CHROM. 6153

Gas chromatographic separation of hexachlorobenzene and the α,- β-, γ- and δ-isomers of hexachlorocyclohexane

Hexachlorobenzene (HCB) is a selective fungicide for the control of bunt of wheat, and was introduced for seed treatment by YERSIN[1] in 1945. It may occur as a residue in feeds and foods[1].

The most widely used stationary phases for gas chromatographic analyses of pesticide residues are the mixture DC-200 + QF-1[2]; OV-17, either alone[3–5] or mixed with QF-1[6]; and SE-30, either alone[7] or mixed with QF-1[8]. The separation of HCB and the α-, β-, γ- and δ-isomers of hexachlorocyclohexane (HCH) on these phases has not been reported.

Some workers reported the separation of the isomers of HCH on several phases[9–11]. It appears that only SIMMONS AND TATTON[11] have described the separation of HCB, α-HCH and γ-HCH along with other compounds. They used cyanosilicone oil XE-60, which cannot separate the β-HCH from the δ-HCH, as was proved during this study.

The primary objective of this work was to find a packing suitable for separating HCB and the α, β-, γ- and δ- isomers of HCH. As a secondary requirement, the packing should allow the separation of these compounds along with the group of chlorinated pesticides that are usually sought in foods, *i.e.*, heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, *o,p'*-DDT and *p,p'*-DDT. A further good reason for having such a packing is that the mixed phase DC-200 + QF-1 cannot separate HCB from the α-isomer of HCH; this is probably the reason why relatively large amounts of α-HCH residues are frequently found in the gas chromatographic determination of pesticide residues in milk and cheese extracts.

Experiments and results

The gas chromatograph used was a Packard, Model 409, instrument connected with a Hewlett-Packard 1 mV strip chart recorder.

The chromatographic operating conditions were as follows. Temperatures: injection block 220°, oven 190°, detector 305°; carrier gas: argon with 10% of methane; flow-rate: 40 cm³/min; electron capture detector: concentric design, ^{63}Ni 10 mCi source, operated with pulsed voltage; pulse width 0.5 μsec, pulse period 50 μsec; sensitivity: 2.5×10^{-10} a.f.s.; attenuation × 8 or × 4.

The chromatographic conditions were the same for all the columns tested. All the columns were made of glass, the injection was "on column" and the columns were packed as described by BONIFORTI[12].

The following three columns were prepared and tested: (1) a coiled glass column, 2.40 m × 4 mm I.D., packed with 5% by weight of OV-1 on 80–100 mesh silanized Gas-Chrom P; (2) a coiled glass column, 2.40 m × 4 mm I.D., packed with 5% by weight of OV-61 on 80–100 mesh silanized Gas-Chrom P; and (3) a coiled glass column, 2.30 m × 3 mm I.D., packed with 3% by weight of XE-60 on 80–100 mesh silanized Anakrom AS.

Table I shows the retention times relative to aldrin of the pesticides under

TABLE I

RETENTION TIMES OF PESTICIDES RELATIVE TO ALDRIN

For description of columns see text.

Column (1)		*Column (2)*		*Column (3)*	
Compound	*Rel. RT*	*Compound*	*Rel. RT*	*Compound*	*Rel. RT*
α-HCH	0.36	HCB	0.39	HCB	0.42
HCB	0.41	α-HCH	0.44	α-HCH	0.89
β-HCH	0.41	γ-HCH	0.58	Aldrin	1.00
γ-HCH	0.44	β-HCH	0.63	γ-HCH	1.28
δ-HCH	0.47	Heptachlor	0.78	Heptachlor epoxide	2.28
Heptachlor	0.78	δ-HCH	0.82	*p,p'*-DDE	2.91
Aldrin	1.00	Aldrin	1.00	Dieldrin	3.54
Heptachlor epoxide	1.25	Heptachlor epoxide	1.50	β-HCH	3.70
p,p'-DDE	1.90	*p,p'*-DDE	2.36	δ-HCH	3.82
Dieldrin	1.90	Dieldrin	2.36	*o,p'*-DDT	4.52
o,p'-DDT	2.60	*o,p'*-DDT	3.66	*p,p'*-DDT	7.50
p,p'-DDT	3.30	*p,p'*-DDT	4.80		
RT aldrin (min)	10.5		15.3		4.9

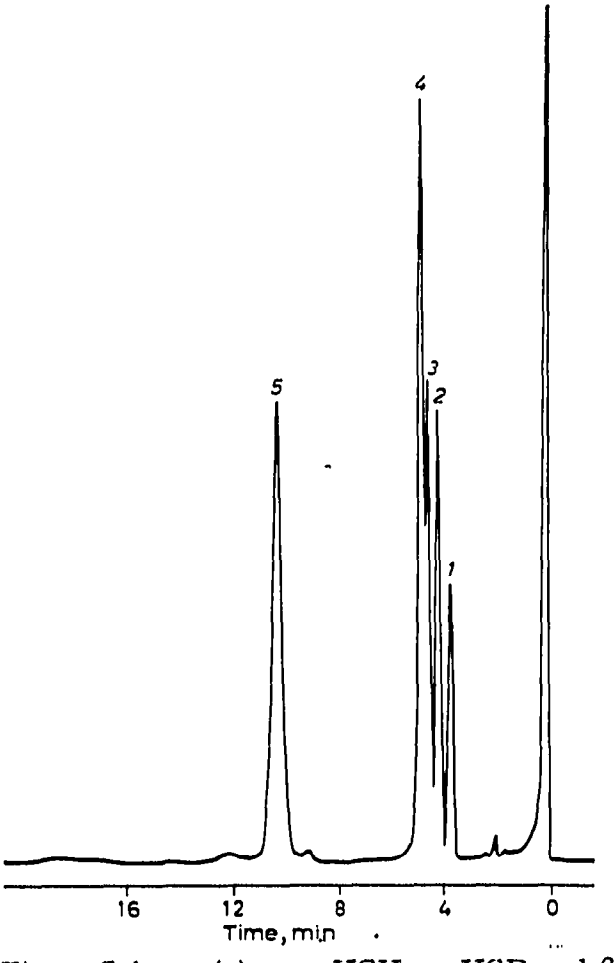

Fig. 1. Column (1): 1, α-HCH; 2, HCB and β-HCH; 3, γ-HCH; 4, δ-HCH; 5, aldrin.

study and the retention time of aldrin for each column. In Figs. 1–3 are shown some examples of separations.

On the basis of the results obtained, it can be said that columns packed with OV-1 stationary phase do not give a good separation of HCB and HCH isomers and cannot separate *p*, *p'*-DDE from dieldrin.

Columns packed with OV-61 stationary phase do not separate β-HCH from γ-HCH very satisfactorily and cannot separate *p*,*p'*-DDE from dieldrin.

XE-60 stationary phase gives a satisfactory separation of the compounds considered except for β-HCH and δ-HCH, which are not well separated and are eluted too close to dieldrin. On the other hand, HCB has a short retention time so that there is a certain risk of overlap with interfering peaks from incompletely cleaned-up extracts.

Hence it was decided to mix the XE-60 stationary phase with other phases in order to improve the separation between β-HCH and δ-HCH and to obtain a longer retention time for HCB. The following two columns were therefore packed and tested: (4) a coiled glass column, 2 m × 4 mm I.D., packed with a 1 + 1 + 1 mixture by weight of the following three packings, which were previously coated: 10% by weight of DC-200 on 80–100 mesh Chromosorb W HP, 7.5% by weight

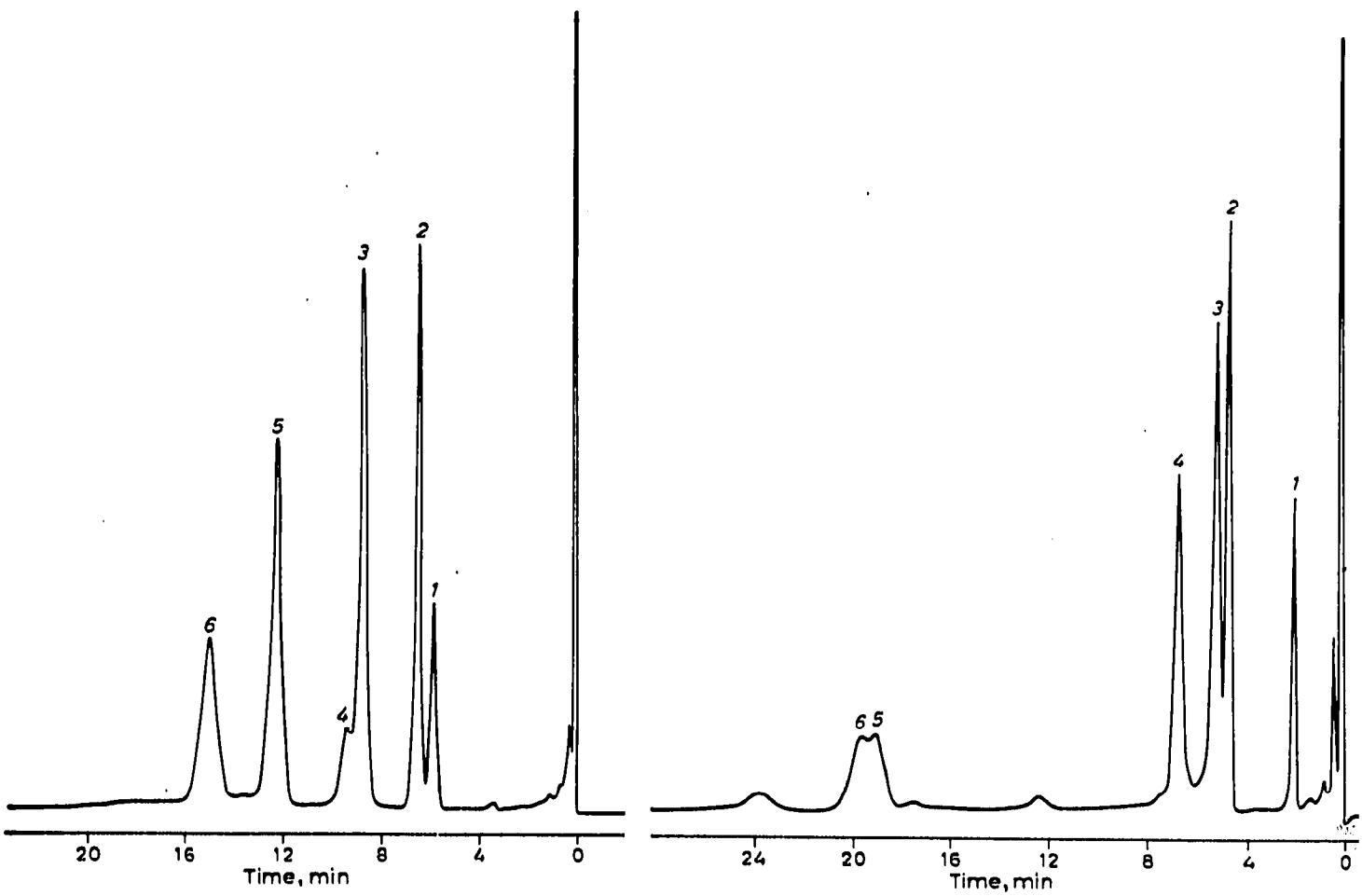

Fig. 2. Column (2): 1, HCB; 2, α-HCH; 3, γ-HCH; 4, β-HCH; 5, δ-HCH; 6, aldrin.

Fig. 3. Column (3): 1, HCB; 2, α-HCH; 3, aldrin; 4, γ-HCH; 5, β-HCH; 6, δ-HCH.

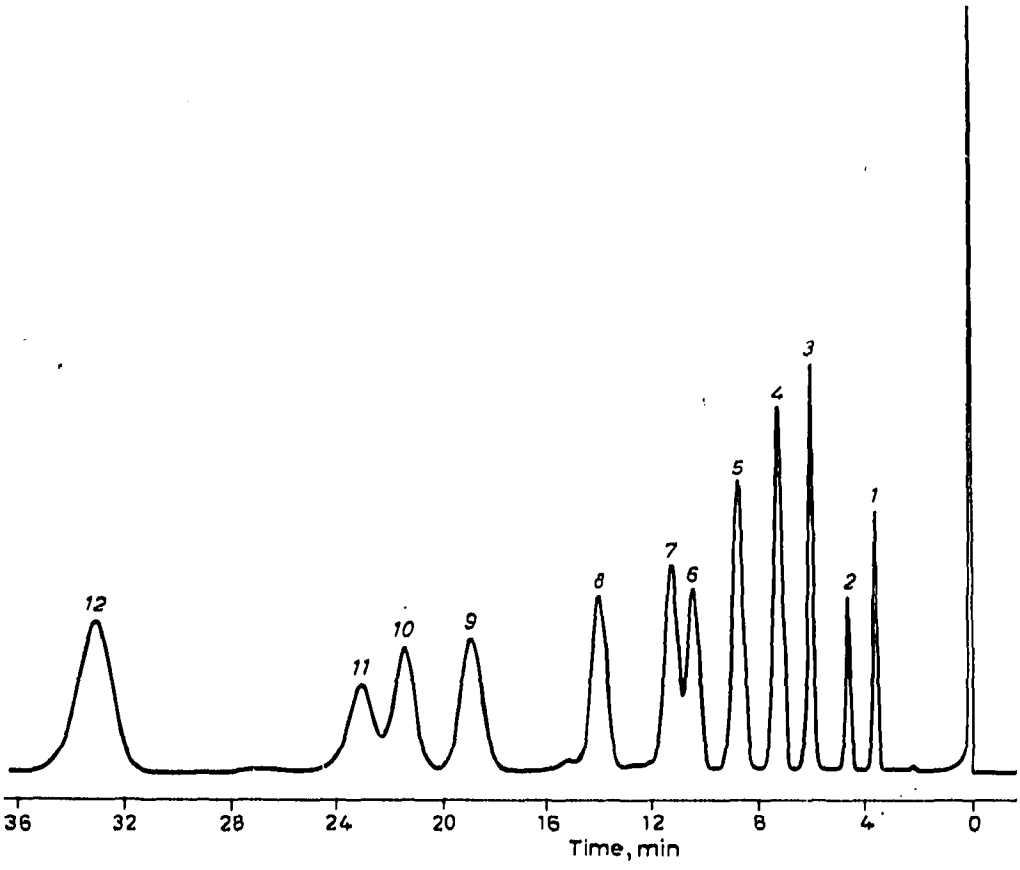

Fig. 4. Column (4): 1, HCB; 2, α-HCH; 3, γ-HCH; 4, heptachlor; 5, aldrin; 6, β-HCH; 7, δ-HCH; 8, heptachlor epoxide; 9, *p,p'*-DDE; 10, dieldrin; 11, *o,p'*-DDT; 12, *p,p'*-DDT.

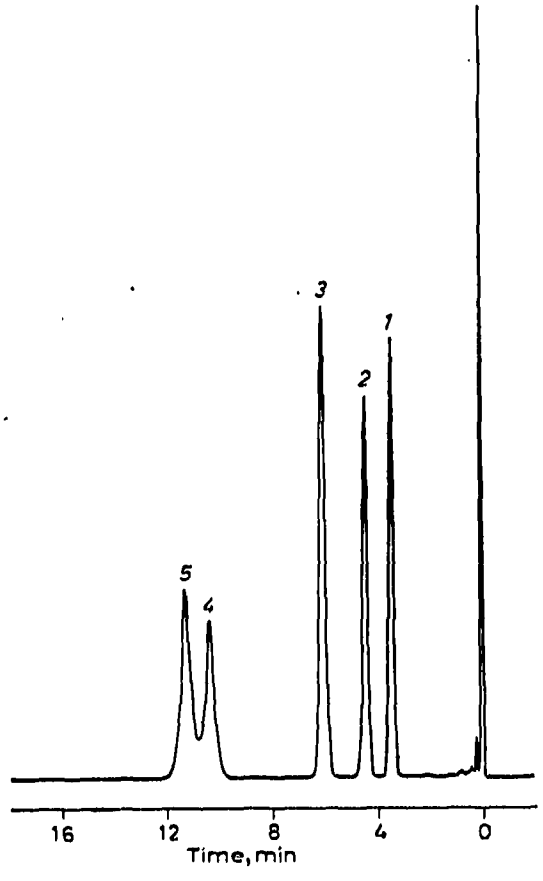

Fig. 5. Column (4): 1, HCB; 2, α-HCH; 3, γ-HCH; 4, β-HCH; 5, δ-HCH.

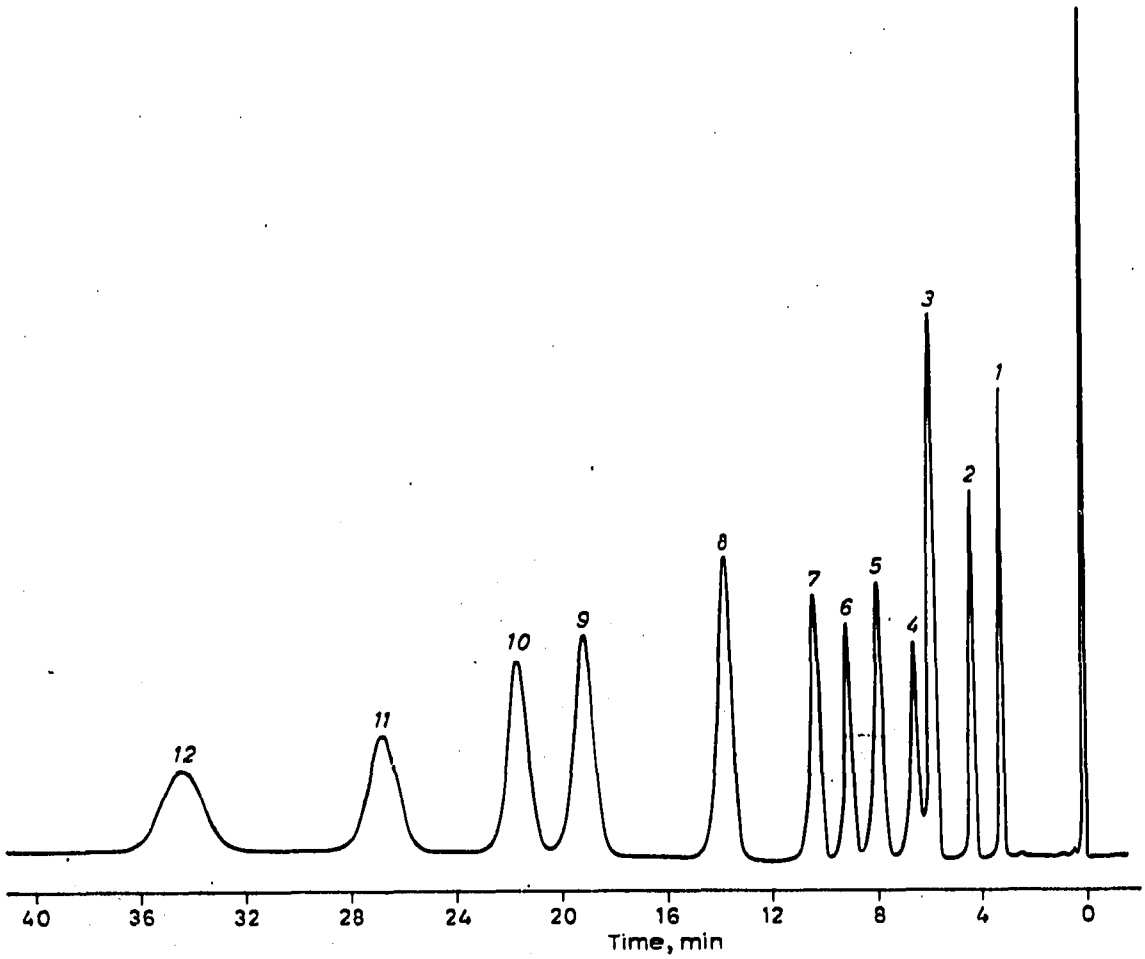

Fig. 6. Column (5): 1, HCB; 2, α-HCH; 3, γ-HCH; 4, heptachlor; 5, aldrin; 6, β-HCH; 7, δ-HCH; 8, heptachlor epoxide; 9, *p,p'*-DDE; 10, dieldrin; 11, *o,p'*-DDT; 12, *p,p'*-DDT.

TABLE II

RETENTION TIMES OF PESTICIDES RELATIVE TO ALDRIN

Compound	*Column (4)*	*Column (5)*
HCB	0.41	0.40
α-HCH	0.53	0.54
γ-HCH	0.70	0.73
Heptachlor	0.83	0.82
Aldrin	1.00	1.00
β-HCH	1.20	1.15
δ-HCH	1.29	1.31
Heptachlor epoxide	1.60	1.74
p,p'-DDE	2.16	2.42
Dieldrin	2.46	2.74
o,p'-DDT	2.65	3.39
p,p'-DDT	3.78	4.36
RT aldrin(min)	8.9	7.9

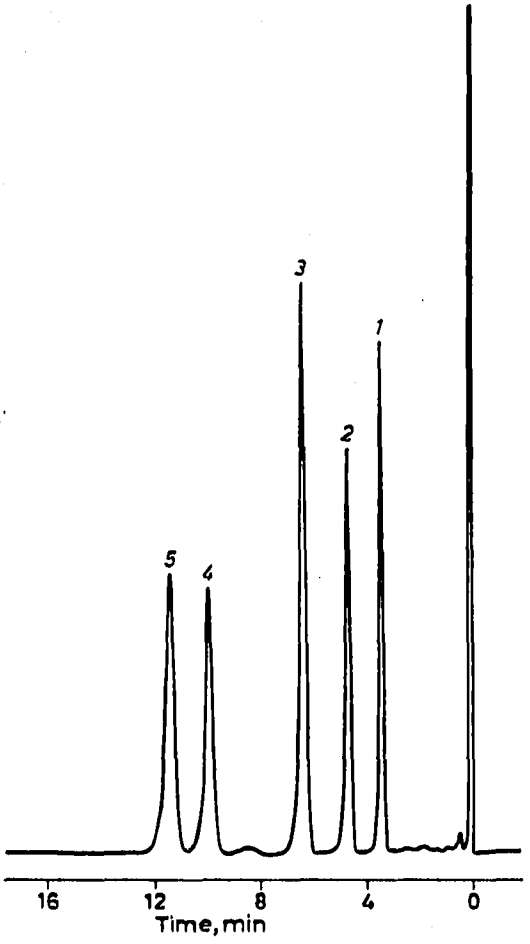

Fig. 7. Column (5): 1, HCB; 2, α-HCH; 3, γ-HCH; 4, β-HCH; 5, δ-HCH.

of QF-1 on 80–100 mesh Chromosorb W HP and 3 % by weight of XE-60 on 80–100 mesh silanized Anakrom AS; and (5) a coiled glass column, 2 m × 3 mm I.D., packed with a 1 + 1 + 0.5 mixture by weight of the following three packings, which were previously coated: 3 % by weight of OV-61 on 80–100 mesh silanized Gas-Chrom P, 7.5 % by weight of QF-1 on 80–100 mesh Chromosorb W HP and 3 % by weight of XE-60 on 80–100 mesh silanized Anakrom AS.

Table II shows the retention time of aldrin and the retention times relative to aldrin of the compounds studied for each column under the above chromatographic conditions. In Figs. 4–7 are shown some examples of separations. There was a good separation of the pesticides studied in an overall time of about 35 min.

Conclusions

In the course of this work some stationary phases were studied either alone or in mixtures in order to achieve the separation of HCB and the α,-β-, γ- and δ-isomers of HCH. Furthermore, the behaviour of each packing was also examined with regard to the possibility of analysing in a single run these pesticides and the group of chlorinated pesticides that are usually sought in foods.

On the basis of the results obtained, it can be concluded that columns (4)

and (5) give the best results; they give a good separation of twelve chlorinated pesticides: HCB, α-HCH, γ-HCH, heptachlor, aldrin, β-HCH, δ-HCH, heptachlor epoxide, *p,p'*-DDE, dieldrin, *o,p'*-DDT and *p,p'*-DDT.

XE-60 is the stationary phase that produces the good separation of HCB, α-HCH and γ-HCH, but the separation of β-HCH from δ-HCH is achieved only by using a mixed phase. The optimum ratio of XE-60 to the other phases is considered to be that in column (5). On this column, the separation of HCB and HCH isomers is achieved in a few minutes under the described chromatographic conditions.

Some analyses of samples of milk and cheese on columns (4) and (5) were carried out and a peak was identified as HCB. The identification was made from the retention time of the standard compound and confirmed by the extraction value between hexane and acetonitrile.

Packings such as those described above should enable the gas chromatographic determination of chlorinated pesticide residues in foods to be extended to HCB and enable α-HCH to be determined more precisely than is possible on the mixed phase DC-200 + QF-1.

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