Optimization of Sample Introduction Parameters for Determinations of Pesticides by Capillary Gas Chromatography Using a Two Column, Two Detector System

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A study was undertaken to determine if another injection mode could be substituted for splitless injection in the trace analysis of pesticide samples. This technique often leads to problems such as carryover, poor repeatability, and breakdown of labile pesticides. Two capillary injectors were compared: a hot splitless injector and a temperature- programmable injector, in which the sample is introduced into a glass insert under nonvaporizing conditions. With each injector, 2 columns of different polarity were installed and a test sample containing a variety of pesticides was split between the 2 columns immediately after introduction. The effects of changing parameters such as injector temperature, injection speed, method of installation of the 2 columns, and method of filling the syringe were examined for splitless injection. It was concluded that optimization is more difficult with splitless injection. Additionally, a comparison of precision and discrimination data demonstrated superior performance with the nonvaporizing temperature-programmable injector.

The simplest method for obtaining optimum resolution with capillary columns is to introduce the sample in a very narrow band (1, 2). This is achieved by using a split injector at a high split ratio. Unfortunately, most environmental applications involve trace analysis and require the introduction of a large sample containing very low levels of analytes.

Several injectors are available for trace analysis, but hot splitless injection is still the most common method for determining pesticides. Some problems with optimizing splitless injection were described by Snell et al. (3). In the present study, the difficulties in optimizing splitless injection for pesticide analysis are demonstrated.

The temperature-programmable injector, which was described in detail previously (4), was designed for fused silica columns of 0.10–0.53 mm id. The analyst has a choice of 3 glass inserts; in this study, the high-performance insert for columns with an id up to 0.32 mm was used. The samples were injected under nonvaporizing conditions into the glass insert, then the injector was rapidly heated to transfer the sample onto the column. The splitless injector and the temperature-programmable injector were compared for area count precision and for their performance in avoiding discrimination against pesticides of low volatility.

Experimental

Apparatus and Reagents

(a) *Gas chromatograph.*—Model 3600 (Varian Chromatography Systems, Walnut Creek, CA) equipped with a conventional split/splitless injector and a temperature-programmable injector (SPI), nitrogen–phosphorus and electron capture detectors (TSD and ECD) and model 8100 AutoSampler. Data from the 2 detectors were acquired simultaneously with a Varian 654 chromatography data system.

(b) *Chromatographic conditions.*—Columns (J&W Scientific, Rancho Cordova, CA), 15 m × 0.25 mm DB-17 (connected to the TSD) and 15 m × 0.25 mm DB-5 (connected to the ECD). Temperature program: 40°C, 20°C/min to 280°C, hold 2 min. Carrier gas: He, 59 cm/s at 40°C. Injector: splitless, 280°C, open splitter at 1 min, or SPI, sample injected at 40°C, 100°C/min to 280°C, hold 11.6 min. Detectors heated to 300°C; TSD, range 10^{-12}; ECD, range 10. Autosampler conditions: 1 μL sample with upper and lower air gaps and 1 μL solvent plug (hexane) injected at 0.5 μL/s, needle residence time 6 s.

The 2 columns were inserted into the split/splitless injector by passing them both through a 0.8 mm graphite ferrule. For comparison, the columns were also connected to a "Press-Fit" splitter (Schmidlin AG, Zug, Switzerland), and a 50 cm piece of a DB-17 column was used to connect the splitter to the injector.

A 50 cm piece of uncoated "deactivated" fused silica was used initially, but the pesticides tailed. The DB-17 inlet splitter, pre-column, and column were later moved to the SPI.

(c) *Sample.*—Pesticide standards (Chem Service Inc., West Chester, PA) were weighed, dissolved in glass-distilled acetone (1.0 mg/mL), and diluted 500:1 (v/v) with glass-distilled hexane.

The retention time of each pesticide was determined and the test mixture was prepared in hexane. Table 1 lists the compounds in the samples with retention times. The rationale was to examine a mixture of "model" pesticides: early and late eluters; thermolabile, and relatively stable compounds.

Determination

(a) *Splitless injection.*—The parameters that were varied initially were injector temperature (220°C vs 280°C), injection speed (0.5 μL/s and 5 μL/s), and post-injection needle residence time (0 and 6 s). For the remainder of the splitless study, the injector temperature was 280°C, injection speed was 0.5 μL/s, and post-injection residence time was 6 s.

(b) *Distribution of the sample between the 2 columns.*—Screening of pesticides with 2 columns and 2 detectors is valuable as a qualitative tool. The splitting of the sample between the columns must be repeatable. Zolone, a fairly late eluting compound with rather poor area count repeatability, was chosen for this part of the study. The test sample was injected into the hot splitless injector while the 2 columns were installed directly in the column, as described above, by using the Press-Fit splitter. Area counts on the 2 detector channels were compared for 10 runs.

(c) *Solvent-flush technique with splitless injection.*—The autosampler was programmed for 3 injection modes of the 1 μL sample as follows: (*1*) sample in the needle (no air gaps or solvent plug); (*2*) sample in the syringe barrel (sandwiched between a 0.8 μL air gap in the needle and bottom of the barrel and a 0.5 μL air gap under the plunger); (*3*) solvent-

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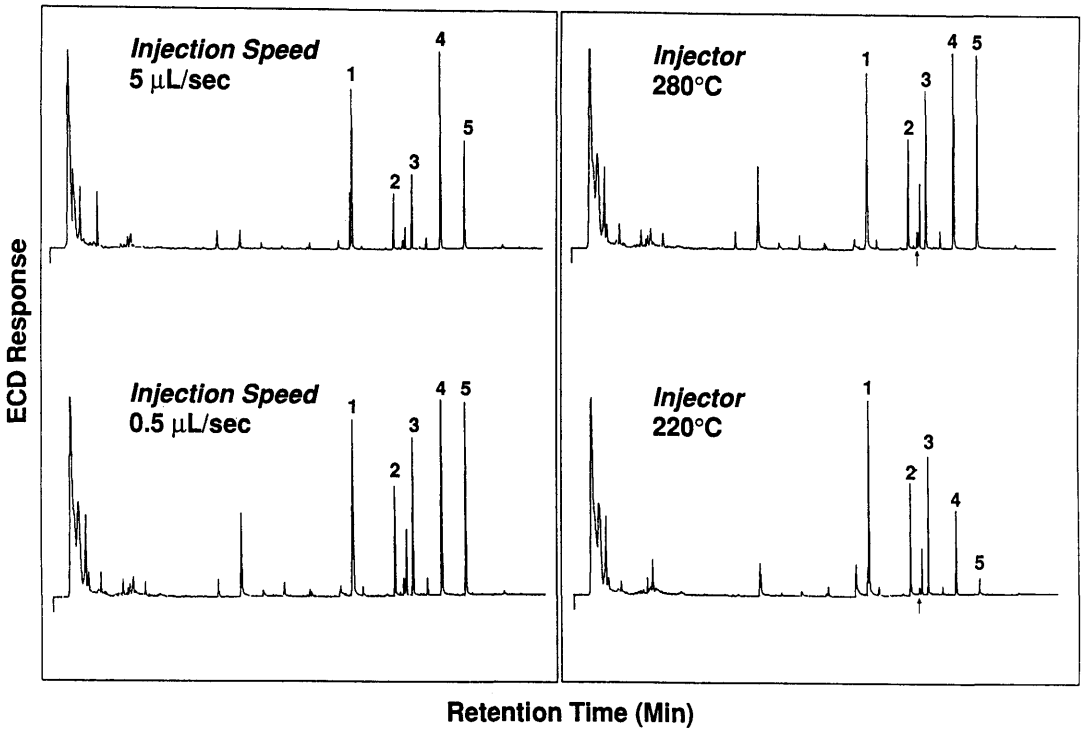

Figure 1. Chromatograms of pesticide standards with the ECD and hot splitless injection. The chromatogram on the left shows the effect of varying injection speed with injector temperature at 280°C. The chromatogram on the right shows the effect of changing the injector temperature with the slower injection rate. The peaks are (1) malathion, (2) endrin, (3) DDT, (4) Zolone, and (5) Co-Ral. The very small peak after endrin is endrin aldehyde.

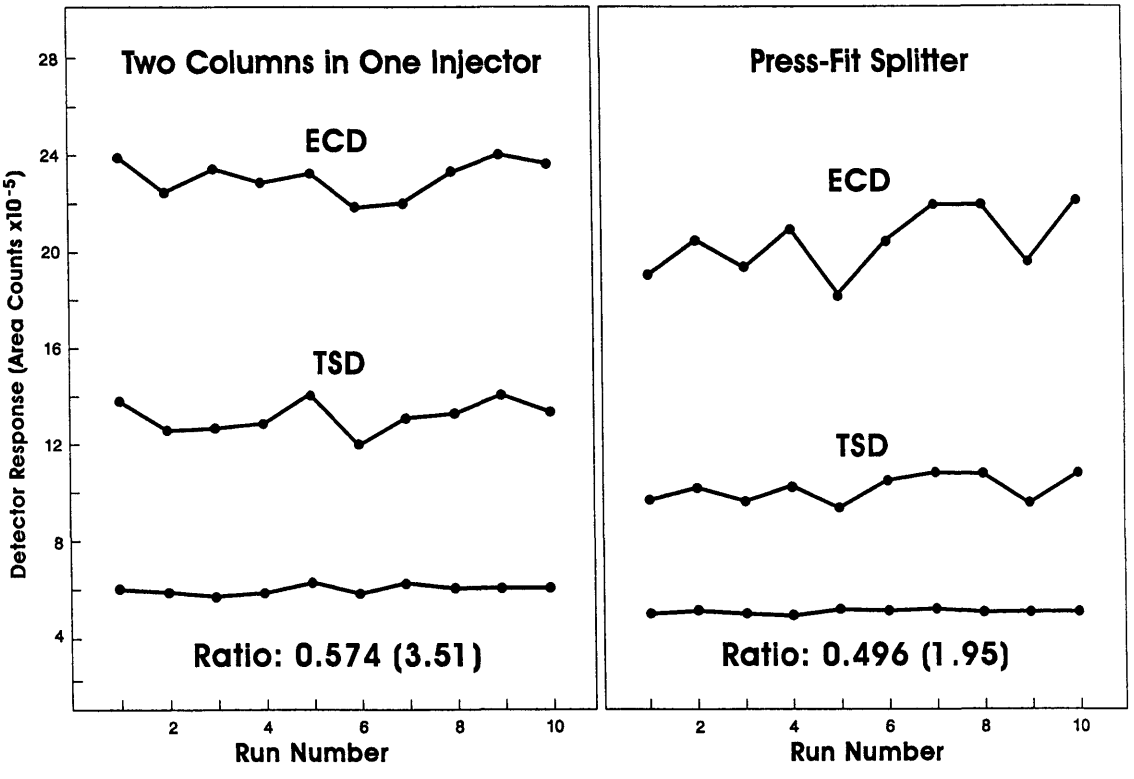

Figure 2. The distribution of Zolone between two 15 m × 0.25 mm columns in splitless injection. Left: Both column were inserted through 1 ferrule into the injector. Right: Columns connected to an inlet splitter. Each point represents the detector response from a single run. The numbers in parentheses next to the ratios are RSD (n = 10).

Table 1. Compounds present in the sample with their retention times[a]

Compound	Concn, ng/μL hexane	RT, min DB–5/ECD	RT, min DB–17/TSD
Simazine	2	7.56	8.48
Atrazine	2	7.65	8.40
Aminocarb	2	—	7.77
Carbaryl	2	8.44	9.71
Aldrin	0.2	8.77	—
Malathion	2	8.77	9.61
Endrin	0.2	10.04	—
DDT	0.2	10.57	—
Zolone	2	11.43	12.46
Co–Ral	2	12.12	13.50

[a] A blank indicates no detector response.

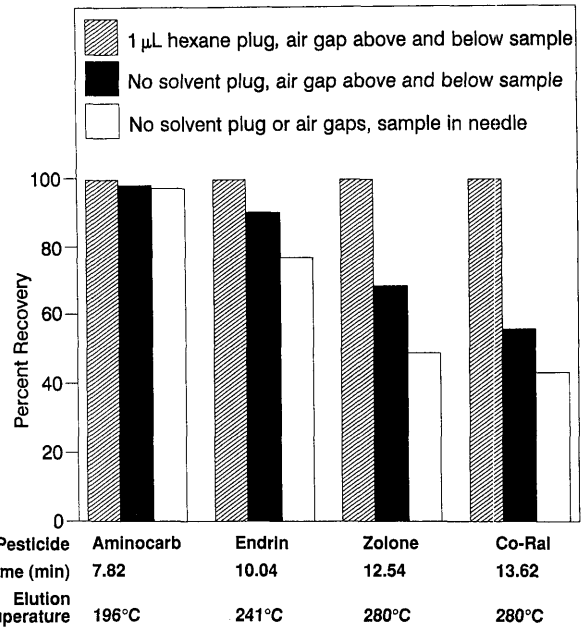

Figure 3. Effect of solvent flush technique on detector response for pesticides eluting at different times. Injection mode: hot splitless. GC conditions are in the text. The results for each pesticide are shown as percent recovery relative to recovery with a solvent plug and air gaps.

flush: same as (*2*), but with 1 μL hexane between the 0.5 μL air gap and the plunger.

(d) *Temperature-programmed injection.*—The 2 columns with the Press-Fit splitter and pre-column were removed from the splitless injector and installed in the SPI. Repeatability of this system was compared to repeatability of injection into the splitless injector with both methods of column installation.

Results and Discussion

(a) *Hot splitless injection.*—Figure 1 illustrates the effects of injector temperature and injection speed. Note that the higher temperature was required for good recovery of the late eluters, but approximately 5–10% of the endrin tended to decompose to endrin aldehyde (*5*) at 280°C. The slower injection speed was beneficial in recovering late eluters. Although it was not evident here, slow injection of samples > 1 μL is often desirable with low-capacity capillary columns to minimize peak splitting and solvent tailing (*6*). Unfortunately, slow injection is difficult to effect reproducibly with manual injection. The longer residence time tended to improve precision somewhat, but the presence of a stainless steel needle in the hot injector body would probably exacerbate the decomposition of labile compounds. The response ratio of Zolone on the ECD and TSD was consistent with 2 columns inserted into 1 injector, and even more precise with the Press-Fit splitter (Figure 2). Figure 3 demonstrates the higher recovery of late eluters with the solvent flush technique.

(b) *Temperature-programmed nonvaporizing injection.*—Data comparing the precision with the splitless injector and the SPI are presented in Table 2. The SPI gave better precision and recovery of high boilers than hot splitless injection. The decomposition product of endrin, endrin aldehyde, was not detected after injection into the SPI under normal nonvaporizing conditions, although 5–10% decomposition was observed upon injection into a hot SPI or splitless injector.

Identification of pesticides in environmental samples can be facilitated by injection into 2 fused silica columns of different polarity; in many cases, identification can be reinforced by the use of 2 different detectors.

Table 2. Comparison of area count precision with 2 columns in the splitless injector and the SPI for selected pesticides eluting at different retention times

Pesticide	RT, min	Area counts × 10^{-2} (% RSD, n = 8) Splitless 2 columns in 1 injector	Splitless Press-fit splitter	SPI Press-fit splitter
Aminocarb[a]	7.79	5676 (1.88)	3416 (1.91)	3177 (1.03)
Endrin[b]	10.06	6771 (1.69)	8082 (2.05)	8451 (1.43)
Zolone[a]	12.54	13347 (6.58)	10119 (5.96)	12061 (3.60)
Co-Ral[a]	13.62	9700 (13.0)	5687 (11.6)	12905 (2.24)

[a] TSD.
[b] ECD.

In conclusion, it was stated earlier that the purpose of this study was to determine the feasibility of substituting another injection mode for splitless injection in trace analysis of pesticide samples. If splitless injection is the only technique available, reasonable precision can be obtained, especially with the more volatile compounds. However, optimization will be more time-consuming and compromises between recovery and thermal breakdown will be required when developing the chromatographic method. Therefore, the use of the nonvaporizing temperature-programmable injector is recommended.

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Determination of Mirex in Human Blood Serum Containing Polychlorinated Biphenyls by Using Packed Column Gas Chromatography

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An analytical method has been developed that uses electron capture/gas-liquid chromatography to determine Mirex in serum containing polychlorinated biphenyls (PCBs) (Aroclor 1260). With this method, 0.2 ppb Mirex can be determined in 4 mL serum that also contains 10 ppb PCBs. The method provides approximately 70% recovery of Mirex at 1.0 and 3.5 ppb. The coefficients of variation are 4.5 and 4.6% at 1.0 and 3.5 ppb, respectively. In a cooperative study with the Ohio Department of Health, the Centers for Disease Control used this method to determine the extent of exposure of Salem, OH, residents to Mirex. Confirmation of Mirex was obtained by using high resolution gas chromatography and high resolution mass spectrometry.

The Ohio Department of Health was concerned about the possible exposure of residents in Salem, OH, to Mirex from a defunct chemical manufacturing facility. Studies by the federal and state Environmental Protection Agency already documented the presence of Mirex in off-site sources, such as fish and sediments in a creek downstream from the plant. To determine the significance of this exposure, we had to compare the prevalence of Mirex detection among the Salem cohorts with the prevalence among control subjects and with the prevalence rate in the U.S. population, which is less than 1% (1). The epidemiological significance of the results of this study is reported elsewhere (2).

The basis of this report is the analytical method used to determine Mirex in the presence of more ubiquitous analytes, such as polychlorinated biphenyls (PCBs). Other researchers have encountered the analytical problems presented by the presence of PCBs and Mirex in the same sample. The use of capillary gas chromatography (3) and UV irradiation (4) has eliminated or minimized the interference of PCBs with the quantitation of Mirex. In this paper, we report the results of our attempt to limit this interference and our quantitation of the extent of PCB interference with Mirex by the Webb and McCall PCB Peak 332 (5). We have used selective adsorption chromatography and electron capture/gas-liquid chromatography as the primary mode of determination. Results were later confirmed by gas chromatography/mass spectrometry (GC/MS). We used linear regression analysis to compare the data obtained by the 2 techniques.

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Experimental

Acquisition of Specimens

Forty-four people participated in the study and provided overnight fasting blood samples. A standard red-top vacutainer tube was used to collect ca 15 mL blood from each participant by venipuncture. All blood samples clotted at room temperature for 30 min. The serum was separated by low-speed centrifugation (2500 × *g* for 15 min) at 4°C, recovered by aspiration, transferred to solvent-rinsed vials, and stored frozen at −20°C until analysis.

Preparation of Quality Control Pools

Serum pools, to be used for method validation and to monitor quality control (QC) during the analysis of unknowns, were prepared according to previously recorded procedures (6, 7). In vivo QC pools of PCBs, as Aroclor 1260 (AR 1260), were prepared from serum taken from a goat dosed with AR 1260 and then added to base bovine serum. Four serum pools were prepared: (*1*) a pool of base bovine serum that contained no analyte above background concentrations; (*2*) a pool containing 1 ppb Mirex (spiked in vitro) plus 10 ppb AR 1260 (in vivo); (*3*) a pool containing 3.5 ppb Mirex (spiked in vitro)