QUALITATIVE SCREENING FOR POLICYCLIC AROMATIC SULFUR HETEROCYCLES

Andrei Niculae, Marcela Niculescu

National Research & Development Institute for Industrial Ecology,
90-92 Panduri Road, district 5, 050663 Bucharest, ecoind@incdecoind.ro

ABSTRACT

There is increasing interest in isolation, identification and quantification of polyaromatic sulfur heterocycles (PASH) in view of their importance in environmental forensics, toxicology, geochemistry, etc.

Although EU regulations are still in process for this class of compounds, the mutagenic and carcinogenic effects of some PASH compounds determined a major analytical development, all efforts being conducted to a higher level of interest of monitoring water resources and possible soil contamination.

Over the past decade, gas chromatography coupled with mass spectrometry was a powerful tool for characterization of PASH compounds in environmental samples.

To be able to identify possible water and soil contamination with PASH compounds, a new method was necessary to be developed, to have the opportunity to take action and promote a new step in Romanian environmental protection.

For this purpose, we developed a GCMS method using a low resolution mass spectrometer for identification and quantification of some PASH compounds including ***phenanothro[3,4b]thiophene*** with ***mutagenic effect*** J.Heterocycl.Chem.17(1980)1259).

Keywords: policyclic aromatic sulfur heterocycles, PASH , sulfur compounds, oil, soil, air, waste;

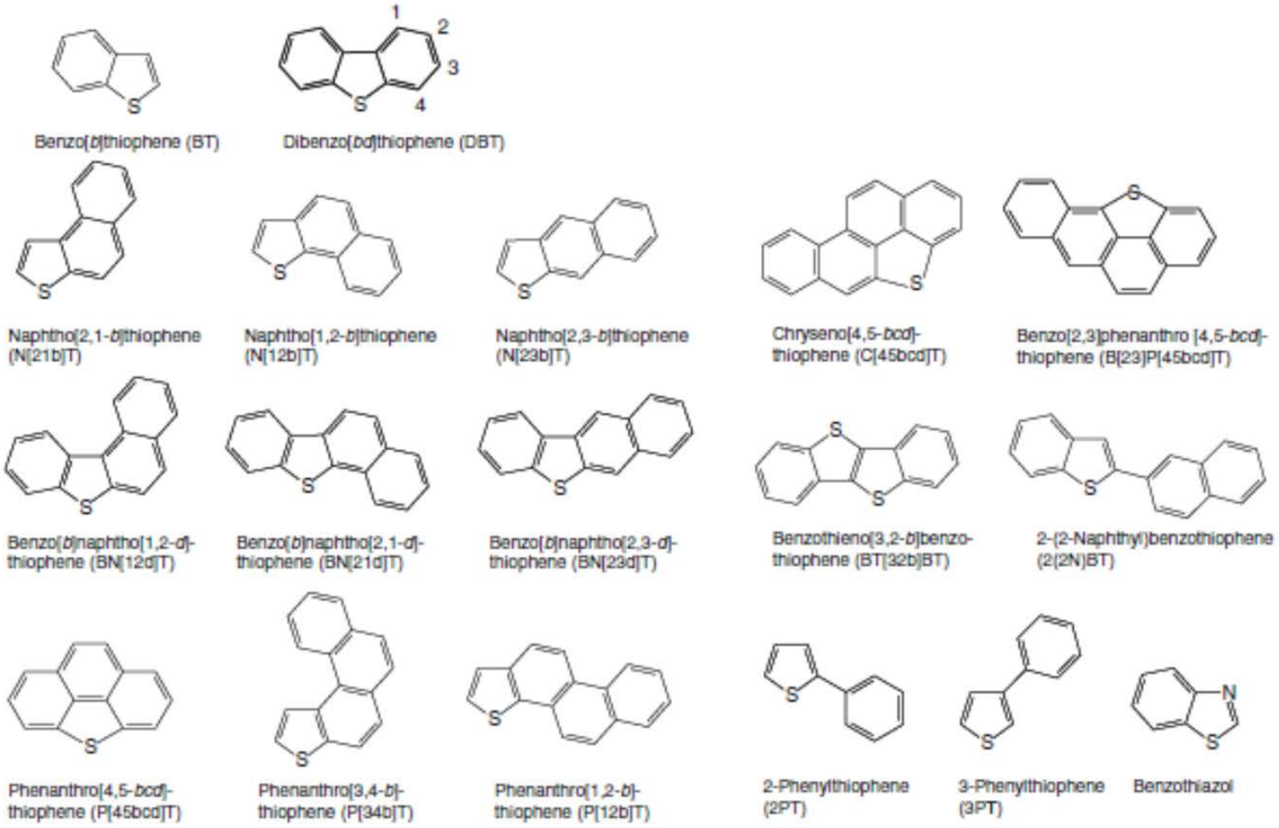
INTRODUCTION

Few compounds have been so extensively studied by environmental scientists as polycyclic aromatic hydrocarbons (PAH). The carcinogenic properties of some PAHs, as well as their ubiquitous occurrence, whether deriving from man's activities or from natural sources, have stimulated intense studies on all aspects of this group of compounds. They range from their formation, mainly under thermal conditions, their occurrence in the environment, the exposure of humans to them through various routes, to the details of their carcinogenesis and biotransformation. Apart from the health aspects associated with them, these compounds are also of fundamental interest as markers for environmental processes, e.g. pollution through petroleum spills or combustion sources. Under appropriate conditions, they can be very persistent in the environment; they have even be found even in 2.5-billon-year-old shales [1]
and in carbonaceous chondrites—meteorites that are thought to be among the most primitive materials in the solar system [2]. Although PAHs have received enormous attention, the fact that they are usually accompanied by related compounds that sometimes convey information different from or complementary to that of the PAHs is often overlooked. Many environmental studies would benefit from including heterocyclic PAH analogues—mainly those containing sulfur, oxygen or nitrogen—as analytes,
particularly since the extra work involved would be fairly small: these compounds can be sampled together with the PAHs and measured using more or less the same analytical
techniques. They also derive from similar sources: petrogenic (fossil material) or pyrolytic (combustion or thermal) sources. It seems that the introduction of the so-called EPA PAHs, a list of 16 PAHs that the US Environmental Protection Agency issued in 1982 and considers to be priority pollutants, has narrowed the focus of many analysts to the compounds on that list. Consequently, although heterocyclic polycyclic aromatic compounds are present in the sample, they are often not reported and the information they could convey is therefore neglected.

Indeed, a strong case has been made for including heteroaromatic compounds and alkylated derivatives in particular in environmental analyses, since focusing on parent PAHs alone (such as the EPA priority pollutants) can lead to a drastic underestimation of the contaminant levels in environmental matrices and the potential toxic effects of the pollutants [3, 4]. In a risk assessment study of polycyclic aromatic compound (PAC) contamination from petroleum released into a lake and the exposure of intertidal sediments to spilled oil, heterocyclic compounds and alkylated PACs were also included [5]. It was demonstrated that if this is not done (i.e. if traditional analytical approaches are used), the risk to wildlife can be underestimated by a factor of 40–70.

In this review, the sulfur-containing analogues of the PAHs, the PASHs, will be our focus. A review was published in book form in 1990 [6], but the field has grown
enormously since then. In Fig. 1, the parent structures of some of the more commonly encountered PASHs are depicted. Indeed, the physicochemical properties of the PASHs resemble those of the PAHs so much that it is difficult to separate the two compound classes. Therefore, much of what has been said about the analysis of PAHs elsewhere will be equally applicable to the PASHs, such as sample collection, work-up, etc., so we will focus mainly on those aspects that differ or expand on such works here.

Since much of the information searched for in a sample is contained in the concentrations of individual compounds, very often PASH speciation is the goal of a study, not the determination of the sum of all PASHs, meaning that a chromatographic separation will be incorporated before quantification. Some importance will therefore be placed on ways of separating both PASHs from PAHs and also specific PASH species from each other. Such separations can be vital to the validity of the data: if a PAC quantification is attempted, major problems can ensue since PAHs and PASHs can easily seriously interfere with each other. It should be noted that this interference occurs both ways: not only will PASH quantifications be erroneous unless the influence of PAHs is taken into account, but PAH quantifications will also be incorrect. This issue, often overlooked, will be touched on below. Then, various techniques used for the detection and quantification of PASHs will be discussed in "Detection and quantification of PASHs". After that, we will describe how PASHs can be used as markers for oil pollution, thermal processes and vehicular traffic ("PASHs as marker compound"). Not only alkyl-substituted PASHs, but also some other derivatives of PASHs with polar functionalities have been discovered in environmental samples. They will be treated separately in "Oxidized derivatives of PASHs". Polychlorodibenzothiophenes (PCDT) have also been found in the environment [7], together with their oxygen heterocyclic cousins, the polychlorodibenzofurans and -dioxins. Since PCDTs have the same nominal mass as their corresponding chlorinated polychlorodibenzodioxins, high-resolution mass spectrometry is necessary to distinguish between these two classes of compounds. An alternative approach involves oxidizing the thiophenes to the sulfones and chromatographically separating them from the oxygen heterocycles [8] before GC–MS analysis.



EXPERIMENTAL

Chemicals and reagents

Standards: Standard solutions of each compound were purchased from University of Munster, Germany. Each solution of PASH had a concentration of 0.1 mg/mL in methanol.
For each PASH compound, a stock solution have been prepared (2000 µg/L) and stored in dark 4°C before use.
Solvents: hexane of GC purity grade from *Merck* (Germany); Anhydrous sodium sulphate, *p.a.*, purchased from *Chimopar (*Romania).

Instrumentation

The Agilent GCMS equipment was used; 6890N GC with CTC Combi Pal autosampler coupled with MSD 5975B mass spectrometer with quadrupole was considered the best option for a qualitative screening analysis of PASH compounds.

Extraction procedure

The pretreatment step water samples contaminated with PASH compounds, was performed by liquid-liquid extraction (LLE) of the analytes from aqueous samples using hexane. Taking into account the fact that PASH compunds are nonpolar compounds, the pH of the aqueous samples was not adjusted.
For LLE, the ratio aqueous solution: organic solvent was of 20:1 (*v/v*). The extraction step was performed with 15 minutes of mechanical shaken. The organic extract was passed through a short column (15 cm) with anhydrous sodium sulphate (5g), in order to retain possible water traces. The extraction was repeated two more times with fresh solvent and dried with a anhydrous sodium sulphate.
The extract was evaporated at low pressure till maximum 1 mL using a rotary evaporator. The residue was dissolved in 1 mL of hexane and analyzed by GCMS.

RESULTS AND DISCUTIONS

Chromatographic conditions

Gas chromatograph parameters:
GC column : DB-5, 50 mm, 0.2 mm , 0.33 µm stationay phase.
Oven: intial temperature 50^0 C for 0.5 min with 15^0 C /min to 320^0 C for 12 min.
Inlet: split mode, 5:1 split ratio, 260^0C temperature.
Carrier: He 1.5 mL/min with vacuum compensation
Transfer line: 300^0C

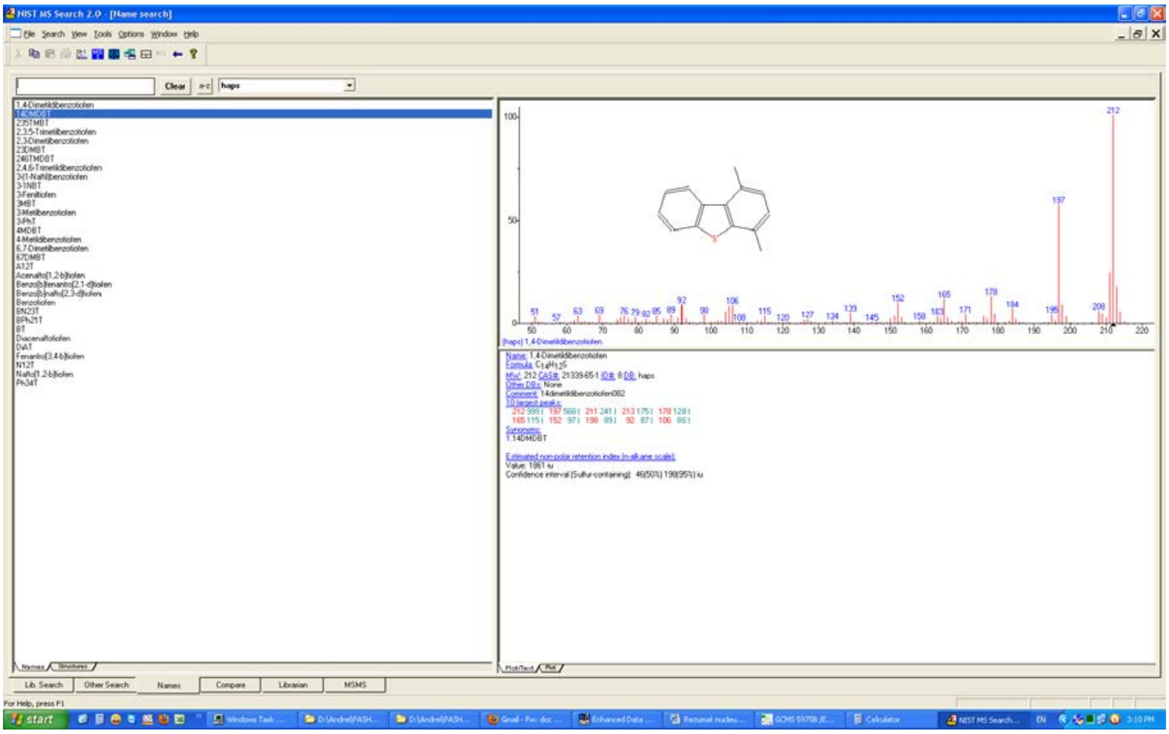
Mass spectrometer parameters: Full scan mode
Source temperature: 250^0 C
Quadrupole temperature: 200^0 C
EI enegy: 70 eV
Repeller : 23 V
Ion focus: 87 V

EntLens : 35 V
EMvoltage: 2035 V

Autosampler parameters:
Injection volume: 1 µL
Preclean cycles :2
Postclean cycles: 2
Injection speed: 50 µL/s
Preinjection delay: 100 ms
Postinjection delay: 500 ms

Mass spectrum and new library for PASH compounds

The individual PASH solution were analyzed in full scan mode, and every compound had been characterized by a mass spectrum.
A new library was created based on the new mass spectrums obtained for PASH compounds.



1,4-Dimetildibenzotiofen
14DMDBT
235TMBT
2,3,5-Trimetilbenzotiofen
2,3-Dimetilbenzotiofen
23DMBT
246TMDBT
2,4,6-Trimetildibenzotiofen
3-(1-Naftil)benzotiofen
3-1NBT
3-Feniltiofen
3MBT
3-Metilbenzotiofen
3-PhT
4MDBT
4-Metildibenzotiofen
6,7-Dimetilbenzotiofen
67DMBT
A12T
Acenafto[1,2-b]tiofen
Benzo[b]fenantro[2,1-d]tiofen
Benzo[b]nafto[2,3-d]tiofen
Benzotiofen
BN23T
BPh21T
BT
Diacenaftotiofen
DiAT
Fenantro[3,4-b]tiofen
N12T
Nafto[1,2-b]tiofen
Ph34T

[haps] 1,4-Dimetildibenzotiofen

Name: 1,4-Dimetildibenzotiofen
Formula: $C_{14}H_{12}S$
MW: 212 CAS#: 21339-65-1 ID#: 8 DB: haps
Other DBs: None
Comment: 14dimetildibenzotiofen082
10 largest peaks:
212 999 | 197 968 | 211 241 | 213 175 | 178 128 |
165 115 | 152 97 | 198 89 | 92 87 | 106 86 |
Synonyms:
1.14DMDBT

Estimated non-polar retention index (n-alkane scale):
Value: 1861 iu
Confidence interval (Sulfur-containing): 46(50%) 198(95%) iu

Method Validation

The established protocol LLE-GCMS was validated by the analysis of contaminated water samples and blank samples .
The necessary performance parameters for a qualitative screening method were studied.
- detection capability
- repeatability
- selectivity/specificity
- applicability

Limits of detection (LODs) and Repeatability

The limit of detection LOD was calculated based on the Chemstation software capabilities to calculate the S/N ratio.

The LOD was calculation wad based on the formula:

LOD = 3 x Noise

The LODs for all compounds were calculated for a concentration of each compound of 2000 µg/L.

Repeatability was calculated based on 10 injection of the same compound solution and calculate as RSD%.

Compound	Abbreviation	Molecular mass (g/mol)	Molecular formula	Relative retention time (min)	S/N Ratio 2 mg/L	Detection limit LOD	Repeatability r
Benzotiofen	BT	134	C_8H_6S	9.90	2672	2.24 µg/L	1.04
3-Metilbenzotiofen	3-MeBT	148	C_9H_8S	11.02	2409	2.49 µg/L	1.45
3-Feniltiofen	3-PhT	160	$C_{10}H_8S$	11.95	3442	1.74 µg/L	1.17
2,3-Dimetilbenzotiofen	2,3-DMeBT	162	$C_{10}H_{10}S$	12.09	2165	2.77 µg/L	1.13
6,7-Dimetilbenzotiofen	6,7-DMeBT	162	$C_{10}H_{10}S$	12.15	1047	5.73 µg/L	2.67
2,3,5-Trimetilbenzotiofen	2,3,5-TMeBT	176	$C_{11}H_{12}S$	13.07	2297	2.61 µg/L	1.86
Nafto[1,2-b]tiofen	N12T	184	$C_{12}H_8S$	15.05	1016	5.9 µg/L	2.32
4-Metildibenzotiofen	4-MeDBT	198	$C_{13}H_{10}S$	15.75	740	8.1 µg/L	1.55
1,4-Dimetildibenzotiofen	1,4-DMeDBT	212	$C_{14}H_{12}S$	16.75	1212	4.95 µg/L	2.25
Acenafto[1,2-b]tiofen	AT	208	$C_{14}H_8S$	17.20	1188	5.05 µg/L	1.79
2,4,6-Trimetildibenzotiofen	2,4,6-TMeDBT	226	$C_{15}H_{14}S$	17.14	1374	4.36 µg/L	2.21
Benzo[b]nafto[2,3-d]tiofen	BN23T	234	$C_{16}H_{10}S$	19.70	1752	3.42 µg/L	1.88
Fenantro[3,4-b]tiofen	Ph34T	234	$C_{16}H_{10}S$	19.73	1880	3.19 µg/L	1.51
3-(1-Naftil)benzotiofen	3-1NBT	260	$C_{18}H_{12}S$	19.26	2045	2.93 µg/L	1.45
Benzo[b]fenantro[2,1-d]tiofen	BPh21T	284	$C_{20}H_{12}S$	27.24	606	9.9 µg/L	3.6
Diacenaftotiofen	DiAT	332	$C_{24}H_{12}S$	20.6	-	-	-

RSD% = (Standard Deviation x 100) / Average

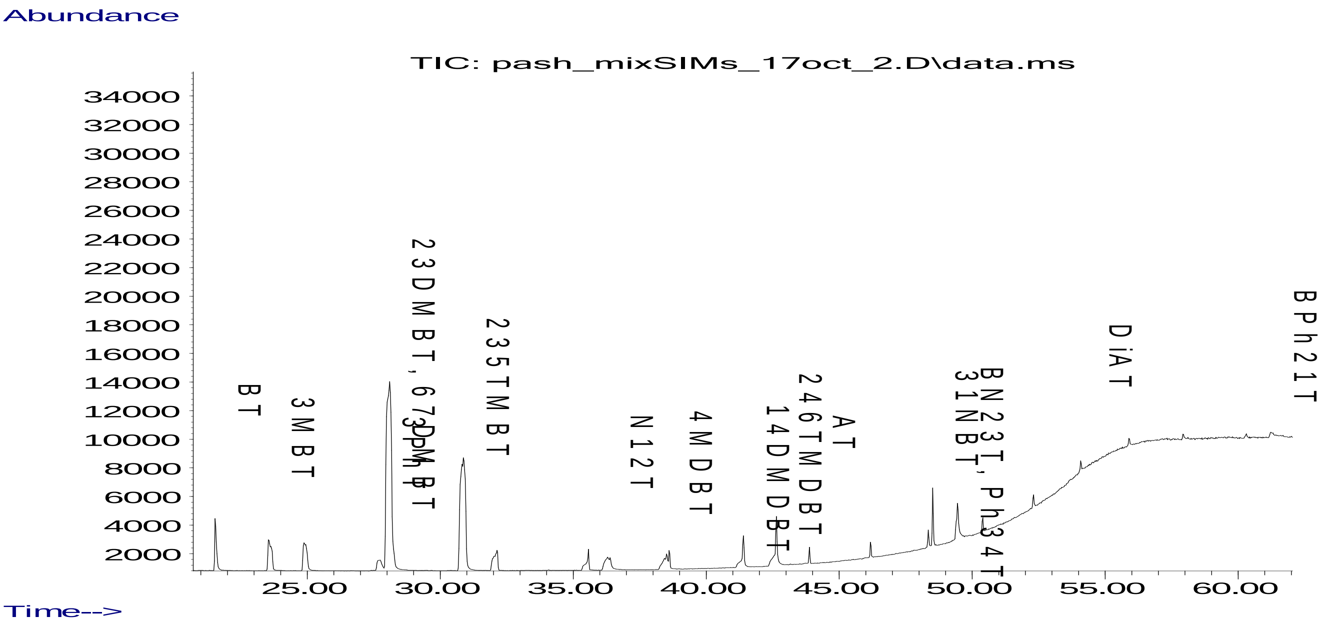
Selectivity/Specificity

The specificity was studied based on blank samples and contaminated samples.

Because of selectivity of the mass spectrometer in SIM mode in a narrow time window, the interferences with other compounds (PAH, PCB) are almost absent.

Applicability

The study was conducted on water samples contaminated with 10 µg/L of PASH compounds. The LLE and GCMS analysis showed a promising potential. A SIM (single ion monitoring) method was developed to verify the applicability of a screening method. A mix solution of 16 PASH compounds (10 ppb) was injected. Almost all 16 PASH compounds were separated at base line.



Conclusions

The study of PASH compounds as markers for environmental pollution or as compounds with a high grade of toxicity it is welcomed for future development and implementation of new reglementations in EU.

The developed method showed a satisfactory detection limit for a low resolution quadrupole mass spectrometer with a high potential for development of a qualitative and quantitative method on a high resolution mass spectrometer.

We consider this study as a step forward for new research and development on this kind of compounds.

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