COMPARISON OF DIFFERENT METHODS OF PURIFICATION OF ENVIRONMENTAL SAMPLES FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS

Guerriero E, Rotatori M, Mosca S, Rossetti G, Pomponio S
CNR – Institute for Atmospheric Pollution Via Salaria, Km 29,300, 00015 Monterotondo Scalo (Rome)

Abstract
The Stockholm Convention on Persistent Organic Pollutants (POPs) was signed in May 2001 by 127 countries. Currently, 12 substances are regulated by the convention, and the work on finding new candidate chemicals to the convention has started. One group of substances in focus is polycyclic aromatic hydrocarbons (PAH). In this study, the development of a simple methodology for the determination of several PAHs, using the isotope dilution technique by GC/MS was performed. In this work, an assessment of a new automated clean-up system based on gel permeation chromatography (AccuPrep MPS™, J2 Scientific) combined with an in-line concentration system (AccuVap™, J2 Scientific) was performed. The system is used for the purification of a wide range of samples (pesticides, mycotoxins, antibiotics etc ...) in different matrices such as foods, tissues, plants and environmental samples (soil, sludge, hazardous waste as described by the method EPA, then enlarging the purification of dioxins, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). The suitability of the new automated system in the analysis of real samples was evaluated, by comparing chromatographic traces and values obtained by analyzing different rates of the same sample, purified by the methods in exam: Florisil, silica and GPC.

Introduction
The Stockholm Convention on Persistent Organic Pollutants (POPs) was signed in May 2001 by 127 countries. Currently, 12 substances are regulated by the convention, and the work on finding new candidate chemicals to the convention has started. One group of substances in focus is polycyclic aromatic hydrocarbons (PAH). PAH is a large group of compounds consisting of molecules containing two or more fused benzene rings. They are formed during all types of incomplete combustion of organic matter, and they exhibit the characteristic POPs properties: persistence, bio-accumulation, adverse effects and potential for long-range environmental transportation to a certain extent. Many of the PAHs are carcinogenic, they are also believed to exhibit reproductive effects, as well as immune system inhibiting properties, genotoxicity and mutagenecity[1].
Directive 2004/107/EC[2] – acknowledged in Italy by 152/07 national directive[3] – states that benzo(a)pyrene (B[a]P) should be used as a marker for the carcinogenic risk of polycyclic aromatic hydrocarbons in ambient air, with a target value of 1ng/m^3 for the total content in the PM_{10} fraction averaged over a calendar year. Moreover, to assess the contribution of benzo(a)pyrene in ambient air, each Member State shall monitor other relevant polycyclic aromatic hydrocarbons at a limited number of measurement sites. These compounds shall include at least: benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene.
The development of innovative analytical methods for determination of PAHs has been and is of fundamental importance, the high carcinogenicity of these compounds. The quali-quantitative analysis of PAHs is an important challenge due to the low concentration at which these hydrocarbons may be present. The whole analytical process can be divided into three basic steps: extraction or enrichment of PAHs from the matrix, purification of the extract or isolation, final determination.
In this study, the development of a simple methodology for the determination of PAHs listed above, using the isotope dilution technique by GC/MS was performed. The sample to analyze, with a content of PAHs in traces, should undergo a clean-up procedure that affords an enrichment, reducing at the same time physical or chemical interferents. These could be present at a concentration that does not give observable physical problems, however there may be a "temporary poisoning" of the GC/MS (injector, liner, column ...) or chromatographical interferences, which can make difficult the quantification of the analytes of interest. Traditionally techniques that provide long steps on packed columns with different materials such as silica gel, Florisil, alumina, Sephadex LH-20 are employed or alternative techniques of thin-layer chromatography (TLC)[4]. In this work, an assessment of a new automated clean-up system based on gel permeation chromatography (AccuPrep MPS™, J2 Scientific)

combined with an in-line concentration system (AccuVap™, J2 Scientific) was performed. The system is used for the purification of a wide range of samples (pesticides, mycotoxins, antibiotics etc ...) in different matrices such as foods[5], tissues[6], plants and environmental samples (soil, sludge, hazardous waste as described by the method EPA[7], then enlarging the purification of dioxins, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)[8,9,10,11]. In addition to benefits related to the reduced time of analysis, this technique allows also a reduction of sample handling and volumes of solvents in use. It involves a reduction of cost of analysis, risk of external contamination and professional exposure to the chemical agents. In terms of recovery and repeatability of the three clean up procedures, the results of a test (done standardizing the steps of extraction and analytical determination) have shown that the preferred method is the GPC.

Materials and methods

Samples

Several samples (n=12) added of a known amount of standard mixture of perdeuterated PAHs (IS-L429, Wellington Laboratories, Canada), were subjected to a Soxhlet extraction with hexane and, subsequently, rates of extract were subjected to three different types of purification, as follows in the next section.

Clean-up

The manual method requires the packing of a 6ml commercial polypropylene column with polypropylene frit (Supelco, USA) with silica (Polygoprep 60-130, Macherey-Nagel, Germany); the semi-automatic method uses a Dual Layer Florisil®/Na_2SO_4 SPE Tube, 2g/2g/6mL (Supelco, USA) on a system vacuum Visiprep (Supelco, USA).

As for the automated system, (J2 Scientific, USA) it consists of three modules, as schematically shown in Fig. 1:

• AccuPrep MPS™: pump, glass chromatographic "Express column" (J2 Scientific, USA) containing the polymer resin styrene-divinylbenzene Biobead SX-3, the injector, a six-way valve, and a loop in line with the column where the sample is loaded);

• autosampler (a needle connected to a 10 mL syringe, capable of sucking, dispense and inject the sample, two racks - a sampling and storage - and a station for washing and drying);

• AccuVap Inline ™ (syringe for taking and releasing of the sample and the solvent evaporation chamber, pump).

The addition of the Inline AccuVap™ module as evaporation system eliminates the need to evaporate the sample manually, increasing the repeatability of the analysis.

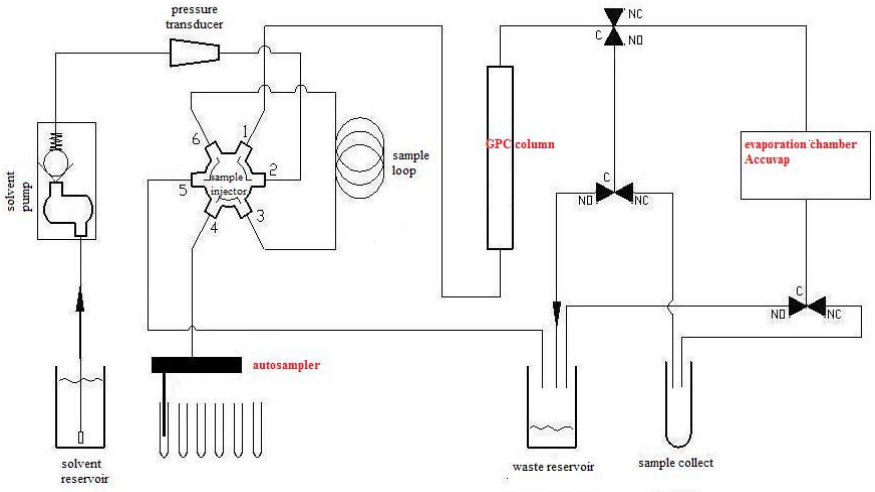

Figure 1 – scheme of the automated clean up system

Operating mode

Florisil – the cartridge is washed with 4 ml of methylene chloride/hexane (1:1) and conditioned with 4 ml of hexane. The extract (500µl) in hexane is loaded on the column and washed with 6 ml of hexane. 6 ml of

methylene chloride/hexane (1:1) are collected and manually concentrated at about 40°C under nitrogen flow to a final volume of 0.5 ml.
Silica – The polypropylene column is manually packed with 3g of silica. The column is washed with 20 ml of methylene chloride/hexane (1:1) and conditioned with 20 ml of hexane. The extract (500µl) in hexane is loaded on the column and washed with 20 ml of hexane. 20 ml of methylene chloride/hexane (1:1) are collected and manually concentrated at about 40°C under nitrogen flow to a final volume of 0.5 ml.
GPC – The extract of a volume of 2.5 ml is injected into the GPC system by using dichloromethane as mobile phase. The first 16 minutes fraction is dumped (early eluting interferences and co-extractives are discarded to waste); the second fraction (11 minutes), containing purified PAHs, is collected. The washing fraction (containing late eluting interferences) is discarded to waste. The automated combined system of GPC and evaporator automatically concentrates the second fraction up to a volume of about 0.5 ml, while it comes out the GPC column.
The extracts obtained from each purification were analyzed by gas chromatography with electron-impact source and ion trap analyzer (Trace GC + PolarisQ, Thermo-Fisher Scientific) in single ion monitoring (SIM).

Results and discussion
The suitability of the new automated system in the analysis of real samples was evaluated, by comparing chromatographic traces and values obtained by analyzing different rates of the same sample, purified by the methods in exam, labeled with a mixture of a known quantity of deuterium standard PAHs, in order to perform the quantitative determination by the method of isotopic dilution. Polycyclic aromatic hydrocarbons of toxicological significance, as suggested by D.Lgs. 152/07 were considered: benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd) pyrene and dibenzo(a, h)anthracene, in addition to BaP.

Qualitative evaluation: chromatograms
Since BaP is the only PAH with a limit prescribed by law, chromatograms of the same sample purified with the three systems: manual (silica), semi-automated (Florisil) and automated (GPC), relative to BaP perdeuterated (*m/z* 264) and the corresponding native (*m/z* 252), are shown (fig. 2 and 3). It may be noted in Fig. 2 that GPC purification is better than the other two methods. In particular, the interfering peak at 28.82 min, is present only after the purification on silica and on Florisil. It could be due to an interfering compound present in the polypropylene column or frit.
The interfering peak on the ion current of the labeled standard affects the quantitative determination of the standards themselves, as it is found in high concentration. As for the ion current chromatogram of *m/z* 252, relative to native BaP (Fig. 3), there is a good chromatography for all the three purifications. The peak at 29.07 min, pointed as BeP, is the benzo(e)pyrene isomer, well separated from the more toxic BaP, and whose presence does not affect the quantitative analysis.

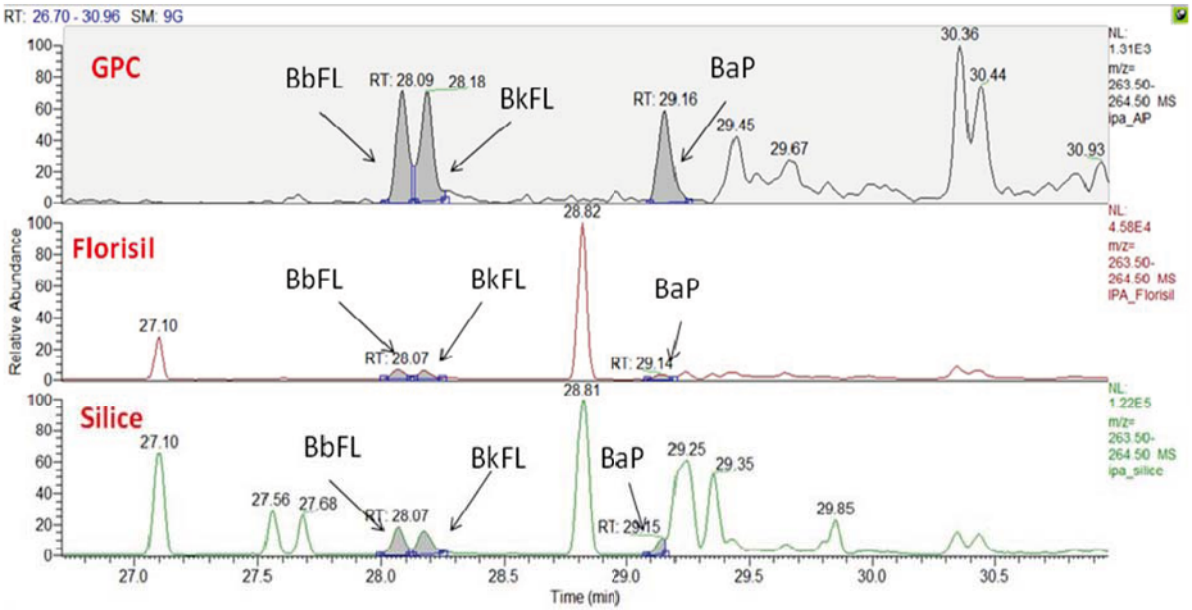

Figure 1 - Effect of different types of clean-up on the chromatogram GC/MS report on the specific *m/z* 264 isomers. Acronyms: BbFL = benzo(b)fluoranthene, BkFL = benzo(k)fluoranthene, BaP = benzo(a)pyrene)

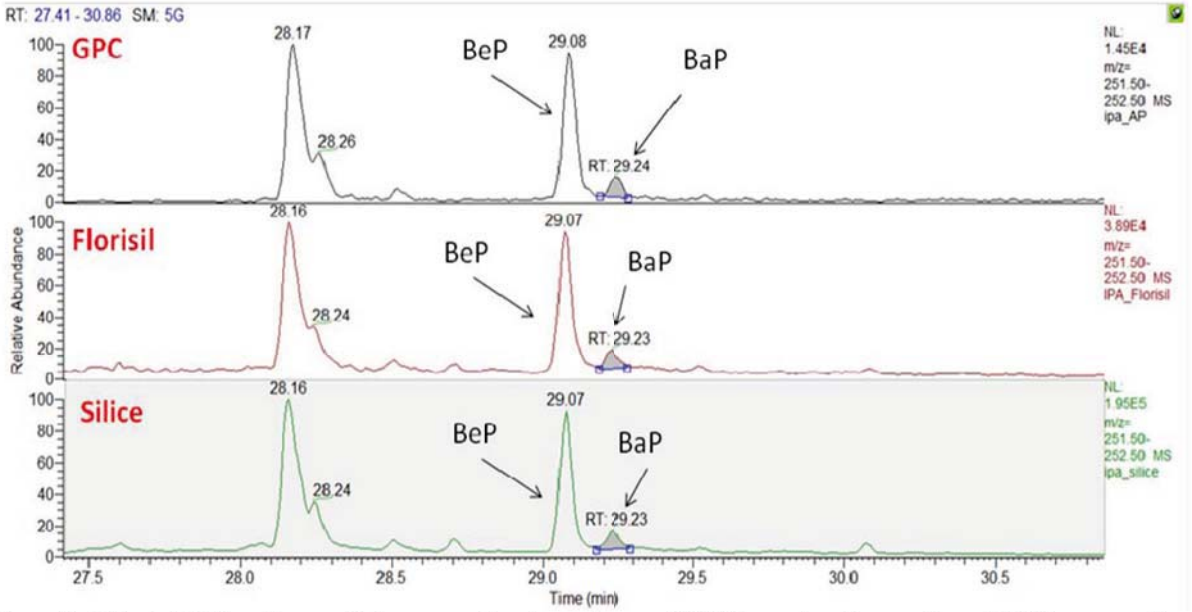

Figure 2 - Effect of different types of clean-up on the chromatogram GC/MS report on the specific *m/z* 252 isomers natives. Acronyms: BeP = benzo(e)pyrene, BaP = benzo(a)pyrene

Quantitative evaluation: recovery

The estimated recovery was determined by subjecting the three analytical procedures rates of six different samples (n = 12), and calculating the recovery of standard extraction/purification compared with a standard "syringe" added before each injection (L429-RS , Wellington Laboratories, Canada). The values are between 75 and 105% for all three processes for clean-up (Table 1).

	Florisil	Silica	GPC
	Recoveries %		
benzo(a)anthracene	87±2	98±3	101±3
benzo(b)fluoranthene	88±3	84±5	79±4
benzo(j)fluoranthene	94±1	92±3	83±2
benzo(a)pyrene	99±4	91±2	96±2
indeno(123,cd)pyrene	103±2	87±3	95±3
dibenzo(a,h)anthracene	91±3	85±4	81±1

Table 1 - Recovery rate of standard extraction/purification with three different methods of clean-up

As a control, in addition, discarded fractions of silica and Florisil, containing interferents were injected. In both were found, in addition to interfering, lighter polycyclic aromatic hydrocarbons (from naphthalene to phenanthrene) in a not negligible percentage. The dumped fractions before and after the collected one on the GPC did not show this kind of trouble.
Method blank were analyzed for each clean up system: they gave negligible concentrations. It can be assumed that no memory effect can be seen, even in a complex system such the automated GPC.

Conclusions

The comparison between the different methods of purification of PAHs showed that the GPC, although not highly selective, is a technique suitable for the purpose. From a quantitative viewpoint, the GPC has performance similar to the most often used cleanup methods based on silica gel or Florisil. From a qualitative point of view, the GPC has shown that in ambient-air samples the chromatographic interferents are eliminated more effectively. Further advantage of the GPC used in this work (Accuprep + Accuvap) is the total automation of the purification: the in-line concentration allows the samples being processed without a technical operator and the autosampler can inject the samples on the GPC column, as it can be used for hundreds of samples without the need to replace it, in contrast to the SPE cartridges, which are for single use (one sample, one cartridge).
As a final remark, the collected fraction on the GPC selected for separating PAHs from interfering compounds, is suitable for the analysiof other organic micropollutants such as PCDD/Fs, PCBs, pesticides. With the common SPE purification techniques for PAHs, based on polarity (Florisil, silica, alumina), these compounds are eluted in different fractions with different solvents. It is therefore possible to determine different organic compounds, analyzing rates of the same GPC eluate.

References

1. Edlund S., *IIIEE Reports* 2001:09, Lund University
2. Directive 2004/107/EC of the European parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air
3. D. Lgs. 3 agosto 2007, n. 152 – Attuazione della direttiva 2004/107/CE concernente l'arsenico, il cadmio, il mercurio, il nichel e gli idrocarburi policiclici aromatici nell'aria ambiente
4. Peltonen K., Kuljukka T. *J. Chrom. A* 710 (1995) 93-108
5. Ahmed, F. E. Analyses *Trends Anal Chem* 20 (2001) 649-661
6. Frenich, A.G., Bolaños, P.P., Vidal, J.L.M. *J Chrom A* 1153 (2007) 194-202
7. US EPA SW-846 Method 3640A– Gel-permeation Cleanup 1994
8. Liu H., Zhang Q., Song M., Jiang G., C. Z. *Talanta* 70 (2006) 20-25
9. Ahmed, F. E. *Trends Anal Chem* 22 (2003) 170-185
10. Helaleh Murad I.H., Al-Omair A., Nisar A., Gevao B. *J Chrom A* 1083 (2005) 153-160
11. Guerriero E., Rotatori M., Mosca S., Rossetti G., Manni A. *Organohalogen Compounds* 70 (2008) 501-504