Sample pre-fractionation of environmental and food samples using LC-GC multidimensional techniques

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Multidimensional techniques are very powerful tools for unraveling naturally-occurring complex samples. The potential of multidimensional techniques is reviewed, also, as a substitute for the preparation step, with special attention to the hyphenation of liquid chromatography (LC) with gas chromatography (GC). An excursus on the evolution of hyphenated GC-based techniques and the main features of the LC-GC instruments is presented, before focusing on the more recent applications, discussing the performance of the LC separation as a preparation step. An overview of the potential of both comprehensive LC×GC and LC-GC×GC is reported.

Keywords: Environmental sample; Food sample; LC-GC; LC×GC; Liquid chromatography-gas chromatography; Multi-dimensional technique; Normal-phase liquid chromatography (NPLC); Reversed-phase liquid chromatography (RPLC); Sample fractionation; Sample preparation

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1. Introduction

The unraveling of naturally-occurring complex samples has always been the driving force behind improvement and innovation in separation science. Significant steps forward were made by replacing packed columns with capillary columns [1] increasing the separation power 10-fold, and by coupling gas chromatography (GC) with a time-of-flight (ToF) mass spectrometry (MS) [2]. Such powerful techniques are often insufficient for full understanding of complex samples. More efficient purification/separation would be necessary for easier, more reliable data interpretation. The main way explored to boost the separation power has been the exploitation of different separation mechanisms by multidimensional chromatography, with both heart-cutting and comprehensive chromatography techniques [3,4]. Such an approach is an interesting alternative, especially when the existing technologies (e.g., different column technologies), pushed to their limit, are still insufficient for complex samples. The coupling of the same form of chromatography (both heart-cutting and comprehensive), such as liquid chromatography (LC-LC, LC×LC) and GC (GC-GC, GC×GC), or of two different forms of chromatography (e.g., LC-GC, LC×GC) has been investigated over the years, both off-line and on-line.

Considering GC-based multidimensional techniques, the first coupling was presented by Simmons and Snyder in 1958 [5]. Two 50-m capillary columns were connected using a pneumatically-operated diaphragm six-port valve for the analysis of a dilute hydrocarbon gas mixture. Different types of transfer devices have been developed since then, and can be classified in three main groups, namely in-line valve, out-line valve, and valve-less systems. Furthermore, a cryotrap located before the second column may be a useful option to increase both sensitivity and peak capacity by focusing the band entering the second column. The main step forward in such a technique was made in 1968 by Deans with the introduction of pressure switching [6], which

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led to several advantages (e.g., eliminating temperature limitations, artifact formation and memory effects, avoidance of the direct contact between the sample compounds and the mechanical part of the valve, and keeping band broadening very low). Basically, multidimensional GC (MDGC) allows to isolate a limited number of target analytes from interfering compounds by transferring selected cuts to a second conventional column with a different separation mechanism. Thus, the total peak capacity is the sum of the peak capacity (n) of the first column (n_1) with the peak capacity of the second column (n_2). Few reviews have been published on MDGC [7–10], however two of them were published very recently (2012), so it seems wiser to direct readers to those rather than to summarize the state of the art herein.

Another important step was the development of a comprehensive GC system, first published in 1991 by Liu and Phillips [11]. The difference with the MDGC system is the use of a short second column (1–2 m) to enable receiving continuous and sequential cuts from the first column. The heart of the system is the transfer device, called modulator (usually cryogenic), which enables the accumulation and re-injection of the chromatographic bands eluting from the first column into the second one. Several advantages can be obtained using such a system:

(1) great increase in the separation power, the total peak capacity is theoretically equal to the product of n_1 and n_2;
(2) improved selectivity;
(3) speed (in terms of number of peaks per unit time);
(4) sensitivity (when a cryogenic modulator is employed, deriving from the band compression occurring at the modulator);
(5) structure, formation of a chemical class pattern easily recognizable in the two-dimensional (2D) plot.

A large number of applications employed such a technique, and several reviews have been published on the topic [12–15]. To some extent, comprehensive GC can be considered as a pre-fractionation, since it enables further separation of the target compounds from the hundreds of peaks that may be present in a complex sample. However, it cannot prescind from a preparation step before the GC injection, therefore the attention will be focused on the GC×GC applications preceded by a preparative LC separation, and it will be discussed after the main subject of the present review, the hyphenation of LC with GC. LC-GC is the multidimensional technique most suitable to be discussed in a special issue devoted to sample preparation. Two reviews were recently published on LC-GC, but one is devoted only to mineral-oil (MO) application [16] and the other focused on the transfer technique used in different applications [17]. In the present review, a special attention will be devoted to LC separation, which, in most cases, represents a pre-cleaning step, avoiding previous sample-preparation steps.

Coupling LC with GC was first published by Majors in 1980 [18] for the analysis of atrazine in sorghum samples. The first automated system was presented in 1987 by Ramsteiner [19] for the analysis of pesticides in biological matrices. In 1989, Carlo Erba (Italy) introduced the first commercial LC-GC instrument, called Dualchrom 3000. In 1991, the system was coupled for the first time with an MS detector for the analysis of polycyclic aromatic hydrocarbons (PAHs) in vegetable oil [20].

Another interesting step was made in 2000 by Quigley and co-workers [21], who developed for the first time a comprehensive system (LC×GC) to analyze volatile organic compounds (VOCs) in water. Since the first application in 1980, the attention to such an approach had slowly increased reaching its maximum in the second part of the 1990s. Then, it suddenly decreased, but it has never been completely abandoned. Recently, the number of papers published has been increasing, mainly related to the hot-topic of MO migration from recycled cardboard and printing ink into foods, for which the LC-GC technique is the most suitable analytical approach.

LC-GC is a very powerful technique that assures an efficient sample clean-up and/or group-type separation of the analytes, thus it is very suitable for complex samples and when high sensitivity and selectivity are required. Furthermore, the on-line system is faster, more sensitive, and it allows to minimize sample manipulation and the related risks (e.g., sample loss, cross-contamination, and artifact formation due to atmospheric oxygen or moisture).

An LC-GC instrument is mainly composed of three parts LC, GC, and the interface to transfer the fraction from the LC into the GC. The role of the GC is to perform analytical separation before detection [performed using flame-ionization detection (FID) or MS]. The role of LC and the interface is much more delicate and it depends on the specific application.

2. LC-GC instrument

2.1. LC dimension

The LC step can be a simple separation of the target compounds from the bulk of the matrix, or the separation efficiency and the selectivity of the LC column can be exploited to perform a selective clean-up, concentration or fractionation of the sample. The main LC-separation mechanisms have been intensively explored over the years [16,17].

Normal-phase (NP) chromatography has been employed most, since the eluents used are suitable for GC. Few applications using size-exclusion chromatography (SEC) have been published, but this technique involves