Analytica Chimica Acta 838 (2014) 93–101

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A new measure of orthogonality for multi-dimensional chromatography

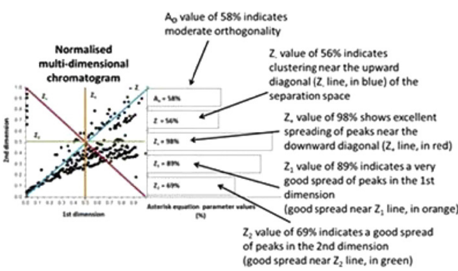
Michelle Camenzuli [a,b,*], Peter J. Schoenmakers [a,b]

[a] van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, Amsterdam 1098 XH, The Netherlands
[b] COAST, Science Park 904, Amsterdam 1098 XH, The Netherlands

HIGHLIGHTS

- A new method for measuring orthogonality in multidimensional separations is introduced.
- Our method also diagnoses areas where peaks are clustered in the separation space.
- The new method comprises of a number of equations which are easily implemented in Microsoft Excel.
- We applied the method to 8 computer-generated and 2 experimental multidimensional chromatograms.
- The method compared favorably against established methods.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 February 2014
Received in revised form 22 May 2014
Accepted 27 May 2014
Available online 2 June 2014

Keywords:
Multi-dimensional chromatography
LC × LC
GC × GC
Orthogonality
Separation science

ABSTRACT

Multi-dimensional chromatographic techniques, such as (comprehensive) two-dimensional liquid chromatography and (comprehensive) two-dimensional gas chromatography, are increasingly popular for the analysis of complex samples, such as protein digests or mineral oils. The reason behind the popularity of these techniques is the superior performance, in terms of peak-production rate (peak capacity per unit time), that multi-dimensional separations offer compared to their one-dimensional counterparts. However, to fully utilize the potential of multi-dimensional chromatography it is essential that the separation mechanisms used in each dimension be independent of each other. In other words, the two separation mechanisms need to be orthogonal. A number of algorithms have been proposed in the literature for measuring chromatographic orthogonality. However, these methods have their limitations, such as reliance on the division of the separation space into bins, need for specialist software or requirement of advanced programming skills. In addition, some of the existing methods for measuring orthogonality include regions of the separation space that do not feature peaks. In this paper we introduce a number of equations which provides information on the spread of the peaks within the separation space in addition to measuring orthogonality, without the need for complex computations or division of the separation space into bins.

© 2014 Elsevier B.V. All rights reserved.

* Corresponding author at: van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, Amsterdam 1098 XH, The Netherlands. Tel.: +31 20 525 7040.
E-mail address: M.Camenzuli@uva.nl (M. Camenzuli).

http://dx.doi.org/10.1016/j.aca.2014.05.048
0003-2670/© 2014 Elsevier B.V. All rights reserved.

1. Introduction

When analysing very complex samples, it is essential to use a technique that is capable of providing the maximum separation power possible. Multi-dimensional chromatographic techniques, for example comprehensive two-dimensional liquid chromatography (LC × LC), are much more powerful than their one-dimensional counterparts. This is because of the larger peak capacity that multi-dimensional techniques afford in a reasonable time [1,2]. The high peak capacities arise from the combination of two or more separation techniques within the one system. However, the choice of separation mechanisms in each dimension has a large effect on whether the high peak capacity of the corresponding multi-dimensional system can be effectively exploited. In order to attain the maximum effective peak capacity, the separation mechanisms in each dimension must be independent from each other. In other words, the dimensions must be chromatographically orthogonal [3]. Multi-dimensional techniques that use orthogonal separation mechanisms are capable of fully exploiting the various chemical and physical properties of the sample to obtain better separations [3].

The importance of chromatographic orthogonality is not restricted to multi-dimensional chromatography. In the pharmaceutical industry, part of the validation process for quality-control methods requires the development of two separation methods, the separation mechanisms of which must be chromatographically orthogonal. This ensures that the quantification of impurities within the sample is as accurate as possible [4].

The importance of chromatographic orthogonality has led to the development of a variety of methods for its measurement, particularly in multi-dimensional chromatography. Perhaps the most widely known methods involve dividing the separation space into bins.

The number of bins containing peaks is counted and related back to the total number of bins within the separation space [5,6]. Another variation of the bin-counting method has been proposed by D. Stoll, an author of [6]. It involves drawing a box around the part of the separation space which contains peaks. The bins within this box are counted and reported as a proportion of the total number of bins within the separation space. These methods are elegant in their simplicity and are effective. However, they are strongly affected by the decision the user must make with regards to the total number of bins to use in the division of the separation space [7]. The total number of bins in these methods is meant to be ideally equal to the total number of components within the sample. With complex samples, which contain hundreds or perhaps even thousands of peaks, determining the total number of components in the sample is not straightforward. Peaks often co-elute within such samples, making estimates of the total number of peaks (and, thus, the ideal number of bins) quite error-prone. Furthermore, the width of the bins is determined by the peak width, which is assumed constant. This assumption or requirement is fine for temperature programmed elution in GC and gradient elution in LC. However, in GC × GC the second-dimension separation is usually performed in (near-) isothermal mode and in LC × LC the second dimension may also involve isocratic elution to eliminate the need to equilibrate the column between runs. It is well known that the peak width in isothermal GC or isocratic LC is not constant but increases with increasing retention time. This could pose a problem for the selection of the bin width. Another variant of the bin-counting methods is the fractal approach [8]. Although it relies on bin counting, it is implemented in quite a different manner. This approach is based on the mathematical concept of fractals which relates to the scaling of self-similar objects. In the case of multi-dimensional separation, these self-similar objects are bins. The implementation of this approach involves applying a number of bins which scales with regards to the length/height of the bins. The logarithm of the number of bins required to cover the used separation space is plotted against the logarithm of the length/height scaling parameter. The slope of this plot is multiplied by −1 which results in a value of dimensionality [8]. For a completely orthogonal two-dimensional separation, a dimensionality value of 2.00 is obtained. A completely non-orthogonal two-dimensional separation would have a dimensionality value of 1.00 [8]. Because the dimensions of the bins is scaled, the fractal approach does not rely on correct determination of the peak width which gives it a potential advantage over more established bin methods. However it still shares the other limitations of the bin-counting methods. That is, the number of bins must be appropriate for the number of sample components and it is not possible to automate the fractal approach, at this stage. It is important to note that the fractal approach loses the simplicity with which the Gilar and Stoll bin-counting methods can be implemented. This ease of implementation is one of the strong aspects of the Gilar and Stoll bin-counting methods.

There are other methods that do not require the division of the separation space into bins. Such methods include measures derived from information theory [9], the minimum-convex-hull method and the kernel method [10]. The information-theory approach is based on determining the amount of mutual information shared by the two dimensions. Such mutual information includes the peaks which cluster along the right-leaning (upward) diagonal of the separation space. The proportion of mutual information compared to the total separation space 'entropy', or the total spread of peaks, is expressed by the term synentropy. In information theory an orthogonal separation would have a synentropy value of 0% [9]. The downfall of this technique for measuring orthogonality is its reliance on the assumption that peaks only cluster along the upward diagonal. This is certainly the most common form of clustering, but not the only possible one. The spreading angle method of Liu, Patterson and Lee [11] also shares this limitation. In this case, the measure of orthogonality is the amount of separation space used. To calculate this, two vectors corresponding to the retention times of each dimension are determined. These vectors are used to create a correlation matrix which is then used to determine the correlation or peak spreading angle. Once this angle is known, a fan-like shape is constructed with its apex located at the origin of the separation space. The spreading angle determines the width of the apex. The area enclosed within the fan describes the area of the separation space occupied by peaks [11]. It is clear that a fan with an apex located at the origin assumes that peaks tend to only cluster around the upward diagonal of the separation space. This limitation of the spreading angle method has been pointed out previously [12,13].

Unlike in informational theory and the peak spreading angle, peaks are not assumed to only cluster along the diagonal in the convex-hull and kernel methods. The latter methods are often used in ecological home-range studies and have recently been applied to multi-dimensional chromatography [10]. Although, these methods are reported to be quite effective, the minimum-convex-hull method includes parts of the separation space which do not include peaks, thus biasing the measure of orthogonality. This is not so much the case in the kernel method. In the kernel method, an area around each peak is blurred to form a kernel. The summed area covered by the kernels is the indicator of the degree of orthogonality in this method. The analyst sets a threshold which is a multiple of the height of an individual kernel. However, it should be noted that the selection of an appropriate threshold is difficult [10] and appears to be done empirically. The amount by which the summed kernel area exceeds the set threshold is the measure of separation space coverage which is directly related to

Download English Version:

https://daneshyari.com/en/article/1163917

Download Persian Version:

https://daneshyari.com/article/1163917

Daneshyari.com