DESIGN AND APPLICATION OF AN ISOTOPE PATTERN CALCULATOR FOR MICROSOFT WINDOWS

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The isotope pattern calculator (IPC) that we developed calculates the expected isotopic masses and abundances for molecular fragments consisting of the following elements: H, B, C, Cl, Br, I, F, O, S, P, As, Se, N, and Si. The natural abundances for all these elements are incorporated in the program. The program was developed because it is difficult to manually calculate the isotope pattern for fragments of species like $C_{12}H_5Cl_3Br_2O_1$. The program is especially useful in cases where mass spectra of a certain compound or interfering compounds are not available[1]. Incorporated in MS interpretation software, the program could help in the identification of compounds by providing possible element combinations and the detection of an erroneous spectrum in the data base[2]. For Selective Ion Detection (SID), the program can help the user to choose the optimum ion traces for analysis.

The IPC program was written in Borland C++, version 3.1 and runs under Microsoft Windows 3.1. When the user clicks the IPC icon, the screen of Fig. 1 appears. To calculate isotope masses and abundances, the user types (or clicks the button using the mouse) the formula of the fragment into the molecular formula box. Then the user clicks the EQUAL button or presses the ENTER key; the mass, absolute frenquency and relative frequency are shown in the main window. The pattern can be displayed on the screen and/or printed.

The program is based on the known natural isotopic frequency of the elements. If the elemental composition of a particular fragment at mass M is known, then, by using a multinomial distribution, one can compute the intensity contribution from the isotopes at mass M+1, M+2 and so on. In general, when a large number of different elements compose a fragment, intensity contribution at masses higher than M may also be significant. The expressions for the expected intensity of the isotopic peaks used in the present work are derived using a multinomial distribution and are given below.

Monoisotopic elements. For these elements, e.g., fluorine (F), phosphorus (P), there will be no isotopic contribution at masses higher than M. For a fragment at mass M consisting of N atoms, the number of peaks will be 1 and can be written in the form of

$$NoP = \frac{(N+1-1)!}{(1-1)!N!} = 1.$$ where, NoP is the number of peaks, N is the number of atoms and N! is factorial of N.

Two isotope - elements. These are elements which possess two stable natural isotopes. The group can be divided into two subgroups. The first subgroup includes the elements

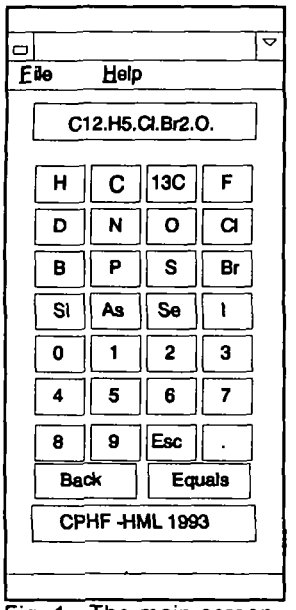

Fig. 1: The main screen of IPC

whose second major isotope is one mass unit higher. The second subgroup includes the elements whose second major isotope is two mass units or more higher than the first one. For example, the second isotope of carbon (C) and hydrogen (H) is one mass unit higher than the first one, but for chlorine (Cl) and bromine (Br), the second isotope is two mass units higher than the first one. If the isotope ratio of these elements is represented as a:b, for a fragment at mass M consisting of N atoms of this element, the number of peaks will be N+1 and can be written in the form

NoP = $\frac{(N+2-1)!}{(2-1)!N!}$. The formula is valid for two subgroups. For the first subgroup, the relative abundance intensities at mass M+1, M+2, M+3, ..., M+N in that cluster are given by the following binomial expansion[3]:

$$(a+b)^n = C_n^0\ a^n + C_n^1\ a^{n-1}b + C_n^2\ a^{n-2}b^2 + \cdots + C_n^{n-1}ab^{n-1} + C_n^n b^n = \sum_{k=0}^{n} C_n^k a^{n-k}b^k$$

where, C_n^k are binomial coefficients and $C_n^k = \frac{n!}{r!(n-r)!}$. For the second subgroup, the above formula represents the relative abundance of the peaks at M, M+2, M+4, M+6,⋯, M+2n.

It is easy to remember the binomial coefficients with the help of Pascal's triangle shown in Fig 2. For example, the binomial coefficients will be 1, 3, 3, 1, when n = 3.

Multi isotope elements. These are elements which possess more than 2 isotopes, e.g. oxygen (O) and sulfur (S). The number of peaks can be presented as

NoP = $\frac{(NoA+NoI-1)!}{(NoI-1)!(NoA)!}$. Where, NoP is the number of peaks, NoA is the number of atoms and NoI is the number of isotopes. The relative abundance of the peaks in the cluster can be represented by the multinomial expansion,

$$(a + b + \cdots + f)^n = \sum_{p+q+\ldots s=n} \frac{n!}{p!q!\ldots s!} a^p b^q \ldots f^s ,$$

where p, q, ..., s are non-negative integers with the property that p+q+⋯+s = n. In fact, Pascal's triangle can also show the number of peaks. Two sloping rows of numbers have been marked in Fig 2. The first sloping row shows the number of peaks for the fragment consisting of a 2 isotope element, e.g. a fragment with 1 chlorine atom will have 2 peaks and a fragment with 2 atoms will have 3 peaks. The other sloping row shows the number of peaks for the fragment consisting of a 4 isotope element, e. g. a fragment with 1 sulfur atom will have 4 peaks and a

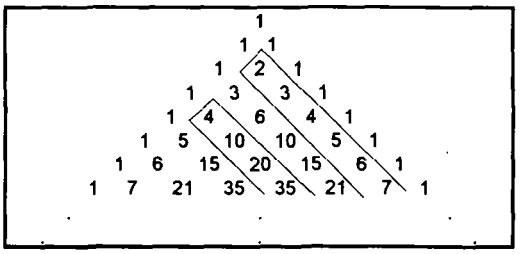

Fig. 2: Pascal's triangle

fragment with 2 atoms will have 10 peaks.

Multi-element combination. According to the multiplication rule[4], a fragment consisting of different elements will have multiple peaks derived from product of the peak number of the different elements involved, for example, S_2O_3 will have 10*10 = 100 peaks theoretically. Some peaks can be neglected due to weak intensities. The peak number can be presented by,

$$NoP = \frac{(NoA1+NoI1-1)!}{(NoI1-1)!(NoA1)!} * \frac{(NoA2+NoI2-1)!}{(NoI2-1)!(NoA2)!}$$

where NoA1, NoA2 are the number of atoms of the first element and the number of atoms of the second element, respectively. NoI1 and NoI2 are the number of the isotopes of the first and second elements, respectively. The relative abundance of the peaks in the cluster can be derived from the product of the two corresponding multinominal expansions.

One very important application of GC/MS is in deducing the elemental composition of the compound investigated. IPC can be used to confirm elemental compositions which satisfy the constraints of the mass of the fragment as well as the reported intensities of the isotopic peaks. We used the IPC program to identify and correct erroneous spectra in a commercial library.

IPC can aid the analyst to choose the optimum ion traces for the analysis. Selective Ion Detection (SID) is a common technique used in GC/MS analysis. In this mode, the retention time and the isotope ratios are critical parameters to distinguish the compounds. For example, in the case of polyhalogenated dibenzofurans (PXDFs), we often find some interferences. These interfering compounds elute at the same retention time as the analytes on a DB-5 column. Other polar columns were tested to separate the analytes and the interferences, however, they can not be used due to the very long retention time for PBDFs. IPC was used to calculate the isotope ratios of the analytes and the possible interferences. Aided by other information, we concluded that the interferences in this case were polychlorobiphenyls (PCBs)[5]. Table 1 shows the isotope ratios and masses of the molecular ions of PXDFs and PCBs.

Table 1: Mass and intensity of the molecular ions of PXDFs and PCBs

PXDFs and PCBs	M	I	M+2	I	M+4	I	M+6	I	M+8	I
Br_2DF	323.879	51	325.877	100	327.875	50				
Cl_5PCB	323.883	61	325.880	100	327.878	65	329.875	21	331.873	3
Br_2Cl_1DF	357.840	44	359.837	100	361.835	70	363.833	14		
Cl_6PCB	357.844	51	359.841	100	361.839	82	363.836	36	365.833	9
Br_2Cl_2DF	391.801	44	393.803	100	395.800	98	397.794	32	399.792	4
Cl_7PCB	391.805	44	393.803	100	395.800	98	397.797	53	399.794	17
Br_2Cl_3DF	425.762	31	427.759	92	429.757	100	431.754	50	433.752	12
Cl_8PCB	425.766	34	427.764	88	429.761	100	431.758	65	433.755	27
Br_2Cl_4DF	459.723	24	461.720	78	463.718	100	465.715	64	467.713	22
Cl_9PCB	459.728	26	461.725	77	463.722	100	465.719	76	467.716	37
Br_2Cl_5DF	493.684	19	495.681	69	497.679	100	499.676	77	501.673	34
$Cl_{10}PCB$	493.689	21	495.686	68	497.683	100	499.680	87	501.677	49

Besides PCBs, many other compounds could interfere with the determination of polychlorined dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Interferences coextracted from samples will vary considerably from source to source, depending on the nature of the site being sampled. Interfering compounds may be present

at concentrations several orders of magnitude higher than the PCDDs and PCDFs. The most frequently encountered interferences are polychlorinated terphenyls (PCTs), polychlorinated naphthalenes (PCNs), polychlorinated biphenylenes (PCBPs), polychlorinated diphenyl ethers (PCDPEs), polychlorinated methoxy biphenyls (m-PCBs), polychlorinated methylthiobiphenyls (mt-PCBs), polychlorinated benzylphenyl ethers (PCBPEs), polychlorinated xanthenes (PCXs), polychlorinted xanthones (PCXos), and organochlorinated pesticides. Because very low levels of PCDDs and PCDFs are measured by EPA method 1613, the elimination of interferences is essential. Even after a very careful clean-up, some of these interferences can still be present in the samples. Then, the information of the mass and intensity of the ions monitored are important for the identification of PCDDs and PCDFs. The molecular ions of the possible interfering compounds, as point out by Buser[6], calculated by IPC are listed in Table 2. With the help of the exact mass and isotope pattern, we can minimize false positive identification of PCDDs and PCDFs.

Table 2: Possible interfering compounds and their molecular ions in EI-HRMS analysis of PCDDs and PCDFs

Compounds	Interfering		Interfering	Molecular ions with different chlorination				
	PCDDs	PCDFs	ions	tetra	penta	hexa	hepta	octa
PCDDs	-	-	-	319.8965	353.8576	387.8186	421.7796	455.7407
PCDFs	-	-	-	303.9016	337.8627	371.8237	405.7847	439.7457
PCBs	+	-	M^+-Cl_2/-Cl_4	289.9224	323.8834	357.8444	391.8054	425.7665
PCTs	-	+	M^++6	365.9537	399.9147	433.8757	467.8367	501.7978
PCNs	-	+	M^++6	263.9067	297.8677	331.8287	365.7898	399.7508
PCBPs	+	-	M^+	287.9067	321.8677	355.8287	389.7898	423.7508
PCDPEs	-	+	M^+-Cl_2	305.9173	339.8783	373.8393	407.8004	441.7614
m-PCBs	+	+	M^+/M^+-50	319.9329	353.8939	387.8550	421.8160	455.7770
mt-PCBs	+	-	M^+-50	335.9101	369.8711	403.8321	437.7932	471.7542
PCBPEs	+	-	M^+	319.9329	353.8940	387.8550	421.8160	455.7770
PCXs	+	-	M^++2	317.9173	351.8783	385.8393	419.8004	453.7614
PCXos	-	+	M^+-CO	331.8965	365.8576	399.8186	433.7796	467.7406

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