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Comprehensive two-dimensional gas chromatography using large sample volume injection for the determination of polynuclear aromatic hydrocarbons in complex matrices

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Abstract

The sensitivity of a gas chromatography (GC) analytical method can be substantially enhanced by injecting large sample volumes. The novel Large Volume Splitless injection technique was used in combination with comprehensive two-dimensional GC (GC × GC), taking advantage of the improved detectability and the very high separation capability. An experimental version of Thermo Electron TRACE GC Ultra using a cryogenic dual jet modulator was utilized with a fast flame ionization detector (FID) for the analysis of low ppbs level of PAHs compounds in very complex matrices. Experimental data (relative standard deviation of 1% evaluated on a standard mixture) demonstrate the reliability of the whole system. A dedicated data system is presented for acquiring and managing GC × GC data: three-dimensional and color plot visualization, peaks integration, identification and quantitation are functions available with the software.

Keywords: Gas chromatography, comprehensive two-dimensional; Large-volume injection; Splitless injection; Injection methods; Polynuclear aromatic hydrocarbons

1. Introduction

Traces analysis and the demand for lower and lower detection limits is growing especially for environmental samples in pollution control. A simple way of increasing the sensitivity without having to concentrate the sample extract, is to inject higher sample volumes reducing, at the same time, the sample preparation complexity.

To fully exploit the advantages of large volume injection, a good separation of target compounds from matrix interferences is needed.

The very high separation power offered by the comprehensive two-dimensional gas chromatography (GC × GC) [1] allows, even in case of very complex matrices, to well separate the target compounds from the interferences. Thanks to the peak compression obtained during the modulation step [2], low detection limits are reached even with a non selective detector as flame ionization detector (FID).

Since the first dimension column of a GC × GC system is a conventional capillary column, it is possible to

Abbreviations: CSR, concurrent solvent recondensation; LV, large volume; SL, splitless; SSL, split/splitless; PTV, programmed temperature vaporizing; OC, on-column; FID, flame ionization detector; RT, retention time; PAH, polynuclear aromatic hydrocarbon

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use the available techniques for injecting large sample volumes. Use of PTV injector in solvent split mode for increasing the injected volume in conjunction with GC × GC was reported [3]: a sample volume of 10 μl was introduced to enhance analyte detectability.

Within the conventional one dimensional GC, PTV solvent split technique is the most common approach to large sample volumes injection for the determination of semi-volatile compounds as PAHs, especially if contained in complex or dirty matrices [4,5]. This technique is actually robust towards contaminants and to be preferred in respect to large volume on-column techniques which are more suitable for relatively clean samples and for volatile and/or thermally labile compounds. On the other hand, PTV solvent split injection involves the optimization of several parameters. Besides, loss of more volatile PAHs, such as those characterized by two or three rings, was reported [4] as a function of the PTV initial temperature.

In this paper, the possibilities offered by the new automatic large volume splitless injection based on concurrent solvent recondensation (CSR) [6,7], combined with the GC × GC technique, are illustrated for the determination of PAHs at low ppb levels.

1.1. Large sample volume splitless injection

The new LVI technique consists in the injection of sample volumes up to 50 μl in splitless mode by using a low dead volume SSL injector and a precolumn for sample recondensation and desolvation. Since the sample is injected in a permanently hot vaporization chamber, using a liner with a small packing of deactivated glass wool at its bottom, this technique is robust towards contaminants which are trapped into the liner itself. This technique involves rapid injection of large volumes in splitless mode, preventing sample evaporation inside the syringe needle and therefore avoiding discrimination of high boiling compounds [8]. The technique is also suitable for volatile compounds since the sample is completely transferred into the analytical column. Moreover, it has the advantage of being a very simple technique since the processes involved are self-regulated.

The CSR splitless injection overcomes the limitation of sample volume to few microliters of classical splitless injection by combining a restricted evaporation rate inside the injector with a strong increase of the transfer rate of sample vapors into the precolumn (Fig. 1). The sample is injected rapidly, keeping the needle cool by inserting it for a short distance into the injector; in these conditions the sample leaves the needle and travels through the injector as a liquid band [9]. The liquid is then collected on a small packing of glass wool positioned just above the precolumn inlet where evaporation takes place almost from a “single droplet”. Since all the heat necessary to evaporate the solvent must be transferred to a small region, the evaporation process is quite slow (it can take many seconds) [10]. As soon as the solvent starts to evaporate, the volume of vapors expands into the liner, displacing and compressing the carrier gas. If accessible volumes outside the liner are minimized, a strong pressure increase is observed and the carrier gas acts as a buffer preventing the sample vapors to escape from the vaporizing chamber. This “auto pressure surge” effect is self-regulated and essential to quickly drive the first vapors to the precolumn. Besides, as the solvent vapors are recondensed into the precolumn, a strong suction effect is produced greatly enhancing the transfer rate from the injector to the precolumn.

From an operational point of view, the initial oven temperature must be maintained below the pressure corrected boiling point of the solvent.

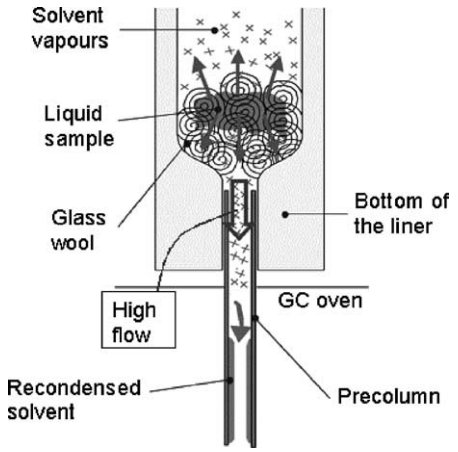


Fig. 1. Concurrent solvent recondensation (CSR) mechanism for large sample volume splitless injection (LVSL). A combination of a slow sample evaporation from the packing retaining the liquid with a high transfer rate into a precolumn generated by solvent recondensation.

It is already known that solvent recondensation in the cool column inlet strongly increases the flow rate from the vaporizing chamber to the recondensation point, accelerating the sample transfer in splitless injection [11]. What was not yet realized is the fact that the transfer rate can reach the rate of vapor formation into the hot injector. Since these two processes occur concurrently, the vapors volume no longer limits the injection volume. Practically, there is a limit of about 50 μl to avoid too long precolumns and an excessive duration of the solvent evaporation from the precolumn which determines the solvent peak width.

The sample components are then trapped by the solvent recondensed inside the precolumn. When the solvent starts evaporating from the precolumn, the volatile components are focused on the rear side of the liquid layer due to the solvent effect and the chromatographic process will not start until the solvent is fully evaporated. The higher boiling compounds are instead refocused into the first column by the retention gap effect [12] due to the increased retention power of the stationary phase.

In a GC × GC system, the second dimension column acts as a restriction, leading to a higher pressure drop and consequently to a decreased carrier gas linear velocity into the precolumn. Therefore, in order to limit the duration of the solvent peak, a higher carrier gas flow rate is necessary. On the other hand, the resulting high inlet pressure allows to start at a higher oven temperature that is still effective for solvent recondensation. The higher oven temperature helps to speed up the solvent evaporation in the precolumn reducing the solvent peak duration.

A software has been developed in order to assist the operator in choosing the correct operative parameters for sample injection and solvent evaporation. In particular, once the dimensions of the precolumn are chosen, the software calculates the achievable injection volume and the solvent peak duration as a function of the inlet pressure and the starting oven temperature.

2. Experimental

2.1. Instrumentation

An experimental version of Thermo Electron TRACE GC Ultra (TRACE 2DGC) was used to perform comprehensive two-dimensional GC. The TRACE 2DGC system was provided with the dual-stage CO_2 jet modulator, as proposed by Beens et al. [13] driven by a dedicated electronics which ensured the synchronization between the valves activation and the signal acquisition. A fast FID was used as detector, capable of producing a digital signal at a sampling rate up to 300 Hz. The injection system was a low dead volume split/splitless injector containing a 105 mm × 5 mm i.d. glass liner. The minimization of dead volumes inside the injection system and the relative pneumatic lines, ensures optimal auto pressure surge and fast vapors transfer processes.

The liner was tapered at the bottom to leave a vaporizing chamber of 80 mm in length and provided with 8 mm of deactivated glass wool (Supelco, Bellefonte, USA) at the liner tapering. The first dimension column was a RTX-5 (Restek, Bellefonte, USA) 30 m long, with an internal diameter of 0.32 mm and a phase thickness of 0.25 μm. It was connected through a deactivated press-fit (Restek) to a BPX-50 second dimension column (SGE Europe, UK), 0.8 m long with an internal diameter of 0.1 mm and a phase thickness of 0.1 μm.

To perform the Large Sample Volume injection and retain the liquid solvent, 5 m of deactivated precolumn (MEGA, Legnano, Italy) with an internal diameter of 0.32 mm were used and connected through a deactivated press-fit to the GC × GC columns set. A schematic representation of the GC × GC system adapted for LVSL injection is shown in Fig. 2.

Large volume splitless injections were performed with the AS2000 Autosampler (Thermo Electron) equipped with a 50 μl syringe (Hamilton, Bonaduz, Switzerland). The injection rate was set to 100 μl/s and the needle insertion depth into the injector was set to 30 mm, which corresponds to insert the needle for about 5 mm into the vaporization chamber.

2.2. Operating conditions

The oven temperature was programmed at 3 °C/min from 75 °C (6 min isotherm) to 330 °C. The carrier gas (helium) was supplied at 3.0 ml/min during the first isotherm and then it was linearly programmed to 0.8 ml/min in 7 min. The initial higher flow rate was necessary for speeding up solvent evaporation from

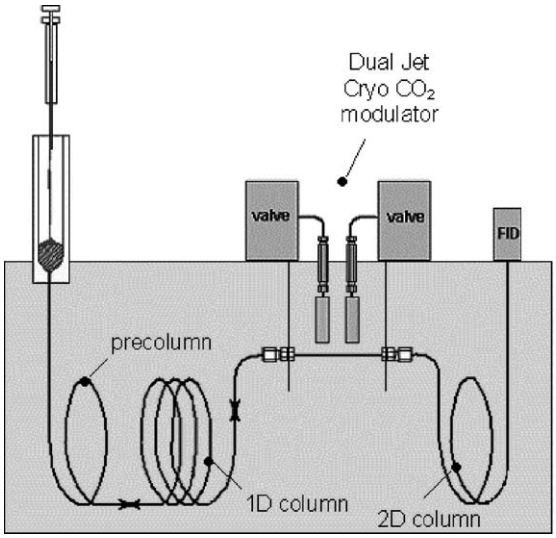


Fig. 2. Schematic diagram of the GC × GC system adapted for LVSL injection.

the precolumn while the lower flow rate used during the analysis was chosen to permit a suitable velocity for proper modulation on the second dimension column inlet.

The SSL injector was set to a temperature of 270 °C and the FID detector was set to a temperature of 300 °C.

A standard mixture of 19 PAHs was obtained from Restek (8270 Calibration Mix) and diluted with *n*-hexane to a concentration of 0.02 ng/μl each. Thirty micro-litres were injected in splitless mode with a splitless time of 1 min. To make the auto pressure surge effective, also the septum purge outlet was closed during the splitless period. Previous studies [7] demonstrated that solvent evaporation and vapors transfer are completed in few tens of seconds, if the CSR splitless injection is performed correctly. For this study higher splitless time did not lead to higher peak areas, showing that the transfer of analytes was completed.

In order to check the detectability level, a certified mixture of PAHs in composite diesel fuel obtained from Restek and diluted 1:10,000 with *n*-hexane was used.

A lower dilution (1:2500) was prepared for validating the quantitative results. The original concentration of each compound is reported in Table 1.

Table 1
LVSL–GC × GC analysis of Restek certified mixture of PAHs in composite diesel fuel

Restek certified solution			Results[a] (ppm)
Component	Mean concentration (ppm)	Uncertainty[b] (ppm)	
Naphthalene	90	±10.2	82
1-Methylnaphthalene	269	±37.7	262
2-Methylnaphthalene	180	±21.1	200
Acenaphthylene	14	±1.2	11
Acenaphthene	20	±2.2	18
Fluorene	32	±6.8	40.5
Phenanthrene	47	±6.9	38

Comparison with quantitative results obtained with external standard calibration. Injected volume 30 μl splitless.
[a] Obtained with external standard calibration.
[b] Restek data: calculated as the 95% confidence limit from the standard deviation of three analyses.

The GC × GC analyses were carried out with a modulation cycle time of 4 s.

2.3. Software

A specific software, Splitless Large Volume Assistant (Thermo Electron), was used for assisting the optimization of Large Volume Splitless Injection operative parameters.

Data acquisition and data handling were performed with an experimental software version for GC × GC (HyperChrom) based on Thermo Electron Chrom-Card Data System. The software is capable to visualize the generated data set as a three-dimensional chromatogram or as a color plot and perform peaks integration, identification and quantitation. A component table can be created for identification and quantitation of target compounds by graphically enclosing the spot with a square box. All the peaks (slices) within the drawn box are identified as target compound and each peak area is added up to obtain the total area used for quantitation.

3. Results and discussion

When LVSL injection is applied to two-dimensional comprehensive GC, operative parameters need to be optimized in order to limit the solvent peak duration

due to the low carrier gas linear velocity into the pre-column consequent to the addition of the narrow bore second dimension column to a conventional capillary column. With the aid of the Splitless Large Volume Assistant software it was possible to easily establish the correct conditions of head pressure and starting oven temperature in order to obtain an acceptable solvent peak duration. The GC × GC color plot resulting from the injection of 30 μl of a standard mixture of 19 PAHs in *n*-hexane is reported in Fig. 3. A solvent peak duration of 3.4 min was obtained, fitting well with the value calculated by the software.

By combining Large Volume Splitless Injection and comprehensive two-dimensional GC, thanks also to peak compression obtained during the modulation step, it is possible to detect traces at low ppb levels contained in complex matrices, even using a non selective detector as fast FID. The very high peak capacity attainable with the GC × GC technique allows to reach such low detection limits even in case of heavily contaminated samples granting the separation of target compounds from matrix interferences. It can be stated that, in this case, the high separation power achieved with comprehensive two-dimensional technique can make up for the detector selectivity of a conventional GC–MS system, with the advantageous possibility of relying on FID response factors. However, it must be pointed out that using a 50% phenyl polysiloxane as second dimension column of a GC × GC system, the separation of critical pairs of PAHs, such

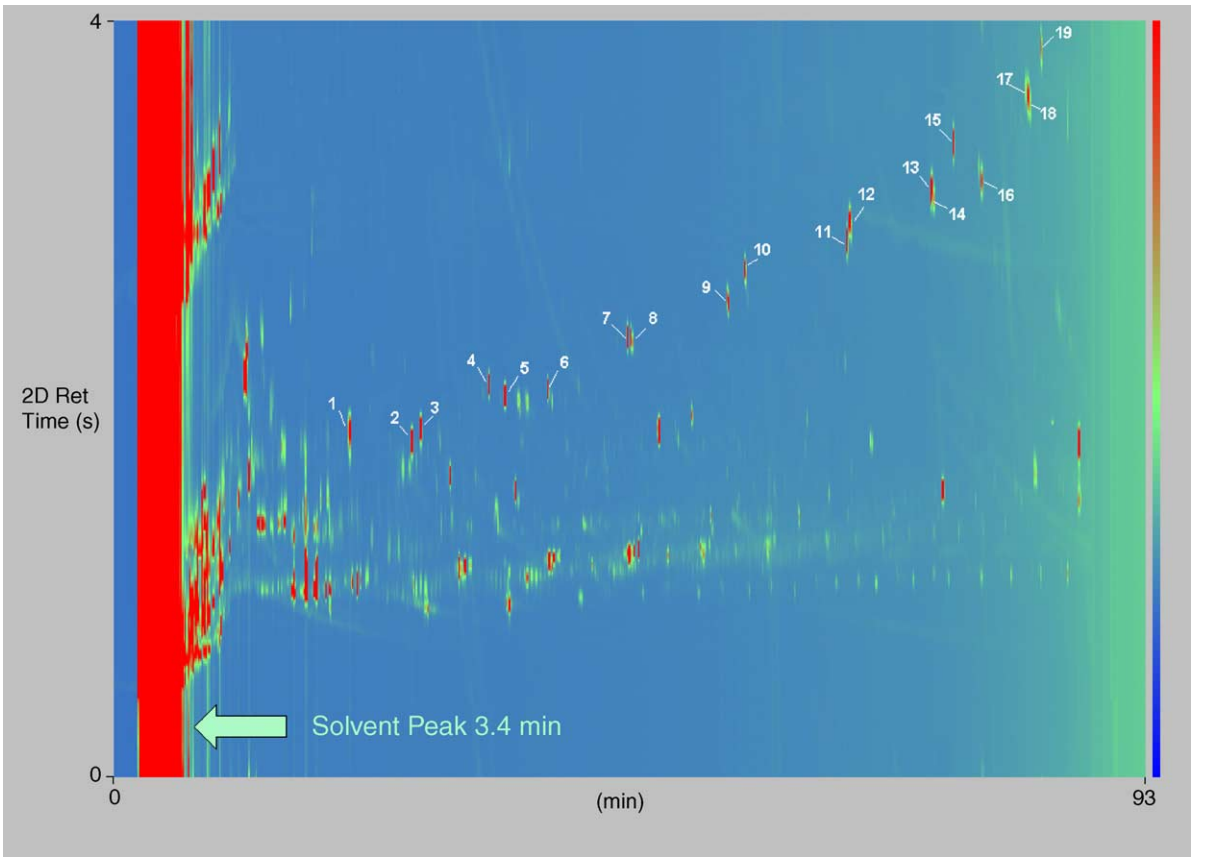


Fig. 3. Splitless injection of 30 μl of a standard mixture of 19 PAHs in *n*-hexane at a concentration of 0.02 ng/μl each: (1) naphthalene; (2) 1-methylnaphthalene; (3) 2-methylnaphthalene; (4) acenaphthylene; (5) acenaphthene; (6) fluorene; (7) phenanthrene; (8) antracene; (9) fluoranthene; (10) pyrene; (11) benzo(*a*)antracene; (12) chrysene; (13) benzo(*b*)fluoranthene; (14) benzo(*k*)fluoranthene; (15) benzo(*a*)pyrene; (16) 3-methylcholanthrene; (17) indeno(1,2,3-*c*,*d*)pyrene; (18) dibenzo(*a*,*h*)antracene; (19) benzo(*g*,*h*,*i*)perylene.

as benzo(*k*)fluoranthene/benzo(*b*)fluoranthene and indeno(1,2,3-*c*,*d*)pyrene/dibenzo(*a*,*h*)anthracene cannot be fully achieved, due to their similar volatility and polarity.

With the addition of the fast TOF-MS detector to the GC × GC system, it is possible to increase much more the amount of information available from a single analysis, especially in case of heavy peaks overlay, as third-order data can be obtained. Nevertheless, the coupling with TOF-MS was not considered in this work since the aim was to highlight the possibilities offered by a simple system, such as GC × GC–FID.

3.1. GC × GC versus GC

In order to evaluate the gain in sensitivity resulting from the focusing effect of the modulation, a comparison between the single and the two-dimensional chromatograms was done by repeating the same splitless injection of 30 μl of a standard mixture of PAHs with and without the modulation process (Fig. 4).

Since the GC × GC conditions are not optimized for the single dimension analysis, the comparison was done with a different temperature programming rate and a different acquisition rate (the conditions are reported in the legend of Fig. 4). Depending on the number of modulations per peak, an improvement in signal to noise ratio of about four to five times is obtained, as illustrated in Table 2. The data reported in the table also confirm that the total area counts of each compound is maintained after the modulation.

3.2. Detectability with GC × GC–FID

In order to check the detectability of the system, 30 μl of a certified mixture of PAHs in composite diesel fuel diluted 1:10,000 in *n*-hexane were injected in splitless conditions. Fig. 5 shows the color plot and the three-dimensional visualization of three PAHs compounds to highlight the separation: the resulting signal to noise ratios show a detectability for traces at a concentration level of few ppbs.

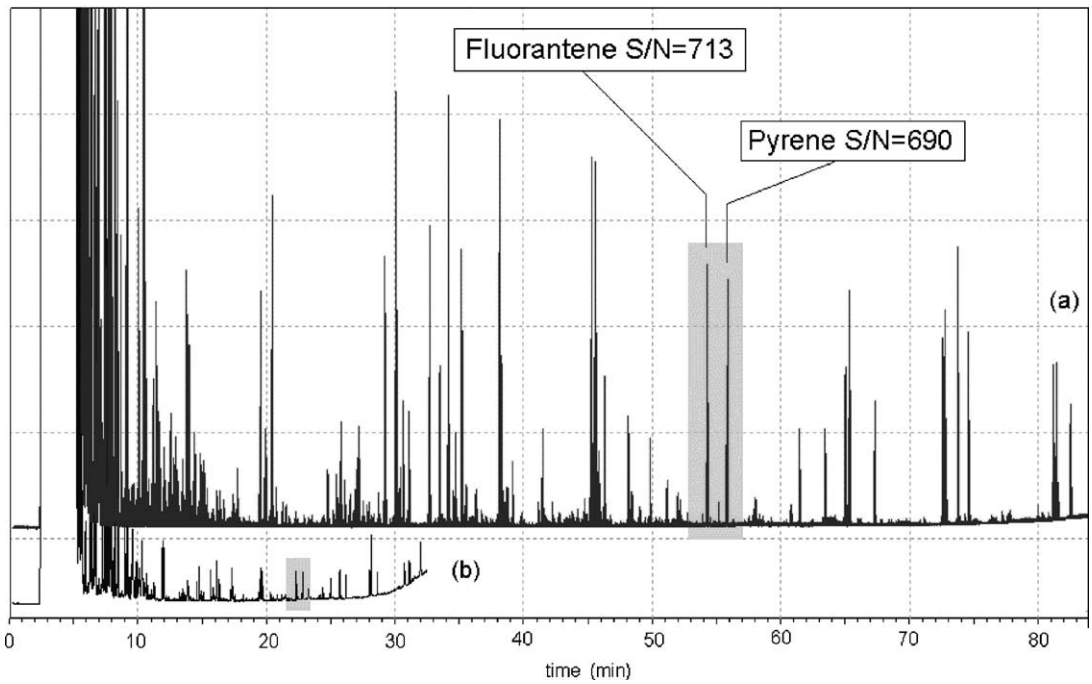


Fig. 4. Comparison between single dimension and two-dimensional PAHs standard mixture analysis: (a) with a modulation cycle time of 4 s; oven temperature program 75 °C (6 min), 3 °C/min to 330 °C; acquisition rate 100 Hz; (b) without modulation; oven temperature program 75 °C (6 min), 10 °C/min to 330 °C; acquisition rate 10 Hz.

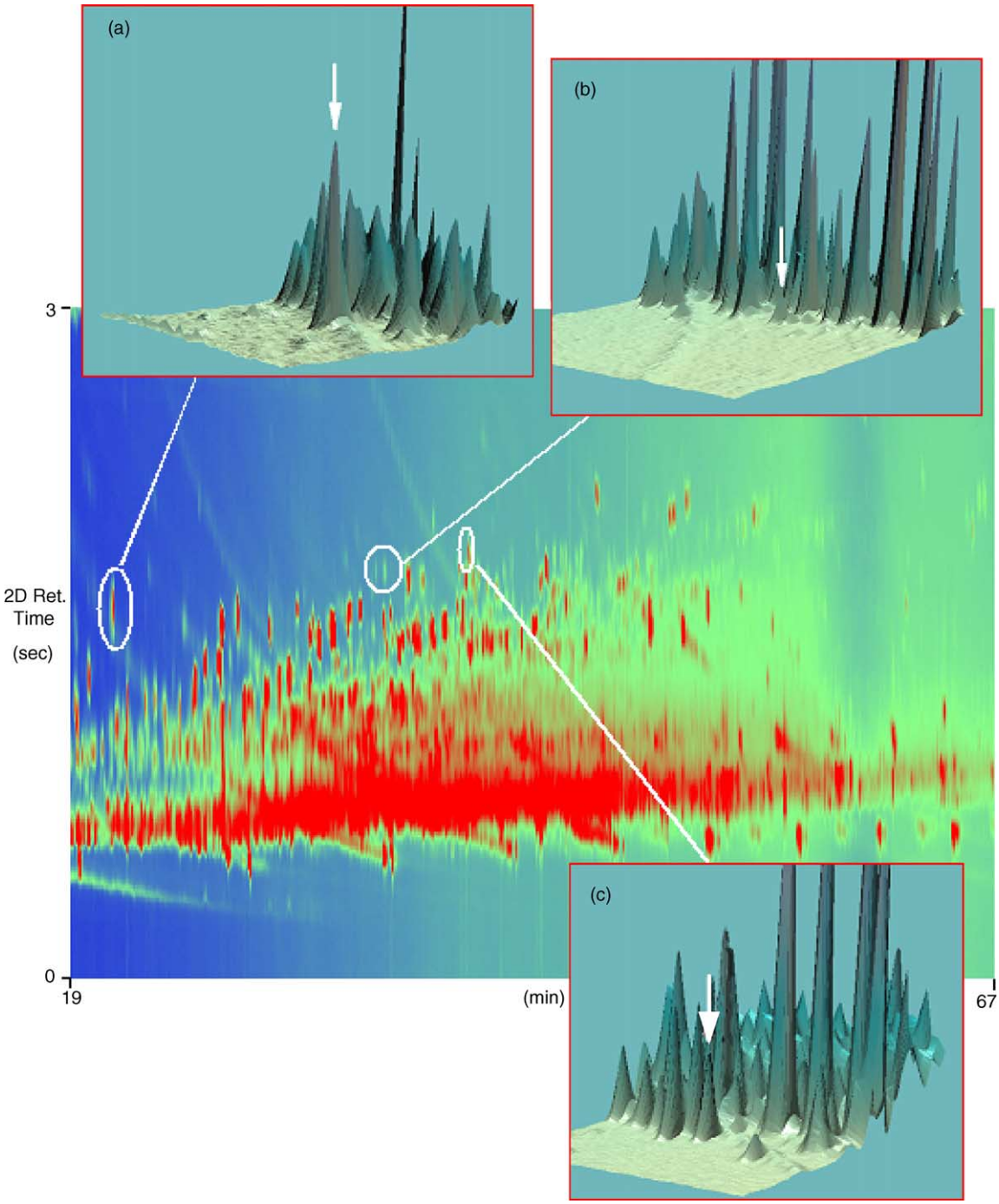


Fig. 5. Splitless injection of 30 μl of a certified mixture of PAHs in composite diesel fuel diluted 1:10,000 in *n*-hexane. (a) Naphthalene 9 ppb, S/N = 140; (b) acenaphthene 2 ppb, S/N = 27; (c) fluorene 3.2 ppb, S/N = 55.

Table 2
LVSL–GC and LVSL–GC × GC analysis of PAHs standard mixture

Compound	S/N ratio		Peak areas (10^3 counts)	
	GC[a]	GC × GC[b]	GC	GC × GC
Naphthalene (**1**)	180	1001	955	976
Fluorene (**6**)	185	915	740	752
Fluoranthene (**9**)	177	713	737	739
Pyrene (**10**)	170	690	727	753
Chrysene (**12**)	160	607	701	728
Benzo(*a*)pyrene (**15**)	145	455	613	661
Benzo(*g*,*h*,*i*)perylene (**19**)	130	415	536	626

Comparison between single and comprehensive two-dimensional analysis. Injected volume 30 μl splitless.

[a] Without modulation; oven temperature programming rate 10 °C/min; acquisition rate 10 Hz.

[b] Modulation cycle time 4 s; oven temperature programming rate 3 °C/min; acquisition rate 100 Hz.

3.3. Repeatability of LVSL–GC × GC

An evaluation of the total area counts repeatability when LVSL injection is applied, was performed on five consecutive injections of 30 μl of PAHs standard mixture diluted in *n*-hexane in order to have a concentration of 0.02 ng/μl for each compound. Data for selected PAHs compounds are collected in Table 3, showing an averaged relative standard deviation of absolute peak areas of about 1%. The total area counts was calculated by the data system as the sum of four to five slices per peak.

In order to establish the first and the second dimension retention times of a target compound, represented in the color plot as a single spot, the highest slice is considered by the software. The two-dimensional chromatogram is constructed by the software by cutting the raw data every modulation cycle time and by stacking each cut along the second dimension axis. Therefore, the first dimension retention time is necessarily a number which is a multiple of the modulation cycle time while the second dimension retention time is evaluated considering the absolute retention time on the raw data minus the first dimension retention time. With the second dimension retention time data it is possible to evaluate the standard deviation as a measure of the variation of retention time on both dimensions, since the temperature at which the fast second dimension separation is performed in case of temperature programmed oven depends on the elution time from the first column. The calculated standard deviation for second dimension retention time is much lower than the peak width obtained from the second dimension column, permitting a reliable identification. The repeatability data evaluated on five consecutive injections are collected in Table 4.

3.4. Quantitation

In order to validate the capability to obtain reliable quantitative results, the certified solution of PAHs in diesel fuel was diluted 1:2500 in *n*-hexane and injected

Table 3
LVSL–GC × GC analysis of PAHs standard mixture

Compound	Peak areas (10^3 counts)	
	Mean	R.S.D.[a] (%)
Naphthalene (**1**)	976	1.10
Fluorene (**6**)	752	1.40
Fluoranthene (**9**)	739	0.44
Pyrene (**10**)	753	0.49
Chrysene (**12**)	728	0.91
Benzo(*a*)pyrene (**15**)	661	1.40
Benzo(*g*,*h*,*i*)perylene (**19**)	626	1.59
Average		1.05

Absolute total area counts repeatability data. Injected volume 30 μl splitless.

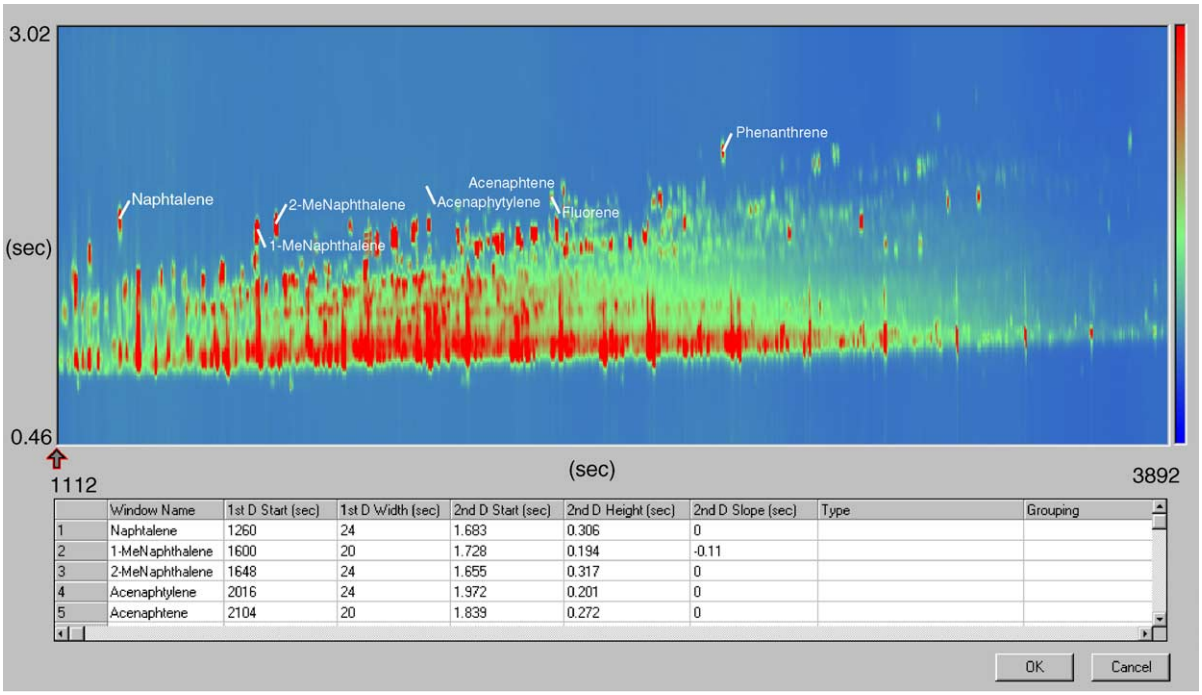
[a] Evaluated on five injections.

Table 4
LVSL–GC × GC analysis of PAHs standard mixture

Compound	PW at 10% h (ms)	Retention time two-dimensional	
		Mean[a] (s)	S.D.[a] (ms)
Naphthalene (**1**)	130	1.876	6
Fluorene (**6**)	120	2.050	10
Fluoranthene (**9**)	170	2.537	21
Pyrene (**10**)	170	2.670	20
Chrysene (**12**)	190	2.937	15
Benzo(*a*)pyrene (**15**)	210	3.353	21
Benzo(*g*,*h*,*i*)perylene (**19**)	240	3.870	26

Second dimension retention times repeatability data. Injected volume 30 μl splitless.

[a] Evaluated on five injections.



	Window Name	1st D Start (sec)	1st D Width (sec)	2nd D Start (sec)	2nd D Height (sec)	2nd D Slope (sec)	Type	Grouping
1	Naphtalene	1260	24	1.683	0.306	0		
2	1-MeNaphthalene	1600	20	1.728	0.194	-0.11		
3	2-MeNaphthalene	1648	24	1.655	0.317	0		
4	Acenaphtylene	2016	24	1.972	0.201	0		
5	Acenaphtene	2104	20	1.839	0.272	0		

Fig. 6. Splitless injection of 30 μl of a certified mixture of PAHs in composite diesel fuel diluted 1:2500 in *n*-hexane. View of the chromatogram from the component table window.

as unknown sample (Fig. 6). The quantitative results, as reported in Table 1 in comparison with certified data, were obtained after external calibration with a standard solution of PAHs (0.02 ng/μl each) and calculated according to the dilution.

4. Conclusions

The combination of the new splitless large volume injection technique with the comprehensive two-dimensional gas chromatography offers a very powerful tool for traces analysis in complex mixtures. It is possible to achieve a complete separation of target compounds from the matrix and detect them at a concentration of few ppbs by using a solid and reliable detector as the fast FID.

Injections of large sample volumes (up to 50 μl) are feasible in a very simple and reliable way by using a low dead volume split–splitless injector in splitless mode, since the LVSL injection technique involves a fully self-regulated process.

The upper limit is mainly determined by the duration of solvent evaporation in the precolumn and the dimensions of the precolumn needed for retaining the recondensed vapors.

A software has been developed to assist the operator in choosing the correct operating parameters and predict the solvent peak duration. Experimental results demonstrate the performances and the reliability of the system utilized for GC × GC technique.

Also HyperChrom software for comprehensive two-dimensional GC has been proved to be a very good tool for managing GC × GC data allowing suitable visualization, conventional peak integration and reliable identification and quantitation of target compounds.

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