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RAPID ANALYSIS OF DICHLORAN, LINDANE, PCNB AND TCNB RESIDUES IN LETTUCE BY AUTOMATED GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Residues of the pesticides dichloran (2,6-dichloro-4-nitroaniline), lindane, PCNB (quintozene) and TCNB (tecnazene) are extracted from lettuce samples with ethyl acetate. An internal standard is added. The extracts obtained are diluted with *n*-hexane and analyzed by automated gas–liquid chromatography, with electron capture detection. Chromatographic data are processed on a programmable desk-top calculator. A program has been developed for this purpose that can also be applied to other multi-component residue analyses. The system is suitable for screening large series of samples. The accuracy of the analysis is at least as good as can be obtained with manual analysis.

INTRODUCTION

Gas–liquid chromatography (GLC) employing highly sensitive and selective detectors is a well established technique for pesticide residue analysis in a variety of sample materials. Owing to the many possibilities created by this technique, the demand for residue determinations is still increasing. Large series of samples, for both inspection and research purposes, may be submitted to laboratories involved in this type of analytical work. When many samples of the same nature have to be investigated, automation of the analytical procedure may become attractive.

Lettuce from glasshouses is one of the crops that is examined on a large scale for pesticide residues at this laboratory. Residues of organophosphorus pesticides in lettuce are screened in crude extracts by thin-layer chromatography (TLC) using an enzyme inhibition technique[1]. If positive results are obtained, the crude extracts are examined further by GLC with a flame photometric detector. Usually, a limited number of samples give positive results, so the amount of analytical work to be done by GLC can be carried out manually.

For chlorinated pesticides, the situation is different, however. Residues of these compounds (dichloran, lindane, PCNB and TCNB) are frequently found on glasshouse lettuces and are analyzed by GLC with electron capture detection. A screening by TLC (with silver nitrate detection) does not save time because, in contrast to the TLC system for organophosphorus compounds, a clean-up of crude extracts is

necessary. Moreover, most extracts do contain organochlorine pesticides, which reduces the usefulness of a preliminary screening. For this reason, the number of GLC analyses for residues of chlorinated compounds is considerable.

In this laboratory, several thousands of lettuce samples have to be analyzed in about 6 months. In order to ensure efficient handling of such large series, a rapid analytical method has been developed, employing automated GLC. A description of the method, and the equipment used, is given in this paper.

EXPERIMENTAL

The method has been developed for the following four pesticides: dichloran (2,6-dichloro-4-nitroaniline; a fungicide); lindane (the gamma-isomer of hexachlorocyclohexane; an insecticide); PCNB (pentachloronitrobenzene, quintozene; a fungicide); TCNB (2,3,5,6-tetrachloronitrobenzene, tecnazene; a fungicide).

Residues of these compounds are extracted from lettuce samples with ethyl acetate, which is also suitable as a solvent for organophosphorus compounds[2]. After dilution with *n*-hexane, the extracts are analysed directly (without clean-up) by GLC. The GLC analysis, from sample injection to printing out the analytical report, is performed automatically. The modular construction of the chromatograph and the data processing system are shown in Fig. 1. A more detailed description of the methods and apparatus used is given in the following sections.

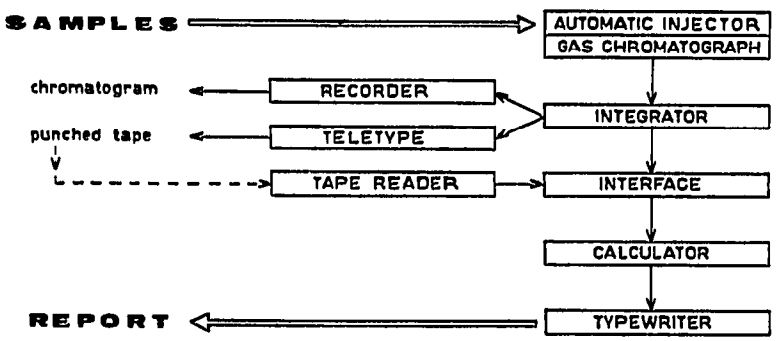

Fig. 1. Automated gas chromatography and data processing.

Extraction

Each lettuce sample, consisting of six heads, is homogenized in a 15-l food cutter. A sub-sample of 50 g of homogenate is macerated by an Ultra Turrax mixer for 1 min with 100 ml of ethyl acetate (Merck, Darmstadt, G.F.R., Cat. No. 9623). The extraction liquid contains an internal standard (pure heptachlor epoxide, 4 μg/ml). After centrifuging, an aliquot of the organic phase is diluted 100 times with *n*-hexane (Merck, Cat. No. 9688, redistilled in glass). An automatic dilution apparatus is used for this purpose. The diluted sample (volume 1 ml) is collected in a sample vial for the automatic injector (see below). The vial is capped manually with a PTFE-coated rubber septum.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS USED

Parameter	*Condition*
Instrument	Tracor 550
Detector	Electron capture, nickel 63 source
Detector operation	Pulse mode; 50 V; pulse rate, 150 μsec; pulse width, 7 μsec
Recorder range	1 mV
Attenuation	$10^2 \times 8$ ($8 \cdot 10^{-10}$ A full scale)
Column	1 m × 4 mm I.D., Pyrex, packed with 3% OV-225 on Gas-Chrom Q, 80–100 mesh
Injection port temperature	200°
Oven temperature	190°
Detector temperature	275°
Carrier gas	Nitrogen, 35 ml/min
Purge gas	Nitrogen, 65 ml/min
Sample injection	Hewlett Packard 7670A, horizontal automatic sampler
Sample volume	5 μl
Analysis cycle	13 min

Gas–liquid chromatography

The chromatographic conditions used are listed in Table I. The automatic sampler is mounted on the back of the gas chromatograph so as to avoid blocking of the instrument panels at the front. Mounting the sampler in this position requires two modifications to the Tracor instrument: the injection port has to be moved to the rear, and the shape of the glass column has to be altered.

The sample tray of the automatic sampler, which can accommodate 36 vials, is filled as follows: in positions Nos. 1, 7, 13, 19, 25 and 31 are placed vials containing a standard *n*-hexane solution of dichloran, lindane, PCNB, TCNB and the internal standard. The remaining positions are filled with vials containing extracts of the samples.

Integrator

Retention times and peak areas are measured with an Infotronics (Boulder, Colo., U.S.A.) CRS 204 digital integrator. The sampler starts the integrator at the moment of injection. The position number of the sample injected is directed by means of the integrator to the interface as well as the Teletype. The sampler control unit stops integration after a pre-set time of 12 min, switching the integrator to the "reset" mode. The calculator is then commanded to retrieve data of the finished chromatogram from the buffer memory of the interface (see below). On the Teletype, this command is printed as a letter "M" (see Fig. 3). The data printed by the Teletype are also recorded on paper tape in ASCII code.

Interface

The interface unit (TM 5000, Techmation, Amsterdam, The Netherlands) converts the integrator and tape reader output into a code acceptable to the calculator. The integrator output of one complete chromatogram is stored in the interface before feeding the calculator. The capacity of the buffer memory is 2000 bcd characters or about 75 peaks. The tape reader (TM 5020-2A, Techmation) has a capacity of 70 characters per second.

Calculator

The chromatographic data obtained are processed by a Wang 720 C programmable electronic desk calculator. The output is printed by a Wang 701 typewriter.

Program

A computation program has been worked out for the calculator, performing the following functions.

(1) Chromatographic data of one run are retrieved from the interface or punched tape and entered in the registers of the calculator.

(2) In standard chromatograms (identified by positions Nos. 1, 7, 13, 19, 25 or 31), relative retentions and response factors are computed, taking the internal standard as a reference. Taking lindane as an example, the response factor is calculated with the following equation:

$$\text{Response factor}_{\text{lindane}} = \left(\frac{\text{concentration}}{\text{peak area}}\right)_{\text{lindane}} \cdot \left(\frac{\text{peak area}}{\text{concentration}}\right)_{\text{internal standard}}$$

Concentrations are expressed in micrograms per millilitre and peak areas in integrator counts. The values obtained are stored and used for processing data of the following five samples. After this a new standard follows, the standard retention data and response factors are updated, and so on.

(3) In sample chromatograms (always containing the internal standard peak), relative retentions are also computed. Peaks are identified when fitting in the "window" (plus or minus 1.5% of the relative retention) of a standard peak. Residue levels, expressed in parts per million, are computed with the equation:

$$\text{ppm lindane} = \frac{(\text{peak area} \cdot \text{response factor})_{\text{lindane}}}{(\text{peak area})_{\text{internal standard}}} \cdot \begin{pmatrix}\text{ppm internal standard}\\ \text{added to lettuce}\end{pmatrix}$$

The use of an internal standard compensates for dilution and injection errors. As a check, the recovery of the internal standard is computed and printed for each sample.

(4) Residue levels exceeding official tolerances are marked with asterisks in the report.

A number of basic data are part of the program, *viz.*:

concentrations of the standard solution;

amount of internal standard (in ppm) added to the lettuce samples;

names of the peaks and their elution order;

text to be printed out in the report.

Changes required, for instance, when a new standard solution with different concentrations is taken into use, are easily made in the program.

The program has the following checks built-in for the prevention of false results:

Indication of overflow when too many peaks are present in the chromatogram (more than 18);

application of threshold values for peak areas in standard chromatograms;

indication of the number of peaks found in the standard run;

rejection of samples where no internal standard peak is found (this may happen when it lies outside its window, or when its area is below a certain threshold value).

RESULTS AND DISCUSSION

Typical chromatograms of a standard solution and of a sample of residue-free lettuce are shown in Fig. 2. The OV-225 column is very effective in separating lindane and PCNB. This separation is difficult to achieve on silicone phases such as OV-1 and OV-210, which are frequently used in pesticide residue analysis.

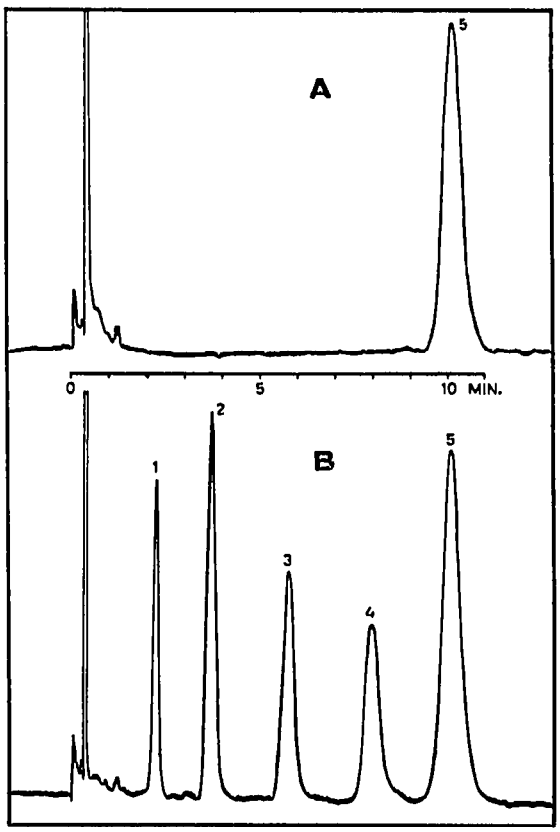

Fig. 2. Typical chromatograms obtained under the conditions specified in Table I. (A) Residue-free lettuce, analyzed as described in the text (peak No. 5 is the internal standard). The amount injected is equivalent to 0.025 mg of lettuce. (B) Standard mixture: peaks Nos. 1 to 5 are, respectively: TCNB (0.032 ng), PCNB (0.072 ng), lindane (0.072 ng), dichloran (0.072 ng) and heptachlor epoxide (0.200 ng).

Figs. 3 and 4 are examples of Teletype output and of the final report, produced by the calculator–typewriter combination,[1] respectively. By using a slightly modified program, it is also possible to produce a summary report, presenting the residue figures in a tabular form.

As shown in Fig. 1, data processing may occur either on-line or off-line. In the on-line mode, the calculator is connected to the interface. After loading the program in the calculator and starting the sampler, the system is ready for unattended operation, including print-out of the report. When the end stop indicator in the sample tray is reached, the sampler and recorder are stopped.

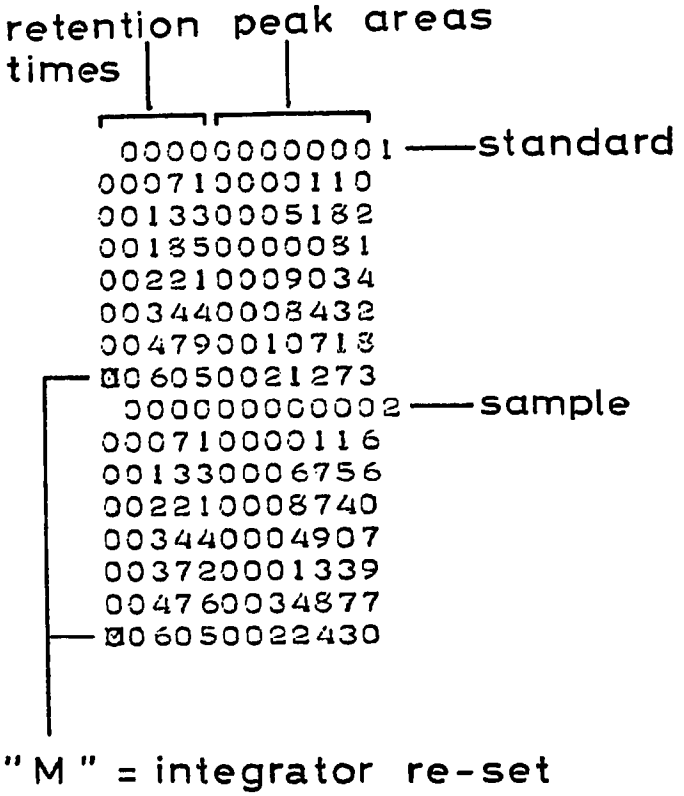

Fig. 3. Teletype output.

In the off-line mode, the same functions are performed, but the interface and calculator are disconnected. In this case, the calculator is available for other computation work or it may be switched on-line to another GLC channel. Chromatographic data are recorded on paper tape, which may be processed later on, using the tape reader.

Small fluctuations in GLC performance do not affect the accuracy of the system,

Lettuce analysis Date: Nov. 14, 1973

STANDARD No. 1 has been stored (5 peaks)

SAMPLE No. 2

Relative retention	Area	Response factor	Identity	ppm
0.2198	6756	0.656	TCNB	1.58
0.3652	8740	0.847	PCNB	2.64
0.5685	4907	0.908	lindane	1.59
0.7867	34877	0.714	dichloran	8.89
1.0000	22430	0.999	internal standard	8.00
			Recovery: 105%	

Fig. 4. Sample report; samples Nos. 1 and 2 are the same as in Fig. 3.

as relative retentions and responses are used and standard mixtures are injected regularly.

The GLC part of the system has proven to be stable, which makes it possible to apply relatively narrow identification windows of plus or minus 1.5% of the relative retentions.

The linear dynamic range of a nickel-63 electron capture detector is limited (about 50). In the present study, the main interest is quality control of lettuce with regard to pesticide residues. For this reason, the highest accuracy is required for residue levels near the tolerances (residue tolerances in The Netherlands are: dichloran, 10 ppm; lindane, 2 ppm; PCNB, 3 ppm (for lettuce); TCNB, 2 ppm). This may be effected by choosing the proper sample dilution (100-fold). If good accuracy at elevated residue levels (two to three times the tolerance) is required, the extracts must be diluted further. Alternatively, a smaller volume may be injected (this possibility is available in the autosampler). Good accuracy at low residue levels (below *ca.* 10% of the tolerance) requires the injection of more concentrated sample extracts. In this case, a clean-up is usually necessary and for this purpose crude extracts may be shaken with an adsorbent[3]. This aspect, however, lies outside the scope of this paper.

Residues of heptachlor epoxide might interfere in the analysis, as it is used as an internal standard. Field applications of heptachlor are not allowed, however, and in the past, before the present method with an internal standard was adopted, no residues of this pesticide had been found in glasshouse lettuce.

In order to determine the accuracy of the whole procedure, a lettuce sample (ten heads) containing "field residues" of dichloran, lindane, PCNB and TCNB was chopped as usual in a food cutter. Ten sub-samples of 50 g were analyzed by the technique described and the results are given in Table II. The sample cannot be regarded as representative for normal horticultural practice, as it was treated with the four pesticides for the purpose of this experiment. The mean relative standard deviation of the analyses reported in Table II is 10%. This is an overall value, including the variation between sub-samples of the homogenate.

The variation of the GLC analyses alone is smaller. The mean relative standard deviation of peak areas, expressed in integrator counts, obtained from repeated injections of standard solutions is about 2%. This accuracy is satisfactory; it is actually comparable with, or even better than, that usually obtained by manual analysis.

Known amounts (1–3 ppm) of dichloran, lindane, PCNB and TCNB, added to lettuce at the extraction stage, were recovered quantitatively (100 $\pm$ 5%) after carrying out the analysis as described. It can be concluded, therefore, that quantitative measurement with the internal standard technique is adequate.

Since its installation, over 3000 lettuce samples have been analyzed with good performance with the system described. Among the other advantages of automation, the increased efficiency of the instruments obtained by unattended operation during non-working hours is particularly noteworthy.

The advantages of the data processing system described are its modular build-up and the simplicity of the calculating unit. No specialized computer technicians are required. The calculator program developed is also applicable to other GLC analyses. In this laboratory, for instance, it is also used for screening 14 different organochlorine pesticide residues in animal feedstuffs.

With the development of the method described above, most attention was paid

TABLE II

ANALYSIS OF TEN SUB-SAMPLES FROM A LETTUCE HOMOGENATE

Sub-sample No.	*TCNB (ppm)*	*PCNB (ppm)*	*Lindane (ppm)*	*Dichloran (ppm)*
1	1.58	2.64	1.59	8.89
2	1.39	2.61	1.41	8.46
3	1.47	2.81	1.43	8.34
4	1.31	2.16	1.23	6.79
5	1.42	2.21	1.34	8.37
6	1.43	2.61	1.45	8.70
7	1.60	3.03	1.49	8.52
8	1.12	2.18	1.15	7.42
9	1.60	2.71	1.57	8.57
10	1.52	2.78	1.51	8.36
Mean	1.4	2.6	1.4	8.2
Standard deviation	0.15	0.30	0.14	0.64
Relative standard deviation	10.7%	11.5%	10.0%	7.8%

to the automation of GLC and data processing, and to the accuracy obtainable with this system. No clean-up studies were made, because this was found to be unnecessary for lettuces. With other crops, the situation may be different, however. For this reason, investigations concerning automation of extraction and clean-up processes have been initiated.

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