Journal of Chromatography, 135 (1977) 235–240

CHROM. 9880

Note

Separation, detection and densitometric determination of chlorinated insecticides on silica gel and aluminum hydroxide papers

JOSEPH SHERMA and KEITH BLOOMER

Chemistry Department, Lafayette College, Easton, Pa. 18042 (U.S.A.)

(First received November 5th, 1976; revised manuscript received December 9th, 1976)

Silica gel and aluminum oxide layers are most widely used for the separation and detection[1–5] and *in situ* quantitation[6,7] of chlorinated insecticides, and pesticides in general, by thin-layer chromatography (TLC). Because commercial chromatography papers loaded with silica gel and aluminum oxide are simple and convenient to use and much less expensive than commercial pre-coated layers or hand-coated plates[8], a comparative evaluation of the chromatographic analysis of a group of important chlorinated insecticides on these papers and thin layers was undertaken. Separations are in general better by TLC than by paper chromatography (PC), but in some systems the results are virtually identical. Although differences in R_F values are observed, sequences of compounds are usually the same. Chromogenic detection by a modified silver nitrate–UV method is equally sensitive on papers and layers. The successful use of silica gel paper for the densitometric determination of pesticides is demonstrated by analysis of a fortified natural water sample.

We believe this to be the first evaluation of silica gel and aluminum oxide chromatography papers for pesticide analysis and for the densitometry of any type of compound.

EXPERIMENTAL

The chlorinated insecticides studied included α-BHC, *p',p'*-DDD, *o,p'*-DDT, aldrin, dieldrin, endrin and methoxychlor. Standard solutions of each pesticide were prepared by dissolving standards, received from the USEPA repository, in pesticide-grade *n*-hexane at a level of 100 ng/μl for spotting with a 1-μl Drummond microcap pipette. Solvent was allowed to evaporate between multiple applications to the same origin, so that all initial zones were of similar size.

Quantum Q-3 pre-coated aluminum oxide thin layers and Q-4, Q-5 and Analtech Uniplate pre-coated silica gel layers (20 × 20 cm) were tested and compared with 20 × 20 cm sheets of the impregnated cellulose papers Whatman AH-81 (7.5% Al_2O_3) and SG-81 (22% SiO_2). The layers and papers, as received from the manufacturer, were impregnated with silver nitrate chromogenic reagent prior to spotting of the sample.

The stock chromogenic solution contained 20 g of silver nitrate in 100 ml of distilled water, while the dipping solution included 4 ml of stock solution, 100 ml of

acetone, 10 ml of water, 6 ml of concentrated ammonia and 8 drops of phenoxyethanol. The dipping solution was mixed immediately before use. The paper or layer was dipped into the solution (in a Thomas–Mitchell dip tank for the layer or a shallow glass dish for the paper) for 60 sec and then dried in a dark hood with the aid of a stream of cool air from a hair dryer for up to 5 min.

The spots were applied at once, and ascending development of layers was carried out with an appropriate solvent contained in the bottom of a paper-lined saturated rectangular TLC tank. Papers were fastened with tounged plastic clips into a cylindrical shape and developed in a round Shandon Unikit tank in a similar manner. The tanks were placed in a dark cabinet during development to preclude darkening of the paper from exposure to light. After a 10-cm distance of development past the origin (12.5-cm total), the chromatogram was dried in the dark with a hair dryer, and exposed at once to ultraviolet light from a Hanovia No. 679A 450-W mercury vapor lamp, placed 10 in. above the chromatogram. Layers were exposed for 5 min and papers for 1 min on the front, 1 min on the back and finally a further 1–2 min on the front. Spots began to form within 1 min of initial exposure and were finally black-grey against a light-brown background.

For quantitation, layers were covered with a clean glass plate and paper chromatograms were sandwiched between two clean glass plates, and scanning was performed at once with a Kontes Chromaflex fiber-optics densitometer in the double-beam mode using the visible wavelengths emitted by the long UV source. The densitometer was equipped with a Kontes baseline corrector and a Bausch and Lomb VOM 6 recorder. Recorder chart peaks were photocopied, the copies cut out and weighed, and calibration graphs plotted as weight in grams times attenuation setting *versus* nanograms of pesticide spotted. Details of the proper use of the Kontes scanner have been previously published[7,9–11].

Water was collected from a small creek flowing into the Delaware River near Easton, Pa., and 500 ml was fortified to 10 ppb with *o,p'*-DDT by adding 1 ml of *n*-hexane containing 5000 ng of the pesticide. The sample was shaken well to dissolve the pesticide. The spiked water was extracted with three 25-ml volumes of pesticide grade *n*-hexane in a separatory funnel, and the combined extracts were filtered through Whatman PS phase separating paper to remove any traces of water and collected in a beaker. The extract was evaporated just to dryness on a warm hot-plate, transferred with minimum washing with *n*-hexane into a calibrated micro-evaporative concentrator tube, and evaporated just to dryness under a stream of nitrogen. The residue was dissolved in 0.10 ml of *n*-hexane and a 10-μl aliquot was applied, using a 2-μl microcap, to silica gel paper together with 300-, 500- and 700-ng standard spots for densitometric evaluation.

RESULTS AND DISCUSSION

Pre-dipping layers and papers as described above was found far superior to either dipping or spraying with the silver nitrate reagent after chromatographic development. Analtech and Q-4 gypsum-bound silica gel layers were essentially equivalent and provided more sensitive detection of the pesticides than did the organic-bound Q-5 silica gel or Q-3 aluminum oxide layers. Silica gel paper provided better detection than aluminum oxide paper. An amount of 100 ng was easily detected

on silica gel G layers and silica gel paper, 100 ng was only weakly detectable on aluminum oxide papers, and minimum detection was 400 ng on aluminum oxide layers. The background color was light golden brown in all cases. Developed zones were somewhat more compact and well defined on layers compared with their corresponding papers, but differences in the darkness of the spots was the main determinant of detection sensitivity. Solvent development times were about 20 min for both papers and silica gel layers and 35 min for the aluminum oxide layers.

Table I shows R_F values relative to the compound *p,p'*-DDD (R_{DDD}) for six chlorinated insecticides in five solvents commonly used for the TLC analysis of these compounds. Absolute R_F values for DDD are included.

TABLE I

R_{DDD} VALUES* FOR PESTICIDES ON SILICA GEL AND ALUMINUM OXIDE LAYERS AND PAPERS

*System***	*DDD****	*DDT*	*BHC*	*Aldrin*	*Dieldrin*	*Endrin*	*Methoxychlor*
A-1	0.13	2.3	1.2	3.2	0.31	0.38	0.0
A-2	0.23	1.7	1.2	2.2	0.87	1.2	0.57
A-3	0.39	1.4	1.0	1.5	0.87	0.97	0.72
A-4	0.57	1.0	0.93	1.1	0.53	0.67	0.47
A-5	0.69	0.97	0.96	0.94	0.96	0.99	0.96
B-1	0.10	2.6	1.4	3.0	0.50	0.90	0.40
B-2	0.15	2.0	1.3	2.3	0.80	1.2	0.67
B-3	0.25	1.6	1.2	1.8	0.88	1.2	0.92
B-4	0.48	1.1	0.94	1.0	0.65	0.73	0.71
B-5	0.57	1.0	0.98	0.98	1.0	0.98	1.0
C-1	0.53	1.2	1.0	1.3	1.0	1.0	0.84
C-2	0.53	1.2	1.0	1.3	0.96	1.0	0.75
C-3	0.52	1.2	1.0	1.3	0.94	0.98	0.77
C-4	0.70	1.0	0.97	1.0	0.94	0.96	0.94
C-5	0.67	1.0	1.0	1.0	1.0	1.0	1.0
D-1	0.44	1.4	1.2	1.4	0.89	1.1	0.72
D-2	0.55	1.2	1.0	1.2	1.0	1.0	0.89
D-3	0.56	1.1	1.0	1.2	1.0	1.0	0.93
D-4	0.66	1.0	1.0	1.0	1.0	1.0	1.0
D-5	0.62	1.0	0.95	0.95	0.98	0.97	0.97

* R_F relative to *p,p'*-DDD.

** Letters designate the stationary phase: A = silica gel layer; B = aluminum oxide layer; C = silica gel paper; D = aluminum oxide paper. Numbers designate the chromatographic solvent: 1 = *n*-heptane; 2 = 2% acetone in *n*-heptane; 3 = 1% methanol in *n*-hexane; 4 = 50% benzene in *n*-hexane; 5 = 10% ethanol in benzene.

*** Absolute R_F value.

In general, there was a greater variation in R_{DDD} with change in solvent for a given pesticide on a layer compared with the corresponding paper. In some cases, however, such as dieldrin on the aluminum oxide media or α-BHC on silica gel, there was little difference in the patterns. Likewise, in general, there was greater resolution of the pesticides by a given solvent by TLC than by corresponding PC (*e.g.*, with *n*-heptane), but again in some systems (1% *n*-hexane in methanol, silica gel papers and layers) the differences were minor. The best separations on all media were with

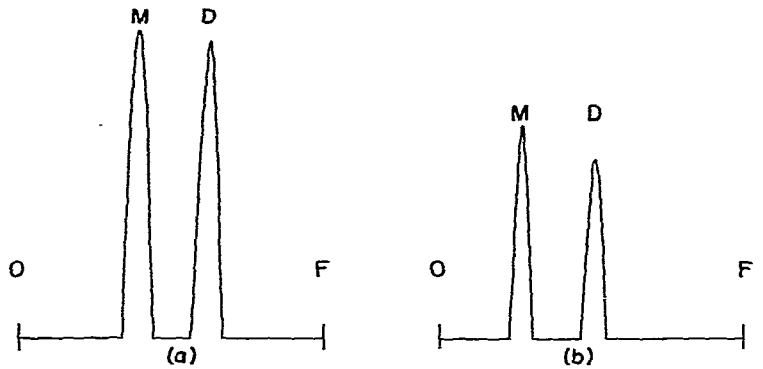

Fig. 1. Densitometer scans of a mixture containing 500 ng each of methoxychlor (M) and DDT (D) resolved on silica gel paper (a) and a silica gel thin layer (b). O is the origin of each chromatogram and F is the solvent front. In both cases, the scan speed of the chromatogram was 10 cm/min, recorder chart speed 5 in./min and attenuation ×50.

n-heptane and 2% acetone in *n*-heptane. Small differences were observed between separations on silica gel layers compared with aluminum oxide layers and silica gel papers compared with alumina papers. The choice here should be based on detection efficiency as described earlier.

To illustrate relative resolution and efficiency on silica gel papers and layers, Fig. 1 shows scans of 500 ng each of methoxychlor and DDT standards separated on the two media by development with 2% acetone in *n*-hexane. The major differences evident from these scans are the more narrow spots, lower R_F values and slightly better resolution on the layer. The R_F values and lengths and widths of the actual spots were as follows: on paper, methoxychlor R_F 0.39, 0.95 cm, 0.55 cm and DDT 0.62, 0.90 cm, 0.91 cm; on the layer, methoxychlor 0.26, 0.52 cm, 0.42 cm and DDT 0.50, 0.64 cm, 0.64 cm. Plate numbers calculated according to the usual equation

$$N = 16\left(\frac{V_R}{W}\right)^2$$

where V_R cm is the distance on the recorder chart from the origin to the peak center and W cm is the width of the peak were 255 for methoxychlor and 621 for DDT on paper and 211 and 539, respectively, on the layer. The higher numbers for the paper reflect the effect of the higher absolute R_F values (higher V_R) of the pesticides on the calculation method and obviously (see Fig. 1) do not indicate higher efficiency for the paper. The larger peak areas (Fig. 1) obtained on the paper for 500 ng of each of the pesticides is due to the greater slope of the calibration graph compared with the thin layer (see below). The higher slope but lower *y*-intercept on paper means that very low pesticide levels will result in greater peak areas from layers, but that higher levels will result in greater areas from paper chromatograms.

That the added adsorbent was mainly responsible for results on the papers was proved by developing the pesticides on Whatman No. 1 pure cellulose paper with several different solvents. No separations were achieved, and zones were diffuse and tailed. However, differences between loaded papers and thin layers arise mainly

from the presence of cellulose in the former and their fibrous nature[12]. Although absolute R_F values differ between loaded papers and layers, sequences of migration are almost always the same. It is concluded that when maximum resolution is required, such as in screening an unknown sample extract, layers should be chosen rather than paper. When the problem of separation is not foremost, as might be the case in routine screening or quantitation for a certain pesticide in less complex samples, PC may be a valid alternative to TLC. The difference in R_F for a given compound between papers and layers can be used as an aid in identifying unknown pesticides by developing a sample in several solvents on both types of supports.

The ability to quantitate residues by densitometry on silica gel paper and comparisons with silica gel layers were studied. Triplicate generation of calibration graphs on paper yielded the following averages as determined by computer analysis of data: slope, 0.0026; y-intercept, 0.019; linearity constant (r), 0.993 (Fig. 2). These values can be compared with the following values on thin layers: slope, 0.0012; y-intercept, 0.11; r, 0.961. To test reproducibility, six initial zones containing 500 ng each of DDT were spotted on paper, and the relative standard deviation of the areas of the scanned

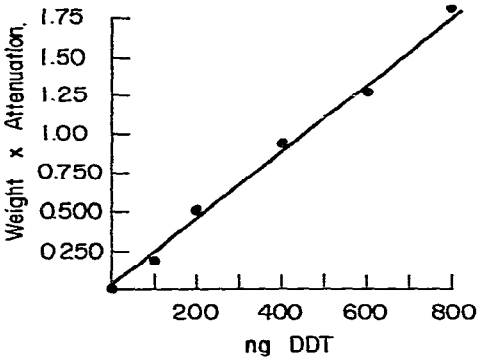

Fig. 2. Typical calibration graph for 100–800 ng of *o,p'*-DDT on paper loaded with silica gel.

spots was 11%. A natural water sample was fortified with 10 ppb of DDT and analyzed directly without clean-up of the extract. A recovery of 81% was obtained by interpolation of the area of the sample scan from a calibration graph constructed from standards developed on the same chromatogram. Except for a faint dark residue at the origin, no other spots appeared in the extract. These results, which were not optimized in terms of extraction or work-up of the sample, plus the excellent detection sensitivity described earlier, indicate that silica gel papers are equally as suitable as silica gel thin layers[6,7] for the densitometry of chlorinated pesticides at residue levels. The same quantitative techniques can be used for analysis of samples other than water after extraction of the compounds of interest and any necessary clean-up of the extract prior to spotting on the paper.

ACKNOWLEDGEMENT

The authors are indebted to Mr. Eric Nyberg of Kontes, Inc., for support and encouragement during this research.

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