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A SCREENING METHOD FOR ORGANOCHLORINE PESTICIDE RESIDUES USING THIN-LAYER CHROMATOGRAPHY*

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SUMMARY

A screening method is described for the analysis of organochlorine pesticides in fats and vegetables by semi-quantitative thin-layer chromatography. The sample is cleaned up by the dimethyl sulphoxide partitioning process, by chromatography on a small column of alumina, or by a combination of both these methods. Thin-layer chromatography is performed on Alumina G incorporating silver nitrate as a sensitive and selective visualising reagent, and residues estimated by comparison of spot sizes with those of standards. Residues as low as 0.01 p.p.m. can be estimated in some cases.

INTRODUCTION

Thin-layer chromatography has been used in this laboratory in the analysis of organochlorine pesticide residues mainly for confirmation of identity[1] or as a clean-up stage prior to gas chromatographic examination[2,3]. It has been used quantitatively for the analysis of herbicide residues[4,5] and organophosphorus pesticide residues[6]. The present work extends these methods to the determination of organochlorine pesticide residues in fats and vegetables. As in the case of the herbicide residue methods, determination is based on estimation of spot areas on the developed chromatoplates.

MORLEY AND CHIBA[7] have described the application of thin-layer chromatography, without preliminary clean-up, as a rapid screening method for organochlorine pesticide residues in wheat, apples and lettuce. For most types of sample, however, some purification of the sample extract is essential before proceeding to thin-layer chromatographic analysis. This is especially so in the case of fatty samples, for which a thorough clean-up process cannot be omitted without considerably reducing the sensitivity of detection and producing distorted spots with abnormal R_F values. Less rigorous clean-up processes would be necessary if a very sensitive and selective method were to be used for visualising the residues separated on the thin-layer plate. It would then suffice to take an aliquot of the sample extract equivalent to only a relatively

small amount of sample, resulting in interference from co-extractives being correspondingly less. By using the thin-layer chromatographic procedure described below, the clean-up processes were reduced to two simple procedures, elution from a column of prepared alumina, or a dimethyl sulphoxide (DMSO) partition method[8]. The latter method was used in preference to similar processes involving acetonitrile[9] or dimethylformamide[10] because it is much more effective in eliminating fat from samples.

The use of silica gel chromatoplates and a silver nitrate spray, with or without 2-phenoxyethanol, followed by irradiation with ultraviolet light, has long been established as a technique for the detection of organochlorine compounds on the chromatoplate. The process is not particularly sensitive—the detection limit is about 0.5 μg—and discoloration of large areas of the plates occurs unless precautions and care are taken. Coupled with the normal clean-up processes, which restrict the amount of extract that can be applied to the plate, the low levels of pesticides often encountered in routine residue analysis, below about 0.05 p.p.m., cannot be detected.

However, recent claims of detection limits of 5–20 ng (refs. 11–14) have shown that the thin-layer chromatographic method can be made much more sensitive. The reagent can either be applied as a spray or be incorporated into the slurry used in the preparation of the chromatoplates. KOVACS[11] claims that high sensitivity can be achieved with a spray of silver nitrate containing 2-phenoxyethanol, but all the other workers reporting good results[12–14], have preferred to incorporate the reagent into the adsorbent. Since this latter approach is more convenient and ought to give more reproducible results, it was adopted in the present work, and attention was directed towards finding other adsorbents that would give higher sensitivity with the silver nitrate technique, while permitting less rigorous clean-up processes to be used.

The sensitivity obtainable with the silver nitrate on Silica Gel G is limited by the fact that the treated adsorbent rapidly darkens during irradiation, presumably because of the presence of impurities. KOVACS[11] claims good results with a highly purified grade of silica gel, Silica Gel G-HR, and in the present work similar results have been obtained using a related adsorbent, Silica Gel HR. The purified adsorbent darkened only slowly during the irradiation process and, by the use of prolonged exposures, 10 ng of most organochlorine pesticides could be detected. γ-BHC behaved exceptionally in that spots were slow to develop and the limit of detection was about 100 ng. Another disadvantage was that chromatoplates of Silica Gel HR containing silver nitrate turned appreciably brown in about a day after preparation.

Much better results were obtained with Alumina G, as chromatoplates composed of this adsorbent and silver nitrate showed little tendency to darken either during storage or the irradiation process. The detection limit was 10 ng for all pesticides although spots of γ-BHC were again particularly slow to develop.

FEHRINGER AND OGGER[12] claim that the sensitivity of silver nitrate as a visualising agent on alumina is markedly improved by pre-washing the adsorbent with very dilute nitric acid and incorporating hydroquinone into the layer. However, we did not find these measures helpful, and the presence of the hydroquinone proved disadvantageous in that the darkening of the chromatoplates during storage was considerably accelerated.

METHOD

Apparatus

Top-drive macerator, for analysis of milk and vegetables.

Centrifuge with 250-ml bottles, for analysis of milk and vegetables.

Chromatographic columns of internal diameter about 7 mm.

Kuderna-Danish evaporator[15] with a volume of 100 ml.

Collection tube for above evaporator, of volume about 6 ml and having a tapered bottom with a 0.5-ml graduation mark.

Micro-Snyder column[16] to fit collection tube.

Glass tubes of approximate dimensions 27 × 3.5 mm I.D. and graduated to 0.05 ml.

Thin-layer chromatographic equipment.

Pipettes graduated to 2 μl. "Microcaps" from the Drummond Scientific Company (U.S.A.) are very suitable.

Source of U.V. light: 2 Phillips 15-W TUV lamps in a reflector are suitable

Reagents

Hexane, reagent grade.

Acetone, general purpose reagent.

Dimethyl sulphoxide, reagent grade.

Sodium sulphate, anhydrous, granular.

Sodium sulphate, 2% (w/v) aqueous solution.

Prepared alumina, dry at 500° for 4 h, cool and add 5% water.

Silver nitrate, 0.4% (w/v) aqueous solution.

Alumina G for TLC, supplied by E. Merck (Darmstadt) is suitable.

Evaporation of solutions

Solutions may be concentrated to about 4 ml in a Kuderna-Danish evaporator[15] fitted with a splash head and heated on a steam bath. Concentration may be continued down to about 0.25 ml by replacing the Kuderna-Danish evaporator on the collection tube with a micro-Snyder column[16]. An anti-bumping granule is essential at this stage to permit rapid evaporation while avoiding losses. To concentrate to 0.05 ml, transfer the solution from the collection tube to a small graduated tube and warm on a water bath in a gentle stream of air.

Dimethyl sulphoxide clean-up

Extract the sample extract with 3 portions of 20 ml of DMSO. Wash the combined DMSO extracts with 10 ml of hexane and back-extract the hexane wash with 10 ml of DMSO. Add the combined DMSO extracts to 200 ml of 2% aqueous sodium sulphate solution and extract the mixture with 2 portions of 10 ml of hexane. Dry the combined hexane extracts briefly with anhydrous sodium sulphate and concentrate the solution to 0.5 ml.

Clean-up by chromatography on alumina

Partially fill a chromatographic column with hexane and push a small plug of cotton wool (about 25 mg) through the liquid to the bottom of the column. Dust in 2 g of prepared alumina through a funnel and, when this has settled, add a small

amount of sand as a protective layer. Drain the hexane until the level falls to that of the sand and add the sample extract in about 1 ml of hexane. Elute with hexane and collect 20 ml of eluate in a Kuderna-Danish evaporator. Concentrate the eluate to 0.5 ml or 0.05 ml according to the level of residues being sought.

Extraction and clean-up of samples for analysis of residues down to about 0.1 p.p.m.

Butter fat. Perform the DMSO clean-up on a solution of 25 g of butter fat in 15 ml of hexane.

Milk. Macerate 25 ml of milk with 10 ml of acetone and 20 ml of hexane, centrifuge and draw off the upper layer. Repeat the extraction twice with further 20-ml portions of hexane. Combine the extracts, dry with anhydrous sodium sulphate and concentrate to about 10 ml. Carry out the DMSO clean-up on this solution, using one-half of the volumes quoted above, and concentrate the final solution to 0.5 ml.

Mutton fat. Grind 50 g of chopped mutton fat with 25 g of sand, add about 100 ml of hexane and warm on a steam bath until the fat dissolves. Decant the hexane and wash the residue with more hot solvent. Make the extract and washings up to 200 ml at 20°, and place in a refrigerator at 4° for 1 h to precipitate some of the fat. Withdraw 100 ml of the supernatant liquor and subject it to the DMSO clean-up, using double the volumes quoted above. Concentrate the final solution to 0.5 ml.

Carrots, onions, and peas. Macerate 25 g of vegetables with a mixture of 10 ml of acetone and 20 ml of hexane, centrifuge and decant the upper solvent phase. Repeat the extraction twice with further 20-ml portions of hexane. Combine the hexane extracts, dry with anhydrous sodium sulphate and concentrate to about 1 ml. Carry out the prepared alumina clean-up described above and concentrate the final eluate to 0.5 ml.

Further clean-up of samples for analysis of residues down to about 0.01 p.p.m.

Butter fat, milk, and mutton fat. Subject the hexane solution obtained from the DMSO treatment to the further clean-up on prepared alumina, concentrating the eluate to 0.05 ml.

Carrots, and onions. Concentrate the eluate from the alumina clean-up to 0.05 ml. It is necessary to draw off the solution from a precipitate which forms during this evaporation.

Peas. Concentrate the eluate from the column of prepared alumina to about 5 ml and extract it with 3 portions of 5 ml of DMSO. Add the extracts to 100 ml of 2% sodium sulphate solution and extract the mixture with 2 portions of 5 ml of hexane. Dry the combined extracts with anhydrous sodium sulphate and concentrate the solution to 0.05 ml.

TLC analysis of sample extracts

Prepare 250-μ thick layers of Alumina G from a slurry formed by shaking 30 g of adsorbent with 45 ml of 0.4% (w/v) aqueous silver nitrate solution for 2 min. Activate the layer by heating the prepared chromatoplates in an oven at 100° for 2 h. Divide the layer into tracks by scribing lines parallel to one edge and 3 cm apart; scribe another line at right angles to the others and 12 cm from one edge so as to limit the distance travelled by the solvent front. Spot the sample solutions, one sample per track, 10 cm below this last line as single applications of 2 μl. Also apply a standard

mixture of pesticides at several levels in the 10–100 ng range, again using only single applications of 2 μl. This range of standards corresponds to residue concentrations, in the samples, of 0.1–1.0 p.p.m. or 0.01–0.1 p.p.m. according to whether the final bulk of the sample solution after clean-up was 0.5 or 0.05 ml. Develop the chromatogram with hexane.

To reveal the organochlorine pesticides, irradiate with strong U.V. light for up to 20 min, occasionally exposing the adsorbent to water vapour from a steam bath to assist development of the spots[13].

Characterize each spot, shown by the sample solution, by comparison with the R_F values given by the standard pesticides. In nearly all cases, interference from co-extracted material should be so small as to allow very close correlation of R_F values. The weight of residue in each spot should be estimated by comparison of its area with those of the corresponding standards, ignoring any difference in spot intensity. Calculate the level of each residue in the original sample in p.p.m. by dividing the weight of pesticide contained in the corresponding standard spot, expressed in ng, by 100 or 1000, according to whether the final bulk of the sample extract after clean-up was 0.5 ml or 0.05 ml.

TABLE I

R_F VALUES OF SOME ORGANOCHLORINE COMPOUNDS

Adsorbent, Alumina G (250 μ), incorporating silver nitrate as visualising agent; developing solvent, hexane; temperature, 20°.

Compound	R_F *value*
Aldrin	0.70
p,p'-DDE	0.68
Heptachlor	0.65
p,p'-DDT	0.52
α-BHC	0.29
p,p'-TDE	0.25
γ-BHC	0.24
Heptachlor epoxide	0.15
Endrin	0.14
Dieldrin	0.11
β-BHC	0.04

RESULTS AND DISCUSSION

The R_F values of some organochlorine pesticides on Alumina G with hexane as developing solvent are shown in Table I. This system seems to be the most generally useful for the thin-layer chromatography of small amounts of these pesticides, although as can be seen, some compounds are not resolved by it. γ-BHC and p,p'-TDE can be separated if a silicone oil is added to the alumina but this drastically reduces the sensitivity. Excellent separation of these two pesticides is possible on Silica Gel HR with hexane as developing solvent, but again sensitivity to γ-BHC is much poorer than on Alumina G. Heptachlor and p,p'-DDE can be resolved satisfactorily on Alumina G containing dimethylformamide by developing with "isooctane" (2,2,4-trimethylpentane)[17].

The usual linear relationship was observed between the square root of spot area and the logarithm of the weight of pesticide in the spot[4,5,18]. Quantitative results can be obtained by application of this relationship but a calibration graph is required for each chromatoplate. Because this necessarily lengthens the procedure, the method is not suitable for screening numerous samples. A simpler procedure was therefore adopted in which quantities present were estimated by visual comparison of spot areas.

The proposed methods were tested by experiments in which samples were spiked with a mixture of γ-BHC, p,p'-DDE, p,p'-DDT, and dieldrin at 0.5 p.p.m. and 0.05 p.p.m. The R_F values of the recovered pesticides always corresponded closely with those of the standard compounds. Recoveries as judged visually by spot sizes were usually in the range of 80–100% but were about 50% for some pesticides in the case of carrots and onions. The reason for these low recoveries is that coextractives streaked across the lower part of the chromatoplate and interfered with the development of spots from pesticides with low R_F values. Apparently, carotenes from carrots and organic sulphides from onions were the main source of interference. Repeated application of the clean-up methods showed that the interfering substances could not be separated from the pesticides by these means. A yellow oil in the pea extract could not be separated from the pesticides by chromatography on an alumina column, but fortunately this substance did not significantly interfere with the analysis of pesticides down to 0.1 p.p.m. and was readily eliminated by a simplified version of the DMSO clean-up procedure, to enable residues down to 0.01 p.p.m. to be estimated.

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