Journal of Chromatography, 83 (1973) 439–446

CHROM. 6807

THE POTENTIAL USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN RESIDUE ANALYSIS

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SUMMARY

High-performance liquid chromatography is introduced as a new procedure for the determination of pesticides or pharmaceutically active ingredients in plant or animal materials. The most important factors influencing the efficiency of a residue method, *e.g.* detection limit, reproducibility and linearity, are comparable to those of gas chromatography, if suitable column packings and sensitive detectors are used. Moreover, liquid chromatography has the advantage that less volatile substances and substances that decompose rapidly at high temperatures can be detected. The exact identification of residues using more than a single analytical method will become more important in the future. Thus, liquid chromatography can also find its place in "confirmatory analysis". The results of experimental investigations with pesticides confirm that high-performance liquid chromatography can be regarded as an alternative or a supplementary method to conventional methods such as gas chromatography.

INTRODUCTION

In the analysis of pesticides, there are numerous methods of detection and determination, the most important of which are colorimetry and gas chromatography (GC). In recent years, special detectors have been developed for the GC detection of insecticidal chlorinated hydrocarbons and phosphoric acid esters. With an electron capture detector and a phosphorus detector, respectively, these compounds can be detected with a 10- to 1000-fold better sensitivity than with the commonly used flame ionization detector. Gas chromatography, however, is not applicable to substances with very high boiling-points and for compounds that undergo thermal decomposition.

Furthermore, in pesticide analysis it is desirable to have more than one method for the identification of an active constituent. As mentioned in detail later, liquid chromatography (LC) may be a very valuable supplement to gas chromatography.

The most important parameters that characterize a quantitative residue method are:

(1) *Detection limit* —which generally should be better than 0.1 ppm with respect to the plant material tested.

(2) *Reproducibility* —which can be expressed in terms of the standard deviation. This term includes the extraction and clean-up procedure as well as the characteristics of the determination method.
(3) *Recovery* —which is measured after fortifying untreated samples of biological material with the active constituent, and subsequent extraction, clean-up and determination.

EXPERIMENTAL AND RESULTS

In our experiments in applying liquid chromatography to pesticide analysis, we used two commercially available chromatographs. We used monochromator detectors for the ultraviolet and visible spectral ranges modified for high-performance liquid chromatography. In all cases the column packing was a new support developed by E. Merck (Darmstadt, G.F.R.). This adsorbent, Perisorb® A, is a porous-layer bead which may be used both in adsorption and partition chromatography.

Table I shows that for the insecticide DDT and the herbicide 2,4-D methyl esters, for example, the detection limit in gas–liquid chromatography using an electron capture detector is about 100–1000 times better than in liquid chromatography, using a photometric detector.

TABLE I

SENSITIVITY IN GAS AND LIQUID CHROMATOGRAPHY

Same conditions of noise and same sample volumes.

Substance	*Relative detector response, GLC:LC*
DDT	~1000:1
2,4-D ester	~ 100:1

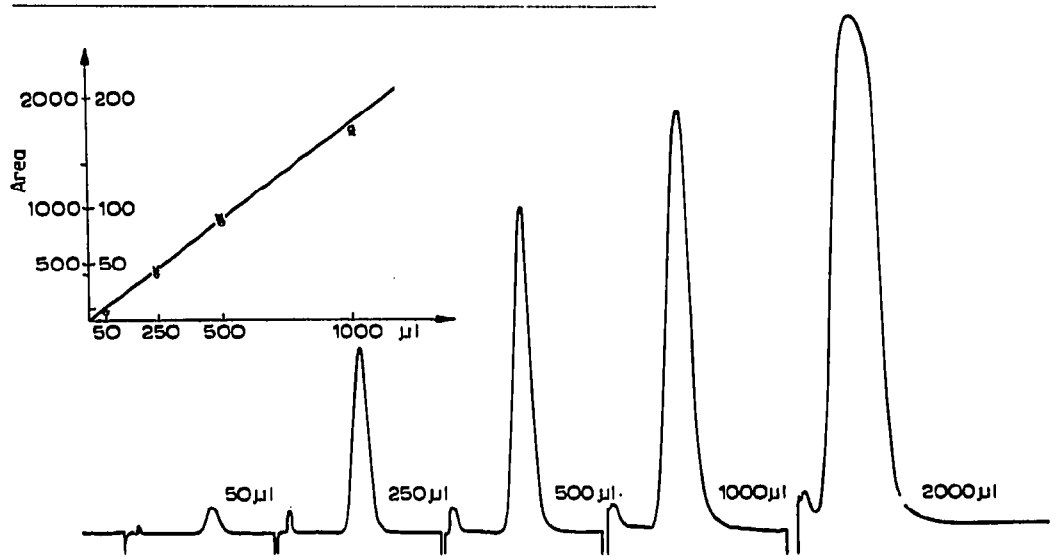

Fig. 1. Detector response as a function of sample volume. Sample: dithianone (1,4-dithiaanthroquinone-dicarbonitrile-2,3) at two concentrations (×, 10^{-9} and ○, 10^{-8} g/µl). Column: Perisorb® A; 500 × 2 mm. Detector: Zeiss PMQ II; 254 nm, modified for column chromatography.

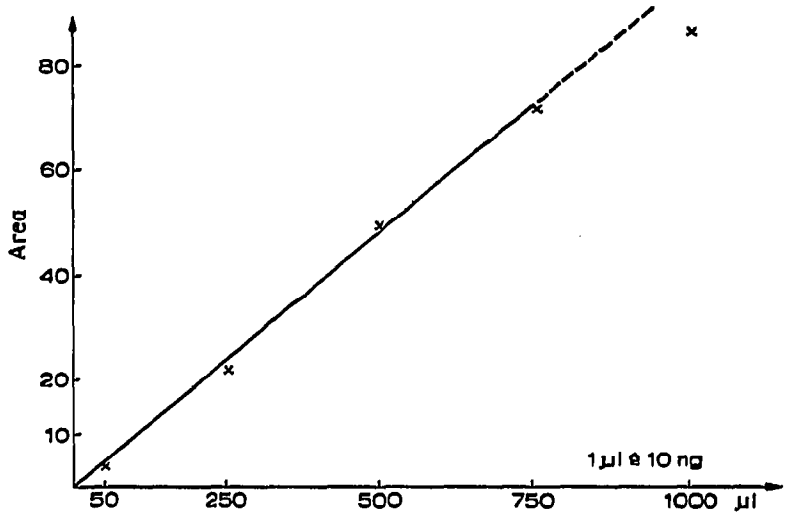

Fig. 2. Detector response as a function of sample volume, with MCPA as the sample. Column: Perisorb® A; 1500 × 2 mm. Mobile phase: *n*-hexane–acetic acid (92.5:7.5).

In order to obtain better detectability in liquid chromatography, more sensitive detectors can theoretically be constructed, but up to now none are commercially available. Another method to improve the detectability is to inject much larger volumes than usual[1]. As Fig. 1 shows, by using a modified preparative Hewlett-Packard (formerly Hupe and Busch) valve it was possible to inject from 50 µl to 1500 µl of a solution of the fungicide dithianone without any loss in linearity or peak symmetry, although a low capacity support was being used. With a volume of 2000 µl, however, the calibration curve became non-linear.

The injection of a high volume is possible if the substance is dissolved in the mobile phase of the chromatographic system. In this case, the substance is relatively strongly retained at the top of the column filling. After this concentration step, the chromatography proceeds according to the equilibrium of adsorption and desorption in the system slected.

With the aid of injections of large volumes the detection limit of dithianone, for example, was 100–200 times better compared with that with the usual 5- or 10-µl injection.

A second example with the herbicide methylchlorophenoxyacetic acid in partition chromatography gave exact linearity up to 750 µl, as shown in Fig. 2. Standard deviations of eight repeated injections with six different volumes were in the range ± 1.5%. With normal syringes injecting volumes of 5 or 10 µl, the standard deviation was higher, *viz.*, 3–8%.

Since in liquid chromatography it is possible to apply large volumes, the detectability becomes comparable with that in gas chromatography, because in gas chromatography the injection volume is much more limited. Defining the detection limit as double the noise level, we obtained absolute amounts of about 5 ng as the minimum detectable amount. This is shown in Fig. 3 as an example for dithianone, which cannot be determined by gas chromatography. In Table II, the detection limits for other pesticides, for example, DDT, methylchlorophenoxyacetic acid and

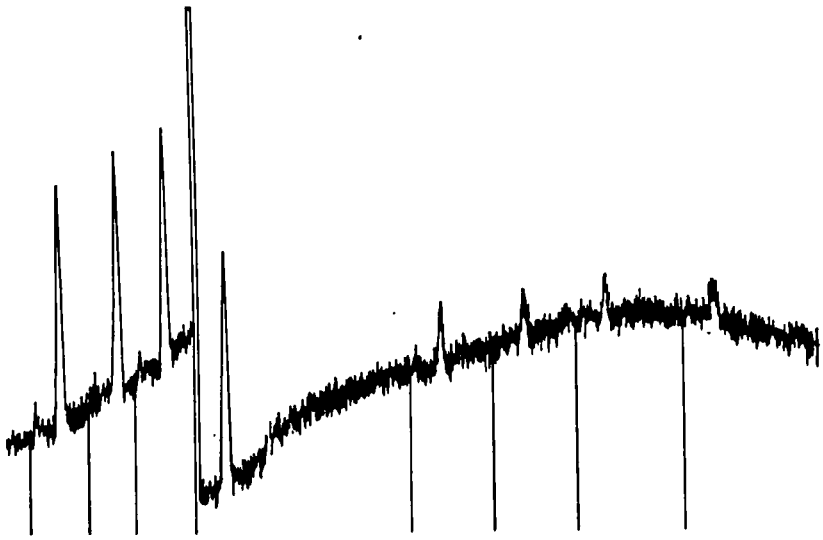

Fig. 3. Minimum detectable sample. Left: 50 ng of dithianone injected; right: 5 ng of dithianone injected. Instrument: Siemens S200 P with PM 4-CHR as detector. Column: Perisorb® A; 500 × 3 mm. Mobile phase: *n*-heptane–ethyl acetate (95:5).

TABLE II

MINIMUM DETECTABLE SAMPLE FOR SOME PESTICIDES IN LIQUID CHROMATOGRAPHY

Pesticide	*Minimum detectable amount (ng)*	*Detector*	
		Type	*Wavelength (nm)*
p,p'-DDT	~5	Zeiss, PMQ II	210
Dithianone	~5	PM 4-CHR	254
MCPA	~5	PM 4-CHR	278
2,4-D	~5	PM 4-CHR	278
PCB (Chlophen A 40)	~1	Zeiss, PMQ II	210

2,4-dichlorophenoxyacetic acid, are also shown to be 5 ng in liquid chromatography.

As far as the second parameter of a pesticide analysis method is concerned, *i.e.*, the linearity, we found that DDT could be determined within a concentration range of three orders of magnitude without any loss in linearity. Amounts of 0.01–10 μg of DDT in 10 μl of solution were injected (Fig. 4). In general, the linearity is limited by the optical density and not by the column.

For residue analysis, it is necessary to separate the pesticide from interfering substances of biological origin before determination. Fig. 5 shows the separation of dithianone residues on apples. Prior to the analysis, the biological material was extracted and purified according to a colorimetric method described by Sieper and Pies[2]. Using liquid chromatography, the determination includes a further effective purification step. The chromatogram of a blank extract of apples illustrated that there was

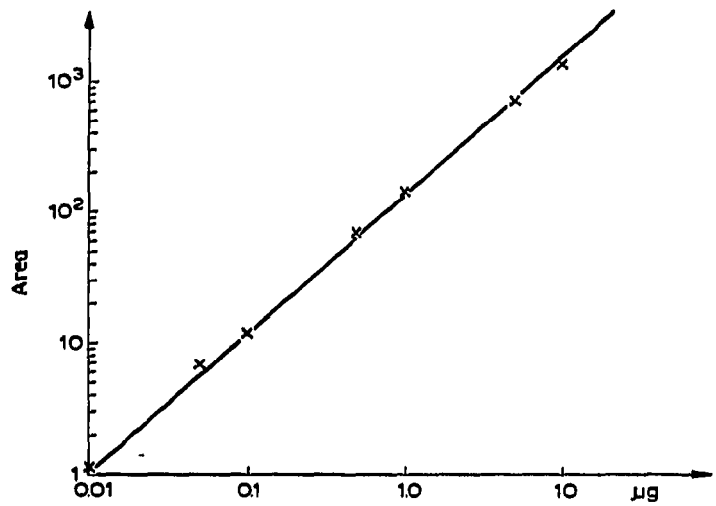

Fig. 4. Linearity with increasing amounts of DDT. Detector: Zeiss PMQ II; 210 nm. Column: Perisorb® A; 500 × 2 mm. Injection volume: 10 µl.

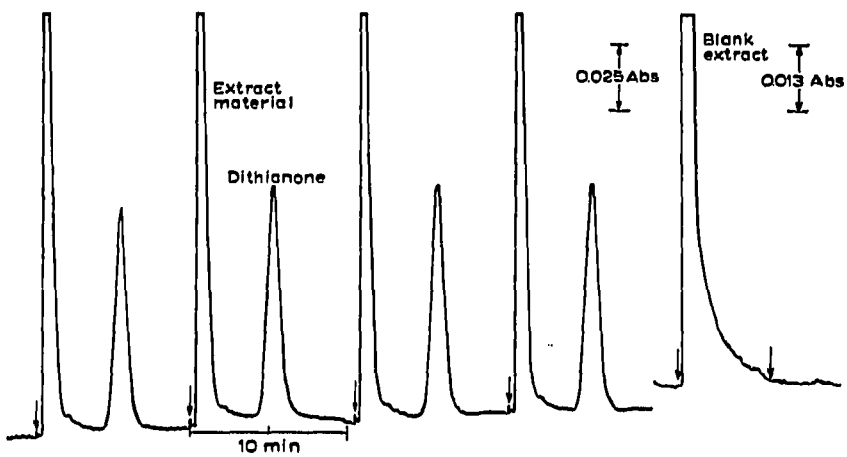

Fig. 5. Separation of dithianone from biological material. Column: Perisorb® A; 500 × 2 mm. Detector: Zeiss PMQ II; 254 nm. Mobile phase: *n*-heptane–ethyl acetate (96.5:3.5). Injection volume: 250 µl. Right-hand peak: Blank extract without dithianone, detected with double the sensitivity of that in the left-hand chromatograms.

no response to the retention time of dithianone, even at higher sensitivity of the detector.

With apples fortified with 0.1 and 0.02 ppm of dithianone, we obtained recoveries of about 80% by means of standard curves with external standards. Repeated analyses resulted in a standard deviation of about 10% over the whole procedure (Fig. 6).

In the introduction, we mentioned that in many instances gas chromatography is not applicable to residue analysis if the compounds are non-volatile or do under-

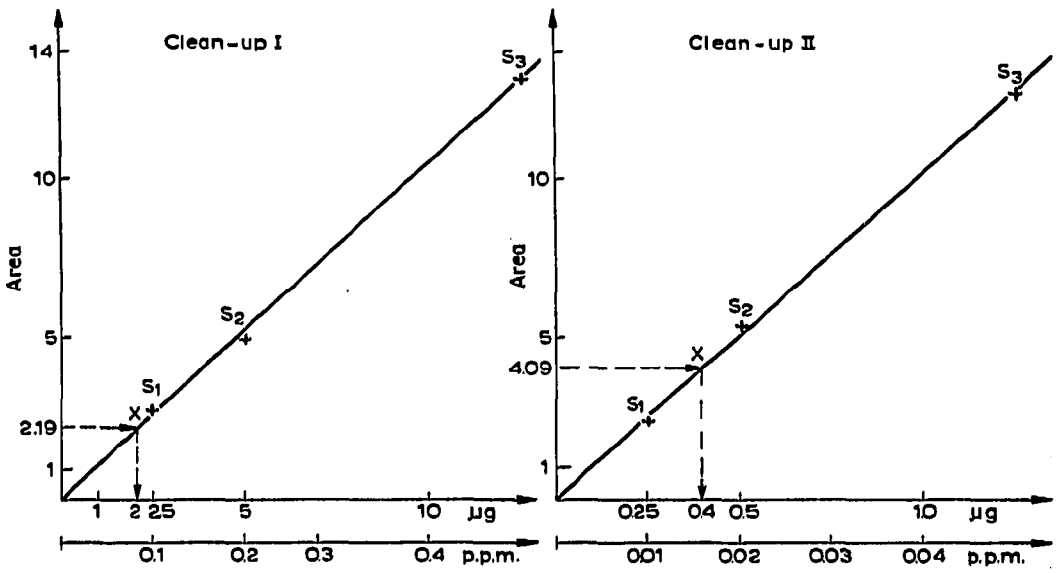

Fig. 6. Left: Quantitation of dithianone on apples fortified with 0.1 ppm of active ingredients. S_{1-3}: external standards. Right: Same, with 0.02 ppm of dithianone.

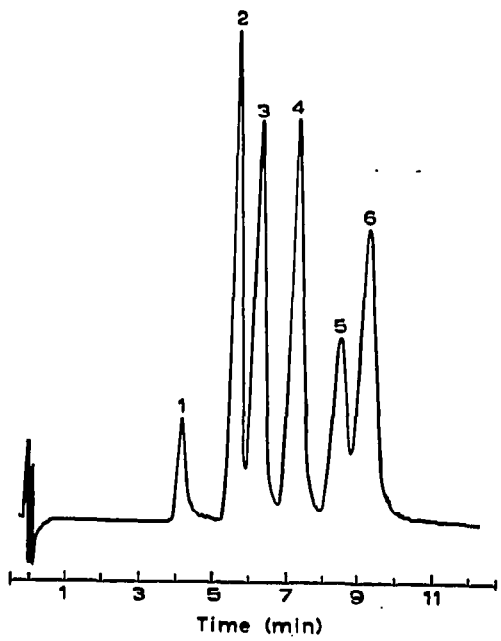

Fig. 7. Selectivity in liquid chromatography. Separation of phenoxy acids as example of non-volatile substances. Column: Perisorb® A; 1500 × 2 mm. Mobile phase: *n*-hexane-acetic acid (92.5:7.5). Instruments: Not commercial, with PM4-CHR (278 nm) as detector. 1 = Solvent; 2 = Dichlorprop; 3 = MCPB; 4 = MCPA; 5 = 2,4,5-T; 6 = 2,4-D.

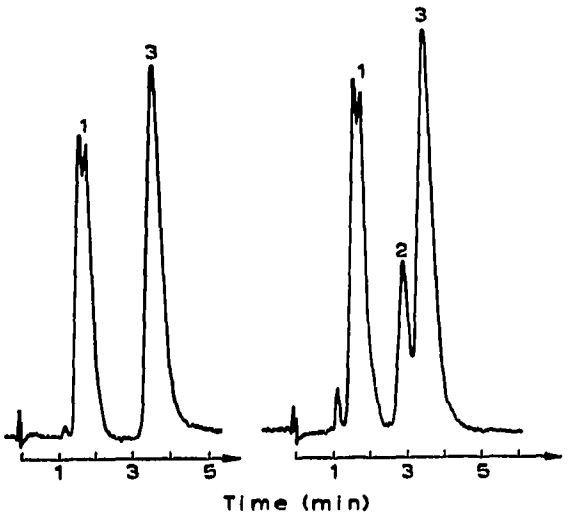

Fig. 8. Separation of PCBs and DDT in liquid chromatography as an example of a confirmatory analysis. Column: Perisorb® A; 500 × 2 mm. Mobile phase: *n*-hexane (anhydrous). Instruments: UFC 1000 with a modified PMQ II (210 nm) as detector. 1 = PCBS; 2 = *o,p'*-DDT; 3 = *p,p'*-DDT.

go thermal decomposition. With phenoxy acids (see Fig. 7), gas chromatography can be used only after conversion of the compounds into the corresponding ester derivatives, whereas liquid chromatography can be applied directly. Furthermore, the chromatograms show a good separation of the chemically very similar herbicides dichlorprop, MCPB, MCPA, 2,4,5-T and 2,4-D.

Nowadays, the exact identification of a pesticide using gas chromatography with an electron capture detector has led to a severe problem. The so-called PCB compounds, a group of polychlorinated biphenyls which are found in widely varying locations because of their widespread use as hydraulic oils, plasticisers and additives for dyestuffs, can readily be mistaken for chlorinated hydrocarbon pesticides. Additional methods are therefore necessary. Besides thin-layer chromatography, liquid chromatography can be used successfully as a supplementary method, as can be seen in the chromatogram in Fig. 8. With Perisorb® A as column packing and *n*-hexane as eluent, the PCBs are separated as a group from *o,p'*-DDT and *p,p'*-DDT.

It is necessary in residue analysis to choose the optimal separation system. Highly active adsorbents, *i.e.*, adsorbents with a large surface area, lead to long retention times and flat peaks. The concentration per volume unit of such peaks is relatively low, and such low concentrations give poor detector response. The detection limit therefore becomes more insensitive in residue analysis. On the other hand, because of the small amounts injected in residue analysis, irreversible adsorptions on high-capacity supports are relatively high.

It is for the above reasons that for residue analysis a system has to be selected that gives relatively small capacity values for the substances to be separated and determined. In our experiments, therefore, we used Perisorb® A as the support.

ACKNOWLEDGEMENTS

We should like to express our sincere thanks to Messrs. M. Witzgall and G. Reitzel for the careful execution of the experimental investigations.

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