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A critical look at the definition of multidimensional separations☆

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ABSTRACT

Multidimensional (MD) separations, especially comprehensive two-dimensional (2D) separations such as comprehensive 2D LC (LC × LC), and comprehensive 2D GC (GC × GC), are potentially powerful separation techniques. It is important to have a clear definition of MD techniques to better understand the scope and boundaries of the subject. Widely accepted definitions of MD Separations have their roots in the definition proposed by Giddings. Giddings also added several comments that clarified the scope of his definition. However, some researchers extend Giddings' definitions beyond their intended scope. Doing so disqualifies such comprehensive 2D techniques as LC × LC, GC × GC and 2D TLC from being considered as 2D techniques. In other instances, extended treatment of Giddings' definition is used as a basis to justify design-parameters of comprehensive 2D separations despite the fact that these parameters lead to sub-optimal implementations. We believe that the shortcomings in the definition and its popular interpretations are serious enough to warrant attention, especially by those interested in designing optimal instrumentation for MD separations like comprehensive 2D GC. After discussion of the weaknesses in the currently used definitions, we propose to define *n*-dimensional analysis as one that generates *n*-dimensional displacement information. We believe that this definition captures the spirit of Giddings' definition while avoiding the problems associated with its popular interpretations.

1. Introduction

In any field of study, definitions provide context to frame discussion. Sometimes definitions provide a needed focus to development of related theory and even applicable instrumental development. If definitions are overly constraining, exclusionary, or misapplied, then they can cause problems. Such is the case with a commonly presented definition of multidimensional (MD) separations, and especially comprehensive two-dimensional (2D) separations such as LC × LC [1,2] and GC × GC [3]. The common impression is that to consider a separation to be multidimensional, requires the following two conditions:

1. Separation mechanism in each step must be "orthogonal".
2. Separation gained in the first dimension must not be lost in subsequent steps.

These requirements are typically attributed to Giddings' definitions of MD separations [4–6]. In Giddings early discussion of MD separations [4], he stated, "two-dimensional (2D) separations are those techniques in which a sample is subject to two displacement *processes oriented at right angles to one another*." Later, he rephrased that aspect to "components in a mixture are subjected to two or more separation steps (mechanisms) in which their displacements depend on *different factors*" [5], or "two *largely independent separative displacements*" [6] (All *italics* are ours.).

In his later definitions of MD separations, Giddings also added a second constraint which required that [5] "the separation must be structured such that whenever two components are adequately resolved in any one displacement step, they will generally remain resolved throughout the process" Giddings was very explicit that the purpose of the second constraint was to "rule out purely tandem arrangements of two or more columns" where (as illustrated in Fig. 1) "the resolution gained in one column can be partially or entirely nullified"

These two attributes of Giddings' definition are the basis for the oft repeated definition of multidimensional separations. Unfortunately, extension of Giddings' second constraint beyond its intended goal to exclude tandem columns excludes many multidimensional separation techniques that are done today.

2. Materials and methods

Concepts illustrated in the comprehensive 2D GC separation illustrated in Figs. 2 and 3 are derived from Ref. [7].

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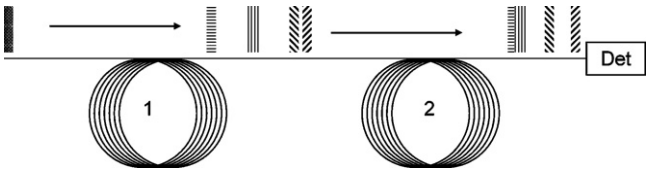

Fig. 1. In tandem serial column configurations, such as this, effluent from one stage flows directly to the next stage. Retention information relating to the first stage is not acquired. The retention information acquired relates to the net combination of both steps. In the illustration, 1st-dimension separation of horizontal and vertical striped peaks is degraded by subsequent passing through 2nd-dimension column.

The background and details about the allergens example in Figs. 4 and 5 are described in Ref. [8].

3. Discussion

Consider the following three examples. Fig. 2 shows chromatograms that can be obtained from a typical comprehensive 2D GC experiment. The top chromatogram was generated by direct connection of the primary column to a mass spectrometer. The flow and temperature program rate used was typical of GC × GC conditions frequently used [9]. On average, peak standard deviation was 2.1 s [7]. For the 2D experiment, elute of the primary column was thermally modulated every 6 s into a polar secondary column. That increased standard deviation of the 1st-dimension modulated and reconstructed peaks to 3.7 s [7] causing nearly 70% increase in the 1st-dimension peak width and more than 40% loss in the 1st-dimension peak capacity. This is a significant violation of the extended interpretation of the second constraint in Giddings' definition.

Should one conclude, therefore, that the separation resulted in 2D density plot of Fig. 2c was NOT a 2D separation?

In our opinion, such a conclusion would serve little useful purpose. It ignores the fact that the separation yielded 2D displacement information regarding the sample, and it is at odds with previously accepted concepts of MD separations [1,10,11].

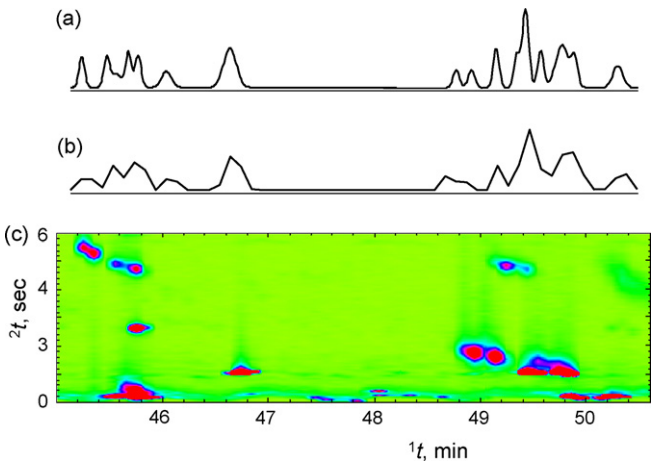

Fig. 2. Analysis of semivolatiles from Ref. [7]. (a) Chromatogram obtained from the system with modulation turned off. (b) A reconstruction of the 1st-dimension chromatogram from thermally modulated GC × GC analysis. (c) Density plot presentation of GC × GC chromatogram. The standard deviation of peaks in (a) was 2.1 s. The standard deviation of peaks in (b) was 3.7 s [5,6] – nearly a 70% increase in the 1st-dimension peak width and more than 40% loss in the 1st-dimension peak capacity.

Fig. 3 illustrates a 2D TLC separation. Development in the first axis leads to spot expansion in all directions as a function of mobility. Development in the second axis does the same, and in doing so degrades the 1st-dimension separation (as illustrated on the side). This also does not comply with the extended interpretation of the second constraint.

Figs. 4 and 5 illustrate a practical example of sequential heartcutting in the analysis of allergens in a perfume sample. Fig. 4 illustrates the GC configuration used for heartcutting portions of the primary column effluent to a column of different polarity. Unlike more typical heartcut configurations, the secondary column in this example is a low thermal mass (LTM) oven module that can be temperature programmed separately from the primary column.

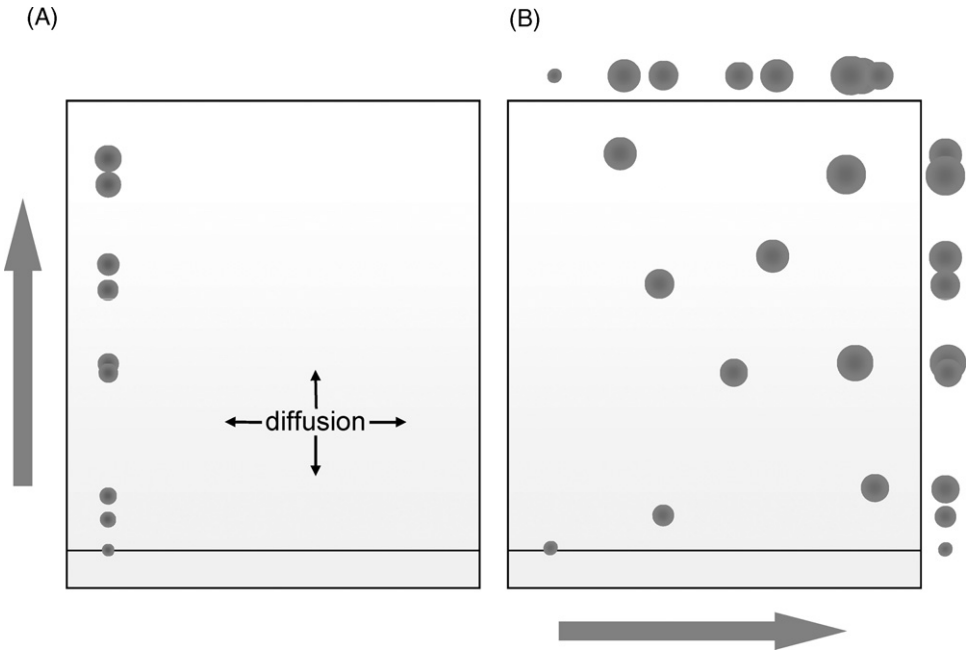

Fig. 3. Hypothetical 2D TLC example. (A) Development in the first axis separates many components and the spots spread. (B) Subsequent separation in the perpendicular axis. The quality of peak separation achieved in the 1st dimension axis was degraded through development in the second dimension (reconstruction to the right) even though overall peak separation improved in 2D space.

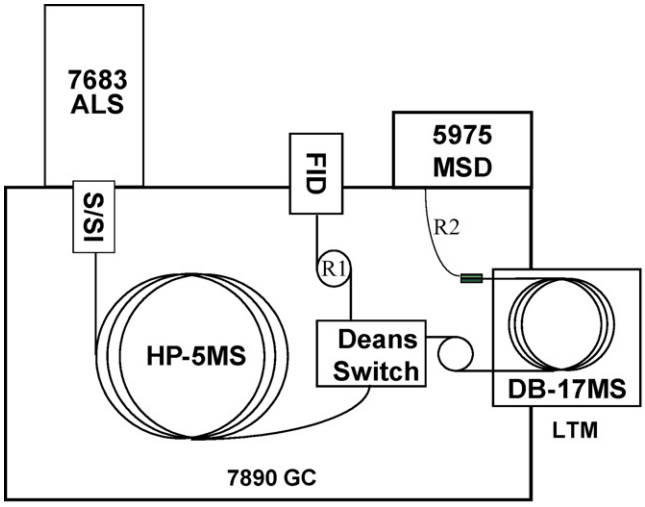

Fig. 4. System configuration used for multiple heartcut experiment. The 2nd dimension column is a Low Thermal Mass column module, allowing independent temperature control. Columns and conditions listed in [8].

Fig. 5a shows the chromatogram from the primary column. The single column was unable to separate target allergens from sample matrix. Heartcuts of the poorly separated regions (outlined with dashed lines) were heartcut to the secondary column. In preliminary experiments, when the secondary column was housed in the same oven as the primary column, separation was insufficient to resolve all target allergens. By maintaining the secondary column at low temperature until the last heartcut was completed, then programming the secondary column temperature, two previously hidden target allergens (Lyral 1 and Lyral 2) were fully resolved, as illustrated in Fig. 5.

In this example, all separated compounds from the primary column are recombined at the head of the secondary column through thermal focusing. Each subsequent heartcut is recombined with prior heartcuts. So, primary column separations for the heartcuts are totally destroyed through the process. As was the case for the other examples, the separation in this example resulted in useful 2D displacement information regarding a sample, even though none complied with the extended interpretation of Giddings' definition.

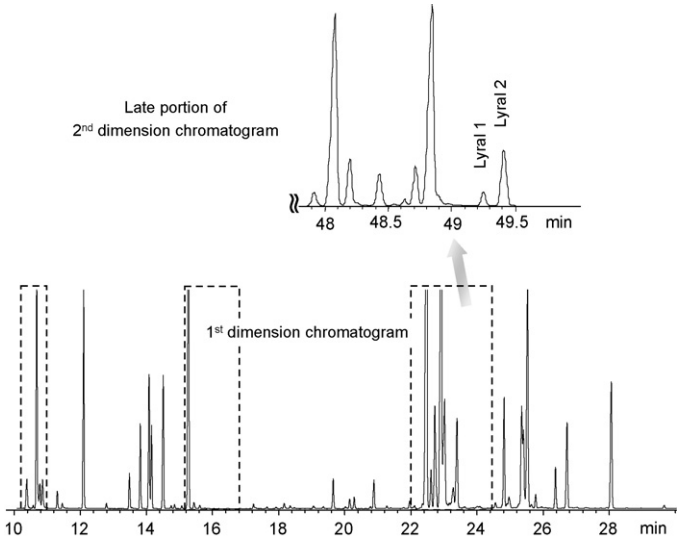

Fig. 5. Sequential heartcut example using the configuration of Fig. 4. Lower chromatogram shows separation of a perfume sample on the 1st-dimension column (HP-5MS) using FID. Times of the three heartcuts are outlined with dashed lines. Heartcuts were sequentially focused on DB-17 2nd column at low temperature, after which its temperature was increased to elute heartcut components. Inset chromatogram is of the latter part of the secondary column temperature program wherein components originally in the third heartcut are now separated.

The weakness of the existing definitions of *n*-dimensional separations comes from its very structure. The first requirement in Giddings' definition states: "Multidimensional separation ... requires ... that components be subjected to two or more largely independent separative displacements." This requirement accommodates all arrangements consisting of two or more largely independent separative steps including those (like tandem arrangements) that do not provide additional dimensions of information. To compensate for this weakness in the definition, Giddings added a second constraint: "whenever two components are adequately resolved in any one displacement step, they will generally remain resolved throughout the process." This constraint excludes tandem arrangements, but it also unfortunately excludes other arrangements that *do* provide multidimensional displacement information. They are excluded simply because the MD displacement information is acquired at the expense of reduced resolution obtained in prior separative steps, as illustrated in our previous examples.

The following proposed definition is based not on counting the number of internal separative steps involved in an MD analysis, but on the outcome of the analysis as a whole.

3.1. An n-dimensional (nD) analysis is one that generates n-dimensional displacement information

The definition implies that, for example, a GC or LC analysis is 2-dimensional if the output, y, of its detector can be expressed as a function, $y({}^1t, {}^2t)$, of two *time-coordinates* (*displacement coordinates*) ${}^1t$ and ${}^2t$, as in Fig. 2c. The output of 2D thin layer chromatography (TLC) could be a function $y({}^1x, {}^2x)$ of displacement *distances* ${}^1x$ and ${}^2x$. Functions like $y({}^1t, {}^2t)$ and $y({}^1x, {}^2x)$ are 3D objects. However, the dimensionality of analysis is defined not by dimensionality of its output, but only by dimensionality of its displacement space.

Going back to our previous examples, one can conclude that the tandem arrangement of Fig. 1 is a 1D analysis because its output is a function, $y(t)$: of 1D displacement, t. As mentioned earlier, Fig. 2c is graphical presentation of a chromatogram, $y({}^1t, {}^2t)$, in 2D displacement space. Therefore, the GC analysis that resulted in this chromatogram was a 2D analysis. The same is true for 2D TLC analyses resulting in the 2D spot-distribution map of Fig. 3.

Testing the example of heartcut GC analysis (Figs. 4 and 5) against the definition is a little more difficult because, at the time of this writing, we did not have access to the original data to present it in 2D displacement space. We can suggest that the peak distribution map of this analysis might look like the one shown in Fig. 6. If that is the case then our three-heartcut example is indeed a 2D one.

Several features of the proposed definition deserve attention.

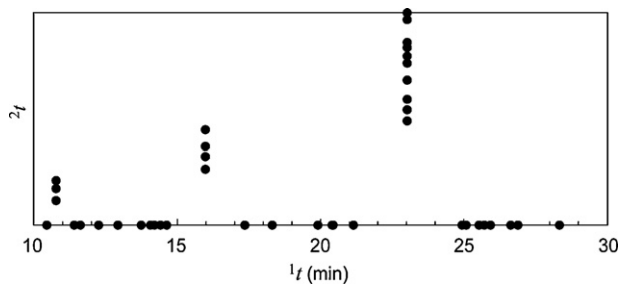

Fig. 6. 2D peak distribution map of a heartcut analysis with the cuts at 11, 16 and 23 min.

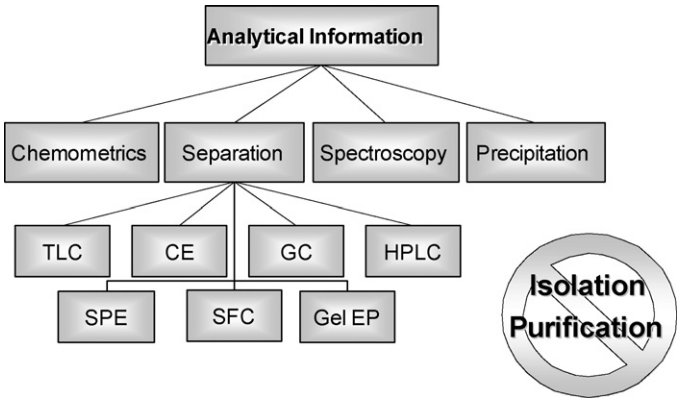

Fig. 7. The term "analysis" is used in the proposed definition instead of "separation" to keep the focus analytical information. Analytical displacement information can be gained from a number of techniques in addition to chromatographic separation. Preparative separations, with a goal of isolating material, are excluded from the definition even though multiple steps of separation might be involved because the do not provide analytical information at each step, they provide physical material.

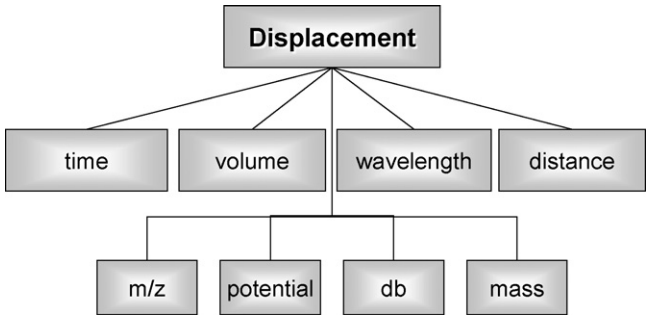

Fig. 8. "Displacement information" is used instead of terms like "retention time" in the proposed definition because the information can take several forms depending on the technique used for that particular dimension. Displacement relates to the gradient or scale relevant to the individual technique.

The definition speaks of *analysis* – a separation that generates *information* regarding a sample. This point is graphically illustrated in Fig. 7. The definition does not apply to *preparative* separations producing *purified substances*. The number of *separative steps* that it takes to isolate the substance is a meaningful concept. However, it is not as meaningful to speak of dimensionality of the substance (the outcome of a preparative separation).

*n*D displacement space where the separation obtained by *n*D analysis takes place can be also called *n*D *separation space* of that analysis. In that context, the 2D time-space of GC × GC or LC × LC analysis is its 2D *separation space*, Fig. 2c.

The new definition accommodates many existing concepts.

The definition does not mention *displacement* (*separation*) *steps*. It does not deny that *n*D analysis might consist of *n* separative displacement steps, but it also does not require the existence of exactly *n* steps. For example, there could be three tandem columns in the first dimension and two tandem columns in the second dimension of a comprehensive 2D GC analysis; a total of five steps.

The definition also does not require that, if *n*D analysis consists of *n* displacement steps, then the entire sample be subjected to all *n*D steps. In *comprehensive* *n*D analysis like GC × GC and LC × LC, the entire sample or representative fraction thereof is subjected to *n* separative steps. The term *comprehensive* has been proposed by Bushey and Jorgenson [2]. Notations such as GC × GC and LC × LC are adaptations of the pattern *chromatography* × *chromatography* proposed by Giddings [4]. Traditionally, notations like GC × GC and LC × LC imply *comprehensive* 2D techniques. In non-comprehensive techniques like 2D heartcutting, only a subset of the sample is subjected to the second step.

Although the proposed definition speaks of displacement information (examples illustrated in Fig. 8), it is silent regarding *orthogonality* of the separative steps. An important (in most cases, obligatory) part of existing definitions of *n*D separation is *orthogonality* (total independence from each other) of all *n* separation mechanisms. Being intentionally silent about the displacement mechanisms, the newly proposed definition is also silent about concepts of orthogonality.

The orthogonality of two displacement mechanisms is typically illustrated by a peak distribution map similar to one in Fig. 9a while the absence of the orthogonality is illustrated by the peak distribution map similar to one in Fig. 9b. Although the latter offers less efficient *utilization* of the separation space, and, as a result, possibly fewer resolved peaks, the separation space in Fig. 9b, and, therefore, the analysis that resulted in it is 2D analysis according to the newly proposed definition and in agreement with the earlier established understanding of a concept of 2D analysis [1,10,11].

Distribution of spots in MD space is considered to be *statistically uniform* (Fig. 9a). if the likelihood of having a spot in a subspace of that space does not depend on the location of that subspace within the space. Otherwise, the distribution is *non-uniform* (Fig. 9b).

Although statistically uniform peak distribution in 2D separation space is frequently used to illustrate two orthogonal (mutually independent) separative mechanisms, the two concepts – orthogonality of the displacement mechanisms and statistically uniform peak distribution – are different. Consider a map, Fig. 10, of the temperature pairs $({}^1T, {}^2T)$ yielding the same retention factor, k_0, in two columns, #1 and #2, for each *solute* – component of some test-mixture. In the case of independence of retention mechanisms in these columns, the temperatures, ${}^1T$ and ${}^2T$, at which each solute has retention factor k_0 in columns #1 and #2 are independent from each other. This is reflected in *statistically uniform* distribution of $({}^1T, {}^2T)$ pairs in Fig. 10.

Suppose that columns #1 and #2 are the 1st- and the 2nd-dimension columns, respectively, in a single-oven temperature-programmed GC × GC analysis with heating rate of about 10 °C per 1st-dimension hold-up time ramping from 50 °C to 350 °C (10 °C per hold-up time is optimal heating rate for stand-alone column [12,13]). In this case, retention factors, ${}^1k_R$, of all solutes eluting from column #1 are close to 2 (${}^1k_R \approx 2$) [7,12,14–16]. Suppose that, in Fig. 10, $k_0 = 2$, and consider a point at (100 °C, 350 °C) in the upper left corner of the map. It represents a solute that elutes from column #1 at temperature close to 100 °C. That solute will not elute from column #2 until close to the end of the entire GC × GC analysis when oven temperature gets above 300 °C. In fact, the majority of

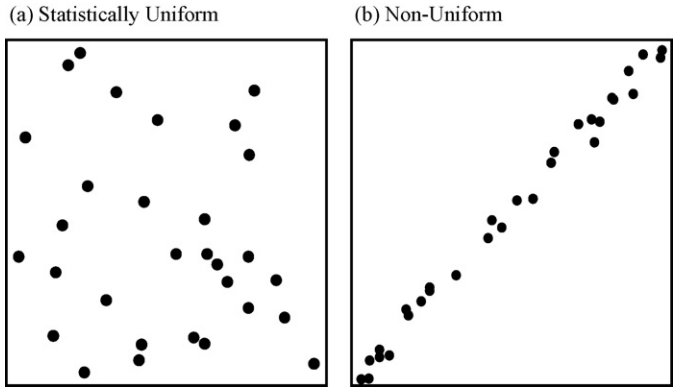

Fig. 9. Graphical representation of the statistics of spot distribution in a 2D plane. The statistically uniform distribution in (a) offers a better utilization of separation space and higher likelihood of resolving any two spots than the non-uniform distribution in (b).

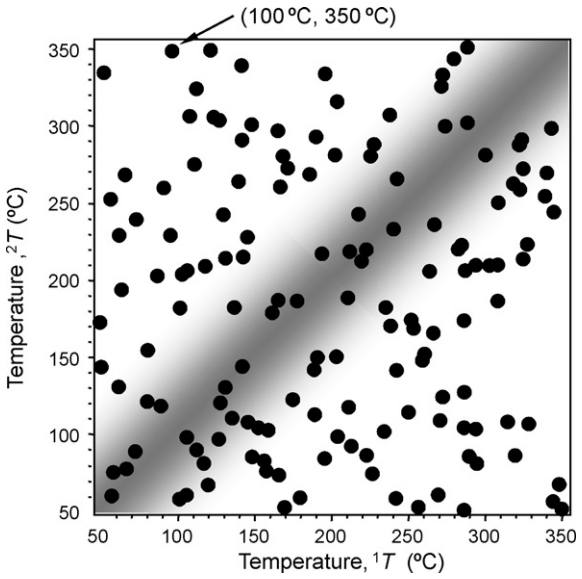

Fig. 10. Distribution map of temperature pairs (^1T, ^2T) corresponding to the same retention factor, k_0, for all components of a test-mixture in two columns, #1 and #2. Each (^1T, ^2T) pair is represented by a dot. Thus, the dot at (100 °C, 300 °C) represents a solute with $k = k_0$ at 100 °C in column #1 and at 350 °C in column #2. The shaded portion reflects the correlation in (^1T, ^2T) that is more typical of GC.

solutes mapped in the upper left corner above the shaded stripe around main diagonal in Fig. 10 will *wrap around* [17] several times before eluting from column #2 as abnormally wide peaks. On the other hand, solutes in the lower right corner of the map in Fig. 10 (below the shaded stripe) will elute from column #1 at temperatures that are too high to cause any noticeable retention in column #2. As a result, these peaks will appear in 2D chromatogram as a cluster of *unresolved* peaks distributed along the line representing *hold-up time* in column #2.

The above example, however, is unrealistic. A dominant factor of retention in GC is solute volatility, which is correlated with molecular size and weight. In any column (*polar* or *apolar*), if other factors are equal, larger molecules tend to be more retained than smaller molecules. This means that, even if two GC columns have different polarities, their retention mechanisms are *strongly correlated* in such a way that (^1T, ^2T)-pairs in Fig. 10 tend to be mapped along the main diagonal (shaded portion in Fig. 10). This highly correlated distribution of the (^1T, ^2T)-pairs indicating high interdependence of retention mechanisms is conducive of statistically more uniform peak distribution than the distribution resulted from totally independent separation mechanisms.

These observations illustrate that the concept of independence/interdependent of separation mechanisms is related to but different from the concept of statistically uniform/non-uniform peak distribution in the separation space. Independent mechanisms can result in non-uniform peak distributions, while highly interdependent mechanisms can result in statistically uniform distributions.

In the proposed definition of MD analysis, judgments of quality (e.g., possible loss of the 1st-dimension separation, statistics of peak distribution in the separation space, and so forth) are not included the definition and are thereby relegated to a set of metrics relating to performance of MD analyses along with other metrics such as peak capacity, detection limit, sample capacity, etc.

4. Conclusion

A definition of multidimensional analysis is proposed that avoids contradictions found in prior definitions. The proposed definition covers only analytical separations. It is based on the dimensionality of the results. Factors affecting quality of separation (possibility of partial loss of the 1st-dimension separation, statistics of peak distribution in the separation space, and so forth) are not included in the definition. However the definition does not exclude the use of these metrics.

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