Determination of Organochlorine Pesticides and PCBs in Fish using off-line Supercritical Fluid Extraction, HPLC separation and Gas Chromatography

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1. Introduction

Organochlorine pesticides and polychlorinated biphenyls (PCBs) are usually extracted from environmental and biological samples using various solvent extraction techniques. These extraction procedures and the subsequent clean-up systems are time-consuming and labour-intensive, and often involve large volumes of environmentally harsh or harmful solvents.

In recent years the use of supercritical fluid extraction (SFE) in analytical chemistry has been projected as a powerful alternative sample preparation technique[1-3] providing extraction efficiency comparable to conventional solvent extraction methods inspite of its greatly reduced sample preparation time. The use of non-toxic, non-inflammable and inexpensive supercritical carbon dioxide fluid (extraction medium) gives the technique an added advantage. So far only very few researchers have, with limited success, extended this technique to fish matrices. This paper presents results of studies involved with pre-extraction preparation of fish samples, optimization of extraction and collection strategies in SFE, and HPLC separation, prior to GC analysis of organochlorine compounds (OCCs) in the samples. Off-line optimization has been utilized because it is simpler to perform and more versatile than on-line optimization ,particularly when working with trace analysis of multiple residues in complex samples of biological origin which often contain a considerable amount of extractable matter[4-5].

2. Method

The SFE system consisted of ISCO SFX 2-10 and the HPLC instrument was the same as reported earlier[6]. Due to their relative high concentrations of native organochlorine compounds Baltic sea trout, herring, salmon and eel were chosen as test materials. Water reduction was performed by freeze- or air-drying. The dried homogenates were ground with a mechanical grinding device to obtain a fine powder.

SFE was performed using an optimized pressure of 300 atmospheres, temperature 50°C, carbon dioxide density 0.90 g/ml and a flow rate of 1.8 ml/min for 30 minutes (about 50 ml). Samples were packed in extraction cells 2.5, or 10 ml. For fat-free extractions samples were packed together with basic alumina (activated at 250°C over night).

Analytes were trapped in a test tube filled with about 10 ml n-hexane at temperature 0-5°C. The grinding of the samples after drying created a homogenous and easily permeable extraction bed which could be more efficiently extracted by SFE with minimal channeling. The maximum sample amount that could be extracted (with aluminium oxide fat absorbent) depends on the amount of fat in the sample.

3. Results and Discussion

Depending on the pre-extraction strategies, the results obtained with the SFE-based procedure compare very well with the conventional extraction techniques. The applicability of the method was evaluated for some PCB congeners and chlorinated pesticides down to parts per billion levels. The fish samples were pre-treated by either freeze- or air-drying prior to SFE extraction, and the results seem to indicate that pre-extraction sample preparation influences the degree of recoveries of the analytes (Fig.1), particularly the low-chlorinated pesticides like hexachlorobenzene and hexachlorocyclo-hexane. Fig. 2 shows the relative recoveries of some PCBs from different freeze-dried fish species.The matrix seemed to contribute significantly to the chequered recoveries of the target substances and this was even pronounced on the pesticides. The extraction efficiencies in all cases are expressed in terms of percentage recovery of the native concentrations relative to the conventional extraction values on fresh samples.

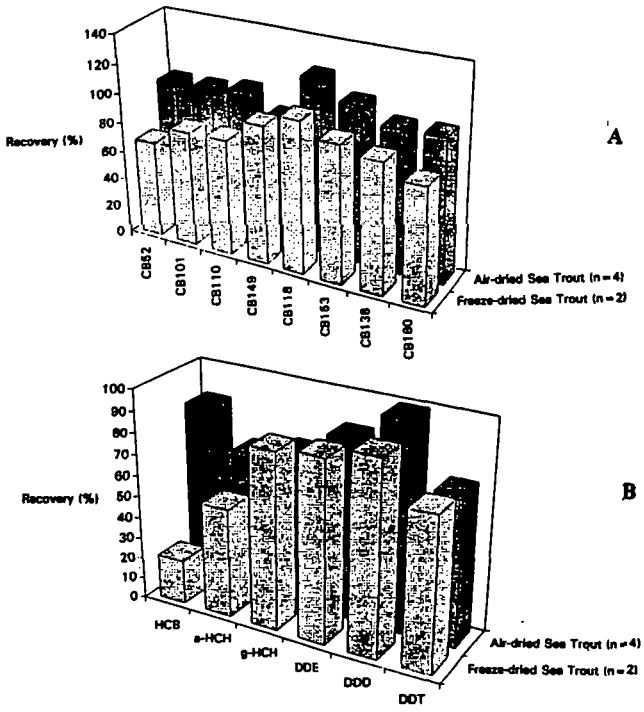

Fig. 1 Effect of pre-tretment on the recoveries of A) PCB congeners and B) chlorinated pesticides from sea trout

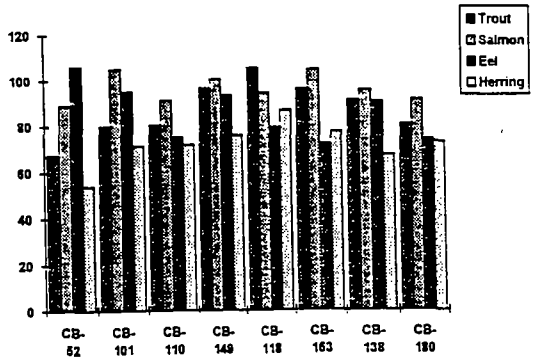

Fig. 2 Recoveries of some PCB congeners from freeze-dried sea trout, salmon, eel and herring by SFE relative to the conventional solvent extraction

Results and recoveries obtained by Bϕwadt et al[7] were based entirely on lyophilized fish tissue extracted by both SFE and soxhlet techniques. The soxhlet procedure should have been performed even on fresh fish tissue and the results compared with those of the SFE-based lyophilized samples. Table 1 shows a comparison of such results from sea trout.

In order to increase the recoveries of the analytes, some extractions were performed using methanol (5%) as a modifier. Inclusion of aluminium oxide in the extraction cell gave fat-free extracts which further enhanced the capability of the technique. It was, however, not possible to get fat-free extracts with a modifier.

Table 1 Recoveries of some target substances from SFE-based freeze-dried Sea Trout relative to values obtained by conventional solvent extraction (CSE) of both the freeze-dried and the fresh sample.

Target subst. PCBs & pest.	52	101	110	118	138	149	153	180	HCB	α-HCH	γ-HCH	DDE	DDT
CSE on **dried** Sea Trout = 100%	132	125	105	112	114	104	95	110	91	101	83	75	102
CSE on **fresh** Sea Trout = 100%	64	87	91	114	100	98	101	90	12	48	85	78	84

The flow diagram for cleanup and separation procedures which have hitherto been operative in our laboratory is now being changed (Fig. 3) to reflect the new technique, including both the SFE and the HPLC separation of the PCB congeners. The silica gel column is still retained because of so many co-eluants which otherwise could interfere with the final GC quantitation, particularly when working with trace analysis of multiple residues in complex samples of biological origin. The HPLC separation on hypercarb column, which has replaced the tedious columns of SP-1 and carbopack C, has greatly

improved the system. The results from the entire study will be published later and will include even the planar PCB congeners which are not shown here.

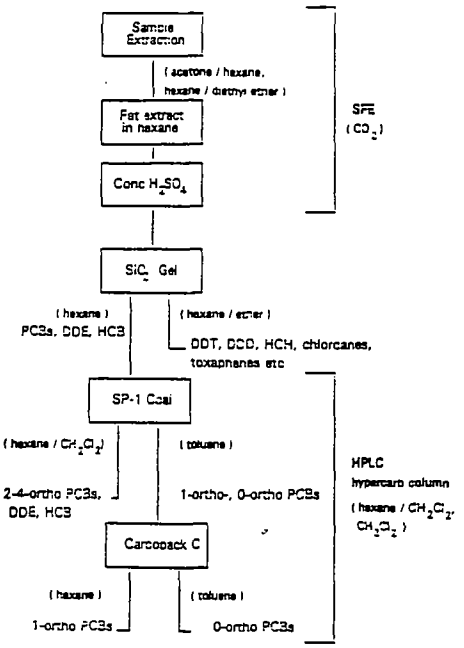

Fig. 3 Schematic diagram of the cleanup/separation process

4. References

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