DETERMINATION OF PCB IN BIOLOGICAL SAMPLES USING ON-LINE SFE-GC

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ABSTRACT

On-line supercritical fluid extraction (SFE) with high resolution capillary gas chromatography was used to extract polychlorinated biphenyls (PCB) from biological samples. This methodology allows a more selective extraction of the PCB from the lipid matrix than conventional solvent extraction. A high detectability was achieved due to quantitative transfer of the extracted species into a retention gap. At this stage the extracted species were cryogenically focused prior to normal GC analysis, using electron capture detection. Basic alumina was utilized as the selective sorbent. In order to test the recovery of the system, off-line extraction with a C_{18} sorbent was performed.

INTRODUCTION

The properties of supercritical fluids have a potential for allowing rapid and selective extraction of analytes from solid matrices compared to conventional solvent extraction techniques.The methodology may thus significantly shorten the total time of extraction and clean-up of complex samples. Another advantage is that expensive and potentially toxic organic solvents can be replaced by non-toxic CO_2. The goal of this study was to achieve a selective exctraction of the PCB from biological samples using as little clean-up as possible.

EXPERIMENTAL

Sample preparation

Samples spiked with Aroclor standard or individual PCB-congeners were mixed and homogenized with anhydrous sodium sulfate (1:3, w/w) in a household mixer or in a morter. The dry salt-like mixture was placed in the extraction chamber. Basic alumina, same amount as the sample, was placed in a separate column placed after the extraction cell.

SFE-GC system

The instrumentation consisted of a SFE-GC system, SFE-30 and SFC-3000 from Carlo Erba Instrumentations (Figure 1). The supercritical fluid extract was decompressed through a restrictor inserted into a retention gap. The analytes were collected in the retention gap by a cryofocusing trap. Reinjection of the trapped components into the GC capillary column was performed by thermal desorption.

The transfer from the supercritical to the gaseous state was obtained by a linear fused silica restrictor, 20cm X 28µm ID. At 14.5 MPa the CO_2 gas flow through the restrictor was 133 ml/min. The temperature at the outlet of the restriction zone was 250 °C. Helium was added as a make up gas at the restriction zone. Extracted compounds were transferred, in the gaseous state, via a fused silica line , 90cm X 0.53 mm ID kept at 300 °C.

The cold trap, which was cooled by liquid nitrogen, was mostly kept at -30 °C during the collection step. After 40 min. the cooling was shut down, the heating started and the collected material transferred to the analytical column. During extraction , CO_2 and helium gases were removed by opening a valve. During the transfer to the analytical column this valve was closed. The GC column used was a DB-5, 30m x 0.25µm ID, 0.25 µm film thickness. The initial GC oven temprature was 60°C, which was kept for 1 min, then increased to 200°C at 20°/min, then to 280°C at 3.5°/min. The electron capture detector, ECD-40, from Carlo Erba Instruments was kept at 350°C.

SFE off-line.

For recovery testing of the SFE step, the restrictor was directly introduced into a Waters Sep Pak C_{18} cartridge. The cartidge was simultaneously flushed with warm water in order to adsorb the eluting component quantitatively to the C_{18} silica. The adsorbent was eluted with hexane and the eluate analysed by GC-ECD.

RESULTS AND DISCUSSION

The recovery data are given in Table 1. All recoveries were between 70 and 86 %. The data were obtained at 14.5 MPa and 60°C and are based on six parallels. Extraction under these conditions yielded satisfactory recovery of analytes while maintaining selective extraction of the PCB from the lipid matrix. The off-line system was utilized in the recovery testing of the SFE step. By controlling the density of the CO_2 and by using basic alumina as a selective adsorbent, it was possible to extract most of the PCBs from the lipid matrix. The conditions chosen seem to be a good compromise between recovery and selectivity. A better recovery of the analytes is possible by employing higher pressure, but then more interfering components become solubilized in the supercritical fluid phase. Small sample sizes of approximately 450-900 mg were utilized in the studies.

Figure 2 shows a chromatogram of crab hepatopancreas spiked with Aroclor 1260. A blank run analysed at the same conditions as the samples, did not show any interfering background from the system (Figure 3). The methodology has so far been tested on biological samples with different fat contents such as crab hepatopancreas, cod liver and cod fillet. Increasing fat content did not seem to influence the analysis negatively.

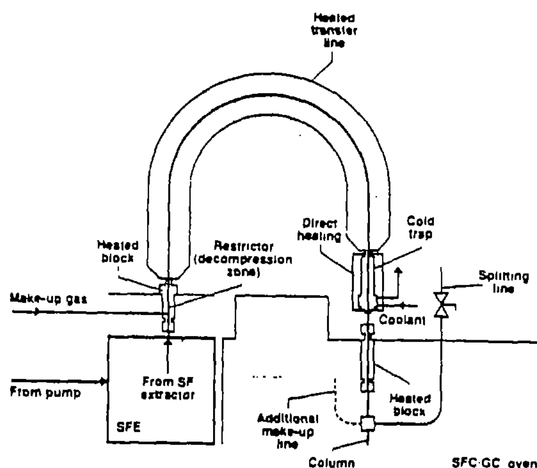

Fig.1. Schematic diagram of the SFE-GC system

Tabel 1. Recovery of PCB from biological samples after spiking the sample with selected PCB congeners, then extraction with supercritical CO_2 and analysis with GC-ECD.

Congener	28	118	138	153	180	170	209*	O.C.N **
Recovery %	70	72	83	77	84	86	76	76
Relative standard deviation	8	5	6	0.5	15	12	2.1	5.6

* based on 2 parallels
** O.C.N. = Octachloronaphthalene

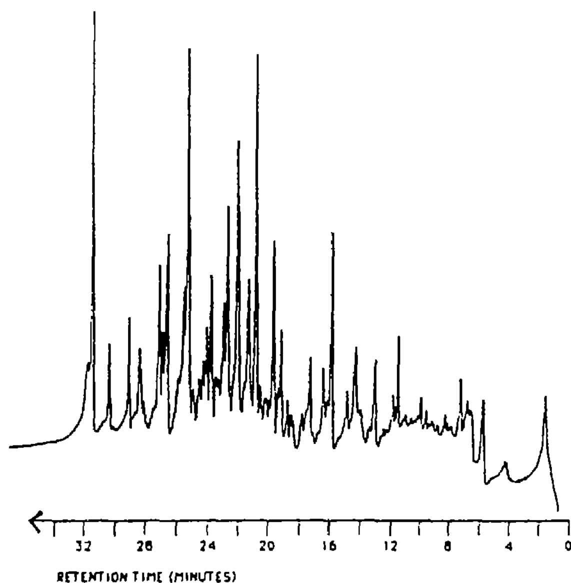

Figure. 2.
SFE-GC-ECD chromatogram of crab hepatopancreas spiked with Aroclor 1260. The extraction was performed with CO_2 at 14.5 MPa and 60°C for 45 min.
GC conditions : 60°C(1)-20°C/min -200° 3.5°C/min-280°C

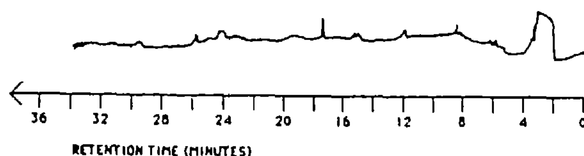

Figure. 3. Blank obtained at the same conditions as those in fig.2