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Use of deactivated fused-silica capillary precolumns in pesticide analysis by gas chromatography with electron-capture detection[☆]

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Abstract

The advantages and disadvantages of coupling a retention gap of fused-silica between the injection port and the chromatographic column are discussed. The influence on the peak width and height of several factors such as the solvent (*n*-hexane, acetone, ethyl acetate and methanol), the gap (length, inner diameter, deactivation mode), the injection volume and the pesticide concentration has been examined. Those factors have very different incidences so, it is not possible to extract a general recommendation about the use of gaps. For this reason, checking its viability in each particular case is more advisable. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In gas chromatography (GC) using splitless injection mode, the focusing of the analytes by the so-called thermal and solvent effects, is a critical factor to be considered in order to obtain taller and narrower peaks. It enhances the resolution and the sensitivity achieved [1]. In addition to these effects, the use of precolumns (retention gaps without stationary phase and adequately deactivated) is an interesting alternative to reduce the bandwidth originated by the dispersion of the analytes that are thermally re-condensed on the stationary phase. This

is more noticeable for solvents of high polarity, which produce bigger vapor volumes [1–6].

The use of an uncoated and deactivated precolumn is nowadays necessary in the development of high-performance liquid chromatography (HPLC)–GC couplings [7–10] and in the injection of big volumes of sample in GC by programmed temperature vaporization (PTV) or by an on-column injection [11–14]. All of them are used in environmental analysis. A reduction of the influence of the “matrix effects” in pesticide quantitation has also been reported when a large sample volume is injected in the presence of a retention gap [15].

In this work the possibilities offered by different retention gaps in analyzing pesticide residues by splitless GC–electron-capture detection (ECD) with a conventional column have been studied. The different peak heights and widths obtained by solv-

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ing pesticides in *n*-hexane, acetone, ethyl acetate and methanol, and injecting different volumes and concentrations with and without precolumns have been compared, in an attempt to deduce recommendations for general use.

2. Experimental

2.1. Reagents

Dichlobenil (1), propachlor (2), chlorpropham (3), dicloran (4), δ -hexachlorocyclohexane (δ -HCH) (5), triallate (6), vinclozolin (7), alachlor (8), dichlofluanide (9), chlorpyrifos (10), *z*-chlorfenvinphos (11), dinobuton (12), 4,4'-DDE (13), endosulfan B (14), bromopropylate (15), tetradifon (16), acrinathrin (17) and permethrin (18) pesticide standards were obtained from Riedel-de Haën (Hannover, Germany) and Promochem (Wessel, Germany). Residue-analysis grade methanol, ethyl acetate, acetone and *n*-hexane were supplied by Lab-Scan (Dublin, Ireland).

Stock solutions of each pesticide, and pesticide mixtures, were prepared in their corresponding injection solvent to carry out the study. Solutions were kept in darkness at 4°C.

2.2. Precolumns and parameters studied

The influence of the following parameters was assayed: the precolumn length (5, 10 and 15 m), the inner diameter (I.D., 0.25, 0.32 and 0.53 mm), two types of deactivation of the fused-silica tubing: non polar (methyl deactivated) and intermediate polarity (phenylmethyl deactivated), the injection volume (1, 3 and 5 μ l), the pesticide concentration (0.1, 0.7 and 1.4 mg/l) and finally, the injection solvent: *n*-hexane, ethyl acetate, acetone and methanol.

The precolumns of different length, inner diameter, and deactivation were supplied by Supelco (Bellefonte, PA, USA) and Sugelabor (Madrid, Spain).

Pesticide mixtures were injected several times. The first time without using retention gap and the others, after coupling the different assayed precolumns. The variation of the height and width of the chromatographic peaks obtained by injecting each

mixture with precolumns was calculated. It has been expressed as a percentage in relation to the data obtained without coupling precolumns.

Injections were always performed in triplicate to obtain an average. A HP3365 Chemstation from Hewlett–Packard (Avondale, PA, USA) provided the peak height and width data.

2.3. Gas chromatographic analysis

A Hewlett–Packard 5890 gas chromatograph equipped with a HP7673 auto-sampler, an ECD system and a 30 m \times 0.32 mm capillary column coated with a 0.25 μ m thick film of 5% phenylmethylpolysiloxane (HP-5), also from Hewlett–Packard, were used. Deactivated silica-fused precolumns were placed between the injection port and chromatographic column, coupling both columns by a 1:1 fused-silica connector from Ohio Valley Specialty Chemical (Marietta, OH, USA). The oven temperature program was as follows: initial temperature 50°C, held for 1.5 min, 8°C/min ramp to 160°C, and finally a 1.4°C/min ramp to 270°C. The carrier gas (He) flow-rate was 2.4 ml/min, measured at 50°C. Splitless injection was carried out at 220°C, and the purge valve was on at 1 min. Argon–methane (90:10, v/v) was used as auxiliary gas for the ECD system, the temperature of which was 300°C. Gases were obtained from Carbueros Metálicos (Barcelona, Spain).

3. Results and discussion

3.1. Influence of the deactivation mode

The peak heights and widths obtained for several pesticides, using non polar and semipolar retention gaps, are shown in Tables 1 and 2. As can be seen, the response is so varied that it is not possible to assume a general behavior to establish a direct relationship between the peak characteristics and the precolumn type. Nevertheless, it is true that the non polar precolumn gives taller and narrower peaks than the semipolar one, for almost all the compounds studied. It is also observed in the tables that using different solvents, the peak shape changes as well,

Table 1

Peak height (counts) obtained in the injection of 3 μ l of a pesticide mixture (0.7 mg/l each one) solved in the four solvents, using 15 m \times 0.32 mm I.D. precolumns ($n=3$)

Pesticide	Peak height (counts)							
	Solvent: hexane		Solvent: methanol		Solvent: ethyl acetate		Solvent: acetone	
	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar
Dichlobenil	234 175	244 536	94 396	157 716	105 737	196 176	104 160	186 682
Propachlor	42 229	29 124	–	17 798	20 187	22 753	21 805	21 467
Chlorpropham	6503	8109	–	2623	1432	5404	2096	6549
Dicloran	173 104	208 765	55 592	74 021	87 674	118 662	107 494	115 278
Triallate	208 765	112 080	162 863	76 407	183 638	86 082	192 890	94 366
Dichlofluamid	222 140	92 422	1628	–	150 606	52 601	107 141	58 515
Chlorpyrifos	166 127	98 072	56 630	51 621	130 968	64 466	153 131	79 005
4,4'-DDE	398 336	159 937	299 614	78 992	306 648	95 000	348 755	103 548
Endosulfan A	452 124	205 386	293 813	86 177	371 295	120 898	425 436	139 522
Acrinathrin	46 608	44 765	1665	3947	27 372	21 504	55 369	29 759
Permethrin	9488	5793	7411	2408	6282	3491	9153	3771

–: Not detected.

although the relative distribution of heights and widths is nearly the same.

The peak widths obtained when using non polar precolumns were about 15% lower in comparison with the ones obtained when precolumns were not used. This reduction was only observed for a few compounds when semipolar precolumns were used and the effect was stronger for those compounds that elute later.

That behavior was similar when the inner diameter

was changed from 0.32 mm to 0.25 mm, whereas when the inner diameter was 0.53 mm, the differences between the deactivation modes were considerably reduced.

It was also observed that working with semipolar precolumns implied an increase in the retention times of the compounds, in quantities between 2 and 5 min for a 15 m length gap; the selectivity also varied because the elution order was changed for some compounds, which in certain cases produced over-

Table 2

Peak width (min) obtained in the injection of 3 μ l of a pesticide mixture (0.7 mg/l each one) solved in the four solvents, using 15 m \times 0.32 mm I.D. precolumns ($n=3$)

Pesticide	Peak width (min)							
	Solvent: hexane		Solvent: methanol		Solvent: ethyl acetate		Solvent: acetone	
	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar	Precolumn: non polar	Precolumn: semipolar
Dichlobenil	0.126	0.128	0.312	0.142	0.299	0.122	0.425	0.138
Propachlor	0.115	0.193	–	0.199	0.154	0.170	0.174	0.183
Chlorpropham	0.126	0.200	–	0.180	0.201	0.183	0.169	0.241
Dicloran	0.271	0.232	0.628	0.269	0.480	0.249	0.521	0.249
Triallate	0.111	0.221	0.136	0.243	0.133	0.217	0.134	0.223
Dichlofluamid	0.110	0.275	0.145	–	0.132	0.309	0.131	0.325
Chlorpyrifos	0.128	0.302	0.163	0.328	0.186	0.306	0.159	0.315
4,4'-DDE	0.149	0.271	0.187	0.304	0.176	0.282	0.168	0.296
Endosulfan A	0.158	0.333	0.203	0.358	0.168	0.338	0.171	0.360
Acrinathrin	0.196	0.263	0.245	0.374	0.230	0.284	0.230	0.286
Permethrin	0.196	0.297	0.339	0.442	0.308	0.377	0.232	0.324

–: Not detected.

lapping, mainly for the couples triallate/ δ -HCH, vinclozolin/alachlor and dinobuton/chlorfenvinphos.

3.2. Influence of precolumn size

The average variation of the peak height when injecting different volumes of pesticide mixtures, using semipolar precolumns of various lengths and inner diameters, is shown in Table 3. The results are the average values for the pesticides studied, and are expressed as a percentage in relation to those heights obtained when the same mixture is injected without precolumn. As it can be seen, for 0.32 and 0.53 mm I.D. gaps, the peak height rose with the increase in the precolumn length, as a consequence of the lower dispersion into the chromatographic column. Thus, when a mixture of 0.7 mg/l in methanol was injected, the average values for peak height increased by about 24 and 45% for semipolar precolumns of 5 and 15 m length, respectively.

For precolumns of 0.25 mm I.D. the increase in the length did not imply an increase in the signal. Sometimes, the height for certain peaks even diminished.

In relation to the effect of the inner diameter, it can also be observed that the heights increased as the precolumn got wider. So, the use of short precolumns, with 0.25 mm I.D., to enhance the peak

height and to reduce the broadening, seemed to be the worst choice.

3.3. Influence of the injection volume

Regarding the injection volume, the influence was not so clear, an increase in the peak height was not always found when larger volumes were injected using precolumns, as can be seen in Table 3. For those pesticides with a lower chromatographic response (pyrethroids like acrinathrin and permethrin) the highest increases in peak height were obtained for injections of 1 μ l and 3 μ l.

It is necessary to take into account that with the gaps used, especially for those ones of lower capacity, it was not possible to avoid splitting of peaks when a large volume was injected. For this reason, gaps of 0.25 and 0.32 I.D. and up to 15 m are not advisable for injection volumes higher than 3 μ l. In the case of using precolumns of 15 m \times 0.53 mm, a volume up to 3 μ l might be injected without a great distortion of the chromatographic peaks.

Although there was a general increase in the peak height when precolumns were used, it is interesting to remark that the values for the peak height obtained injecting directly 3 μ l, were usually higher than the ones obtained when a sample of 1 μ l was injected with the gap installed. The same considerations

Table 3

Mean increase of peak height (in percentage) obtained in the injection of 1, 3 and 5 μ l of a pesticide mixture (0.7 mg/l each one) using semipolar precolumns of several lengths and diameters ($n=3$)

	Peak height								
	0.25 mm I.D.			0.32 mm I.D.			0.53 mm I.D.		
	5 m	10 m	15 m	5 m	10 m	15 m	5 m	10 m	15 m
Hexane, 1 μ l	1.1	1.8	2.0	13	10	21	6	17	27
Methanol, 1 μ l	0.8	2.2	2.0	12	22	30	15	24	34
Ethyl acetate, 1 μ l	1.5	1.6	1.9	10	19	27	14	21	29
Acetone, 1 μ l	1.5	2.1	4.0	10	16	25	13	19	30
Hexane, 3 μ l	1.0	2.4	2.8	13	24	31	9	23	35
Methanol, 3 μ l	3.1	3.0	3.9	21	33	45	21	33	46
Ethyl acetate, 3 μ l	1.5	1.7	2.5	17	24	41	24	31	40
Acetone, 3 μ l	1.8	2.8	3.0	14	30	40	21	36	47
Hexane, 5 μ l	0.9	2.4	3.0	9	28	33	13	31	45
Methanol, 5 μ l	1.8	3.0	4.0	24	33	46	36	45	54
Ethyl acetate, 5 μ l	0.8	2.4	3.8	12	18	38	15	40	39
Acetone, 5 μ l	0.5	2.7	3.7	14	32	42	20	39	46

could be made when 5 μl were injected without gap and the results were compared with the injection of 3 μl with gap. Thus, using a 15 m \times 0.53 mm I.D. precolumn, the peak heights obtained for an injection of 3 μl without precolumn were 40–80% higher than when 1 μl of the same mixture was injected with precolumn. This tendency was similar for non polar or semipolar precolumns.

Using non polar precolumns for pesticide mixtures solved in *n*-hexane, ethyl acetate or acetone, the reduction of peak width was proportional to the injected volume increase. This reduction was lower when methanol was used as a solvent.

When we tried to separate two compounds that eluted very closely, both the change in the peak width and the possible modification in the selectivity had to be considered at the same time, before selecting a precolumn. In Fig. 1 some chromatograms for a mixture in acetone, analyzed with and without a semipolar gap of 15 m \times 0.53 mm I.D. are shown. The broadening originated in most of the peaks by the use of a semipolar precolumn can also be seen.

3.4. Effects of solvent and concentration

The mean variations of the peak height obtained in the injection of 3 μl of a pesticide mixture (solved in the four solvents and for three levels of concentration) on a non polar precolumn of 15 m \times 0.53 mm I.D., and always with respect to the injection without precolumn, are shown in Table 4. It can also be appreciated that the highest increases in the height were obtained with the most polar solvents, and that the concentration is another factor to be taken into account. Thus, the use of gaps turned out to be more useful when low concentrations were injected.

The reduction in the bandwidth was higher when ethyl acetate or acetone were used, and also for lower concentrations, as can be seen in Table 5.

In Table 6, the percentages of variation of the peak heights depending on the concentration and using acetone as a solvent are summarized. It can be observed that the peak height variation is different for each pesticide analyzed. Furthermore, compounds that are very sensitive to active sites, such as dichlobenil and dicloran (those with a great band-

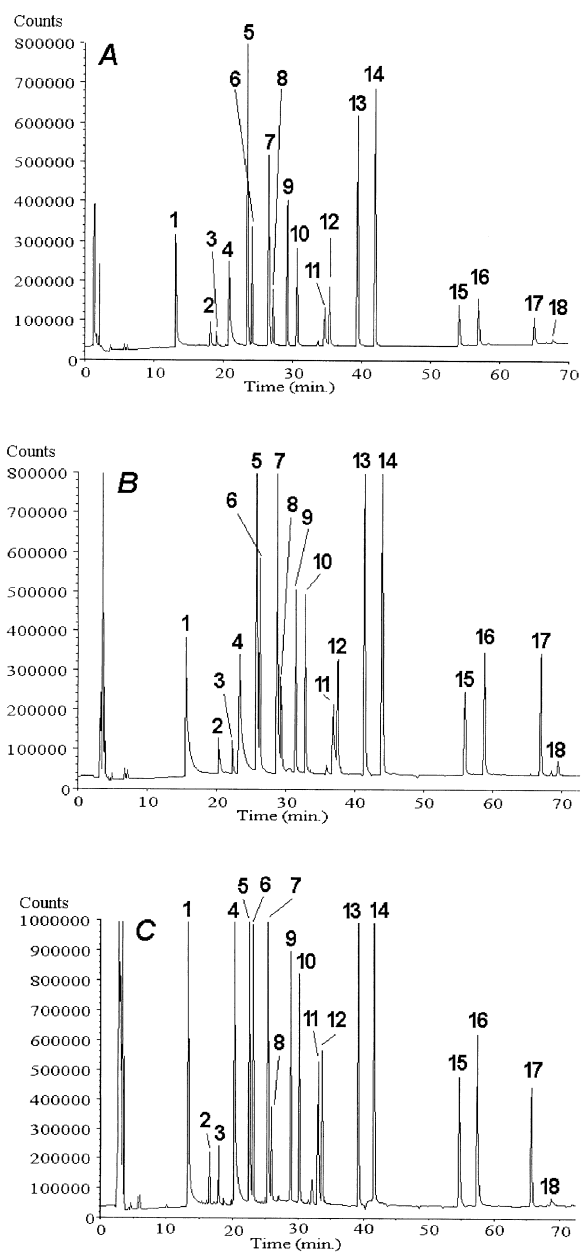


Fig. 1. Chromatogram of a pesticide mixture (0.7 mg/l each one) dissolved in acetone. Injection volume: 3 μl . See Section 2.1 for peak identification. (A) Without precolumn. (B) With a 15 m \times 0.53 mm I.D. semipolar precolumn. (C) With a 15 m \times 0.53 mm I.D. non polar precolumn.

width and tail, as can be observed in Fig. 1), have lower increases in the peak height in comparison with the other compounds.

Table 4

Mean increase (in percentage) of the peak height obtained in the injection of 3 μ l of a pesticide mixture dissolved in *n*-hexane, methanol, acetone and ethyl acetate, and for three concentrations ($n=3$)

	Peak height increase (%)		
	0.1 mg/l	0.7 mg/l	1.4 mg/l
Hexane	64	49	27
Methanol	84	66	32
Ethyl acetate	89	70	34
Acetone	77	63	21

Table 5

Mean decrease (in percentage) of the peak width (values +) obtained in the injection of 3 μ l on a 15 m \times 0.53 mm I.D. non polar precolumn in comparison with those obtained without precolumn ($n=3$)

	Peak width decrease (%)		
	0.1 mg/l	0.7 mg/l	1.4 mg/l
Hexane	1.9	-0.2	-1.5
Methanol	6.9	0.2	-0.6
Ethyl acetate	20	17.1	14.9
Acetone	12	3.5	1.1

3.5. Incidence of the precolumn manufacturing

All the data shown above were recorded with precolumns from Supelco. Some experiments were made with similar precolumns obtained from Sugelabor, and it could be observed that there was a

Table 6

Increase of the peak height (in percentage) obtained in the injection of 3 μ l on a 15 m \times 0.53 mm I.D. non polar precolumn in comparison with the heights obtained without using a precolumn

	Peak height increase (%)		
	0.1 mg/l	0.7 mg/l	1.4 mg/l
Dichlobenil	67	28	-46
Propachlor	61	51	42
Chlorpropham	64	20	29
Dicloran	21	1	-75
Endosulfan B	70	42	14
Bromopropylate	212	156	121
Tetradifon	190	189	102
Acrinathrin	94	58	25
Permethrin	317	330	142

Data for several pesticides dissolved in acetone at different concentrations. Values (-) mean a decrease in the peak height when a precolumn is used ($n=3$).

Table 7

Mean increase of peak height (in percentage) obtained in the injection of different volumes of a pesticide mixture (0.7 mg/l each) using a 15 m \times 0.53 mm I.D. precolumn from another supplier ($n=3$)

	Peak height increase (%)		
	1 μ l injected	3 μ l injected	5 μ l injected
Hexane	96	36	15
Methanol	9	26	52
Ethyl acetate	73	62	35
Acetone	66	31	20

change in the chromatographic behavior of several pesticides, with modifications not only in the retention times, but also in the peak shape.

The results obtained for a 15 m \times 0.53 mm precolumn from another manufacturer can be observed in Table 7. The experiment was carried out injecting 1 μ l, 3 μ l and 5 μ l of a mixture of pesticides, 0.7 mg/l in each, solved in the four solvents. The results are different in comparison with the first precolumns assayed. It was stated for the first precolumns that, in general, the peak height increased when the injection volume rose. However, for the precolumns of the other manufacturer, the peak height was favored as the injection volume diminished, the worst results were those which belong to an injection of 5 μ l; for methanol, the results were very similar for both brands of precolumns.

This different performance could be attributed to the different silica classes used in the manufacturing process and to the different procedure of deactivation.

4. Conclusions

The coupling of an uncoated and deactivated retention gap allows one to enhance analyte focusing and to increase the height of the chromatographic peaks, but only under specific conditions. The assumption of general rules to predict the usefulness of coupling a retention gap is not advisable, because of the very different behavior observed depending on the compounds to be separated and on the working

conditions. The experimental verification of the precolumn efficacy is strongly recommended.

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References

- [1] K.J. Hyver (Ed.), High Resolution Gas Chromatography, 3rd ed., Hewlett-Packard, 1983.
- [2] K. Grob, J. Chromatogr. 213 (1981) 3.
- [3] K. Grob, J. Chromatogr. 213 (1981) 13.
- [4] K. Grob, J. Chromatogr. 237 (1982) 15.
- [5] P. Sandra, M. van Roelenbosch, M. Verzele, C. Bicchi, J. Chromatogr. 279 (1983) 279.
- [6] K. Grob, J. Chromatogr. 324 (1985) 252.
- [7] F. Modeste, M. Coude, P. Devaux, J. High Resolut. Chromatogr. 19 (1996) 535.
- [8] A.J. Bulterman, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 16 (1993) 397.
- [9] A.J.H. Louter, U.A.Th. Brinkman, R.T. Ghijsen, J. Microcol. Sep. 5 (1993) 303.
- [10] L. Mondelo, P. Dugo, G. Dugo, A.C. Lewis, K.D. Bartle, J. Chromatogr. A 842 (1999) 373.
- [11] H.J. Stan, M. Linkerhanger, J. Chromatogr. A 727 (1996) 275.
- [12] R. Tamilarasan, P.L. Morabito, L. Lamparski, P. Hazelwood, A. Butt, J. High. Resolut. Chromatogr. 17 (1994) 689.
- [13] S. Ramalho, T. Hankemeier, M. Dejong, U.A.Th. Brinkman, R.J.J. Vreuls, J. Microcol. Sep. 7 (1995) 383.
- [14] T. Hankemeier, P.C. Steketee, R.J.J. Vreuls, U.A.Th. Brinkman, Fresenius' J. Anal. Chem. 364 (1999) 106.
- [15] M. Godula, J. Hajslova, K. Alterova, J. High Resolut. Chromatogr. 22 (1999) 395.