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Gas chromatography of 209 polychlorinated biphenyl congeners on an extremely efficient nonselective capillary column

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ABSTRACT

The gas chromatographic–mass spectrometric (GC–MS) separation of all 209 polychlorinated biphenyl (PCB) congeners was studied on an extremely efficient 80 m × 0.1 mm i.d. capillary column coated with a 0.1 μm film of poly(5%-phenyl methyl)siloxane stationary phase. The quality of the separation and the number of resolved and coeluting peaks were compared to predictions according to the statistical overlap theory (SOT) and to literature data on PCB separations obtained by one-dimensional and comprehensive two-dimensional GC (GC × GC) and GC–MS. Mass spectral and chemometric deconvolution procedures were used to resolve overlapping peaks. On the highly efficient column, 195 PCB congeners were resolved in 96 min separation time using spectral and chemometric deconvolution. This number is comparable to the best separations described in GC × GC–MS mode. The novel method was developed for spectral deconvolution of overlapped PCB congeners which was verified determining the most toxic, dioxin-like PCBs both in the model mixture of 209 PCBs as well as in the Aroclor 1242 and Aroclor 1254 formulations.

1. Introduction

The chromatographic separation of polychlorinated biphenyls (PCBs) has challenged analytical chemists since these chemicals were found in environmental samples more than 40 years ago [1]. The set of 209 possible PCB congeners, from the three monochloro biphenyl isomers to decachloro biphenyl, is commercially available and their separation also provides an interesting tool to evaluate separation power of different chromatographic methods.

The methods of choice in congener-specific PCB analysis are capillary gas chromatography (CGC) with electron capture detection (ECD) or CGC with mass spectrometric (MS) detection performed on a single column, on dual parallel columns, on two columns coupled in series as well as on two columns working in two-dimensional (GC–GC) and comprehensive GC (GC × GC) mode.

Fused silica open tubular capillary columns, coated with stationary phases of various polarity and/or selectivity are used for GC separation of PCBs [2–6]. The basic methodology for the separation of PCB congeners, as described by Mullin et al. [2] has not changed substantially over the years. Based on the separation of individual PCB congeners, it was claimed that on a 50 m × 0.20 mm fused silica capillary column coated with poly(5%-phenyl methyl)siloxane (SE-54), 187 of the total 209 PCBs could be resolved. Cochran and Frame found, however, that the number of (sufficiently) resolved PCBs on this stationary phase is much lower, and that only 90–100 congeners can be resolved if the whole mixture of 209 congeners is analyzed in a single run [6].

Due to a number of important coelutions, especially those that involve a toxic or regulated congener, on capillary columns coated with poly(5%-phenyl methyl)siloxane, researchers have explored alternative phases for PCB separations. Silarylene phases (polysiloxanes containing alkyl, phenyl and *p*-phenylene substituents, e.g., DB XLB, from J&W Scientific, Folsom, CA, USA) as well as carborane backbone phases (polysiloxanes containing methyl and metacarborane CB_{10} $H_{10}C$ substituents, e.g., HT-8 from SGE Inc., Austin, TX, USA) enabled to resolve more than 190 CBs using mass spectrometric deconvolution of overlapped peaks [7]. Also serial coupled columns, resulting in "tuned" selectivities, were used to obtain optimized resolution of PCB congeners [8,9].

The analysis of PCB was further investigated by separating PCB samples on two parallel capillary columns [7,10,11] and dual-column GC, in which the sample is injected simultaneously into two columns coated with different stationary phases, is therefore recommended by the US Environmental Protection Agency (EPA) in their Method 1668 for analysis of toxic PCBs [12]. It is claimed that a column pair consisting of a DB-1 (J&W Scientific) and a SPB-Octyl (Supelco, Bellefonte, PA, USA) is "capable of resolving all 209 PCB congeners".

From reviews by Larsen [3] and Cochran and Frame [6], it can, however, be concluded that no single capillary column is able to resolve all 209 PCB congeners.

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For resolution of PCB congeners overlapping on a single capillary column, also two-dimensional GC (GC–GC) and comprehensive GC (GC × GC) were used. For the isolation of coplanar PCB congeners in technical PCB mixtures (e.g., Aroclors), for instance, two-dimensional GC with heart-cutting is probably still the best and most robust method, resulting in excellent quantification in a short analysis time [13–15].

More recently, the potentially extremely high resolving power of comprehensive GC was also applied to PCB analysis [16–21]. The most advanced papers on the GC × GC separation of PCBs were published by Harju et al. [19] and Focant et al. [20] where the resolution of respectively 176 and 192 PCB congeners was reported.

The separation efficiency of columns and column combinations (in GC × GC) is usually evaluated by a number of "resolved" PCBs. It is, however, impossible to appraise the separation quality of capillary columns or column combinations in most published papers where very high numbers of resolved PCBs are reported without showing the actual separation or resolution criteria (e.g.: "Are they baseline resolved or can only peak maxima be distinguished? Chromatographically resolved or resolved also by mass spectrometric deconvolution?").

The aim of this paper is to show the perspectives and limitations of separation of PCBs on an extremely efficient capillary column coated with poly(5%-phenyl methyl)siloxane. An 80 m narrow bore (0.10 mm i.d.) column with a theoretical plate number of 800,000 is probably the most efficient single-dimension GC column that can be used on commercially available equipment. Retention times and elution order of all 209 PCB congeners were determined and the resolution of all neighboring peaks (or peak clusters) was calculated. Mass spectral and chemometric deconvolution procedures were applied to resolve chromatographically non-separated PCBs. Statistical overlap theory (SOT) was used to evaluate the separation of PCBs on this column.

2. Theory

2.1. Characterization of column separation efficiency in linear temperature programmed gas chromatography (LTPGC)

Peak capacity (n_c) is used to describe the column separation efficiency both in isothermal as well as in temperature programmed GC. It is defined as the maximum number of peaks that can fit with required resolution factor R_s into an available separation space, being the interval between the gas-hold-up time (t_M) and the retention time of the last peak ($t_{R,n}$). The peak capacity calculated for resolution factor $R_s = 1.00$ in this separation space can be defined as theoretical peak capacity $n_{c,t}$ and, for LTPGC runs, $n_{c,t}$ can be calculated from Eq. (1):

$$n_{c,t} = \frac{t_{R,n} - t_M}{\bar{w}_b} \quad (1)$$

where $\bar{w}_b$ is the mean peak width at base [22].

In practice, however, the full separation space is usually not exploited, and therefore, instead of theoretical peak capacity $n_{c,t}$, the practical peak capacity $n_{c,p}$ should be used as it expresses more realistically the column separation efficiency in the $t_{R,1} - t_{R,n}$ separation space for a given application:

$$n_{c,p} = \frac{t_{R,n} - t_{R,1}}{\bar{w}_b} \quad (2)$$

where $t_{R,1}$ is the retention time of the first eluting peak. In case of PCBs, for instance, all congeners will elute in a window between the first eluting monochloro biphenyl and the last eluting PCB (decachloro biphenyl on majority columns). This window is narrower than the total analysis time (temperature window) would suggest.

As indicated, the peak capacity is determined by the minimum required resolution for two adjacent peaks. R_s depends on the retention time difference (Δt_R) between the peaks and the average peak width ($\bar{w}_b$) as given by Eq. (3):

$$R_s = \frac{t_{R,j} - t_{R,i}}{2\sigma_i + 2\sigma_j} = \frac{\Delta t_R}{4\bar{\sigma}} = \frac{\Delta t_R}{\bar{w}_b} \quad (3)$$

where $t_{R,i}$ and $t_{R,j}$ are retention times and σ_i and σ_j are peak standard deviation of the first (i) and second (j) eluting peak in the considered peak pair, $\bar{\sigma}$ is the mean value of standard deviations of the peaks and $\bar{w}_b$ is the mean value of peak width at base. In this respect, $R = 1$, corresponds to a 4σ separation.

2.2. Statistical overlap theory (SOT)

The statistical overlap theory (SOT), has been introduced by Davis and Giddings, about 25 years ago [23–25]. In SOT, a separation is considered as a combination of several separations, in which the number of sample components and their retention times (or other measure of positions) vary in accordance with some probability distribution and frequency. In the simplest theory, the components are randomly distributed in the separation time interval in accordance with a homogeneous Poisson's distribution process.

The peak capacity plays an essential role in the SOT, which is often used to characterize a column separation quality. The saturation factor α is a measure of overlap and is defined as the number of components in the sample m divided by the peak capacity [26]. The theoretical saturation factor α_t can be calculated from Eq. (4):

$$\alpha_t = \frac{m}{n_{c,t}} \quad (4)$$

As for peak capacity, also a practical saturation factor α_p can be defined:

$$\alpha_p = \frac{m}{n_{c,p}} \quad (5)$$

Based on the saturation factor α and the number of solutes in the mixture m, the average number of peaks p appearing on the chromatogram can be predicted according to Davis [27]:

$$p = m \times e^{-\alpha} \quad (6)$$

For the calculation of the number of singlets and multiplets Eqs. (7)–(9) are used:

number of singlets:

$$s = m \times e^{-2\alpha} \quad (7)$$

number of doublets:

$$s = m \times e^{-2\alpha} \times (1 - e^{-\alpha}) \quad (8)$$

number of any multiplets can be found from the general equation:

$$t = m \times e^{-2\alpha} \times (1 - e^{-\alpha})^{\nu} \quad (9)$$

The exponent ν can be calculated from the equation $\nu = n - 1$ where n characterizes number of peaks in a cluster (e.g., for triplet $\nu = 2$, etc.).

3. Experimental

3.1. PCB standards

The PCB standard solutions were purchased from AccuStandard (New Haven, CT, USA). Nine multi-congener solutions (C-CS-01–C-CS-09), containing subsets of all native 209 PCB congeners at concentrations of 10 μg/mL in iso-octane, were used.

Aliquots of the nine multi-congener solutions (C-CS-01–C-CS-09) were mixed to produce a solution containing all 209 congeners