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Flow-modulation low-pressure comprehensive two-dimensional gas chromatography

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ABSTRACT

The present paper contains research data relative to an approach herein defined as flow-modulation (FM) low-pressure comprehensive two-dimensional gas chromatography, abbreviated as "GC × LP GC". The abbreviation LP is positioned before the second GC abbreviation because LP conditions were generated across a mega-bore second-dimension column (10 m × 0.53 mm ID), it being connected to a quadrupole mass spectrometer (qMS). Flow modulation was performed with MS-compatible gas flows (7–8 mL/min), following recent research work [7]; using such an approach, the main disadvantage of flow modulation, specifically the generation of excessively high second-dimension flows, is avoided. A further noteworthy aspect of the investigation was the use of a long accumulation loop (*e.g.*, 51 cm), a modification that greatly improved the general post-modulation peak shape quality. FM GC × LP GC–qMS applications on pure standard compounds, as well as on milk and fish oil fatty acid methyl esters, are shown and discussed.

1. Introduction

Over the last two decades it has been widely demonstrated that comprehensive two-dimensional gas chromatography (GC × GC), compared to one-dimensional GC, is characterized by: (I) increased peak capacity and selectivity; (II) enhanced sensitivity (mainly in cryogenically modulated experiments); (III) increased identification potential, due to the formation of ordered chromatogram patterns of homologous compounds (*e.g.*, alkanes, monoaromatic hydrocarbons, etc.). With regard to modulation this is usually performed with a cryogenic device, or in alternative with a flow modulator [1]. The interest in flow modulation (FM) stems from its low hardware and operational costs. On the contrary, cryogenic devices are characterized by high costs in terms of hardware and operation, with the latter aspect valid for modulators requiring cryogenic fluids. The main advantage of cryogenic modulation (over FM) is the generation of very narrow analyte bands, prior to the injection onto the second column.

The most effective FM device, in the present authors' opinion, was first reported by Seeley *et al.* in 2006 [2], and was formed of three deactivated fused-silica columns, two microvolume T-unions and a two-way solenoid valve (located outside the GC oven), connected to an auxiliary gas source. The outlet ports of the valve were linked to the unions by using two of the fused-silica columns, while the third acted as sample loop, and bridged the two unions. One of the T-unions was linked to the first-dimension outlet, while the other was connected to the second-column inlet. It is worthy of note that Amirav worked on an altogether similar flow-modulation concept during the same time period [3].

The FM process proposed by Seeley *et al.* was characterized by an accumulation step, and a much briefer re-injection stage [2]. Major disadvantages were a complicated method optimization process (more than in cryogenic modulation GC × GC) and the generation of high second-dimension gas flows. In fact, since the first experiment it has been widely accepted that the operation of such modulators requires high gas flows (*e.g.*, 20–25 mL/min) to efficiently re-inject the content of the accumulation loop onto the second column [4–6]. However, in a recent investigation it was demonstrated that efficient flow modulation can be performed at greatly reduced gas flows (6–8 mL/min) by extending the

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re-injection period [7]. Such an approach is applied in the present research.

The use of a mega-bore column, under vacuum-outlet conditions, is a well-known option for fast and very-fast GC experiments. The presence of low-pressure (LP) intra-column conditions causes an increase in analyte-gas diffusion coefficients and, consequently, of the optimum gas linear velocity. An additional advantage is an increased sample capacity, while the limited column efficiency can be considered as a disadvantage [8]. A series of very-fast LP GC applications, using a 10–15 m × 0.53 mm ID column, have been reported in the literature [9–11]. In such experiments, a restrictor was positioned between the injector and the analytical column, to avoid sub-ambient pressure reaching the injection system. The concept of very-fast LP GC is herein extended to the field of GC × GC, by using a 10 m × 0.53 mm ID secondary column (GC × LP GC). A restrictor was used between the flow modulator and the second dimension. Low-pressure conditions were generated by connecting the outlet of the secondary column to a rapid-scanning quadrupole mass spectrometer (qMS). To the best of the present authors' knowledge, such a second-dimension configuration has never been reported in FM GC × GC–MS analyses.

In all GC × GC experiments, second-wide primary-column analyte bands, with a specific concentration profile, enter the modulator; in cryogenic systems concentration profiles are nullified (apart for highly volatile compounds) due to intense cooling. Cryogenic modulation generates very narrow analyte plugs, suitable for very fast analysis on a short micro-bore column in the second dimension [1]. When using a flow modulator equipped with a loop, concentration profiles are maintained during the accumulation period. Such a factor can have a negative effect on peak shape as discussed by Harvey and Shellie [12]. It will be herein demonstrated that the use of a long accumulation loop has a beneficial effect on peak shape in FM GC × GC.

2. Materials and methods

2.1. Standard compound, samples and sample preparation

The C_{10} *n*-alkane was provided by Sigma-Aldrich/Supelco (Bellefonte, PA, USA), and diluted in *n*-hexane prior to injection. The menhaden oil was purchased in a store, while the UHT whole cow milk was attained from a supermarket, both in Messina.

Milk lipids were extracted using the Folch extraction method [13]. Fish oil fatty acid methyl esters (FAMEs) were prepared as previously published [5]. The same derivatization process was used for the extracted milk fat.

2.2. FM GC × LP GC–qMS analyses

All FM GC × LP GC–qMS applications were performed on a system consisting of two independent Shimadzu GC2010 gas chromatographs (GC1 and GC2), and a QP-2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The two GC ovens were linked through a heated transfer line. The primary GC was equipped with an AOC-20i auto-injector and a split-splitless injector (310 °C).

The primary column (situated in GC1), a Supelcowax-10 (100% polyethylene glycol) 30 m × 0.25 mm ID × 0.25 μm d_f (Sigma-Aldrich/Supelco),was connected to position 1 of a 7-port wafer chip (SGE, Ringwood, Victoria, Australia), after passing through the heated transfer line. An SLB-5ms 10 m × 0.53 mm ID × 0.10 μm d_f capillary [(silphenylene polymer, practically equivalent in polarity to poly(5% diphenyl/95% methylsiloxane)] (Sigma-Aldrich/Supelco) was used as second analytical column. A 1.5 m × 0.25 mm ID segment of uncoated column was used as restrictor, it being connected on one side to the wafer chip (port 6) and on the other to the second analytical column. The connection between the restrictor and the column was made by using an MXT union connector (Restek Corporation, Bellefonte, PA, USA). All capillaries were supplied by Sigma-Aldrich/Supelco. Position 7 of the interface was blocked by using an adequate nut.

Method I conditions (C_{10} alkane and milk fat FAMEs): inj. vol.: 0.2 μL (C_{10}) and 0.5 μL (FAMEs); split ratio: 200:1; GC1/GC2 temp. program: 50–280 °C at 5 °C/min; initial inlet pressure: 45.5 kPa; initial auxiliary pressure: 3.9 kPa; in both dimensions the linear velocity was maintained constant. Stainless steel accumulation loops measuring 20 cm and 46 cm (×0.71 mm OD × 0.51 mm ID) were used (SGE). Modulation period: 3.1 s (accumulation period 2.7 s/injection period 0.4 s).

Method II conditions (fish oil FAMEs): inj. vol.: 0.6 μL; split ratio: 100:1; GC1/GC2 temp. program: 150–280 °C at 1.5 °C/min; initial inlet pressure: 79.2 kPa; initial auxiliary pressure: 35.8 kPa; in both dimensions the linear velocity was maintained constant. The accumulation loop (an uncoated silica capillary was used) measured 51 cm × 0.71 mm OD × 0.53 mm ID. Modulation period: 5.0 s (accumulation period 4.6 s/injection period 0.4 s).

Quadrupole MS conditions: ionization mode: electron ionization (70 eV). Interface and ion source temperatures: 250 and 200 °C, respectively. Mass range: 45–360 *m/z*; scan frequency: 25 Hz. Mass spectral database matching was carried out by using the FAMEs Fatty Acid Methyl Esters: Mass Spectral Database (Shimadzu Europe, Duisburg, Germany).

Data were acquired using the GCMSsolution software (Shimadzu). Bidimensional chromatograms were generated by using the ChromSquare software v. 2.0 (Shimadzu).

3. Results and discussion

3.1. Preliminary considerations and initial results

After experimentally demonstrating that the use of gas flows in the 6–8 mL/min range can produce efficient modulation [7], the next research objective was to extend the concept of very fast LP GC to the field of GC × GC. The initial intention was to use a 10 m × 0.53 mm ID column, because such capillaries have been proven to work ideally under such gas flow conditions [8]. A 1.5 m × 0.25 mm ID restrictor was positioned before the analytical column to avoid sub-ambient pressure conditions reaching the wafer chip. The accumulation loop dimensions were 20 cm × 0.51 mm ID. Initial optimization applications were carried out on a single compound, namely C_{10} *n*-alkane. Under the experimental conditions applied (see Method I in Section 2.2), the He average linear velocity (ALV) in the first dimension was 12.9 cm/s (initial flow: 0.44 mL/min). During the accumulation period (2.7 s), the loop ALV was 3.7 cm/s. With regard to the injection pulse (0.4 s), the flow exiting the modulator was calculated to be about 7 mL/min, while the second-dimension ALV was ~170 cm/s. In short, both ideal very-fast LP GC and MS-compatible gas flow (the maximum qMS pumping capacity was 15 mL/min) conditions were used. Three modulated peaks relative to the C_{10} *n*-alkane are shown in Fig. 1a. Apparently, the modulation process worked well.

A further issue herein considered is peak shape. In fact, after a careful glance of the three peaks illustrated in Fig. 1a, it can be seen that the shapes of both the second and third modulated peak are not satisfactory, with both peaks presenting a shoulder on the left side of the base. The asymmetry factor (*As*) was measured for the third modulated peak at 10% of its height, and a value of 0.75 was derived. The same parameter was not measured for the second modulated peak because the shoulder is located below the 10% peak height mark.