of a mixture of isotopically labelled compounds consists of ions arising from all the isotopomers present. The intensities of the observed peaks in a particular ion cluster arise from a superimposition of ions arising from each isotopomer, paying due regard to the change in mass and also to any differences in volatility, ionization, fragmentation, etc, which might be occasioned by the isotopic substitution. A simple example of this process is given by the molecular ion of a hypothetical mixture of deuterated isotopomers shown in the Scheme.

.[pic]

Figure 1: Contribution of isotopomeric forms to the observed molecular ion of a labelled compound consisting of a 4:2:1 mixture of unlabelled, monodeuterated and dideuterated isotopomers.

In this case the observed mass spectrometric molecular ion is considered as a simple summation of the appropriate ion intensities. This situation is indeed typical of the molecular ions of most labelled compounds, since in the majority of cases isotopic effects on volatilization, ionization, fragmentation, etc, are rarely of a primary bond-breaking nature and hence may be ignored, at least as a first approximation. In other cases the experimental MS conditions (e.g. the ionization voltage) or data collection regime (e.g. integration of all scans for GC-MS or HPLC-MS peaks) may need to be adjusted to minimize any isotope effects observed.

Operation of the NAIC program.

The program has a number of functionalities as shown in Figure 2. Initially, data on the intensities of the peak cluster constituting the MS ion of interest is either input or calculated. As shown above, these peak intensities arise from contributions from naturally-abundance isotopes of the elements present in the ion. Hence the intensities of the various peaks in the cluster can be calculated from the molecular formula of the ion and the known natural abundances of the appropriate isotopes. NAIC can carry out this calculation for simple molecules containing C, H, O, N, P, I and F, however more comprehensive web-based facilities[2,3] are freely available which provide for more complex molecular species. Data from these systems can be easily incorporated during the use of NAIC. However, direct experimental measurement of the intensities of the ions in the unlabelled material is likely to give more reliable analyses since the only assumption made in this case is of the similar behaviour of the labelled and unlabelled compound under the MS conditions. Once the data from the unlabelled compound and the corresponding data for the labelled compound has been calculated, or input, the program then addresses the determination the contribution of each isotopomeric component in the labelled compound to the ion cluster intensities.

The analysis of an ion such as the one in Figure 1 may be approached in a number of ways but the simplest method (and essentially the one used by NAIC) is by solving a series of over-determined simultaneous equations, which, taken together, express the contributions of the ion clusters from each isotopomer to the overall intensities of the peaks in the MS ion cluster.

Having extracted and quantified the peak patterns for each isotopomer present in the labelled compound ion the calculation of the percentage contribution of each isotopomer to the overall ion merely requires a simple summation of the constituent peaks for that isotopomer and expression of the result as a percentage of the total ion cluster intensity. The resultant data is then easily converted to the atom% abundance of the isotope by a summation of the isotopic contributions to

the total, whilst noting that isotopomers with increasing numbers of isotopic atoms will contribute to a correspondingly increased degree to the overall abundance i.e.

$$\text{Atom\% abundance, A\%} = 100 \times \sum_{i=0}^{i=n} (A_i/n)$$

Where A is the fractional abundance of each isotopomer, i is the number of isotopic atoms present in the isotopomer and n is the maximum number of possible isotopic replacements in the molecule.

In the case of the molecular ion of a radioactively labelled compound this abundance value can then be converted to specific radioactivity (S) provided that the molar specific activity (M) of the radioactive isotope at 100% abundance is known, as it is for most common nuclides, *via* the simple relation

$$S = A\% \times M.$$

[pic]

Figure 2. Simplified operation of the NAIC program

The interface between users and the NAIC program is shown in Figure 3. It is is composed of various input and output objects. Initially two choices must be made using the Choices Dialog form (Bottom right). First, since the isotopic pattern of the unlabelled compound may be calculated either from the molecular formula or by entering the experimental intensities from the MS, a decision on these options is required from the user. Second, the type of the isotope in the labelled compound must be specified as either differing from the light isotope by one mass unit e.g. ^2H, ^{13}C, ^{15}N, ^{17}O or differing by two mass units e.g, ^3H, ^{14}C, ^{18}O.

If the isotopic pattern of the unlabelled compound is to be determined from the molecular formula, then the formula is selected in the Data Entry form (Bottom right) which allows the input of the number of atoms of each element present. The elements supported in the current release are C, H, N, O, P, I, F. If, instead, the isotopic pattern of the unlabelled is to be provided from the experimental MS or from the output of a web-based public domain calculator,[2,3] then the data on the intensities of the peaks in the ion cluster is entered in the Data Entry form under the heading of 'Molecular ion intensities'. The peak intensities from the corresponding ion cluster of the labelled compound are also input in the same Data Entry form, this time under the 'Intensities from labelled spectra' heading. Lastly, there is an option to specify the total number of label atoms which is to be used when calculating the atom% abundance.

When data entry is complete the 'Calculate' button is selected. The results are then output in the Results window (Top). The output comprises; the natural abundance isotope corrected MS ion data, a graphical display of the corrected spectrum, the calculated atom% abundance and the molecular weight (if using the molecular formula option). An option to export the data to an excel

sheet is also provided. NAIC accepts floating point values for the Molecular ion intensities and for the Intensities from labelled spectra input parameters, and integers for all the other parameters.
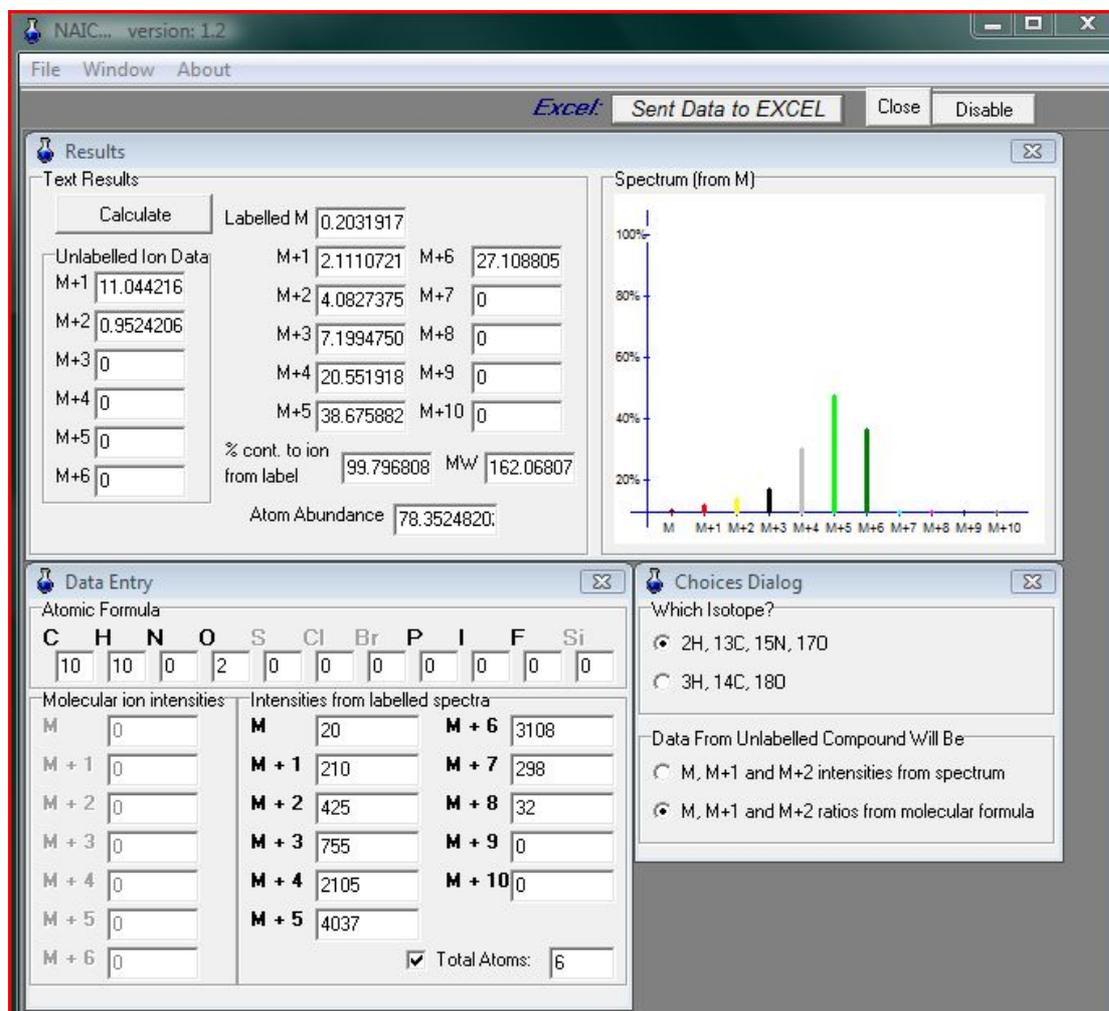
Figure 3. Graphical user interface of NAIC

Further information and program limitations.

Provision of extensive programming data in this paper would be inappropriate for the Journal. Hence further technical information on the detailed construction of the NAIC program has been lodged at a publically accessible website.[8]

A number of limitations of the program should be noted. First, the unlabelled ion data entry is limited to values from M to M+6 whilst the labelled ion data is limited to values from M to M+10. These limits will enable analysis of the overwhelming bulk of labelled organic compounds. However it is worth noting that the IsoPat2 spreadsheet[7] embraces a larger range of values. Second, the algorithm for the calculation of the natural abundance MS ion intensities is only approximate, though quite appropriate for the analysis of small molecules ($<C_{50}$ or so). For high precision calculations relating to larger molecules a more accurate algorithm such as those

available in the web-based public domain calculators[2,3] should be utilised and the resulting natural abundance isotope pattern then entered into NAIC.

The NAIC program can be obtained[9] from w.lockley@surrey.ac.uk.

References

1. There are isotope pattern calculators in several MS control programs: For a typical example see: MassLynx Version 4.0 User's Guide, p457-458 via http://www.waters.com [19 May 2009].

2 There are several free isotope calculators or open-source software sources: (a) Sheffield ChemPuter isotope pattern calculator, M Winter: http://winter.group.shef.ac.uk/chemputer/. [24 Feb 2009]; (b) Molecular weight calculator, M. Monroe: http://www.alchemistmatt.com/mwtwin.html. available at http://ncrr.pnl.gov/software/. ([27 Jan 2009] ;(c) IsoPro3, M. Senko: via: http://www.oc.uni-koeln.de/ms-oc/download/isopro3.zip [27 Jan 2009] [27 Jan 2009]; (d) Molecular Weight and Isotopic Abundance calculator. W. Zheng: http://www.ifa.hawaii.edu/~zhengwj/Applets/IsoPattern.html; [27th Jan 2009]; (e) Isotope cluster. http://www.colby.edu/chemistry/NMR/IsoClus.html. [27th Jan 2009].

For a comprehensive Windows-based calculator and an excellent review of web-based and windows-based calculators see: K. Massila, R. D. Stein, S. M. Suhaizan, A. A. Azlianor, *Proc. World. Acad. Sci. Eng. Tech*. **2007**; *20*, 146-149. ISSN 1307-6884.

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3. Isotope Pattern Calculator v4.5, Junhua Yan: http://www.geocities.com/junhuayan/pattern1.htm. [24 Feb 2009]

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7. C.C. Gruber, G. Oberdorfer, C. V. Voss, J. M. Kremsner, C. O. Kappe, W. Kroutil, *J Org Chem*, **2007**; *72*, 5778-5783.

8. For more detailed background information on the NAIC program algorithms, etc, please see: http://www.surrey.ac.uk/Chemistry/NaicLMSP/
9. NAIC is free to users after registration via e-mail. Efforts will be made to correct any errors or bugs brought to the attention of the authors, however, the use of the program and any errors occasioned by its use are entirely the responsibility of the user.

Input the
maximum
number of
isotopic atoms
present

Input peak
intensity
data from
labelled ion
cluster

[pic]".HWw~€""–? ¦§¼½¾ÁÍÕÖ×ØÙ@   I   üóçóçÞçüÑÃÑ¶¬¶?¶?Œ¶'¶u?¶dUFhmÆh 2
B*[pic]CJaJphhmÆhmEB*[pic]CJaJph
hmÆhÑ0*B*[pic]CJH*[pic]aJphhmÆhb]n5?B*[pic]phhmÆ5?B*[pic]ph#hmÆhmE5?B*[pic]CJ
H*[pic]aJ phhmÆhmE5?B*[pic]H*[pic]phhÖÊ5?B*[pic]phhmÆhmE5?B*[pic]phCalculate or
input

ion cluster intensities
from the formula
of the unlabelled ion

Calculate
atom%
abundance

Calculate the
percentage of
each isotoper
present

Extract cluster
pattern for each
isotoper
present

Input peak

intensity data
from the
unlabelled ion
cluster

M+5

M

Unlabelled Molecular Ion

M+2

Dideuterated
Molecular Ion

M+1

Monodeuterated Molecular Ion

M

Resultant
Observed
Molecular
Ion