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Short Communication

Supercritical fluid extraction of organotins from biological samples and speciation by liquid chromatography and inductively coupled plasma mass spectrometry

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ABSTRACT

Supercritical fluid extraction is used to extract tributyltin and triphenyltin from biological samples. The extraction conditions with carbon dioxide as supercritical fluid (methanol modifier used) are optimized for the organotins from fish tissue certified reference material. The total extraction time is found to be approximately 15 min. The recovery studies at the optimal conditions shows a recovery of 44% for tributyltin and 23% for triphenyltin. The reproducibilities for both the compounds extracted are within 2% R.S.D. The optimum conditions obtained are also used to extract tributyltin and triphenyltin from tuna fish obtained from a local grocery store.

INTRODUCTION

Extractions of trace organic analytes is usually performed by liquid solvents or with a Soxhlet apparatus. These extractions are generally time-consuming and the most error-prone step of an analytical procedure. Recent publications [1–5] have demonstrated the potential of using supercritical fluid extraction (SFE) as an alternative to the time-consuming, less efficient, and less quantitative conventional extraction procedures. Supercritical fluids have several characteristics that make them useful for the rapid and quantitative extraction and recovery of analytes. Properties of supercritical fluids that are attractive from an extraction viewpoint include: diffusion coefficients, density, and viscosity. Diffusion coefficients of supercritical fluids (between 10^{-4} and 10^{-3} cm^2 s^{-1}) are considerably greater than those of liquids (less than 10^{-5} cm^2 s^{-1}) which leads to more efficient and rapid extractions from a variety of sample matrices [6]. The low viscosity and the absence of surface tension in supercritical fluids facilitate pumping and fluid flow in the extraction process. Solvent strength is a function of the density of a supercritical fluid. Densities of supercritical fluids are closer to those of liquids enabling the greater interactions on a molecular level necessary for the solubilization process. With supercritical fluids, densities can be easily controlled by adjusting temperature

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and pressure, and thereby enhancing the solvating power which facilitates extraction of a host of analytes of varying polarity and molecular size [6]. Temperature or pressure changes, near the critical point of the supercritical fluid, can change solute solubilities [6] by as much as a factor of 100, or even 1000. Their lower viscosities and higher solute–fluid binary diffusion coefficients improve mass transfer from solid or liquid matrices, thus decreasing the overall extraction time. The use of modifiers can also increase the solvent strengths of the supercritical fluid for the extraction of polar and high-molecular-mass solutes. Moreover, the use of fluids that have low critical temperatures, such as, CO_2, N_2O and SF_6, allow extractions under thermally mild conditions, thereby protecting thermally labile analytes. The use of a non-toxic supercritical fluid as an extraction solvent offers many advantages over conventional liquid organic solvents in terms of solvent disposal and long term exposure of laboratory personnel to the extracting medium. Supercritical fluids thus provide a greater flexibility in optimizing the extraction conditions for a specific solute or a class of solutes from a complex matrix by changing the extraction pressure and/or temperature.

The analytes of interest are usually extracted by the supercritical fluid and are subsequently analyzed by an appropriate on-line or off-line analytical method. There are also several reports where SFE is directly interfaced with analytical chromatography. There are a number of examples of on-line SFE coupled with supercritical fluid chromatography [7–12] and gas chromatography [13–15] which include several environmental, pharmaceutical and industrial applications. With SFE directly coupled to a chromatographic technique, there is no sample handling required between the extraction and the chromatography stage, and the extraction effluents can be quantitatively and reproducibly transferred for the analysis. On-line SFE requires the SFE parameters, analyte trapping conditions, and the chromatographic separation conditions be understood before a successful analysis can be done. Also, once the on-line analysis is completed, the extract is no longer available for further evaluation using different techniques. Since, supercritical fluids undergo expansive (*i.e.* Joule–Thomson effect) cooling during decompression, even volatile components can be quantitatively and effectively collected off-line in various common solvents. The principal advantage of off-line SFE is that it does not require previous knowledge of the operating conditions and also allows the extract to be analyzed by any appropriate technique, even it is not readily interfaced to SFE. Organotin compounds have numerous applications [16–19], for example, mono and diorganotins are used to stabilize poly(vinyl chloride) (PVC) polymers. Triorganotins are used as biocides, catalysts, wood preservatives, fire retardants and reducing agents, as well as in the pharmaceutical, ceramic and glass industries. Tin compounds have also been used in the production of cans for food storage. These organotin compounds are of high environmental and toxicological concern, since they are released into the environment. A large fraction of the total organotin compounds used as biocides and algicides in antifouling paints have directly entered into the aquatic environment. Tributyltin and triphenyltin have been used as antifouling paints for fish nets and ship hulls. Pollution by triphenyltin is a more serious problem than pollution by tributyltin, since it accumulates in the lipophilic tissues in the fish.

This short communication describes the extraction of tributyltin (TBT) and triphenyltin (TPT) compounds from a fish tissue certified reference material and tuna fish that was obtained from a local grocery store. The extracts obtained were speciated by liquid chromatography with inductively coupled plasma mass spectrometry (LC–ICP-MS).

EXPERIMENTAL

Biological samples

Fish tissue (certified reference material No. 11) was obtained from National Institute for Environmental Studies, Japan Environmental Agency. The tuna fish was from canned tuna obtained at a local grocery store. Tuna was dried in the oven at 90°C and homogenized before the extraction procedure.

Reagents

SFE/SFC-grade carbon dioxide (with the helium head space option) was obtained from Air products (Middletown, OH, USA). Optima grade methanol (Fisher Scientific) was used for extractions. HPLC grade methanol (Fisher Scientific), deionized distilled water (Barnstead, 18 MΩ), reagent grade glacial acetic acid (Fisher Scientific) and certified ACS ammonium acetate (Fisher Scientific) were used to prepare the mobile phase.

SFE instrumentation

The SFE instrument is the Isco model 260D (Lincoln, NE, USA) supercritical fluid extractor. It consists of two pumps: one for the primary fluid, SFE/SFC-grade carbon dioxide and the other for the modifier, optima grade methanol. The temperature and pressure were adjusted to the desired value. The system was operated by first placing the sample into the extraction cell and warming the sample for about 5 min before the extraction process. The extraction procedure was started by letting the extraction cell pressurized with the supercritical fluid. During this process which is known as the static extraction, there is no outflow of the fluid. Static extraction was carried on for three minutes and the valve was then moved to the extract position to facilitate dynamic extraction. During dynamic extractions, the fluid continuously flows through the cell and the extracts were directly collected by placing the extraction cell outlet restrictor in 5 g of methanol. For all the extractions the fluid flow was maintained around 0.65 ml/min. The extracts obtained were injected directly into the chromatographic system.

LC–ICP-MS operating conditions

The extracts were analyzed by employing ion-pair reversed-phase LC–ICP-MS, following the procedure described previously [20]. The HPLC system consisted of a Model 300DX (Dionex, Sunnyvale, CA, USA) A Plasma Quad PQS (Fisons, VG Elemental, Winsford, Cheshire, UK) ICP-MS was used. The PRP-1 column was used with a guard column obtained from Anspec (Ann Arbor, MI, USA). Mobile phase of pH 6 containing methanol–water–acetate (94:5:1) (*i.e.* 0.046 *M* acetic acid and 0.012 *M* of ammonium acetate) was used for the analysis. Sodium pentane sulfonate (0.004 *M*) was used as the ion-pairing agent. The flow-rate of the mobile phase was 1 ml/min. About 2–3% of oxygen was added to the argon nebulizer gas to prevent carbon accumulation on the sampler and skimmer. A forward power of 1500 W was used with an argon coolant flow of 14 l/min, auxiliary flow of 1 l/min, and nebulizer gas flow of 0.8 l/min. The spray chamber was cooled to about −18°C, to enhance solvent condensation. The tin major isotope at m/z 120 (32.37% abundance) was monitored.

RESULTS AND DISCUSSION

Initial studies were performed to find the optimal conditions for the extraction of TBT and TPT in fish tissue. The parameters that were optimized include: extraction time, extraction temperature, extraction pressure and the amount of methanol content which is used as the modifier.

Optimization of the extraction time

The optimum extraction time depends on the pressure, temperature and the flow-rate of the fluid through the extraction cell. For unknown samples, the extraction time can be found by conducting successive extractions. Fig. 1 shows the chromatograms obtained for four successive extractions that were done with 0.14 g of fish tissue at 80°C and 6000 p.s.i. (1 p.s.i. = 6894.76 Pa), with a flow-rate of 0.65 ml/min. The first chromatogram represents a 3-min static extraction, followed by a 12-min dynamic extraction to yield a 15-min total extraction time. The rest of the chromatograms are from the dynamic extractions. These chromatograms indicate that a large amount of the soluble components can be extracted in first 15 min. This suggests that optimization of other parameters involved, might lead to a successful completion of extraction within a 15-min time period. Further optimization was performed by extracting for 20 min (3-min static extraction followed by a 17-min

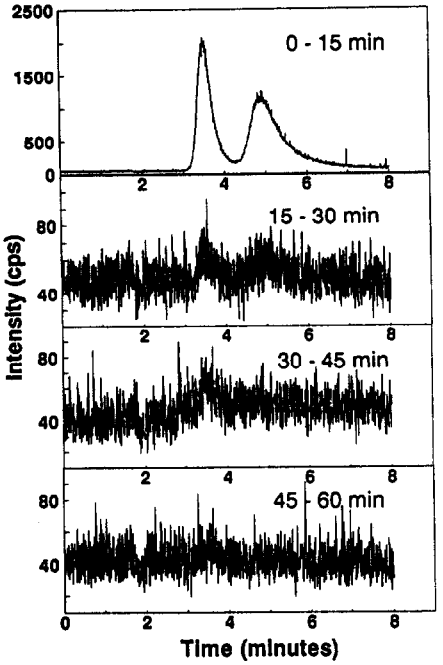

Fig. 1. Chromatograms of tin compounds from 0.14 g fish tissue extract demonstrating the optimization of the extraction time. The intensity scale should be noted.

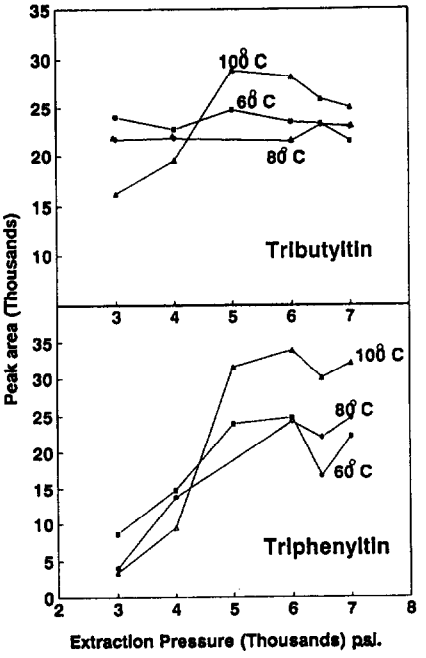

Fig. 2. Optimization of the extraction temperature and pressure for TBT and TPT from 0.14 g of fish tissue (supercritical fluid = CO_2, flow-rate = 0.65 ml/min; static extraction time = 3 min; dynamic extraction time = 17 min; total extraction time = 20 min).

dynamic extraction) at each set of extraction conditions.

Optimization of extraction temperature and pressure

The extractions were performed at temperatures 60, 80 and 100°C. At each temperature, the pressure was varied from 3000 to 7000 p.s.i. These studies were all done without any modifier present in the supercritical fluid carbon dioxide and the flow-rate of the fluid was again maintained at 0.65 ml/min. The effect of temperature and pressure on extraction is shown in Fig. 2. These results indicate that higher amounts of both TBT and TPT were extracted at a temperature of 100°C, when the pressure is higher than 4000 p.s.i. The optimum extraction pressure was found to be at 6000 p.s.i. at all temperatures for TPT, whereas for TBT, the optimum pressure was found to be between 6000 and 6500 p.s.i. Further extractions were done with extraction pressure of 6000 p.s.i. at a temperature of 100°C.

Optimization of the amount of modifier

The use of a binary phase system offers greater flexibility, since the modifier identity and concentration can be easily altered, thereby allowing the adjustment of supercritical fluid for extraction conditions. The modifier used in these extractions was methanol. The addition of methanol to CO_2 increases the solvent polarity, and enhancing the extraction of polar components (TBT and TPT). The effect of the amount of methanol content on extraction is shown in Fig. 3. These observations indicate an increase in the extraction of both TBT and TPT with the increase in the percentage of the modifier used.

Thus, the optimum conditions for TBT and TPT extractions using supercritical carbon dioxide with methanol were found to be CO_2 with

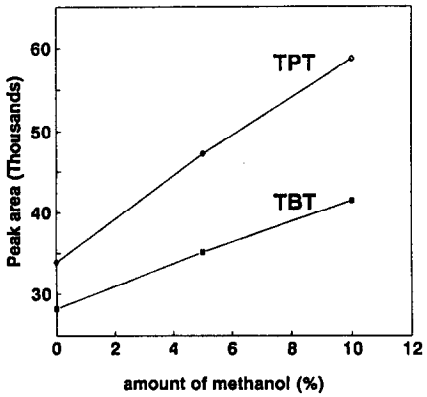

Fig. 3. Effect of the amount of methanol for extractions of TBT and TPT from 0.14 g fish tissue (extraction temperature = 100°C; extraction pressure = 6000 p.s.i.; flow-rate = 0.65 ml/min; static extraction time = 3 min; dynamic extraction time = 17 min; total extraction time = 20 min).

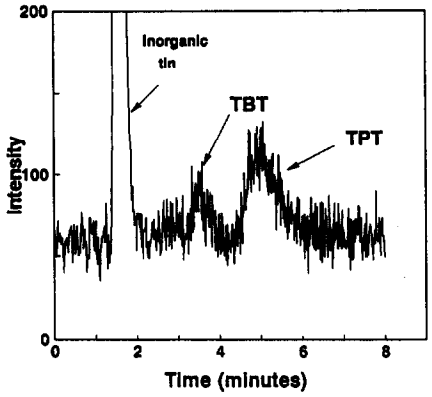

Fig. 4. Chromatogram of tin compounds from the tuna fish extract.

10% methanol, at a temperature of 100°C and a pressure of 6000 p.s.i.

Recovery and reproducibility

The recovery study of these compounds at optimal conditions shows a recovery of 44% (±1.4) for TBT and 23% (±0.9) for TPT, when 0.14 g of fish tissue was extracted. The precision of the extraction method was evaluated by three replicate sample analysis. The recovery study was also done with differing amount of sample sizes using small (0.5 ml and 6.9 mm I.D.), medium (2.5 ml and 7.6 mm I.D.) and large (10 ml and 15.1 mm I.D.) extraction cells. The results are shown in Table I. These results suggest that by keeping the extraction cell small with respect to the sample volume, the degree of sample contact with the extraction fluid increases which is reflected by greater recoveries.

SFE was also employed for extracting organotins from tuna fish. An appreciable amount of inorganic tin was found to be present in the tuna fish extract, which could result from the soldered can that is used to store the tuna. The chromatogram obtained from the tuna fish SFE is shown in Fig. 4. The amount of TBT and TPT were estimated to be about 1.7 ng/g (±2.2) and

TABLE I

RECOVERY OF TBT AND TPT FROM FISH TISSUE (CERTIFIED REFERENCE MATERIAL) BY SUPERCRITICAL FLUID EXTRACTION

Extraction cell	Amount of sample	Recovery of TBT (%) (mean ± S.D., $n = 3$)	Recovery of TPT (%) (mean ± S.D., $n = 3$)
Small (0.5 ml and 6.9 mm I.D.)	0.14 g	44 ± 1.5	23 ± 0.9
Medium (2.5 ml and 7.6 mm I.D.)	0.6 g	39 ± 2.3	22 ± 2.8
Large (10 ml and 15.1 mm I.D.)	3.0 g	31 ± 4.4	17 ± 1.8

3.4 ng/g (±1.6) respectively in 0.2 g of dried tuna fish.

CONCLUSIONS

The potential of using SFE for extracting organotins from fish tissue and tuna fish was demonstrated. It was observed that the total extraction time can be reduced between 15 to 20 min. The amount of sample used was reduced to 0.14 g as compared to 2.5 to 5 g of sample with the solvent extraction method [20]. The reproducibility of the recoveries with SFE were within 2%. However, the low recoveries obtained by SFE suggests procedural modifications, which might include addition of a complexing agent and/or investigating other supercritical fluid/modifier combinations.

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