To the Graduate Council:

I am submitting herewith a thesis written by Min Huang entitled " Simultaneous Determination of Multi Pesticide Residues in Vegetables". I have examined the final paper cope of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Sharon L. Melton

Sharon L. Melton, Major Professor

We have read this thesis
and recommend its acceptance:

John R Mount

John R. Mount, Professor

Arnold M Saxton

Arnold M. Saxton, Professor

Accepted for the Council:

Vice Provost and Dean of Graduate Studies

SIMULTANEOUS DETERMINATION OF MULTI PESTICIDE RESIDUES IN VEGETABLES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Min Huang

May 2002

DEDICATION

This thesis is dedicated to my parents

Mr. Chao-dun Huang

and

Mrs. Zhen-rong Pu

who have been giving me unlimited love

and invaluable encouragement.

ACKNOWLEGEMENTS

I would like to express my deepest gratitude to my advisor, Dr. Sharon L. "Momma" Melton, professor of Food Science and Technology, for her guidance, patience, encouragement, and friendship throughout this study. I acknowledge my great indebtedness to her for her invaluable suggestions and constructive criticisms during the course of the present investigation. Not only did she take great pains, but also gave skillful guidance throughout the tenure of the whole project.

I would also like to express my appreciation for my committee members, Dr. John R. Mount and Dr. Arnold M. Saxton, for their advice and assistance.

Special thanks are extended to Bush Brothers & Company (Dandridge, TN) for providing the graduate research assistantship and funding this project. I would especially like to thank Kay Trigiano and Teri Ingle for their friendship and valuable assistance.

Last, but not the least, my sincere appreciation are extended to my husband, Ge Zhang, my lovely daughter, Tracy Xinyi Zhang, and my mother-in-law, Ji-qin Qi, who did not mind my indisposition to give them all the attention they deserve. Their strong support and understanding made me courageous to complete the present study.

ABSTRACT

The objectives of this experiment were to determine the accuracy (percentage recovery or PR) and precision (coefficient of variation or CV) of three different cleanup methods for the measurement of seven pesticides in different vegetables (turnip greens, dried beans and cabbage) using selective ion monitoring (SIM) on a gas chromatograph-mass spectrometer (GC-MS). The cleanup methods for an acetonitrile extract of the vegetables included (1) a Florisil solid phase extraction (SPE), (2) a double SPE using C_{18} and amino propyl cartridges, and (3) a direct injection of the extract. The pesticides included ethalfluralin, bromoxynil, dimethoate, alachlor, bentazon, pendimethalin and esfenvalerate. The limits of detection for each pesticide in the acetonitrile extract of turnip greens also were determined.

Three of the pesticides, ethalfluralin, pendimethalin and esfenvalerate, were recovered at measurable amounts by methods 1 and 3, while dimethoate was recovered by methods 2 and 3, and alachlor was recovered by all methods in all vegetables. Ethalflluralin, alachlor, pendimethalin and esfenvalerate, were recovered at high enough percentages in method 1 to be measured in each vegetable, but method 2 should be used for quantitation of dimethoate. Although bromoxynil and bentazon could be measured by GC-MS SIM, neither one was recovered by any of the 3 methods from the acetonitrile extracts.

The CVs for the four pesticides in method 1 and for dimethoate in method 2 ranged from 5.6 to 38.4% and, overall, were of similar magnitudes as those reported by other researchers. The limits of detection of the pesticides in the turnip greens

acetonitrile extract matrix ranged from 0.00075 (alachlor) to 0.19 ppm (dimethoate) and were, with the exception of dimethoate, lower than the limits of detection reported by others using SIM.

TABLE OF CONTENTS

LIST OF TABLES

TABLES **PAGE**

CHAPTER I

INTRODUCTION

Nutrition educators are encouraging consumers to increase the consumption of the fresh fruits and vegetables directly as a source of fiber, nutrition and anti-carcinogens. However, the health concern about the effects of pesticide residues has created fear about consuming fresh products, especially if they are treated with pesticides (Dittus et al., 1993). Therefore, rapid and accurate methods for detecting pesticide residues in fresh products are necessary to monitor the usage of pesticide and provide a pesticide low-risk or risk-free fresh market.

Traditionally, multiresidue methods (MRMs) use organic solvents, such as acetone, acetonitrile or ethyl acetate for extraction of pesticides from the food products. Since these methods provide high recoveries of pesticides with a wide range of polarity, the Association of Analytical Chemists (AOAC) has recommended these methods for several decades, and they are still used widely as standard methods with some improvements made over time (Lehotay, 2000). For more than 5 years, the Food Science and Technology Department at The University of Tennessee (FST-UT) successfully analyzed fresh vegetable samples from Bush Brothers & Company (Dandridge, TN) for selected pesticides using a modified AOAC (1990) method proposed by Yang (1993) and an improved method reported by Shao (1997).

With each pesticide method modification made at FST-UT, new pesticides were added and old pesticides were removed from the MRMs to reflect the changes in pesticides used to produce Bush Brothers & Company vegetables. Currently, pesticides other than those analyzed are used to produce vegetables processed by Bush Brothers & Company. Seven such pesticides, listed in Table 1, are used currently in the production of Bush Brothers & Company vegetables. No research reports were found in which all of the listed pesticides were analyzed together by one method. In order to extend the number of pesticides that can be monitored by the improved MRMs used by FST-UT now, the reliability of such MRMs for detecting the seven pesticides listed in Table 1 needs to be determined.

Although the current MRM used by FST-UT is reliable and accurate for specific pesticides (method 1 in Table 2), there is still a problem determining very polar pesticides, such as naled, carbofuran, fenamiphos, and azinphosmethyl (Shao, 1997). Several of the pesticides in Table 1 are very polar, such as bentazon, bromoxynil, and dimethoate. A new MRM for determination of 251 pesticide residues in fruits and vegetables was reported by Fillion et al. (2000), which included acetonitrile extraction and double solid-phase extraction (SPE) cleanup steps using a C_{18} and an amino propyl cartridges after extraction. Four of the pesticides listed in Table 1 (alachlor, dimethoate, ethalfluralin and pendimethalin) were included in the 251 pesticides analyzed by Fillion et al. (2000). Before applying this new MRM to routine multiresidue analysis by FST-UT, the reliability of this double SPE cleanup method was determined for all the pesticides in Table 1.

Even though the new MRM reported by Fillion et al. (2000) provides high

Table 1-- Pesticides used for production of Bush Brothers & Company vegetables during the year 2000-2001.

Compound	Type	Polarity
Alachlor	Herbicide	Non-polar
Bentazon	Herbicide	Polar
Bromoxynil	Herbicide	High Polar
Dimethoate	Insecticide	Polar
Esfenvalerate	Insecticide	Non-polar
Ethalfluralin	Insecticide	Non-polar
Pendimethalin	Herbicide	Non-polar

Table 2 -- Least squares means[ab] for percent recoveries (PR) and coefficients of variation (CV) for 11 insecticides from different vegetables and different cleanup methods (Shao, 1997).

Compound	Method [c]	PR (%) in vegetable			CV (%)	Possible reasons for too low/high PR
		Corn	Beans	Spinach		
Naled	1	7	3	5	9.2~13.1	Rapid hydrolysis
	2	5	2	4	8.1~13.4	
Phorate	1	65	85	69	3.2~8.9	
	2	61	143	103	7.1~10.1	
Carbofuran	1	3	8	3	6.7~14.9	High polarity, thermal
	2	45	338	265	1.1~9.4	Interference
Dyfonate	1	84	102	107	7.4~14.2	
	2	98	181	238	1.3~12.7	Pigments or lipids
Disulfoton	1	23	46	34	5.1~7.9	Not elute with EE/PE
	2	67	109	54	7.3~7.6	
Methyl-	1	37	87	33	9.3~11.2	Bound some components
parathion	2	47	306	318	2.9~13.0	Interference
Malathion	1	55	79	41	4.8~13.0	
	2	114	253	228	4.0~4.9	Interference
Chlorpyrifos	1	164	139	137	2.2~6.3	
	2	106	203	201	2.5~9.0	Interference
Fenamiphos	1	1	8	9	2.5~9.0	High polarity
	2	28	245	226	5.6~9.8	Interference
Azinphos-	1	3	16	2	4.8~8.1	High polarity, high BP[d]
methyl	2	30	345	152	8.7~9.4	

[a] n=3.
[b] $p<0.05$.
[c] 1= Acetonitrile extract with Florisil cleanup; 2= Ethyl acetate-acetone extract with reverse phase cleanup.
[d] BP= boiling point.

recoveries for most pesticides, several post-extraction cleanup steps were required before gas chromatographic analysis. Undoubtedly, the possibility of introducing a sample directly into the gas chromatograph (GC), without additional cleanup steps, would significantly reduce the time needed to complete the analysis and, perhaps, enhance of its percentage recovery and sensitivity for each pesticide listed in Table 1. However, the reliability of a direct injection for the pesticides listed in Table 1 needed to be determined.

I. OBJECTIVES

Therefore, the objectives of this research are as follows:

1. To determine the reliability (accuracy and precision) of the current FST-UT extraction and cleanup method (Yang, 1993) for the seven pesticides listed in Table 1 in selected vegetables (turnip greens, dried beans, and cabbage).
2. To determine the reliability (accuracy and precision) of a modified double SPE cleanup method (Fillion et al., 2000) for each pesticide listed in Table 1 in acetonitrile extracts of the previously listed vegetables.
3. To evaluate the reliability of direct injection of acetonitrile extract without additional cleanup for the analysis of each pesticide in Table 1.
4. To compare the reliability (accuracy and precision) of the two cleanup methods and the direct injection method for each pesticide listed in Table 1 for each vegetable.
5. To determine the sensitivity of the method of analysis for these pesticides in an extract of a vegetable without cleanup procedures.

CHAPTER II

LITERATURE REVIEW

I. PESTICIDES APPLICATION AND HUMAN IMPACT

Pesticides are chemical substances used to kill or control pests for maximum yield of high quality fruits and vegetables at minimum cost to both growers and consumers and with minimum health risk to humans. There are three major types of pesticides: (1) herbicides, for killing weeds or inhibiting plant growth, (2) insecticides, for preventing or destroying any insects that may destroy crops or gardens, and (3) fungicides, being used to destroy or inhibit fungi which usually cause plant diseases. Pesticides are used mainly for three purposes: (1) agriculture, (2) industry, commercial establishments, and government, and (3) home and garden. Over two decades, more than two thirds of the amount of pesticides used have been in the agriculture sector (Kamrin, 1997).

The earliest record of pesticides being used was about 1000 B.C.. The world's largest market for pesticides is the United States (Ware, 1989). The use of pesticides provides a plentiful and widely available food supply in the U. S. However, the use of pesticides in food affects consumers' concerns about food safety and environmental contamination. Pesticide residues in food was the number one factor impacting confidence level of consumers about food safety (Scroggins, 1991). The widespread use and disposal of pesticides by farmers, institutions, and the general public provide many

possible sources of pesticides in the environment. The release of pesticides into the environment may be followed by a very complex series of events that can transport the pesticide through the air or water, into the ground, or even into living organisms. The most important route of distribution or the extent of distribution is different for each pesticide. The distribution depends on the formulation of the pesticide (what it is combined with) and how and when it is released. However, significant gaps remain in the knowledge of pesticide movement and its fate in the environment. Therefore it is best to minimize unnecessary release of pesticides into the environment. The lower the level of pesticides released, the safer our environment will be.

Although most of the food we consume may contain low levels of pesticide residues, the food supply of U. S. is the one of safest in the world. In the U. S., pesticides are regulated by a myriad of laws and agencies to protect the public from unreasonable risks. No less than 14 different Federal Acts control the aspects of the manufacture, registration, distribution, use, consumption, and disposal of pesticides (Kamrin, 1997). The bulk of pesticide regulation falls under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This legislation governs the registration, distribution, sale, and use of pesticides. The Environmental Protection Agency (EPA) is responsible for the administration of this Act and for establishing rules and regulations consistent with the Act's intent. The EPA is responsible under FIFRA for registering new pesticides to ensure that, when used according to label direction, they will not pose unreasonable risks to human health or the environment. The EPA regulates the safety of the food supply by setting tolerance levels, or maximum legal limits, for pesticides on food commodities and in animal feed available for sale in the U. S. The purpose of the tolerance program is to

ensure that consumers are not exposed to unsafe levels of pesticide residues in food. To evaluate the risks posed by pesticides in the diet, the EPA follows standard risk assessment guidelines. The Agency uses different procedures for cancer risks and non-cancer risks. Under the new Food Quality Protection Act these tolerances must result in a reasonable certainty of no harm from aggregate exposure to the pesticide. In addition, this Act requires that safety for infants and children be explicitly addressed in tolerance setting and also that the EPA develop and implement a screening program for pesticides that may be endocrine disruptors (Kamrin, 1997). Pesticide tolerances, set by EPA, are enforced by the Food and Drug Administration (FDA), which monitors all domestically produced and imported foods traveling in interstate commerce except meat, poultry, and some egg products. The FDA conducts a Total Diet Study also known as a Market Basket Study, which measures the average American consumer's daily intake of pesticide residues from foods that are bought in typical supermarkets and grocery stores, and prepared or cooked as they would be in a household setting. The findings of the ongoing Total Diet Study show that dietary levels of most pesticides are less than 1% of the RfD (Reference Dose, once known as the Acceptable Daily Intake). The USDA's Food Safety and Inspection Service (FSIS) conducts monitoring meat and poultry products. Each year, the FSIS conducts 10,000 to 20,000 pesticide residue analyses. Currently, less than 1% of these tests show illegal residues, and the violation rate has been declining steadily over the last 2 decades (Kamrin, 1997).

Historically, environmental contamination by pesticides has been subject to less regulatory scrutiny than food supply contamination. Until recently little regulatory emphasis was placed on the monitoring and establishment of specific levels of pesticides

in the environment once a pesticide was registered for use. Requirements for monitoring are found in the Endangered Species Act, in the discharge limits for point source under the Clean Water Act, and in the Maximum Contaminant Levels (MCLs) in drinking water from both surface and groundwater sources.

II. CHARACTERISTICS OF THE SEVEN PESTICIDES TO BE ANALYZED

Bush Brothers & Company has a yearly contract with The University of Tennessee Department of Food Science and Technology for pesticide analysis in vegetables they process. Specific pesticides are used to produce these vegetables, and the pesticides used change with time as more and more pesticides are banned in the U.S. With these changes in pesticides used, it is necessary to develop and adapt analytical procedures to analyze as many of the new pesticides in a multiple residue method (MRM) as possible. It is helpful to know the chemical formulation and characteristics, as well as the stability, of these new pesticides. This following information about new pesticides was reported by Chem Service, Inc. on the *Material Safety Data Sheet* for each pesticide and by Kamrin (1997) in his book *Pesticide Profiles*.

Alachlor ($C_{14}H_{20}ClNO_2$; MW=269.8) is a selective systemic herbicide, absorbed by germinating shoots and by roots. Technical grade alachlor is a white crystalline solid. It is slightly soluble in and breaks down rapidly in water. It is incompatible with strong bases and acids and sensitive to heat. The Oral LD50 (rat) for alachlor is 1200 mg/kg. Alachlor can be used to control annual grasses and broadleaf weeds in field corn,

soybeans and peanuts. This compound is one of the most highly used herbicides in the U.S. with over 50 million pounds applied in 1990.

Bentazon ($C_{10}H_{12}N_2O_3S$; MW=240.3) is a post-emergence and contact herbicide. Technical grade bentazon is a colorless crystalline solid. It is slightly soluble in water. The Oral LD_{50} (rat) for bentazon is 1100 mg/kg. Bentazon is used to selectively control broadleaf weeds and sedges in beans, rice corn, peanuts, mints and others.

Bromoxynil ($C_7H_3Br_2NO$; MW=276.93) is a post-emergence herbicide which controls annual broadleaf weeds. Technical grade bromoxynil is a colorless crystalline solid and odorless. It is very slightly soluble in water. The Oral LD_{50} (rat) for bromoxynil is 190 mg/kg. Bromoxynil is used effectively to control the weeds in cereal crops, corn, sorghum, onions, flax, mint, turf and onion croplands.

Dimethoate ($C_{14}H_{18}N_4O_3$; MW=290.36) is a systemic organophosphate insecticide used to kill mites and insects on contact. Technical grade dimethoate is a colorless crystalline solid with stench odor. It is slightly soluble in water and decomposes under alkaline conditions. It is sensitive to light and heat. The Oral LD_{50} (rat) for dimethoate is 215 mg/kg. Dimethoate is used against a wide range of insects.

Esfenvalerate ($C_{25}H_{22}ClNO_3$; MW=419.93) is an insecticide used on vegetable crops, tree fruits and nut crops. Technical grade esfenvalerate is a colorless crystalline solid. It decomposes under alkaline conditions. The Oral LD_{50} (rat) for esfenvalerate is 75 mg/kg. Esfenvalerate may be mixed with a wide variety of other types of pesticides.

Ethalfluralin ($C_{13}H_{14}F_3N_3O_4$; MW=333.30) is a yellow to orange crystalline solid of technical grade. It is insoluble in water and sensitive to light. The Oral LD_{50} (rat) for ethalfluralin was not found.

Pendimethalin ($C_{13}H_{19}N_3O_4$; MW=281.35) is a selective pre-emergence and early post-emergence herbicide. Technical grade pendimethalin is a yellow to orange crystalline solid. It is insoluble in water. The Oral LD_{50} (rat) for pendimethalin is 1050 mg/kg. Pendimethalin is used to control most annual grasses and certain broadleaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts, and sunflower.

III. GENERAL PESTICIDE ANALYSIS

Official Pesticide Analysis

General pesticide analysis methods include "multiresidue methods" (MRMs) by which a number of pesticides can be analyzed simultaneously, and "single residue method" (SRM) which only analyze a single pesticide.

Currently, the FDA and USDA use the five most common MRMs as the official pesticide monitoring (PAM, 1994). The Mills method (Mills, 1959), MOG method (Mills et al., 1963), Storherr method (Storherr et al., 1971), Luke method (Luke et al., 1975; Luke et al., 1981), and Krause method (Krause, 1980) can detect over 300 pesticides and related compounds. Each of them is also included in the AOAC (1990) *Official Methods of Analysis.*

The general MRMs have basic five steps: sampling, extraction, cleanup, separation and detection. These steps include using acetone or acetonitrile as the extraction solvents, using liquid-liquid partition (LLP) and chromatographic separation techniques to remove the interfering compounds, and finally employing gas

chromatography (GC) or high-performance liquid chromatography (HPLC) with selected detectors to identify pesticides.

Determination by Gas Chromatography/ Mass Spectrometer

All the MRMs used in the United States today involve either GC or HPLC as the determinative step. GC analysis is the best analytical tool for the simultaneous determination of the most pesticides. In GC analysis, the sample is injected and vaporized before it is carried into a column where the various components are separated into individual components before eluting into the detector. GC is normally used for samples that are volatile and thermally stable. The classical packed column allowed analysis of around 20 pesticides simultaneously. With the development of capillary columns, capillary GC (cGC) has become an important tool for pesticide analysis. Capillary column can analyze over 200 pesticides at one time. The advantages of capillary column over packed column also include higher resolving power, lower retention times, lower bleed, longer life and greater reproducibility (Yang, 1993). Capillary column has replaced the packed column gradually during the last 20 to 30 years.

The detectors used in GC analysis affect the results. The use of selective detectors in GC reduces the amount of cleanup necessary for the removal of interfering co-extracted components in the analysis. Detectors with a high degree of selectivity used in conjunction with GC for analysis pesticide are the electron capture detector (ECD) for residues containing electronegative containing groups, the nitrogen-phosphorus detector (NPD) for organic P and N compounds, the Hall electrolytic conductivity detector

(HECD) for organic S, Cl and N compounds, and the alkali flame ionization detector (AFID) (OTA, 1988). The disadvantages of these detectors are that separate detection systems are required for better identification and quantitation of different pesticides. Secondly, compounds with the same retention time as a pesticide may be identified as that pesticide (false positive results). Thirdly, they are restricted because of the relatively narrow range of analyses that each can detect (Parrilla and Vidal, 1996; Jing and Amirav, 1997). In multiclass, multiresidue methods, several GC injectors are often required when these selective detectors are used to determine the gamut of GC-amenable pesticides. Furthermore, cleanup of extracts is necessary for improving the ruggedness and reliability of the GC system. Nonvolatile matrix components build up in the injection liner and capillary column and reduce performance of the system until maintenance is performed.

The use of mass spectrometer (MS) in a universally selective detection approach with GC analysis permits the detection and confirmation of a wide range of compounds. The combination of MS with GC, so called GC-MS, was introduced by Holmes and Morrell (1957) and Gohlke (1959), and has become one of the most powerful techniques for identifying compounds. GC-MS can overcome the problems mentioned before and is quite often used by regulatory agencies to determine pesticide residues in food (Luke et al., 1988). But at the same time, cleanup of complex extracts is still important to avoid contamination of the injection liner, capillary column and MS source with matrix components.

The analysis of samples using mass spectrometric detection is dependent on successful ionization. Electron impact (EI) and the chemical ionization (CI) are the most common ionization methods in MS (Mattern and Rosen, 1992). In EI, high energy

electrons bombard gaseous pesticide molecules causing them to become unstable and to degrade into ions of various mass/charge (M/z) ratios that give structural information characteristic for that compound (Mattern and Rosen, 1992). In CI, ionization is achieved by the reaction of the sample with ionized gas such as methane or isobutene (Mattern and Rosen, 1992). Although GC-MS with electron impact ionization (GC-EIMS) is the most widely used technique in pesticide analysis (Torres et al., 1996), it may be limited by its sensitivity. However, selective ion monitoring (SIM), one of the GC-EIMS modes, can greatly improve the sensitivity problem (Stan, 1989).

Pesticide Residues Analysis with Selective Ion Monitoring

The mass spectrometer can be used in two different modes, cyclic scanning and selective ion monitoring (SIM). In cyclic scanning, the mass spectrometer acquires a series of mass spectra over a wide range of M/z ratios (i.e., from 35 to 500) continuously during the GC separation process. In SIM, the MS detects and measures only a few ions of selected M/z during the GC run, but MS sensitivity is increased over that for cyclic scanning. The M/z of the ions measured in SIM are selected to be as specific for a given pesticide as possible. These ions often are the most abundant ions formed during electronic ionization of the pesticide by the MS. The combined data from SIM and the retention time provide the optimum in detection sensitivity and reliability with respect to the identification and analytical measurement of the pesticide residue that can be achieved. Since 1980's, SIM has become possible for many more analysts through the availability of cheaper GC-MS instruments with quadrupole or ion trap detectors (Stan, 1989).

During the last 10 years, GC-EIMS with SIM method has been used for the measurement of pesticide residues in MRMs more frequently. Stan (1989) used GC-EIMS with SIM to analysis 18 pesticides in green peppers with sensitivities at parts per billion (ppb) levels. In 1990, Benfenati et al. used SIM with GC-EIMS for simultaneous analysis of 50 pesticides in water samples with sensitivities below sub-ppb levels. Liao et al. (1991) used GC-EIMS with SIM mode to analyze 143 pesticides in crop samples with good recovery on all pesticides. Miyahara et al. (1994) used GC-EIMS with SIM to analyze pesticide residues in 9 crop samples. Recently, Fillion et al. (2000) reported the analysis of 250 pesticides in fresh vegetable samples using GC-EIMS. Even the N-methylcarbamate pesticides, which are usually investigated by a HPLC method, can also be determined by GC-EIMS with SIM mode. In addition to identification and confirmation, the SIM mode with GC-MS can be applied to quantification. The Food Science and Technology Department at the University of Tennessee has used the GC-EIMS with SIM mode to analyze pesticide residues in vegetable products from Bush Brothers & Company over 15 years with good results (Melton, 1996).

IV. METHOD DEVELOPMENT FOR PESTICIDE ANALYSIS

Improved Extraction and Cleanup

Traditionally, the MRMs use organic solvents, such as acetone, acetonitrile or ethyl acetate of extraction the food products. Since these methods can provide high recoveries of pesticides over a wide range of polarity, the Association of Analytical Chemists (AOAC) has recommended these methods for decades. They are still used

widely as a standard method with improvements made over time (Lehotay, 2000). A multiresidue method was described for the determination of 251 pesticide and degradation product residues by gas chromatographic analysis in various fruit and vegetable samples (Fillion et al., 2000). Extraction of the sample with acetonitrile is followed by a salting-out step. Then co-extractives are removed by passing a portion of the acetonitrile extract through an octadecyl (C_{18}) solid-phase extraction (SPE) cleanup cartridge and then, in a second cleanup, through a carbon cartridge coupled to an amino propyl cartridge. Determination is by GC-MS in the selected-ion monitoring mode, or by LC with post-column reaction and fluorescence detector (FD). Limits of detection range between 0.02 and 1.0 mg/kg for most compounds. Over 80% of the compounds have a limit of detection of < 0.04 mg/kg. Even though the multiresidue method can provide high recoveries for most pesticides, several post-extraction cleanup steps are generally required before gas chromatographic analysis. These cleanup techniques, which often include solid-phase extraction (SPE), liquid-liquid partitioning, and/or gel permeation chromatography (GPC), make up the bulk of the cost, time, and labor in analytical methods (Lehotay, 2000).

In recent years, supercritical fluid extraction (SFE) has been found to be a viable alternative to organic solvents in the extraction of pesticide residues from non-fatty foods. Valverde-Garcia et al. (1996) extracted pesticide-spiked tomato and pepper samples with supercritical carbon dioxide assessing different extraction efficiencies. Under most extraction conditions, pesticide recoveries were greater than 80%. SFE provided a more selective extraction process for pesticides without requirement of post-extraction cleanup steps before analysis. Furthermore, the SFE approach is semi-

automated, uncomplicated, waste-free and inexpensive. However, a result of the greater selectivity is a narrower range of polarities in pesticides that have complete recoveries in SFE. Two or more sets of SFE extraction conditions or more selective detectors are needed in SFE to cover the wide range of polarity that liquid organic solvents can encompass.

Solid phase micro-extraction (SPME) is a relatively new technique, which is capable of extracting and concentrating organic molecules of analytical interest from aqueous samples prior to quantitation by GC or HPLC methods. For example, it was reported the application of SPME to analysis of pesticides residues in juices, fruits and vegetables (Volante et al., 2000). SPME technique allows the fiber to capture the organic compounds at trace levels without the use of solvent, and the concentration of the extracted analyses occur in the small volume of the fiber itself. Volante et al. tested nearly one hundred active compounds, with two types of adsorbent phases (polydimethylsiloxane and carbowax/divinylbenzenz). GC-NPD was used to detect organophosphate insecticides, and GC with ECD was employed to detect fungicides and organochlorines. The recovery test was performed by GC-MS. The procedure was further tested analyzing real samples. A direct extraction was used for the fruit juices or beverages by simple immersion of the fiber. However, for whole vegetable or fruit, the matrix was too thick for a direct extraction by fiber insertion. Even after making a homogenized aqueous dispersion, it was unlikely that water alone was able to extract the pesticide residues from the vegetable or fruit tissues. A modified method was used by treating the sample with ultra-sound in a small amount of acetone before suspending it in water and immersing the fiber. Some active compounds still showed very low response

or remained undetectable by this method. These compounds included some organophosphates (acephate, dimethoate and others), pyrethroids (permethrin, fenvalerate and others) and other pesticides (metomil, captan, captafol and others) (Volante et al., 2000). Even now, SPME still can not compete in sensitivity and the numbers of pesticides analyzed with traditional MRMs based on extraction with large quantities of solvent. However, the facts that the SPME does not require large amounts of expensive pure solvents and that the extraction and determination procedure is relatively simple make this technique particularly interesting.

Recently, a fast and sensitive new method - direct sample introduction (DSI), or "dirty sample injection" was used for pesticide analysis in food products (Liao et al., 1991; Jing and Amirav, 1997; Lehotay, 2000). One of the DSI methods is on-column injection, where the sample enters the chromatographic column as a liquid without any cleanup procedure. Liao et al. (1991) used acetonitrile extraction to determine pesticide residues in different crop samples without any cleanup steps. The average recovery of 143 compounds in 13 crops was 92%. Lehotay (2000) analyzed 22 pesticide residues in a mixed apple, green bean, and carrot extracts. The average recoveries were 103±7% with relative standard deviation of ±14.5%, and limits of detection were <2 ng/g for nearly all pesticides studied.

HPLC and IEMMA

In pesticide residue analysis, the use of HPLC is particularly useful for pesticides which are not directly amenable to GC determination. These pesticides include those that lack thermal stability or are not sufficiently volatile without further derivatization. A

reversed-phase HPLC method with acetonitrile-methanol water mobile phase gradient and photodiode-array detection was described for simultaneous determination of 21 pesticides (Parrilla and Vidal, 1996). The pesticides were extracted with acetone and then partitioned from the vegetable sample with dichloromethane. Sample cleanup was accomplished by SPE using both C_{18} and Florisil SPE columns. Average recoveries from green beans ranged from 70.0 to 110.1%. Detection limits of less than 0.1 mg/kg were obtained. However, the HPLC method still has the complex cleanup steps, and this technology has not advanced to the type of column that allows resolution and analysis of a large number of pesticides (>50) even with advent of LC-MS.

Both GC and HPLC methods require expensive instruments and toxic reagents. Also highly skilled personnel and sophisticated laboratories are required for the effective operation for these methods. Because of the above problems, other methods for pesticide analyses have been developed. An immunological method- immobilized enzyme modulator microassay (IEMMA) was developed for the detection of pesticide in fresh produce (Lui et al., 1996). The principle of the IEMMA depends on the fact that many enzymes give a classic Michaelis-type kinetic reaction. That means that in a relatively large volume, and at a defined substrate concentration where mixed order kinetics occur, the initial reaction rate V_0 would be constant over time. As the substrate is consumed, the concentration drops, and the enzyme reaction rate begins to slow down. The sample is analyzed in each modulator well, which has a predetermined enzyme activity baseline level. Then the modulator is monitored in a fluorometric, photometric or luminescent microtiter plate reader. Analysis relies on the slope of the enzyme kinetic reaction. Thus the assay is independent of the starting time of the reaction and the exact volume of the

substrate added in each well. The enzyme has been found to be extremely sensitive to carbofuran, detecting < 0.1 mg/kg in all vegetable with high reproductivity. In a situation where it is not feasible or economical to use sensitive chromatographic techniques or flow injection analysis system, IEMMA offers a rapid, cheap, reproducible and environmental friendly method for pesticide detection.

However, so of all the methods used for pesticide analyses, the most reliable methods for the widest range of pesticides is GC-MS with SIM analysis of organic solvent extract, with and without cleanup. The availability of economical GC-MS instruments further strengthens its usefulness in pesticide analysis and will be the instrument used in the current study.

CHAPTER III

MATERIAL AND METHODS

I. EXPERIMENTAL DESIGN AND PLAN

The selective ion monitoring (SIM) mode of the gas chromatograph-mass spectrometer (GC-MS) was used to detect 7 pesticides (Table 1) in three standard solutions, each containing different concentrations of each of the seven pesticides but the same concentration of anthracene-d10, the internal standard. Two replications of the SIM analysis of each standard solution were completed during the time that the samples were being analyzed. The relative response factor, RR_x, for each pesticide to the internal standard, anthracene-d10, was calculated. The explanation on how to calculate RR_x is given later. The model for RR_x was as follows:

$$RR_x = \mu + S + \varepsilon$$

where μ = mean RR_x, S = concentration of pesticide in standard solution, and ε = error. Testing RR_x at different pesticide concentrations determined the possible dependence of RR_x on the concentration range of each pesticide used.

The experimental design of the recovery of each of the seven pesticides in Table 1 was a randomized complete block design with split plot treatment. There were three vegetables in the whole plot: cabbage, turnip greens and dried beans, and three replicate blocks. For each vegetable, enough raw materials (approximately 1200 g) were obtained

at one time to complete all planned analyses. For each replication, each of the three vegetable samples was spiked with a known amount of each of the seven pesticides (Table 1) and the internal standard, anthracene-d10. The amounts of each pesticide and the internal standard added to each vegetable are given later. The spiked vegetable was extracted by acetonitrile as described by Yang (1993). The "spike" was added after the addition of the acetonitrile to 50-g of vegetable and before blending as described by Yang (1993). An unspiked sample of each vegetable was also analyzed in the same manner as the spiked samples to serve as a baseline for determining the percentage recovery of each pesticide in each vegetable-replication combination. The acetonitrile extract of each spiked vegetable-replication or the unspiked sample was split into three portions, with each portion receiving one of the subplot treatments. These treatments were: (1) the current FST-UT method which involves ethyl ether/petroleum ether (EE/PE) partitioning before Florisil cleanup (Yang, 1993) and GC-MS SIM analysis; (2) a new MRM method, which consists of double solid phase extraction (SPE) cleanup before GC-MS SIM analysis; and (3) direct injection of an extract without cleanup (dirty injection). The percent recovery (PR) of each pesticide residue for a single replication was calculated as a measure of accuracy. The model for PR was as follows:

$$PR = \mu + V + R\,(V) + T + V \times T + \varepsilon$$

where μ = mean PR, V = vegetable, R = replication, T = cleanup method, and ε = error. Preliminary analysis found replications to be similar, so they were pooled into the whole plot error.

II. MATERIALS AND INSTRUMENTATION

Materials and Samples

The pesticide standards: ethalfluralin, bromoxynil, dimethoate, alachlor, bentazon, pendimethalin and esfenvalerate, were purchased from Chem Service, Inc. (West Chester, PA). The purity of each of these pesticides was more than 98%. The internal standard, anthracene-d10, (98% pure) was bought from Sigma Chemical Co., (St. Louis, MO).

For SPE, Florisil Sep-Paks, C_{18} Sep-Paks, and the Carbon Cartridge, all were purchased from Phenomenex Co. (Torrance, CA). Ashless filter paper was obtained from Scheicher & Schueel (Keene, NH). Celite, anhydrous sodium sulfate and sodium chloride were bought from Fisher Scientific Co., (Springfield, NJ). All materials, except the SPE cartridges, were a special grade for pesticide analysis. The solvents: acetonitrile, petroleum ether, ethyl ether and acetone, were pesticide grades, and all were purchased from Fisher Scientific Co..

Turnip greens and dried beans were obtained from Bush Brothers & Company (Dandridge, TN) and were mixed thoroughly upon being received. A head of cabbage was bought from a local supermarket in Knoxville, TN, and sliced into 50-g wedges. All vegetables were sealed in heavy polyethylene freezer bags and stored at -18°C until used. The turnip greens and dried beans were mixed thoroughly again before being sampled for use, and a cabbage wedge was picked at random as a single sample.

Instrumentation and Conditions for Analysis

A Model 17A gas chromatograph (Shimadzu Scientific Instrumental Inc., Columbia, MD) interfaced with a Shimadzu Model QP-5000 mass spectrometer (MS) was used to analyze all standards and samples. The GC was fitted with a split/splitless injector and a 30-m × 0.25-mm i.d. XTI-5ms fused silica capillary column (Restek Corp., Bellefonte, PA). The GC temperature program was set as follows: The initial column temperature of 70°C was held for 2.5 min, then increased to 220°C at 10°C/min, where it was held for 30 min before being increased at the same rate to 280°C with a hold for 5 min to clean the column. The injector temperature was maintained at 250°C. A split injection of 1:10 was used for sample analysis with a flow rate of 1.1 mL/min of high purity helium as the carrier gas.

The Shimadzu Model QP-5000 MS used in this study was equipped with a quadrupole mass analyzer and was operated in the electron impact (EI) mode over a mass range selected between 40-400 M/z at 70 eV in the GC-EIMS mode. The GC transfer line and ion source temperatures were kept at 250°C. There were two desired MS modes used in this study: the EIMS, the cyclic scan mode, for standard analysis to get the retention time and most abundant ions for each of the pesticides and internal standard; and the EIMF, the selected ion monitoring (SIM) mode, for sample analysis to detect each of the pesticides and the internal standard using at least 2 ions of known M/z per compound. The MS was checked by perfluorotributylamine (PFTBA) during analysis to maintain good performance. PFTBA is one of the most frequently used compounds for calibration and tuning of a quadrupole analyzer (Yang, 1993).

Determination of Retention Times and Ions for SIM

A GC-EIMS analysis of a standard solution containing all seven pesticides and the internal standard, anthracene-d10, (Standard Solution 1, Table 3) was run using previously described conditions. The retention time (RT), and the most abundant ion and the alternate ion (M/z) were determined for each of the seven pesticides and the internal standard from the respective GC-EIMS chromatogram and mass spectrum. After determining the retention time and the most abundant and alternate ions for each compound of interest, the same GC-MS conditions used for GC-EIMS were used for SIM analysis of all pesticide standards and vegetable samples prepared for analysis.

III. METHODS

Analysis of Retention Times and M/z Ratios of Selected Ions

Standard solution 1 containing known concentrations of the seven pesticides and internal standard (Table 3) were analyzed by GC-EIMS, and the retention time and the M/z ratios of two of the ions were determined for each compound. These retention times and M/z ratios of the ions were used to construct a SIM program that was used to analyze all pesticide standard solutions (Table 3) and spiked and unspiked vegetables samples under the same GC-MS conditions as previously stated. The concentration of each pesticide in each standard solution was selected such that the SIM analysis could be run at a single detector voltage on the MS that was within ± 0.2 volts of the detector voltage 1.0 eV set by autotuning the MS with PFTBA. The Shimadzu MS gives the best quantitative data in this voltage range (Schafer, 2001).

Table 3 -- Concentrations (μg/μL) of the seven pesticides and internal standard in the three standard solutions.

Compound	Standard solution		
	1	2	3
Ethalfluralin	0.10	0.07	0.04
Bromoxynil	0.50	0.35	0.20
Dimethoate	0.25	0.175	0.10
Alachlor	0.10	0.07	0.04
Bentazon	0.50	0.35	0.20
Pendimethalin	0.25	0.175	0.10
Esfenvalerate	0.50	0.35	0.25
Anthracene-d10[a]	0.25	0.25	0.25

[a]Internal standard.

Acetonitrile Extraction

Dried beans were soaked with distilled water overnight and mixed greens and cabbage were chopped prior to extraction. Fifty (50.00) grams of soaked or chopped sample, 100-mL acetonitrile, and if the sample was spiked, 1mL of a standard solution containing levels of each pesticide and internal standard shown in Table 4, and 5-g Celite were placed in a 0.45-L Mason jar and blended on an Osterizer blender (Sunbeam Co., McMinnville, TN) at high speed for 2 min. The mixture was filtered with suction into a 250-mL graduated cylinder through a 12-cm Buchner funnel containing 11-cm diameter ashless filter paper. At least 93 mL of the acetonitrile extraction for each sample was obtained. The amount of each pesticide used for spiking vegetable samples was chosen so that the mass spectrometer could be run at the same condition as the standards were run.

Description of Three Treatments

For each vegetable sample, the acetonitrile extract was split into three 30-mL portions and each portion was stored under nitrogen at -18°C until used. A 20-mL of the first 30-mL portion was cleaned up by the method of Yang (1993), another 30-mL portion was cleaned up by a modified double SPE method of Fillion and others (2000), and the third 30-mL portion was not cleaned up but a sample of it was injected directly for SIM analysis.

The method of Yang (1993) or method 1 was as follows. A 20-mL portion was transferred to a 500-mL separatory funnel, and 50 mL of 6% ethyl ether (EE) in petroleum ether (PE) (v/v) was added. The funnel and contents were shaken vigorously

Table 4 -- Concentration (μg/μL) of the seven pesticides and internal standard used to spike vegetables in the analysis of GC-EIMS.

Compound	Concentration (μg/μL)
Ethalfluralin	0.020
Bromoxynil	0.075
Dimethoate	0.050
Alachlor	0.020
Bentazon	0.075
Pendimethalin	0.050
Esfenvalerate	0.075
Anthracene-d10[a]	0.050

[a]Internal standard.

for 1 min, and the phases allowed to be separated. The aqueous layer was discarded, and 150 mL of distilled water: saturated NaCl solution (5:1, v/v) was added to the funnel. After the funnel and its contents were shaken, the aqueous layer was discarded, and the EE/PE layer was washed twice with 25 mL of a 1:1 water: saturated NaCl solution (v/v) solution, each time discarding the aqueous layer. The water was removed from the EE/PE layer by passing it through a filter funnel containing 15-g anhydrous sodium sulfate over glass wool. A 25-mL aliquot of the extract was then evaporated to near dryness under nitrogen flow on a steam bath, and the dried residue was dissolved in 1.0 mL of 6% EE/PE and loaded onto a 1-g Florisil Sep-Pak Cartridge attached to a vacuum manifold (Baxter Scientific Products, Stone Mountain, GA). The samples were then eluted with 10-mL 6% PE/EE followed by 10-mL 15% EE/PE and 10-mL 35% EE/PE. The elutes were combined and dried to near dryness under nitrogen flow on a steam bath, and the residue dissolved in 2.5-mL acetone and stored at -18°C until SIM analysis.

A modification of the double SPE cleanup method of Fillion et al. (2000) or method 2 was done as follows. The C_{18} SPE tube was preconditioned by passing 2-mL acetonitrile through the tube and then passing approximately 2 mL of the vegetable acetonitrile extract (top layer) through the tube and discarding the eluate. Next, 15 mL of the acetonitrile extract was loaded into the C_{18} cartridge, and eluted by gravity into a 15-mL centrifuge tube. Only 13-mL of the elute was collected to which enough sodium sulfate was added to bring the volume to 15 mL, and the tube was capped and shaken well. A 10-mL aliquot of the elute, which was equivalent to 5 g of the original vegetable sample, was transferred to a 50-mL beaker, where it was evaporated to 0.5 mL under nitrogen flow. Then, an amino propyl SPE cartridge was attached to the bottom of a

carbon SPE tube, and both tubes were pre-conditioned by passing 2-mL acetonitrile-toluene (3+1, v/v) through the tubes. The 0.5-mL residue in the beaker was quantitatively transferred to the carbon cartridge by rinsing with 2-mL acetonitrile-toluene (3+1, v/v) three times. The SPE cartridges were eluted using the rest of 20-mL acetonitrile-toluene (3+1, v/v) into another 50-mL beaker. This eluate was evaporated to near dryness under nitrogen flow on a steam bath. A 10-mL aliquot of acetone was added to the concentrated eluate two different times with subsequent evaporation to a low volume (<0.5 mL) under nitrogen flow on the steam bath each time. The concentrated, cleaned-up extract (eluate) was finally diluted to 2.5 mL with acetone and stored at -18°C until analyzed by SIM. The modification is that instead of evaporating a filtrate or eluate on a rotary evaporator as done by Fillion et al. (2000), the filtrate or elute was evaporated under a nitrogen flow on a steam bath.

For direct injection or method 3, a 1.0-μL injection of the acetonitrile extract of each vegetable in each replication was analyzed by SIM. This acetonitrile extract did not receive any additional cleanup. This method is known as dirty-injection and also was studied for other pesticides by Yang (1993).

IV. METHOD RELIABILITY AND CALCULATIONS

Analysis of Relative Response Factors

Three standard solutions, each containing different concentrations of the seven pesticides, were prepared. After analysis by GC-EIMF in the SIM mode, the average

relative response factor (RR_x) was determined for each pesticide by the following equation:

$$RR_x = \frac{A_x \times C_s}{A_s \times C_x}$$

where A_x = peak area of pesticide, A_s = peak area of internal standard, C_s = concentration of internal standard and C_x = concentration of pesticide.

Pesticide Concentrations in Spiked Samples

The concentration of each pesticide for each vegetable-replication-treatment combination was determined using the following equation:

$$C_x = \frac{A_x \times C_s}{A_s \times RR_x}$$

where A_x = peak area of pesticide in sample chromatogram, A_s = peak area of internal standard in sample chromatogram, C_s = concentration of internal standard added to 50-g sample, and RR_x = relative response factor.

The area of each peak was based on the total ion count of the most abundant ion and alternate ion at the M/z ratio selected for each component analyzed. However, the A_s for all method 2 samples was adjusted to the respective A_s of method 1 samples for each vegetable-method-replication combination. This was done because every A_s value for a sample from method 2 consistently had a 50-60% lower A_s value than the corresponding sample A_s value from method 1, even though each sample had equivalent levels of internal standard. The difference in A_s between the two methods was tested for significance as described later.

In addition, the RR_x used differed from sample to sample for the same pesticide depending on the pesticide peak area ratio A_x/A_s in the sample chromatogram. If the pesticide peak area ratio in the sample was less than that of the pesticide peak area ratio of the lowest concentration in the standard solutions, then the RR_x calculated for that lowest concentration was used. If the sample pesticide peak area ratio was between the standard peak area ratios of the lowest and the second highest concentrations in the standard solutions, then an average of their relative response factors was used to calculate the concentration in the samples. If the sample pesticide peak area ratio was between the standard peak area ratios of the second highest and the highest concentrations in the standard solutions, then an average of those relative response factors was used to calculate the concentration in the sample. However, if the sample peak ratio was higher than the standard peak ratio of the highest concentration in the standard solutions, the concentration of the pesticide in the sample was estimated using the RR_x calculated from the standard solution with the highest pesticide concentration.

Percentage Recoveries and Coefficients of Variation

The percentage recovery (PR) is a measurement of accuracy of a method with a PR closest to 100% being the most accurate. The PR of each pesticide for each vegetable-replication-treatment combination was calculated by the following equation:

$$PR(\%) = \frac{(C_x - Y)}{Z} \times 100$$

where C_x = concentration of pesticide in spiked sample, Y = concentration of pesticide in unspiked sample, and Z = concentration of pesticide added to spiked sample.

For each pesticide, the standard deviation in PR (δ_{PR}) was determined by the averaged PR across replications for each vegetable-method combination. The coefficient of variation (CV) in PR, as a measure of precision, was calculated by the following equation:

$$CV(\%) = \frac{\delta_{PR}}{PR_{av}} \times 100$$

where δ_{PR} = standard deviation in PR across replications, and PR_{av} = average percentage recovery across replications.

The smaller the CV is, the more precise the method is (Pomeranz and Meloan, 1987). Only those CVs where PRs of pesticides in each vegetable-method combination were close to or in excess of 50% were reported.

Determination of Practical Sensitivity

While the best quantitative results may be obtained within ± 0.2 volts of the MS detector voltage (1.0 eV) set by autotuning with PFTBA in this experiment (Schafer, 2001), a more sensitive setting for the detector (1.8 eV) normally is used in routine screening for pesticides in vegetables. Standard solution 1 (Table 3) was diluted 1:1000 with the acetonitrile extract of unspiked turnip greens, and the peak area was obtained for each pesticide when run at a MS detector voltage of 1.8 eV using SIM analysis. Then, for each pesticide, the concentration resulting in a peak two times the baseline noise (peak area = 10,000) was calculated by interpolation and reported in µg pesticide/g turnip greens (ppm) as the estimated limit of detection (Pomeranz and Meloan, 1987).

Statistical Analysis

The relative response factor (RR_x) for each pesticide was analyzed by the Mixed Procedure in the SAS system (SAS Institute Inc., 1999). The fixed effects were pesticide concentration (S). Significant differences of least-squares means ($p<0.05$) were separated by pair wise test (PDIFF).

A paired t-test was used to determine the difference in A_s between method 1 and method 2 (SAS Institute Inc., 1999). A significant difference was defined at the level of $p<0.05$.

The percentage recovery (PR) for each pesticide also was analyzed by the Mixed Procedure in SAS. The fixed effects were vegetable (V), method (T), and V×T, while the error term for determining significance for vegetable (V) included replication (R) and R×V. The error terms for determining the significance of method (T) and V× T included T× R and R×V×T. Significant differences of least-squares means ($p<0.05$) were separated by the pair wise test (PDIFF).

CHAPTER IV

RESULTS AND DISCUSSION

I. METHODS

Analysis of Retention Times and Selected Ions

The retention time and the mass to charge ratios (M/z) of the selected ions for each pesticide and the internal standard were determined by gas chromatography-mass spectrometry (GC-MS) using the cyclic scan mode (GC-EIMS) are listed in Table 5. The mass spectrum for each compound is shown in Appendix A. The spectrum for any one pesticide is comparable to that given by Hites (1992).

Programming the Mass Spectrometer

The retention time, the M/z ratios of the most abundant and alternate ions for each pesticide and the internal standard were used to program the mass spectrometer for selective ion monitoring (SIM). The time period and M/z ratios of the ions set for each compound are shown in Table 6. This program was used to detect the pesticide residues in the vegetable samples by GC-MS using SIM mode (GC-EIMF).

Table 5 -- Retention times and mass/charge ratios (M/z)[a] for the selected ions of 7 pesticides and internal standard in GC-MS analysis.

Compound	Retention time (min)	Most abundant ion (M/z)	Alternate ion (M/z)
Ethalfluralin	10.69	55	276
Bromoxynil	10.96	277	88
Dimethoate	11.33	87	125
Alachlor	12.75	45	160
Bentazon	13.88	119	198
Pendimethalin	14.36	252	162
Esfenvalerate	22.84	167	125
Anthracene-d10[b]	11.87	188	94

[a] M/z = mass/charge ratio where charge = +1.
[b] Internal standard.

Table 6 -- GC-MS program for analysis of 7 pesticides and internal standard.

Compound	Time period (min)	Ion monitored (M/z)[a]	
		Most abundant ion	Alternate ion
Ethalfluralin	9.46 – 10.84	55	276
Bromoxynil	10.86 – 11.20	277	88
Dimethoate	11.21 – 11.60	87	125
Alachlor	12.60 – 13.70	45	160
Bentazon	13.73 – 14.15	119	198
Pendimethalin	14.16 – 19.50	252	162
Esfenvalerate	22.50 – 24.50	167	125
Anthracene-d10[b]	11.72 – 12.59	188	94

[a] M/z = mass/charge ratio where charge = +1.
[b] Internal standard.

II. METHOD RELIABILITY EVALUATION

Relative Response Factors

The analysis of variance for the relative response factor (RR_x) for each pesticide at different concentrations (Table 3) in the standard solutions is presented in Appendix B. Differences were found in the relative response factor for pesticide concentration ($p<0.05$) for four of the seven pesticides. Time of analysis had no significant effect on the relative response factor. However, there were significant concentration × time interactions for ethalfluralin, dimethoate, alachlor and bentazon.

The least squares means and standard errors of the relative response factors for different concentrations of each pesticide versus the internal standard are given in Table 7. The relative response factors changed ($p<0.05$) across concentration for ethalfluralin, bromoxynil, alachlor, and pendimethalin. These results were the reason that concentrations of each pesticide in the vegetables were calculated using different values of the relative response factor as described previously in Material and Methods. The differences in the relative response factors across concentration for any pesticide were because of the nonlinear response of the mass spectrometer in the SIM mode.

The least squares means of the relative response factor and standard error for the concentration at each time of analysis for each pesticide with a significant interaction are shown in Table 8. The significant interactions for ethalfluralin, alachlor, and bentazon occurred because there was a significant decrease in RRx at concentration level 2 between Times 1 and 2, but not for concentration levels 1 and 3, where RRx increased between Times 1 and 2. The significant interaction for dimethoate occurred for the same

Table 7 -- Least squares means[a] and standard errors (SE) for relative response factor of pesticide to internal standard for each concentration level[b] of each pesticide in standards.

Compound	Concentration level			SE
	1	2	3	
Ethalfluralin	0.3143c	0.6632a	0.5420b	0.0390
Bromoxynil	0.2567c	0.4137a	0.2833b	0.0040
Dimethoate	0.3167	0.3898	0.2828	0.0181
Alachlor	1.2418b	1.6743a	1.1088b	0.0660
Bentazon	0.1837	0.1859	0.1182	0.0170
Pendimethalin	0.1552c	0.2440a	0.1934b	0.0071
Esfenvalerate	0.0408	0.0520	0.0657	0.0103

[a]Least squares means (n=2) followed by unlike letters in a row are different ($p<0.05$).
[b]See Table 3 for the concentration of each pesticide at each level.

Table 8-- Least squares means[a] for relative response factors (RRx) of pesticides to internal standard average across replications for each concentration level and time analyzed when interaction of concentration and time was significant ($p<0.05$).

Compound	Time	Concentration level		
		1	2	3
Ethalfluralin	1	0.3760b	0.4785a	0.4982a
	2	0.3911b	0.3901b	0.5283a
Dimethoate	1	0.4555c	0.5163ab	0.5135ab
	2	0.5096abc	0.4689bc	0.5467a
Alachlor	1	1.4750bc	1.6489a	1.6118ab
	2	1.6739a	1.4433c	1.6912a
Bentazon	1	0.2359bc	0.2939ab	0.3025a
	2	0.2577abc	0.2266c	0.3100a

[a] For any one pesticide, least square means (n=4) followed by unlike letters are different ($p<0.05$).
[b] Standard error for ethalfluralin is 0.0180; for dimethoate, 0.0184; for alachlor, 0.0557; and for bentazon, 0.0211.

reason as the other pesticides, but the difference in RRx at concentration level 2 was not significantly different.

Internal Standard Peak Area Differences

The peak area of the internal standard, anthracene-d10, of a single sample-replication combination cleaned up by method 2 was much less when compared with that of the same sample-replication combination in method 1. The mean area (n=9) of the internal standard peaks for method 2 (2,155,936) was less ($p<0.006$) than the mean area (n=9) for method 1 (4,105,317). Obviously, not all of the anthracene-d10 was recovered in the double SPE cleanup method 2. A paired comparison T-test for the internal standard areas between method 1 and 2 is given in Appendix C. This was the reason that the areas of the internal standard for those samples in method 2 were corrected before pesticide levels were calculated as explained previously in Materials and Methods.

Percentage Recoveries

No measurable amounts of any of the seven pesticides (Table 1) were found in unspiked vegetable samples. Therefore, it was not necessary to correct the pesticide concentrations present in the spiked samples prior to calculation of the percentage recovery for each pesticide.

The analysis of variance for the percentage recovery (PR) for each pesticide from different vegetables and cleanup methods is presented in Appendix D. The least squares means and the standard errors for the PR of each pesticide for each vegetable are presented in Table 9. Significant differences in PR were found among the vegetables

Table 9 -- Least squares means[a] and standard errors (SE) for percentage recoveries for each pesticide averaged across cleanup method for each vegetable.

Compound	Vegetable			SE
	Greens	Dried beans	Cabbage	
Ethalfluralin	26.4	28.3	32.7	2.0
Bromoxynil	0.0b	0.3b	14.9a	3.9
Dimethoate	35.7c	53.0b	73.9a	3.6
Alachlor	56.4b	62.6b	89.0a	5.5
Bentazon	0.0	4.3	3.4	2.6
Pendimethalin	67.9b	70.3b	88.3a	5.2
Esfenvalerate	157.4a	102.5b	61.4c	9.0

[a] Least squares means (n=9) in a row followed by unlike letters are different ($p<0.05$).

for bromoxynil, dimethoate, alachlor, pendimethalin, and esfenvalerate. Cabbage had highest recoveries of four of these latter pesticides but lowest recovery among the vegetables for esfenvalerate. The highest recovery of esfenvalerate was found in greens. Two pesticides, bromoxynil and bentazon had zero to <15% recoveries from the three vegetables, while the highest recoveries among the vegetables were found for pendimethalin and esfenvalerate (Table 9). The least squares means and standard errors for PR of each pesticide for each cleanup method are presented in Table 10. Cleanup method had a significant effect on PR for all pesticides except bentazon. Interactions ($p<0.05$) between vegetables and cleanup methods were found for bromoxynil, dimethoate, alachlor, and esfenvalerate (Appendix D). The least squares means and standard error of PR for each combination of vegetable and cleanup method for each pesticide are given in Table 11. The PDIFF mean separation showed differences ($p<0.05$) among the nine combinations for each pesticide except bentazon (Appendix D). The differences in PR found among vegetables, cleanup methods and any significant interactions will be presented for each pesticide separately.

Ethalfluralin. The recoveries of ethalfluralin were significantly different among the three methods (Appendix D-1). The recovery of ethalfluralin was highest by using acetonitrile extraction with the Florisil cleanup method (method 1) (Yang, 1993) in all vegetables and lowest in the acetonitrile extract with double solid phase extraction cleanup (method 2) (Table 10). PR in method 3, the method without any cleanup also was lower ($P<0.05$) than the PR in method 1, the Florisil cleanup. Ethalfluralin may have bound to co-extractives, which were present in method 3 samples during storage, but were not present in method 1 samples. Part of the acetonitrile extract not cleaned up

Table 10 -- Least squares means[a] and standard errors (SE) for percentage recoveries for each pesticide averaged across vegetables for each cleanup method[b].

Compound	Cleanup method			SE
	1	2	3	
Ethalfluralin	52.7a	7.0c	27.8b	2.0
Bromoxynil	0.0b	14.9a	0.3b	3.9
Dimethoate	0.0c	123.7a	39.0b	3.3
Alachlor	81.7a	89.6a	36.8b	5.5
Bentazon	4.3	3.4	0.0	2.6
Pendimethalin	117.9a	25.1c	83.4b	5.2
Esfenvalerate	91.0b	2.4c	227.9a	9.0

[a] Least square means (n=9) in a row followed by unlike letters are different ($p<0.05$).
[b] 1=acetonitrile extract with Florisil cleanup; 2=acetonitrile extract with double SPE cleanup; 3=direct injection without cleanup steps.

Table 11 -- Least squares means[a] and standard error (SE) of percentage recovery (PR) for each pesticide from different spiked vegetables and cleanup method.

Compound	Method[b]	Vegetable			SE
		Greens	Dried beans	Cabbage	
Ethalfluralin	1	49.3a	53.3a	55.6a	3.4
	2	4.2d	1.2d	15.4c	
	3	25.8bc	30.5b	27.0b	
Bromoxynil	1	0.0b	0.0b	0.0b	6.7
	2	0.0b	0.0b	44.6a	
	3	0.0b	0.8b	0.0b	
Dimethoate	1	0.0f	0.0f	0.0f	5.7
	2	58.4c	130.0b	182.7a	
	3	48.7cd	29.2e	39.1de	
Alachlor	1	66.5c	115.9b	62.6c	9.6
	2	62.5c	42.2cd	164.1a	
	3	40.3cd	29.7d	40.3cd	
Bentazon	1	0.0	13.0	0.0	4.6
	2	0.0	0.0	10.1	
	3	0.0	0.0	0.0	
Pendimethalin	1	114.5ab	121.2a	118.0a	8.9
	2	12.2e	6.1e	57.1d	
	3	77.0cd	83.6cd	89.8bc	
Esfenvalerate	1	72.0d	127.4c	73.5d	15.7
	2	0.0e	0.0e	7.3e	
	3	400.3a	180.1b	103.3cd	

[a] For any one pesticide, least squares means (n=3) followed by unlike letters are different at level $p<0.05$.

[b] 1=acetonitrile extract with Florisil cleanup; 2=acetonitrile extract with double SPE cleanup; 3=direct injection without cleanup steps.

(method 3) as well as the cleaned up extracts were stored at –18°C for up to one month before GC-MS analysis. The vegetable pigments and other components soluble in acetonitrile would have more opportunity to interact with ethalfluralin in method 3 than in the method 1.

Cleanup methods using SPE with a Florisil cartridge was similar to the cleanup procedure of Mattern et al. (1991) except for eluting solvents. In the cleanup method of Mattern et al. (1991), the extract was loaded on a 1-g Florisil column and eluted with two 1-mL aliquots of ethyl ether/dichloromethane/petroleum ether (1:1:1). Mattern et al. (1991) reported 110.9% recovery with a 14.5% coefficient of variation (CV) for ethalfluralin from corn. This is over two times the mean PR of ethalfluralin found from dried beans, greens and cabbage using cleanup method 1 (Table 10) in my study. A more polar eluting solvent than the 35% ethyl ether in petroleum ether used in my study or one similar to that used by Mattern et al. (1991), perhaps would improve the recovery of ethalfluralin from the vegetables. However, the standard error of the PR for ethalfluralin shows that this method 1 is precise (Table 10). This high degree of precision allows a correction factor to be applied to levels of ethalfluralin determined using cleanup method 1 to correct to 100% recovery and an accurate estimate of the ethalfluralin level present.

Fillion et al. (2000) reported a 75.9% recovery with an 8.2% CV of ethalfluralin from fruits and vegetables. Cleanup method 2 was a modified method of double SPE cleanup used by Fillion et al. (2000). The modification was that instead of evaporating the eluted pesticides from each SPE cartridge on a rotary evaporator, they were evaporated on a steam bath under a flow of nitrogen. This difference might have caused more heat to be applied to the pesticide fraction with subsequent destruction of

ethalfluralin and its low recovery in cleanup method 2. Also, Lehotay (2000) observed that more cleanup steps in pesticide analysis generally reduces the recovery significantly and leads to more error in analysis.

Bromoxynil. Types of vegetable, cleanup method and the vegetable × cleanup method interaction affected the PR of bromoxynil significantly (Appendix D-2). The highest recovery of bromoxynil was from cabbage (Table 9). Bromoxynil had a higher PR for cleanup method 2 than for the other two cleanup methods. However, for any cleanup method the recovery was either zero or so low that the methods used in my study cannot be recommended for the analysis of bromoxynil in vegetables. The significant interaction between vegetables and cleanup method occurred because the only recovery of bromoxynil was from cabbage extract that received a double SPE cleanup method (Table 11).

The reason for the recovery of bromoxynil from cabbage might be due to some contaminant having ions of the same M/z as those (M/z of 277 and 88) used to detect and measure bromoxynil. Prater (1980) found that cabbage waxes with high molecular weights have similar polarities as organochlorine pesticides, and co-elute from a nonpolar gas chromatographic column at similar times as several of those pesticides. The GC column used in my study was non-polar, and the possibility exists that these waxes or some other co-extracted contaminants, and not bromoxynil, were measured by gas chromatography-mass spectrometry.

The fact that no bromoxynil was recovered from vegetables by my methods agrees with Cessna (1990), who reported that bromoxynil, an acidic herbicide, forms conjugates with plant substituents and requires special extraction procedures. Cessna

(1990) used ethanol/water (80:20, v/v) solvent to extract bromoxynil conjugates from onions followed by hydrolysis of the extract to free bromoxynil from the conjugates before partitioning into diethyl ether. Since I used an acetonitrile extraction of vegetables followed by different cleanup methods and did not use any hydrolysis in the extraction, the bromoxynil was not freed from the conjugates and was not analyzed during GC-EIMF analysis.

Dimethoate. Vegetable, cleanup method and the vegetable × cleanup method interaction all affected the PR of dimethoate significantly (Appendix D-3). Cabbage had the highest PR for dimethoate and greens had the lowest PR (Table 9). PR averaged across vegetable for each method ranged from 0 to 123.7 (Table 10). Dimethoate was not recovered when cleanup method 1 was used. This lack of recovery is probably because the solvent used to elute pesticides from the Florisil SPE cartridge was not polar enough to elute dimethoate. The most polar solvent used in cleanup method 1 was 35% diethyl ether in petroleum ether. Parrilla and Vidal (1996) cleaned up a spiked green bean extract containing dimethoate using a Florisil SPE cartridge, but they eluted dimethoate with acetonitrile. Parrilla and Vidal (1996) found 88-89% recovery of dimethoate in their study. Lee et al. (1991) extracted dimethoate from spiked samples of potatoes, tomatoes, or oranges and removed nonpolar co-extractives by passing the acetonitrile extracts through a C_{18} SPE cartridge. Lee and others (1991) reported 78.3 to 153.2 % recovery of dimethoate from the samples. Barwick et al. (1991), who extracted vegetables with acetonitrile, used ENVI Carb SPE to remove co-extractives. These latter researchers eluted dimethoate with acetonitrile/ toluene (3:1, v/v) and found 53 to 79% recovery of dimethoate from dimethoate-fortified vegetable samples. In each of these successful

studies of dimethoate recovery using a single SPE cartridge, the eluting solvent was more polar than the 35% diethyl ether in petroleum ether used in my study. Possibly, increasing the polarity of the solvent used to elute pesticides in cleanup method 1 would elute dimethoate. Additional research is needed to determine if this is true.

In cleanup method 2, the PR of dimethoate was dependent upon the vegetable and ranged from 58.4% from greens to 182.7% from cabbage (Table 11). The recovery of dimethoate in excess of 100% from dried beans and cabbage could indicate that some co-extractive from these two vegetables may have had ions of M/z 87 and/or 125 and eluted at the same time as dimethoate in the SIM analysis. Fillion and others (2000), who used ions of M/z 87, 229 and 143 to measure dimethoate by SIM analysis, reported 90.5% recovery of dimethoate from fruits and vegetables. However, it is not that unusual to see pesticide recoveries above 100% in scientific research reports. Lee et al. (1991) reported a recovery of 153.2% for dimethoate from oranges.

The low recoveries (29.2 to 48.7%) of dimethoate from vegetables extracts with no cleanup (method 3, Table 11), may indicate that dimethoate either degraded or bound to some co-extractives from dried beans and cabbage during the one-month cold storage of the acetonitrile extract. These co-extractives most likely were removed by cleanup method 2 prior to storage of samples. Further research is needed to investigate the stability and recovery of dimethoate from acetonitrile extracts of vegetables stored at -18°C.

In my study, dimethoate could be determined using cleanup method 2; however, a suitable correction factor would have to be applied for each vegetable (Table 11) for accurate results (100% recovery). The PR from cleanup method 2 had a standard error of

5.7 (Table 11), making it a precise enough method to allow correction factors to be applied.

The interaction between vegetable and cleanup method can be explained that for method 2, PR for the dried beans was larger than that for greens, and the PR for the cabbage was the highest of all. However in method 3, the PR for the dried beans was significantly lower than the PR for the greens while the PR for the cabbage was intermediate and not significantly different from either the PR in method 1 or 3 (Table 11).

Alachlor. Vegetable, cleanup method and the interaction of cleanup method × vegetable significantly affected the PR of alachlor (Appendix D-4). Cabbage had the highest PR for alachlor while greens and dried beans both had the lowest PR (Table 9). The values of PR by cleanup method 1 and 2 were not significantly different and were greater than 80% (Table 10). The PR of alachlor by cleanup method 3 or direct injection without any cleanup was significantly lower than the recoveries for methods 1 or 2.

However, the level of PR for any one method was dependent upon the type of vegetable. In method 1, dried beans had the highest PR with the recoveries for greens and cabbage being only half as large (Table 11). For method 2, cabbage had the highest PR and greens and dried beans had lower recoveries. For method 3, the lowest PR was for dried beans with a trend to have higher recoveries for the greens and cabbage (Table 11).

The 66.7 and 62.6% recovery of alachlor from greens and cabbage, respectively, for cleanup method 1 agree fairly well with the 72.5 % recovery from corn reported by Mattern et al. (1991). These investigators used the same extraction and a cleanup method

similar to method 1 for measuring alachlor in corn. The recovery of 115.9% of alachlor from dried beans is excellent and compares well with the 109.5% of alachlor from beans reported by Mattern et al. (1991). My results combined with an acceptable standard error of 9.6 indicate that alachlor can be analyzed using cleanup method 1. In the case of greens and cabbage, a correction factor should be applied to make the results accurate (100% recovery), but probably no factor is needed for dried beans.

Averaged across vegetables, the PR of 89.6 for cleanup method 2 (Table 10) compares well with the mean recovery, 91.6%, of alachlor from fruits and vegetables reported by Fillion et al. (2000). But when the percentage recovery of alachlor is determined for each vegetable in cleanup method 2 (Table 11), low recoveries for greens and dried beans are offset by an extremely high percentage recovery of alachlor from cabbage. Alachlor is a neutral pesticide (Bucheli et al., 1997), and cabbage has waxes that cannot be removed by differences in polarity from nonpolar pesticides (Prater, 1980). These waxes, which are esters of long chain acids (>32C) and alcohols (>18C), interfere with the analysis such pesticides by gas chromatography using electron capture detection (Prater, 1980). These waxes also may have interfered with the SIM analysis in my study, especially since the ions of M/z 45 and 160 were used to estimate alachlor. This is contrast to the M/z 188 and 160 used by Fillion et al. (2000) to measure alachlor by SIM in fruits and vegetables.

The low percentage recoveries of alachlor from the vegetables for method 3 (Tables 10 and 11), in which the extracts were not cleaned up (also called dirty injection), can be explained as before for ethalfluralin. The neutral alachlor either bound with co-

extractives during storage at -18°C or degraded during the storage period of method 3 samples before SIM analysis.

Results of my study show that alachlor can be analyzed by the current method (cleanup method 1) used for pesticide analysis of vegetables from Bush Brothers & Company. However, correction factors must be used for accurate quantification of alachlor, particularly in greens and dried beans.

Bentazon. The PR of bentazon was not significantly different among vegetables or cleanup methods or the vegetable × method interaction (Appendix D-5). Average recoveries of bentazon across vegetable or method ranged from 0.0 to 4.3% (Tables 9 and 10). The very low PR of bentazon by cleanup methods 1 and 2 indicates that it is a polar compound that was not eluted from the SPE cartridges in my study. The 0% PR in method 3 (Table 10) indicates that either the bentazon was bound by co-extractives from the vegetables or degraded during storage. Nemoto and Lehotay (1998) reported that bentazon was a polar herbicide that was difficult to extract, isolate and quantify from a soybean matrix. These latter researchers also reported that it took a variety of cleanup techniques to isolate bentazon, and they used HPLC analysis in quantitation of bentazon. Bentazon, however, has been determined by gas chromatography in water and extracts of cheeses (Molinari et al., 1998) and extracts of leeks (Cessna, 1985). Cessna (1985) reported that a N-methyl derivative of bentazon was made prior to GC analysis, but Molinari et al. (1998) did not make any derivative. I was able to analyze bentazon with GC-MS in a standard solution without making a derivative. Therefore, analysis of bentazon directly by GC was not the problem, but in the extraction and the cleanup methods. My results indicate that bentazon, like bromoxynil, requires an analysis method

other than the current methods at The University of Tennessee used to screen for pesticides in vegetables from Bush Brothers & Company.

Pendimethalin. Cleanup method and vegetable ×method interaction affected PR of pendimethalin ($p<0.05$), but vegetable was significant only at $p<0.06$ (Appendix D-6). However when least squares means of PR for vegetables were separated by the PDIFF test, cabbage had a higher PR than the other two vegetables (Table 9). Averaged across cleanup method, the PR of pendimethalin ranged from 68.7 from greens to 88.3 from cabbage (Table 9). Cleanup method 2 had the lowest recovery (25.1%) while cleanup method 1 had the highest (118.7%) (Table 10). The low recovery of pendimethalin by cleanup method 2 is in disagreement with Fillion et al. (2000), who had a similar cleanup method as method 2. Fillion et al. (2000) found a mean 81.8% recovery of pendimethalin after a double SPE cleanup method of an acetonitrile extracts of vegetables and fruits. Cleanup method 2 was a modified cleanup method of Fillion et al. (2000) in that the eluates from the SPE cartridges were dried to near dryness on a steam bath under a flow of nitrogen instead of being evaporated on a rotary evaporator. Fillion et al. (2000) did not report the temperature at which the eluates were evaporated; therefore, it is impossible to know if this modification represents a higher temperature and thus a greater potential for pendimethalin degradation. However, as Lehotay (2000) reported generally the more extensive cleanup methods give lower PR.

Pendimethalin could be analyzed using either cleanup method 1 or by direct injection of the acetonitrile extract (method 3) because of the good recovery from both methods (Table 11). The 83.4% recovery from cleanup method 3 is similar to the pendimethalin 81-84% recoveries reported by Tsiropoulos and Miliadis (1998) from

onions and those (81.8-88.0%) reported by Fillion et al. (1995, 2000), while the 117.9% recovery for cleanup method 1 (Table 11) is higher. Fillion et al. (1995, 2000) used SIM analysis to measure pendimethalin choosing M/z 252 as the target ion and 281 as the qualifier ion. Pendimethalin was analyzed using both M/z 252 and 161 ions in my study. The inclusion of M/z 161 with M/z 252 may have increased the response for pendimethalin for cleanup method 1. However, why SIM would have given increased response for cleanup method 1 and not for cleanup method 3, which was direct injection of acetonitrile extract, is unknown. Because of their precision (low standard error, Table 11), either cleanup method 1 or 3 with an appropriate correction factor to adjust to 100% can give an accurate measurement of pendimethalin in vegetables. Both methods are currently used for analysis of pesticides in Bush Brothers & Company samples.

Esfenvalerate. Vegetable, cleanup method and vegetable × method interaction significantly affected the PR of esfenvalerate (Appendix D-7). The PR averaged across cleanup methods ranged from 61.4 from cabbage to 157.8 from greens (Table 9). The PR of esfenvalerate averaged across vegetables ranged from 2.4 for cleanup method 2 to 227.9 for cleanup method 3.

Cleanup method 1 had a 91.3% recovery of esfenvalerate and is the method of choice for its analysis in vegetables. The PR found for cleanup method 1 agrees well with the 87% recovery reported for esfenvalerate from almond twigs (Zalom et al., 1998). Zalom et al. (1998) used hexane extraction followed by Florisil SPE cleanup and quantification by gas chromatography-electron capture detection for analysis of esfenvalerate.

The instability of esfenvalerate may be the cause of the low recovery in cleanup method 2 (Table 10), in which the SPE eluates were dried on a steam bath. Esfenvalerate is not very stable. Zabik et al. (2000) reported that production of apple juice or apple-sauce destroyed 97.8% of the esfenvalerate in fortified apples. The extremely high PR for esfenvalerate in cleanup method 3 (Table 10) will be explained during the discussion of the vegetable × method interaction.

The significant vegetable × method interaction for esfenvalerate was because for cleanup method 1 the highest recovery was found for dried beans and lower but similar PR were found for greens and cabbage (Table 11). In comparison, for cleanup method 3, the highest recovery was for greens followed by that for dried beans and the lowest recovery was for cabbage (Table 11). In cleanup method 3, the recovery for each vegetable was different ($p<0.05$). No significant differences existed in PR among vegetables for cleanup method 2, for which the recoveries were essentially zero.

The high recoveries of esfenvalerate for greens and dried beans in cleanup method 3 (Table 11) indicate that some co-extractive was interfering with the detection of the pesticide. Ions of M/z 167 and 125 were monitored by SIM for the analysis of esfenvalerate. Therefore, one or more of these ions from the co-extractive had to be present during the elution of esfenvalerate from the GC column resulting in the large PR calculated for the greens and dried beans. This interference was not found for cabbage, in which the PR was very near 100 (Table 11).

The best single method to measure esfenvalerate across all vegetables is cleanup method 1. However, a factor to correct the PR to 100 for each vegetable study is needed to make the method more accurate. However, my results indicate that no cleanup is

necessary to analyze esfenvalerate in cabbage, because the PR is near 100 in cleanup method 3 (Table 11). Both cleanup methods 1 and 3 are used at The University of Tennessee in the analysis of pesticide in vegetables from Bush Brothers & Company.

Coefficients of Variation

Selected coefficients of variation (CVs) are presented in Table 12 for those pesticides, which had PRs ranging from approximately 50 up to 400% (Table 11). In Table 12, CVs are given for ethalfluralin by cleanup method 1, for dimethoate by method 2, for alachlor by method 1 in all vegetables and by method 2 in greens and cabbage, for pendimethalin by methods 1 and 3, and for esfenvalerate by methods 1 and 3.

Mattern et al. (1991), who used a similar cleanup method to cleanup method 1 and SIM to analyze ethalfluralin in corn, reported a 14.5% CV. Lower CVs were found here for recoveries of ethalfluralin from greens and cabbage by cleanup method 1, but a larger CV for dried beans (Table 12). Mattern et al. (1991) also measured the PR of alachlor from corn by the same method and found a 13.0% CV. Compared with this CV value, I found lower CVs for alachlor for cleanup method 1 in greens and cabbage but a higher CV in beans (Table 12).

Only two pesticides, dimethoate and alachlor, were recovered in sufficient amounts to be analyzed by cleanup method 2. Cleanup method 2 was similar to the double SPE method used by Fillion et al. (2000), who used SIM analysis and reported a 10.6% CV for dimethoate and an 8.0% CV for alachlor in their recovery from selected fruits and vegetables. CVs for the recovery of dimethoate ranged from 10.9 to 16.3% among the vegetables (Table 12); these CVs are comparable to that reported by Fillion et

Table 12 -- Least squares means for coefficient of variations for pesticides recovered in sufficient quantities to be analyzed in selected vegetables[a] by selected cleanup methods.

Compound	Method[b]	Vegetable		
		Greens	Dried beans	Cabbage
Ethalfluralin	1	5.6	25.8	7.7
Dimethoate	2	16.3	10.9	12.5
Alachlor	1	3.9	33.7	2.8
	2	18.9	-----	2.1
Pendimethalin	1	12.1	1.3	12.9
	3	22.7	24.4	22.8
Esfenvalerate	1	22.4	38.4	23.5
	3	12.1	6.1	27.6

[a]Percentage recoveries of pesticide-method combination of these vegetables are above 50%, see Table 11.

[b] 1=acetonitrile extract with Florisil cleanup; 2=acetonitrile extract with double SPE cleanup; 3=direct injection without cleanup steps.

al. (2000). Compared with the 8.0% CV for alachlor reported by Fillion et al (2000), the 2.1% CV for the recovery of alachlor in cabbage was smaller, but the 18.9% CV for alachlor in greens was larger.

CVs for method 3 or "direct injection" without cleanup are given for pendimethalin and esfenvalerate in Table 12. The >20 % CVs for the percentage recovery of pendimethalin in my study are in excess of 6.1% reported by Lehotay (2000). However, Lehotay (2000) used a special injector, in which large volumes of sample could be directly injected and the solvent evaporated at low temperatures, before the injector was heated to vaporize the pesticide residues. The CVs (1.3 to 12.9%) for recovery of pendimethalin by cleanup method 1 are smaller than the CVs for method 3 (Table 12); thus, analysis of pendimethalin by cleanup method 1 was more precise.

For esfenvalerate, cleanup method 1 had poorer precision (higher CVs) than cleanup method 3 or "direct injection". However, for method 3, the 400% recovery of esfenvalerate from greens and 180% recovery from dried beans (Table 11) indicate that interferences existed with the SIM analysis of esfenvalerate in the acetonitrile extract of those vegetables. Perhaps, cleanup method 1 with lower PRs of esfenvalerate from these vegetables (Table 11) would give more accurate estimates than would method 3, even though it is not as precise (Table 12).

Ethalfluralin, alachlor, pendimethalin and esfenvalerate can be analyzed fairly accurately in the vegetables studied by using cleanup method 1, particularly if the level found is adjusted to 100% recovery for each pesticide. Cleanup method 1 is the current method that is used for analysis of selected pesticides by The University of Tennessee Food Science and Technology Department in vegetables from Bush Brothers & Company

(Dandridge, TN). Therefore, these four pesticides could be added to the list of pesticides analyzed by the current method. For dimethoate, cleanup method 2 should be used to analyze accurately and precisely (Tables 11 and 12) followed by GC-MS with SIM.

Limits of Detection

The estimated practical detection limit for each of the seven pesticides in an acetonitrile extract of turnip greens at a detector voltage of 1.8eV using SIM analysis is presented in Table 13. The 1.8eV is the detector sensitivity at which all samples from Bush Brothers & Company are analyzed routinely for selected pesticide residues. Greater sensitivity or higher detector voltages results in excess noise in the baseline and failure to recognize small peaks.

Mattern et al. (1991) reported detection limits of 0.05 ppm for ethalfluralin and 0.10 ppm for alachlor in an acetonitrile extract. Fillion et al. (2000) reported a limit of detection of 0.02 ppm for both ethalfluralin and alachlor, but did not report the type of matrix in which the detection limits were determined. My detection limit in an acetonitrile extract of turnip greens for ethalfluralin (Table 13) was smaller than those reported by Mattern et al. (1991) or Fillion et al. (2000). Detection limit for alachlor in the turnip green extract matrix (Table 13) was much smaller than those reported by the latter two groups of researchers. Both Mattern et al. (1991) and Fillion et al. (2000) used SIM analysis for the detection and measurement of the pesticides.

Fillion et al. (2000) reported the detection limits for dimethoate as 0.03 ppm and for pendimethalin as 0.02 ppm. My detection limit for dimethoate was approximately 6.5

Table 13 -- Estimated limits of detection (μg/g or ppm) for seven pesticides in the acetonitrile extract of turnip greens.

Compound	Detection limit (ppm)
Ethalfluralin	0.0052
Bromoxynil	1.06
Dimethoate	0.19
Alachlor	0.00075
Bentazon	1.01
Pendimethalin	0.0099
Esfenvalerate	0.039

times greater than that found by Fillion et al. (2000), but for pendimethalin, my detection limit was two times smaller (Table 13).

Therefore, three of the pesticides (ethalfluralin, alachlor and pendimethalin by cleanup method 1), which I investigated, can be detected at or below reported limits of detection by other researchers. These results combined with their adequate recovery and coefficients of variation (Tables 10, 11 and 12) indicate that they can be added to the pesticides analyzed by the current multiple residue method used to screen vegetables from Bush Brothers & Company (Melton, 2002). Esfenvalerate, which was recovered well from all vegetables for cleanup method 1 (Table 11), also has a small detection limit (Table 13) on the order of that of the latter three pesticides. Esfenvalerate also can be added to the pesticides list that can be analyzed by the same current multiple residue method used to screen Bush Brothers & Company vegetables.

At the present time dimethoate, which had a higher detection limit than that reported by Fillion et al. (2000), can be measured in the same acetonitrile extract without cleanup (method 3, Table 11). However, for dimethoate to have adequate recovery from the different vegetables, cleanup method 2 must be used (Table 11).

The remaining two pesticides, bromoxynil and bentazon, have limits of detection of approximately 1 ppm in the turnip greens extract. However, neither of these pesticides could be measured in the acetonitrile extract by any cleanup methods investigated (Table 10); therefore, another method must be used for their measurement in vegetables.

CHAPTER V

SUMMARY AND IMPLICATIONS

The five objectives of this study were fulfilled. The accuracy and precision of three different cleanup methods of acetonitrile extracts of three vegetables (turnip greens, dried beans and cabbage) were determined and compared for seven pesticides: ethalfluralin, bromoxynil, dimethoate, alachlor, bentazon, pendimethalin, and esfenvalerate. The pesticides were measured using an internal standard, anthracene-d10, and gas chromatography-mass spectrometry in the selective ion monitoring (SIM) mode. The accuracy was estimated by the percentage recovery (PR) of each pesticide, which was analyzed as a function of type of vegetable, cleanup method and their interaction. Coefficients of variation (CVs) were determined as a measurement of precision for those pesticides recovered at or greater than 50% in each vegetable-cleanup method combination. The estimated sensitivity or limit of detection (ppm) of the SIM analysis at a detector voltage of 1.8 eV for each pesticide also was determined in a matrix of an acetonitrile extract of turnip greens.

The design of the experiment was a randomized complete block design with split plot treatment and replication. Three replications were run. Each vegetable was the whole plot, and the split was the division of the acetonitrile extract for each vegetable into portions for the three cleanup methods: (1) the Florisil solid phase extraction (SPE), (2) a double SPE procedure, and (3) no cleanup or direct injection. Enough of each

vegetable was obtained prior to the start of the experiment to complete all planned analyses. A baseline analysis for each of the seven pesticides in each vegetable was determined. Then, each vegetable in each replication was spiked with a known concentration of each pesticide at the same time as the acetonitrile extraction. After each portion of the acetonitrile was subjected to a cleanup method, the level of each pesticide was measured by SIM analyses and the PR calculated. After all replications were completed, the CVs for each pesticide in each vegetable-method combination were calculated where PR was about 50% or greater. The detection limit for each pesticide was determined as the μg of pesticide per gram turnip greens or ppm that resulted in a peak area two times an estimated peak area of the baseline noise.

Results of this study indicate that five of the pesticides: ethalfluralin, dimethoate, alachlor, pendimethalin, and esfenvalerate, were recovered at high enough percentages to be measured in each of the three vegetables studied. Ethalfluralin, alachlor, pendimethalin and esfenvalerate can be measured using the Florisil cleanup method, a method currently used by The University of Tennessee Food Science and Technology Department for screening pesticides in a vegetable sample from a local processor. Dimethoate can be measured in the same acetonitrile extract of each vegetable using the double SPE cleanup method. However, for the most accurate estimate of the amount of each pesticide present, a correction factor adjusting the PR determined in this experiment to 100% should be applied for each vegetable.

However, further research is needed to find methods of analysis for bromoxynil and bentazon. Although these two pesticides could be measured by gas chromatography-mass spectrometry using SIM analysis, none of the cleanup methods, including direct

injection of an acetonitrile extract of the vegetables, recovered meaningful amounts of either pesticide.

Implications of my study indicate that four of the pesticides, ethalfluralin, alachlor, pendimethalin, and esfenvalerate can be added to the current multiresidue method used at The University of Tennessee for pesticide monitoring of vegetables without changing the method. However, additional cleanup of the current vegetable extract is necessary to measure dimethoate.

LIST OF REFERENCES

LIST OF REFERENCES

Ambrus, A. and Their, H.P. 1986. Application of multiresidue procedures in pesticides residues analysis. Pure & Appl. Chem. 58: 1035-1062.

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists. 15th Ed. Washington, D.C.

Barwick, V.J., Ellison, S.L.R., Lacey, S.J., Mussel, C.R. and Lucking, C.L. 1999. Evaluation of a solid phase extraction procedure for the determination of pesticide residues in foodstuffs. J. Sci. Food Agric. 79: 1190-1106.

Benfenati, E., Tremolada, P., Chiappetta, L., Frassanito, R., Bassi, G., Di Toro, N., Fanelli, R., and Stella, G. 1990. Simultaneous analysis of 50 pesticides in water samples by solid phase extraction and GC-MS. Chemosphere. 21: 1411-1421.

Bucheli, T.D., Gruebler, F.C., Muller, S.R. and Schwarzenbach. 1997. Simultaneous determination of neutral and acidic pesticides in natural waters at the low nanogram per liter level. Anal. Chem. 69(8): 1569-1576.

Cessna, A. 1985. Gas chromatographic analysis of the herbicide bentazon in leeks. J. Agric. Food Chem. 33(1): 108-110.

Cessna, A. 1990. Gas chromatographic analysis of bromoxynil in onions. J. Agric. Food Chem. 38(9): 1844-1847.

Dittus, K.L., Hillers V.N., and Beerman, K.N. 1993. Attitudes and behaviors about pesticide-residues, susceptibility to cancer, and consumption of fruits and vegetables. J. Nutri. Edu. 25(5): 245-250.

Fillion, J., Hindle, R., Lacroix, M. and Selwyn, J. 1995. Multiresidue determination of pesticides in fruit and vegetables by gas chromatography-mass-selective detection and liquid chromatography with fluorescence detection. J. AOAC Intern. 78 (3): 1252-1266.

Fillion, J., Sauve, F., and Selwyn, J. 2000. Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection. J. AOAC Intern. 83(3): 698-713.

Gohlke, R.S. 1959. Time-of-flight mass spectrometry and gas-liquid partition chromatography. Anal. Chem. 31: 535-541.

Hites, R.A. 1992. Handbook of Mass Spectra of Environmental Contaminants. 2nd Ed. CRC Press, Inc., Boca Raton, FL.

Holmes, J.C. and Morrell, F.A. 1957. Oscillographic mass spectrometric monitoring of gas chromatography. Applied Spectroscopy. 11: 86-87.

Jing, H.W. and Amirav, A. 1997. Pesticide analysis with the pulsed-flame photometer detector and a direct sample introduction device. Anal. Chem. 69 (7): 1426-1435.

Kamrin, M.A. 1997. Pesticide profiles: toxicity, environmental impact, and fate. CRC Press, Inc., Boca Raton, FL.

Krause, R.T. 1980. Multiresidue method for determining N-methylcarbamate insecticides in crops, using high performance liquid chromatography. J.A.O.A.C. 63: 1114-1124.

Lee, S.M., Papathakis, M.L., Hsiao-Ming, C.F., Hunter, G.F., and Carr, J.E. 1991. Multipesticide residue method for fruits and vegetables: California Department of Food and Agriculture. Fresenius J. Anal. Chem. 339: 376-383.

Lehotay, S.J. 2000. Analysis of pesticides residues in mixed fruit and vegetable extracts by direct sample introduction/gas chromatography/tandem mass spectrometry. J. AOAC Intern. 83 (3): 680-697.

Liao, W., Joe, T., and Cusick, W.G. 1991. Multiresidue screening method for fresh fruits and vegetables with gas chromatographic/mass spectrometric detection. J.A.O.A.C. 74: 554-565.

Lui, J., Tan, M., Liang, C., and Ying, K.B. 1996. Immobilized enzyme modulator microassay (IEMMA) for the detection of pesticide in fresh produce. Analytica Chimica Acta. 329 (3): 297-304.

Luke, M.A., Froberg, J.E., and Masumoto, H.T. 1975. Extraction and clean-up of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. J.A.O.A.C. 58: 1020-1026.

Luke, M.A., Froberg, J.E., Doose, G.M., and Masumoto, H.T. 1981. Improved multiresidue gas chromatographic determination of organophosphorus, organonitrogen and organohalogen pesticides in produce, using flame photometric and electrolytic conductivity detectors. J.A.O.A.C. 64: 1187-1195.

Luke, M.A., Masumoto, H.T., Carins, T., and Hundlfy, H.K. 1988. Levels and incidences of pesticide residues in various foods and animal feeds analyzed by the Luke multiresidue methodology for fiscal years 1982-1986. J.A.O.A.C. 71: 415-420.

Mattern, G.C. and Rosen, J.D. 1992. The use of ion trap mass spectrometry for multiresidue pesticide analysis. In Emerging Strategies for Pesticide Analysis, ed. T. Cairns and J. Sherma, pp. 261-261. CRC Press, Boca Raton, FL.

Mattern, G.C., Liu, C.H., Louis, J.B., and Rosen, J.D. 1991. GC/MS and LC/MS determination of 20 pesticides for which dietary oncongenic risk has been estimated. J. Agric. Food Chem. 39 (4): 700-704.

Melton, S.L. 1996. Report of pesticide residue analysis in Bush Brothers & Co. vegetables (Jan.1-Dec.31, 1995), The University of Tennessee, Knoxville.

Mills, P.A. 1959. Detection and semiquantitative estimation of chlorinated organic pesticide residues in foods by paper chromatography. J.A.O.A.C. 42: 734-740.

Mills, P.A., Onley, J.H., and Gaither, R.A. 1963. Rapid method for chlorinated pesticide residues in nonfatty foods. J.A.O.A.C. 46: 186-191.

Miyahaara, M., Okada, Y., Takeda, H., Aoki, G., Kobayashi, A., and Saito, Y. 1994. Multiresidue procedures for the determination of pesticides in food using capillary gas chromatographic, flame photometric, and mass spectrometric techniques. J. Agric. Food Chem. 42: 2795-2802.

Molinari, G.P., Fontana, G. and Carrara, G. 1998. Evaluation of herbicide migration from water to Gorgonzola and Mozzarella cheeses in industrial processing. Food Add. and Contam. 12 (2): 195-201.

Nemoto, S. and Lehotay, S.J. 1998. Analysis of multiple herbicides in soybeans using pressurized liquid extraction and capillary electrophoresis. J. Agric. Food Chem. 46 (6): 2190-2199.

OTA. 1988. Contemporary analytical techniques for pesticide residues in food. In Pesticide Residues in Food: Technologies for Detection, pp. 21-33. U.S. Congress. Office of Technology Assessment, OTA-F-398, U.S. Government Printing Office, Washington, D.C.

PAM. 1994. Pesticide Analytical Manual Volume I: Multiresidue Methods, 3rd Ed. Food and Drug Administration, Washington, D.C.

Parrilla, P. and Vidal, J.L.M. 1996. HPLC determination of pesticides in green bean samples after SPE clean-up. Chromatographia. 43 (5/6): 265-270.

Prater, A. 1980. Analysis of organochlorine and organophosphate pesticide residues in cabbage. M.S. Thesis, The University of Tennessee, Knoxville.

Pomeranz, Y. and Meloan, C.E. 1987. Food Analysis: Theory and Practice. 2nd Ed. AVI Publishers Co. Westport, CT.

SAS Institute Inc. 1999. SAS User's Guide: Statistics, 1999ed. SAS Institute Inc., Cary, NC.

Schachterle S. and Feifel, C. 1996. Pesticide residue analysis in fresh produce by gas chromatography - tandem mass spectrometry. J. Chromatography A. 754 (1-2): 411-422.

Schafer, L. 2001. Personal communication. Shimadzu Scientific Instrumental Inc., Columbia, MA.

Scroggins C.D. 1991. Consumer attitudes toward the use of pesticides and food safety. In Pesticide residues and Food Safety, ed. B.G. Tweedy, H.J. Dishburger, L.G. Ballantine, and J. McCarthy, pp.50-55. A.C.S., Washington, D.C.

Shao C.C. 1997. Reliability of methods for extraction of vegetables and clean-up for selective ion monitoring of selected organophosphate and N-methylcarbamate insecticides. M.S. Thesis, The University of Tennessee, Knoxville.

Stan, H.J. 1989. Application of capillary gas chromatography with mass selective detection to pesticide residue analysis. J. Chromatogr. 467: 85-98.

Storherr, R.W., Ott, P., and Watts, R.R. 1971. A general method for organophosphorus pesticide residues in nonfatty foods. J.A.O.A.C. 54: 513-516.

Torres, C.M., Pico, Y., and Manes, J. 1996. Determination of pesticides residue in fruit and vegetables. J. Chromatogr. A. 754: 301-331.

Tsiropoulos, N.G. and Miliadis, G.E. 1998. Field persistence studies on pendimethalin residues in onions and soil after herbicide postemergence application in onion cultivation. J. Agric. Food Chem. 46 (1): 291-295.

Valverde-Garcia, A., Fernaddez-Alba, A. R., Contreras, M., and Aguera, A. 1996. Supercritical fluid extraction of pesticides from vegetables using anhydrous magnesium sulfate for sample preparation. J. Agric. Food. Chem. 44 (7): 1780-1784.

Volante M., Pontello, M., Valoti, L., Cattabeo, M., Boanchi, M., and Colzani, L. 2000. Application of solid phase micro-extraction (SPME) to the analysis of pesticide residues in vegetables. Pest Manag. Sci. 56 (7): 618-636.

Ware G.W. 1989. The Pesticide Book. Thomson Publications, Fresno, CA.

Yang R. 1993. Mass selective detection of 11 pesticides in vegetable extracts with and without cleanup. M.S. Thesis, The University of Tennessee, Knoxville.

Zabik, M.J., El-Hadidi, M.F.A., Cash, J.N., Zabik, M.E. and Jones, A.L. 2000. Reduction of azinphos-methyl, chlorpyrifos, esfenvalerate, and methomyl residues in processed apples. J. Agric. Food Chem. 48 (9): 4199-4203.

Zalom, F.G., Stimmann, M.W., Arndt, T.S., Walsh, D.B., Pickel, C. and Krueger, W.H. 2001. Analysis of permethrin (cis and trans-isomers) and esfenvalerate on almond twigs and effects of residues on predator mite Galendromus occidentals (Acari: Phytoseiidae). Environ-entomol. 30 (1): 70-75.

APPENDICES

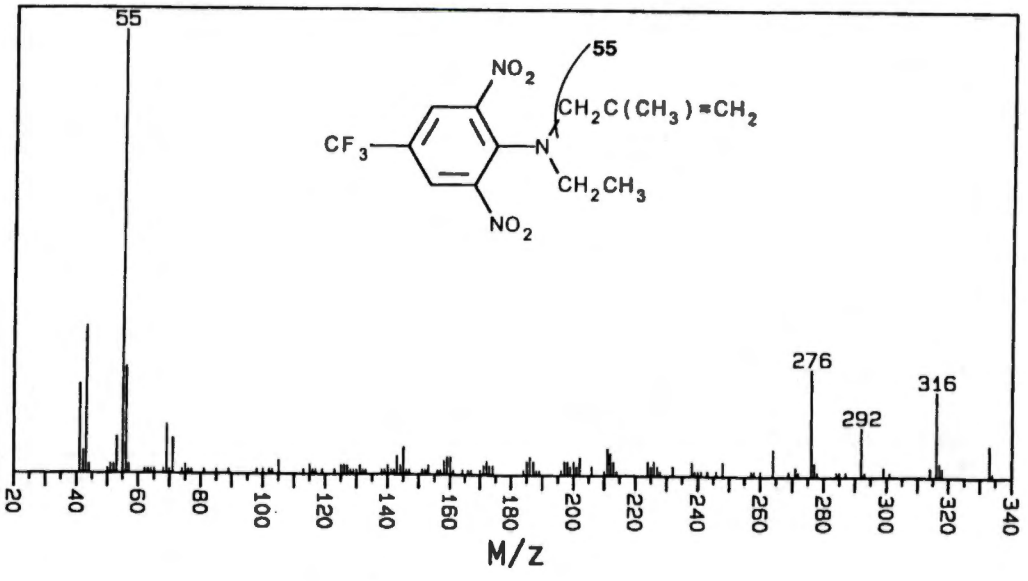
APPENDIX A.

Mass spectra of 7 pesticides and internal standard.

Ethalfluralin, Merck No: 3671

CAS No: 55283-68-6, Formula: $C_{13}H_{14}F_3N_3O_4$, MW: 333.0936

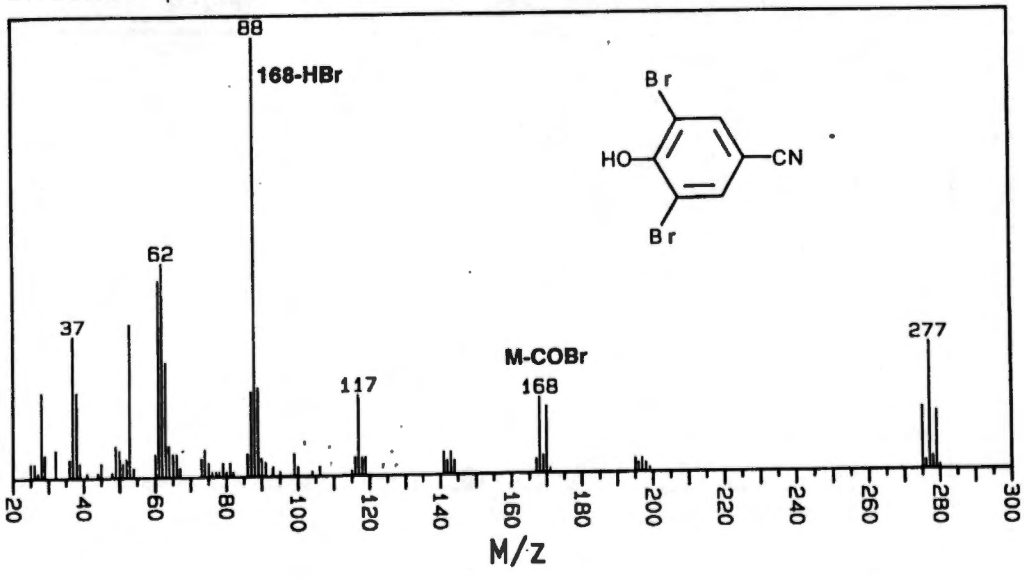
Intense peaks: 55 (100), 43 (33), 276 (24), 56 (24)



Bromoxynil, Merck No: 1431

CAS No: 1689-84-5, Formula: $C_7H_3Br_2NO$, MW: 274.8582

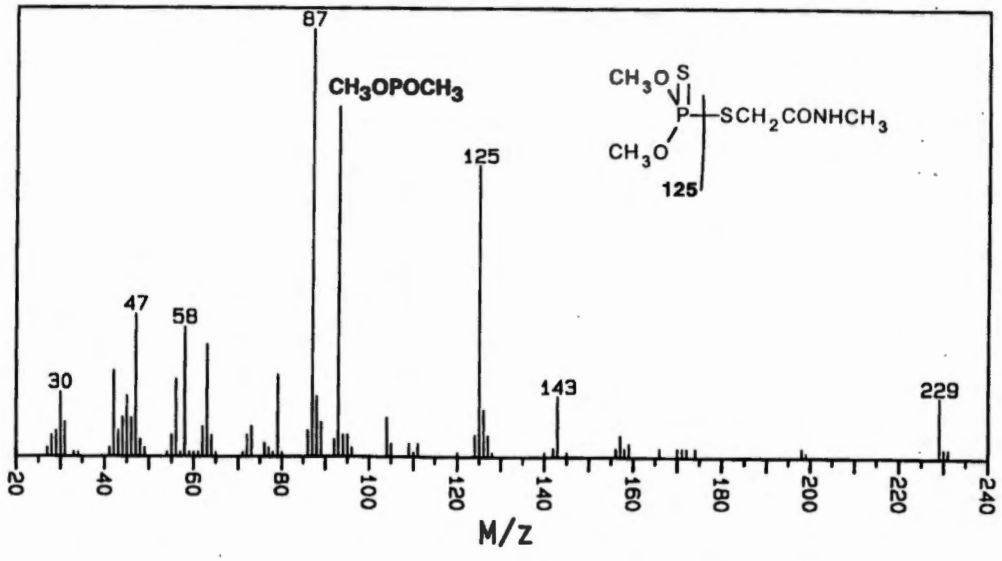
Intense peaks: 88 (100), 62 (49), 61 (45), 53 (35)



Dimethoate, Merck No: 3209

CAS No: 60-51-5, Formula: $C_5H_{12}NO_3PS_2$, MW: 228.9996

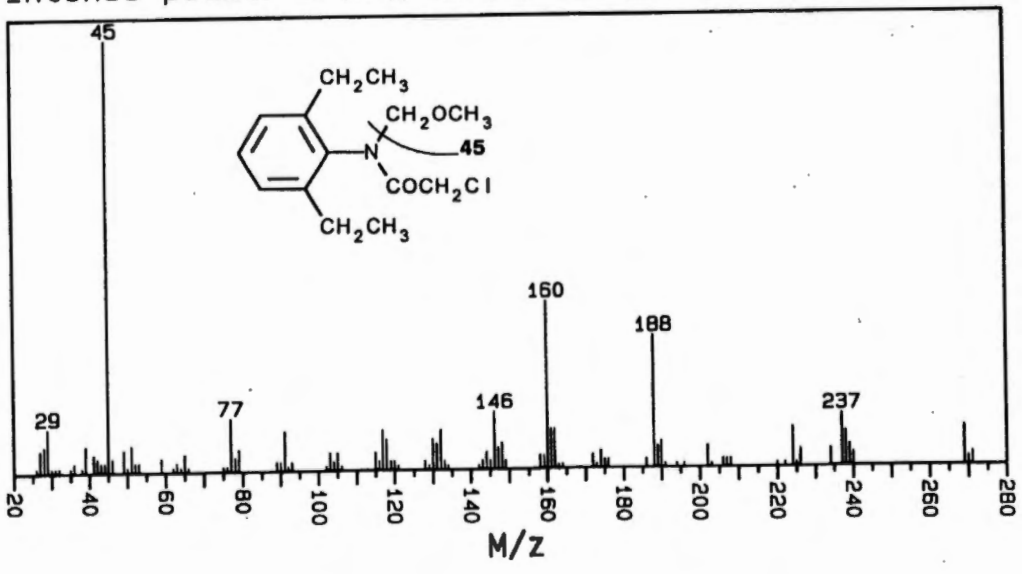
Intense peaks: 87 (100), 93 (82), 125 (68), 47 (33)



Alachlor, Merck No: 193

CAS No: 15972-60-8, Formula: $C_{14}H_{20}ClNO_2$, MW: 269.1182

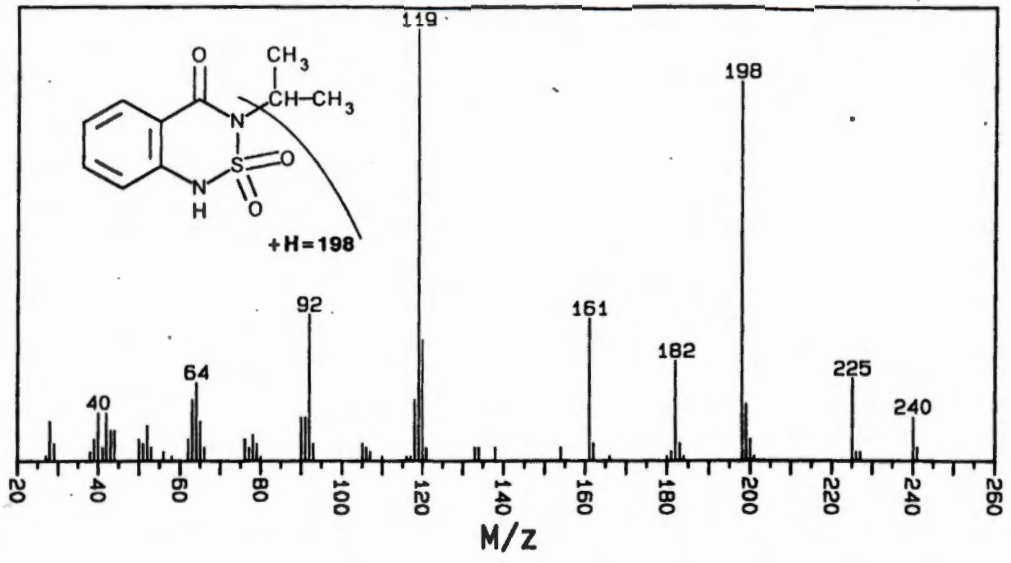
Intense peaks: 45 (100), 160 (38), 188 (30), 146 (13)



Bentazon, Merck No: 1060

CAS No: 25057-89-0, Formula: $C_{10}H_{12}N_2O_3S$, MW: 240.0569

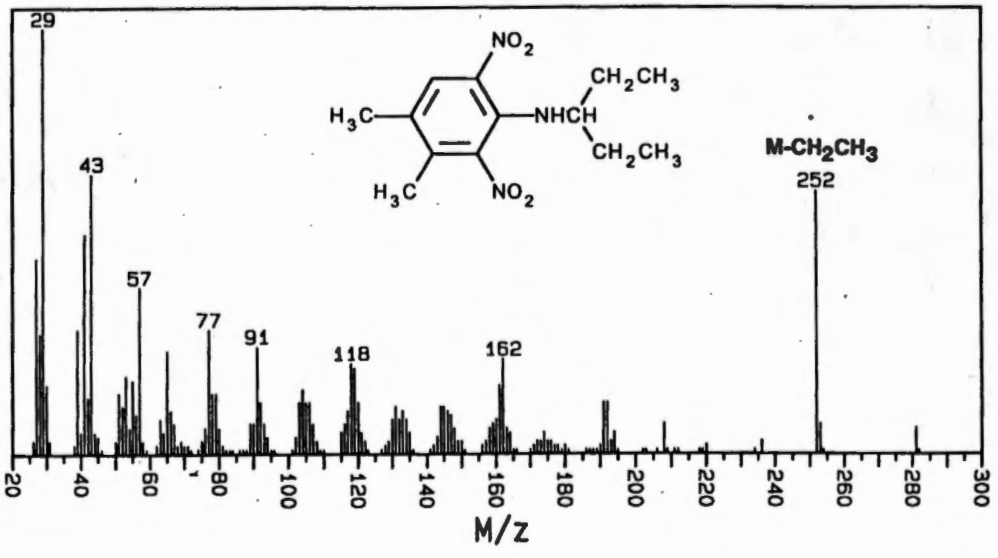
Intense peaks: 119 (100), 198 (88), 92 (34), 161 (33)



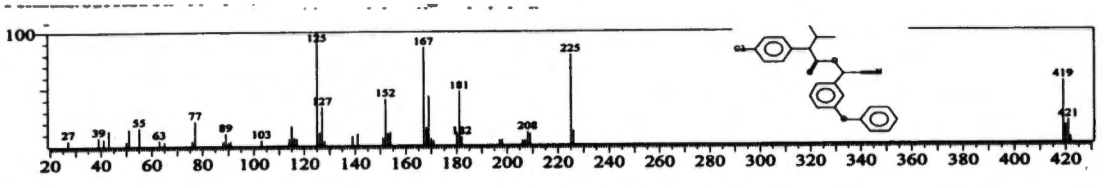
Pendimethalin, Merck No: 7026

CAS No: 40487-42-1, Formula: $C_{13}H_{19}N_3O_4$, MW: 281.1375

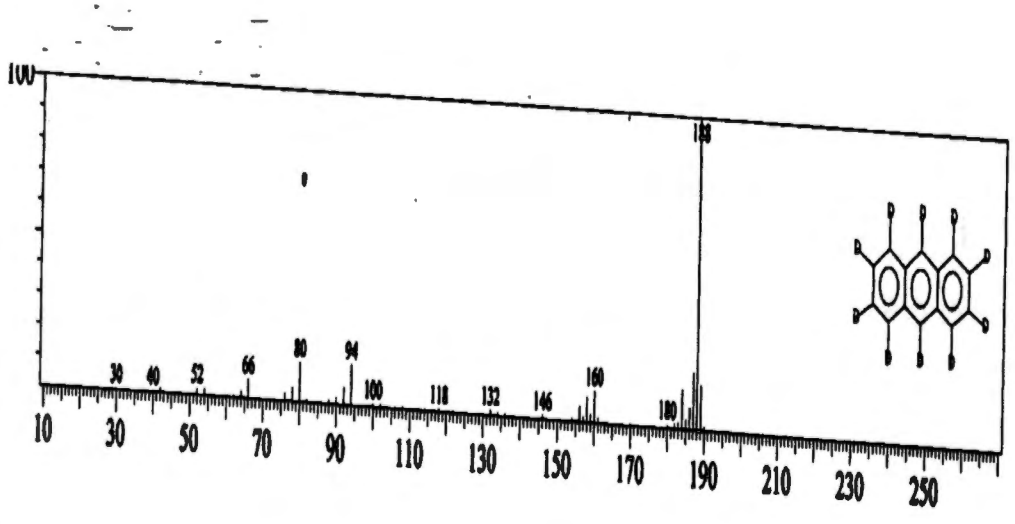
Intense peaks: 29 (100), 43 (66), 252 (62), 41 (52)



Esfenvalerate
CAS No: 66230-4-4 Formula: $C_{25}H_{22}ClNO_3$ MW: 419
Intense peaks: 125(100) 167(86) 225(79) 181(47)



Anthracene-d10
CAS No: 1719-06-8 Formula: $C_{14}D_{10}$ MW: 188
Intense peaks: 188(100) 43(20) 189(18) 80(17)



APPENDIX B.

The analysis of variance for the relative response factors of 7 pesticides to the internal standard

B-1 The analysis of variance for the relative response factors of pesticide ethalfluralin to the internal standard

---------- PKNO=1 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	40 70 100
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	9.24E-6
Residual	0.003035

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	20.62	0.0463
rep	1	2	0.01	0.9410

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	40		0.3143	0.03901	2	8.05	0.0151
conc	70		0.6632	0.03901	2	17.00	0.0034
conc	100		0.5420	0.03901	2	13.89	0.0051
rep		1	0.5046	0.03186	2	15.84	0.0040
rep		2	0.5084	0.03186	2	15.96	0.0039

B-2 The analysis of variance for the relative response factors of pesticide bromoxynil to the internal standard

---------- PKNO=2 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	200 350 500
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	0.000031
Residual	4.964E-9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	449.68	0.0022
rep	1	2	38.30	0.0251

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	200		0.2567	0.003961	2	64.83	0.0002
conc	350		0.4137	0.003961	2	104.45	<.0001
conc	500		0.2833	0.003961	2	71.54	0.0002
rep		1	0.3321	0.003234	2	102.69	<.0001
rep		2	0.3038	0.003234	2	93.93	0.0001

B-3 The analysis of variance for the relative response factors of pesticide dimethoate to the internal standard

---------- PKNO=3 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	100 175 250
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	4.277E-7
Residual	0.000654

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	9.13	0.0987
rep	1	2	15.85	0.0577

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	100		0.3167	0.01809	2	17.51	0.0032
conc	175		0.3898	0.01809	2	21.55	0.0021
conc	250		0.2828	0.01809	2	15.64	0.0041
rep		1	0.3714	0.01477	2	25.15	0.0016
rep		2	0.2882	0.01477	2	19.52	0.0026

B-4 The analysis of variance for the relative response factors of pesticide alachlor to the internal standard

---------- PKNO=5 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	40 70 100
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	0.000075
Residual	0.008645

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	20.05	0.0475
rep	1	2	14.34	0.0632

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	40		1.2418	0.06603	2	18.81	0.0028
conc	70		1.6743	0.06603	2	25.36	0.0016
conc	100		1.1088	0.06603	2	16.79	0.0035
rep		1	1.4860	0.05391	2	27.56	0.0013
rep		2	1.1973	0.05391	2	22.21	0.0020

B-5 The analysis of variance for the relative response factors of pesticide bentazon to the internal standard

---------- PKNO=6 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	200 350 500
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	3.299E-7
Residual	0.000574

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	5.16	0.1625
rep	1	2	8.64	0.0989

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	200		0.1837	0.01695	2	10.84	0.0084
conc	350		0.1859	0.01695	2	10.97	0.0082
conc	500		0.1182	0.01695	2	6.97	0.0200
rep		1	0.1913	0.01384	2	13.83	0.0052
rep		2	0.1338	0.01384	2	9.67	0.0105

B-6 The analysis of variance for the relative response factors of pesticide pendimethalin to the internal standard

---------- PKNO=7 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	100 175 250
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	9.884E-9
Residual	0.000099

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	42.54	0.0230
rep	1	2	4.84	0.1589

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	100		0.1522	0.007051	2	21.59	0.0021
conc	175		0.2440	0.007051	2	34.61	0.0008
conc	250		0.1934	0.007051	2	27.44	0.0013
rep		1	0.2055	0.005757	2	35.70	0.0008
rep		2	0.1876	0.005757	2	32.59	0.0009

B-7 The analysis of variance for the relative response factors of pesticide esfenvalerate to the internal standard

---------- PKNO=8 ----------

The Mixed Procedure

Class Level Information

Class	Levels	Values
conc	3	200 350 500
rep	2	1 2

Covariance Parameter Estimates

Cov Parm	Estimate
conc*rep	4.461E-8
Residual	0.000211

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
conc	2	2	1.47	0.4054
rep	1	2	7.64	0.1098

Least Squares Means

Effect	conc	rep	Estimate	Standard Error	DF	t Value	Pr > |t|
conc	200		0.04082	0.01028	2	3.97	0.0579
conc	350		0.05196	0.01028	2	5.06	0.0370
conc	500		0.06567	0.01028	2	6.39	0.0236
rep		1	0.06921	0.008391	2	8.25	0.0144
rep		2	0.03642	0.008391	2	4.34	0.0492

APPENDIX C.

T- test for RRx of the internal standard
between method 1 vs. 2

The Mixed Procedure

The MEANS Procedure

Analysis variable: diff

T Value	Pr > [t]
5.49	<.0001

APPENDIX D.

The analysis of variance for the percentage recoveries of 7 pesticides from different vegetables and cleanup methods.

D-1 -- Analysis of variation for percent recovery of ethalfluralin from different vegetables extracted and cleanup by different methods

The Mixed Procedure
Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	2.66	0.1492
mmet	2	12	135.76	<.0001
mveg*mmet	4	12	1.77	0.1988

---------- Effect=mveg Method=LSD(P<.05) Set=1 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
1	1	1	_	26.4242	1.9661	A
2	1	2	_	28.3349	1.9661	A
3	1	3	_	32.6776	1.9661	A

---------- Effect=mmet Method=LSD(P<.05) Set=2 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
4	1	_	1	52.7181	1.9661	A
5	1	_	2	6.9650	1.9661	C
6	1	_	3	27.7537	1.9661	B

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=3 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
7	1	1	1	49.2875	3.4054	A
8	1	1	2	4.2207	3.4054	D
9	1	1	3	25.7644	3.4054	BC
10	1	2	1	53.2917	3.4054	A
11	1	2	2	1.2475	3.4054	D
12	1	2	3	30.4656	3.4054	B
13	1	3	1	55.5750	3.4054	A
14	1	3	2	15.4267	3.4054	C
15	1	3	3	27.0311	3.4054	B

D-2 -- Analysis of variation for percent recovery of bromoxynil from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	4.80	0.0569
mmet	2	12	4.80	0.0294
mveg*mmet	4	12	4.93	0.0139

---------- Effect=mveg Method=LSD(P<.05) Set=4 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
16	2	1	_	-178E-17	3.8830	B
17	2	2	_	0.2622	3.8830	B
18	2	3	_	14.8617	3.8830	A

---------- Effect=mmet Method=LSD(P<.05) Set=5 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
19	2	_	1	6.06E-16	3.8830	B
20	2	_	2	14.8617	3.8830	A
21	2	_	3	0.2622	3.8830	B

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=6 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
22	2	1	1	-178E-17	6.7256	B
23	2	1	2	-533E-17	6.7256	B
24	2	1	3	1.78E-15	6.7256	B
25	2	2	1	-603E-17	6.7256	B
26	2	2	2	-603E-17	6.7256	B
27	2	2	3	0.7867	6.7256	B
28	2	3	1	9.62E-15	6.7256	B
29	2	3	2	44.5850	6.7256	A
30	2	3	3	2.52E-15	6.7256	B

D-3 -- Analysis of variation for percent recovery of dimethoate from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	27.84	0.0009
mmet	2	12	414.77	<.0001
mveg*mmet	4	12	49.94	<.0001

---------- Effect=mveg Method=LSD(P<.05) Set=7 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
31	3	1	_	35.6866	3.6289	C
32	3	2	_	53.0359	3.6289	B
33	3	3	_	73.9243	3.6289	A

---------- Effect=mmet Method=LSD(P<.05) Set=8 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
34	3	_	1	1.78E-14	3.2888	C
35	3	_	2	123.68	3.2888	A
36	3	_	3	38.9678	3.2888	B

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=9 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
37	3	1	1	0	5.6964	F
38	3	1	2	58.3853	5.6964	C
39	3	1	3	48.6744	5.6964	CD
40	3	2	1	3.55E-15	5.6964	F
41	3	2	2	129.96	5.6964	B
42	3	2	3	29.1511	5.6964	E
43	3	3	1	4.97E-14	5.6964	F
44	3	3	2	182.70	5.6964	A
45	3	3	3	39.0778	5.6964	DE

D-4 -- Analysis of variation for percent recovery of alachlor from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	9.74	0.0131
mmet	2	12	26.35	<.0001
mveg*mmet	4	12	23.23	<.0001

---------- Effect=mveg Method=LSD(P<.05) Set=10 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
46	5	1	_	56.4470	5.5455	B
47	5	2	_	62.5795	5.5455	B
48	5	3	_	89.0159	5.5455	A

---------- Effect=mmet Method=LSD(P<.05) Set=11 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
49	5	_	1	81.6505	5.5455	A
50	5	_	2	89.5935	5.5455	A
51	5	_	3	36.7985	5.5455	B

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=12 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
52	5	1	1	66.4917	9.6051	C
53	5	1	2	62.5004	9.6051	C
54	5	1	3	40.3489	9.6051	CD
55	5	2	1	115.85	9.6051	B
56	5	2	2	42.1850	9.6051	CD
57	5	2	3	29.7022	9.6051	D
58	5	3	1	62.6083	9.6051	C
59	5	3	2	164.10	9.6051	A
60	5	3	3	40.3444	9.6051	CD

D-5 -- Analysis of variation for percent recovery of bentazon from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	0.74	0.5148
mmet	2	12	0.74	0.4963
mveg*mmet	4	12	1.79	0.1955

---------- Effect=mveg Method=LSD(P<.05) Set=13 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
61	6	1	_	-444E-18	2.6437	A
62	6	2	_	4.3411	2.6437	A
63	6	3	_	3.3732	2.6437	A

---------- Effect=mmet Method=LSD(P<.05) Set=14 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
64	6	_	1	4.3411	2.6437	A
65	6	_	2	3.3732	2.6437	A
66	6	_	3	-592E-18	2.6437	A

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=15 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
67	6	1	1	-133E-17	4.5790	A
68	6	1	2	0	4.5790	A
69	6	1	3	0	4.5790	A
70	6	2	1	13.0233	4.5790	A
71	6	2	2	-101E-17	4.5790	A
72	6	2	3	-101E-17	4.5790	A
73	6	3	1	1.89E-15	4.5790	A
74	6	3	2	10.1197	4.5790	A
75	6	3	3	-77E-17	4.5790	A

D-6 -- Analysis of variation for percent recovery of pendimethalin from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	4.68	0.0597
mmet	2	12	82.57	<.0001
mveg*mmet	4	12	2.85	0.0716

---------- Effect=mveg Method=LSD(P<.05) Set=16 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
76	7	1	_	67.8853	5.1610	B
77	7	2	_	70.2906	5.1610	B
78	7	3	_	88.3059	5.1610	A

---------- Effect=mmet Method=LSD(P<.05) Set=17 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
79	7	_	1	117.90	5.1610	A
80	7	_	2	25.1296	5.1610	C
81	7	_	3	83.4485	5.1610	B

---------- Effect=mveg*mmet Method=LSD(P<.05) Set=18 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
82	7	1	1	114.49	8.9390	AB
83	7	1	2	12.1989	8.9390	E
84	7	1	3	76.9711	8.9390	CD
85	7	2	1	121.19	8.9390	A
86	7	2	2	6.0800	8.9390	E
87	7	2	3	83.6067	8.9390	CD
88	7	3	1	118.04	8.9390	A
89	7	3	2	57.1100	8.9390	D
90	7	3	3	89.7678	8.9390	BC

D-7 -- Analysis of variation for percent recovery of esfenvalerate from different vegetables extracted and cleaned up by different methods

Class Level Information

Class	Levels	Values
mveg	3	1 2 3
mrep	3	1 2 3
mmet	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mveg	2	6	28.42	0.0009
mmet	2	12	157.82	<.0001
mveg*mmet	4	12	36.32	<.0001

---------- Effect=mveg Method=LSD(P<.05) Set=19 ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	Letter Group
91	8	1	_	157.43	9.0407	A
92	8	2	_	102.51	9.0407	B
93	8	3	_	61.3698	9.0407	C

---------- ADJUSTMENT=LSD(.05) BYGROUP=20 Effect=mmet ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	DF	t Value	Pr > |t|	Let Grp
94	8	_	1	90.9874	9.0407	12	10.06	<.0001	B
95	8	_	2	2.4439	9.0407	12	0.27	0.7915	C
96	8	_	3	227.88	9.0407	12	25.21	<.0001	A

---------- ADJUSTMENT=LSD(.05) BYGROUP=21 Effect=mveg*mmet ----------

Obs	mpkno	mveg	mmet	Estimate	Standard Error	DF	t Value	Pr > |t|	Let Grp
97	8	1	1	72.0200	15.6589	12	4.60	0.0006	D
98	8	1	2	-853E-16	15.6589	12	-0.00	1.0000	E
99	8	1	3	400.27	15.6589	12	25.56	<.0001	A
100	8	2	1	127.42	15.6589	12	8.14	<.0001	C
101	8	2	2	-142E-16	15.6589	12	-0.00	1.0000	E
102	8	2	3	180.10	15.6589	12	11.50	<.0001	B
103	8	3	1	73.5189	15.6589	12	4.70	0.0005	D
104	8	3	2	7.3317	15.6589	12	0.47	0.6480	E
105	8	3	3	103.26	15.6589	12	6.59	<.0001	CD

VITA

Min Huang was born on May 25, 1969, at Chengdu, P. R. China. She is the daughter of Mr. Chao-dun Huang and Ms. Zhen-rong Pu. She graduated from Chengdu Shi-Shi Middle School and then majored in Cereals & Oils Science and Technology at Wu'xi University of Light Industry, P. R. China receiving her Bachelor of Science Degree in June 1991. After graduating, she served as the assistant engineer at the Chengdu Vegetable Oil Mill for several years. Before she came to the United States, she also worked in the business area at XEROX (China) Ltd., Chengdu Office.

In the spring 2000, Min started her graduate studies in Food Science and Technology at The University of Tennessee, Knoxville. She was a Graduate Research Assistant while working on her degree. She is a member of Gamma Sigma Delta, the Honor Society of Agriculture, and a member of the Institute of Food Technologists.

Min is married to Ge Zhang, and they have a 6-month old daughter, Tracy Xinyi Zhang.