Fundamental considerations of efficiency and speed of chemical separation applied to the analysis of dioxins and related substances

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1. Introduction

In chemical analysis at sufficiently low concentrations, all samples are complex. Quantitative measurement of any one substance then requires powerful chemical separation methods to discriminate against the large number of possible interfering substances that may be present at very low concentrations. Substances present at high concentration may also interfere because even a very small sensitivity toward them results in a relatively large signal. Powerful chemical separation and discrimination methods require time and effort, and consequently, are expensive.

The detection and quantitation of dioxins and related substances at trace levels is a challenging analytical problem. Many possible interferences must be removed or discriminated against before the final measurement can be made using a very sensitive detection device. In conventional methods, an extensive sample clean up procedure is followed by a long-column gas chromatographic separation with further selectivity provided by a high-resolution mass spectrometric detector. [1] Each step in the procedure is designed to discriminate against possible interferences from other substances that may be present so that the final quantitative measurement can be made with the greatest possible precision and accuracy. The time and effort required for this procedure results in a very high cost per analysis which limits the number of analyses that can be done and limits our understanding of the environmental and toxicological effects of these substances.

2. Theory

Chemical separation becomes more efficient as the number of independent separation steps or mechanisms applied to the problem is increased. Any one separation mechanism can discriminate efficiently only against substances which differ significantly from the target substance by the chemical properties relevant to the particular mechanism. Substances which differ only slightly from the target substance are difficult or impossible to separate. It is usually better to combine several separation methods rather than to try to increase the separation power of any one single method. Thus, the analysis of dioxins and related substances is done using several clean-up separations, followed by a high-resolution gas chromatographic separation, and finally, a high-resolution mass discrimination at the

detector. [1] Obviously, no wet chemical method could be expected to alone provide sufficient separating power for this analysis. Nor is high-resolution mass spectrometry alone sufficient to discriminate against whatever might be present in the samples. Nor can gas chromatography, even with an exceedingly long and powerful column, do the job. Only the combination of these several different separation methods can provide the required discriminating power.

Gas chromatography, given sufficient plate count, can separate substances which differ only very slightly in chemical properties from the target substance. Thus, some variety of gas chromatography is often chosen to separate the substances which most closely resemble the target substance in overall properties.

Unlike most separation methods, the resolving power of chromatography is readily adjustable over a wide range. We can substantially increase the number of substances that can be separated and the resolution between any pair of substances by lengthening the column to increase the plate count. In simple separations, the improvement obtained can be directly proportional to column length because extra-column components often limit the efficiency of separation. However, at some point improvement in separating power of any chromatographic method must become column limited and then is only proportional to the square root of column length. [2] Attempting to obtain substantial increases in separating power in this regime can be quite expensive in terms of instrument time. If the number of samples to be analyzed is large and the instrument is expensive (both are true in dioxin analysis), then this square root efficiency relationship of chromatography can become a major limitation.

If a chromatographic separation is operating in the regime where its resolving power is proportional to the square root of time and the chromatographic analysis time is significant to the overall analysis cost, then we should consider using a multi-dimensional chromatographic separation. Just as we can obtain much greater efficiency by combining several different separation or discrimination techniques in an analysis, we can obtain greater efficiency within the chromatographic separation by combining two or more columns of differing selectivity. The first column provides a separation based on one molecular property, such as volatility, while the second column provides separation based on an independent property, such as polarity or shape. Neither column need provide the entire separation and so each can be substantially shorter than would be otherwise required.

If one target substance in a complex mixture is to be determined, then these ideas lead to the heart-cutting method of gas chromatography. In this method, the first column provides a quick, partial separation based on one molecular property. The peak containing the target substance is then transferred to a second column for separation based on an independent property. Heart-cutting methods are often used for separations that would be impossible otherwise because the length of column and time required would be far beyond practicality if attempted as a single column technique. Heart-cutting is also potentially useful to increase the speed of existing long-column separations, but is often overlooked in this application.

If more than one target substance is to be determined,then the heart-cutting variety of multi-dimensional chromatography becomes impractical. It has been used to determine multiple substances in complex mixtures. For example, Gordon and his group used it to characterize a tobacco essential oil by taking heart-cuts from a series of first column chromatograms. [3)]

Comprehensive two-dimensional gas chromatography is a powerful multi-dimensional chromatographic separation method. [4-7)] Two columns are connected serially to provide two independent separations as in heart-cutting. Unlike heart-cutting, however, it is applicable to the determination of an entire complex mixture. All sample emerging from the first column is transferred to the second column using a thermal modulator. The second column separates the components of each transferred portion to generate a series of high-speed secondary chromatograms. At each point in the primary chromatogram where one would normally record a single value, the secondary column generates a complete high-speed gas chromatogram instead. The method is analogous to GC / MS in which mass spectra are generated at each point in the chromatogram. Like GC / MS, the data is truly two-dimensional. Plotting the two-dimensional data using one independent axis for primary column retention, a second independent axis for secondary column retention, and a third dependent axis for detector signal gives a comprehensive two-dimensional gas chromatogram as illustrated in Figure 1.

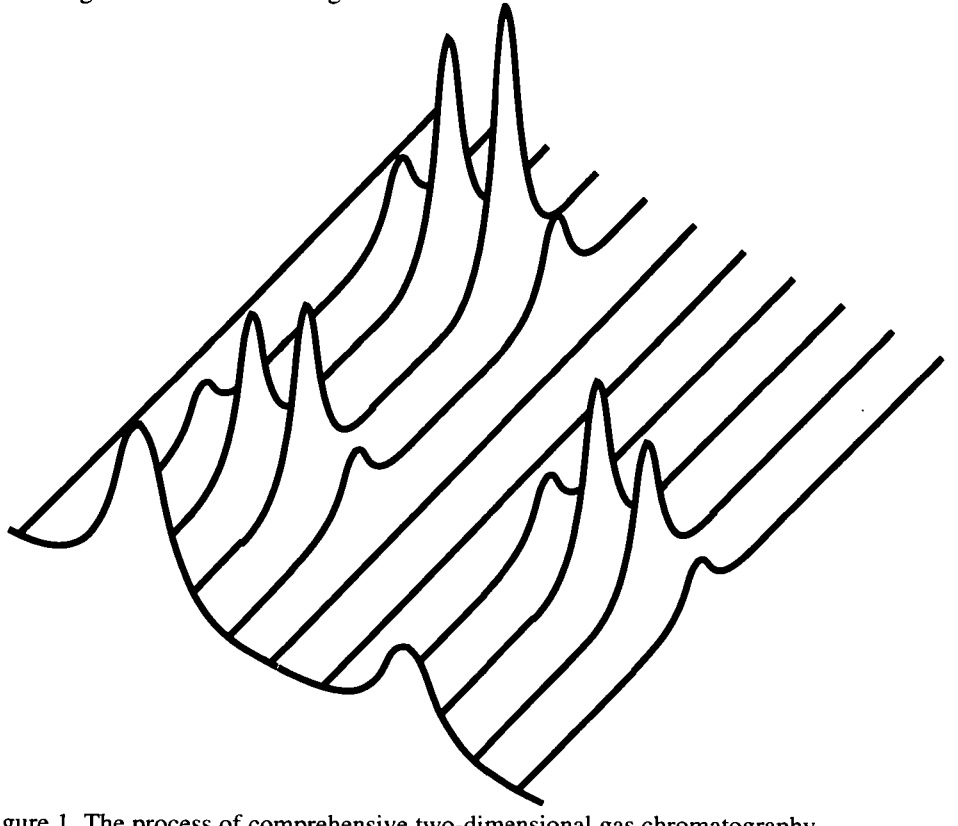

Figure 1. The process of comprehensive two-dimensional gas chromatography.

Replacing the long-column gas chromatographic separation used in a conventional dioxin analysis with this new method, can substantially improve speed and resolving power of the chromatographic portion of the procedure. A very long column is required in conventional methods for dioxin analysis because a substantial number of substances must be separated and several critical pairs exist within this group. Changing the stationary phase does not help much because that just moves peaks around creating different critical pairs and the column must still be long to separate them. A long column requires a slow temperature program, and thus, the chromatographic separation requires a long time. The much shorter primary column used in a comprehensive two-dimensional separation can be programmed at a much greater rate to get the analysis done much faster. Of course, the short primary column can no longer separate the critical pairs, but the secondary column, which uses a different and independent mechanism, can separate them. The secondary column is also short and fast because it never sees a mixture containing more than a few substances at any one time; the major separation based on volatility is provided by the primary column. The speed of the secondary column also provides an improved sensitivity because sharper chromatographic peaks are taller peaks.

3. References

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