Journal of Chromatography A, 1384 (2015) 115–123

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Two-dimensional correlation analysis of the reproducibility of high-performance liquid chromatography columns☆

József Simon[a], Attila Felinger[a,b,*]

[a] Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary
[b] MTA–PTE Molecular Interactions in Separation Science Research Group, Ifjúság útja 6, H-7624 Pécs, Hungary

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 18 January 2015
Accepted 20 January 2015
Available online 24 January 2015

Keywords:
Two-dimensional correlation
Reproducibility
Principal component analysis
Liquid chromatography

ABSTRACT

Two-dimensional (2D) correlation analysis is a well-established tool in spectroscopy. Despite its versatility in various measurement systems, 2D correlation has not yet become popular in separation science. 2D correlation is seldom used in chromatography; only a few a studies can be found on this topic and most of those publications report about gel chromatography. In the present study, 2D correlation analysis is applied to chromatograms. In this study, a simple method is built for studying the similarities and dissimilarities between a number of chromatograms. We present the applicability of the method by two examples, where the repeatability and reproducibility of the analytical and nonlinear measurements in HPLC are evaluated and demonstrated. In order to validate the results of 2D correlation analysis, they are compared to principal component analysis (PCA). We confirm the equivalence in the interpretation of the results obtained with the two methods of calculation. The results confirm that 2D correlation can be a successful chemometric tool in chromatography.

1. Introduction

The concept of 2D spectroscopy was first introduced in the field of nuclear magnetic resonance (NMR) in 1976 [1]. It was a breakthrough in analytical chemistry, and opened a number of ways for promising possibilities. Ten years later, 2D spectroscopy outgrew the boundaries of resonance spectroscopy, when Noda introduced the perturbation based 2D correlation spectroscopy (2D-COS) [2]. With that method a number of chemical phenomena became available for analysis and interpretation with vibrational spectroscopy. 2D correlation spectroscopy gained large interest and became very popular in infrared (IR), ultraviolet (UV) and visible (VIS) spectroscopy. It only took a few years to realize that 2D spectroscopy was clearly a volatile technique that could be applied in a wider perspective.

The construction of the generalized method was the next step in the evolution of 2D-COS [3]. The fundamental step of the calculation was based on Fourier transform, i.e. the intensity variations had to be built by sinusoidal components.

In 2000 Noda published a study [4], which introduced an entirely new calculation method for 2D correlation, the discrete Hilbert transform. This procedure replaced the Fourier transform with matrix operations, lifted all the boundaries of the previous technique, but provided the same results (see Fig. 1). With that, an ultimate tool was made available, which could be applied to any kind of measurement data [5]. Not surprisingly, 2D correlation has spread rapidly from vibrational spectroscopy to new analytical problems, such as mass spectrometry, microscopy or chromatography.

Despite its wide range of usefulness, the application of 2D correlation calculation in chromatography is still very rare. The first breakthrough was the work of Izawa et al. [6]. In their study, the foundations of the two-dimensional correlation gel permeation chromatography (2D-GPC) were established [6]. Another milestone was the application in gas chromatography (GC) published by Hyde at al. [7]. They used hetero-correlation analysis with GC-IR spectroscopy. There are several publications in this field, but 2D correlation has not really gained popularity in chromatography, and there may be much more potential in it.

In this study, we employed 2D correlation analysis to evaluate the repeatability and reproducibility of HPLC measurements both at analytical and preparative scales. The analytical data are extended in the second dimension using a number of 1D signals, thus a matrix (chromatomatrix) is created. Otherwise hidden information can be extracted from the chromatomatrix. Thus, a number

☆ Presented at the 30th International Symposium on Chromatography (ISC 2014), Salzburg, Austria, 14–18 September 2014.

* Corresponding author at: Department of Analytical and Environmental Chemistry and Szentágothai Research Center, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary. Tel.: +36 72 501500x24582; fax: +36 72 501518.
E-mail address: felinger@ttk.pte.hu (A. Felinger).

http://dx.doi.org/10.1016/j.chroma.2015.01.058
0021-9673/

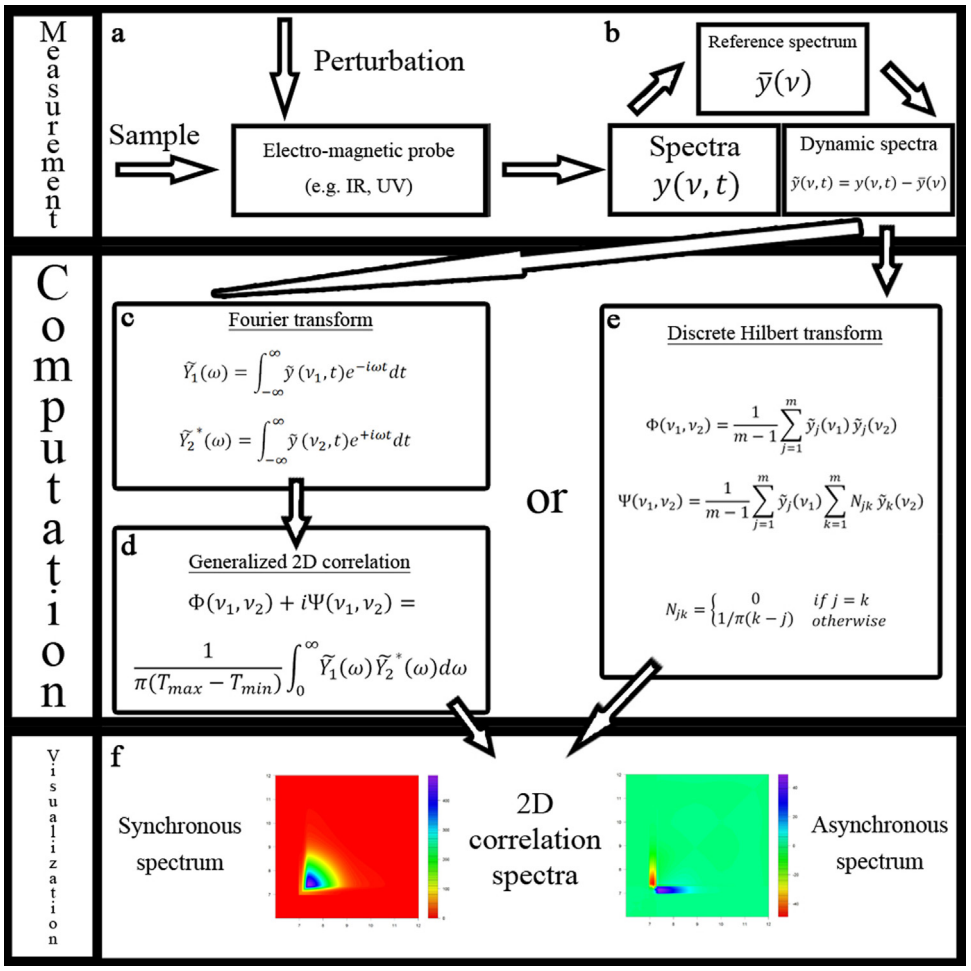

Fig. 1. The process of 2D correlation: (a) measurement system, (b) dynamic spectra, (c) Fourier transform, (d) the generalized correlation function, (e) discrete Hilbert transform, (f) synchronous and asynchronous spectra [5].

of chromatograms obtained by replicate measurements on several columns can be simultaneously evaluated. Our primary aim was to build a simple but versatile algorithm, which can be easily applied to any chromatographic use.

With the 2D correlation method, we present two comparison studies of HPLC columns, and compare the results with the outcome of previously published PCA study performed by Felinger et al. [8,9] on the same data sets given by the systematic and extensive work of Kele and Guiochon [10–15] on the reproducibility of reversed phase packing materials in analytical chromatography. Furthermore, we employed 2D correlation analysis to the experimental data reported by Gritti et al. [16,17,9] on the reproducibility of reversed phase packing materials under nonlinear conditions.

2. Theory

2.1. Discrete Hilbert transform [18]

In the original concept of 2D-COS, dynamic spectra are recorded and processed. Dynamic spectra are obtained when a number of spectra are recorded over time, usually after some perturbation. The raw spectral data (y) measured in time between T_{min} and T_{max} with m spectra are

$$y(\nu, t_j) \quad j = 1, 2, \ldots, m \tag{1}$$

where ν is the frequency, and t_j is

$$t_j = T_{min} + \frac{(T_{max} - T_{min})(j-1)}{m-1} \tag{2}$$

Usually the average of the spectra is used as a reference spectrum ($y'(\nu)$), although there is no universal rule how to pick the reference spectrum.

$$y'(\nu) = \sum_{j=1}^{m} \frac{y(\nu, t_j)}{m} \tag{3}$$

The dynamic spectrum is then calculated as

$$\tilde{y}(\nu, t_j) = y(\nu, t_j) - y'(\nu) \tag{4}$$

From this set of data, a matrix is formed

$$\mathrm{Y} = \begin{bmatrix} \tilde{y}(\nu_1,t_1) & \tilde{y}(\nu_2,t_1) & \cdots & \tilde{y}(\nu_n,t_1) \\ \tilde{y}(\nu_1,t_2) & \tilde{y}(\nu_2,t_2) & \cdots & \tilde{y}(\nu_n,t_2) \\ \vdots & \vdots & \ddots & \vdots \\ \tilde{y}(\nu_1,t_m) & \tilde{y}(\nu_2,t_m) & \cdots & \tilde{y}(\nu_n,t_m) \end{bmatrix} \tag{5}$$

Using this matrix, the calculation of 2D correlation can be clearly simplified compared to the original, Fourier transform based approach.