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

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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 (1D) (typically 15-30 m long, 0.32-0.25 mm $d_c$), and a short, narrow-bore second dimension (2D) 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 2D separation.

In this study, improved gas linear velocities in both chromatographic dimensions were achieved by coupling the 1D column with two parallel 2D 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.

Key-words:
Two-dimensional comprehensive gas chromatography-mass spectrometry; parallel dual secondary column-dual detection; dual 2D pattern alignment, outlet pressure correction, second dimension linear velocity optimization, essential oil analysis
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 (1D) (typically 15–30 m long and 0.32–0.25 mm dc), and a short, narrow-bore second dimension (2D) column (typically 0.5–2 m 0.1 mm dc). Thanks to the short narrow-bore 2D 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 2D 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 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 1D 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 2D.

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 1D apolar 30 m × 0.25 mm dc column, connected to a 1 m × 0.10 mm dc polar 2D and to an uncoated capillary of 30 cm × 0.10 mm dc, 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/sec in the 1D and 2D 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 1D flow is periodically halted and during each pause the 2D 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 2D 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 2D stationary phase selectivity, even at higher temperature rates, and at the same time increasing 2D 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 2D stationary phase selectivity. At the same time, reliable quasi-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 2D columns [14], and (b) multiple capillary columns in parallel as 2D [15]. With monoliths, efficiency and column flow can be optimized independently, but at the cost of poor separation efficiency. However, multi-2D columns appear to be a good alternative; the carrier gas flow is divided over multiple-parallel 2D flow paths, enabling both dimensions to be fully exploited at the same time. Unfortunately, as the authors themselves state, coupling the 1D to the multi-2D 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 2D, 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 1D column with two parallel 2D 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 2D capillaries to narrow bands entering the 2D [17]. Three different column set-up were tested: the first, Set-up I, included a primary column connected with two parallel 2Ds of different lengths (1.6 m x 0.1 mm $d_c$ to MS and 1.4 m x 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 2D columns (1.4 m x 0.1 mm $d_c$) and an auxiliary pressure controller to deliver a supplementary flow of carrier gas at the outlet of the 2D 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 2D column (total length including modulation loop: 1.4 m x 0.1 mm dC) 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 (I3) 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 hours [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. The Flame Ionization Detector (FID) conditions were: base temperature 280°C, H2 flow 40 mL/min; air flow 240 mL/min; make-up (N2) 450 mL/min; sampling frequency 150 Hz.
Injections of the essential oil, and of the two model mixtures, as well as those for $I'_s$ determination samples, were by HT280T sampler (HTA, Brescia, Italy) under the following conditions: split/splitless injector, split mode, split ratio 1/50, injector temperature 280°C, injection volume 0.1 µL of undiluted essential oil and 1µL of the n-HydStd1 and FFSTd2 model mixtures at 100 mg/L. The oven temperature was programmed as follows: 50°C (1 min) to 270°C at 3.0°C/min and to 290°C at 10°C/min (10 min).

Flow/pressure optimization was checked on a standard solution of tridecane, tetradecane and pentadecane (n-C13 to n-C15) at 100 mg/L analyzed in isothermal conditions at 150°C. Head-pressure values are reported in Table 1.

2.3 Thermal modulator parameters

The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation, Houston, TX) cooled with liquid nitrogen controlled by Optimode™ V.2 (SRA Instruments, Cernusco sul Naviglio, MI, Italy). Hot jet pulse time was set at 250 ms, modulation time was 5 s and cold-jet total flow progressively reduced with a linear function, from 40% of Mass Flow Controller (MFC) at initial conditions, to 5% at the end of the run. Loop dimensions were chosen on the basis of the expected carrier linear velocities, to ascertain that at least two stage-band-focusing releases were performed for each modulation. Thus, for all Set-ups, the first 0.6 m of the $^2$Ds war wrapped in the metal slit of the modulator.

2.4 Column connections and auxiliary control module

Connections between the primary and the two secondary columns (Set-ups I and II), and between the secondary column and the deactivated capillaries for FID/MS effluent splitting (Set-up III) was via a SilFlow™ GC 3 Port Splitter (SGE Ringwood, Victoria, Australia). The auxiliary pressure controller consisted of a one channel Pneumatics Control Module (G2317A) connected to a Quick Swap unit (G3185, Agilent, Little Falls, DE, USA) with a restrictor capillary of 0.17 m x 0.1 mm d.. A diagram of the system configuration is provided as Supplementary File (Supplementary Figure 1 - SF1).

Column set configurations are listed in Table 1, together with carrier gas head pressures and calculated linear velocities [18,21].

2.5 Data acquisition and 2D plot elaboration

Data were acquired by Agilent MSD ChemStation ver D.02.00.275 and processed using GC Image GC×GC Software version 2.1b1 (GC Image, LLC Lincoln NE, USA).

3. Results and discussion

3.1 Some theoretical aspects
Conventional GC×GC configurations with thermal modulators imply that the two columns of the set are connected in series and the volumetric flow rates and linear velocities in the two capillaries are correctly calculated; the pressure drop across the total length must also be estimated [8,18].

The outlet column volumetric flow \( F_{o(C)} \) can be derived by the Poiseuille equation (Eq. 1)

\[
F_{o(C)} = \frac{6\pi r^4 (p_i^2 - p_o^2) T_{ref}}{16 \eta L p_o} \text{ Equation 1}
\]

where \( r \) is the column radius, \( \eta \) the dynamic viscosity of the carrier gas at a given temperature, \( L \) is the column length, \( p_i \) and \( p_o \) are the absolute inlet and outlet pressures, and \( T_{ref} \) is the reference temperature (typically 298K) and \( T \) is the absolute operative temperature.

The pressure at any point \( z \) along the column can be calculated according to Equation 2:

\[
p_z = \sqrt{p^2 - \left(\frac{z}{L}\right) (p^2 - 1)} \text{ Equation 2}
\]

The linear velocity at the column outlet \( u_o \) is:

\[
u_o = \frac{r^4 (p_i^2 - p_o^2)}{16 \eta L p_o} \text{ Equation 3}
\]

where \( r \) is the column radius, \( \eta \) the dynamic viscosity of the carrier gas at the operating temperature, \( L \) is the column length, \( p_i \) and \( p_o \) are the absolute inlet and outlet pressures. The average velocity is proportional to the outlet velocity corrected by the compression factor \( j \):

\[
u = u_o \cdot j \text{ Equation 4}
\]

where

\[
j = \frac{3 (p^2 - 1)}{2 (p^3 - 1)} \text{ Equation 5}
\]

The average linear velocity along each separation dimension can be estimated by combining the above functions. Table 1 reports average linear velocities, calculated at 333 K (60°C), together with inlet pressure \( (p_i) \), midpoint pressure \( (p_o) \) at the connection between primary and secondary column(s) in kPa (over pressure) and hold-up times (s). In the case of Set-up II, the data do not include the adjustment of outlet pressure by the auxiliary flow controller.

3.2 Parallel dual secondary columns operating at different outlet pressures (GC×2GC-MS/FID)
The first part of this study was carried out on dual parallel columns of identical inner diameter (i.e., 0.10 mm). It consisted of an almost equivalent length in terms of flow resistance. **Set-up I** was inspired by the "split-flow" configuration proposed by Tranchida et al. [17], with the sole difference that the outlet of the split capillary (an OV1701 capillary column with 0.10 µm) was connected to a FID detector (atmospheric pressure). Compared to a conventional configuration (**Set-up III**), where one of the two dimensions has to operate very far from its optimum performance, whatever the head pressure, in **Set-up I** close-to-optimal linear velocities in both chromatographic dimensions are applied, i.e. t1 at about 34 cm/s and the t2s at about 180 cm/s (see **Table 1**).

Differences in secondary column length were expected to condition the separation in terms of absolute retention and peak-widths. However, the resulting 2D patterns were expected to be consistent, although not identical. According to Schutjes et al. [21], who rearranged the Golay plate height equation in terms of dimensionless parameters (i.e., \( \xi = \frac{H}{H_{\text{min}}} \) and \( \nu = \frac{\bar{u}}{\bar{u}_{\text{opt}}} \)) operating at \( \frac{p_i}{p_o} \gg 1 \) (i.e., vacuum outlet), the maximum efficiency is reached for a close interval of average linear velocities around \( \bar{u}_{\text{opt}} \). Conversely, when \( \frac{p_i}{p_o} \) approaches unity (i.e. at ambient pressure), the experimental curve of \( \frac{H}{H_{\text{min}}} \) as a function of \( \frac{\bar{u}}{\bar{u}_{\text{opt}}} \) is flatter, and enables a better separation efficiency. With **Set-up I** lower efficiencies were expected for the MS branch [21] also in consequence of the longitudinal diffusion effect.

The experimental results confirmed these hypotheses: **Figure 1** shows the raw chromatogram overlaid with 2D plots of linear hydrocarbons from C13 to C15 analyzed in isothermal conditions (i.e., 150°C) at 296 kPa head-pressure with **Set-ups I and II**. System hold-up times, measured experimentally with methane injections at 80% of MFC cold jet regulation, were 1.905 min and 0.86 s in the t1 and t2 respectively, in fair accordance with the expected values. Alkanes showed an absolute retention time shift (MS vs. FID) of -0.18 s for n-C13 and of -0.36 s for n-C15. Although minimized by the lower retention in the second dimension due to the temperature of the isothermal analysis, a much larger mismatch was expected for temperature programmed conditions and strongly retained analytes.

**Figure 2** reports 2D plots (**Fig. 2a** full scan MS and **Fig. 2b** FID plots) of *Artemisia umbelliformis* essential oil, analyzed with **Set-up I**. The consistency of the 2D patterns of the two detectors is evident; the structured patterns of mono-terpenoid (m) and sesqui-terpenoid (s) hydrocarbons are clearly organized, and separated from the oxygenated derivatives (mox and sox) and from other secondary metabolites (mono terpenoid esters - mest). More polar compounds (carbonyl derivatives, alcohols and esters) having greater affinity for the second dimension stationary phase were more strongly retained along the t2 branch towards MS (higher retention factors - k).

The magnitude of the retention time shift is better illustrated in **Figures 3a and 3b**, which show t2 retention time absolute differences (FID vs. MS) for: **(3a)** n-alkane hydrocarbons from n-C9 to n-C25 and **(3b)** fourteen volatiles of interest in flavor and fragrance applications. For the n-alkanes, where retention in the t1 is negligible, absolute differences in retention times in no case exceeded (-)0.15 s (i.e., 3% as relative %
difference over 5 seconds of 2D separation time); conversely, 2D retention shifts for more polar compounds (Fig. 3b) were larger with differences between MS and FID patterns ranging from the (-)0.12 s of α-pinene to the (-) 0.68 s of vanillin (i.e., 2.38 and 13.6 % of relative difference). Marked differences were recorded for the more polar analytes (benzyl alcohol, cinnamyl alcohol, vanillin, isoeugenol and isoeugenyl acetate) that suffered from the wrap-around phenomenon.

3.3 Parallel dual secondary columns operating at equivalent (atmospheric) outlet pressures (GC×2GC-MS/FID)

The study continued, adopting two secondary columns with the same number of theoretical plates and the same equivalent lengths, in terms of flow resistance; in addition a correction of the pressure drop across dimensions was operated by an auxiliary flow/pressure controller (EPC) connected to a microfluidic device installed between the outlet of the 2DMS column and the MS transfer line (restrictor) [18]. In Set-up II, the two 2D columns were both 1.4 meters long (0.6 meters at the head of each column were wrapped to form the modulation loop) thus leaving available 0.8 meters of each column for separation. At the end of the 2D to MS, 0.17 m x 0.1 mm d.c. of deactivated silica capillary (restrictor) was used to compensate for differences in flow resistance (Table 1: Set-up II - auxiliary off conditions). Additional helium flow was delivered by setting the auxiliary EPC at 40 kPa (5.7 psi relative) to adjust the outlet pressure towards MS. The compensation was minimal, because of the low resistance of the two parallel 2Ds.

The outlet pressure correctness was verified by isothermal analysis (i.e., 150°C) of linear hydrocarbons from C13 to C15 at 296 kPa head-pressure; Figure 1b shows the raw chromatograms overlaid with the FID 2D plot resulting from an outlet pressure correction towards MS of 40 kPa. System hold-up times were 1.91 min and 0.88 s in the 1D and 2D respectively. Alkanes did not show any retention time shift. Experiments without outlet pressure correction were also run with test mixtures and under programmed temperature conditions; the relative difference between 2D retention times was on average 0.6 % for n-alkanes and 5.65 % for the FFStd2 model mixture. Figures 3c and 3d show absolute differences in time values in detail. Again, wrapped-around analytes showed higher discrepancies between 2D elution times, due to accumulation of the delay error across subsequent modulations. However, with pressure compensation, the retention shift in no case exceeded 1.1 % for linear hydrocarbons and 4% (cinnamyl alcohol) for the FFStd2 model mixture components. These values are in agreement with those reported by Shellie et al. [18], although most of the analytes investigated in that study had lower retention in both dimensions.

Figures 2c and 2d show the 2D plots (Fig. 2c full scan MS and Fig. 2d FID plot) of Artemisia umbelliformis essential oil, analyzed with Set-up II with auxiliary outlet compensation. As is clear, the 2D patterns are in this setup highly consistent, the structure is maintained, and the chromatographic space properly occupied. Experiments run without any outlet pressure correction (data not shown) produced 2D patterns with very
few differences from those shown, and this approach would be a good alternative when an additional EPC is not available, or turbo pumping systems do not tolerate high outlet flows. In such cases, adaptive algorithms (called transforms) for pattern recognition, like those used for template matching procedures [23] in targeted and untargeted data elaboration, can successfully compensate for 2D retention times shifts, and consistently transfer identification from MS to FID.

### 3.4 Single secondary column with dual parallel detection (GCxGC-MS/FID)

To evaluate the practical advantages that can be obtained by operating at near-optimal linear velocities, with two parallel columns and two detection systems, an additional setup (Set-up III) consisting of a single 2D column (1.4 m x 0.1 mm \(d_c\)) connected to two parallel detectors was tested. Pressure/flow conditions adopted were a compromise between optimal conditions in both dimensions, and were allowed to run at 23 cm/s and 240 cm/s in the 1D and in the 2D, respectively. As expected, with Set-up III 1D retention times slightly increased, reflecting the higher elution temperatures that resulted, while those in the 2D decreased, due to the consequent loss of retention. Figures 4a, 4b and 4d show differences in retention times from Set-up I to Set-up III.

For *Artemisia umbelliformis* essential oil, although the separation structure was maintained, the overall resolution was lower. Figures 2e and 2f show the 2D patterns resulting from Set-up III. In this case, a concurrent reduction of the temperature rate and of the modulation period might be expected to produce better results, although analysis time is longer.

### 3.5 Practical advantages of the optimized GC×2GC-MS/FID platform

Some aspects deserve a brief discussion, to outline the practical advantages on real-world samples deriving from a GC×2GC-MS/FID platform, in terms of both dual 2D column and dual detection. *Artemisia umbelliformis* essential oil was selected as a case study, since its detailed quantitative profiling is interesting for botanical classification, as well as in the light of quality aspects relating to its use to prepare a highly-prized Alpine liqueur, called “genepi”, characterized by a bitter taste and a distinctive aroma [24]. These sensory properties can be ascribed to terpenoids, in particular to \(\alpha\)- and \(\beta\)-thujones, the main components of the volatile fraction for the aroma profile, and to sesquiterpene lactones with a cis-eudesmanolide skeleton (5-desoxy-5-hydroperoxy-5-epitelekin; 5-desoxy-5-hydroperoxytelekin and umbellifolide) for its bitterness [25]. The debate on the toxicity of thujones is still open [26], and European Union legislation has fixed a limit of 35 mg/kg on the total amount of these compounds in alcoholic beverages [27]. Thujone-free chemotypes of *A. umbelliformis* have been selectively bred to overcome this issue, and diagnostic fingerprints have been defined by combining biomolecular characterization with chemical profiling of informative secondary metabolites [25]. In any case, a detailed profiling of the volatile fraction is necessary to assess both sensory quality and safety of the aerial parts that are used to prepare the liqueur.
The first aspect to be considered is the separation power of GC×2GC-MS/FID. Resolution reflects the adequacy of the separation conditions adopted for a given group of target analytes, and becomes fundamental for samples where several informative peaks in variable abundances elute in a given region of the chromatographic space. Extra-chromatographic phenomena, e.g. column overloading, may in these cases condition correct separation, i.e. identification/quantitation. For example, when 2D overloading occurs, minor peaks eluting in the proximity of highly abundant components, with large peak-width, may be lost, together with the information they carry. The 2D dual column doubles the 2D loadability, thus limiting 2D overloading and loss of significant minor peaks due to this phenomenon. At the same time, the higher efficiency due to the average linear velocity closer to the optimal value, and the enhanced 2D stationary phase selectivity, increase the system orthogonality, improving occupation of the chromatographic plane.

For instance, the calculated α-thujone half-height peak width in the FFStd2 model mixture at 100 mg/L, was 120 ms (see Table 2). In *A. umbelliformis* essential oil, α- and β-thujones are the two most abundant peaks, each with a peak width of 480 ms, that dramatically overloads the 2D; in Set-up III, where the second dimension loadability is halved compared to Set-up II, they coelute in 1D-GC with two minor components, i.e. nonanal and 2-methylbutyl isovalerate. Apparent resolution values (R) estimated on the raw chromatogram, and referred to the most abundant modulation for all compounds, were 1.93 for the 2-methylbutyl isovalerate/α-thujone pair with Set-up II, and 1.53 with Set-up III, while for the nonanal/α-thujone pair they were 1.28 for Set-up II, but coeluted in Set-up III (Figure 5).

A second practical aspect to consider for in attempting an overall evaluation of the potential of a GC×2GC-MS/FID system concerns quantitation reliability: this exploits the synergisms of dual detection operating by different principles. MS is known to provide a fundamental contribution to unequivocal analyte identification, while FID offers a wide dynamic range of linearity and a very high frequency of acquisition, thereby improving the accuracy of 2D peak (areas) volumes. Moreover, the correct alignment of the two patterns obtained with both Set-up II and Set-up III enables one to consider the data set from the two detectors as a single integrated system, thus cross-validating the results. These considerations are confirmed by experimental data on the FFStd2 mixture. Table 2 shows 1D and 2D retention times and their absolute errors (2D Error in seconds), Normalized 2D Volumes for MS (TIC current) and FID signals (normalization was done on geranyl acetate), half-height peak-width (50% peak width (ms)) and the number of points per peak (MS operated at 28 Hz and FID at 150 Hz) for the analytes of FFStd2 mixture with Set-up II and Set-up III.

These results demonstrate that the chromatographic efficiency (expressed as half-height peak-width) is comparable for the two setups. It has to be stressed that Set-up III had to operate at 2D flow conditions close to those adopted for the two-parallel-column system; if higher head-pressures had been applied, peak-widths would have been narrower. The number of points-per-peak was, in consequence, similar for
Setup II and Setup III for each detector, while mass quantitative descriptors (Normalized 2D Volumes) from the two detectors were consistent.

However, the potential of dual detection can concretely be perceived with real-world samples (e.g. A. umbelliformis essential oil). In these applications, the consistency of acquired MS spectra is fundamental since identification is mainly based on commercial spectral libraries. Table 3 reports the 1D Linear Retention Indices (experimental and reference values [28]), the MS match factors resulting from the NIST Identity Spectrum Search algorithm (NIST MS Search 2.0 ver. d) on spectra collected in commercial databases, and/or on spectra obtained by analyzing reference compounds, and the Signal-to-Noise (Peak-to-Peak S/N as calculated by the Agilent algorithm - SNR) estimated on the highest modulation of each 2D peak of the components characterizing A. umbelliformis essential oil.

For the selected analytes, the quality of the spectral match, as well as the S/N values were comparable between Set-up II and III. Higher S/N values would be expected for a conventional configuration, because of the sharper peaks generated at faster flow rates. Moreover, within the experimental conditions applied here, the 2D peak widths generated were comparable (Table 2) and in accordance with the results recently obtained by Tranchida et al. [29].

Data reported in Table 3 also show that the GC×2GC-MS/FID platform provides enhanced information, because the concurrent presence of two detectors not only provides contemporary analyte identification and quantitation, but also offers internal cross-validation of results. It is also important to note that the international guidelines for quantitative gas chromatography of volatile flavoring substances and essential oils [30-32] indicate Relative Response Factors (RRF) (i.e. external standard calibration with internal standard normalization) as the most suitable approach to obtain consistent quantitative data in these matrices, in particular with MS detection. However, for complex samples consisting of hundreds of potentially informative peaks, a full quantitative assessment by RRFs cannot be applied in practice. The internal normalization approach performed on the FID signal, also known as analyte percent normalization [31], is therefore accepted. In this case, the composition error is minimized by an appropriate selection of internal standard(s) and FID response factors [33-35] making true quantitation by RRF necessary only for those compounds that are limited by law (e.g. α- and β-thujone). FID also opens the possibility of applying the approach introduced by de Saint Laumer et al. [36] where analytes’ RRFs on FID signal are estimated on the basis of combustion enthalpies. With this approach, target analytes can be quantified through estimated RRFs, with accuracy errors limited to a few % points even without external standard calibration. Parallel dual detection thus seems to be very promising for reliable and simple qualitative component identification, and to quantify markers of complex samples of natural origin.

4. Conclusions
The advantages of using a dual-secondary-column dual-detection system in an integrated platform for GC×GC have been discussed, and some practical aspects concerning the tuning of experimental conditions to obtain consistent separation patterns from both dimensions have been addressed. These systems can operate at close-to-optimal 2D linear velocities, and double the secondary column loading capacity, with positive effects on overall system orthogonality and resolution. Experimental data also indicate that the GC×2GC-MS/FID system provides consistent results, both in terms of analyte identification (reliability of spectra and MS matching) and quantitation, also affording internal cross-validation of quantitation accuracy.

The choice of different setups, in terms of 2D column dimensions and flow conditions, should take into consideration some critical aspects, including the auxiliary flow correction, which should be compatible with the turbo pumping capacity and the required sensitivity. The outlet pressure correction adopted in the present study was minimal, and compatible with both system-limiting factors. These data open the way to investigating further applications, where system orthogonality and loading capacity are key-factors for successful separations.

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Caption to Figures

Figure 1: 2D plots (upper part) and raw chromatograms of $n$-C13-$n$-C15 linear hydrocarbons, analyzed in isothermal conditions at 150°C, 296 kPa head-pressure and 5s of modulation period. 1a: Set-up I; 1b: Set-up II with the outlet pressure correction as indicated in the text.

Figure 2: 2D plots of Artemisia umbelliformis essential oil, analyzed with Set-up I (2a full scan MS and 2b FID signals), Set-up II (2c full scan MS and 2d FID signals) and Set-up III (2e full scan MS and 2f FID signals). Chemical classes: $m$: mono-terpene hydrocarbons, $s$: sesqui-terpene hydrocarbons, $mox$: oxygenated monoterpenoids, $sox$: oxygenated sesquiterpenoids, $mest$: mono terpenoid esters.

Figure 3: $^{1}$D retention time absolute differences (FID vs. MS) for: 3a: $n$-alkanes from $n$-C9 to $n$-C25, 3b: fourteen volatiles of interest for the flavor and fragrance field.

Figure 4: $^{1}$D (4a) and $^{2}$D (4b) retention time variations for Set-up I, Set-up II (with and without outlet pressure correction) and Set-up III.

Figure 5: 2D plots of Artemisia umbelliformis essential oil, the magnified region corresponds to the elution area of 2-methylbutyl isovalerate, nonanal and α-thujone. 5a: the separation pattern obtained from Set-up II, and the corresponding raw chromatogram, 5b: Set-up III separation. Apparent resolution values are reported in the text.
Caption to Tables

**Table 1**: Column configurations, column head pressure ($p_i$) and midpoint pressure (i.e., estimated pressure at the junction between the 1D column and the two secondary columns - $p_z$), estimated linear velocities in the 1D and two 2Ds ($\bar{u}, \bar{u}_{ms}, \bar{u}_{fid}$), hold-up times and calculated split-ratio.

**Table 2**: 1D (min) and 2D (sec) retention times, 2D absolute errors (sec), half-height peak-width (ms), number of scans/points per (modulated) peak, normalized 2D Volumes (normalization on geranyl acetate) obtained by analyzing the FFStd2 model mixture with Set-up II and Set-up III.

**Table 3**: Artemisia umbelliformis essential oil target analytes listed, together with experimental and tabulated [28] Linear Retention Indices in the 1D ($I^T_s$), MS match factors resulting from the NIST Identity Spectrum Search algorithm, Signal-to-Noise values (Peak-to-Peak S/N as calculated by the Agilent algorithm - SNR) estimated on the highest modulation of each 2D peak, Normalized 2D Volumes (normalization was done on the Internal Standard n-C12) for Set-ups II and III.
Table 1

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<th>2D column(s)</th>
<th>Carrier gas (He)*</th>
<th>Auxiliary EPC correction</th>
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<td>30 m, 0.25 mm d_c, 0.25 μm d_f</td>
<td>SE52 (95% polydimethylsiloxane, 5% phenyl) Mega (Legnano, Milan, Italy) to MS detector: 1.6 m - to FID detector: 1.4 m</td>
<td>p_i: 296.0 KPa</td>
<td>p_i: 296.0 KPa</td>
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<td>column dimensions: 0.1 mm d_c, 0.10 μm d_f</td>
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<td>p_2: 182.6 KPa</td>
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<td>1: 34.3 cm/s</td>
<td>2: 195 - hold-up: 0.8 s</td>
<td>2: 178 - hold-up: 0.8 s</td>
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<tr>
<td></td>
<td>1:ū: 34.3 cm/s</td>
<td>2:ū: 195 - hold-up: 0.8 s</td>
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<td>split ratio (MS/FID): 50:50</td>
<td>split ratio (MS/FID): 50:50</td>
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| **Set-up II** | 30 m, 0.25 mm d_c, 0.25 μm d_f | SE52 (95% polydimethylsiloxane, 5% phenyl) Mega (Legnano, Milan, Italy) to MS detector: 1.4 m - to FID detector: 1.4 m | p_i: 296.0 KPa | p_i: 296.0 KPa |
|              | column dimensions: 0.1 mm d_c, 0.10 μm d_f | OV1701 (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl) deactivated capillary to MS detector: 0.17 m, 0.1 mm d_c Mega (Legnano, Milan, Italy) to MS detector: 1.4 m - to FID detector: 1.4 m | p_2: 181.9 KPa | p_2: 182.6 KPa |
|              | 1: 34.5 cm/s | 2: 198 - hold-up: 0.8 s | 2: 177 - hold-up: 0.8 s | 1: 34.5 cm/s |
|              | 1:ū: 180 - hold-up: 0.8 s | 2:ū: 180 - hold-up: 0.8 s | split ratio (MS/FID): 51:49 |
|              | 1:ū: 180 - hold-up: 0.8 s | 2:ū: 180 - hold-up: 0.8 s | split ratio (MS/FID): 50:50 |

| **Set-up III** | 30 m, 0.25 mm d_c, 0.25 μm d_f | SE52 (95% polydimethylsiloxane, 5% phenyl) Mega (Legnano, Milan, Italy) column dimensions: 1.4 m, 0.1 mm d_c, 0.10 μm d_f | p_i: 280.0 KPa | p_i: 280.0 KPa |
|                | OV1701 (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl) deactivated capillaries for effluent splitting to parallel detectors: to MS detector: 0.4 m, 0.1 mm d_c - to FID detector: 0.25 m, 0.1 mm d_f Mega (Legnano, Milan, Italy) | p_2: 205.1 KPa | p_2: 205.1 KPa |
|                | 1: 22.8 cm/s | 2: 240 - hold-up: 0.6 s | split ratio (MS/FID): 50:50 |
|                | 1:ū: 22.8 cm/s | 2:ū: 240 - hold-up: 0.6 s |

*: reported values were calculated on the basis of reference equations and are just approximations of real ones
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<th>Number of scans</th>
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<th>MS (TIC signal)</th>
<th>FID signal</th>
<th>1D (min)</th>
<th>2D (sec)</th>
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*a*: Adams Essential Oils database Ref. 28  
*b*: partial coelution  
*c*: authentic standards ad-hoc synthesized Ref. 25
Set-up I

- TIC MS
- FID

C13

8.15 8.25 8.35 8.45 8.55 8.65 8.75 8.85 8.95 min

C14

5.75 5.80 5.85 5.90 5.95 6.00 6.05 6.10 6.15 6.20 min

C15

4.38 4.42 4.46 4.50 4.54 4.58 4.62 min

Set-up II

- TIC MS
- FID

C13

8.45 8.55 8.65 8.75 9.05 9.15 min

C14

6.10 6.15 6.20 6.25 6.30 6.35 6.40 6.45 6.50 min

C15

4.45 4.50 4.55 4.60 4.65 4.70 4.75 4.80 4.85 min
α-Tujone
Nonanal
2-Methylbutyl valerate

Abundance

20.00 20.04 20.08 20.12 20.16 20.20 20.24 min

20.00 20.04 20.08 20.12 20.16 20.20 20.24 min
1. Loop-Type thermal modulator
   (Zoex Corporation, Houston, TX)

2. Microfluidic 3-port splitter
   (Sil-flow™- SGE Ringwood, Victoria, Australia)

3. Outlet pressure compensation
   Microfluidic device (Quick-Swap™- Agilent)