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Journal of Chromatography A, 889 (2000) 281–286

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Solid-phase extraction of sugar cane soot extract for analysis by gas chromatography with flame ionisation and mass spectrometric detection

Gisele C.M. Zamperlini[a], Mary Santiago-Silva[a,*], Wagner Vilegas[b]

[a]*Analytical Chemistry Department, Institute of Chemistry, UNESP, P.O. Box 355, BR 14801-970 Araraquara (SP), Brazil*
[b]*Organic Chemistry Department, Institute of Chemistry, UNESP, P.O. Box 355 Araraquara (SP), Brazil*

Abstract

The incomplete combustion of biomass is one of the most important sources of emissions of organic compounds into the atmosphere, like polycyclic aromatic hydrocarbons (PAHs) which show genotoxic activity. Since environmental samples generally contain interferents and trace amounts of PAHs of interest, concentration and clean-up procedures are usually required prior to the final chromatographic analysis. This paper discusses the performance of Sep-Pak cartridges (silica gel and RP18) on clean-up of sugar cane soot extract. The best results were obtained with a silica Sep-Pak cartridge. The recoveries ranged from 79% (benzo[*b*]fluoranthene) to 113% (benzo[*e*]pyrene).

Keywords: Sugar cane; Extraction methods; Polynuclear aromatic hydrocarbons

1. Introduction

In Brazil, after the 1970s, sugar cane production expanded greatly, mainly due to PROÁLCOOL, the program developed by the Brazilian Government for the production of ethanol used as automotive fuel. Brazil has 25% of sugar cane plantation worldwide and is the major sugar cane producer [1]. Nowadays, our country has 4 500 000 hectares of sugar cane plantations, 324 plants of ethanol production and more than 1 100 000 rural workers involved in the sugar cane industry. At harvesting time, from May to November every year, the plantation is burnt in order to make the process of harvesting easier and also to increase the sugar content by weight, due to water evaporation. Biomass burning introduces several compounds into the atmosphere, including carcinogenic/mutagenic compounds, like polyaromatic hydrocarbons (PAHs). Sixteen PAHs are included in the priority pollutants list of the US Environmental Protection Agency (EPA)[2], while the US National Institute of Occupational Health and Safety (NIOSH) includes 17 of these compounds in its list [3]. The additional compound in the NIOSH list is benzo[*e*]pyrene.

The main problem with the determination of PAHs is the complexity of environmental matrices and the presence of many interfering substances with the PAHs which cannot be completely removed by repeated extraction and purification procedures. Since environmental samples generally contain interferents and trace amounts of PAHs of interest, concentration and clean-up procedures are usually required prior to the final chromatographic analysis. C_{18} cartridges or discs have been used for water

*Corresponding author. Fax: +55-16-222-7932.
E-mail address: mssqam@iq.unesp.br (M. Santiago-Silva).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00291-0

samples [4,5], soil [6] and particulates [6,7]. Alumina and XAD-2 have been used in soil samples [8,9], and Florisil in sediment and particulate material [10]. Amino (NH_2–), XAD-2 and silica [9,11,12] cartridges have also been used. Aiming to find a simple and rapid methodology for efficient clean-up of the complex sugar cane soot extract, in this work we studied the behaviour of Sep-Pak cartridges, silica and C_{18}.

2. Experimental

2.1. Conditions

Spectroscopy-grade cyclohexane and chromatography-grade methanol and methylene chloride were purchased from Merck (Darmstadt, Germany). Silica (Sep-Pak cartridges, 690 mg, 8 μm) and C_{18} (Sep-Pak cartridges, 360 mg, 80 μm) micro-columns were purchased from Millipore (Bedford, MA, USA). PAH standards (NIOSH list) were purchased from Aldrich Chemie (Steinheim, Germany), with the exception of indeno[1,2,3-*cd*]pyrene, which was not available. Stock mixtures of PAH standards were made up from the individual solutions in cyclohexane and used as external standards.

GC–flame ionisation detection (FID) analysis was performed with a Shimadzu GC-14B chromatograph (Japan), using a capillary column CBP-5 (J&W Scientific, Folsom, CA, USA) (25 m×0.33 mm I.D., 0.25 μm), programmed from 130°C (2 min) to 175°C at rate of 3°C min^{-1}, then 15°C min^{-1} until 240°C (15 min), and finally 10°C min^{-1} to 290°C, with a 10-min final hold. The injector temperature was 250°C in the split mode (manual injection, split 1:10, 1 μl injected). The detector temperature was held at 300 °C. Hydrogen at 2.5 ml min^{-1} was used as the carrier gas and nitrogen (35 KPa) as the make-up gas.

The analyses by GC–MS were done with a Fisons MD800 equipment (quadrupole detector, Thermo Instruments, Manchester, UK), using a fused-silica capillary column 30 m×0.25 mm I.D. DB-5 (film thickness 0.25 μm) (J&W Scientific). The oven temperature was programmed from 90°C (1 min) to 120°C at a rate of 10°C min^{-1}, and then from 120 to 310°C at 4°C min^{-1}, with a 20-min final hold. Helium at a flow-rate of 3 ml min^{-1} was used as the carrier gas. The injector temperature was 280°C in the splitless mode (3.0 μl; hot needle technique), the split valve being closed for 48 s. The transfer line and ion source temperatures were 280°C and 200°C, respectively. The analysis was performed by electron ionisation (70 eV), scanning from 100 to 500 mass units at 1 s per decade. Data were acquired also in the single ion monitoring (SIM) mode, selecting the ions shown in Table 1.

2.2. Optimization of solid-phase extraction (SPE) clean-up

Sep-Pak cartridges, C_{18} and silica, have been evaluated. Reversed-phase C_{18} retains PAHs, which are eluted by apolar solvent, while the behaviour of the normal-phase silica is the inverse, eluting PAHs first and retaining polar contaminants.

At first we studied silica cartridges. The sequence study included: (1) optimization of the conditioning volume, using the same solvent of the standard solutions, cyclohexane (C_6H_{12}); (2) optimization of the flow-rate and elution volume; and (3) determination of PAH recovery.

Low-polarity solvents such as hexane, methylene chloride and their mixtures are often used for fractionation on silica-gel cartridges. In this work we have evaluated the use of cyclohexane as eluent, since it does not show significant polarity differences.

Silica-gel cartridges were conditioned with aliquots of 5 ml C_6H_{12}. Each fraction was concentrated almost to dryness under a N_2 stream, redissolved with 200 μl C_6H_{12} and analysed by GC–FID. 5-ml fractions were collected and analysed until no more chromatographic peaks were observed.

After conditioning the silica-gel cartridge with 25 ml C_6H_{12}, it was spiked with 200 μl of a PAH standard solution (7.5 μg ml^{-1}). The cartridge was then eluted with cyclohexane (first fraction); cyclohexane–methylene chloride (DCM) (4:1) (second and third fractions). Several different elution volumes were tested, as follows:

- First elution: 1.00, 1.50 and 2.00 ml C_6H_{12}.
- Second elution: 1.00, 1.50, 2.00 and 2.50 ml C_6H_{12}–DCM (4:1).
- Third elution: 0.50 ml C_6H_{12}–DCM (4:1).

Table 1
Selected ions (m/z) for MS-SIM detection

Window	m/z	Compound
1	166	Fluorene
	178	Phenanthrene, anthracene
	184	Thiophenes
	192	Methylphenanthrenes
	198	Methyldibenzothiophenes
	202	Fluoranthene, pyrene, acephenanthrylene
	206	Dimethylphenanthrenes
	212	Pyrene deuterated
	216	Methylfluoranthenes/pyrenes, benzofluorenes
	226	Benzo[*ghi*]fluoranthene, cyclopenta[*cd*]pyrene
	228	Benz[*a*]anthracene, crysene/triphenylene
	234	Benzonaphthothiophenes, rethene
2	252	Benzofluoranthenes, benzopyrenes, perylene
3	276	Indeno[7,1,2,3-*cdef*]crysene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene
	278	Dibenzoanthracenes, picene, benzo[*b*]crysene
	288	Benzo[*ghi*]perylene deuterated
	300	Coronene
	302	Dibenzopyrenes

Each fraction was evaporated almost to dryness under a N_2 stream and redissolved in 200 μl C_6H_{12} for GC–FID analysis.

Flow-rates were also changed between 0.6, 1.0, 3.0 and 6.0 ml min^{-1}.

C_{18} cartridges were conditioned with aliquots of 5 ml methanol. Each fraction was concentrated almost to dryness under a N_2 stream, redissolved with 200 μl C_6H_{12} and analysed by GC–FID. Three 5-ml fraction were collected and analysed until no more chromatographic peaks were observed.

After conditioning the C_{18} cartridge with 15 ml methanol, it was spiked with 200 μl PAH standard solution 3.5 μg ml^{-1} in acetonitrile (CH_3CN) and elution with 2×500 μl of CH_3CN (first fraction), 2×500 μl of C_6H_{12}–DCM (4:1) (second fraction) and 4×500 μl of C_6H_{12} (third fraction).

The sugar cane soot utilized in this work was collected on the sugar cane field after the process of cane burning (Araraquara region, São Paulo, Brazil). For study of the behaviour of silica-gel cartridges with sugar cane soot extract, we used a sugar cane soot sample spiked with 100 μl PAH standard solution 15 μg ml^{-1}. This material was extracted using DCM–MeOH (4:1) in a Soxhlet apparatus (1.0 g soot, 24 h, 200 ml solvent). The extract was fractionated on a silica-gel cartridge obtaining three fractions: first elution (1.5 ml C_6H_{12}), apolar compounds; second elution [2.5 ml C_6H_{12}–DCM (4:1)], PAH fraction; and third elution [2.5 ml C_6H_{12}–DCM (4:1)]. After concentration almost to dryness under a N_2 stream and redissolution with 500 μl isooctane, fraction analysis was done using GC–MS in the SCAN and SIM modes.

3. Results and discussion

Chromatographic analyses showed that C_6H_{12} has extracted substances contained in silica-gel cartridges. Some compounds showed retention time near those of PAHs. It was necessary to rinse the cartridge with 25 ml cyclohexane to fully eliminate those interferents.

Concerning the recovery of PAHs with silica-gel cartridges, the best results were obtained with elution volumes of 1.5 ml cyclohexane (first fraction) and 2.5 ml cyclohexane–DCM (4:1) (second fraction). No significant variation was observed with different flow-rates, but lower ones improved the PAH recoveries, mainly for the low-polarity compounds. Therefore, a 0.6 ml min^{-1} rate was chosen in all subsequent analyses.

For RP18 cartridges, elution with 2×500 μl

CH_3CN eluted almost all PAHs. Only small amounts of PAHs were eluted with C_6H_{12}–DCM (4:1). However, several polar contaminants were also eluted with CH_3CN. To avoid this, we have added water to the standard solution, in a 3:5 proportion [6], but the results were not satisfactory.

Due to the difficulty of obtaining a PAH enriched fraction without polar interferents with RP18 cartridges, we chose silica-gel Sep-Pak cartridges for clean-up of sugar cane soot extract.

A recovery study was performed using a procedure that included: (1) silica cartridge conditioning (25 ml C_6H_{12}); (2) spiking with 200 μl standard solution 5 μg ml^{-1}; (3) first elution (1.5 ml C_6H_{12}), apolar compounds; (4) second elution (2.5 ml C_6H_{12}–DCM (4:1), PAH fraction; (5) concentration of second fraction under a N_2 stream, almost to dryness; (6) redissolution in 500 μl cyclohexane; and, finally, GC–FID analysis.

Recovery results (six replicates) are shown in Table 2. High RSD values are due to the concentration step, as was checked experimentally. In general, PAH recoveries vary between 80 and 100%. More volatile compounds, such as naphtalene, acenaphtylene and acenaphtene, show lower recoveries.

Concerning sugar cane soot extract on silica-gel cartridge, the chromatograms obtained for each fraction showed the absence of PAHs in the first and third fractions. In the second, PAHs were eluted with some medium polarity substances. Fractionating improved the chromatographic profile of the soot extract, separating in different fraction substances with very similar retention times. Fig. 1 presents GC–MS-SIM chromatograms for standard solution, whole sugar cane soot extract and SPE fractions.

4. Conclusion

In this work we can conclude that an RP18 cartridge is not suitable for the clean-up of sugar cane soot extract, aiming to isolate PAHs for analysis by GC–FID or GC–MS, because it was impossible to obtain a PAH fraction without more polar compounds. For silica-gel cartridges the best results are obtained after conditioning with 25 ml cyclohexane for removal of all low-polarity compounds from the

Table 2
Recovery of PAHs on silica cartridge clean-up (based on second fraction)

Peak number	PAH	Recovery[a] (%)		
		Range	Average±SD	RSD[b] (%)
1	Naphtalene	0–13.3	7.80±4.80	62.2
2	Acenaphtylene	41.0–67.9	55.1±8.90	12.2
3	Acenaphtene	43.2–79.5	65.7±12.8	19.5
4	Fluorene	63.2–99.0	80.8±13.5	16.7
5	Phenantrene	75.3–88.5	82.2±4.9	6.0
6	Anthracene	81.6–114	100±11	11.8
7	Fluoranthene	88.4–107	98.6±8.0	8.2
8	Pyrene	82.0–103	90.2±7.5	8.3
9	Benzo[*a*]anthracene	92.2–109	98.3±8.1	8.3
10	Crysene	81.7–109	100±9.6	9.6
11	Benzo[*b*]fluoranthene	39.7–129	79.0±35.7	45.2
12	Benzo[*k*]fluranthene	51.5–126	88.6±29.6	33.4
13	Benzo[*e*]pyrene	86.4–149	113±21	19.0
14	Benzo[*a*]pyrene	80.3–101	91.2±8.8	9.6
15	Dibenzo[*a,h*]anthracene	76.1–102	86.0±9.2	10.7
16	Benzo[*ghi*]perylene	86.2–112	102±9	9.2

[a] Six replicates.
[b] RSD, relative standard deviation = (SD/average)×100.

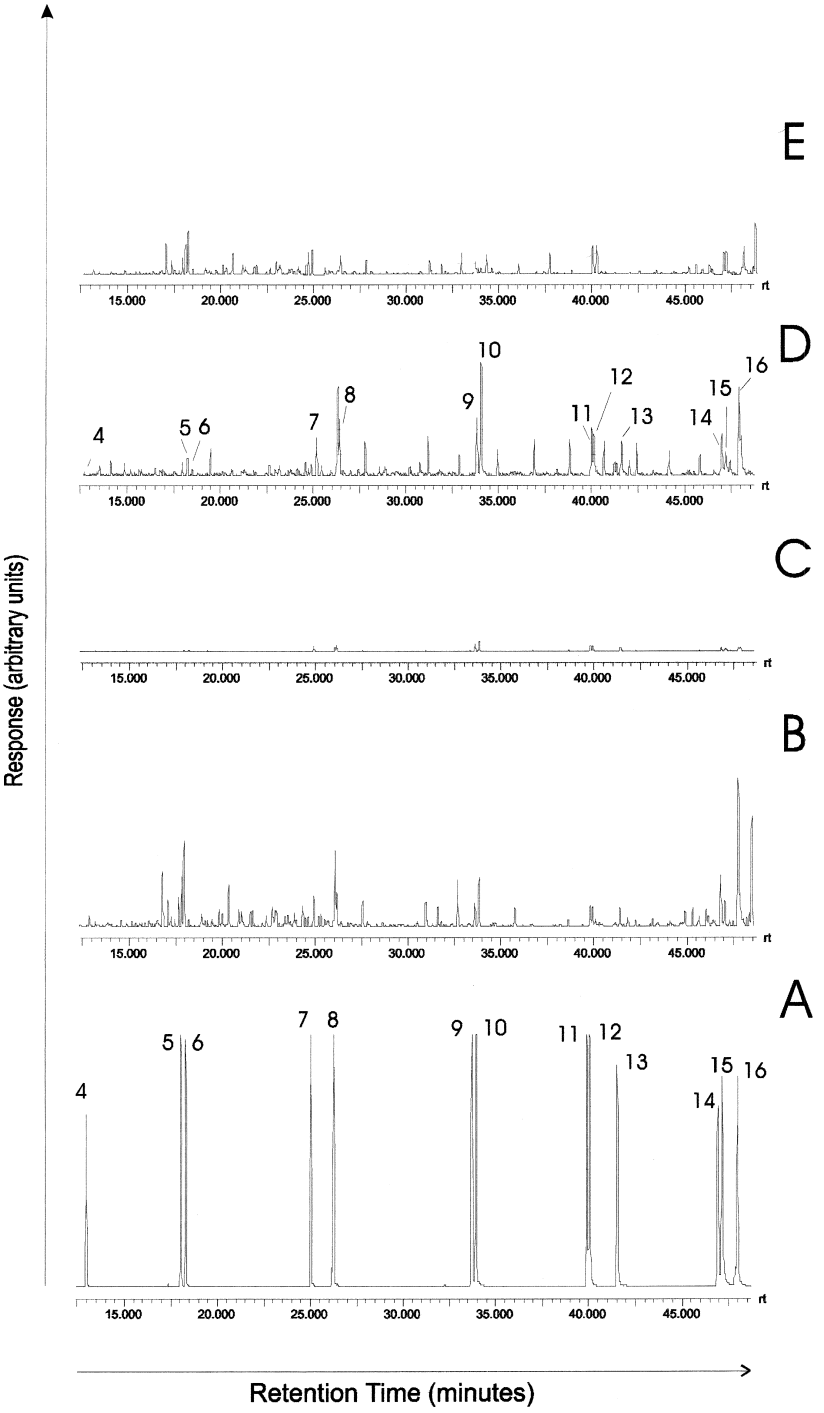

Fig. 1. GC–MS-SIM reconstructed chromatograms (TIC). (A) PAH standard solution, 1 ng ml^{-1}; (B) whole (spiked) sugar cane soot extract (1 g, 200 ml cyclohexane, Soxhlet, 24 h); (C–E) first, second and third silica cartridge fractions of the same sugar cane soot extract, respectively. The conditions of analysis are detailed in the text. For peak identification see Table 2.

cartridge. The sequential elution was optimized with 1.50 ml cyclohexane (first fraction, aliphatic compounds); 2.50 ml cyclohexane–methylene chloride (4:1, v/v) (second fraction, aromatic compounds) and 2.50 ml cyclohexane–methylene chloride (4:1, v/v) (third fraction, polar compounds). A study with sugar cane soot spiked with 1.5 μg PAH standard mix per gram of sample, made it possible to verify that the second fraction was more enriched with PAHs. This shows that this clean-up procedure is suitable for GC–MS analysis of sugar cane soot extract.

Acknowledgements

The authors would like to thank CNPq (Brazilian National Council for Research and Development) for a master grant to G.C.M. Zamperlini; and to FAPESP (Foundation for Research Support of São Paulo State, Brazil), for financial support (proc. 92/2912-9 and 98/01514-6).

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