OPTIMIZING MATRIX DIGESTION AND CLEAN UP FOR THE DETERMINATION OF ORGANOTIN COMPOUNDS IN FISH AND MUSSELS

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Introduction

The widespread distribution of organotin compounds in the environment is mainly caused by their extensive use as additives in the PVC production and as biocides in agriculture and in antifouling paints. The latter use is a substantial source for the contamination of seawater with organotins and has resulted in high levels in marine biota by accumulation[1,2]. The high toxicity of some of those compounds to non-target aquatic organisms which are part of the food chain is of major concern for the human health and requires appropriate analytical methods for quantifying undesirable concentrations in fish and mussels[3].

Most of the analytical procedures available are either focussed on the determination of organotins in sewage sludge, soil and sediments, or they cover only a small group of compounds with a similar polarity. Therefore, the aim of this study was to develop a method which is suitable for determining a pattern of organotins of polarities as broad as possible in marine organisms by using the same analytical procedure. For this purpose, special emphasis was given to the digestion of the matrix and to the clean up of the extract.

Methods and Materials

For the non-destructive but efficient extraction of the organotins from the homogenized samples, four different ways were tested: ultrasonic treatment with methanol, the same in the presence of acetic acid, alkaline digestion with a methanolic tetraethylammonium hydroxide solution and enzymatic digestion using both lipase (type VII) and protease (type XIV).

In most cases, diethylammonium diethyldithiocarbamate (DEA-DDC) was added as a complexing agent in order to improve extraction efficiency[4]. In the extract, the organotins were alkylated with sodium tetraethylborate to form the volatile tetrasubstituted derivatives. They were partitioned from the aqueous phase either with n-hexane or with cyclohexane/ethyl acetate (1+1, v/v). For the clean-up of the organic phase, silica gel 60 or Florisil were used[4-13].

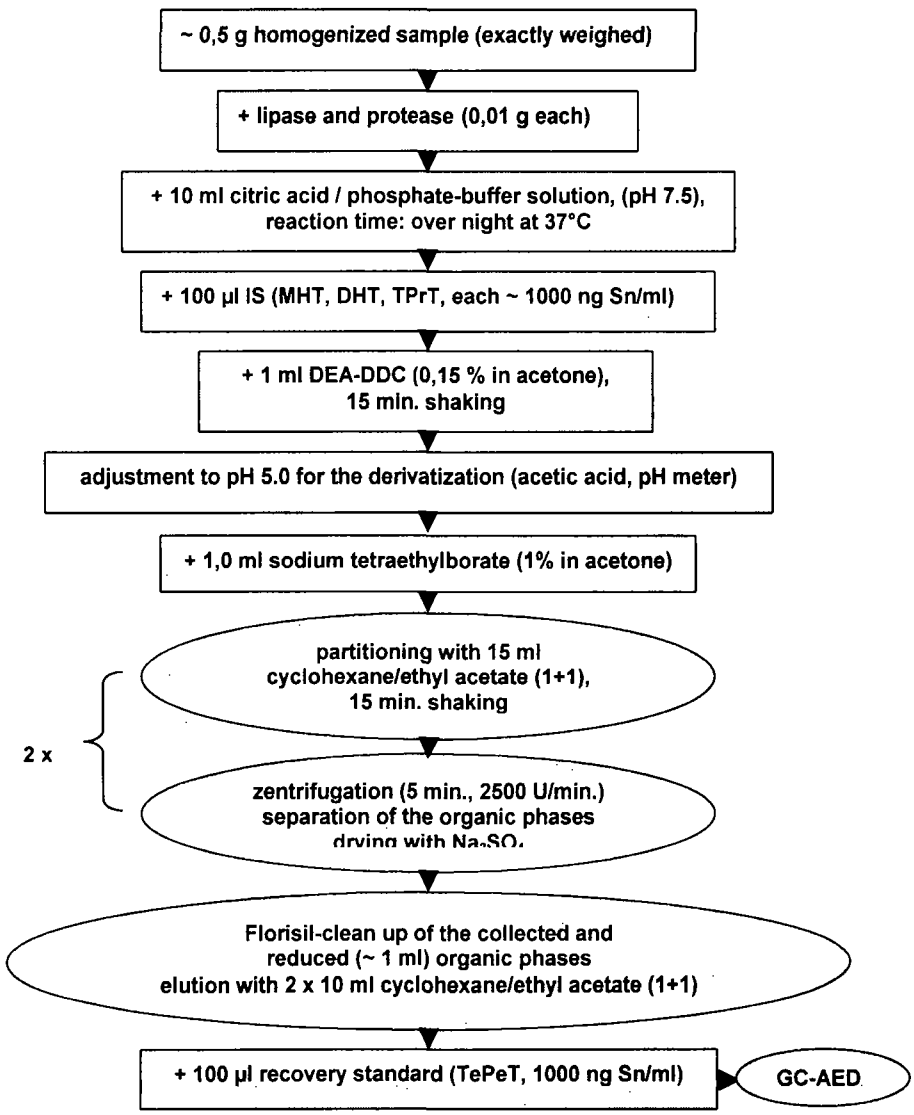
Finally the analytes were determined by gas chromatography using a 60 m DB-5 capillary column and an atomic emission detector (Sn channels 301 and 303 nm). For quantitation, monoheptyltin trichloride (MHT), diheptyltin dichloride (DHT), tripropyltin chloride (TPrT) were used as internal standards for each substitution level and tetrapentyltin (TePeT) for checking the recovery. The complete procedure was in-house validated by analysing the certified reference material CRM 477 (freeze dried mussel tissue).

Results and Discussion

The best results were obtained by enzymatic hydrolysis of the fish and mussel tissues, jointly with complexing the organotins before derivatization, partitioning with cyclohexane/ethyl acetate (1+1) and purifying the extract on a Florisil column. Fig. 1 shows the flow chart of the final analytical method.

Ultrasonic treatment alone was not effective enough to extract each analyte from the matrix, and in the presence of acetic acid, the extracts contained too many interferences. With tetraethylammonium hydroxide, the recoveries obtained for butyltins in the certified reference material were similar to those from enzymatic hydrolysis. With the enzymes, however, the recoveries for all other analytes were even higher.

Fig. 1 Analytical method for the determination of organotins in biological tissue



For the partitioning step, n-hexane and cyclohexane/ethyl acetate (1+1) were compared for a standard mixture containing mono- to tetraalkylated butyltins and phenyltins as well as mono- to trialkylated octyltins. The best recoveries were obtained with the cyclohexane/ethyl acetate mixture for the phenyltins and the low substituted mono- to dialkyltins. In addition, the number of partitioning steps and the amounts of solvent were optimized: Two steps of each 15 ml cyclohexane/ethyl acetate mixture were required for partition and 20 ml for elution of the Florisil column. Florisil (heated at 600°C) removed more interferences from the extract than did untreated silica gel 60.

The precision of the method was determined by analysis (eight replicates each) of two different fish tissues and the certified reference mussel tissue CRM 477. The coefficients of variation obtained for the analytes were between 2,3 % and 15,0 % in the reference material and 6,7 % to 13,4 % or 28,4 % to 35,1 %, respectively, in both fish tissues. The high values in the latter fish tissue are caused by the low residue levels in the sample material, which were close to the detection limits.
The trueness of the method follows from the results obtained for the reference material containing certified concentrations of mono-, di- and tributyltin. Table 1 demonstrates that both certified and found levels do match well. The somewhat lower value found for dibutyltin resulted from high blanks observed in the gas chromatogram.

Table 1: Accuracy

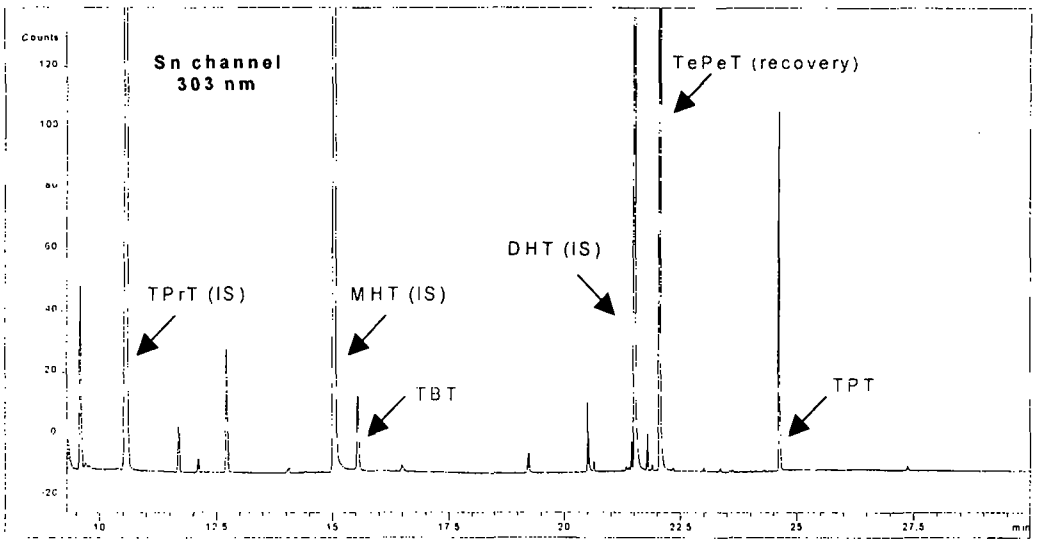
	concentration mg/kg kation in dry weight	
	analysed value	certified value
monobutyltin	1,45	1,50 +/- 0,27
dibutyltin	0,88	1,54 +/- 0,12
tributyltin	1,85	2,20 +/- 0,19

The limits of detection for the butyltins and phenyltins which are both relevant in samples from the environment were in the range of 1 - 3 µg/kg Sn related to fresh weight.

When 20 samples of freshwater fish were analysed with the new method, three of the samples contained tributyltin in the range of 15 - 44 µg/kg kation and five samples contained triphenyltin in the range of 42 - 109 µg/kg kation (related to fresh weight in both cases).

A typical chromatogram obtained from the analysis of a fish sample is depicted in Fig. 2.

Fig. 2: Typical chromatogram for a fish sample



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