A Simplified Clean-Up Technique for Organochlorine Residues at the Microliter Level

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The extreme sensitivity of the electron capture detector to organochlorine pesticides is not generally exploited to the full. After extraction and clean-up, the pesticide residues are usually dissolved in large volumes of solvents (ml) of which only some 5-10 µl are taken for the gas chromatographic determination.

In order to study the occurrence of traces of organochlorine residues in samples of freshwater algae, the efficiency of the electron capture detector had to be utilized maximally. This report describes a procedure in which the sample extract is cleaned-up at the microliter level. For the whole operation, that included two separate clean-up processes and a fat-content determination, 350 µl of extract is sufficient.

MATERIAL AND METHODS

Methods for the extraction of the sample are chosen in accordance to the amount and type of sample under investigation (DALE and MILES 1970, BENVILLE and TINDLE 1970, JENSEN and AHLING 1970, BURKE et al. 1971). The resulting extract (in hexane) is concentrated to the desired volume (350 µl), and divided into three portions for different treatment.

With a pipette-filler, one portion of the extract (approx. 50 µl) is sucked up in a Pyrex glass tube (ID 2 mm, 100 mm in length), followed by an equal amount of fuming sulphuric acid (10% sulphuric trioxide). With the pipette-filler still attached, the glass tube is sealed in a microflame. By gently tapping (or centrifuging) the tube, the contents fall to the base of the sealed capillary. After that, the other end of the tube is sealed. At this point, the procedure may be interupted for

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considerable time (days) without affecting the final result.

The contents of the capillary tube are thoroughly mixed by means of a vibrator and/or ultra-sound equipment. This treatment eliminates most of the material interfering in the gas chromatographic analysis.

After mixing, the phases are easily separated by centrifugation (3000 rpm) and the hexane layer is retained, and the sulphuric acid discarded, simply by cutting the tube just above the acid phase. The part of the tube containing the hexane phase is resealed in a microflame. The clean-up procedure is thereby completed and the extract can then be stored for subsequent gas chromatographic analysis. In order to avoid extraneous peaks in the beginning of the chromatograms, a small amount of silica gel may be added to the hexane extract in the capillary tube. This treatment does not reduce efficiency of the recovery of organochlorine residues.

For confirmatory purposes, a part of the remaining original extract is cleaned-up by treatment with potassium hydroxide. A specially designed pipette with one end sealed (Fig. 1), is gently flamed and the open end immediately immersed in a solution of 5% potassium hydroxide in propanol. As the pipette cools, it sucks in some 50 µl of the solution. After centrifugation in order to transfer the potassium hydroxide solution to the base of the pipette, the above operation is repeated, this time with the same amount of hexane extract, The pipette is then sealed and the contents heated on a water bath for 10 min.

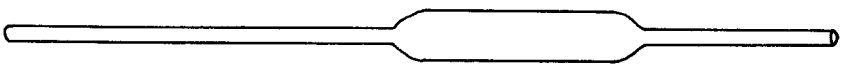

Fig. 1. Pipette used in the clean-up procedure.

After heating, the pipette is opened at the shorter end and approx. 5 ml water are injected by means of an ordinary syringe with a long needle.

The pipette is sealed again and, by means of a vibrator, the contents are mixed.

The phases are separated by centrifugation (3000 rpm), so that the hexane fraction collects at the longer end of the pipette. The hexane layer is removed by cutting off this part of the pipette, and after sealing, the sample can be safely stored for awaiting analysis.

The remainder of the extract (200 µl) is taken for gravimetric determination of the extractable lipids in the sample. Using a calibrated Biopette dispenser, the extract is placed in tared plastic vials (tara 60-70 mg), and the hexane is allowed to evaporate. The weighings are carried out with an electromagnetic balance, capable of registering 1/1000 of mg.

RESULTS AND DISCUSSION

Fatty as well as non-fatty samples have been subjected to clean-up by the present method. Depending on the extraction procedure employed, the recovery of residues from fortified samples has been in the range 78-94%. The losses involved in the cleaning-up procedures are negligible, owing to the fact that all operations are performed in sealed tubes and thus no organochlorine residues can escape. The major losses occur in the extraction, and the only way of improving recovery seems to lie in a careful choice of solvents and a skilled extraction technique.

By the treatment with potassium hydroxide, p,p'-DDT and p,p'-DDD are almost quantitatively converted to p,p'-DDE and p,p'-DDMU (1-chloro-2,2-bis (p-chlorophenyl)ethylene). The conversion products thus confirm the identity of the original products. Products lost in the sulphuric acid treatment can also be estimated. Treatment with neither potassium hydroxide nor sulphuric acid affects the polychlorinated biphenyls (WESTÖ 1970).

Determination of the extractable lipid fraction in the muscle tissues of a fish and in the breast muscles of a bird resulted in 0.6% (S.D. 0.08, n = 10) and 6.4% (S.D. 0.1, n = 10) respectively.

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