

***** Information for IsoPatrn *****

PACKAGE CONTENTS:

There are four files in this package.

- 1) IsoPatrn.exe - the binary executable file that performs the calculations.
- 2) IsoPatrn.chm - the help file that supplements the executable file.
- 3) readme.pdf - this file.
- 4) checksums.md5 - the md5 checksum file. This file can be used to determine whether any corruption has taken place in downloading the IsoPatrn package of files, see below.

OPERATING SYSTEM REQUIREMENTS:

"IsoPatrn" requires a "Windows" 32-bit operating system. Thus it will run under any version of Windows from Windows 98 to present Windows versions, including 64-bit operating systems, and under Windows NT V4.0. It will not run under versions of Windows earlier than Windows 98.

FILE PLACEMENT AND USE:

The software does not come with installation or uninstallation programs. Once the .exe and .chm files have been created by decompressing the downloaded .zip file, these are ready for use. It is highly recommended that the .exe and .chm files be placed in their own subfolder (subdirectory), perhaps named "IsoPatrn", in the "Program Files" or "Program Files (x86)" folder (directory). You may have to be logged on as administrator to accomplish this. IsoPatrn can then be run by double-clicking on "Program Files\IsoPatrn\IsoPatrn.exe" in "Windows Explorer" or by clicking on Run from the Start Menu and selecting IsoPatrn.exe in the "Run" pop-up window that appears. A desktop shortcut can be generated for "IsoPatrn.exe" in the usual fashion, if desired (see Windows Help if you are unsure how to do this). IsoPatrn can then be run by double-clicking on this shortcut. It is not necessary to place the .exe and .chm files in a separate folder, they can be accessed from any location as long as they are in the same folder.

A problem has sometimes been observed with the HTML Help system. The "Windows" Help Viewer sometimes creates an index file with a ".chw" extension. In theory this should not cause a problem. However, under certain circumstances this index file displays only the index and none of the Help contents. To avoid this it is recommended that the downloaded ".zip" file be decompressed by special decompression software such as "Winzip" or "7-Zip" rather than the Windows operating system. This problem has not been observed with IsoPatrn placed in a subfolder of the "Program Files" folder.

To uninstall IsoPatrn, simply delete all the files listed above and any folders that contained only these files. Note: If the "Windows" HTML Help Viewer has created an index file with a ".chw" extension, simply delete this file also.

CHECKING FOR CORRUPTION DURING DOWNLOADING:

The file "checksums.md5" (an ASCII text file) contains the 128-bit checksums, in hexadecimal format, for the "IsoPatrn.exe" and "IsoPatrn.chm" files, calculated using the Message Digest number 5 (md5) algorithm. These same checksums should be calculated for the decompressed files you have downloaded using an appropriate program (see, for example, <http://www.fastsum.com/>). If the calculated checksums and those in the .md5 file do not agree, corruption has occurred and the downloaded program should not be used.

THE "IsoPatrn" PROGRAM:

The code for IsoPatrn was written in Visual C++ (with some inline assembly code) and compiled using the Microsoft Visual Studio 2005 compiler system. In order to avoid errors for very large molecules, all floating-point math routines use 80-bit values instead of the usual 64-bit values and all calculations are done with a mantissa precision of 64 bits.

The algorithm used is the "classical" polynomial expansion algorithm, very similar to that described by Yerger [1]. Pruning is not employed, since writing a pruning algorithm for molecules of widely differing molecular weights is a difficult task. Instead, a feature is included that allows the calculation to be stopped upon reaching a specified percentage of completion, excluding species of high molecular weight, which are usually present in very low amounts, from the calculation. This causes no errors in the calculated fractional abundances of the lighter isotopic arrangements and, as long as all the isotopic arrangements contributing to the base peak are included, no errors will be observed in the relative percentages of the lighter peaks.

This approach has the advantages of being universal - it can be used for all the elements and can be applied to "synthetic" elements - and its application is straight forward. The main disadvantage is speed. Faster methods of calculation have been proposed [2,3]. However, for modern personal computers with clock speeds above 1 GHz, even the polynomial expansion method provides rapid calculations except for very large molecules or molecules containing elements with large numbers of isotopes.

The method of performing the calculations for product ion isotope clusters is similar to that proposed by Singleton *et al.* [4].

IsoPatrn can provide two types of data output. The first type might be referred to as general or "low resolution" data. This type of output is always provided for each calculation and for all types of scans. The intensity values provided by the program are calculated assuming that the instrumental resolving power is such that peaks for ions differing by one integer mass unit can be completely resolved, but that none of the peaks for ions with the same integer mass can be resolved. For this type of data no attempt is made to take into account the differing exact masses of ions with the same integer mass or to calculate peak positions, widths, or shapes. The peak positions (m/z values) are only approximate, except for the $[A+0]$ peak, and the relative intensity values more accurately represent peak areas than peak heights.

For certain types of scans (see below) the individual ions making up a "low resolution" peak can be listed. This type of data might be referred to as "high resolution" and is output only when this option is selected by the user. The accurate mass of every ionic component in such data is given along with the individual fractional abundance of the component. If the option to list such components is chosen, the spectrum corresponding to a specific instrument resolving power can be calculated and plotted. To do this it is assumed that the instrument will provide a Gaussian peak shape for a single ionic component (the individual Gaussian peaks are summed to produce the overall peak). This spectrum should represent the actual experimental peak in shape, width, and height provided that the instrument provides Gaussian peak shapes and the correct resolving power is chosen.

TYPES OF CALCULATIONS:

Simple mass spectra:

IsoPatrn provides peak intensities for isotope clusters in simple spectra in a straight forward manner. Using the positively charged molecular ion of carbon tetrachloride as an example, the program lists ten peaks, ranging in integer mass-to-charge ratio from 152 to 161, in the isotope cluster. Some of these peaks may not be observed experimentally. Both general data and individual ion listing are provided for this type of scan.

MS/MS Spectra:

Three types of MS/MS spectra are commonly encountered: precursor ion spectra, neutral loss spectra, and product ion spectra. Not all instruments readily provide all three types of spectra. In all of

these cases more than one spectrum may be obtained as a result of the ability to select multiple product ions, neutral losses, or precursor ions for observation. This leads to the ability to observe isotope clusters within a spectrum and to compare the peak intensities between different spectra. Refer to Singleton *et al.* [4] or Ramaley and Cubero Herrera [5] for a more detailed discussion of these phenomena and to de Hoffman and Auriel [6] for an example of the use of different product ion MS/MS spectra in elucidating a fragmentation mechanism.

In the discussion below it will be assumed that the precursor ion is the molecular ion of carbon tetrachloride, the product ion is CCl_3^+ , and the neutral loss is a chlorine atom.



Precursor Ion MS/MS spectra:

There are eight different isotopic configurations for CCl_3^+ , the product ion, ranging in integer m/z from 117 to 124. Any one of these may be selected as the product ion for which precursor ion spectra may be calculated. Thus there are eight possible precursor ion spectra. Each spectrum will have two peaks in its isotope cluster, separated by two mass units, with intensities representative of the fractional abundances of the isotopes of chlorine, the neutral loss. The integrated intensities of each of the eight spectra will be representative of the intensities in the isotopic cluster of the product ion, CCl_3^+ .

In general there will be as many different spectra as there are peaks in the isotope cluster of the product ion and the integrated intensities of these spectra will be representative of the intensities of the peaks in the isotope cluster of the product ion. The number and intensities of the peaks in the isotope cluster in an individual spectrum will be those of the peaks in the "isotope cluster" of the neutral loss.

The instruments that provide precursor ion spectra usually have two mass filters in tandem (*e.g.* a triple quadrupole instrument) and the second of these filters is set at a constant m/z value while the first is scanned. This arrangement provides an isotope cluster for each spectrum with peaks that all have the same composition (and thus the same shape) regarding the ionic components. In the CCl_4 case each of the two peaks has the same composition (a single ion), but a different intensity. Since all peaks within a spectrum have the same composition, IsoPatrn does not list individual peaks for precursor ion spectra. The composition and overall peak shape can be determined by performing an analysis for a regular spectrum (see above) of the product ion and selecting the appropriate $[\text{A}+\text{i}]$ ion for examination. For example, to determine the composition of the peaks in the precursor spectrum of CCl_3^+ at 119 m/z , do a regular analysis for CCl_3^+ and observe the results for the $[\text{A}+2]^+$ ion.

Neutral Loss MS/MS spectra:

There are two different isotopic configurations of the neutral fragment, 35 or 37 mass units. Either of these losses may be selected as the basis of an MS/MS spectrum. Thus there are two possible neutral loss spectra. Each spectrum will have eight different peaks in the isotope cluster arising from the isotopic configurations of the product ion. The integrated intensities of the two spectra will be representative of the fractional abundances of the isotopes of chlorine, the neutral fragment.

In general there will be as many different spectra as there are peaks in the "isotope cluster" of the neutral fragment and the integrated intensities of these spectra will be representative of the intensities of those peaks. The number and intensities of the peaks in the isotope cluster of an individual spectrum will be those of the peaks in the isotope cluster of the product ion.

Instruments that provide neutral loss spectra usually scan both mass filters, thus providing isotope cluster peaks with different compositions. IsoPatrn thus can provide a listing of the individual peak components in such spectra.

Product Ion MS/MS spectra:

There will be ten possible product ion spectra, one for each peak in the isotope cluster of CCl_4^+ , the precursor ion. In general there will be as many product ion spectra as there are peaks in the isotope cluster of the precursor ion. The integrated intensities of these spectra will be representative of the peaks in the isotope cluster of the precursor ion. The number of peaks in each individual spectrum will depend

on the particular parameters involved. For the example of CCl_4 , there will be either one or two peaks in the isotope cluster of each spectrum. The intensities of these peaks will not be indicative of any isotopic fractional abundances, but rather will depend on the number of ways of forming the product ion from the precursor ion.

IsoPatrn can provide individual ionic component listings for the peaks in product ion spectra, but only does so for four stages of filtering (up to MS/MS/MS/MS spectra). The ionic peak components for any number of stages can be obtained by using the two-stage MS/MS data analysis, but entering, as the precursor ion, the ion selected in the second-to-last (penultimate) stage of filtering and, as the product ion, the ion selected in the last filter. This procedure will provide accurate compositions and relative intensities, but the fractional abundances provided will be too high.

Not all of the peaks in the isotope cluster within an individual spectrum nor all of the different spectra will necessarily be experimentally observable.

COMMENTS:

Comments, suggestions, and criticisms will be gladly received and perhaps even used to improve future versions of IsoPatrn. These should be forwarded by email to Lou Ramaley at Louis.Ramaley@Dal.CA.

REFERENCES:

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