CHROMSYMP. 132

RESIDUE ANALYSIS OF PESTICIDES IN FOOD BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH CAPILLARY COLUMNS AND PARALLEL DETECTION WITH FLAME-PHOTOMETRIC AND ELECTRON-CAPTURE DETECTION

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SUMMARY

   Two-dimensional gas chromatography on two capillary columns is used for pesticide residue analysis in food samples. Both columns are connected by effluent splitting to the selective flame-photometric and electron-capture detectors, which are linked on-line to independent data processing channels. About 100 halogenated and organophosphorus pesticides, together with three internal standards, are screened on the first column with methylsilicone as the stationary phase. The compounds are recognized by their retention data and response to the two selective detectors. In a second analysis, peaks eluted from the first column are transferred by means of pneumatic switching to the second column with phenyl cyanopropyl methylsilicone as the stationary phase. This cutting of narrow fractions can be executed with high accuracy and reproducibility ("live chromatography") by using time programming of the cutting valve. The final identification of all investigated pesticides is achieved by evaluating the set of linked retention data and, additionally, the response ratio of the two detectors characteristic for each compound. The application of the method to two real food samples, fortified with chlorinated and organophosphorus pesticides, is demonstrated.

INTRODUCTION

   The reliable gas chromatographic (GC) identification of any compound at trace concentrations in complex matrices depends very much on the efficiency of the columns used. A commercial supply of less fragile high-performance capillary columns seems to be a prerequisite for their introduction into laboratories executing routine analyses in the field of food control and environmental protection. Facing the multitude of substances contaminating our food and environment, the determination of retention data on one capillary column in connection with selective detection is definitely not sufficient for the identification of any compound, and a confirmatory analysis on another column of different polarity is necessary. Parallel detection with two detectors of different selectivity is an additional method of enhancing the information about the substances to be identified.

Another approach for enhancing the information about the identity of un-known substances is the application of two-dimensional GC. This technique was developed as early as 1968 by Deans[1], who introduced pneumatic switching of two columns. For a long time its practical application was restricted to monitoring pro-cess streams in industry. Only a few groups of chromatographers have used two-dimensional GC for the determination of components in complex mixtures[2-5]. A re-view of the technique and its application was given by Bertsch[6]. Practical and the-oretical aspects based on information theory were discussed by Ševčík[7].

In this paper, we report the application of a commercial gas chromatograph, designed for two-dimensional work with "live switching", to pesticide multiresidue analysis and the application of the method to food samples fortified with several pesticides. Modifications necessary for the simultaneous detection of pesticides with two selective detectors are described.

EXPERIMENTAL

Instrumentation

A Sichromat 2 gas chromatograph (Siemens, Berlin, F.R.G.) consisting of two separately heated oven units, equipped with a glass capillary split/splitless injector and the two detectors for selective detection [electron-capture (ECD) and flame-photometric (FPD) detection], was used. The instrument is furnished with a device allowing pneumatic switching in order to transfer selected fractions of effluent from the first column to the second. The heart of the switching device is a T-piece of sophisticated design, which permits column switching with immediate detection of the selected GC fraction. This technique, called "live switching", is depicted sche-matically in Fig. 1.
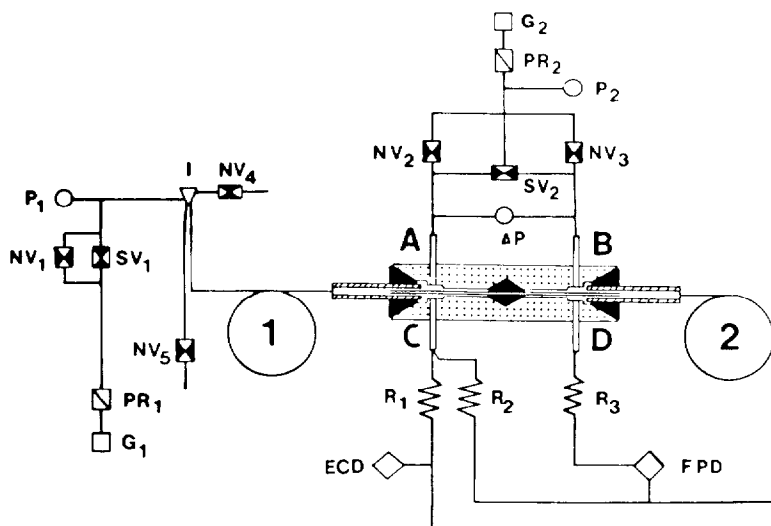

Fig. 1. Flow diagram of the gas chromatographic system. G = gas supply; PR = pressure regulator; P = pressure gauge; SV = solenoid valve; NV = needle valve; ΔP = differential pressure gauge; R = restriction; I = injector; FPD, ECD = detectors; A, B = switch gas entry; C, D = effluent exit.

The system works with slight pressure differences between both ends of the T-piece, generated by means of two make-up gas lines (A, B). The gas flows through these make-up gas lines are adjusted with the two needle valves, NV_2 and NV_3, and the pressure difference, Δp, is indicated by the manometer. Any gas flow from one end to the other must pass the tiny platinum–iridium capillary mounted in the centre and inserted loosely into the two capillary columns. The smallest I.D. of capillary columns to be applied is 0.3 mm. For capillary columns of smaller I.D. a connection via a short glass capillary was established by applying silver chloride to the junction[8]. This type of connection was also used for constructing the two effluent splitters[9] in the system described. Both the effluent stream from the first column, directed to the detectors via outlet C, and that from the second column are split into nearly equal portions. In this way, all chromatograms can be recorded in parallel from the ECD and FPD signals.

Operation of the system

The direction of flow inside the platinum–iridium capillary connecting the first and second column can be changed at will by simply opening and closing the external solenoid valve, SV_2. By using a higher pressure in line B than in line A the effluent from column 1 is directed via outlet C to the two detectors, resulting in conventional chromatograms with parallel detection. As long as the pressure at A exceeds that at B, the effluent from column 1 enters column 2, a process usually called "cutting". A third mode of operation of major importance in our analytical procedure is "back-flushing" the first column, which can be performed by closing SV 1.

After cutting a selected fraction from the first column into the second, back-flush of the first column is required in order to prevent interference of peaks eluted from the second column with more retarded compounds from the first column.

Gas chromatography

Helium was used as both carrier gas and make-up gas, adjusted at P_1 to 2.65 bar and at P_2 to 0.95 bar. Temperatures were set at 290°C for the injection port, 270°C for the FPD and 280°C for the ECD instrument.

The temperature programme for oven 1 was 2 min at 100°C, increased at 25°C/min to 180°C, held for 1 min at 180°C, increased at 10°C/min to 230°C, isothermal at 230°C. The temperature programme for oven 2 was 2 min at 100°C, increased at 25°C/min to 180°C, held for 4 min at 180°C, increased at 10°C/min to 200°C, isothermal at 200°C.

The FPD fuel gas consisted of 80 ml/min of hydrogen and 80 ml/min of air. The ECD instrument was operated with 20 ml/min of argon containing 10% of methane as purge gas.

For the restrictor lines R_1 and R_2 fused-silica tubing (0.5 m × 0.15 mm I.D.) was used (SGE). The restrictor line R_3 serving for fine adjustment of the flow balance in the T-piece as well as the effluent splitting lines at the end of the second column were made from fused-silica capillary of 0.2 mm I.D.

An SP-2100 fused-silica capillary column (17 m × 0.2 mm I.D.), supplied by Hewlett-Packard, was used as the first column; the second was a laboratory-prepared soda-lime glass capillary column coated with OV-225 (20 m × 0.32 mm I.D.)[10].

Injections of 1 μl were carried out splitless into the "cold" column at 100°C; after 60 sec, the split of the carrier gas was reopened.

Data processing

The data system of the Sichromat 2 is designed for dual-channel operation. Although the data of the two channels are simultaneously processed on-line, only one chromatogram can be plotted. In this paper channel 1 (KANR 1) is always connected to the ECD and channel 2 (KANR 2) to the FPD instrument. For multi-component analysis the set-up of various retention time catalogues is of particular importance and may be outlined briefly. The retention time catalogue (RKNR) involves five parameters, of which name of compound (maximum 8 symbols), retention time and labelling of internal standards are printed in the report.

Six different retention time catalogues had to be established for correct recognition and quantitation of all calibrated compounds. They include the different retention times on the first and on both columns for chlorinated pesticides and organophosphorus pesticides as well as the different response factors for the latter on both detectors.

Clean-up

The clean-up followed the procedure of Becker[11]. The internal standards were added to the homogenized food samples before the first solvent extraction was carried out. The final concentration of the purified extracts was the equivalent of 2 g of food in 1 ml.
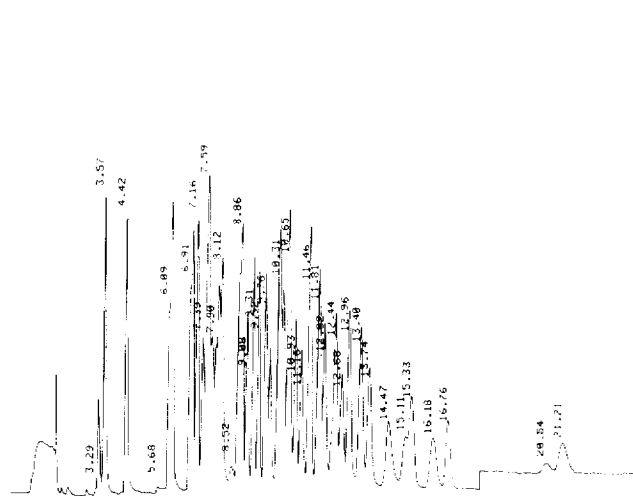
Materials

The pesticides used as test substances were purchased at a purity of 97–99% from Dr. Ehrenstorfer (Augsburg, F.R.G.). Solvents and chemicals for the clean-up of food samples were of analytical-reagent grade obtained from Merck (Darmstadt, F.R.G.). O-Phenyl dimethylthiophosphinate (ISTD 1) and O-2-naphthyl dimethylthiophosphinate (ISTD 2) were used as internal standards; their preparation has been described elsewhere[12].

RESULTS AND DISCUSSION

Features of the method

The determination of a pesticide residue in food samples of unknown history requires the retention data on at least two columns of different polarity and a selective detector. The reliability of a positive identification depends on the accuracy of the retention data and the specificity of the detector signal. Using ECD, all compounds carrying electron-capturing groups are detected. Chlorinated pesticides are only one class of ECD-active compounds present in the environment and in food samples. Other well known chemical classes include polychlorinated biphenyls, polybrominated naphthalenes, polychlorinated dibenzodioxins and phthalate esters. The last group is found frequently in food samples after clean-up for multiresidue analysis.

Fig. 2 shows the separation of 51 chlorinated pesticides, including the two internal standards 1,2,3-trichlorobenzene and aldrin, on the SP-2100 column. The report was calculated from a calibration table for chlorinated pesticides by using the internal standard method with aldrin as reference compound. Recognition and quantitation of 36 compounds by one chromatographic analysis in about 20 min demonstrates the high performance of modern capillary column technology. However,
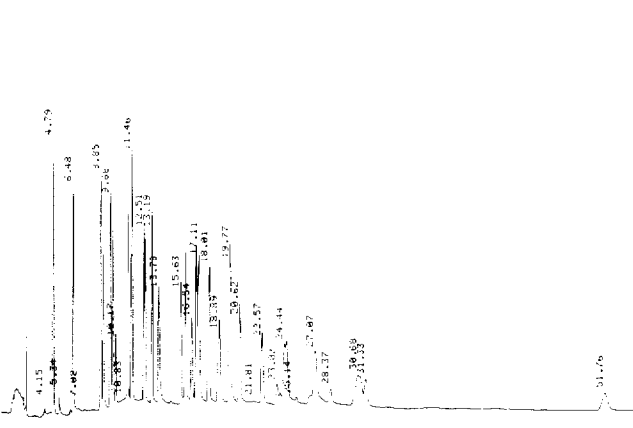
Fig. 2. Chromatogram and report of 51 chlorinated pesticides and 2 internal standards on the first column (ECD). RET. TIME = Retention time in min.

the separation is not sufficient for the identification of all pesticides. This is announced in the report by printing a slash following the coded pesticide name. In all these instances another known pesticide exhibits the same retention time under the specified chromatographic conditions. However, we did not intend to elaborate a GC method achieving maximum resolution of all pesticides under investigation. In daily pesticide analysis, screening methods are of great importance as they allow discrimination between samples free from and probably contaminated with pesticide residues within a reasonable time.

In Fig. 3 the same mixture of chlorinated pesticides is chromatographed on both columns, connected in series by switching the gas flow to the cut position. Most

Fig. 3. Chromatogram and report of 51 chlorinated pesticides and 2 internal standards on the first and second columns in sequence (ECD).

TABLE I

RELATIVE RETENTION TIMES (RRT) OF CHLORINATED PESTICIDES IN TWO-DIMENSIONAL GAS CHROMATOGRAPHY

No.	Pesticide	Test (ng/μl)	First column		Second column	
			RRT	Name	RRT	Name
1	1,2,3-Trichlorobenzene	5	0.372	123-TCB	0.360	123-TCB
2	Chlorthiamid	1	0.462	DCBENIL/	0.492	DCBENIL/
3	Dichlobenil	1	0.462	DCBENIL/	0.493	DCBENIL/
4	Chlorfenprop-methyl	3	0.639	TECNAZE/	0.690	CFENPRO
5	Tecnazene	1	0.648	TECNAZE/	0.673	TECNAZE
6	Propachlor	5	0.651	TECNAZE/	0.774	PROPAC
7	Trifluralin	2	0.723	TRIFLUR	0.752	TRIFLUR
8	α-BHC	1	0.733	A-HCH	0.848	A-HCH
9	Hexachlorobenzene	1	0.753	HCB	0.733	HCB
10	Dicloran	1	0.776	DCAN	1.047	DCAN
11	Lindane	1	0.795	LINDAN/	0.964	LINDAN
12	Quintozene	1	0.799	LINDAN/	0.868	QUINTOZE/
13	β-BHC	2	0.812	B-HCH	1.302	B-HCH/
14	Dichlone	2	0.822	CTALONI/	n.n.	
15	Chlorothalonil	1	0.831	CTALONI/	n.n.	
16	Tri-allate	3	0.851	TRIALAT	0.868	QUINTOZE/
17	Metribuzin	1	0.916	VINCZOL/	1.301	B-HCH/
18.	Vinclozolin	1	0.922	VINCZOL/	1.188	VINCZOL
19	Heptachlor	1	0.931	HEPTAC/	0.948	HEPTAC
20	Dinoseb acetate	2	0.951	HEPTAC/	n.n.	
21	Dichlofluanid	2	0.975	DCFLUAN	1.369	ENDOSFA/
22	Aldrin	1	1	ALDRIN	1	ALDRIN
23	Chlorthal-dimethyl	1	1.025	CTAL-ME/	1.217	CTAL-ME
24	Triadimefon	1	1.025	CTAL-ME/	1.438	TRIADIM/
25	Nitrothal-isopropyl	3	1.041	NTR-IPR	1.257	NTR-IPR
26	Captan	2	1.077	CAPTAN/	1.940	CAPTAN
27	Heptachlor-epoxide	1	1.085	CAPTAN/	1.283	HEPTCOX
28	Folpet	2	1.093	FOLPET	1.811	FOLPET
29	Procymidone	5	1.113	CINOMET/	1.873	PROCYMI
30	Chinomethionat	1	1.113	CINOMET/	1.502	DDE/
31	Chlorbenside	1	1.114	CINOMET/	1.435	TRIADIM/
32	Endosulfan	1	1.146	ENDOSFA	1.368	ENDOSFA/
			1.257	BINAPAK/	2.327	BUPIRIM/
33	Chlorfenson	1	1.168	CFENSON	2.060	CFENSON
34	Dieldrin	1	1.199	DDE/	1.567	DELDRIN
35	p,p'-DDE	1	1.206	DDE/	1.504	DDE/
36	Bupirimate	5	1.234	ENDRIN/	2.337	BUPIRIM/
37	Endrin	1	1.236	ENDRIN/	1.716	ENDRIN
38	Barban	5	1.238	ENDRIN/	n.n.	
39	Binapacryl	1	1.262	BINAPAK/	n.n.	
40	Chlorobenzilate	5	1.289	DDD/	2.160	CBENZIL
41	p,p'-DDD	1	1.305	DDD/	2.350	DDD
42	Tetrasul	1	1.328	T.SUL	1.859	T. SUL
43	Fenazaflor	5	1.358	FENAZFL	n.n.	
44	p,p'-DDT	2	1.404	DDT	2.384	DDT
45	Captafol	2	1.439	CAPTAFO	n.n.	
46	Oxycarboxin	2	1.518	OXYCARB	n.n.	
47	Iprodione	2	1.568	IPRODIO	n.n.	
48	Methoxychlor	2	1.608	METOXYC	3.949	METOXYC
49	Tetradifon	1	1.696	T.DIFON	/	
50	Mirex	1	1.757	MIREX	/	
51	Permethrin	5	2.167	PERMETR	/	
			2.298	PERMETR	/	

of the compounds are eluted within 32 min, but the less volatile methoxychlor takes 52 min and a few pesticides are not eluted even after 60 min. In addition to those less volatile compounds, several more polar and labile pesticides are also found to be undetectable in the effluent from the second column. A compilation of the retention data of all 51 chlorinated compounds is given in Table I.

Considering the pesticides recognized on the basis of calibrated retention data, it is evident that nearly all compounds can be identified by means of two-dimensional analysis. Only for the small number of pesticides that cannot yet be analysed on both columns must other confirmatory tests be applied.

Fig. 4 gives an example demonstrating the utility of the cut operation by using the same complex mixture of pesticides as in Figs. 2 and 3. The first part of the chromatogram represents the separation of the compounds eluted early from the first column and conducted via the restrictor lines R_1 and R_2 directly to the detectors. According to the concept of "live switching" the detectors now serve for monitoring the chromatogram. The fraction between 9.20 and 10.20 min was transferred to the second column, followed immediately by actuating the back-flush of the first column. As mentioned above, back-flushing of the first column is necessary in order to prevent peak interferences by low-boiling compounds eluted later from the first column. In the chromatogram in Fig. 4, the appearance of an unexpected peak at 10.37 min illustrates the fact that back-flushing comes into operation with a time delay of about 30 sec. This is the time elapsed until the flow in the first column is reversed. However, cutting is executed immediately. As can be quoted from the report in Fig. 2, the fraction transferred to the second column consists of five calibrated pesticides, specified as DCFLUAN, ALDRIN, CTAL-ME/ and NTL-IPR. The slash following CTAL-ME indicates a second compound having the same retention time. In fact, five well resolved peaks are recorded in the chromatogram in Fig. 4, beginning with that at 13.22 min. The report in Fig. 4 is established by using the calibration values

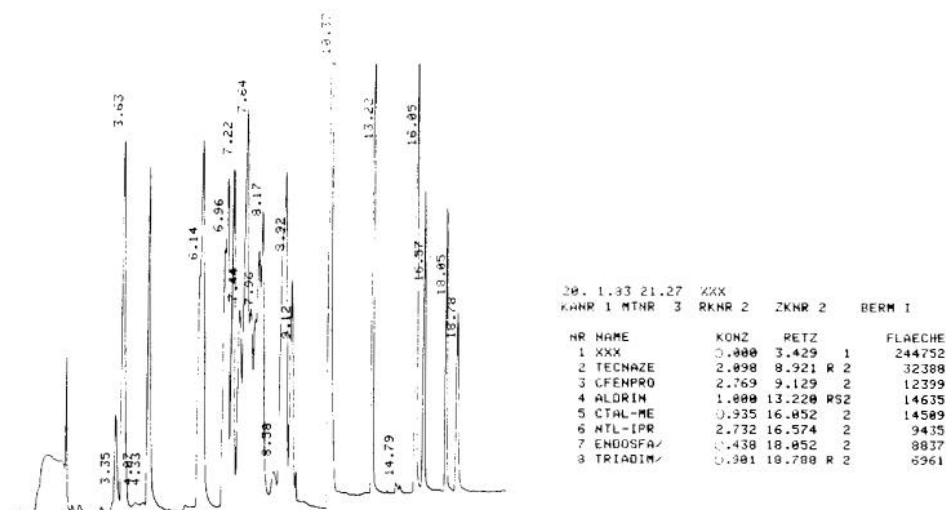| 20. 1.83 21.27 XXX				
KHNR 1 MTNR 3 RKNR 2		ZKNR 2		BERM I
NR NAME	KONZ	RETZ		FLAECHE
1 XXX	0.000	3.429	1	244752
2 TECNAZE	2.098	8.921	R 2	32388
3 CFENPRO	2.769	9.129	2	12399
4 ALDRIN	1.000	13.220	RS2	14635
5 CTAL-ME	0.935	16.052	2	14589
6 NTL-IPR	2.732	16.574	2	9435
7 ENDOSFA/	0.438	18.052	2	8837
8 TRIADIM/	0.301	18.788	R 2	6961

Fig. 4. Identification of a fraction of five chlorinated pesticides by cutting to the second column. Cut, 9.20–10.20 min; back-flush, 10.30 min (ECD).

for the second column. Therefore, all information about peaks from the first column must be disregarded. This means that the printed report contains useful values for Nos. 4 (ALDRIN) to 8 (TRIADIM/). These statements must be decoded by means of Table I, together with those in the report in Fig. 2 (Nos. 15–18). When this procedure is followed, the following pesticides are identified as being present in the cut fraction: dichlofluanid, aldrin, chlorthal-dimethyl, triadimefon and nitrothal-isopropyl.

Fig. 5 shows a chromatogram of the recorded FPD signal demonstrating the separation of 57 organophosphorus pesticides and the two internal standards. Two reports were established by using calibration tables for organophosphorus pesticides in the two channels connected to the ECD and FPD instrument. In the report of the FPD channel (KANR 2) 41 peaks are indicated, two of them representing the internal standards ISTD 1 and ISTD 2. A compilation of all organophosphorus pesticides with their retention data on both columns and their code names is given in Table II.

A second report in Fig. 5 is set up by using the calibration data in the ECD channel (KANR 1); all organophosphorus pesticides indicated respond to the electron-capture detector. The parallel response of a pesticide to both detectors yields additional information about its identity.

Application to food samples

In the following, examples are given of the application of the procedure to real food samples, fortified with several pesticides. The pesticides were selected to demonstrate the superiority of two-dimensional GC to conventional methods.
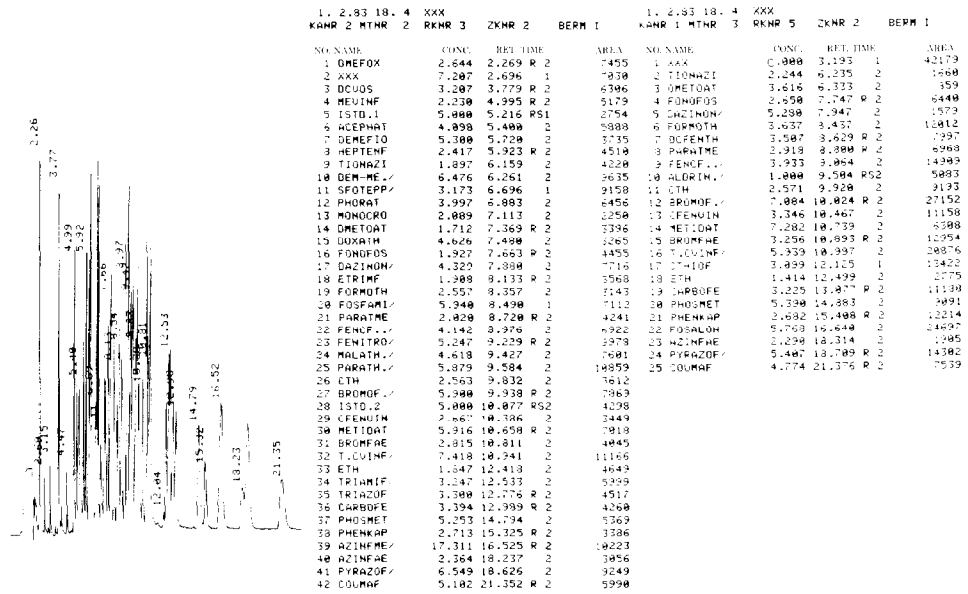

Fig. 5. Chromatogram and reports of 57 organophosphorus pesticides and 2 internal standards on the first column (FPD and ECD).

TABLE II

RELATIVE RETENTION TIMES OF ORGANOPHOSPHORUS PESTICIDES IN TWO-DIMENSIONAL GAS CHROMATOGRAPHY

No.	Pesticide	Test (ng/µl)	First column		Second column	
			RRT	Name	RRT	Name
1	Trichlorphon	5			0.108	TRICFON
2	Dimefox	2	0.216	DMEFOX	1.057	DMEFOX
3	Dichlorvos	2	0.371	DCVOS	0.212	DCVOS
4	Mevinphos	2	0.497	MEVINF	0.294	MEVINF
			0.497	MEVINF	0.307	MEVINF
5	Acephate	3	0.556	ACEPHAT	n.n.	
6	Demephion	5	0.567	DEMEFIO	0.389	TIONAZI/
7	Heptenophos	2	0.585	HEPTENF	0.371	HEPTENF
8	Thionazin	2	0.608	TIONAZI	0.386	TIONAZI/
9	Demeton-S-methyl	3	0.617	DEM-ME/	0.429	DEM-ME/
10	Omethoate	2	0.641	DEM-ME/	n.n.	
11	Sulfotep	2	0.668	SFOTEPP/	0.426	DEM-ME/
12	Dicrotophos	2	0.666	SFOTEPP/	0.540	DCROTOF
13	Phorate	3	0.675	PHORAT	0.417	PHORAT
14	Monocrotophos	2	0.734	MONOCRO	0.700	MONOCRO
15	Dimethoate	2	0.739	DMETOAT	0.692	DMETOAT
16	Dioxathion	5	0.744	DOXATH	0.509	DOXATH
			0.858	DOXATH	0.563	DOXATH
17	Fonofos	2	0.752	FONOFOS	0.481	FONOFOS
18	Diazinon	2	0.771	DAZINON/	0.455	DAZINON
19	Disulfoton	2	0.775	DAZINON/	0.493	ETRIMF/
20	Etrimfos	2	0.801	ETRIMF	0.491	ETRIMF/
21	Formothion	3	0.828	FORMOTH	0.822	FORMOTH
22	Phosphamidon	3	0.843	FOSFAMI/	0.759	BROM-FAE/
23	Dichlofenthion	2	0.841	FOSFAMI/	0.522	DCFENTH
24	Parathion-methyl	2	0.869	PARATME	0.729	PARATME/
25	Fenchlorphos	2	0.885	FENCF/	0.570	FENCF
26	Paraoxon	2	0.888	FENCF/	0.809	PARATH/
27	Pirimiphos-methyl	2	0.912	PIRIMME/	0.592	PIRIMME
28	Fenitrothion	2	0.916	PIRIMME/	0.781	FENITRO
29	Demeton-S-methyl sulphone	3	0.917	PIRIMME/	n.n.	
30	Malathion	2	0.932	MALATH	0.726	PARATME/
31	Amidithion	3	0.936	AMIDITH	1.057	AMIDITH
32	Fenthion	2	0.950	PARATH/	0.717	FENTH
33	Chlorpyrifos	2	0.952	PARATH/	0.618	CPYRIF
34	Parathion	2	0.954	PARATH/	0.807	PARATH/
35	Chlorthion	3	0.972	CTH	0.925	CTH
36	Trichloronate	2	0.979	BROMOF/	0.608	TRICNAT
37	Bromophos	3	0.979	BROMOF/	0.682	BROMOF
38	Chlorfenvinphos	3	1.008	CFENVIN	0.758	BROM-FAE/
			1.026	CFENVIN	0.834	CFENVIN
39	Methidathion	5	1.051	METIDAT	1.127	METIDAT/
40	Bromophos-ethyl	3	1.060	BROMFAE	0.758	BROM-FAE/
41	Tetrachlorvinphos	3	1.085	T.CVINF/	1.048	T.CVINF
42	Ditalimfos	2	1.088	T.CVINF/	1.147	DTALIMF

TABLE II (*continued*

No.	Pesticide	Test (ng/µl)	First column		Second column	
			RRT	Name	RRT	Name
43	Vamidothion	3	1.090	T.CVINF/	n.n.	
44	Chlorthiophos	3	1.186	CTHIOF	1.124	METIDAT/
			1.204	FENSFTH/	1.169	CTHIOF
			1.233	TRIAMIF/	1.291	CTHIOF
45	Fensulfothion	3	1.206	FENSFTH/	2.251	FENSFTH
46	Ethion	2	1.214	ETH	1.348	ETH
47	Triamiphos	2	1.234	TRIAMIF/	1.760	TRIAMIF
48	Triazophos	3	1.259	TRIAZOF	2.380	TRIAZOF
49	Carbophenothion	3	1.276	CARBOFE	1.416	CARBOFE
50	Phosmet	5	1.471	PHOSMET	n.n.	
51	Phenkapton	3	1.490	PHENKAP	2.136	PHENKAP
52	Phosalone	5	1.616	AZINFME/	/	
53	Azinphos-methyl	5	1.620	AZINFME/	/	
54	Azinphos-ethyl	3	1.783	AZINFAE	/	
55	Pyrazophos	3	1.808	PYRAZOF/	/	
56	Dialifos	5	1.815	PYRAZOF/	/	
57	Coumaphos	5	2.062	COUMAF	/	
–	ISTD 1	5	0.518	ISTD.1	0.323	ISTD.1
–	ISTD 2	5	1	ISTD.2	1	ISTD.2

In Fig. 6a and b the screening run of a pesticide analysis on zucchini on the first column is exemplified. The ECD signal, together with two reports, is shown in Fig. 6a. Many peaks appear in the chromatogram, one of them representing the internal standard aldrin and the others pesticides or substances from the matrix. However, the number of peaks suspected of being pesticides is reduced because in the reports only calibrated substances are indicated. Consequently, in the report based on the calibration table for chlorinated pesticides (RKNR 1) a few compounds are stated, whereas in the report for organophosphorus pesticides responding to the electron-capture detector (RKNR 5) only one pesticide is recognized, namely bromophos ethyl (BROMFAE). This result is in accord with the plot of the FPD signal obtained with a second injection and shown in Fig. 6b. The corresponding report indicates only bromophos ethyl as a calibrated pesticide.

Confirmation of the suspected compounds was carried out by transferring the corresponding fraction from the first to the second column, where further separation is accomplished. The results plotted in Fig. 6c (ECD signal) and d (FPD signal) verify the presence of bromophos ethyl and chlorfenson (CFENSON) in the sample, whereas all the other suspected pesticides could not be confirmed. The results may be misinterpreted as confirmation of dichlofluanid also, but cutting a narrow fraction resulted in an uncalibrated peak at 14.52 min.

An organophosphorus pesticide with electron-capturing properties such as bromophos ethyl is calibrated in both channels and, consequently, quantitated with two different internal standards. The parallel reports in Fig. 6a–d exhibit satisfactory agreement of the concentration of this compound.

a
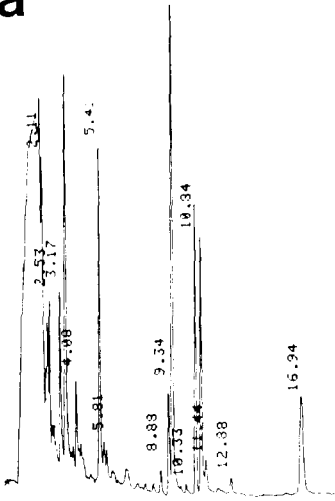
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27. 1.83 19.20  ZUCCHINI
KANR 1 MTNR  3  RKNR 1   ZKNR 2    BERM I

NO. NAME        CONC.    RET. TIME        AREA
 1 XXX          0.000    2.116    1      33736
 2 VINCZOL/     0.041    8.888 R  2        429
 3 DCFLUAN      0.265    9.346    2       2059
 4 ALDRIN       1.000    9.547 RS2        12374
 5 CFENSON      0.567   11.160    2       5956
 6 DDE..../     0.070   11.445 R  2        899


27. 1.83 19.20  ZUCCHINI
KANR 1 MTNR  3  RKNR 5   ZKNR 2    BERM I

NO. NAME        CONC.    RET. TIME        AREA
 1 XXX          0.000    2.116    1      37584
 2 ALDRIN./     1.000    9.547 RS2        12374
 3 BROMFAE      0.567   10.845 R  2       5494
```
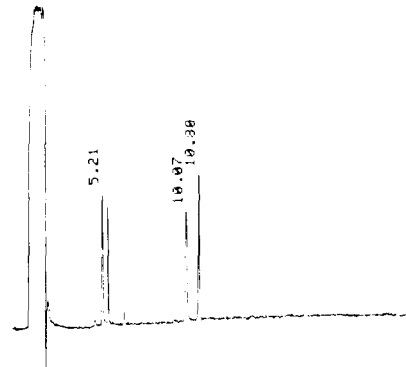
b

```
27. 1.83 22. 7  ZUCCHINI
KANR 2 MTNR  2  RKNR 3   ZKNR 2    BERM I

NO. NAME        CONC.    RET. TIME        AREA
 1 ISTD.1       1.000    5.219 RS1        2426
 2 XXX          0.000    5.540    1        699
 3 ISTD.2       1.000   10.074 RS2        2906
 4 BROMFAE      0.599   10.805    2       2910
```

c
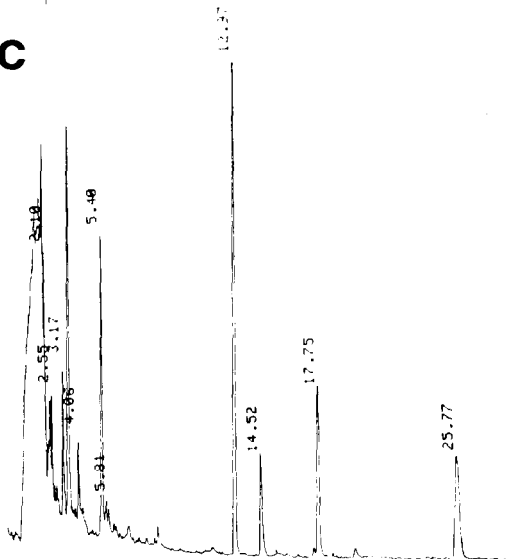
```
28. 1.83 10.37  ZUCCHINI
KANR 1 MTNR  3  RKNR 2   ZKNR 2    BERM I

NR NAME         KONZ     RETZ          FLAECHE
 1 XXX          0.000    2.104    1      24249
 2 ALDRIN       1.000   12.973 RS2       11763
 3 ENDOSFA/     0.327   17.758    2       5304
 4 CFENSON      0.484   25.774    2       5974


28. 1.83 10.37  ZUCCHINI
KANR 1 MTNR  3  RKNR 6   ZKNR 2    BERM I

NR NAME         KONZ     RETZ          FLAECHE
 1 XXX          0.000    2.104    1      24249
 2 ALDRIN ./    1.000   12.973 PS2       11763
 3 BROMFAE/     0.617   17.758    2       5304
 4 OTALIME      1.806   25.774 R 2       5974
```

Fig. 6.

d

```
27. 1.83 21.33  ZUCCHINI
KANR 2 MTNR  2   RKNR 4    ZKNR 2    BERM I

NO. NAME              CONC.    RET. TIME      AREA
  1 DCVOS            0.099    5.223 R 2       1087
  2 XXX              0.000    5.547   1        031
  3 BROMFAE/         0.577   17.754   2       5494
  4 ISTD.2           1.000   22.650  RS2      4692
```
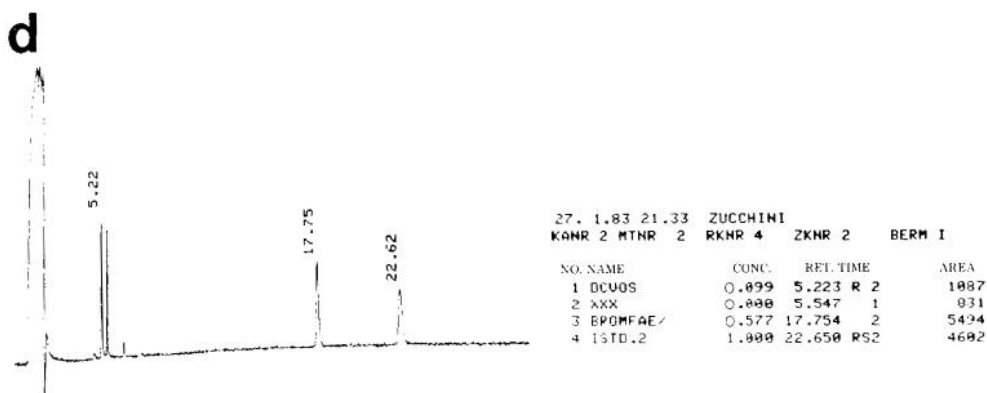
Fig. 6. Identification of pesticide residues in zucchini. Chromatograms on the first column: (a) ECD; (b) FPD. Two-dimensional chromatograms with cutting between 8.50 and 11.70 min, back-flush starting at 11.75 min: (c) ECD; (d) FPD.

The example in Fig. 7 is likely to be confusing at first sight. Oranges were selected because they yield chromatograms with many matrix peaks. Therefore, the report of the ECD signal (Fig. 7a) contains many peaks at low concentration levels, which may be due to pesticides residues. A decision about the occurrence of the suspected compounds can be made only after the confirmatory analysis on the second column, shown in Fig. 7c. After having transferred the fraction between 9.20 and 12.60 min from the first to the second column, no chlorinated pesticide is recognized in the report, with the exception of the internal standard aldrin. In the same way the fraction between 7.40 and 8.20 min, when analysed, did not confirm any pesticide.

Evaluating the FPD signal in Fig. 7b, only one peak is found, together with the two internal standards and recognized as PARATH./. From Table II, it is understood that this peak may consist of parathion, chlorpyrifos or fenthion. After transferring the specified fraction to the second column, the peak from the first column appears well separated into two peaks (Fig. 7d). The corresponding report indicates CPYRIF and PARATH./, confirming chlorpyrifos and parathion as being present in the sample. Both of these organophosphorus pesticides respond to the electron-capture detector. Therefore, a cross-check of the results on channel 1 should confirm the findings. In fact, chlorpyrifos and parathion (PARATH./) are printed in the report, based on the calibration table of organophosphorus pesticides in the ECD channel (RKNR 6).

Comparison with other methods

Routine pesticides residue analysis is commonly performed on a set of gas chromatographs, equipped with selective detectors and packed columns of different polarity. The individual pesticides are identified by comparison of retention data obtained in two or three different chromatographic systems, operated in parallel. In addition, by comparing the characteristic response of many pesticides to various selective detectors, useful information may be provided.

In the last decade, a few laboratories have introduced high-performance capillary columns, taking advantage of the higher separation efficiency and, simultane-
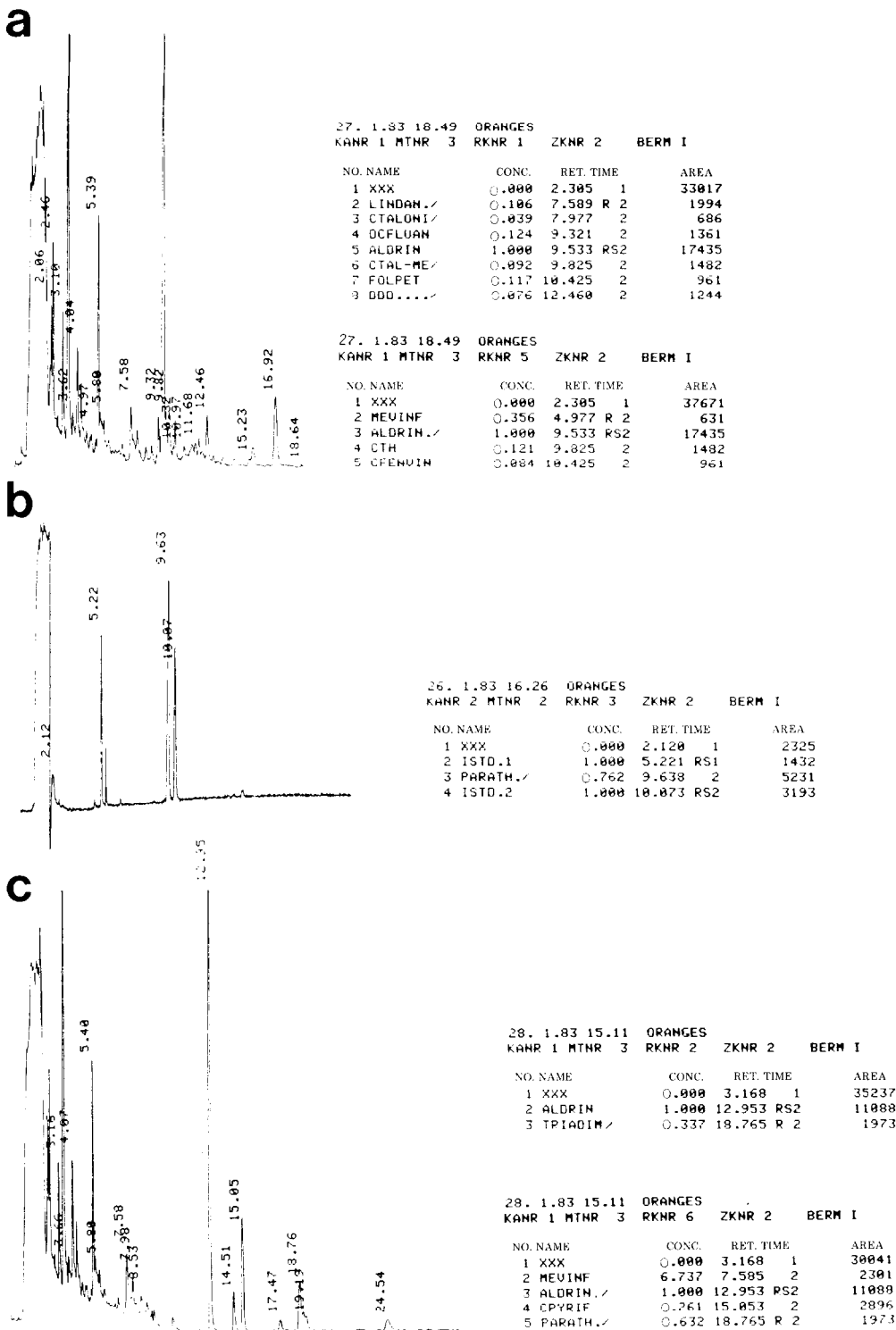
a

```
27. 1.83 18.49   ORANGES
KANR 1 MTNR  3   RKNR 1    ZKNR 2    BERM I

NO. NAME          CONC.    RET. TIME      AREA
 1 XXX            0.000    2.305    1     33017
 2 LINDAN./       0.106    7.589 R  2      1994
 3 CTALONI/       0.039    7.977    2       686
 4 DCFLUAN        0.124    9.321    2      1361
 5 ALDRIN         1.000    9.533 RS2      17435
 6 CTAL-ME/       0.092    9.825    2      1482
 7 FOLPET         0.117   10.425    2       961
 8 DDD..../       0.076   12.460    2      1244


27. 1.83 18.49   ORANGES
KANR 1 MTNR  3   RKNR 5    ZKNR 2    BERM I

NO. NAME          CONC.    RET. TIME      AREA
 1 XXX            0.000    2.305    1     37671
 2 MEVINF         0.356    4.977 R  2       631
 3 ALDRIN./       1.000    9.533 RS2      17435
 4 CTH            0.121    9.825    2      1482
 5 CFENVIN        0.084   10.425    2       961
```

b

```
26. 1.83 16.26   ORANGES
KANR 2 MTNR  2   RKNR 3    ZKNR 2    BERM I

NO. NAME          CONC.    RET. TIME      AREA
 1 XXX            0.000    2.120    1      2325
 2 ISTD.1         1.000    5.221 RS1       1432
 3 PARATH./       0.762    9.638    2      5231
 4 ISTD.2         1.000   10.073 RS2       3193
```

c

```
28. 1.83 15.11   ORANGES
KANR 1 MTNR  3   RKNR 2    ZKNR 2    BERM I

NO. NAME          CONC.    RET. TIME      AREA
 1 XXX            0.000    3.168    1     35237
 2 ALDRIN         1.000   12.953 RS2      11088
 3 TPIADIM/       0.337   18.765 R  2      1973


28. 1.83 15.11   ORANGES
KANR 1 MTNR  3   RKNR 6    ZKNR 2    BERM I

NO. NAME          CONC.    RET. TIME      AREA
 1 XXX            0.000    3.168    1     30041
 2 MEVINF         6.737    7.585    2      2301
 3 ALDRIN./       1.000   12.953 RS2      11088
 4 CPYRIF         0.261   15.053    2      2896
 5 PARATH./       0.632   18.765 R  2      1973
```
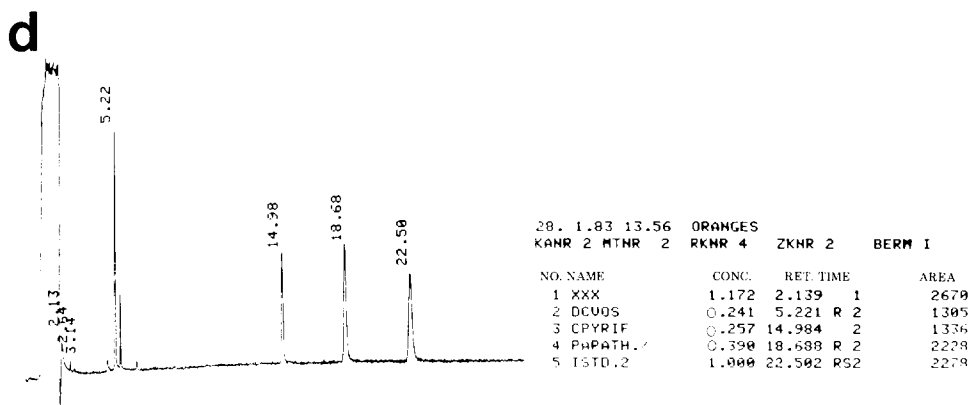
Fig. 7.

Fig. 7. Identification of pesticide residues in oranges. Chromatograms on the first column: (a) ECD; (b) FPD. Two-dimensional chromatograms with cutting between 9.20 and 12.60 min, back-flush starting at 12.70 min: (c) ECD; (d) FPD.

ously, the much greater accuracy of the measured retention data[13-23]. However, even the higher resolution of one long high-performance capillary column is not sufficient to identify the individual pesticides by using selective detectors. One way to solve the problem is to enhance enormously the specificity of the detecting unit, and this can be achieved only by the direct connection of such a chromatographic system to a mass spectrometer utilizing its high specificity for differences in chemical structure. This technique enables the analyst to identify unequivocally pesticide residues in biological samples[24-27]. When sophisticated detection techniques such as selected-ion monitoring or selective ionization procedures are applied, substances in trace concentrations can be determined without being separated from other chromatographically overlapping compounds. Another approach is to use two properly selected capillary columns that always supply parallel detection on two selective detectors in conjunction with data processing, adapted to the analytical problem[28]. The technique of two-dimensional GC is a new method for solving the problem by enhancing the chromatographic resolution. According to information theory, a combined interrelated assay of data yields much more information than sequential sets of data[7]. Undoubtedly, the most elegant and efficient technical achievement is the pneumatic column switching[6]. In our system, additional information is derived from the parallel detection on two selective detectors.

CONCLUSIONS

    The two-dimensional GC system described, equipped with one injection port, two capillary columns and two selective detectors, has proved to be more efficient for pesticide residue analysis than methods applying packed or capillary columns in two or more conventional instruments. Results are obtained more reliably in a shorter time with instrumentation requiring lower expenditure for purchase and maintenance.
    The two reported examples demonstrate that the method is best qualified for the determination of trace amounts of pesticide residues in food samples, even if the

clean-up proves to be inadequate for particular samples. Therefore, we believe that this technique is an attractive alternative to the conventional GC systems now used in routine analysis.

At the present stage, further elaboration of the method is limited by the built-in microprocessor, which cannot be programmed to the analyst's special needs. The method described here is in a preliminary state, but has the potential for far-reaching automation.

ACKNOWLEDGEMENT

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