한국환경위생학회지(1993), 제19권 제1호
Kor. J. Env. Hlth. Soc. (1993), Vol. 19, No. 1 pp. 90~93

綜 說

Analysis of Pesticide Contaminants in Food

Jong-Gyu Kim
Department of Public Health, Keimyung University, Taegu 704-701, Korea

식품 중 잔류농약의 분석

김 종 규
계명대학교 자연과학대학 공중보건학과

Increasing Public Concern

Pesticide residue is probably one of the fastest growing problems in regard to environmental contamination. Pesticide use in agriculture in this century has produced certain benefits, including a decrease in crop waste and an increase in crop yields and food quality. However, pesticide use also creates problems of having effects on the environment and remaining in food chain. The presence of pesticide residue in food, water, and soil has aroused public concern over potential health hazards. Despite information provided by national and private level agencies suggesting that food is safe, consumer groups worldwide are demanding assurance as to the safely of agricultural products.

Pesticide Analysis

1. Accuracy is important

In order to check levels of pesticides, food processors and regulatory agenicies utilize a variety of analytical methods. Because pesticide residues are normally found at low levels, assay procedures must be highly accurate and sensitive to ensure that all pesticides, metabolites, and breakdown products are detected.

Such procedures fit into one of two categories; single-residue analysis and multi-residue analysis. The single-residue methods (SRMs) measure a single pesticide and often its metabolites and are used less frequently and viewed as less desirable than multi-residue methods (MRMs). MRMs are preferred because they help us minimize the cost of assessing food safety, and environmental impact.

2. Sample prepartion

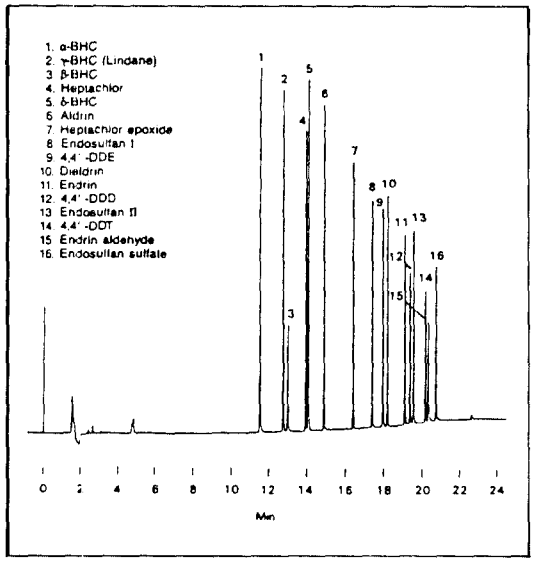
Analytical methodology has been developed for extraction of pesticide residues in a variety of food samples using liquid-liquid partition and column chromatography prior to qualitative and quantitative analysis by gas chromatography (GC) or high-performance liquid chromatography (HPLC). Cleanup techniques have been focused on he separation of pesticides from lipids which interfere analysis. The ordinary cleanup metods that have been adopted, such as the Association of Official Analytical Chemists (AOAC) methods and the Japanese official methods, are based on acetonitrile-hexane partition or dichloromethane extraction and adsorption chromatography. Although the majority of the lipids are removed by the partition technique, the lipid removal remains a problem.

This sample cleanup approach is also time consuming and often results in losses of compounds. This problem should be overcome by developing alternatives to these laborious sample preparations.

3. Separation and quantitation

Several types of chromatography have been uti-

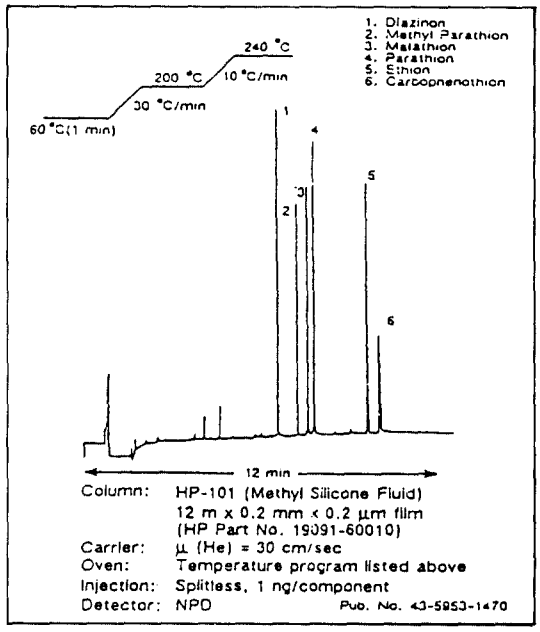
* 이 내용은 한국식품과학회 주최 위생분과위원회 워크샵(1993. 2. 19)에서 발표되었음.



SPB-608 fused silica capillary column, 30m × 0.25mm ID, Film Thickness: 0.25μm, Col. Temp.: hold 4 min at 150°C, then to 290°C at 8°C/min. and hold 5 min., Linear Velocity: 25 cm/sec. He, set at 290°C (a halogenated gas such as chloromethane or chlorotrifluoromethane is used to determine linear velocity by ECD), Make-up Gas: N_2, 60ml/min., Det.: ECD, Range: 10^{-11}, Atten.: ×16, Sample: 0.8μl decane containing 160pg each pesticide, splitless mode, 45 sec. hold time.

Source : Supelco

Fig. 1. 16 chlorinated pesticides separate in 20 minutes.



Source : Hewlett-Packard

Fig. 2. Organophosphorus pesticides.

lized for the determination of pesticides, including thin-layer chromatography (TLC) for qualitative screening, and GC and HPLC for quantitative analysis.

Historically, most methods for pesticides have utilized GC. Compound-specific detectors such as electron capture (organochlorines) and nitrogen-phosphorus (organophosphates) afforded sensitivity and specificity for the typical pesticides of the 1960s and 1970s. The new pesticides of the 1980s and 1990s, however, are less amenable to GC.

The advantage of HPLC is its ability to separate a wide polarity range of compounds at room temperature. Its weakness has been the lack of specific and sufficiently sensitive detectors for pesticides, though adaptation of post-column reactions with highly sensitive fluorescence detectors is supporting the proliferation of HPLC methods for pesticide residue.

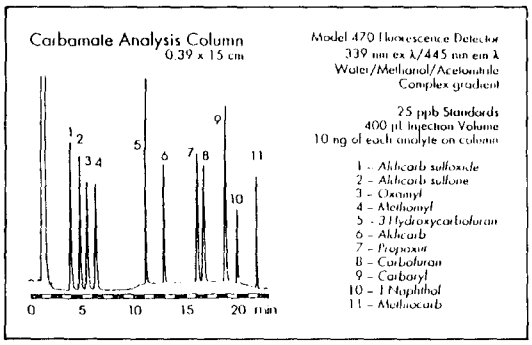
One method that has been approved for regulatory use is the method for N-methylcarbamates. N-methylcarbamates are insecticides with widespread use and varying degrees of mammalian toxicity. The method is a first-action official method of (AOAC) and is based on a method developed by Richard Krause. A chromatogram of the N-methylcarbamates in the MRM is shown in Fig. 3.

Crisis : The Sampling Bottleneck

1. Sample cleanup

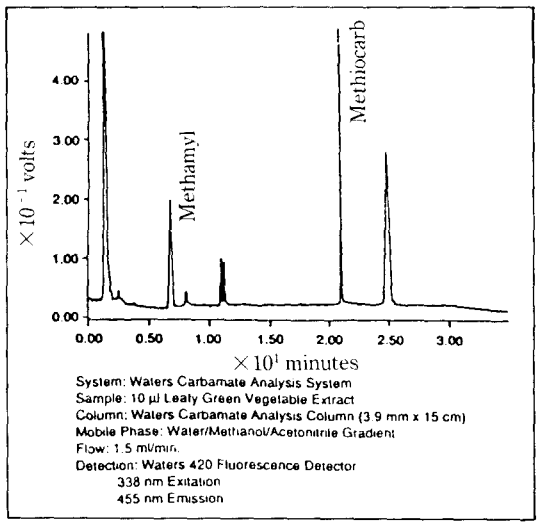
One limitation of any residue analysis from food is the time required for sample preparation. Analytical methods for pesticides usually require costly high-purity halogenated solvents and time-consuming liquid-liquid extractions and evaporation steps. Also halogenated solvents used in the procedures need to be disposed of in an environmentally acceptable manner. Methods that can result in shorter analytical procedures and lower reagent consumption without compromising quantitative requirements would be desirable.

Faced with too many samples, scientists and researchers are convinced that faster preparation and analytical methods are needed. Although



Source : Millipore Corporation

Fig. 3. LC separation of 25 ppb standards.



Source : Millipore Corporation

Fig. 4. HPLC carbamate pesticide analysis.

GC/MS and HPLC methods are faster today than ever before, even faster analytical methods are needed.

2. Faster sample cleanup

Solid-phase extraction is seen as a way to reduce analysis time, solvent consumption and overall costs in pesticide analysis. Fig. 4 depicts a vegetable simply extracted in acetonitrile and salt water, dried, reconstituted in methanol and passed across a C_{18} Sep-pak cartridge. The procedure is rapid and can be adapted to HPLC or GC analysis.

3. State of the art : Rapid and direct field testing

The function of a sample preparation is to reduce the range of components in the mixture to a manageable number, so that the analytical method and detector have a chance for accuracy. Detection specificity has been a key factor in the successful history of GC for pesticide analysis, but it has become less useful as newly developed pesticides exhibit greater thermal lability due to pesticide manufacturers recongnizing that degradation in the environment is a key to safety. The ideal analytical method would be less instrument-intensive and have a detection mechanism sufficiently specific that any pre-analysis preparations would be limited to simple dissolution and filtration.

Immunoassay methods, developed initially for used in high volume clinical chemistry labs like those found in hospitals, are ideal candidates for use in crop screening for pesticides. Much like human fluids and tissues, plants and fruits are highly complex mixtures that contain trace chemicals that must be monitored. The situation is much analogous to therapeutic drug monitoring in hospitals. Millipore has developed such methods for environmental use.

In practical use, the sample solution is introduced into a tube which was previously coated with an immobilized enzyme that is specific in its reaction to the target compounds. The enzyme remains immobilized in the container after the sample solution is discarded, and a second reagent is added which will react with available (not bound with target compounds) enzyme to form a colored solution. The solution is read manually by comparison to color charts or is read in a spectrophotometer. Greater color intensity is equivalent to low target compound presence, conversely the absence of color indicates a high concentration of the target compounds. In biochemical terms, the target compound competes with the enzyme conjugate for immobilized antibody sites. Some commercially available kits containing highly specific antibodies to pesticides have been introduced and are a possible tool for screening samples.

Determination of pesticide residue by the combination of solid phase extraction, immunoassay kits, GC, and LC are the current state-of-the art in pesticide analysis.

Future Prospects

GC will remain the leader in pesticide residue analysis. However, the current generation of pesticides are readily biodegradable and thermally labile compound, the type of analytes more amenable to HPLC. Developments in HPLC technology, including more selective and sensitive detectors will help the present problems. Capillary electrophoresis (CE), a rapidly emerging technology, is already in use in pesticide research laboratories and may have a place in the residue labs as well. Solid phase extraction with highly reproducible sample recovery and rapid analytical tests by immunoassay will displace some current procedures.

References

1) Official Methods of Analysis, 12th ed., AOAC, Arlington, VA. Chapter 29, 1975.
2) Aoki, Y., Takeda, M. and Uchiyama, M., *J. Assoc. Off. Anal. Chem.* **58**, 1286-1293 (1975).
3) Official Methods of Analysis, 14th ed., First supplement, AOAC Arlington, VA. 1985.
4) Krause, R. T. and August, E. M., *J. Assoc. Off. Anal. Chem.* **66**(2), 234-240 (1983).
5) Ting, K. C. *et al., Bull. Environ. Contam. Toxico.* **33**, 538-547 (1984).
6) Bogus, E. R. *et al., J. Agric. Food Chem.* **33**, 1018-1021 (1985).
7) Frost, B. A., Environmental Lab Magazine, Waters. 1989.
8) Tuinstra, L. G. M. Th, Traag, W. A. and Van Munsteren, A. J., *J. Chromatogr.* **395**, 307-315 (1987).
9) Adachi, K., Ohokuni, N. and Mitsuhashi, T., *J. Assoc. Off. Anal. Chem.* **67**(4), 798-800 (1984).
10) Thurman, E. M. *et al. Anal. Chem.* **62**, 2043-2048 (1990).
11) Bushway, R. J. *et al. J. Assoc. Off. Anal. Chem.* **75**(2), 323-327 (1991).