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Short communication

# Analysis of priority pollutants in environmental samples by on-line supercritical fluid chromatography cleanup–cryo-trap–gas chromatography–mass spectrometry

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**Abstract**

On-line coupling of a supercritical fluid chromatography cleanup system with gas chromatography–mass spectrometry (GC–MS) was performed using a custom-made cryo-trap cell. The cleanup system was based on a high-performance liquid chromatography (HPLC) column packed with about 100 mg of Florisil. This coupling considerably reduces the effect of high boiling compounds on the chromatographic efficiency in the determination of organic pollutants in environmental samples. Moreover, it enables multistep elution from the cleanup column by the addition of a suitable supercritical $CO_2$ modifier, which was performed automatically. The system was proved for the determination of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls in sediment reference material samples, and in spiked sea-water samples. In particular, a three-step elution of selected PAHs, which were the most retained, from the Florisil column was performed at 40°C and 25 MPa, at a supercritical $CO_2$ flow of 0.4 ml/min for a total elution time of 10 min. The recovery of all contaminants considered was always better than 75%, with a relative standard deviation of about 15%. 

*Keywords:* Environmental analysis; Sample handling; Sediments; Water analysis; Polynuclear aromatic hydrocarbons; Polychlorinated biphenyls; Organochlorine compounds; Pesticides

## 1. Introduction

The analytical procedures applied for the determination of organic micropollutants in environmental samples are generally based on the off-line extraction of analytes from the sample by a suitable apparatus, followed by cleanup of the extract and, finally, instrumental analysis. Two different approaches can be followed to improve the detection limit of these procedures, namely, on-line coupling of the extraction apparatus with the separation/detection system, and the injection of high volumes of organic extract into the gas chromatograph. The former is generally confined to the coupling of a supercritical fluid extraction (SFE) system with gas chromatography–mass spectrometry (GC–MS) [1–3], whereas several techniques have been developed for the latter [4]. The most useful are the use of an on-column interface with partially concurrent solvent evaporation [5], the loop-type interface with fully concurrent solvent evaporation [6,7], and the programmed temperature vaporiser (PTV) [8,9]. Moreover, solid-phase microextraction (SPME) can be a valid alternative for water samples with a low total organic carbon content, since it can be directly coupled with both gas chromatography [10] and high-performance liquid chromatography (HPLC) [11].

This paper deals with the determination of some

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classes of priority organic micropollutants, i.e. polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorobiphenyls (PCBs), in organic extracts of environmental samples by a combined instrumentation based on the on-line coupling of a supercritical fluid chromatography (SFC) cleanup system with GC–MS using a custom-made cryo-trap accumulation cell [1]. The system allows a volume of organic extract as large as 100 μl or more to be loaded onto a HPLC column packed with a suitable stationary phase. The analytes are eluted by supercritical $CO_2$, to which a modifier can be added when necessary, and are trapped in the cryo-trap accumulation cell, which allows the quantitative transfer of the analytes into the gas chromatographic column. The performance of this hyphenated instrumentation for the determination of PAHs, OCPs and PCBs in organic extracts of environmental samples is discussed.

## 2. Experimental

### 2.1. Apparatus and reagents

A chromatographic system (SFC 3000, Carlo Erba Strum., Italy), equipped with an automatic cold on-column injection port was used. The cleanup column was made with a HPLC column (3×0.46 cm I.D.) packed with 100 mg of activated Florisil (60–100 mesh size). $CO_2$ was employed as the supercritical fluid and was delivered by a syringe pump (SFC 300, Carlo Erba, Italy). Both six-port and eight-port valves were used to connect the cleanup column, syringe pump, modifier auxiliary pump and cryo-trap cell. The latter was made with a suitably shaped Pyrex glass tubing [1]. A mass spectrometry Model 5989 Engine (Hewlett-Packard Italiana, Italy) was used as a detector. Chromatographic separation was performed on chemically bonded fused-silica capillary columns, HP-5 MS (Hewlett-Packard Italiana), 30 m×0.25 mm I.D., 0.25 μm film thickness. The oven temperature programme was: initial temperature, 50°C; isothermal for 2 min; then 10°C/min up to 200°C; 2°C/min up to 250°C and 10°C/min up to 280°C, isothermal for 20 min. The carrier gas was helium. *n*-Hexane, toluene, acetone and methanol were of pesticide grade and were supplied by Carlo-Erba (Italy). PAH and OCP standard solutions were supplied by AccuStandards (USA), and PCB standard solutions were supplied by Supelco (USA). Working standard solutions were prepared in pesticide grade *n*-hexane supplied by Carlo Erba (Table 1). Marine sediment reference materials for PCBs (HS-2) and harbour marine sediment reference materials for PAHs (HS-6) were supplied by the National Research Council of Canada. Florisil (synthetic magnesium silicate), 60–100 mesh size, was supplied by Carlo Erba, and was activated at 450°C for about 4 h.

### 2.2. Off-line SFC cleanup system

Fig. 1 shows a diagram of the system employed. The following operations were performed in the off-line mode:

Table 1
Standard solutions in *n*-hexane

| | Solution 'A' (μg/ml) | Solution 'B' (μg/ml) | Solution 'B1' (μg/ml) | | Solution 'C' (ng/ml) | Solution 'D' (ng/ml) | Solution 'D1' (ng/ml) |
|---|---|---|---|---|---|---|---|
| PAHs | | | | PCBs and OCPs | | | |
| Phenanthrene (PHE) | 27.9 | 0.11 | 5.1 | PCB52 | 198.0 | 4.8 | 99.0 |
| Anthracene (ANT) | 23.3 | 0.09 | 4.9 | PCB138 | 372.0 | 9.1 | 180.0 |
| Fluoranthene (FLU) | 24.5 | 0.09 | 4.9 | PCB153 | 410.5 | 10.0 | 201.0 |
| Pyrene (PYR) | 20.5 | 0.08 | 4.0 | PCB156 | 189.0 | 4.6 | 110.0 |
| Benzo(*a*)anthracene (BaA) | 28.8 | 0.12 | 5.1 | PCB180 | 636.7 | 15.5 | 291.0 |
| Crysene (CRY) | 25.7 | 0.10 | 5.0 | α-HCH | 100.0 | 2.4 | 50.5 |
| Benzo[*a*]pyrene (BaP) | 50.1 | 0.10 | 10.0 | γ-HCH | 107.0 | 2.6 | 50.5 |
| Benzoperylene (BPE) | 50.6 | 0.11 | 10.0 | HCB | 99.1 | 2.3 | 50.0 |
| | | | | *p,p′*-DDD | 362.3 | 8.8 | 175.0 |
| | | | | *p,p′*-DDT | 572.6 | 13.9 | 280.0 |

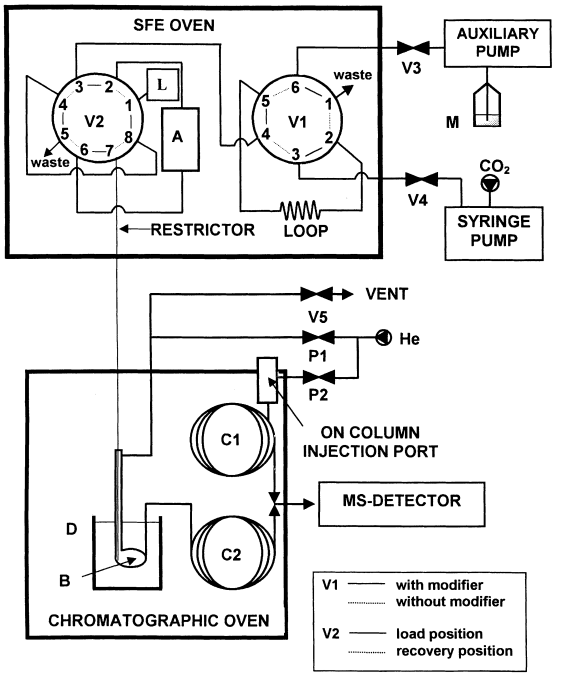


Fig. 1. On-line SFC–cryo-trap–GC–MS system.

- *Sample loading*. Valve connections and pump status were as follows: V1 (2–3, 4–5, 6–1), V2 (1–2, 3–4, 5–6, 7–8); V3 (auxiliary pump) off, V4 (syringe pump) off. A syringe containing the volume of sample to be loaded, typically 100 μl, was connected to port no. 1 of the valve V2. During the injection, the sample passed into column A and was discharged by port no. 6 (waste). In this way, all of the analytes were retained on the Florisil stationary phase. Then, a nitrogen line was connected to port no. 1, and a gentle flow of $N_2$ was allowed to pass into the column for about 2 min in order to eliminate the solvent.
- *Elution and recovery of the analytes*. Valve connections and pump valve status were as follows: V1 (2–3, 4–5, 6–1), V2 (1–2, 3–4, 5–6, 7–8); V3 off, V4 on. After solvent elimination was completed, the valve V2 was switched to the new position, and V4 was switched on. Supercritical $CO_2$, delivered at a selected flow and pressure by the syringe pump, was passed into column A, which was located in the SFE oven kept at a selected temperature. In the off-line mode, the supercritical fluid reached a vial (B) containing about 2 ml of hexane by a restrictor (30 cm×25 μm I.D.). The vial was cooled at about 0°C, and the restrictor tip was immersed in the solvent. Supercritical $CO_2$ expanded in the solvent, where it left all of the compounds that were eluted. The cooling bath was used both to increase analyte recovery and to buffer the temperature of the restrictor tip, which might become as low as −30/−40°C due to expansion of the supercritical $CO_2$. At this low temperature, occlusion of the restrictor may occur due to dry-ice formation and analyte condensation inside it. For highly retained analytes that are not eluted by supercritical $CO_2$, a modifier should be added. In our system, this was performed in the following way: the auxiliary pump valve, V3, was switched on, and the loop, L, was loaded. Then, V1 was switched to the following position: 1–2, 3–4, 5–6. In this way, supercritical $CO_2$ took the modifier into column A. After about 30 s, V1 was switched to the first position, and the system was ready for another addition of modifier, if needed.

Finally, the solvent was analysed by GC–MS.

### 2.3. On-line SFC cleanup–cryo-trap–GC–MS system

Fig. 1 shows a diagram of the system employed for the analysis by the on-line SFC cleanup–cryo-trap–GC–MS system. Two columns (C1 and C2) were mounted in the chromatographic oven. C1 was connected to the on-column injection port of the chromatograph, and C2 to the cryo-trap cell. Both columns were connected to the MS detector by a Y-shaped press–fit connector. Sample loading and elution of the analytes were the same as before, but, in this case, the restrictor (30 cm×25 μm I.D.) was positioned into the cryo-trap cell B, and the vent valve, V5, was switched on. Supercritical $CO_2$ eluted column A, and then expanded in the cryo-trap cell, where it left all of the compounds eluted and went out as gas through the vent valve. At the end of the elution time, V2 was switched off. About 20 s later, the vent valve, V5, was switched off and the helium

valve, P1, was switched on. Then, 1–2 μl of a suitable standard solution in hexane containing the same analytes that needed to be determined in the sample were injected into column C1 by the on-column injection port and, finally, the oven temperature program was started. All of the volatile compounds were quantitatively transferred from the cryo-trap cell to the chromatographic column C2 by the helium flow, whereas non-volatile compounds were left in the cell without affecting the chromatographic efficiency. In this way, the chromatogram showed the peak of the target analyte that was present in the sample and was eluted from column C2, along with that of the same analyte that was present in the injected standard solution and was eluted from column C1, within a few minutes (Fig. 2). This allowed the recovery to be more rapidly evaluated for each analyte in the same run.

### *2.4. Extraction of PAHs, PCBs and OCS from real samples*

The extraction procedure of organic micropollutants from both marine sediment and sea-water samples has been described elsewhere [12,13]. Briefly, sediment samples were extracted twice with a 1:1 (v/v) hexane–acetone mixture in an ultrasonic bath for about 30 min. The organic extract was separated from the sample, reduced at about 3 ml, and treated with activated copper powder mixed with mercury for sulphur removal. The extract was then filtered, treated with $Na_2SO_4$ for water elimination, reduced to about 0.5 ml, and finally analysed. Sea-water samples were extracted with hexane using a custom-made high-performance liquid–liquid extraction system that allowed the extraction of 10 l of sample with 10 ml of solvent (L. Zoccolillo, private communication). The extract was treated with $Na_2SO_4$ for water elimination, reduced to about 0.5 ml, and finally analysed.

## 3. Results and discussion

A set of measurements was performed by the off-line SFC cleanup system in order to evaluate the efficiency of analyte retention by the Florisil column, and to establish the experimental conditions for their quantitative elution by supercritical $CO_2$. The following experimental parameters were taken into consideration: the temperature of the SFE oven

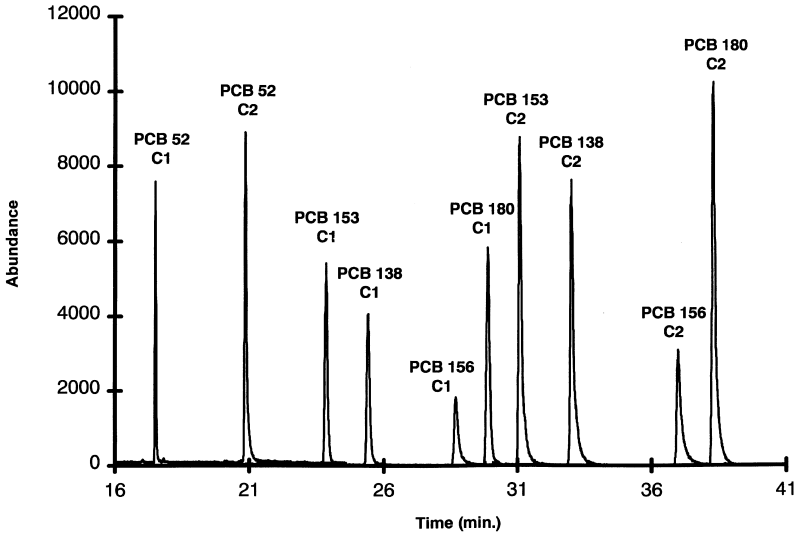


Fig. 2. Gas chromatogram of standard solution D1 (2 μl injected in the GC–MS system, column C1) and D (100 μl injected in the SFC–cryo-trap–GC–MS system, column C2). (For experimental conditions, see text).

where the column was installed, the pressure and flow of supercritical $CO_2$, and the elution time. In particular, a supercritical $CO_2$ flow of 0.4 ml/min was always used, whereas the temperature, the pressure, and the elution time ranged between 40–90°C, 20–30 MPa and 2–15 min, respectively. Quantitative recoveries were obtained for all of the selected PAHs, with the exception of benzoperylene (BPE), at 25 MPa, 40°C and an elution time of 15 min. At 25 MPa, the recovery of BPE, which was the compound that was most retained by the Florisil column, increased from about 25% to about 60% when the temperature was decreased from 90 to 40°C, and the density increased from 0.63 to 0.88 g/ml. Since the recovery of BPE was still unsatisfactory, a further increase in the density or a longer elution time was needed. As an alternative, the addition of a suitable modifier to supercritical $CO_2$ was considered. In fact, quantitative recoveries of all PAHs were obtained by eluting the Florisil column for 10 min with 0.4 ml/min of supercritical $CO_2$ at 40°C, 20 MPa, and making two additions of 15 μl of acetone as a modifier 3 and 6 min after beginning the elution. These findings also showed that a quantitative retention of analytes from 100 μl of sample by the cleanup column packed with about 100 mg of activated Florisil was obtained. The same experimental conditions were then transferred to the on-line system. Table 2 compares the recoveries obtained by the two systems for the eight selected PAHs. In particular, PAHs were recovered by the on-line SFC–cryo-trap–GC–MS system with an increasing percentage from the most to the least volatile, due to the loss of analytes during solvent evaporation in the cryo-trap cell. The recoveries of PHE, ANT and FLU were 64, 71 and 84%, respectively. Measurements were then performed to know the performance of our system for the determination of PCBs and OCPs under the same experimental conditions as for PAHs. Table 2 shows the percentage recoveries of some selected OCPs and PCBs, respectively. As expected, the recovery or these two classes of contaminants, which are less retained on the Florisil column, was always higher than 80%, with or without the addition of the modifier. Fig. 2 shows a typical gas chromatogram as obtained by the on-line system for a PCB standard solution. As already stated, the peak of an analyte, for example PCB153, from the cryo-trap cell follows, in the chromatogram, the peak of the same analyte from the standard solution injected in column C1. It is worth mentioning that the band width at the inflection points of the PCB153 peak is 4.4 s for the direct on-column injection (column C1) and 5.6 s for the cryo-trap cell (column C2), which is a very acceptable band-broadening of the on-line system. Replicate measurements using very diluted solutions showed that 0.2 pg/μl in the final extract can be determined with a relative standard deviation of about 15%.

Table 2
Percentage recoveries of polychlorobiphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) from standard solutions: (A) Off-line SFC cleanup and analysis by GC–MS; (B) On-line SFC–cryo-trap–GC–MS[a]

| Compound | % Recovery (SD) | |
|---|---|---|
| | A (Off-line) | B (On-line) |
| PCBs | | |
| PCB 52 | 70 (5) | 84 (4) |
| PCB 153 | 87 (4) | 91 (6) |
| PCB 138 | 92 (4) | 96 (6) |
| PCB 156 | 93 (4) | 75 (3) |
| PCB 180 | 82 (3) | 76 (4) |
| OCPs | | |
| α-HCH | 76 (8) | 85 (3) |
| γ-HCH | 77 (8) | 98 (9) |
| HCB | 84 (3) | 81 (4) |
| *p,p′*-DDD | 93 (2) | 93 (3) |
| *p,p′*-DDT | 81 (4) | 75 (3) |
| PAHs | | |
| PHE | 79 (4) | 64 (4) |
| ANT | 90 (2) | 71 (4) |
| FLU | 88 (2) | 84 (5) |
| PYR | 87 (2) | 86 (4) |
| BaA | 84 (4) | 95 (4) |
| CRY | 93 (3) | 108 (2) |
| BaP | 89 (1) | 102 (2) |
| BPE | 89 (4) | 94 (4) |

[a] For experimental details, see text. The standard deviations for five measurements are given in brackets.

Finally, the performance of the on-line system was verified on organic extracts of real samples. In particular, several aliquots of the HS-2 marine sediment reference materials for PCBs and the HS-6 harbour marine sediment reference materials for PAHs, and a sea-water sample were extracted following the procedure above described. The hexane extract of a sea water sample was spiked with about

2 pg/μl of each compound. Tables 3 and 4 show the results relevant to the determination of selected PAHs and PCBs in the extracts by the on-line SFC cleanup–cryo-trap–GC–MS system. These results show that the percentage recoveries of both PAHs and PCBs from the sea-water spiked hexane extract is always better than about 80%. The recovery is slightly lower (about 70%) for the determination of PAHs in reference sediment samples.

In conclusion, it was shown that the use of supercritical $CO_2$ was very useful for combining on-line the cleanup of up to 100 μl of an organic extract loaded onto a 0.4-ml HPLC column packed with a suitable stationary phase and the quantitative transfer of the analytes in the chromatographic column for the determination by GC–MS, particularly for PAHs, PCBs and OCPs in environmental matrices. The key component is the accumulation cell where the analytes were trapped after elution from the cleanup column. The optimised design of the cell also allowed the use of a supercritical $CO_2$ modifier when needed, without significantly affecting the final recovery of the analytes, even at concentrations as low as 0.2 pg/μl in the organic extract.

Table 3
Determination of selected PAHs and PCBs in certified marine sediment samples using the on-line SFC–cryo-trap–GC–MS system[a]

| | Concentration | | |
|---|---|---|---|
| | Certified | Found | Recovery (%) |
| PAHs[b] | | | |
| PHE | 3.0±0.6 mg/kg | 2.1 mg/kg | 70 |
| FLU | 3.5±0.6 mg/kg | 2.2 mg/kg | 65 |
| BaA | 1.8±0.3 mg/kg | 1.6 mg/kg | 87 |
| CRY | 2.0±0.3 mg/kg | 1.6 mg/kg | 80 |
| BaP | 2.2±0.4 mg/kg | 2.2 mg/kg | 100 |
| BPE | 1.8±0.7 mg/kg | 1.2 mg/kg | 65 |
| PCBs[c] | | | |
| PCB138 | 6.9±0.5 μg/kg | 6.1 μg/kg | 88 |
| PCB153 | 6.1±0.7 μg/kg | 5.4 μg/kg | 88 |
| PCB180 | 3.7±0.3 μg/kg | 3.0 μg/kg | 81 |

[b] CRM-HS-6.
[c] CRM-HS-2.
[a] For experimental conditions, see text.

Table 4
Determination of selected PAHs and PCBs in a spiked hexane extract of sea-water samples using the on-line SFC–cryo-trap–GC–MS system[a]

| | Concentration (μg/kg) | | |
|---|---|---|---|
| | Spiked | Found | Recovery (%) |
| PAHs | | | |
| PHE | 2.0 | 1.7 | 85 |
| FLU | 2.0 | 1.6 | 80 |
| PYR | 2.0 | 1.7 | 85 |
| BaA | 2.0 | 1.8 | 110 |
| CRY | 2.0 | 1.9 | 95 |
| BaP | 2.0 | 1.7 | 85 |
| BPE | 2.0 | 2.1 | 105 |
| PCBs | | | |
| PCB28 | 2.0 | 1.4 | 70 |
| PCB52 | 2.0 | 1.6 | 80 |
| PCB118 | 2.0 | 1.7 | 85 |
| PCB138 | 2.0 | 1.6 | 80 |
| PCB153 | 2.0 | 1.8 | 90 |
| PCB180 | 2.0 | 1.8 | 90 |

[a] For experimental conditions, see text.

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## References

[1] R. Fuoco, A. Ceccarini, S. Lottici, Anal. Chim. Acta 346 (1997) 81.
[2] S.B. Hawthorne, D.J. Miller, J.J. Langenfeld, J. Chromatogr. Sci. 28 (1990) 2.
[3] M. Lohleit, K. Bächmann, J. Chromatogr. 505 (1990) 227.
[4] H.G.J. Mol, H.G.M. Janssen, C.A. Cramers, J. Vreuls, J. Chromatogr. A 703 (1995) 277.
[5] A. Venema, J.T. Jelik, J. High Resolut. Chromatogr. 19 (1996) 234.
[6] T. McCabe, J.F. Hiller, J. High Resolut. Chromatogr. 12 (1989) 517.
[7] T. Hankemeier, H. Van Der Laan, J. Vreuls, J. Chromatogr. A 732 (1996) 75.
[8] H.J. Stan, M. Linkerhagner, J. Chromatogr. A 727 (1996) 275.

[9] H.G.J. Mol, M. Althuizen, H.G. Janssen, J. High Resolut. Chromatogr. 19 (1996) 69.
[10] Z. Zhang, M.H. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[11] J. Chen, J. Pawliszyn, Anal. Chem. 67 (1993) 2530.
[12] R. Fuoco, M.P. Colombini, C. Abete, Ann. Chim. (Rome) 81 (1991) 383.
[13] R. Fuoco, M.P. Colombini, C. Abete, Int. J. Environ. Anal. Chem. 55 (1994) 15.