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Note

Minimizing blank values in chlorinated hydrocarbon analyses

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The concentrations of chlorinated hydrocarbons dissolved in seawater are low (in the nanogram per litre range). In order to reduce the relative importance of blank values, the extraction of larger amounts of ocean water (up to hundreds of litres) has been reported. However, the larger amounts of particulate matter in estuaries and near-coastal areas limit the amount of water available for extraction to only a few litres and low blank values are then critical.

The separation of fats and other interfering compounds in the determination of chlorinated hydrocarbons can be performed with chromatographic columns of neutral alumina and silica, resulting in good separations with small amounts of adsorbents[1]. The use of basic alumina[2] results in more effective removal of fats and better yields of β-hexachlorocyclohexane.

We have found that the adsorbents used in these procedures contain components that interfere in the determination of some chlorinated hydrocarbons at the concentration level encountered in seawater and even at higher levels such as those in animal tissues. This paper describes a simple treatment of the adsorbents that results in considerably lower blank values.

EXPERIMENTAL

The adsorbent columns were prepared according to Holden and Marsden[1] and Greve and Grevenstuk[2]. However, in order to achieve a satisfactory separation of polychlorinated biphenyls (PCBs) and pesticides, silica was dried overnight. All glassware was heated at 350° overnight and washed with *n*-hexane. Solvents were distilled over a 1.5-m column packed with glass balls (2-mm diameter). Glass-wool was heated at 350° overnight, and sodium sulphate was Soxhlet extracted with pentane for 8 h, dried at 350° and kept dry.

The chromatograms were obtained with a Tracor Model MT-220 gas chromatograph with a nickel-63 electron-capture detector equipped with a 1.8 m × 0.64 cm O.D. borosilicate column packed with 1.5% SP 2250 and 1.95% SP 2401 on 100–120-mesh Supelcon (AW DMCS). The injector, detector and column temperatures were 225, 280 and 215°, respectively. A septum washer was used.

RESULTS

Extracts of seawater in *n*-hexane, evaporated to 1 ml, were injected into the chromatographic column before and after elution from either a silica or an alumina column. Some peaks in the chromatograms obtained from the direct injections were

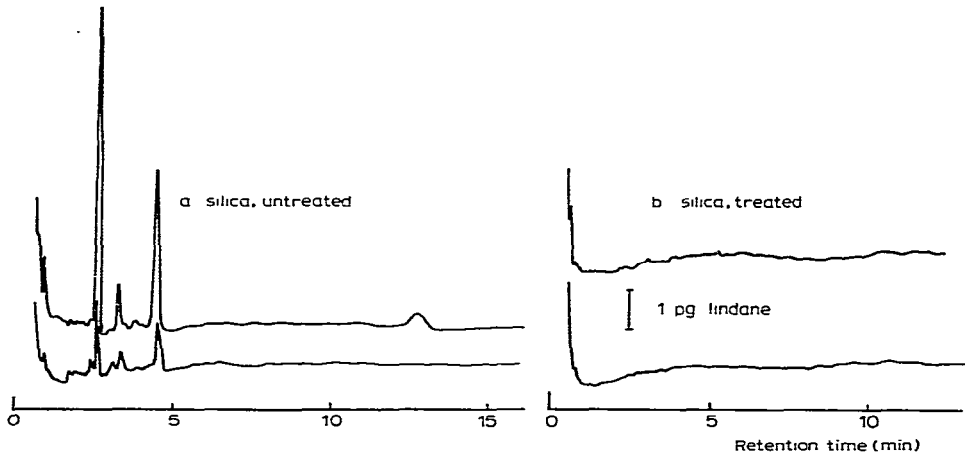

Fig. 1. Chromatogram of *n*-hexane having passed through a silica column that had (b, lower trace) and had not (a, lower trace) been treated with dichloromethane (7 ml of *n*-hexane eluate concentrated to 1 ml) and of a 10% diethyl ether in *n*-hexane solution having passed through the column successively that had (b, upper trace) and had not (a, upper trace) been treated with dichloromethane (13.5 ml of eluate concentrated to 1 ml). Volume injected, 2 μl; attenuation, $16 \cdot 10^2$.

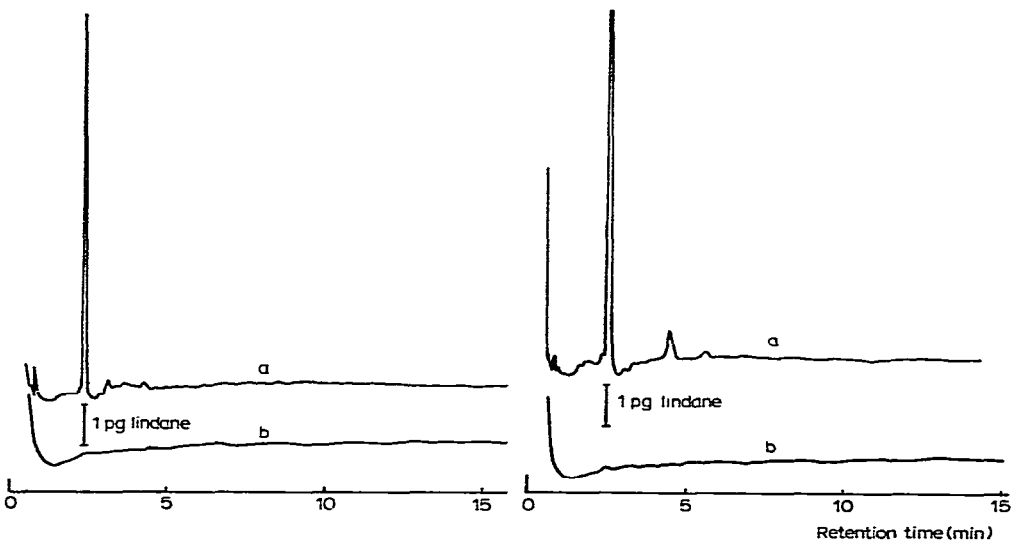

Fig. 2. Chromatograms of *n*-hexane having passed through an alumina column that had (b) and had not (a) been treated with dichloromethane (13.5 ml of *n*-hexane eluate concentrated to 1 ml). Right, neutral alumina; left, Woelm basic alumina. Volume injected, 2 μl; attenuation, $16 \cdot 10^2$. Corresponding response of 1 pg of lindane indicated at its retention time is shown. Solvent peak omitted.

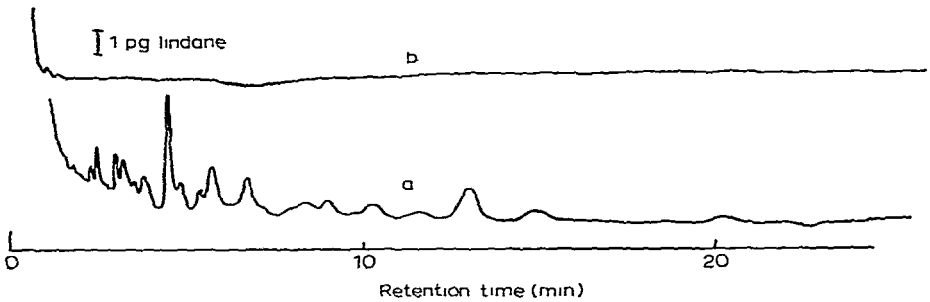

Fig. 3. Chromatograms of *n*-hexane, concentrated from 150 to 1 ml in a Kuderna–Danish evaporator equipped with three-ball Snyder column. (a) Glassware thoroughly cleaned and rinsed afterwards with acetone; (b) same procedure except that the glassware was heated at 350° overnight with successive rinsing with *n*-hexane. Volume injected, 2 μl; attenuation, $8 \cdot 10^2$.

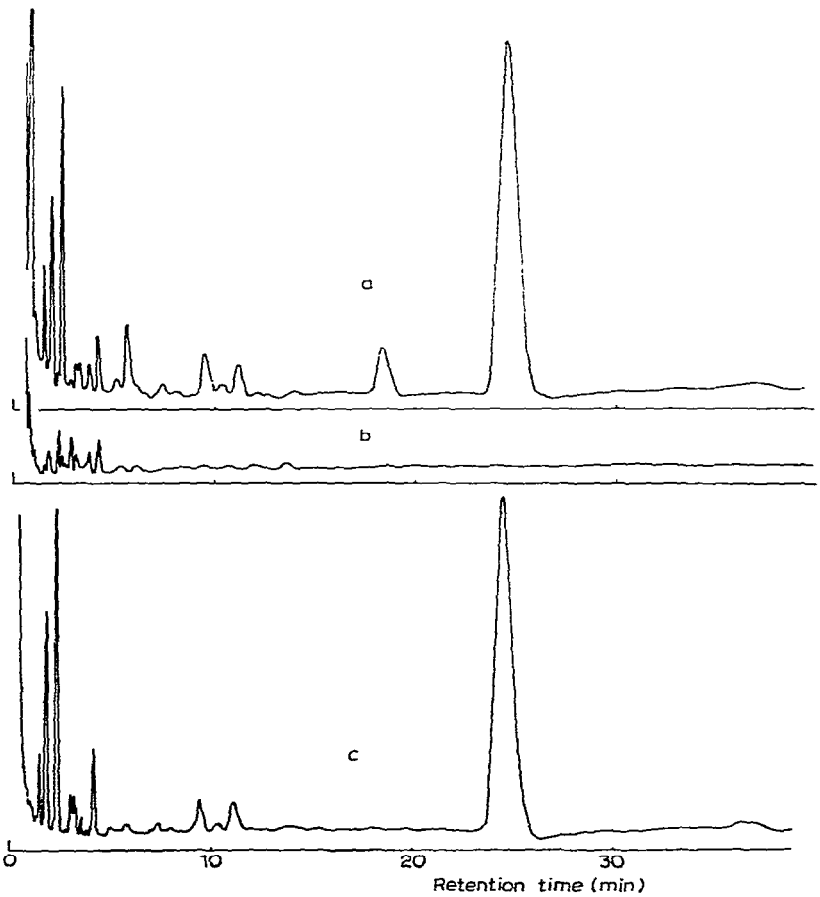

Fig. 4. Chromatograms of seawater extracts in *n*-hexane: 150 ml were concentrated to 1 ml and injected without clean-up or separation (a), this 1 ml was eluted over treated (dichloromethane) silica with 6.5 ml of *n*-hexane, concentrated to 1 ml and injected (b). The successive eluate with 13.5 ml 10% diethyl ether in *n*-hexane was concentrated to 1 ml and injected (c). Volume injected, 2 μl; attenuation, $16 \cdot 10^2$.

missing after the column treatment, and other peaks were considerably higher or new. The effects were pronounced for a compound with the retention time of lindane. It was found that the adsorbents used are responsible for the new peaks (Figs. 1a and 2a), provided that the equipment and chemicals used have been eliminated as source of contamination by appropriate measures (Fig. 3). Subtraction of blank values for actual samples is not justified because of their highly irreproducible character. Repeated washings with *n*-hexane did not remove the interfering substances from the columns; treatment with 10 ml of dichloromethane followed by 10 ml of *n*-hexane resulted in satisfactory blank chromatograms without the column activity being changed (Figs. 1b and 2b). The effect of the treated silica column on an actual seawater sample is shown in Fig. 4. The blank value for the more involved procedure used in the analysis of biological samples is represented in Fig. 5.

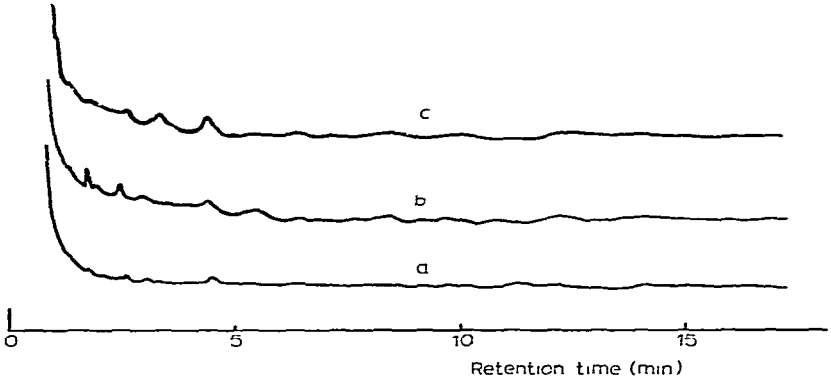

Fig. 5. Results when solvents were manipulated through the whole procedure of extraction and separation processes as used in the analysis of biological tissues. Alumina and silica were treated with dichloromethane. (a) Blank of the "extract" after alumina separation, concentrated to 1 ml; (b) first fraction of the eluate of this 1 ml over silica with 6.5 ml of *n*-hexane concentrated to 1 ml; (c) second fraction of the eluate with 13.5 ml of 10% diethyl ether in *n*-hexane, concentrated to 1 ml. Volume injected, 2 μl; attenuation, $16 \cdot 10^2$.

DISCUSSION

Some peaks that are given by seawater extracts are absent after the silica separation that is required in the Holden and Marsden procedure[1] (Figs. 4b and 4c). They can, however, be eluted with a larger portion of 10% diethyl ether in *n*-hexane. Hence the analysis of extracts of natural waters before and after clean-up and separation procedures is useful for tracing peaks that may otherwise remain undected in the Holden and Marsden procedure, which was, in fact, developed for a particular series of compounds. Studies on the nature of these unknown substances are in progress. The nature of the compounds that are introduced by the column is also largely unknown. Their effect disappears, however, after treatment of the columns with dichloromethane.

The interfering peaks could also be removed with diethyl ether, but the activity of the alumina and silica is then changed. As the lipid–chlorinated hydro-

carbon and PCB–pesticides separation becomes dependent on smaller amounts of solvent, we prefer the use of dichloromethane.

We have found that the introduction of basic alumina[2] can make the Holden and Marsden procedure[1] even more convenient. Elution is more rapid and the method of preparation of the adsorbent in the required state of activity is slightly more convenient. It should also be treated with dichloromethane in order to remove interfering substances.

If contamination during sampling and extraction is avoided, it is possible with the present method of separation to determine sub-nanogram per litre concentrations of chlorinated hydrocarbons in seawater extracts from only a few litres of sample. Also, the convenience and accuracy of their determination in biological samples is improved.

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