Automation of Pesticide Analysis

by HOWARD G. APPLEGATE[1]
Department of Plant Sciences
Texas A&M University
College Station, Texas
and
GEORGE CHITTWOOD
Barber-Colman Company
Pasadena, Texas

Frequently the bottleneck in pesticide analysis is detection and quantification via gas-liquid chromatography. Not only do the samples take time to elute from the column but the necessity of running standards and spiked sample limits the time available

---

1/ The research was supported by Grants AP00028 and CC00272, U. S. Public Health Service, Technical Article No. 7174, Texas Agricultural Experiment Station, College Station, Texas.

Bulletin of Environmental Contamination & Toxicology,
Vol. 3, No. 4, 1968, published by Springer-Verlag New York Inc.

to run actual samples. If an eight hour day is adhered to the gas chromatograph, usually the most expensive piece of equipment in the laboratory, stands idle for two-thirds of a day. If an automatic method of injection could be devised so that samples could be injected over a 24 hour period not only would a bottleneck be removed but the gas chromatograph could be utilized to its fullest capacity. This paper will describe an automatic injection system which has worked well for us.

Materials and Methods

The system is basically that described by Ruchelman (1) modified to fit our particular chromatograph and needs. A slotted Teflon wheel in an aluminum housing is rotated by a motor (Figure 1). Each slot holds a stainless steel gauze (Dixon gauze rings, 1/8" x 1/8", 100 mesh) which drops through a port into a glass thimble (Figure 2.). The lower end of the thimble is perforated and fits into the top of the chromatographic column. Both the lower end of the thimble and the top of the chromatographic column are enclosed in a brass heating block which, in turn,

is encased in an insulation block (Figure 1.). The heating block is connected to the temperature controller of the former injection port of the chromatograph. All joints are sealed with O rings and clamps.

The Pyrex thimble has two injection ports. The 90° port is connected via stainless steel tubing to the carrier gas. The 45° port is stoppered with a septum and allows liquid samples to be injected with a syringe.

To prepare samples for injection, clean gauzes are placed in depressions in glazed porcelain spot plates. A measured amount of the sample in hexane is dropped onto the gauze with a syringe. A dryer evaporates the hexane and the sample, by capillarity, is drawn onto the gauze. If needed, several applications can be made. The dry gauzes are inserted into slots on the Teflon wheel. During the time the cover is off the wheel, carrier gas is kept passing at 2 psi through the injection apparatus and column. The cover is placed on the wheel and tightened after all gauzes are in place.

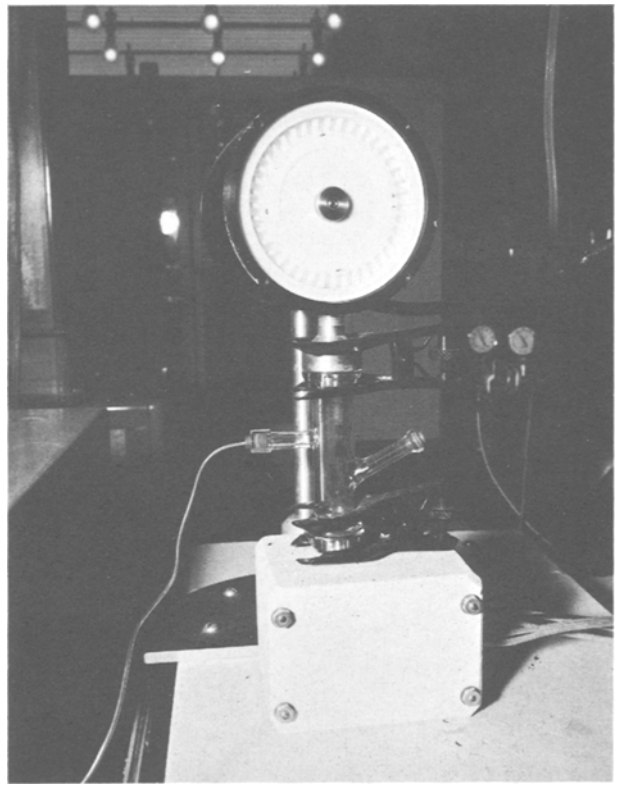

Figure 1. The automatic injection device with its cover removed to show the slotted Teflon wheel. Sample impregnated gauzes are placed in the slots. Tubing leading to the 90° arm on the thimble brings carrier gas to the Teflon wheel, the thimble and the column. The 45° arm allows manual injection. The top of the column is visable above the insulation covering the brass heating block.

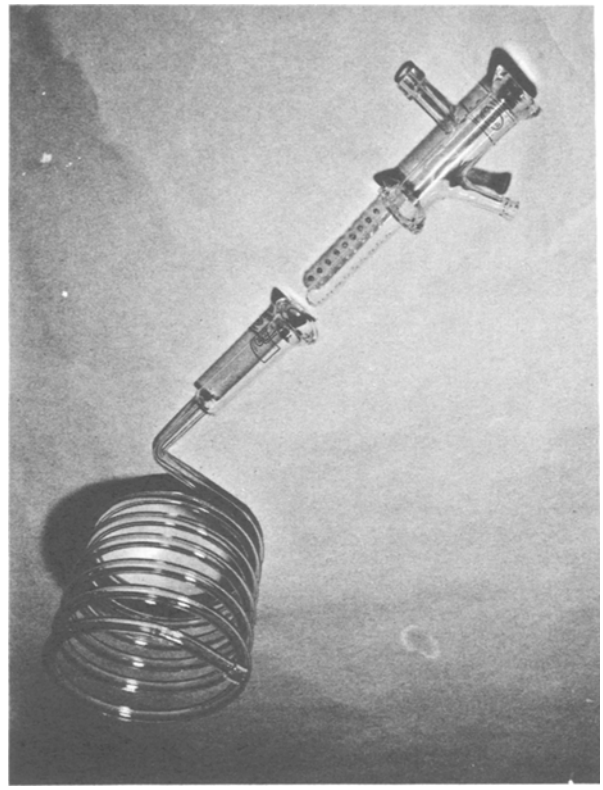

Figure 2. The thimble showing the perforations in its lower portion. The lower portion fits into the wide neck of the column.

Carrier gas pressure is increased to operating pressure and the wheel is turned on. It should be noted that once the wheel cover is in place the entire system from gauzes to column outlet is in the carrier gas atmosphere.

Normally, the slot next to the port leading to the thimble is left vacant. This gives a period of at least 15 minutes before the first gauze drops into the thimble. This time period has been found adequate to allow the baseline to stabilize.

Gauzes drop into the thimble every 15 minutes. The sample is vaporized and carried into the column by carrier gas. By changing the gear ratio or by placing gauzes into alternate slots the time of drop can be varied.

By use of a syringe with a three inch needle liquid samples can be injected into the thimble. The long needle allows the sample to be injected below the carrier gas port.

The chromatograph to which the automatic injection system was attached was a Barber-Colman model 5360. The column was 10% DC 200 on Anakrom ABS 80/100 mesh. Column effluent was split between an electron

capture detector (220 mc of tritium) and a sodium thermionic detector. A saturated solution of KCl was used to coat the grid. Nitrogen gas flow was 200 cc/min and air flow was 450 cc/min. Hydrogen flow was adjusted to give 2 x 10^{-8} amps standing current. The thimble temperature was 220°C, column temperature was 200°C and detector temperature was 210°C. The electrometer of the sodium thermionic detector was maintained at sensitivity 300 and attenuation 1; that of the electron capture detector was at sensitivity 100 and attenuation 1.

Quantification was by means of an Aerograph 476 digital integrator with a Victor heavy duty printer.

Results

The chromatograph of a standard mixture vaporized from a gauze is shown in Figure 3 while Figure 4 shows the same mixture injected manually with a syringe. The manually injected sample shows low, broad, poorly defined peaks compared to the automatically injected sample. This is probably due to the large volumn of the thimble into which the liquid can expand after being vaporized.

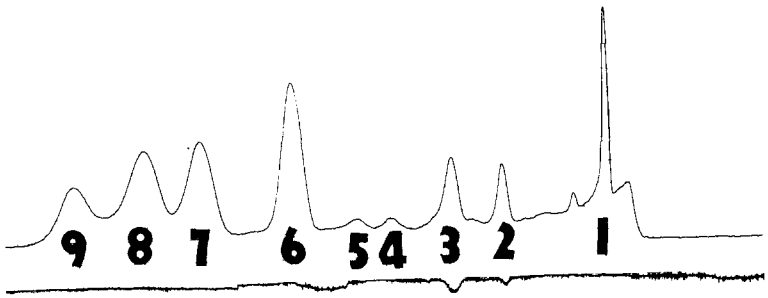

Figure 3. Mixture of pesticides vaporized from a coil. Two µl of solution were deposited on the coil. Chromatographic conditions are given in the text. The mixture consists of 0.1 ppm of methyl parathion, parathion, p,p-DDE, p,p-DDD, o,p-DDT and p,p-DDT. Peaks are: 1 Hexane, 2 Methyl parathion, 3 Parathion, 4 o,p-DDE, 5 o,p-DDD, 6 p,p-DDE, 7 p,p-DDD, 8 o,p-DDT, 9 p,p-DDT.

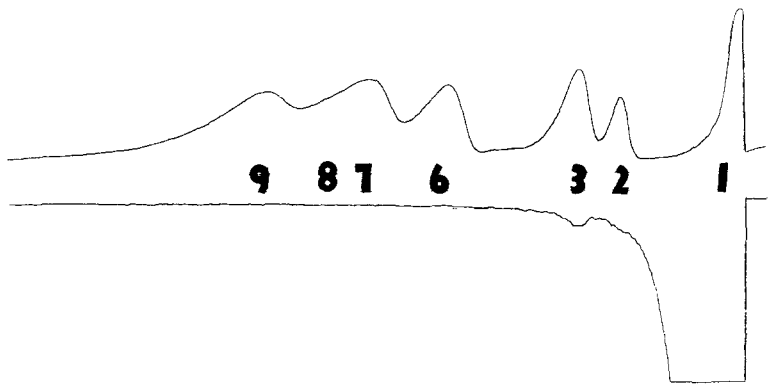

Figure 4. Mixture of pesticides injected manually. Two μl were injected. Chromatographic conditions are given in the text. Peaks are identified in legend of Figure 3.

Quantification of various concentrations of standard mixtures injected automatically are given numerically in Table 1 and as graphs in Figures 5 and 6. It is obvious the auromatic method of injection is suitable for quantification.

TABLE I

INTEGRATION VALUES FOR A STANDARD MIXTURE

ppm	MP*	P*	DDE*	DDD*	DDT*
0.01	2,258	9,446	70,489	105,899	50,474
0.1	37,101	45,675	194,578	223,381	180,091
1.0	57,951	64,580	262,965	321,757	246,879
10.0	84,889	93,447	353,188	419,571	333,581

* MP = 0,0-dimethyl 0-p-nitrophenyl phosphorothioate

P = 0,0-diethyl 0-p-nitrophenyl phosphorothioate

DDE = 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene

DDD = 2,2-bis (p-chlorophenyl) -1,1-dichloroethane

DDT = 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane

Chromatographic conditions are given in the text. Integrator settings are: Attenuation 4, activation level 500, baseline corrector 7, slope sensitivity 3, peakwidth 30.

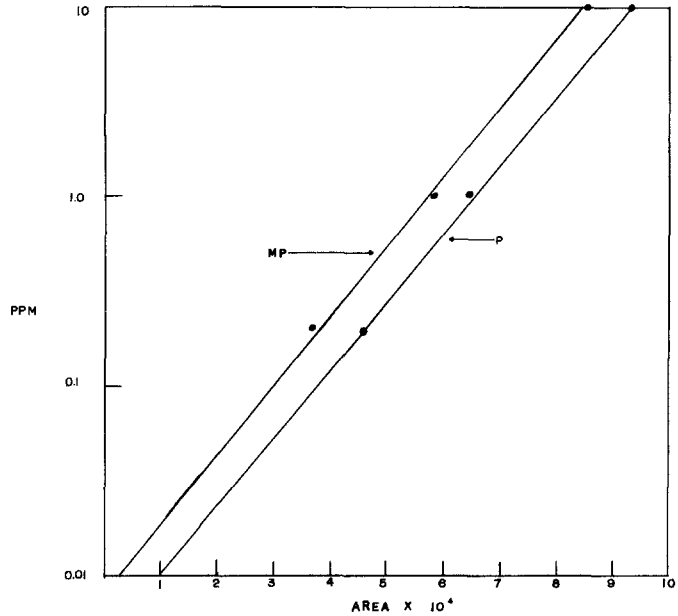

Figure 5. Graphic representation of data in Table 1.

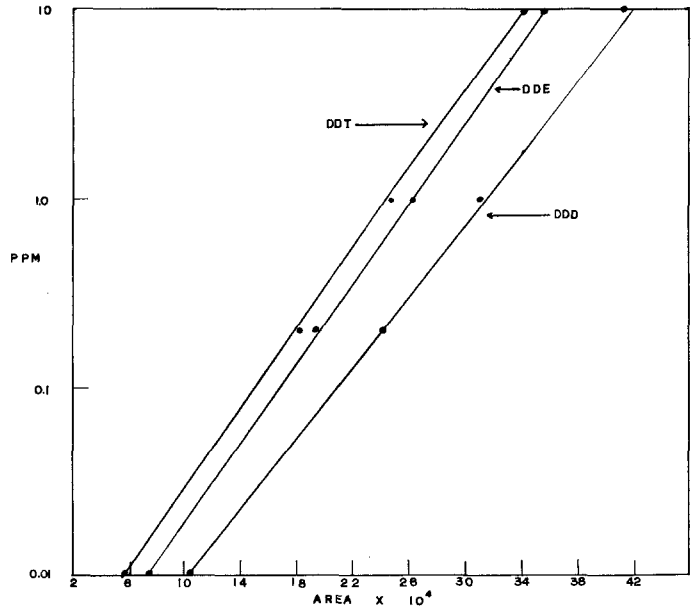

Figure 6. Graphic representation of data in Table 1.

The effect of time the gauze is in the Teflon wheel is given in Table 2. Two gauzes were impregnated with a standard mixture at 1 ppm and placed in the Teflon wheel. One gauze dropped through the port and was vaporized 30 minutes later; the other gauze dropped and was vaporized 12 hours later. Integrator values for the sample dropped last were compared to those for the first dropped gauze. It can be seen that there was little, if any, breakdown of compounds in the wheel.

TABLE 2

Effect of Time on Pesticide Breakdown in the AutoInject

Compound	Hours	
	1/2	12
Methyl Parathion	1 ppm	1.08 ppm
Parathion	1 ppm	0.97 ppm
DDE,p,p	1 ppm	0.97 ppm
DDD	1 ppm	0.95 ppm
DDT,p,p	1 ppm	1.09 ppm

Discussion

When the idea of automating gas-liquid chromatographic analysis of pesticides was first discussed

in this laboratory it was felt the use of the stainless steel gauzes of Ruchelman would lead to pesticide breakdown. Accordingly, glass capillaries were cut to fit the slotted Teflon wheel. They were filled with a measured amount of sample, dried and placed in the wheel. The difficulties of cutting the capillaries to the desired size and then filling them led us to try the stainless steel gauzes. A comparison of results between the two methods led to the conclusion that there was no difference between using glass capillaries and stainless steel gauzes.

It is believed that two factors led to the stability of the pesticides on the stainless steel gauzes: a) the gauzes are in a nitrogen atmosphere once the cover has been replaced on the wheel; b) the stainless steel gauzes never become hot until they drop into the thimble. (The outside of the metal housing of the Teflon wheel reaches 36°C after 24 hours operation)

This technique has been developed to analyize pesticides from a specific area. In that area only DDT, methyl and ethyl parathions are used. Compared

to lindane these compounds have heavier molecular weights. The stability of lighter molecular weight pesticides has not been investigated.

A comparison between the samples injected manually and those injected automatically shows a great difference. The manual injection method cannot be used to quantify the amounts of pesticide present. It can be used to determine rough retention times and what is present without breaking into the system. This is one advantage of this system over that described by Ruchelman. Thus manual injection can be used to determine the parameters needed to obtain best results from automatic injection.

By means of simple time clocks the automatic injection system, the chromatographic recorder and the digital integrator can be programmed to turn on or off at pre-determined times.

Two difficulties have been encountered in using the system. The gauzes must be tightly rewound prior to use. A fine needle-nosed pair of tweezers has been found satisfactory for rewinding. If the rewinding is omitted there is a tendency for the

gauzes to stick in the Teflon slots. All protruding wires must be cut flush with the two edges of the gauze. Otherwise they tend to catch under the Teflon lugs as the wheel turns.

The second difficulty is the poor response of manually injected samples. It is felt if the injection port was at a more acute angle to the thimble (60° rather than 45°) thus allowing the syringe needle to go deeper into the thimble better response might be obtained. In addition, injecting a smaller sized sample has helped to sharpen the peaks.

The chromatographs shown here (Figures 3 and 4) are representative samples. No effort was made to pick the best chromatographs available. The quantification data shown in Figures 5 and 6 show the best quantification we have obtained and do not represent our average data. There are periods when the chromatograph is operating at its optimum. These periods occur with no changes being made in the operating parameters and last from a few hours (usually) to a few days (rarely). During these periods quantification data such as are presented here are obtained

routinely. During most of the time, however, the chromatograph is operating at less than optimum. During these periods it is impossible to fit an acceptable straight line to the experimental points derived from a wide concentration range. During these less than optimum operating periods we use a 100 fold concentration range rather than a 1000 fold range.

Sources of Equipment

The Teflon wheel and motor were manufactured by Scientific Systems Corporation, Baton Rouge, Louisiana.

The glassware was manufactured by Glass Engineering Company, Houston, Texas.

The brass heating block was manufactured by Barber-Colman Company, Pasadena, Texas.

The stainless steel gauzes were obtained from Dr. M. W. Ruchelman, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas.

References

1. M. W. RUCHELMAN, J. Gas Chromatog. 4,265 (1966).