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Parallel dual secondary column-dual detection: A further way of enhancing the informative potential of two-dimensional comprehensive gas chromatography☆

Luca Nicolotti[a], Chiara Cordero[b,*], Davide Bressanello[b], Cecilia Cagliero[b], Erica Liberto[b], Federico Magagna[b], Patrizia Rubiolo[b], Barbara Sgorbini[b], Carlo Bicchi[b]

[a] Faculty of Chemistry, Technical University of Munich, Lise-Meitner-Strasse 34, 85354 Freising, Germany
[b] Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro Giuria 9, I-10125 Turin, Italy

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ABSTRACT

Comprehensive two-dimensional gas chromatography (GC × GC) coupled with Mass Spectrometry (MS) is one of today's most powerful analytical platforms for detailed analysis of medium-to-high complexity samples. The column set usually consists of a long, conventional-inner-diameter first dimension (^{1}D) (typically 15–30 m long, 0.32–0.25 mm d_c), and a short, narrow-bore second dimension (^{2}D) column (typically 0.5–2 m, 0.1 mm d_c) where separation is run in a few seconds. However, when thermal modulation is used, since the columns of a set are coupled in series, a flow mismatch occurs between the two dimensions, making it impossible to operate simultaneously at optimized flow conditions. Further, short narrow-bore capillaries can easily be overloaded, because of their lower loadability, limiting the effectiveness of ^{2}D separation.

In this study, improved gas linear velocities in both chromatographic dimensions were achieved by coupling the ^{1}D column with two parallel ^{2}D columns, having identical inner diameter, stationary phase chemistry, and film thickness. In turn, these were connected to two detectors: a fast quadrupole Mass Spectrometer (MS) and a Flame Ionization Detector (FID). Different configurations were tested and performances compared to a conventional set-up; experimental results on two model mixtures (*n*-alkanes and fourteen medium-to-high polarity volatiles of interest in the flavor and fragrance field) and on the essential oil of *Artemisia umbelliformis* Lam., show the system provides consistent results, in terms of analyte identification (reliability of spectra and MS matching) and quantitation, also affording an internal cross-validation of quantitation accuracy.

1. Introduction

Comprehensive two-dimensional gas chromatography (GC × GC) coupled with Mass Spectrometry (MS) is one of the most powerful analytical platforms now available for the detailed analysis (identification and quantitation) of medium-to-high complexity samples. Compared to one-dimensional systems, it offers remarkable separation power and unmatched peak capacity [1,2]; the possibility of applying different separation mechanisms in the two chromatographic dimensions produces rationalized 2D patterns, suitable as sample fingerprints for classification and identification purposes [3].

The most common GC × GC column sets consist of a long, conventional-inner-diameter first dimension (^{1}D) (typically 15-30 m long and 0.32–0.25 mm d_c), and a short, narrow-bore second dimension (^{2}D) column (typically 0.5–2 m 0.1 mm d_c). Thanks to the short narrow-bore ^{2}D column, the separation is run in a few seconds, both minimizing wrap-around phenomena and contributing to the high efficiency of the system. However, when thermal modulation is used, since the columns of a set are coupled in series, a flow mismatch occurs between the two dimensions; this makes it impossible to operate simultaneously at optimized flow conditions. In addition, short narrow-bore capillaries can easily overload, because of their lower loadability, limiting ^{2}D separation effectiveness [4,5]. The configuration and optimization of a GC × GC set-up is thus a crucial, but also a complex step, since separation in the two

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* Corresponding author. Tel.: +39 011 6707662; fax: +39 011 2367662.
E-mail address: chiara.cordero@unito.it (C. Cordero).

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dimensions is differently influenced in the two separation dimensions by carrier gas flow, temperature, and modulation period. With regard to the flow regime, in their earlier publications Phillips et al. [6,7] indicated a possible way of optimizing carrier gas flow by splitting part of the flow from ^{1}D to waste, prior to modulation. They adopted a Tee union to connect the two analytical columns, and a short capillary segment enabling the diversion of about 30% of the primary column flow to waste, thus applying flows closer to the optimal in both dimensions, and reducing overloading of the ^{2}D.

In 2007, Tranchida et al. [8] included a flow splitter in a classical GC × GC-FID system. The method, called "split-flow" comprehensive 2D-GC, consisted of a ^{1}D apolar 30 m × 0.25 mm d_c column, connected to a 1 m × 0.10 mm d_c polar ^{2}D and to an uncoated capillary of 30 cm × 0.10 mm d_c, using a Y press fit. The carrier gas (hydrogen) linear velocities were regulated thanks to a manually-operated split valve, connected to the uncoated capillary. Experimental results on Fatty Acids Methyl Esters (FAMEs) from a cod oil sample showed that, with a 35:65 (FID) split-flow ratio and 146.3 kPa head pressure, gas velocities close to optimal could be obtained (i.e., about 35 and 213 cm/s in the ^{1}D and ^{2}D respectively) with a positive effect on separation efficiency and resolution (+50% for a selected critical pair) while maintaining structured chromatograms.

Other straightforward solutions have been proposed to overcome this critical issue, which is known as flow-mismatch in the two dimensions. In stop-flow GC × GC [9–11] the ^{1}D flow is periodically halted and during each pause the ^{2}D separation continues, by delivering carrier gas via an auxiliary pressure controller. This latter set-up enables column flow to be independently regulated, thus optimizing the separation in both dimensions.

Another possibility is to adopt wider ^{2}D capillaries [12,13]; if columns of a set have the same inner diameter, flow conditions closer to optimal can be applied in both dimensions, improving the exploitation of the ^{2}D stationary phase selectivity, even at higher temperature rates, and at the same time increasing ^{2}D column loadability [12]. Experimental results on medium-complexity samples of interest in the flavor and fragrance field, with homologous d_c column sets, show that the mean loss of peak capacity (by a factor of 3; System Separation Measure - $S_{GC\times GC}$) is partially or fully compensated, thanks to better exploitation of ^{2}D stationary phase selectivity. At the same time, reliable quali-quantitative results are achieved, by complying with the minimal modulation requirements (Modulation Ratio criterion - M_R) [13]. More recently, Peroni et al. evaluated two alternative solutions: (a) the use of monolithic ^{2}D columns [14], and (b) multiple capillary columns in parallel as ^{2}D [15]. With monoliths, efficiency and column flow can be optimized independently, but at the cost of poor separation efficiency. However, multi-^{2}D columns appear to be a good alternative; the carrier gas flow is divided over multiple-parallel ^{2}D flow paths, enabling both dimensions to be fully exploited at the same time. Unfortunately, as the authors themselves state, coupling the ^{1}D to the multi-^{2}D is, in practice, rather a complex procedure, limiting the feasibility of such set-ups in routine use.

As discussed by Peroni and Janssen [16], the optimum linear velocities in both dimensions are reduced when the second dimension operates at high outlet pressure. The proposed set-up includes a restrictor at the outlet of the ^{2}D, prepared by melting the end of the column with a high-temperature hydrogen flame (1800 °C) until closure, and then partially re-opening it, by grinding it with sandpaper, to obtain the desired flow. The elevated outlet pressure conditions resulted in flatter *Van Deemter* curves at higher velocities, causing a slower loss of efficiency at higher inlet pressures. Experimental results indicated that this system configuration is characterized by a slightly improved resolution for a given column set, compared to conventional pressure drops, but that the analysis time is longer.

In the present study, improved gas linear velocities in both chromatographic dimensions were achieved by coupling the ^{1}D column with two parallel ^{2}D columns having identical inner diameter, stationary phase chemistry, and film thickness, in turn connected to two detectors: a fast quadrupole Mass Spectrometer (MS), and a Flame Ionization Detector (FID). The system was equipped with a loop-type thermal modulator; cryotrapping and refocusing were set at the head of the ^{2}D capillaries to narrow bands entering the ^{2}D [17]. Three different column set-up were tested: the first, *Set-up I*, included a primary column connected with two parallel ^{2}Ds of different lengths (1.6 m × 0.1 mm d_c to MS and 1.4 m × 0.1 mm d_c to FID) but operating at an almost equal nominal flow (comparable hold-up times) although subjected to different outlet pressures. The second system configuration, *Set-up II*, included two identical ^{2}D columns (1.4 m × 0.1 mm d_c) and an auxiliary pressure controller to deliver a supplementary flow of carrier gas at the outlet of the ^{2}D connected to the MS detector. The latter was inspired by the system proposed by Shellie et al. [18], in which GC × GC-FID and GC × GC-TOF-MS chromatograms were successfully matched, obtaining almost identical 2D patterns thanks to the adjustment of inner and outlet pressures. Lastly a conventional set-up was taken as a reference, i.e. *Set-up III* consisted of a single ^{2}D column (total length including modulation loop: 1.4 m × 0.1 mm d_c) connected to two parallel detectors, via splitting capillaries.

The performance of each *Set-up* are evaluated by analyzing two model mixtures (n-alkanes (HydStd1) and 14 medium-to-high polarity volatiles in the flavor and fragrance field (FFStd2)), and the *Artemisia umbelliformis* Lam. essential oil. The potentials and limits of each set-up are also discussed in terms of separation performances and in view of the practical information that can be derived from each single analytical run.

2. Experimental

2.1. Samples and solvents

Pure standards of *n*-alkanes (from *n*-C9 to *n*-C25) for system evaluation, flow/pressure optimization and Linear Retention Indices (I^T_S) determination were from Sigma–Aldrich (Milan, Italy).

Pure standards of α-pinene, benzaldehyde, benzyl alcohol, α-thujone, camphor, carvone, cinnamyl alcohol, geranyl acetate, vanillin, coumarin, isoeugenol, isoeugenyl acetate, benzyl benzoate, and sclareol, were from Sigma-Aldrich (Milan, Italy). The two model mixtures (i.e., HydStd1 and FFStd2) for system evaluation were prepared by mixing single component Standard Mother Solutions, at 10 g/L in dichloromethane, and adjusting the final volume up to 100 mg/L. Solvents were all HPLC-grade, from Riedel-de Haen (Seelze, Germany).

Artemisia umbelliformis Lam. essential oil (EO) was prepared following the method of the European Pharmacopoeia [19]. Ten grams of dried aerial parts from experimental cultivations run in different alpine valleys were suspended in 250 mL of water in a 500 mL flask for 1 h, and then submitted to hydrodistillation in a Clevenger micro-apparatus for 2 h [20]. The resulting EO was left to stabilize for 1 h, then recovered and analyzed directly.

2.2. GC × GC instrument set-up

GC × GC analyses were run with a system configured as follows: a HT280T multipurpose sampler (HTA, Brescia, Italy) was integrated with an Agilent 6890 GC unit coupled to an Agilent 5975C MS detector (Agilent, Little Falls, DE, USA) operating in EI mode at 70 eV. The GC transfer line was set at 280 °C. A *Standard Tune* was used and the scan range was set to *m/z* 40–300 with a scanning rate of 12,500 amu/s to obtain a spectra generation frequency of 28 Hz.