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Enhanced resolution comprehensive two-dimensional gas chromatography applied to the analysis of roasted coffee volatiles

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ABSTRACT

The present research is based on the full exploitation of the separation power of a 0.05 mm internal diameter (ID) capillary, as a comprehensive two-dimensional (2D) GC (GC × GC) secondary column, with the objective of attaining very high-resolution second dimension separations. The aim was achieved by using a split-flow system developed in previous research [P.Q. Tranchida, A. Casilli, P. Dugo, G. Dugo, L. Mondello, Anal. Chem. 79 (2007) 2266], and a dual-oven GC × GC instrument. The column combination employed consisted of a polar 30 m × 0.25 mm ID column connected, by means of a T union, to a detector-linked high-resolution 1.1 m × 0.05 mm ID apolar analytical column and to a 0.33 m × 0.05 mm ID retention gap; the latter was connected to a manually operated split valve. As previously demonstrated, the use of a split valve enables the regulation of gas flows through both analytical columns, generating the most appropriate gas linear velocities. Comprehensive 2D GC experiments were carried out on Arabica roasted coffee volatiles (previously extracted by means of solid-phase microextraction) with the split-valve closed (equal to what can be defined as conventional GC × GC) and with the split-valve opened at various degrees. The reasons why it is absolutely not effective to use a 0.05 mm ID column as second dimension in a conventional GC × GC instrument will be discussed and demonstrated. On the contrary, the use of a 0.05 mm ID column as second dimension, under ideal conditions in a split-flow, twin-oven system, will also be illustrated and discussed.

1. Introduction

Comprehensive two-dimensional (2D) GC (GC × GC), a method which has been on the chromatographic scene since the beginning of the 90s, can be considered one of the most revolutionary inventions in the GC field [2]. The 2D technique enables a great increase in peak capacity, if compared to single-column GC separations. Typically, and briefly, GC × GC separations occur on two capillaries connected in series, and characterized by distinct selectivities. A transfer device, defined as modulator, is located between the two columns and has the fundamental function of cutting and concentrating first dimension column effluent bands; the latter, after the focusing stage, are injected onto a short micro-bore capillary. The whole process, which lasts typically 4–8 s, is carried out continuously throughout the GC × GC analysis. During the second-dimension run time, the modulator is engaged in the subsequent modulation process. Comprehensive two-dimensional GC has been thoroughly described elsewhere [3,4].

The combination of a long 0.25 mm internal diameter (ID) + a short 0.10 mm ID column, with thermal modulation, has always been by far the most preferred choice made by analysts in this field. However, the full potential of GC × GC is hardly ever expressed when using the conventional set-up because mobile phase velocities are generally satisfactory (usually near to optimum) in the first dimension and higher than ideal in the second dimension [1,5,6]. The main consequence is that in many GC × GC separations a great amount of the exploitable chromatographic space remains unoccupied.

In previous research, a GC × GC system, defined as "split-flow", was developed: a primary 0.25 mm ID column was connected to a short 0.10 mm ID secondary one and to a 0.3 m retention gap, by using a T press fit. The second-dimension analytical column passed through the modulator, while the retention gap outlet was linked to a manual split valve. The column flows were regulated in both

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dimensions by simply adjusting the split valve [1]. The validity of the new approach was demonstrated by analyzing petrochemical and fatty acid methyl ester (FAME) samples.

The present research is based on the use of a 0.05 mm ID column as GC × GC secondary column, with the objective of attaining very high-resolution second dimension separations. However, such a micro-bore column has been employed rarely as second column [7,8] and never under optimized conditions. In order to achieve such an aim, a split flow, twin-oven, GC × GC system was employed, characterized by a 30 m (0.25 mm ID) polar primary column (located in GC1) connected to a 2 m segment of 0.25 mm ID retention gap (necessary for cryogenic loop-type modulation); the latter was connected, *via* a T-union, to a 1.1 m × 0.05 mm ID apolar analytical column and to a 0.33 m × 0.05 mm ID retention gap, both located in GC2. The outlet of the 0.05 mm ID retention gap was connected to a needle split-valve, situated in GC2. As demonstrated previously, column flows can be regulated at the conjunction point by adjusting the split valve. Conventional and split-flow GC × GC experiments were carried out on a very complex matrix, *viz*., roasted Arabica coffee volatiles, extracted by using solid-phase microextraction (SPME).

2. Experimental

2.1. Sample and sample preparation

The Arabica ground coffee was a commercially-available one, produced in Italy (the producer will not be reported for industrial secrecy reasons). No preliminary treatment was applied before the SPME process.

The C_{16} alkane and $C_{12:0}$ FAME, used for efficiency calculations, were supplied by Sigma–Aldrich (Milan, Italy). Solutions, at the 100 ppm level, were prepared in *n*-hexane.

2.2. SPME process

About 2 g of ground coffee bean were transferred to a 10 mL vial and then sealed. A Shimadzu autosampler (AOC-5000; Kyoto, Japan) was used for the headspace-SPME operations. The SPME triple phase 50/30 μm fiber (divinylbenzene/carboxen/polydimethylsiloxane) was purchased from Supelco (Milan, Italy). The fiber was appropriately conditioned before use. The extraction procedure was carried out according to previously optimized conditions [9]. Briefly, the samples contained in the vials were heated at 60 °C for 10 min (preincubation) and agitated (clockwise–anticlockwise alternate rotation) at 500 rpm. After this preliminary equilibrium procedure, the SPME needle was inserted in the sealed vial and the fiber exposed to the coffee for 40 min at the same temperature and agitation speed. After this process, the fiber was thermally desorbed in the GC injection port for 1.0 min at 250 °C in the splitless mode (after 1 min, a 100:1 split ratio was applied).

2.3. GC × GC-FID operational conditions

All comprehensive 2D GC applications were carried out on a Shimadzu GC × GC system consisting of two independent GC2010 gas chromatographs, and a flame ionization detector (FID) (280 °C). Data were acquired using the GCsolution software (Shimadzu). Bidimensional chromatograms were generated by using the ChromSquare software (Chromaleont, Messina, Italy). The two GC ovens were linked through a heated (280 °C) transfer line (Shimadzu). The primary GC (GC1) was equipped with an AOC-20i auto-injector and a split-splitless injector (280 °C). The primary column (situated in GC1), an Omegawax 250 30 m × 0.25 mm ID, 0.25 μm film thickness [poly(ethyleneglycol)], was connected to a 2 m × 0.25 mm ID retention gap segment (Supelco) by using an SGE SilTite mini-union (Ringwood, Victoria, Australia). The retention gap was passed through the heated transfer line and connected, by using a fixed outlet capillary column splitter (SGE), to a custom-made SLB-5ms 1.1 m × 0.05 mm ID, 0.05 μm film thickness (silphenylene polymer) capillary and to a 0.33 m × 0.05 mm ID retention gap (both columns, provided by Supelco, were located in the second oven, defined onwards as GC2). The outlet of the 0.05 mm ID retention gap was connected to a manually-controlled valve, namely an OSS-2 outlet splitter system (SGE). A scheme of the split-flow, twin-oven GC × GC instrument is illustrated in Fig. 1. The 2 m retention gap was used to create a double-loop, necessary for cryogenic modulation. The latter was carried out in GC2 and was applied every 6 s by using a dual-stage loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (325 °C) was 375 ms. The FID was operated as follows: H_2 flow: 50.0 mL/min; air flow: 400.0 mL/min; make up (He): 50.0 mL/min; sampling frequency: 125 Hz and 250 Hz in the split-flow and conventional GC × GC applications, respectively.

GC1 oven program was optimized elsewhere [9]; optimized conventional GC × GC: GC1 and GC2 temperature program: 60 °C (5 min) to 230 °C at 1.5 °C/min, to 280 °C (2 min) at 50 °C/min. Initial H_2 pressure (constant linear velocity): 614 kPa.

Optimized split-flow GC × GC: GC1 temperature program: 60 °C (5 min) to 230 °C at 1.5 °C/min, to 280 °C (2 min) at 50 °C/min. GC2 temperature program: a −20 °C offset was applied. Initial H_2 pressure (constant linear velocity): 292 kPa.

First-dimension chromatographic efficiency calculations for conventional and split flow GC × GC: a $C_{12:0}$ FAME 100 ppm solution

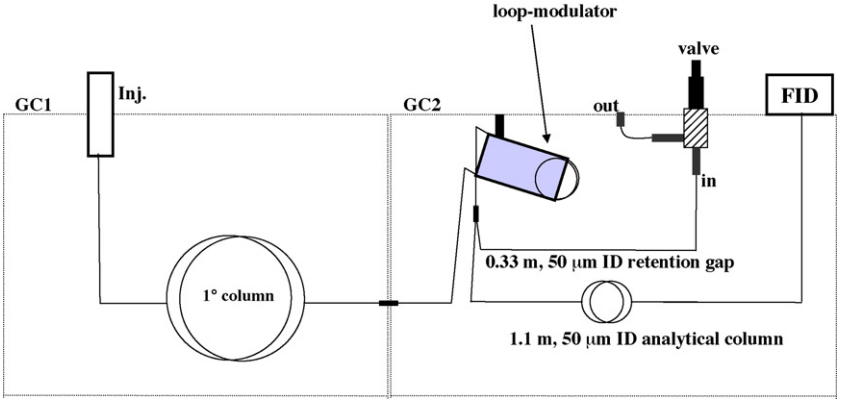

Fig. 1. Scheme of the split-flow, twin-oven GC × GC instrument.

was injected at an isothermal temperature of 160 °C, at variable H_2 pressures.

Second-dimension chromatographic efficiency was calculated with approximation through analysis carried out on a 5 m × 0.05 mm ID, 0.05 μm film thickness SLB-5ms capillary. The latter was installed in a GC2010 gas chromatograph. A C_{16} alkane 100 ppm solution was injected at an isothermal temperature of 165 °C, at variable H_2 pressures.

3. Results and discussion

3.1. GC × GC with a secondary apolar 0.05 mm ID column: analysis of Arabica coffee volatiles under twin-oven conditions

In the field of GC × GC analysis, the 0.25 mm ID + 0.1 mm ID column set-up has been, by far, the most commonly employed combination. The main reason is related to the requirements of relatively widened first dimension bands, and rapid high-resolution second-dimension chromatography. However, in capillary GC it is well-known that a reduction in ID, enables a substantial increase in column efficiency: for columns with a high phase ratio, the minimum *H* value approaches the column ID. Consequently, a 1 m × 0.05 mm ID × 0.05 μm capillary generates a doubled theoretical plate number, with respect to a 1 m × 0.1 mm ID × 0.1 μm column, if both are operated under ideal conditions.

In the present research, a 1.1 m, 50 μm ID apolar column was employed as second dimension. The column segment was attained from a custom-made 5 m capillary; a 30–90 cm/s hydrogen Golay plot, relative to the latter and before length reduction, was constructed by injecting a C_{16} hydrocarbon at an isothermal temperature of 165 °C ($n = 3$). The lowest *H* value, namely 0.055 mm, was attained at a 70 cm/s velocity; through simple calculations it is possible to derive that the 5 m column generates *circa* 18,000 N/m, and a 1.1 m segment would generate *circa* 20,000 N, if operated under optimum conditions. At a 90 cm/s linear velocity, the same column generated *circa* 15,000 N/m ($H = 0.067$ mm).

At this point, it appears that the use of a 0.25 mm ID + 0.05 mm ID combination, in GC × GC, would be a convenient choice. However, very few GC × GC applications have been carried using such an approach, the reasons being essentially two: first, the low sample capacity of a 0.05 mm ID column. It is clear that great attention must be devoted to the injection of adequate sample amounts, to avoid column overloading as much as possible. It can be argued that the main price to pay is the loss in sensitivity. However, as band broadening is greatly reduced by decreasing the column ID, then sensitivity should be essentially maintained, even if lower sample amounts are injected: in 50 μm capillary fast-GC work, no sensitivity loss was observed compared to a conventional 250 μm column, even if greatly reduced sample amounts were analyzed [10]. The second reason, the most important one in the authors' opinion, is that excessively high linear velocities are generated in the second-dimension, nullifying the initial anticipated benefits. On the basis of previously described calculation procedures, considering conventional GC × GC conditions, and using the columns reported in the experimental section, a H_2 linear velocity of ~10 cm/s in the first dimension, will correspond to a linear velocity of ~375 cm/s in the second dimension [1,5,6]. Therefore, an extremely slow first-dimension separation will result in excessively fast second-dimension ones. It could be argued, in truth, that a linear velocity of ~5 cm/s in the first dimension, will correspond to an improved linear velocity (~185 cm/s) in the second dimension. Such an option appears to be an unpractical one (as will be shown later), because analyses times, already of considerable length at 10 cm/s, would be excessively long. A conventional GC × GC first-dimension Golay plot was constructed with the assumption that the band-broadening contribution of the 0.05 mm ID second-dimension, on the final *N* values, can be considered as negligible. The unmodulated applications carried out at 5, 7.5, 10, and 12.6 cm/s (the instrumental pressure limit), analyzing a $C_{12:0}$ FAME at an isothermal temperature of 160 °C ($n = 3$), generated *N* values of *circa* 94,000, 153,000, 164,000, and 127,000, respectively. Observing this data, it is clear that the optimum first-dimension linear velocity is rather "slow", *viz*., 10 cm/s and that column efficiency is very good ($H = 0.183$ mm); in previous GC × GC research, it has been observed that the high first-dimension pressure conditions (the main pressure drop occurs in the second dimension) reduces the analyte diffusion coefficients, and consequently, optimum velocities are far lower than in monodimensional GC [5].

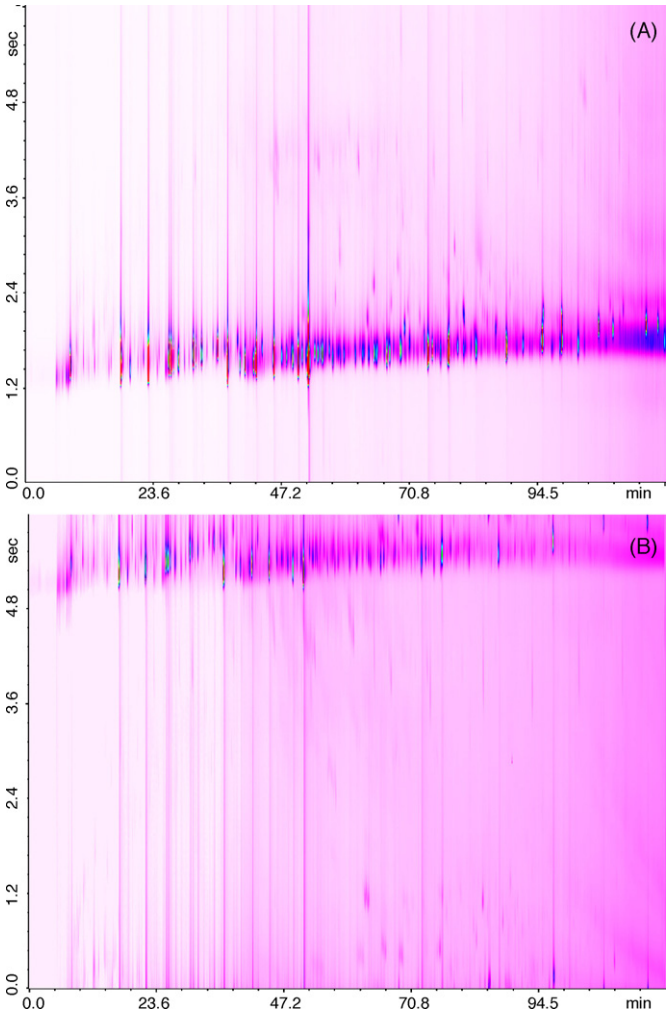

Fig. 2. (A) Conventional SPME-GC × GC-FID analysis of roasted coffee volatiles; (B) with a −20 °C offset applied in GC2.

On the basis of the Golay-plot results, and on the necessity to carry out applications in an acceptable time, the conventional GC × GC experiments (split valve closed) were achieved at a 10 cm/s 1D velocity. The sample analyzed was a very complex one, *viz*., coffee headspace volatiles, extracted by using SPME. The extraction conditions, the (inverted) polar–apolar stationary-phase combination, as well as the GC × GC temperature program, have been optimized in previous SPME-GC × GC research [9]. The bidimensional chromatogram relative to the conventional SPME-GC × GC coffee experiment is shown in Fig. 2A. As can be seen, it took about 115 min to elute the final compounds of interest. The SPME fiber was

inserted into the GC injector, operated in the splitless mode, which is very common in SPME procedures. Although the secondary column does suffer from moderate overloading, due to the excessive band width of several peaks across the chromatogram this does not appear to be the main problem of the present experiment. In fact, the almost complete lack of second-dimension separation is clearly evident: nearly all coffee volatiles are located in a 1-s wide band. The excessively high 2D linear velocity (~375 cm/s) was the main reason behind this unsatisfactory result; in order to improve the overall separation, an additional experiment was carried out with a −20 °C offset applied to GC2 (Fig. 2B). Although the availability of a secondary oven does enhance the flexibility of the GC × GC methodology it was of little use in this case: although analyte capacity factors increased, the coffee volatiles were situated in a widened horizontal band in the 4.5–6 s zone of the bidimensional chromatogram. As aforementioned, the use of a lower head pressure would not be a practical solution, as it would extend the already long analysis time. It could also be argued that the oven temperature gradient could be slowed down, or a thicker stationary phase could be employed. Such solutions would certainly increase the degree of interaction between the analytes and stationary phase. In this respect, a great deal of experiments have been carried out, but with limited benefits; in fact, the gas linear velocity remains very high.

From the results herein reported, the reasons why the employment of 0.05 mm ID columns in GC × GC has been rarely reported are obvious and require no further comment.

3.2. GC × GC with a secondary apolar 0.05 mm ID column: analysis of Arabica coffee volatiles under split-flow, twin-oven conditions

At this point, various split-flow GC × GC experiments were carried out by manually-regulating the split valve at different stages. The best operational conditions were attained with the split-valve completely opened: linear velocities of *circa* 22 and 100 cm/s were calculated in the first and second dimension, respectively [1]. On the basis of Golay-plot results, such gas velocity values can be considered as ideal: unmodulated applications were carried out at 10, 20, 30, and 40 cm/s, analyzing a $C_{12:0}$ FAME at an isothermal temperature of 160 °C ($n = 3$); the calculated N values were *circa* 109,000, 116,000, 73,000, and 47,000, respectively. Observing this data, it is clear that the highest column efficiency ($H = 0.259$ mm), attained at a 20 cm/s gas velocity, was rather lower than that observed in the conventional GC × GC experiment. With regards to the second-dimension linear velocity, this can be considered approximately as ideal: optimum linear velocities increase as the column length is shortened (5 m *vs.* 1.1 m), due to the increase of the analyte-gas diffusion coefficient [11]. It must be added that 2D efficiency was not evaluated by directly installing a 1.1 m segment of column in a gas chromatograph, because injector-derived band broadening would have greatly influenced the overall efficiency value. A further advantage was that approximately 23.1% of the first-dimension gas flow reached the FID, while the remaining part was directed to waste. The 2D chromatogram, relative to the split-flow GC × GC experiment, is illustrated in Fig. 3A. As can be observed, a great improvement has been attained in terms of the chromatography performance. However, it is also evident that the main analyte band is spread in the 5–6 s and 0–1 s zone of the chromatogram; in truth, it appears that the volatiles located in the 5–6 s zone have been subjected to a single wrap-around (retention times = 11–12 s), whereas those present in the 0–1 s zone have undergone the same process twice (retention times = 12–13 s). A further experiment was carried out with a −20 °C offset applied to GC2, with the aim of positioning the main analyte band within the upper and lower chromatogram boundaries and attaining a more extensive occupation of the 2D space. Wrap-around, a phenomenum which occurs more often than not in GC × GC, is entirely acceptable if the main analytical aim is reached, in this case the resolution of the highest number of volatiles per unit of time; the 2D chromatogram relative to this split-flow GC × GC application is shown in Fig. 3B. Observing the latter figure, it is immediately evident that the: (I) the coffee analytes occupy a more vast zone of the 2D space; (II) the main component band is nicely located within the 2D plane; (III) the main peaks are characterized by tailing. The latter aspect can be considered as a negative one, related to column overloading, and dependent on the obliged injection conditions (typically splitless using SPME). Nevertheless, fine tuning of the GC × GC temperature programs and gas linear velocities have provided an overall satisfactory result. Considering the conventional and split-flow experiments the following aspects can be emphasized, namely, (a) the exploitation of the 2D space has greatly increased, (b) group-type patterns appear, and (c) the number of resolved peaks is much higher. The first two advantages are evident observing Fig. 4; the latter is an expansion derived from Fig. 3B in which 50 peaks were identified (Table 1) on the basis of acquired knowledge in previous GC × GC research on coffee [9,12]. In particular, the presence of a pyrazine group pattern is now visible (the 2D zone defined by the ellipse): pyrazines with the same degree of C substitution (*e.g.*, dimethylpyrazines) are situated along horizontal bands. Moreover, almost the entire available 2D space is now occupied.

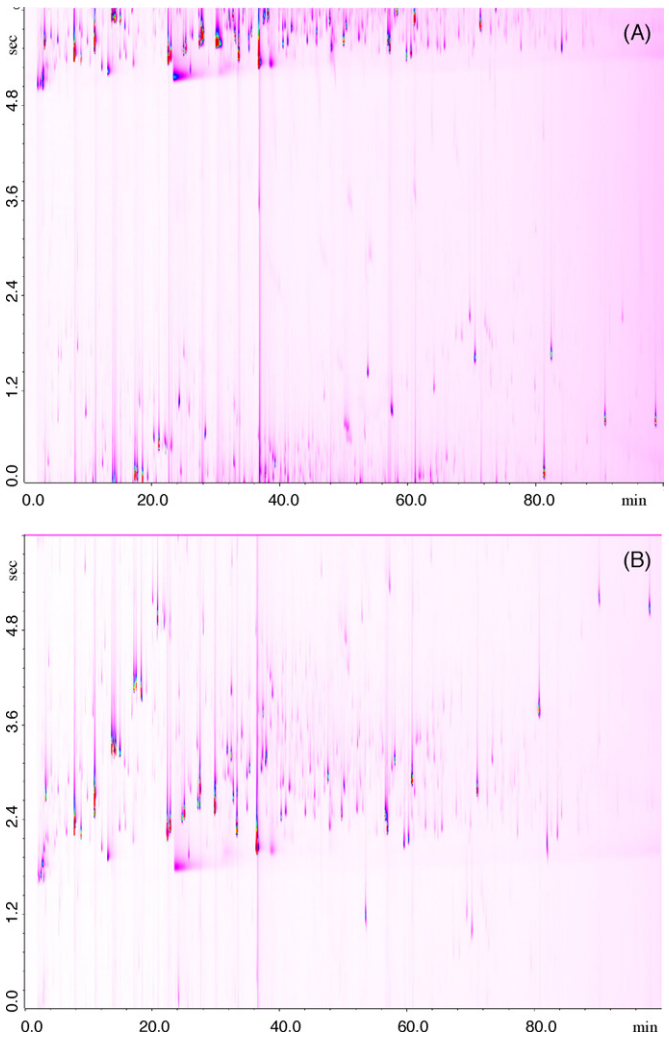

Fig. 3. (A) Split-flow SPME-GC × GC-FID analysis of roasted coffee volatiles; (B) with a −20 °C offset applied in GC2.

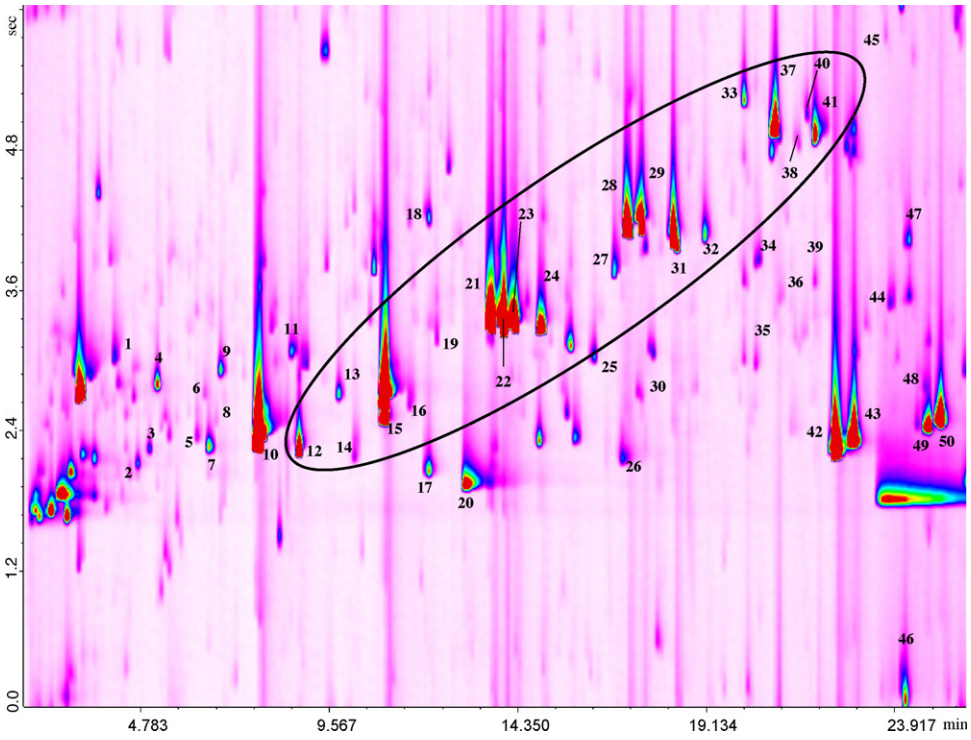

Fig. 4. Chromatogram expansion derived from Fig. 3B, highlighting the pyrazine group-type pattern (refer to Table 1 for peak identification).

The impressive increase in the number of resolved peaks (c) in the split-flow application is highlighted in Fig. 5: in the conventional GC × GC 12.6 min expansion (5A) there is essentially no degree of separation in the second dimension, with only *circa* 10% of the 2D space occupied. Considering the same compound elution range in the split-flow experiment (5B), *circa* 70% of the 2D space is now exploited, with an impressive improvement in terms of resolution.

The general analytical sensitivity was satisfactory; although it is true that *ca*. 75% of the injected material was vented to split, it is also true that the limited band broadening contrasted the injection of low sample amounts onto the second-dimension.

Finally, it could be correctly observed that the term "enhanced resolution" used in the present work is not appropriate because the performance of the 0.25 mm × 0.05 mm ID combination was not compared to that of the more conventional 0.25 mm × 0.1 mm ID set (under split-flow conditions). It must be note that the direct comparison has been made in the analysis of a fuel sample, fully demonstrating the superiority of the proposed method. A paper, based on practical and theoretical considerations, has been very recently submitted for evaluation [13].

Table 1
Volatile coffee compounds identified in the split-flow GC × GC experiment.

Peak	Compound	Peak	Compound
1	3-Hexanone	26	1-Hydroxy-2-butanone
2	2,3-Pentanedione	27	3-Ethylpyridine
3	Phenol	28	2-Ethyl-6-methylpyrazine
4	Hexanal	29	2-Ethyl-5-methylpyrazine
5	3-Penten-2-one(E)	30	D,L-Butandiol diacetate
6	Acetyl butyryl	31	Trimethylpyrazine
7	*n*-Methylpyrrole	32	*n*-Propylpyrazine
8	3,4-Hexanedione	33	2,6-Diethylpyrazine
9	2-Vinyl-5-methylfuran	34	Tetrahydro-2-furanmethanol
10	Pyridine	35	Vinylpyrazine
11	2,4,5-Trimethyloxazole	36	2,3-Dimethyl-2-cyclopenten-1-one
12	Pyrazine	37	2,5-Dimethyl-3-ethylpyrazine
13	Furan-2-methoxymethyl	38	2,3-Diethylpyrazine
14	3-Methyl-3-buten-1-ol	39	1-Octen-3-ol
15	2-Methylpyrazine	40	2,5-Diethylpyrazine
16	4-Methylthiazole	41	2,6-Dimethyl-3-ethylpyrazine
17	Acetoin	42	Furfural
18	2,3-Octanedione	43	Acetol acetyl
19	3-Methylpyridine	44	2-Furfurylmethylsulfide
20	Acetol	45	2-Butyl-3-methylpyrazine
21	2,5-Dimethylpyrazine	46	2,3-Diethyl-6-methylpyrazine
22	2,6-Dimethylpyrazine	47	2,3,4-Trimethyl-2-cyclopenten-1-one
23	2-Ethylpyrazine	48	Furaneol
24	2,3-Dimethylpyrazine	49	Furfuryl formate
25	2-Methyl-5-cyclopenten-1-one	50	2-Acetylfuran

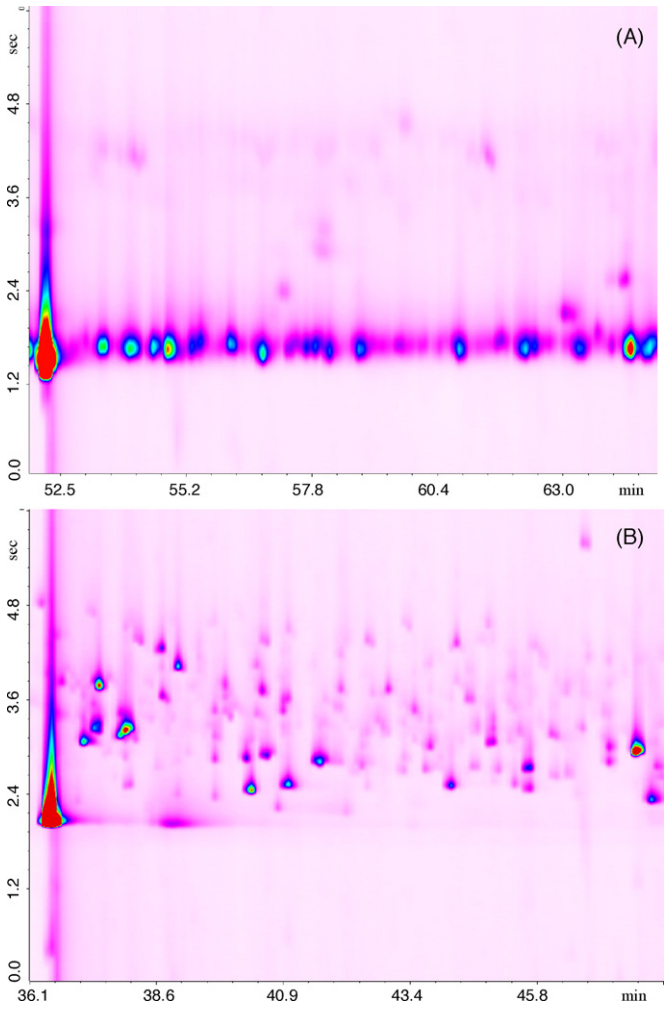

Fig. 5. (A) 12.6 min chromatogram expansion derived from Fig. 2A and, (B) 10.8 min chromatogram expansion derived from Fig. 3B.

4. Conclusions

The beneficial effects of using a highly flexible GC × GC system have been illustrated in the present research. Fine tuning of the first and second-dimension temperature programs and gas flows enabled the development of an optimized enhanced-resolution GC × GC method for the analysis of coffee volatiles. To the best of the authors' knowledge this is the first time that a second-dimension high resolution 0.05 mm ID capillary, as well as a 0.25 mm ID one, have been used under ideal conditions in a GC × GC instrument.

In general, there appears to be a considerable amount of room for improvement in the field of comprehensive 2D gas chromatography and future research will be devoted to the filling of some of these existing gaps.

Acknowledgments

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