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AUTOMATED CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS OF PESTICIDE RESIDUES IN FOOD

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SUMMARY

A method for multiresidue pesticide analysis in food is described. After a conventional clean-up, gas chromatographic analysis is performed in a gas chromatograph equipped with two fused-silica capillary columns coated with methylsilicone SP 2100 and methylphenylsilicone OV-17. The effluent from each column is split to electron-capture and nitrogen–phosphorus detectors, which are connected to a dual channel integrator. Therefore, from each gas chromatographic run parallel records of signals from the two detectors are obtained. Calibration of the system is carried out for the SP 2100 column with three test mixtures covering all pesticides. Additionally, four internal standards are included, two responding to the electron-capture detector and the other two to the nitrogen–phosphorus detector. Automated analysis is performed with test mixtures and food samples on the SP 2100 column overnight as a screening procedure. After selection of positive samples a confirmatory test and quantitation are carried out manually applying appropriate test mixtures according to the results of the screening runs.

INTRODUCTION

In the analysis of pesticide residues in foods using gas chromatography with selective detectors, a major problem is the large number of compounds to be detected. The efficiency of packed columns permits the separation of only a limited number of substances. The determination of a pesticide from its retention time on one column is definitely not sufficient. Therefore, analysis on one or two further columns with stationary phases of different polarity is necessary in order to confirm the identity of a compound detected on the chromatogram. This procedure is time consuming and requires a whole set of gas chromatographs.

Capillary columns exhibit excellent efficiencies which allow the separation of complex mixtures and the determination of the retention times of compounds with high accuracy and reproducibility. The high resolution facilitates the differentiation of substances belonging to the same structural class, such as organophosphorus pesticides (OP) or chlorinated pesticides (CP).

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Early applications of capillary columns demonstrated their tremendous separation power in the analysis of polychlorinated biphenyl (PCB) isomers[1] and their differentiation from CP of the DDT group[2–5]. In the investigation of environmental pollution with persistent chlorinated pesticides and other contaminants, PCB and TCDD isomers being the most prominent examples, capillary gas chromatography with glass and fused-silica columns with an electron-capture detector is now becoming the standard method. Some impressive examples of this technique are the analysis of TCDD isomers in soil by Buser[6,7] and CP and PCB isomers in river sediments[8] and marine fish[5,9]. Frequently, gas chromatography–mass spectrometry (GC–MS) is used for the identification of individual components of mixtures after their separation on capillary columns[6–8,10]. The analysis of CP and PCB isomer residues in food was first described by Schulte *et al.*[4], who demonstrated the resolution of DDD and DDT from PCB isomers. The occurrence of these PCB isomers frequently leads to incorrect results for these two pesticides with packed columns. The large number of compounds belonging to the OP group requires the highest separation power available in gas chromatography.

Several workers have described the analysis of OP residues on capillary columns. Krijgsman and Van de Kamp[11] reported the retention times of 59 OP compounds on a 50-m glass capillary column coated with SE-30 and demonstrated the separation of a mixture of 21 components. Stan[12,13] utilized a 20-m SE-54 column for the identification of 23 OP compounds mainly in foods with GC–MS. Hild *et al.*[14] described the analysis of 26 OPs including several metabolites in vegetables using a 25-m glass capillary coated with DEGA.

The utility of commercially available capillary columns is usually demonstrated by results for test mixtures of CPs and OPs in the catalogues. Although capillary gas chromatography is now readily available to the routine laboratory, not all laboratories in food control make use of it.

Automatic glass capillary gas chromatographic analysis of PCB and CP residues in food samples applying splitless injection and a short packed pre-column with a 25-m capillary column coated with an apolar silicone phase was described by Tuinstra and Traag[15]. Recently, the analysis of chlorophenoxy acid herbicide residues in flour using a 60-m SE-30 glass capillary column was developed by Gilsbach and Thier[16].

In this paper, we describe the determination of 83 pesticide residues in fruits and vegetables using two fused-silica capillary columns coated with SP 2100 and OV-17 in one gas chromatograph. The instrument is equipped with electron-capture and nitrogen–phosphorus detectors, an automatic sampler and a dual-channel integrator.

EXPERIMENTAL

Instrumentation

The gas chromatographic analysis was carried out on an HP 5880 A gas chromatograph (Hewlett-Packard) equipped with two injection ports for capillary columns and the two selective detectors. A fused-silica capillary column coated with SP 2100 (25 m × 0.2 mm I.D.) was installed in the first capillary inlet and connected to both detectors via a home-made effluent splitter. A second fused-silica column coated

with OV-17 (6 m × 0.3 mm I.D.) was installed in the second injection port and also connected to both detectors via the effluent splitter.

Data from the two detectors were processed simultaneously and reported on two separate terminals. The HP 5880 A was also equipped with an HP 7671 A autosampler for 36 sample bottles.

Effluent splitter

The effluent splitter was constructed using fused silver chloride as a thermally stable and chemically inert cement[17]. The two fused-silica capillary ends were sealed into a glass sleeve. In order to protect and strengthen the splitter, a piece of shrinkable PTFE tubing was fixed on to the glass sleeve.

Gas chromatography

For both capillary columns helium was used as the carrier gas, and the temperatures of the injection ports and the detectors were 250 and 300°C, respectively. The sample volumes were 1 μl with the autosampler and with the manual injection. The injection was carried out splitless into the "cold" column at 100°C, according to the method of Grob and Grob[18]. After 30 sec the carrier gas splitting was restarted and after 60 sec a temperature programme was started: 30°C/min to 150°C, held for 2 min, 3°C/min to 205°C, 30°C/min to 240°C, 2°C/min to 260°C, held for 10 min, then cooled to the initial temperature. The gas flow-rates, optimized to achieve maximum sensitivity, were as follows: carrier gas, helium at 60 cm/sec through the SP 2100 column and 120 cm/sec through the OV-17 column; purge gases, helium at 20 ml/min for the nitrogen–phosphorus detector and argon–10% methane at 25 ml/min for the electron-capture detector.

Data processing

The microprocessor-controlled HP 5880 A possesses a series of features that are of significance for the methods described here, including peak integration, peak recognition with peak name annotation and various calibration procedures.

Materials

The pesticides were purchased from Dr. Ehrenstorfer (Augsburg, F.R.G.) as test substances with a purity of 97–99%. Solvents and chemicals for clean-up of food samples were Merck (Darmstadt, F.R.G.) products of z.A. quality.

The fused-silica capillary column coated with SP 2100 was supplied by Hewlett-Packard (Avondale, PA, U.S.A.). The fused-silica capillary coated with OV-17 was a product of Quadrex Scientific (Weybridge, Surrey, U.K.).

Preparation of internal standards

With reference to the derivatization method of Jacob *et al.*[19], a series of thiophosphinates were synthesized from various phenolic compounds. The phenol (40 mg) was mixed with 800 μl of a 5% (v/v) solution of dimethylthiophosphinic chloride in dry diethyl ether and 800 μl of a 20% (v/v) solution of triethylamine in diethyl ether were added. The reaction mixture was kept for 2 h at 40°C in a stoppered vial. The precipitate was removed by filtration and the clear filtrate was evaporated to dryness in a nitrogen stream. The product was recrystallized from diethyl ether–hexane (1:1).

The white crystals were dissolved in hexane and drained through a small column containing 3 g of alumina topped with 3 g of a mixture of charcoal and silica gel (1:10, w/w). After this final purification step each product showed only a single peak on the chromatograms at the concentrations at which it was applied as an internal standard.

Clean-up of food samples

The clean-up followed the procedures of Becker[20] and Specht and Tillkes[21]. Internal standards were added to the homogenized food samples before the first solvent extraction step was started. The final concentration of the purified extracts was the equivalent of 2 g of food in 1 ml.

RESULTS AND DISCUSSION

Evaluation of internal standards

From a series of phenolic compounds substituted with alkyls and halogens, dimethylthiophosphinates were synthesized and checked for suitability as internal standards for pesticide analysis. Our first intention was to look for two internal standards responding to both of the selective detectors. However, no compound was found that did not interfere with either the CPs or the OPs. Therefore, we decided to look for internal standards separately for compounds active towards the electron-capture and nitrogen–phosphorus detectors and showing no cross-activity. Aldrin and 1,2,3-trichlorobenzene were found to serve the purpose as internal standards for the halogenated hydrocarbons. For the OPs, O-phenyl dimethylthiophosphinate (PT) and O-2-naphthyl dimethylthiophosphinate (NT) were found to be well suited (see Fig. 1). Aldrin is a well known insecticide that has been banned from use in Europe for a long time; in the last years there have been no positive reports of aldrin residues in fruit and vegetables. The other three compounds are not likely to be found in the environment. Internal standards are mainly used for compensating for the losses of the sample substances during clean-up and for correcting the deviations of injection volumes and variations in detector response. To serve this purpose, the internal standard should exhibit properties very similar to those of the substances to be analysed. In a mixture the peak of the internal standard should lie in the centre of the chromatogram. These postulates are satisfied by aldrin for the halogenated pesticides and NT for the organophosphates. NT exhibits excellent retention properties because it falls in the centre of the chromatogram in a retention gap.

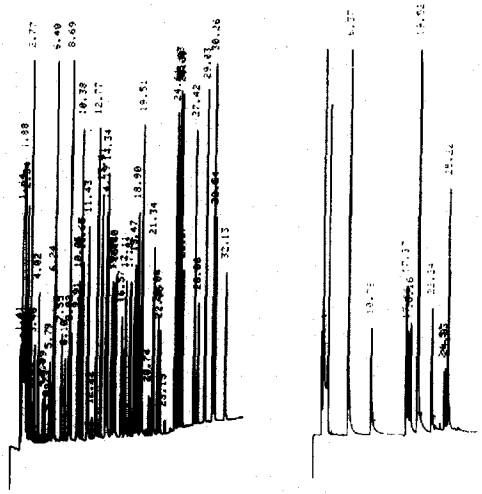
Aldrin and NT are used as primary internal standards and all calculations in the internal standard method are related to these substances. However, there are three reasons to add a second internal standard. First, the pesticides differ considerably in volatility and the recovery of the most volatile compounds may be insufficient if during the evaporation steps insufficiently high temperatures were erroneously applied. Loss of lindane is the most prominent example in residue analysis. Therefore, the volatile compounds 1,2,3-trichlorobenzene and PT were used. Second, with the great variety of food samples of unknown origin, it cannot be assumed that no co-extracted substance will interfere with the internal standard in the chromatogram, in which case the quantitation of all calibrated pesticides is biased. However, with the use of a second internal standard this bias is indicated. Third, some of the pesticides are sensitive to hydrolysis, which may occur in unfavourable samples. NT and PT

have been found to be reliable indicators of such unfavourable conditions owing to their sensitivity against hydrolysis. In routine work it is our experience that agreement of the quantitation of the controlling second standard within $\pm 15\%$ indicates reliable analysis conditions.

Calibration with test mixtures

It seems trivial for good laboratory practice in residue analysis to include the daily calibration of the GC method with all 83 pesticides. Therefore, our first aim was to prepare three calibration mixtures containing a maximum number of pesticides that are well separated, including the internal standards. Good resolution of all peaks is necessary for the recognition of all separated compounds by means of their retention times. This selection should be carried out taking advantage of the high separation efficiency of the capillary columns and the software capabilities of the instrument.

The calibration procedure is executed as follows. After running the first test mixture containing 38 OPs and the two internal standards PT and NT, a calibration table is generated and stored in the memory of channel 1 connected to the nitrogen–phosphorus detector. This happens by assignment of names and concentration values of the individual pesticides to the peaks in the chromatogram via the alphanumeric keyboard. The next step is to run a second test mixture, containing six OPs and the two internal standards under the same conditions and adding their data to the existing calibration table. The two chromatograms and the calibration table are shown in



STANDARD MIXTURE FOR ORGANOPHOSPHORUS PESTICIDES
ISTD

RT	CAL	AMOUNT	NAME
3.09	1	2.000	DIMEFOX
4.02	2	2.000	DICHLOFVOS
5.73	3	2.000	MEVINPHOS
6.48	4	2.000	PT
7.55	5	4.000	DEMEPHION
8.10	6	2.000	HEPTENOPHOS
8.69	7	2.000	THIONAZOMETH
8.92	8	2.000	DEMETON-S-ME
9.91	9	2.000	DICROTOPHOS
10.78	10	2.000	SULFOTEP
10.65	11	2.000	PHORATE
11.43	12	2.000	DIMETHOATE
12.21	13	6.000	DIOXATHION
12.77	14	3.000	FONOFOS
13.41	15	2.000	DIAZI-DISULF
14.19	16	2.000	ETRIMFOS
14.34	17	4.000	FORMOTHION
15.05	18	4.000	PHOSPHAMIDON
15.35	19	2.000	DICHLOFENTHI
15.60	20	2.000	PARATHION-ME
16.57	21	3.000	FENCHL. PARAO
17.11	22	2.000	FENITROTHION
17.82	23	2.000	MALATHION
18.47	24	2.000	PARATH-DURSE
18.90	25	4.000	CHLORTHION
19.51	26	ISTD 1	NT
20.74	27	2.000	CHLORFENVIN
21.34	28	2.000	METHIDATHION
22.04	29	2.000	BROMOPHOS-ET
22.35	30	2.000	DITALIMFOS
24.61	31	4.000	FENSULFOTHI
25.03	32	2.000	ETHION-TRIAM
25.38	33	2.000	TRIAZOPHOS
25.67	34	2.000	CARBOPHENOT
27.42	35	4.000	PHOSMET
28.06	36	2.000	PHENTHARTON
29.03	37	4.000	PHOSALAZINME
30.26	38	4.000	AZINPHOSETH
30.54	39	4.000	DIALI PYRAZ
32.13	40	4.000	COUMAPHOS
6.37	4	2.000	PT
10.78	41	4.000	MONOCROTOPH
17.37	42	2.000	PIRIMIPHOSME
17.66	43	4.000	AMIDITHION
19.16	44	2.000	FENTHION
19.52	26	ISTD 1	NT
23.24	45	4.000	TETRACH-VINI
24.57	46	4.000	CHLORTHIOPH
24.83	47	4.000	CHLORTHIOPH
25.22	48	4.000	CHLORTHIOPH

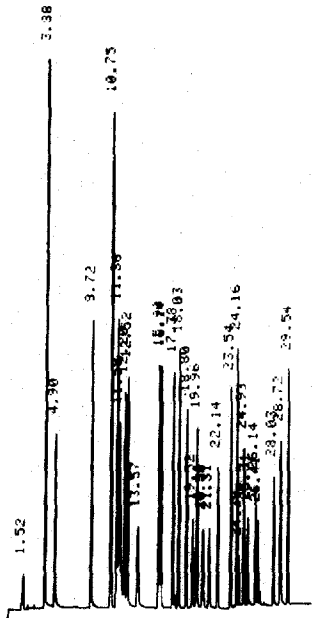
Fig. 1. Gas chromatogram of 44 organophosphorus pesticides and two internal standards in two calibration mixtures on an SP 2100 fused-silica capillary column. Nitrogen–phosphorus detector. For report, see Table I.

Fig. 1. The purpose of dividing the test mixture of OPs into two parts is to extend the number of calibrated peaks. On this account, the second mixture is composed of OPs not separated sufficiently from other pesticides in the first mixture to achieve a reproducible quantitative calibration. The difference in the retention times of critical pairs of this type, however, is large enough to result in reliable recognition of these compounds by the computer. An experimental check of the criteria mentioned can be made by repeated injections of the two test mixtures.

In the same way a calibration table for 27 chlorinated pesticides is created and stored in channel 2 connected to the electron-capture detector (Fig. 2).

Using the internal standard method the computer first tries to identify the peak of the internal standard within a given retention time window. In this reference peak window the largest peak is identified as the internal standard without further checking. As the reliable positive identification of the internal standard is the basis of the entire calibration and recognition procedure, this compound must be well separated from all other components of the mixture. A very important feature of the internal standard method for complex mixtures is the automatic correction of small changes in the gas chromatographic conditions. After identifying the internal standard all retention time values in the calibration table are recalculated on the basis of the actual retention time of the internal standard.

As mentioned above, it is not possible to include all pesticides responding to each detector in one calibration mixture. Our selection took into consideration four chromatographic features: (a) complete separation, (b) no separation, (c) insufficient resolution of two peaks and (d) interference of a third substance with the



STANDARD MIXTURE FOR CHLORINATED PESTICIDES
ISTD

RT	CAL	AMOUNT	NAME
3.88	1	2.000	1, 2, 3-TCB
4.90	2	2.000	DICHLOBENIL
3.72	3	2.000	TECNAZENE
10.75	4	4.000	ALPHA-HCH
11.36	5	2.000	HEXACHLOROBE
11.58	6	4.000	DICHLORAN
12.20	7	2.000	LINDANE
12.52	8	2.000	QUIN\BETAHCH
13.57	9	2.000	CHLOROTHALON
15.90	10	4.000	VINCLOZOLIN
16.14	11	2.000	HEPTACHLOR
17.38	12	4.000	DICHLOFLUANI
18.03	13	ISTD 1	ALDRIN
18.80	14	2.000	CHLORT\TRIAD
19.52	15	2.000	NITROTHAL-IS
19.96	16	4.000	CAPTAN
20.58	17	8.000	FOLPET
21.31	18	4.000	PROCYMIDONE
22.14	19	2.000	ENDOSULFAN I
23.54	20	2.000	DIELDRIN\DDE
24.16	21	2.000	ENDRIN\BUPIR
24.39	22	2.000	ENDOSULF- II
24.93	23	2.000	DDD
25.31	24	2.000	TETRASUL
26.14	25	2.000	DDT
26.41	26	4.000	CAPTAFOL
28.03	27	4.000	METHOXYCHLOR
28.72	28	4.000	TETRADIFON
29.54	29	4.000	MIREX

Fig. 2. Gas chromatogram of 27 chlorinated pesticides and two internal standards on an SP 210 fused-silica capillary column. Electron-capture detector. For report, see Table II.

complete resolution of two peaks. All completely separated OPs (a) are included in the first test mixture. From critical pairs (b, c) only one OP is present, and in case (d) the interfering substance is omitted. The remaining compounds (c, d) are combined in the second test mixture, but a completely non-separated critical pair (b) is represented only by one component in the calibration procedure. The existence of those "obscure" compounds is indicated in the calibration table by a second name after the calibrated pesticide (Figs. 1 and 2, Tables I and II). Again the discrimination between the separation conditions (b) and (c) or (d) was undertaken not from theoretical considerations but from experimental results.

All pesticides to be determined are compiled in Tables I and II. As usual in pesticide residue analysis, the compounds are listed according to their relative retention times (RRT) on the main SP 2100 capillary column.

Analysis of residues in foods

A daily routine analysis series of food samples by autosampling is started by injection of the three calibration mixtures on to the main SP 2100 column. The pesticides are recorded in parallel in two channels according to their responses in the two detectors. The CPs (Table II) are recalibrated in channel II. Some of these components show a response in the nitrogen–phosphorus detector and are registered also in channel I but not calibrated. These pesticides, indicated in Table II, are reported in channel I as uncalibrated peaks exhibiting the same retention time as a calibrated peak in the report of channel II. In the same way most of the OPs (Table I) were recorded on both channels but calibrated only in channel I. Many of the OPs generate positive signals in the electron-capture detector.

Fig. 3 shows the chromatograms of the two test mixtures of OPs both containing the internal standard aldrin. A multitude of OPs produce signals in the electron-capture detector, but only a few of them are recognized erroneously as CPs, as demonstrated in the internal standard report in Fig. 3. These falsely identified CPs may be a source of error when the results are not checked critically. Some of these false CPs, however, can be eliminated immediately because of the difference between the actual and "expected" retention times. When comparing the results on the two channels a decision can be made on the basis of coincidence of retention times. To facilitate daily routine operation, two additional columns considering the signals on the other channel are included in Tables I and II. In column F all pesticides which may be designated falsely are indicated. The columns headed ECD and NPD report on positive signals in the other channel.

On the other hand, the accord of signals in both channels is a valuable criterion for the positive identification of a substance, and the lack of such an expected coincidence can indicate the absence of a suspected pesticide residue.

The final confirmation is performed on the OV-17 column installed in the same gas chromatograph in the second injection port and connected via the effluent splitter to both detectors in the same way as the SP 2100 column. Chromatography on the OV-17 column is carried out without calibration because the memory of the microprocessor can be loaded with only one calibration table per channel. The few positive samples in daily routine work are injected manually.

After selecting the likely candidates by means of all information from the first chromatographic run, appropriate calibration mixtures are prepared from standard

TABLE I

RELATIVE RETENTION TIMES (RRT) OF 53 ORGANOPHOSPHORUS PESTICIDES ON TWO COLUMNS DETECTED WITH A NITROGEN–PHOSPHORUS DETECTOR

No.	*Pesticide*	*Name calibrated**	*F***	*ECD****	*SP 2100* §	*OV-17* §
1	Dimefox	DIMEFOX			0.158	–
2	Dichlorvos	DICHLORVOS		+	0.206	0.107
3	Mevinphos	MEVINPHOS		+	0.294	0.288
4	PT	PT			0.328	0.292
5	Demephion	DEMEPHION			0.387	0.345
6	Heptenophos	HEPTENOPHOS			0.415	0.353
7	Omethoate	THIONA/OMETH		+	0.441	0.413
8	Thionazin	THIONA/OMETH		+	0.445	0.369
9	Demeton-S-methyl	DEMETON-S-ME			0.457	0.384
10	Dicrotophos	DICROTOPHOS			0.508	0.483
11	Sulfotep	SULFOTEP			0.532	0.421
12	Phorate	PHORATE		+	0.546	0.401
13	Monocrotophos	MONOCROTOPH			0.552	0.591
14	Dimethoate	DIMETHOATE		+	0.586	0.557
15	Dioxathion	DIOXATHION		+	0.626	0.522
16	Fonofos	FONOFOS		+ +	0.654	0.479
17	Diazinon	DIAZI/DISULF		+	0.687	0.528
18	Disulfoton	DIAZI/DISULF		+	0.688	0.521
19	Etrimfos	ETRIMFOS		+	0.727	0.572
20	Formothion	FORMOTHION		+ + +	0.735	0.758
21	Phosphamidon	PHOSPHAMIDON		+	0.771	0.741
22	Dichlofenthion	DICHLOFENTHI		+ +	0.787	0.590
23	Parathion-methyl	PARATHION-ME		+ +	0.799	0.722
24	Paraoxon	FENCHL/PARAO		+	0.841	0.807
25	Fenchlorphos	FENCHL/PARAO		+ + +	0.849	0.665
26	Fenitrothion	FENITROTHION		+ +	0.877	0.833
27	Pirimiphos-methyl	PIRIMIPHOS-ME	+		0.890	0.792
28	Amidithion	AMIDITHION		+	0.905	0.954
29	Malathion	MALATHION		+	0.913	0.909
30	Fenthion	FENTHION			0.930	0.914
31	Dursban	PARATH/DURSB		+ +	0.940	0.813
32	Parathion	PARATH/DURSB		+ +	0.946	0.875
33	Chlorthion	CHLORTHION		+ + +	0.969	0.943
34	NT	NT			1.000	1.000
35	Chlorfenvinphos	CHLORFENVIN		+ + +	1.063	1.075
36	Methidathion	METHIDATHION	+	+	1.094	1.203
37	Bromophos-ethyl	BROMOPHOS-ET		+ + +	1.129	1.015
38	Vamidothion	TETRACH/VAMI			1.134	1.396
39	Tetrachlorvinphos	TETRACH/VAMI		+ + +	1.139	1.222
40	Ditalimfos	DITALIMFOS		+ +	1.145	1.277
41	Chlorthiophos	CHLORTHIOPH		+	1.259	1.426
42	Chlorthiophos	CHLORTHIOPH		+	1.272	1.460
43	Chlorthiophos	CHLORTHIOPH		+ + +	1.292	1.566
44	Fensulfothion	FENSULFOTHI		+	1.261	1.672
45	Ethion	ETHION/TRIAM		+ +	1.283	1.567
46	Triamiphos	ETHION/TRIAM			1.288	1.697
47	Triazophos	TRIAZOPHOS			1.297	1.870
48	Carbophenothion	CARBOPHENOT		+ + +	1.316	1.600
49	Phosmet	PHOSMET		+ +	1.405	2.126
50	Phenkapton	PHENKAPTON		+ + +	1.438	1.977

TABLE I *(continued)*

No.	*Pesticide*	*Name calibrated**	*F***	*ECD****	*SP 2100*[§]	*OV-17*[§]
51	Azinphosmethyl	PHOSA/AZINME		+	1.487	2.355
52	Phosalone	PHOSA/AZINME		+ + +	1.488	2.208
53	Azinphosethyl	AZINPHOSETH		+ +	1.551	2.509
54	Pyrazophos	DIALI/PYRAZ		+ +	1.564	2.478
55	Dialifos	DIALI/PYRAZ		+ +	1.565	2.509
56	Coumaphos	COUMAPHOS		+ +	1.647	2.689
57	Bromophos[§§]	–	+	+ + +	0.990	0.864

* Plot of names with up to 12 alphanumeric signs after calibration on the SP 2100 column.
** False designation caused by CPs in the sample containing nitrogen.
*** Positive response in the electron-capture detector: + < + + < + + +.
§ RRT: retention times relative to NT (O-2-naphthyl dimethylthiophosphinate) as internal standard.
§§ Bromophos is omitted from the calibration mixtures because of overlapping with the internal standard NT.

solutions by dilution. These calibration mixtures contain the internal standards and the suspected pesticides at the approximate concentration estimated in the screening run. The actual residue concentration in the food sample is calculated by direct peak-area comparison; the internal standard can be used for determination of the recovery.

Analysis of a food sample

The following example is given to demonstrate the application of the method to a food sample. Pears were fortified with three pesticide residues, two of them belong to critical pairs. Fig. 4 shows the chromatograms and reports of the two channels. In the record of the nitrogen–phosphorus detector channel four peaks can be observed; the two larger ones are the internal standards PT and NT and the two smaller peaks are components of the two critical pairs parathion–dursban and tetrachlorvinphos–vamidothion. The chromatogram obtained with the electron-capture detector contains four larger peaks, two of them being the internal standards 1,2,3-TCB and aldrin and a third being recognized by the computer as captan. The other peaks are not calibrated compounds originating from the food matrix, but two of them coincide with the OPs in the other channel. From this accord between retention time and response to both detectors, tetrachlorvinphos is the most likely candidate of one of the critical pairs, whereas no discrimination between parathion and dursban can be made. The final confirmation performed on the OV-17 column is demonstrated in Fig. 5.

A first run of the sample resulted in relative retention times in accord with captan, dursban and tetrachlorvinphos using the values listed in Tables I and II. On the basis of the screening results shown in Fig. 4, a calibration mixture was composed containing 2 μg each of the three internal standards PT, NT and aldrin, 1 μg of dursban, 8 μg of captan and 2 μg of tetrachlorvinphos per millilitre. The chromatograms of the calibration run and the sample run are shown in Fig. 5 and the quantitative results and recovery calculations are summarized in Table III.

As described above, the concentrations in the sample were calculated by comparison of the peak areas in the calibration run with those in the sample run for all

TABLE II

RELATIVE RETENTION TIMES (RRT) OF 30 CHLORINATED PESTICIDES ON TWO COLUMNS DETECTED WITH AN ELECTRON-CAPTURE DETECTOR

No.	*Pesticide*	*Name calibrated**	*F***	*NPD****	*SP 2100*[§]	*OV-17*[§]
1	1,2,3-TCB	1,2,3TCB			0.215	0.150
2	Dichlobenil	DICHLOBENIL		+ + +	0.272	0.318
3	Tecnazene	TECNAZENE	+	+	0.484	0.598
4	α-HCH	ALPHA-HCH			0.596	0.736
5	Hexachlorobenzene	HEXACHLOROBE			0.630	0.667
6	Dichloran	DICHLORAN		+ + +	0.642	0.822
7	Lindane	LINDANE	+		0.677	0.815
8	β-HCH	QUIN/BETAHCH			0.693	1.046
9	Quintozene	QUIN/BETAHCH			0.694	0.795
10	Chlorothalonil	CHLOROTHALON		+ +	0.752	1.154
11	Vinclozolin	VINCLOZOLIN		+ +	0.882	1.132
12	Heptachlor	HEPTACHLOR			0.895	0.898
13	Dichlofluanid	DICHLOFLUANI		+ + +	0.964	1.384
14	Aldrin	ALDRIN			1.000	1.000
15	Chlorthal	CHLORT/TRIAD	+		1.043	1.423
16	Triadimefon	CHLORT/TRIAD	+		1.050	1.496
17	Nitrothal-isopropyl	NITROTHAL-IS	+	+ + +	1.083	1.444
18	Captan	CAPTAN			1.107	1.918
19	Folpet	FOLPET			1.141	1.961
20	Procymidone	PROCYMIDONE	+	+ +	1.182	1.860
21	Endosulfan I	ENDOSULFAN I	+		1.228	1.564
22	Dieldrin	DIELDRIN			1.306	1.798
23	DDE	DDE			1.316	1.963
24	Endrin	ENDRIN/BUPIR			1.340	1.993
25	Bupirimate	ENDRIN/BUPIR			1.346	2.555
26	Endosulfan II	ENDOSULF II			1.353	2.291
27	DDD	DDD	+		1.383	2.510
28	Tetrasul	TETRASUL	+		1.404	2.443
29	DDT	DDT			1.450	2.700
30	Captafol	CAPTAFOL			1.465	3.249
31	Methoxychlor	METHOXYCHLOR	+		1.555	3.612
32	Tetradifon	TETRADIFON			1.593	3.777
33	Mirex	MIREX			1.638	3.000

* As in Table I.

** False designation caused by OPs in the sample.

*** Positive response in the nitrogen–phosphorus detector: + < + + < + + +. The absolute response depends very much on the operating conditions of the detector.

[§] RRT: retention times relative to aldrin.

compounds including the internal standards (external calibration). The recovery factors calculated for the two internal standards therefore include all losses during the clean-up and all deviations in volumes including the injection into the gas chromatograph.

Evaluation and discussion of the method

The chromatograms in Figs. 1 and 2 demonstrate the high efficiency of the capillary columns applied. All components in the three calibration mixtures are well separated, which is a prerequisite for quantitative calibration and also reliable recog-

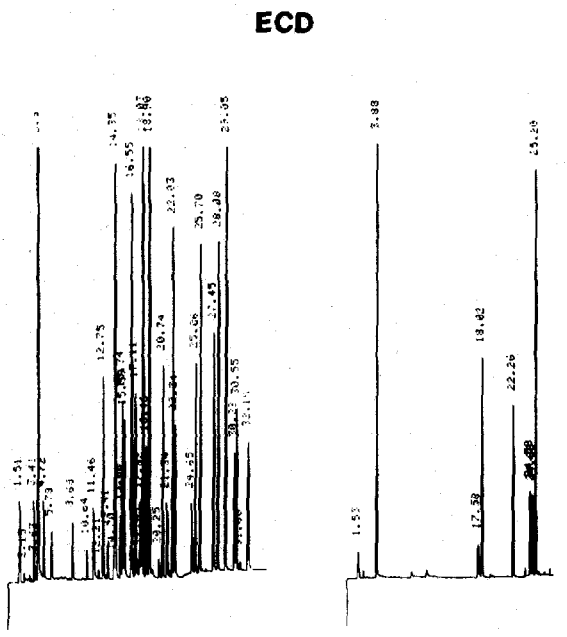


Fig. 3. Gas chromatogram of 44 organophosphorus pesticides and four internal standards in two calibration mixtures on an SP 2100 fused-silica capillary column. Electron-capture detector. For report, see text and Tables I and II.

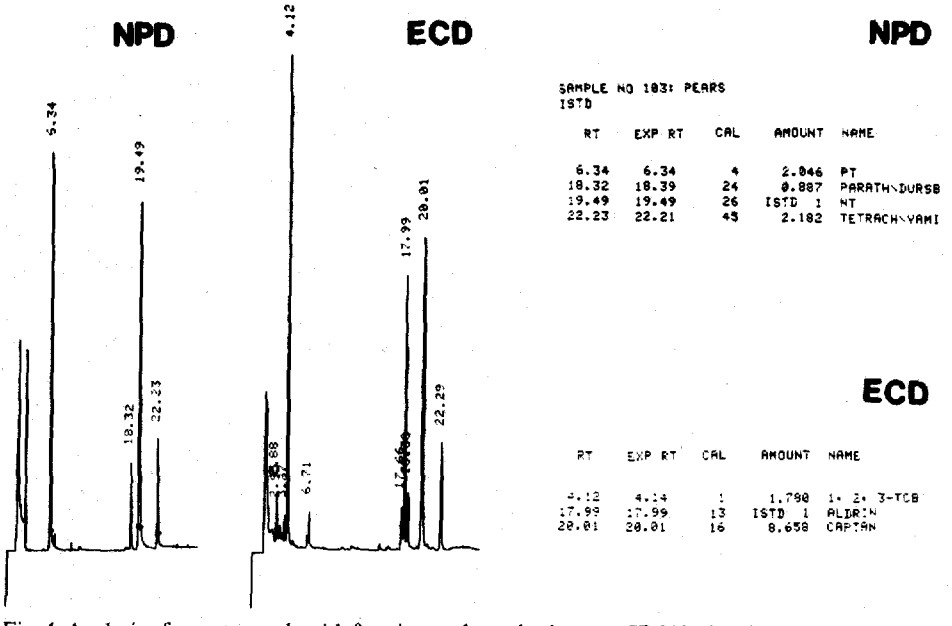


Fig. 4. Analysis of a pear sample with four internal standards on an SP 2100 fused-silica capillary column. Parallel detection on nitrogen–phosphorus and electron-capture detectors.

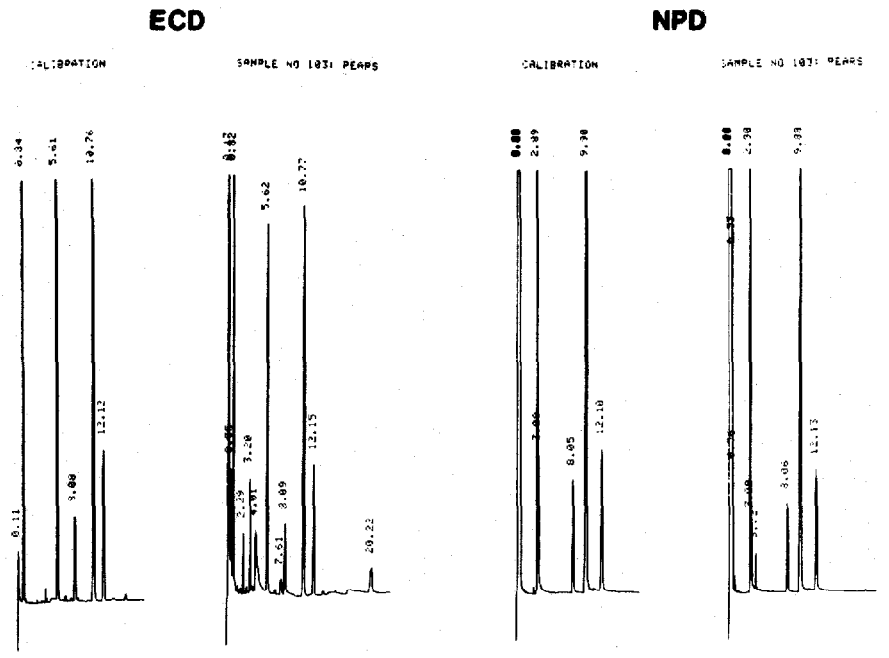


Fig. 5. Confirmation and quantitation of pesticide residues found on an OV-17 fused-silica capillary column. Parallel detection on nitrogen–phosphorus and electron-capture detectors.

nition of the individual compounds. The internal standards selected exhibit the essential features of similar properties in the clean-up procedure, chemical stability, response comparable to those of the pesticides with the selective detectors and chromatographic retention data on all columns which cause no interference with any pesticide. In Table I all OPs included in the two calibration mixtures and all OPs to be determinated are listed together with the two internal standards. Seventeen of the 53

TABLE III

RESULTS OF RESIDUE ANALYSIS IN PEARS

Substance	*Retention time (min)*	*Expected* (ng/μl)*	*Found (ng/μl)*		*Recovery§ (%)*	*Result (ppm)*	
			*ECD***	*NPD****		*ECD***	*NPD****
Aldrin	5.61	2.0	1.70		85		
Dursban	8.08	1.0	0.88	0.77		1.03	0.96
NT	9.90	2.0	1.60		80		
Captan	10.76	8.0	7.17			8.44	
Tetrachlorvinphos	12.12	2.0	1.69	1.62		1.99	2.02

* 100 g of pears were fortified with 200 μg each of aldrin, NT and tetrachlorvinphos, 100 μg of dursban and 800 μg of captan; 1 ml of the gas chromatographic sample is equivalent to 1 g of food.

** ECD = electron-capture detector.

*** NPD = nitrogen–phosphorus detector.

§ Including volume errors of 1 μl for the calibration mixture and 1 μl for the sample.

OPs cannot be characterized by their retention times on the SP 2100 column because of overlapping. Eight critical pairs consisting of two unresolved compounds are indicated in Table I. Seven of these critical pairs are certainly resolved on the OV-17 column, as can be recognized from the relative retention times given in Table I. For four critical pairs additional information can be drawn from the different responses of the two compounds in the electron-capture detector. The most critical pair, diazinon–disulfoton, however, cannot be differentiated in this way. These two must be finally confirmed by using other methods.

The last critical pesticide of this class is bromophos. When starting to develop this method $2\frac{1}{2}$ years ago, bromophos was found near to the internal standard NT but completely separated on the SP 2100 column. After 2 years we had to replace this first column by a new one which exhibits very similar properties to the first one. Although the two columns fulfilled expectations with regard to the reproducibility of manufactured fused-silica columns, the new one did not separate bromophos from NT; the peak coincidence is not complete and a partially separated peak or a shoulder precedes the NT peak if bromophos is present. The situation described here is characteristic of transformations of highly sophisticated multi-component separations between capillary columns of the same type. With the new conditions bromophos must also be identified by its high response to the electron-capture detector. However, on the electron-capture detector channel bromophos forms a critical pair with the CP nitrothal-isopropyl also. Therefore, a peak in the chromatogram indicating nitrothal-isopropyl must always be analysed on the OV-17 column where a differentiation of these two compounds can be easily achieved.

All 30 CPs studied are listed in Table II together with two internal standards. Only eight compounds form four critical pairs of unresolved peaks on the SP 2100 column, three of which are easily resolved on the OV-17 column. However, with the pair quintozene–β-HCH a problem arises. Although both compounds show different retentions on OV-17, β-HCH cannot be determined because of overlap with the internal standard aldrin on this column. When β-HCH is present in a sample it produces a peak on the SP 2100 but frequently no signal is observed on the OV-17 column. This behaviour indicates the likely presence of this compound in the sample. For confirmation the analysis must be repeated without adding aldrin to the sample.

Particular attention must be focused on the fact that many pesticides respond to both selective detectors. Critical evaluation of all information from the microprocessor is necessary to avoid misinterpretation. The chromatograms and reports shown in Fig. 3 illustrate this problem and in Tables I and II the columns headed F are incorporated to support the analyst's work. It must be emphasized that these false designations change with alterations in the chromatographic conditions and the variation of the selected retention time windows. However, again all these alterations are easy to identify by critical interpretation of the calibration runs on test mixtures.

In most laboratories the routine analysis of pesticide residues in food samples is carried out today on packed columns using a series of gas chromatograms equipped with columns of different polarity. Official methods[22,23] are based on packed columns and recent textbooks on pesticide analysis[24,25] neglect the enormous advantages offered by capillary gas chromatography. Because of the limited resolution power of packed columns it is not possible to differentiate all of the 83 pesticides studied here on two columns. Even if all possibilities of rational use of the instruments,

for example by effluent splitting, are exploited, a minimum of two gas chromatographs with four detectors and integrator channels is necessary to obtain a similar amount of information about a food sample to that given by the method described here. Most pesticide residue laboratories, however, use a battery of more than four instruments.

A recent comprehensive description of gas chromatographic pesticide analysis in monitoring of food and environmental samples was given by Ambrus and coworkers[26,27]. Their papers included the experience of 20 laboratories in Hungary with more than 10,000 field and market samples. Two short columns packed with OV-101 and OV-22 are applied for the first screening and three further columns for confirmatory analyses. Summarizing their experience in daily work, Ambrus *et al.*[27] stated that the most efficient, packed columns cannot separate more than 20–22 peaks using temperature programming. Comparing these results with those presented here (Figs. 1 and 2), the superiority of capillary columns is well demonstrated. One advantage of packed columns over fused-silica capillary columns is having a free choice of stationary phases of all types and polarities. Until recently the production of fused-silica capillary columns was restricted to more apolar phases. However, the selection of a methylsilicone phase (OV-101) and a phenylmethylsilicone phase (OV-22) as the most appropriate for packed columns by the Hungarian group is very similar to our choice of SP 2100 and OV-17. This emphasizes the suitability of these stationary phases for multiresidue analysis of pesticides. With reference to the data handling the method was restricted intentionally to a gas chromatograph equipped with a common microprocessor integrator.

Owing to the application of effluent splitting with both columns, parallel reports are always generated by the two detectors, permitting further calculations and logical decisions not taken advantage of using the method described here. As the instrument used in this work is complete with options for BASIC programming, the method has been improved by data processing, which will be published elsewhere[28,29].

CONCLUSIONS

Automated capillary gas chromatography with parallel detection of the column effluent split to an electron-capture and a nitrogen–phosphorus detector is a very suitable technique for the multiresidue analysis of pesticides in food samples. Using splitless injection, the detection sensitivity for most pesticides is in the lower ppb range, as necessary for monitoring legislative norms. In screening food samples for pesticide residues, dual-channel on-line data processing is a valuable aid to the analyst in selecting samples suspected to be contaminated. The application of microprocessors that can be programmed according to the analyst's special needs will further facilitate decision making in the screening procedure. The final confirmatory test and the quantitation, however, have to be carried out by the analyst manually using appropriate test mixtures for each sample composed individually on the basis of the screening results. The entire pesticide analysis can be performed with only one gas chromatograph and two capillary columns with different polarities connected to the two selective detectors. The method described here has been used successfully for more than 2 years in the routine analysis of food samples.

ACKNOWLEDGEMENT

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NOTES ADDED IN PROOF

(1) By mistake the trade name dursban was used instead of the general name chlorpyrifos throughout this paper.

(2) In parallel to this method a pesticide analysis applying two-dimensional capillary gas chromatography was developed in our group[30–33].

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