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ULTRATRACE ANALYSIS (BELOW p.p.b.*) BY COUPLING CENTRIPETAL THIN-LAYER CHROMATOGRAPHY AND GAS CHROMATOGRAPHY

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SUMMARY

A method for the determination of ultratrace amounts of lindane by centripetal thin-layer chromatography combined with gas chromatography under common laboratory conditions is described. The method was used for the determination of pesticide contents in the dry material obtained from cabbage from a trial field. The accuracy of the analysis under the conditions easily available in routine work is 0.1 p.p.b. The sensitivity limit of the method is 0.001 p.p.b. with analytical precision.

INTRODUCTION

There is no doubt that ultratrace analysis is currently a considerable problem in analysis, the importance of which is increasing rapidly. Chromatographic techniques are suitable for this application not only because of their separating power but also because of their concentration ability. There are several examples in gas chromatography (GC), *e.g.*, the method of chromatographic equilibration[1] and the method of reversed-phase chromatography[2], which are applicable at or below the p.p.b. level. Recently, the new technique of centripetal thin-layer chromatography (TLC) has been developed[3], which can be combined with column chromatographic techniques. The aim of this paper is to show the possibilities and limitations in ultratrace analysis of the combination of centripetal TLC with GC.

EXPERIMENTAL

Thin-layer centripetal chromatography

Preparation of the layer. A glass disc (diameter 240.0 ± 0.05 mm; thickness 10.0 ± 0.02 mm), in the centre of which a glass filter (diameter 6 mm; thickness 4 mm; porosity 15–40 μm) was sealed with PTFE tape, was set into a circular opening (diameter 240.2 ± 0.05 mm) in an auxiliary glass plate (5000 × 3000 × 8 mm), which was equipped with three adjustable screws and with a level indicator. A 30:60 (w/w) aqueous suspension of Silica Gel G (E. Merck, Darmstadt, G.F.R.) was spread over the plane formed by the glass disc and the auxiliary glass plate. The thickness of the layer was levelled to 0.5 mm with a glass rod. The disc, removed from the opening in the auxiliary plate, was spanned along its circumference with

* Throughout this article the American (10^9) billion is meant.

a 17-mm wide stainless-steel wire gauze (2500 mesh/cm^2; mesh diameter 0.112 mm), and with a stainless-steel rim (thickness 0.5 mm) of the same dimensions. The layer, arranged in this way, was dried overnight at ambient temperature.

Dosing. A PTFE connector (Hamilton Co., Whittier, Calif., U.S.A., Part No. 86506) was fixed into a central circular opening (diameter 4 mm; depth 6 mm) on the reverse side of the disc. The whole system was installed on the horizontally rotating circular plate of the gear box of an electric motor, the revolution of which was adjusted in such a way that the resulting speed of rotation of the disc was 200 r.p.m.

A 200-ml volume of a model solution of lindane in light petroleum (concentration 5×10^{-10} g/ml) was applied on to the rotating layer at a distance of 1.5 cm from the edge of the disc through the thin capillary of an injector by means of a controlled excess pressure of gas. The amount of liquid flowing on to the layer was adjusted with a manostat in such a way that the solvent would evaporate rapidly from the layer during the application of the solution and thus not cause unwanted broadening of the sample circle on the rotating line of origin.

Development. The development of the plate is carried out in a circular stainless-steel chamber consisting of six compartments, which are separated from each other and arranged in a circle. These circular compartments of the chamber are used for gradient separations with the vapour of selected solvents. The disc, with a coated layer, is placed with the layer facing downwards in the developing chamber. The solvent rises owing to capillary action along the whole of the circumference of the disc between the steel wire gauze and rim and penetrates into the sorbent layer. Here it separates substances in the shape of concentrically separated zones. The solvent, containing the compounds under analysis, is then aspirated under reduced pressure from the centre of the disc through the glass filter and via a connecting capillary into a fraction collector. In our work, all of the inner segments of the developing chamber were connected to each other and the whole space was filled with benzene–hexane (85:15) as developing solvent.

General arrangement of the instrument and the procedure. A flow diagram of the whole centripetal chromatograph is shown in Fig. 1. The solvent is led by gas pressure (N_2) from a reservoir (3) through a capillary resistor (4) into the circular compartments of the developing chamber (5), thus balancing continuously the loss of the solvent which penetrates into the layer (6, top). The required level in the developing chamber could be adjusted with an adjustable overflow tube (7) through which superfluous solvent occasionally flows out. The process of collecting fractions under analysis and adjusting the reduced pressure manometrically is demonstrated on the right of Fig. 1. The flow-rate of the solvent through the layer was approximately constant in the first stage of the separation process and fluctuated around 1 cm/min. The velocity of the eluate being collected was 0.1 ml/min in the second stage of the separation process.

Lindane (Poly Science Corp., U.S.A., Analytical Standards Kit No. 51A) was used as a model compound. Approximately 4 ml of developing solvent were required for the elution of lindane from the layer. The solvent was aspirated from the centre of the disc into the fraction collector by means of a controlled reduced pressure produced with a water pump (20 mm Hg). The total concentration of lindane in this eluate was therefore 50 times greater than that in the solution originally deposited on the layer.

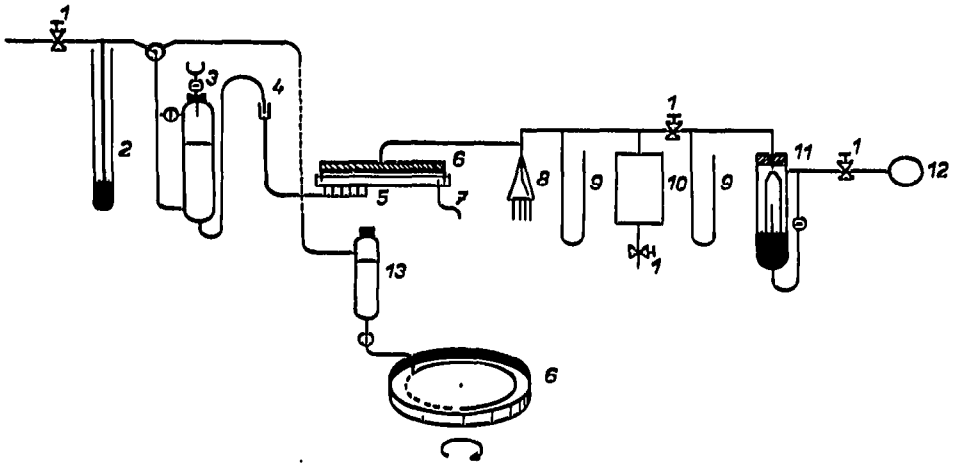

Fig. 1. Flow diagram of centripetal thin-layer chromatography. 1 = Needle valve; 2 = mercury manostat; 3 = solvent reservoir; 4 = capillary resistor; 5 = developing chamber; 6 = glass disc; 7 = overflow tube; 8 = sample collector; 9 = mercury manometer; 10 = vacuum buffering vessel; 11 = cartesian manostat; 12 = water jet pump; 13 = sample injector.

Gas chromatography

All of the chromatographic measurements were carried out on a Hewlett-Packard Model 402 gas chromatograph equipped with an electron-capture detector (^{63}Ni; radiation activity 2 mCi; pulse interval 150 μsec). A glass column, length 4 ft. and I.D. 3 mm, was packed with 3.8 % UCCW 98-HP on Chromosorb G, AW-DMCS, 80–100 mesh (Hewlett-Packard, U.S.A.). Technical-grade nitrogen (Technoplyn Ostrava, Czechoslovakia), which was not specially purified prior to use, was used as the carrier gas at a flow-rate of 40 ml/min. The temperature of the column and injection port was maintained at 200° and the detector temperature was 250°. A 5-μl volume of the mixture under analysis was injected into the column with a Hamilton 7005 syringe (Whittier, Calif., U.S.A.).

Determination of lindane in cabbage. The procedure was applied to the determination of lindane in an extract of cabbage from a trial field. The cabbage seedlings were first grown for 3 weeks in a soil containing lindane and then transplanted into

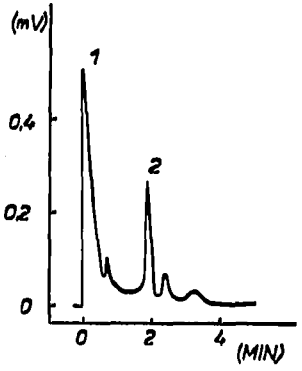

Fig. 2. Gas chromatogram of a lindane-rich fraction from centripetal thin-layer chromatography. 1 = Solvent; 2 = lindane fraction.

an uncontaminated soil and allowed to grow to a weight of 900 g. For analytical purposes, each plant was sorted into outer green leaves and inner leaves and each part was analyzed separately. The dried material was extracted with light petroleum[4]. The extracts obtained, each with a volume of 100 ml, were, without any preceding purification, applied on to the layer and analyzed under the conditions described above. A typical gas chromatogram of a lindane-enriched fraction of the eluate from the centripetal chromatograph is shown in Fig. 2. The residual amount of lindane per gram of dried material found in the outer leaves was 2.25×10^{-7} g and that found in the inner leaves was 4.3×10^{-9} g.

DISCUSSION

When both of these chromatographic techniques are combined, the properties of this non-classical arrangement of the thin layer become favourable. In the centripetal development of an analyzed compound, dilution of the compound does not occur during the chromatographic process, as is common in circular chromatography, and its concentration per unit of layer area in fact increases.

A long and rotating line of origin on the thin layer of the centripetal chromatograph enables large volumes of the solutions of analyzed compounds to be applied. The solvent, which is specially chosen, is evaporated during the rotation of the disc.

The reproducibility of separations in the centripetal chromatograph is determined by the preparation of the thin layer and by the constancy of withdrawal of the eluate from the centre of the layer. The presence of any inhomogeneity results in eccentricity of the separated rings. These distortions have an unfavourable influence on the simultaneous elution of the total amount of the analyzed compound.

The maximum sensitivity obtained by this method can easily be increased. It must be emphasized that our analyses were carried out under conditions that are available in any laboratory. The poured and dried layer of the centripetal chromatograph was not specially treated before analysis. The carrier gas was not purified to remove trace amounts of oxygen and water vapour, which decrease the sensitivity of the electron-capture detector.

The limiting sensitivity obtained under the conditions used was 0.1 p.p.b., which is acceptable for the lindane contents in the inner leaves of the cabbage examined (5 p.p.b.). The work under the special conditions of centripetal chromatography, with prior concentration of the extract, enables two additional orders of magnitude to be obtained so that a sensitivity limit 0.001 p.p.b. can be achieved with analytical precision.

CONCLUSION

The combination of flat-bed and column techniques, suggested in this paper and represented by thin-layer centripetal chromatography plus GC with an electron-capture detector, will be most useful for ultratrace analysis. The possibility of processing relatively large volumes of solvents containing minute amounts of compound to be analyzed, without prior purification, will permit the wider utilization of the technique in the analysis of extracts of biological materials.

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DISCUSSION

PROCHÁZKA: The high resolution of thin circular zones in ring chromatograms after the conventional radial (centrifugal) development has been attributed to the relatively slow flow-rate and low cross-section of the mobile phase at the periphery. As your centripetal method is the opposite of the usual "centrifugal" development, I wonder whether the resolution will not be blurred by the widening of the zones or excess of mobile solvent phase?

MARTINŮ: The method was devised mainly in order to separate low concentrations of high-R_F substances under analysis from the less mobile bulk components. Improved resolution is not claimed.

HAIS: Chromatography in which the sorbent bed is much wider near the origin than on the opposite side has been proposed several times, *e.g.*, by I. KRZECZKOWSKA of Lublin, in the form of "conical chromatography", or by LACOURT, in the form of triangular sheets. These earlier proposals need no mechanical rotating parts.

I wonder whether instead of GC, the effluent could not be immediately led on top of a "liquid" chromatography column?

JANÁK: On the first comment, although the rotating device makes VAN DIJK's technique a little expensive and delicate, the rotating arrangement itself makes possible the continuous sampling of large volumes of dilute solutions relatively quickly without greater broadening of the starting spot, a procedure which is quite difficult to perform by conical or other types of flat-bed chromatographic techniques. On the second comment, in my plenary lecture the application of flat-bed plus column techniques has been mentioned and a combination of TLC and liquid chromatographic techniques has been advocated. I am sure that positive results can be obtained from such couplings. Nevertheless, in our present problem of determining a halogenated pesticide, GC with an electron-capture detector is more convenient owing to the sensitivity, which is much higher than that of any of the present liquid chromatographic detectors.