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Optimisation of a programmed split–splitless injector in the gas chromatographic–mass spectrometric determination of organochlorine pesticides

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Abstract

Large-volume injection techniques in gas chromatography are used to compensate for the at times limited detection sensitivity of mass spectrometric detection. In this work a programmed split–splitless injector in solvent split mode was employed to determine organochlorine pesticides in environmental samples. The injection conditions were selected by a Plackett-Burman design followed by a central composite design. The LODs obtained in the optimum conditions were compared with those obtained with splitless-MS and splitless-ECD. Finally, the method was applied to a soil sample.

Keywords: Injection methods; Large-volume injection; Pesticides

1. Introduction

Analysis of organochlorine pesticides in environmental samples requires the use of highly sensitive and selective detectors such as ECD, because of the toxicity of the pesticides and the low levels allowed by law. In recent years, GC–MS has become a useful tool in pesticide residue detection [1,2] because it offers simultaneous identification and quantification of a large number of pesticides, avoiding successive analyses with different selective detectors [3]. Nevertheless, the very high sensitivity needed for the analysis of environmental matrices makes it necessary to improve the limits of detection reached by GC–MS.

The use of large-volume injection techniques increases the sensitivity, allowing the determination of pesticides at much lower concentration levels, and eliminates the re-concentration step in the extraction, avoiding a possible source of loss of the most volatile compounds [4].

Different approaches are available for achieving large-volume injection in capillary gas chromatography: on column injection [5,6], programmed-temperature-vaporisation (PTV) injection [7–11], or splitless injection with solvent diversion [3]. On-column injection is the simplest and most reliable of these techniques, however, contamination of the

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column inlet with non-volatile sample materials is frequent. With programmed temperature sample introduction, the negative effect of column contamination can be more or less avoided, because non-volatile products are retained in a vaporisation chamber without reaching the analytical column [12]. The PSS (programmed split–splitless) injector is a PTV system.

This paper describes the optimisation of PSS injector for the gas chromatographic–mass spectrometric determination of 21 organochlorine pesticides, improving the sensitivity of this kind of detection. The injector operates in solvent split mode. The split vent is open when the sample is introduced in the cold liner. After eliminating the solvent, the valve is closed (solvent venting time) and then fast warming of the liner causes vaporisation of the analytes. These are transferred to the oven when the valve is again open (transfer time) [7]. There are several factors that can affect vaporisation efficiency. The employment of statistical techniques such as Taguchi experimental design [13], simplex optimisation [14], or Plackett-Burman designs, helps to find the best injection conditions. In this work, different factors (heating rate, split-vent flow, solvent venting time, transfer time, initial PSS temperature and inlet liner empty or packed with glass wool) were introduced in a Plackett-Burman design to determine which of them affect the vaporisation efficiency of the studied pesticides. The significant factors were then optimised by a central composite design.

Detection limits (LODs) obtained with PSS were compared with LODs obtained with splitless injection coupled with MS and with splitless injection coupled with ECD.

A soil sample was analysed by PSS-MS and splitless-MS to examine the proposed method.

2. Experimental

2.1. Apparatus

A Perkin-Elmer (Norwalk, CT, USA) GC Autosystem equipped with autosampler and PSS injector was employed for the chromatographic separation of the compounds. The column was a fused-silica capillary column 30 m×0.25 mm×0.25 μm (film thickness), 5% diphenyl methyl siloxane HP-5 (Palo Alto, CA, USA). Compounds were detected by a Perkin-Elmer Q-Mass 910 mass spectrometer scanning in SIM (selected ion monitoring) mode. Statgraphics Plus 4.0 for Windows software was employed for the analysis of results.

2.2. Materials

n-Hexane (95%) super purity solvent was purchased from Romil (Cambridge, UK). Appendix IX Organochlorine Pesticide Mix, 2 mg ml^{-1} in toluene:hexane (50:50) and individual standards of α-chlordane, γ-chlordane, and endrin ketone were from Supelco (Bellefonte, PA, USA). Isodrin was from ChemService (West Chester, USA). A working standard mix of pesticides of 0.4 μg ml^{-1} was prepared by dilution in *n*-hexane and stored in a refrigerator (4 °C).

2.3. Operating conditions

Helium (99.999%) was used as carrier gas, at 11 p.s.i. of constant pressure at the head of the column measured at initial temperature (80 °C) (1 p.s.i.= 6894.76 Pa). The GC oven temperature programme was: 80 °C (1 min) increased at 30 °C min^{-1} to 180 °C (held for 3 min), and then increased at 3 °C min^{-1} to 270 °C.

The mass spectrometer was used scanning in SIM (selected ion monitoring) mode according to Table 1. Ionisation voltage was 70 eV; transfer line temperature 290 °C; ion source temperature 240 °C and multiplier voltage 1700 V. The PSS was operated as a cold injector (with the solvent split mode) or as a classic hot injector in the splitless mode.

The volume injected was 20 μl because this is the maximum assayed volume that can be injected without peak splitting in a single injection. Larger sample volumes are injected normally with multiple injections of small volumes (e.g. 40 μl in eight injections of 5 μl [11], or 100 μl in ten injections of 10 μl [15]) to prevent losses of sample in the split. The standard solution had a concentration of 0.4 μg ml^{-1}. A 50-μl syringe (model 805; Hamilton, Reno, NV, USA) with a needle length of 7 cm was employed. The injection speed was normal (the injection takes ~1 s). A PSS quartz liner with 2-mm

Table 1
MS working conditions in SIM mode

Pesticides	t_R	Quant. ion	Confirm. ion	Pesticides	t_R	Quant. ion	Confirm. ion
α-HCH	7.99	181	219	*p,p′*-DDE	18.23	246	318
β-HCH	8.78	181	219	Dieldrin	18.28	263	237
γ-HCH	9.02	181	219	Endrin	19.36	263	–
δ-HCH	10.04	181	219	β-Endosulfan	20.02	195	–
Heptachlor	11.69	272	237	*p,p′*-DDD	20.53	235	–
Aldrin	13.21	263	293	Endrin aldehyde	20.86	67	–
Isodrin	14.55	193	263	Endosulfan sulfate	22.19	272	237
Heptachlorepoxide	15.06	353	237	*p,p′*-DDT	22.64	235	165
γ-Chlordane	16.24	373	237	Endrin ketone	25.74	67	317
α-Chlordane	16.90	373	–	Methoxychlor	26.05	227	–
α-Endosulfan	16.90	237	241				

I.D. was employed. These parameters were selected according to previous studies [16].

For splitless-MS the experimental conditions were: injector temperature 290 °C; empty liner (2 mm); split-vent flow 20 ml min^{-1}; and splitless time 1 min. For splitless-ECD conditions were: injector temperature 300 °C; empty liner (2 mm); split-vent flow 7.7 ml min^{-1}; and splitless time 1.2 min.

3. Results and discussion

3.1. Selection of significant factors

A Plackett-Burman $2^7 \times 3/32$ design, with six experimental factors and a dummy was carried out, using Statgraphics Plus 4.0 for Windows routine, to establish which factors may be statistically significant. Table 2 shows the experimental design matrix corresponding to the considered factors.

Table 2
Factors of the Plackett-Burman design

Run	A	B	C	D	E	F	G
1	Yes	250	100	0.1	5	80	–
2	No	100	100	0.8	5	50	–
3	No	250	10	0.1	1	80	–
4	Yes	250	10	0.8	1	50	–
5	Yes	100	100	0.1	1	50	–
6	No	100	10	0.8	5	80	–
7	No	250	100	0.1	5	50	–
8	Yes	100	100	0.8	1	80	–
9	No	250	100	0.8	1	80	–
10	Yes	250	10	0.8	5	50	–
11	No	100	10	0.1	1	50	–
12	Yes	100	10	0.1	5	80	–

A: inlet liner packing (glass wool); B: heating rate (°C min^{-1}); C: split-vent flow (ml min^{-1}); D: solvent venting time (min); E: transfer time (min); F: initial PSS temperature (°C); G: dummy.

Analysis of the results (average of six injections) produce the Pareto charts (P=95.0%). In these charts, the length of each bar is proportional to the standardised effect. The standardised effect is the estimated effect divided by its standard error, which is equivalent to computing a *t*-statistic for each effect. The vertical line on the plot judges the effects that are statistically significant. Bars that extend beyond the line correspond to effects that are statistically significant at the 95% confidence level.

The Pareto charts obtained for each pesticide show that split-vent flow, transfer time and presence of packing in the inlet liner are significant factors for most of the studied pesticides. Two-factor interactions were not found. As examples, the Pareto charts obtained for lindane and β-endosulfan are show in Fig. 1.

The values of the non-significant factors were selected according to their positive or negative influence in the response. The fastest heating rate gives a high response as well as a better peak shape. The solvent venting time selected was 0.1 min (negative influence), and initial PSS temperature was 80 °C (positive influence).

In spite of achieving better results (higher response) in the design when an empty liner was employed, glass-wool packing was selected because of the better reproducibility and peak shape obtained, as is apparent from the chromatograms in Fig. 2.

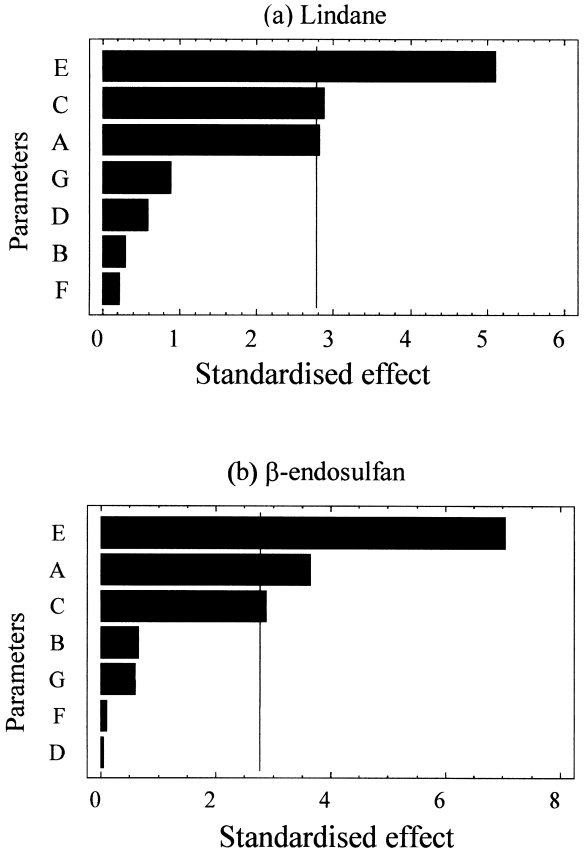

Fig. 1. Standardised Pareto chart obtained for (a) lindane and (b) β-endosulfan. A: Inlet liner packing (glass wool); B: heating rate (°C min^{-1}); C: split-vent flow (ml min^{-1}); D: solvent venting time (min); E: transfer time (min); F: initial PSS temperature (°C); G: dummy. Scale in the *x*-axis is the standardised effect for each factor.

3.2. Optimisation of significant factors

The next step was the optimisation of split-vent flow and transfer time (the significant factors) by a central 2^2+star orthogonal design resulting in ten randomised runs with 4 df. Table 3 shows the values established for each factor in each experiment.

The results of the experiments (average of six injections) were analysed by the response surfaces and are very similar for all the studied pesticides. The response increases when transfer time increases and when the split-vent flow decreases. As an example Fig. 3 shows the estimated response surface obtained for lindane and β-endosulfan.

The optimum values obtained for each pesticide can be seen in Table 4. A better response of the less volatile compounds is obtained at high split-vent flows probably due to the greater elimination of solvent without loss of analytes. Nevertheless in this case more volatile compounds could be in part eliminated with the solvent. To achieve a better response of the less volatile compounds (with the smallest peaks), a split-vent flow of 50 ml min^{-1} and a transfer time of 4.5 min were selected.

3.3. Comparison with splitless-MS and splitless-ECD

The instrumental detection limits obtained with the proposed PSS injection method were compared with those obtained with splitless injection coupled with MS and with LOD obtained with splitless injection coupled with a more selective detection method such as ECD. The splitless conditions employed were selected by an univariate study and were described in the Operation conditions paragraph of the Experimental section.

For each injection method, LODs calculated as $\bar{x}_b + 3s_b$(average value and standard deviation of the blank, respectively), are shown in Table 5. LODs have been improved by two orders of magnitude with PSS injection, although they are still higher than those obtained with ECD. These values nevertheless show the suitability of PSS-MS for pesticide analysis in environmental samples, with advantages over ECD.

The RSD with the proposed method are between 2.5 and 8% for all the studied pesticides.

3.4. Application of the proposed injection method to the analysis of a soil sample

A soil sample from a contaminated area was extracted by sonication with hexane:acetone (1:1) and analysed with the proposed PSS injection and with splitless injection (Fig. 4). The presence of α-HCH and β-HCH were detected in concentrations of 0.014 and 0.010 μg g^{-1}, respectively, in the PSS injection. In splitless-MS, β-HCH was not detected.

4. Conclusions

Split-vent flow, transfer time and the presence of

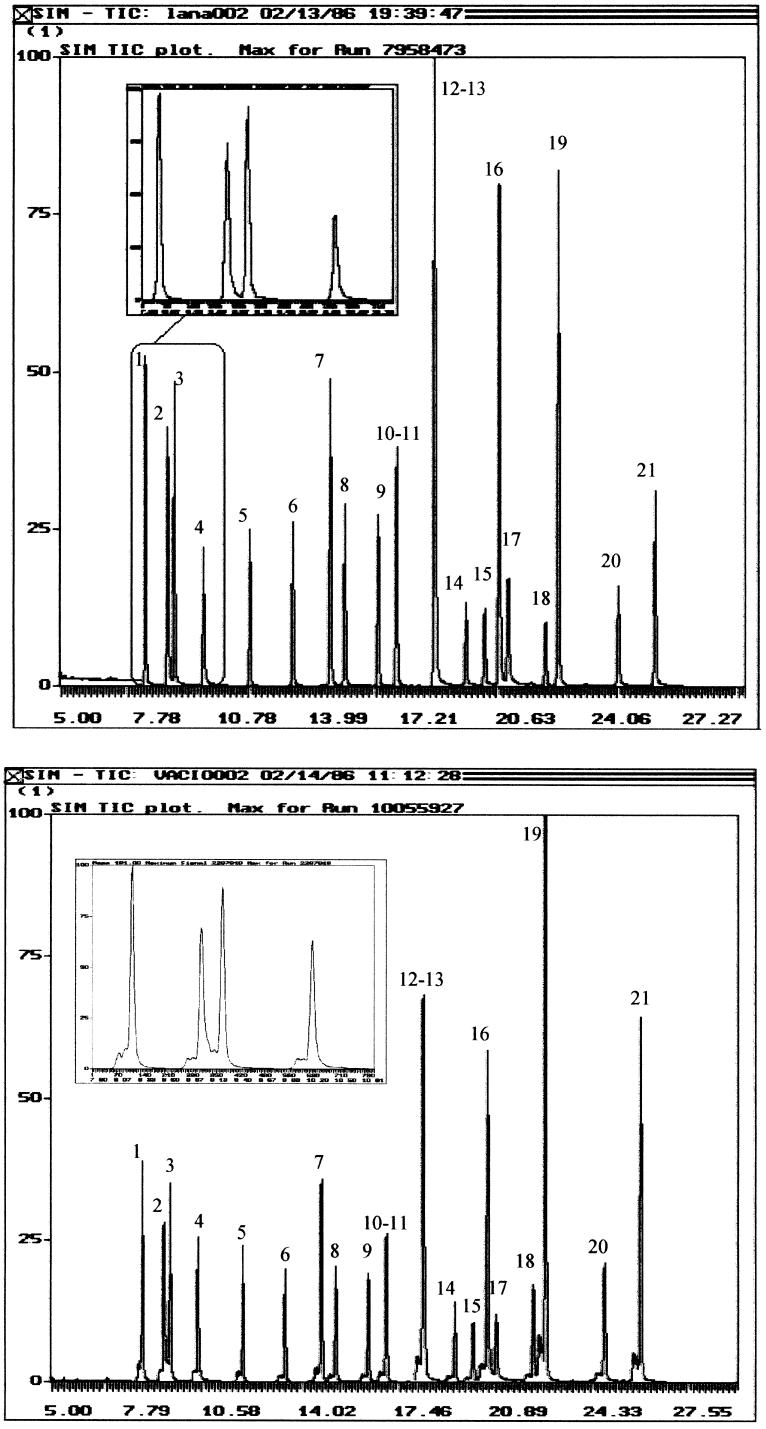

Fig. 2. Chromatograms obtained (and zooms in small window) with PSS injection of a standard solution with (top) and without (bottom) glass wool. 1: α-HCH; 2: γ-HCH; 3: β-HCH; 4: δ-HCH; 5: heptachlor; 6: aldrin; 7: isodrin; 8: heptachlorepoxide; 9: γ-chlordane; 10: α-chlordane; 11: α-endosulfan; 12: *p,p′*-DDE; 13: dieldrin; 14: endrin; 15: β-endosulfan; 16: *p,p′*-DDD; 17: endrin aldehyde; 18: endosulfan sulfate; 19: *p,p′*-DDT; 20: endrin ketone; 21: methoxychlor.

Table 3
Compositional values

Run	C	E
1	104.5	3
2	20	5
3	5.5	3
4	90	5
5	55	0.17
6	20	1
7	55	5.8
8	55	3
9	90	1
10	55	3

C: split-vent flow (ml min^{-1}); E: transfer time (min).

packing in the inlet liner were statistically significant factors for the PSS solvent split mode injection. These factors were optimised using a central composite design. Split-vent flow was fixed at 50 ml min^{-1} and transfer time at 4.5 min. The effect of glass wool filling the inlet liner was negative but it was used because of the better reproducibility of the injections.

Final injection conditions for 20 μl were: injector temperature programme: 80 °C (0.1 min) increased at 200 °C min^{-1} to 290 °C (held for 11 min), and then decreased at 200 °C min^{-1} to 80 °C. The split-vent programme was as follows. The valve was initially closed and was opened at 1 min. The liner (2-mm internal diameter) was packed with glass wool, injection speed was normal, and a 7-cm needle was used. LODs using the PSS injection and MS detection were better than those obtained with splitless injection and the same detection, but worse than

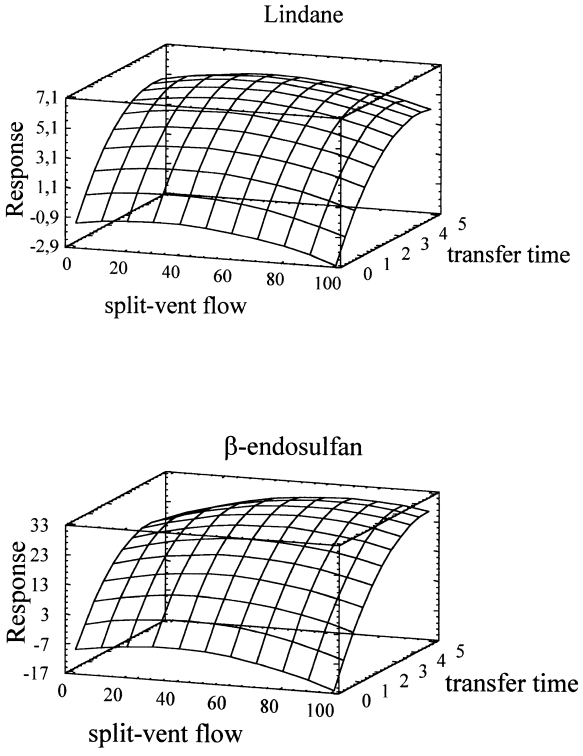

Fig. 3. Estimated response surface obtained for lindane and β-endosulfan.

LODs obtained using splitless-ECD with the equipment assayed in the present work. The detection limits are nevertheless sufficient for the concentrations of pesticides present in environmental sam-

Table 4
Optimum values for split-vent flow (ml min^{-1}) and transfer time (min)

Pesticides	Split-vent flow	Transfer time	Pesticides	Split-vent flow	Transfer time
α-HCH	46.7	4.2	*p,p′*-DDE	62.0	4.6
β-HCH	48.1	4.2	Dieldrin	54.6	4.4
γ-HCH	39.6	4.2	Endrin	64.8	4.8
δ-HCH	41.2	4.2	β-Endosulfan	65.2	4.5
Heptachlor	34.6	4.1	*p,p′*-DDD	57.8	4.7
Aldrin	40.4	4.3	Endrin aldehyde	57.3	4.3
Isodrin	42.8	4.3	Endosulfan sulfate	55.1	4.7
Heptachlorepoxide	43.7	4.1	*p,p′*-DDT	49.1	4.4
γ-Chlordane	30.9	4.4	Endrin ketone	56.5	4.2
α-Chlordane	37.5	4.3	Methoxychlor	54.6	5.2
α-Endosulfan	54.4	4.2			

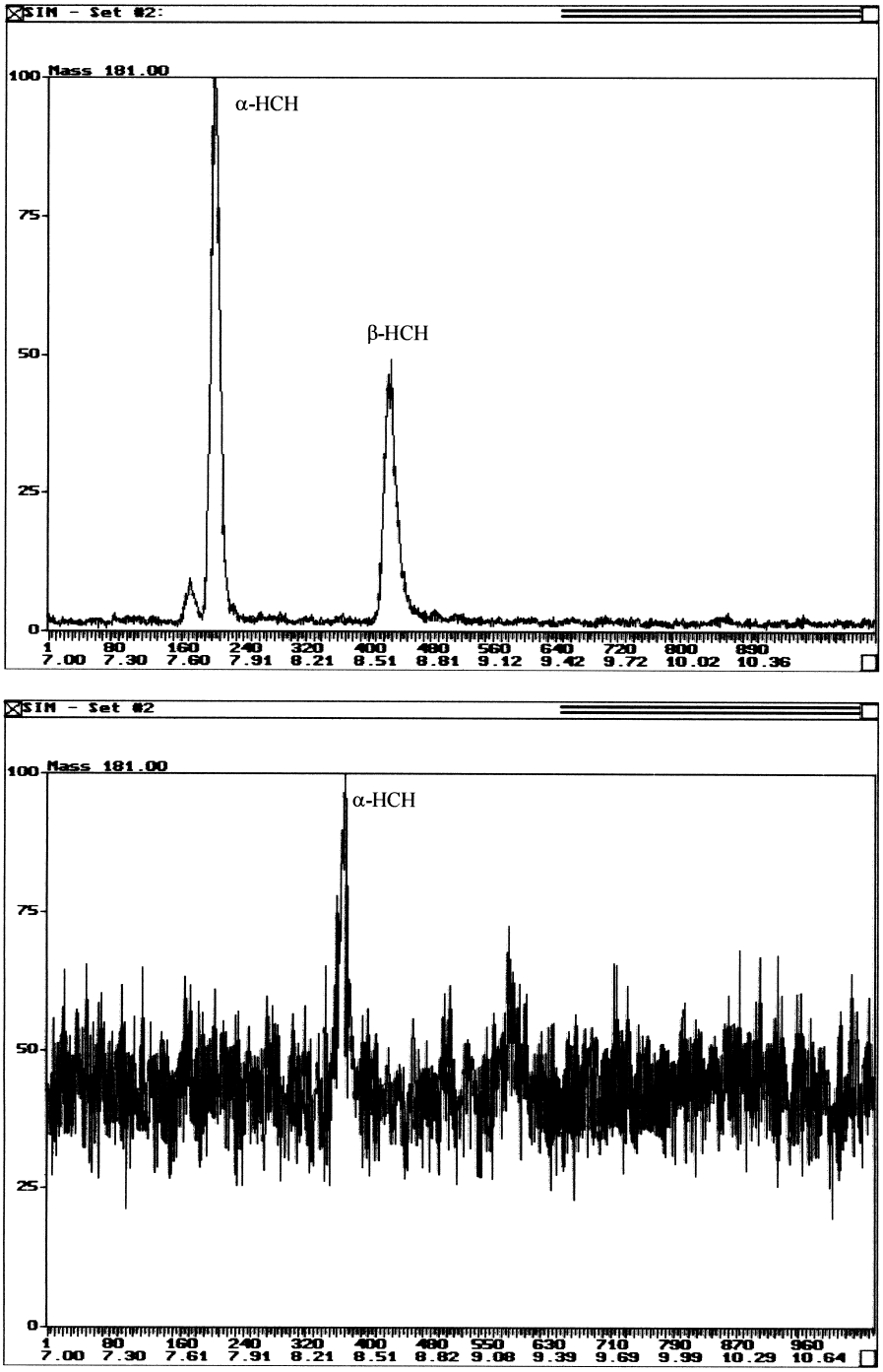

Fig. 4. Chromatograms obtained by PSS-MS injection (top) and splitless-MS injection (bottom) of the same soil sample. The volumes injected were 20 and 1 μl, respectively, and a fused-silica capillary column 30 m×0.25 mm×0.25 μm, 5% diphenyl methyl siloxane HP-5 and GC–MS in SIM mode (m/z: 181) were employed.

Table 5
LODs ($\mu g\ l^{-1}$) for PSS (20 μl) and splitless injection (1 μl) coupled with MS and ECD

Pesticides	PSS-MS	Splitless-MS	Splitless-ECD	Pesticides	PSS-MS	Splitless-MS	Splitless-ECD
α-HCH	3.2	74	0.065	*p,p′*-DDE	1.5	66	0.099
β-HCH	3.0	84	0.176	Dieldrin	1.9	77	0.102
γ-HCH	3.4	78	0.074	Endrin	5.4	54	0.143
δ-HCH	4.7	103	0.078	β-Endosulfan	2.8	43	0.128
Heptachlor	7.4	306	0.085	*p,p′*-DDD	2.9	117	0.160
Aldrin	2.3	78	0.075	Endrin aldehyde	1.1	37	0.171
Isodrin	2.1	10	0.080	Endosulfan sulfate	3.4	156	0.155
Heptachlorepoxide	2.6	140	0.090	*p,p′*-DDT	2.0	242	0.196
γ-Chlordane	2.2	143	0.089	Endrin ketone	5.5	11	0.127
α-Chlordane	2.2	145	0.091	Methoxychlor	2.2	265	0.528
α-Endosulfan	2.5	139	0.100				

ples and the use of MS detection is interesting because of its higher qualitative information.

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