

Technical Report

On-line LC-GC×GC-MS/MS: A Powerful Unified Separation-science Tool

In-depth Analysis of a Very Complex Mixture

Mariosimone Zoccali¹, Peter Q. Tranchida¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

Abstract:

This Technical Report demonstrates the potential of the on-line combination of high performance liquid chromatography (HPLC), cryogenically modulated comprehensive two-dimensional gas chromatography (GC×GC), and triple quadrupole mass spectrometry (MS/MS). The selectivity of the HPLC dimension enabled the separation of chemical classes; each fraction, transferred to the GC×GC instrument via a syringe-based interface, was subjected to a specific programmed temperature vaporizer (PTV) GC×GC-MS/MS untargeted or targeted analysis.

Keywords: liquid-gas chromatography, comprehensive gas chromatography, triple quadrupole mass spectrometry

1. Introduction

The detailed characterization, or untargeted analysis, of very complex mixtures of organic volatiles (and semi-volatiles) represents one of the main challenges in the field of analytical chemistry. Sample complexity, as such, is not only related to a potentially high number of constituents (e.g., ≥ 1000), but also to the range of different chemical classes (from apolar to highly polar) and concentration levels (from trace amounts to highly concentrated).

Consequently, the satisfactory profiling of a very complex mixture would require: 1) enhanced separation power; 2) high selectivity; 3) increased sensitivity; 4) wide dynamic range. High-resolution gas chromatography (GC) methods, such as comprehensive 2D GC (GC×GC), are advisable for the untargeted analysis of a complex sample.

In terms of mass spectrometry (MS) unit-mass devices such as single quadrupole (quad MS) or low-resolution time-of-flight (LR ToF MS) are sufficient for the scopes of an untargeted experiment. In a targeted experiment, the analyst has an *a priori* knowledge of the name and number of solutes requiring investigation. In targeted GC-MS experiments, the use of quad MS, LR ToF MS, high-resolution (HR) ToF MS and triple quadrupoles (MS/MS) is popular.

The combination of GC×GC, with quad MS, LR ToF MS, HR ToF MS, and MS/MS systems generates very powerful analytical tools that can be employed in both untargeted and targeted applications. However, as will be seen, the separation power of comprehensive 2D GC is often insufficient, and many co-elutions still occur at the end of the analytical process.



Fig. 1 LC-GC×GC-MS/MS instrumentation

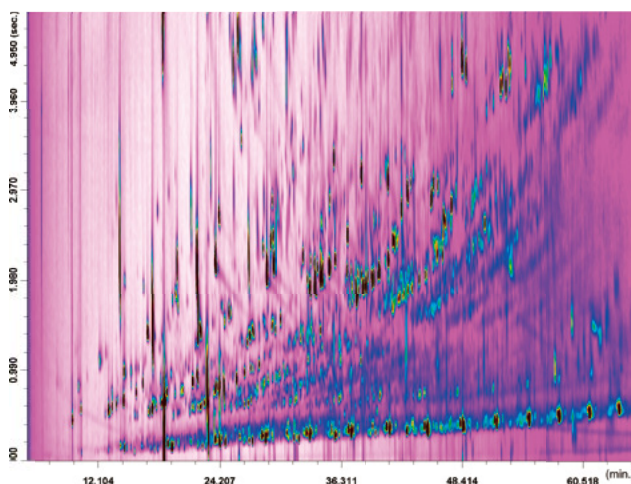


Fig. 2 TIC-GC×GC-MS chromatogram of coal tar

Probably, the best choice to improve selectivity, due to the high number of stationary phases, is the exploitation of an HPLC pre-separation step. HPLC is very useful for the performance of polarity-based separations (e.g., hydrocarbons, aromatic constituents); after, simplified fractions can be subjected to a GC-based analysis, injecting large sample volumes, enabling the detailed analysis of complex samples.

The potential of the novel LC-GC×GC-MS/MS combination was evaluated in the analysis of a highly challenging sample, namely coal tar (Figs. 1-2). The normal-phase LC step was successful in the separation of the following groups of compounds: I) non aromatic hydrocarbons, II) aromatic compounds (with and without S), III) oxygenated constituents. Each LC fraction was subjected to a specific untargeted or targeted GC×GC-MS/MS approach. For example, the coal tar S-containing compounds were pinpointed through MRM analysis, while full-scan MS information was sufficient for the hydrocarbons.

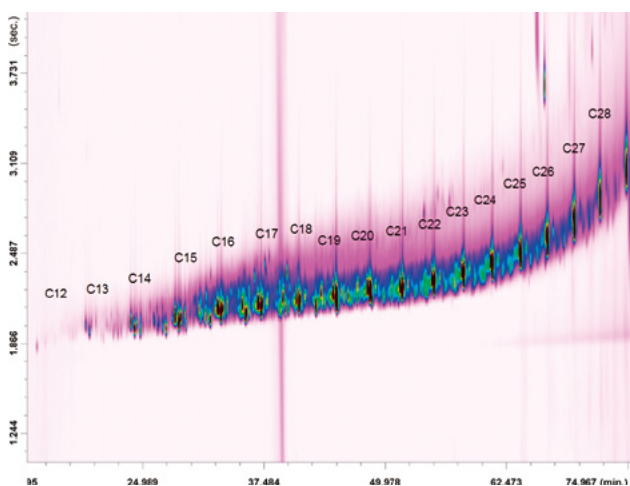


Fig. 3 TIC LC-GC×GC-MS/MS chromatogram of the 1° fraction

2. Experimental

2-1. Materials and Methods

All solvents were HPLC grade from Sigma-Aldrich. All standard sulphur compounds were from Sigma-Aldrich. Prior to LC analysis, the sample was diluted 1:200 (v/v) in dichloromethane.

2-2. Shimadzu Instrumentation

- CBM-20A controller
- LC-30AD dual-plunger pump
- DGU-20A online degasser
- SPD-M20A photodiode array detector
- CTO-20A column oven
- SIL-30AC autosampler
- AOC-5000 auto injector equipped with a dedicated dual side-port syringe employed as transfer device
- Optic-4 injector (GL Sciences)
- Two GC-2010 gas chromatographs
- Zoex dual-stage loop-type cryogenic modulator
- GCMS-TQ8040 triple quadrupole mass spectrometer
- D1 column: SLB-5ms 30 m × 0.25 mm ID × 0.25 μm d_f (Supelco)
- Loop: 1.5 m × 0.18 mm ID uncoated capillary
- D2 column: Supelcowax-10 1 m × 0.1 mm ID × 0.1 μm d_f (Supelco)

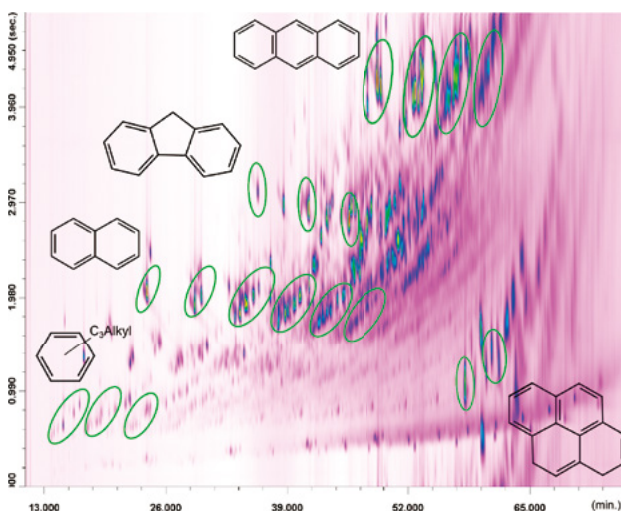


Fig. 4 TIC LC-GC×GC-MS/MS chromatogram of the 2° fraction

3. Chromatographic method

3-1. LC conditions

Column	: SUPELCO SIL LC-Si (100 × 3.0 mm ID × 5.0 μm d_p)
Flow rate	: 0.35 mL/min
Mobile phases	: (A) <i>n</i> -hexane and (B) dichloromethane
Gradient mode	: 0-6.5 min 100% A; from 6.5 to 7.5 min 100% B (until the end of the analysis)

3-2. LC fractions

- I) non aromatic hydrocarbons were transferred from 1.35 to 1.85 min (175 μ L)
- II) aromatic compounds (with and without S) were transferred from 1.85 to 7.00 min (1802 μ L)
- III) oxygenated compounds were transferred from 8.95 to 14.10 min (1802 μ L)

3-3. GC \times GC analysis

I) non aromatic hydrocarbons (Fig. 3)
 Split ratio 200:1 for 1 min, then splitless for 1.5 min, then split 150:1
 GC1 and GC2 ovens : from 50°C to 280°C at 3°C/min
 Carrier gas : He
 Inlet pressure : 175.8 kPa (constant linear velocity mode)
 Modulation period : 5 seconds.
 Duration of the hot pulse (300°C) was 300 msec
 MS ionization mode : electron ionization
 Scan speed : 20,000 u/sec
 Mass range : 45-360 m/z
 Acquisition frequency : 33 Hz
 Ion source temperature : 250°C
 Interface temperature : 280°C

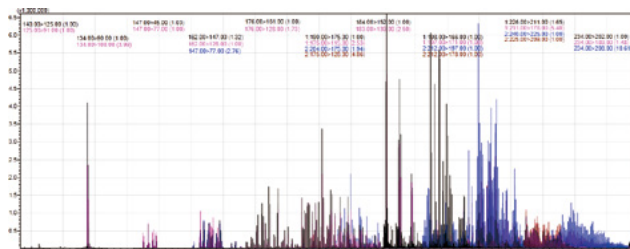


Fig. 5 LC-GC \times GC-MS/MS MRM

II) aromatic compounds (with and without S) (Figs. 4-8)
 Split ratio 250:1 for 1 min, then splitless for 1.5 min, then split 150:1
 GC1 oven : from 50°C to 280°C at 3°C/min
 GC2 oven : from 60°C to 280°C (3.3 min) at 3°C/min
 Carrier gas : He
 Inlet pressure : 175.8 kPa (constant linear velocity mode)
 Modulation period : 5 seconds.
 Duration of the hot pulse (300°C) was 300 msec
 MS ionization mode : electron ionization

Full scan mode

Scan speed : 20,000 u/sec
 Mass range : 45-360 m/z
 Acquisition frequency : 33 Hz
 Ion source temperature : 250°C
 Interface temperature : 280°C

MRM mode

Argon (200 kPa) was employed as collision gas
 For MRM transitions and collision energies see Fig. 5

III) oxygenated compounds (Fig. 9)
 Split ratio 250:1 for 1 min, then splitless for 1.5 min, then split 150:1
 GC1 oven : from 50°C to 280°C at 3°C/min
 GC2 oven : from 110°C to 280°C (20 min) at 3°C/min
 Carrier gas : He
 Inlet pressure : 175.8 kPa (constant linear velocity mode)
 Modulation period : 5 seconds.
 Duration of the hot pulse (300°C) was 300 msec
 MS ionization mode : electron ionization
 Scan speed : 20,000 u/sec
 Mass range : 45-360 m/z
 Acquisition frequency : 33 Hz
 Ion source temperature : 250°C
 Interface temperature : 280°C

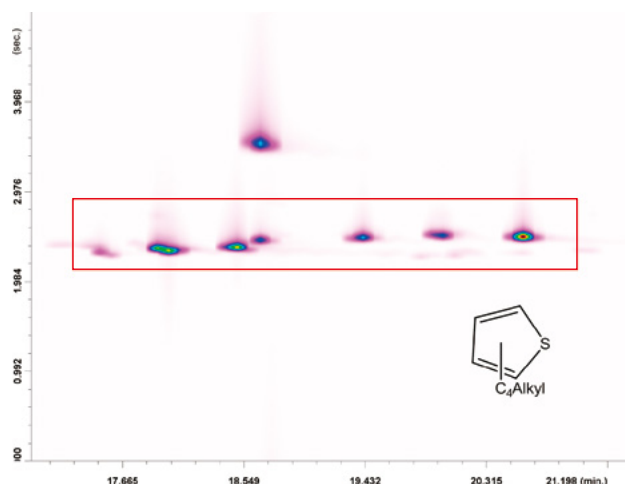


Fig. 6 LC-GC \times GC-MS/MS MRM of C4-T isomers

3-4. Shimadzu Software

- 5D Solution ver. 1.0.9
- GCMSSolution ver. 4.20
- LCSolution ver. 1.25
- Chromsquare ver. 2.1

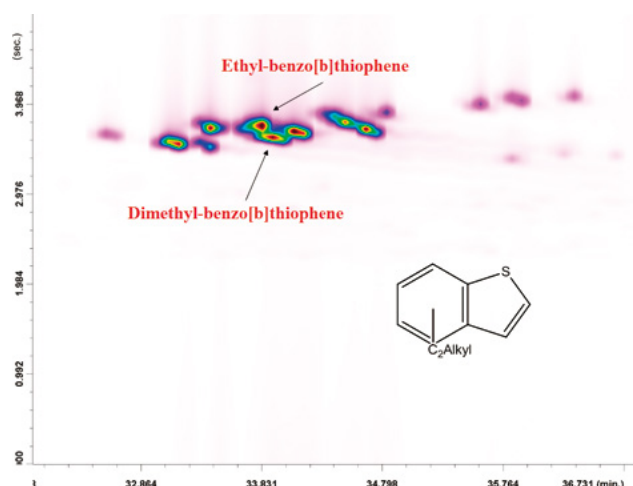


Fig. 7 LC-GC×GC-MS/MS MRM of C2-BT isomers

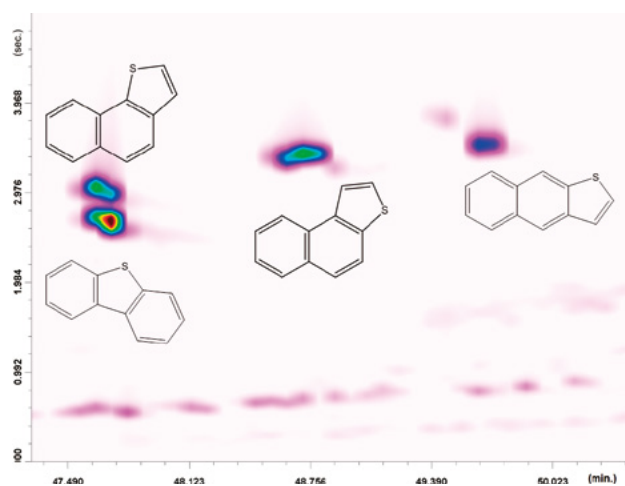


Fig. 8 MRM LC-GC×GC-MS/MS chromatogram of DBT isomers

4. Results and Discussion

As can be observed in the figures, the pre-separation step was very useful in generating simplified sub-samples of coal tar, which is an extremely complex sample. The purification step made quali/quantitative analysis easier. All the fractions were analyzed in the full-scan mode; moreover, the second fraction was analyzed in the MRM mode for the determination of the sulphur compounds.

With regards to the first fraction, containing non aromatic hydrocarbons, a series of linear, branched and cyclic hydrocarbon compounds (from C12 up to C28) is shown in Fig. 3.

For the analysis of the aromatic compounds, a positive off-set of 10°C was used, which enabled a full exploitation of the entire 2D space, with only residual wrap-around of the pyrene group. As evident in Fig. 4, this is not a problem, because it occupies an area of the chromatogram free of analytes. The use of a greater positive off-set would reduce the wrap-around of the pyrene group but also the intra-class separation. Different classes of aromatic compounds are well separated and can be easily identified, from one up to four aromatic rings. With regards to the sulphur compounds (Figs. 5-8), 129 compounds, belonging to 14 sub-classes, were identified.

Finally the third fraction containing the oxygenated compounds was analyzed in the full scan mode. A positive off-set of 60°C was applied to reduce the extensive band broadening of the O-containing compounds in the second dimension.

A well-organized chromatogram, illustrated in Fig. 9, was generated, with the furan, phenol, indenol and naphthol groups of simple identification.

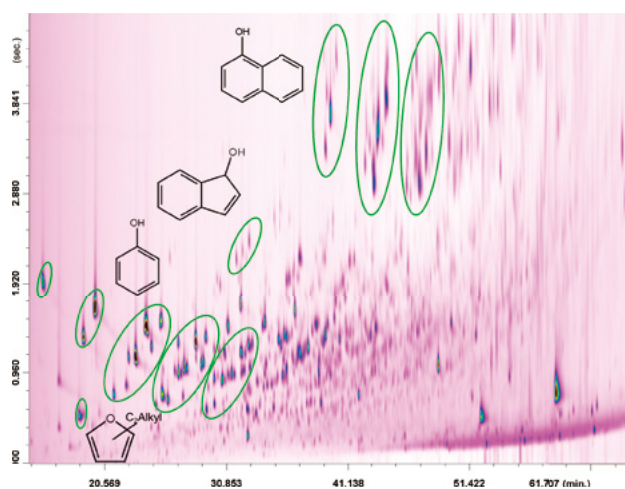


Fig. 9 TIC LC-GC×GC-MS/MS chromatogram of the 3rd fraction

5. Conclusions

In conclusion, the proposed four-dimensional technique is certainly very powerful. The high selectivity and peak capacity, due to the combination of the LC, cryogenic GC×GC, and the MS/MS system, allowed the targeted and untargeted analysis of a very complex sample.