Chapter 5

Automation in Today's Pesticide Laboratory

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INDIVIDUAL COMPONENT AUTOMATION

Automation has greatly increased analytical productivity of pesticide residue laboratories, and most such laboratories today use some type of automated equipment. Computers, for instance, have made the identification and quantification of pesticides easier. Automated geI permeation chromatography and autoinjection of samples onto chromatography have allowed unattended work to take place day and night and permitted analysts to do additional work.

The Role of Auomation

Despite such advances in automation, the prospect of designing a fully automated analytical laboratory remains an ideal (box 5-A). The procedures for analyzing a food sample are time-consuming, and many steps must still be done manually. A major percentage of the total analysis time is spent in preparation, extraction, and cleanup. Food is generally subsampled, cut into manageable pieces if necessary, and subsequently blended with solvent to extract the pesticide. The sample is then filtered or centrifuged, and the extract is either partitioned with another solvent or concentrated by evaporation. An optional cleanup step to isolate the pesticide may be required. Finally, the sample is injected into a gas or liquid chromatography for analysis.

Automating the sample preparation and extraction steps would generate the greatest time savings, but these steps are the most difficult to automate because many types of samples require different preparation (10). Consequently, improvements in automation have focused primarily on the cleanup and determination stages of pesticide residues in food analysis.

Several types of automated equipment can be used in the cleanup step. Gel permeation chromatography (GPC) can be automated (16) (for a description of gel permeation chromatography see ch. 3); in fact, FDA and FSIS use automated GPC, primarily for fatty foods. Requirements for quick results may pose a problem, however, because automated GPC processes only one sample at a time.

Further automation of the cleanup step may be possible with the recent development of an

Box 5-A.—The Ideal, Fully Automated Analytical Laboratory

A fully automated laboratory, now existing only on paper, is one that would automatically process a sample from its entrance into the laboratory through the production of a written final report. An automated process of this type would move the sample through a series of operations whereby it could be subsampled, chopped, ground, blended, filtered, centrifuged, and extracted. The extract then could be evaporated, partitioned, redissolved, diluted, dried, chemically treated, subsampled and chromatographed, Data from the chromatography would go to a computer, which would identify the sample, perform calculations on its abundance, graph the results, collate it with other data, and produce a hardcopy. Leftover sample or sample extracts would automatically be moved back to a refrigerator, freezer or other proper storage area, where it would be available for reanalysis if the computer data did not meet certain quality assurance/quality control standards.

Only very few regulatory laboratories have experience with robotic automation systems. Given the current cost and capability of automation instrumentation and technology, it is not yet possible to automate regulatory laboratories totally.

evaporation device that can be connected to the automated GPC. This evaporation device replaces the gel permeation solvent with one more suitable for gas chromatography, concentrates the sample through evaporation, and deposits each sample into a sealed vial, which then can be injected into a chromatography for analysis. Such a device can process various types of pesticides with excellent reproducibility and recoveries (3). At present, FDA has not used automated evaporation equipment, in part because it does not want to use its capital budget to replace still functional manual evaporators and concentrators. FSIS laboratories do, however, use such equipment.

Another automated device for cleaning up food extracts is the DuPont Autoprep System. This device, used by some FSIS labs, uses centrifugal force rather than gas pressure or vacuum, as is done by other devices designed for this purpose. As many as 12 samples can be processed at a time, only small volumes for each wash are required (1 to 5 milliliters), and the pesticide is effectively concentrated for analysis by chromatography or other means.

The detection step has also been automated. Samples to be analyzed using gas or liquid chromatography can be loaded on sample trays holding as many as 100 miniature vials and capped to seal-in volatile organic solvents and pesticides. These trays can be refrigerated to prevent the decomposition of thermally unstable pesticides. Automated sample injectors, also known as autosamplers, can then inject the sample into an automated chromatography for unattended analysis. Autosamplers have the added advantage of being more precise in their volumetric sampling than a chemist, resulting in higher quality analytical data. Autosamplers, however, do not appear to be used for the majority of food samples at regulatory laboratories. In some cases, they are considered slower and more expensive than hand injection (9).

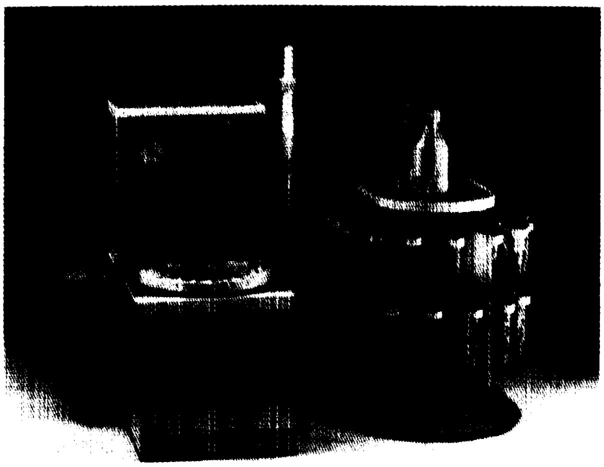


Photo credit: Analytica/ Blo-Chemistry *Laboratories, Inc.*

ABC Laboratories' GPC/Autovap@ system combines a gel permeation chromatography module with an evaporation module to allow automated sample cleanup and concentration for a maximum of 23 sample extracts.

Automation of the detection step has been greatly facilitated by computerized data processing. Gas and liquid chromatography are equipped with computers known as integrators. Integrators determine the retention time of an unknown chemical, necessary for its identification, and the quantity of the chemical. The integrator can then provide this information in report form. An integrator can be programmed to identify any specified retention time, allowing easier analysis of a specific pesticide. Mass spectrometer and infrared detectors are equipped with computers for sample identification that can search a library to match a sample to a known mass spectra. Data processing's importance is seen as increasing with the development of the laboratory information management system (LIMS). The LIMS goes beyond recording data; it produces tables that could be included in reports, it tracks samples, and it provides an electronic "paper trail" for fulfilling the requirements of "good laboratory practices.'" In addition, a properly designed LIMS can be linked with virtually any type of analytical instrument from any manufacturer and can be used to collect and interpret data from it. Pesticide residue laboratories have not

'These are standards describing the quality of instrumental, procedural, analytical, and personnel performance prescribed for laboratories conducting studies that support or are intended to support applications for research or marketing permits for (a) pesticide products regulated by the EPA (40 C.F.R. Section 160) and (b) products regulated by the FDA (21 C.F.R. Section 58).

yet adopted LIMS because of its early stage of application and its high cost (4).

Further improvements in analytical methods are possible through automation, but some constraints exist. Given that much of the automated equipment including robots has high capital costs, Federal regulatory laboratories with low or fluctuating capital budgets may have difficulty purchasing such equipment. Second, manual procedures may be faster than automated ones on a small scale, although automation may provide other benefits, e.g., reducing analyst exposure to hazardous solvents. Therefore, decisions to increase the use of automated equipment must consider the goals of monitoring programs and the moneys available. For example, if increased sample throughput were the primary goal of a monitoring program, then further advances in automation maybe necessary before its adoption.

MULTIPLE COMPONENT AUTOMATION-ROBOTICS

Robotics is a special type of automation that allows mechanical manipulation of an object in a multitask computer-assisted, and reprogrammable manner (6). In the laboratory, the robot uses systems technology to allow multiple devices to perform such simple laboratory operations as weighing, dissolving, diluting, extracting, and so forth. The laboratory robot is a mechanical extension of a computer that allows it to do physical work as well as to process data.

Laboratory robotics is based on the Laboratory Unit Operations (LUOs) concept. LUOs are individual processes that can be linked to each other by hardware and by computer software to achieve a workable, fully automated analytical procedure. Table 5-1 explains most of the LUOs that robotic systems can now perform. The most popular laboratory robotics system is produced by the Zymark Corporation and is a modular system that combines robotics, programmable computers, and peripheral instruments to carry out laboratory procedures (box 5-B). In this system, the robot itself does little work but simply moves the sample from one workstation to another *where* the various operations are performed.

Robotics in the Pesticide Residue Laboratory

The presence of automated chromatography in the laboratory now permits their overnight

Table 5.1 .—Laboratory Unit Operations (LUOs) of Robotic Systems

LUO Class	Definition	Example
Weighing	Quantitative measurement of sample mass	Direct measurement using a balance
Homogenization	Reducing sample particle size and creating a uniform sample	Sonication, homogenization, grinding, etc.
Manipulation	Physical handling of laboratory materials	Moving test tube from rack to balance, capping, uncapping
Liquid Handling	All physical handling of liquids—reagents and samples	Dispensing reagents, pipetting sample, large-volume transfer of liquids
Conditioning	Modifying and controlling the sample environment	Timing (start and stop), temperature (heat and control), atmosphere (vacuum or gas blanket), agitation (mix, stir, vortex, shake)
Measurement	Direct measurement of physical properties	pH, conductivity, absorbance, fluorescence, etc.
Separation	Coarse mechanical and precision separations	Filtration, extraction (liquid-liquid, liquid-solid), centrifugation, precipitation, distillation, recrystallization, electrophoresis
Control	Use of calculation and logical decisions in laboratory procedures	Adding calculation volume of solvent based on sample weight
Data Reduction	Conversion of raw analytical data to usable information	Peak integration, spectrum analysis, molecular weight distribution
Documentation	Creating records and files for retrieval	Notebooks, listings, computers

SOURCE: Zymark Corporation, “Laboratory Robotics Handbook,” Hopkinton, MA, 1988.

Box 5-B.—Zymate's PyTechnoIogy Robotics System

Zymate's PyTechnology concept of organizing wedge-shaped modules around the central robot is shown in fig. 5-1. In the PyTechnology robotics system, there are *48* positions available, the typical module requiring 2 to 5 positions. Each module is called a PySection and is available for such LUOs as those in table 5-I. Custom modules can also be obtained on special order, designed to meet the user's specifications. Any PySection is simply locked into position with wing nuts, and electrical connection is made at the base of the robot through premounted contacts.

Control of the robot, connected PySections, and other peripheral analytical instrumentation is accomplished through the Zymate controller, consisting of a keyboard, disk drive, and an Easy Lab Controller. The EasyLab Controller houses the central processing unit (CPU), a memory board, and module card for each laboratory station connected to the system. A second personal computer can be interfaced with the Easy Lab Controller to allow simultaneous acquisition of data and user interaction.

Analytical procedures are programmed into the system via Easy Lab Software. Using this software, the chemist programs a series of defined tasks using a "top down" approach. This program has three levels of instructions: the top-level program, the mid-level program, and the robot commands. As the chemist proceeds downward toward the robot commands level, the instructions to the controller become increasingly detailed, so that the last instruction might be something like "open fingers. " In addition to having all the software available upon delivery for immediate startup and running "real-world" analytical procedures, the software also can be custom programmed.

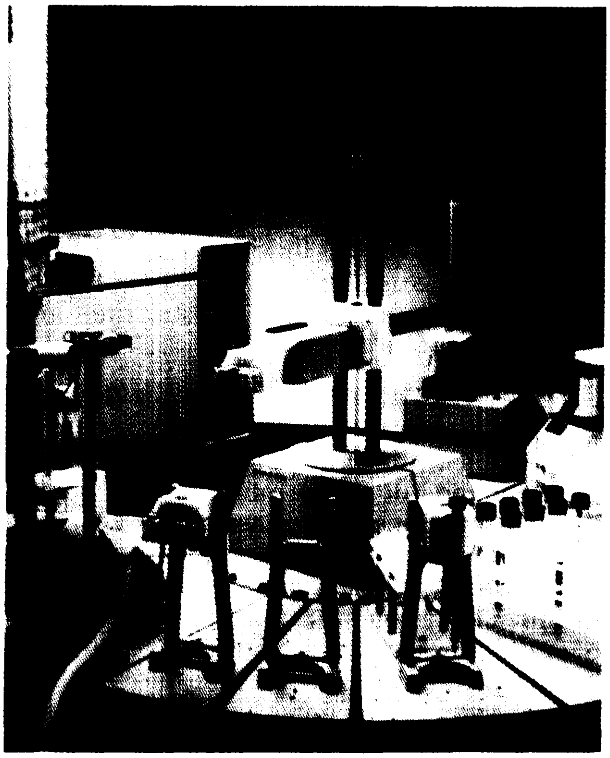


Photo credit: Zymark Corporation

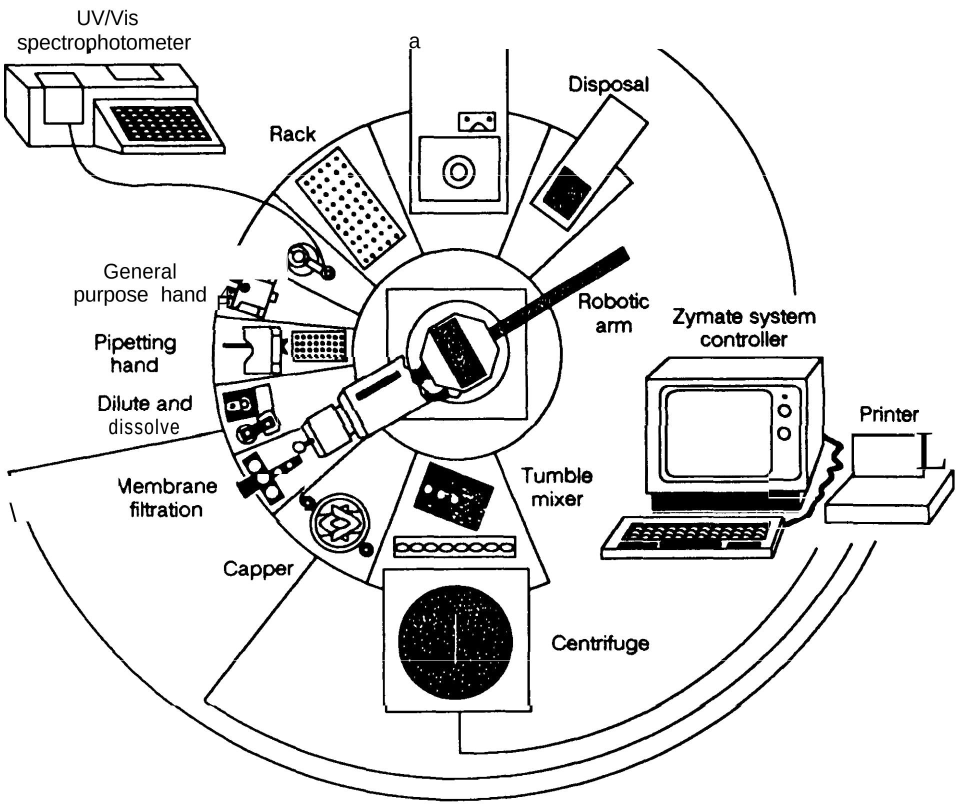
The robot acting as an arm and hand, moves the sample to various modules for different processing steps.

and weekend use; however, many analytica steps are still done manually by highly skilled technicians who perform the tasks of weighing, chopping, blending, filtering, partitioning, and evaporating. If such steps could be done with robotics, these technicians would be free to perform more creative tasks such as data interpretation and method development.

The use of robotics for routine pesticide residue analysis in foods is just beginning. Chemists at the Residues and Environmental Chemistry Section of the Plant Protection Division of Jealotts Hill Research Station in England have successfully devised a robotics system to analyze the pyrethroid insecticide Karate in apples. Portions of apples and pears are carried through weighing, extraction, partitioning, solid phase extraction (SPE) cleanup, concentration, and evaporation steps. Table 5-2 compares recovery data for apples and pears by a robot and by a human. The robot gave more consistent recoveries for all samples studied.

A robotic system for the determination of the herbicide tridiphane in rat chow has been developed as part of a toxicology study on that chemical (7). Recoveries, however, were generally lower using robotics (86.5 percent recov-

Figure 5-1.–schematic Drawing of Zymate Robotic System



SOURCE" Zymark Corporation, "Laboratory Robotics Handbook," Hopkinton, MA, 19SS

Table 5-2.—Comparison of Robot and Human Generated Data

	Robot		Human	
Sample no.	Internal standard recovery (%)	Residue (mg/kg)	Internal standard recovery (%)	Residue (mg/kg)
1	83	0.10	119	0.10
2	84	0.11	127	0.12
3	83	0.09	111	0.07
4	81	0.08	127	0.07
5	88	0.08	127	0.09

SOURCE I Laws and R Jones, "Generic Sample Preparation System for Automation of Pesticide Analysis, Advances m *Laboratory Automatic Robotics* VOl 4 (Hopkinton MA Zymark Corp 1984)

cry) than by the manual method ($93.0 percent recovery), and 4% hours were required to process 10 samples by robotics compared with 3% hours for the manual procedure. On the other hand, the robot can work 24-hour days, whereas the technician normally works only 8. Furthermore, robotic recoveries were more consistent.

A robotic system has been used to isolate a synthetic pyrethroid insecticide from extracts of soil, sediment, fish, and mussel at levels as low as 1 part per billion. Small SPE cartridges packed with Florisil were used to accomplish this, and observed recoveries averaged 85 percent or more for these sample types (I),

Neither FDA nor FSIS uses or is testing the use of robots for analyzing pesticide residues in food, although both agencies are monitoring advances in robotic technology. Health and Welfare Canada is evaluating two robots in its laboratories: one to carry out the liquid-liquid partition step in the Luke method and the other to carry out the extraction and cleanup steps on milk samples undergoing a multiresidue test for organochlorines (12). Early results show the latter robot to be equivalent in accuracy to manual preparation while doubling the weekly output of samples (12).

Two Principles for Successful Use of Robotics

In a recent survey of numerous firms that have installed four or more robotics systems, two principles were mentioned that many felt were necessary for successful incorporation of robotics in a laboratory (8):

- A single motivated and well-qualified person must be given the responsibility of seeing that a system is installed and put into operation in a productive way. That person should be given enough resources and time so that his or her efforts are not diluted with other responsibilities. It may be necessary to hire a chemist with some electronics training or experience, since robotics relies on electronic and computer technologies. Analysts could also be retrained through on-the-job short instruction that would allow persons to improve their understanding of how things are done within the framework of the modern robotics system.
- The selection of an initial application or part of a complex application should have well-understood chemistries so that rapid startup with quickly measurable productivity is realized.

Benefits and Limitations of Robotics in the Analytical Laboratory

The benefits of using robotics in a laboratory include improved test precision, morale, worker safety, and "product" quality (2). Robotics in a regulatory laboratory doing pesticide residue analyses or method development has other advantages as well. It provides exacting timing and uniform sample handling, which ensure precision and accuracy (5). Analytical methods are transportable from laboratory to laboratory, since they are stored on computer diskette and executed by instrumentation that is identical wherever they are implemented (14). Moreover, an electronic "paper trail" is left on the computer for all analytical operations performed on a sample (6, 14). (See Kropscott et al. in appendix B for additional discussion.)

Currently, attention is being focused on designing robots for methods development and the subsequent method optimization. This would lead the way toward a robot specifically designed for pesticide regulatory laboratories.

One common mistake made by those attempting to use robotics in the laboratory is to assume that robots are designed to simulate humans. Robots have a great deal of difficulty with some operations because their parts simply do not have the degree of freedom that a human hand does, for instance. They also do not move as fast nor do they have the load-carrying capacity of a human. For example, robots have trouble moving and processing large fruits or vegetables. They are unable to manipulate some laboratory glassware that is currently in vogue, such as large separator funnels or evaporative devices. They are better able to manipulate small tubes, pipettes, flasks, and similar containers.

Before robotics can be used in existing MRMs such as the Luke and the Mills-Onley-Gaither procedures, smaller samples, smaller amounts of solvent, and more sophisticated evaporative techniques must be demonstrated to be effective. when necessary, the newer cleanup techniques that reduce sample size requirements, such as SPE cartridges, need to be evaluated. Innumerable successes in the drug and petrochemical industries have demonstrated that great gains can be made in productivity when procedures can be miniaturized.

Robots have significant costs similar to costs of conventional computer systems—estimates range from $60,000 to $120,000 for the purchase of the robot and the renovation of the laboratory (5, 15). In addition to the cost, significant time must be given to adapting the robot to the needs of the laboratory and to familiarizing laboratory personnel with its use. Older model robots had startup times of 3 to 6 months (15), and the Health and Welfare Canada robot now doing milk samples took about a year to set up (11). However, startup times should decrease dramatically with improvements in the robots such as pre-programming and with increased familiarity with their use (13). Lastly, robots are subject to mechanical and electrical breakdowns and require a continuous power supply.

Robotics then should not be viewed as a cure-all for those regulatory agencies now inundated with food samples, but rather as a supplement to conventional manual techniques now being used. It is expensive to acquire, requires a new way of thinking to use it effectively, and suffers from the limitations listed above. On the other hand, it can measurably improve the overall operation of the analytical laboratory.

CHAPTER 5 REFERENCES

1. Akkari, K. H., "Robotic Residue Sample Purification by Solid-Phase Extraction, " *Fifth International Symposium on Laboratory Robotics,* Boston, MA, 1987, Abstr. In: *Advances in Laboratory Automation Robotics,* vol. 4 (Hopkinton, MA: Zymark Corp., 1988).
2. Curley, J. E., "Making Robots Fly," *Advances in Laboratory Automation Robotics,* vol. *2* (Hopkinton, MA: Zymark Corp., 1985).
3. Hopper, M. L., and Griffitt, K. R., "Evaluation of An Automated Gel Permeation Cleanup and Evaporation System for Determining Pesticide Residues in Fatty Samples," *J. of Assoc. Off. Anal. Chem.* 70(4):724-726, July/August 1987.
4. Joe, T., Chemistry Laboratory Services, California Department of Food and Agriculture, Sacramento, CA, personal communication, June 3, 1988.
5. Kropscott, B., Dow Chemical Company, Midland, MI, comment at the OTA workshop "Technologies to Detect Pesticide Residues in Food, " Mar. 15, 1988.

*6. Kropscott, B. E., Peck, C. N., and Lento, H. G., "The Role of Robotic Automation in the Laboratory, " OTA commissioned paper, Spring 1988.

7. Kropscott, B. E., and Dittenhafer, M. L., "Automated Feed Analysis—an Application for Toxicology, " *Advances in Laboratory Automation Robotics,* vol. 1 (Hopkinton, MA: Zymark Corp., 1984).
8. Laboratory Robotics Handbook (Hopkinton, MA: Zymark Corp., 1988),
9. Luke, M., FDA Pesticide Laboratory, Los Angeles, CA, personal communication, Apr. 26, 1988.
10. McCullough, F., ABC Laboratories, Columbia, MO, personal communication, May 3, 1988.
11. Newsome, W. H., Health Protection Branch, Health and Welfare Canada, personal communication, May 6, 1988.

*12. Newsome, W. H., and Graham, G. F., "Pesticide Residue Monitoring in Canada," OTA commissioned paper, Spring 1988.

13. Paul, A., Zymark Corporation, Hopkinton, MA, comment at the OTA workshop "Technologies to Detect Pesticide Residues in Food, " Mar. 15, 1988.
14. Paul, A., Zymark Corporation, Hopkinton, MA, personal communication, May 10, 1988.
15. Peck, C., Dow Chemical Company, Midland, MI, comment at the OTA workshop "Technologies to Detect Pesticide Residues in Food, " Mar. 15, 1988.
16. Tindle, R. C., and Stallings, D, L., "Apparatus for Automated Gel Permeation Cleanup for Pesticide Residue Analysis," *Analytical chemistry* 44(11):1768-1774, September 1972.

*These reference papers are contained in appendix B.