Spring 5-7-2019

A More Efficient Method for Extracting and Analyzing Pesticides in Baby foods

Oluwatobi Fabunmi

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This project was supported by the Department of Chemistry and Biochemistry at Kennesaw State University. Also, fractions of this research work were presented at the Southeastern Regional Meeting of the American Chemical Society and The Federation of Analytical Chemistry and Spectroscopy Societies.

During my quest of a Master in the Chemical Sciences, I am deeply thankful to God. To my committee chair, Dr. Huggins Msimanga, I am thankful for his guidance and supervision while working on this project. He has been a major source of motivation and inspiration. Also, my other principal investigator Dr. Christopher Dockery, I am thankful of the effort you put into making this research work a success. To Dr. Thomas Leeper, my other committee member, thank you for your contribution to this project. Many thanks to Dr. Koether, my colleagues, most especially Opeyemi Omosebi, Latanya Downer, and Dianne Diatta. Many thanks to Georgia Department of Agriculture for sharing the results obtained from the Pesticide analysis carried out using the QuEChERS method, as the research was based on obtaining a better result through method development.

I would love to thank my parents and family who have encouraged and motivated me throughout this journey. My sincere appreciation goes to Uncle Bimbo, Aunt Iyabo, Uncle Tunde, Aunt Jibola, Timi and Tade. It is with your love and support that I have completed and achieved this dream of mine with great success.

Finally, I would love to thank the laboratory staff and IT department for their prompt availability during the research work. I am thankful to Ben Huck and Matthew Rosenberg for their support in providing all that was needed for the research work. The successful completion was a collective effort and I am sincerely grateful.

ABSTRACT

Pesticides that are used to control pests such as insects, rodents, bacteria, mold, and fungus in food production end up in the fruits and vegetables that we consume. Clearly, concentration levels of pesticides must be carefully monitored. Successful monitoring of the concentrations is critically dependent upon pesticide extraction efficiency, the pesticide structure and the matrix (food product) in which the pesticide is found. Variables such as polarity, solubility, and pH must be investigated. A common approach to develop analysis methods involves spiking food products with pesticides and evaluating method efficiency by calculating percent recoveries from the foods. Sample results from the Georgia Department of Agriculture Labs (Tifton, Ga) showed in some cases, a range of 84% to 140% recoveries for some pesticides on the lower end of ppm concentration levels. These recoveries were obtained from fruit extracts such as peaches, bananas, carrots, and green beans that provided very complex matrices. In this study, recovery range of 95% to 105% is our plausible goal to establish the efficiency of our extraction technique.

We propose to develop a method that will improve percent recoveries by modifying the QuEChERS methods. This proposal entails spiking fruit matrices with known amounts of pesticides and studying percent recoveries by quantifying the extracts with standard instruments like High-performance liquid chromatography-photodiode array (HPLC-PDA), Gas chromatography-mass spectrometer (GC/MS), and Ultra-performance liquid chromatography-ultraviolet (UPLC/UV). The focus will be on improving the extraction process. Starting with eight pesticides, we will study structural differences in the pesticides via middle-infrared spectroscopy to establish extraction compatibility. Also, relative polarities under different pH conditions will be determined using reverse-phase HPLC/UV. This aspect will help with optimizing the organic solvents to be ultimately used for extraction. Lastly, the optimized conditions will be used to analyze bulk pesticides using HPLC-PDA and UPLC-multiwavelength detector (UPLC-MWD) for comparison.

An efficient method was developed by modifying the QuEChERS method using liquid-liquid extraction and the percent recoveries were satisfactory and showed a good precision.

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ACRONYMS

ACN	Acetonitrile
ANOVA	Analysis of variance
ATR	Attenuated total reflectance
DF	Degree of freedom
EPA	Environmental Protection Agency
FTIR	Fourier Transform Infrared
GC	Gas chromatography
GDOA	Georgia Department of Agriculture
HPLC	High performance liquid chromatography
IR	Infrared
LC	Liquid chromatography
LIBS	Laser Induced Breakdown Spectroscopy
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
mL	milliliter
MS	Mass spectrometry
MRL	Maximum Residue Limits
MWD	Multi-wavelength detector
NIR	Near infrared
OCPs	Organochlorine pesticides
OPPs	Organophosphate pesticides
PC	Principal Component

PCA	Principal Component Analysis
PDA	Photodiode array
PLSDA	Partial Least Squares Discriminant Analysis
ppm	Parts per million
QuEChERS	quick, easy, cheap, effective, rugged, and safe
R^2	Square of the correlation coefficient
RPM	Revolutions per minute
RSD	relative standard deviation
SPE	Solid-phase extraction
UPLC	Ultra Performance Liquid Chromatography
UV/Vis	Ultraviolet / visible
VF	Volumetric flask
v/v	Volume/volume
w/w	Weight/weight
ZnSe	Zinc selenide

CHAPTER 1. BACKGROUND TO PESTICIDES

1.1 Introduction

Pesticides are chemical substances used in the agricultural and food industry to control pests. The use of pesticides is of importance as it improves food production and protects plants from pests and weeds. Pesticide is a general term which includes herbicide, rodenticide, insecticide and fungicide depending on the target pest to be controlled. Many pesticides are associated with health issues due to their carcinogenic nature as well as environmental hazards. [1-2]

The most widely used pesticides in pest control are the organochlorine, organophosphorus and carbamate pesticides. Other classes of pesticides include the neonicotinoid, triazine and phenyl urea. This class of pesticides are associated with various health effects due to their ability to inhibit the function of the enzyme cholinesterase.[4]

Extensive review on various classes of pesticides can be found in several publications based on their uses and applications internationally. Pesticides serve various purposes which include occupational pesticide use, commercial applications to foodstuffs, and household applications such as the insecticides which are specific to insect control.[4-5]

1.2 History of Pesticides

Historical background of pesticides dates back to the beginning of agriculture and it became pronounced as a result of increased pest population which was directly proportional to decreasing soil fertility.[6] Paul Muller discovered that DDT was an effective insecticide. DDT belongs to the class of organochlorine pesticides and it was dominant. However, by 1975 DDT were replaced in the U.S. by organophosphate and carbamates. The original definition of pesticide in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) came from earlier California law. According to FIFRA, an organism is declared as a pest if it is deleterious to man or the

environment. In 1997, FIFRA laid out foundation for the regulation of pesticide by the federal government. Currently, pesticides are regulated by three federal agencies which are the US Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA).[7-9]

1.3 Common Uses of Pesticides

After world war II, there was an emergence in pesticide use, firstly with the introduction of DDT, BHC, aldrin, dieldrin and endrin. DDT was especially favored for its broad-spectrum activity against insect pests of agriculture and human health.[11]Pesticides are used to control pests such as ticks, mites and insects. In agriculture and lawns, pesticides control weeds, insects and crop diseases.

1.4 Classification of pesticides based on Chemical structure

Pesticides are classified according to the chemical nature of their active ingredients. The chemical composition of pesticides is significant to researchers because it gives an idea about the efficacy, physical and chemical properties of the pesticides. Chemical classification of pesticides is based on their functional group, namely, organochlorines, organophosphorus, carbamates, pyrethrin and pyrethroids.[10]

Organochlorine pesticides (OCs) are organic compounds with five or more chlorine atoms attached to it while organophosphate pesticides (OPs) contain a phosphate group as its basic structural framework. Some of the widely used organophosphate pesticides are malathion, parathion and diazinon. Carbamates are another class of organic pesticide derived from carbamic acid. Pyrethrin's are naturally occurring organic pesticides derived from the Chrysanthemum flower. Figure 1 is a structural display of the studied pesticides. Table 1 summarizes the physical properties of studied pesticides.

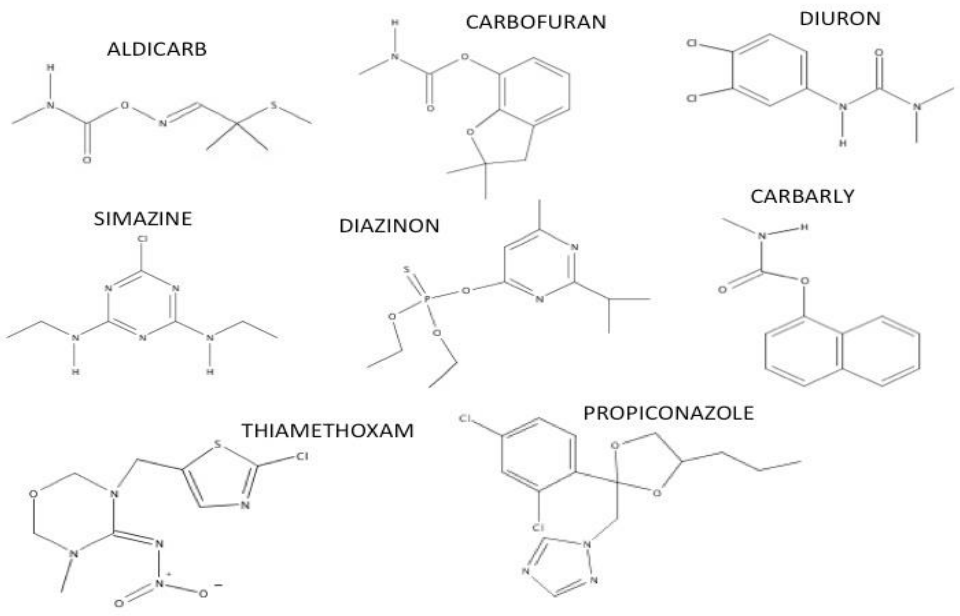


Figure 1. Chemical structure of selected pesticides.

1.5 Pesticide toxicity

Pesticide toxicity is measured by their lethal dose (LD_{50},) which is the dose needed to kill 50 percent of laboratory test animals (usually measured as milligrams of poison per kilogram body weight). The smaller the LD_{50}, the more toxic the poison. Also, chronic risk resulting from pesticides intake is difficult to measure as it is based upon the type of pesticide, length of exposure, dose and genetic differences among the organism involved.[10] The dermal lethal dose of studied pesticides are shown in Table 2. Biological activity of pesticides specific to a target pest is mainly influenced by its physical and chemical properties. The physical properties of individual pesticides determine its mode of action, dosage, mode of application and its interaction in the environment.

Table 1. Type and physical properties of studied pesticides**.**

Pesticide Name	Type	Physical Properties	Health Effects	Route of entry
Aldicarb	Carbamate pesticide	White crystals with a slightly sulfurous odor	Super toxic: probable oral lethal dose for humans	Inhalation, skin and by ingestion
Carbaryl	Carbamate pesticide	White crystalline solid	Highly toxic, may be fatal if inhaled, swallowed or absorbed through the skin	Inhalation, skin absorption, ingestion, skin and/or eye contact
Carbofuran	Carbamate pesticide	Odorless white crystalline solid	Fatal if inhaled, fatal if swallowed, very toxic to aquatic life with long lasting effects	Inhalation, skin absorption, ingestion, skin and/or eye contact
Diuron	Phenyl urea	White crystalline solid	Suspected of causing cancer	Inhalation, ingestion, skin and/or eye contact
Diazinon	Organophosphorus pesticide	Toxic colorless liquid	Eye and skin irritant, may cause gastrointestinal symptoms	Inhalation, ingestion, skin
Simazine	Triazine pesticide	solid	Suspected of causing cancer, very toxic to aquatic life with long lasting effects	Inhalation or by ingestion
Thiamethoxam	Neonicotinoid insecticide	Crystalline powder	Harmful if swallowed and very toxic to aquatic life	Inhalation or by ingestion
Propiconazole	Triazole fungicide	Yellowish odorless liquid	Harmful if swallowed, may cause an allergic skin reaction and very toxic to aquatic life	Inhalation or by ingestion

Table 2. Shows the maximum residue limit and lethal dose of studied pesticides.

Pesticide	Commodity	Maximum residue limits (banana) mg/kg	LD_{50} – Dermal
Aldicarb	Bananas	0.20	>2000
Carbaryl	Bananas	0.10	850
Carbofuran	Bananas	0.10	>3000
Diuron	Bananas	0.10	>5000
Diazinon	Bananas	0.20	3600
Simazine	Bananas	0.20	>3100
Thiamethoxam	Bananas	0.30	>2000
Propiconazole	Bananas	0.20	>4000

CHAPTER 2. CURRENT METHODS FOR PESTICIDES ANALYSIS

2.1 Introduction

Broad research has been carried out on multiresidue method for pesticides determination in fruits and vegetables using various extraction techniques and instrumentation methods. These researches can be found in various publications.[12-16] Standard techniques used for the determination of pesticides in fruits and vegetables are gas chromatography (GC) coupled with mass spectrometry (MS), electron-capture detector (ECD), flame-photometric detector (FPD), nitrogen phosphorus detector (NPD), thermionic specific detector (TSD), liquid chromatography (i.e. HPLC, LC-MS). Each of these techniques are employed for the determination of various pesticide classes, such as the organophosphorus and the organonitrogen pesticides.[17-21]

In the past, liquid-liquid extraction (LLE) was employed as the main extraction technique in pesticide analysis. However, there is need to cut down on cost of analysis as LLE consumes more organic solvent and is more laborious. Recently, solid phase extraction (SPE), supercritical-fluid extraction and solid phase microextraction are modern techniques been used for the analysis of pesticide residues due to its efficiency and reduced use of organic solvents for extraction. SPE method is of great advantage as it can be used for multiple preconcentration of pesticides, clean-up and water removal from the sorbent by air vacuum. Recent advances in determination of pesticides present in environmental samples are also been done using capillary electrophoresis (CE).[22] The current trend has shifted to the use of QuEChERS method for the extraction and preconcentration of pesticides. The simplicity of the method and ease of development make it very attractive for multiresidue pesticide determination. The sample preparation in this technique is followed by a quantitative analysis of the pesticides using these instruments such as LC-MS, UPLC and GC-MS.

2.2 Spectroscopy-based method

Spectroscopy is the science concerned with the investigation and measurement of spectra produced when materials interact with or emits electromagnetic radiation.[22] It is a commonly used technique employed for quantitative and qualitative analysis in various fields such as the food, agriculture and textile industry. Spectroscopy method is widely used due to its non-destructive mode of analysis as well as its durability, accuracy of measurement and reproducibility. It has been used in various research studies for the detection of pesticides residue in fruits and vegetables.[2]

2.2.1 Principles of spectroscopy

Spectroscopy works on the principle that all atoms and molecules absorb and emits light at certain wavelengths. It is a term used to refer to the measurement of radiation as a function of wavelength after the radiation from the source interacts with the sample. There are different spectroscopy techniques depending on the type of compound of study and the region in which atoms absorb and emit within the electromagnetic region, such as Ultraviolet-visible absorption spectroscopy (UV-Vis), infrared absorption spectroscopy (IR), and Raman spectroscopy. These techniques use optical materials to disperse and focus the radiation and are often identified as optical spectroscopies.[22]

2.2.2 Ultraviolet-Visible Spectroscopy

Ultraviolet-Visible (UV-Vis) spectroscopy is the commonly used analytical technique for detecting numerous molecules. The UV-Vis radiation (I_0) is passed through the sample and the unabsorbed light (I) is measured. The signal (absorbance) is given by ($-\log_{10}(I/I_0)$). The absorbance of a compound as a function of the wavelength range gives the spectrum which is unique to the compound. For a UV/Vis region the wavelength ranges from about 200 – 800 nm. Molecules containing double bonds and triple bonds are normally UV active.[22]

2.2.3 Fourier-transform infrared spectroscopy (FTIR/ATR)

Fourier transform infrared spectroscopy (FTIR) is an analytical technique used to obtain infrared spectrum of samples. Infrared techniques are capable of qualitative and quantitative analysis. This technique relies on the fact that molecules absorb at specific frequencies which is solely dependent on their chemical structure. There are three IR regions, near infrared (NIR) which covers 0.75 to 2.5μm wavelengths, mid-IR which covers 2.5 to 20 μm and far infrared regions which spans from 20 to 200 μm. For a molecule to be IR active, the molecule must have a non-zero dipole moment, thus not all molecules are IR active. FTIR can also detect the presence of specific functional groups in a sample and provide a unique fingerprint for it. When FTIR is coupled to attenuated total reflectance (ATR) an infrared spectrum is made capable of acquiring IR spectra directly on various phases of the sample (liquids, solids and gaseous). An advantage to the FTIR/ATR technique is that it provides faster sampling with minimal or no preparation step, non-destructive and excellent sample-to-sample reproducibility. The limitation involves ensuring as good as possible optical contact between the sample and the internal reflection element (IRE). Figure 2 summarizes the mode of action of the ATR accessory.

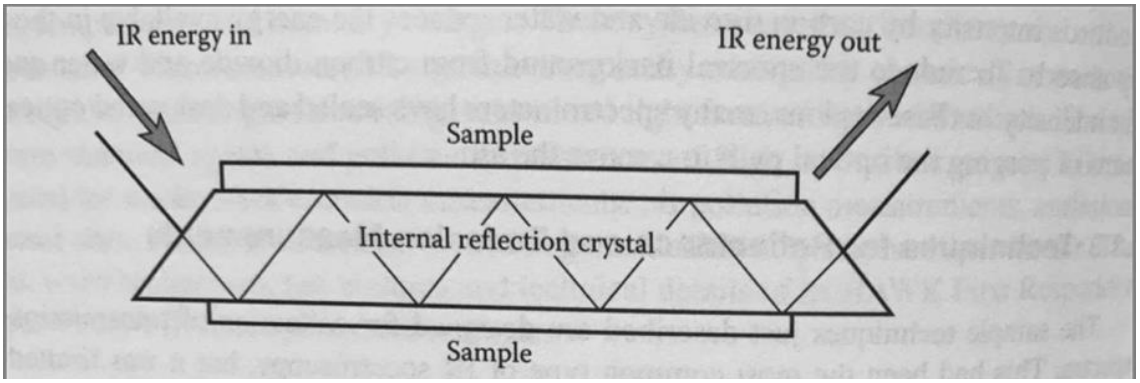


Figure 2. Schematic diagram of ATR sampling accessory.[23]

2.2.4 Laser-induced Breakdown Spectroscopy (LIBS)

Laser-induced breakdown spectroscopy is an atomic/ionic emission spectroscopic technique which uses a focused pulsed laser beam to generate plasma from the sample, also called laser-induced plasma spectroscopy.[24] The plasma contains atoms, ions and free electrons which emit electromagnetic radiation as the plasma cools down. The emitted light is resolved by a spectrometer to form a spectrum. Recently, LIBS has become an emerging analytical technique for characterization and identification of materials. This technique covers a broad range of elements, including lighter elements such as H, Be, Li, C, N, O, Na, K, Mg and Phosphorus.[24]

Recently, LIBS has been used for the detection of phosphorus and chlorine containing pesticide residues on fruit surfaces. This is as a result of the characteristic peaks of phosphorus at 213.62 nm, 214.91 nm, 253.6 nm and 255.33 nm and the characteristic peak of chlorine at 837.59 nm which are the major elements found in both organophosphorus pesticides and organochlorine pesticides. An advantage to the LIBS technique is due to consumption of a small amount of sample during the ablation process, it is non-destructive in nature and involves minimal or no sample preparation. A major limitation is the variation in the laser ablation and resultant plasma which affects reproducibility as well as detection limits (ppm).[29-30] Figure 3 shows the schematic representation of major components of a LIBS instrument.

2.3 Chromatography methods

Chromatography is an analytical method of separation in which components to be separated are distributed between two phases, stationary phase and mobile phase. The compounds to be separated are carried in the mobile phase through the stationary phase in a column. For successful separation, the affinities of the compounds towards the stationary phase or mobile phase must be different. The components of the sample interact with stationary phase and separate into bands.

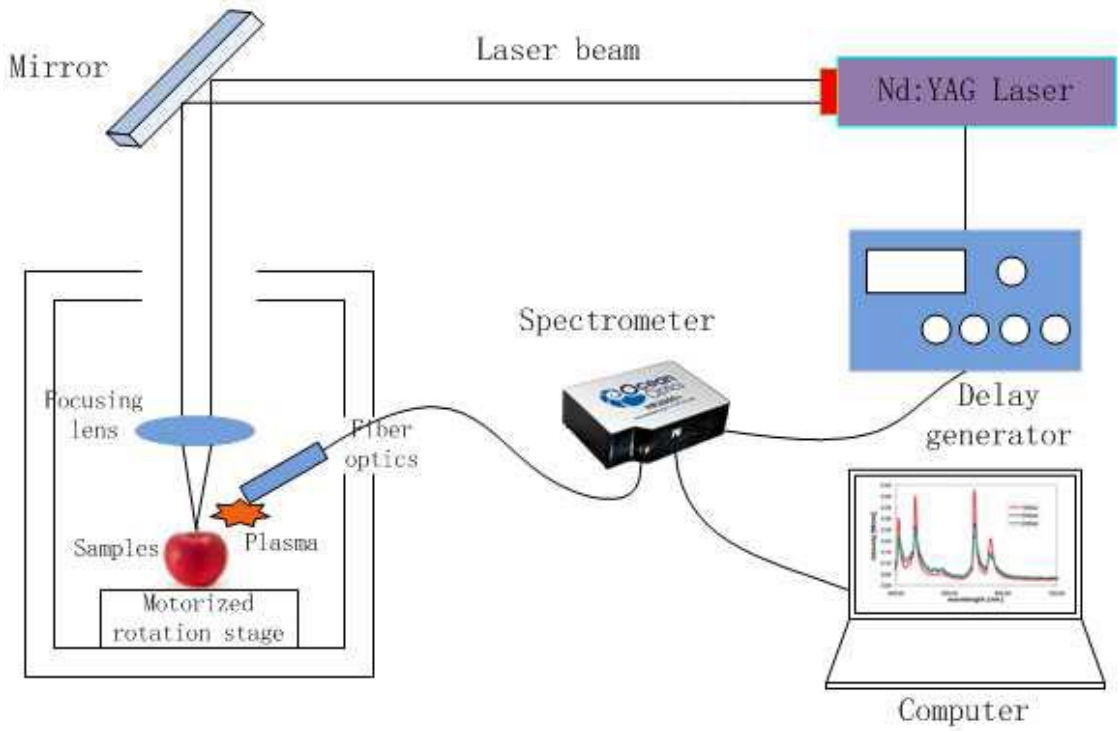


Figure 3. Schematic representation of the Laser induced breakdown spectroscopy.[11]

The samples appear at the end of the column in order of their interaction with the two phases.[5] The compounds that interact least elute first while the one that interacts most with the stationary phase elutes lastly. The mobile phase can either be gas or liquid, and the stationary phase is either liquid or solid. Gas chromatography (GC) has a gaseous mobile phase and a solid or liquid stationary phase. GC is suitable for separating thermally stable, volatile organic and inorganic compounds. High performance liquid chromatography (HPLC) has a liquid mobile phase and a solid stationary phase. It is used extensively for the separation of thermally unstable and organic compounds of different polarities. Based on the different polarities of compounds two modes of HPLC are commonly used, the normal phase and reverse phase. For normal phase HPLC, the stationary phase is a polar and the mobile phase is non-polar while for reverse phase HPLC the stationary phase is non-polar, and the mobile phase is a polar.

2.3.1 Principles of Liquid Chromatography

Liquid Chromatography (LC) is essentially a separation technique in which sample mixture in a liquid phase is subjected to a competitive distribution between two phases, one of which is a moving liquid and the other is a stationary solid (silica or alumina).[7]

The separation mechanism involves the use of pumps to push a liquid solvent containing a mixture of samples through a column made up of a solid adsorbent material. Each component in the sample mixture will interact with the adsorbent material present in the column in a slightly different way. Thus, different flow rates in the various components will cause the components to separate as they elute out of the column.

2.3.2 Mode of Operation

HPLC works by pumping at high pressure a sample (analyte) dissolved in an organic solvent which is the mobile phase through a column with a chromatographic packing material which is called the stationary phase. Compound separation can either be through isocratic elution, where the composition of the mobile phase is held constant or gradient elution in which the composition of the mobile phase is changed during the separation toward conditions favoring analyte dissociation from the stationary phase. On exiting the column, the eluents pass through a detection system, such as a UV-Visible detector where the signal is produced. The detector generates a signal corresponding to the quantity of analyte emerging from the column, which is then transferred to and recorded by the HPLC computer program and data is made available for subsequent preview and analysis. For a set of analytes of different concentrations, corresponding peak areas are used to create a calibration point for quantitative analysis.

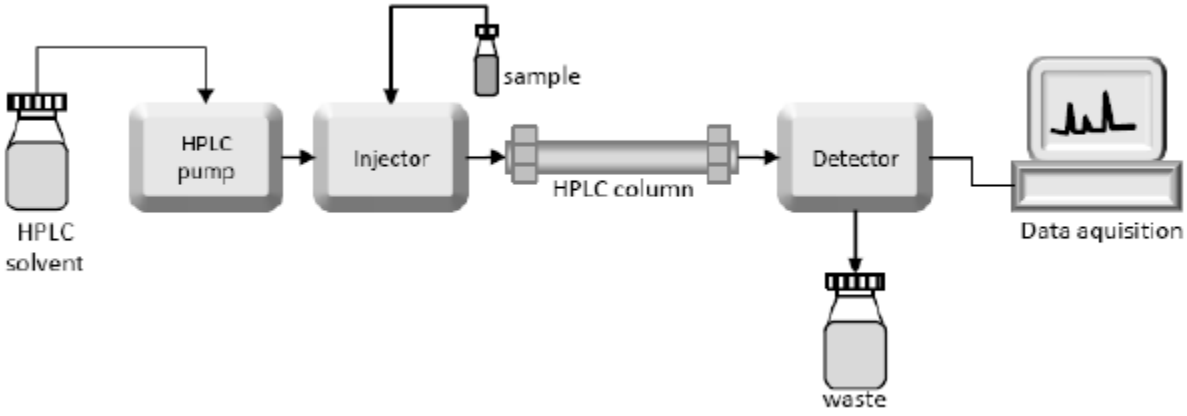


Figure 4. Schematic diagram of High-Performance Liquid Chromatography (HPLC) System.[6]

2.4 Ultra-Performance Liquid Chromatography

Ultra-Performance Liquid Chromatography is a chromatographic separation technique with unique possibilities in liquid chromatography. The UPLC is designed to withstand high system back-pressures and equipped with a C_{18} packed with 1.5 – 2 µm particles used in connection with the system. Separation on UPLC is performed under very high pressures (up to 100 MPA) without negative impact on the analytical column.[25]

2.4.1 Principle of Ultra Performance Liquid Chromatography

The UPLC principle is based on the use of a stationary phase consisting of particles less than 2.5 µm unlike HPLC columns filled with about 3 – 5 µm particles. The principles are governed by the Van Deemter equation, which is an empirical formula that

describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency. Given the equation, H = A +B/v + Cv

Where; A, B, and C are constants. v is the linear velocity, the carrier gas flow rate.

The A term represents 'eddy' mixing and is independent of velocity. It is smallest when the packed column particles are small and uniform. The B term represents natural diffusion or axial diffusion tendency of molecules. This effect is reduced at high flow rates and so this term is divided by v. The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time delay involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to stay behind molecules in the mobile phase. Thus, term is proportional to *v*.

2.5 Data processing and analysis

2.5.1 MS Excel

The preprocessing of spectra data set prior to analysis was done using MS Excel. Chemometric techniques require accurate experimental data to achieve good result for discrimination and comparison. Common preprocessing techniques used for spectroscopic data collected include, baseline correction, normalization, analysis of variance, (ANOVA), linear regression and correlation analysis. Firstly, baseline correction and normalization to unity ensure that the replicated spectra from the same instrument are of equal magnitude for true comparison. Also, if one is to compare a spectrum of acetaminophen with that of aspirin, normalization of the two spectra will highlight the structural differences in the two molecules. Normalization to unity ensures that intensity values for the total spectrum range from zero to unity. Normalization was carried out by dividing each absorbance point by the maximum absorbance point in the total

spectrum. However, preprocessing techniques tend to alter the original data set, optimum care is important when applying these changes.

ANOVA and correlation coefficients are employed to evaluate data precision within a batch and to obtain spectra similarities or differences within-group or between-groups. ANOVA is used to test multiple means, perform pairwise comparisons, and introduce within-group variance. In MS Excel, there are two types of ANOVA: one-way and multi-way. However, only one instrumental method will be used at a time in this study, one-way is appropriate. The two-way or multi-way analysis would be utilized when more than one instrument or method is used, while comparisons must be made between their differing units of measure.

2.5.2 Principal component analysis (PCA)

Principal component analysis is the most widely used chemometric technique. PCA is also sometimes referred to as single value decomposition (SVD) or eigenvector analysis.[26] In the PCA model, an orthogonal set of correlated variables is transformed into a set of linearly uncorrelated variables called principal components (PCs). These uncorrelated variables are then projected onto a two- or three-dimensional plot to achieve the following such as

1) summarizing and visualizing the data set,
2) multivariate classification and discriminant analysis and
3) discovering quantitative relationships among the variables.

PCA produces very quickly, a data summary showing how similar/different each of the observations are to one another, as well as any deviation from the groups in the data set. The data table for this analysis is the matrix obtained through each collected IR spectrum (intensity vs. wavenumber). While the matrices are quite large, they are not a challenge for PCA analysis.

This technique is extremely useful when the dimensionality of the measurements is large and the samples themselves exist in small dimensional space; meaning it handles data matrices with many more variables than observations extremely well. It can also handle data that are noisy or highly collinear (correlated). This small dimensional space relates to the number of principal components that are needed in order to define the information in the data set to the noise in the spectra. The number of principal components in the data set are less than or equal to the number of original variables. Determining the number of relevant PCs for inquiry is one of the greatest challenges, as choosing too many or too few could disrupt the interpretation of the data. Therefore, the process of dimensionality reduction through PCA must take place in order to make the data more easily visualized, thereby, reducing the time and possible memory required for analysis and helping to eliminate irrelevant or redundant features and to reduce noise. In summary, PCA highlights the underlying structure of a matrix by reducing the dimensionality of that matrix and summarizing the results into score plots. Mathematically, PCA decomposes the $\boldsymbol{X}$ matrix into a two meaningful matrices, $\boldsymbol{T}$ and $\boldsymbol{P}$, where $\boldsymbol{T}$ is the score matrix and $\boldsymbol{P}$ is the loading matrix as summarized in equation (1).

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^{t} + \boldsymbol{E}_{x} \qquad (1)$$

In Eq. (1), $\boldsymbol{T}$ is the score matrix and $\boldsymbol{P}^{t}$ is the transpose of the loadings, and $\boldsymbol{E}_{x}$ is the residual matrix.

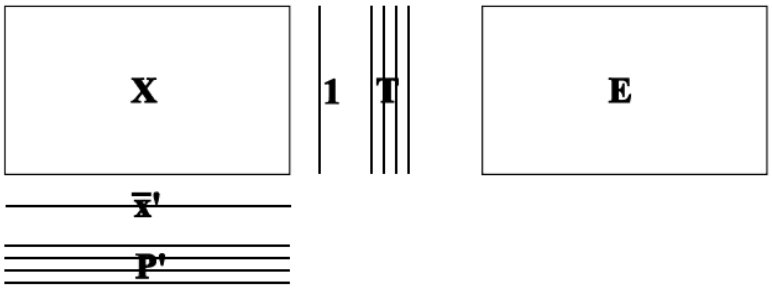


Figure 5. Summary sketch of PCA.

CHAPTER 3. DATA COLLECTION AND METHODOLOGY

3.1 Instrumentation

FTIR analysis was carried out using a Perkin Elmer Frontier 400 FTIR spectrometer equipped with a universal attenuated total reflectance accessory, which uses a diamond crystal in contact with ZnSe. LIBS analysis was performed using a LIBS 2000+, Ocean Optics Inc. equipped with a pulsed laser, glass fiber optics, a delay generator, spectrometer, and a housed sample chamber equipped with rotating sample holder. A Q-switched Nd:YAG laser emitting at 1064 nm and a repetition rate of 10 Hz was used as an excitation source. UV-Vis analyses were performed using the Cary 4000 high performance UV-visible spectrophotometer with photometric performance in the 175 – 900 nm range, controlled by the Cary WinUV software and equipped with temperature control and multicell holders.

HPLC analyses were performed using a Waters 2795 Alliance HT separation HPLC system equipped with an auto sampler and a photodiode array detector (Waters 2996) was employed. HPLC separation was achieved using a Luna C_{18} column (100 x 4.6 mm, i.d., 5µm particle size, Waters, Milford MA, US), with a mobile phase flow rate of 0.5 mL min^{-1} at a column temperature of 24.1℃ and sample maintained at 22℃. The HPLC operating pressure was 600 psi under isocratic conditions and a reverse phase C_{18} stainless steel column with matching guard column as a stationary phase and a mixture of acetonitrile – water as the mobile phase was used. UPLC analyses were performed using a Shimadzu Ultra High-Performance LC system (Shimadzu, US). UPLC separation was achieved using a Hypersil GOLD C_{18} column (100 x 4.6 mm, i.d., 1.7 µm particle size), maintained at 30℃. The Shimadzu operating pressure was at 6000 psi using a binary gradient pump to deliver the solvent mixtures into the column. Isocratic elution and a run time of twelve minutes. The mobile phase was a mixture of water in solvent A and acetonitrile in solvent

B, flow rate of 1.0mL/min making the pumps to deliver 0.60mL/min for solvent A and 0.40mL/min for solvent B. Mobile phase was degassed automatically upon setting the parameters. The injection volume was set at 10.0 µL. Determination was performed using a Shimadzu multiwavelength detector (MWD)

3.2 Materials

Pesticide standards (diazinon, thiamethoxam, aldicarb, simazine, carbofuran, carbaryl, diuron and propiconazole) were purchased from Sigma-Aldrich chemical company (St.Louis, MO) (Chemical structures shown in Figure 1). Working standards solutions were prepared by dissolving original stock solutions in acetonitrile and diluting to 50 ppm. An internal standard solution was prepared by dissolving 20 mg of diuron in acetonitrile and diluting to 8 ppm with acetonitrile. The internal standard was used to compensate for possible loses encountered during preparation steps.

Acetonitrile (HPLC grade) were obtained from Fisher Chemical. All the solvents were filtered prior to use. Ultra-pure water (18Ω) was prepared in the Biochemistry laboratory at Kennesaw State University using a Milli-Q water purification system. PSA/Carbon cartridges and sorbents were purchased from Waters Corporation (Milford, MA). A 0.45 µm PTFE filter (Fisher brand) was used for the filtration of the extraction solvent and pesticide standards in acetonitrile. QuEChERS sorbent (composed of homogeneous mixture of sodium acetate and magnesium sulfate), vortex instrument (Vortex Genie 2), Rotavapor (B.U.CHI), Eppendorf centrifuge 5430 R, weigh balance (Mettler Toledo), spatula, mortar/pestle, and pellet presser.

Gerber Banana baby food pellets were purchased from Walmart Store in Kennesaw Georgia.

3.3 Experimental methods

3.3.1 Structural comparison and pesticide detectability using FTIR and LIBS

Infrared spectra of all eight pesticides were collected using Spectrum v5.0.2 software was used to produce the spectra for exporting to MS Excel. A force gauge setting of 70-75 was used to reduce noise observed in lower settings. Sample collection was done over the mid infrared range (4000 – 650 cm^{-1}) at a resolution of 4cm^{-1} averaged over seven spectra per sample batch. These settings produced a spectrum with 3351 wavenumbers (variables). Initially, spectra for each of the samples were obtained singly; the first batch obtained was one spectrum per pesticide standard, then subsequent 9 spectra per sample without reloading. Spectra were recorded in the absorbance mode. When samples are analyzed without reloading, instrument precision is put to test as well as real life sampling in an analytical field where only few samples will be available. Spectra averaging per sample batch are done to increase the signal to noise ratio that can be observed when only one scan is obtained. With the use of a spatula, a small scoop of each original pesticide standards was placed on a clean FTIR-ATR crystal beam and wiped away using a Kim-wipe, which removed contaminants left as a result of cleaning with methanol; an additional scoop was then placed on the crystal beam for identification. After the spectra were collected for each of the eight pesticide samples (aldicarb, thiamethoxam, diazinon, diuron, carbaryl, carbofuran, simazine and propiconazole) with the above settings, spectra data obtained were analyzed by MS Excel and PCA.

For FTIR/ATR experiments, about ten grams of homogenized Gerber baby food was spiked with five concentrations of Diuron pesticide (0.1 ppm, 0.5 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm). Infrared spectra of unspiked homogenized Gerber baby food and pesticide-spiked Gerber baby food was collected. Five spectra per sample batch were collected without reloading. Using a

spatula, a small portion of both unspiked Gerber banana sample and pesticide-spiked Gerber banana samples were placed on a clean FTIR/ATR crystal for identification after using a Kim-wipe and methanol to remove any contaminants that must have accumulated on the crystal. After the spectra were collected for each of the six sample groupings (unspiked homogenized Gerber banana, 0.1 ppm, 0.5 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm pesticide-spiked homogenized Gerber banana) with the above instrument settings, spectra data obtained were analyzed by MS Excel and PCA.

For LIBS experiments, about one gram of grounded baby food was spiked with three levels of concentrations of Diazinon pesticide (20.0 ppm, 40.0 ppm and 50.0 ppm) and left to dry for few hours so that the Diazinon was sufficiently absorbed into the powdered Gerber banana prior to conversion into pellet using a pellet maker. The ground powder was pelletized to provide a uniform surface to obtain uniform LIBS emission. LIBS spectra of unspiked and pesticide-spiked banana baby food were collected LIBS use a short laser pulse to create micro plasma on the sample surface. The Q-switch delay time was optimized at 4.0 following a precision study carried out on the various delay times. The emitted light from plasma was collected by a collimating lens and transferred to a broadband spectrometer measuring emission lines from 200 – 965 nm with a spectral resolution of 0.1 nm. During measurements, the sample holder was manually rastered to provide a fresh surface of a pellet sample. Eight spectra were collected for each of the four sample groupings (unspiked Gerber banana, 20.0 ppm, 40.0 ppm and 50.0 ppm) with the above instrument settings, spectra data obtained were analyzed by MS Excel and PCA. Spectra precision were obtained by calculating %RSD using the formula,

$$\% \ RSD = (S.D/mean) \times 100 \qquad (2)$$

3.3.2 Relative polarities and pH effects on pesticides using reverse phase HPLC-PDA

A mixture of 5.0 ppm pesticide standards were prepared in acetonitrile from the stock standard solution. Several compositions of mobile phase using acetonitrile/ultra-pure water were tried out to achieve resolved peaks of the pesticides. The mobile phase was prepared by degassing and vacuum filtering prior to use. Reversed phase HPLC-PDA was used for the relative polarities study using a C_{18} Luna column at a flow rate of 0.5 mL/min and a column temperature of 30℃. Samples were run under isocratic elution.

For pH effect studies, the mobile phase was made up in different pH phosphate buffer solutions. Purpose was to determine what optimum pH could be used for extraction. Phosphate buffers were prepared using a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate. The mobile phase mixture was degassed, vacuum filtered to get rid of particles and pH was confirmed using a pH meter prior to analysis. The pH for analysis ranged from 2.1 – 10.1 inclusive of the mobile-phase which has a pH of 4.85. A mixture of pesticide standards of 12.5 ppm was run via reverse phase HPLC-PDA using the mobile phase buffered at different pH ranges. The retention time (min) of each pesticide at their optimum wavelength was recorded against the pH been analyzed and a graph was plotted to monitor the changes observed as pH increases.

3.3.3 LODs and LOQs using reverse phase UV-Vis detection

For LOD and LOQ determinations, absorbances of individual pesticides using a range of 0.5 ppm – 10.0 ppm concentration of individual pesticide working standard solution in acetonitrile were obtained using acetonitrile as a blank. The absorbances, measured at λ_{max}, were obtained by

placing the standard solutions in a quartz cuvette and obtaining the spectra using the Cary 4000 Spectrophotometer. The absorbances of individual pesticides plotted against the concentrations to obtain a linear calibration curve. The data analysis tool kit on MS Excel was then used to obtain regression statistics which gives the standard deviation of the response (S_y) and the slope of the calibration curve (S) at levels approximating the LOD. The parameters obtained from the regression statistics was used for the determination of LOQ also. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated as:

$$\boldsymbol{LOD = 3.3\ (S_y / S)} \qquad (3)$$

$$\boldsymbol{LOQ = 10\ (S_y / S)} \qquad (4)$$

3.3.4 Percent recoveries at different levels of concentration using (HPLC-PDA, UPLC-UV data)

Five different calibration standards of a mixture of thiamethoxam, aldicarb, simazine, carbofuran, carbaryl, diuron, and propiconazole were prepared in acetonitrile. Their concentration range was between 2.0 – 12.5 ppm. The linear calibration curve was obtained by plotting peak areas of individual pesticides as a function of the concentration using the internal standard method. Diuron pesticide was used as the internal standard. The internal standard was used to correct for instrument fluctuations from one injection to the next.

For sample preparation, about ten grams of homogenized banana pellet was weighed into a 50 mL centrifuge tube. Ground sample was spiked with an appropriate volume of pesticide standard solution to give a spiking level equivalent to 2.5 ppm, 1.0 ppm and 0.5 ppm respectively. About 30-35 mL of Acetonitrile was introduced into the spiked banana sample, homogenized in the centrifuge tube and shaken thoroughly to prevent clumping which can interfere with the extraction. This step was followed by the addition of pre-weighed QuEChERS sorbents made up

of sodium acetate and magnesium sulfate. The contents were shaken thoroughly for about three minutes. Sample present in the centrifuge tube is then centrifuged at about 4000 rpm for fifteen minutes. The supernatant which contains the analyte of interest present in the acetonitrile layer was then transferred into the round bottom flask for evaporation. This was done to evaporate the organic solvent leaving behind the residue analyte containing the pesticides. The residue analyte was then reconstituted into 2.0 mL of Acetonitrile and vortexed vigorously. About 2.0 mL of the reconstituted sample was filtered using a 0.45 µL microtex filter, transferred into an ampule and analyzed by HPLC-PDA and UPLC/UV. The best mobile phase composition obtained was used to acquire chromatograms for this study. Mobile phase was degassed and filtered using a sonicator and vacuum filter prior to use to get rid of particles that may clog the column during separation analysis. Isocratic elution was employed throughout the analysis at a run time of twenty minutes for both the calibration standards and the spiked food samples of various concentrations. The injection volume was set at 10.0 µl and the eluents were monitored by the UV detection wavelengths at 254 nm for HPLC-PDA and four different wavelengths of 210nm, 220 nm, 248 nm, and 263 nm for UPLC-MWD.

Percent recoveries of pesticides from the banana baby food were determined by four replications at three different spiking levels (2.5 ppm, 1.0 ppm, and 0.5 ppm) using the internal standard calibration curve in Equation 6.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Structural comparison of the pesticides

Structural comparison analysis was important to carry out since all studied pesticides will be subjected to a common extraction method. Figure 6 shows a stacked plot of all the spectra with aldicarb, carbaryl and carbofuran showing spectra similarities at a wavenumber of 1716 cm^{-1}, which depicts the presence of the carbonyl groups functional groups. Aldicarb, carbaryl and carbofuran belongs to the same pesticide class which is known as the carbamates and further reinforces the similarities observed in the spectra. Thiamethoxam shows a distinct spectrum compared to others as there is no vibrational activity between wavenumber 3650 – 1600 cm^{-1}, thiamethoxam belongs to the neonicotinoid class of pesticides and has a distinct chemical structure with the presence of two different electronegative atoms attached to the ring structure as shown in Figure 1. Diuron shows the C-Cl stretching vibration at a wavenumber between 1080 – 1000 cm^{-1} and diazinon with a C-S stretching vibrations present in the region 800 – 600 cm^{-1}. Additional structural comparisons can be further reinforced via ANOVA, correlation coefficients, and PCA.

4.1.1 Analysis of variance and correlation coefficients

Analysis of variance (ANOVA) was used to analyze the IR spectra of all pesticides. Table 3 summarizes the information. For the variation across the pesticides, row 2, show F_{calc} (24.381) value that was higher than F_{crit} (2.010) with 7 degrees of freedom (DF) at 95% confidence level, and a p-value of 0.000 indicating that the spectra across the pesticide are significantly different.

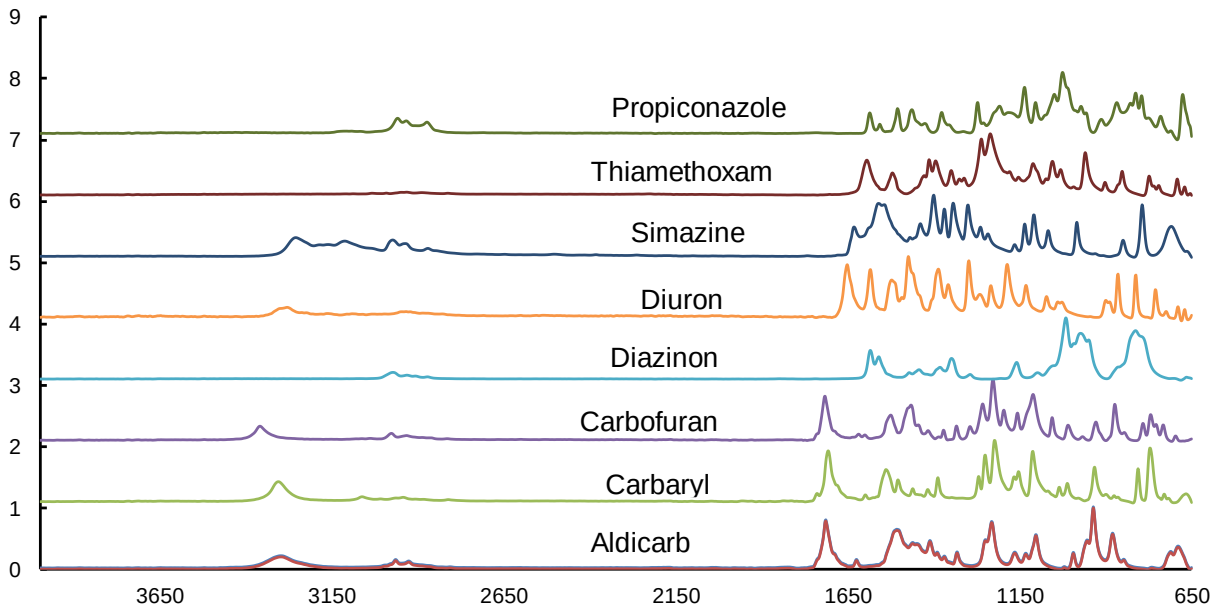


Figure 6. FTIR stacked plot spectra of all pesticide samples. Spectra obtained using Perkin Elmer Spectrum 400 FTIR/ATR for wavenumbers 4000-650cm^{-1}.

The F_{calc}, in rows 3-10, for diazinon (0.006), thiamethoxam (0.023), aldicarb (0.016), simazine (0.021), carbofuran (0.057), carbaryl (0.038), diuron (0.210) and propiconazole (0.015) are all less than the $F_{crit,}$ (1.939) at a 95 % confidence level, 8 degrees of freedom and p-value > 0.989, indicating there are no significant differences between the 8 spectra (each repeated 9 times). Percent RSD obtained for within group analysis is ≤ 5% and depicts a good spectra precision. The correlation values in Table 4, rows 2 – 9, further show the variations present for between group analysis, as they vary greatly in an overall range of least correlated (0.099) to most correlated (0.752). The least correlation shown by carbofuran and diazinon is expected due to the distinct functional groups present in their structure as well as the class of pesticides in which they belong. Carbaryl and carbofuran exhibits the most correlation as expected, this is resulting from the amino functional group present in their structures and both belong to the same class of pesticides (carbamates). Further analysis was carried out to conclude if all pesticides can be subjected to the same extraction method using the PCA.

Table 3. Summary of ANOVA results for the eight different pesticides (Diazinon, Thiamethoxam, Aldicarb, Simazine, Carbofuran, Carbaryl, Diuron, and Propiconazole) as well as the pesticides within a group (All Pesticide Types).

Objects	Mean	% RSD	F-Calc	F-Crit	DF	P-Value
All Pesticide Types	0.089	20.917	24.381	2.010	7	0.000
Diazinon	0.063	0.452	0.006	1.939	8	1.000
Thiamethoxam	0.084	0.672	0.023	1.939	8	1.000
Aldicarb	0.084	0.523	0.016	1.939	8	1.000
Simazine	0.122	0.534	0.021	1.939	8	1.000
Carbofuran	0.077	1.018	0.057	1.939	8	1.000
Carbaryl	0.084	0.825	0.038	1.939	8	1.000
Diuron	0.110	1.712	0.210	1.939	8	0.989
Propiconazole	0.092	0.507	0.015	1.939	8	1.000

4.1.2 Principal Component Analysis

When the 72 x 3351 matrix of the eight spectra (each repeated 9 times) was subjected to PCA, the score plot in Figure 7 was obtained and showed that carbofuran, carbaryl, propiconazole, thiamethoxam, and aldicarb are more closely grouped together in the upper left quadrant as compared to diazinon, simazine, and diuron. This strong grouping can be attributed to the presence of the amino and carbonyl functional groups attached to carbofuran, carbaryl, and aldicarb. The rationale for propiconazole and thiamethoxam grouping with the carbamates is not clear. Diazinon, simazine and diuron appears far apart from other pesticides and this observation is also supported by their correlation coefficients, all less than 0.5 versus the other pesticides.

Table 4. Correlation table on the averages for each of the pesticide types for comparison. MS Excel v15.19.1

	Aldicarb	Carbaryl	Carbofuran	Diazinon	Diuron	Prop-iconazole	Simazine	Thia-methoxam
Aldicarb	1.000							
Carbaryl	0.613	1.000						
Carbofuran	0.669	0.752	1.000					
Diazinon	0.141	0.168	0.099	1.000				
Diuron	0.381	0.325	0.460	0.194	1.000			
Propiconazole	0.265	0.371	0.415	0.686	0.385	1.000		
Simazine	0.370	0.296	0.318	0.263	0.534	0.247	1.000	
Thiamethoxam	0.498	0.564	0.590	0.289	0.508	0.502	0.486	1.000

Structurally, the grouping of diazinon, simazine, and diuron displayed in the score plot may be explained as follows:- Diuron contains a phenyl group in its structure linked to a nitrogen atom of a urea group while simazine structure shows the presence of a heterocyclic nitrogen ring which is unique as compared to others. Diazinon uniquely contains O-P=S functional groups, with five-CH_3 groups. Simazine contains a -C-N- ring, whereas diuron contains a -C=C- ring. Therefore, all pesticides can be subjected to the same extraction method as all pesticides fall within the T^2 at 95% confidence level.

4.2 Relative polarities and pH effects on the pesticides

Initially, several mobile phase compositions were tried out to resolve the peaks, starting with 50:50 v/v acetonitrile/water. Finally, a 40:60 v/v acetonitrile/water resolved the seven peaks successfully. Diazinon spectrum was not evident in the chromatogram and this could be as a result of breakdown of diazinon under warm storage temperature as opposed to keeping it refrigerated. Figure 8 shows the relative polarities of all the pesticide studied, thiamethoxam shows a shorter

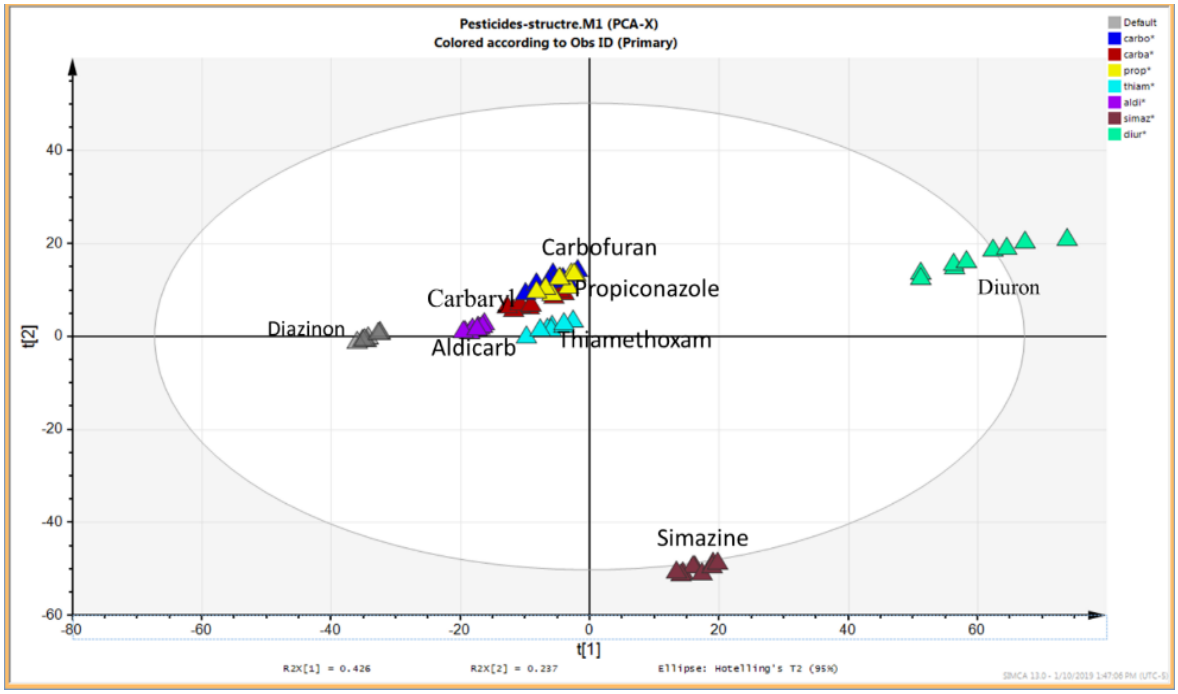


Figure 7. PCA score plot of all pesticides. Hoteling's T^2 at the 95% confidence level: PCA was performed using SIMCA v13.0.2.0.

elution time which depicts a more polar pesticide while propiconazole shows a longer retention time and thus, the least polar pesticide.

The relative polarities exhibited by the pesticides are mainly due to their chemical structure, thiamethoxam structure has oxadiazole-4-imine ring, where rings consist of atoms of different electronegativity (N and O), but in propiconazole, the structure consists of symmetric triazole ring, dioxolidine ring and benzene ring. Propiconazole rings contain symmetric structures and not much polarity is expected. Under the isocratic conditions used, all the pesticides elute within 18 minutes run-time.

Table 5 and Figure 9 show the retention times of pesticides as a function of the buffered mobile phase. Thiamethoxam which is a more polar pesticide shows a shorter retention time than

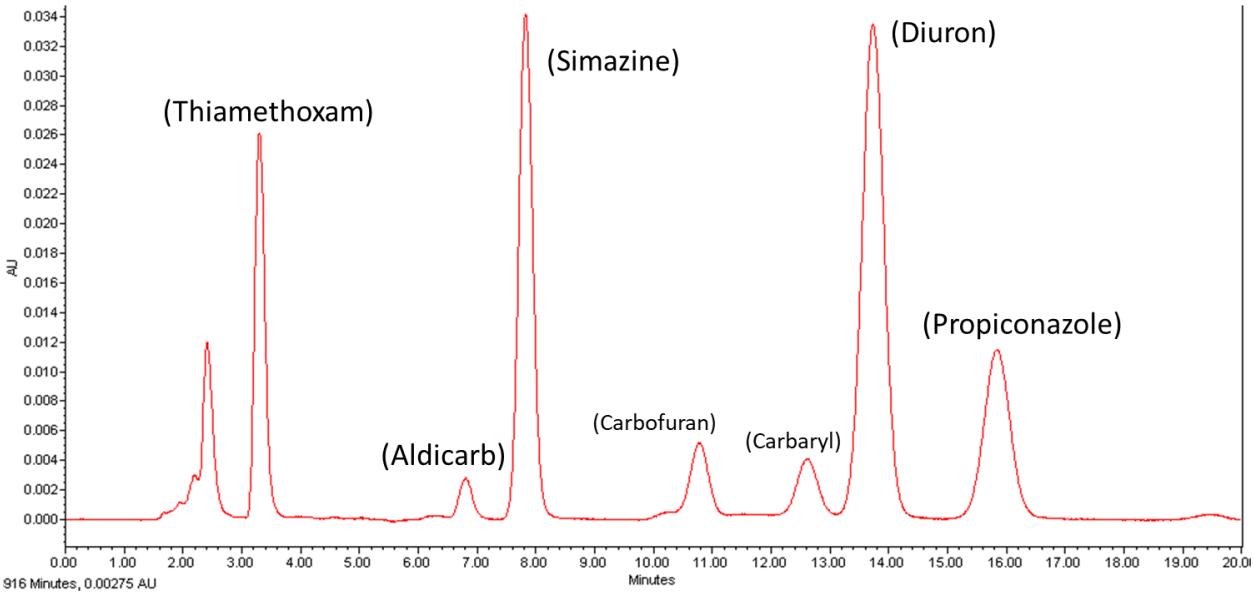


Figure 8. Showing relative polarities of studied pesticides on the HPLC-PDA Chromatogram.

propiconazole which is the least polar with a longer elution time further reinforces the relative polarities as shown in Figure 8. Thiamethoxam, aldicarb and simazine showed little or no fluctuations in retention times at different pHs of the mobile phase. However, carbofuran, carbaryl, diuron and propiconazole showed a decrease in retention time around pH 9 to 10. As shown in Figure 9, if chromatograms are acquired at pH 5.5 for instance, pesticides will show a longer elution time. However, at pH 9-10 there was a slight convergence which gives a clue that extraction of all studied pesticides will be optimum under basic condition. In summary, Figure 9 provides two important pieces of information:

1) All the eight pesticides can be easily resolved when the 40:60 acetonitrile: water mobile phase is buffered around pH 5.5.
2) For effective liquid-liquid extraction of a mixture of the pesticides, a more basic (pH 9-10) acetonitrile would be more suitable. This is probably the reason why in the "QuEChERS" method, sodium acetate is added in the extraction step to set the pH around 9-10.

Table 5. Showing the retention time (min) and the Buffer pH at pesticide optimum wavelengths

λ-max	253	248	222	281	222	251	212
pH	Thiamethoxam	Aldicarb	Simazine	Carbofuran	Carbaryl	Diuron	Propiconazole
2.1	3.342	7.108	7.450	11.588	13.884	15.730	18.079
3.2	3.338	7.066	8.114	11.455	13.711	15.414	17.790
4.3	3.397	7.268	8.319	11.823	14.174	15.845	18.444
4.9	3.505	7.591	8.669	12.558	15.257	17.171	20.101
7.1	3.343	7.078	8.179	11.486	13.708	15.426	17.675
8.0	3.239	6.487	7.431	10.138	11.883	13.043	15.085
9.2	3.150	5.998	6.861	8.988	10.308	11.189	12.640
9.5	3.268	6.646	7.617	10.488	12.337	13.544	15.142
10.1	3.244	6.546	7.489	10.256	12.041	13.159	14.167

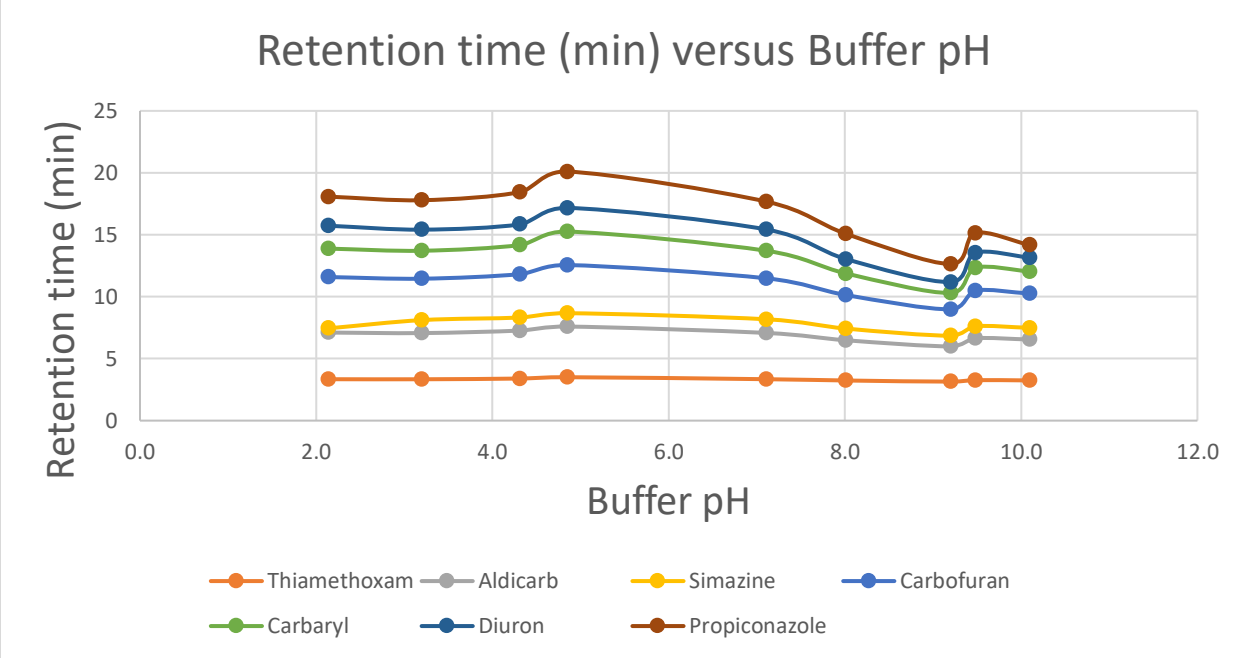


Figure 9. Showing graph of Retention time (min) versus pH of pesticides.

4.3 Presence or absence of pesticides in foods using FTIR/ATR and LIBS

When analyzing a food product for pesticides, it is worthwhile to know beforehand whether the food contains pesticides or not. Figure 10 shows the spectra overlay of unspiked banana sample versus 0.1, and 10.0 ppm pesticide spiked banana. At a vibration mode around 1050cm^{-1}, there is an increase in the intensity of 10.0 ppm spiked concentration. However, not much difference can be observed in the plot thus there is need to subject the spectra collected to statistical techniques using statistics to reveal any underlying differences. ANOVA results in Table 6 summarize the analysis of multiple IR spectra of spiked food at different levels in the 650 – 1800 cm^{-1} range. The "All Groups" row in Table 6 shows $F_{calc} = 8.053$, which is greater than $F_{crit} = 1.430$ with 34 degrees of freedom (DF), at 95% confidence level, and a p-value of 0.000. This is to say that overall, the spiked and unspiked banana samples are significantly different. The F_{calc}, in Table 6, rows 3 – 8, for unspiked banana (0.004), 0.1 ppm pesticide-spiked banana (0.010), 0.5 ppm pesticide-spiked banana (0.027), 2.0 ppm pesticide-spiked banana (0.090), 5.0 ppm pesticide-spiked banana (0.011) and 10.0 ppm pesticide-spiked banana (0.015) are all less than the F_{crit} (2.375) at a 95% confidence level, 7 degrees of freedom and a p-value greater than 0.986, indicating there is good precision within group. Good precision is highly desirable if one is to successfully compare several objects (spectra).

The correlation table (Table 7) summarizes the relationship between the unspiked banana sample and the various spiked concentrations. Recall that the closer a correlation value is to 1.00, the stronger the association. Each sample is correlated with itself in a correlation value of 1.00 and they are found on diagonals in the correlation table. All correlation values are equal to or greater than 0.90 which is indicative of the existence of a strong correlation; the least correlation exists between unspiked banana and 10.0 ppm pesticide-spiked banana (0.987). Correlation coefficients

alone cannot distinguish highly similar spectra. To further identify the variation between these highly correlated samples, PCA was employed.

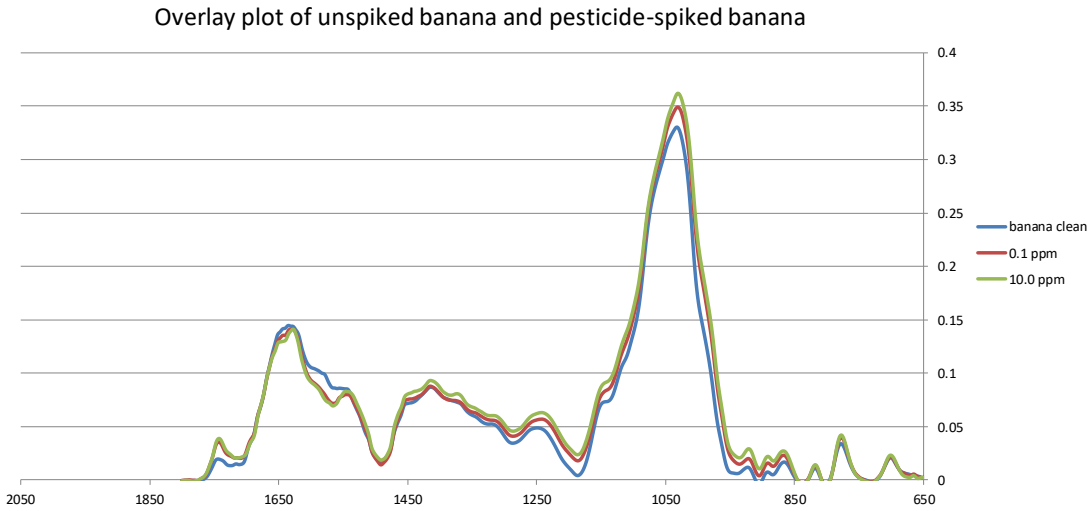


Figure 10. FTIR Overlay plot showing variations of unspiked banana versus concentrations of Pesticide-spiked banana samples (0.1 ppm, and 10.0 ppm) obtained in the IR region 650 – 1800 cm^{-1}.

Table 6. FTIR summary of ANOVA results for the six different samples (Unspiked banana, 0.1 ppm, 0.5 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm pesticide-spiked banana) as well as the samples within a group.

Objects	**Mean**	**% RSD**	**F-Calc**	**F-Crit**	**DF**	**P-Value**
All Groups	0.067	13.263	8.053	1.430	34	0.000
Unspiked Banana	0.064	0.289	0.004	2.375	4	1.000
0.1 ppm Pesticide-spiked Banana	0.069	0.471	0.010	2.375	4	1.000
0.5 ppm Pesticide-spiked Banana	0.071	0.762	0.027	2.375	4	0.999
2.0 ppm Pesticide-spiked Banana	0.073	1.339	0.090	2.375	4	0.986
5.0 ppm Pesticide-spiked Banana	0.074	0.499	0.011	2.375	4	1.000
10.0 ppm Pesticide-spiked Banana	0.073	0.561	0.015	2.375	4	1.000

Table 7. FTIR correlation table on the averages for each of the six different samples (Unspiked banana, 0.1 ppm, 0.5 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm pesticide-spiked banana) for comparison. MS Excel v15.19.1.

	Unspiked banana	0.1 ppm pesticide-spiked	0.5 ppm pesticide-spiked	2.0 ppm pesticide-spiked	5.0 ppm pesticide-spiked	10.0 ppm pesticide-spiked
Unspiked banana	1.000					
0.1 ppm pesticide-spiked	0.992	1.000				
0.5 ppm pesticide-spiked	0.992	1.000	1.000			
2.0 ppm pesticide-spiked	0.988	0.999	0.999	1.000		
5.0 ppm pesticide-spiked	0.989	1.000	0.999	1.000	1.000	
10.0 ppm pesticide-spiked	0.987	0.999	0.999	0.999	1.000	1.000

4.3.1 Principal Component Analysis of the IR spectra

Figure 11 shows the score plot of all six objects (unspiked banana, spiked banana at five levels of pesticide concentration). All the six objects were successfully grouped, with the unspiked banana sample far apart on the right quadrant of the score plot away from the various concentrations of the pesticide-spiked banana samples. The 0.1 ppm spiked sample is nearest to the unspiked banana sample, because it resembles the unspiked banana more than the 10.0 ppm spiked banana. A 0.1 ppm spiked banana can be discriminated from an unspiked banana sample using IR spectra and PCA. Further increase in the spiking concentration as observed with 10.0 ppm and 20.0 ppm shows a farther distance apart from the unspiked banana sample, thus they appear in opposite quadrants of the score plot.

Figure 12 shows the LIBS spectra overlay plot of unspiked banana sample versus 20.0, 40.0, and 50.0 ppm pesticide spiked banana. At a wavelength of around 589nm, there is an increase in the intensity of 50.0 ppm spiked concentration which depicts a high spiking concentration different from the unspiked banana sample. However, not much difference can be observed in the plot thus there is need to subject the spectra collected to statistical techniques using means, ANOVA and correlation coefficients. The variation summaries for LIBS as shown in Table 8, row 2, have F_{calc} (3.846) value higher than the F_{crit} (2.613) with 3 degrees of freedom (DF), at 95% confidence level, and a p-value of 0.000 which is indicative that the means on the averages obtained for the four groups are significantly different.

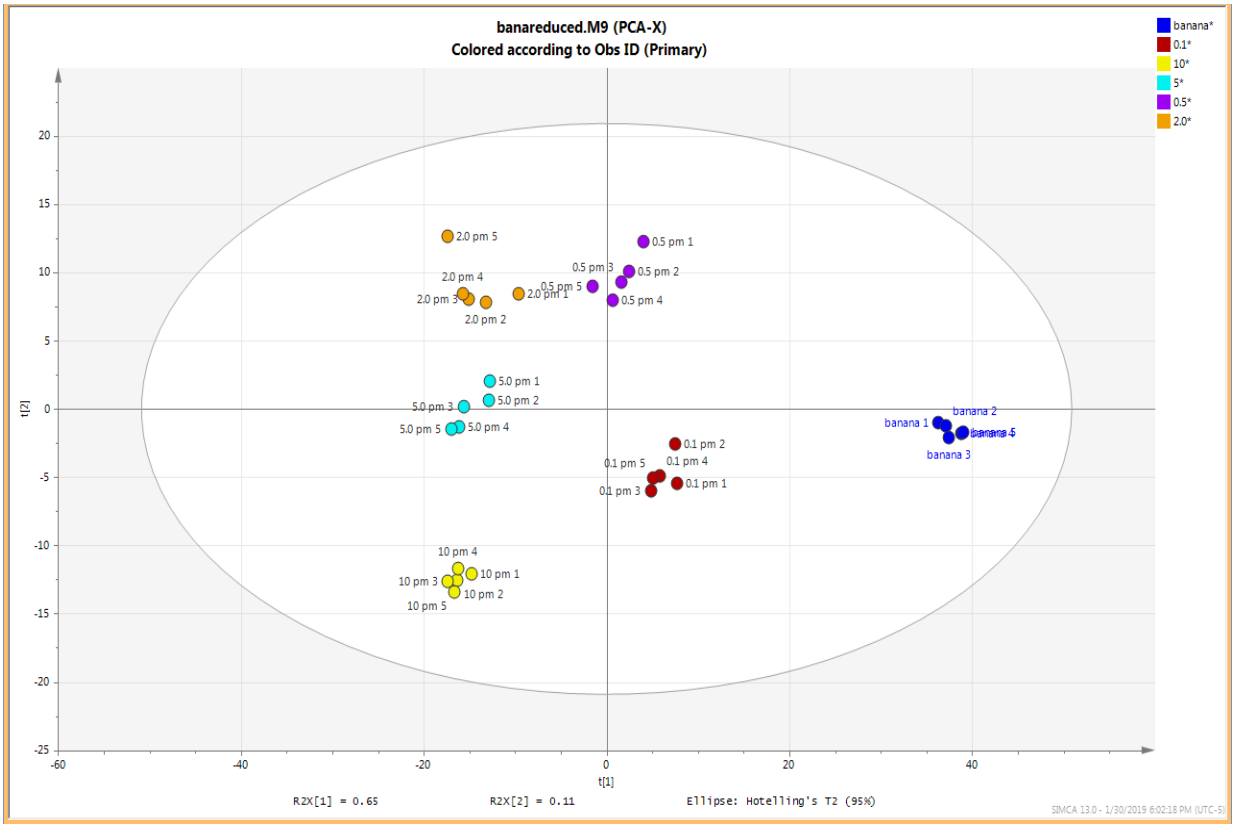


Figure 11. FTIR PCA score plots of the first and second principal components for unspiked banana sample and the various spiking concentrations showing possible discriminations at 95% confidence level: (Unspiked banana, 0.1 ppm, 0.5 ppm, 2.0 ppm, 5.0 ppm and 10.0 ppm pesticide-spiked banana).

The disparities in the means tend to be far apart as the concentration of Diazinon pesticide increases from 40.0 ppm to 50.0 ppm, but not so obvious for 20.0 ppm concentration because the concentration is negligible to cause a significant difference. The F_{calc}, in Table 8, rows 3 – 6, for unspiked banana (1.780), 20.0 ppm pesticide-spiked banana (0.133), 40.0 ppm pesticide-spiked banana (0.335), and 50.0 ppm pesticide-spiked banana (0.086) are all less than the F_{crit} (2.014) at a 95% confidence level, 7 degrees of freedom and a p-value greater than 0.087, indicating there are no significant differences between the 8 spectra collected within each group. In addition, overall % RSD of about 7.00 shows a good spectroscopic precision.

The Correlations in Table 9, rows 2 – 5, further supports the dissimilarities present for the within group analysis. The least correlated of (0.570) to most correlated (0.986) which is again indicative of the concentrations of the pesticide-spiked in the banana sample. Unspiked banana is strongly correlated to 20.0 ppm pesticide-spiked banana because the concentration is negligible to give a distinct correlation. However, at an increased concentration of 50.0 ppm pesticide spiked, there is a least correlation when compared to the unspiked banana. To further highlight the difference, it will require the use of multivariate statistical techniques using the PCA and PLS-DA if possible, for a better visual discrimination between the unspiked banana and the three spiking levels of the pesticide-spiked banana.

4.3.2 Principal Component Analysis (LIBS)

PCA may provide additional information in discerning spectra that show high similarities. Figure 13 is a score plot of unspiked banana sample along with pesticide-spiked banana (20.0 ppm, 40.0 ppm and 50.0 ppm).

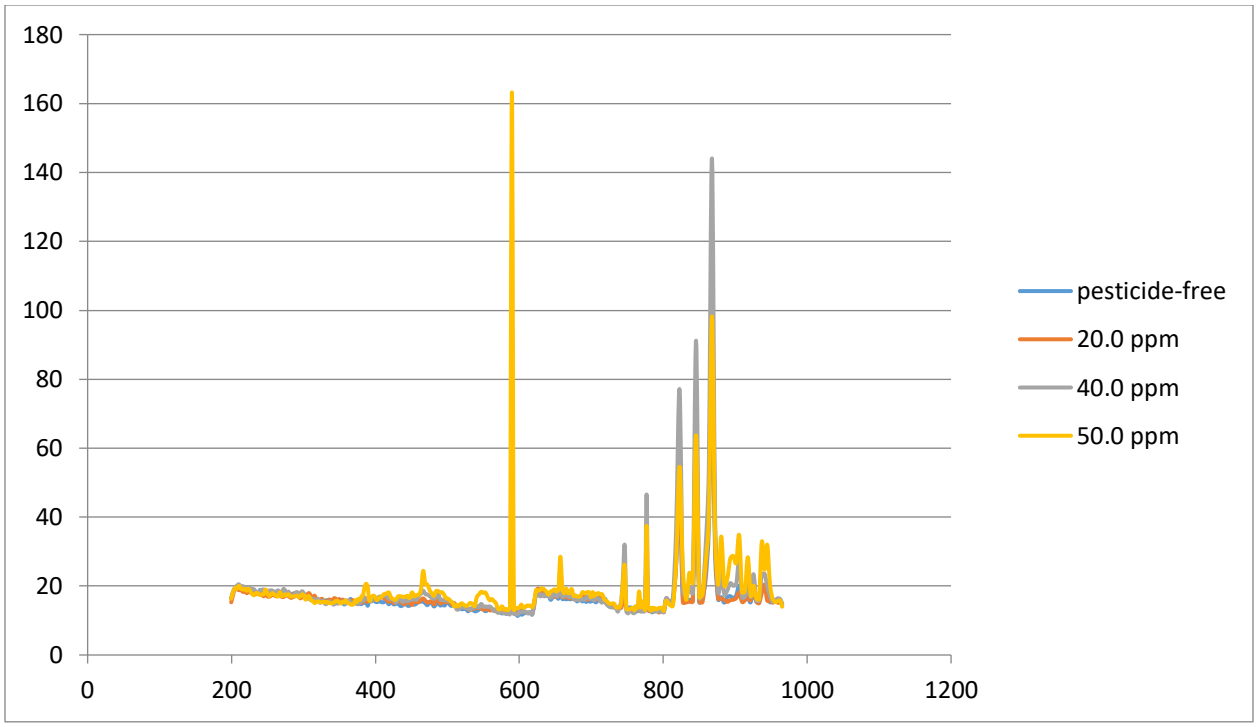


Figure 12. LIBS Overlay plot showing variations of Unspiked banana sample versus concentrations of Pesticide-spiked banana samples (20.0 ppm, 40.0 ppm, and 50.0 ppm) obtained in the UV-Vis region 200 – 965 nm.

Table 8. LIBS summary of ANOVA results for the four different samples (Unspiked banana, 20.0 ppm. 40.0 ppm and 50.0 ppm pesticide-spiked banana) as well as the samples within a group.

Objects	Mean	% RSD	F-Calc	F-Crit	DF	P-Value
All Groups	17.210	6.235	3.846	2.613	3	0.000
Unspiked banana	16.205	3.266	1.780	2.014	7	0.087
20.0 ppm spiked banana	16.481	0.987	0.133	2.014	7	0.996
40.0 ppm spiked banana	17.625	2.195	0.335	2.014	7	0.938
50.0 ppm spiked banana	18.530	1.220	0.086	2.014	7	0.999

Table 9. LIBS correlation table on the averages for each of the four different samples (Unspiked banana, 20.0 ppm. 40.0 ppm and 50.0 ppm pesticide-spiked banana).

	Unspiked banana	20.0 ppm spiked banana	40.0 ppm spiked banana	50.0 ppm spiked banana
Unspiked banana	1.000			
20.0 ppm spiked banana	0.986	1.000		
40.0 ppm spiked banana	0.982	0.978	1.000	
50.0 ppm spiked banana	0.629	0.570	0.683	1.000

Three clusters are evident in the score plot. The “Clean Banana” merging with the 20.0 ppm spiked banana, the 40.0 ppm and 50.0 ppm spiked banana away from the unspiked banana. The scattering of object members in each group may reflect imprecision in the instrument, or heterogeneity in the preparation of the target sample. concentration, the unspiked is close but still well separated on the score plot. At 20.0 ppm concentration, the unspiked is close but still well separated on the score plot. This is because the 20.0 ppm concentration is indistinguishable from the unspiked banana negligible to cause a significant discrimination. By increasing the pesticide concentration to 50.0 ppm, a significant difference is observed as compared to the unspiked banana. At 50.0 ppm pesticide spiked, the discrimination can be well established when compared to the unspiked banana as it appears on the right quadrant of the score plot far apart from the unspiked banana sample.

Both FTIR and LIBS technique showed satisfactory results for detecting presence or absence of pesticides in food. However, FTIR gave a lower level of detectability at 0.1 ppm compared to LIBS level of detectability at 20.0 ppm. Also, FTIR technique showed a better spectra precision and discrimination between spiked and unspiked banana when compared to LIBS and this is as a result of the fluctuations in the laser beam pulse used as an excitation source in the LIBS instrument as well as sample preparation (homogeneous mixture).

4.4 Limit of detection (LOD)

Table 10 summarizes the detection and quantification limits obtained for each pesticide at their optimum wavelength. Under the chromatographic conditions selected and extracting 10 gram of banana baby food, the limit of detection for the studied pesticides ranged from 0.10 mg/kg to 1.40 mg/kg while the limit of quantification varied from 0.3 mg/kg to 4.2 mg/kg. The results obtained for quantification were slightly higher than the maximum residue limits (MRLs) for these

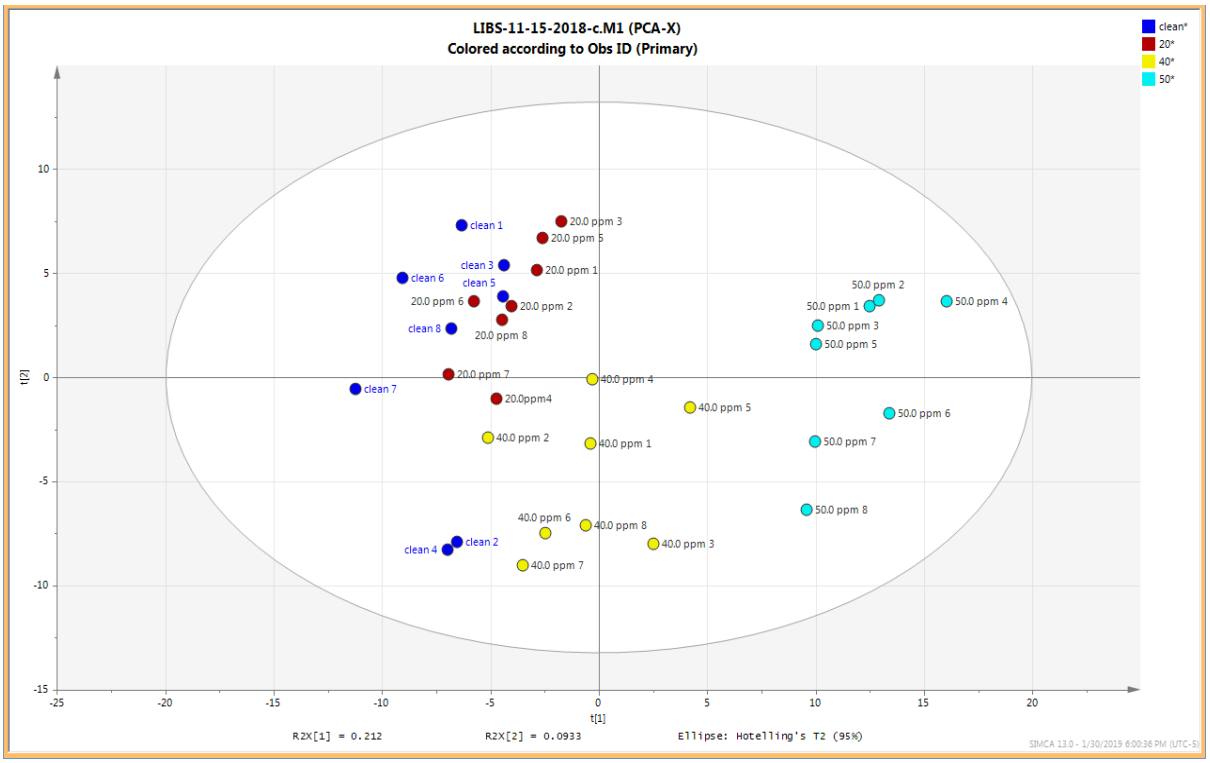


Figure 13. PCA score plots of the first and second principal components for unspiked banana sample and the various spiking concentrations showing possible discriminations at 95% confidence level: 5 total scores per sample, 3351 wavenumbers. (Unspiked banana, 20.0 ppm, 40.0 ppm and 50.0 ppm pesticide-spiked banana).

pesticides established by the USA Global MRL database on fruits and vegetables, which range between 0.10 mg/kg – 5.00 mg/kg. The lowest concentration that can be quantitated using the UV-Vis was obtained at a concentration of around 1.0 ppm. Also, the detector response was linear in the range of concentrations studied, and the correlation coefficients for the pesticides ranged from 0.980 – 1.000 (Table 10). Sensitive detection of pesticides in banana baby food using this method will require the use of more sensitive instrumentation such as LC-MS/MS.

Table 10. Showing Optimum wavelengths (nm), limits of detection (LOD), limits of quantification (LOQ), and calibration data of the studied pesticides.

Compound	λ max (nm)	Slope	R^2	LOD (mg/kg)	LOQ (mg/kg)
Thiamethoxam	253	0.061	1.000	0.1	0.4
Aldicarb	248	0.010	0.996	0.8	2.5
Simazine	221	0.166	0.997	0.3	0.8
Carbaryl	215	0.269	0.980	1.4	4.2
Carbofuran	222	0.014	1.000	0.1	0.4
Diuron	251	0.111	0.999	0.2	0.7
Propiconazole	220	0.062	0.991	0.1	0.3

4.5 Percent recoveries at different levels of concentration using HPLC-PDA and UPLC-UV

Acetonitrile extraction with different clean up procedures were used for the analysis of pesticides in banana baby food sample. Using the QuEChERS procedure with the SPE clean-up, we encountered strong interferences compromising both identification and quantification of the pesticides at lower concentration levels. However, the modified QuEChERS procedure with the LLE clean-up and pre-concentration step gave a satisfactory percent recovery and was effective in the removal of interference during the extraction process. The sodium acetate which is a conjugate base was used during the extraction process and made up for the stability of basic pH sensitive analyte.

The HPLC/PDA and UPLC-MWD optimized conditions were employed for the analysis of percent recovery. Generally, researchers have made use of acetonitrile for the extraction of pesticides in fruits and vegetables solely because of the solvent's high polarity.[26-28] Other commonly used nonpolar solvents for polar pesticide extraction include chloroform, toluene, hexane and dimethyl ether.

Table 11 and 12 summarizes the average percent recovery of all pesticides at a spiking level of 0.5, 1.0 and 2.5 mg/kg from banana baby food. Average recoveries obtained were

satisfactory and ranged from 62.30 to 123.0 % and 85.90 – 115.1 % using HPLC-PDA and UPLC-UV respectively. Also, the precision obtained after four repeated analysis were acceptable as % RSD was ≤ 20. Percent recovery obtained for simazine using HPLC-PDA was low and this can be a result of the spectra overlap observed at the monitoring wavelength between the spectrum of dodecenyl succinic anhydride present in the banana baby food and simazine pesticide as shown in Figure 14. Percent recovery results obtained using UPLC-MWD gave a better precision as compared to analysis done using HPLC-PDA and this can be attributed to the fact that UPLC operates under a higher pressure (psi) and produces narrower peaks with better resolutions. Also, simazine showed a significant increase in percent recovery using the UPLC-UV. This may likely be since wavelengths selected during analysis using the multiwavelength detector subdued the interference caused by dodecenyl succinic anhydride, thus there was no peak eluting closely to simazine on the chromatogram.

Figure 15 shows a representative chromatogram of unspiked banana baby food extract and a banana baby food spiked at 0.5 mg/kg analyzed by HPLC with a PDA detector. The chromatographic program used for the separation allows a good resolution of the pesticide mixture under twenty minutes and twelve minutes using the HPLC-PDA and UPLC-MWD respectively. The chromatogram observed from the blank banana extract showed a peak at a retention time of 9.0 minutes known to be dodecenyl succinic anhydride (DDSA) corn starch which is part of the active ingredients of the banana baby food sample. The peak was obtained by collecting eluates at the retention time and diluting with acetonitrile prior to confirmation using GC-MS, which gave an 87 % match of the identified interferent. No additional interference compounds were present in the extract as the clean-up step was efficient in achieving a satisfactory percent recovery as compared with some literature review articles.[29-34]

Table 11. Showing percent recoveries obtained at three spiking levels using modified QuEChERS method and HPLC-PDA.

n=4			
	2.5 (mg/kg)	**1.0 (mg/kg)**	**0.5 (mg/kg)**
Compound	**Banana**	**Banana**	**Banana**
Thiamethoxam	105.3 ± 6.0	114.6 ± 11.6	107.8 ± 1.5
Aldicarb	104.5 ± 4.2	107.7 ± 11.0	88.5 ± 12.1
Simazine	62.3 ± 19.3	59.6 ± 9.0	55.3 ± 9.3
Carbofuran	100.8 ± 9.1	104.8 ± 6.6	90.3 ± 2.7
Carbaryl	110.8 ± 4.9	123.1 ± 4.6	111.3 ± 5.6
Propiconazole	104.9 ± 8.7	104.2 ± 5.3	98.7 ± 6.7

*Results are mean of four replicates ± % RSD

Table 12. Showing percent recoveries obtained using modified QuEChERS method and UPLC-MWD.

n=4			
	2.5 (mg/kg)	**1.0 (mg/kg)**	**0.5 (mg/kg)**
Compound	**Banana**	**Banana**	**Banana**
Thiamethoxam	97.4 ±0.6	93.8 ±1.8	87.4 ±1.0
Aldicarb	115.1 ±6.6	102.1 ±2.6	88.1 ±2.5
Simazine	91.9 ±1.7	90.5 ±3.1	105.3 ±2.9
Carbofuran	90.0 ±2.4	88.9 ±4.2	85.9 ±4.4
Carbaryl	94.3 ±0.8	93.7 ±5.1	97.7 ±5.9
Propiconazole	97.3 ±1.4	96.7 ±2.8	95.7 ±5.10

*Results are mean of four replicates ± % RSD

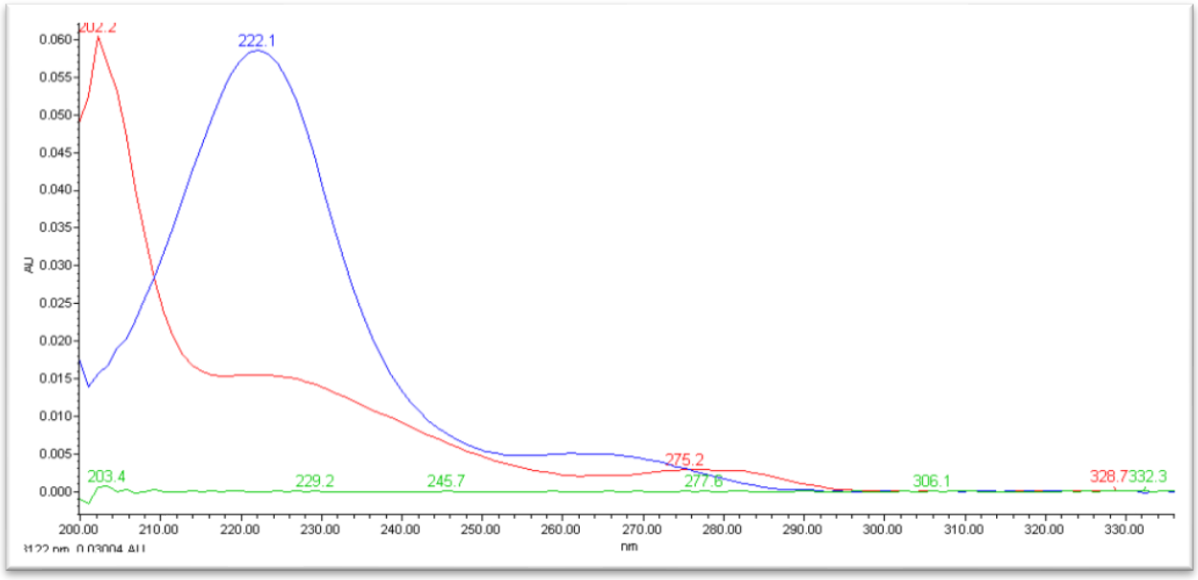


Figure 14. HPLC spectra overlay of dodecenyl succinic anhydride (red) and simazine pesticide (blue).

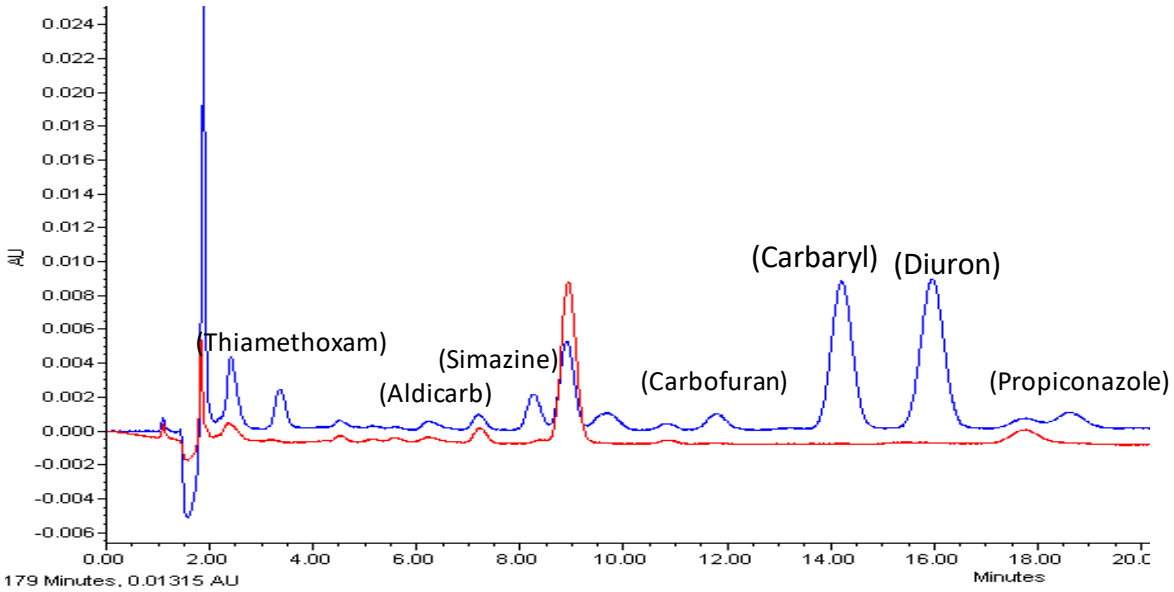


Figure 15. HPLC-PDA chromatogram overlay of unspiked banana baby food extract (red) and banana spiked at 0.5 mg/kg (blue).

CHAPTER 5. CONCLUSION

The HPLC-PDA and UPLC-MWD results for pesticide analysis were successfully developed. Modification of QuEChERS method was simple, suitable and reliable for the successful determination of pesticides in banana baby food at low concentration levels. The modified QuEChERS procedure gave satisfactory percent recoveries and data precision compared to the results obtained from the Georgia Department of Agriculture (Tifton, GA). When determining pesticide of different classes, it is important to carry out a structural comparison and pH study to ensure that the proposed extraction method can accommodate for all pesticides. The study of relative polarities and pH-dependence of retention times assisted in the optimization of the extraction method. A significant advantage was that UPLC-MWD gave a shorter analysis time and better peak resolution as compared to HPLC-PDA which gave a longer analysis time. Also, percent recovery data obtained using UPLC-MWD gave better data precision as compared to HPLC-PDA. The limit of detection obtained were slightly higher than the MRLs stipulated by the USA Global MRLs database for each pesticide in banana matrix.

Additionally, by combining Chemometrics techniques with spectra precision obtained from unspiked banana baby food and spiked banana baby food using FTIR and LIBS, it was possible to discriminate pesticide-infected food from the un-infected. FTIR and LIBS technique provided an efficient method that is non-destructive and requires no preparation step for establishing the presence or absence of pesticide in baby food sample and PCA provided a strong statistical argument for the visual cluster analysis.

However, there were challenges in the sample preparation step for LIBS analysis. This was rectified using a pellet presser to produce pellets of the same size and height in order to obtain a better spectra precision. Also, with FTIR, detection limit for the discrimination was as low as 0.1

ppm whereas for LIBS the lowest detection limit was at 20.0 ppm. Hopefully, this study in its entirety can be extended to other food products.

References

1.Nicolopoulou-Stamati, P.; Maipas, S.; Kotampasi, C.; Stamatis, P.; Hens, L. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Frontiers in public health **2016**, 4, 148.

2. Anonymous public health.

3. Matzner, S. Introduction; 2018;

4. Repetto R, Baliga S. Trends and patterns of pesticide use: In Pesticides and the immune system; Public Health Risks, 3-8. World Resources Institute; Washington, D.C.: 1996.

5. Department of Environmental Protection. Chemical evaluated for carcinogenicity, Office of pesticides programs. Washington, DC.: 2002.

6. April; 2017 Pesticide Toxicity and Hazard.

7. EPA (US Environmental Protection Agency). 1992. Incentives for Development and Registration of Reduced Risk Pesticides. Federal Register. 57(139):32140–32145.

8. EPA (US Environmental Protection Agency). 1998. Status of Pesticides in Registration, Reregistration, and Special Review (Rainbow Report). Washington, DC: EPA, Office of Pesticide Programs.

9.EPA (US Environmental Protection Agency). 1999. What are biopesticides. Office of Pesticide Program. [Online]

10. Buchel, K. H. (1983). Chemistry of Pesticides, John Wiley & Sons, Inc. New York, USA

11. Anonymous Edwards, Clive D; 2012; Vol. 2, pp 753.

12. States: History; Benefits; Risks Pesticide Usage in the United.

13. Sivaperumal, P.; Anand, P.; Riddhi, L. Rapid determination of pesticide residues in fruits and vegetables, using ultra-high-performance liquid chromatography/time-of-flight mass spectrometry. Food Chemistry 2015, 168, 356-365.

14. Parrilla Vázquez, P.; Hakme, E.; Uclés, S.; Cutillas, V.; Martínez Galera, M.; Mughari, A. R.; Fernández-Alba, A. R. Large multiresidue analysis of pesticides in edible vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy, cheap, effective, rugged and safe methodology followed by gas chromatography–tandem mass spectrometry. Journal of Chromatography A, 2016, 1463, 20-31.

15. Sivaperumal, P.; Anand, P.; Riddhi, L. Rapid determination of pesticide residues in fruits and vegetables, using ultra-high-performance liquid chromatography/time-of-flight mass spectrometry.Food Chemistry 2015, 168, 356-365.

16. Petrarca, M. H.; Fernandes, J. O.; Godoy, H. T.; Cunha, S. C. Multiclass pesticide analysis in fruit-based baby food: A comparative study of sample preparation techniques previous to gas chromatography–mass spectrometry. Food Chemistry 2016, 212, 528-536.

17. Camino-Sánchez, F. J.; Zafra-Gómez, A.; Ruiz-García, J.; Bermúdez-Peinado, R.; Ballesteros, O.; Navalon, A.; Vílchez, J. L. UNE-EN ISO/IEC 17025:2005 accredited method for the determination of 121 pesticide residues in fruits and vegetables by gas chromatography–tandem mass spectrometry. Journal of Food Composition and Analysis 2011, 24, 427-440.

18.Štajnbaher, D.; Zupančič-Kralj, L. Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry. Journal of Chromatography A **2003**, 1015, 185-198.

19. AnonymousContents. Food Chemistry **2009**, 114, vi.

20. LAL, A.; TAN, G.; CHAI, M. Multiresidue Analysis of Pesticides in Fruits and Vegetables Using Solid-Phase Extraction and Gas Chromatographic Methods. Analytical Sciences **2008**, 24, 231-236.

21.Payá, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. Anal Bioanal Chem **2007**, 389, 1697-1714.

22.Fenik, J.; Tankiewicz, M.; Biziuk, M. Properties and determination of pesticides in fruits and vegetables. Trends in Analytical Chemistry **2011**, 30, 814-826.

23. Chang, P.; Hsieh, M.; Chiu, T. Recent Advances in the Determination of Pesticides in Environmental Samples by Capillary Electrophoresis. International journal of environmental research and public health **2016**, 13, 409.

24. Robinson, James W.; Skelly Frame, Eileen M.; Frame II, G. M. Undergraduate Instrumental Analysis, Seventh Ed.; CRC Press: Boca Raton, 2014.

25.Markiewicz-Keszycka, M.; Cama-Moncunill, X.; Casado-Gavalda, M. P.; Dixit, Y.; Cama-Moncunill, R.; Cullen, P. J.; Sullivan, C. Laser-induced breakdown spectroscopy (LIBS) for food analysis: A review. Trends in Food Science & Technology **2017**, 65, 80-93.

26. Nováková, L.; Matysová, L.; Solich, P. Advantages of application of UPLC in pharmaceutical analysis. Talanta **2006**, 68, 908-918

27. Willard, H. H.; Merritt, L. L.; Dean, J. A. Instrumental methods of analysis; Princeton, N.J., Van Nostrand 1965]; 4th ed: 1965

28. Msimanga, H. Z.; Lam, T. T. H.; Latinwo, N.; Song, M. K.; Tavakoli, N. Reduction of interferences in the analysis of Children's Dimetapp using ultraviolet spectroscopy data and target factor analysis. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **2018**, 192, 159-167.

29. Bernardi, G.; Kemmerich, M.; Ribeiro, L. C.; Adaime, M. B.; Zanella, R.; Prestes, O. D. An effective method for pesticide residues determination in tobacco by GC-MS/MS and UHPLC-MS/MS employing acetonitrile extraction with low-temperature precipitation and d-SPE clean-up. Talanta **2016**, 161, 40-47.

30. Melo, A.; Cunha, S. C.; Mansilha, C.; Aguiar, A.; Pinho, O.; Ferreira, Isabel M. P. L. V. O. Monitoring pesticide residues in greenhouse tomato by combining acetonitrile-based extraction with dispersive liquid–liquid microextraction followed by gas-chromatography–mass spectrometry. Food Chemistry **2012**, 135, 1071-1077.

31. Wilkowska, A.; Biziuk, M. Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chemistry **2011**, 125, 803-812.

32. Kaihara, A.; Yoshii, K.; Tsumura, Y.; Nakamura, Y.; Ishimitsu, S.; Tonogai, Y. Multiresidue Analysis of Pesticides in Fresh Fruits and Vegetables by Supercritical Fluid Extraction and HPLC. *Journal of Health Science* **2000**, *46*, 336-342.

33. S Kontou; D Tsipi; V Oreopoulou; C Tzia Determination of ETU in Tomatoes and Tomato Products by HPLC-PDA. Evaluation of Cleanup Procedures. *Journal of agricultural and food chemistry* **2001**, *49*, 1090-1097.

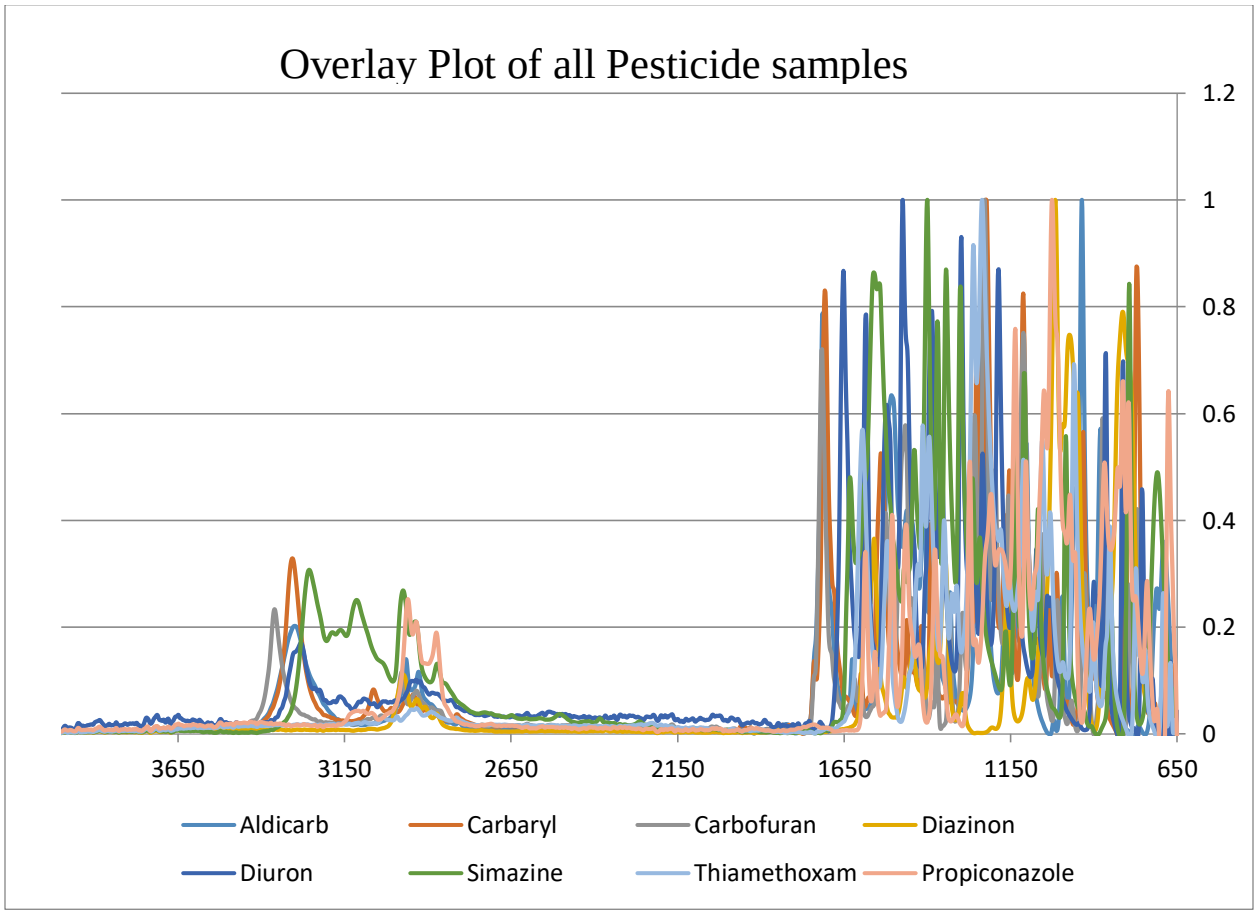
34. Hirotaka Obana; Msahiro Okihashi; Kazuhiko Akutsu; Yoko Kitagawa; Shinjiro Hori Determination of Acetamiprid, Imidacloprid, and Nitenpyram Residues in Vegetables and Fruits by High-Performance Liquid Chromatography with Diode-Array Detection. *Journal of agricultural and food chemistry* **2002**, *50*, 4464-4467.

APPENDIX A

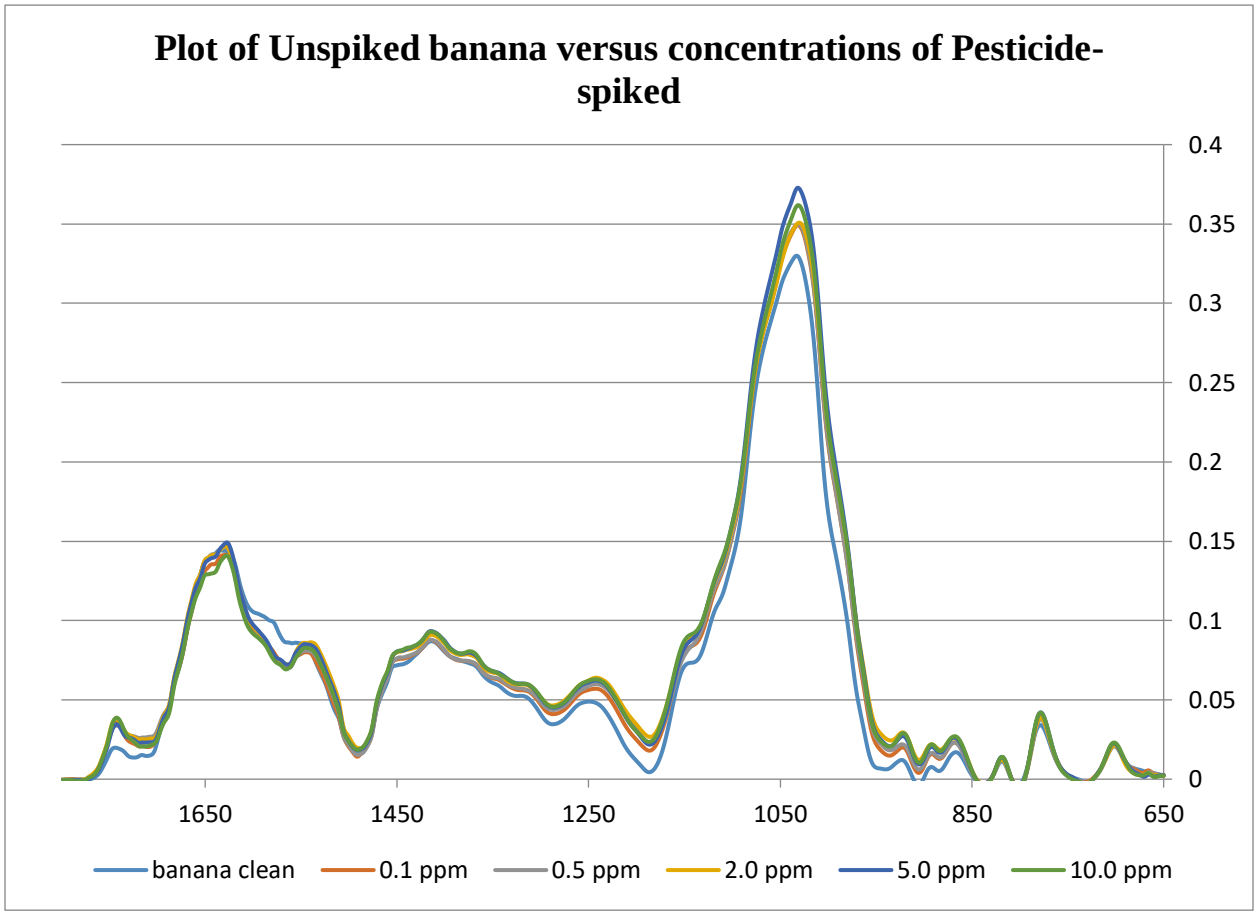
A.1. Selected pesticides and their % recoveries from Georgia Agriculture Laboratory, Tifton Georgia

Pesticide(s)	**Bananas (% Recovery)**
Aldicarb	84-99.7
Carbofuran	7.9-130.7
Diuron	65.8-113
Propiconazole	72.6-146.0
Simazine	85.4-108.9
Thiamethoxam	84.3-114

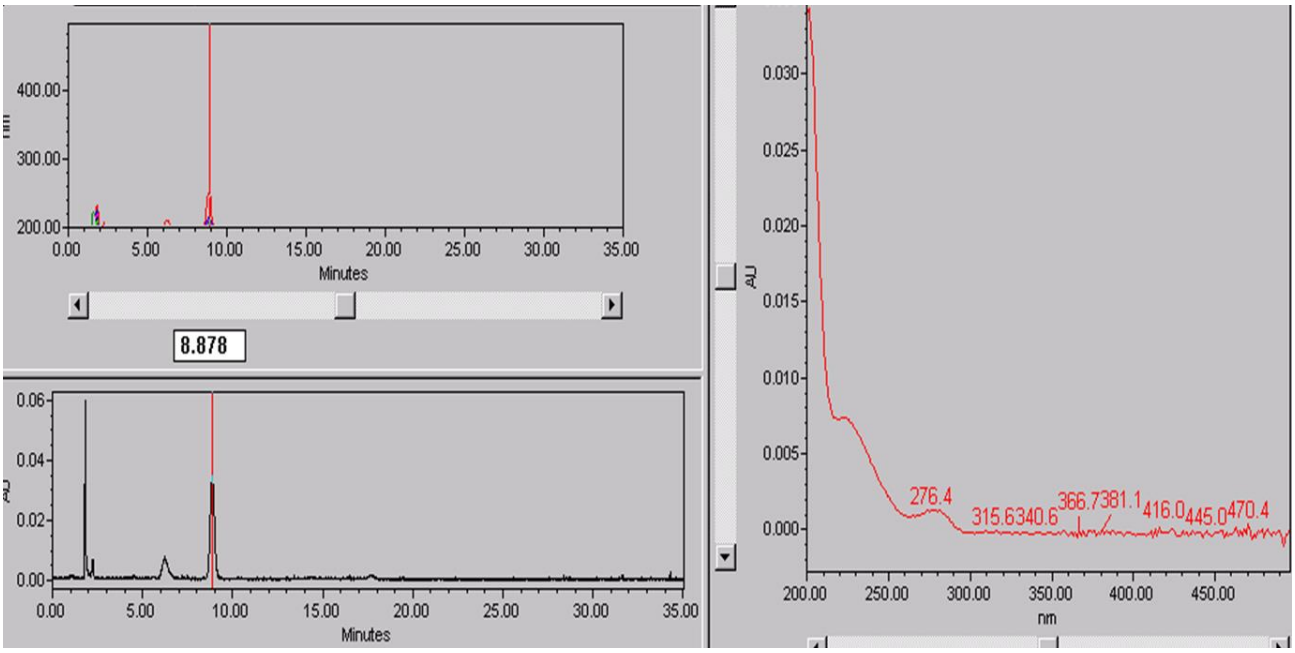
A-2. Overlay Summary Plot of FTIR spectra of the pesticide samples, for visual comparison using a Perkin Elmer Spectrum 400 FTIR/ATR for wavenumbers 4000-650cm^{-1}.



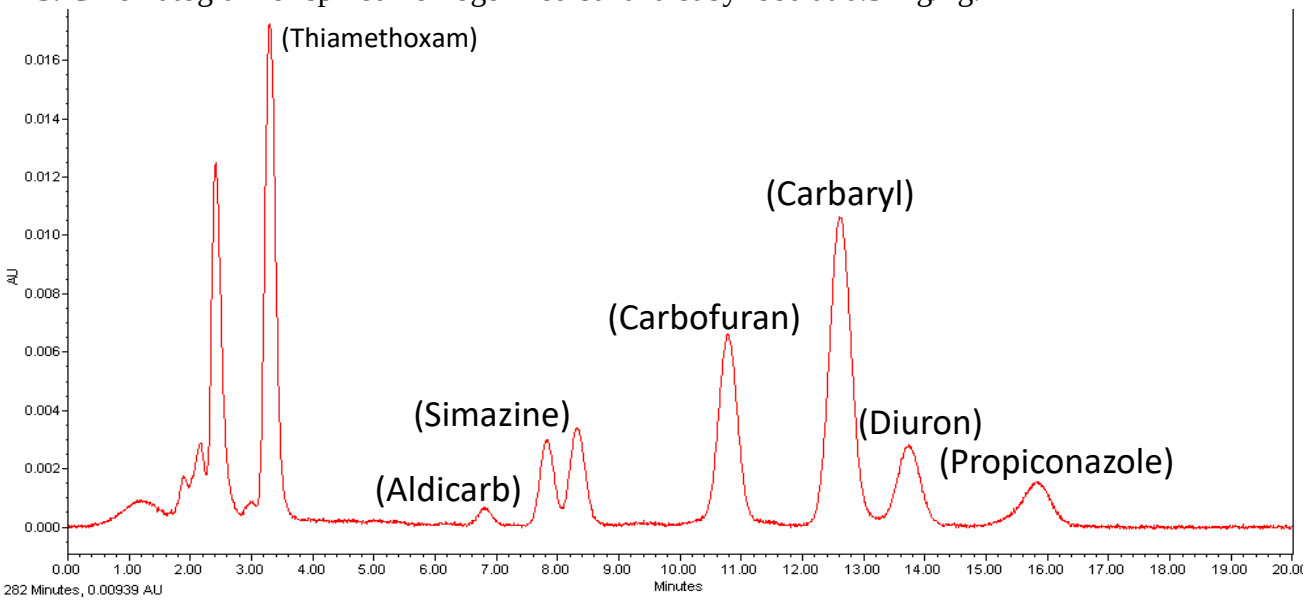
A-3. Overlay Summary Plot of FTIR spectra of homogenized unspiked banana sample and various concentrations of pesticide-spiked banana sample.



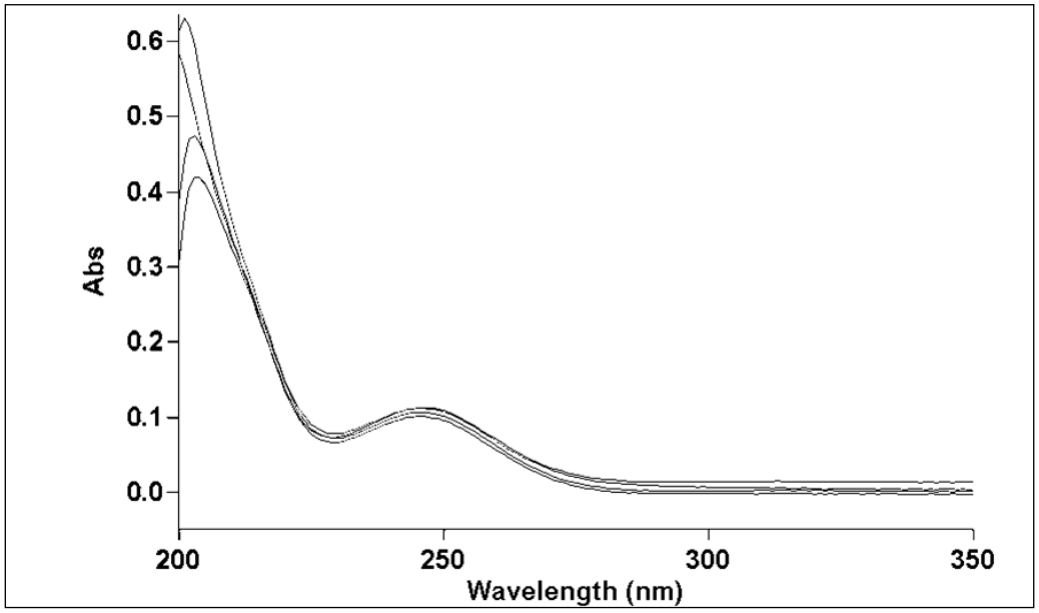
A-4. Chromatogram and spectrum of blank homogenized banana sample under established HPLC/PDA conditions.



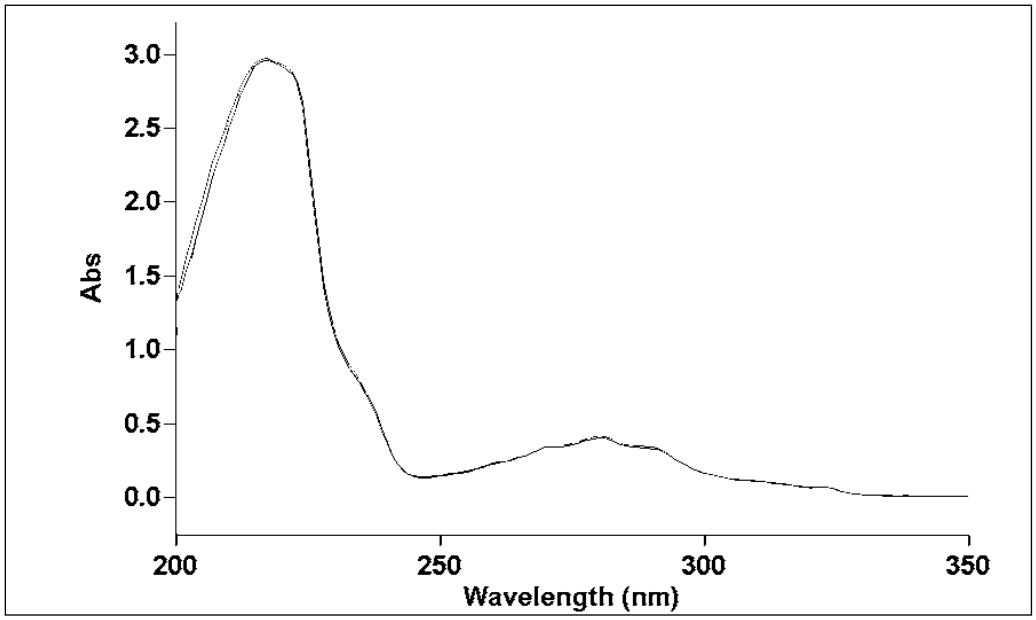
A-5. Chromatogram of spiked homogenized banana baby food at 0.5 mg/kg.



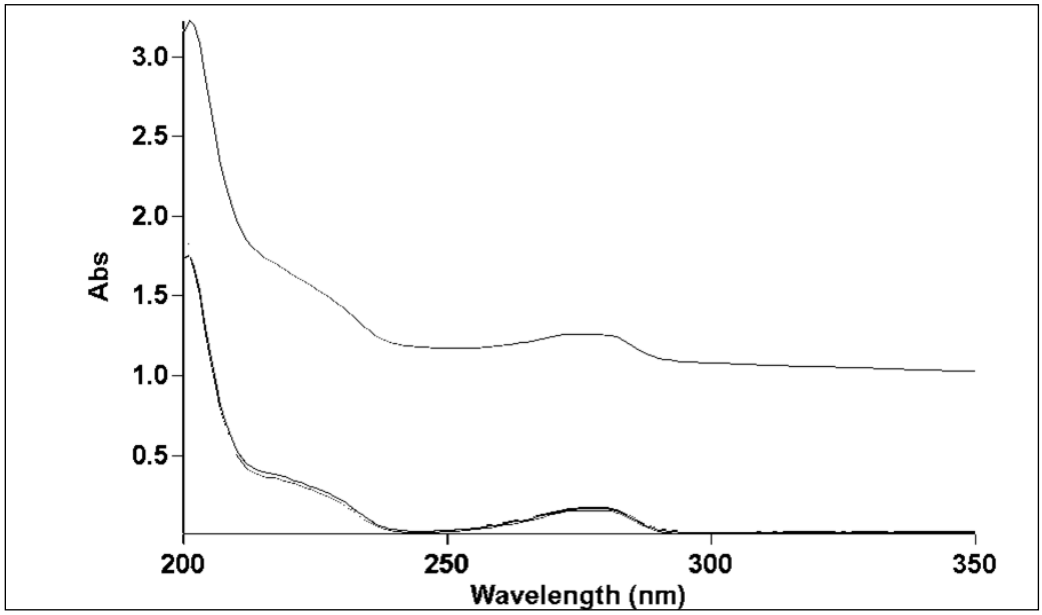
A-6. UV-Vis absorption spectra of Aldicarb pesticide.



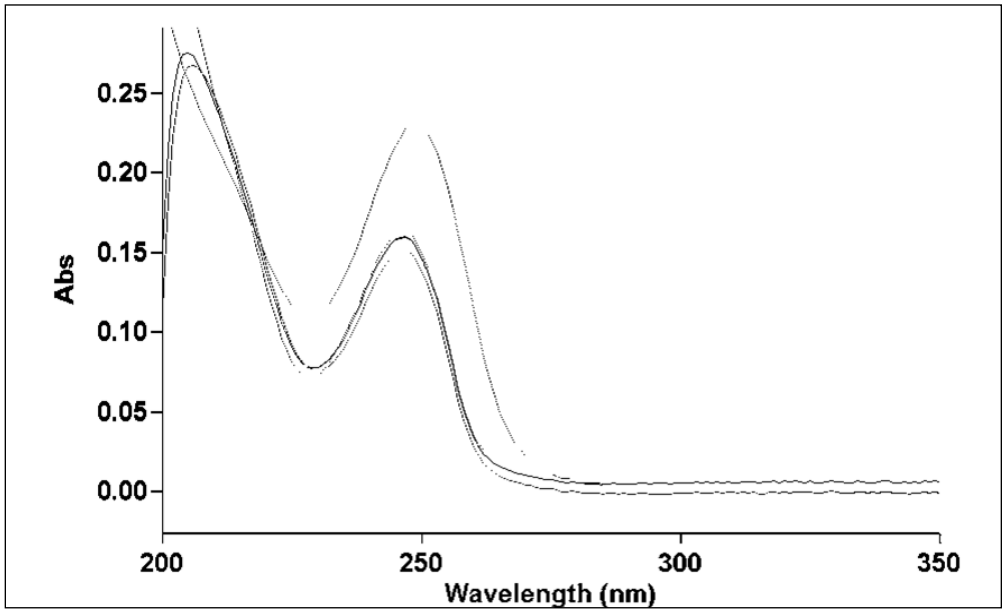
A-7. UV-Vis absorption spectra of Carbaryl pesticide.



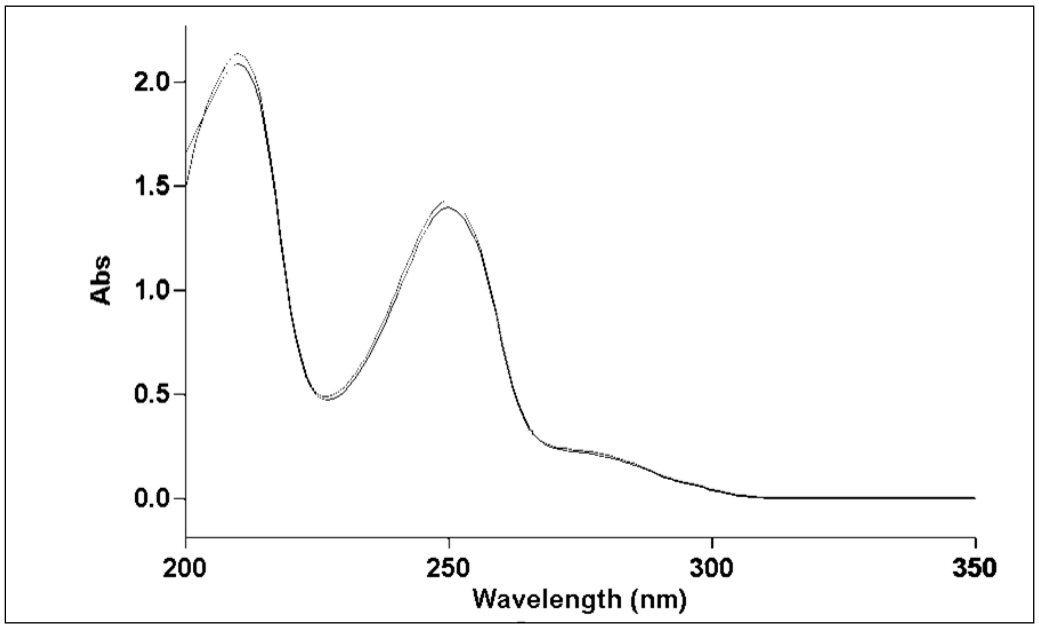
A-8. UV-Vis absorption spectra of Carbofuran pesticide.



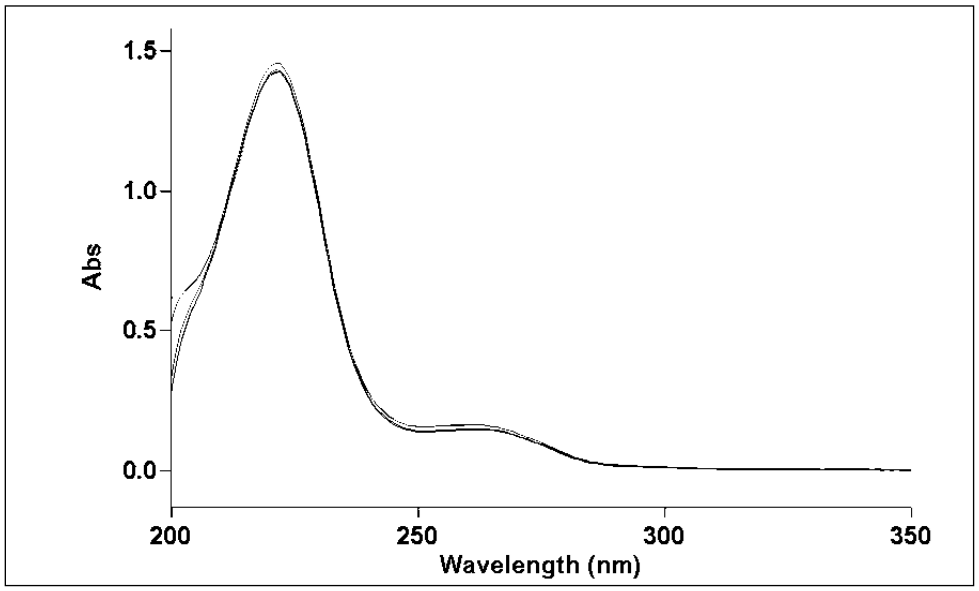
A-9. UV-Vis absorption spectra of Diazinon pesticide.



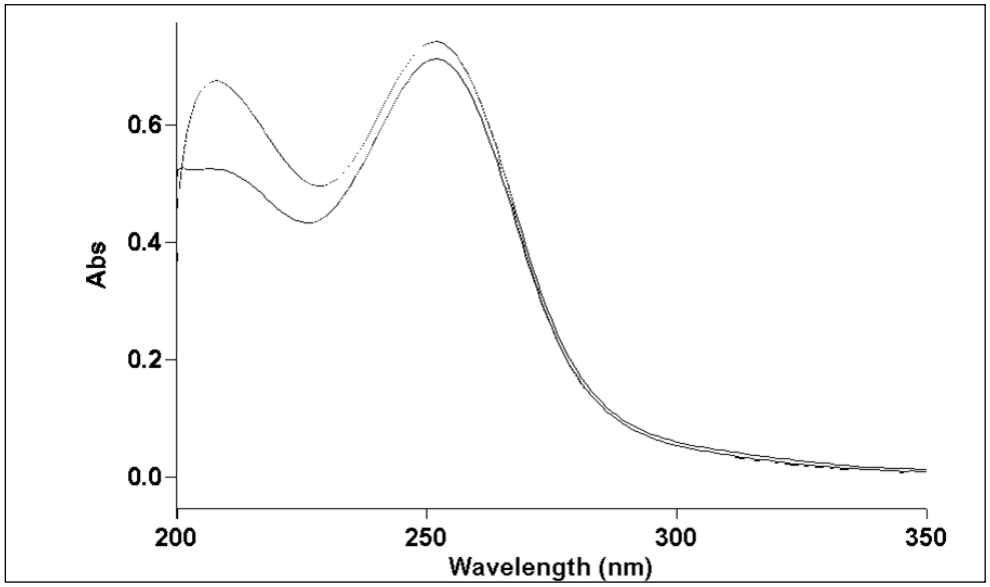
A-10. UV-Vis absorption spectra of Diuron pesticide.



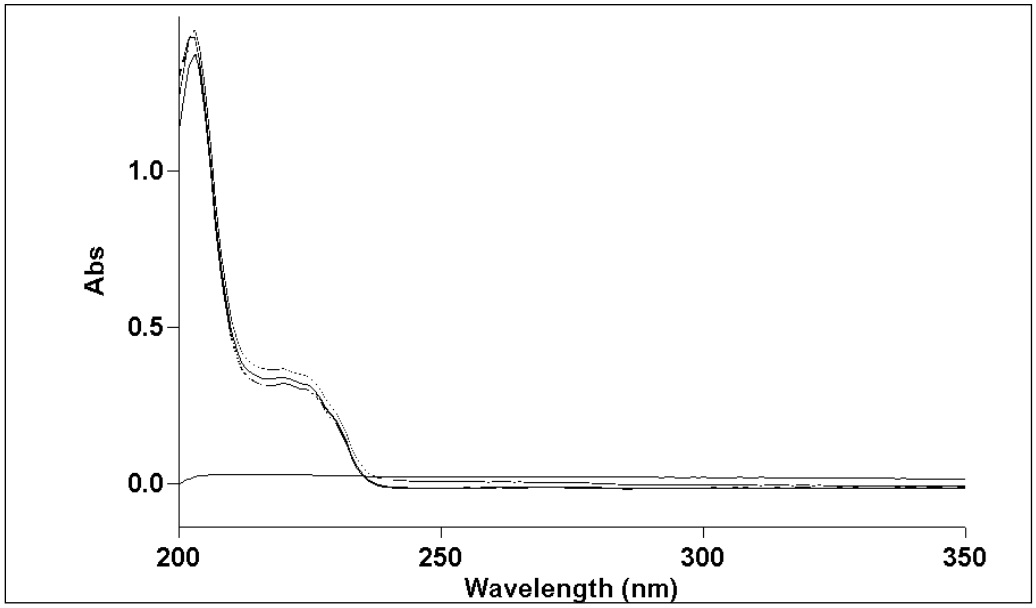
A-11. UV-Vis absorption spectra of Simazine pesticide.



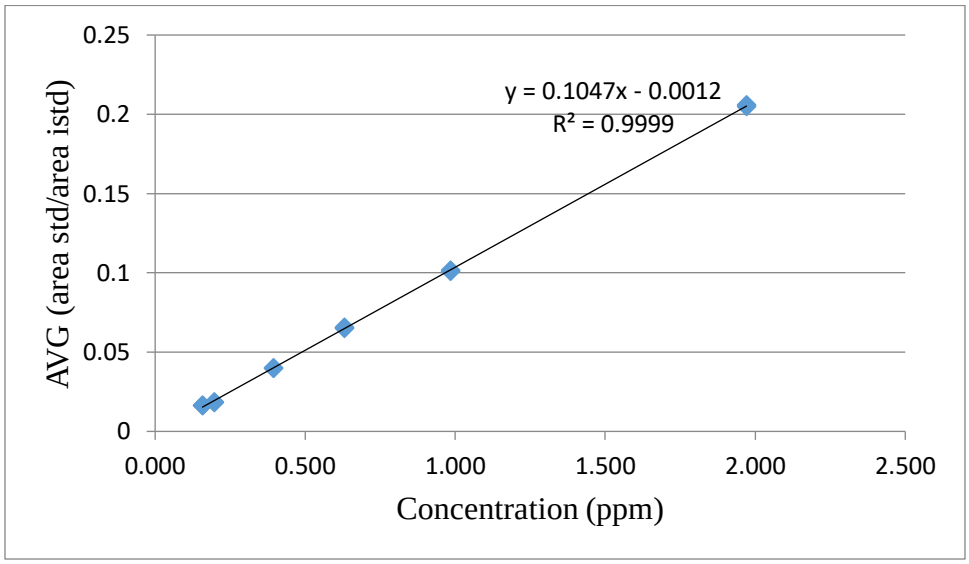
A-12. UV-Vis absorption spectra of Thiamethoxam pesticide.



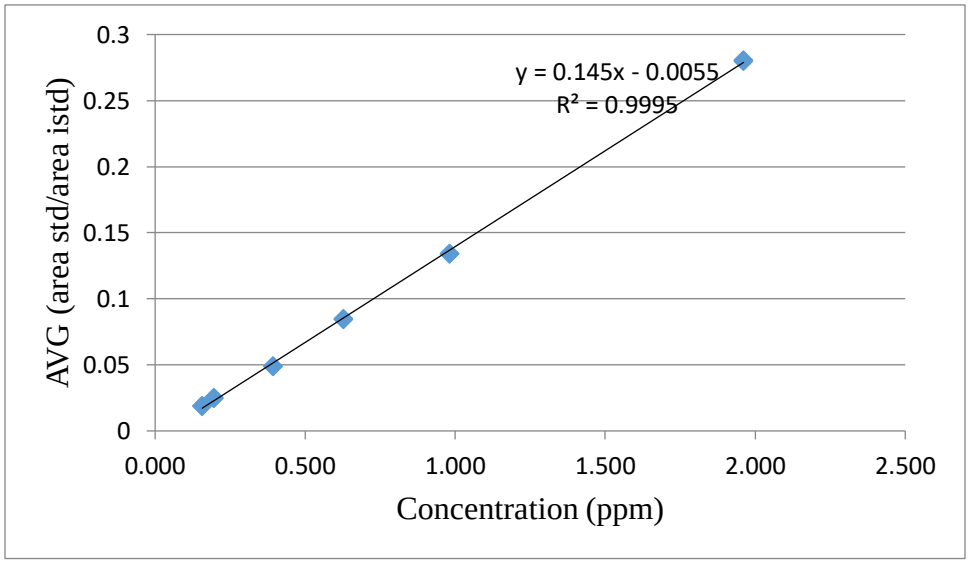
A-13. UV-Vis absorption spectra of Propiconazole pesticide.



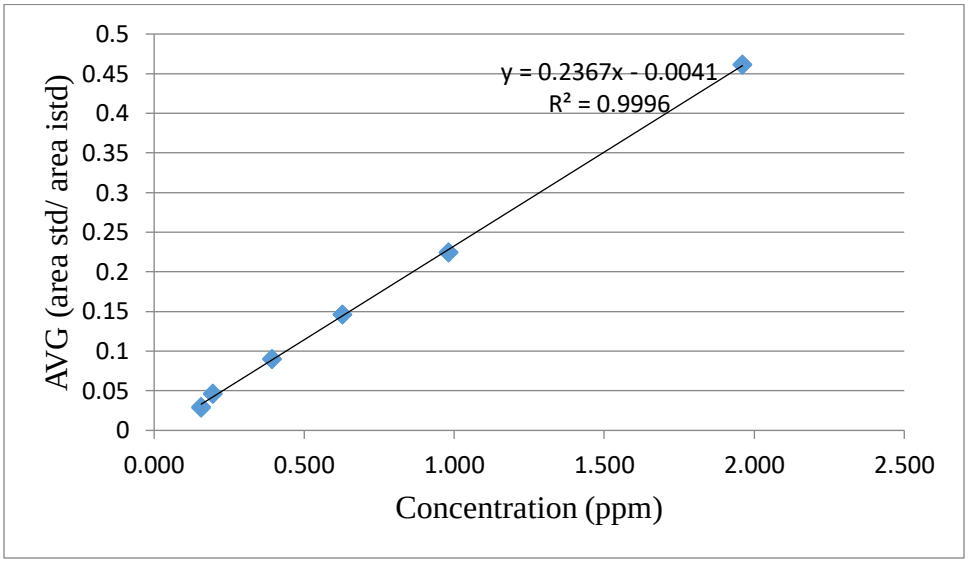
A-14. Calibration curve for Aldicarb pesticide



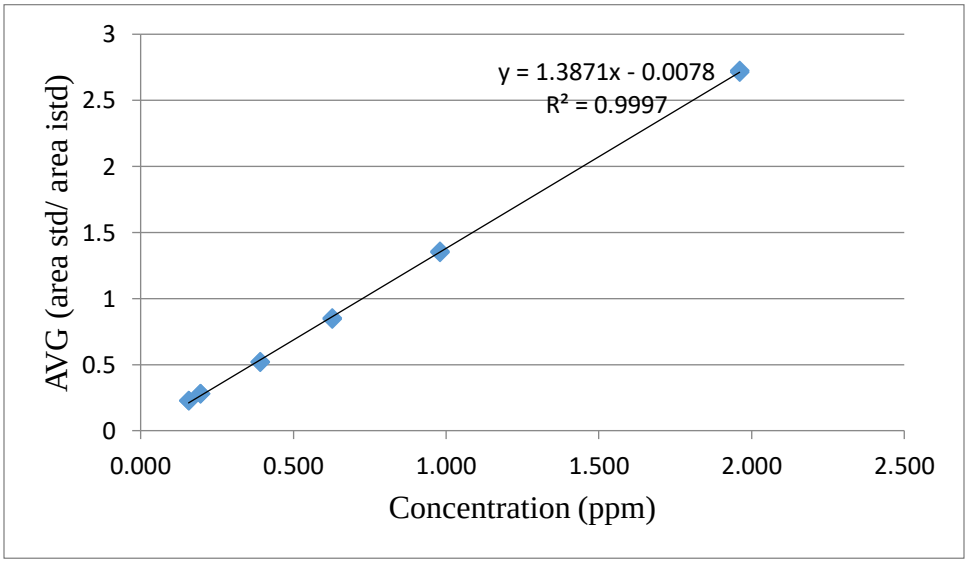
A-15. Calibration curve for Carbofuran pesticide



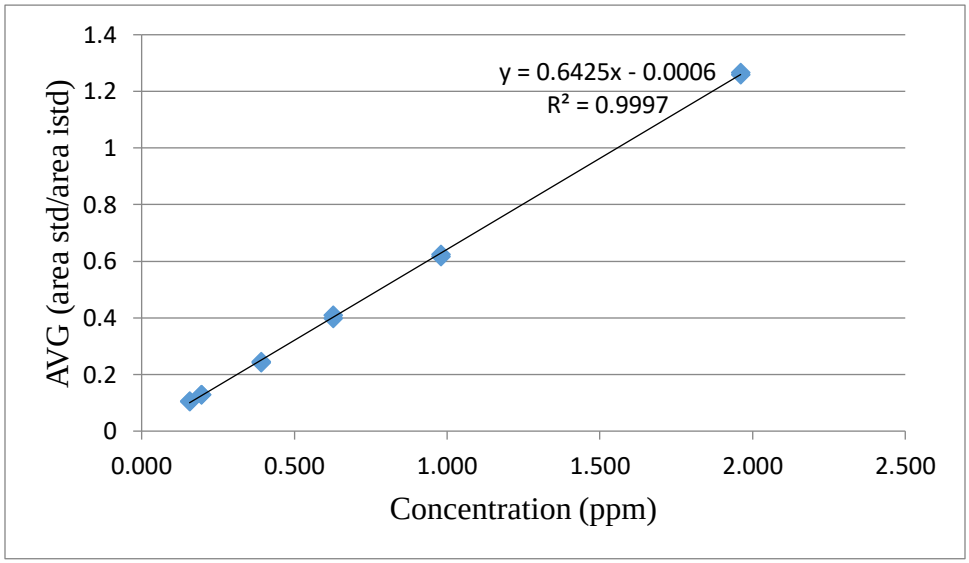
A-16. Calibration curve for Carbaryl pesticide.



A-17. Calibration curve for Simazine pesticide.



A-18. Calibration curve for Thiamethoxam pesticide.



A-19. Calibration curve for Propiconazole pesticide.