USING SFE FOR SIMULTANEOUS DETERMINATION OF PCBs AND PAHs IN VARIOUS MARINE SPECIES AND SEDIMENTS.

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Introduction

SFE is particularly suitable for analyses of organic micropollutants such as PCBs(1-4), PAHs (5), PCDDs+PCDFs (6) and pesticides in various matrices. By combining a selective extraction with the process of recovering the solutes from the supercritical fluid, it performes an efficient clean-up of a complex matrix and allows the direct GC injection of the extract. On the other hand, due to the narrow polarity range of the extract, the extraction is selective and generally each of the classes of compounds listed above requires its specific extraction conditions; we have however set up a SFE based method for the simultaneous determination of both PAHs and PCBs in mollusk samples. It was then used in our lab in the past three years on many different matrices, including fish, human blood, vapor-phase air pollutants, marine sediments and food of animal origin. Here we present an overall assessment of its applications to marine matrices based mainly on the study of the recovery yields of the isotopically labeled internal standards used in the analysis, and discuss the differences observed in the analyses of 28 different sediments and 47 fish and mollusk samples. We think of expanding later the present extended abstract into a more detailed evaluation of all the applications of this method.

Materials and methods

Wholly ^{13}C labeled CB congeners were obtained from Cambridge Isotope Laboratories (CIL, Andover, Mass.) as solutions. From the solutions of the pure compounds a mixture (mix A) was prepared in 2,2,4-trimethylpentane containing the following 14 PCBs:15; 28; 52; 77; 101; 105; 118; 126; 138; 153; 169; 178; 180; 202.

For PAH analysis, a mixture (mix B) of seven wholly deuterated compounds (Anthracene; Benz[a]anthracene; Chrysene; Benzo[k]fluoranthene; Benzo[a]pyrene; Indeno[1,2,3-c,d]pyrene; Indeno[1,2,3-c,d]pyrene; Dibenz[a,h]anthracene) was used as internal standard: crystalline compounds, were obtained from CIL.

The analytical procedure was reported in detail elsewhere. Briefly, the biota samples were homogenized, spiked with labeled internal standards, freeze-dried; the sediment samples were dried, sifted and spiked with labeled internal standards; from this point the analytical procedure was the same for both classes and included SFE extraction, further purification on silica gel and GC-MS injection. The spiking level was adjusted to the expected level of the analytes in the sample. Sixty-one PCB congeners are searched in the analysis, together with eight selected PAHs and DDE.

The complete extraction method consists of two extraction steps: an initial 20-min extraction in the dynamic or continuous flow mode with CO_2 modified with methanol (3%), followed by a 5-min extraction with unmodified CO_2 (density = 0.60 g/ml; pressure = 311 bar; chamber temperature = 120°C; trap material = ODS; rinse solvent = hexane. For sediment analysis,

prior to the dynamic extraction, a 20 min static extraction is performed. This allows elemental sulfur to react with activated copper powder. The purity of CO_2 was increased during the analyses, from 99.999% to 99.9999% to decrease the PAH procedural blank.
The extract was further purified on a silica gel column prepared by packing 5 cm of silica gel in a disposable Pasteur pipette. The eluate was concentrated to 1 µL *n*-tetradecane, and taken to the final volume of 50 µL with a solution containing the injection standard ($^{13}C_1$ chlordane).
Determination was performed on a HP5989A GC-MS system equipped with a PTV injector and a capillary gas chromatographic column (50 m-long 0.2 mm-i.d. HP Ultra 2). The GC and MS condition are described elsewhere (7).
The recovery yields were determined in the following way: solutions containing 61 PCB unlabeled congeners, a portion of mix A, 16 unlabeled PAHs, a portion of mix B, labeled and unlabeled DDE were prepared, concentrated to 1 µL in *n*-tetradecane and taken-up to 100 µL with the solution of injection standard. These calibration/recovery solutions were injected three to four times daily. Each batch of samples had its own calibration/recovery solution with the internal standard concentration calculated to grossly match the internal standard content of the portion of sample analyzed. The responses were normalized by means of the injection standard. The normalized responses of the internal standards in the samples were further adjusted to keep into account the exact portion of the sample analyzed and finally compared to the normalized responses of the same standards in the calibration/recovery solution.

Results and discussion
The accuracy of this method, as assessed through certified reference materials, laboratory reference materials and interlaboratory comparison, the precision on replicate analyses, the advantages in terms of procedural blank were discussed in previous papers (7-8); the results obtained on the samples were also already reported (9-13). In this chapter we will discuss the variability of the recovery yields of the labeled internal standards, which we think may reflect differences among matrices. SFE seems indeed to be very sensitive even to slight matrix differences.
It should however be considered that the results reported span through a very wide contamination range (up to more than four orders of magnitude) and that the fortification levels of the isotopically labeled internal standards varied accordingly, whilst the amount of sample analyzed varied, for matrices of the same kind, less than an order of magnitude. Moreover, these results were obtained in the course of two years and may be slightly affected by the long-term variability.
Sediments: the method was applied to 28 samples from different areas, varying from low contaminated spots to heavily contaminated industrial sites, with analyte concentrations ranging from 0.3 to 5600 ng PCB /g and from 0.7 to 10300 ng BaP/g.
In Table 1 the recovery yields of the isotopically labelled internal standards obtained in the analyses of sediments are reported.
Biota: the method was applied to 47 different fish and mollusk samples, with a fat content ranging from 0.5% to 8.7% and contamination levels from 177 to 3 ng/g whole weight for PCB and from 12 to 0.1 ng/g whole weight for Benzo[a]Pyrene. In Table 2 the recovery yields of the isotopically labeled internal standard used in the analyses of biota samples are reported. Nine different species of mollusk and fish were analyzed.

Table1: Mean recovery yields of the isotope labeled internal standards, their coefficient of variation, their minimum and maximum values measured in 28 sediment samples.

Labeled compound	Mean	CV	min	max
T3CB 28 $^{13}C_{12}$	84%	28%	42%	130%
T4CB 52 $^{13}C_{12}$	78%	21%	47%	110%
P5CB 101 $^{13}C_{12}$	89%	20%	50%	113%
P5CB 118 $^{13}C_{12}$	95%	16%	65%	134%
P5CB 105 $^{13}C_{12}$	92%	20%	62%	127%
H6CB 153 $^{13}C_{12}$	81%	20%	48%	107%
H6CB 138 $^{13}C_{12}$	91%	20%	65%	140%
H7CB 178 $^{13}C_{12}$	81%	28%	29%	120%
H7CB 180 $^{13}C_{12}$	76%	25%	46%	113%
O8CB 202 $^{13}C_{12}$	57%	31%	25%	94%
DDE	113%	26%	69%	178%
Benz[a]anthracene	74%	33%	39%	148%
Chrysene	68%	30%	37%	109%
Benzo[k]fluoranthene	67%	37%	27%	113%
Benzo[a]pyrene	62%	43%	19%	110%
Indeno[1,2,3-c,d]pyrene	54%	28%	30%	90%
Dibenz[a,h]anthracene	52%	36%	21%	98%

Table 2: Mean recovery yields of the isotope labeled internal standards, their coefficient of variation, their minimum and maximum values measured in 47 fish and mollusk samples.

Labeled compound	Mean	CV	min	max
T3CB 28 $^{13}C_{12}$	61%	29%	22%	112%
T4CB 52 $^{13}C_{12}$	66%	22%	25%	92%
P5CB 101 $^{13}C_{12}$	81%	23%	44%	123%
P5CB 118 $^{13}C_{12}$	79%	18%	52%	107%
P5CB 105 $^{13}C_{12}$	84%	19%	54%	111%
H6CB 153 $^{13}C_{12}$	71%	26%	24%	105%
H6CB 138 $^{13}C_{12}$	77%	17%	49%	105%
H7CB 178 $^{13}C_{12}$	69%	28%	21%	105%
H7CB 180 $^{13}C_{12}$	72%	28%	24%	108%
O8CB 202 $^{13}C_{12}$	65%	33%	11%	103%
DDE	85%	20%	42%	113%
Benz[a]anthracene	75%	38%	19%	133%
Chrysene	88%	24%	54%	154%
Benzo[k]fluoranthene	75%	41%	24%	161%
Benzo[a]pyrene	49%	48%	11%	94%
Indeno[1,2,3-c,d]pyrene	48%	46%	8%	102%
Dibenz[a,h]anthracene	52%	51%	11%	140%

The variability of the recovery yields obtained, indicated by the values of the coefficient of variation, is rather high, especially for PAHs. Other features of these sets of data are the decreasing trend of the mean recovery yields from PCBs to PAHs and, inside the latter class, with increasing molecular weight. Among the PCBs the octachlorobiphenyl 202 generally shows comparatively low recoveries.

The lower recoveries of the PAH may be due to their higher polarity and stronger interaction with the matrix: the use of methanol as an organic modifier of the supercritical extracting fluid is necessary for their extraction, but it does not always allow a complete efficiency. It is useful to report that two apparently similar mussel samples displayed a significantly different behavior: in the first one the PAH extraction was complete and repeatable with no methanol, in the second 3% was necessary. The use of methanol also results in a more complex extract which needs a further purification step on a Pasteur pipette packed with silica gel deactivated with 3% water. With pure CO_2 the extract only needs concentration before injection, but PAH recovery is generally low in these conditions.

In the fourth column of the Tables (PAH maximum recovery yields), values far exceeding 100% are found; they can be explained with the presence of interfering compounds: only the molecular mass is monitored for PAHs, and it may sometimes be interferred; it is, however, a rare event.

These results seem to indicate that this method, presumably due to matrix interactions, shows for PAHs a considerable variability, which makes it reliable only when the isotope dilution technique is used. For PCBs the variation is much lower, and may be considered acceptable taking into account the wide range of contamination covered. Also in this case the use of a rich set of isotopically labeled internal standard gives higher reliability to the analyses.

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