Environmental Applications of Comprehensive Two-Dimensional Gas Chromatography

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Abstract

Comprehensive two-dimensional gas chomatograms of well defined sample mixtures tend to form highly structured chromatograms with peaks distributed over the retention plane in chemically meaningful patterns. It is possible to predict stationary phase combinations which will produce these meaningful patterns and to predict the patterns themselves from one-dimensional gas chromatographic retention index data. The polychlorinated biphenyls form a well defined group of substance which can be separated into chemically meaningful groups. Here the meaningful groups are determined by the number of chlorines and the position of a peak within a group is determined by the pattern of chlorines on the biphenyl structure.

Introduction

Comprehensive two-dimensional gas chromatography (GCxGC) is a high resolution separation method especially well suited to the analysis of complex mixtures. (1-5) This method provides two independent dimensions of separation and results in a gas chromatogram in which peaks are distributed over a retention plane rather than along a retention line. Each substance forms a peak on this retention plane with two retention times determined by two different molecular properties of the substance. Molecular properties that can by used in separation include volatility, polarity, and shape.

GCxGC has significant advantages over one-dimensional GC and over conventional heart-cutting techniques. Included among these advantages are: greatly increased peak capacity for more complete separation of complex mixtures; two retention times for each substance for substantially more reliable peak identification; improved detection limits due to sharper peaks; improved quantitation reliability due to reduced peak overlap and the availability of true empty baseline near every peak; chromatograms that can, at least in principle, be interpreted from fundamental principles; and the presence of distinctive patterns that can be employed to recognized particular substances or mixtures present in samples.

There are numerous environmental analysis problems for which GCxGC might be useful. Here we present one example analysis, that of the polychlorinated biphenyls, which illustrates some of the advantages of the technique. Xu presents a review of previous two-dimensional methods of PCB analysis. (6)

Theory

The primary mechanism of GC retention on most stationary phases is volatility. A nonpolar substance on a nonpolar column interacts with the stationary phase almost entirely based on its volatility. Volatility of a substance is its tendency to enter the gas phase, which is related to its molecular size or weight. The volatilities of PCB congeners are related to the number of chlorines and their relative positions on the biphenyl structure.

With a polar or shape selective column, however, retention is based on a composite of volatility and specific interaction effects. The retention index is then the sum of two contributions, a volatility retention index and a specific retention index. The specific retention index of a substance may be computed by subtracting the volatility retention index of a substance on a nonpolar phase from the total retention index of the same substance on a specific phase of interest. (7)

Recently, Frame (8) reported 27 sets of relative retention times of all 209 PCB congeners on high-resolution GC or GC/MS instruments using 20 different stationary phases. The database provides PCB congener elution orders and predicted coelutions on a variety of phases and is useful in congener identification. Here, we use Frame's PCB retention database to predict and interpret GCxGC chromatograms. (8) In a two-dimensional chromatogram using a nonpolar first column and a specific selectivity second column, retention in the first dimension is related to sample substance volatility while retention in the second dimension is determined only by specific interactions between sample subsances and the stationary phase. Retention is independent in the two dimensions and, thus, the second dimension is independent of volatility. (4) It is easy to predict first dimension retention from one-dimensional retention index data; first dimensions retention is directly proportional to retention on an appropriate nonpolar column. It is also possible to compute retention in the second dimension from one-dimensional retention index data; subtracting the portion of retention index due to volatility from the composite retention index leaves only the retention index due to the specific selectivity interation between sample substance and stationary phase. This specific retention index should be directly related to retention in the second dimension of a GCxGC chromatogram.

To implement this procedure, we introduce a new PCB index system and compute indices of all 209 PCB congeners on several stationary phases from Frame's PCB retention data compilation. This PCB index is defined based on the number of chlorines and their relative positions of attachment on the biphenyl rings. Among each group of PCB congeners with a particular number of chlorines, the one least retained on Frame's system 1 (DB1) is chosen as a reference. The ten selected PCB reference compounds are listed in Table 1. Notice that for congener groups with even numbers of chlorines, the most structure-symmetrical ones are coincidently chosen as references.

Table 1. Reference compounds selected from among the polychlorinated biphenyls.

Congener group	IUPAC No.	Substitution pattern	PCB index
1	1	2	100.00
2	4	2,2'	200.00
3	19	2,2',6	300.00
4	54	2,2',6,6'	400.00
5	104	2,2',4,6,6'	500.00
6	155	2,2',4,4',6,6'	600.00
7	188	2,2',3,4',5,6,6'	700.00
8	202	2,2',3,3',5,5',6,6'	800.00
9	208	2,2',3,3',4,5,5',6,6'	900.00
10	209	2,2',3,3',4,4',5,5',6,6'	1000.00

The PCB indices are calculated from Frame's database of temperature-programmed relative retention times using the following equation:

$$I = 100\frac{RRT - RRT_n}{RRT_{n+1} - RRT_n} + 100n$$

where I is the PCB index in the range of 100.00 to 1000.00; RRT is relative retention time from Frame's database; n is the number of chlorines (from 1 to 10); RRT_n and RRT_{n+1} are the relative retention times of PCB references with n and n+1 chlorines, respectively.

Results and Discussion

Figure 1 is constructed by the plotting retention index differences on CNBP (p,p-cyanobiphenyl, Frame's system 16) minus DB1 (Frame's system 1) versus PCB indices on DB1. Retention indices on DB1 provide a volatility separation axis. Differences in indices on CNBP remove volatility and provide a polarity separation axis. A GCxGC chromatogram with DB1 and CNBP stationary phase combination would be expected to provide the same volatility and polarity separations as computed here and resemble the distribution of points presented here. The plot in Figure 1 should simulate a GCxGC chromatogram.

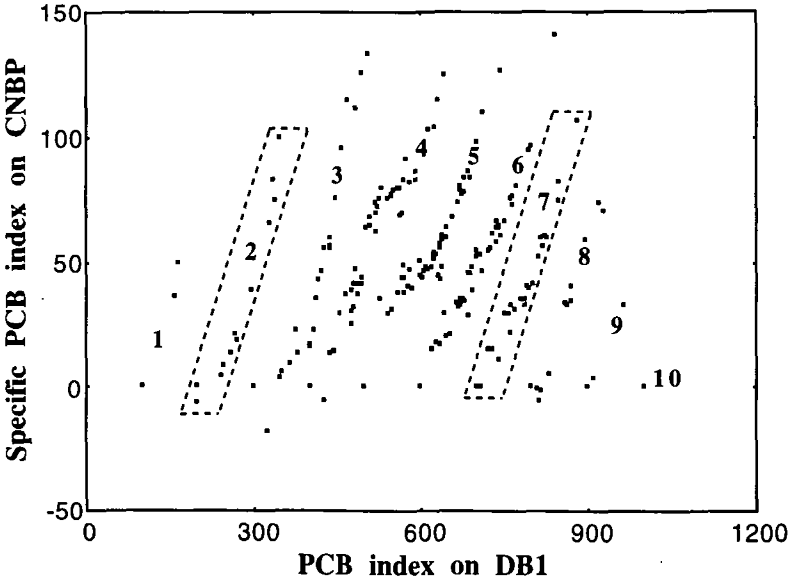

Figure 1. A predicted two-dimensional gas chromatogram obtained from one-dimensional retention index measurements.

The first interesting features of Figure 1 is that congeners are distributed over the retention plane rather than along a diagonal as would be expected for correlation in retention. As addressed in the section on theory, the second dimension retention of a GCxGC is a relatively "pure" measure of specific selectivity. In this simulated chromatogram, the relative second dimension retention (PCB index difference between CNBP and DB1) is solely based on polarity. Since the first dimension separation depends solely on volatility, the two dimensions are independent and thus the PCB's distribute over the retention plane.

Figure 1 also shows linearly distributed groups of peaks. Careful examination reveals that these lines of peaks correspond to ten PCB congener groups (labled **1** to **10**) with 1 to 10 chlorines attached. These linearly shaped groups all have approximately the same slope as indicated by groups **2** and **7**. The number of chlorines for different groups varies systematically along the nonpolar first dimension. These features are related to PCB congener structures and separation mechanisms on the two columns. In any one group, the same factor, position of chlorines, affects both volatility and polarity. A one-dimensional gas chromatographic separation of the PCB's does not separate the mixture into chlorine-number groups because retention is a composite of both volatility and polarity. Separating the two effects into two orthogonal retention axes, however, allows separation of the PCB's into groups based on chlorine-number. Within any one group, volatility and polarity are correlated and thus, each group forms a roughly linear distribution of PCB congener positions on the retention plane. Congeners in different groups have different numbers of chlorines and so have different volatilities as a group. Thus, volatility and polarity are not well correlated among the PCB congeners as a whole. The individual chlorine-number groups systematically distribute along with the nonpolar dimension according to chlorine-number only if the second dimension of separation is present to prevent overlap between chlorine-number groups.

Congener groups based on chlorine-number are not separated on either the DB1 column or the CNBP column. One-dimensional gas chromatography can not get chlorine-number groups because one-dimensional gas chromatography can not differentiate volatility separation from specific separation. To get chlorine-number groups by differentiating volatility and polarity, we must have two independent dimensions of separation which is provided by GCxGC.

The details of peak distribution over a GCxGC retention plane vary with choice of the two stationary phases involved. All phase combinations available from Frame's (8) retention index data were examined. Several combinations gave chlorine-number group separations. Most phase combinations did not separate the PCB congeners into any useful pattern.

Generating real GCxGC chromatograms of PCB mixtures using some of the phase combinations predicted to give good separations did in fact give such separations. Peaks in these two-dimensional gas chromatograms are approximately located as predicted in the retention plane. The computed retention patterns provide peak identification for the real GCxGC chromatograms of PCB mixtures.

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