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Note

Evaluation of automated splitless and manual on-column injection techniques using capillary gas chromatography for pesticide residue analysis

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Capillary gas chromatography (GC) with wall-coated open tubular columns is gaining growing acceptance in the analysis of complex mixtures at trace level concentrations. One of the most important practical fields is pesticide residue analysis in food. The prerequisite for utilizing the enormous separation power of capillary columns for trace analysis was the development of new injection techniques. These techniques have in common the separation of the solvent from the sample components without introducing the sample as a broad band. The advantages and restrictions of the various known sampling techniques were discussed by several authors[1,2]. Under the aspects of quantitative determination and separation of labile compounds under gentle conditions, the direct on-column injection technique of Grob[3,4] proved to be the superior technique[5,6]. However, this sampling method needs special equipment and technical skill. Until recently it was not possible to adapt the on-column technique to autosampling. On the other hand the splitless injection technique first introduced by Grob[7] is very easy to handle and can be performed with any commercial autosampling device. Therefore, to apply both techniques for different purposes is considered to be a good compromise in many laboratories. In our laboratory the screening analysis for pesticide residues in food is automated using splitless injection. The analyses are executed overnight. The results of this screening are based on daily calibration of all pesticides incorporated in the method. The residue concentrations found in the screening can be used as reliable quantitative estimations. Finally, quantitative determination of the pesticide residues is carried out with the on-column sampling method by applying freshly prepared pesticide calibration mixtures[8,9].

In this paper we describe some experiments to study the reliability of splitless and on-column injection techniques for pesticide residue analyses. These were carried out on capillary columns that were in use for more than six months in daily routine analysis. Our results reflect the reliability and reproducibility of the methods under actual conditions of food control.

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EXPERIMENTAL

Instrumentation

The GC analysis was carried out on a gas chromatograph HP 5880 A (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with two injection ports for capillary columns and two selective detectors. Electron-capture and nitrogen–phosphorus detectors were used in parallel. Signals were processed on the built-in two-channel integrator. One injection port is designed for splitless the other one for on-column injection. Both injection ports were supplied by Hewlett-Packard. The splitless injector is connected to an autosampler (HP 7671 A).

Installation of capillary columns

Two fused-silica capillary columns, coated with "bonded phase" dimethylsilicone BP-1 (SGE, Ringwood, Australia) 25 m × 0.2 mm I.D. and methylphenylsilicone BP-10 (SGE) 12 m × 0.2 mm I.D., were mounted alternately to the on-column injector or splitless injector. Both columns were joined in an effluent splitter[9] connecting both selective detectors with a split ratio of 1:1.

Gas chromatography

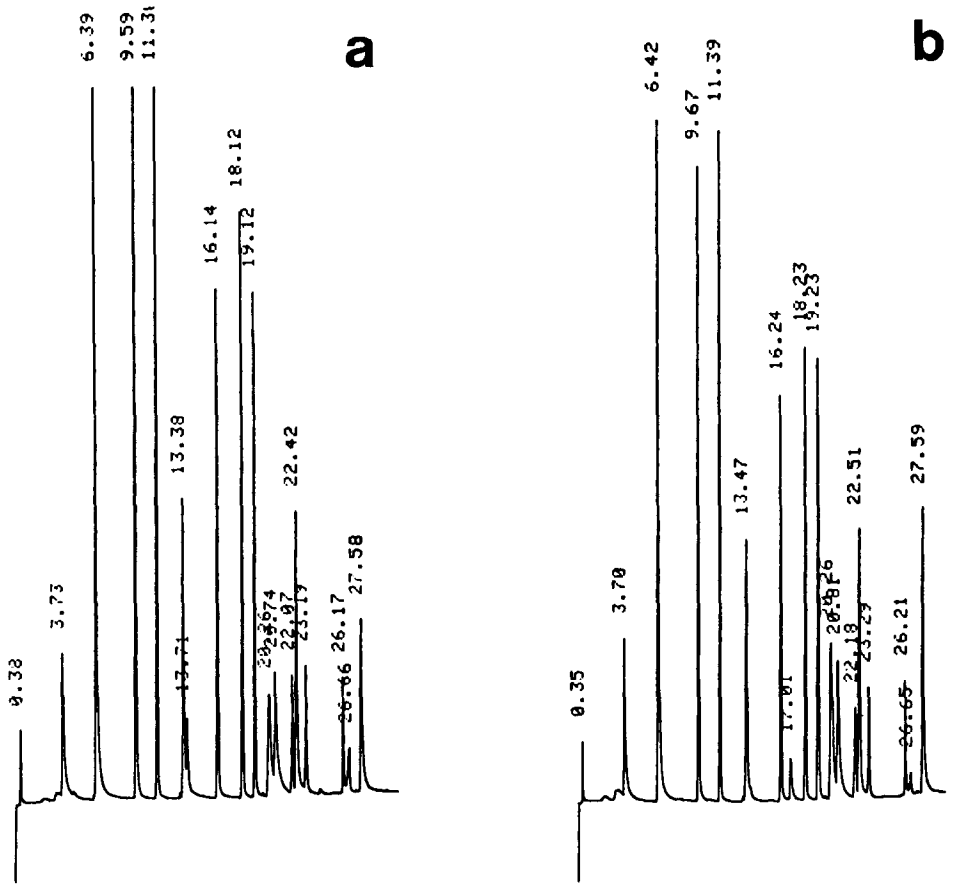
Helium was used as the carrier gas and make-up gas in the nitrogen–phosphorus detector. The electron-capture detector was purged with argon and 10% methane. Temperatures were set to 300°C for the detectors and 240°C for the splitless injector. The sample volumes always were 2 μl. Splitless injection according to Grob and Grob[3,4] onto the "cold" column at 90°C was carried out with the split valve closed for 30 sec. The on-column injection was carried out with a 5-μl syringe and a fused-silica needle at 90°C; 1 min after injection the following temperature programme was started: 30°C/min to 150°C; 2 min; 3°C/min to 205°C; 10°C/min to 240°C; 2°C/min to 260°C; 10 min; stop; cool to the initial temperature (90°C).

Materials

Pesticides were purchased from Dr. Ehrenstorfer, Augsburg, F.R.G. in 97–99% purity. Internal standards for determination with nitrogen–phosphorus detection, O-phenyl dimethylthiophosphinate (PT) and O-2-naphthyl dimethylthiophosphinate (NT), were synthesized as described[8]. The pesticide test mixtures were prepared with iso-octane as solvent at concentrations of 2 μg/ml.

RESULTS AND DISCUSSION

Two pesticide mixtures were composed of relevant chlorinated and organophosphorus compounds. They reflected the wide range of volatility, polarity and reactivity in both chemical groups. Both mixtures contained internal standards. The GC conditions were the same for both injection techniques, resulting in nearly identical retention times for the individual components of the mixtures. In Fig. 1 two chromatograms of the mixture of chlorinated pesticides are shown. The left one was obtained using the splitless, the other one using the on-column injection technique. Most of the peaks show a greater tailing than reported in other papers and manufacturers' advertisements, but these chromatograms were obtained on a column that



RT	AREA	NAME	RT	AREA
3.73	36062.90	DICHLOBENIL	3.70	29213.40
6.39	129917.00	HCB	6.42	105475.00
9.59	102156.00	LINDAN	9.67	85832.40
11.30	107025.00 +	ALDRIN	11.39	89190.20 +
13.38	51763.40	VINCLOZOLIN	13.47	47544.50
16.14	75132.50	ENDOSULF I	16.24	64003.30
18.12	83330.80	DIELDRIN	18.23	71526.00
19.12	75598.70	ENDRIN	19.23	73601.40
20.26	35370.20	CHLORFENSON	20.26	48036.60
20.74	43877.70	TETRASUL	20.81	41634.60
22.07	17568.40	ENDOSULF II	22.18	15250.50
22.42	54630.20	DDD	22.51	52566.90
23.19	19925.00	DDT	23.29	18507.00
26.17	13660.30	METHOXYCHLOR	26.21	13896.30
26.66	11324.60	CAPTAFOL	26.65	5704.96
27.58	43681.50	TETRADIFON	27.59	61579.40

Fig. 1. Chromatograms of chlorinated pesticides on a BP-10 column. (a) Splitless injection with autosampler; (b) on-column injection manually.

was in use for more than six months in daily routine work. This means that the chromatograms reflect the performance of the columns after 600–800 injections of biological samples.

The experimental data of replicate injections applying splitless and on-column sampling are compiled for both pesticide mixtures in Tables I and II. All data were calculated with the internal standard method. This procedure is recommended for practical residue analysis with capillary columns because it eliminates the volume deviation between single injections and fluctuations in detector response.

TABLE I

TEN REPLICATE INJECTIONS OF CHLORINATED PESTICIDES ON TWO COLUMNS: AUTOSAMPLER SPLITLESS AND MANUAL ON-COLUMN INJECTIONS

Relative response calculated with aldrin as an internal standard.

No. Pesticide	*Splitless injection*				*On-column injection*			
	BP 1	*R.S.D. (%)*	*BP 10*	*R.S.D. (%)*	*BP 1*	*R.S.D. (%)*	*BP 10*	*R.S.D. (%)*
1 Dichlobenil	0.370	3.2	0.380	0.7	0.380	3.5	0.310	1.6
2 HCB	1.080	1.2	1.205	0.2	1.135	0.6	1.110	1.4
3 Lindan	0.990	1.4	0.980	0.4	1.030	0.9	1.000	0.3
4 Vinclozolin	0.490	1.5	0.475	0.6	0.565	0.5	0.580	0.6
5 Endosulfan I	0.650	1.2	0.690	0.4	0.725	0.3	0.727	0.5
6 Chlorfenson	0.395	2.4	0.345	1.7	0.580	3.1	0.615	1.1
7 Dieldrin	0.745	1.2	0.765	0.3	0.855	0.4	0.815	0.5
8 Endrin	0.635	1.1	0.615	0.6	0.875	1.3	0.870	0.8
9 DDD	0.485	1.6	0.515	0.9	0.585	3.0	0.675	1.3
10 Tetrasul	0.315	1.6	0.415	1.0	0.515	1.9	0.500	2.2
11 DDT	0.255	3.6	0.225	2.5	0.380	2.4	0.310	3.9
12 Methoxychlor	0.140	5.5	0.110	3.1	0.290	3.2	0.200	4.6
13 Tetradifon	0.360	3.4	0.290	2.0	0.700	1.8	0.630	5.1
Mean R.S.D. (%)		2.26 ± 1.29		1.16 ± 0.91		1.7 ± 1.16		1.8 ± 1.58

The response factors relative to the internal standard aldrin are listed for thirteen chlorinated pesticides in Table I. The mixture was composed from relevant substances representing the whole range of volatility, the differences in polarity and reactivity of this chemical group of pesticides. The mixture includes endrin, a compound proposed as a sensitive indicator for the inertness of chromatographic systems[10,11]. Chlorfenson and DDT give further examples for sensitivity to polar spots, mainly to be expected in the insert liner.

The relative response factors of all chlorinated pesticides have been found to be of good reproducibility and independent of the column used. Their standard deviations do not differ very much for both injection methods, resulting in a mean value of less than 2%. This means there is no difference in precision between the two sampling methods for this chemical class.

In Table II the relative response factors and their standard deviations are compiled for fourteen organophosphorus pesticides. This chemical class of pesticides exhibits a greater diversity in its chemical properties than the chlorinated hydrocarbons.

TABLE II

TEN REPLICATE INJECTIONS OF ORGANOPHOSPHORUS PESTICIDES ON TWO COLUMNS: AUTOSAMPLER SPLITLESS AND MANUAL ON-COLUMN INJECTIONS

Relative response calculated with NT as an internal standard.

No. Pesticide	*Splitless injection*				*On-column injection*			
	BP 1	*R.S.D. (%)*	*BP 10*	*R.S.D. (%)*	*BP 1*	*R.S.D. (%)*	*BP 10*	*R.S.D. (%)*
1 PT	0.780	3.5	0.742	2.4	0.574	1.3	0.556	2.2
2 Heptenophos	0.526	2.0	0.512	2.0	0.444	1.7	0.403	1.9
3 Phorate	0.699	2.5	0.710	1.9	0.569	0.8	0.547	1.7
4 Diazinon	0.649	2.5	0.574	2.4	0.482	1.7	0.450	1.4
5 Dimethoate	0.781	5.4	0.854	2.2	0.501	1.8	0.528	1.4
6 Parathion-Me	0.608	1.7	0.559	1.7	0.566	1.3	0.531	1.5
7 Malathion	0.576	2.5	0.540	1.9	0.473	0.8	0.452	1.3
8 Parathion	0.562	1.5	0.494	1.2	0.507	0.5	0.456	0.5
9 Bromophos-Et	0.499	3.2	0.426	1.1	0.405	2.3	0.366	0.6
10 Methidathion	0.338	4.1	0.350	1.8	0.401	1.7	0.403	2.0
11 Ethion	0.795	1.4	0.726	1.9	0.704	0.7	0.680	1.4
12 Phosalone	0.158	7.4	0.143	5.9	0.320	1.5	0.286	2.3
13 Azinphos-Et	0.152	8.8	0.155	7.1	0.495	1.8	0.467	2.6
14 Coumaphos	0.050	5.8	0.052	11.8	0.276	1.2	0.240	3.3
Mean R.S.D. (%)		3.7 ± 2.3		3.2 ± 3.0		1.36 ± 0.52		1.7 ± 0.74

In accordance with the results obtained from the chlorinated pesticides, the relative response factors of all compounds are not affected by the column when using the same injection technique. The standard deviations of all pesticides, however, were on average considerably smaller for the on-column technique. The increase in the standard deviation applying splitless injection shows the lack of precision and reflects mainly the larger thermal burden during the evaporation process in the insert liner. Therefore, the less volatile compounds such as azinphos-ethyl and coumaphos, and the more polar compounds such as dimethoate, exhibit higher deviations within repetitive injections.

The ratio of the relative response factors was chosen as a parameter for comparing the two injection techniques. This quotient indicates the differences in the peak areas when identical test mixtures are analysed on the same columns. If the two techniques are equivalent the ratio for the individual compounds must be *ca.* 1.0. These ratios obtained from the experimental series are plotted against the retention times in Figs. 2 and 3. The bars of the chlorinated pesticides in Fig. 2 demonstrate a discrimination of late eluting compounds with splitless sampling on both columns.

From the plot of the organophosphorus pesticides in Fig. 3a considerable fluctuation of the quotients can be seen.

The internal standard (NT) eluting in the second half of the chromatogram is apparently discriminated. The ratio of non-discriminated compounds must be *ca.* 1.3 in the scale related to NT. This assumption corresponds with the distribution of the ratios for the organophosphorus pesticides on both columns. From the plots it is evident that the latest eluting compounds are so much discriminated with splitless

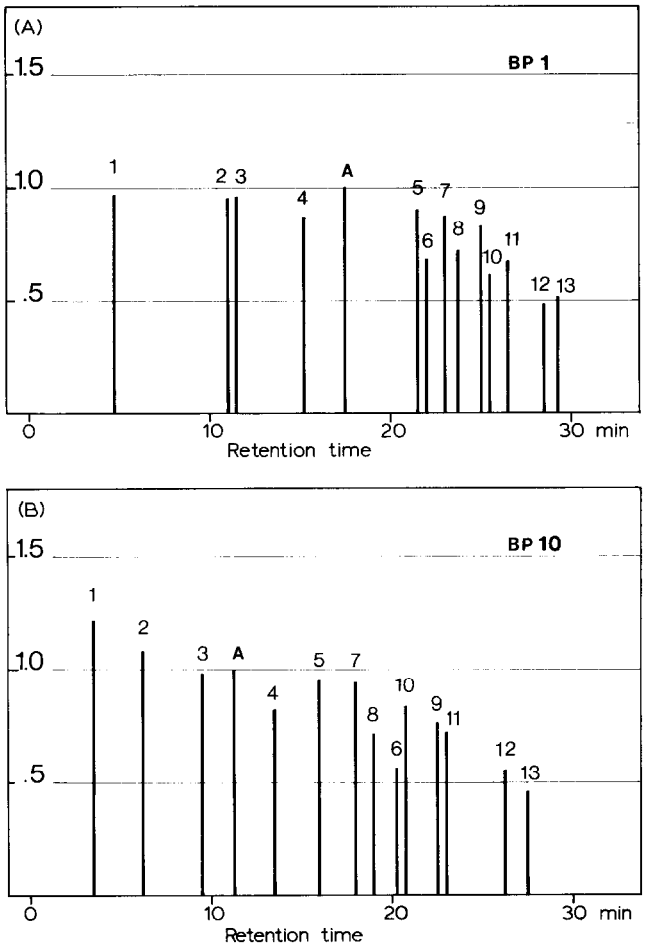


Fig. 2. Ratio of relative response factors of splitless and on-column injection for chlorinated pesticides on two columns. Internal standard is aldrin (A) with quotient 1.0. Pesticides are numbered as in Table I.

sampling that a sensitive detection and a quantitative determination becomes difficult at trace level. The same conclusion results from the absolute low response and high standard deviation of these compounds as documented in Table II.

When comparing the ratios of the individual pesticides calculated for both columns a remarkable accord was observed. In the group of organophosphorus esters all deviations between the corresponding ratios were less than 8%. The ratios of the chlorinated pesticides, however, vary more with the largest deviations calculated for tetrasul (36%) and dichlobenil (26%). In summary, these observations agree with the assumption that there is no effect of the capillary column used on the differing responses obtained with both sampling methods.

Recently a similar evaluation of splitless and on-column injection techniques for the determination of priority micropollutants was executed by Onuska *et al.*[6]. They studied mixtures of chlorinated benzenes and PCB mixtures. These mixtures

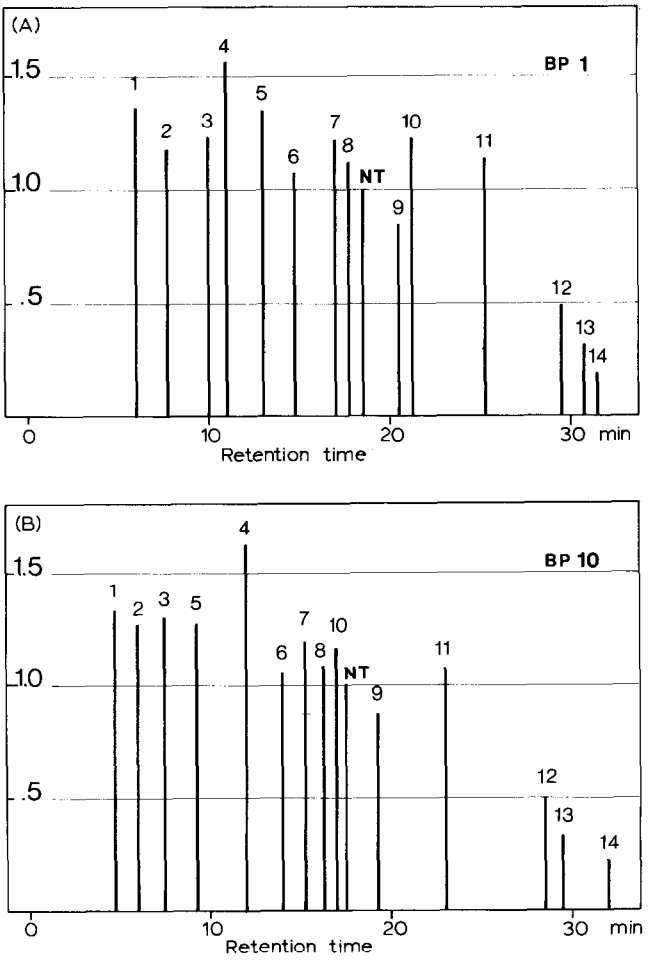


Fig. 3. Ratio of relative response factors of splitless and on-column injection for organophosphorous pesticides on two columns. Internal standard is O-2-naphthyl dimethylthiophosphinate (NT) with quotient 1.0. Pesticides are numbered as in Table II.

cover a wide range of volatility but the individual components are much more uniform considering polarity, chemical reactivity and thermal stability. At low picogram levels the percent relative standard deviations for ten chlorinated benzene isomers were in the range 1.0–2.9, averaging 2.0 for manual on-column injection, and somewhat greater with autosampler splitless injection. The data presented here show averages of the relative standard deviations in the same range but with greater variations. This precision is remarkable for mixtures of such great chemical diversity and can be achieved only with the internal standard method, which eliminates the volume error and long-term drifts in detector response. Turner and Freeman[12] compared cool on-column and automated splitless injection using polynuclear aromatic hydrocarbons as test samples. They calculated the relative response of the compound with respect to a saturated hydrocarbon and applied exactly the same instrumentation as

in our study. As documented in the literature for various classes of compounds, high-molecular-weight discrimination with splitless sampling was evident for polynuclear aromatic hydrocarbons. The discriminated compounds exhibited greater relative standard deviations. On-column sampling yielded a constant relative response for all compounds with a precision of less than 1% and an average of 0.5%. Splitless injection resulted in precision levels ranging up to more than 4% with an average of 2.8%. Calculating the quotient of the response of splitless to on-column injection as in this study for the latest eluting compound coronene results in a value 0.423. This ratio is greater than those of the latest eluting organophosphorus pesticides, although the boiling point of coronene is much higher than those of the late eluting organophosphates. The difference in discrimination behaviour reflects the variation in thermal stability. The results presented in this study support the general view that the portion of discrimination caused by thermal degradation, adsorption and chemical reaction in the insert liner yields a loss of precision.

CONCLUSIONS

The study presented here confirms the results and conclusions of other groups that on-column injection is the most suitable method to quantify the components of a complex mixture with a wide range of volatility. The discrimination of later eluting pesticides is clearly documented by calculating the ratio of the registered peak areas with both sampling methods. With the homologous series of alkanes and isomers of growing substitution as polychlorinated benzenes or biphenyls the discrimination was found mainly to reflect the decrease of volatility with increasing molecular weight. The pesticide mixtures applied to this study cover a considerable heterogenicity in chemical structures. Therefore, in general, the discrimination increases with retention time but with fluctuations. These must be explained for the individual compounds with differing thermal stability, adsorption to polar surfaces in the insert liner and chemical reactivity that result in breakdown. For splitless sampling, however, a remarkable reproducibility of the relative response factors for nearly all pesticides was found. This makes automated splitless sampling a suitable method for screening in residue analysis, provided the internal standard method is applied and a daily calibration is performed with appropriate test mixtures.

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