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Isotopic ratio of molecular patterns via gas chromatography–mass spectrometry with selected-ion monitoring as a chemometric tool

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Abstract

Information on isotopic ratios for molecular fragment ions obtained via a chromatographic process is helpful for determining the elemental formulae of the components of an eluted mixture. It can be used in chemometric detectors to determine chlorine, bromine or sulphur. If the isotopic ratios are obtained from the whole mass spectra, the precision is not of much use for this purpose. More precise isotopic ratios were obtained with acquisition data in the selected-ion monitoring mode and use as statistics in a chemometric approach. The isotopic ratios vary with the chromatographic peak and they depend on the GC–MS acquisition conditions. These disadvantages may be removed by setting optimum acquisition parameters. The technique was verified on polychlorinated biphenyls.

1. Introduction

Compounds with biological activity, such as toxic compounds and drugs, are conveniently studied by combined gas chromatography–mass spectrometry (GC–MS). Low-resolution mass spectrometry for the analysis of organic compounds has been used for the determination of elemental compositions from the isotopic distributions of molecular ions [1–7].

Because isotopic ratios from the measurement of the whole mass spectra were used, the precision was not very useful for determining the number of C, H, N and O atoms in a molecule. Better results were obtained for chlorinated compounds [8–11], because the isotopic occurrences of heavier isotopic ions are higher than those of isotopic ions of compounds containing only C, H, N and O atoms. The precise measurement of isotopic ratios can be used for determining the numbers of chlorine atoms in measured ions. Worse results are obtained with compounds containing sulphur atoms, but in many instances it is possible to determine the number of atoms of sulphur in a molecule from the isotopic distribution [7].

There are a growing number of systems [9–11] using GC–MS analysis for determining the number of atoms of chlorine, bromine and sulphur in components of a complex mixture. They involve selective chromatographic detectors based on the computer analysis of GC–MS data. The first step is the GC–MS analysis of a complex mixture, which does not require selective detection, but it

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is necessary to use high-performance gas chromatography. The second step involves the selection of compounds containing chlorine, bromine or sulphur by the application of chemometric software to multivariate mass spectrometric data in the chromatographic process. The best results have been obtained for chlorinated compounds. LaBrosse and Anderegg [10] described four types of errors in chlorine-selective detection. Sulphur-selective detectors are not so successful [12], because contributions of sulphur to the $(M+2)^+$ ion interfere with the contributions of hydrogen, carbon, oxygen and nitrogen.

The purpose of this paper is to demonstrate how the observed isotopic intensities for the molecular and fragment ions are changed via chromatographic peaks. We believe that GC–MS with selected-ion monitoring (SIM) is the method of choice for improving the accuracy and precision of isotopic ratios and is better than acquiring data for the whole mass spectra (scan mode). The isotopic ratio observations were applied to the analysis of compounds that give molecular patterns, with high relative intensities [polychlorinated biphenyls (PCBs)]. Strategies for obtaining better accuracy of intensities and removing the experimental errors are discussed. Studies of some of the experimental factors affecting the measurement of relative intensities are reported.

2. Experimental

GC–MS analyses were made on Hewlett-Packard 5977B and 5988A GC–MS systems. A fused-silica column (30 m × 0.25 mm I.D., 0.25 μm) coated with DB-5 was used throughout all experiments. Splitless injection (250°C) was employed. The GC oven temperature programme was 2 min at 50°C increased at 20°C/min to 164°C and then at 4°C/min to 280°C. The carrier gas was helium at a linear velocity of 30 cm/s. The quadrupole mass spectrometer was operated in the electron impact (EI) mode. The ionizing voltage was 70 eV. Acquisition was done in the SIM mode. The selected ions were M^+, $(M+2)^+$, $(M+4)^+$, etc.

Delor 104 (a mixture of PCBs) was purchased from Slovak Metrological Institute (Bratislava, Slovak Republic). The individual PCB congeners were synthesized at the Drug Research Institute (Modra, Slovak Republic).

3. Results and discussion

The computer program RATIO for computing isotopic ratios in chromatographic processes was written in macroprogramming language on a PASCAL MS Chemstation, software version 3.1.1. It computes the ratio of isotopic ions to the ion with the highest intensity via chromatographic peaks.

The principle of the SIM process on quadrupole analysers (HP 5977B and HP 5988A) is illustrated in Fig. 1. Instead of scanning the whole mass spectra and recording information on all relative intensities, the quadrupole mass filter can be programmed to select a few specific m/z values for measurement. The "dwell" time is the amount of time spent monitoring a specific m/z value during SIM. The SIM scan cycle time is the time required to complete one SIM scan and begin another. Fig. 1 shows that the SIM scan

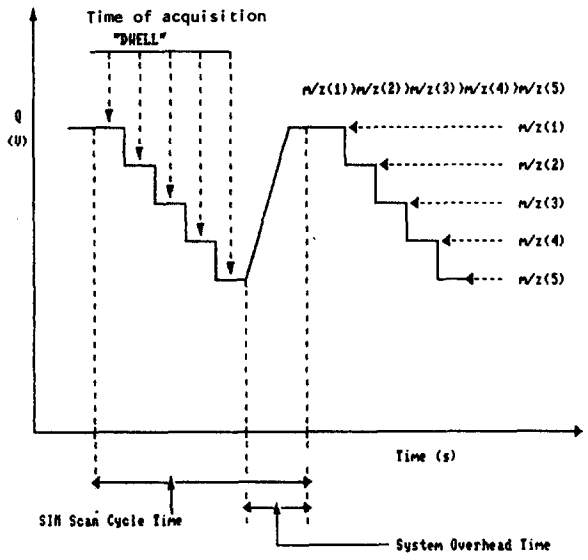

Fig. 1. Selected-ion monitoring process.

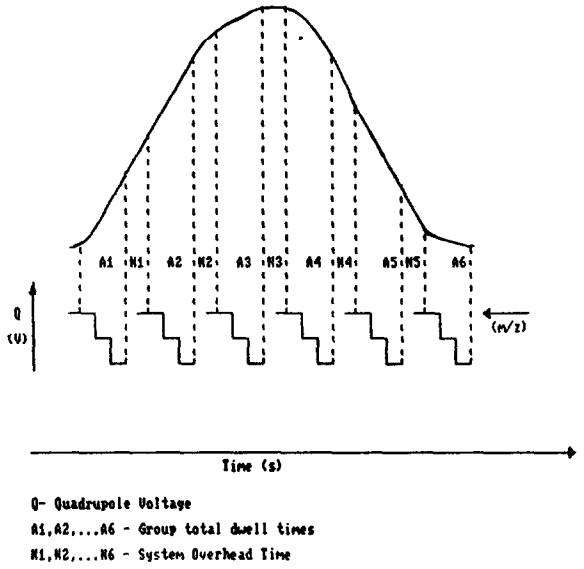

Fig. 2. Monitoring three ions as the GC peak elutes.

cycle time is numerically equal to the sum of the individual m/z dwell times plus the system overhead time. The system overhead time is the time between setting the system from the lowest to the highest m/z value. Fig. 2 shows the monitoring of three ions, which were monitored as the chromatographic peak eluted. In this example, the dwell time is equal to the system overhead time. When the quadrupole mass filter is tuned to transmit each of the selected m/z values, digitized information is accumulated for the duration of the user-specified dwell time. After completing the dwell time period, the accumulated count total is divided by dwell time and stored. The SIM scan cycle, *i.e.*, the dwell time followed by the system overhead time, is repeated until the user-specified run time is completed or the run is terminated by the user.

The principles of acquisition help us to understand the variability of the relative intensities of isotopic satellites during a chromatographic peak. The intensities of the individual ions in one SIM scan cycle time are not acquired in the same time, but their acquisition time is shifted by a few ms (dwell) (Fig. 2). The time of the first-acquired ion with the highest m/z belongs to the next acquired ions in one cycle in the control computer. The result is that the measured relative intensities are not the same as the theoretical relative intensities measured at the time of the highest m/z.

Isotopic ratios of the $(M+2)^+$ ion of a PCB with one atom of chlorine in a molecule in the SIM mode were measured. Graphical plots of isotopic ratio with various dwell times are shown in Fig. 3. The ratio y_{M+2}/y_M of the relative

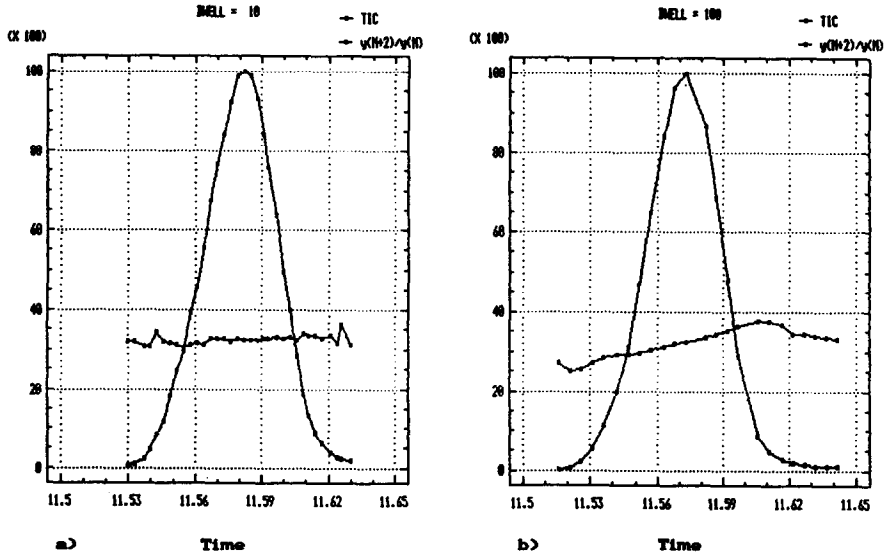

Fig. 3. Isotopic ratio via chromatographic peak. Time spent: (a) 10 and (b) 100 ms.

intensity of an ion with a lower m/z (y_M) to that of an ion with a higher m/z (y_{M+2}) is increased via the chromatographic peak. This increase depends on the time spent on the monitoring ion. A longer time spent causes a larger increase in the isotopic ratio of the ion with higher m/z via the chromatographic peak (Fig. 3). This effect can be explained as follows. The isotopic ratios y_{M+2}/y_M on the increasing slope of the chromatographic peak are lower than the theoretical isotopic ratio, because y_M is acquired later and their intensities are higher than if they had been acquired in the same time as y_{M+2}. The differences between the measured and theoretical ratio y_{M+2}/y_M is minimal at the top of the chromatographic peak because the measured y_M is almost equal to the theoretical value, although it is measured later than y_{M+2}. The y_{M+2}/y_M ratio on the decreasing slope of the peak is higher than the theoretical value because y_M is lower than if measured in the same time as y_{M+2}.

Another and possibly better example of intensity errors is presented in Fig. 4. The mass chromatograms of ions M^+ and $(M+2)^+$ are normalized on the maximum of their peak height. The mass chromatogram of the ion with lower m/z (y_M) is shifted to the left from the mass chromatogram of the ion with higher m/z (y_{M+2}) by the time spent on one ion (Dwell time value). If the relative intensities of both ions were measured in the same time, the normalized mass chromatogram would be ideally overlapping. These two negative influences can be removed by the measurement of intensities with the lowest time spent on selected ions (10 ms). The isotopic ratios fluctuate around the mean value and average values can be statistically computed.

There is no problem in calculating the theoretical ratios of isotope intensities of the molecular ion from the distribution of naturally occurring isotopic elements. It is possible to compare the theoretical and experimental relative intensities of the isotopic satellite of the molecular ion via the chromatographic process. The program for calculating the theoretical ratio of isotopic ions was written in BASIC on an HP 9000 Model 310 computer. The algorithm is the same as in the program used by Kavanagh [1].

We observed average relative intensities of individual congeners of PCBs (congeners 3, 8, 28, 52, 101, 138, 180, 203, 209) with 1, 2, 3, 4, 5, 6, 7, 8 and 10 atoms of chlorine in the molecule and they were compared with the theoretical intensities.

The data were averaged from 7 to 13 measurements of relative intensities of isotopic ions of congeners of PCBs. It was observed that the measurements of the average values of relative intensities of isotopic ions were not equal to the theoretical relative intensities. The errors between the theoretical and measured relative intensities were caused by the time shift in measuring the abundances of ions. A linear correction of the differences between theoretical and experimental relative intensities was suggested.

The relative errors D_0, D_1, $D_2, \ldots, D_i, \ldots, D_n$ between theoretical x_0, x_1,

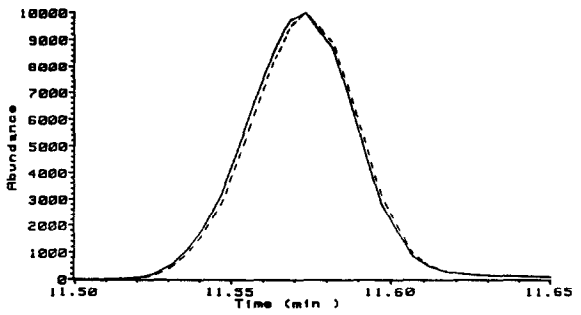

Fig. 4. Shift of mass chromatograms. Solid line: ion 188 u; dotted line: ion 190 u.

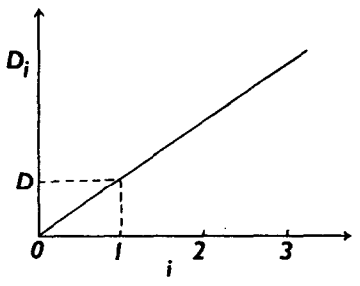

Fig. 5. Linear correlation of experimental error on the order of the measured ion.

$x_2, \ldots, x_i, \ldots, x_n$ ($x_0 = 100$) and experimental intensities $y_0, y_1, y_2, \ldots, y_i, \ldots, y_n$ ($y_0 = 100$), where i is the order of measured ion to the most intense ions ($i = 0$) of the molecular pattern, which can be negative if the molecule contains atoms of chlorine and (or) bromine, may be expressed as

$$D_0 = \frac{y_0 - x_0}{y_0} = 0 \quad (1)$$

$$D_1 = \frac{y_1 - x_1}{y_1} = D \quad (2)$$

$$D_2 = \frac{y_2 - x_2}{y_2} = 2D \quad (3)$$

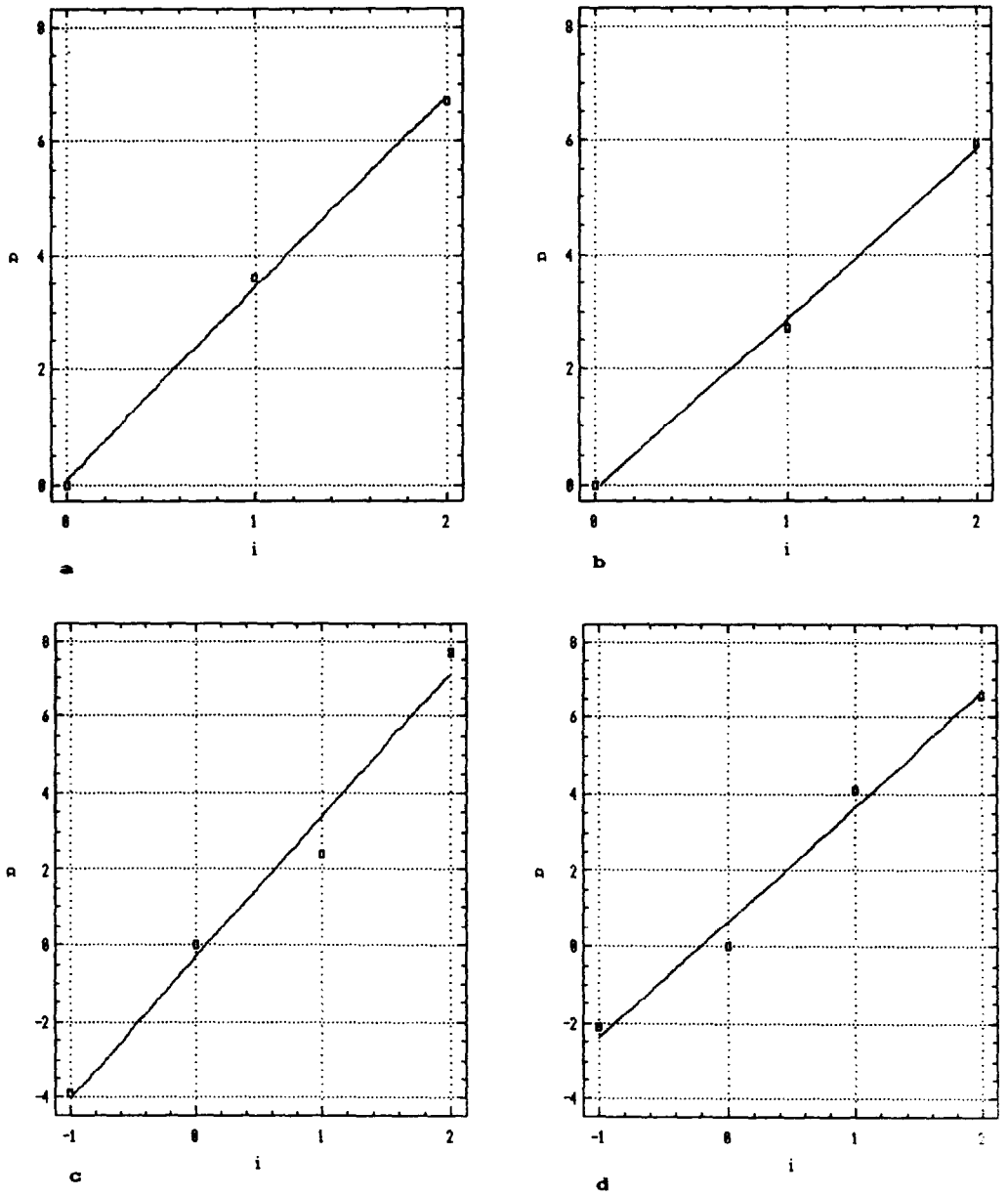

Fig. 6. Linear function errors D_i on ions i for PCBs with (a) 2, (b) 3, (c) 4 and (d) 5 atoms of chlorine.

$$\vdots$$

$$D_i = \frac{y_i - x_i}{y_i} = iD \quad (4)$$

$$\vdots$$

$$D_n = \frac{y_n - x_n}{y_n} = nD \quad (5)$$

and

$$D = D_0 - D_1 = D_1 - D_2 = D_i - D_{i-1} = D_n - D_{n-1} \quad (6)$$

where D is a correction factor. This value may be used for the characterization of the relative

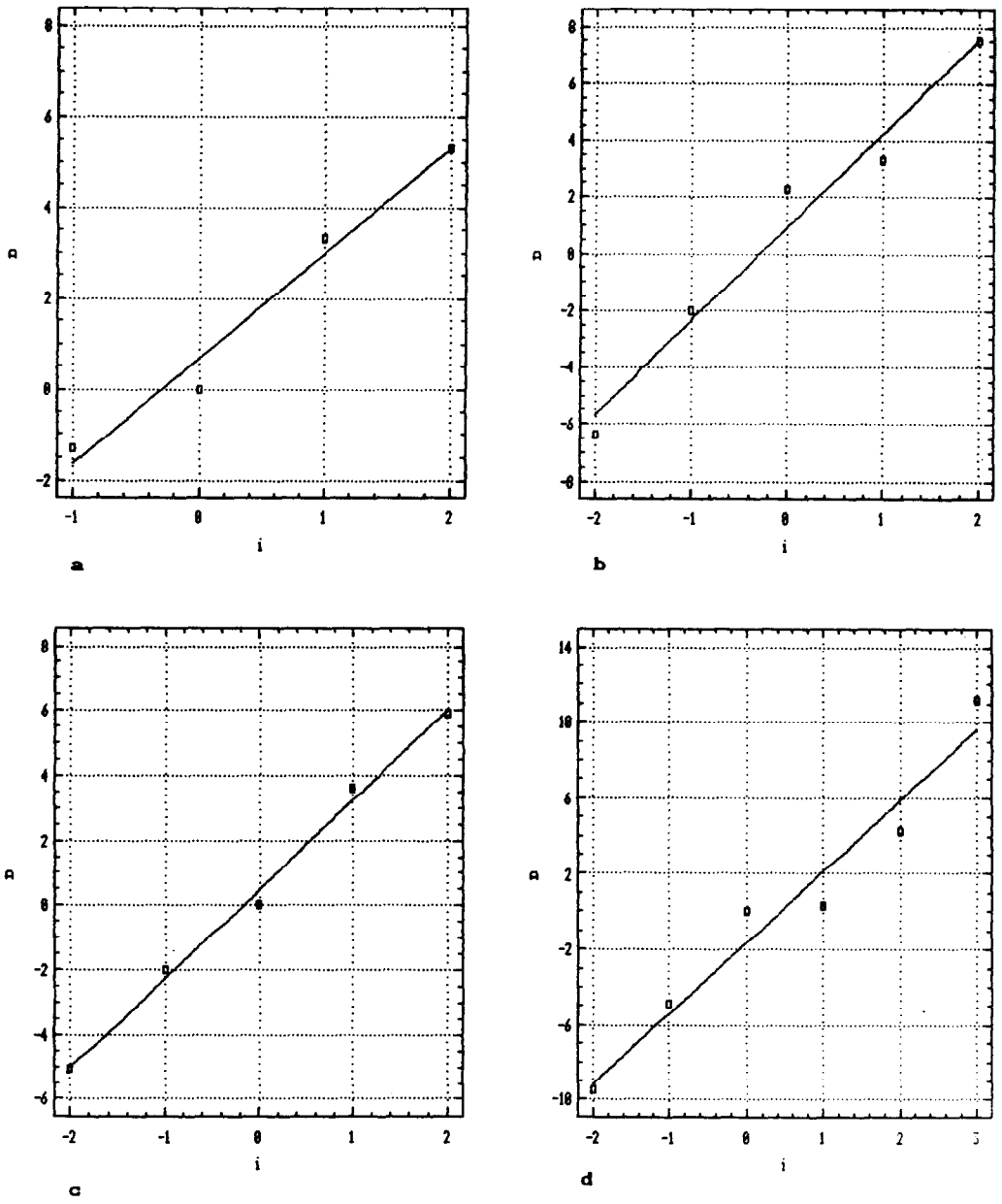

Fig. 7. Linear function errors D_i on ions i for PCBs with (a) 6, (b) 7, (c) 8 and (d) 10 atoms of chlorine.

error between experimental and theoretical intensities for two neighbouring ions.

In general, the error between theoretical and experimental intensities of isotopic pattern can be approximated by linear regression (Fig. 5):

$$D_i = a + Di \quad (7)$$

where a and D are coefficients of linear regression. If $a = 0$, then the experimental relative intensities must be corrected by the value of Di.

Table 1
Values of y_i, x_i, S.D., R.S.D., n, D_i, D and r for PCBs

N_0	M	y_i	x_i	S.D.	R.S.D.	n	D_i	D	r
1	188	10000	10000				0		
	190	3206	3323	68	2.1	10	+3.7	3.7	1
2	222	10000	10000				0		
	224	6336	6562	269	4.2		+3.6		
	226	1035	1104	52	5.0	10	+6.7	3.4	0.9991
3	256	10000	10000				0		
	258	9549	9802	289	3.0		+2.7		
	260	3051	3231	143	4.7	7	+5.9	3.0	0.9988
4	290	7981	7667	366	4.6		−3.9		
	292	10000	10000				0		
	294	4797	4912	121	2.6		+2.4		
	296	1006	1084	45	4.5	8	+7.7	3.7	0.9896
5	324	6247	6142	117	1.9		−2.1		
	326	10000	10000				0		
	328	6272	6530	113	1.8		+4.1		
	330	2010	2143	60	3.1	10	+6.6	3.0	0.9825
6	358	5191	5123	112	2.2		−1.3		
	360	10000	10000				0		
	362	7884	8148	390	4.9		+3.3		
	364	3374	3552	132	3.9	11	+5.3	2.3	0.9877
7	392	4695	4393	151	3.2		−6.4		
	394	9806	10000	230	2.4		−2.0		
	396	10000	9767				+2.3		
	398	5141	5310	111	2.2		+3.3		
	400	1617	1738	72	4.4	11	+7.5	3.3	0.9848
8	426	3553	3377	95	2.7		−5.1		
	428	8959	8782	178	2.0		−2.0		
	430	10000	10000				0		
	432	6288	6515	227	2.3		+3.6		
	434	2510	2659	149	3.1	13	+5.9	2.8	0.9970
10	494	2326	2105	123	5.3		−9.5		
	496	7193	6838	199	2.8		−4.9		
	498	10000	10000				0		
	500	8646	8673	242	2.8		+0.3		
	502	4737	4942	132	2.8		+4.3		
	504	1739	1934	64	3.7	12	+11.2	3.8	0.9779

N_0 = number of chlorine atoms in a molecule; M = nominal mass of ion; y_i = experimental relative intensities; x_i = theoretical relative intensities; S.D. = standard deviation; R.S.D. = relative standard deviation (%); n = SIM scan cycles; D_i = relative error of y_i as percentage of the base peak of the molecular pattern; D = correction factor; r = correlation coefficient of linear function for PCBs.

This theoretical procedure was verified by comparing measured and computed relative intensities. The plot of the linear function errors D_i on ions i for individual PCB congeners with two to ten atoms of chlorine are in Figs. 6 and 7. Table 1 gives measured (y_i) and theoretical relative intensities (x_i), standard deviations (S.D.) and relative standard deviations (R.S.D.) in percentages from n SIM scan cycles, values (D_i) in percentages of the base peak of the molecular pattern, correction factors (D) and correlation coefficients (r) of the linear function for PCBs with N_0 atoms of chlorine in a molecule and m/z equal to M. The correction factor D was computed from each PCB congener. The highest and the lowest values of D were statistically tested and the difference between these two values was not statistically significant. The average value of the correction factor was 3.2% and was applicable with both the HP 5977B and HP 5988A instruments.

The best way of computing the correction factor was from one GC–SIM-MS experiment of PCB congeners. PCB congeners were separated and the molecular ions with their isotopic satellites were acquired. Fig. 8 shows the chromatogram obtained with PCB congeners (Delor 104). Although the chromatographic peaks of some congeners were not resolved, this did not affect the results. The average value of the correction factor D was computed from all the congeners (Fig. 8). The correction factors and correlation coefficients of the linear regression (r) for PCB congeners with N_0 atoms of chlorine in the molecule are given in Table 2. The average correction factor D of 3.0% is similar to the average correction factor computed for individual PCBs and the difference is not statistically significant.

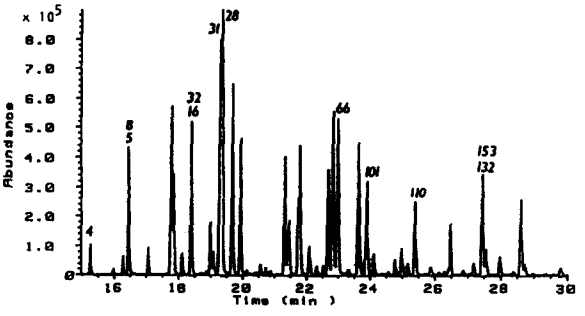

Fig. 8. Chromatogram of PCB mixture (Delor 104).

Table 2
Values of D and r for PCB congeners

N_0	Congeners	M	D	r
2	4	222	2.6	0.9806
2	5 + 8	222	2.9	1.0000
3	16 + 32	256	2.9	0.9414
3	28 + 31	256	2.5	0.8940
4	66	290	3.6	0.9326
5	110	324	3.2	0.9926
5	101	324	2.9	0.9998
6	132+ 153	358	3.3	0.9441

N_0 = number of chlorine atoms in a molecule; D = correction factor; r = correlation coefficient of linear function.

4. Conclusions

A knowledge of experimental errors in the relative intensities of ions helps in the determination of the relative intensities with sufficient accuracy for the determination of the elemental composition of ions. The measurement of relative intensities in the SIM mode give better results than those obtained in the scan mode. Greater sensitivity, accuracy and precision of measured intensities are the advantages of the proposed method.

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